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Gastrointestinal helminthic parasites of habituated wild chimpanzees (*Pan troglodytes verus*) in the Taï NP, Côte d'Ivoire – including characterization of cultured helminth developmental stages using genetic markers

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Sonja Metzger

Tierärztin

aus München

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Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Georg von Samson-Himmelstjerna
Zweiter Gutachter:	Univ.-Prof. Dr. Heribert Hofer
Dritter Gutachter:	Univ.-Prof. Dr. Achim Gruber

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Abbreviations

A. lumbricoides	*Ascaris lumbricoides*
A. suum	*Ascaris suum*
AIC	Akaike information criterion
am	ante meridiem
Amp	Ampicillin
B. anthracis	*Bacillus anthracis*
B. mucronata	*Bertiella mucronata*
B. studeri	*Bertiella studeri*
BAER	Baerman concentration
bp	base pair
bv	biovar
C. brochieri	*Capillaria brochieri*
C. brumpti	*Concinnum brumpti*
C. hepatica	*Capillaria hepatica*
C. atys	*Cercocebus atys*
C. diana	*Cercopithecus diana*
C. polykomos	*Colobus polykomos*
Cdian	*Cercopithecus diana*
Chisq	chi-square
cox1 (= COI)	cytochrome oxidase I
Cpoly	*Colobus polykomos*
D. dendriticum	*Dicrocoelium dendriticum*
D. lanceatum	*Dicrocoelium lanceatum*
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
DIR	direct examination
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
DRC	Democratic Republic of Congo
E. anthropopitheci	*Enterobius anthropopitheci*
E. bipillatus	*Enterobius bipillatus*
E. foecunda	*Enterobius foecunda*
E. vermicularis	*Enterobius vermicularis*
EBO-CI	Ebola filovirus sybtype Côte d`Ivoire
E. coli	*Escherichia coli*
e.g.	exempli gratia
epg	eggs per gram feces
et al.	et alii
F	female
fam.	family
FEAS	Formalin-ethyl-acetate sedimentation

FSE	Formol-saline-ether sedimentation
GLMM	generalized liniar mixed model
GRASP	Great Apes Survival Partnership
GTR	global time reversal
H. diminuta	*Hymenolepis diminuta*
H. nana	*Hymenolepis nana*
HKY	Hasegawa, Kishino and Yano
HMFC	Harada-Mori fecal cultures
HMPV	human metapneumovirus
i.e.	id est
IFNy	interferon gamma
ind	individual
individ	individual
ITS	internal transcribed spacer
IUCN	International Union for Conservation of Nature,
JC	Jukes and Cantor
Kato	Kato-Katz method
LB medium	Luria-Bertani medium
LCV	lymphocryptovirus
L1-	first-stage
L2-	second-stage
L3-	third-stage
L4-	fourth-stage
M	male
Mang	mangabey
max.	maximum
McM	McMaster quantitative flotation
ML	maximum likelyhood
Molec	molecular methods
MPI EVA	Max Planck Institute for Evolutionary Anthropology
mtDNA	mitochondrial DNA
MWSF	Modified Wisconsin Sugar Flotation
N. americanus	*Necator americanus*
N. excilidens	*Necator excilidens*
N. congolensis	*Necator congolensis*
nad4 (= ND4)	nicotinamide dehydrogenase subunit 4
NaNO3-FL	Sodium Nitrate Flotation
NNI	nearest-neighbor interchange
no.	number
NP	national park
O. acuelatum	*Oesophagostomum acuelatum*
O. apiostomum	*Oesophagostomum apiostomum*
O. bifurcum	*Oesophagostomum bifurcum*
O. blanchardi	*Oesophagostomum blanchardi*

O. brumpti	*Oesophagostomum brumpti*
O. dentigerum	*Oesophagostomum dentigerum*
O. polydentatum	*Oesophagostomum polydentatum*
O. quadrispinulatum	*Oesophagostomum quadrispinulatum*
O. stephanost.	*Oesophagostomum stephanostomum*
P. badius	*Piliocolobus badius*
P. caucasica	*Physaloptera caucasica*
P. gombensis	*Probstmayria gombensis*
P. gorilla	*Probstmayria gorilla*
P. vivipara	*Probstmayria vivipara*
P. t. ellioti	*Pan troglodytes ellioti*
P. t. schw.	*Pan troglodytes schweinfurthii*
P. t. trog.	*Pan troglodytes troglodytes*
P. t. verus	*Pan troglodytes verus*
Pbad	*Piliocolobus badius*
PCR	polymerase chain reaction
PCV	packed cell volume
pers. comm.	personal communication
ppg	propagules (= eggs + larvae) per gram feces
PtroAdV	*Pan troglodytes* adenovirus
PtvPyV	*Pan troglodytes verus* polyoma virus
rDNA	ribosomal deoxyribonucleic acid
rpm	rounds per minute
rRNA	ribosomal ribonucleic acid
RSV	respiratory syncytical virus
SAF	sodium acetate-acetic acid-formalin solution
SED	Sedimentation
SFVcpz	simian foamy virus (chimpanzee)
SFVwrc	simian foamy virus (red colobus)
spp.	species
SPR	subtree pruning and regrafting
SSC	Species Survival Commission
S. fuelleborni	*Strongyloides fuelleborni*
S. planiceps	*Strongyloides planiceps*
S. ratti	*Strongyloides ratti*
S. stercoralis	*Strongyloides stercoralis*
STLV-1	simian t-cell leukemia virus 1
subfam.	subfamily
T. deminutus	*Ternidens deminutus*
T. retortaeformis	*Trichostrongylus retortaeformis*
T. simiae	*Ternidens simiae*
T. trichiura	*Trichuris trichiura*
TAE	tris-acetate-EDTA
TCP	Taï Chimpanzee Project

U	units
U.S.	United States of America
UNESCO	United Nations Educational, Scientific & Cultural Organization
unident.	unidentified
VDM	volumetric dilusion method
VIF	variance inflation factors
WHO	World Health Organization
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside

I INTRODUCTION

I.1 Background

Chimpanzees (*Pan troglodytes*), our closest living relatives apart from bonobos (*Pan paniscus*), have been studied for decades – in captivity as well as at various field sites (e.g. Gombe/ Tanzania, Mahale/ Tanzania, Taï/ Côte d'Ivoire (Goodall, 1986; Nishida 1990; Boesch & Boesch-Achermann, 2000)). Nevertheless, our current knowledge of the pathogens and parasites of wild chimpanzees in their natural habitat is still remarkably limited (Leendertz et al., 2006a, Gillespie et al., 2008b). While this is particularly true as for the majority of viruses and bacteria which only fairly recently have become a focus of more intense research, considerable knowledge gaps also still exist regarding the gastrointestinal helminths parasitizing different wild chimpanzee populations.

The reasons for these knowledge gaps are manifold. First of all, even to date a great part of our information regarding the spectrum of helminths able to infect chimpanzees is still derived from parasitological studies of captive individuals or populations (e.g. Yamashita, 1963; Myers & Kuntz, 1972) and whilst valuable, the results of such studies are not necessarily transferable to wild chimpanzee populations in their natural environment (Ashford et al., 2000).

Secondly, even though parasitological surveys of a number of wild chimpanzee populations have been conducted, the overall information derived from these studies is somewhat limited. Reason for this is that many of the surveys were more or less only snapshot inventories, based on relatively few, partly unassigned samples collected over fairly short periods of time and thus might potentially have failed to detect seasonal or low prevalence infections. Repeated and more comprehensive surveys by contrast, have so far only been conducted on a few populations (Gombe, Mahale, Kanyawara) of eastern chimpanzees (*Pan troglodytes schweinfurthii*) in Tanzania and Uganda (e.g. File et al., 1976; Kawabata & Nishida, 1991; Huffman et al., 1997; Ashford et al., 2000; Murray et al., 2000; Bakuza et al., 2009; Krief et al., 2005 & 2010; Gillespie et al., 2010).

The species *Pan troglodytes* however comprises four different currently recognized subspecies – *Pan troglodytes schweinfurthii, Pan troglodytes troglodytes* in central Africa, *Pan troglodytes ellioti* in Nigeria and Cameroon, and *Pan troglodytes verus* in West Africa (Oates et al., 2008). Each of these subspecies again consists of a number of – meanwhile often geographically isolated – wild populations which live in a variety of different habitats and climatic conditions, ranging from primary rainforest to woodland savannah and are subjected to varying degrees of human impact (Oates et al., 2008). As a consequence, also the numbers and spectra of gastrointestinal helminths, the different populations are exposed to and infected with, might vary considerably (e.g. McGrew et al., 1989).

Another reason for our still limited knowledge is the inherent difficulty to reliably and unequivocally identify the various gastrointestinal helminths parasitizing wild chimpanzees to species level. Diagnostic deworming, as often performed in humans or domestic animals, is inherently not possible in wild chimpanzees. Hence, the recovery of morphologically more or less distinct adult worms is invariably restricted to opportunistic necropsies of deceased chimpanzees or the incidental finding of shed helminths or worm fragments in collected feces. DNA-based diagnostic methods like polymerase chain reaction (PCR) and sequencing are meanwhile standard practice for the diagnosis of viruses and bacteria and are also increasingly employed for the identification and characterization of helminthic parasites of humans and livestock (e.g. Morgan, 2000; Gasser et al., 2008a+b; Bott et al., 2009). Their application in the field of wildlife- and particularly wild primate helminthology however, appears to be still in its infancy.

As a result, all parasitological surveys of wild chimpanzee populations conducted so far – apart from a few exceptions (Hasegawa et al., 2010; Krief et al., 2010) – were more or less exclusively based on conventional coprological examination techniques. Given the lack of unique, species-specific morphological features of most helminth early developmental stages (e.g. Newton et al., 1998b; Schnieder et al., 1999; Bott et al., 2009), unequivocal identification of the respectively detected helminth types to species or even genus level was therefore largely not possible (e.g. Ashford et al., 2000; Muehlenbein, 2005; Gillespie et al., 2010), particularly if no fecal cultures had been performed as part of the diagnostic process.

But why do we need to know more about the gastrointestinal parasitic helminths affecting wild chimpanzees in their natural habitat at all? Chimpanzees, like all great apes, belong today to the most endangered animals of our planet, and disease alongside with habitat loss and poaching is one of the major threats to their long-term survival (Leendertz et al., 2006; Oates et al., 2008).

Gastrointestinal helminths are clearly less likely than certain viruses, such as for example Ebola virus, RSV and HMPV (Formenty et al., 1999; Köndgen et al., 2008), or bacteria like *B. cereus* bv *anthracis* (Leendertz et al., 2004) to cause acute fatalities and severe outbreaks of disease in wild chimpanzees. At least at low intensities, they might not cause any clinical symptom at all (e.g. Toft, 1982; Krief et al., 2008). Nevertheless, depending on their respective numbers, they might still be able to exert a considerable negative impact on the health and fitness of infected chimpanzees (e.g. Toft, 1982; Huffman, 1997; Gillespie, 2006; Gillespie et al., 2010). This might be either through direct pathological effects, such as tissue damage and blood loss (e.g. Orihel, 1971; Toft, 1982; Krief et al., 2008) or indirectly – as observed in other vertebrate hosts – for instance by reducing the host's body condition through nutrient malabsorption, reduced food intake (Coop & Holmes, 1996; WHO, 2012) and increased energy expenditure (e.g. Munger & Karasov, 1989; Coop & Holmes, 1996; Robar et al., 2011).

Parasitic gastrointestinal helminths might furthermore also alter and possibly impair the immune response and resistance of their hosts towards other pathogens, such as for example certain protozoa, viruses or bacteria (e.g. Brady et al., 1999; Cox, 2001; Khamal & El Sayed Khalifa, 2006; Pathak et al., 2012). Additionally, worm-induced tissue lesions in the host's lungs or gastrointestinal tract, as frequently caused by migrating larvae and/or adult specimens of various helminth species (e.g. *Strongyloides spp., Necator spp., Oesophagostomum spp.*) might potentially serve as entry ports for other pathogens and thus might cause or contribute to the onset of severe bacterial or viral infections or aggravate the course of infection. Toft (1982) for example reviews a case of fatal bacterial secondary infection in a zoo chimpanzee supposed to have resulted from a primary *Trichuris sp.* infection.

More profound knowledge, including species-specific identification of the gastrointestinal helminths affecting different wild chimpanzee populations in their natural environment, and the compilation of comprehensive baseline data on the helminth spectra and infection parameters from each population might therefore help to increase our understanding of the general health status of wild chimpanzees and the recurrent occurrence of severe outbreaks of disease in various populations (Gillespie, 2006; Leendertz et al., 2006a; Gillespie et al., 2008b).

Due to their close phylogenetic relationship, the general risk of pathogen transmission between humans and chimpanzees is considerable (Wolfe et al., 1998; Davies & Pedersen, 2008; Gillespie et al., 2008b; Köndgen et al., 2008). As this risk is inherently likely to be higher in areas with close spatial contact between people and chimpanzees (Daszak et al., 2001; Dobson & Foufopoulos, 2001; Chapman et al., 2005) as compared to areas with little contact, detailed helminthological baseline data from different wild chimpanzee populations might furthermore provide a valuable index of the degree of direct anthropogenic impact the individual populations are subjected to (Chapman et al., 2005 & 2006; Gillespie et al., 2010), At the same time, they might also provide some information regarding the presence or respectively absence of potentially zoonotic, human-pathogenic helminth species, such as for example *O. bifurcum* (Krief et al., 2010).

Additionally, comprehensive baseline data are an essential reference and prerequisite for the detection of any changes in the pattern and spectrum of gastrointestinal helminth infection in the different wild chimpanzee populations over time. Given the aforementioned potentially considerable detrimental effects of gastrointestinal helminths on the health and fitness of their chimpanzee hosts, such changes might be valuable indicators of alterations in the respective population's overall health status and disease susceptibility and thus might be able to serve as sentinels of potential increases in the general infection and disease susceptibility of individual chimpanzee populations (Gillespie et al., 2010). At the same time, they might furthermore also act as indicators of potential changes in the degree of direct human impact on the different populations over time (Gillespie et al., 2010).

I.2 Study objectives

The overall objective of the present study was to collate comprehensive and up-to-date baseline data on the spectrum and parameters of gastrointestinal helminth infections in the Taï chimpanzee population through a helminthological multi-year and multi-method survey of samples from individually known study group members – as a reference for the long-term health monitoring of this chimpanzee population, as well as to contribute to the general knowledge and understanding of gastrointestinal helminthic parasites affecting wild chimpanzees in their natural habitat and the impact of helminth infections on the health and fitness of infected individuals.

The study is composed of three main parts, assesses and describes the current spectrum of parasitic gastro-intestinal helminths of three human-habituated groups of wild chimpanzees in the Taï NP, and determines respective infection parameters and the impact of host- intrinsic and seasonal factors.
Employed in this study were four different conventional coprological examination methods including fecal cultures as well as molecular, DNA-based techniques (i.e. polymerase chain reaction and sequencing) in order to allow for a more precise and reliable identification of the spectrum of helminths, particularly the diversity of strongyle nematodes, affecting the study population.
For comparative purposes, the molecular analysis of the different chimpanzee strongyle and *Strongyloides* developmental stages was partly supplemented with sequence data from morphologically indistinguishable helminth developmental stages cultured from opportunistically collected fecal samples of sympatric monkeys (*Piliocolobus badius, Cercopithecus diana, Cercocebus atys*).

Overall study composition

1. Assessment and identification of the spectrum of parasitic gastrointestinal helminths affecting the Taï chimpanzee study population using four different conventional coprological methods and morphological description of detected and cultured helminth developmental stages

2. Determination of helminth infection parameters (including a method comparison) and investigation of potential host-intrinsic and seasonal influencing factors.

3. Molecular validation and specific identification of the spectrum of strongyle nematodes and *Strongyloides spp.* by means of diagnostic DNA sequences – including comparison with respective sequences obtained from morphologically indistinguishable helminth developmental stages from sympatric monkeys.

II Literature Overview

II.1 Taï National Park (Taï NP)

II.1.1 Location and climate

Taï National Park (5°15'-6°07'N, 7°25'-7°54'W) is situated in south-west Côte d'Ivoire on the border with Liberia (see Figure 1), between the Cavally River in the west and the Sassandra River in the east. The park which at present covers a total area of about 536,000 ha – comprising a core zone of 330,000 ha, a buffer zone of 20,000 ha as well as, since 2006, the adjacent N`zo Reserve in the north (UNEP-WCMC, 1982-2011) – first obtained protection in 1926 when the area was declared a `Forest and Wildlife Refuge´. In 1972 it obtained national park status. Five years later, a peripheral zone around the original area was added to the park and in 1982, the Taï NP was proclaimed UNESCO Natural World Heritage Site, as one of the last major remnants of primary tropical lowland forest in West Africa (UNEP-WCMC, 1982-2011).

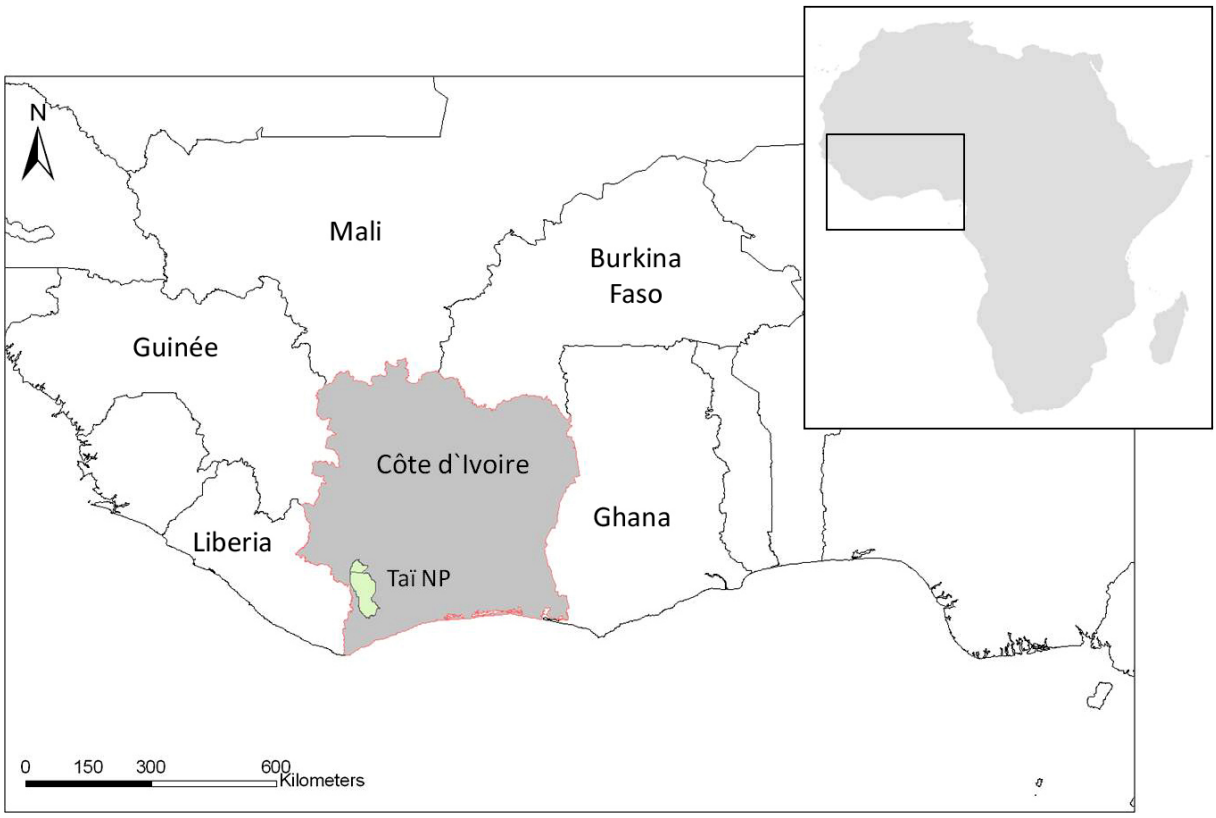

Figure 1. *Location of Côte d`Ivoire and Taï National Park*

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Altitude within the park ranges from ca. 80 m to 396 m above sea level, with Mt. Niénokoué (located roughly in the center of the park) as the highest peak (McGraw &

Zuberbühler, 2007). Altitude within the study area of the present study ranges between ca. 150 m and 264 m above sea level.

The climate in the area is generally characterized by two rainy seasons (March-July and September-December) intermitted by two, not clearly defined dry seasons with a monthly precipitation of less than 20 mm (Boesch & Boesch-Achermann, 2000). Annual rainfall, which has decreased considerably since the 1960s (Servat et al., 1997; Kühl et al., 2012), averages about 1500 mm (Kühl et al., 2012), and the mean relative humidity exceeds 80%. Average daily temperatures vary between ca. 24-28°C (Boesch & Boesch-Achermann, 2000), but in February maximum diurnal temperatures can occasionally reach up to 31°C. Minimum nocturnal temperatures can drop as low as 15-16°C for a few days in January due to a brief, but accentuated influence of the dry northeasterly Harmattan wind during this period of the year. Throughout the rest of the year, minimum nocturnal temperatures range between ca. 21-23°C.

II.1.2 Vegetation and fauna

Vegetation in the park is predominantly primary dense evergreen ombrophilous forest of a Guinean type, characterized by tall trees (40 to 60 m) and varying degrees of undergrowth (Boesch & Boesch-Achermann, 2000; UNEP-WCMC, 1982-2011). The park edges and the in 1977 added peripheral zone by contrast consist at least in parts of secondary forest and old coffee and cacao plantations (Boesch & Boesch-Achermann, 2000). Right beyond its boundaries, the park is surrounded by plantations and farmland. The forest is traversed by a network of small rivers and streams that dry out almost completely in the dry season, but can swell up considerably and temporarily flood adjacent areas during the rainy seasons (Boesch & Boesch-Achermann, 2000; UNEP-WCMC, 1982-2011).

The Taï National Park is home to an exceptional variety of birds and mammals, including several threatened species. Overall, a total of 12 different non-human primates have been recorded, including western chimpanzees (*Pan troglodytes verus*) and 11 species of monkeys, such as for example western red colobus (*Piliocolobus badius*), black-and-white colobus (*Colobus polykomos*), Diana monkey (*Cercopithecus diana*), and sooty mangabey (*Cercocebus atys*). Other noteworthy, although meanwhile mostly rather rare resident mammal species are leopard, golden cat, forest elephant, red river hog, giant forest hog, pygmy hippopotamus, giant pangolin, water chevrotain, bongo, and buffalo. Additionally, also a great variety of forest duikers occur in the park (UNEP-WCMC, 1982-2011). Presence and density of the different resident mammal species however, seem to vary considerably in different areas of the park – largely presumably as a result of respective local variations in the degree of poaching. Campbell et al. (2011) for example observed considerably higher primate and duiker encounter rates within the research area of the Taï Chimpanzee Project as compared to adjacent areas within the park, whereas the reversed pattern was observed as for signs of poaching.

II.1.3 Human pressure and impact on the park

Even though the Taï forest is a National Park, proclaimed World Heritage Site and still constitutes a relatively large block of predominantly intact primary forest, the human pressure and impact it is exposed to is considerable. Human population around the park has increased dramatically over the last decades – from for instance eight inhabitants per km² in the Taï sous-préfecture close to the study site in 1971, to 135 inhabitants per km² in 1991 (Boesch & Boesch-Achermann, 2000). This population growth has led to a high increase in the demand for arable land in the area and consequently to heavy local and regional deforestation up to the park border. Due to a similar rise in the demand for bushmeat, also poaching inside the park, which reportedly has always existed to some extent, has increased considerably (Boesch & Boesch-Achermann, 2000) and is now one of the most serious conservation problems faced by the park.

Other severe negative human impacts on the park include illegal logging and farming as well as artisan gold mining (Boesch pers. comm.). Furthermore, increasing degradation of and human encroachment into the forest, particularly into the surrounding buffer zone, which is generally not respected by local farmers, has been reported (McGraw & Zuberbühler, 2007).

II.2 Chimpanzees

II.2.1 Status

All four currently recognized chimpanzee subspecies (*Pan troglodytes verus, Pan troglodytes troglodytes, Pan troglodytes schweinfurthii* and *Pan troglodytes ellioti* (previously *Pan troglodytes vellerosus*)) are listed as endangered in the IUCN Red list of threatened species (Oates et al., 2008). Reason for the decline of chimpanzee numbers is a range of factors, such as deforestation, poaching and bush meat trade as well as disease. Chimpanzees are endangered in all of their range countries, but Côte d'Ivoire is an extreme example, as the country's chimpanzee population (subspecies *P. t. verus*) has decreased by as much as 90% between 1989/90 and 2007 (Campbell et al., 2008). The population in the Taï NP – according to recent estimates overall only about 361 (230 to 568) individuals (N'Goran et al., 2008) – is the last remaining viable wild chimpanzee population in the whole country and thus for the survival of the species of great importance.

II.2.2 Group sizes and composition

The chimpanzee community surveyed in this study has been observed and studied by researchers of the Taï chimpanzee project (TCP) for more than 30 years and is currently made up of three separate study groups (north-, south- and east group) of habituated and individually known chimpanzees. A fourth habituated group (middle group) no longer exists, as presumably only one to three group members are still

alive. The habituation process was first started by Swiss primatologist Christophe Boesch in 1979 with the so-called north group. Habituation of south- and east group began in 1989 and 2000 respectively (Boesch & Boesch-Achermann, 2000; Boesch et al., 2008). At the time of sample collection for the present study, all members of north- and south group, as well as all adult and adolescent male chimpanzees and a number of females and their offspring from east group were well habituated to the presence of human observers and could be followed at close range.

At the beginning of the present study in June 2007, south group consisted of 38 individuals, north group of 16 individuals and east group of about 47 individuals (Table 1). The precise number of chimpanzees in the latter group could not be established, as not all female group members and their offspring were fully identified at the time.

Table 1. *Composition of the three habituated chimpanzee study groups in June 2007*

	South: 38		**North: 16**		**East: 47***	
Age class**	**M**	**F**	**M**	**F**	**M**	**F**
Adult	4	11	0	4	5	17
Adolescent	4	3	1	2	1	1
Juvenile	4	1	3	1	5	7
Infant	7	4	2	3	7	4
Total	**19**	**19**	**6**	**10**	**18**	**29**

* estimated number

By the end of the field work for this study, in March 2010, group sizes and composition had changed considerably (Table 2) – mainly due to two severe disease outbreaks with multiple deaths in April 2009 (anthrax/ east group) and December 2009 respectively (pneumonia/ south group), as well as various other causes, such as births, immigrations and emigrations of female individuals, but also poaching and leopard attacks. In a number of cases however, the reason for the disappearance of individual chimpanzees could not be determined.

Table 2. *Size and composition of the three habituated chimpanzee study groups in March 2010*

	South: 23		**North: 16**		**East: 45***		
Age class**	**M**	**F**	**M**	**F**	**M**	**F**	**Unknown**
Adult	3	5	0	6	4	13	0
Adolescent	4	2	1	0	2	4	0
Juvenile	5	2	3	2	4	5	0
Infant	1	1	2	2	6	3	4
Total	**13**	**10**	**6**	**10**	**16**	**25**	**4**

* estimated number

** definition of chimpanzee age classes (adopted from Boesch & Boesch-Acherman, 2000):

	Males	Females
Adult	> 15 years	> 13 years
Adolescent	10- 15 years	10- 13 years
Juvenile	5- 9 years	5- 9 years
Infant	0- 4 years	0- 4 years

II.2.3 Territories and ranging behavior

Even though chimpanzees live in groups with defined territories, the individual group members are not constantly found together. Chimpanzee groups are fission-fusion societies (Goodall, 1986; Chapman et al., 1993; Boesch & Boesch-Acherman, 2000) and depending on the season as well as the type and amount of food available, individual animals, females and their offspring or subgroups of variable size and composition regularly split from the rest of the group and travel and forage independently within the group's territory for days up to several weeks.

Adolescent females usually permanently emigrate from the group they are born in at the age of about 11 years and join another chimpanzee community to avoid inbreeding with close male relatives, whereas chimpanzee males stay in their natal groups for life (Boesch & Boesch-Achermann, 2000).

The territories of the three study groups vary in size from 17-17.5 km^2 (north group), 16.8-22 km^2 (south group) (Herbiger et al., 2001) to about 28 km^2 (east group). The territories of the neighboring south and east group slightly overlap and intergroup encounters occasionally occur.

The vegetation in all three territories, despite their relative proximity to the western park border (ca. 5 to 10 km), consists largely still of relatively undisturbed primary forest, although the territory of the north group had partly been affected by some degree of logging and forest encroachment in the 1970s (Kühl et al., 2012). As a result, this territory has a generally more dense vegetation and undergrowth than the territories of the east and south group which had not or respectively only to a much lesser extent been affected. Unlike other areas in the Taï NP, none of the three territories seems to be currently affected by illegal logging, gold mining or forest encroachment. Signs of poaching, particularly empty shotgun shells, by contrast are repeatedly seen.

I.2.4 Diet and hunting behavior

Chimpanzees are omnivores. Even though their main diet is largely vegetarian and consists of a great variety of fruits, leaves, piths, nuts and seeds, they also regularly consume animal protein in the form of ants, termites or fresh meat (Goodall, 1986; Boesch & Boesch-Achermann, 2000). Chimpanzees are known to actively hunt and consume a number of different mammal species, including duikers and wild pigs. In the Taï NP however, they exclusively prey on monkeys, mainly western red colobus (*Piliocolobus badius*): ca. 81% and black- and- white colobus (*Colobus polykomos*):

ca. 12%. Other more rarely hunted monkey species include Diana monkeys (*Cercopithecus diana*): 1.7%, olive colobus (*Procolobus verus*) ca. 1.7%, and sooty mangabeys (*Cercocebus atys*): 0.4% (Boesch & Boesch, 1989). Monkeys are hunted throughout the year, but main `hunting season´ with sometimes several hunts per day, are the months with high rainfall.

Normally, all edible parts of the prey, including some bones, brain, stomach, and intestines are consumed by the chimpanzees, but the amount of meat and organs eaten per individual varies considerably depending on sex, age, rank, and group size. On average, adult male chimpanzees consume distinctly more meat and entrails per day than adult females. Subadults and juveniles normally only consume very small amounts (Boesch & Boesch-Achermann, 2000).

II.2.5 Contact with humans

All three chimpanzee study groups are followed on a daily basis by human observers at close range from dawn till dusk. In order to avoid interference with the chimpanzees' natural behavior and to minimize the risk of anthropozoonotic pathogen transmission, TCP researchers and field assistants try to keep a minimum distance of seven meters at all times. Additionally, all people following the chimpanzees wear surgical face masks as well as dedicated clothing and are obliged to adhere to a comprehensive hygiene and quarantine protocol (see Leendertz et al., 2006a and Boesch, 2008 for details). Unlike chimpanzees at other field sites (e.g. Budongo (own observation), Gombe (Wallauer, pers. comm.)), chimpanzees from the three study groups only very rarely enter the research camps.

Contact of the three study groups with people other than TCP project staff and researchers is relatively limited, but does occur, even though all three groups live entirely inside the national park and, as opposed to chimpanzees at some other field sites, do not leave the park to crop raid. While tourism is currently basically non-existent in the area, and access to the park for the local population is restricted, various people still regularly enter the forest including the territories of the three study groups. Apart from poachers who repeatedly access the park illegally, also researchers and field assistants from other research projects in the park regularly enter or traverse the chimpanzee territories, particularly parts of the south group territory which overlaps with the home range of a habituated study group of sooty mangabeys. Additionally brigades of park rangers from the regional forestry department patrol the area for several days at a time on a regular basis.

II.2.6 Pathogens and diseases affecting Taï chimpanzees

Disease, as mentioned before, is one of the major threats to the conservation and long-term survival of wild chimpanzee populations. As for the Taï chimpanzee population this threat is very prominent. Within the last two decades, all three chimpanzee groups surveyed in the present study have been repeatedly hit by severe and fatal outbreaks of infectious bacterial and viral diseases. Particularly anthrax (Leendertz et al., 2004b) as well as several severe outbreaks of respiratory disease, caused by human-transmitted respiratory viruses (RSV, HMPV) (Köndgen et al., 2008), have led to a considerable decline of the chimpanzee numbers in all three groups. Identification and diagnosis of the different pathogens was possible thanks to a permanent chimpanzee health and disease monitoring project (`Projet Santé´) established by the Taï Chimpanzee Project in 1998 in cooperation with the Robert Koch Institute (RKI) in Berlin under the auspices of Dr. Fabian Leendertz. A number of other bacteria and viruses found to infect the three study groups are listed in Table 3. Diagnosis was generally made from tissue samples obtained from deceased individuals at necropsy and/or from non-invasively collected sample material (feces, urine, fruit wadges) of the different study group members.

Table 3. *Some viruses and bacteria detected in necropsy samples and/or non-invasively collected sample material from habituated Taï chimpanzees*

Pathogen	Course of infection	Pathogenic yes/no	References
VIRUSES			
Filoviridae			
EBO-CI (= TEBOV)	acute	yes*	Formenty et al., 1999
Retroviridae			
STLV-1	chronic	no reports	Leendertz et al., 2004a
SFVcpz, SFVwrc	chronic	no reports	Morozov, 2009; Leendertz et al., 2008
Herpesviridae			
LCV1	chronic	no reports	Ehlers et al., 2003
Paramyxoviridae			
RSV	acute	yes***	Köndgen et al., 2008
HMPV	acute	yes***	Köndgen et al., 2008
Adenoviridae			
PtroAdV4, -5, -8	chronic	no reports	Wevers et al., 2011
Hepadnaviridae			
HBV	chronic	no reports	Leendertz, 2004
Polyomaviridae			
PtvPyV1a+ b,	chronic	no reports	Leendertz et al., 2011
PrvPyV2a,b,c			
BACTERIA			
Bacillus cereus bv. *anthracis*	acute/peracute	yes**	Leendertz et al., 2004b; Klee et al., 2010
Streptococcus pneumoniae	acute/chronic	yes***	Chi et al., 2007; Köndgen et al., 2008
Pasteurella multocida	acute/chronic	yes***	Köndgen et al., 2011

* fatal Ebola outbreaks reported in 1992 and 1994 (Formenty et al., 1999)

** repeated fatal anthrax outbreaks and individual cases between 2002 & 2011 (Leendertz et al., 2004 and own observations)

*** involved in repeated fatal and non-fatal outbreaks of respiratory disease between 1999 and 2009 (Köndgen et al., 2008; Köndgen et al., 2011 and Leendertz, personal communication)

II.3 Helminth classification and taxonomy

The taxonomic classification of helminths, especially nematodes which traditionally was solely based on comparative morphological studies at light-microscopic level (Gibbons, 2010) is rather confusing and subject of continuous adaptations and revisions. Among the reasons for this perpetual revision process are the immense number of nematodes on our planet (so far about 25,000 different species have been described many of which are parasitic (De Ley & Blaxter, 2002)), the ongoing discovery and description of new species and genera as well as the continuous refinement of examination techniques (Gibbons, 2010). In recent years, the application of molecular methods, such as the phylogenetic analysis of 18S rRNA gene sequences, has intensified this ongoing adaptation and revision process even

further and has led to the development of a whole new classification system for nematode helminths, combining morphological characteristics with molecular phylogenetic data (Blaxter et al., 1998; De Ley, 2000; De Ley & Blaxter, 2002).

The systematic of helminths employed in this study (Table 4) is based on the taxonomic classification of nematodes and platyhelminths as utilized by Schnieder et al., 2006.

Table 4. *Systematic classification of chimpanzee parasitic helminths employed in this study*

Phylum				
NEMATODA				
	Class			
	# ENOPLEA			
		Subclass		
		DORYLAIMIA		
			Order	
			• Trichinellida	
				Family	**Genus**
				Capillariidae	*Capillaria*
				Trichuridae	*Trichuris*
	# CHROMADOREA			
		Subclass		
		RHABDITIA		
			Order	
			• Tylenchida	
				Suborder	
				+ Panagrolaimoina	
				Superfamily	**Family**	**Genus**
				Strongyloidoidea	Strongyloididae	*Strongyloides*
			• Spiruida		
				+ Rhabditina	
				Strongyloidea	Ancylostomatidae	
					Subfamily	**Genus**
					Bunostominae	*Necator*
				Strongylidae		
					Chabertiinae	*Oesophagostomum*
						Ternidens
				Trichostrongylidae		
					Trichostrongylinae	*Trichostrongylus*
				+ Oxyurina		
				Oxyuroidea	Oxyuridae	*Enterobius*
				+ Ascaridina		
				Cosmocercoidea	Atractidae	*Probstmayria*
				Ascaridoidea	Ascarididae	
					Ascaridinae	*Ascaris*
				+ Spirurina		
				Superfamily	**Family**	**Genus**
				Physalopteroidea	Physalopteridae	*Physaloptera*
				Spiruroidea	Gongylonematidae	*Gongylonema*

Phylum				
PLATYHELMINTHS				
	Subphylum			
	## CERCOMEROMORPHA			
		Class		
		# CESTODEA		
			Subclass	
			EUCESTODIA	
				Order
				• Cyclophyllida
				Suborder
				+ Cyclophyllidina
			Superfamily	**Family**	**Genus**
			Anoplocephaloidea	Anoplocephalidae	*Bertiella*
			Hymenolepidoidea	Hymenolepididae	*Hymenolepis*
Phylum				
PLATYHELMINTHS				
	Subphylum			
	## TREMATODA			
		Class		
		# DIGENEA		
				Order
				• Plagiorchiida
				Suborder
				+ Xiphidiina
			Superfamily	**Family**	**Genus**
			Gorgoderoidea	Dicrocoeliidae	*Dicrocoelium*
					Concinnum

II.4 Gastrointestinal helminthic parasites reported in wild chimpanzees

II.4.1 Overview of reported helminth diversity

Parasitological surveys of wild chimpanzees, as mentioned before, have been conducted over the last decades at a number of field sites. For an overview of the respective types of gastrointestinal helminths found to affect the different study populations, see Tables 5 & 6.

For wild *Pan troglodytes ellioti* (previously *P. t. vellerosus)* however, so far no published systematic parasitological studies seem to exist, merely the occurrence of *Oesophagostomum stephanostomum* in a study population in the Gashaka Gumti NP, northeastern Nigeria has been described (Fowler et al., 2007). Additionally, a population of captive and semi-captive individuals at the Afi Mountain Primate Conservation Area in Calabar, Nigeria was reportedly (Mbaya & Udendeye, 2011) found to be parasitized by a number of helminth species: *Ascris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Strongyloides stercoralis, Ancylostoma duodenale, Hymenolepis nana,* and *Schistosoma mansoni.* Whether or not all

chimpanzees examined during this study, actually belong to the subspecies *P. t. ellioti* is however unclear.

As for the chimpanzee population in the Taï National Park, one previous parasitological study (Roduit, 1999) conducted between August 1994 and August 1995 employing conventional coprology ($ZnCl_4$-flotation, SAF-sedimentation, simple sedimentation, McMaster quantitative flotation, and fecal cultures) reported the presence of a total of 13 different helminth taxa.
Among these were one family of trematodes (Dicrocoeliidae fam.), one cestode genus (*Bertiella spp*.) as well as 11 taxa of nematodes. Six of these could be identified to genus level: *Oesophgostomum spp., Ternidens spp., Strongyloides spp., Trichuris spp., Capillaria spp.,* and *Gongylonema spp*. (whereby the identity of the latter reportedly remained unconfirmed), three to family level (Ancylostomatidae fam., Ascarididae fam., Oxyuridae fam.), and two to order level (Strongylida order, Spiruina order – translating to Strongyloidea superfam. and Spiruina suborder employing the classification used in the present study). A small number of adult *Capillaria* and *Enterobius* specimens excreted by the chimpanzees or obtained from opportunistic necropsies could morphologically be identified as *C. brochieri* and *E. anthropopitheci* respectively. All examined fecal samples (N= 478) and adult worms had been obtained from chimpanzees of the north group.

In addition to the various helminth types reported from parasitological surveys of wild chimpanzees (see above and Tables 5 & 6), a number of other gastrointestinal helminths (e.g. *Streptopharagus spp., Anatrichosoma spp., Protospirura muricola and, Chitwoodspiura spp*.) have been identified and described by several authors in captive, captured or reintroduced chimpanzees (e.g. Yamashita, 1963; Jessee et al., 1970; Graber & Gevrey, 1981; Petrzelkova et al., 2006 & 2010; Myers & Kuntz, 1972).

Method abbreviations used in Tables 5 & 6:

Baer= Baerman concentration (= Baermann technique)
DIR= direct examination
FEAS= formalin-ethyl-acetate sedimentation (stain: Lugol's solution)
FSE= formol-saline-ether sedimentation (= formalin-ether-concentration)
HMFC= Harada Mori fecal culture
Kato= Kato-Katz method
McM= McMaster quantitative flotation
Molec= molecular methods
NaNO3-FL= $NaNO_3$-flotation
SED= sedimentation
VDM= volumetric dilution method (iodine stain)
ZnSO4= $ZnSO_4$-flotation

Table 5. *Overview of parasitic helminths described in wild populations of eastern chimpanzees (P. t. schweinfurthii)*

Authors	Wrangham, 1995 Ashford et al., 2000 Krief et al., 2005 Krief et al., 2010	Mühlenbein, 2005	Zommers et al., 2012	Kawabata, 1991 Huffman et al., 1997 Hasegawa et al., 2010 Kooriyama et al., 2012	File et al., 1976 Murray et al., 2000 Bakuza et al., 2009 Gillespie et al., 2010
Country	Uganda	Uganda	Uganda	Tanzania	Tanzania
Fieldsite	Kanyawara	Ngogo	Budongo	Mahale	Gombe
Species	*P. t. schw.*	*P. t. schw.*	*P. t. schw.*	*P. t. schw.*	*P. t. schw.*
No of exam. samples	123, 252, 295	121	435	153, 161/156/86, 254	78, NA, 170, 1038
Method**	DIR, McM, FSE, VDM, Baer, Molec	FEAS	NaNO3-FL, SED	DIR, McM, FSE, Molec	DIR, FSE, NaNO3-FL, SED, ZnSO4, HMFC
# NEMATODES					
unidentified strongyles	**X**	**-**	**X**	**-**	**X**
Oesophagostomum spp.	**-**	**X**	**X**	**X**	**X**
O. stephanostomum	**X**	**-**	**-**	**X**	**-**
O. bifurcum	**X**	**-**	**-**	**-**	**-**
unidentified hookworm	**-**	**-**	**-**	**-**	**X**
Necator spp.	**X**	**-**	**X**	**-**	**X**
N. americanus	**-**	**-**	**-**	**-**	**-**
Trichostrongylus spp.	**-**	**-**	**X**	**-**	**-**
Strongyloides spp.	**X**	**X**	**-**	**X**	**X**
S. fuelleborni	**X**	**-**	**X**	**X**	**X**
S. stercoralis	**-**	**-**	**-**	**X**	**-**
Probstmayria spp.	**X**	**-**	**X**	**-**	**-**
P. gombensis	**-**	**X**	**-**	**X**	**X**
Enterobius spp.	**X**	**-**	**-**	**-**	**-**
E. anthropopitheci	**-**	**-**	**-**	**-**	**-**
Trichuris spp.	**-**	**-**	**X**	**X**	**X**
T. trichiura	**-**	**-**	**-**	**X**	**-**
Ascaris spp.	**-**	**-**	**X**	**-**	**X**
A. lumbricoides	**-**	**-**	**-**	**-**	**-**
Physaloptera spp.	**-**	**-**	**-**	**-**	**X**
P. caucasica	**-**	**X**	**-**	**-**	**X**
Gongylonema spp.	**-**	**-**	**-**	**-**	**-**
unidentified nematodes	**X**	**-**	**-**	**X**	**X**
# CESTODES					
Bertiella spp.	**X**	**-**	**X**	**X**	**X**
B. studeri	**X**	**-**	**-**	**-**	**-**
Hymenolepis spp.	**-**	**X**	**-**	**-**	**-**
# TREMATODES					
Dicrocoeliidae fam.	**-**	**-**	**X**	**-**	**X**
D. lanceatum	**-**	**-**	**-**	**X**	**-**
# ACANTHOCEPHALA					
Prostenorchis spp.	**-**	**-**	**-**	**X**	**-**

Table 6. *Overview of parasitic helminths described in wild P. t. troglodytes and P. t. verus populations at other field sites*

Authors	Howells et al., 2011	Mc Grew et al., 1989	Bakarr et al., 1991	Lily et al., 2002	Landsoud-S., 1995
Country	Senegal	Senegal	Sierra Leone	CAR	Gabon
Fieldsite	Fongoli	Mt Assirik	Tiwai	Mondika	Lope
Species	*P.t. verus*	*P.t. verus*	*P.t. verus*	*P. t. trog.*	*P.t. trog.*
No of exam. samples	132	70	7	23	66
Method**	NaNO3-FL, SED	DIR, FSE, ZnSO4, HMFC	DIR, HMFC	wet mounts	DIR, FSE, Kato, Baer
# NEMATODES					
unidentified strongyles	-	-	-	-	X
Oesophagostomum spp.	-	-	-	X	-
O. stephanost.	-	-	-	-	-
O. bifurcum	-	-	-	-	-
unidentified hookworm	X	-	-	X	-
Necator spp.	-	-	-	-	-
N. americanus	-	-	X	-	-
Trichostrongylus spp.	-	-	-	-	-
Strongyloides spp.	-	-	X	X	X
S. fuelleborni	X	X	-	-	-
S. stercoralis	-	-	-	-	-
Probstmayria spp.	-	-	-	-	-
P. gombensis	-	-	-	-	-
Enterobius spp.	-	-	-	-	-
E. anthropopitheci	-	X	-	-	-
Trichuris spp.	X	-	-	-	-
T. trichiura	-	-	-	-	-
Ascaris spp.	X	-	-	X	-
A. lumbricoides	-	-	-	-	X
Physaloptera spp.	X	X	-	-	-
P. caucasica	-	-	-	-	-
Gongylonema spp.	-	-	-	-	X
unidentified nematodes	X	-	-	-	-
# CESTODES					
Bertiella spp.	X	-	-	-	-
B. studeri	-	-	-	-	-
Hymenolepis spp.	-	-	-	-	-
# TREMATODES					
Dicrocoelium spp.	-	-	-	-	X
D. lanceatum	-	-	-	-	-
# ACANTHOCEPHALA					
Prostenorchis spp.	-	-	-	-	-

II.4.2 Helminth life cycles, morphology and pathogenicity

The following section gives a short overview of the morphology, life cycles, and pathogenicity of the different types of gastrointestinal helminths found to parasitize wild chimpanzees.

II.4.2.1 NEMATODES (`roundworms´)

II.4.2.1.1 Superfamily Strongyloidea (`strongyle´ nematodes)

Strongyle nematodes, i.e. nematodes of the superfamily Strongyloidea, belong to the most common gastrointestinal parasites of wild chimpanzees and have been discovered in almost all surveyed chimpanzee populations (see Tables 5 & 6).

Their eggs are generally more or less oval shaped with a thin and smooth shell consisting of an outer layer and an inner membrane and – depending on their stage of development – contain a varying number of blastomers or an already developed larva (Thienpont et al., 1990). As the eggs of all strongyle genera are morphologically very similar and overlap in size, reliable differentiation and identification of individual strongyle eggs to genus, let alone species level is usually not possible (e.g. Myers et al., 1971; Blotkamp et al., 1993; Newton et al., 1998b; Gasser et al., 2008b). Microscopic screening of cultured, morphologically distinct L3-larvae (Little, 1981) is thus crucial to reliably distinguish between the different strongyle genera. Reliable identification to species level on the basis of L3 morphology by contrast, is generally not possible (Newton et al., 1998b; Gasser et al., 2008b). For this, detailed microscopic examination of adult worm specimens and/or the application of molecular techniques (e.g. PCR) are indispensable.

+ Genus *Oesophagostomum*

Helminths of the genus *Oesophagostomum* (`nodular worms´) are common parasites of ruminants, pigs and primates worldwide (Steward & Gasbarre, 1989), and respective infections have been reported from the majority of surveyed wild chimpanzee populations (see Table 5 and 6). Humans have initially been thought to be only accidental hosts (Anthony & Mc Adam, 1972), but in the 1990s, locally highly prevalent patent infections of people with *O. bifurcum,* CREPLIN, 1849, were detected in Ghana and northern Togo (Poldermann et al., 1991). *Oesophagostomum* species isolated from wild chimpanzees include *O. bifurcum* as well as *O. stephanostomum,* STOSSICH, 1904 (e.g. Krief et al., 2010, Gasser et al., 1999*).* Other species reported from chimpanzees – if mostly from captive individuals – are *O. blanchardi (*RAILLIET % HENRY, 1912), *O. brumpti (*RAILLIET & HENRY, 1905), *O. apiostomum* (WILLACH, 1891), *O. dentigerum* (RAILLIET & HENRY, 1906), and *O. polydentatum* (SCHNEIDER & KREIS, 1934) (see e.g. Yamashita, 1963; Myers & Kuntz, 1972). The respective nomenclature however is apparently somewhat questionable. While *O. brumpti* and *O. apiostomum* as described in some publications might actually be synonymous to *O. bifurcum*, *O. apiostomum* as

described in other publications might in fact be identical to *O. acuelatum*, LINSTOW, 1879 (Chabaud & Larivière, 1958; Polderman & Blotkamp, 1995).

Oesophagostomum species have a direct life cycle. The adult worms are 50-250 mm in length (Marcus, 1982), possess a small buccal capsule with a perioral corona radiata, and live in the lumen of the large intestine of their host. After a prepatent period of around one month (Anderson, 2000), the adult females start producing eggs (in *O. bifurcum*, about 5000 eggs/ worm per day (Krepel & Polderman, 1992)), which are passed in the host's feces.

Freshly excreted eggs are ca. 43-75 x 33-48 µm in size (Goldsmid, 1982), contain about 16-32 blastomers and, at optimal temperatures, develop to L1-stage within 24 hours (Anderson, 2000). Development of the larvae up to the infectious L3-stage in a moist environment takes 5 to 7 days. The L3-larvae are quite resilient and reportedly able to survive long periods of desiccation (Polderman & Blotkamp, 1995). Infection of the host occurs most probably through ingestion of the infectious L3-larvae (Anderson, 2000), although successful experimental trans-cutaneous infection of calves has been reported (Mayhew, 1939). Ingested L3-larvae burrow into the submucosa of the small or large bowel of their host, where they become encapsulated and develop to L4-larvae which return to the gut lumen, molt again, and become adults (Marcus, 1982). Overall, development from L3-larva to adult worm normally takes about two weeks (Anderson, 2000), but can be extended to several months through hypobiosis of the still encapsulated L4-larvae (Eckert et al., 2008).

Pathology of *Oesophagostomum* infection ranges from no or only mild signs to severe clinical manifestations and is mostly associated with perforation and invasion of the intestinal submucosa by the L3-larvae, which in most species causes an inflammatory reaction and the formation of distinct nodules around the larvae – hence the name `nodular worm´ (Marcus, 1982). In humans, this inflammatory reaction can be severe with the formation of multiple intestinal and sometimes ectopic abscesses (`multi-nodular disease´) or the development of unilocular inflammatory abdominal masses (`Dapaong tumor´) leading to abdominal pain, fever, diarrhea, and weight loss and potentially also to peritonitis and bowel obstruction (Storey et al., 2000).

In chimpanzees, clinical manifestation seems to differ considerably between wild chimpanzees and captive individuals (Krief et al., 2008). Whereas chimpanzees in the wild reportedly exhibit no or only mild symptoms, despite the presence of multi-nodular intestinal abscesses, several severe cases of *Oesophagostomum*-induced disease have been observed in captive individuals (Krief et al., 2008). Suspected reason for this apparent difference (Krief et al., 2008) is the repeatedly described self-medication behavior of wild chimpanzees (e.g. Huffman et al., 1997, Boesch & Boesch-Achermann, 2000; Fowler et al., 2007).

+ Genus *Ternidens*

Ternidens deminutus, RAILLIET & HENRY, 1909, also known as `false hookworm´ (Goldsmid, 1968) is the only currently recognized species of its genus and a locally highly prevalent but overall still relatively little described parasite infecting people and non-human primates in parts of Africa, Asia, and the Pacific islands (Amberson & Schwarz, 1958; Schindler et al., 2005). *T. deminutus* is closely related to the genus *Oesophagostomum.*

The existence of a second *Ternidens* species, *T. simiae,* parasitizing monkeys in Celebes, Sulawesi has been reported (Yamaguti, 1954), but could not be confirmed (Goldsmid & Lyons, 1973). Based on observed morphological variations in adult *T. deminutus* specimens from different hosts (Goldsmid & Lyons, 1973) and recent genetic evidence, the potential existence of host-specific genetic variants or cryptic species has been suggested (Schindler et al., 2005), but confirmation is pending.

T. deminutus infections of African non-human primates have so far mostly been observed in parasitologically surveyed baboons and other monkey species. Accounts of respective infections in chimpanzees by contrast are scarce, and the seemingly only description of *Ternidens* infections in wild chimpanzees in their natural habitat to date stems from the Taï National Park (Roduit, 1999). All other reports come from chimpanzees in captivity (Graber & Gevrey, 1981; Yamashita, 1963*;* Myers & Kuntz, 1972).

The life cycle of *T. deminutus* is currently still largely unknown. Proposed route of infection is ingestion of infectious L3-larvae (Goldsmid, 1968), but, as respective infection experiments were inconclusive and experimental trans-cutaneous infection of susceptible hosts unsuccessful (Sandground, 1929 & 1931; Blackie, 1932), the involvement of an arthropod intermediate hosts seems likely (Amberson & Schwarz, 1952, Goldsmid, 1982). Following ingestion, the L3-larvae are thought to enter the intestinal submucosa of their host, where they develop to L4-larvae and form nodules. The L4-larvae then supposedly return to the intestinal lumen where they molt and complete their development (Sandground, 1931; Goldsmid, 1968).

The adult worms are about 4.5-17 mm long (Goldsmid & Lyons, 1973; Goldsmid, 1982) and live in the large intestine of their hosts. The females produce eggs which are passed in the host's feces. In human hosts, a mean egg production between ca. 3,500 and 7,000 eggs/worm per day has been observed (Goldsmid, 1971). Freshly excreted eggs are 70-94 x 40-60 µm in size and contain mostly eight (or occasionally only 1-4) blastomeres (Goldsmid, 1982), although Sanground (1929) reports a minimum of 16 blastomeres and an egg size of 72-103 x 37-45 µm. Development to L3-larvae takes about 8 to 10 days (Goldsmid, 1971).

Like its life cycle, also the pathogenicity of *T. deminutus* is still largely unclear. Repeatedly reported for instance have been cystic nodules in the intestinal wall of infected monkeys (Toft, 1982) as well as inflammatory pseudotumors and abscesses,

similar to the ones caused by *O. bifurcum* in the intestinal wall of infected humans (Anthony & McAdam, 1972; Hemsrichart, 2005). Furthermore, evidence exists that adult worms consume blood and thus might potentially cause chronic blood loss and hypochromic anemia in their hosts. Whether the worms are active blood-suckers (like hookworms) or only feed on blood oozing from tissue lesions they have inflicted is however unresolved (Goldsmid, 1982).

+ Genus *Necator*

Infections with so-called `hookworms´ of the genus *Necator* have been observed in a number of wild chimpanzee populations (see Tables 5 & 6). *Necator* species identified as chimpanzee parasites include *N. congolensis,* GEDOELST, 1916*,* and *N. exilidens,* LOOSS, 1912 (Ackert & Payne, 1923; Yamashita, 1963; Myers & Kuntz, 1971; Orihel, 1971; Graber & Gevrey, 1981). Additionally, also *N. americanus,* STILES, 1902, one of the most common gastrointestinal parasites of humans in the tropics and subtropics, is reportedly able to infect and parasitize chimpanzees (Orihel, 1971).

Necator species have a direct life cycle (Brooker et al., 2005). Infectious stages are the L3-larvae which develop in the environment from excreted eggs within 5 to 10 days (WHO Expert Committee, 1987) and actively penetrate the skin of their host before completing a visceral migration. After reaching the lumen of the small intestine, the larvae develop to adult worms which are species dependant ca. 5.4-13.5 mm in length (Ackert & Payne, 1923) and live attached to the intestinal mucosa of the small intestine where they suck blood. After a prepatent period of about 42 to 54 days (Orihel, 1971), the adult females start to produce eggs which are passed with the host's feces. Freshly excreted eggs are about 64-76 x 36-40 µm (*N. americanus;* Thienpont et al., 1990) or respectively ca. 60 x 40 µm in size (*N. congolensis*; Ackert & Payne, 1923) and contain 4-8 blastomeres. The L1-larvae hatch temperature dependant within about 24 to 48 hours (Brooker et al., 2005). Maximum life expectancy of adult *N. americanus* in human hosts is reportedly several years (e.g. Kendrick, 1934; Brooker et al., 2005; WHO Expert Committee, 1987).

The pathological effects of *Necator* infections in wild chimpanzees and other wild non-human primates are unclear. In captive, experimentally *N. americanus*-infected chimpanzees however, several cases of severe anemia have been observed (Orihel, 1971) conforming to the clinical symptoms caused by *N. americanus* in heavily infected humans, particularly children (Hotez et al., 2003; Hotez et al., 2005). Signs of anemia (i.e. reduced PCV and hemoglobin levels) have also been reported from experimentally *N. americanus*-infected captive common marmoset monkeys (*Callithrix jacchus*). Further symptoms observed in these animals were peripheral alveolar hemorrhage and fluid accumulation in the lungs, caused by the visceral migration of the *Necator* L3-larvae.

In heavily infected children, severe *N. americanus*-induced hypochromic anemia might lead to serious growth retardation as well as to intellectual and cognitive impairments (Hotez et al., 2003; Hotez et al., 2005).

II.4.2.1.2 Genus *Strongyloides*

Apart from the above described strongyle nematodes, helminths of the genus *Strongyloides* belong to the most frequently reported parasitic gastrointestinal helminths in wild and captive chimpanzees, and all surveyed wild chimpanzee populations (see Table 5 & 6) were found to harbor respective infections. Most common are infections with *S. fuelleborni*, LINSTOW, 1905, the primary species parasitizing chimpanzees and other old-world primates (e.g. Olsen et al., 2009), but also infections with *S. stercoralis*, BAVAY, 1876, the prevailing *Strongyloides* species in humans in most parts of the world, have been observed (e.g. Hasegawa et al., 2010). Likewise, patent infections of humans with *S. fuelleborni* have been described (e.g. Brown & Girardeau, 1977, Ashford & Barnish, 1989; Hasegawa et al., 2010).

Strongyloides spp. have a direct heterogonic life cycle, involving a parasitic generation of adult females – which live in the small intestines of their host and propagate through parthenogenesis – and facultatively of one or more non-parasitic generations of sexually reproducing free-living adult male and female worms in the environment. Infection occurs through infective L3-larvae which actively penetrate the intact skin of their host and subsequently complete a visceral migration before reaching the host's small intestine, where they develop to parthenogenic adult females (Schad, 1989). These are species-dependent ca. 2-4 mm long (Little, 1966), live embedded in the mucosal epithelium and, after a prepatent period of 5 to 7 days (*S. fuelleborni;* Flynn, 1973), produce eggs which are passed in the host's feces (Schad, 1989). Freshly shed *S. fuelleborni*, eggs are oval to almost rectangular in shape, thin-shelled, about 43-63 x 30-48 µm in size (Goldsmid, 1982) and contain a developed larva. In *S. stercoralis* by contrast, eggs generally hatch already in the intestine of their host, and thus mainly L1-larvae are excreted (Schad, 1989).

Shed *Strongyloides* eggs or L1-larvae respectively develop in the environment to free-living adults or else directly to infective L3-larvae. In *S. stercoralis*, permanent autoinfection through L1-larvae penetrating the intestinal wall or perianal skin of their host can occur (Schad, 1989). In *S. fuelleborni,* the existence of such a mechanism is unknown (Ashford & Barnish, 1989; Olsen et al., 2009). For *S. fuelleborni* on the other hand, the possibility of transmammary passage of larvae from nursing women to their suckling infants has been described (Brown & Girardeau, 1977).

All larval stages of *S. fuelleborni* and *S. stercoralis* are morphologically indistinguishable. Cultured free-living adult females of both species by contrast can reliably be differentiated on the basis of several specific morphological characteristics (e.g. Little, 1966; Speare, 1989).

In the absence of respective accounts in the literature, the pathology and clinical symptoms caused by *S. fuelleborni* and *S. stercoralis* in wild chimpanzees are largely unclear. In infected captive chimpanzees, humans, and other non-human primates symptoms seem to vary considerably depending on the respective infection intensities as well as the immune status of the infected hosts. Mild infections with each one of the two species seem to be largely asymptomatic (Lim et al., 2004; Olsen et al., 2009). Heavy infections by contrast might cause acute to chronic diarrhea, nausea, and anorexia (e.g. Brack, 1987; Grove, 1989; Hasegawa et al., 2010) as well as respiratory symptoms, urticaria, and pruritus induced by migrating larvae (Brack, 1987; Grove, 1989).

In human hosts with compromised cellular immunity (induced for instance by HTLV1-infection or long-term corticosteroid treatment), *S. stercoralis* infections might furthermore lead to severe strongyloidiasis with fulminant hyperinfection and generalized dissemination of *S. stercoralis* larvae in the organism and organs of the host causing potentially fatal respiratory symptoms, fever, and secondary bacteremia (Lim et al., 2004; Vladmudi et al., 2006). In captive chimpanzees and several other non-human primates, a number of similar cases of fatal disseminated *S. stercoralis* infection have been described (as reviewed by Toft, 1982 and Genta & Grove, 1989). The most prominent clinical symptom and immediate cause of death in the majority of these cases was acute respiratory distress resulting from extensive pulmonary hemorrhages. Although none of the respectively affected chimpanzees and other primates had a history of corticosteroid treatment, all individuals reportedly appeared to be either very young or to have a potentially otherwise reduced immune response (Genta & Grove, 1989). In *S. fuelleborni*-infected hosts with compromised immunity by contrast disseminated strongyloidiasis doesn't seem to occur.

II.4.2.1.3 Genus *Capillaria*

Reports of *Capillaria* infections in chimpanzees as well as other non-human primates are rare. Troisier et al. (1928) describe the *C. hepatica*, BANCROFT, 1893, infection of two chimpanzees from the Kindia region in Guinea, while Graczyk et al. (1999) report the detection of respective infections in habituated wild mountain gorillas (*Gorilla gorilla beringeri*) in the Parc National de Volcans in Rwanda. Additionally, infections of captive chimpanzees (Yamashita, 1963) as well as a few individual cases of *C. hepatica* infections in captive monkeys and lemurs have been described (Pizzi et al., 2008; Zordan et al., 2012).

C. hepatica, primarily a parasite of rodents worldwide, but also able to infect other mammals including humans and non-human primates, parasitizes the liver of its host (Banzon, 1982). Infection occurs through ingestion of infective, embryonated eggs with contaminated soil, food or water. Egg development to infective stage occurs in the environment, following the death and decomposition of the infected host. As the adult worms live and reproduce in the liver of their host, no eggs are passed in the host's feces. Instead, diagnosis is made through liver biopsy or liver examination at

necropsy (Troisier et al., 1928, Graczyk et al., 1999; Pizzi et al., 2008). Excretion of *C. hepatica* eggs in primate feces might potentially occur following the ingestion of infected liver tissue (Banzon, 1982) and is no sign of patent *C. hepatica* infection of the respective primate. *C. hepatica* eggs are about 48-62 x 29-37 µm in size and barrel-shaped with a striated shell and two shallow polar plugs (Thienpont et al., 1990).

Infections of non-human primates with *C. hepatica* are usually asymptomatic (EAZW, 2008), but also a few cases of sudden death associated with severe *C. hepatica*-induced hepatitis have been described (Pizzi et al., 2008; Zordan et al., 2012). Serious and sometimes fatal liver disorders have also been observed in *C. hepatica*-infected humans (Banzon, 1982).

Infection with a different *Capillaria* species, namely with *C. brochieri* has been reported by Justine (1988) from a wild caught bonobo (*Pan paniscus*) deceased from diarrhea in Zaïre (now DRC). Life cycle, epidemiology and pathogenicity of this parasite are unknown. Adult worms recovered from the intestines of the deceased bonobo were about 11.6-13.3 mm (males) and 22.3-25.9 mm (females) long. Eggs obtained from the genital tract of the recovered female worms were barrel-shaped with two polar plugs and a rough, striated two-layered shell and about 38-43 x 18-22 µm in size.

Morphologically similar, only slightly larger eggs (43-50 x 21-23 µm and 45-55 x 23-35 µm respectively) had also been detected by Hasegawa et al. (1983) in fecal samples from healthy wild bonobos at Wamba, DRC as well as by Roduit (1999) in fecal samples from habituated chimpanzees in the Taï National Park. From Taï NP, also the recovery of two adult worms conforming to the morphological descriptions of *C. brochieri (*Justine, 1988) has been reported. Both worms had been found at necropsy in the intestines of a deceased infant chimpanzee (Roduit, 1999). Reports of *C. brochieri* infections in other chimpanzee populations or captive individuals are lacking.

II.4.2.1.4 Genus *Trichuris*

Infection of wild or captive chimpanzees with nematodes of the genus *Trichuris* (so-called `whipworms´), has been reported by several authors (see Tables 5 & 6); Myers & Kuntz, 1972). Identified species in all cases where identification to species level had been performed was *T. trichiura,* LINNAEUS, 1771 – one of at least 70 species of its genus and a common parasite of humans and non-human primates, particularly in the tropical and subtropical parts of the world (Barriga, 1982).

T. trichiura has a direct life cycle, and infection occurs through ingestion of infective embryonated eggs which hatch and develop following ingestion without visceral migration (Bundy & Cooper, 1989). The adult worms are about 26-48 mm in size (Ooi et al., 1993) and live in the caecum of their host where they bury into the mucosa with their hair-like anterior end and feed on blood and enterocyte material (Bundy &

Cooper, 1989). The characteristic eggs which are produced after a prepatent period of ca. 2 to 3 months and excreted with the host's feces (in humans ca. 3,000-20,000/female worm per day (Bundy & Cooper, 1989)), are about 50-61 x 23-29 µm in size, yellow-brown and lemon-shaped, with two prominent polar plugs and a thick, smooth, two-layered shell (Barriga, 1982).

Pathology and clinical symptoms caused by *T. trichiura* depend on the severity of infection (Bundy & Cooper, 1989; Barriga, 1982). Whereas light *Trichuris* infections in chimpanzees and other non-human primate seem to be typically asymptomatic (Toft et al., 1982), considerable clinical symptoms such as anorexia and mucoid diarrhea as well as several fatal cases of severe enteritis, bacterial secondary infection and ileal intussusceptation have been observed in heavily infected captive individuals (e.g. Toft, 1982; Hennessy et al., 1994). In heavily *T. trichiura* infected humans, also severe hemorrhagic diarrhea, hypochromic microcytic anaemia and rectal prolaps have been described (Barriga, 1982).

II.4.2.1.5 Genus *Enterobius*

Members of the genus *Enterobius* (so-called `pinworms´ or `threadworms´) are small, parasitic nematodes with a worldwide distribution parasitizing a wide range of mammalian hosts, including humans and non-human primates (Anderson, 2000). *Enterobius* infections of chimpanzees have been reported from individuals in captivity (e.g. Yamashita, 1963; Myers & Kuntz, 1972) as well as from various wild populations (see Tables 5 & 6; Roduit, 1999). Wild and free-ranging chimpanzees, although identification to species level was not always performed, appear to be mainly infected with *E. anthropopitheci,* GEDOELST, 1916 (Hasegawa & Udono, 2007), a reportedly specific parasite of chimpanzees and bonobos (Hugot, 1993). Infections of captive chimpanzees by contrast seem to be usually caused by the `human pinworm´ *E. vermicularis,* LINNAEUS, 1758 (Hasegawa & Udono, 2007), a common nematode parasites of humans, particularly children worldwide (Eckert, 1998). Other reportedly chimpanzee-parasitic *Enterobius* species include *E. vermicularis microbulbus,* YAMASHITA & KONNO, 1957 *(*Yamashita & Konno, 1957)*, E. bipillatus,* GEDOELST, 1916, BAYLIS, 1923, and *E. foecunda,* LINSTOW, 1879 (e.g.; Yamashita, 1963; Myers & Kuntz, 1972).

Enterobius species have a direct life cycle. Infection occurs through ingestion of infective eggs, which hatch in the small intestine and develop to adult worms (Eckert, 1998). These are about 1.5-13 mm in size and live in the large intestine of their host (Hasegawa & Udono, 2007; Eckert, 1998). After mating, the males die whereas the female worms, following a prepatent period of ca. 15 to 35 days (as reviewed by Anderson, 2000), travel to the anus of their host where they deposit their eggs on the perianal skin before they die as well. The deposited, characteristic eggs are ca. 53-58 x 26-29 µm (*E. anthropopitheci;* Hasegawa et al., 2005) or 50-60 x 20-32 µm (*E. vermicularis;* Thienpont et al., 1990) in size, flattened on one side, translucent, thick-shelled, and contain a morula or folded larva. They develop and become

infectious within hours (Anderson, 2000), cause considerable pruritus and are mechanically spread by the host, become airborne or might be directly ingested by a new hosts (e.g. upon grooming). Additionally, also auto- as well as retroinfections might occur (Eckert, 1998; Anderson et al., 2000) or eggs might appear in the host's feces.

Pathogenicity associated with *Enterobius* species varies considerably dependent on the severity of infection. Light infections are reportedly largely asymptomatic, apart from causing irritation and anal pruritus, which however might occasionally lead to self-mutilation as well as increased restlessness and aggression of the infected host (as reviewed by Toft, 1982). Heavy infections by contrast might lead to severe clinical manifestations. In captive chimpanzees, several fatal cases of heavy *E. vermicularis* infection, characterized by extensive ulcerative enterocolitis, peritonitis, and necrogranulomatous lymphadenistis have been described (e.g. Toft, 1982, Murata et al., 2002).

II.4.2.1.6 Genus *Ascaris*

Ascaris or `roundworm´ infections have been reported from several wild and captive chimpanzee populations or individuals (see e.g. Tables 5 & 6; Graber & Gevrey, 1981, Petrzelkova et al., 2010, Myers & Kuntz, 1972), whereby *Ascaris lumbricoides,* LINNAEUS, 1758, was diagnosed in all but one cases where determination to species level had been performed. In one European zoo population by contrast *A. suum* was identified (Nejsum et al., 2006 & 2010). *A. lumbricoides* is primarily a human parasite infecting more than 1.2 billion people worldwide (de Silva et al., 2003), whereas the morphologically more or less indistinguishable *A. suum,* GOEZE, 1782 (Nejsum, 2006; Dold & Holland, 2011) is mainly a parasite of pigs. Recent molecular evidence however suggests that the two taxa are a single species and that *A. suum* should be considered a synonym of *A. lumbricoides* (Leles et al., 2012).

The ascarid life cycle is direct and infection occurs through ingestion of infective embryonated eggs (Dold & Holland, 2011). Once ingested, the eggs hatch. The larvae penetrate the intestinal mucosa and conduct a visceral migration and maturation phase in the lungs, before reaching the small intestine of their host where they develop to adult male and female worms. These are 15-20 and 20-35 cm respectively in size (*A. lumbricoides*; Dold & Holland, 2011) and feed on intestinal content. The reported life span of *A. lumbricoides* is ca. 12 to 18 months (Eckert, 1998). The female worms produce characteristic eggs which are excreted in the host`s feces, become infective after several weeks, and can remain viable in the soil for up to 15 years (WHO, 1967). The eggs are gold-brown, ca. 45-75 x 35-50 µm in size, round to oval shaped with a very thick and course layered shell and granular content (Thienpont et al., 1990).

The clinical manifestation of *A. lumbricoides* infection in chimpanzees and other non-human primates seems to vary depending on the severity of infection. Whereas

light infections are reportedly in general fairly innocuous and of little clinical significance (Toft, 1982), several fatal cases have been described in heavily infected captive non-human primates, including chimpanzees. In all deceased chimpanzees, death was associated with the presence of numerous worms, intestinal blockage, and worm migration into the bile duct and liver (Toft, 1982). In heavily infected humans, migrating larvae are known to cause pulmonary hemorrhages as well as inflammatory infiltrations and associated eosinophilia referred to as Löffler's syndrome (Eckert, 1998).

II.4.2.1.7 Genus *Probstmayria*

Infections of chimpanzees with nematodes of the genus *Probstmayria* have mostly been reported from the study populations in Gombe and Mahale, Tanzania as well as from the Kanyawara and Ngogo field sites in Uganda (see Table 5) and only few other records of infection exist (e.g. File, 1976; Myers & Kuntz, 1972). Primary chimpanzee-parasitic species seems to be *P. gombensis*. File, 1976 (File, 1976). Another species reportedly able to infect chimpanzees is *P. vesiculata* (Vuylsteke, 1956).

The life cycle of *P. gombensis* is largely unknown, but other members of the family Atractidae are believed to multiply over generations within the same host (File, 1976). Adult *P. gombensis* are very small, ca. 1.4-1.6 mm (male) and 1.8-1.83 mm (female) respectively in length, and the adult females excrete no eggs but larvae which resemble the adult worms in size and morphology and are passed in the host's feces (File, 1976). Infection supposedly occurs through ingestion of these excreted larvae (File, 1976).

Studies and reports regarding the pathogenicity of *P. gombensis* and *P. vesiculata* in infected chimpanzees are lacking. *Probstmayria* infections in other host species, for example *P. vivipara,* PROBSTMAYR, 1865*,* infections in horses and donkeys however appear to be largely asymptomatic, although the worms at high intensities might cause considerable pruritus and mucosal irritation (Schnieder et al., 2006). Also for the gorilla-parasitic *P. gorilla,* KREIS, 1955, reportedly no signs of pathogenicity have been observed (Rothman & Bowman, 2003).

II.4.2.1.8 Genus *Physaloptera* (Genus *Abbreviata*)

Physaloptera spp. and *Physaloptera* (= *Abbreviata*) *caucasica,* LINSTOW, 1902, respectively infections have been reported from surveyed wild chimpanzee populations in Tanzania (Gombe), Uganda (Ngogo), and Senegal (Mt. Assirik and Fongoli) (see Tables 5 & 6).

P. caucasica is a spirurid nematode parasitizing the digestive tract of non-human primates as well as occasionally of humans. The life cycle of this parasite is largely unknown. Infection probably occurs through ingestion of beetles, crickets or other arthropods containing infective larvae (Kraus et al., 2003). The potential involvement

of second intermediate host or parathenic host has been suspected (Toft & Ebenhard, 1998). The adult worms which morphologically resemble *Ascaris spp*. are up to 10 cm long and live in the esophagus, stomach, and small intestine of their host where they are firmly attached to the mucosa (Kraus et al., 2003). The female worms produce eggs which upon excretion in the feces of the host are about 40-50 µm in size, translucent, thick-shelled, and contain an embryo (Kraus et al., 2003).

Described clinical symptoms associated with heavy *P. caucasica* infections in non-human primates include abdominal pain, bloody diarrhea or melena, vomiting, and anorexia (Toft & Ebenhard, 1998; Kraus et al., 2003) caused by multiple mucosa lesions and inflammation. Records of the clinical manifestation of *Physaloptera* infections in chimpanzees are lacking.

II.4.2.2 TREMATODES (`flukes´)

II.4.2.2.1 Family Dicrocoeliidae

Infections of wild chimpanzees with dicrocoeliid trematodes have been reported from a number of field sites (see paragraph II.4.1). Additionally, infections of captive and wild caught chimpanzees have been described (Yamashita, 1963; Graber & Gevrey, 1981). Two dicrocoeliid trematode species reportedly able to infect chimpanzees are *D. lanceatum,* STILES & HASSALL, 1898 *(= D. dendriticum,* LOOSS, 1899*)* (Yamashita, 1963, Myers & Kuntz, 1972; Huffman et al., 1997) and *Concinnum brumpti,* RAILLIET, HENRY & JOYEUX, 1912 (= *Eurytrema brumpti,* R, H & J, 1936) (e.g. Ralliet et al., 1912; Stunkard & Goss, 1950; Yamashita, 1963; Graber & Gevrey, 1981). In most reported cases of dicrocoeliid trematode infections of chimpanzees, particularly of wild chimpanzees however no identification of the respective parasites to genus and species level had been performed.

The life cycle of dicrocoeliid trematodes is complex and involves at least two intermediate hosts (Samuel et al., 2001). The small (ca. 8 x 2 mm (Samuel et al., 2003)), lancet-shaped adults of *D. dendriticum* (respectively *D. lanceatum*) live in the liver and bile ducts of their definite hosts which are primarily wild and domestic ruminants, but also other herbivore mammals, including humans and non-human primates (Samuel et al., 2001; Rack et al., 2004). The female flukes produce eggs which are passed with the host's feces. The eggs are ca. 38-45 x 22-30 µm in size, dark brown, have an asymmetric oval shape, a thick shell with inconspicuous operculum, and contain a miracidium (Thienpont et al., 1990).

C. brumpti eggs are morphologically similar (Graber & Gevrey, 1981). Following ingestion by a suitable molluscean host, the miracidia hatch, penetrate the intestinal wall, and settle in the hepatopancreas of the mollusc, where they develop to cercariae which are subsequently excreted and consumed by ants serving as second intermediate hosts (Samuel et al., 2001). Inside the abdomen of the ants, development to metacercariae occurs which, following ingestion of the second

intermediate hosts by the definite host, hatch and migrate to the definite host's liver and bile duct, where they develop to adult flukes (Samuel et al., 2001).

Reports regarding the pathogenicity and clinical manifestation of dicrocoeliid trematode infections in chimpanzees and other non-human primates are lacking. In wild cervids and other wild mammalian hosts infections reportedly tend to be mild and largely asymptomatic. In chronically infected domestic ruminants by contrast considerable pathology including liver fibrosis and extensive bile duct hyperplasia has been described (Samuel et al., 2001). Symptoms observed in *D. dendriticum* infected human hosts include: abdominal pain, weight loss, and chronic relapsing diarrhea (Rack et al., 2004).

II.4.2.2.2 Other Trematodes

Infections of wild chimpanzee populations with other types of tremtodes have so far not been reported. In captive and semi-captive chimpanzees however infections with *Schistosoma mansoni* (Mbaya & Udendeye, 2011; Standley et al., 2013) as well as *Schistosoma haematobium* (De Paoli, 1965) have been described.

II.4.2.3 CESTODES (`tapeworms´)

II.4.2.3.1 Genus *Bertiella*

Cestodes of the genus *Bertiella* are primarily parasites of non-human primates, rodents, dermopterans, and marsupials. Two species, namely *B. studeri*, BLANCHARD, 1891 and *B. mucronata*, MEYNER, 1895, also sporadically infect humans (Denegri et al., 1998; Galan-Puchades et al., 2000). In wild chimpanzees, tapeworm infections seem to be generally fairly rare and have only been recorded from a few study populations (see paragraph II.3). Apart from one case where *Hymenolepis*-like eggs were detected (Mühlenbein et al., 2005), all reported tapeworm infections were found to be caused by *Bertiella spp*. or respectively *B. studeri* (Krief et al., 2005). From captive chimpanzees, also a few cases of infections with *B. mucronata* have been described (Myers & Kuntz, 1972), a parasite which generally seems to be geographically restricted to the Americas and Cuba.

Bertiella species have an indirect, heteroxenous life cycle which to date is still only partly known. Infection of the definite host occurs through ingestion of cysticercoid larvae-containing orbatid mites which serve as intermediate hosts. Following ingestion, the larvae migrate inside the intestines of their host and develop to adult tapeworms which are about 150-450 mm in lengths, 6-15 mm wide (Stunkard, 1940), and can live for up to several years (Thompson et al., 1967). Reproduction occurs through shedding of eggs and/or egg containing proglottids (often several at a time) (e.g. Stunkard, 1940) which are passed with the host's feces and are subsequently consumed by suitable orbatid mites. *Bertiella* eggs isolated from fecal material or gravid proglottids are spherical, about 49-60 x 40-46 µm (*B. studeri*) and 40-46 x

36-40 µm (*B. mucronata*) in size (Denegri & Perez- Serrano, 1997), and contain a hooked oncosphere enclosed by a characteristic pyriform apparatus and a chitineous embryonic membrane (Stunkard, 1940).

Pathogenicity of *Bertiella* infections in non-human primates seems to be generally rather low. Toft (1982) states, that although *Bertiella* specimens may be present in large numbers in the non-human host, clinical disease or enteric lesions are seldom associated with these infections. Clinical symptoms occasionally observed in *Bertiella*-infected human hosts include abdominal pain, intermittent diarrhea, non-specific gastroenteritis, constipation, loss of appetite and weight as well as general fatigue. In rare cases, severe, recurrent abdominal pain with intermittent vomiting has been described (Denegri & Perez- Serrano, 1997).

II.4.2.3.2 Genus *Hymenolepis*

While infections of captive and/or semi-captive chimpanzees with *Hymenolepis spp.* or respectively *H. nana* have been described by several authors (Toft, 1982; Mbaya & Udende, 2011; Akpan et al., 2010), the significance of tapeworms belonging to this genus as parasites of wild chimpanzees seems to be rather limited. The only record of *Hymenolepis* infections in wild chimpanzees so far comes, as mentioned above, from a population of habituated wild chimpanzees at Ngogo/ Uganda (Mühlenbein, 2005), and the diagnosis (according to the author of the respective study) was not absolutely certain due to the absence of visible hooklets within the oncosphere of the examined eggs.

In humans, particularly in children in arid countries of the tropics and subtropics by contrast infections with *H. nana*, the so-called `dwarf tapeworm´, are very common (WHO, 1987; Eckert, 1998). The small adult worms reach a maximum length of ca. 1 to 4 cm, rarely up to 9 cm (Kayser et al., 1998) and live in the small intestine of their host. Infection occurs either directly through ingestion of infective eggs or indirectly through (accidental) ingestion of infected arthropod intermediate host (e.g. flour beetles or fleas) (WHO, 1987). Additionally, internal auto-infection has been described (WHO, 1987; Eckert, 1998). Following ingestion, the eggs hatch and the released oncoshperes penetrate the villi of the small intestine of the host where they develop to cysticercoid stage. The cysticercoids return to the intestinal lumen, attach to the intestinal wall with their scoleces, and within 2 to 3 weeks develop to adult worms (Eckert, 1998). These have a life span of 4 to 6 weeks, live in the ileal portion of the small intestine, and produce gravid proglottids. The spherical or ovoid-shaped eggs, which have a diameter of 30-47 µm, a thin membraneous shell, and contain an oncosphere with three pairs of hooklets (Sadaf et al., 2013), are excreted with the host's feces or might already hatch in the intestines of the host (WHO, 1987; Eckert, 1998). Also described, but less common are human infections with another *Hymenolepis* species, namely *H. diminuta*.

Just as for *Bertiella spp.,* the pathogenicity of *Hymenolepis spp*. in infected non-human primates seems to be fairly low, and even highly infected individuals seem to rarely show *Hymenolepis*-induced enteric lesions and/or clinical symptoms. Likewise, *Hymenolepis* infections of humans, particularly adults seem to be mostly asymptomatic. In heavily infected humans, particularly children however, as reviewed by Sadaf et al. (2013), various symptoms such as pruritus ani, diarrhea, abdominal pain, anorexia, headache, and dizziness have been observed.

II.4.2.3.3 Other Cestodes

Infections of wild chimpanzees with other types of cestodes have so far not been reported.
In captive and semi-captive chimpanzees by contrast infections with *Taenia spp.* (Myers & Kuntz, 1972) as well as *Dipylidium spp*. (Akpan et al., 2010) have been described.

II.5 Impacts of host-intrinsic and seasonal factors on parameters of parasitism

Parameters of parasite infections (e.g. infection intensity, species richness, species prevalence) are generally neither uniform across an infected host population nor constant over time. Instead they are influenced by a variety of parasite-related (e.g. parasite ecology), host-intrinsic (e.g. host sex and age), and/or environmental factors (e.g. season and climate).

II.5.1 Influence of host sex

Although it is not always clear whether or not observed sex biases are genuine or merely sampling or other artifacts (Wilson et al., 2002), influence of host sex on parasitic infection parameters has been reported across a range of parasite taxa and vertebrate host species, including humans and non-human primates (e.g. Hausfater & Watson, 1976; Zuk, 1990; Zuk & McKean, 1996; Gillespie et al., 2010). Most commonly observed, especially in nematode-infected mammals, is a tendency of male individuals to exhibit higher rates of parasite prevalence and/or infection intensity than the respective females (Poulin et al., 1996; Zuk & McKean, 1996; Ferrari et al., 2004, Krief et al., 2005, Gillespie et al., 2010). At the same time however, also the opposite scenario has been described (e.g. Hausfater & Watson, 1976, Polderman et al., 1991; Monteiro et al., 2007), indicating that the type and extent of observed sex biases may vary between different host species and parasite taxa (Poulin et al., 1996; Wilson et al., 2002).

In gastrointestinal helminth-infected non-human primates, the respective host sex-related biases appear to be equally inconsistent (Nunn & Altizer, 2006). Whereas some authors conducting helminthological surveys of wild primate populations found higher helminth prevalence in male study group members (e.g. Roduit, 1999; Gillespie et al., 2005; Gillespie et al., 2010), others observed higher prevalence rates

in females (e.g. Monteiro et al., 2007; Hausfater & Watson, 1976) or else no respective differences between the sexes (e.g. Stoner, 1996; Gillespie et al., 2004; Gillespie et al., 2010). Some authors counted higher parasite loads in male primates (e.g. Roduit, 1999; Krief et al., 2005), others in female individuals (e.g. Hausfater & Watson, 1976).

Although they are certainly often intertwined (e.g. Grear et al., 2009), the causes supposedly responsible for generating sex biases in parasitism rates are usually divided into two categories – ecological differences between the host sexes and physiological, mostly hormonal, differences (Zuk & McKean, 1996; Wilson et al., 2002).
Ecological differences like sex-specific behavior, diet composition or morphological variations (e.g. body size/ body mass), may result in varying exposure of male and female hosts to parasite infectious stages or may render one of the sexes a more attractive host (e.g. Zuk & McKean, 1996; Klein, 2000; Wilson et al., 2002).
Physiological respectively hormonal differences between male and female hosts by contrast are mainly associated with hormone-dependent sex-specific variations in the immune response towards parasitic infections and/or direct hormonal effects on parasite development and reproduction (Zuk & McKean, 1996; Wilson et al., 2002; Escobedo et al., 2005). Overall however, these associations are rather complex and to date still only poorly understood. Furthermore, different hormonal effects might interact (e.g. Ezenwa et al., 2012) and/or vary for different host-parasite systems.

Testosterone for instance is generally known to depress both cell-mediated as well as humoral immune responses in male hosts (Alexander & Stimson, 1988). In experimentally infected female mice for example, testosterone has been found to induce persistent susceptibility to *Plasmodium chabaudi* malaria by altering liver gene-expression leading to changes in liver metabolism including a decreased production of antibodies and IFNy (Delic et al., 2010). Moreover, testosterone has been shown to stimulate and enhance the growth and development of certain parasites (Addis, 1946; Harder et al., 1992). On the other hand however, also a diminishing effect of testosterone on the fertility and reproductive capacity of for instance *Schistosoma haematobium* has been described (Remoué et al., 2002), and Morales-Montor et al. (2002) observed that testosterone was able to directly enhance the cellular immune response against *Taenia crassiceps* in experimentally infected mice.

Estrogens by contrast, while reportedly inhibiting some cell-mediated immune responses, are believed to enhance humoral immunity and thus to exert a protective effect on female hosts (Alexander & Stimson, 1988; Klein, 2004). A concept that occasionally is referred to as `female supremacy paradigm´, but which seemingly does not apply to all host-parasite systems (Morales-Montor et al., 2004). In mice for instance, estrogens reportedly favor growth and development of the cestode parasite *Taenia crassiceps* (Morales-Montor et al., 2002).

Progestines (e.g. progesterone) can have both stimulatory as well as suppressive effects on the host immune system, but are typically regarded as being immunosuppressive (Klein, 2004). Consequently, progestines may also reduce female immunity against parasitic infections – at least in some host-parasite systems (Dobson, 1966; Zuk & McKean, 1996). On the other hand however, also direct antiparasitic effects of progesterone, for example on the molting-rate of *Trichinella spiralis* larvae, have been described (Hernandez-Bello et al., 2011).
The involvement of prolactine in the periparturient relaxation of immunity and associated rise in fecal egg counts observed in female individuals of several host species is still unclear (Chartier et al., 1998) and might possibly also vary for different hosts and parasites. While some authors found a positive association of increased fecal egg counts with high prolactin concentrations, the results of other studies were ambiguous or the studies found no association (Rahman & Collins, 1992; Chartier et al., 1998).

As high levels of stress hormones (e.g. glucocorticoids) are known to have various suppressing effects on the immune system, they are also likely to reduce immunity against parasitic infections in mammal and other vertebrate hosts (Zuk & McKean, 1996). The respective amount of stress they are exposed to (Zuk & McKean, 1996) as well as the production of stress hormones and the interaction between these hormones and the immune response may however differ between male and female hosts (Wilson, 2002; Klein 2004) which again might lead to differences in the susceptibility to parasitic infections between the two sexes. Overall however, the role of stress hormones as mediators of sex differences with respect to parasitic infections has reportedly not yet been adequately explored (Klein, 2004).

II.5.2 Influence of host age

Influence of host age on parameters of parasitic infection has been reported from parasitological studies of various vertebrate host species (e.g. Bell & Burt, 1991; Horii et al., 1982; Kahn et al., 2010), including humans and non-human primates (e.g. Dancesco et al., 2005, Müller-Graf et al., 1997). The observed type of influence however varies. Whereas comparatively higher parameters of parasitism have frequently been found in younger hosts (e.g. Müller-Graf et al., 1997; Horii et al., 1982; Dancesco et al., 2005, Kahn et al., 2010), also the opposite effect, such as for instance higher helminth prevalence, diversity or intensity of infection in older as compared to young hosts has been reported (Bell & Burt, 1991, Gillespie et al., 2010; Turner & Getz, 2010).
A potential reason for this apparent discrepancy is that the responsible epidemiological mechanisms might be highly specific to the respective host-parasite interaction and thus might vary considerably between different parasites and different host species or respectively -populations (Wilson et al., 2002).

Older hosts for example might show higher levels of parasite species richness and infection intensity because they are likely to have been exposed to more parasites

during their respective lifetime than younger individuals of the same host population (Bell & Burt, 1991; Morand & Harvey, 2000).

This prolonged or respectively repeated parasite contact might on the other hand however have triggered the immune system of the older hosts to such an extent that they have build up a certain level of resistance (i.e. a certain level of acquired immunity) and as a result exhibit lower parameters of infection (e.g. Dobson et al., 1990, Wilson et al., 2002) than young individuals whose naïve immune system has not had time yet to acquire any resistance.

Through coevolution with their host, parasites have developed various adaptation mechanisms, such as immune evasion (e.g. reviewed by Schmid-Hempel, 2008) which in parasitic helminths for example might lead to a decrease in virulence and increased host-tolerance (e.g. Schierack et al., 2003). The buid-up of an effective host immune response and resistance against certain parasites might potentially however be rather slow (e.g. Yazdanbakhsh & Sacks, 2010).

The often comparatively higher infection susceptibility of young hosts might be further increased by their generally still low innate immunity and overall low immune response (Nunn & Altizer, 2006; Fallon et al., 2003; Nel et al., 2011) resulting from their not yet fully developed immune system. A similarly low overall immune response might on the other hand however also be observed in very old hosts towards the end of their lives as a result of their generally decreasing immune function (Morand & Harvey, 2000). Infants by contrast might temporarily be protected against parasitic infection by maternal antibodies (Nunn & Altizer, 2006).

Changes in the host's hormonal status at different life stages (e.g. changes in testosterone and estrogene levels and/or levels of circulating adrenal androgens such as dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) at the onset of puberty) are a further potential cause of age-dependent discrepancies in parasite susceptibility or respectively -resistance within a host population (e.g. Fulford et al., 1998).

Apart from immunological differences between younger and older hosts however, also other factors, such as age-specific variations in parasite exposure, might lead to significant discrepancies in the parameters of parasite infection between different host age classes (e.g. Bundy, 1988a). Cause of such age-specific variations might for instance be differences in diet composition and/or behavior between different age classes (e.g. more frequent geophagy in children (Bundy, 1988a) or more frequent water contact in juvenile baboons (Müller-Graf et al., 1997)).

II.5.3 Influence of seasonality and seasonal climatic variations

Considerable seasonal variations in infection parameters are a commonly observed phenomenon in many host-parasite systems. As reviewed by Altizer et al. (2006), there are a number of biological mechanisms by which seasonality and seasonal variations in temperature, rainfall, and for instance resource availability can impact host-parasite interactions and as a consequence lead to respective parasite-, host-, site-, and/or habitat-specific variations in observed patterns of parasitism.

On the host level these biological mechanisms might for instance include seasonal variations in host exposure to infected intermediate hosts or infective parasite developmental stages in the environment. Additionally, seasonal variations in immune response and infection intensity might play a role which again might be caused by seasonal changes in for example host behavior, abundance, density and diet, as well as reproductive status, stress level, and body condition (Altizer et al., 2006).

On the parasite level, seasonal changes in climatic components like rainfall, humidity, UV-radiation, and temperature might for instance have a substantial influence on the development, survival, and infectivity of helminth eggs and larvae in the environment and thus on the degree of host exposure to respective infective helminth developmental stages (e.g. Pietrock & Marcogliese, 2003, Tembeley, 1998, Kraglund et al., 2001). The relative tolerance of the eggs and larvae of different helminth species to changes in individual climatic components might however vary considerably (Pietrock & Marcogliese, 2003).

II.6 Molecular markers used for the identification and differentiation of helminth parasites

As outlined before (see paragraph I.1), the application of molecular methods such as PCR and sequencing for the identification of helminth parasites of non-human primates, particularly chimpanzees, is to date still rather rare, and the majority of helminthological surveys of wild ape and monkey populations still primarily rely on conventional coprologogical methods. As for the diagnosis and characterization of helminth parasites of humans and domestic livestock by contrast (e.g. Morgan, 2000; Gasser et al., 2008a+b; Bott et al., 2009), DNA-based diagnostic techniques have already become a more or less routinely employed diagnostic tool.

Through application of molecular methods, the limitations of morphological helminth identification – and thus the major disadvantage of conventional coprology – can be overcome. The selection of suitable molecular markers however is crucial and depends on the taxonomic level at which the identification of the respective helminths should occur (de Gruijter, 2005). Different DNA regions exhibit variable magnitudes of sequence variation and, while for the identification of a given helminth to species level for instance markers with considerably higher inter- than within-species

variation are needed, markers with a significant amout of within-species variation are necessary for more specific identification (e.g. to strain level) (de Gruijter, 2005).

Molecular markers commonly utilized for the identification and characterization of helminth parasites and other helminths are for example nuclear ribosomal internal transcribed spacer ITS-1 and ITS-2 as well as various mitochondrial gene regions (e.g. nad4, cox1).

II.6.1 Nuclear ribosomal DNA markers

The non-coding first and second nuclear ribosomal internal transcribed spacers (ITS-1 and ITS-2) are particularly employed for the identification and diagnosis of known, well-differentiated helminth and especially nematode species.

The ITS region, located between the 18S and 28S ribosomal DNA genes (see Figure 2), is one of the most variable gene regions and contains a high number of insertations and deletions (Blouin et al., 1998). This high variability in conjunction with relatively low levels of intra-specific polymorphism (generally < 1.5% (Gasser et al., 2008a)) renders the two ITS subunits excellent markers for this particular application (Blouin, 2002). Another advantage of the two markers is their relative shortness and repetitive nature, rendering any diagnostic PCR assay sensitive (Gasser, 2001). Moreover, respective universal primers that work with most nematodes are readily available (Blouin, 2002).

Reportedly less suitable by contrast is the use of ITS-1 and ITS-2 sequences for the detection of cryptic helminth species (Blouin et al., 1998). Reason for this is that, despite the high overall variability of the ITS gene region, respective sequence differences between very closely related helminth species are often very small (Blouin, 2002).

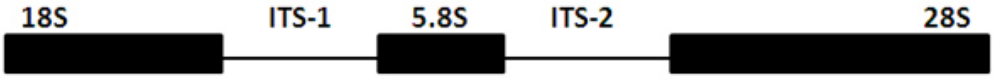


Figure 2. *Schematic representation of the ribosomal internal transcribed spacer (ITS) region**

*adapted & reprinted from Molecular and Cellular Probes, 11(3), Romstad A, Gasser RB, Monti JR, Poldermann AM, Nansen P, Pit DSS, Chilton NB, Differentiation of *Oesophagostomum bifurcum* from *Necator americanus* by PCR using genetic markers in spacer ribosomal DNA, 169-176, Copyright (1997); with permission from Elsevier.

II.6.2 Mitochondrial DNA markers

Mitochondrial DNA generally evolves faster than nuclear DNA, and mtDNA sequences reportedly accumulate substitutions much more quickly than for example ITS sequences (Blouin, 2002). Additionally, interspecific mtDNA sequence differences among even the most closely related helminth species (in nematodes,

marker dependent, typically around 10-20% (Blouin et al., 1998)) are distinctly outside the range of respective within-species sequence differences, which generally range from about a fraction of one percent to 2% (Blouin et al., 1998). Maximum reported within-species difference between two specimens from the same interbreeding population has been 6% (Blouin et al., 1998; Blouin, 2002).

As a result, mtDNA markers such as for example the nicotinamide dehydrogenase subunit 4 (nad4= ND4) sequences or cytochrome oxidase I (cox1= COI) sequences (see Figure 3) are likely to be very useful for determining the relationship between very closely related helminth species (Blouin et al., 1998) and to be more suitable markers for the detection of potential cryptic species than ITS sequences – especially if only a small number of specimens are examined (Blouin et al., 1998; Blouin, 2002).

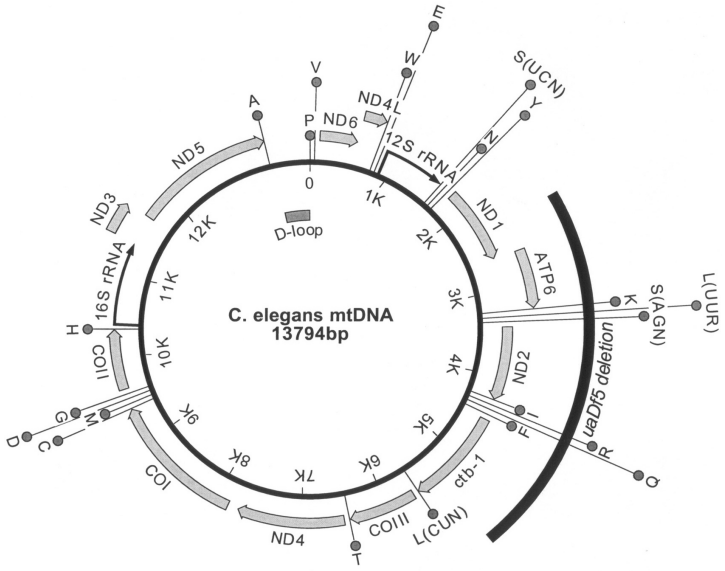


Figure 3. *Gene map of the Caenorhabditis elegans mtDNA**

* reprinted from Lemire, 2005; Mitochondrial genetics. In WormBook (ed) The *C elegans* Research Community, WormBook. doi/10.1895/wormbook.1.25.1; http://www.wormbook.org;
with permission from the author

III METHODS & MATERIAL

III.1 Study site

Field work and sample collection for the present study was conducted inside Taï National Park, Côte d'Ivoire within the research area of the Taï Chimpanzee Project (TCP). This chimpanzee field research project was founded in 1979 by Swiss primatologist Christophe Boesch and is presently run by the Department of Primatology of the Max Planck Institute for Evolutionary Anthropology (MPI EVA), Leipzig, Germany.

The study site, comprising the territories of currently three habituated groups (south-, east- and north group) of wild western chimpanzees (*P. t. verus*), three research camps (south-, east- and north camp), and a small field lab, is located inside the forest about 5 to 10 km from the western park boundaries and about 15 to 20 km linear distance from Taï, the nearest local town. All three chimpanzee groups are followed and observed on a daily basis by MPI EVA researchers and/or local field assistants.

III.2 Collection & processing of chimpanzee fecal samples

Fecal samples were collected and exported with permission of the Ivorian authorities. Importation took place according to German (and respectively U.S.) veterinary regulations for import of organic material.

III.2.1 Period and frequency of sample collection

Fecal samples were collected from chimpanzees belonging to the three study groups during three field seasons: season 1: June 2007-April 2008, season 2: September 2008-August 2009, season 3: December 2009-March 2010, whereby samples collected during the latter season were mainly used to culture L3-larvae and free-living adult *Strongyloides* specimens for molecular analysis.

Main study group was the so-called south group, and the majority of samples analyzed in the present study were obtained from this chimpanzee community. Fecal samples were gathered from adult, adolescent as well as juvenile group members, whereby it was attempted to collect a minimum of two samples per month from each adult and adolescent individual. Because of logistical constraints as well as due to the chimpanzees' behavior and group dynamics this was however not always possible. Especially during the months with high rainfall and relatively scarce and scattered main food sources (May, June, July, and August), the group was often split up, and individuals or subgroups were occasionally not seen for several weeks.

Samples from juvenile and particularly from infant chimpanzees were in general collected more opportunistically, as defecation of individuals of these age groups was

less readily observed and sample collection thus more intricate. Nevertheless, it was attempted to obtain a minimum of one fecal sample per months from at least some individuals. Feces from infants younger than two years could hardly (only in two cases) be collected at all, as individuals of this age are almost constantly carried on the belly or back of their mothers, and defecation could thus either not be observed or feces were deposited onto the fur of the mothers.

Fecal samples from adult, adolescent, and juvenile chimpanzees of the north and east community respectively were collected opportunistically, and from several individuals of both groups none or only one sample could be obtained throughout the study period.

III.2.2 Method of sample collection

Chimpanzees were followed and observed on a daily basis from around six o'clock in the morning, when the chimpanzees usually leave their nests, for a period of approximately 8 to 12 hours. Chimpanzees were individually known and could readily be distinguished by their morphological characteristics. To minimize the risk of pathogen transmission from researchers and research assistants to the chimpanzees, care was taken to keep a minimum distance of seven meters between human observers and the apes at all times.

Fecal samples were collected from visually identified individuals directly after defecation, once the respective chimpanzee had deliberately moved a minimum of seven meters away. Under no circumstances chimpanzees were disturbed or chased off. In most cases, samples could be collected within one or two minutes following defecation. Maximum time span between defecation and sample collection was ca. 30 minutes. Upon collection, care was taken to avoid collecting any fecal matter contaminated with soil or vegetation. Feces were categorized according to their size as small, medium or large. Consistency was assessed on a scale from 1 to 5 (see Table 7 for details). Samples were collected in plastic bags labeled with chimpanzee name, defecation- and collection time as well as size and consistency, placed in a thermos flask chilled with cold packs, and transported back to the field lab within hours. The field lab was based at the so-called south camp, one of the research camps of the Taï Chimpanzee Project, situated at the periphery of the south group territory.

Table 7. *Key of fecal consistency (adapted from Goldsmid, 1971)*

5 = formed stool (cylindrical, cannot be stirred)
4 = mushy-formed stool (cylindrical, but can be stirred)
3 = mushy stool (soft and takes shape of container)
2 = mushy-diarrheic stool (softer than above, but cannot be poured)
1 = diarrheic stool (liquid enough to be poured)

III.2.3 Sample preservation and storage

In the field lab, fecal samples were immediately placed in a gas-powered fridge until further processing. Each sample was given a unique identification code (e.g. S1-1SUM), consisting of `S1´ for samples collected between June 2007 and April 2008, `S2´ for samples collected between August 2008 and August 2009 and `S3´ for samples collected between December 2009 and February 2010, a consecutive number and a three letter abbreviation representing the name of the sampled chimpanzee (e.g. SUM= Sumatra). Within hours samples were macroscopically examined for adult nematodes and tapeworm proglottids. If present, tapeworm proglottids were rinsed in clean boiled and filtered water to wash off any adherent fecal matter and stored individually in tubes containing 70% ethanol.

3.0 grams of fecal material of each sample were weighed on a digital 0.01-gram scale and transferred to labelled plastic cups using disposable plastic spatula. In order to avoid cross- contamination, care was taken to avoid swapping spatula between the different samples. To prevent blowflies from laying their eggs onto the samples, plastic cups were covered immediately with tin foil and stored in the fridge until further processing and examination using a modified Wisconsin Sugar Flotation (MWSF).

For fecal cultures employing the Harada-Mori technique (Harada & Mori, 1955), two filter paper strips (ca. 12 cm x 1 cm) per fecal sample were coated one third of their length with a layer of fecal material (about 1 to 1.5 mm thick) using a disposable plastic spatula. Each paper strip was placed into a conical 15 or 50 ml test tube filled with 2 to 3 ml of filtered water in such a way that its lower end was in contact with the water. The other end was folded over the lip of the tube to prevent the paper strip from sliding all the way into the water, and each tube was loosely closed with a screw cap. Tubes were labelled with date and sample number and incubated upright at ambient temperature for 7 to 10 days (maximum 21 days). To avoid desiccation of the fecal material, water level in the tubes was checked regularly and if necessary, carefully topped up using disposable plastic pipettes.

For later ex-situ analysis, an additional aliquot (~ 5 ml) of fecal material from each sample was placed into a 15 ml test tube filled with 5 ml of 10% formalin. Tubes were closed tightly with screw caps, labelled with date, sample number and chimpanzee name and shaken vigorously, to maximize contact between the fixative and fecal material. Tubes were stored and transported in a dark environment at ambient temperatures and 120 selected tubes were sent to the Gillespie Lab, Emory University, Atlanta, USA for further processing and analysis within a maximum of two years of collection.

III.3 Collection and processing fecal samples from sympatric monkeys

III.3.1 Sample collection

Fecal samples from unhabituated sympatric monkeys (*Piliocolobus badius, Colobus polykomos, Cercopithecus diana, Cercocebus atys*) in the chimpanzee territories were collected opportunistically throughout the study period. The majority of samples were collected in south group territory. None of the sampled monkeys was individually known, and also sex-determination was generally not possible. Repeated sampling of the same individuals can thus not be ruled out. Feces were collected in labeled plastic bags as described above within a few (ca. 1 to 5) minutes following observed defecation. Monkey species as well as location, date and time of defecation and sample collection were recorded, and samples were transported back to the field lab within hours. Transport occurred in a thermos flask on chilled cold packs.

Additionally, on several occasions during the study period, intestinal content remains from monkeys, which had been killed and consumed by the chimpanzee study groups, could be obtained. The samples were collected in labeled plastic bags once the chimpanzees had moved off (i.e. several minutes up to ca. one hour after the death of the monkey) and were transported to the field lab as described above.

Samples of intestinal content were also collected if possible during monkey necropsies. On-site necropsies of all primate and non-primate mammal species found dead in the research area were routinely performed as part of the chimpanzee long-term health monitoring and were always carried out under high security standards (Leendertz et al., 2006a). Time and cause of death of the dissected monkeys were generally unknown. Intestinal content samples were collected in labeled 15 ml test tubes without preservatives and transported to the field lab immediately after the necropsy.

III.3.2 Sample processing

Each sample was assigned a unique code (e.g. S2-9Mang) consisting of a continuous number and abbreviations for the respective sampling season (e.g. S2; see paragraph III.2.3 for details) and monkey species (i.e. Pbad= *Piliocolobus badius*, Cdian= *Cercopithecus diana*, Cpoly= *Colobus polykomos*, Mang= `mangabey´ or *Cercocebus atys*). Afterwards, Harada-Mori fecal cultures were prepared from each sample as described above for chimpanzee fecal samples.
Additionally, for later analysis (beyond the scope of the present study) one aliquot of each sample was preserved in 10% formalin and, if enough fecal matter was available, a second, unpreserved aliquot was stored in liquid nitrogen.

III.4 Recording of weather data

Weather data were collected on a daily basis (mostly between about 4:30 am and 8:00 am) by resident researchers and project staff. Daily cumulative precipitation was measured using a standard plastic rain-gauge positioned in a forest clearing within the perimeter of the main research camp (south camp) at a height of about one meter above the ground. Daily maximum and minimum ambient temperatures were recorded using a standard battery-powered digital thermometer/ hygrometer installed on the veranda of one of the main buildings in the same camp.

III.5 Sample screening using conventional coprological methods

III.5.1 Modified Wisconsin Sugar Flotation (MWSF)

III.5.1.1 Method description

Fecal samples were examined in the forest lab using a slightly adapted `Modified Wisconsin Sugar Flotation´ (MWSF) (Cox & Todd, 1962; Pittman et al., 2010) with Sheather's solution. This particular flotation method had been employed as it allows a qualitative and quantitative assessment of the nematode and cestode developmental stages present in each examined fecal sample. Furthermore, this method does not produce any toxic waste – an imperative criterion as the respective lab work was carried out inside a national park – and all necessary equipment and consumables were readily available in the forest lab or could be purchased locally.

Sheather's solution (specific gravity 1.27) was prepared by adding 454 grams of table sugar to 355 ml of very hot filtered water. The mixture was stirred until all sugar was dissolved, left to cool, and kept refrigerated until use.

3.0 grams** of fresh, unpreserved fecal material per sample were each placed into a plastic cup and mixed well with 10 ml of Sheather's solution. A funnel and conventional kitchen strainer were placed into a conical 15 ml test tube, and the feces-sugar solution mixture was poured through the strainer and funnel into the tube. All remaining liquid was squeezed out from the fecal material left in the strainer with a disposable plastic spatula.

Funnel and strainer were removed, and the test tube was closed with a screw cap and centrifuged for 1.5 minutes at maximum speed and ca. 1077 x *g* with a hand centrifuge (Hettich Lab Technology, Tuttlingen, Germany). Afterwards, using a disposable plastic pipette, fresh Sheather's solution was carefully added to the tube to create a slight meniscus, and a glass cover slip (18 x 18 mm) was placed on top. After five minutes flotation time the coverslip was removed and transferred to a labelled glass slide.

The slide was placed immediately under a trinocular compound microscope with a x10 ocular (Zeiss Axiostar, Carl Zeiss AG, Germany), and the entire cover slip was

scanned for nematode and cestode developmental stages in a meandering fashion using the x40 objective lens of the microscope. Trematode eggs could not be detected using this method, as they don't float on account of their relatively high weight. Helminth eggs and hatched L1-larvae present were counted and if possible identified to genus level based on their morphological characteristics like shape, size, contents, shell thickness, and colour.

Respective reference data were obtained from the literature (e.g. Goldsmid, 1982; Sanground, 1929; Thienpont et al., 1990; Justine, 1988; Hasegawa & Udono, 2007; Stunkard, 1940). Eggs and hatched L1-larvae of the different nematode genera belonging to the superfamily Strongyloidea however, due to their great morphological similarity (Blotkamp et al., 1993; Newton et al., 1998b; Gasser et al., 2008b), could not reliably be distinguished and were thus collectively classified as `strongyles´. The different types of eggs and larvae recovered from each sample were recorded, and representatives of each type were measured (width and length in micrometers) with a calibrated ocular micrometer and photographed using a digital camera (Nikon Coolpix) mounted onto the trinocular tube of the microscope.

Furthermore, the number of detected parasitic specimens or `propagules´ (Turner & Getz, 2011) per gram feces (ppg) was determined by dividing the total number of helminth eggs and hatched L1-larvae present under the cover slip by 3**. Ppg-values were not determined for samples containing a lot of detritus.

*** In rare cases (25 out of 857 samples) more or less fecal material than 3.0 grams had to be used (maximum 4.65 grams, minimum 2.0 grams). The respective ppg-values were calculated accordingly by dividing the respective total number of helminth eggs and L1-larvae present under the cover slip by the respective sample weight*

III.5.1.2 Number and distribution of examined samples

Altogether, a total of 857 fresh, unpreserved fecal samples obtained between January 2008 and August 2009 from 72 chimpanzees (35 M, 37 F) were examined using this method (Table 8). 446 samples had been collected from male individuals, 411 samples from females. The number of samples examined per individual ranged from 1 to 61 (median: 7; quartiles: 3/ 20.5; mean: 11.9 +/- 12.0). For the main study group (south group), the median number of samples examined per individual was 20 (quartiles: 5.5/ 27; mean: 18.9 +/- 12.8; range 1 to 61).

Table 8. *Distribution of fecal samples examined using Modified Wisconsin Sugar Flotation (N=857)*

Study group	Adult (n= 489)		Adolescent (n= 180)		Juvenile (n= 152)		Infant (n= 36)	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
South (n_S= 719)	159	263	105	42	92	26	19	13
North (n_N= 77)	2	33	12	9	11	8	1	1
East (n_E= 61)	25	7	9	3	11	4	0	2
Total (N= 857)	**186**	**303**	**126**	**54**	**114**	**38**	**20**	**16**

Propagule counts were performed on 806 of the overall examined 857 samples (1 to 58 samples per individual chimpanzee; median: 5; quartiles: 3/ 20; mean: 11.19 +/- 11.45) (Table 9).

Table 9. *Distribution of fecal samples on which progagule counts were performed (n=806)*

Study group	Adult (n= 462)		Adolescent (n= 171)		Juvenile (n= 139)		Infant (n= 34)	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
South (n_S=678)	152	245	100	41	85	25	17	13
North (n_N= 68)	2	31	11	7	9	6	1	1
East (n_E= 60)	25	7	9	3	10	4	0	2
Total (N= 806)	**179**	**283**	**120**	**51**	**104**	**35**	**18**	**16**

III.5.2 Harada-Mori Fecal Cultures (Harada & Mori, 1955)

III.5.2.1 Method description

Harada-Mori Fecal Cultures were performed on site in order to supplement MWSFs and to allow for a better and more reliable strongyle and *Strongyloides* differentiation and identification.

After an incubation period of 7 to 10 (max. 21) days at ambient temperature, the filter paper strip from each fecal culture tube was discarded, and the water in the conical tube bottom decanted into a Petri dish and examined for L3-larvae and free-living adult nematodes using a magnifying glass. If larvae or free-living adults were present, 6 to 8 drops of water were transferred onto glass slides (two drops per slide) using a disposable plastic micropipette.

Slides were immediately placed under a trinocular compound microscope with 10x ocular (Zeiss Axiostar, Carl Zeiss AG, Germany) and examined using the x20 and x40 objective lens of the microscope. If possible, all L3-larvae present were differentiated to genus level according to their morphological characteristics following the identification key compiled by Little (1981). Identification to species level was generally not possible due to the inherent morphological similarity between the larvae

of different species belonging to one genus (Newton et al., 1998b; Gasser et al., 2008b). Recovered free-living adult females of the family Strongyloididae by contrast could be identified to species level based on characteristic morphological features of the vagina and perivulval area as described by Speare (1989).

Representatives of all types of larvae and free-living adults detected in each sample were measured (width and length in micrometers) using a calibrated ocular micrometer and photographed using a camera (Nikon Coolpix) mounted onto the trinocular tube of the microscope.

III.5.2.2 Number and distribution of examined samples

Due to logistical reasons, fecal cultures could only be performed for 793 of the 857 fecal samples examined using MWSFs (Table 10). 410 of these samples had been collected from male chimpanzees, 383 samples from females. Overall, 1 to 54 fecal cultures per individual (median: 6; quartiles: 3/20; mean: 11.3 +/- 10.9) had been performed from fecal samples of a total of 71 chimpanzees (35 M, 36 F).

Table 10. *Distribution of performed Harada-Mori fecal cultures (n=793)*

Study group	**Adult** (n= 451)		**Adolescent** (n= 166)		**Juvenile** (n= 140)		**Infant** (n= 36)	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
South (n_S= 665)	142	244	97	40	85	25	19	13
North (n_N= 67)	2	31	11	6	8	7	1	1
East (n_E= 61)	25	7	9	3	11	4	0	2
Total (N= 793)	**169**	**282**	**117**	**49**	**104**	**36**	**20**	**16**

III.5.3 Sodium-Nitrate Flotation and Sedimentation

III.5.3.1 Method description

+ Sodium-Nitrate Flotation

Formalin-fixed fecal samples were analyzed in the Gillespie Lab, Emory University, Atlanta, in November 2009, using Sodium-Nitrate Flotations ($NaNO_3$-flotations) as described by Gillespie (2006) and advocated by the Great Ape Survival Plan (GRASP) and the IUCN/ SSC Primate Specialist Group (Gillespie et al., 2010; Leendertz, 2010). Due to logistical constraints (for example, no electric centrifuge was available in the forest lab) this method could not be performed on site. In combination with sedimentations it was therefore additionally included into the methodological repertoire of the present study in order to comply with the general recommendations for primate endoparasite surveys (Gillespie, 2006) and thus to render the results of this study more comparable.

About 3 to 4 grams of formalin-fixed fecal material from each sample was transferred from its container to a plastic centrifuge tube. The tubes were filled two thirds with

distilled water and the fecal material homogenized with disposable wooden applicators. To remove the formalin from the samples, the tubes were centrifuged at 1220 x *g* (1400 rpm) for 10 minutes (centrifuge: Allegra® X-12, Beckman Coulter, Inc., USA; rotor: SX4750A ARIES^{TM}) and the supernatant poured off. The fecal pellets were then re-suspended in $NaNO_3$ ($NaNO_3$-solution: 1 liter of distilled water + 564 grams of sodium nitrate) and more $NaNO_3$ was added to each tube to form a slight meniscus. A microscope cover slip (18 x 18 mm) was placed on the lip of each tube, and the tubes were centrifuged for 10 minutes at 1220 x *g* (1400 rpm).

Afterwards the cover slips were removed, and each slip was carefully transferred to a glass microscope slide labeled with the respective sample number. Because of the rapid crystal formation of the $NaNO_3$-solution, all slides were immediately examined for helminth eggs and larvae using the x10 and x40 objective lenses of a compound microscope with x10 ocular (Leica DM750, Wetzlar, Germany). Helminth eggs were identified to genus level according to their morphological characteristics (see paragraph III.5.1.1). Due to their close morphological similarity, eggs of nematodes belonging to the superfamily Strongyloidea however were not identified to genus level, but jointly classified as `strongyles´.

+ Sedimentation

The same set of formalin-fixed fecal samples screened with $NaNO_3$-flotations as described above was also subjected to a sedimentation process (Gillespie, 2006) using the fecal pellets left over from the previous flotation procedure. Sedimentations were mainly performed to assess the presence of trematode helminths, which, as trematode eggs don't float is only possible employing this method.

Each fecal pellet was transferred from its tube into a 50 ml plastic beaker and mixed well with 40 ml of soapy water (soapy water was prepared by adding ca. 4 to 5 milliliters of liquid hand soap to one liter of tap water). The suspension was filtered through a double layer of cheese cloth held over the lip of the beaker into a 60 ml glass tube with conical bottom. The cheesecloth was rinsed with soapy water to transfer all fine fecal material into the glass tube. The remaining fecal pellet in the cheesecloth was discarded and the feces-soap-water mix in the glass tube was left to sediment for 10 to 20 minutes.

The supernatant liquid was pipetted off using a disposable plastic pipette and the sediment rinsed back into the plastic beaker with more soapy water and re-filtered through the cheesecloth into the glass tube. After rinsing the cheesecloth again to transfer all fine fecal material back into the glass tube, the cheesecloth was discarded and the feces-soap-water suspension left to sediment for another 10 to 20 minutes. Five drops of sediment were transferred to a glass microscope slide labeled with the number of the respective sample, mixed well with one drop of iodine solution and covered with two glass cover slips (18 x 18 mm) placed side by side. Slides were placed under a compound microscope with x10 ocular (Leica DM750, Wetzlar,

Germany) and screened thoroughly for helminth eggs and larvae using the x10 and x40 objective lenses of the microscope. Helminth eggs were again identified and classified as described before.

III.5.3.2 Number and distribution of examined samples

Overall, a total of 120 formalin-fixed samples (Table 11), which had been collected between June 2007 and August 2009 from 30 south group chimpanzees (14 M, 16 F), were screened with the combination of $NaNO_3$-flotations and sedimentations. The number of examined samples per individual ranged from 3 to 6 (median: 4; quartiles: 4/ 4; mean: 4.0 +/- 0.6). Samples from chimpanzees of the north and east group were not examined.

Fresh and unpreserved aliquots of 96 of these 120 samples had been examined before using MWSFs.

Table 11. *Distribution of fecal samples examined using $NaNO_3$- flotations and Sedimentations (N=120)*

	Sex		
Age class	**M**	**F**	**Total**
Adult	20	42	66
Adolescent	16	8	20
Juvenile	16	6	22
Infant	4	8	12
Total	**56**	**64**	**120**

III.6 Statistical data analysis

III.6.1 Descriptive statistics

Means, standard deviations, medians and quartiles were calculated in R (R Core Team, 2013) or Excel (Microsoft Office Excel 2007 for Windows). Helminth prevalence values and confidence intervals were calculated using the program Quantitative Parasitology 3.0 (Rozsa et al., 2000).

Graphs were plotted in R (R Core Team, 2013) or using Microsoft Office Excel 2007 for Windows (histograms).
Graphs in Figures 14 to 18 were created by R. Mundry (Department of Primatology; MPI of Evolutionary Anthropology, Leipzig).

III.6.2 Inferential statistics

Statistical comparison of helminth prevalence values and assessment of host-sex and sampling intensity-dependent prevalence differences was done using Fisher's exact test.

Comparison of conventional examination techniques (MWSF versus Harada-Mori fecal cultures) and type of sample preservation (formalin-fixed versus unpreserved) was done with McNemar's test using GraphPad QuickCalcs (GraphPad Software, Inc., USA; www.graphpad.com), whereby respective P-values were computed using a chi-square approximation with Yates' correction. Ppg-values obtained from formalin-fixed and unpreserved fecal aliquots were compared using Wilcoxon matched-pairs signed rank test. Both, Fisher's exact test and Wilcoxon matched-pairs signed rank test were performed employing GraphPad InStat 3.10 for Windows software (Graphpad Software, Inc., USA, 2009).

The impact of seasonality (season*, rainfall**, maximum temperature**) and/or host intrinsic factors (chimpanzee sex, -age, and -group) on helminth reproductive output (ppg) and cumulative helminth morphotype richness, by contrast were investigated in R (R Core Team, 2013) using the following two statistical models. Included in the respective analysis were only fecal samples for which complete data sets (i.e. information for all covariates) were available.

* `Season´ was integrated into the model by first transforming the date (i.e. the date of defecation and sample collection) into a circular variable using the equation: season= 2*pi*date/365 (where date was the index number of the day) and then including the sine and cosine of season into the models.

** Weather data utilized, comprised the values for rainfall and maximum temperature averaged over a period of 30 days between day 60 and day 30 prior to the collection of each fecal sample in order to account for external helminth egg/larvae development as well as prepatence. All rainfall and temperature data had been recorded in south camp as described before. See Annex, Figures A1 & A2 for an overview of recorded weather data between November 2007 and August 2009.

Models were built and run with the help and guidance of R. Mundry (Department of Primatology; MPI of Evolutionary Anthropology, Leipzig).

III.6.2.1 Model 1 (response variable: individual cumulative helminth morphotype richness)

The influence of host intrinsic factors (sex, age and group) on the cumulative helminth richness per individual (i.e. on the total number of different helminth morphotypes each individual chimpanzee was found to be infected with employing MWSFs and Harada-Mori fecal cultures) was analyzed through non-linear minimization using a Newton-type algorithm. Fixed effect predictor variables included into the model were chimpanzee group, -sex and -age, as well as number of samples examined from each individual. `Age´ as used in this model was the age of the respective chimpanzee at the date of its last fecal sample considered in this analysis. Categorial predictor variables (sex and group) were manually dummy-coded prior to running the analysis. This model simultaneously assessed helminth richness per

individual as a function of its sex, age and group while accounting for the total number of samples collected per individual.
The model was fitted in R (R Core Team, 2013) employing the nlm function and run for all 72 chimpanzees (35 M, 37 F) screened during the course of this study, including data from all samples examined from each individual using Harada-Mori fecal cultures and/or MWSFs (range: 1-61 samples/individual, mean: 11.9 +/- 12.0, median: 7; quartiles: 3/ 20.5).

The significance of the overall effect of the predictor variables as a whole (except the number of samples collected per individual) was tested comparing the full model with a null model comprising only the number of samples per individual using a likelihood ratio test (Dobson, 2002). The individual effects were tested by comparing the full model with an accordingly reduced model (i.e. with a model excluding the respective predictor variable(s)) using a likelihood ratio test.

III.6.2.2 Model 2 (response variable: ppg)

The influence of seasonality and host intrinsic factors on the number of helminth propagules (i.e. the number of helminth eggs and L1-larvae (Turner & Getz, 2010)) per gram feces (ppg) was investigated using a generalized linear mixed model (GLMM) (Baayen, 2008) with Gaussian error structure and identity link function. Chimpanzee sex and age, maximum temperature, rainfall, and season** were employed as fixed effects predictor variables. To control for potential ppg deviances caused by variations in defecation time and fecal consistency, these two factors were included as additional fixed effect variables. Ppg-values had been determined employing MWSFs as described before.

As the number of fecal samples for which data for all predictor variables were available was very small for chimpanzees from both east and north group (5 and 4 samples respectively), only samples (n=607) collected from south group members were considered in this analysis (Table 12).

These 607 fecal samples had been collected from a total of 38 south group chimpanzees (19 M, 19 F) and examined using a combination of MWSFs and fecal cultures. The number of examined samples per chimpanzee ranged from 1 to 53 (median: 17.0; quartiles: 4.5/ 22.8; mean: 16.0 +/- 11.3).

Table 12. *Distribution of fecal samples from south group members included in the model (n=607)*

	Sex		
Age class	**M**	**F**	**Total**
Adult	138	225	363
Adolescent	88	36	124
Juvenile	75	19	94
Infant	14	12	26
Total	**315**	**292**	**607**

A GLMM rather than a general linear model was utilized due to the need to incorporate `individual´ as a random effect in order to account for the non-independence of multiple samples collected from the same chimpanzees during the study period.

The need to include temporal autocorrelation into the model was tested for by deriving an 'autocorrelation term' and including it into the model. This autocorrelation term was derived, separately for each data point, by averaging the residuals of all other data points derived from the same individual. The contribution of the other residuals was weighted by the time lag between the two sampling dates. The weight function had the shape of a Gaussian function with a mean of zero and a standard deviation determined such that the likelihood of the full model with the derived autocorrelation term included as a fixed effect was maximized. Since the autocorrelation term revealed significance ($P<0.05$), it was included in the final model.

The model was fitted in R (R Core Team, 2013) using the function lmer of the R-package lme4 (Bates et al., 2013). Prior to running the analysis, the response variable of the model (ppg) was log transformed to achieve approximate normality, and the numerical fixed effect predictor variables (i.e. chimpanzee age, rainfall, maximum temperature, defecation time, and fecal consistency) were z-transformed to a mean of zero and a standard deviation of one to render them more easily interpretable (Schielzeth, 2010).

The fulfillment of the assumptions of normally distributed and homogeneous residuals was checked by visually inspecting a qqplot and the residuals plotted against fitted values (both indicated no obvious deviations from these assumptions). Model stability was examined by excluding individuals one by one from the data set and comparing the derived estimates with those obtained for the full model. Overall, this examination indicated no unduely influential cases to exist. Variance Inflation Factors (VIF, Field, 2005) were derived using the function vif of the R-package car (Fox & Weisberg, 2011) applied to a standard linear model excluding the random effect and indicated no severe issues with multicollinearity (maximum VIF: 4.25).

The significance of the full model as compared to the null model (comprising only the intercept, the random effect as well as defection time and fecal consistency) was established using a likelihood ratio test (R function anova with argument test set to `Chisq´). Included to keep the type I error rate at the nominal level of 0.05 were random slopes of all fixed effects except sex within individual, but not the correlations between random slopes and random intercepts (Schielzeth & Forstmeier, 2009; Barr et al., 2013). P-values for the individual effects were based on likelihood ratio tests (r function drop1 with argument test set to `Chisq´), whereas the significance of the overall effect of season was tested comparing the full model with a reduced model excluding the sine and cosine of date (R function anova with argument test set to `Chisq´).

III.7 Preservation of larvae and free-living adult helminths for DNA extraction

Helminth larvae and free-living adults cultured from fresh unpreserved chimpanzee and monkey fecal samples in the forest lab were examined, measured, and photographed under a compound microscope as described before.

Subsequently, a number of representatives of all different types of larvae and free-living adult worms found in the fecal cultures were each transferred in small volumes (~100 to 500 µl) of filtered water to individual 1.2 or 2.0 ml cryotubes using disposable plastic micropipettes and with the aid of a magnifying glass. Care was taken not to place more than one individual larva or free-living adult into each tube. Cryotubes were labeled with a continuous number, sample code, chimpanzee name or monkey species, type and measurements of the respective larva or free-living adult worm, and were immediately placed in liquid nitrogen. Tubes were transported to Germany on dry ice and stored at -70°C until further processing at the Tannich Lab, Bernhard Nocht Institute, Hamburg.

III.8 DNA extraction

Genomic DNA was isolated from individual L3-larvae or free-living adult specimens using a QIAamp® Mini Kit (Qiagen) according to the manufacturer's protocol with the following modifications:

Amounts of proteinase K, ATL buffer and AL buffer used in the extraction process were determined according to the respective volume of each sample:
Cryotubes containing individual larvae or free-living adults were thawed at room temperature. Volume of liquid (water + larva/ free-living adult) in each tube was assessed using a 1000 µl pipette and the amounts of ATL buffer and proteinase K necessary for DNA extraction from each sample, were calculated using the formulas:

Volume of ATL buffer (ml) = (100 x sample volume) divided by 80
Volume of proteinase K (ml) = (20 x sample volume) divided by 80

Calculated volumes of ATL buffer and proteinase K were added to each cryotube and the tubes incubated at 56°C for a minimum of 60 minutes. See Annex, Table A1 for a detailed list of sample volumes and incubation periods.
Volumes of AL buffer and ethanol added in later steps of the extraction process were calculated for each sample with the formula:

Volume of AL buffer/ ethanol (ml) = Volume of ATL buffer x 2

DNA from each sample was eluted in 200 µl elution buffer and stored in labeled micro test tubes at -20°C until further processing.

III.9 Polymerase chain reaction (PCR)

III.9.1 Strongyle nematodes

Genomic DNA of two different gene regions from third-stage larvae, morphologically identified as members of the superfamily Strongyloidea, was amplified by PCR using specific primer sets (see below). PCRs were performed in PeQlab Primus 25 advanced or MWG Biotech Primus thermocyclers. For each PCR, no-DNA samples were included as negative controls.

III.9.1.1 Ribosomal ITS-2

The ribosomal ITS-2 region including primer-flanking sequences (20 bp at the 3` end of the 5.8S and 70 bp at the 5` end of the 28S rDNA) of the individual strongyle larvae was amplified using primer set NC1 and NC2 (Gasser et al., 1993). See Figure 4 for primer location.

NC1: 5'-ACGTCTGGTTCAGGGTTGTT-3' (forward)
NC2: 5'-TTAGTTTCTTTTCCTCCGCT-3' (reverse)

Primer NC2 is conserved across a broad range of organisms, whereas NC1 is specific for parasitic nematodes of the suborder Strongylida (superfam. Strongyloidea) (Gasser et al., 1998).

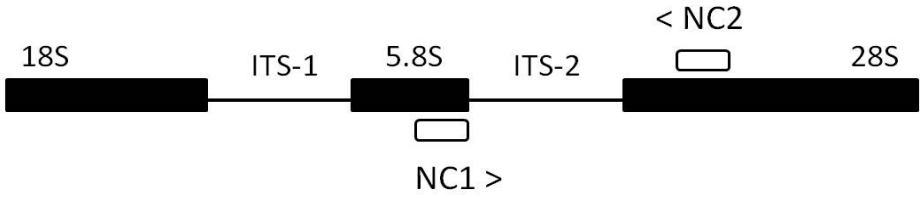


Figure 4. *Schematic representation of part of the rDNA transcriptional unit and relative location of primers NC1 and NC2**

*adapted & reprinted from Molecular and Cellular Probes, 11(3), Romstad A, Gasser RB, Monti JR, Poldermann AM, Nansen P, Pit DSS, Chilton NB, Differentiation of *Oesophagostomum bifurcum* from *Necator americanus* by PCR using genetic markers in spacer ribosomal DNA, 169-176, Copyright (1997); with permission from Elsevier.

PCR was performed in 50- or 100 µl using 10 pmol of each primer, 350 µM of each dNTP, 1.75 mM $MgCl_2$ and 5U (10U were used in the 100 µl mix) Expand long enzyme (see Table 13 for details) under the following conditions:

2 min	94°C	initial denaturation
35 cycles:		
10 sec **	94°C	denaturation
30 sec	50°C	annealing
2 min	68°C	extension
10 min	68°C	final extension

** If PCR was performed in 100µl, this denaturation step was prolonged to 15 sec

Table 13. *PCR- mixes (Primers: NC1 and NC2)*

50 µl- Mix:

			µl / rxn	**final concentration**
Aqua dest			26.25	
dNTPs	10 mM each		1.75	350 µM
NC1-Primer		10 pmol/µl	5.00	1 µM
NC2-Primer		10 pmol/µl	5.00	1 µM
Larval DNA			6.00	
Expand long buffer 1*			5.00	$MgCl_2$ 1.75mM
Expand long enzyme*			1.00	5 U
Total volume			**50.00**	

100 µl- Mix:

			µl / rxn	**final concentration**
Aqua dest			54.50	
dNTPs	10 mM each		3.50	350 µM
NC1-Primer		10 pmol/µl	10.00	1 µM
NC2-Primer		10 pmol/µl	10.00	1 µM
Larval DNA			10.00	
Expand long buffer 1*			10.00	$MgCl_2$ 1.75mM
Expand long enzyme*			2.00	10 U
Total volume			**100.00**	

*Expand long Template PCR System, Roche Diagnostics Deutschland GmbH, Mannheim

III.9.1.2 12s RNA mtDNA

The mitochondrial gene for the 12s RNA of each strongyle larvae (see Figure 5 for gene location) was amplified by PCR using primer set 12s Pannem-F and 12s Pannem-R1.

12s Pannem-F: 5'-GTTCCAGAATAATCGGCTA-3' (forward)*
12s Pannem-R1: 5'-GCAATTGATGGATGATTTG-3' (reverse)*

*Primers designed by Prof. Tannich, Bernhard Nocht Institute, Hamburg (personal communication)

Primers 12s Pannem-F and -R1 had been designed as pannematode primers, but in a preliminary trial using the same PCR conditions as described below, had failed to produce an amplicon from the DNA of a free-living male of *Strongyloides spp.* obtained from one of the performed fecal cultures. In the same trial however, an amplification product had been produced from DNA of a specimen of *Strongyloides ratti* (DNA obtained from Bernhard Nocht Institute, Hamburg).

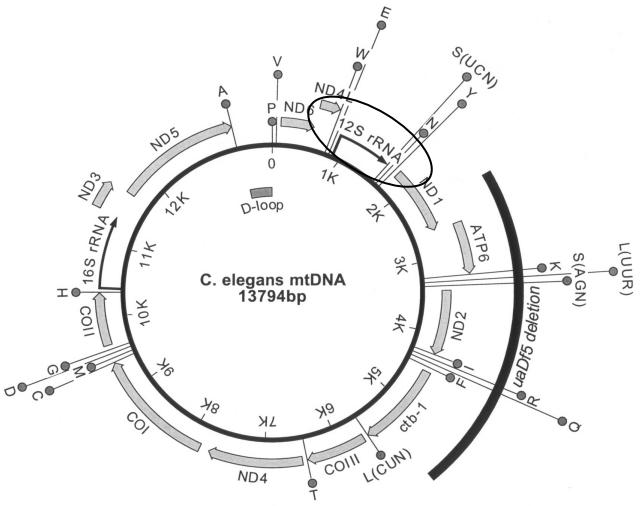


Figure 5. *Gene map of the Caenorhabditis elegans mtDNA & location of the 12s rRNA gene*

* reprinted from Lemire, 2005; Mitochondrial genetics. In WormBook (ed) The *C elegans* Research Community, WormBook. doi/10.1895/wormbook.1.25.1; http://www.wormbook.org;
with permission from the author

PCR was performed in 100 µl, using 10 pmol of each primer, 350 µM of each dNTP, 1.75 mM $MgCl_2$ and 10U Expand long enzyme (see Table 14) under the following conditions:

2 min	94°C	initial denaturation
8 cycles:		
15 sec	94°C	denaturation
30 sec	50°C – 0.5°C/cycle	annealing
2 min	68°C	extension
30 cycles:		
15 sec	94°C	denaturation
30 sec	48°C	annealing
2 min	68°C	extension
10 min	68°C	final extension

Table 14. *PCR-mix (Primers: 12s Pannem F+R1)*

100 µl- Mix:

			µl / rxn	**final concentration**
Aqua dest			54.50	
dNTPs	10 mM each		3.50	350 µM
12s Pannem-F-Primer		10 pmol/µl	10.00	1 µM
12s Pannem-R1-Primer		10 pmol/µl	10.00	1 µM
Larval DNA			10.00	
Expand long buffer 1*			10.00	$MgCl_2$ 1.75mM
Expand long enzyme*			2.00	10 U
Total volume			**100.00**	

*Expand long Template PCR System, Roche Diagnostics Deutschland GmbH, Mannheim

To optimize PCR, different concentrations of $MgCl_2$ (1.75 mM, 2.75 mM, 2.75 mM + detergent) and dNTPs (350 µM, 500 µM) had been tested in preliminary trials, but no apparent differences in PCR performance had been observed.

III.9.2 Genus *Strongyloides*

The mitochondrial COI gene (cytochrome c oxidase subunit 1 gene) of L3-larvae and free-living adult worms morphologically identified as *Strongyloides spp*. was amplified using primer set COI-Pannem-F and COI-Pannem-R:

COI-Pannem-F: 5‘- AT(A/G)ATTGGTGG(T/G)TTTGGTAA-3‘ (forward)**
COI-Pannem-R: 5‘- AGC(T/C)CA(A/C)ACTACACAACC-3‘ (reverse)**

**Primers designed by Prof. Tannich, Bernhard Nocht Institute, Hamburg (personal communication)

Primer sets NC1/ NC2 and 12s Pannem-F/ 12s Pannem-R1 were not utilized for DNA amplification from these larvae and free-living adult specimens, as primer NC1 is specific for nematodes belonging to the suborder Strongylida (superfam. Strongyloidea) (Gasser et al., 1998) and primers 12s Pannem F/ 12s Pannem R had in a preliminary trial (see paragraph III.9.1.2) failed to produce an amplicon from the DNA of a male free-living *Strongyloides sp.* specimen cultured from a Taï chimpanzee fecal sample.

PCRs were performed in PeQlab Primus 25 advanced (or MWG Biotech Primus) thermocyclers. For each PCR, no-DNA samples were included as negative controls.

PCR was performed in 100 µl, using 10 pmol of each primer, 350 µM of each dNTP, 1.75 mM $MgCl_2$ and 10U Expand long enzyme (see Table 15) under the following conditions:

2 min	94°C	initial denaturation
8 cycles:		
15 sec	94°C	denaturation
30 sec	50°C – 0.5°C/cycle	annealing
2 min	68°C	extension
30 cycles:		
15 sec	94°C	denaturation
30 sec	48°C	annealing
2 min	68°C	extension
10 min	68°C	final extension

Table 15. *PCR-mix (Primers: COI-Pannem-F+R)*

100 µl- Mix:

			µl / rxn	final concentration
Aqua dest			54.50	
dNTPs	10 mM each		3.50	350 µM
COI-Pannem-F-Primer		10 pmol/µl	10.00	1 µM
COI-Pannem-R-Primer		10 pmol/µl	10.00	1 µM
Larval DNA			10.00	
Expand long buffer 1*			10.00	$MgCl_2$ 1.75mM
Expand long enzyme*			2.00	10 U
Total volume			**100.00**	

*Expand long Template PCR System, Roche Diagnostics Deutschland GmbH, Mannheim

III.10 Visualization of amplification products

A proportion (10 µl) of each PCR product was mixed with 2 µl loading buffer 6x Orange G (0.4% Orange G (Sigma-Aldrich, Germany) + 15% Glycerol) and examined by electrophoresis on ethidium bromide-stained 2% (w/v) agarose-TAE gels (40 mM Tris pure, 5 mM sodium acetate, 1.25 mM Na2- EDTA, pH 7.9) using GeneRuler™ 100bp DNA Ladder (Fermentas GmbH, St. Leon-Rot, Germany) as size markers.

Gels were photographed and visually assessed for band presence/ absence and -intensity under ultraviolet light using Gel Doc 2000 Apparatus (Bio-Rad Laboratories, CA, USA) and Multi-Analyst™ Software (Bio-Rad Laboratories).

III.11 Purification of PCR products

PCR products were purified using a NucleoSpin Extract II Protein and Nucleic Acid Purification Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol.
To assure removal off all primer dimers, buffer NT was diluted 1/ 6 with sterile water.
Purification success was subsequently verified by electrophoresis on ethidium-bromide stained 2% agarose-TAE gels as described above.

Purified PCR products producing strong bands upon electrophoresis were used as templates for direct sequencing, whereas amplicons producing faint bands were subjected to a cloning step (see Annex, Tables A2 & A3).

III.12 Cloning

1 to 4 µl of purified DNA from each sample were cloned into competent *E. coli* (TOP10) using a TOPO TA Cloning® Kit with pCR® 2.1.-TOPO® vector (Invitrogen Corporation, CA, USA) according to the manufacturer's protocol. Transformed cells

were cultured overnight at 37°C on LB/Amp plates with X-gal added for blue-white screening. 5 to 10 white (i.e. insert containing) colonies from each plate were selected and picked using 10 µl pipette tips. Selected colonies were used to each inoculate 2 ml of LB/ Amp broth and were incubated shaking over night at 37 °C.

Plasmids were subsequently purified according to the manufacturer's protocol using a FastPlasmid™ Mini Kit (5 Prime, Hamburg, Germany) and screened for presence of correct DNA inserts: 5 µl of purified plasmid DNA from each clone were enzymatically digested with 1 µl FastDigest® EcoRI and 2 µl FD buffer (Fermentas GmbH, St. Leon-Rot, Germany) for 10 minutes at 37°C and examined and visualized by electrophoresis on ethidium-bromide stained 2% agarose-TAE gels as described above.

III.13 Sequencing

III.13.1 Sequencing of cloned PCR products

20 µl of purified plasmid DNA from 1 to 4 positive clones per sample/PCR product (see Annex, Tables A2 & A3) were sent to Eurofins MWG Operon (Ebersberg, Germany) for sequencing with standard universal primer M13rev (-29).
Sequences were received online and plasmid- and primer sequences were removed from the respective inserts using MacVector software (MacVector, Inc., NC, USA).

III.13.2 Direct sequencing

Purified PCR products having produced strong bands upon electrophoresis (see Annex, Tables A2 & A3) were sent to the Robert Koch Institute (Berlin, Germany), where 1 µl of each PCR product was subjected to sequencing using the Sanger method. Sequencing was performed in both directions employing the same forward and reverse primers as used for PCR amplification.
Sequences were received online and assembled using SeqMan Pro Version 8.1.5(3), Lasergene® software (DNASTAR Inc., Madison, USA). Primers were removed manually.

III.14 Sequence- and phylogenetic analysis

III.14.1 Sequence analysis

5' and 3' ends of each sequence (`chimpanzee-´ and `monkey-isolates´) were determined by comparison with those of a range of published sequences from strongylid nematodes (Gasser et al., 1999a, Schindler et al., 2005) and *Strongyloides fuelleborni* (Hasegawa et al., 2010) respectively. Subsequently, sequences were compared with published nematode reference sequences in the GenBank nucleotide database through blast search employing BLASTN 2.2.25 software (Zhang et al.,

2000, Altschul et al., 1990; blast.ncbi.nlm.nih.gov/Blast.cgi) with default settings, but optimized for highly similar (megablast) or more dissimilar (discontinuous megablast) sequences.

Sequences were aligned in SeaView (SeaView Program, Version 4.2.8 (Gouy et al., 2010), Laboratoire de Biometrie et Biologie Evolutive CNRS/ Universite Lyon, Lyon, France) using Muscle software (Edgar, 2004a+b).

Alignments were then manually edited in SeaView before being reduced to unique sequences (haplotypes) using the FaBox program (Villesen, 2007). Given the good quality of the alignment, none or only minor manual editing of the respective nucleotide alignments was necessary. Pairwise comparisons of the different sequences in each alignment were made on the level of respective sequence differences (D) using the formula D= 1- (M/L), whereby M is the number of alignment positions at which the two aligned sequences have a base in common, and L is the total number of alignment positions over which the two sequences are compared (Chilton et al., 1995). Alignment gaps were treated as equivalent to substitutions, and both, M and L were calculated using BLASTN 2.2.25 software (Zang et al., 2000, Altschul et al., 1990).

III.14.2 Phylogenetic analysis

Published ITS-2 and mtDNA reference sequences from specimens of the families Ancylostomatidae, Chabertiidae, Trichostrongylidae, Cooperiidae, Haemonchidae, and Strongyloididae (see Annex, Tables A24 to A27) were obtained from the GenBank nucleotide database (Altschul et al., 1990) and added to the respective alignments using SeaView (SeaView Program, Version 4.2.8 (Gouy et al., 2010)) and Muscle Software (Edgar, 2004a+b). Cropping of individual sequences was performed as necessary.

The nucleotide substitution models to which the different alignments were a respective best fit were subsequently determined using the jModeltest program (version 0.1.1) (Guindon & Gascuel, 2003; Posada, 2008). Three nucleotide substitution models/ schemes (Jukes and Cantor [JC], Hasegawa, Kishino, and Yano [HKY], and global time reversible [GTR]) were examined along with rate variation (+I, +G, +I+G) and base frequency (+F) modelling. The respectively most favourable model was selected through comparison of model likelihoods according to the Akaike information criterion (AIC).

Phylogenetic analysis for each alignment were performed in a maximum likelihood (ML) framework on the dedicated PhyML webserver (http://www.atgc-montpellier.fr/phyml/) (Guindon & Gascuel 2003; Guindon et al., 2005) under the respective selected model. Equilibrium frequencies, topology, and branch lengths were optimized. The starting tree was determined using the BioNJ program, and both nearest-neighbor interchange (NNI) and subtree pruning and regrafting (SPR) algorithms of tree search were used (keeping the best outcome). Individual branch

robustness was assessed by performing non-parametric bootstrapping with 500 replicates and obtained bootstrap values were transformed and given as percentages. Figures depicting the inferred phylogenetic trees were created using the FigTree program (version 1.3.1; http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Photoshop Elements 2.0 (Adobe Systems Incorporated, 1990-2002).

IV RESULTS

IV.1 Spectrum of parasitic gastrointestinal helminths determined using conventional coprology

To assess the spectrum of gastro-intestinal helminths affecting the three study groups, a total of four different conventional coprological techniques had been employed. Prior to any microscopic examination all collected fecal samples had been screened macroscopically for tapeworm proglottids, adult helminths or worm fragments.

IV.1.1 Macroscopic examination

Whereas macroscopic screening revealed no adult nematodes, trematodes, or respective worm fragments in any of the collected fecal samples (N= 857), shed tapeworm proglottids containing eggs which morphologically most closely resembled *Bertiella spp.* (Galán-Puchades et al., 2000) were detected in a small number of samples (n= 5).

IV.1.2 Coprological microscopic examination

IV.1.2.1 Modified Wisconsin Sugar Flotation (MWSF)

In the overall 857 fecal samples examined with this method, eggs of five different helminth genera were detected, including four nematodes (*Strongyloides spp., Trichuris spp., Capillaria spp., Enterobius spp.*) and one cestode (*Bertiella spp.*) (Figure 6a-j). Eggs and occasionally hatched L1-larvae of strongyle nematodes (i.e. of nematode helminths belonging to the superfamily Strongyloidea) were also found but, due to their great morphological similarity, could not be determined to genus level. The considerable size variation of these eggs however indicated the presence of several different genera (see Table 17). As they don't float in Sheather`s solution, no trematode eggs could be discovered using this method.
Mite eggs in different stages of development as well as individual adult mites (Figure 7a-c) were additional findings in some of the examined fecal samples.

IV.1.2.2 Sodium Nitrate Flotation and Sedimentation

The spectrum of nematodes and cestodes detected with the combination of $NaNO_3$-flotations and sedimentations from the overall 120 fecal samples examined with these methods was identical to the above described spectrum of helminths found using MWSF.
Additionally, upon sedimentation, eggs of trematodes belonging to the family Dicrocoeliidae were detected in several samples (see Figure 6a-j). Unequivocal identification of these eggs to genus level however was not possible.

IV.1.2.3 Harada-Mori Fecal Cultures

By culturing aliquots of fresh, unpreserved fecal material from a total of 793 fecal samples using the Harada-Mori technique, the abovementioned difficulty to reliably identify strongyle nematodes to genus level based on egg morphology was largely overcome. Overall, L3-larvae of three different strongyle genera (*Ternidens spp., Oesophagostomum spp.* and *Necator spp.*) were detected employing this method. Additionally several trichostrongylid L3-larvae were found which could not unequivocally be identified to genus level but morphologically most closely resembled respective larval stages of helminths belonging to the genus *Trichostrongylus* (see Figure 8a-h).

As for the detected *Oesophagostomum* larvae, two morphotypes (A and B) could be distinguished on the basis of their respective tail-sheath length:

+ type A: short tail-sheath
+ type B: long tail-sheath

Also detected were L3-larvae and some free-living adult specimens (Figure 9a-d), of the genus *Strongyloides* (superfamily Strongyloidoidea), whereby all free-living adult females could readily be identified as *S. fuelleborni* specimens based on the species-specific morphology of their vulva and peri-vulval area (Speare, 1989).

Supplemental findings in some of the examined fecal cultures were several types of helminth larvae and adult worms (see Figure 10a-d) which very likely constitute different developmental stages or adult specimens of free-living soil nematodes.

IV.1.3 Overall spectrum of detected parasitic helminth genera and morphotypes

Overall, combining macroscopic screening and all four methods of conventional coprology employed in this study, developmental stages of a total of 11 different morphotypes of parasitic helminths (representing 10 helminth genera) were detected and identified from the collected fecal material of the three chimpanzee study groups – including nine nematodes (representing 8 genera), one cestode and one trematode (Table 16 and Figures 6a-j, 8a-h and 9a-d). Identification to species level based on distinct morphological criteria was only possible for a small number of cultured free-living adult female specimens of *S. fuelleborni.*

Table 16. *Overall spectrum of helminths determined employing conventional coprology***

NEMATODES		
# CHROMADOREA (class); Rhabditia (subclass)		
• Tylenchida (order)		
Strongyloidoidea (superfam.)		
	Strongyloididae fam.	*Strongyloides spp.*
		S. fuelleborni
• Spiruida (order)		
Strongyloidea (superfam.)		
	Ancylostomatidae fam.	*Necator spp.*
	Strongylidae fam.	*Oesophagostomum spp.*
		Oesophagostomum sp. (type A)
		Oesophagostomum sp. (type B)
		Ternidens spp.
	Trichostrongylidae fam.	*Trichostrongylus*-like genus not identified
Oxyuroidea (superfam.)		
	Oxyuridae fam.	*Enterobius spp.*
# ENOPLEA (class); Dorylaimia (subclass)		
• Trichinellida (order)		
	Capillariidae fam.	*Capillaria spp.*
	Trichuridae fam.	*Trichuris spp.*
CESTODES		
# CESTODEA (class); Eucestodia (subclass)		
• Cyclophyllida (order)		
Anoplocephaloidea (superfam)		
	Anoplocephalidae fam.	*Bertiella spp.*
TREMATODES		
# DIGENEA (class)		
• Plagiorchiida (order)		
Gorgoderoidea (superfam.)		
	Dicrocoeliidae fam.	genus not identified

** Taxonomic classification as employed in Schnieder et al. (2006)

Figure 6a-j. *Helminth eggs detected using MWSF, $NaNO_3$-flotation and sedimentation*

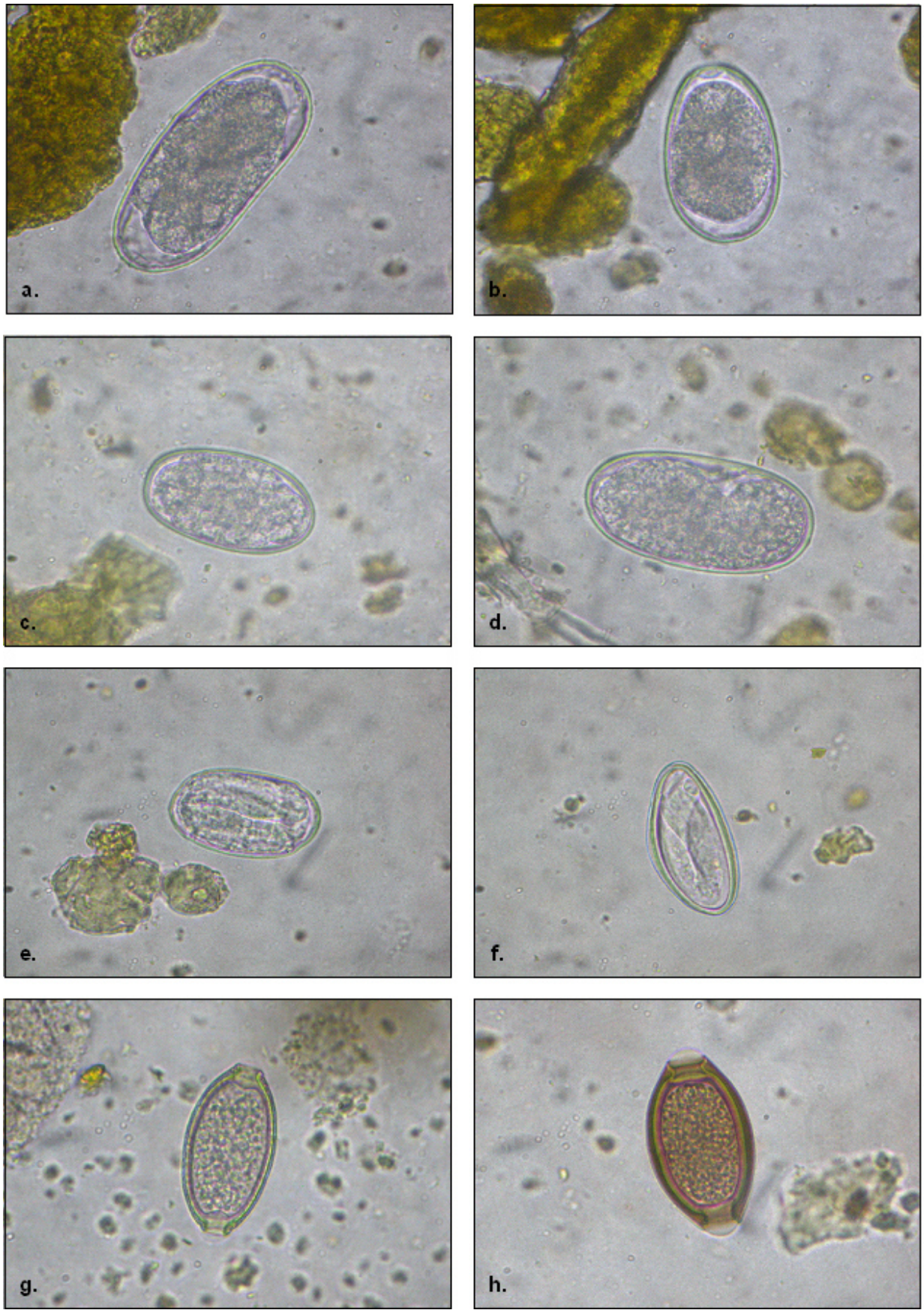
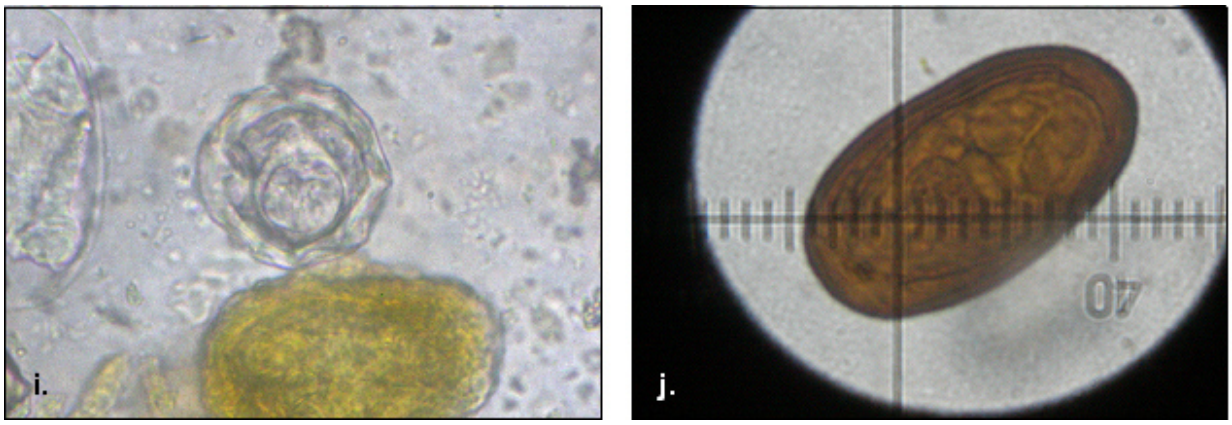


Figure 6a-j. *Helminth eggs detected using MWSF, $NaNO_3$-flotation and sedimentation (continued)*

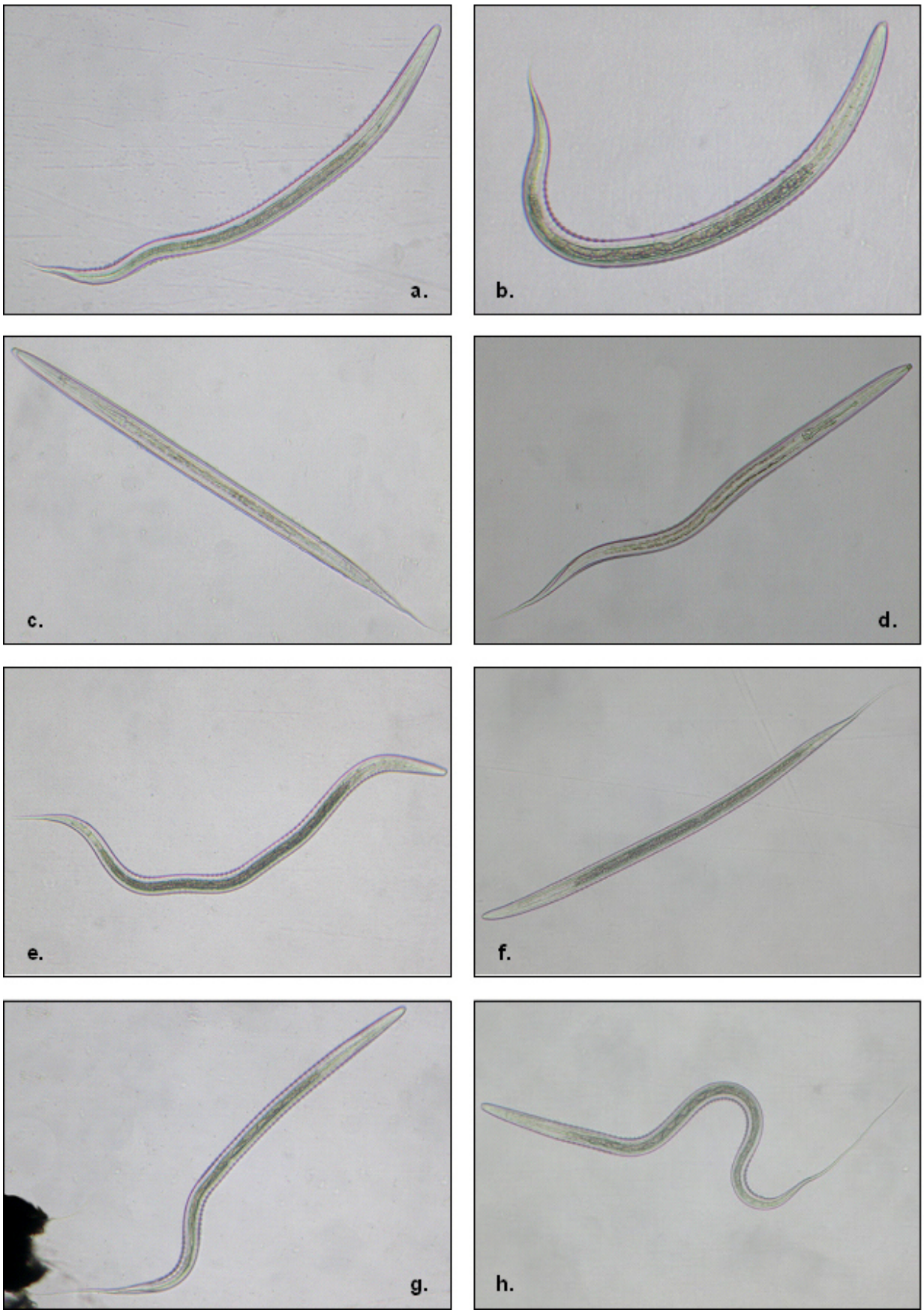


a. Strongyle egg (87.5 x 40.0 µm)
b. Strongyle egg (65.0 x 37.5 µm)
c. Strongyle egg (60.0 x 35.0 µm)
d. Strongyle egg (75.0 x 40.0 µm)
e. *Strongyloides spp.* (50.0 x 30.0 µm)
f. *Enterobius spp.* (55.0 x 25.0 µm)
g. *Capillaria spp.* (47.5 x 30.0 µm)
h. *Trichuris spp.* (57.5 x 32.5 µm)
i. *Bertiella spp.* (47.5 x 47.5 µm)
j. Dicrocoeliid egg (40.0 x 20.0 µm)

Figure 7a-c. *Unidentified mite and mite eggs detected in fecal samples examined with MWSF*

a. Unidentified mite egg (125.0 x 70.0 µm)
b. Unidentified mite egg (125.0 x 70.0 µm)
c. Unidentified mite (body: 370 x 180 µm)

Figure 8a-h. *Strongyle L3-larvae recovered from Harada-Mori fecal cultures*



a. *Ternidens spp.* (620x30 µm)

b. *Ternidens spp.* (580x30 µm)

c. *Necator spp*. (640x25 µm)

d. *Necator spp.* (630x30 µm)

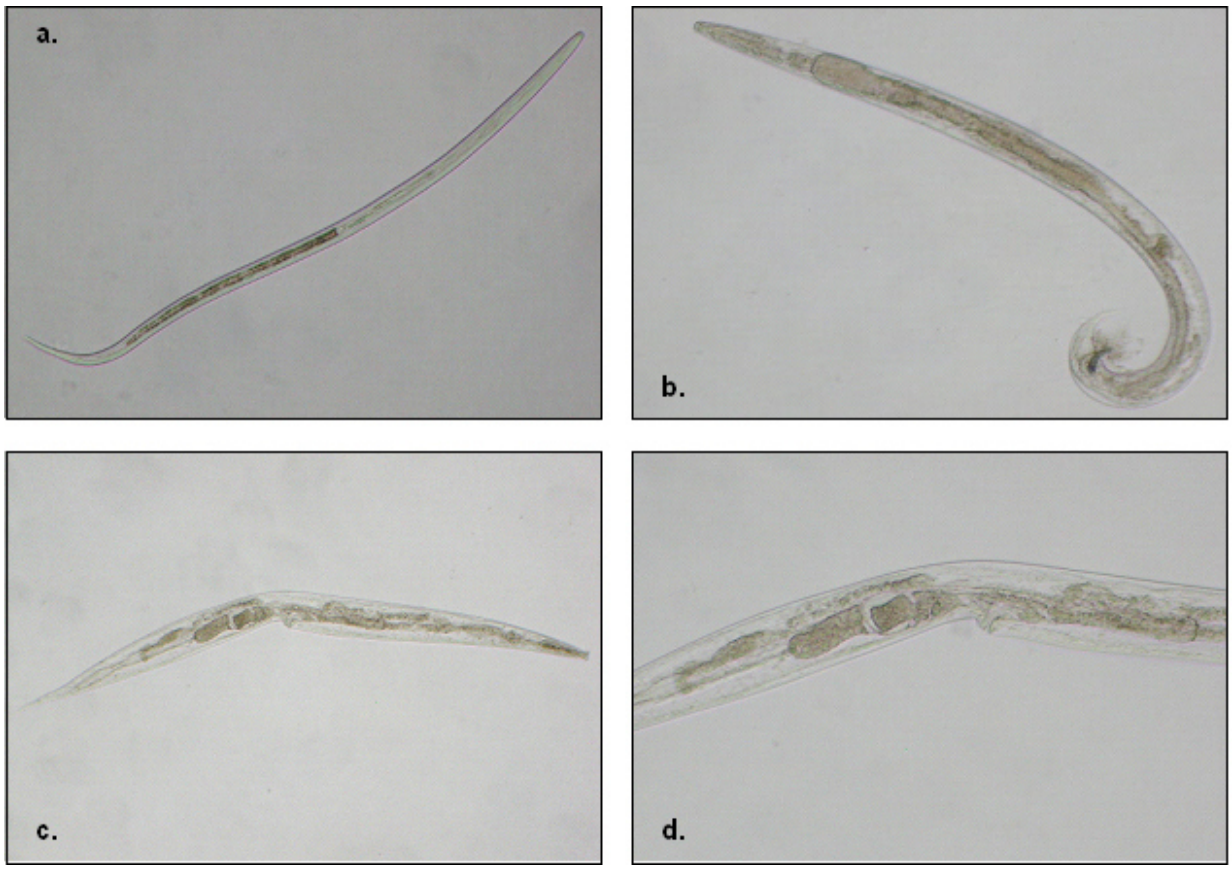
e. Trichostrongylidae fam. (780x30 µm)

f. *Oesophagostomum spp.* type A (820x30 µm)

g. *Oesophagostomum spp.* type A (800x30 µm)

h. *Oesophagostomum spp.* type B (900x30 µm)

Figure 9a-d. *Strongyloides L3-larvae and free-living adult specimens recovered from Harada-Mori fecal cultures*

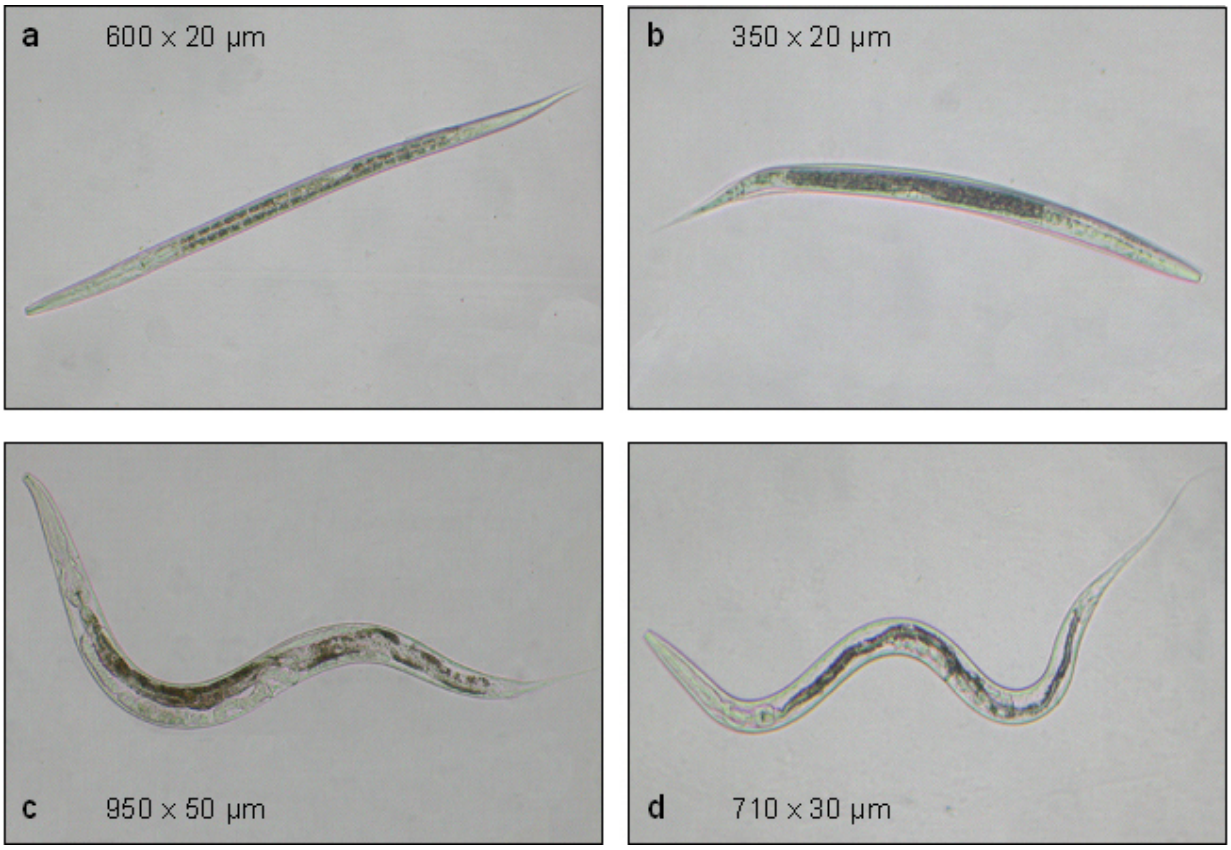


a. *Strongyloides* L3-larvae (650x20 µm)

b. *Strongyloides* free-living adult male (850x50 µm)

c.+ d. *S. fuelleborni* free-living adult female (1150x70 µm)

Figure 10a-d. *Larval stages (a+b) and adult specimens (c+d) of unidentified soil nematodes recovered from Harada-Mori fecal cultures*



IV.2. Morphometrics and morphological description of helminth developmental stages

Measurements of nematode and cestode eggs were taken from specimens recovered from unpreserved fecal material using MWSFs, whereas measurements of trematode eggs were taken from specimens obtained from formalin-preserved fecal samples through sedimentation. Measured nematode L3-larvae and free-living adults had been recovered from Harada-Mori fecal cultures.

IV.2.1 NEMATODES

IV.2.1.1 Strongyle nematodes (Superfamily Strongyloidea)

+ Eggs

Overall, measurements had been taken from a total of 3224 strongyle eggs, obtained from 732 out of the overall 857 chimpanzee fecal samples examined during this study (1 to 12 eggs per sample; mean: 4.4 +/- 1.8). The 732 samples had been collected from a total of 71 different chimpanzees (35 M, 36 F). All detected strongyle eggs (see Figure 6a-d) were elliptical in shape with more or less flattened poles, a thin smooth, non-pigmented shell and contained a variable number of blastomers

(< 8 blastomers, n= 213; 8-32 blastomers, n= 979; or > 32 blastomers, n= 1547) or an already developed larva (n= 485).

Egg size varied considerably, suggesting the presence of several different strongyle genera and/or species (see Table 17). Measured egg length ranged from 42.5 to 162.5 µm (mean: 72.0 +/- 7.9), respective width from 22.5 to 62.5 µm (mean 41.3 µm +/- 4.6).

Table 17. *Morphometrics of measured strongyle eggs in comparison with respective reference values for different strongyle genera adopted from literature references*

length/width (µm)	22.5	25.0	27.5	30.0	32.5	35.0	37.5	40.0	42.5	45.0	47.5	50.0	52.5	55.0	57.5	60.0	62.5	No. of eggs
42.5							1											1
47.5			1	1														2
50.0				2														2
52.5				1		2	1											4
55.0	1			1		6	2	1										11
57.5				2	13	12	31	5	2	1	1							67
60.0		1		2	9	37	61	18	1	2	1							132
62.5				3	11	74	234	30	5	8	4	1						370
65.0			1	1	16	68	84	20	16	12	5	3						226
67.5					8	58	50	39	36	34	13	4	2					244
70.0					9	49	44	32	90	86	22	4		3				339
72.5				1	11	36	25	42	126	114	52	9	1	1				418
75.0					9	21	25	54	199	169	48	11	3		1			540
77.5					7	12	6	30	108	96	35	9	1					304
80.0					3	5	10	33	72	74	25	15		2		1		240
82.5			1	1		1	6	16	29	39	16	9	3	1				122
85.0					1	4	1	3	17	35	29	9	2					101
87.5							5	5	11	22	16	4	1		1			65
90.0								1	3	4	5	2						15
92.5								1	1	2	3		1					8
95.0											2							2
97.5												1						1
100.0											1		1					2
107.5												2						2
112.5											1	1						2
117.5								1								1		2
120.0											1							1
162.5																	1	1
Total no. of eggs	1	1	3	15	97	385	586	331	716	698	280	84	15	7	2	2	1	3224

*** Colour code: Egg-size reference values from the literature*

	Oesophagostomum spp.	egg size: ca. 43-75 x 33-48 µm (Goldsmid, 1982)
	Ternidens deminutus A	egg size: ca. 70-94 x 40-60 µm (Goldsmid, 1982);
	Ternidens deminutus B	egg size: ca. 72-103 x 37-45 µm (Sandground, 1929)
	Necator americanus	egg size: ca. 64-76 x 36-40 µm (Thienpont et al., 1990)
	Trichostrongylus spp.	egg size: ca. 70-125 x 30-55 µm (Thienpont et al., 1990)

+ L3-larvae

Altogether, morphometric data had been gathered from a total of 1310 cultured filariform strongyle L3-larvae, including 642 *Ternidens*-, 196 *Necator*-, 570 *Oesophagostomum*- (429 type A-, 153 type B larvae) and two trichostongylid (*Trichostrongylus*-like) larvae.

IV.2.1.1.1 Genus *Ternidens*

Length of the overall 642 measured *Ternidens* L3-larvae (see Figure 8a+b), which had been cultured from a total of 481 fecal samples (1 to 3 larvae per sample; mean: 1.3 +/- 0.6) collected from 57 different chimpanzees (29 M, 28 F), ranged from 480 to 780 µm (mean: 602.2 µm +/- 36.5). Maximum width was 20 to 30 µm (mean: 29.9 µm +/- 0.8). Each larva was covered with an undulated cuticular sheath consisting of the unshed cuticle of the previous larval stage. The posterior end of this sheath was somewhat elongated and tapering to a thread-like tip. The esophagus of each larva comprised about one quarter of the larva's respective body length and was separated from the zigzagged intestinal lumen by a pair of elongated sphincter cells. The tips of the larvae's tails were pointed. All larvae exhibited a typical sluggish movement compared to the very fast moving *Strongyloides* larvae.

IV.2.1.1.2 Genus *Necator*

The overall 196 measured *Necator* L3-larvae (see Figure 8c+d), which had been cultured from a total of 180 fecal samples (1 to 4 larvae per sample; mean: 1.1 +/- 0.4) collected from 49 chimpanzees (21 M, 28 F), ranged in length from 470 to 750 µm (mean: 611.6 µm +/- 48.4). Maximum body width was 15 to 30 µm (mean: 26.5 µm +/- 2.6). Each larva was covered with a striated cuticular sheath consisting of the unshed cuticle of the previous larval stage. The posterior end of the sheath was pointed rather than tapering to a fine end. The esophagus of each larva ended in a conspicuous esophageal bulb and comprised about one quarter of the length of the respective larva's body length excluding the sheath. The intestinal lumen of each larva was straight and at the esophago-intestinal junction about as wide as the esophageal bulb. The tips of the larvae's tails were pointed.

IV.2.1.1.3 *Genus Oesophagostomum*

Each of the overall 570 *Oesophagostomum* L3-larvae (type A & type B larvae) (see Figure 8f-h) from which measurements and morphometric data had been collected, possessed an esophagus comprising about one quarter of its body length (excluding the sheath). The intestinal lumen was zigzagged due to the triangular shape of the intestinal cells, and there were no sphincter cells between the esophagus and the intestinal cells. Like the examined *Necator*- and *Ternidens* L3-larvae, each larva was enclosed in a cuticular sheath, consisting of the unshed cuticula of the L2-stage. The posterior end of this sheath i.e. the tail-sheath was either:

+ **short** (91.5 to 139.6 µm; n_{typeA}= 25; mean: 116.2 +/- 15.1) as in **type A larvae** (see Figure 9f+g) or
+ **long** (142.9 to 285.2 µm; n_{typeB}= 24; mean: 210.5 +/- 37.1) as in **type B larvae** (see Figure 9h) and tapering to a fine point.

Total body length (including tail-sheath) of the 429 measured type A larvae ranged from 650 to 900 µm (mean: 766.5 µm +/- 49.4), total length (including tail-sheath) of the overall 153 measured type B larvae ranged from 710 to 1200 µm (mean: 991.4 µm +/- 89.2). Maximum body width was 25 to 40 µm (mean: 30.0 µm +/- 1.2) and 30 to 40 µm (mean: 30.1 µm +/- 0.9) respectively.

Measured type A larvae had been recovered from a total of 70 cultured chimpanzee fecal samples (1 to 3 larvae per sample; mean: 1.2 +/- 0.4) collected from 59 different chimpanzees (32 M, 38 F). Type B larvae had been cultured from a total 140 fecal samples (1 to 3 larvae per sample; mean: 1.1 +/- 0.3) collected from 44 different chimpanzees (22 M, 22 F).
Similar to the examined *Ternidens* and *Necator* larvae, both type A and type B larvae displayed conspicuously sluggish movement as compared to the very fast moving *Strongyloides* L3-larvae.

IV.2.1.1.4 Family Trichostrongylidae

The two measured trichostrongylid (*Trichostrongylus*-like) larvae (see Figure 8e), which had been cultured from the feces of two different female chimpanzees, were 710 and 780 micrometers long. Maximum width of both larvae was 30 µm. Each larva was enclosed in an undulated cuticular sheath consisting of the unshed cuticula of its precedent second larval stage. The posterior end of this sheath was relatively short and not tapering to a fine point.

The esophagus of each larva comprised about one quarter of its respective body length (excluding the sheath); the intestinal lumen was zigzagged and the tip of each larva's tail rather blunt than pointed. The larvae's movements were again rather sluggish.

IV.2.1.2 *Genus Strongyloides* (Superfamily Strongyloidoidea)

+ Eggs

All examined eggs were oval-shaped with two blunted poles and a thin, smooth, non-pigmented shell (see Figure 6e) and contained a folded larva. Measurements had been taken from a total of 366 *Strongyloides* eggs recovered from 244 chimpanzee fecal samples (range: 1-2 eggs per sample, mean: 1.1 +/- 0.2), which had been collected from 59 different chimpanzes (32 M, 27 F). Recorded egg length ranged from 35.0 to 62.5 µm (mean: 52.5 µm +/- 2.5), egg width from 20.0 to 47.5 µm (mean: 31.3 µm +/- 1.3).

+ L3-larvae

Overall, measurements had been obtained from 400 filariform L3-larvae cultured from a total of 351 fecal samples (range: 1-2 larvae per sample; mean: 1.1 +/- 0.4) which had been collected from 53 different chimpanzees (32 M, 21 F). All measured larvae had a slender body, a long esophagus comprising about one half of their respective body-length and were lacking a cuticular sheath (see Figure 9a). Body length ranged from 480 to 810 µm (mean: 633.1 µm +/- 52.5). Maximum body width was 15.0 to 30.0 µm (mean: 20.0 µm +/- 1.0). The tip of the tail of each larva appeared notched. Unlike the examined *Ternidens* and *Oesophagostomum* L3-larvae, all examined *Strongyloides* larvae were generally moving very fast.

+ Free-living adult males

Since the majority of respective specimens were dead and autolytic, measurements could only been taken from two intact free-living males (see Figure 9b) which had been recovered from the cultured feces of two chimpanzees (1 M, 1 F). The two adult worms were 800 and 850 µm long and had a spindle-shaped body with a rhabditiform esophagus and a ventrally curved tail carrying two markedly bowed spicules. Maximum body width of both worms was 50.0 µm.

+ Free-living adult females

Morphometric measurements had been taken from a total of five intact free-living adult females morphologically identified as *S. fuelleborni,* which had been recovered from cultured fecal samples of four male chimpanzees. Dead and autolytic worms had not been measured.

All five measured specimens had a spindle-shaped body with a rhabditiform esophagus and a marked post-vulval constriction and ranged in length from 1000 to 1150 µm (mean: 1078 µm +/- 52.3). Maximum width was 60.0 to 70.0 µm (mean: 64 µm +/- 4.9) (see Figure 9c-d). The prominent vulva of each female was located near the midpoint of its body and showed a characteristic posterior rotation (Speare, 1989). The uterus of each female contained a variable amount of more or less developed eggs.

IV.2.1.3 Genus *Trichuris* (Family Trichuridae)

The overall 26 measured *Trichuris* eggs were all barrel-shaped with two prominent achromatic pole plugs, a granular content, and a thick smooth shell, consisting of two layers: a thinner yellowish inner layer and a thick brownish outer layer (see Figure 6h). The eggs had been obtained from 26 fecal samples collected from 18 different chimpanzees (12 M, 6 F). The lengths of the measured eggs ranged from 50.0 to 62.5 µm (mean: 58.8 µm +/- 3.8); the width from 20.0 to 32.5 µm (mean: 25.6 µm +/- 2.1).

IV.2.1.4 Genus *Capillaria* (Family Capillariidae)

All 24 measured *Capillaria* eggs were barrel-shaped with shallow achromatic and translucent polar plugs, granular content and a thick shell consisting of two layers: a continuous thin yellow inner layer and a thicker radially striated outer layer (see Figure 6g). The eggs had been recovered from a total of 24 chimpanzee fecal samples collected from 16 different study group members (7 M, 9 F). Egg length ranged from 45.0 to 60.0 µm (mean: 51.0 µm +/- 3.8), egg width from 22.5 to 30.0 µm (mean: 25.0 µm +/- 1.4).

IV.2.1.5 Genus *Enterobius* (Family Oxyuridae)

All *Enterobius* eggs were asymmetrically ovoid, flattened on one side and contained a folded larva (see Figure 6f). All eggs had a smooth, thick and un-pigmented shell consisting of two thin visible layers. Egg length ranged from 45.0 to 70.0 µm (mean: 55.5 µm +/- 2.4), egg width from 25.0 to 32.5 µm (mean: 27.8 µm +/- 2.1). Measurements had been taken from a total of 55 Enterobius eggs recovered from 55 fecal samples representing 30 different chimpanzees (16 M, 14 F).

IV.2.2 CESTODES

Genus *Bertiella* (Family Anoplocephalidae)

Overall 59 *Bertiella* eggs recovered from 59 chimpanzee fecal samples had been measured. The samples had been collected from a total of 27 chimpanzees (13 M, 14 F). All recovered eggs were achromatic and spherical in shape and contained a hooked embryo enclosed in a distinct, bifurcated pyriform apparatus and a rugged irregular inner embryonic membrane (see Figure 6i). Egg diameter ranged from 35.0 to 52.5 µm (mean: 47.5 µm +/- 0.0).

IV.2.3 TREMATODES

Fam. Dicrocoeliidae

Measurements were taken from a total of four eggs recovered from fecal samples of three different male chimpanzees. All four eggs were dark brown, thick-shelled and elliptical in shape with an inconspicuous operculum (see Figure 6j). Egg length ranged from 40.0 to 47.5 µm (mean: 43.1 µm +/- 3.2), egg width from 17.5 to 20.0 µm (mean: 19.4 µm +/- 1.1).

IV.3 Parameters of helminth infection

IV.3.1 Overall frequency of helminth infection (i.e. overall level of infection)

The overall proportion of individuals found to be infected with at least one type of parasitic helminths from the total number of chimpanzees (N= 72) screened during the course of this study using a combination of MWSFs and Harada-Mori fecal cultures (n= 71) or MWSFs only (n= 1), was 100% (95% confidence limits: 0.9479 to 1.0000). In other words, all 72 chimpanzees screened for gastrointestinal parasites, excreted eggs and/or larvae of one or more types of parasitic helminths at least once during the study period.

The total percentage of examined fecal samples found to be positive for developmental stages of at least one type of parasitic helminths was 99.5% (95% confidence limits: 0.9880 to 0.9990), with one or more helminth eggs and/or larvae detected in 853 out of a total of 857 examined unpreserved fecal samples. Three of the four helminth-negative samples however had only been screened with MWSFs and no subsequent fecal cultures had been performed.

The proportion of individuals found to be infected with at least one helminth type from the overall 30 south group chimpanzees screened with the combination of $NaNO_3$-flotations and sedimentations was equally 100% (95% confidence limits: 0.8885 to 1.0000), as was the percentage of the overall 120 examined formalin-fixed samples in which one or more helminth eggs and/or larvae had been detected (95% confidence limits: 0.9687 to 1.0000).

IV.3.2 Helminth prevalence

IV.3.2.1 NEMATODES

Prevalence values for the different nematode genera and morphotypes were determined from 793 fresh and unpreserved chimpanzee fecal samples which had been examined using a combination of MWSFs and Harada-Mori fecal cultures (see III.5.2.2). The samples had been collected from a total of 71 chimpanzees (35 M, 36 F) representing all age classes and all three study groups.

The overall most commonly detected gastrointestinal worms were nematodes belonging to the superfamily Strongyloidea (i.e. `strongyles´). Strongyle developmental stages were present in 792 (99.5%) of all examined samples, and each of the 71 screened chimpanzees was found to shed respective eggs and/or larvae. Prevalence of the different strongyle genera however varied considerably. The most prevalent (i.e. the most frequently excreted) strongyles were specimens of the genus *Ternidens.* Respective L3-larvae were cultured from 86.8% of all examined fecal samples and most screened chimpanzees (95.8%) were found to be infected. The four other strongyle types (*Oesophagostomum* type A & type *B, Necator spp.* and trichostrongylid strongyles) by contrast were significantly less common (see

Tables 18 & 19), with infection rates (i.e. proportions of infected chimpanzees) between 85.9% (*Oesophagostomum* type A) and as little as 15.5% (trichostrongylid strongyles). The respective percentages of positive fecal cultures differed considerably as well, although the variation was somewhat less pronounced ranging from 49.8% (*Oesophagostomum spp.*) to 1.4% (trichostrongylid strongyles).

Prevalence of the four `non-strongyle´ nematode genera (*Strongyloides spp., Trichuris spp., Capillaria spp. & Enterobius spp.*) also differed significantly (see Tables 18 & 19). Determined infection rates ranged from 88.3% (*Strongyloides spp.*) to as little as 22.5% (*Capillaria spp.*) of all examined chimpanzees. The respective percentages of examined fecal samples in which developmental stages were found varied only slightly less ranging from 64.0% (*Strongyloides spp.*) to just 3.0% (*Capillaria spp.*).

Table 18. *Prevalence of nematode-positive fecal samples determined using a combination of MWSFs and Harada-Mori fecal cultures*

	Total number of examined samples (N=793)			
Helminth type	**No of pos samples**	**Prev of pos samples**	**95 % CI Sterne's exact method**	**Significance of prevalence difference****
All `strongyles´	792	99.5 %	0.9928 to 0.9998	
+ *Ternidens spp.*	688	86.8 %	0.8419 to 0.8898	T#O_A, T#O_B, T#N, T#TS, T#S, T#E, T#Tr, T#C
+ *Oesopghagostomum spp.*	519	65.4 %	0.6261 to 0.6873	
type A (*short tail*)	395	49.8 %	0.4628 to 0.5334	O_A #T, O_A #O_B, O_A #N, O_A #TS, O_A #S, O_A #E, O_A #Tr, O_A #C
type B (*long tail*)	149	18.8 %	0.1620 to 0.2167	O_B #T, O_B #O_A, O_B #N, O_B #TS, O_B #S, O_B #E, O_B #Tr, O_B #C
+ *Necator spp.*	214	27.0 %	0.2395 to 0.3019	N#T, N#O_A, N#O_B, N#TS, N#S, N#E, N#Tr, N#C
+ Trichostrongylid strongyles	11	1.4 %	0.0073 to 0.0249	TS#T, TS#O_A, T#O_B, TS#N, TS#S, TS#E, TS#Tr, TS#C
Strongyloides spp.	521	64.0 %	0.6230 to 0.6898	S#T, S#O_A, S#O_B, S#N, S#TS, S#E, S#Tr, S#C
Enterobius spp.	53	6.7 %	0.0509 to 0.0868	E#T, E#O_A, E#O_B, E#N, E#TS, E#S, E#Tr, E#C
Trichuris spp.	27	3.4 %	0.0232 to 0.0490	Tr#T, Tr#O_A, Tr#O_B, Tr#N, Tr#TS, Tr#S, Tr#E, **Tr–C**
Capillaria spp.	24	3.0 %	0.0200 to 0.0445	C#T, C#O_A, C#O_B, C#N, C#TS, C#S, C#E, **C–Tr**

** ` # ´ indicates a significant difference (P<0,05; two-tailed; Fisher's exact test; 95% significance) level) between the prevalence of the respective helminth types;

` **–** ´ indicates a non-significant prevalence difference (P>0.05; two-tailed; Fishers exact test 95% significance level)

For explanation of abbreviations see legend of Table 19

Table 19. *Prevalence of nematode-infected chimpanzees determined using a combination of MWSFs and Harada-Mori fecal cultures*

	Total number of screened chimpanzees (N=71)			
Helminth type	**No of infect ind**	**Prev of infect ind**	**95 % CI Sterne's exact method**	**Significance of prevalence difference****
All `strongyles´	71	100 %	0.9472 to 1.0000	
+ *Ternidens spp.*	68	95.8 %	0.8824 to 0.9883	**T–O_A**, T#O_B, T#N, T#TS, **T–S**, T#E, T#Tr, T#C
+ *Oesopghagostomum spp.*	65	91.5 %	0.8283 to 0.9625	
type A (*short tail*)	61	85.9 %	0.7551 to 0.9248	**O_A–T**, O_A #O_B, **O_A–N**, O_A #TS, **O_A–S**,O_A #E, O_A #Tr, O_A #C
type B (*long tail*)	46	64.8 %	0.5284 to 0.7550	O_B #T, O_B #O_A, **O_B–N**, O_B#TS, O_B #S, O_B #E, O_B #Tr, O_B #C
+ *Necator spp.*	54	76.1 %	0.6486 to 0.8465	N#T, **N–O_A**, **N–O_B**, N#TS, **N–S**, N#E, N#Tr, N#C
+ Trichostrongylid strongyles	11	15.5 %	0.0847 to 0.2592	TS#T, TS#O_A, T#O_B, TS#N, TS#S, TS#E, **TS–Tr**, **TS–C**
Strongyloides spp.	63	88.3 %	0.7904 to 0.9471	**S–T**, **S–O_A**, S#O_B, S#N, S#TS, S#E, S#Tr, S#C
Enterobius spp.	29	40.8 %	0.2957 to 0.5283	E#T, E#O_A, E#O_B, E#N, E#TS, E#S, **E–Tr**, E#C
Trichuris spp.	18	25.4 %	0.1606 to 0.3656	Tr#T, Tr#O_A, Tr#O_B, Tr#N, **Tr–TS**, Tr#S, Tr#E, **Tr–C**
Capillaria spp.	16	22.5 %	0.1387 to 0.3372	C#T, C#O_A, C#O_B, C#N, **C–TS**, C#S, C#E, **C–Tr**

** ` # ´ indicates a significant difference (P<0,05; two-tailed; Fisher's exact test; 95% significance level) between the prevalence of the respective helminth types;
` **–** ´ indicates a non-significant prevalence difference (P>0.05; two-tailed; Fisher's exact test; 95% significance level)

Abbreviations in Tables 18 & 19:

T= *Ternidens spp.*, O_A = *Oesophagostomum* type A, O_B = *Oesophagostomum* type B, N= *Necator spp.*, TS= trichostrongylid strongyles, S= *Strongyloides spp.*, E= *Enterobius spp.*, Tr= *Trichuris spp.*, C= *Capillaria spp.*

Overall, infections with all different nematode types were detected in both male and female chimpanzees as well as in each of the four age classes. Only exceptions were the *trichostrongylid* strongyles*,* as no respective L3-larvae were found in any of the fecal cultures performed on fecal material from infant chimpanzees.

Developmental stages of most nematode types were furthermore also found in fecal samples of chimpanzees from all three study groups. Only exceptions were again the

trichostrongylid strongyles of which L3-larvae were only detected in cultured fecal material from south group chimpanzees as well as the genus *Capillaria*, eggs of which were only found in fecal samples from south and north group members.

IV.3.2.2 CESTODES

The prevalence of *Bertiella spp.,* the only cestode type detected in this study, was determined from the same sample set as used to assess the above-mentioned nematode prevalence values (see IV.3.2.1). In total, 26 of all 71 screened chimpanzees (i.e. 36.6%; 95% CI: 0.2593 to 0.4858) were found to be infected, even though *Bertiella* eggs were present in only 7.2% of all examined fecal samples (i.e. in 57 of 793 samples; 95% CI: 0.0553 to 0.0919) and shed progottides were detected in the feces of only five (1 M, 4 F) of the overall 26 infected individuals. Overall, *Bertiella* infections were diagnosed in both male and female chimpanzees as well as in individuals from all four age classes and each of the three study groups.

IV.3.2.3 TREMATODES

Trematode prevalence values by contrast, were established from the results of the examination of 120 formalin-fixed chimpanzee fecal samples (samples from south group members only) using a combination of $NaNO_3$- flotations and sedimentations as described before (see III.5.3.2). Overall, dicrocoeliid trematode eggs were found in 2.5% of the examined samples (i.e. in 3 out of 120 samples; 95% CI: 0.0069 to 0.0732) translating to an infection rate of 10.0% (i.e. 3 positive of 30 screened chimpanzees; 95% CI: 0.0279 to 0.2632). All three infected individuals were males, two of which were adults; the third individual was a four-year-old infant.

IV.3.2.4 Influence of sampling intensity on nematode and cestode prevalence

The nematode and cestode infection rates presented above had been established from a dataset including 71 chimpanzees irrespective of the actual number of fecal samples examined from each individual which, as described before, varied between 1 and 54 samples per chimpanzee (median: 6; quartiles: 3/ 20; mean: 11.3 +/- 10.9; see III.5.1.1).

If by contrast only the 36 chimpanzees (16 M, 20 F) from whom a minimum of six fecal samples had been examined were included (6 being the overall median number of samples screened per individual) (total no. of examined samples: 697; 6-54 samples per individual; median: 20; quartiles: 9.8/ 25.3; mean: 19.4 +/- 9.8), observed infection rates were in general notably higher (Table 20). Significant differences (Fisher's exact test; $P_{Oesoph\_typeA} = 0.016$; $P_{Oesoph\_typeB} < 0.0001$ and $P_{Necator} = 0.03$; all two-tailed; 95% significance level) were seen with respect to the rates of chimpanzees infected with *Oesophagostomum* type A and type B (100%: 36 out of 36 individuals (type A & B) versus 85.9%: 61 out of 71 individuals (type A) and

64.8%: 46 out of 71 individuals (type B)) as well as regarding the rate of *Necator* infections (94.4%: 34 out of 36 individuals versus 76.1%: 54 out of 71 individuals).

Comparison of infection rates including all 71 screened chimpanzees with an even further limited dataset, comprising only individuals from whom a minimum of 12 samples had been examined (n= 25; 13 M, 12 F), revealed significantly higher infection rates also for trichostrongylid strongyle-, *Enterobius*- and *Bertiella*-infections (Fisher's exact test; P_{Trich} = 0.022; P_{Ent} = 0.010 and P_{Bert} = 0.005; all two-tailed; 95% significance level) (Table 20).

Table 20. *Influence of sampling intensity on chimpanzee helminth infection rates determined using a combination of MWSFs and Harada-Mori fecal cultures*

	Total number of screened chimpanzees							
	>/= 1 sample/ ind **(N=71)**		>/= 6 samples/ ind **(n=36)**			>/= 12 samples/ ind **(n=25)**		
Helminth type	No of infect ind	Prev of infect ind	No of infect ind	Prev of infect ind	**P-value**	No of infect ind	Prev of infect ind	**P-value**
All `strongyles´	71	100.0 %	36	100.0 %	n.s.	25	100.0 %	n.s.
Ternidens spp.	68	95.8 %	36	100.0 %	n.s.	25	100.0 %	n.s.
Oesophagostomum spp	65	91.5 %	36	100.0 %	n.s.	25	100.0 %	n.s.
type A (short tail)	61	85.9 %	36*	100.0 %	0.016	25	100.0 %	n.s.
type B (long tail)	46	64.8 %	36**	100.0 %	<0.0001	25**	100.0 %	0.0002
Necator spp.	54	76.1 %	34*	94.4 %	0.030	24*	96.0 %	0.035
Trichostrong. strongyles	11	15.5 %	10	27.8 %	n.s.	10*	40.0 %	0.022
Strongyloides spp.	63	88.3 %	33	91.7 %	n.s.	24	96.0 %	n.s.
Trichuris spp.	18	25.4 %	13	36.1 %	n.s.	11	44.0 %	n.s.
Capillaria spp.	16	22.5 %	10	27.8 %	n.s.	9	36.0 %	n.s.
Enterobius spp.	29	40.8 %	22	61.1 %	n.s.	18*	72.0 %	0.010
Bertiella spp.	26	36.6 %	20	55.6 %	n.s.	18**	72.0 %	0.005

* significant difference (P<0.05; two-tailed; Fisher's exact test; 95% significance level) compared to the full dataset (N=71 chimpanzees)

** highly significant difference (P<0.01; two-tailed; Fisher's exact test; 95% significance level) compared to the full dataset (N=71 chimpanzees); n.s. = non significant

IV.3.2.5 Variations in helminth prevalence between male and female chimpanzees

Infections with all identified types of nematodes and cestodes were found in both male and female chimpanzees. Respective prevalence values however varied between the sexes (Table 21).

A very marked difference was seen with respect to the proportion of examined fecal samples from male and female chimpanzees found to be positive for nematodes of the genus *Strongyloides.* Whereas respective developmental stages were detected in

most fecal samples collected from male chimpanzees (i.e. in 391 out of 410 samples (95.4%)), *Strongyloides* eggs and/or larvae were found in significantly fewer samples, i.e. in only about one third of all examined samples (130 out of 383 samples (33.9%)) which had been obtained from female chimpanzees (Fisher's exact test; P<0.0001; two-tailed; 95% significance level).

However, when the prevalence of *Strongyloides*-positive fecal samples in male versus female chimpanzees was compared separately for each of the four chimpanzee age classes, the observed male bias was only significant in adult (Fisher's exact test; P<0.0001; two-tailed; 95% level of significance) and to a lesser extent in adolescent individuals (Fisher's exact test; P< 0.0001; two-tailed; 95% level of significance).

While *Strongyloides* developmental stages were detected in 160 out of 169 fecal samples (94.7%) from adult males as well as in 111 out of 117 samples (94.8%) from adolescent males, only 45 out of 282 samples (16.0%) from adult females and 35 out of 49 fecal samples (71.4%) from adolescent females were *Strongyloides*-positive. In infant and juvenile chimpanzees by contrast almost all fecal samples from both male and female individuals were found to be *Strongyloides*-positive (infants: 20 out of 20 samples (males), 15 out of 16 samples (females); juveniles: 100 out of 104 samples (males), 35 out of 36 samples (females)).

A significant, although less pronounced overall male bias was also observed as to the prevalence of *Trichuris* positive fecal samples. While *Trichuris* eggs were detected in 21 (5.1%) out of 410 examined fecal samples collected from male chimpanzees, significantly fewer, only six (1.6%) out of 383 samples from female individuals were found to be *Trichuris*-positive (Fisher's exact test; P= 0.0058; two-tailed; 95% confidence level).

Necator L3-larvae by contrast were significantly more often detected in fecal samples from female chimpanzees (Fisher's exact test; P= 0.0002; two-tailed; 95% level of significance). While repective larvae were recovered from 126 (32.9%) out of 383 cultured samples from female individuals, only 87 (21.2%) out of 410 fecal cultures from male chimpanzees were found to contain any *Necator* L3-larvae.

The differences between the sexes with regard to the corresponding infection rates on the other hand were overall less pronounced (see Table 22) and at a significance level of 95% generally non-significant.

Only exception was the rate of individuals infected with *Strongyloides spp*. which differed significantly between male and female chimpanzees (Fisher's exact test: P= 0.0051; two-tailed; 95% level of significance) if all 71 screened individuals were considered (100%, i.e. 35 out of 35 individuals in male chimpanzees versus 77.8%, i.e. 28 out of 36 individuals in females), whereby all female chimpanzees found to be *Strongyloides* negative were adult individuals.

Table 21. *Prevalence of helminth-positive fecal samples in male versus female chimpanzees*

	Total number of examined fecal samples (N= 793)						
	Male chimpanzees (n= 410)			Female chimpanzees (n= 383)			
Helminth type	**No of pos samples**	**Prev of pos samples**	**95% CI**	**No of pos samples**	**Prev of pos samples**	**95% CI**	**P-value***
All `strongyles´	410	100.0 %	0.9909- 1.0000	382	99.7 %	0.9850- 0.9998	n.s.
Ternidens spp.	353	86.1 %	0.8235- 0.8917	334	87.2 %	0.8345- 0.9024	n.s.
Oesophagostomum spp.	259	63.2 %	0.5830- 0.6781	259	67.6 %	0.6268- 0.7221	n.s.
type A (short tail)	203	49.5 %	0.4464- 0.5439	192	50.1 %	0.4504- 0.5522	n.s.
type B (long tail)	68	16.6 %	0.1327- 0.2058	81	21.2 %	0.1730- 0.2556	n.s.
Necator spp.	87**	21.2 %	0.1742- 0.2546	126**	32.9 %	0.2832- 0.3784	0.0002
Trichostrong. strongyles	5	1.2 %	0.0049- 0.0287	6	1.6 %	0.0069- 0.0335	n.s.
Strongyloides spp.	391**	95.4 %	0.9285- 0.9712	130**	33.9 %	0.2936- 0.3889	<0.0001
Trichuris spp.	21**	5.1 %	0.0329- 0.0776	6**	1.6 %	0.0069- 0.0335	0.0058
Capillaria spp.	8	2.0 %	0.0092- 0.0385	16	4.2 %	0.0255- 0.0674	n.s.
Enterobius spp.	22	5.4 %	0.0350- 0.0801	31	8.1 %	0.0571- 0.1132	n.s.
Bertiella spp.	23	5.6 %	0.0373- 0.0826	34	8.9 %	0.0636- 0.1223	n.s.

* Fisher's exact test; ** highly significant difference (P<0.01; two-tailed; 95% significance level)

Table 22. *Comparison of helminth infection rates between male and female chimpanzees*

	Total number of screened chimpanzees (N= 71)						
	Male chimpanzees (n= 35)			Female chimpanzees (n= 36)			
Helminth type	**No. of pos ind.**	**Prev of pos ind.**	**95 % CI**	**No. of pos ind.**	**Prev of pos ind.**	**95 % CI**	**P-value***
All `strongyles´	35	100.0 %	0.9043- 1.0000	36	100.0 %	0.9070- 1.0000	n.s.
Ternidens spp.	33	94.3%	0.8047- 0.9897	35	97.2 %	0.8523- 0.9985	n.s.
Oesophagostomum spp.	34	97.1%	0.8481- 0.9985	31	86.1 %	0.7105- 0.9436	n.s.
type A (short tail)	32	91.4%	0.7743- 0.9762	29	80.6 %	0.6407- 0.9069	n.s.
type B (long tail)	23	65.7 %	0.4856- 0.8046	23	63.9 %	0.4720- 0.7805	n.s.
Necator spp.	23	65.7 %	0.4856- 0.8046	30	83.3 %	0.6798- 0.9248	n.s.
Trichostrong. strongyles	5	14.3 %	0.0581- 0.2978	6	16.7 %	0.0752- 0.3202	n.s.
Strongyloides spp.	35**	100.0 %	0.9043- 1.0000	28**	77.8 %	0.6124- 0.8937	0.0051
Trichuris spp.	13	37.1 %	0.2258- 0.5433	6	16.7 %	0.0752- 0.3202	n.s.
Capillaria spp.	7	20.0 %	0.0958- 0.3696	9	25.0 %	0.1342- 0.4158	n.s.
Enterobius spp.	12	34.3 %	0.1954- 0.5144	17	47.2 %	0.3171- 0.6406	n.s.
Bertiella spp.	13	37.1 %	0.2258- 0.5433	13	36.1 %	0.2195- 0.5280	n.s.

* Fisher's exact test; ** highly significant difference (P<0.01; two-tailed; 95% significance level)

If by contrast only chimpanzees from whom a minimum of six samples (n= 36 individuals: 16 M & 20 F) had been examined were included in the calculation, infection rates started to converge (100%, i.e. 16 out of 16 individuals in males versus 85.0%, i.e. 17 out of 20 individuals in females) and the respective difference between the sexes was no longer significant (Fisher's exact test: P= 0.2381; two-tailed; 95% level of significance).

IV.3.2.6 Method comparison

Upon performing the different conventional coprological examination methods employed in this study, a number of differences regarding the respective helminth detection rates were noted.

IV.3.2.6.1 Comparison of nematode detection rates between MWSF and Harada-Mori fecal cultures

The proportion of chimpanzees (N= 71) found to be infected with *Strongyloides spp*. using Harada-Mori fecal cultures was marginally higher (61 out of 71 individuals; 85.9%; 95% CI: 0.7551 to 0.9248) than the respective proportion obtained using MWSFs (60 out of 71 individuals; 84.5%; 95% CI: 0.7408 to 0.9153), but slightly lower than the infection rate determined with the combination of both methods (63 out of 71 individuals; 88.3%; 95% CI: 0.7904 to 0.9471). Both differences however were clearly non-significant at a significance level of 95% (McNemar's test with continuity correction; P= 1.0; two-tailed; chi-square= 1.0; df= 1 and P= 0.4795; two-tailed; chi-square= 0.5; df= 1).
The observed rates of strongyle-infected individuals were 100% (95% CI: 0.9472 to 1.0000) with either one method and thus identical.

Further comparison of the two examination methods however revealed a significantly higher sensitivity of Harada-Mori fecal cultures to detect *Strongyloides*-positive fecal samples. In other words, *Strongyloides* developmental stages were found in significantly more fecal samples (McNemar's test with continuity correction; P<0.0001; two-tailed; chi square= 90.374; df= 1; 95% confidence level) when the samples were examined by means of Harada-Mori fecal cultures (493 positive out of 793 samples; 62.2%; 95% CI: 0.5871 to 0.6551) than when they were screened with MWSFs (362 positive out of 793 samples; 45.6%; 95% CI: 0.4218 to 0.4917).

Nevertheless, from a number of samples (n= 28) in which *Strongyloides* eggs had been detected upon examination with MWSFs, no respective larvae and/or free-living adult specimens could be cultured. Hence, the overall detection rate of *Strongyloides*-positive fecal samples was highest (521 out of 793 samples; 64.0%; 95% CI: 0.6230 to 0.6898) employing a combination of fecal cultures and sugar flotations. The respective difference was again significant (McNemar's test with continuity correction; P<0.0001; two-tailed; chi square= 26.036; df= 1; 95% significance level).

Fecal samples positive for strongyle nematodes on the other hand were significantly more reliably identified employing MWSFs as opposed to Harada-Mori fecal cultures (McNemar's test with continuity correction; P<0.0001; two-tailed; chi-square= 38.025; df= 1; 95% significance level). While strongyle eggs were detected in 783 of all 793 performed sugar flotations (98.7%; 95% CI: 0.9769 to 0.9932), respective L3-larvae could only be cultured from 743 of these samples (93.7%; 95% CI: 0.9176 to 0.9522). The significantly highest detection rate (792 out of 793 fecal samples, 99.9%; 95% CI: 0.9928 to 0.9998) however was again obtained using a combination of both methods (McNemar's test with continuity correction; P<0.0077; two-tailed; chi-square= 7.111; df= 1; 95% significance level).

IV.3.2.6.3 Comparison of helminth detection rates between fresh and formalin-preserved sample aliquots using MWSFs

To compare helminth detection rates obtained from fresh, unpreserved feces and fecal material preserved in 10% formalin using the same examination method, an additional sample set consisting of 45 chimpanzee fecal samples from a total of 17 south group members (10 M, 7 F) was collected between December 2009 and March 2010 and analyzed on site using MWSFs (1 to 5 samples per individual; median 3; quartiles: 1/ 4; mean: 2.65 +/- 1.32). From each sample both, one fresh and unpreserved aliquot of fecal material as well as one formalin-fixed aliquot of equal weight were examined. Sample collection, preservation and analysis were performed as described before.

Results of the comparison were as follows: prevalence values obtained from formalin- preserved aliquots were overall largely similar to the respective values established from fresh and unpreserved fecal material (see Tables 23 & 24). A marked difference however was observed with regard to the genus *Strongyloides.* Whereas *Strongyloides* eggs were detected in 51% (n= 23) of all examined unpreserved fecal samples, they were significantly less prevalent (8.9%; n= 4 positive samples) in the respective formalin-fixed aliquots (McNemar's test with continuity correction; P<0.0001; two-tailed; chi-square= 17.053; df= 1; 95% significance level). The difference between the respective proportions of *Strongyloides*-infected individuals was similarly notable (70.6%; 12 out of 17 chimpanzees versus 23.5%; 4 out of 17 individuals) and at a 95% confidence level also significant (McNemar's test with continuity correction; P= 0.0133; two-tailed; chi-square= 6.125; df= 1).

Detection rates for strongyle nematodes on the other hand were almost equal. Strongyle eggs were detected in all 45 examined unpreserved fecal samples, but also in as many as 43 (95.6%) of the formalin-preserved aliquots. The proportion of the individual chimpanzees found to be infected with strongyle nematodes was 100% (N= 45) regardless of whether unpreserved or formalin-fixed sample aliquots had been screened.

Table 23. *Prevalence of helminth-positive fecal samples determined from unpreserved versus formalin-fixed fecal material employing MWSFs*

	Unpreserved feces (N=45)			Formalin-fixed feces (N=45)			
Helminth type	**No of pos samples**	**Prev pos samples**	**95% CI Sterne's exact method**	**No of pos samples**	**Prev of pos samples**	**95% CI Sterne's exact method**	**P-value**
'Strongyles'	45	100.0 %	0.9168- 1.0000	43	95.6 %	0.8480- 0.9920	n.s.
Strongyloides spp.	23**	51.1 %	0.3657- 0.6569	4**	8.9 %	0.0310- 0.2087	<0.001
Capillaria spp.	3	6.7 %	0.0185- 0.1854	3	6.7 %	0.0185- 0.1854	n.s.
Enterobius spp.	3	6.7 %	0.0185- 0.1854	5	11.1 %	0.0449- 0.2418	n.s.
Bertiella spp.	4	8.9 %	0.0310- 0.2087	3	6.7 %	0.0185- 0.1854	n.s.

** highly significant difference (P<0.01); McNemar's test with continuity correction
n.s.= non significant; 'strongyles' = Strongyloidea superfam.

Table 24. *Prevalence of helminth-infected chimpanzees determined from unpreserved versus formalin-fixed fecal samples employing MWSFs*

	Total number of screened chimpanzees (N=17)						
	Unpreserved feces			Formalin-fixed feces			
Helminth type	**No of pos individ**	**No of pos individ**	**95% CI Sterne's exact method**	**No of pos individ**	**Prev of pos individ**	**95% CI Sterne's exact method**	**P-value**
'Strongyles'	17	100.0 %	0.8039- 1.0000	17	100 %	0.8039- 1.0000	n.s.
Strongyloides spp.	12*	70.6 %	0.4559- 0.8762	4*	23.5 %	0.0847- 0.4887	0.0133
Capillaria spp.	2	11.8 %	0.0214- 0.3497	3	17.6 %	0.0214- 0.3497	n.s.
Enterobius spp.	3	17.6 %	0.0499- 0.4165	4	23.5 %	0.0847- 0.4887	n.s.
Bertiella spp.	2	11.8 %	0.0214- 0.3497	1	5.9 %	0.0031- 0.2873	n.s.

* significant difference (P<0.05); McNemar's test with continuity correction);
n.s.= non significant; 'strongyles' = Strongyloidea superfam.

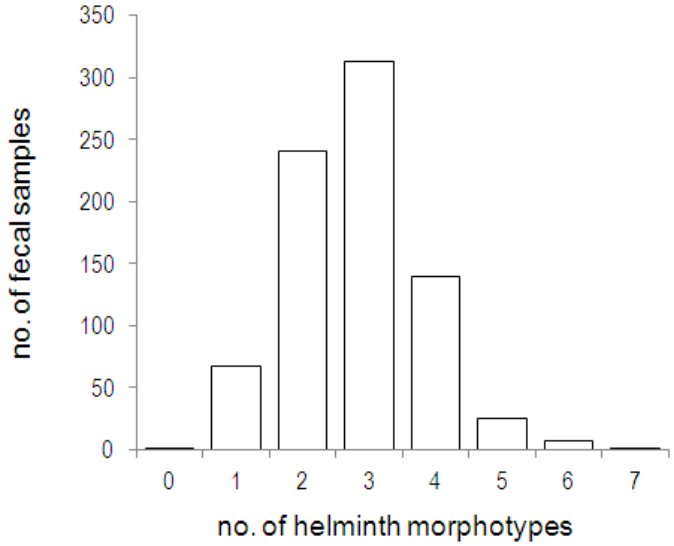
The number of eggs and/or larvae (= `propagules´ (Turner & Getz, 2010)) per gram feces (ppg) in each sample however varied considerably between the two sets of sample aliquots and was overall significantly lower in the formalin-fixed aliquots (range: 0 to 148; median: 6,0; quartiles: 2/ 9; mean: 11.1 +/- 23.2) as compared to the aliquots of non-preserved fecal material (range: 3 to 250; median: 50.0; quartiles: 20/ 72; mean: 54.2 +/- 45.8) (Wilcoxon matched-pairs signed rank test; P<0.0001; two-tailed; 95% significance level).

IV.3.3 Helminth morphotype richness and prevalence of multiple infections

IV.3.3.1 Overall sample helminth morphotype richness

The respective number of unique, light-microscopically differentiated nematode and cestode genera and morphotypes detected in each of the overall 793 fecal samples examined using a combination of MWSFs and Harada-Mori fecal cultures ranged from 0 to 7 helminth types per sample (median: 3; quartiles: 2/ 3; mean 2.8 +/- 1) (see Figure 11).

Figure 11. *Frequency distribution of observed helminth morphotype richness in 793 fecal samples*



Bars denote the respective number of fecal samples found to contain developmental stages of a certain number of different helminth morphotypes

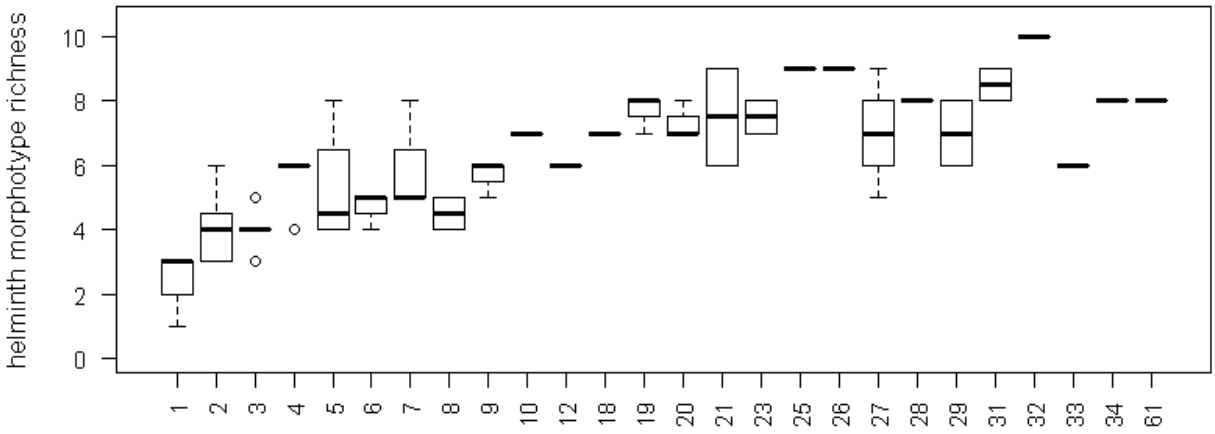
IV.3.3.2 Cumulative individual helminth morphotype richness

The cumulative individual helminth morphotype richness of the different study group members (N= 72) by contrast, i.e. the respective total number of unique nematode and cestode types each individual chimpanzee was found to be infected with ranged from 1 to 10 (median: 5; quartiles: 4/ 7; mean: 5.5 +/- 2.1) (see Figure 12).

From each chimpanzee between 1 and 61 fecal samples had been examined (median: 7; quartiles: 3/20.5; mean: 11.9 +/- 12.0; see III.5.1.2) out of a total of 857 fecal samples screened during the study period with a combination of MWSFs and Harada-Mori fecal cultures (n= 793) or MWSFs only (n= 64).

If the latter 64 samples, for which for no fecal cultures had been performed, were excluded from the calculation, the respective cumulative individual helminth morphotype richness of the remaining 71 chimpanzees ranged between 1 and 9 morphotypes per individual (median: 5; quartiles: 4/ 7; mean: 5.5 +/- 2.0).

Figure 12. *Boxplot of cumulative helminth morphotype richness values observed at different sampling intensities determined from 857 fecal samples*



Boxes represent the respective inter-quartile ranges, bold horizontal bars the median. Whisker ends indicate the min. and max. observed cumulative helminth richness-values that are not outliers, outlier values are depicted as circles.

X-axis labels indicate the respective number of fecal samples examined from each of the overall 72 screened chimpanzees.

IV.3.3.3 Influence of host-intrinsic factors on cumulative individual helminth morphotype richness

Whether or not host-intrinsic factors, i.e. host sex, age and/or group-affiliation had a significant impact on the determined individual cumulative helminth (nematode & cestode) morphotype richness of the screened study group members, was investigated using model 1.
The model was run for all 72 chimpanzees using data from all 857 fecal samples examined with a combination of MWSFs and fecal cultures, or MWSFs only (see above). The results of the model were as follows.

There was no obvious overall effect of the predictor variables (sex, age and group) as a whole on the cumulative helminth morphotype richness (full null model comparison: χ^2= 0.57, df= 4, P= 0.966) of the different study group members. Correspondingly, also the individual effect of chimpanzee sex was found to be non-significant (χ^2= 0.0001, df= 1, P= 0.978). Equally, no significant individual impact on the observed cumulative helminth morphotype richness could be attributed to either age (χ^2= 0.13, df= 1, P= 0.715) or group affiliation (χ^2= 0.44, df= 2, P= 0.804) of the screened chimpanzees.

IV.3.3.4 Prevalence of multiple infections

Whereas developmental stages of more than one helminth genus or morphotype were detected in only 725 (91.4%) out of the 793 samples examined with the combination of MWSF and Harada-Mori fecal cultures, the overall prevalence of multiple infections among the screened individuals (N= 71) was 98.6%. In other words, all chimpanzees except one female screened with both methods during the course of this study were found to harbor infections with more than one helminth type. From this particular female however, overall only one sample had been examined.

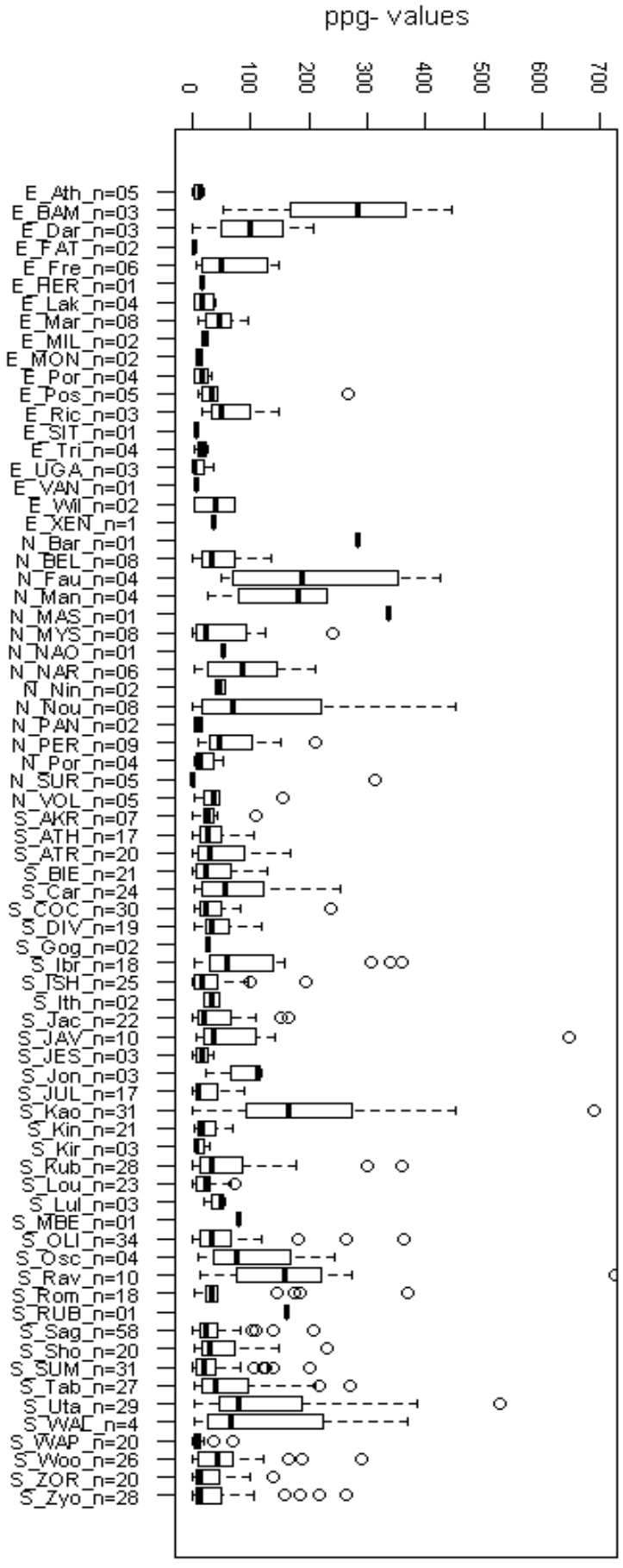
The majority of fecal samples (i.e. 58 out of 68 samples (85.3%)) in which eggs and/or larvae of only one helminth genus or morphotype or no helminth developmental stages were found, had been collected from female chimpanzees, only 10 samples (14.7%) from male chimpanzees.

IV.3.4 Propagule output (ppg-values)

Propagule output, i.e. the number of helminth propagules (= eggs and L1-larvae) per gram feces (ppg-values), had been determined for a total of 806 out of the overall 857 fecal samples from 72 chimpanzees (35 M, 37 F) examined using MWSFs. Obtained ppg-values ranged from 0 to 1172.0 propagules per gram feces (median: 27.8; quartiles: 11.0/ 75.7; mean 64.3 +/- 97.7) (Figure 13) and showed an aggregated frequency distribution.

Median individual ppg-values of the different study group members, calculated from all fecal samples from each individual for which fecal propagule counts had been performed (1 to 58 samples per individual; median: 5; quartiles: 3/ 20; mean: 11.19 +/- 11.45) ranged from 1.33 to 337.0 propagules per gram feces (Figure 13) and showed a similarly aggregated frequency distribution.

Figure 13. *Boxplot of the respective individual helminth propagule output (ppg) of all 72 screened chimpanzees determined from a total of 806 fecal samples*



Boxes represent the respective inter-quartile ranges, bold horizontal bars the median.

Whisker ends indicate the min. and max. ppg-values that are not outliers, outlier values are depicted as circles.

X-axis labels

indicate group affiliation (N= north group, E= east group, S= south group and name (first 3 letters) of each screened chimpanzee, as well as the number of fecal samples examined from the respective individual.

Name abbreviations in all-capital letters indicate female chimpanzees

IV.3.5 Influence of seasonality and host intrinsic factors on fecal propagule output (i.e. on the number of helminth propagules per gram feces (ppg))

Whether or not host sex and -age and/or seasonal factors, including rainfall and maximum temperature (see Annex, Figures A1 to A2 for rainfall and temperature recorded during the study period), had a significant effect on the observed fecal helminth propagule output, i.e. on the number of helminth eggs and/or L1-larvae per gram feces (ppg) found in the examined samples from the different study group members, was investigated using a Generalized Linear Mixed Model (GLMM). As additional potential influencing factors, defecation time as well as fecal consistency had been included into the model.

The model (model 2) was run for a total of 607 fecal samples collected from 38 south group chimpanzees (19 M, 19 F). Samples from north and east group members had not been included. The outcomes of the model were as follows:

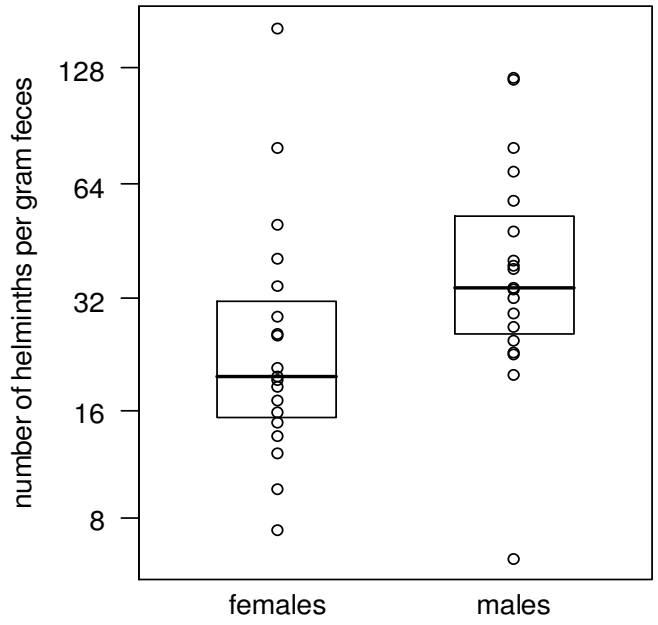
Overall, the full model was highly significant as compared to the null model (see III.6.2.2) (likelihood ratio test: χ^2= 94.65, df= 6, P<0.001). More specifically, male chimpanzees showed significantly higher ppg-values than females (Table 25 & Figure 14). Host age by contrast revealed no significant influence on the number of detected helminth propagules per gram feces (Table 25 & Figure 15).

Table 25. *Results of the GLMM (model 2) with number of helminth propagules per gram feces as the response*

Term	Estimate	SE	χ^2	df	P
Intercept	3.165	0.115	(1)	(1)	(1)
Rain	0.247	0.076	10.301	1.000	0.001
Maximum temperature	-0.326	0.070	21.090	1.000	<0.001
Sex (0=F; 1=M)	0.520	0.167	8.298	1.000	0.004
Age	-0.084	0.080	1.088	1.000	0.297
Defecation time	0.025	0.049	0.242	1.000	0.622
sin(day)	0.068	0.128	(1)	(1)	(1)
cos(day)	-0.627	0.086	(1)	(1)	(1)
Fecal consistency	-0.093	0.044	4.392	1.000	0.036
autocorrelation term	0.164	0.043	13.850	1.000	<0.001

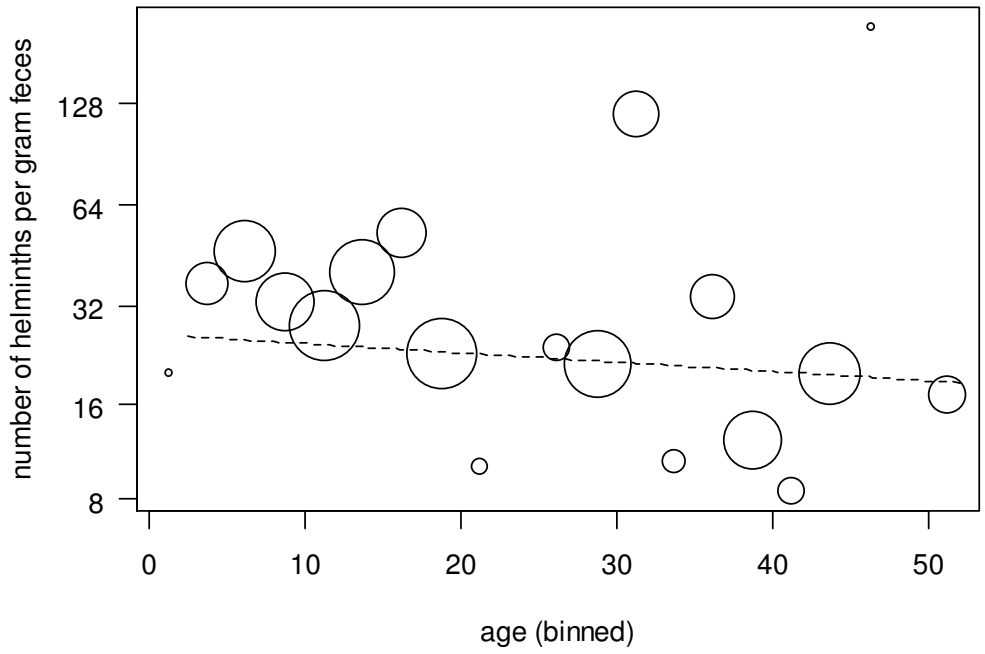
(1): not indicated because of having no interpretation (see III.6.2.2)

Figure 14. *Number of helminth propagules per gram feces in female (n= 19) and male (n= 19) chimpanzees*



Shown are the means per individual (after log-transforming the values +1, back-transformed to the original scale; each individual is represented by one point) as well as medians and quartiles.

Figure 15. *Number of helminth propagules per gram feces as a function of chimpanzee age*

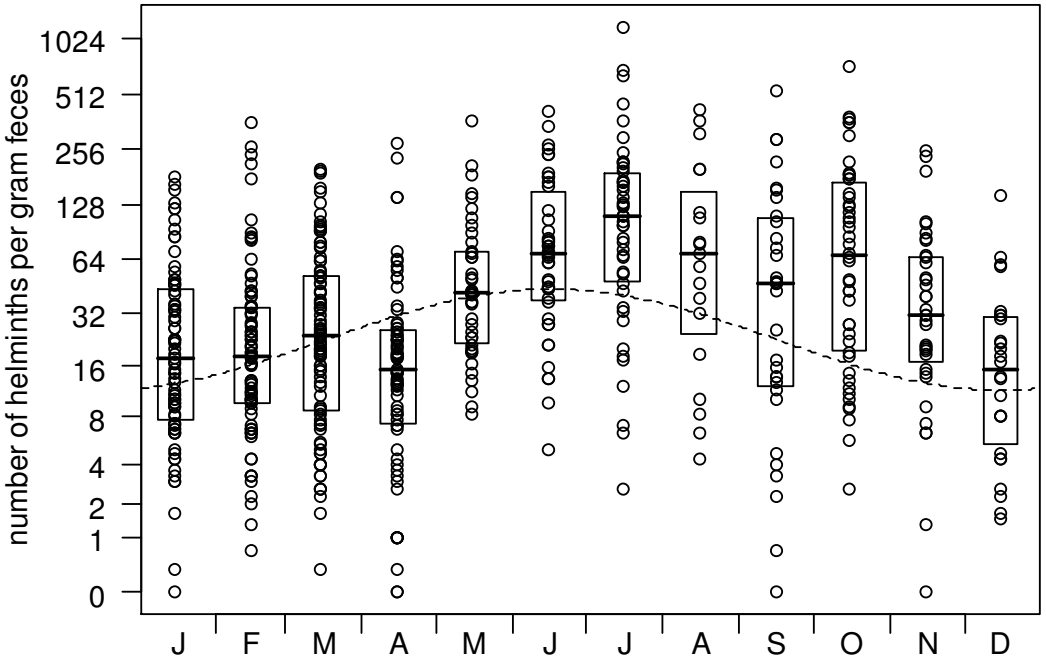


Shown are the means per age class. Age classes have a width of 2.5 years and are represented by their mid value (means were calculated as for individuals; see Figure 14). The area of the points corresponds to sample size per age class (range: 1 to 67), and the dashed line represents the fitted model.

Regarding the influence of seasonality on the number of helminth propagules per gram feces, the model showed a significant overall effect of season (likelihood ratio test: χ^2= 42.21, df= 2, P<0.001) with a peak at the months around July (Figure 16) as well as a significant impact of maximum temperature (Table 25), whereby higher maximum temperatures were generally associated with lower ppg-values (Figure 17). Rainfall on the other hand was found to have a significant positive impact on the observed number of propagules (Table 25 & Figure 18).

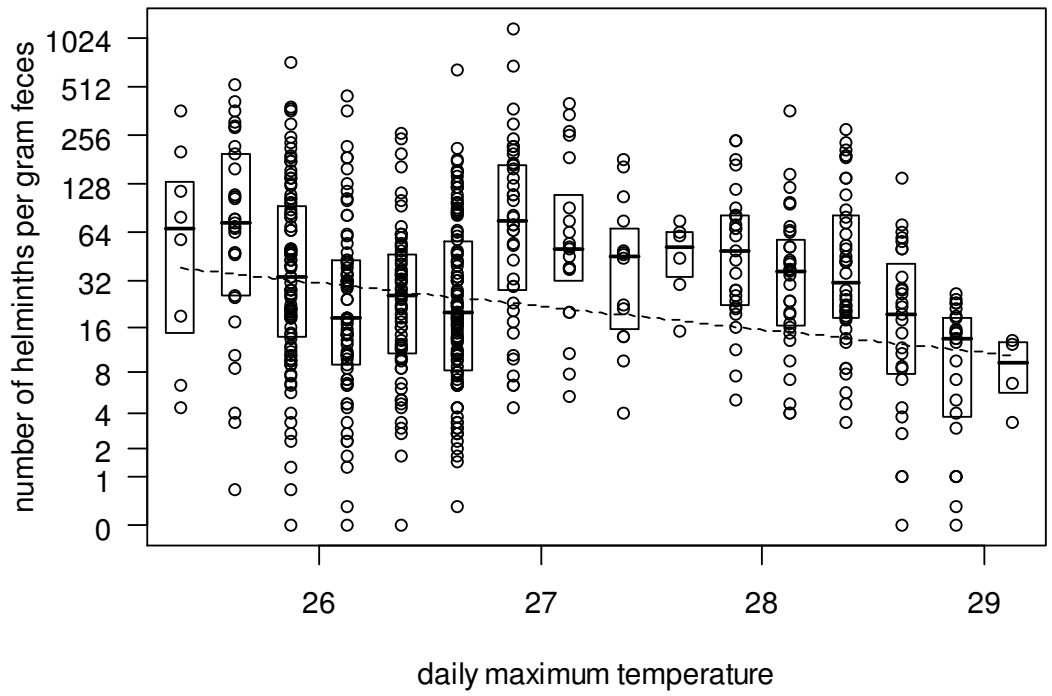
Defecation time did not show a significant influence on the number of excreted helminth eggs and L1-larvae per gram feces (Table 25), but in feces with more solid consistency fewer propagules were found (Table 25).

Figure 16. *Number of helminth propagules per gram feces as a function of sampling date*



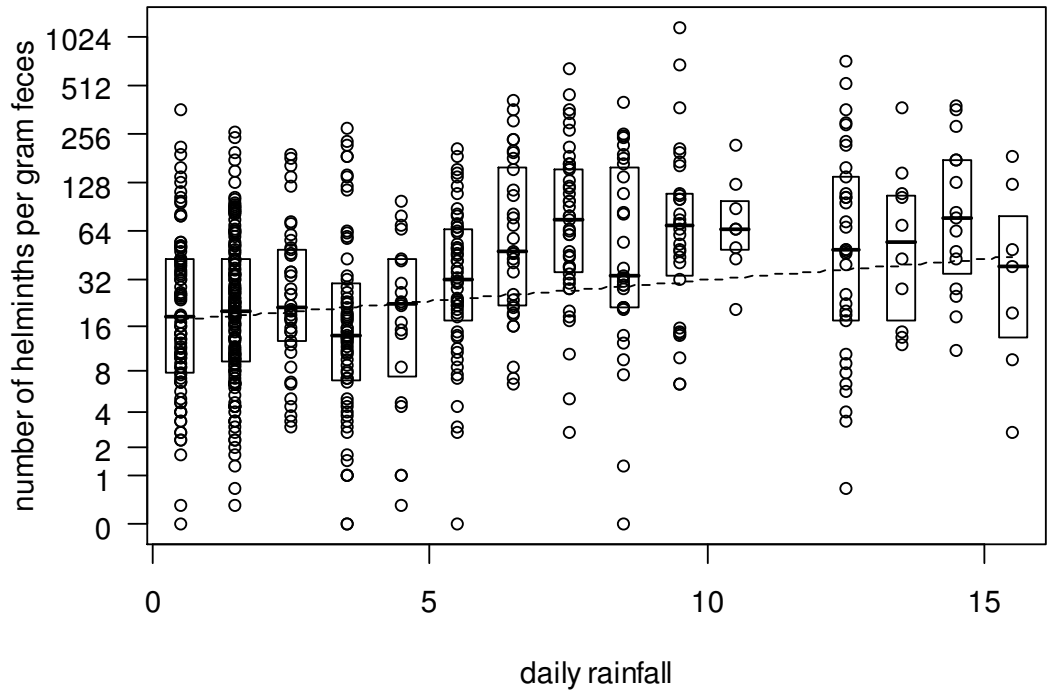
Shown are the individual propagule counts as well as medians and quartiles, aggregated per month, and the dashed line represents the fitted model.

Figure 17. *Number of helminth propagules per gram feces as a function of daily maximum temperature (binned in 0.25° bins)*



Shown are the individual counts as well as medians and quartiles, aggregated per month, and the dashed line represents the fitted model.

Figure 18. *Number of helminth propagules per gram feces as a function of daily rainfall (binned in 1 mm bins)*



Shown are the individual counts as well as medians and quartiles, aggregated per month, and the dashed line represents the fitted model.

IV.4 Identification and characterization of cultured strongyle L3-larvae and *Strongyloides* specimens using genetic markers & comparison with sequences of helminth developmental stages from sympatric monkeys

Goal of this part of the study was to generate and analyze diagnostic DNA sequences from cultured L3-larvae and fee-living adult specimens in order to allow for validation and more precise identification of the spectrum of strongyle nematodes and *Strongyloides* species harboured by the chimpanzee study population. Genetic comparison with morphologically similar nematode L3-larvae and/or free-living adult worms cultured from opportunistically collected fecal material of sympatric monkeys was conducted when possible.

Overall, DNA-extraction and PCRs were performed on a total of 34 L3-larvae of three strongyle genera (*Ternidens spp.*, n= 11; *Oesophagostomum spp.*, n= 14; *Necator spp.*, n= 9), on two *Trichostrongylus*-like larvae as well as on 18 *Strongyloides spp.* specimens (10 L3-larvae, 3 free-living adult females; 5 free-living adult males) isolated from cultured fecal material of south group chimpanzees.

Additionally, a total of 23 L3-larvae (*Ternidens spp.*, n= 3, *Oesophagostomum spp.*, n= 13; *Strongyloides spp.,* n= 7) and six free-living adult *Strongyloides spp.* (males, n= 2; females, n= 4) cultured from feces of sympatric monkeys (*Piliocolobus badius, Cercopithecus diana, Cercocebus atys*) were examined.

Size of the different **ITS-2 amplicons** obtained using **primer pair NC1 + NC2** ranged between 306 and 437 bp. Length of the respective sequences after primer removal was between 266 and 397 bp.

The different **12s RNA mtDNA amplicons** generated using **primers Pannem F+R1** ranged in size between 510 and 519 bp. Sequence length excluding the primers was between 472 and 481 bp long.

All *Strongyloides* **COI mtDNA amplicons** obtained using **primer pair Pannem Col F+R** had an identical size of 645 bp. Net sequence length of all respective sequences after primer removal was 607 bp.

Sequence names, e.g. 14_S3-2CAR_ITS2, are composed of a consecutive lab number (e.g. 14), the code of the fecal sample the respective larva or free-living adult worm had been cultured from (e.g. S3-2) as well as a letter code indicating from which chimpanzee or respectively sympatric monkey species the sample had been collected (e.g. CAR= `Caramel´, Pbad= *Piliocolobus badius*, Cdian= *Cercopithecus diana*, Mang= *Cercocebus atys*). An abbreviation (ITS2, 12s or COI) indicates the respective sequence type.

IV.4.1 Identification of strongyle nematode L3-larvae by ITS-2 and 12s RNA mtDNA sequencing

IV.4.1.1 *Ternidens* L3-larvae (Subfam. Chabertiinae)

IV.4.1.1.1 Overview of examined larvae and success rates of DNA extraction and PCR amplification

Overall, ITS-2 (second internal transcribed spacer) amplicons and sequences could successfully be generated from eight (out of 11 examined) *Ternidens* L3-larvae recovered from cultured chimpanzee fecal samples (see Annex, Table A4). One of these larvae (69_S3-1_IBR) was lacking a cuticular sheath and was thus potentially a second-stage larva. All other larvae were typical L3-larvae as described before. From larvae 14_S3-2_CAR and 19_S3-3_TAB, two ITS-2 amplicon clones each were sequenced. From all other larvae only one clone was sequenced or the PCR product was sequenced directly.

From three examined *Ternidens* larvae no ITS-2 sequences could be determined. In one case PCR produced an amplicon of the right size, but subsequent repeated cloning was unsuccessful, in both other cases DNA extraction failed.

Amplification and sequencing of the mitochondrial (mt) 12s RNA region was successfully performed for six of the eight *Ternidens* larvae from which ITS-2 sequences had been obtained, whereas PCR failed to produce respective amplicons from the DNA of the two other larvae (20_S3-11_UTA & 69_S3-1_IBR) (see Annex, Table A4). Instead, a sequence was obtained from larva 70_S3-1_IBR (also potentially a second-stage larva), from which, due to repeated unsuccessful cloning, no ITS-2 sequence could be generated. Sequencing (one attempt) of the respective mtDNA amplicon however could only successfully be performed in one direction (R).

IV.4.1.1.2 Analysis of ITS-2 sequences

Each of the nine *Ternidens* ITS-2 nucleotide sequences (chimpanzee-isolates) had a length of 266 bp, a CG-content of 43.6% to 44.0% and represented one of two different haplotypes. Five sequences belonged to haplotype a, four sequences to haplotype b (see Annex, Table A12). The two sequences (14_S3-2CAR_ITS2_A & -B) obtained from the two ITS-2 amplicon clones of larva 14_S3-2_CAR were 100% identical and belonged to haplotype b. The two ITS-2 sequences obtained from larva 19_S3-3_TAB were equally identical and belonged to haplotype a.

Pairwise comparison of haplotype a and b revealed a sequence difference of 0.8% between the respective sequences. Overall, two single-base nucleotide differences were detected: a transition (G/A) at position 60 and a transversion (T/A) at position 156.

BLAST search revealed a maximum similarity of 100% (Identities= 216/216, Gaps= 0/216) and 99.1% (Identities= 214/216, Gaps= 0/216) respectively between the two

haplotypes and the ITS-2 sequence of a *T. deminutus* recovered from a mona monkey (*Cercopithecus mona*) in Ghana (GenBank accession no. AJ888729.1).

IV.4.1.1.3 Analysis of 12s RNA mtDNA sequences

Each of the six fully sequenced *Ternidens* 12s RNA mtDNA nucleotide sequences had a length of 480 bp, a CG-content ranging from 21.9 to 22.7% and represented one of overall five different haplotypes (I-V) (see Annex, Table A14). The sequence of larva 70_S3-1_IBR (which had only been successfully be sequenced in one direction) by contrast was invariably shorter (431 bp), had a CG-content of only 21.8%, but was 100% identical to sequences 11_S3-7BIE_12s and 14_S3-2CAR_12s (haplotype I) over its entire length.

Alignment and comparison of the five sequence haplotypes revealed a total of nine polymorphic sites, at positions 39, 46, 49, 50, 53, 98, 404, 411 and 415 respectively. Polymorphism was caused by single-base transitions (A/G) at seven alignment positions and single-base transversions (G/T, A/T) at two sites. Pairwise sequence differences between the five haplotypes were, like the variations between the respective ITS-2 sequences (see above) overall fairly low, ranging from 0.4% to 1.0% (see Table 26).

BLAST search revealed a maximum similarity of 91.3% to 92.0% between the different haplotypes and the mtDNA nucleotide sequence from a specimen of *Oesophagostomum dentatum* (GenBank accession no. GQ888716.1). Respective mtDNA reference sequences of *Ternidens deminutus* specimens were not available for comparison.

IV.4.1.1.4 Comparison with *Ternidens* sequences from sympatric monkeys

ITS-2 as well as 12s RNA mtDNA amplicons and sequences (*C. diana*-isolates) were obtained from two *Ternidens* larvae (93_S2-7_Cdian & 99_S2-7_Cdian) cultured from the feces of a sympatric Diana monkey (*Cercopithecus diana) (*see Annex, Table A5). Both larvae had been obtained from the same fecal sample. For ITS-2 and 12s RNA mtDNA sequence characteristics see Annex, Tables A13 & A15. DNA-extraction from another *Ternidens* L3-larva, isolated from cultured intestinal contents of a Diana monkey, which had been killed and preyed upon by south group chimpanzees, failed.

No *Ternidens* larvae by contrast had been detected in coprocultures performed on fecal material from a total of 53 sympatric red colobus monkeys (*Piliocolobus badius*), three black and white colobus monkeys (*Colobus polykomos*) as well as two sooty mangabeys (*Cercocebus atys*).

Comparison of the two Diana monkey ITS-2 sequences with the respective chimpanzee *Ternidens* ITS-2 sequences, revealed 100% identity of sequence 99_S2-7Cdian_ITS2 with haplotype b of the chimpanzee-isolates. Sequence

93_S2-7Cdian_ITS2, by contrast, was almost (99.6%) identical to the chimpanzee *Ternidens* ITS-2 haplotype a, except for one single-base transition (C/T) at alignment position 205.

Alignment and pairwise comparison of the respective 12s RNA mtDNA sequences (see Table 26) revealed 100% homology of sequence 99_S2-7Cdian_12s to haplotype I of the chimpanzee *Ternidens* isolates. Hence both molecular markers of larvae 99_S2-7_Cdian (cultured from fecal material of a Diana monkey) and 14_S3-2_CAR (cultured from the feces of a south group chimpanzee) were identical. All other pairwise mtDNA sequence differences ranged from two to five single-base substitutions (0.4% to 1.0%).

Table 26. *Pairwise nucleotide differences (in %) between 12s mtDNA sequences of Cercopithecus diana (C. diana) and Taï chimpanzee (P. t. verus) Ternidens L3-larvae over an alignment length of 480 bp*

No	Haplotype/ Host	Sequence name	1	2	3	4	5	6	7
1	**I** / *P. t. verus*	11_S3-7BIE_12s[A]	**						
2	**II** / *P. t. verus*	16_S2-226ATR_12s	1.0	**					
3	**III** / *P. t. verus*	17_S3-9KIN_12s	0.4	0.6	**				
4	**IV** / *P. t. verus*	18_S3-21JUL_12s	0.8	1.0	0.4	**			
5	**V** / *P. t. verus*	19_S3-3TAB_12s	0.8	1.0	0.4	0.8	**		
6	*C. diana*	93_S2-7Cdian_12s	1.0	1.0	0.6	1.0	1.0	**	
7	*C. diana*	99_S2-7Cdian_12s	0.0	1.0	0.4	0.8	0.8	1.0	**

[A] identical sequences: 14_S3-2CAR_12s, [70_S3-1IBR_12s]

IV.4.1.1.5 Comparison with *Ternidens* sequences from other host species

Alignment and pairwise comparison of the chimpanzee and *C. diana Ternidens* isolates with ITS-2 sequences of *T. deminutus* specimens from other primate host species: Mona monkey (*Cercopithecus mona*/ Ghana), olive baboon (*Papio anubis*/ Ghana) and crab-eating macaque (*Macaca fascicularis*/ China) retrieved from the NCBI Nucleotide database revealed sequence differences of 0% to 6.1% over an alignment length of 216 bp (see Table 27).

Respective 12s mtDNA sequences were not available for comparison. Also, as *T. deminutus* constitutes the only currently confirmed species of the genus, no sequences from other *Ternidens* species were available for comparison.

Table 27. *Pairwise nucleotide differences (in %) between ITS-2 sequences of T. deminutus specimens from different host species (based on an alignment length of 216 bp)*

No	Host	Sequence name	1	2	3	4	5	6
1	*P. t. verus*	haplotype a[A]	**					
2	*P. t. verus / C. diana*	haplotype b[B], 99_S2-7Cdian_ITS2	0.9	**				
3	*C. diana*	93_S2-7Cdian_ITS2	0.5	1.4	**			
4	*Cercopithecus mona*	AJ888729.1**	0.0	0.9	0.5	**		
5	*Papio anubis*	AJ888730.1**	2.8	3.7	3.2	2.8	**	
6	*Macaca fascicularis*	HM067975.1**	5.1	6.1	5.6	5.1	4.6	**

** GenBank accession numbers

[A] 11_S3-7BIE_ITS2, 17_S3-9KIN_ITS2, 18_S3-21JUL_ITS2, 19_S3-3TAB_ITS2_A & -B, 20_S311UTA_ITS2

[B] 14_S3-2CAR_ITS2_A & -B, 16_S2-226ATR_ITS2, 69_S3-1IBR_ITS2

IV.4.1.1.6 Phylogenetic analysis

Due to the close genetic relationship between the genus *Ternidens* and the genus *Oesophagostomum* (both subfamily Chabertiinae), the above-described chimpanzee and monkey *Ternidens* sequences were included in the phylogenetic analysis of the respective *Oesophagostomum* sequences (see IV.6.1.2.5).

IV.4.1.2 *Oesophagostomum* L3-larvae (Subfam. Chabertiinae)

IV.4.1.2.1 Overview of examined larvae and success rates of DNA extraction and PCR amplification

The ITS-2 region was successfully amplified and sequenced from the DNA of eight (4 type A larvae with short tail-sheath, four type B larvae with long tail sheath, see Annex, Table A6) of the overall 14 examined *Oesophagostomum* L3-larvae, which had been cultured from chimpanzee fecal samples. DNA extraction from the other six larvae (3 type A-, 3 type B larvae) failed. From type A larva 29_S2-217_OLI and type B larva 37_S2-3_SUM two clones of the ITS-2 amplification product each were successfully sequenced. From all other examined larvae only one amplicon clone or the PCR product directly had been sequenced.

Amplification and sequencing of the mitochondrial 12s RNA region was successfully performed for all eight *Oesophagostomum* larvae (4 type A, 4 type B), from which ITS-2 sequences had been obtained (see Annex, Table A6). From larva 37_S2-3_SUM two clones of the 12s RNA amplification product were successfully sequenced. From all other larvae (including larva 29_S2-217_OLI) again only one clone or the PCR product directly had been sequenced.

IV.4.1.2.2 Analysis of ITS-2 sequences

The five ITS-2 sequences obtained from *Oesophagostomum* **morphotype A** larvae each had a length of 266 bp, a CG-content ranging from 43.6% to 46.2%, and represented one of three different **haplotypes (a-c)**. The two sequences (29_S2-217OLI_ITS2_A & -B) obtained from the two ITS-2 amplicon clones of larva 29_S2-217_OLI were 100% identical and together with sequence 31_S2-324COC_ITS2 belonged to haplotype a.

The five ITS-2 isolates obtained from **morphotype B** larvae by contrast each had a length of 266 bp, a CG-content of 44.2% or 44.6% and could be reduced to two different **haplotypes (d+e),** each comprising two sequences (Annex, Table A13). The two sequences (37_S2-3SUM_ITS2_A & -B) obtained from the two ITS-2 amplicon clones of larva 37_S2-3_SUM were 100% identical and belonged to haplotype e.

BLAST search revealed a maximum similarity of 94.0% and 94.4% respectively between **haplotypes a** and **b** and the ITS-2 sequence of *O. aculeatum* (GenBank accession no. AB586134.1) isolated from a macaque monkey (*Macaca fuscata)* from Japan (Identities= 250/266 and 251/266, Gaps= 0/266).

Haplotype c (38_S3-5ROM_ITS2) by contrast, presented a maximum homology of 98.6% to *O. bifurcum* (GenBank accession no. AF136575.1/AF136575) recovered from a Mona monkey (*Cercopithecus mona*) from Ghana (Identities= 213/216, Gaps= 0/216). The two sequences differed by two single-base transversions (A/T) and a single-base transition (T/C).

Alignment and pairwise comparison of the three haplotypes corroborated a genetic subdivision of the four examined short-tailed type A larvae. While only two single-base nucleotide differences (0.8%), namely a transversion (C/A) at position 54 and a transition (G/A) at position 178, were discovered between **haplotypes a** and **b**, **haplotype c** differed from the other two haplotypes by 6.0% and 6.4% respectively, i.e. by 16 and 17 single-base substitutions (13 transitions, 3-4 transversions).

BLAST search performed for sequence **haplotypes d** and **e** (type B larvae) revealed a maximum identity of 100% and 99.5% respectively to the ITS-2 sequence of an *O. stephanostomum* (GenBank accession no. AF136576.1) isolated from a chimpanzee, *P. t. schweinfurthii* in Tanzania (Identities= 216/216 and 215/216, Gaps= 0/216).

The two haplotypes differed from each other through a transition (A/G) at alignment position 49 but unlike the *O. stephanostomum* GenBank reference sequence (AF136576.1) showed no polymorphism at positions 116, 176 and 197.
Overall, pairwise comparison of all five *Oesophagostomum* haplotypes (a-e) over an alignment length of 266 bp revealed inter-sequence nucleotide differences ranging from 0.4% to 6.4% (see Table 28).

IV.4.1.2.3 Analysis of 12s RNA mtDNA sequences

All nine obtained *Oesophagostomum* 12sRNA mt DNA sequences were 477 or 481 bp long (38_S3-5ROM_12s), had a CG-content of 19.9% to 21.0% and represented each a unique haplotype (I-IX) (see Annex, Table A18).

Alignment and pairwise comparison of the nine sequences/ haplotypes revealed a total of 98 polymorphic stites and pairwise nucleotide differences ranging from 0.2% to 9.1% (2 to 44 single-base substitutions and/or deletions.

Observed respective sequence differences between the four type A larvae varied markedly from 0.6% to 8.9%. Sequence variations between the four type B larvae by contrast were less pronounced and ranged from 0.2% to only 1.1% (see Table 29). The lowest sequence differences (0.2%) were observed between the two sequenced 12s RNA mtDNA amplicon clones of larva 37_S2-3_SUM (sequences 37_S2-3SUM_12s_A & -B), which were identical apart from one single-base transition (T/C) at position 368, as well as between sequence 37_S2-3SUM_12s_B and the sequence obtained from larva 39_S2-770SUM (haplotype IX).

BLAST search revealed for the nine sequences maximum similarities between 90.1% and 91.9% to the mitochondrial DNA of two *O. dentatum* (GenBank accession no. GQ888716.1 & FM161882.1) and one *O. quadrispinulatum* (GenBank accession no. FM161833.1). Respective mitochondrial reference sequences from specimens of *O. stephanostomum* and *O. bifurcum* were not available for comparison.

IV.4.1.2.4 Comparison with *Oesophagostomum* sequences from sympatric monkeys

ITS-2 as well as 12s RNA mtDNA sequences were obtained from two of six examined long-tailed (type B) *Oesophagostomum* L3-larvae cultured from opportunistically collected fecal samples of sympatric red colobus monkeys (*Piliocolobus badius*) as well as from one of three examined short-tailed (type A) *Oesophagostomum* L3-larvae recovered from cultured fecal material of two sooty mangabeys (*Cercocebus atys*) (see Annex, Table A7). DNA extraction from the respective other larvae had failed.

From the ITS-2 amplicon of type B larva 76_S2-6_Pbad two clones were successfully sequenced. The 12s RNA mtDNA amplicon on this larva as well as both amplicons of larvae 79_S2-10_Pbad & 104_S2-9_Mang had been sequenced directly.
No *Oesophagostomum* sequences could be obtained from Diana monkeys (*Cercopithecus diana*), as DNA extraction from all four cultured and isolated larvae was unsuccessful.

Comparison of the sequences obtained from monkey and chimpanzee *Oesophagostomum* larvae revealed a difference of only 0.4% between the mangabey type A larva sequence 104_S2-9Mang_ITS2 and the chimpanzee type A

larva sequence 38_S3-5ROM_ITS2 (haplotype c). Both sequences differed by only one single-base substitution, namely a transition (G/A) at alignment position 128.
The variation between the respective 12s RNA mtDNA sequences of both larvae by contrast was 4.2% (20 single base substitutions: 15 transitions, 5 transversions).

The two sequences (76_S2-6Pbad_ITS A & -B) of the two ITS-2 amplicons of red colobus type B larva 76_S2-6_Pbad were identical and also showed 100% identity to the sequence (79_S2-10_Pbad_ITS2) of the second red colobus type B larva. All three sequences differed from the ITS-2 isolates of the four chimpanzee type B larvae by 13 and 14 single-base substitutions i.e. by 4.9% (haplotype d) and 5.3% respectively (haplotype e).

The respective 12s RNA mtDNA sequences by contrast differed by 10.0% to 10.4% (haplotype e). For an overview of the pairwise sequence differences between all ITS-2 and respectively 12s RNA mtDNA isolates from *Oesophagostomum* larvae of chimpanzees and monkeys see Tables 28 & 29.

Table 28. *Pairwise nucleotide differences (in %) between ITS-2 sequences of Oesophagostomum L3-larvae from Taï chimpanzees (P. t. verus) and sympatric monkeys*

No	Haplotype / Host	Sequence name	1	2	3	4	5	6	7
type A larvae (short tail-sheath)									
1	**a** / *P. t. verus*	29_S2-217OLI_ITS2[A]	**						
2	**b** / *P. t. verus*	30_S2-29ZYO_ITS2	0.8	**					
3	**c** / *P. t. verus*	38_S3-5ROM_ITS2	6.0	6.4	**				
4	*Cercocebus atys*	104_S2-9Mang_ITS2	6.4	6.8	0.4	**			
type B larvae (long tail-sheath)									
5	**d** / *P. t. verus*	33_S2-740UTA_ITS2[B]	5.6	6.0	3.4	3.8	**		
6	**e** / *P. t. verus*	37_S2-3SUM_ITS2[C]	6.0	6.4	3.0	3.4	0.4	**	
7	*Piliocolobus badius*	76_S2-6Pbad_ITS2[D]	7.1	7.5	3.8	4.2	4.9	5.3	**

[A] comprises both sequences: 29_S2-217OLI_ITS2_A&-B;
identical sequence: 31_S2-324COC_ITS2;
[B] identical sequence: 35_S3-1IBR_ITS2;
[C] identical sequence: 39_S2_770SUM_ITS2
[D] comprises both sequences 76_S2-6Pbad_ITS2_A&-B; identical sequence: 79_S2-10Pbad_ITS2

Table 29. *Pairwise nucleotide differences (in %) between 12s mtDNA sequences of Oesophagostomum L3-larvae from Taï chimpanzees (P. t. verus) and sympatric monkeys*

No	Haplotype/ Host	Sequence code	1	2	3	4	5	6	7	8	9	10	11	12
# type A larvae (short tail-sheath)														
1	**I***	29_S2-217OLI_12s	**											
2	**II***	31_S2-324COC_12s	0.6	**										
3	**III***	30_S2-29ZYO_12s	4.2	4.4	**									
4	**IV***	38_S3-5ROM_12s	8.9	8.7	8.1	**								
5	*C. atys*	104_S2-9Mang_12s	9.5	9.5	9.3	4.2	**							
# type B larvae (long tail-sheath)														
6	**V***	33_S2-740UTA_12s	8.5	8.1	7.5	7.7	8.3	**						
7	**VI***	35_S3-1IBR_12s	9.1	8.9	8.5	7.9	8.9	1.1	**					
8	**VII***	37_S2-3SUM_12s_A	8.9	8.7	8.1	8.1	9.1	0.8	1.1	**				
9	**VIII***	37_S2-3SUM_12s_B	8.8	8.6	7.9	7.9	8.9	0.6	0.8	0.2	**			
10	**IX***	39_S2-770SUM_12s	8.7	8.5	7.9	7.6	8.7	0.4	0.6	0.4	0.2	**		
11	*P. badius*	76_S2-6Pbad_12s	11.2	11.7	11.6	10.9	12.5	9.8	10.4	10.2	10.0	10.0	**	
12	*P. badius*	79_S2-10Pbad_12s	11.0	11.5	11.5	10.9	12.6	9.8	10.4	10.2	10.0	10.0	0.6	*

* host: *Pan troglodytes verus*

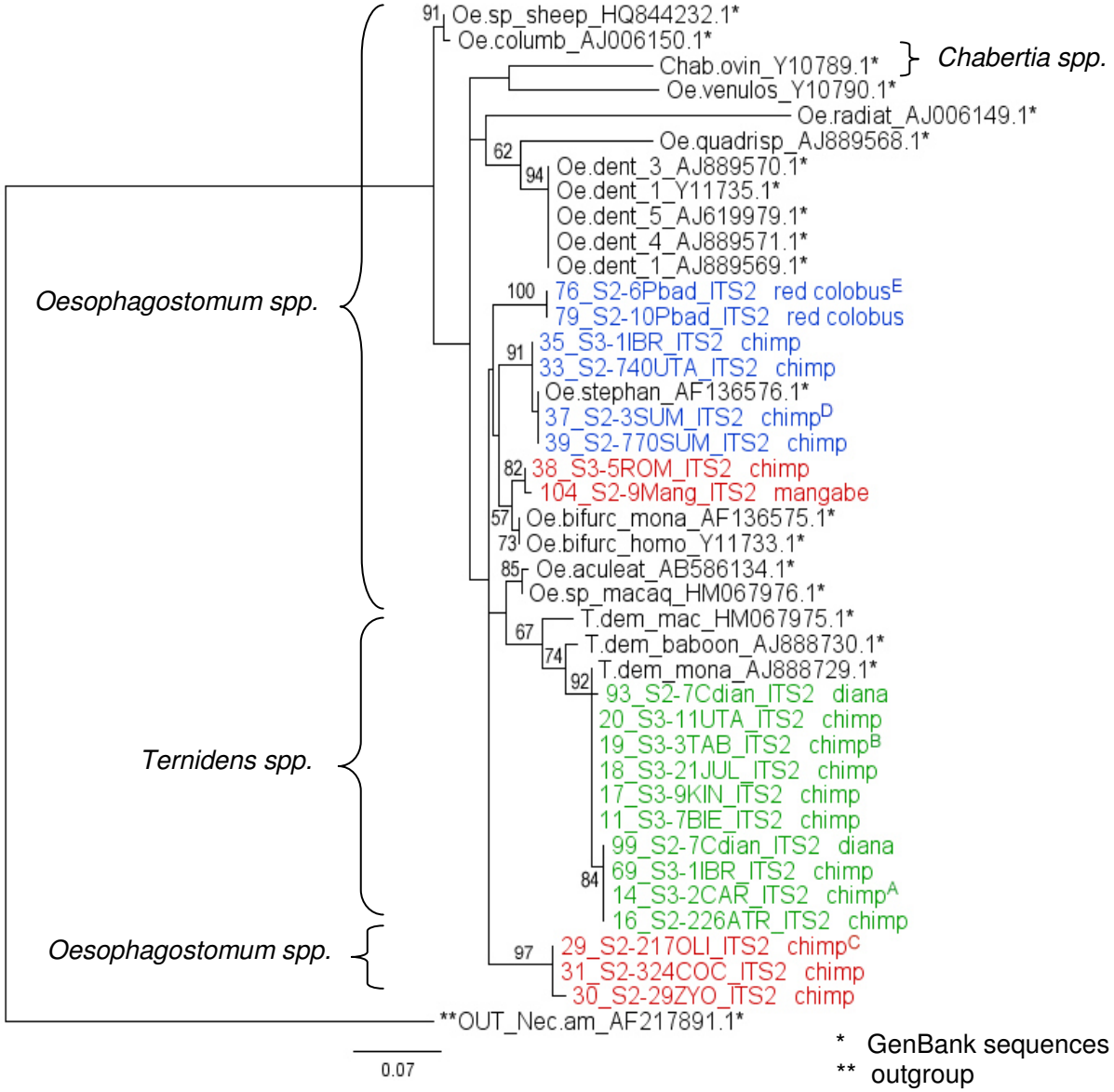
C. atys= Cercocebus atys; *P. badius= Piliocolobus badius*

IV.4.1.2.5 Phylogenetic analysis of *Oesophagostomum* and *Ternidens* sequences

Phylogenetic analyses of the above described chimpanzee and monkey *Oesophagostomum* sequences were performed as described in the methods & material section using a dedicated webserver (http://www.atgc-montpellier.fr/phyml) employing maximum likelihood under the HKY+G model of substitutions (for ITS-2 sequences) and the GTR+I+G model of substitutions respectively (for 12s mtDNA sequences).

Included in the analyses were all above-described chimpanzee and Diana monkey *Ternidens* sequences, as well as a total of 19 ITS-2 and four mtDNA respectively reference sequences from a number of different representatives of the subfamily Chabertiinae obtained from the GenBank database (see Annex, Table A26). Preceding analysis, all sequences were aligned and trimmed to a common alignment length of 361 bp (ITS-2 sequences) and 476 bp respectively (12s mtDNA sequences). As outgroups *Necator americanus* GenBank sequences AF21789.1 (ITS-2) and AJ417719.2 (mitochondrial DNA) respectively had been selected. The two obtained phylogenetic trees are depicted in Figures 19 & 20.

Figure 19. *Phylogenetic tree depicting the positioning of Taï chimpanzee (P. t. verus) and monkey Ternidens and Oesophagostomum larvae within the subfamily Chabertiinae inferred from a 216 bp fragment of their ITS-2 gene region.*

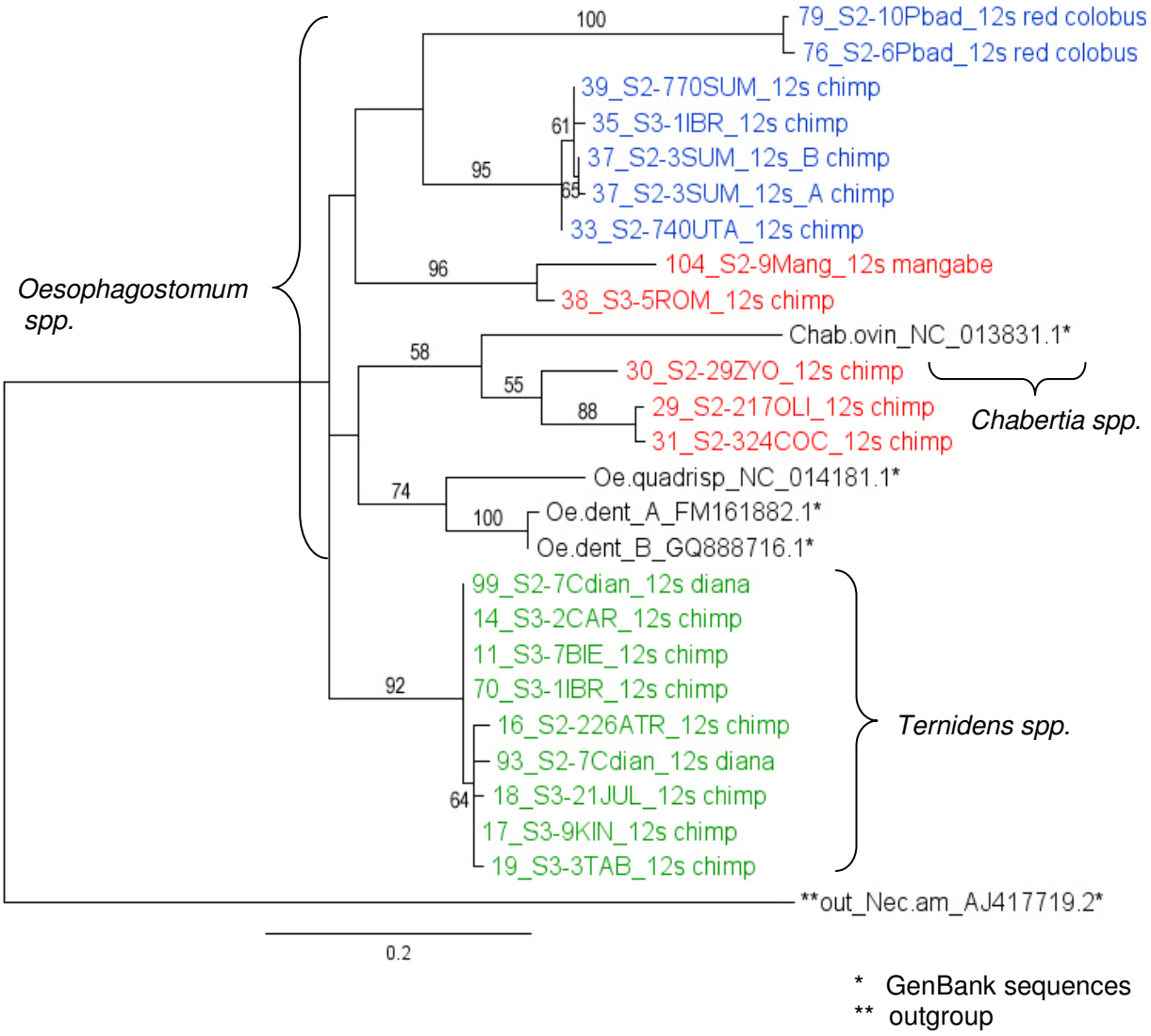


[A]comprises sequences: 14_S3-2CAR_ITS2_A&-B [D]comprises sequences: 37_S2-3SUM_ITS2_A&-B
[B]comprises sequences: 19_S3-3TAB_ITS2_A&-B [E]comprises sequences: 76_S2-6Pbad_ITS_A&-B
[C]comprises sequences: 29_S2-217OLI_ITS2_A&-B

The tree was constructed under the HKY+G model of substitutions using maximum likelihood. Bootstrap values were calculated with 500 replicates and are given in percent. Only values above 50 are shown. Sequences generated in this study are depicted with colored sequence codes (red= sequences of Oesophagostomum type A larvae; blue= sequences of Oesophagostomum type B larvae; green= sequences of Ternidens larvae).

Abbreviations: chimp= *Pan troglodytes verus*; diana= *Cercopithecus diana*;
red colobus= *Piliocolobus badius*; mangabe= *Cercocebus atys*

Figure 20. *Phylogenetic tree depicting the positioning of Taï chimpanzee (P. t. verus) and monkey Ternidens and Oesophagostomum larvae within the subfamily Cabertiinae inferred from a 476 bp fragment of their 12s mtDNA gene region.*



The tree was constructed under the GTR+I+G model of substitutions using maximum likelihood. Bootstrap values were calculated with 500 replicates and are given in percent. Only values above 50 are shown. Sequences generated in this study are depicted with colored sequence codes (red= sequences of Oesophagostomum type A larvae; blue= sequences of Oesophagostomum type B larvae; green= sequences of Ternidens larvae).

Abbreviations: chimp= *Pan troglodytes verus*; red colobus= *Piliocolobus badius*; diana= *Cercopithecus diana*; mangabe= *Cercocebus atys*

IV.4.1.3 *Necator* L3-larvae (Fam. Ancylostomatidae)

IV.4.1.3.1 Overview of examined larvae and success rates of DNA extraction and PCR amplification

The ribosomal ITS-2 genome region of seven out of nine examined morphologically indistinguishable *Necator* L3-larvae, cultured from chimpanzee fecal samples, were successfully amplified and sequenced (see Annex, Table A8). DNA extraction from another two *Necator* larvae failed. From larva 43_S2-330_ZOR four clones of the ITS-2 amplicon were successfully sequenced. From all other examined larvae only one clone or else the PCR product directly had been sequenced.

12s RNA mtDNA sequences were successfully generated from six of the seven *Necator* larvae from which ITS-2 sequences had been obtained but PCR failed to produce an amplicon from the seventh larva (48_S2-4_WAP) (see Annex, Table A8).

IV.4.1.3.2 Analysis of ITS-2 sequences

Their respective characteristics indicated a division of the overall ten obtained *Necator* ITS-2 sequences into two groups consisting of a total of six **haplotypes (a-f)** (see Annex, Table A20). Whereas the ITS-2 sequence of larva 41_S3-4_CAR (**haplotype a**) had a length of only 374 bp and a CG-content of 43.6%, the sequences of the other six larvae (**haplotypes b-f**) were 394 to 397 bp long and had a CG-content of 40.7% to 40.9%.

Three of the four sequences obtained from the four ITS-2 amplicon clones of larva 43_S2-330_ZOR were identical and constituted **haplotype c** (43_S2-330ZOR _ITS2_A-C). The fourth sequence (43_S2-330ZOR_ITS2_D) by contrast differed from the three other clone sequences by three single-base transitions (A/G at position 212, T/C at position 228 and C/T at position 259) but was 100% identical to sequences 47_S3-5ROM_ITS2 and 48_S2-4WAP_ITS2 (**haplotype f**).

This insinuated division of the seven *Necator* ITS-2 sequence haplotypes into two distinct groups was further confirmed by the alignment and pairwise comparison of the eight sequences. While the alignment of **haplotypes b-f** presented a total of eight polymorphic sites (5 single base transitions; 3 single base gaps), and the pairwise sequence differences between these haplotypes were overall rather small (0.3% to 1.5% i.e. 1 to 6 single base substitutions or deletions), the ITS-2 sequence of larva 41_S3-4_CAR (**haplotype a**) differed considerably (14.4% to 15.0%, i.e. by 56 to 59 single-base substitutions and deletions) from the other haplotypes (see Table 30).

Table 30. *Pairwise nucleotide differences (in %) between ITS-2 sequences of Necator L3-larvae from Taï chimpanzees (P. t. verus)*

No	Haplotype	Sequence name	1	2	3	4	5	6
1	**a**	41_S3-4CAR_ITS2	**					
2	**b**	42_S3-9KIN_ITS2	15.0	**				
3	**c**	43_S2-330ZOR_ITS2_A[A]	14.6	1.0	**			
4	**d**	44_S2-772RAV_ITS2	14.4	1.3	0.3	**		
5	**e**	45_S3-18UTA_ITS2	14.4	1.5	0.5	0.8	**	
6	**f**	47_S3-5ROM_ITS2[B]	14.6	0.8	0.8	1.0	1.3	**

[A] identical sequences: 43_S2-330ZOR_ITS2_B, 43_S2-330ZOR_ITS2_C
[B] identical sequences: 48_S2-4WAP_ITS2, 43_S2-330ZOR_ITS2_D

BLAST search, revealed 100% homology (Identities= 366/366, Gaps= 0/366) between the ITS-2 of larva 41_S3-4_CAR and the ITS-2 sequence of a *N. americanus* from a human host in Guatemala (GenBank accession no. AF217891.1).

Maximum identities between this *N. americanus* reference sequence and the ITS-2 sequences (haplotypes b-g) from the six other *Necator* larvae by contrast were considerably lower, ranging from 84.6% to 85.3%, suggesting that these larvae most likely represent developmental stages of a different *Necator* species of which so far no reference sequences are available in the GenBank database.

IV.4.1.3.3 Analysis of 12s RNA mtDNA sequences

All six obtained *Necator* 12s RNA mtDNA sequences ranged in length between 473 and 475 bp, had a CG-content of 22.6% to 23.3% and each represented a unique haplotype (I to VI) (see Annex, Table A21).

Alignment of the six haplotypes revealed a total of 44 polymorphic sites translating to pairwise inter-sequence variations ranging from 0.4% and 7.8% (2 to 37 single-base substitutions).

The 12s RNA mtDNA sequence of larva 41_S3-4_CAR (**haplotype I**), whose ITS-2 sequence had been 100% identical to a *N. americanus* reference sequence (AF217891.1) and had shown marked nucleotide differences upon comparison with the ITS-2 sequences of the other examined *Necator* larvae (see above), differed from the respective mitochondrial sequences (**haplotypes II-VI**) of these larvae by 7.6% to 7.8%.

The pairwise nucleotide differences between the 12s RNA mtDNA sequences of the five other larvae by contrast were as low as 0.4% and 0.8% (see Table 31).

Table 31. *Pairwise nucleotide differences (in %) between mitochondrial 12s RNA sequences of Necator L3-larvae from Taï chimpanzees (P. t. verus)*

No	Haplotype	Sequence name	1	2	3	4	5	6
1	**I**	41_S3-4CAR_12s	**					
2	**II**	42_S3-9KIN_12s	7.8	**				
3	**III**	43_S2-330ZOR_12s	7.8	0.4	**			
4	**IV**	44_S2-772RAV_12s	7.7	0.4	0.4	**		
5	**V**	45_S3-18UTA_12s	7.6	0.8	0.8	0.8	**	
6	**VI**	47_S3-5ROM_12s	7.8	0.4	0.4	0.4	0.8	**

BLAST search revealed for the 12s RNA mtDNA sequence of larva 41_S3-4_CAR (**haplotype I**) maximum similarities of 99.8% and 98.3% respectively to mitochondrial DNA sequences of two *N. americanus* specimens (GenBank accession no. AJ556134.1 & AJ417719.2; Identities= 475/476 and 469/477; Gaps= 1/476 and 4/477) isolated from humans in Togo and China respectively.

Maximum similarity between the 12s RNA mtDNA sequences of the five other *Necator* larvae (**haplotypes II-VI**) and the two *N. americanus* reference sequences by contrast were considerably lower and ranged only between 92.1% and 92.5%.

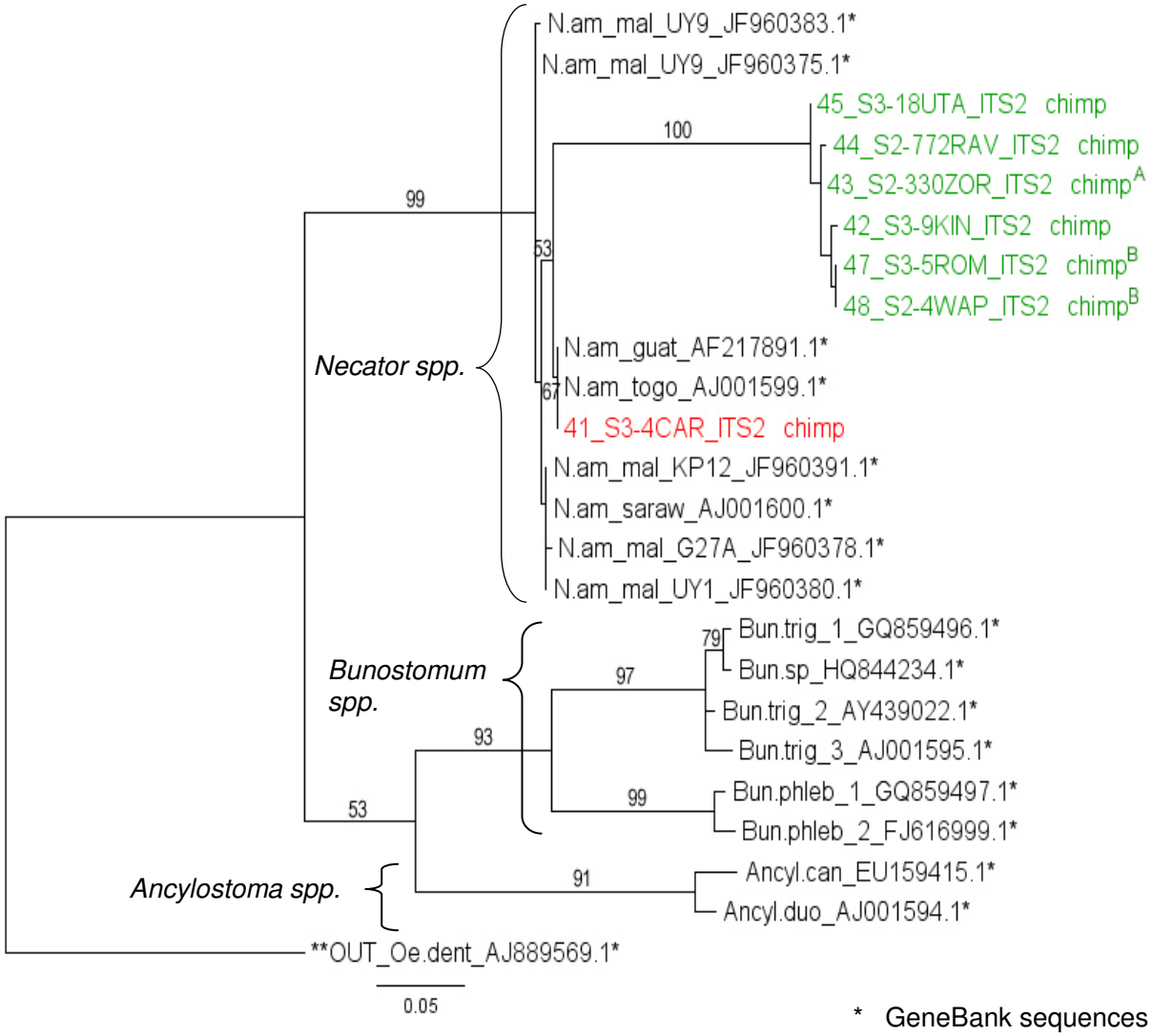
IV.4.1.3.4 Comparison with *Necator* sequences from sympatric monkeys

A comparison between the ITS-2 and 12s RNA mtDNA sequences obtained from *Necator* L3-larvae of chimpanzees and sympatric monkeys was not possible as no respective larvae were found in any of the fecal cultures performed on a total of 66 opportunistically collected monkey fecal samples (*Piliocolobus badius*: n= 53, *Colobus polykomos:* n= 3*, Cercopithecus diana*: n= 8, *Cercocebus atys*: n= 2).

IV.4.1.3.5 Phylogenetic analysis

Phylogenetic analyses of the above-described chimpanzee and monkey *Necator* sequences were performed employing maximum likelihood (http://www.atgc-montpellier.fr/phyml) under the HKY+G model of substitutions.
Included in the analyses were a total of 16 ITS-2 and five mtDNA respectively reference sequences from different representatives of the family Ancylostomatidae (`hookworms´) obtained from the GenBank database (see Annex, Table A27). Preceding analysis, all sequences were aligned and trimmed to a common alignment length of 359 bp (ITS-2 sequences) and 362 bp respectively (12s mtDNA sequences). As outgroups *Oesophagostomum dentatum* GenBank sequences AJ889569.1 (ITS-2) and FM161882.1 (mitochondrial DNA) respectively had been selected. The two obtained phylogenetic trees are depicted in Figures 21 & 22.

Figure 21. *Phylogenetic tree depicting the positioning of Taï chimpanzee (P. t. verus) Necator larvae within the Ancylostomatidae family inferred from their ITS-2 gene region.*

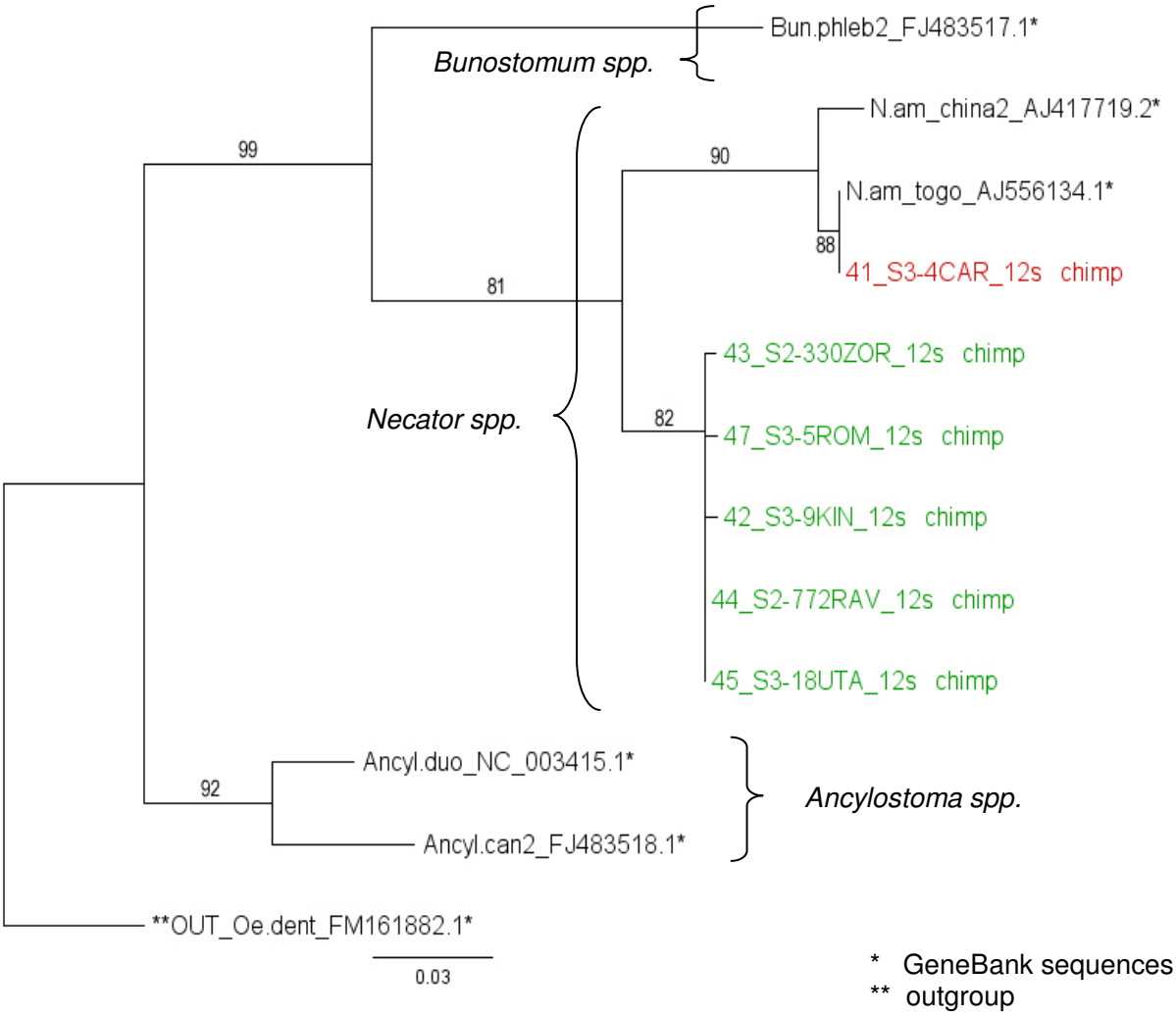


[A] comprises sequences: 43_S2-330ZOR_ITS2_A to -C
[B] identical sequence: 43_S2-330ZOR_ITS2_D

The tree was constructed under the GTR+G model of substitutions using maximum likelihood. Bootstrap values were calculated with 500 replicates and are given in percent. Only values above 50 are shown. Sequence codes of sequences generated in the course of this study are depicted in red (N. americanus) and green (unidentified Necator sp.).

Abbreviations: *chimp = Pan troglodytes verus*

Figure 22. *Phylogenetic tree depicting the positioning of Taï chimpanzee (P. t. verus) Necator larvae within the Ancylostomatidae family inferred from their 12s mtDNA gene region*



The tree was constructed under the HKY+G model of substitutions using maximum likelihood. Bootstrap values were calculated with 500 replicates and are given in percent. Only values above 50 are shown. Sequence codes of sequences generated in the course of this study are depicted in red (N. americanus) and green (unidentified Necator sp.).

Abbreviations*: chimp = Pan troglodytes verus*

IV.4.1.4 Trichostrongylid L3-larvae (Fam. Trichostrongylidae)

IV.4.1.4.1 Overview of examined larvae and success rates of DNA extraction and PCR amplification

Ribosomal ITS-2 sequences as well as 12s RNA mtDNA sequences were successfully generated from the DNA of two trichostrongylid (*Trichostrongylus*-like) L3-larvae cultured from fecal material of two south group chimpanzees (see Annex, Table A9).

IV.4.1.4.2 Analysis of ITS-2 sequences

Both ITS-2 sequences had a length of 292 bp and a CG-content of 33.2% and 33.6% respectively (see Annex, Table A22) and differed from each other by 0.3%, due to a single base transition (T/C) at alignment position 56.

BLAST search revealed a maximum similarity of 92.3% between the ITS-2 sequences of the two trichostrongylid larvae (13_S2-3SUM_ITS2 & 21_S3-12ATH_ITS2) and the ITS-2 sequences of two *Libyostrongylus douglassi* (GenBank accession no. HQ713428.1 & HQ713429.1; Identities= 250/271; Gaps= 6/271 and 4/271).

Maximum similarities between the ITS-2 sequences of the two larvae and respective sequences of *Trichostrongylus* specimen were incidentally even lower, namely 87.3% to the ITS-2 sequence of a *T. retortaeformis* (GenBank accession no. JX046418.1; Identities= 255/292, Gaps= 4/292) as well as 87.0% and 87.3% respectively to the sequence of a *T. vitrinus* (GenBank accession no. AY439027.1; Identities= 254/292 and 255/292; Gaps= 4/292).

IV.4.1.4.3 Analysis of 12s RNA mtDNA sequences

The two 12s RNA mtDNA sequences were 472 (13_S2-3SUM_12s) and 473 bp (21_S3-12ATH_12s) long and had a CG-content of 19.5% and 20.1% respectively (see Annex, Table A23).
Alignment and pairwise comparison of the two sequences over an alignment length of 475 bp revealed nucleotide variations at a total of 11 alignment positions (5 gaps, 5 transitions (A/G), one transversion (T/G)), translating to an overall 12s RNA mtDNA sequence difference of 2.3%.

BLAST search revealed a maximum similarity of 80.3% (Identities= 277/345; Gaps= 23/345) of the 12s RNA mtDNA sequence of larva 13_S2-3_SUM with the respective mitochondrial sequence of a specimen of *Ascaris suum* (GenBank accession no. X54253.1), whereas the sequence of larva 21_S3-12_ATH showed a maximum similarity of 84.4% (Identities= 358/424; Gaps= 22/424) with the respective sequence of an *Ancylostoma caninum* specimen (GenBank accession no. FJ483518.1).

No significant similarity by contrast was found between the 12s RNA mtDNA sequences of both larvae and the full mitochondrial genome of two specimens of *T. vitrinus* (GenBank accession no. NC_013807.1 & GQ888711.1) as well as two specimens of *T. axei* (GenBank accession no. NC_013824.1 & GQ888719.1). Mitochondrial reference sequences of *L. douglassi* were not available in the GenBank database.

IV.4.1.4.4 Comparison with sequences of trichostrongylid larvae from sympatric monkeys

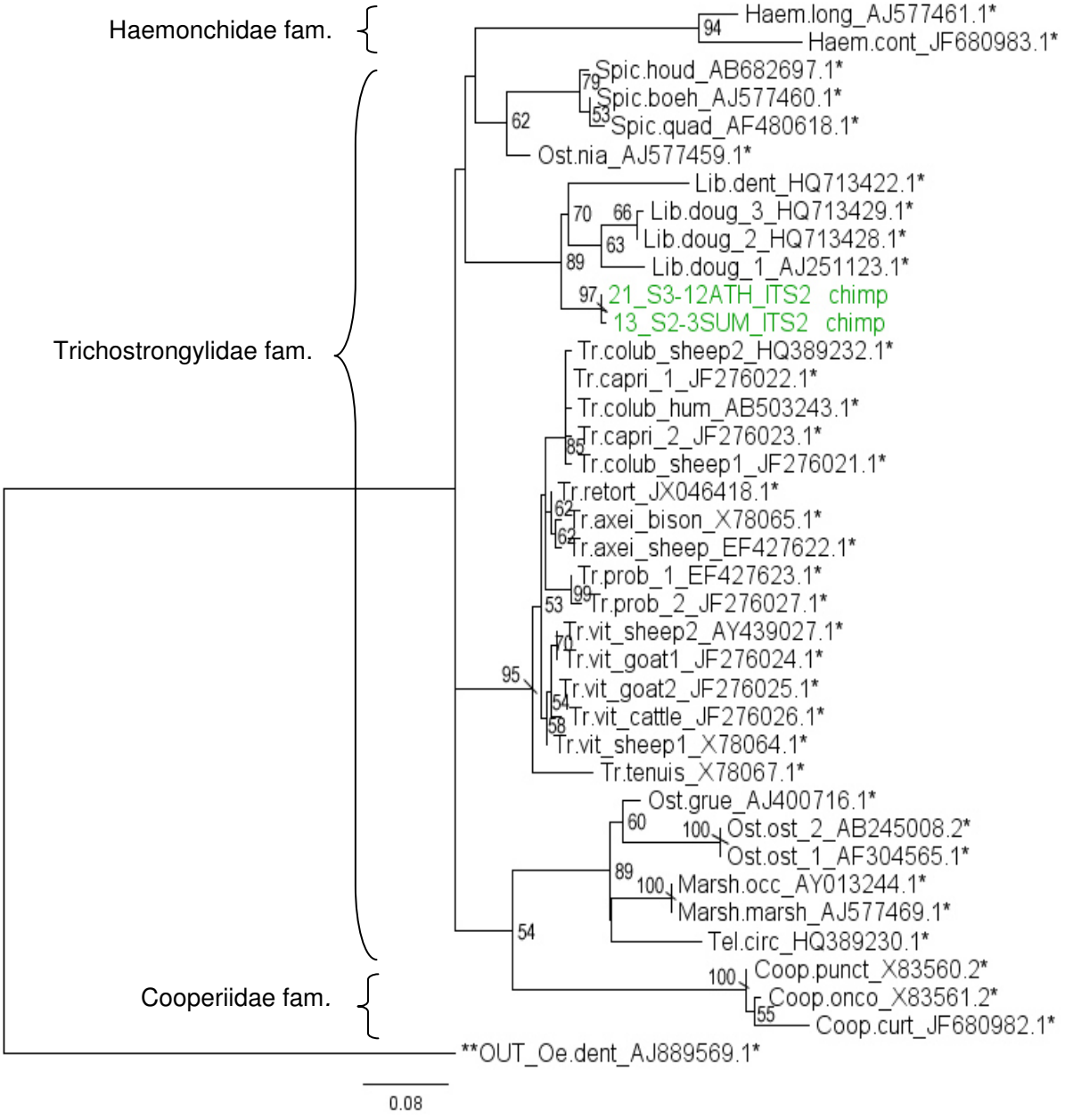
No respective ITS-2 or 12s RNA sequences from sympatric monkeys were available for comparison with the chimpanzee isolates, as no trichostrongylid larvae had been found in any of the 66 fecal cultures performed on opportunistically collected fecal material from four monkey species (*Piliocolobus badius:* n= 53, Colobus polikomos: n= 3, *Cercopithecus diana:* n= 8, *Cercocebus atys:* n= 2).

IV.4.1.4.5 Phylogenetic analysis

Phylogenetic analyses of the above-described sequences were performed employing maximum likelihood (http://www.atgc-montpellier.fr/phyml) under the HKY+G model of substitutions (ITS-2 sequences) as well as the GTR+I+G model of substitutions (12s mtDNA sequences).

Included in the analyses were a total of 31 ITS-2 and five mtDNA respectively reference sequences from different representatives of the superfamily Trichostrongyloidea (Trichostrongylidae fam., Cooperiidae fam., Haemonchidae fam.) obtained from the GenBank database. See Annex, Table A28 for an overview of utilized reference sequences. Preceding analysis, all sequences were aligned and trimmed to a common alignment length of 258 bp (ITS-2 sequences) and 485 bp respectively (12s mtDNA sequences). As outgroups *Oesophagostomum dentatum* GenBank sequences AJ889569.1 (ITS-2) and FM161882.1 (mitochondrial DNA) respectively had been selected. The two obtained phylogenetic trees are depicted in Figures 23 & 24.

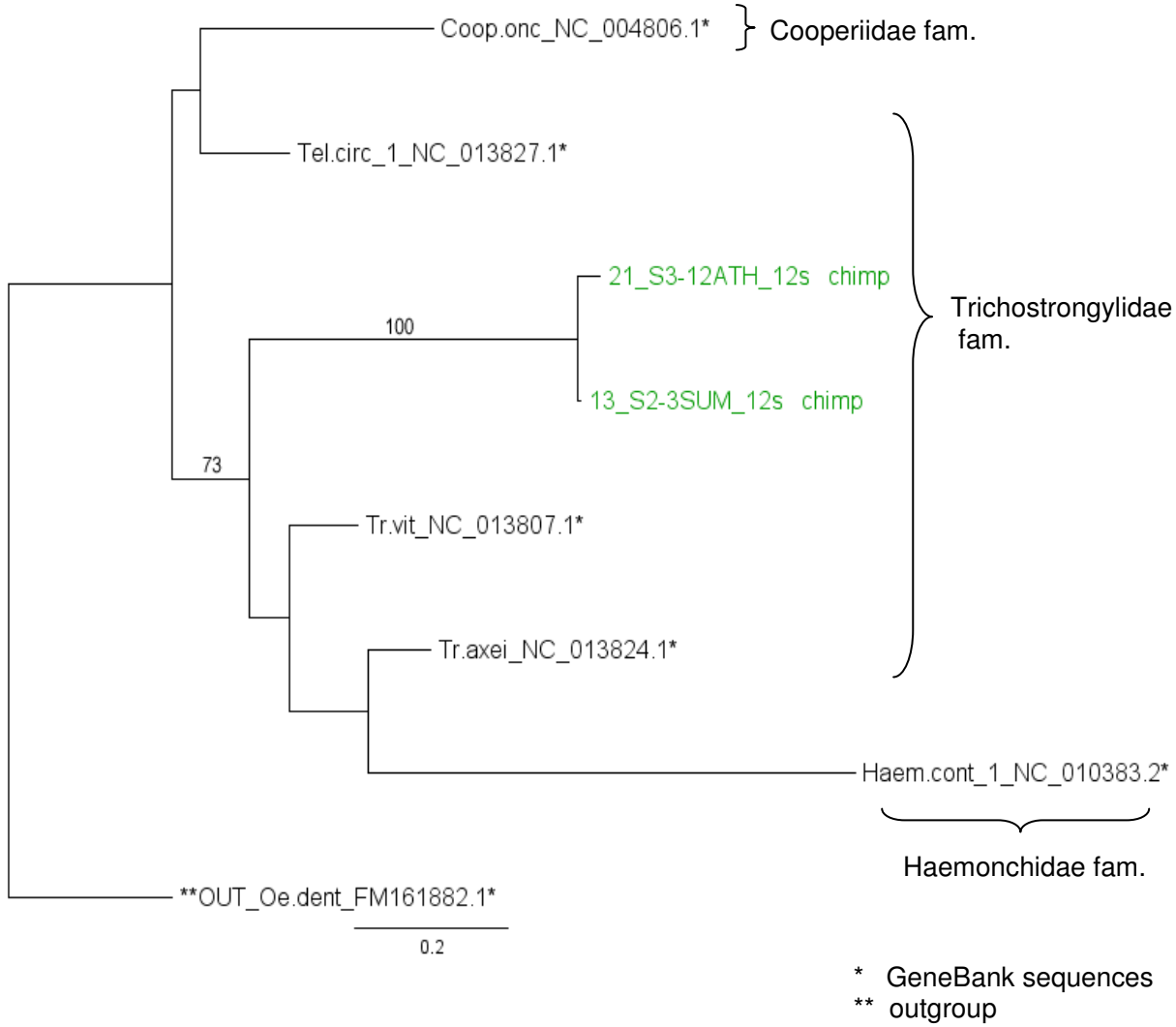
Figure 23. *Phylogenetic tree depicting the positioning of Taï chimpanzee (P. t. verus) trichostrongylid larvae within the superfamily Trichostrongyloidea inferred from their ITS-2 gene region*



The tree was constructed under the HKY+G model of substitutions using maximum likelihood. Bootstrap values were calculated with 500 replicates and are given in percent. Only values above 50 are shown. Sequence codes of the sequences generated in the course of this study are depicted in green.

Abbreviations*: chimp= Pan troglodytes verus*

Figure 24. *Phylogenetic tree depicting the positioning of Taï chimpanzee (P. t. verus) trichostrongylid larvae within the Trichostrongyloidea superfamily inferred from their 12s mtDNA gene region*



The tree was constructed under the GTR+I+G model of substitutions using maximum likelihood. Bootstrap values were calculated with 500 replicates and are given in percent. Only values >/= 50 are shown. Sequence codes of the sequences generated in the course of this study are depicted in green.

Abbreviations: *chimp= Pan troglodytes verus*

IV.4.2 Identification and characterization of L3-larvae and free-living adults of *Strongyloides spp.* using COI mtDNA sequences

IV.4.2.1 Overview of examined larvae and success rates of DNA extraction and PCR amplification

Mitochondrial COI sequences were successfully generated from a total of five out of ten examined *Strongyloides* L3-larvae as well as from two out of five free-living adult male specimens and three out of three free-living adult females which had all been isolated from cultured chimpanzee fecal samples (see Annex, Table A10). From the other eight *Strongyloides* specimen, no sequences could be obtained. While PCR produced a respective amplicon from the DNA of two free-living adult males but subsequent cloning failed, no DNA could be extracted from the other free-living adult male and the five L3-larvae.

The ten obtained sequences each had a length of 607 bp, a CG-content of 30.3% to 32.0% and could be reduced to a total of eight different haplotypes (a-h) (see Annex, Table A24).

IV.4.2.2 Sequence analysis

The eight sequence haplotypes aligned without any gaps and differed from each other through single-base substitutions at a total of 61 alignment positions, translating to pairwise sequence differences between 0.2% (1 single-base substitution) to 6.9% (41 single-base substitutions) (see Table 32).

BLAST search revealed a maximum similarity of 93.9% to 95.5% between **haplotypes a, b, d, f, g and h** and the respective sequence of a *S. fuelleborni* (GenBank accession no. AB526288.1; Identities= 537 to 546/572; Gaps= 0/572) isolated from a chimpanzee (*P. t. troglodytes*) in Gabon. **Haplotypes c and e** (3_S3-7BIE_COI & 55_S2-40UTA_COI) by contrast showed maximum similarity of 94.9% and 96.0% respectively to the sequence of a *S. fuelleborni* (GenBank accession no. AB526285.1; Identities= 538/567 & 549/572; Gaps= 0/567), recovered from a chimpanzee (*P. t. schweinfurthii*) in Mahale/ Tanzania.

Maximum similarities to the mitochondrial DNA sequence of a *S. stercoralis* (GenBank accession no. AJ558163.1) by contrast were considerably lower, ranging from 84.2% (haplotype b) and 84.7% (haplotype f) (Identities= 512/608 & 515/608, Gaps= 2/608).

IV.4.2.3 Comparison with sequences of *Strongyloides* specimens from sympatric monkeys

Overall, COI mtDNA sequences were obtained from a total of five out of eight examined *Strongyloides* specimen, namely from two L3-larvae, two free-living adult females and one free-living adult male which had been cultured from opportunistically collected fecal samples of sympatric red colobus monkeys (*Piliocolobus badius*). DNA extraction from another two adult free-living *Strongyloides* females and one male specimen failed.

Additionally, respective sequences could successfully be generated from three out of three examined *Strongyloides* L3-larvae isolated from cultured fecal material of two Diana monkeys (*Cercopithecus diana*) as well as from two out of two L3-larvae cultured from the feces of two sooty mangabeys (*Cercocebus atys*) (see Annex, Table A11).

The overall ten obtained monkey *Strongyloides* COI mtDNA sequences all had an equal length of 607 bp, a CG-content between 30.3% and 30.6% (see Annex, Table A25) and differed from each other by a maximum of 5.3% (32 single-base substitutions). Four sequences obtained from *Strongyloides* specimens of red colobus monkeys and Diana monkeys respectively were 100% identical (89_S2-10Pbad_COI, 91_S2-6Pbad_COI, 95_S2-7Cdian_COI, 96_S2-7Cdian_COI).

Alignment and comparison of the chimpanzee *Strongyloides* COI mtDNA sequence haplotypes with the *Strongyloides* sequences obtained from the three monkey species, revealed overall pairwise sequence differences between 0% and 6.8% (41 single-base substitutions). The maximum difference between a chimpanzee- and a respective monkey *Strongyloides* sequence was thus slightly smaller (6.8%) than the maximum sequence variation between all sequences obtained from chimpanzee *Strongyloides* specimens (6.9%).

Two red colobus *Strongyloides* isolates (86_S2-14Pbad_COI & 90_S2-6Pbad_COI) were each 100% identical to one of the chimpanzee *Strongyloides* sequences (haplotypes a and h) (see Table 32).

Table 32. *Pairwise nucleotide differences (in %) between COI mtDNA sequences of Strongyloides L3-larvae & free-living adult specimens from Taï chimpanzees (P. t. verus) and sympatric monkeys*

No	Haplotype / Monkey host spp.	Sequence code	1	2	3	4	5	6	7	8	9	10	11	12	13
1	**a*** / *P. badius*	1_S2-44JAC_COI[A]	**												
2	**b***	2_S2-219TAB_COI	0.2	**											
3	**c***	3_S3-7BIE_COI	6.6	6.8	**										
4	**d***	8_S3-1IBR_COI	0.2	0.3	6.8	**									
5	**e***	55_S2-40UTA_COI	2.5	2.6	5.4	2.6	**								
6	**f***	56_S2-52RAV_COI	5.4	5.6	6.9	5.6	5.6	**							
7	**g***	61_S3-12ATH_COI	0.2	0.3	6.8	0.3	2.6	5.6	**						
8	**h*** / *P. badius*	65_S2-286SAG_COI[B]	0.8	1.0	6.4	1.0	3.0	5.6	1.0	**					
9	*P. badius*	73_S2-6Pbad_COI	4.9	5.1	6.8	5.1	5.4	0.8	5.1	5.1	**				
10	*P. badius, C. diana*	89_S2-10Pbad_COI[C]	4.9	5.1	6.4	5.1	5.4	0.5	5.1	5.1	0.3	**			
11	*C. diana*	102_S2-12Cdian_COI	5.1	5.3	6.6	5.3	5.6	0.7	5.3	5.3	0.5	0.2	**		
12	*C. atys*	105_S2-9Mang_COI	0.2	0.3	6.8	0.3	2.6	5.4	0.3	1.0	4.9	4.9	5.1	**	
13	*C. atys*	108_S2-55Mang_COI	0.2	0.3	6.4	0.2	2.3	5.6	0.3	1.0	5.1	5.1	5.3	0.3	**

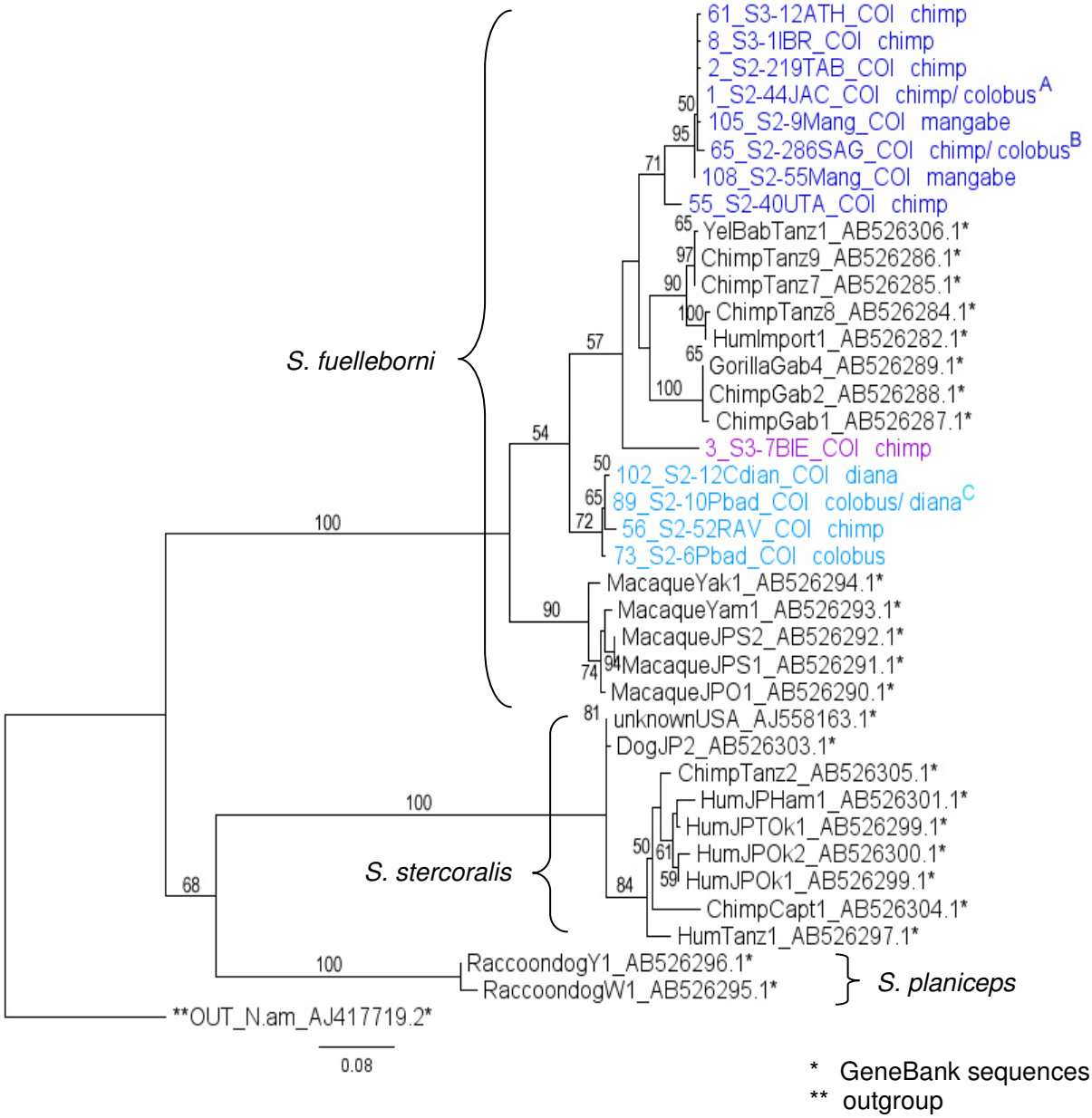
[A] identical sequences: 9_S3-25WAL_COI, 57_S2-286SAG_COI, 86_S2-14_Pbad_COI
[B] identical sequence: 90_S2-6_Pbad_COI
[C] identical sequences: 91_S2-6_Pbad_COI, 95_S2-7_Cdian_COI, 96_S2-7_Cdian_COI
* host: *Pan troglodytes verus*

IV.4.2.4 Phylogenetic analysis of chimpanzee and monkey *Strongyloides* COI sequences

In order to facilitate the systematic positioning of the Taï chimpanzee and monkey *Strongyloides* specimens within the genus *Strongyloide*s, a total of 13 GenBank reference sequences from *S. fuelleborni* specimens of various primate host species as well as respective sequences from other representatives of the genus *Strongyloides* (*S. stercoralis*: n= 9, *S. planiceps:* n= 2) were included into the phylogenetic analysis of the above described chimpanzee and monkey *Strongyloides* COI mtDNA sequences. In accordance with Hasegawa et al. (2010), a respective mtDNA sequence of a specimen of *Necator americanus* (GenBank accession no. AJ417719) had been chosen as outgroup. See Annex, Table A29 for an overview of the utilized reference sequences.

Phylogenetic analysis was performed using maximum likelihood (http://www.atgc-montpellier.fr/phyml) under the GTR+I+G model of substitutions. Preceding analysis all sequences were aligned and trimmed to a common alignment length of 572 bp. The obtained phylogenetic tree is depicted in Figure 25.

Figure 25. *Phylogenetic tree depicting the positioning of Taï chimpanzee (P. t. verus) and monkey Strongyloides specimens within the genus Strongyloides inferred from a fragment of their COI mtDNA gene region*



A identical sequences: 9_S3-25WAL_COI, 57_S2-286SAG_COI, 86_S2-14_Pbad_COI
B identical sequence: 90_S2-6_Pbad_COI
C identical sequences: 91_S2-6_Pbad_COI, 95_S2-7_Cdian_COI, 96_S2-7_Cdian_COI

The tree was constructed under the GTR+I +G model of substitutions using maximum likelihood. Bootstrap values were calculated with 500 replicates and are given in percent. Only values >/= 50 are shown. Sequence codes of the sequences generated in the course of this study are depicted in blue & purple.

Abbreviations: *chimp= Pan troglodytes verus; diana= C. diana; colobus= P.badius; mangabe= C. atys*

V DISCUSSION

V.1 Helminth spectrum and parameters of infection determined using conventional coprology

V.1.1 Method selection

The selection of MWSF as principal coprological examination technique in the present study had largely been dictated by logistical constraints associated with the necessity to perform most analyses on site – that is in a forest lab with limited equipment and no electricity – as well as with the need to utilize locally available and safely disposable consumables.

A selection which might have not been ideal in terms of comparability with other studies, as MWSFs have not previously been employed in any other coprological survey of chimpanzee parasites. However, the subsequent comparative performance of a combination of $NaNO_3$-flotations and fecal sedimentations (Gillespie, 2006), two examination techniques advocated for the parasitological screening of wild primates by the Great Ape Survival Plan (GRASP) and the IUCN/ SSC Primate Specialist Group (Gillespie et al., 2010; Leendertz et al., 2010), revealed MWSF to be an equally apt screening tool for the detection of nematodes and cestodes on the basis that the respectively detected spectra of nematode and cestode eggs were identical. The recovery of trematode eggs by contrast was due to their comparatively high weight only possible employing sedimentations.

Reliable distinction of the different genera of strongyle nematodes affecting the study population on the other hand was, due to the general morphological similarity of all strongyle eggs, not possible with any of the three utilized methods. This inherent shortcoming of all flotation- and sedimentation-based examination techniques however, was compensated for in the present study by the on-site performance of fecal cultures.

V.1.2 Macroscopic screening

The fact that upon macroscopic screening of all 857 samples only very few *Bertiella spp.* proglottids (n= 5) and no adult nematodes or trematodes were found conforms to the observation by other authors that the presence of tapeworm proglottids and even more so of adult nematodes in the feces of wild chimpanzees seems to be generally rather rare (e.g. Huffmann & Caton, 2001, Wrangham, 1995).
On the other hand however, given the small size of the adult specimens of some worm species (e.g. *Strongyloides fuelleborni*: 2.9 to 4.2 mm (Little, 1966)), an at least occasional presence of adult worms or worm fragments in the examined samples could possibly also have been overlooked – particularly as the macroscopic sample screening in the present study had been performed as a quick scan rather than as a thorough examination.

V.1.3 Helminth spectrum and morphology of eggs and larvae

The overall spectrum of gastrointestinal parasitic helminths detected in the present study employing conventional coprology (i.e. macroscopic screening, MWSFs, $NaNO_3$-flotations, sedimentations and fecal cultures), comprised a total of 11 different helminth types, including nine nematodes (representing eight different genera), one cestode, and one trematode.

Important to consider is though that these figures merely represent the numbers of different helminth genera and morphotypes that could be differentiated through light-microscopic examination of their respective developmental stages. Most of the detected helminth genera however comprise several potentially chimpanzee-parasitic species, which, due to the general morphological similarity of their respective eggs and larvae (Blotkamp et al., 1993; Newton et al., 1998b; Gasser et al., 2008b), are largely indistinguishable using conventional coprological methods. As a result, the number of different helminth SPECIES parasitizing the study population might have been considerably higher. The molecular examination of the different types of strongyle larvae cultured in the present study (see paragraphs IV.4 & V.2) confirms this conjecture.

A general validity of the spectrum of gastrointestinal helminths determined for the three chimpanzee groups surveyed in the present study for the entire Taï chimpanzee population can at this point only be assumed. Given the inherent close genetic relatedness and thus presumably similar genetic infection susceptibility of all chimpanzee groups in the park as well as their supposedly largely conformable habitats and exposition to more or less similar environmental and climatic conditions, this assumption seems overall not unreasonable. At the same time however, it cannot be ruled out that variations in direct and indirect anthropogenic impact on different areas of the park (e.g. the degree of forest encroachment and poaching) might have altered the parasite exposure of different Taï chimpanzee groups and thus their respective helminth spectra. Comparative helminthological surveys of chimpanzee groups in different areas of the park will be necessary to assess the de facto existence and/or extent of any potential intra-population variations.

Strongyle nematodes (Strongyloidea superfam.)

Although, as mentioned before, their respective morphology did not allow for unequivocal differentiation and identification, the considerable size range of the different strongyle eggs detected in the present study (see Table 17) indicated the presence of several strongyle genera – which was subsequently verified by the morphological characteristics of the cultured L3-larvae. While the majority of larvae matched the respective morphological descriptions in the literature (Little, 1982) and could thus unequivocally be identified as *Ternidens*-, *Necator*- or respectively *Oesophagostomum* larvae, the genus-affiliation of the few detected trichostrongylid larvae remained unclear. Their observed similarity to published descriptions of

Trichostrongylus L3-larvae (Little, 1982) however suggests their affiliation to this or a closely related genus.

Unequivocal morphological identification of the different strongyle larvae to species level by contrast was generally not possible. Reasons for this are the strongyle larvae's general lack of species-specific morphological features, the reported susceptibility of chimpanzees to several trichostrongyle nematodes, *Oesophagostomum*- and *Necator* species (e.g. Ackert & Payne, 1923; Myers & Kunz, 1972; Krief et al., 2010) as well as the suggested potential existence of a *T. deminutus* species complex (Goldsmid, 1991; Schindler et al., 2005). As for the examined *Oesophagostomum* larvae however, at least a subdivision into two different morphotypes (A and B) was possible without the use of molecular markers due to the larvae's considerable variation in tail sheath length.

Genus *Strongyloides*

While the few free-living adult female *Strongyloides* specimens recovered from the performed fecal cultures could unequivocally be identified as *S. fuelleborni* on the basis of the species-specific morphology of their vulva and peri-vulval area (Speare, 1989), unambiguous morphological identification of the detected *Strongyloides* eggs, L3-larvae and free-living adult males to species level was generally not possible. Concomitant infection of the study population with other *Strongyloides* species particularly with *S. stercoralis,* which is known to be able to parasitize humans as well as wild chimpanzees (Hasegawa et al., 2010), can thus not be ruled out.

Genus *Capillaria*

Even though their respective morphological features did again not allow for unequivocal specific identification, the *Capillaria* eggs detected in the present study are most likely eggs of *C. brochieri*. While being slightly larger than the supposedly *C. brochieri* eggs found in feces of Bonobos at Wamba, Zaire (now DRC) (Hasegawa et al., 1983), they conformed in size with the *Capillaria* eggs found in a previous chimpanzee parasite survey conducted in the Taï NP whose identification as *C. brochieri* eggs had been confirmed through morphological examination of two opportunistically obtained adult specimens (Roduit, 1999). The morphologically similar eggs of *C. hepatica* (Thienpont, 1991), the only other *Capillaria* species known to be able to infect chimpanzees (e.g. Troisier et al., 1982; Banzon, 1982), are furthermore generally not found in fecal samples, as the adult worms live in the liver of infected chimpanzees (Banzon, 1982).

Genus *Trichuris*

The specific identity of the recovered *Trichuris* eggs appears to be somewhat more speculative, even though their size and morphology matched the descriptions published for eggs of *T. trichiura*, a common gastrointestinal parasite of humans and non-human primates and the only species of its genus previously reported as chimpanzee parasite (Yamashita, 1963; Myers & Kuntz, 1972). Due to the general

morphological similarity of all *Trichuris* eggs and adult specimens however (Barriga, 1982), also the infection of the study population with a different, possibly yet undescribed *Trichuris* species cannot be ruled out, particularly as a recent molecular study (Ravasi et a., 2012) reports the existence of two genotypically distinct *Trichuris* types parasitizing humans and at least various monkey species.

Genus *Enterobius*

The assumption that at least some of the morphologically typical *Enterobius* eggs detected in the present study constitute eggs of *Enterobius anthropopitheci* by contrast is probably less speculative, as again an adult specimen of this species had been found and morphologically identified during a previous parasitological survey in the study area (Roduit, 1999). *E. anthropopitheci* is a specific parasite of chimpanzees and bonobos and supposed to be the species most commonly responsible for *Enterobius* infections in wild chimpanzees (Hasegawa & Udono, 2007). At the same time however, also a susceptibility of chimpanzees to several other pinworm species, including *E. vermicularis* has been described (e.g. Yamashita & Konno, 1957; Yamashita, 1963; Myers & Kuntz, 1972). Consequently, particularly given the general morphological similarity of all *Enterobius* eggs, also a concomitant infection of study group members with of other *Enterobius* species seems not unlikely.

Genus *Bertiella*

While all cestode eggs found during the present study could unambiguously be identified as eggs of *Bertiella spp.* on the basis of their typical morphology (Stunkard, 1940), unequivocal identification to species level was again not possible. Measured egg dimensions for example overlap with published egg sizes of two different *Bertiella* species (*B. studeri* and *B. mucronata*) reportedly able to infect chimpanzees (Denegri & Perez-Serrano, 1997).

The *Bertiella* species parasitizing the study population is nevertheless most likely to be *B. studeri,* as this species is a common and widely distributed parasite of non-human primates across Africa and Asia (Galan-Puchades et al., 2000) and hitherto the only one of its genus reported from wild chimpanzees (e.g. Krief et al., 2005). Infections with *B. mucronata* by contrast, of which records in captive chimpanzees exist (Myers & Kuntz, 1972), seem to be geographically restricted to the Americas and Cuba (Galan-Puchades et al., 2000; Denegri & Perez-Serrano, 1997).
The gross morphology of the few detected tapeworm proglottids confirmed the diagnosis *Bertiella spp.* (Stunkard, 1940). Microscopic examination of the proglottids had not been performed.

Family Dicrocoeliidae

The specific identity as well as the genus affiliation of the few, morphologically and regarding their size typical dicrocoeliid eggs detected in the present study is unclear. The family *Dicrocoeliidae* comprises two genera – *Dicrocoelium* and *Concinnum* –

both of which are reportedly able to infect chimpanzees (Huffman et al., 1997; Graber & Gevrey, 1981; Myers & Kuntz, 1972) and, due to the morphological similarity of their respective eggs (Thienpont et al., 1990; Graber & Gevrey, 1981), cannot be distinguished through conventional coprology. The trematode species parasitizing the study population is thus likely to be either *D. lanceatum* or *C. brumpti* as both species, as the respectively only ones of their genera, have been described as chimpanzee parasites before (Myers & Kuntz, 1972). At the same time however, also infections with another, possibly yet undescribed, *Dicrocoelium* or *Concinnum* species cannot be ruled out.

V.1.4 Comparison with the results of a previous chimpanzee parasite survey conducted in the Taï NP

Comparison of the helminth spectrum determined in the present study using conventional coprological methods with the results of a coprological chimpanzee parasite survey conducted in the Taï NP in 1994/95 (Roduit, 1999) revealed a large overlap, but also several discrepancies. Whereas the helminth spectrum determined by Roduit included a *Physaloptera*-like spiruid nematode as well as nematodes belonging to the family Ascarididae and the genus *Gongylonema*, none of these three helminth types was found in the present study. Infections of Taï chimpanzees with trichostrongylid nematodes, as detected in the present study, as well as the presence of two different *Oesophagostomum* morphotypes by contrast have not been reported in the previous survey.

The significance of these apparent differences between the two studies is speculative. On the one hand, they seem to imply that the spectrum of helminth parasites affecting the Taï chimpanzee population has changed over time – a scenario which has been observed before at other chimpanzee field sites (e.g. in Gombe/ Tanzania (Gillespie et al., 2010)) and which expressly underlines the importance of conducting repeated parasitological surveys. On the other hand however, it also has to be considered that at least some of the observed discrepancies might not be genuine.

The prevalence of each of the three only previously detected nematode types for instance had reportedly been extremely low. Overall, only one *Gongylonema*-like egg, the fragment of one adult ascarid worm – respective eggs had not been found – as well as six *Physaloptera*-like eggs had been recovered during the previous study. Accidental ingestion of these helminth eggs and adult worm (fragment) by the respective chimpanzees rather than patent infections of study group members with these nematode parasites seems thus for instance not implausible.

Additionally, also the various methodological differences between the two studies might have caused at least some of the observed discrepancies. Fecal cultures for instance had been included in the diagnostic protocol of both studies. The actual number of cultures performed in the previous study however is unknown. Assuming

that this number might have been rather small, an erroneous failure to detect trichostrongylid larvae and the presence of more than one type of *Oesophagostomum* larvae in the previous study cannot be ruled out – particularly with respect to the very low percentage of trichostrongylid-positive fecal cultures in the present study.

The possibility that the examination techniques employed in the present study might have been unsuitable for the detection of *Ascaris*-, *Gongylonema*- and *Physaloptera*-like eggs by contrast seems less likely, as at least the recovery of *Ascaris*- and *Physaloptera* eggs from chimpanzee feces with one of the methods employed in the present study (i.e. $NaNO_3$-flotations) has been described before (e.g. Howells et al., 2011; Gillespie et al., 2010).

Another notable methodological difference between the two studies is the fact that, mainly for logistical reasons, the majority of samples examined in the present study had been collected from south group chimpanzees, whereas in the previous study only fecal samples from members of the north group (the only fully habituated chimpanzee group at the time) had been screened. This difference might potentially be of relevance as the territories of the two groups, albeit only few kilometers apart, are not completely identical in terms of distance from the park boundary, terrain, and vegetation. The territory of the north group for instance, includes some old plantations and, having been subjected to some degree of logging in the 1970s (Kühl et al., 2010), has a notably denser vegetation and undergrowth than the south group territory. Potentially associated differences in parasite exposure and consequently also in the parasite spectrum of the two chimpanzee groups seem thus again not implausible.

The observation that in the present study trichostringylid larvae were only found in cultured fecal material from south group members seems to support this conjecture. The overall number of examined fecal samples from north-group chimpanzees examined in the present study however had been, comparatively very low (67 samples versus 665 samples from south group chimpanzees).

Despite all aforementioned considerations however, also the possibility that the spectrum of gastrointestinal helminths affecting the Taï chimpanzee population has indeed changed over the last ca. 15 years cannot be ruled out and seems overall not even very unlikely. Like probably most remaining chimpanzee habitats, also the Taï NP has been subjected to increasing human impacts over the last decades. Examples are countrywide deforestation up to the park boundaries, considerable growth of the human population around the park as well as increasingly heavy poaching (Boesch & Boesch-Achermann, 2000; Adou Yao et al., 2005).

While all of these anthropogenic factors certainly have a range of considerable effects on the park's fauna and flora, they might possibly also directly and/or indirectly have influenced and altered the patterns of parasitism in the local chimpanzee population over time (Gillespie et al., 2010). Directly for instance, by

potentially exposing the chimpanzees and/or suitable reservoir- or intermediate hosts in the park to human and/or livestock parasites through a decrease in the physical distance between the chimpanzee territories and human habitation or through increased human presence inside the forest (e.g. Chapman et al., 2005+ 2006).

Indirect influence by contrast might have occurred through alterations of local environmental and climatic conditions (such as for example changes in local rainfall patterns as observed in the Taï NP since the 1960s (Boesch & Boesch-Achermann, 2000; Kühl et al. 2012)) and potentially associated effects on the external development and survival of helminth eggs and larvae as well as on chimpanzee- and/or intermediate- or reservoir host ecology and behavior.

V.1.5 Comparison with the parasite spectra affecting other wild chimpanzee populations

The conventional parasitological screening performed during present study revealed the chimpanzee population in the Taï NP, (or at least the study population) to be parasitized by a minimum of 11 different types of gastrointestinal parasitic helminths.
A finding which is quite remarkable, as considerably lower helminth diversities have hitherto been reported from the majority of coprological parasite surveys of other wild chimpanzee populations (e.g. Kanyawara/ Uganda: seven helminth types (Ashford et al., 2000); Ngogo/ Uganda: five helminth types (Mühlenbein, 2005); Lope/ Gabon: five helminth types (Landsoud-Soukate et al., 1995)). Especially, all published parasitological surveys of other populations of western chimpanzees (*Pan troglodytes verus)* found the respective study groups to harbor infections with only relatively few different types of gastrointestinal helminths (Fongoli/ Senegal: 6 helminth types (Howells et al., 2011), Mt. Assirik/ Senegal: 3 helminth types (McGrew et al., 1989), Tiwai/ Sierra Leone: 2 helminth types (Bakarr et al., 1991)).

Comparison of the respective spectra of gastrointestinal worms with the helminth spectrum detected in the present study unveiled considerable overlap, but at the same time also several notable differences. While infections with a number of helminth types have been reported from all (*Strongyloides spp.* or respectively *S. fuelleborni*), many (*Oesophagostomum spp*.) or at least several other chimpanzee populations (*Necator spp., Trichuris spp*., *Enterobius spp*., *Bertiella spp*., Dicrocoeliidae fam.), infections with *Ternidens spp*. and *Capillaria spp.* respectively *C. brochieri* seem to have only been found in Taï chimpanzees.

Similarly, also the presence of more than one *Oesophagostomum* type appears to have only been observed in the present study as well as in one other wild chimpanzee population (Mahale/ Tanzania (Kief et al., 2010). Infections with a number of helminth types found to parasitize other surveyed wild chimpanzee populations (e.g. *Probstmayria gombensis*, *Ascaris spp*., *Physaloptera spp*.) by contrast have not been detected in the present study and thus seem, at least momentarily, not to occur in the Taï study population.

Potential explanations for these apparent discrepancies in helminth diversity between the different chimpanzee populations are manifold. One reason might for instance be the differing habitats of the different chimpanzee populations. Ranging from primary rainforest (e.g. Taï NP) to woodland savannah (e.g. Fongoli & Mt. Assirik), they each might provide more or less suitable environmental (e.g. vegetation, sympatric fauna) and climatic conditions (e.g. temperature, rainfall, UV-radiation) for the external development and survival of the eggs and/or larvae of certain helmints (e.g. Pietrock & Marcogliese, 2003).

The survival of helminth larvae in the environment depends on adequate moisture and shade (Hansen & Perry, 1994). The local climate in the Taï NP – moderately high temperatures, relatively high rainfall and humidity throughout most of the year in combination with low UV-radiation on the forest floor (Boesch & Boesch-Achermann, 2000) might thus provide a very apt environment for many helminth species. More extreme climatic conditions like for example in the Mt. Assirik and Fongoli area/ Senegal – seasonally very high temperatures (max. temp. > 45°C) and UV-radiation, in combination with low rainfall and humidity (McGrew et al., 1988; Bogart & Pruetz, 2011) by contrast might be less favorable for the survival and development of the eggs and larvae of certain helminths (McGrew et al., 1988). The ideal temperature for larval development of many helminth species for example ranges reportedly between 22°C and 26°C, whereas temperatures over 30°C lead to high larval mortality (Hansen & Perry, 1994). The minimum humidity required for larval development is about 85%, while desiccation from lack of rainfall rapidly kills eggs and larvae of many helminth species (Hansen & Perry, 1994).

Another possible habitat-related factor, which for instance might explain the presence or respectively absence of *Ternidens* infections in the different chimpanzee populations, is the presence or respectively absence of suitable reservoir hosts such as potentially certain sympatric monkey species (Goldsmid, 1982).

In the Taï NP for example, sympatric *Ternidens*-infected Diana monkeys (*Cercopithecus diana*) might possibly constitute a respective reservoir for the local chimpanzee population (see V.2.2). In Kibale forest/ Uganda on the other hand, even though *Ternidens* infections have been described in sympatric baboons (*Papio anubis*) (Benzjian et al., 2008), no respective infections have been reported from the resident chimpanzee populations (e.g. Ngogo (Mühlenbein, 2005), Kanyawara (Ashford et al., 2000; Krief et al., 2005)). An observation which might however be explained by the potential existence of different, possibly host-specific cryptic *T. deminutus* variants or subspecies which has been suggested by different authors (Goldsmid, 1991; Schindler et al., 2005).

Apart from habitat-related variations in environmental and climatic conditions, also a number of other factors might be responsible for or at least contribute to the apparent variations in helminth diversity between the different chimpanzee populations – either for instance by influencing the chimpanzees` parasite exposure and/or by affecting

the infection susceptibility and -resistance. Examples are potential behavioral or dietary variations between the different chimpanzee populations, the populations' genetic infection susceptibility and immune status, concurrent infections with other pathogens, and/or the respective degree of anthropogenic impact the individual populations are subjected to (Chapman et al., 2005; Gillespie et al., 2010). Potential genetic and behavioural differences might be of particular relevance as they are likely to be maintained by the effective spatial separation between at least most of the different chimpanzee populations.

Dietary variations are for example another potential explanation for the apparent absence of *Ternidens* infections from all but the Taï chimpanzee population. Local cultural or ethnological differences regarding the consumption of certain invertebrates (e.g. termites or maggots), which might serve as intermediate hosts for infective *Ternidens* larvae, are one of the hypothesized explanations for the presence or absence of *T. deminutus* infection in different human populations across Africa (Goldsmid, 1982). Invertebrates, especially insects such as ants and termites, are an integral part of the natural diet of all wild chimpanzees (e.g. Boesch & Boesch-Achermann, 2000; McGrew et al., 1988; Tutin & Fernandez; 1993, Bogart & Pruetz, 2011). The respective invertebrate species consumed by chimpanzees from different populations and thus potentially also their exposure to infective *Ternidens* larvae might however vary, albeit further research on the life cycle of *Ternidens spp.* and its route of infection is clearly necessary to confirm this hypothesis.

Additional research is equally necessary to unambiguously verify the aforementioned apparently considerable discrepancies in helminth diversity between the different chimpanzee populations. Even though all observed differences appear to be largely plausible and might have well been caused by any of the aforementioned factors, it also cannot be ruled that at least some of them might not actually be genuine. Reason for this is that, due to the considerable variations in respectively employed examination methods and numbers of examined samples, the results of the different surveys might not be fully comparable (e.g. Ashford et al., 2000; Gillespie, 2006+2010).

Given the morphological similarity of all strongyle eggs (e.g. Blotkamp et al., 1993; Orihel, 1971), failure to perform fecal cultures in various surveys might for instance have caused a considerable underestimation of the true number of different strongyle types affecting the respective chimpanzee populations. Potentially existing *Ternidens* infections and/or infections with trichostrongylid strongyles or multiple *Oesophagostomum* species for example might thus have been easily been overlooked. A scenario which similarly has been suspected by Schindler et al. (2005) as another potential explanation for the apparent absence of *T. deminutus* from many human populations across Africa.

Erroneous underestimation of the spectrum of helminths affecting certain chimpanzee populations might furthermore also have occurred if the numbers of

examined fecal samples had been very small or if the samples had been collected over a relatively short period of time. Reason for this is that possibly existing seasonal or low prevalence infections might have gone undetected in these surveys (e.g. Mühlenbein, 2005).

V.1.6 Helminth prevalence

While each chimpanzee screened during the course of the present study was found to be infected with gastrointestinal helminths, and helminth propagules were detected in almost all examined fecal samples, the respective prevalence of the different helminth genera and morphotypes varied considerably. Whereas the observed prevalence of strongyle and *Strongyloides* infections was generally very high (with the exception of trichostrongyle infections), the prevalence of all other nematodes, *Bertiella spp.,* and dicrocoeliid trematodes was considerably lower. As for trematode infections however the comparatively low number of performed sedimentations (N= 120) has to be considered.

The apparent division into more and less prevalent helminths was even more distinct with regard to the respective proportion of examined fecal samples in which developmental stages of each helminth genus or morphotype had been detected. A finding which conforms to the observation by Huffman et al. (1997) that the prevalence of a parasite species occurring in a host population might be significantly underestimated if the percentage of parasite-positive fecal samples rather than the proportion of infected host is determined.

A similar prevalence underestimation might however also occur if only few samples from each host are examined. As egg production in helminths is generally subjected to considerable day to day and within-day variations (Pit et al., 1999; Hall, 1981), chances to detect low-prevalence and/or low-intensity infections would seem to invariably increase, if several rather than only a few samples from each host are examined.

Accordingly, also the observed prevalence rates in the present study were markedly or even significantly higher if only chimpanzees were considered from whom a minimum of six respectively 12 fecal samples had been examined. This observation seemingly contrasts with the results of a study conducted by Mühlenbein (2005) which suggests that the screening of two or three serial samples per host might suffice to assess the parasite prevalence in a given chimpanzee study population. The author of the study remarked however that this finding might only apply to parasitological short-term studies, as his survey had been carried out over a period of only three month and thus did not account for any potential seasonal variations in helminth occurrence.

Even though the respective prevalence rates of all helminth types detected in the present study increased considerably if only more intensely sampled chimpanzees

were considered, a notable division into highly prevalent helminth genera and morphotypes and less common helminth types was still maintained.

The possibility that each chimpanzee would have been found to be infected with all different helminth types identified in the present study, if enough fecal samples had been examined seems therefore somewhat unlikely. This is particularly the case, as upon examination of a total of 61 samples collected from one adult individual (`Sagu´) throughout the study period neither *Capillaria* eggs nor *Enterobius*- or *Bertiella* eggs had been detected. Nevertheless, on the basis of the data and results obtained in the present study this possibility can still not entirely be ruled out.

Potential reasons for the partly considerable prevalence differences observed in the present study are manifold, including variations in the respective biology and epidemiology of the different helminth types such as for example variations in reproductive output, longevity, survival of developmental stages in the environment, ability to undergo hypobiosis, mode of infection (including potential galactogenic infections), and immunogenicity.

The ability of *Strongyloides spp*. to reproduce and multiply in the environment via one or more generations of free-living adults (Olsen et al., 2009) for instance might lead to a considerable contamination of the forest floor within the chimpanzee territories with infective L3-larvae able to actively penetrate the skin of their host and thus to a proportionally very high risk for all study group members to acquire infections with these parasites.

On the other hand however, also several methodological constraints might have contributed to the observed prevalence differences in the present study. As generally only relatively little fecal material from each sample had been screened, not all samples positive for certain helminth types might have been detected, and the prevalence of these helminths might have been underestimated. This might have been particularly the case as for the seemingly less prevalent helminth types, namely *Capillaria spp., Trichuris spp., Enterobius spp.,* and the unidentified trichostrongylid nematode, as the number of eggs or respectively larvae detected from these helminths in all respectively positive samples had been generally very low (mostly only one or two eggs or larvae per sample).

The seemingly very low prevalence of trichostrongylid strongyles, which incidentally contrasts markedly with the very high observed prevalence of all other strongyle genera and morphotypes, might furthermore also have been caused by two other methodological factors, namely inadvertent misidentification and high larval mortality during culture.

As cultured trichostrongylid and *Ternidens* L3-larvae are morphologically quite similar – at least at relatively low magnification – the possibility cannot be ruled out that a number of trichostrongylid larvae might have been erroneously identified as *Ternidens* larvae. Furthermore, disproportionately high mortality (at L1- and L2-

stage) of one strongyle species in mixed strongyle fecal cultures has been described in the literature (Dobson et al. 1992) and thus might have also played a role in the present study. Potential disproportionately high mortality of cultured trichostrongylid larve in the presence of *Oesophagostomum*-, *Necator*- and/or *Ternidens* larvae for instance might have caused a considerable underestimation of the true prevalence of trichostrongyle-positive fecal samples and infected study group members.

V.1.7 Variation in helminth prevalence between male and female chimpanzees

Just as the spectra of gastrointestinal helminths parasitizing male and female study group members were found to be almost identical (with the exception of dicrocoeliid trematodes of which eggs were only detected in fecal samples of male individuals) also the respective infection rates of both sexes were largely similar.

Even though a highly significant male bias regarding the prevalence of *Strongyloides*-infected individuals was observed if all 71 screened study group members were considered – a finding which conforms to similar observations from other studies (e.g. Gillespie et al., 2010; Roduit, 1999) – this apparent sex bias diminished to non-significance if only chimpanzees were included in the calculation from whom a minimum of six fecal samples had been examined. All other observed helminth prevalence differences between male and female chimpanzees were generally non-significant independent of the number of samples examined from each individual.

One methodological constraint which in this respect however has to be considered is that, when assessing the respective infection rates, it had generally been assumed that each chimpanzee found to excrete developmental stages of a certain helminth type at least once had been infected with this parasite throughout the entire study period (Gillespie et al., 2010). Depending on a number of factors, such as for instance adult life span of the individual helminth types, re-infection rates, and host-immune response towards infections, this assumption might however not have always been correct, as periods of infection of individual hosts might have been intercepted by infection-free periods.

As a consequence, potentially existing more pronounced sex biases, such as for instance permanent infections with certain helminths in one sex versus only intermittently occurring infections in the other sex, might have gone unnoticed. The observed significant differences regarding the numbers of fecal samples from male and female chimpanzees found to contain developmental stages of certain helminths (*Necator spp., Trichuris spp.* and most conspicuously, *Strongyloides spp.*) seemingly corroborate this conjecture, although these differences might have also been caused by other factors. Examples are potential variations in the respective worm burdens of male and female chimpanzees or potential host sex-related variations in the reproductive output of the respective helminths.

About the proximate mechanisms underlying any of the actually observed or else potentially existing sex biases can at this point only be speculated, particularly since other potential confounding factors, such as host age, -rank, and -reproductive statusm had not been included in this comparison. The observation that the detected male bias regarding the prevalence of *Strongyloides*-positive fecal samples appeared to only apply to adult and (to a lesser extent) adolescent chimpanzees if the four chimpanzee age classes were regarded separately underlines the potential impact of these factors.

Explanations for host sex-related differences in helminth prevalence, intensity of infection and/or reproductive output described and hypothesized in the literature, but which might possibly also explain any respective sex biases observed in the present study, are manifold. Examples include ecological differences between male and female hosts (e.g. behavior, diet or body size) leading to discrepancies in parasite exposure or rendering one of the two sexes more attractive hosts for certain parasites (e.g. Klein, 2000; Zuk & McKean, 1996; Bundy, 1988b). Other presumed explanations are sex-specific hormone-dependent variations in the hosts' immune response towards helminth infections and/or diverging direct hormonal effects on parasite development and -reproduction (e.g. Zuk & McKean, 1996; Wilson et al., 2002; Escobedo et al., 2005). While high estrogene levels for instance have been suspected to have an important impact on the up-regulation of host resistance against certain parasites, the opposite effect has been assumed for high testosterone levels (Rivero et al., 2002).

V.1.8 Method comparison

An overall considerable relation between observed helminth prevalence rates, employed examination methods, and type of sample preservation was confirmed by comparing the two main conventional examination techniques utilized in the present study (i.e. MWSFs and Harada-Mori fecal cultures) with regard to their sensitivity to detect strongyle and *Strongyloides spp.* helminth developmental stages, as well as by screening unpreserved versus formalin-fixed sample aliquots.

While the respective overall numbers of strongyle- and *Strongyloides*-infected study group members determined with each of the two methods were largely equal, the detection sensitivity of the two methods with respect to individual strongyle or *Strongyloides*-positive fecal samples differed considerably.
Whereas individual *Strongyloides*-positive fecal samples were significantly more reliably identified employing fecal cultures as compared to MWSFs, the detection of strongyle-positive fecal samples was significantly more reliable using MWSFs.

The reasons for these considerable differences in detection sensitivity between the two methods are unclear. The most likely explanation for the observed comparatively lower sensitivity of Harada-Mori fecal cultures to detect strongyle-positive fecal samples however seem to be suboptimal culture conditions for the hatching and

development of certain strongyle larvae and/or increased L1- or L2-stage mortality of the larvae of certain strongyle species in mixed cultures (Dobson et al., 1992).

The observation that the overall highest infection rates and numbers of strongyle- or respectively *Strongyloides*-positive fecal samples were detected using a combination of fecal cultures and MWSFs rather than using any of the two methods alone, highlights the importance of employing more than one examination method in order to minimize method-related prevalence underestimations and to obtain more reliable prevalence values.

At the same time however, already the repeated examination process and screening of a larger amount of fecal material from each sample (ca. 4.5 to 5.0 grams instead of 3.0 grams) as such might have contributed considerably to the observed increase in detection sensitivity if two instead of one examination techniques were employed to screen each sample.

As formalin appears to reduce the number of detectable helminth eggs in respectively preserved fecal material, erroneous helminth prevalence- as well as intensity underestimations might however (independent of the type and number of utilized examination methods) also occur if formalin-fixed fecal material instead of fresh, unpreserved feces are examined. Such erroneous underestimations, as observed in the present study, seem to be particularly likely as for the genus *Strongyloides* which might be explained by the fact that *Strongyloides* eggs have a thin shell and might thus be more susceptible to the detrimental effects of formalin than thicker-shelled eggs.
In order to avoid any fixative-related prevalence underestimations, all prevalence values in the present study had been determined from unpreserved fecal material.

V.1.9 Helminth morphotype richness and the influence of host-intrinsic factors

As described before, the prevalence rates of all helminth types detected in the present study were markedly higher if the proportions of infected individuals rather than the proportions of positive fecal samples were considered. The observation that also the median cumulative individual helminth morphotype richness of the 72 screened study group members was notably higher than the median number of different helminth morphotypes detected in each examined fecal sample conforms to this finding and might be explained by the same factors. Examples of these factors are temporal and/or helminth species-specific variations in excreted egg numbers, intermittent rather than permanent infections of study group members, potential failure to detect individual positive samples, and increased detection sensitivity upon examination of multiple samples from the same individual.

The reasons for the equally observed considerable inter-individual differences regarding the cumulative individual helminth morphotype richness of the different study group members are somewhat more speculative. While the highly unequal numbers of samples examined from each chimpanzee (1 to 61 samples per

individual) and the associated variable likelihood that actually existing infections would have been detected are certainly one important reason, this notable sampling bias seems still rather unlikely to have been the sole cause.

Reason for this is the observation that the cumulative individual helminth morphotype richness of five different chimpanzees sampled at a similar frequencies (25 to 27 samples per individual) differed notably (5 to 9 different morphotypes), whereby one (`Caramel´) of the three individuals from whom 27 samples had been examined was found to harbor the lowest number of different morphotypes. Furthermore, the cumulative individual helminth morphotype richness of the overall most intensly sampled chimpanzees (`Sagu´; 61 samples, 8 different helminth morphotypes) was equal to the respective morphotype richness of two individuals (`Poseidon´ and `Narcisse´) from whom only a total of five and seven samples respectively had been examined.

As a consequence, also other factors for instance various host-intrinsic factors might have contributed to the considerable inter-individual differences in cumulative individual helminth morphoyte richness observed in the present study. While sex, age and group affiliation of the screened individuals as such were found to have no significant influence, these factors might for instance include genetic constitution, social rank, reproductive state, immune status, and/or stress and hormone levels of the individual chimpanzees (Benavides et al., 2012). The testosterone levels of the individual male study group members for instance seem to differ notably (Deschner pers. comm.), and in Ngogo/ Uganda, Mühlenbein (2006) found higher total helminth and protozoa parasite richness in male chimpazees with high testosterone and cortisol levels as compared to individuals with lower hormone levels (female chimpanzees had not been included in this comparison).

Another potential reason for the apparent considerable helminth richness differences between the individual study group members might have been concurrent infections of individual chimpanzees with other pathogens (e.g. certain viruses, bacteria or protozoa). These co-infecting pathogens again might potentially have caused some degree of immune-suppression in the infected individuals (e.g. by inducing a Th1 immune system bias (Caravalho et al., 2001; Evering & Weiss, 2006)) and thus might have altered his or her susceptibility to infections with certain helminth types or the ability to eliminate respective infections. In a coprological survey of helminth-parasitized people in Salvador/ Brasil for example, the prevalence of *Strongyloides stercoralis* and *Schistosoma mansoni* infections was significantly higher in HTLV-1 positive as compared to HTLV-1 negative individuals, whereas no such difference was found with respect to the prevalence of other helminths, namely *Ascaris lumbricoides* and *Ancylostoma duodenale* (Caravalho et al., 2001).

The cumulative helminth morphotype richness of at least some study group members might furthermore at least in theory also have been influenced by the individuals' respective behavioral or dietary peculiarities. One adult female (`Sumatra´) who had

been found to be parasitized by only relatively few different types of helminths throughout the study period for instance appeared to consume leaves more frequently than other individuals. Systematic quantitative assessment of this behavior and testing of the respective leaves for anti-parasitic components however had not been performed.

The reason for the apparent difference between the findings of the present study (namely the observed absence of a significant impact of host age), and the results of a parasitological survey of two chimpanzee communities in Gombe/ Tanzania (Gillespie et al., 2010) are unclear. While the authors of this survey, like the present study, found no significant difference in the average parasite richness between male and female chimpanzees, they did observe a significant influence of host age, namely significantly higher parasite richness in old (> 30 years) compared to subadult (</= 10 years) chimpanzees during 1 of 2 study years. An observation they associated with potential low pathogenicity and accumulation of different parasite species in the organisms of the old individuals over time (Gillespie et al., 2010). In the present study by contrast no apparent indications for the existence of such a mechanism were seen.

One noteworthy consideration in this respect however is that in the study of Gillespie et al. not only helminths including trematodes, but also protozoa had been included in the analyzed parasite richness values, whereas in the present study only nematodes and cestodes had been considered. Besides, the spectra of nematode helminths found to parasitize the two study populations and thus potentially also their respective immunogenicity differ markedly.

On the other hand however, also the possibility that the respective results of the present study might potentially have been biased by the somewhat skewed distribution of the analyzed sample set cannot be ruled out. For various logistical reasons (see methods & material section), the number of samples from all north and east group chimpanzees as well as from all infant and most juvenile south group members examined and included in the model had been comparatively low. The demography of the main study group (south group) at the same time had been notably skewed towards younger males (< 20 years) and older females (> 30 years), resulting in a proportionally higher number of analyzed fecal samples from adolescent and young adult males as well as from older adult female chimpanzees. As a consequence, potentially de facto existing significant impacts of chimpanzee age or possibly also sex and/or group affiliation of the screened chimpanzees might have been veiled.

V.1.10 Helminth reproductive output and influence of host-intrinsic and seasonal factors

As reported by various authors, parasitic helminths like other macroparasites almost always exhibit aggregated distribution across their host population, with few

individuals harboring disproportionally high worm burdens, whereas the majority of hosts have only relatively few worms (Shaw & Dobson, 1995; Wilson et al., 2002). The salient inter-individual variations in fecal helminth propagule output observed in the present study suggest that this pattern also applies to the helminths affecting the Taï chimpanzee population – although seemingly only for strongyle nematodes and *Strongyloides spp.* whose eggs and/or L1-larvae larvae constituted the vast bulk of detected propagules in each examined fecal sample.

The fecal propagule output of all other helminth types (i.e. *Capillaria spp., Trichuris spp., Enterobius spp., Bertiella spp*.) and thus, particularly given the low proportions of samples in which respective eggs had been found, supposedly also their infection intensities by contrast were generally very low. The reasons for this finding are unclear. Potential causes might for instance be potentially low exposure of study group members to respective eggs or infected intermediate hosts and/or comparatively high general resistance of Taï chimpanzees against infections with these particular helminths.

Additionally, interference competition between co-infecting helminth species (e.g. for attachment space or nutritional resources) leading to numerical suppression of one species or increased immune response against one helminth type, induced by the concomitant presence of another helminth species has been reported from many host-polyparasite systems (e.g. review by Pedersen & Fenton, 2007; Poulin, 2001; Cox et al., 2001; Behnke et al., 2001). Accordingly, also a potential limiting effect of the different highly prevalent strongyle types and/or *Strongyloides spp.* on the numbers and propagule output of all other helminth types parasitizing the three study groups cannot be ruled out.

At the same time however, also the fact that fecal egg- or propagule counts might not necessarily be reliable estimators of the screened hosts' actual worm burdens has to be considered (Anderson & Schad., 1985; Gillespie, 2006). Reason for this is that not only the respective number of reproductively active adult worms harbored by each host, but also various other factors, such as for instance temporal- and inter-specimen fluctuations in egg production and -excretion (Anderson & Schad, 1985), might considerably influence observed epg- or respectively ppg-values. Equally, helminth density-dependent constraints on worm fecundity, variations in egg-laying capacity between different helminth species, fecal consistency as well as the respectively employed examination method, might have a sizeable impact on the number of propagules present in each examined fecal sample (Anderson & Schad, 1985, Hall, 1981). The MWSFs employed in the present study for instance might not necessarily have allowed for equal detection of all helminth eggs and/or L1-larvae present in the respectively examined sample aliquots (e.g. due to the relatively short centrifugation time and use of a hand-centrifuge, the presence of detritus in many samples, and/or potential variations in the floatability of different helminth eggs in Sheather's solution).

 # Host intrinsic factors

Proximate causes for the aforementioned typically aggregated distribution of most parasitic helminths within their host populations suggested in the literature include variations between individual hosts regarding their exposure to parasite infective stages as well as differences in their respective infection susceptibility (Wilson et al., 2002). These individual variations in exposure and susceptibility again are assumed to be induced by a number of different host-specific factors, such as for instance sex, age, body condition, and behavior of the individual hosts (reviewed in Wilson et al., 2002) or immunity-related genetic variations (Stear et al., 2007). Other suggested factors are concurrent infections of individual hosts with other pathogens, such as certain viruses and/or bacteria (e.g. Pathak et al., 2012) or individual variations in stress and/or hormone levels (e.g. Zuk & McKean, 1996).

The relative importance of any of these and/or other influencing factors or of potential interactions between several factors however is to date still largely unclear (Wilson et al., 2002) and might possibly also vary substantially for different hosts and helminth species.

The significant influence of chimpanzee sex, namely the significant male bias towards higher ppg-values and thus possibly also towards higher strongyle and *Strongyloides* burdens observed in the present study, conforms to similar observations from various other helminth-vertebrate host systems (e.g. reviewed in Zuk & McKean, 1996 and Wilson et al., 2002; Poulin et al. 1996) including human- and non-human primate hosts (e.g. Bradly et al., 1992; Krief et al., 2005).

Potential reasons for higher worm burdens in male compared to female hosts described in the literature are manifold. Examples are increased parasite exposure of male compared to female hosts resulting from behavioural and/or physiological differences between the sexes as well as sex-dependent hormone-related effects on infection susceptibility, immune response, and/or helminth development.

Which if any of these factors are responsible for the significant male bias in helminth propagle output observed in the present study remains at this point largely speculative, particularly since no systematic comparative behavioural and hormone data from the different male and female study group members had been obtained during the study period.

Hormonal differences between male and female study group members seem nevertheless to be one of the overall most likely causes, especially as the existence of sex-related hormonal differences in chimpanzees is well know (e.g. Copeland et al., 1985). At the same time however, also a potential influence of other factors, for example of variations in the helminth exposure of male and female individuas cannot be ruled. Even though the overall diet composition, feeding behavior, and habitat use of male and female chimpanzees are generally fairly similar, also some differences between the sexes have been described (Boesch & Boesch-Achermann, 2000), as

has a – albeit comparatively moderate – sexual dimorphism in body size and weight (Smith & Jungers, 1997).

Similarly speculative as the reasons for the observed significant impact of chimpanzee sex on obtained ppg-counts and thus supposedly also on the individual strongyle and *Strongyloides* burdens of the different study group members are the reasons for the equally observed apparent absence of a significant influence of chimpanzee age.

Host age-related notable variations in helminth burdens, possibly caused by age-dependent changes in helminth exposure and susceptibility or acquired resistance of the individual hosts (Wilson et al., 2002), reportedly occur in many host-parasite systems (Wilson et al., 2002; Cattadori et al., 2008). The apparent absence of a significant impact of chimpanzee age on the obtained ppg-values in the present study is thus quite remarkable.

One potential explanation for the absence of an obvious age bias might be the fact that all study group members were infected with multiple helminth species each of which might have been affected differently by the respective age of their host. In rabbits naturally co-infected with two different strongyle nematodes for example, each of the two helminth species had a different age-intensity profile. Whereas *Graphidium strigosum* intensity increased exponentially with host age, *Trichostrongylus retortaeformis* intensity exhibited a convex shape (Cattadori et al., 2008).

On the other hand however, also the potentially poor correlation between helminth propagule output and actual worm burdens as well as a potentially limited detection sensitivity of the employed MWSFs have to be considered. Additionally, the aforementioned notable demographic skew of the south group towards relatively more adolescent and young adult males versus a relatively higher number of older females and the overall very low number of analyzed fecal samples from infant chimpanzees of both sexes might possibly have obscured any potentially existing more pronounced impact of host age.

 # Seasonal factors

Considerable seasonal variations in helminth infection intensities, caused by seasonal changes in host exposure to infected intermediate hosts or infective helminth developmental stages in the environment or resulting from seasonal fluctuations in host infection susceptibility and -resistance, are a commonly observed phenomenon in many host-parasite systems (e.g. reviewed in Altizer et al., 2006). The significant seasonal variations in fecal helminth propagule output in the present study as well as the detected significant impact of rainfall and maximum temperature are in line with these observations.

Changes in host exposure throughout the year are typically caused by seasonal changes in host behavior (e.g. ranging pattern) and diet and/or, on the parasite level, for instance by fluctuations in the numbers of infected intermediate hosts or infective eggs or larvae in the environment. These fluctuations again are generally a result of seasonal variations in climatic conditions such as rainfall, temperature, humidity, and UV-radiation, which can have a considerable impact on the development, survival, and infectivity of helminth developmental stages outside of their host (Altizer et al., 2006; Pietrock & Marcogliese, 2003).

Seasonal changes in host infection susceptibility or resistance against helminth infections by contrast are usually assumed to be induced by fluctuations in host body condition and/or changes in stress levels which again might be caused by seasonal variations in food availability, host diet, and caloric intake and/or for instance by changes in host behavior, reproductive status, host density, and contact rates (Altizer et al., 2006).

Which if any of these factors might have been responsible for the significant seasonal variations in propagule output and thus again possibly in strongyle and *Strongyloides* infection intensities observed in the present study, remains at this point again largely speculative as a potential impact of at least most of the above-mentioned factors or respectively a combination thereof seems plausible.

The Coula (*Coula edulis*) nutcracking season constitutes the period of highest caloric and protein intake for the Taï chimpanzees throughout the year (N'guessan et al., 2009). The apparent overlap – assuming a prepatence period of about one month (e.g. Anderson, 2000) – between the nutcracking season (November-February/ March) and the period of lowest propagule output during the present study (December-April) for example might thus potentially have been caused by a seasonal decrease in the helminth infection susceptibility of the individual study group members resulting from their seasonally good body condition and low nutritional stress.

The observed high propagule output during July on the other hand might accordingly for instance have been caused by a potentially low body condition and increased infection susceptibility of the individual study group members during May and June. Based on the observation that ripe tree fruits (the primary food source of the Taï chimpanzees (e.g. Anderson et al., 2006)) seemed to be scarce during these months and the south group chimpanzees frequently split up and foraged separately, this conjecture seems overall not unlikely. Respective systematic data however had not been collected and N'guessan et al. (2009) actually reported relatively high caloric intake between April and July in their study on daily energy balance and protein gain of the north group chimpanzees.

At the same time however, also another possibility, namely potential seasonal variations in exposure of the individual study group members to infective strongyle and *Strongyloides* developmental stages or infected arthropod intermediate hosts

has to be considered. Particularly given the observed significant impact of both average rainfall and average maximum temperature on the obtained ppg-values, such seasonal variations in helminth exposure might possibly have been caused by seasonal climate-induced fluctuations in the numbers of infective larvae and/or infected arthropod intermediate hosts in the chimpanzee territories.

As the eggs and larvae of many helminth species seem to have limited tolerance for high temperatures and low humidity during their development in the environment (e.g. Hansen & Perry, 1994), the detected general association between high average maximum temperatures, low average rainfall and comparatively low propagule output (after an assumed prepatence period of one month) on the one hand and relatively low average maximum temperatures, high average rainfall and high ensuing propagule output on the other hand corroborates this possibility.

Independent of their respective causative mechanisms, the observed significant seasonal variations, the significant male bias towards higher propagule output as well as various methodological and sample-related factors such as fecal consistency and possibly limited detection sensibility of the employed MWSFs clearly contributed to the considerable ppg-heterogeneities observed in the present study. Nevertheless, even in combination, all of these factors still don't seem to suffice to explain the disproportionately high propagule output and thus potentially strongyle and *Strongyloides* burdens of a few individual chimpanzees throughout the study period (see Figure 13).

As a consequence, other host-intrinsic factors such as for instance individual variations in genetic infection susceptibility (e.g., Stear et al., 2007), individual differences in stress and stress hormone levels (e.g. Krief et al., 2010), general poor body condition, and/or concurrent infections of individual chimpanzees with other pathogens (e.g. Pathak et al., 2012), might have also had an impact. The chimpanzee with the highest recorded propagule output (`Kaos´), a 32-year-old adult male for example had been suffering for years from infected and slow healing wounds and thus might potentially have harbored concurrent chronic bacterial infections. Furthermore, also his general body condition and infection resistance might have been relatively poor.

V.2 Identification and characterization of cultured strongyle L3-larvae and *Strongyloides spp.* specimens using genetic markers

V.2.1 Methodological considerations

DNA-extraction

The proximate cause for the high overall DNA-extraction failure rate in the present study is unclear. One potential cause however might have been the reportedly tough cuticle of adult nematodes and their respective larval stages (Gasser et al., 1993) which might possibly have prevented unfailingly successful DNA-extraction from all

strongyle larvae and *Strongyloides* specimens, employing the Quiagen Mini Kit. Gasser et al. (1993) for example, because of this tough cuticle, found mechanic homogenization using a politron homogenizer to be a more effective method for the extraction of DNA from nematode helminths than simple proteinase K digestion.

At the same time however can also not be ruled out that all or at least some of the assumed failures of DNA-extraction might have actually been PCR failures. As the respective amount of DNA extracted from each larva or free-living adult specimen could not be measured directly, extraction failures had been assumed whenever repeated PCRs with the same primers or consecutive PCRs with two different primer sets failed to produce an amplicon.

As none of the different larvae and free-living adult *Strongyloides* specimens had been washed in distilled water prior to extraction as done by other authors (e.g. von Samson-Himmelstjerna et al., 2002) it seems for instance possible that PCR-failures might have been caused by the presence of inhibitors in the storage fluid (filtered spring water) of the respective larvae or adult worms which had not been inactivated or removed through the extraction process.

 # Primer selection

Primers Pannem F+R1 had been employed to prospect for potential cryptic species as they were able to produce amplicons from the mitochondrial DNA of all types of strongyle L3-larvae examined in the present study. The respective maximum inter-individual sequence variations between different larvae assumed to belong to the same strongyle species based on their respective ITS-2 sequences however were generally relatively small (< 5%) and thus did not allow for an unequivocal distinction between pronounced inter-individual within-species variations and the existence of cryptic species.

The amplification of mitochondrial gene regions featuring more distinct nucleotide differences between cryptic species (mostly > 9%), such as the CO genes or the protein coding loci in the NADH dehydrogenase group, particularly the nad4 locus (Blouin, 2002), might thus have been a better choice. Respective pan-nematode primers however had not been available and according to Blouin (2002) most likely also don’t exist.

 # Sequencing

Another weakness of the present study and mainly a result of budget constraints is the low number of generated sequences – the overall relatively limited number of ITS-2 and/or mtDNA sequences generated from different larvae or free-living adult specimens of each helminth type, but particularly also the very low number of sequences produced from each PCR product. Generally only one sequencing attempt had been made for each directly sequenced amplicon and from each PCR-product that was cloned prior to sequencing, the plasmid inserts of mostly only one or two clones had been sequenced. As a consequence, at least some of the observed

alleged within- or interspecies sequence variations, rather than reflecting genuine nucleotide differences, might actually constitute within-individual point mutations or might have been caused by base-incorporation errors during amplification or by sequencing errors. As the quality of all sequencing chromatograms was generally very good, the latter seems overall however less likely.

V.2.2 *Ternidens* L3-larvae

Ribosomal second internal transcribed spacer sequences are known to be an excellent diagnostic marker for the discrimination and identification of nematode species, independent of their developmental stage (i.e. adult worm, egg or larva) (e.g. Blouin, 2002).

Comparison of the ITS-2 sequences of the different chimpanzee and Diana monkey *Ternidens* L3-larvae examined in the present study with the GenBank nucleotide database confirmed the prior light-microscopic morphological classification of these larvae as *Ternidens* developmental stages and furthermore allowed for their unequivocal specific identification as *T. deminutus* larvae.

As *T. deminutus* is the only currently recognized species of its genus and its ability to infect a range of primate hosts including humans as well as great apes is well documented (Goldsmid, 1982), this finding is not unexspected. The observed 100% and 99.1% homology between the chimpanzee *Ternidens* ITS-2 sequences and the respective GenBank sequence (accession no. AJ888729.1) of a *T. deminutus* specimen from a mona monkey (*Cercopithecus mona*) in Ghana as well as the equally high homology (100% and 99.6%) to the ITS-2 sequences obtained from *Ternidens* larvae from a sympatric Diana monkey is nevertheless noteworthy.

Reason for this is the notable nucleotide difference of 2.8% to 5.1% between the GenBank mona monkey *T. deminutus* sequence and published ITS-2 sequences of *T. deminutus* specimens from two other primate hosts, namely from an olive baboon (*Papio anubis,* accession no. AJ888730.1) and a crab-eating macaque (*Macaca fascicularis,* accession no. HM067975.1), which suggests that *T. deminutus* might actually be a species complex consisting of several cryptic species, and that each primate host species might be infected with a distinct *T. deminutus* variant (Schindler et al., 2005).

This conjecture (Schindler et al., 2005) is based on the reported nucleotide difference of 3.2% between the ITS-2 sequences of two distinct *Oesophagostomum* species, namely *O. bifurcum* from a mona monkey in Ghana and *O. stephanostomum* from a chimpanzee in Mahale/ Tanzania (Gasser et al., 1999a) as well as the typically low levels (</= 1%) of within-species ITS-2 variations described in the literature for many strongyle species (e.g. Romstadt et al. 1997; Newton et al., 1998a+b; Blouin, 2002).

The observed complete or near complete homology between the chimpanzee-, Diana monkey- and mona monkey *T. deminutus* ITS-2 sequences by contrast does not

provide any apparent evidence for the existence of a *T. deminutus* species complex, but rather implies that all three primate host species might have been infected with the same *T. deminutus* variant. The equally identical or near identical 12s RNA mtDNA sequences of the examined *Ternidens* larvae from chimpanzees and Diana monkeys corroborate this assumption, but like the homology of the respective ITS-2 sequences are still no unambiguous proof, as has for instance been shown for *Oesophagostomum bifurcum* specimens from mona monkeys and human hosts in Ghana. Even though the ITS-2 sequences and other genetic markers of the respective worms had revealed no unequivocal nucleotide differences (Gasser et al., 1999a; de Gruijter et al., 2002), further molecular analysis revealed them to constitute two distinct, possibly host-specific *O. bifurcum* variants (de Gruijter et al., 2004, de Gruijter et al., 2005).

Taken together, both, Taï chimpanzees as well as sympatric Diana monkeys harbor infections with *T. deminutus*. Whether or not both primate species are infected with the same *T. deminutus* variant, this parasite is transmitted between them and whether or not Diana monkeys might be its reservoir, remains at this point however speculative, as the genetic markers employed in the present study don't suffice to answer these questions unequivocally.

The validity of the observed absence of *Ternidens* infections in sympatric red colobus monkeys (*Piliocolobis badius*), black and while colobus monkeys (*Colobus polykomos*) and mangabeys (*Cercocebus atys*) remains similarly speculative, as particularly from black and white colobus and mangabeys only very few samples had been examined during the present study. Nevertheless, given that infection with *T. deminutus* might occur through ingestion of infected invertebrate intermediate hosts (Amberson & Schwarz, 1952, Goldsmid, 1971, Goldsmid, 1982), de facto absence of respective infections in the reportedly strictly herbivorous, mainly folivorous red colobus monkeys (Wachter et al., 1997) and black and white colobus monkeys seems overall not unlikely.

V.2.3 *Oesophagostomum* L3-larvae

The prior light-microscopic classification of all 11 examined chimpanzee and monkey *Oesophagostomum* L3-larvae to genus level was invariably confirmed by comparison of their respective ITS-2 sequences with the GenBank database as was the assumption that morphotype A and morphotype B larvae constituted developmental stages of different *Oesophagostomum* species.

Unambiguous identification to species level however was only possible for the four examined chimpanzee *Oesophagostomum* type B larvae, which, based on the homology or near homology of their ITS-2 sequences to the respective GenBank sequence of an *O. stephanostomum* from a chimpanzee in Mahale (Tanzania), were identified as *O. stephanostomum* larvae. A finding which incidentally conforms to the reported infection of two other wild chimpanzee populations (in Tanzania and

Uganda respectively) with this *Oesophagostomum* species (Gasser et al., 1999a; Krief et al., 2010).

By contrast, the specific identity of the four examined chimpanzee *Oesophagostomum* type A larvae, which, despite their morphological similarity upon light-microscopic examination were found to belong to at least two different *Oesophagostomum* species, is less clear.

Notwithstanding the fact that the ITS-2 sequence of larva 38_S3-5_ROM clustered closely with the respective GenBank sequences of *O. bifurcum* specimens from humans and mona monkeys in Ghana (accession no. Y11733.1, AF136575.1) and showed a high similarity of 98.6% to both sequences, the classification of this larva as *O. bifurcum* specimen appears still somewhat speculative.

Even though the discrepancy of 1.4% between the ITS-2 sequence of larva 38_S3-5ROM and the two GenBank *O. bifurcum* sequences might possibly be explained as pronounced within-species variation, it contrasts notably with the observation that no unequivocal nucleotide differences at all were found between the two GenBank sequences themselves (Gasser et al., 1999a) as well as reportedly between the two sequences and the ITS-2 sequences of *O. bifurcum* larvae from fecal material of chimpanzees in Kanyawara/ Uganda (Krief et al., 2010).

As a consequence, also the possibility that larva 38_S3-5ROM might belong to a distinct cryptic species within a potentially existing *O. bifurcum* species complex or might possibly belong to a different, closely related *Oesophagostomum* species cannot be ruled out. The aforementioned observation that examined *O. bifurcum* specimens from humans and mona monkeys in Ghana were found to constitute distinct *O. bifurcum* variants, even though their ITS-2 sequences and other markers had been identical (Gasser et al., 2006; de Gruijter et al., 2002; de Gruijter et al., 2004), corroborates this possibility.

Like the species affiliation of larva 38_S3-5ROM, also the specific identity of the three other chimpanzee *Oesophagostomum* type A larvae (29_S2-217_OLI, 31_S2-324_COC and 30_S2-29_ZYO) remains at this point largely speculative, given their considerable ITS-2 sequence differences (> 6%) to all *Oesophagostomum* species of which ITS-2 sequences had been available in the GenBank database. Many of these *Oesophagostomum* species however are livestock parasites unknown to infect primates. From known primate-parasitic *Oesophagostomum* species by contrast only very few reference sequences are currently available.

As chimpanzees are known to be susceptible to a number of *Oesophagostomum* species of which so far no comparative sequences are available such as for example *O. brumpti* and *O. apiostomum* (Yamashita, 1963; Myers & Kuntz, 1972) both of which occur in Africa, it seems likely that the three type A larvae in question might belong to one of these species. At the same time however, also their affiliation to a

different, possibly yet undescribed *Oesophagostomum* species or species complex cannot be ruled out.

The possibility of a species complex has to be considered with respect to the observed notable 12s RNA mtDNA sequence differences between the three larvae. While their ITS-2 sequences had been largely similar, the 12s RNA mtDNA sequence of 30_S2-29_ZYO differed from the respective sequences of the two other larvae by 4.1%. The mtDNA sequences of larvae 29_S2-217_OLI and 31_S2-324_COC by contrast were largely similar (0.8%).

Given that mtDNA variations among individuals of the same nematode species usually average a fraction of a percent up to 2% (Blouin, 2002), the observed mtDNA differences of more than 4% between larva 30_S2-29_ZYO and the two other larvae seem to be a clear indication for their affiliation to two different cryptic species or species variants. A hypothesis which appears to be further corroborated by the observation that upon phylogenetic analysis the 12s RNA mtDNA sequence of larva 30_S2-29_ZYO occurred on a separate branch compared to the sequences of the other two type A larvae, although the respective branch support was relatively low (55%).

On the other hand however, also within-species mtDNA sequence differences as high as 6% have been described in the literature, for example between a pair of *Ostertagia ostertagi* specimens (Blouin et al., 1998), suggesting that potentially also the 12s RNA mtDNA sequence differences of 4.1% between larvae 30_S2-29ZYO, 29_S2-217OLI and 31_S2-324COC might be no more than somewhat pronounced within-species variations.

Seemingly supported is this suggestion by the observation that mtDNA sequence differences between distinct closely related nematode species seem to be typically in the 10-20% range (Blouin, 2002). To be considered however is that these values for within- and inter-species mtDNA differences (Blouin et al., 1998; Blouin, 2002) have mainly been derived from observed cox1 and nad4 sequence variations. Reference values for respective 12s RNA mtDNA sequence differences by contrast which potentially might be considerably lower are currently not available.

The specific identity of the three *Oesophagostomum* larvae from sympatric monkeys examined in the present study is similary speculative as the identity of the four examined chimpanzee type A larvae.

Based on an ITS-2 difference of only 0.4% mangabey *Oesophagostomum* type A larva 104_S2-9_Mang for instance seems likely to belong to the same *O. bifurcum* variant or cryptic species or else to the same non-*O. bifurcum* species than the morphologically indistinguishable chimpanzee type A larva 38_S3-5_ROM. The 12s RNA mtDNA sequences of both larvae however differed by 4.2% which, as discussed above, might only be a somewhat pronounced within-species variation, but

on the other hand could also indicate that the two larvae belong to different *O. bifurcum* variants or closely related *Oesophagostomum* species.

At this point, without further molecular anylysis, neither of the two possibilities can reliably be ruled out. As Taï chimpanzees and sooty mangabes live in the same habitat, have a similar diet and thus are likely to be exposed to the same infective helminth developmental stages in the environment, infection of both primate species with the same *Oesophagostomum* species seems however not implausible.

The two *Oesophagostomum* type B larvae obtained from sympatric red colobus monkeys by contrast, given sequence differences of 4.9 to 5.3% (ITS-2) and 9.8 to 10.4% respectively (12s RNA mtDNA), clearly don't belong to the same species as the morphologically indistinguishable chimpanzee type B larvae (i.e. are clearly no *O. stephanostomum* larvae). The specific identity of these larvae however remains unclear at this point as does the question as to wether the respective *Oesophagostomum* species is host-specific or might also be infective for the Taï chimpanzees. As also Taï chimpanzees and red colobus monkeys share the same habitat, have an at least partly overlapping diet (Wachter et al., 1997; Boesch & Boesch- Achermann, 2000) and thus might again be exposed to the same infective helminth eggs and larvae, infections of study group members with this *Oesophagostomum* species seem not unlikely. Molecular analysis of more chimpanzee *Oesophagostomum* type B larvae is however necessary to confirm this conjecture.

V.2.4 *Necator* L3-larvae

Just like the morphological classification of all examined *Ternidens* and *Oesophagostomum* L3-larvae to genus level was verified through comparison of their respective ITS-2 sequences with the GenBank database, also the light microscopically diagnosed genus affiliation of all seven examined *Necator* L3-larvae was unequivocally confirmed. Furthermore, based on the observed ITS-2 sequence differences of up to 15% between the seven larvae, the infection of the study population with at least two different *Necator* species was revealed. Given that all seven larvae had been morphologically indistinguishable upon light-microscopic examination, this finding is noteworthy, but on the other hand conforms to the reported susceptibility of chimpanzees to infections with several species of the genus *Necator*, such as *N. concolensis, N. africanus* and *N. americanus* (Orihel, 1971; Ackert & Payne, 1923; Graber & Gevrey, 1981; Yamashita, 1963; Myers & Kuntz, 1971).

Identification to species level was only possible for larva 41_S3-4_CAR which showed 100% and 99.8% respectively identity to GenBank ITS-2 and mtDNA reference sequences of *N. americanus* specimens from human hosts and could thus unequivocally be identified as developmental stage of this *Necator* species.

The specific identity of the other six examined *Necator* larvae by contrast is unclear, as no respective reference sequences were available in the GenBank database. Nevertheless, given the aforementioned susceptibility of chimpanzees to *N. congolensis* and *N. excilidens,* it seems likely that they might belong to one of these two species. Again however also their affiliation to a different, possibly yet undiscribed *Necator* species cannot be ruled out.

Whether or not the observed ITS-2 differences of 0.3% to 1.5% between these six larvae constitute within-species variations or might indicate the existence of different cryptic species is unclear, particularly, as the existence of cryptic species has been suspected for *N. americanus* based on ITS-2 variations of 1.8% between specimens from two geographic localities (Romstadt et al., 1998). However, given the equally observed very low 12s RNA mtDNA sequence differences between the six larvae, within-species variations seem to be the more likely explanation.

The susceptibility of chimpanzees to infections with the primarily human-parasitic hookworm *N. americanus* has, as mentioned before, repeatedly been reported (e.g. Orihel, 1971, Myers & Kuntz, 1971). The majority of these reports however stem from chimpanzees in captivity or wild-caught individuals who had already spent some time in a human environment. As a consequence, these reports provide no evidence for an enzootic existence of *N. americanus* in wild chimpanzee populations, as all infected chimpanzees could have acquired infection from humans (Orihel, 1971). The reported infection of wild chimpanzees on Tiwai Island/ Sierra Leone (Bakarr et al., 1991) is equally no unambiguous proof, as the diagnosis of *N. americanus* in this study had been based on conventional coprology, which as described before does not allow for a reliable species-specific identification of strongyle developmental stages.

The genetically confirmed infection of a wild Taï chimpanzee with *N. americanus* is therefore remarkable and raises the question as to whether this infection was human-transmitted or constitutes a natural infection; two scenarios neither of which can at this point relably be ruled out.

Although the complete and near complete respectively (99.8%) homology between larva 41_S3-4_CAR and the ITS-2 and mitochondrial DNA of *N. americanus* specimens from human hosts (GenBank accession no. AF217891.1 & AJ556134.1) seems to be a clear indication for a human origin of this infection it is still no unambiguous proof. *O. bifurcum* specimens from human hosts and mona monkeys in Ghana for example, as described before, have been found to belong to two different, probably host-specific, genotypic variants despite the absence of unequivocal ITS-2 and mtDNA sequence differences (Gasser et al., 2006; de Gruijter et al., 2004; de Gruijter et al., 2005).

Likewise, genetically distinct, possibly host-specific *N. americanus* variants could potentially also exist in local human hosts and the Taï chimpanzee population or both host species could be naturally infected with the same *N. americanus* variant. As wild

chimpanzees in their natural habitat, harbor infections with various other geohelminths, a natural enzootic presence of *N. americanus* infections in wild chimpanzee populations, including the Taï chimpanzee population, seems not unlikely (Orihel, 1971).

Important to know in this context would be the actual prevalence of *N. americanus* infections among the study population, as in case of an enzootic presence of this parasite, infections of more than one chimpanzee would be expected. The fact that in the present study only one *N. americanus* larva was found is, given the very low overall number of genetically examined *Necator* larvae clearly no conclusive evidence for an infection of only one study group member with this hookworm species.

The possibility of a potential human origin of the observed *N. americanus* infection on the other hand is further supported by the experimentally confirmed ability of infective *N. americanus* larvae cultured from fecal material of infected human hosts to produce patent infections in percutaneously infected chimpanzees (Orihel, 1971). Furthermore, *N. americanus* is reportedly a very common helminthic parasite of man across Africa, including Côte d'Ivoire (prevalence: 50% to 79%, Hotez et al., 2005) and thus very likely also prevalent among the human population in the rural villages around Taï NP. As hence potentially *N. americanus*-infected local people (e.g. poachers), occasionally enter the park and the territories of the three chimpanzee study groups and might possibly also defecate while being in the forest, contamination of the forest floor with human *N. americanus* eggs and larvae and subsequent percutaneous infection of passing chimpanzees is at least theoretically certainly possible.

Overall however, this scenario seems fairly unlikely. One reason is the very localized environmental contamination with *Necator* developmental stages that could possibly be caused by an infected person randomly defecating in the forest in comparison to the fairly large size of the chimpanzee territories (> 16.8 km^2; Herbinger et al., 2001). Second reason is the reportedly limited viability (several weeks; Brooker et al., 2004) of infective *N. americanus* larvae in the environment.

More likely than transmission through random local people entering the forest by contrast would seem a transmission through *N. americanus*-infected TCP (Taï chimpanzee project) researchers or field assistants, as they follow the chimpanzees at close range all day every day. In order to prevent such human-chimpanzee parasite transmissions however, TCP staff and researchers are regularly treated with anthelminthics, and defecation in the forest is prohibited by the project hygiene rules. Yet, it has to be remarked that strict compliance to this regulation is inherently difficult to control, and coprological examinations of researchers and staff are currently not performed.

Taken together, whether the Taï chimpanzee population is naturally infected with *N. americanus* or whether the observed infection of at least one study group member

with this parasite has been human-transmitted remains at this point speculative, as both scenarios seem plausible. To resolve this question, further genetic studies, such as comparison of larva 41_S3-4_CAR with *N. americanus* specimens from human hosts in the Taï area employing more advanced molecular methods, like for instance random amplified polymorphic DNA (de Gruijter et al., 2004) or high resolution DNA fingerprinting (de Gruijter et al., 2005) are thus clearly necessary. Additionally, more detailed studies regarding the prevalence of *N. americanus* among the habituated study population in comparison to its occurrence and prevalence among non-habituated Taï chimpanzees might help to more reliably confirm or reject a potential human origin of the *N. americanus* infection detected in the present study.

Whether or not the observed absence of *Necator* L3-larvae in all fecal cultures performed on fecal material from sympatric monkeys (red colobus monkeys, Diana monkeys, and sooty mangabeys) indicates an overall absence of *Necator* infections in these monkey species in the Taï NP is, given the very low overall number of examined samples, highly speculative. As for the largely arboreal red colobus monkeys, a de facto absence of infection seems overall however not entirely unlikely, as from a number of parasitological surveys of colobus monkeys at other field sites no *Necator* infections have been reported (e.g. Gillespie et al., 2005; Teichroeb et al., 2009, Mbora & Munene; 2006). *Necator* infections have however been reported from red colobus monkeys in Tiwai/ Sierra Leone (Bakarr et al., 1991).

V.2.5 Trichostrongylid L3-larvae

While the phylogenetic analysis of their respective ITS-2 sequences confirmed the classification of the two trichostrongylid larvae (13_S2-3_SUM & 21_S3-12_ATH) as members of the family Trichostrongylidae – which had been suspected on the basis of the larvae's morphological characteristics – unequivocal identification to genus, let alone species level was not possible.

Undisputable however is that the two larvae don't belong to the genus *Trichostrongylus*, as both, their ITS-2 as well as their mtDNA sequences clearly did not cluster with respective GenBank sequences of *Trichostongylus* specimens, but occurred on a branch external to the *Trichostrongylus* clade. Furthermore, also the minimum ITS-2 differences between the two larvae and representatives of the genus *Trichostrongylus (*12.7% to 13%) were considerably higher than the inter-species ITS-2 sequences of 1.3% to 7.6% observed by Chilton et al. (1998) between seven different *Trichostrongylus* species.

A finding that incidentally conforms to the observation that also the morphology of the two larvae was found to not entirely match the descriptions of *Trichostrongylus* L3-larvae given in the literature (Little, 1981).

Instead, the ITS-2 sequences of larvae 13_S2-3_SUM & 21_S3-12_ATH clustered most closely with respective GenBank sequences of two helminth species belonging to the genus *Libyostrongylus* (subfam. Libyostrongylinae) and, upon BLAST search,

showed high maximum homology (92.3%) to the ITS-2 of two *Libyostrongylus douglassi* specimens (GenBank accession no. HQ713428.1 & HQ713429.1).

While these results insinuate that the two larvae most probably belong to the subfamily Libyostrongylinae, the observed relatively high sequence differences (7.7%) and the fact that helminths of the genus *Libyostrongylus* are primarily parasites of ratite birds (Chilton et al., 2001) suggest that they probably don't belong to the genus *Libyostrongylus*, but more likely to a closely related genus, such as for example the genus *Paralibyostrongylus* (subfam. Libyostrongylinae).

Helminths of this genus (e.g. *Paralibyostrongylus hebrenicutus*) are known to parasitize mammalian hosts including lagomorphs and rodents, such as the brush tailed porcupine (*Atherurus africanus*) which incidentally commonly occurs in the Taï NP, but also great apes, specifically mountain gorillas (e.g. Cassone et al., 1992; Durette-Desset et al., 1992; Rothman & Bowman, 2003). Accordingly it seems not unlikely that parasites of this genus might occur in the Taï NP and also cause infections in the local chimpanzee population – particularly as the life cycle of these helminths is direct and infection can occur transcutaneously as well as perorally.

In the absence of any published ITS-2 and mtDNA sequences from *Paralibyostrongylus* species in the GenBank database, the suspected affiliation of larvae 13_S2-3_SUM & 21_S3-12_ATH to this genus as well as their specific identity remain at this point however speculative, especially since no literature records of *Paralibyostrongylus* infections in other wild chimpanzee populations seem to exist.

Also unclear, particularly given the very low overall prevalence of trichostrongylid larvae observed during the present study, remains whether the Taï chimpanzees are a natural hosts of this helminth species or whether they might acquire infections accidentally from other animals or potentially just incidentally ingest and re-excrete respective helminth eggs without developing infections.

V.2.6 *Strongyloides* L3-larvae and free-living adults

Comparison of their respective COI mtDNA sequences with the NCBI database confirmed the prior light-morphological classification of all 20 examined chimpanzee and monkey *Strongyloides* specimens to genus level and furthermore allowed for their unequivocal identification as *S. fuelleborni*. A finding which conforms not only to the observation that all five examined free-living adult female specimens showed species-specific morphological characteristics described for *S. fuelleborni* in the literature (Speare, 1989), but also to the fact that *S. fuelleborni* appears to be the primary *Strongyloides* species parasitizing non-human primates in Africa

At the same time however, also the susceptibility of chimpanzees to the primarily human-parasitic *S. stercoralis* has been described in the literature (e.g. Genta & Grove, 1989). Accordingly, also the presence of at least some *S. stercoralis* specimens wouldn't have been very surprising, especially given the morphological

similarity of *S. fuelleborni* and *S. stercoralis* L3-larvae and free-living adult males as well as the observed infection of at least one study group member with the also primarily human-parasitic *N. americanus*. Additionally, the confirmed concomitant presence of *S. fuelleborni* and *S. stercoralis* has been reported from another wild chimpanzee population (Hasegawa et al., 2010).

To be considered however is that due to the relatively small number of genetically examined *Strongyloides* specimens in the present study, the fact that no *S. stercoralis* specimens were detected in the present study is no proof for the de facto absence of this parasite from the study population.

The relatively high observed COI mtDNA sequence differences of ca. 4% to 7% between all 20 Taï chimpanzee and monkey *S. fuelleborni* specimens and published mtDNA sequences of *S. fuelleborni* specimens from different primate hosts (chimpanzees, gorillas, baboons, and macaques) in Tanzania, Gabon, and Japan (Hasegawa et al., 2010; see this reference for GenBank accession numbers) conform to similar reported sequence variations (5% to >7%) between these three geographic localities.

Remarkable however is that the reported inter-individual sequence differences between the respective *S. fuelleborni* specimens examined at each one of these three localites were comparatively small (up to 2%) (Hasegawa et al., 2010), and that the respective sequences upon phylogenetic analysis formed three distinct, locality-correlated clusters. The COI mtDNA sequences of the 20 Taï chimpanzee and monkey *S. fuelleborni* specimens examined in the present study by contrast showed sequence variations of up to 6.9%, and upon phylogenetic analysis formed three distinct, host species independent within-locality clades, two of which clustered more closely with the *S. fuelleborni* sequences from Tanzania and Gabon than with the third Taï sequence clade.

The reasons for this apparently much higher genetic variability of *S. fuelleborni* specimens from primates in the Taï NP compared to *S. fuelleborni* specimens from Tanzanian, Gabonese, and Japanese primates is unclear.

The conjecture that a different (more or less variable) *S. fuelleborni* subspecies or cryptic species might exist at each geographic locality (Hasegawa et al., 2010) is one at least hypothetical explanation. On the other hand however, given the reportedly quite low numbers of *S. fuelleborni* specimens examined from each of the three localities (Tanzania: n= 6; Gabon: n= 3; Japan: n= 5), it also cannot be ruled out that the actual genetic variability of *S. fuelleborni* in non-human primates in Tanzania, Gabon, and Japan might be considerably higher than observed. A possibility which incidentally appears to be supported by the recently reported very high genetic variability of *S. fuelleborni* specimens from captive, semi-captive and wild Orang Utans (*Pongo pygmaeus*) in East and Central Kalimantan, Borneo/ Indonesia (Labes et al., 2011).

Equally unclear at this point is whether or not all 20 *S. fuelleborni* specimens examined in the present study belong to the same, genetically variable *S. fuelleborni* species variant or whether the apparent differentiation of their respective COI mtDNA sequences into three distinct clades might be an indication for the presence of at least three different cryptic species.

Whether or not Taï chimpanzees and sympatric monkeys are infected with the same *S. fuelleborni* variant(s) or whether host-specific *S. fuelleborni* cryptic species might exist is another speculative question. Given the high homology (up to 100%) between the respective *S. fuelleborni* COI mtDNA sequences from Taï chimpanzees and sympatric monkeys within two of the three observed sequence clades (the third clade contained only one sequence) as well as the direct life-cycle and transcutaneous route of infection of *S. fuelleborni* however, the former possibility rather than the existence of various host-specific variants appears more likely.

V.3 Impact of helminth infections on the health and fitness of the study population

The results of the present study show that all three examined chimpanzee study groups are parasitized by a relatively high number of different gastrointestinal helminths. If and to what extent these parasites might affect and negatively impinge on the individual chimpanzees' health and fitness and, given the reported strong immunmodulatory and -regulatory activity of helminth infections (e.g. Kamal & El Sayed Kalifa, 2006), potentially also on their respective susceptibility and immune response to infections with other pathogens is at this point however largely speculative.

Despite the fact that several of the helminth species found to parasitize the study population, are known to be able to cause considerable pathology in infected hosts (e.g. *O. bifurcum, O. stephanostomum, N. americanus,* (see literature overview)), no evident clinical symptoms clearly attributable to infections with gastrointestinal helminths have been observed throughout the study period in any of the screened chimpanzees, even though in soft feces generally higher numbers of helminth propagules were found. Actual diarrhea however was only occasionally seen and usually occurred simultaneously in all or at least several individuals following the consumption of certain fruits or meat. An association between the occurrence of diarrhea and the excretion of adult worms or tapeworm proglottids, like for instance reported from chimpanzees in Kibale NP/ Uganda (Wrangham, 1995) by contrast has not been noted during the study period. Equally no excretion of bloody feces has been observed.

Potential reasons for the apparent absence of evident clinical symptoms are manifold. Not unlikely for example would seem that the Taï chimpanzees, as they and their natural gastrointestinal helminthic parasites have certainly coexisted over a long period of time, might have developed some degree of resistance against the

pathological effects of these helminths. Krief et al. (2008) on the other hand suspected that deliberate self-medication, i.e. the consumption of plants with mechanical or pharmacological anthelminthic properties, might prevent the development of severe clinical symptoms in wild chimpanzees compared to individuals in captivity who don't have access to respective medicinal plants.

The occasional ingestion of certain leaves (e.g. bristly *Manniophyton fulvum* leaves) for potentially other than nutritional reasons – as they are generally swallowed without chewing and excreted undigested – has incidentally also been reported from several chimpanzee populations including the Taï population (Boesch, 1995) and has also been observed during the present study in most study group members. Whether or not any of the ingested leaves actually possess any anthelmintic potential, is currently however unknown, and further, more targeted studies are necessary to evaluate the extent of self-medication performed by the Taï chimpanzees and its effects on the pattern, pathology and clinical manifestation of helminth infections in this chimpanzee population.

Another at least hypothetical explanation for the observed absence of any obvious clinical symptoms, despite the infection of study group members with potentially quite pathogenic helminths, might be the observation that the overall infection intensities in all screened chimpanzees appeared to be relatively low. Compared to the WHO categories classifying the intensity of gastrointestinal helminth infections in humans, all ppg-values determined in the present study (range: 0 to 1172.0 propagules per gram feces, mean: 54.3 +/- 66.8) clearly qualified as low-intensity infections (range: 0 to 1999 eggs per gram feces) (Montresor et al., 2002).

As a consequence, also the helminth-induced pathology in the infected chimpanzees might have been relatively low and thus not clinically visible. In geohelminth-infected humans for instance, morbidity is usually associated with the number of worms harbored by the individual host, and people with light infections usually show no symptoms (WHO, 2012). To be noted however is that fecal propagule counts, as mentioned before, might not always constitute a very reliable surrogate marker for the actual number of worms harbored by an infected host. Furthermore, assuming the possibility that the MWSFs employed in the present study might have not been able to detect all helminth propagules present in the aliquot of fecal material examined from each sample, also a systematic underestimation of the actual propagule output of all study group members cannot be entirely ruled out.

Nevertheless, the possibility of genuinely low infection intensities in all study group members seems overall not unlikely, particularly considering the relatively large territories and small group sizes of the three study groups and the potentially resulting comparatively low exposure of the individual chimpanzees to infective heminth developmental stages or infected intermediate host.

On the other hand however, it also has to be considered that the overall health and fitness of the study population, despite the absence of any apparent clinical

symptoms, might have still been substantially impaired by the different helminth species parasitizing each chimpanzee. A number of potentially helminth-induced symptoms, such as for example anemia, chronic protein loss (Orihel, 1971; WHO, 2012) or tissue lesions which again might serve as entry ports for other pathogens (Toft, 1982), are difficult or even impossible to detect through non-invasive routine health monitoring and thus might have gone unnoticed during the present study.

Whether or not helminth induced tissue lesions in the gastrointestinal tract of infected chimpanzees might for instance be able to serve as entry ports for orally ingested *B. cereus* bv *anthracis* spores and thus might have facilitated the repeated fatal outbreaks of anthrax observed in the three study groups within the last 15 years (Leendertz et al., 2004; own observations) is at this point purely hypothetical, but seems overall not completely unlikely.

Additionally it cannot be ruled out that infection with multiple gastrointestinal helminth species, through the induction of a Th2 immunity shift (Brady et al., 1999; Cox, 2001; Khamal & El Sayed Khalifa, 2006; Pathak et al., 2012), might have potentially impaired the resistance and immune response of the individual study group members to other pathogens, such as for instance certain viruses, bacteria or protozoa and thus might have increased the virulence of these pathogens in infected individuals.

Bacterial infections in helminth co-infected hosts, as for example reviewed by Pathak et al. (2012), are for instance often associated with greater bacterial proliferation and increased tissue damage, caused by the development of type 2 immune responses against the helminths leading to impairment of the protective type 1 immune response against the bacteria. Likewise, as reviewed by Kamal & El Sayed Khalifa (2006), helminth-induced immunity-shift might also have a negative impact on the outcome of certain viral infections. Human co-infections with *Schistosoma mansoni* and hepatitis C have for instance been associated with immunity-shift-induced increased hepatic viral burdens and increased liver pathology (Kamal et al., 2001 & 2004).

Whether or not the comparatively high number of different helminth types parasitizing the Taï chimpanzee study might for instance have contributed to the observed very severe symptoms and high mortality during the repeated outbreaks of respiratory infections the three study groups have been hit by in recent years (Köndgen et al., 2008), is at this point again purely speculative. Given the aforementioned considerable potential immunmodulatory effects of helminth infections, this conjecture seems overall however not implausible – particularly as from a number of other habituated wild chimpanzee populations (e.g. Fongoli/ Sengal; Budongo/ Uganda) found to be parasitized by notably fewer types of gastrointestinal helminths than the Taï study population (Howells et al., 2011; Asimwe, pers. comm.) so far no outbreaks of respiratory infections or infections with only relatively low morbidity and mortality have been reported (Pruetz, pers. comm.; Asimwe pers. comm.).

Further studies however are clearly necessary to verify this apparent difference in parasite richness between the different chimpanzee populations and its potential impact on the chimpanzees' respective susceptibility to respiratory infections and/or other diseases.

V.4 Zoonotic potential of the helminth types affecting the study population

Just like, as discussed before, infections of study group members with human-transmitted helminths cannot be rule out, also the opposite scenario that is infections of humans, especially of project assistants and researchers, with chimpanzee parasites seems not unlikely.

From one chimpanzee field site in Tanzania for example the infection of one researcher with *S. fuelleborni* has been reported (Hasegawa et al., 2010). Additionally, also the susceptibility of humans to infections with other helminths found in the present study, for example *Trichuris spp*. and *Bertiella spp*. (Hotez et al., 2003; Stunkard, 1940) as well as *O. bifurcum* and *T. deminutus* has been described in the literature (e.g. Polderman et al., 1991; Goldsmid, 1968), whereby especially *O. bifurcum* has the potential to cause considerable pathology in infected people (Polderman & Blotkamp, 1995; Goldsmid, 1982). Whether or not transmissions of this helminth as well as potentially of *T. deminutus* from chimpanzees to humans are indeed possible is at the moment however unclear, as evidence exists that both species might consist of several, potentially host-specific cryptic species (Gasser et al., 2006; Schindler et al., 2005). Nevertheless, particularly with respect to the close genetic relationship between humans and chimpanzees, the possibility of zoonotic transmissions from chimpanzees to humans still has to be considered.

In order to prevent or at least minimize any potential chimpanzee parasite-induced negative effects on the health and wellbeing of the TCP project assistants and researchers, regular parasitological screening of all project members as part of the project's general health and quarantine regime might thus be advisable. That way, the effectiveness of the currently performed anthelminthic treatment of all assistants and researchers could be controlled and treatment intervals, dosages, and employed drugs could be regularly modified and individually adapted as necessary.

This measure would at the same time also help to diminish the risk for any potential future transmission of human parasites from TCP project staff or researchers to the three study groups which, as discussed before, cannot be entirely ruled out due to the inherent difficulty to reliably control the compliance to the strict project rules regarding defecation in the forest. Additionally, the respectively collected stool samples would enable the molecular comparison of the gastrointestinal helminths affecting project staff and Taï chimpanzees and thus might for instance also help to elucidate the origin of the observed *N. americanus* infection of at least one study group member.

V.5 Conclusions

Although the present study, as intended, provided much information and valuable up-to-date baseline data regarding the infection of the Taï chimpanzee population with gastrointestinal helminths, the validity and comparability of these data is somewhat restricted by the various methodological constraints the present study, like probably most parasitological surveys of wildlife populations employing primarily conventional coprology, had been subjected to.

Major constraints were in particular the general inaptitude of conventional coprological examination techniques to allow for reliable specific identification of most helminths as well as the more or less opportunistically collected and thus – in comparison to experimental studies – notably skewed analyzed sample set which could only partly be offset by the additional use of molecular markers, the combination of several conventional examination techniques, the relatively high overall number of examined fecal samples and the relatively long, multi-year and multi-seasonal study period. Additionally, as potential regional variations in the exposure of chimpanzees to infectious helminth developmental stages and/or infected intermediate hosts in different areas of the park might exist, it cannot be ruled out that the results obtained in the present study might not be entirely representative for all chimpanzee groups in the Taï NP.

As a consequence, more detailed and targeted multi-site follow-up surveys employing mainly suitable molecular examination techniques, standardized parasitological long-term monitoring as well as interdisciplinary hormonal and immunological studies will be necessary in order to be able to determine the full helminth spectrum and pattern and origin of helminth infection in this population. Respective studies will furthermore also be needed to more reliably assess potential differences to other wild chimpanzee populations, potential changes in helminth spectrum and infection pattern over time as well as to better understand the impact gastrointestinal helminths have on the health, fitness, and the immune status of the individual chimpanzees. An impact which, as outlined above, particularly in combination with other pathogens might possibly be considerable and thus might ultimately be of sizeable relevance for the conservation and long term survival of the Taï chimpanzee population.

VI SUMMARY

Gastrointestinal helminthic parasites of habituated wild chimpanzees (*Pan troglodytes verus*) in the Taï NP, Côte d'Ivoire - including characterization of cultured helminth developmental stages using genetic markers

Sonja Metzger

Even though chimpanzees have been studied for decades, and disease is known to be one of the major threats to their long-term survival, our knowledge of the pathogens and parasites affecting wild chimpanzees in their natural habitat and their effect on the health and fitness of different chimpanzee populations is still rather limited. The collection of respective baseline reference data and the long-term monitoring of the pathogens and parasites harboured by the different remaining populations of wild chimpanzees, is therefore of great importance – not only to increase our general knowledge and understanding, but ultimately also for the long-term conservation of this great ape species.

In the present multi-year and multi-seasonal study the spectrum of gastrointestinal helminths as well as the pattern and parameters of helminth infection of three study groups of habituated individually known wild western chimpanzees (*Pan troglodytes verus*) in the Taï NP, Côte d'Ivoire were investigated using conventional coprology as well as molecular markers in order to determine detailed and up-to-date helminthological reference data for this chimpanzee population.

Fecal samples were collected between June 2007 and March 2010 from a total of 72 habituated, individually known and identified chimpanzees between ca. 2 and 52 years of age. Unpreserved and/or formalin-fixed sample aliquots were screened on site and/or at the Gillespie Lab, Emory University, Atlanta, macroscopically and employing a total of four conventional coprological examination methods, namely modified Wisconsin Sugar Flotations (MWSFs), Harada-Mori fecal cultures, $NaNO_3$-flotations and sedimentations. Conventional coprological examination revealed the study population to harbour an overall, compared to other wild chimpanzee populations, seemingly very high diversity of gastrointestinal helminths, including a minimum of nine morphologically distinct types of nematodes (representing 8 different genera): *Oesophagostomum spp.* (2 morphotypes), *Ternidens spp., Necator spp.,* Trichostrongylidae fam., *Strongyloides spp.* (*S. fuelleborni*), *Enterobius spp., Trichuris spp., Capillaria spp.,* one cestode: *Bertiella spp.,* and one trematode: Dicrocoeliidae fam.

The prevalence of the different helminth types however was found to vary considerably, as were the examined parameters of helminth infection among the study population. While all 72 coprologically screened chimpanzees were found to be infected with gastrointestinal helminths, substantial, albeit clearly sex- and age-

independent inter-individual variations were observed regarding their respective cumulative helminth morphotype richness. Considerable inter-individual variations were equally noted with respect to the fecal strongyle and *Strongyloides spp.* propagule output of the different study group members, presumably indicating a more or less overdispersed distribution of the respective infection intensities. Additionally however, also a significant male bias towards higher strongyle and *Strongyloides spp.* propagule output, as well as significant seasonal variations were detected. Significant impact of age by contrast was again not seen. The respective underlying causes for all observed considerable and/or significant differences in helminth prevalence and infection parameters could not unambiguously be determined in the present study. Even though a number of potential influencing factors, including various methodological constraints were identified and discussed, their respective true impact needs to be verified in further, more specific studies.

Further studies are equally necessary to assess the validity and underlying causes for the observed seemingly very high helminth diversity in Taï chimpanzees as compared to the majority of other parasitologically screened wild chimpanzee populations. While this difference on the one hand might well be genuine and caused by e.g. environmental or human-induced variations in the parasite exposure of the different chimpanzee populations and/or by differences regarding their genetic susceptibility, it might on the other hand however at least in parts only be a result of the various methodological differences between the individual surveys (e.g. choice of employed methods, sample numbers, and type of preservation). The results of the method comparison performed in the present study underline the latter possibility.

The examination of cultured strongyle larvae and *Strongyloides* specimens in the present study employing molecular markers (ribosomal ITS-2, 12s RNA mtDNA, COI mtDNA) allowed for a much more specific identification of the respective helminth types than had been possible through light-microscopic morphological examination of their eggs and larvae and revealed the study population to be parasitized by at least three different *Oesophagostomum* species, including *O. stephanostomum* and *O. bifurcum*, two different species of the genus *Necator*, including *N. americanus*, one *Ternidens* species (*T. deminutus*), one trichostrongylid species, possibly belonging to the genus *Paralibyostrongylus* or a closely related genus, as well as by at least one *Strongyloides* species (*S. fuelleborni)*.

All examined larvae and free-living adult worms had been cultured on site from unpreserved chimpanzee feces using the Harada-Mori technique; DNA extraction and molecular analyses had been performed at the Department of Molecular Parasitology, Bernhard Nocht Institute, Hamburg.

Comparison of the examined chimpanzee strongyle and *Strongyloides* sequences with respective sequences of helminth larvae and free-living adult *Strongyloides* specimens from sympatric red colobus, Diana monkeys and sooty mangabes (*P. badius, C. diana & C. atys*) as well as sequences from the GenBank data base

indicated potential interspecies- transmissions of helminthic parasites between Taï chimpanzees and sympatric monkeys as well as between humans and chimpanzees. Unequivocal confirmation of these insinuated interspecies transmissions however was not possible. For this, further, more detailed studies employing additional molecular markers and/or other molecular methods are necessary.

The impact of gastrointestinal helminths on the overall health and fitness of the Taï chimpanzee population is unclear. Although no clinical symptoms unequivocally attributable to helminth infections were observed in any of the screened study group members throughout the study period, a considerable negative health impact seems nevertheless not unlikely – particularly with respect to the reported ability of gastrointestinal helminths to induce a Th2 immunity shift in infected hosts and thus to potentially increase the hosts' susceptibility to other pathogens.

Whether or not for instance any connection with the various severe and fatal outbreaks of bacterial and viral disease in the three study groups within the last decades might exist, can at this point however only be speculated.

VII ZUSAMMENFASSUNG

Gastrointestinale parasitische Helminthen habituierter wildlebender Schimpansen (*Pan troglodytes verus)* im Taï Nationalpark, Elfenbeinküste - einschließlich Charakterisierung angezüchteter Entwicklungsstadien mittels genetischer Marker

Sonja Metzger

Obwohl Schimpansen bereits seit Jahrzenten erforscht werden, und Krankheiten eine der größten Bedrohungen für ihr langfristiges Überleben darstellen, ist unser Wissen über die Pathogene und Parasiten wildlebender Schimpansen in ihrem natürlichen Lebensraum wie auch bezüglich ihres jeweiligen Einflusses auf die Gesundheit und Fitness verschiedenere Schimpansenpopulationen noch immer relativ beschränkt. Das Sammeln entsprechender Basis-Referenzdaten, sowie das Langzeitmonitoring der Pathogene und Parasiten der verschiedenen noch verbliebenen Populationen wilder Schimpansen ist daher sowohl für unser allgemeines Wissen und Verständnis, als auch für den langfristigen Erhalt dieser Spezies von großer Bedeutung.

In der vorliegenden mehrjährigen und multisaisonalen Studie wurden das Spektrum gastrointestinaler Helminthen sowie die entsprechenden Infektionsparameter dreier habituierter Gruppen wildlebender Westlicher Schimpansen (*Pan troglodytes verus*) im Taï Nationalpark, Côte d'Ivoire mittels konventioneller parasitologischer Techniken sowie mithilfe molekularer Marker untersucht, um aktuelle und möglichst detaillierte helminthologische Referenzdaten für diese Population zu ermitteln

Kotproben wurden im Zeitraum von Juni 2007 bis März 2010 von insgesamt 72 individuell bekannten Schimpansen im Alter zwischen ca. 2 und 52 Jahren gesammelt.
Unkonservierte und/oder Formalin-fixierte Probenaliquots wurden vor Ort und/oder im Gillespie Labor, Emory University, Atlanta makroskopisch sowie unter Verwendung insgesamt vier verschiedener koprologischer Untersuchungsmethoden (modifizierte Wisconsin Sugar Flotation (MWSF), Harada-Mori Kotkulturen, Natriumnitrat- ($NaNO_3$-) Flotation und Sedimentation) auf das Vorhandensein von Wurmentwicklungsstadien, adulten Würmern sowie Proglottiden hin gescreent. Insgesamt wurde hierdurch eine Infektion der Studienpopulation mit einer, im Vergleich zu anderen wildlebenden Schimpansen-Populationen, scheinbar sehr hohen Diversität an gastrointestinalen Helminthen festgestellt.

Im Einzelnen wurden Eier, Larven und/oder Proglottiden von mindestens neun morphologisch unterschiedlichen Nematoden (*Oesophagostomum spp.* (2 Morphotypen), *Ternidens spp., Necator spp.,* Trichostrongylidae fam., *Strongyloides spp.* (*S. fuelleborni*), *Enterobius spp., Trichuris spp., Capillaria spp.,* einem Cestoden (*Bertiella spp.*) sowie einem Trematoden Typ (Dicrocoeliidae fam.) nachgewiesen.

Sowohl die Prävalenz der einzelnen Wurmtypen, als auch die untersuchten Infektionsparameter variierten allerdings beträchtlich. Obwohl alle 72 insgesamt untersuchten Schimpansen Wurminfektionen aufwiesen, war der jeweilige kumulative Wurmartenreichtum der einzelnen Individuen sehr unterschiedlich, wobei jedoch keine signifikanten Geschlechts- oder Altersunterschiede festgestellt wurden.

Erhebliche individuelle Unterschiede zeigten sich auch bezüglich der Strongyliden- und *Strongyloides* Ei- und Larvenausscheidung der einzelnen untersuchten Schimpansen, was vermutlich auf eine mehr oder weniger aggregative („overdispersed“) Verteilung der jeweiligen Infektionsintensitäten innnerhalb der Studienpopulation schließen lässt. Zudem zeigten männliche Schimpansen eine signifikant höhere Ei- und Larvenausscheidung als weibliche Individuen, und auch signifikante saisonale Unterschiede wurden festgestellt. Eine signifikante Altersabhängigkeit hingegen wurde wiederum nicht beobachtet.

Die jeweiligen Ursachen für die beobachteten erheblichen oder sogar signifikanten Prävalenzunterschiede und Unterschiede bezüglich der einzelnen Infektionsparameter konnten in der vorliegenden Studie nicht eindeutig ermittelt werden. Obwohl eine Reihe potentieller, unter anderem auch methodischer Einflussfaktoren aufgezeigt und diskutiert wurden, muss ihr jeweiliger tatsächlicher Einfluss in zukünftigen, spezifischeren Studien verifiziert und genauer untersucht werden.

Weitere Studien sind ebenfalls notwendig, um die Validität der beobachteten scheinbar sehr hohe Helminthen-Diversität der Taï Schimpansen im Vergleich zu den meisten anderen parasitologisch untersuchten wildlebenden Schimpansenpopulationen sowie die möglichen Gründe hierfür bewerten zu können. Einerseit könnte dieser Diversitätsunterschied nämlich durchaus echt und zum Beispiel durch Umwelt- oder menschlichen Einfluss bedingte Unterschiede in der Parasiten Exposition der einzelnen Schimpansenpopulationen oder auch durch Unterschiede in ihrer jeweiligen genetischen Infektionsempfänglichkeit hervorgerufen sein. Andererseits jedoch kann auch nicht ausgeschlossen werden, dass dieser Unterschied zumindest teilweise lediglich eine Folge der diversen methodischen Unterschiede (z.B. bezüglich Methodenwahl, Probenanzahl, Art der Probenkonservierung) zwischen den einzelnen Surveys ist. Eine Möglichkeit, deren potentielle Bedeutung durch die Ergebnisse des in der vorliegenden Studie durchgeführten Methodenvergleich unterstrichen wird.

Die Untersuchung angezüchteter Strongylidenlarven und *Strongyloides* Spezimen mittels molekularer Marker (ribosomal ITS-2, 12s RNA mtDNA, COI mtDNA) ermöglichte eine deutlich spezifischere Bestimmung der jeweiligen Wurmtypen als durch lichtmikroskopisch morphologische Untersuchung der entsprechenden Eier und Larven möglich gewesen war. Im Einzelnen wurden Infektionen der Studienpopulation mit mindestens drei verschiedenen *Oesophagostomum* Spezies, darunter *O. stephanostomum* und *O. bifurcum,* zwei unterschiedlichen Spezies des

Genus *Necator,* darunter *N. americanus*, einer *Ternidens* Spezies (*T. demintus*), einer nicht spezifisch identifizierbaren Spezies der Familie Trichostrongylidae *(*vermutlich einem Vertreter des Genus *Paralibyostrongylus* oder eines verwandten Genus) sowie mit mindestens einer Spezies (*S. fuelleborni*) des Genus *Strongyloides* diagnostiziert. Larven und freilebende Adultwürmer wurden im Feldlabor vor Ort mittels Harada-Mori Kotkulturen aus frischem, unkonserviertem Schimpansenkot angezüchtet. DNA-Extraktion und molekulare Analysen wurden am Department für molekulare Parasitologie am Bernhard-Nocht-Institut, Hamburg durchgeführt.

Ein Vergleich der untersuchten Schimpansen Strongyliden- und *Strongyloides* Sequenzen mit entsprechenden Sequenzen von angezüchteten Wurmlarven und freilebenden *Strongyloides*-Adultwürmern sympatrischer Roter Stummelaffen, Dianameerkatzen und Rußmangaben (*P. badius, C. diana* und *C. atys*) sowie mit Sequenzen aus der GenBank Datenbank ergab Hinweise auf das potentielle Vorkommen zwischenartlicher Übertragungen von gastrointestinalen Würmern zwischen Taï Schimpansen und sympatrischen Affen einerseits sowie zwischen Menschen und Schimpansen andererseits. Zweifelsfrei bestätigt werden konnte das tatsächliche Vorkommen solcher zwischenartlicher Übertragungen in der vorliegenden Studie allerdings nicht. Hierfür sind weitere Studien unter Einsatz zusätzlich molekular Marker und/oder anderer molekularer Untersuchungsmethoden unbedingt erforderlich.

Der Einfluss den gastrointestinale Helminthen auf die Gesundheit und Fitness der Taï Schimpansenpopulation haben ist unklar. Obwohl bei keinem der untersuchten Studiengruppenmitglieder innerhalb des Studienzeitraums eindeutig auf Wurminfektionen zurückzuführende klinische Symptome beobachtet werden konnten, erscheint ein erheblicher negativer Einfluss, insbesondere im Hinblick auf die in der Literatur beschriebene Fähigkeit gastrointestinaler Würmer, einen Th2-Shift im Immunsytem infizierter Wirte zu induzieren, und dadurch möglicherweise die Anfälligkeit der Wirte gegenüber anderen Pathogenen zu erhöhen dennoch nicht unwahrscheinlich. Über einen beispielsweise potentiellen Zusammenhang mit den innerhalb der letzten Jahrzehnte wiederholt beobachteten schweren und teilweise tödlichen Ausbrüchen viraler und bakterieller Erkrankungen in den drei Studiengruppen kann an dieser Stelle allerdings nur spekuliert werden.

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ANNEX

A.I Temperature and rainfall data

Boxes in Figures A1-A6 represent the respective inter-quartile ranges, bold horizontal bars the respective median. Whisker ends indicate the respective minimum and maximum values that are not outliers, outlier values are depicted as circles.

Figure A1. *Boxplot of recorded daily rainfall from November 2007 to August 2009 (south camp)*

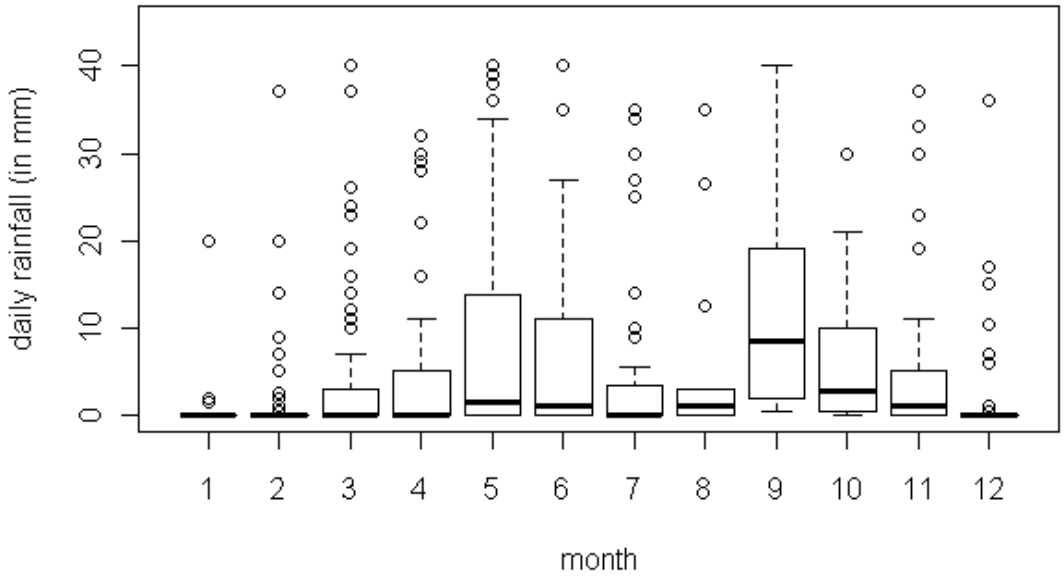
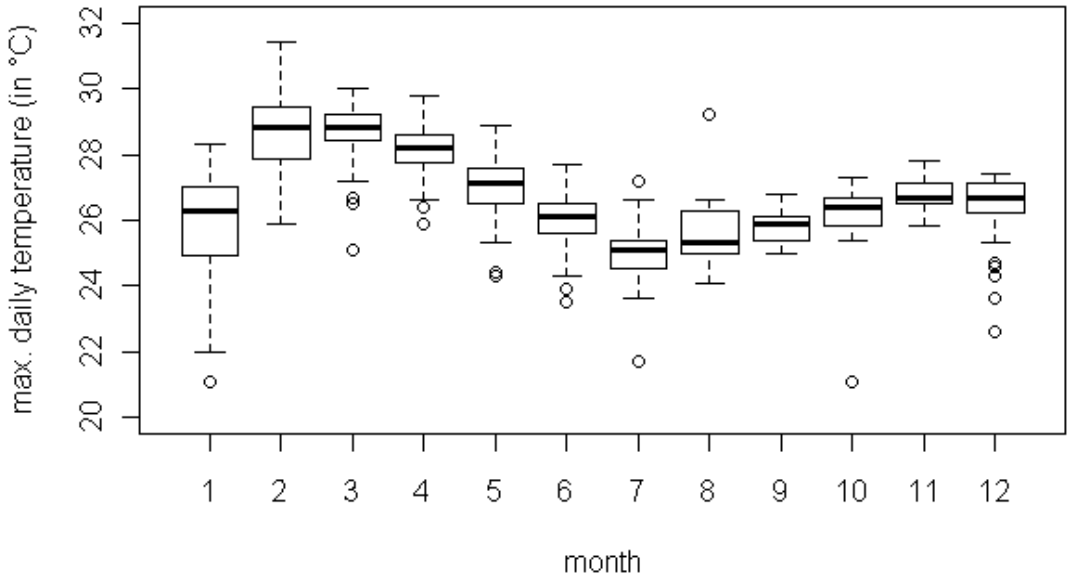


Figure A2. *Boxplot of recorded maximum daily temperatures from November 2007 to August 2009 (south camp)*



A.II DNA-extraction from cultured helminth larvae and free-living adult specimens (using QIAamp® DNA Mini Kit)

Table 1. *Overview of sample volumes and incubation time*

Sample_no	Volume*	Incubation**	Sample_no	Volume*	Incubation**
11_S3-7_BIE	110 µl	over night	45_S3-18UTA	200 µl	2.0 h
14_S3-2_CAR	240 µl	60 min	47_S3-5ROM	220 µl	2.25 h
16_S2-226_ATR	190 µl	90 min	48_S2-4WAP	100 µl	1.75 h
17_S3-9_KIN	500 µl	60 min	13_S2-3SUM	80 µl	over night
18_S3-21_JUL	430 µl	75 min	21_S3-12ATH	220 µl	over night
19_S3-3_TAB	250 µl	105 min	1_S2-44JAC	175 µl	over night
20_S3-11_UTA	330 µl	75 min	2_S2-219TAB	290 µl	2.5 h
69_S3-1_IBR	80 µl	2.0 h	3_S3-7BIE	270 µl	over night
70_S3-1_IBR	80 µl	3.0 h	8_S3-1IBR	80 µl	2.25 h
93_S2-7_Cdian	80 µl	2.5 h	9_S3-25WAL	240 µl	1.75 h
99_S2-7_Cdian	200 µl	2.25 h	55_S2-40UTA	270 µl	2.0 h
29_S2-217OLI	290 µl	2.0 h	56_S2-52RAV	250 µl	1.5 h
31_S2-324COC	150 µl	1.75 h	57_S2-286SAG	120 µl	2.5 h
30_S2-29ZYO	110 µl	2.0 h	61_S3-12ATH	410 µl	2.0 h
38_S3-5ROM	230 µl	1.75 h	65_S2-286SAG	155 µl	3.0 h
33_S2-740UTA	150 µl	over night	73_S2-6Pbad	130 µl	2.5 h
35_S3-1IBR	80 µl	2.0 h	86_S2-14Pbad	410 µl	2.5 h
37_S2-3SUM	120 µl	over night	89_S2-10Pbad	750 µl	1.25 h
39_S2-770SUM	80 µl	3.5 h	90_S2-6Pbad	280 µl	3.0 h
104_S2-9_Mang	120 µl	2.25 h	91_S2-6Pbad	80 µl	1.25 h
76_S2-6_Pbad	80 µl	2.5 h	95_S2-7Cdian	130 µl	2.5 h
79_S2-10_Pbad	260 µl	2.0 h	96_S2-7Cdian	100 µl	1.0 h
41_S3-4CAR	190 µl	2.5 h	102_S2-12Cdian	80 µl	1.25 h
42_S3-9KIN	500 µl	2.0 h	105_S2-9Mang	250 µl	2.25 h
43_S2-330ZOR	140 µl	2.0 h	108_S2-55Mang	100 µl	2.0 h
44_S2-772RAV	280 µl	2.0 h			

* amount of water in which the respective larva or free-living adult specimen had been stored

** time of incubation at 56°C with ATL-buffer and proteinase K

A.III Overview of employed sequencing methods (to III.13)

Table A2. *Overview of obtained strongyle sequences & employed sequencing method*

Examined larva	ITS-2 sequences	Sequencing method**	12s RNA sequences	Sequencing method**
Ternidens spp.				
11_S3-7_BIE	11_S3-7BIE_ITS2	direct	11_S3-7BIE_12s	direct
14_S3-2_CAR	14_S3-2CAR_ITS2_A[A]	cloned	14_S3-2CAR_12s	direct
	14_S3-2CAR_ITS2_B[A]	cloned		
16_S2-226_ATR	16_S2-226ATR_ITS2	direct	16_S2-226ATR_12s	direct
17_S3-9_KIN	17_S3-9KIN_ITS2	direct	17_S3-9KIN_12s	cloned
18_S3-21_JUL	18_S3-21JUL_ITS2	direct	18_S3-21JUL_12s	cloned
19_S3-3_TAB	19_S3-3TAB_ITS2_A[A]	cloned	19_S3-3TAB_12s	cloned
	19_S3-3TAB_ITS2_B[A]	cloned		
20_S3-11_UTA	20_S3-11UTA_ITS2	cloned	none	
69_S3-1_IBR	69_S3-1IBR_ITS2	cloned	none	
70_S3-1_IBR	none	cloned	70_S3-1IBR_12s	direct
93_S2-7_Cdian	93_S2-7_Cdian_ITS2	direct	93_S2-7_Cdian_12s	direct
99_S2-7_Cdian	99_S2-7_Cdian_ITS2	direct	99_S2-7_Cdian_12s	direct
Oesophagostomum spp.				
29_S2-217OLI	29_S2-217OLI_ITS2_A[A]	cloned	29_S2-217OLI_12s	direct
	29_S2-217OLI_ITS2_B[A]	cloned		direct
31_S2-324COC	31_S2-324COC_ITS2	direct	31_S2-324COC_12s	direct
30_S2-29ZYO	30_S2-29ZYO_ITS2	direct	30_S2-29ZYO_12s	direct
38_S3-5ROM	38_S3-5ROM_ITS2	cloned	38_S3-5ROM_12s	cloned
33_S2-740UTA	33_S2-740UTA_ITS2	direct	33_S2-740UTA_12s	direct
35_S3-1IBR	35_S3-1IBR_ITS2	direct	35_S3-1IBR_12s	direct
37_S2-3SUM	37_S2-3SUM_ITS2_A[A]	cloned	37_S2-3SUM_12s_A	cloned
	37_S2-3SUM_ITS2_B[A]	cloned	37_S2-3SUM_12s_B	cloned
39_S2-770SUM	39_S2-770SUM_ITS2	direct	39_S2-770SUM_12s	direct
104_S2-9_Mang	104_S2-9Mang_ITS2	direct	104_S2-9Mang_12s	direct
76_S2-6_Pbad	76_S2-6Pbad_ITS2_A*	cloned	76_S2-6Pbad_12s	direct
	76_S2-6Pbad_ITS2_B*	cloned		
79_S2-10_Pbad	79_S2-10Pbad_ITS2	direct	79_S2-10Pbad_12s	direct
Necator spp.				
41_S3-4CAR	41_S3-4CAR_ITS2	direct	41_S3-4CAR_12s	direct
42_S3-9KIN	42_S3-9KIN_ITS2	cloned	42_S3-9KIN_12s	cloned
43_S2-330ZOR	43_S2-330ZOR_ITS2_A[A]	cloned	43_S2-330ZOR_12s	cloned
	43_S2-330ZOR_ITS2_B	cloned		
	43_S2-330ZOR_ITS2_C[A]	cloned		
	43_S2-330ZOR_ITS2_D[A]	cloned		
44_S2-772RAV	44_S2-772RAV_ITS2	direct	44_S2-772RAV_12s	cloned
45_S3-18UTA	45_S3-18UTA_ITS2	direct	45_S3-18UTA_12s	direct
47_S3-5ROM	47_S3-5ROM_ITS2	cloned	47_S3-5ROM_12s	direct
48_S2-4WAP	48_S2-4WAP_ITS2	cloned	none	

Trichostrongylidae fam.				
13_S2-3SUM	13_S2-3SUM_ITS2	cloned	13_S2-3SUM_12s	cloned
21_S3-12ATH	21_S3-12ATH_ITS2	cloned	21_S3-12ATH_12s	direct

[A] respective multiple amplicon clones from the same larva were identical

** Sequencing method:

+ direct= PCR products sequenced directly at Rober Koch Institut, Berlin using the Sanger method
+ cloned= PCR products cloned into competent *E. coli* (TOP10) with pCR® 2.1.-TOPO® vector; sequenced at Eurofins MWG Operon, Ebersberg with standard universal primer M13rev(-29)

Table A3. *Overview of obtained Strongyloides sequences employed sequencing method*

Examined larva	COI sequence	Sequencing method**
1_S2-44JAC	1_S2-44JAC_COI	cloned
2_S2-219TAB	2_S2-219TAB_COI	cloned
3_S3-7BIE	3_S3-7BIE_COI	cloned
8_S3-1IBR	8_S3-1IBR_COI	cloned
9_S3-25WAL	9_S3-25WAL_COI	cloned
55_S2-40UTA	55_S2-40UTA_COI	cloned
56_S2-52RAV	56_S2-52RAV_COI	cloned
57_S2-286SAG	57_S2-286SAG_COI	cloned
61_S3-12ATH	61_S3-12ATH_COI	cloned
65_S2-286SAG	65_S2-286SAG_COI	cloned
73_S2-6Pbad	73_S2-6_Pbad_COI	cloned
86_S2-14Pbad	86_S2-14_Pbad_COI	cloned
89_S2-10Pbad	89_S2-10_Pbad_COI	cloned
90_S2-6Pbad	90_S2-6_Pbad_COI	cloned
91_S2-6Pbad	91_S2-6_Pbad_COI	direct
95_S2-7Cdian	95_S2-7_Cdian_COI	cloned
96_S2-7Cdian	96_S2-7_Cdian_COI	cloned
102_S2-12Cdian	102_S2-12_Cdian_COI	cloned
105_S2-9Mang	105_S2-9_Mang_COI	cloned
108_S2-55Mang	108_S2-55_Mang_COI	cloned

** Sequencing method:

+ direct= PCR products sequenced directly using the Sanger method at Rober Koch Institut, Berlin
+ cloned= PCR products cloned into competent *E. coli* (TOP10) with pCR® 2.1.-TOPO® vector; sequenced at Eurofins MWG Operon, Ebersberg with standard universal primer M13rev(-29)

A.IV Overview of obtained ITS-2 and 12s RNA mtDNA sequences (to IV.6)

Table A4. *Overview of ITS-2 and 12s RNA mtDNA sequences obtained from chimpanzee Ternidens larvae.*

Examined larvae	Size (µm)	Chimpanzee	ITS-2 sequences	12s sequences
11_S3-7_BIE	590x30	Bienvenue	11_S3-7BIE_ITS2	11_S3-7BIE_12s
14_S3-2_CAR	580x30	Caramel	14_S3-2CAR_ITS2_A &-B	14_S3-2CAR_12s
16_S2-226_ATR	590x30	Atra	16_S2-226ATR_ITS2	16_S2-226ATR_12s
17_S3-9_KIN	650x30	Kinshasa	17_S3-9KIN_ITS2	17_S3-9KIN_12s
18_S3-21_JUL	660x30	Julia	18_S3-21JUL_ITS2	18_S3-21JUL_12s
19_S3-3_TAB	570x30	Taboo	19_S3-3TAB_ITS2_A &-B	19_S3-3TAB_12s
20_S3-11_UTA	600x30	Utan	20_S3-11UTA_ITS2	none
69_S3-1_IBR	550x25	Ibrahim	69_S3-1IBR_ITS2	none
70_S3-1_IBR	540x25	Ibrahim	none	70_S3-1IBR_12s (R only)

Table A5. *ITS-2 and 12s RNA mtDNA sequences obtained from Ternidens larvae of Diana monkeys (Cercopithecus diana)*

Examined larvae	Size (µm)	ITS-2 sequences	12s sequences
93_S2-7_Cdian	640x25	93_S2-7_Cdian_ITS2	93_S2-7_Cdian_12s
99_S2-7_Cdian	620x30	99_S2-7_Cdian_ITS2	99_S2-7_Cdian_12s

Table A6. *Overview of ITS-2 and 12s RNA mtDNA sequences obtained from chimpanzee Oesophagostomum larvae*

Examined larvae	Size (µm)	Type	Chimpanzee	ITS-2 sequences	12s sequences
29_S2-217OLI	820x30	A	Olivia	29_S2-217OLI_ITS2_A &-B	29_S2-217OLI_12s
31_S2-324COC	760x30	A	Coco	31_S2-324COC_ITS2	31_S2-324COC_12s
30_S2-29ZYO	850x30	A	Zyon	30_S2-29ZYO_ITS2	30_S2-29ZYO_12s
38_S3-5ROM	800x30	A	Romario	38_S3-5ROM_ITS2	38_S3-5ROM_12s
33_S2-740UTA	900x30	B	Utan	33_S2-740UTA_ITS2	33_S2-740UTA_12s
35_S3-1IBR	800x30	B	Ibrahim	35_S3-1IBR_ITS2	35_S3-1IBR_12s
37_S2-3SUM	1050x30	B	Sumatra	37_S2-3SUM_ITS2_A &-B	37_S2-3SUM_12s_A &-B
39_S2-770SUM	unknown	B	Sumatra	39_S2-770SUM_ITS2	39_S2-770SUM_12s

Table A7. *Overview of ITS-2 and 12s RNA mtDNA sequences obtained from Oesophagostomum larvae of sympatric monkeys*

Examined larvae	Size (µm)	Type	Monkey species	ITS-2 sequences	12s sequences
104_S2-9_Mang	840x30	A	*C. atys*	104_S2-9Mang_ITS2	104_S2-9Mang_12s
76_S2-6_Pbad	1100x30	B	*P. badius*	76_S2-6Pbad_ITS2_A &-B	76_S2-6Pbad_12s
79_S2-10_Pbad	1000x30	B	*P. badius*	79_S2-10Pbad_ITS2	79_S2-10Pbad_12s

Table A8. *Overview of ITS-2 and 12s mtDNA sequences obtained from Necator larvae*

Examined larvae	Size (µm)	Chimpanzee	ITS-2 sequence	12s sequences
41_S3-4CAR	640x25	Caramel	41_S3-4CAR_ITS2	41_S3-4CAR_12s
42_S3-9KIN	680x25	Kinshasa	42_S3-9KIN_ITS2	42_S3-9KIN_12s
43_S2-330ZOR	570x25	Zora	43_S2-330ZOR_ITS2_A-D	43_S2-330ZOR_12s
44_S2-772RAV	640x25	Ravel	44_S2-772RAV_ITS2	44_S2-772RAV_12s
45_S3-18UTA	590x25	Utan	45_S3-18UTA_ITS2	45_S3-18UTA_12s
47_S3-5ROM	600x25	Romario	47_S3-5ROM_ITS2	47_S3-5ROM_12s
48_S2-4WAP	610x25	Wapi	48_S2-4WAP_ITS2	none

Table A9. *Overview of ITS-2 and 12s RNA mtDNA sequences obtained from chimpanzee trichostrongylid larvae*

Examined larvae	Size (µm)	Chimpanzee	ITS-2 sequence	12s sequences
13_S2-3SUM	710x30	Sumatra	13_S2-3SUM_ITS2	13_S2-3SUM_12s
21_S3-12ATH	780x30	Athena	21_S3-12ATH_ITS2	21_S3-12ATH_12s

Table A10. *Overview of COI mtDNA sequences obtained from chimpanzee Strongyloides specimens*

Specimen	Size (µm)	Type	Chimpanzee	COI sequences
1_S2-44JAC	560x20	L3- larva	Jacobo	1_S2-44JAC_COI
2_S2-219TAB	650x20	L3- larva	Taboo	2_S2-219TAB_COI
3_S3-7BIE	620x20	L3- larva	Bienvenue	3_S3-7BIE_COI
8_S3-1IBR	650x25	L3- larva	Ibrahim	8_S3-1IBR_COI
9_S3-25WAL	520x20	L3- larva	Wala	9_S3-25WAL_COI
55_S2-40UTA	1000x60	free-living F	Utan	55_S2-40UTA_COI
56_S2-52RAV	1150x60	free-living F	Ravel	56_S2-52RAV_COI
57_S2-286SAG	1160x70	free-living F	Sagu	57_S2-286SAG_COI
61_S3-12ATH	750x60	free-living M	Athena	61_S3-12ATH_COI
65_S2-286SAG	800x60	free-living M	Sagu	65_S2-286SAG_COI

Table A11. *Overview of COI mtDNA sequences obtained from monkey Strongyloides specimens*

Specimen	Size (µm)	Type	Host species	COI sequences
73_S2-6Pbad	710x20	L3-larva	*P. badius*	73_S2-6_Pbad_COI
86_S2-14Pbad	850x70	free-living F	*P. badius*	86_S2-14_Pbad_COI
89_S2-10Pbad	600x20	L3-larva	*P. badius*	89_S2-10_Pbad_COI
90_S2-6Pbad	1100x60	free-living F	*P. badius*	90_S2-6_Pbad_COI
91_S2-6Pbad	800x40	free-living M	*P. badius*	91_S2-6_Pbad_COI
95_S2-7Cdian	680x20	L3-larva	*C. diana*	95_S2-7_Cdian_COI
96_S2-7Cdian	610x20	L3-larva	*C. diana*	96_S2-7_Cdian_COI
102_S2-12Cdian	610x20	L3-larva	*C. diana*	102_S2-12_Cdian_COI
105_S2-9Mang	650x20	L3-larva	*C. atys*	105_S2-9_Mang_COI
108_S2-55Mang	660x20	L3-larva	*C. atys*	108_S2-55_Mang_COI

A.V Sequence characteristics (to IV.6)

Table A12. Overview of chimpanzee *Ternidens ITS-2 sequence characteristics.*

Haplotype	Sequence code	Length (bp)	CG-content
a	11_S3-7BIE_ITS2, 17_S3-9KIN_ITS2, 18_S3-21JUL_ITS2, 19_S3-3TAB_ITS2_A&B, 20_S311UTA_ITS2	266	44.0%
b	14_S3-2CAR_ITS2_A&B, 16_S2-226ATR_ITS2, 69_S3-1IBR_ITS2	266	43.6%

Table A13. Overview of Diana monkey (*Cercopithecus diana*) *Ternidens ITS-2 sequence characteristics*

Haplotype	Sequence code	Length (bp)	CG-content
A	93_S2-7Cdian_ITS2	266	44.0%
B	99_S2-7Cdian_ITS2	266	43.6%

Table A14. Overview of chimpanzee *Ternidens 12s RNA mtDNA sequence characteristics*

Haplotype	Sequence code	Length (bp)	CG-content
I	11_S3-7BIE_12s, 14_S3-2CAR_12s, [70_S3-1IBR_12s]*	480 [431]	21.9% [21.8%]
II	16_S2-226ATR_12s	480	22.1%
III	17_S3-9KIN_12s	480	22.3%
IV	18_S3-21JUL_12s	480	22.1%
V	19_S3-3TAB_12s	480	22.7%

* sequenced in one direction (R) only

Table A15. Overview of Diana monkey (*Cercopithecus diana*) *Ternidens 12s RNA mtDNA sequence characteristics*

Sequence code	Length (bp)	CG-content
93_S2-7Cdian_ITS2	480	21,7%
99_S2-7Cdian_ITS2	480	21,9%

Table A16. *Overview of chimpanzee Oesophagostomum ITS-2 sequence characteristics.*

Morphotype	Haplotype	Sequence code	Length (bp)	CG-content
Type A	**a**	29_S2-217OLI_ITS2_A&B, 31_S2-324COC_ITS2	266	46.2%
	b	30_S2-29ZYO_ITS2	266	45.5%
	c	38_S3-5ROM_ITS2	266	43.6%
Type B	**d**	33_S2-740UTA_ITS2, 35_S3-1IBR_ITS2	266	44.7%
	e	37_S2-3SUM_ITS2_A&B, 39_S2_770SUM_ITS2	266	44.4%

Table A17. *Overview of red colobus monkey (Piliocolobus badius) & mangabe (Cercocebus atys) Oesophagostomum ITS-2 sequence characteristics*

Morphotype	Sequence code	Length (bp)	CG-content
Type A	104_S2-9Mang_ITS2	266	43.2%
Type B	76_S2-6Pbad_ITS2_A &-B	266	42.5%
Type B	79_S2-10Pbad_ITS2	266	42.5%

Table A18. *Overview of chimpanzee Oesophagostomum 12s RNA mtDNA sequence characteristics*

Morphotype	Haplotype	Sequence Code	Length (bp)	CG-content
Type A	**I**	29_S2-217OLI_12s	477	20.5%
	II	31_S2-324COC_12s	477	20.3%
	III	30_S2-29ZYO_12s	477	19.9%
	IV	38_S3-5ROM_12s	481	20.2%
Type B	**V**	33_S2-740UTA_12s	477	19.9%
	VI	35_S3-1IBR_12s	477	21.0%
	VII	37_S2-3SUM_12s_A	477	20.3%
	VIII	37_S2-3SUM_12s_B	477	20.3%
	IX	39_S2_770SUM_12s	477	20.3%

Table A19. *Overview of red colobus monkey (Piliocolobus badius) & mangabe (Cercocebus atys) Oesophagostomum 12s RNA mtDNA sequence characteristics*

Morphotype	Sequence code	Length (bp)	CG-content
Type A	104_S2-9Mang_12s	481	21.6%
Type B	76_S2-6Pbad_12s	475	21.3%
Type B	79_S2-10Pbad_12s	477	21.5%

Table A20. *Overview of chimpanzee Necator ITS-2 sequence characteristics*

Haplotype	Sequence code	Length (bp)	CG-content
a	41_S3-4CAR_ITS2	374	43.6%
b	42_S3-9KIN_ITS2	397	40.8%
c	43_S2-330ZOR_ITS2_A-C	396	40.7%
d	44_S2-772RAV_ITS2	396	40.9%
e	45_S3-18UTA_ITS2	394	40.9%
f	47_S3-5ROM_ITS2, 48_S2-4WAP_ITS2 43_S2-330ZOR_ITS2_D	396	40.7%

Table A21. *Overview of chimpanzee Necator 12s RNA mtDNA sequence characteristics*

Haplotype	Sequence code	Length (bp)	CG- content
I	41_S3-4CAR_12s	475	23.2%
II	42_S3-9KIN_12s	473	22.8%
III	43_S2-330ZOR _12s	473	22.8%
IV	44_S2-772RAV _12s	474	22.6%
V	45_S3-18UTA _12s	475	22.7%
VI	47_S3-5ROM_12s	473	22.8%

Table A22. *Overview of chimpanzee trichostrongylid larvae ITS-2 sequence characteristics*

Sequence code	Length (bp)	CG-content
13_S2-3SUM_ITS2	292	33.6%
21_S3-12ATH_ITS2	292	33.2%

Table A23. Overview of chimpanzee *trichostrongylid larvae 12s RNA mtDNA sequence characteristics*

Sequence code	Length (bp)	CG-content
13_S2-3SUM_12s	472	19.5%
21_S3-12ATH_12s	473	20.1%

Table A24. *Overview of chimpanzee Strongyloides COI mtDNA sequence haplotype characteristics*

Haplotype	Sequence Code	Length (bp)	CG-content
a	1_S2-44_JAC_COI, 9_S3-25WAL_COI, 57_S2-286SAG_COI	607	30.5%
b	2_S2-219TAB_COI	607	30.6%
c	3_S3-7BIE_COI	607	32.0%
d	8_S3-1IBR_COI	607	30.3%
e	55_S2-40UTA_COI	607	30.3%
f	56_S2-52RAV_COI	607	31.0%
g	61_S3-12ATH_COI	607	30.6%
h	65_S2-286SAG_COI	607	30,3%

Table A25. *Overview of monkey (Piliocolobus badius, Cercopithecus diana, Cercocebus atys) Strongyloides COI mtDNA sequence haplotype characteristics*

Sequence code	Length (bp)	CG-content
73_S2-6_Pbad_COI	607	30.5%
86_S2-14_Pbad_COI	607	30.5%
89_S2-10_Pbad_COI	607	30.5%
90_S2-6_Pbad_COI	607	30.3%
91_S2-6_Pbad_COI	607	30.5%
95_S2-7_Cdian_COI	607	30.5%
96_S2-7_Cdian_COI	607	30.5%
102_S2-12_Cdian_COI	607	30.3%
105_S2-9_Mang_COI	607	30.5%
108_S2-55_Mang_COI	607	30.6%

A.VI Overview of GenBank reference sequences utilized for phylogenetic analysis

Table A26. *Overview of utilized Chabertiinae subfam. GenBank reference sequences*

Species	Host species	Sequence code	GenBank Access. no.	Locality
+ ITS-2 sequences				
Oesophagostomum spp.				
O. sp.	*Ovis aries*	Oe.sp_sheep_	HQ844232.1	China
O. (Conoveberia) sp.	*Macaca fascicularis*	Oe.sp_macaq_	HM067976.1	China
O. aculeatum	*Macaca fuscata*	Oe.aculeat_	AB586134.1	Japan
O. stephanostomum	*P. t. schweinfurthii*	Oe.stephan	AF136576.1	Tanzania
O. bifurcum	*Homo sapiens*	Oe.bifurc_homo_	Y11733.1	Togo
O. bifurcum	*Cercopithecus mona*	Oe.bifurc_mona_	AF136575.1	Ghana
O. quadrispinulatum	*Sus scrofa*	Oe.quadrisp_	AJ889568.1	China
O. dentatum	*Sus scrofa*	Oe.dent_1_	Y11735.1	unknown
O. dentatum	*Sus scrofa*	Oe.dent_2_	AJ889569.1	China
O. dentatum	*Sus scrofa*	Oe.dent_3_	AJ889570.1	China
O. dentatum	*Sus scrofa*	Oe.dent_4_	AJ889571.1	China
O. dentatum	*Sus scrofa*	Oe.dent_5_	AJ619979.1	China
O. venulosum	*Ovis aries*	Oe.venulos_	Y10790.1	Australia
O. columbianum	*Ovis aries*	Oe.columb_	AJ006150.1	Australia
O. radiatum	*Bos taurus*	Oe.radiat_	AJ006149.1	Australia
Chabertia spp.				
Chabertia ovina	*Ovis aries*	Chab.ovin_	Y10789.1	Australia
Ternidens spp.				
Ternidens deminutus	*Macaca fascicularis*	T.dem_macaq_	HM067975.1	China
Ternidens deminutus	*Papio anubis*	T.dem_baboon_	AJ888730.1	Ghana
Ternidens deminutus	*Cercopithecus mona*	T.dem_mona_	AJ888729.1	Ghana
[***Necator americanus*	*Homo sapiens*	OUT_Nec.am_	**AF21789.1**	Guatemala]
+ mtDNA sequences				
Oesophagostomum spp.				
O. quadrispinulatum	*Sus scrofa domestica*	Oe.quadrisp_	NC_014181.1	China
O. dentatum	*not specified*	Oe.dent_A_	FM161882.1	China
O. dentatum	*Sus scrofa domestica*	Oe.dent_B_	GQ888716.1	Denmark
Chabertia spp.				
Chabertia ovina	*Ovis aries*	Chab.ovin_	NC_013831.1	Australia
[***Necator americanus*	*Homo sapiens*	OUT_Nec.am_	**AJ417719.2**	China]

** outgroups

Table A27. *Overview of utilized Ancylostomatidae fam. GenBank reference sequences*

Species	Host species	Sequence code	GenBank Access. no.	Locality
+ ITS-2 sequences				
Necator sp.				
Necator americanus	*Homo sapiens*	N.am_guat_	AF217891.1	Guatemala
Necator americanus	*Homo sapiens*	N.am_saraw_	AJ001600.1	Sarawak
Necator americanus	*Homo sapiens*	N.am_togo_	AJ001599.1	Togo
Necator americanus	*Homo sapiens*	N.am_mal_G7_	JF960375.1	Malaysia
Necator americanus	*Homo sapiens*	N.am_mal_G27A_	JF960378.1	Malaysia
Necator americanus	*Homo sapiens*	N.am_mal_KP12_	JF960391.1	Malaysia
Necator americanus	*Homo sapiens*	N.am_mal_UY1_	JF960380.1	Malaysia
Necator americanus	*Homo sapiens*	N.am_mal_UY9_	JF960383.1	Malaysia
Bunostomum spp.				
Bunostomum sp.	*Ovis aries*	Bun.sp_	HQ844234.1	China
B. phlebotomum	*Bos taurus*	Bun.phleb_1_	GQ859497.1	China
B. phlebotomum	*Bos taurus*	Bun.phleb_2_	FJ616999.1	South Africa
B. trigonocephalum	*Ovis aries*	Bun.trig_1_	GQ859496.1	China
B. trigonocephalum	*Ovis aries*	Bun.trig_2_	AY439022.1	Scotland
B. trigonocephalum	not specified	Bun.trig_3_	AJ001595.1	not specified
Ancylostoma spp.				
Ancylostoma caninum	not specified	Ancyl.can_	EU159415.1	China
Anyclostoma duodenale	not specified	Ancyl.duo_	AJ001594.1	not specified
[***Oe. dentatum*	*Sus scrofa*	OUT_Oe.dent_	**AJ889569.1**	China
+ mtDNA sequences				
Necator sp.				
Necator americanus	*Homo sapiens*	N.am_china_	AJ417719.2	China
Necator americanus	*Homo sapiens*	N.am_togo_	AJ556134.1	Togo
Ancylostoma spp.				
Ancylostoma duodenale	*Homo sapiens*	Ancyl.duo_	NC_003415.1	China
Ancylostoma caninum	*Canis familiaris*	Ancyl.can_	FJ483518.1	Australia
Bunostomum spp.				
Bunostomum phlebotomum	*Bos taurus*	Bun.phleb_	FJ483517.1	South Africa
[***Oe. dentatum*	not specified	OUT_Oe.dent_	**FM161882.1**	China]

** outgroups

Table A28. *Overview of utilized Trichostrongyloidea superfam. GenBank reference sequences*

Species	Host species	Code	GenBank Access. no.	Locality
+ ITS-2 sequences				
## Fam. TRICHSTRONGYLIDAE				
# Trichostrongylinae subfam.				
Trichostrongylus spp.				
Tr. probolurus	*Ovis aries*	Tr.prob_1_	EF427623.1	Russia
Tr. probolurus	*Ovis aries*	Tr.prob_2_	JF276027.1	Iran
Tr. vitrinus	*Capra aegagrus*	Tr.vit_goat1_	JF276024.1	Iran
Tr. vitrinus	*Capra aegagrus*	Tr.vit_goat2_	JF276025.1	Iran
Tr. vitrinus	*Bos taurus*	Tr.vit_cattle_	JF276026.1	Iran
Tr. vitrinus	*Ovis aries*	Tr.vit_sheep1_	X78064.1	Australia
Tr. vitrinus	*Ovis aries*	Tr.vit_sheep2_	AY439027.1	Scotland
Tr. capricola	*Capra aegagrus*	Tr.capri_1_	JF276022.1	Iran
Tr. capricola	*Capra aegagrus*	Tr.capri_2_	JF276023.1	Iran
Tr. colubriformis	*Ovis aries*	Tr.colub_sheep1_	JF276021.1	Iran
Tr. colubriformis	*Ovis aries*	Tr.colub_sheep2_	HQ389232.1	Iran
Tr. colubriformis	*Homo sapiens*	Tr.colub_hum_	AB503243.1	Laos
Tr. axei	*Ovis aries*	Tr.axei_sheep_	EF427622.1	Russia
Tr. axei	*Bison bison* (zoo)	Tr.axei_bison_	X78065.1	Australia
Tr. tenuis	*Lagopus lagopus*	Tr.tenuis_	X78067.1	Scotland
Tr. retortaeformis	*Oryctolagus cuniculus*	Tr.retort_	JX046418.1	Australia
Ostertagia spp.				
O. ostertagi	not specified	Ost.ost_1_	AF304565.1	not specified
O. ostertagi	not specified	Ost.ost_2_	AB245008.2	Japan
O. nianqingtanggulansis	not specified	Ost.nia_	AJ577459.1	China, Tibet
O. gruehneri	*Rangifer tarandus*	Ost.gue_	AJ400716.1	Norway
Marshallagia spp.				
M. marshalli	not specified	Marsh.marsh_	AJ577469.1	France
M. occidentalis	*Rangifer tarandus.*	Marsh.occ_	AY013244.1	Norway
Teladorsagia sp.				
T. circumcincta	*Ovis aries*	Tela.circ_	HQ389230.1	Iran
Spiculopteragia spp.				
Spic. quadrispiculata	*Cervus elaphus*	Spic.quad_	AF480618.1	Spain
Spic. boehmi	not specified	Spic.boeh_	AJ577460.1	not specified
Spic. houdemeri	*Capricornis crispus*	Spic.houd_	AB682697.1	Japan
# Libyostrongylinae subfam.				
Libyostrongylus spp.				
Lib. douglassi	not specified	Lib.doug_1_	AJ251123.1	France
Lib. douglassi	*Strutio camelus*	Lib.doug_2_	HQ713428.1	Brazil
Lib. douglassi	*Strutio camelus*	Lib.doug_3_	HQ713429.1	Brazil
Lib. dentatus	*Strutio camelus*	Lib.dent_	HQ713422.1	Brazil

## Fam. COOPERIIDAE				
Cooperia spp.				
Cooperia punctata	*Bos taurus*	Coop.punct_	X83560.2	Australia, USA
Cooperia oncophora	*Ovis aries*	Coop.onco_	X83561.2	Scotland
Cooperia curticei	*Ovis aries*	Coop.curt_	JF680982.1	not specified
## Fam. HAEMONCHIDAE				
Haemonchus spp.				
Haemonchus longistipes	not specified	Haem.long_	AJ577461.1	Mauretania
Haemonchus contortus	*Ovis aries*	Haem.cont_	JF680983.1	not specified
[** *Oe. dentatum*	*Sus scrofa*	OUT_Oe.dent_	**AJ889569.1**	China]

+ mtDNA sequences

## Fam. TRICHOSTRONGYLIDAE				
Trichostrongylus spp.				
Tr. axei	*Ovis aries*	Tr.axei_	NC_013824.1	Australia
Tr. vitrinus	*Ovis aries*	Tr.vit_	NC_013807.1	Australia
Teladorsagia spp.				
Tel. circumcincta	*Ovis aries*	Tel.circ_	NC_013827.1	Australia
## Fam. COOPERIIDAE				
Cooperia spp.				
Cooperia oncophora	*Bos taurus*	Coop.onc_	NC_004806.1	Netherlands
## Fam. HAEMONCHIDAE				
Haemonchus spp.				
Haemonchus contortus	not specified	Haem.cont_	NC_010383.2	not specified
[** Oe. dentatum	not specified	OUT_Oe.dent_	**FM161882.1**	China]

** outgroups

Table A29. *Overview of utilized Strongyloides spp. GenBank reference sequences*

Species	Host species	Code	GenBank Access. no.	Locality
S. fuelleborni	*Homo sapiens*	HumImport1_	AB526282.1	returned from Tanzania
S. fuelleborni	*Pan troglodytes schweinfurthii*	ChimpTanz8_	AB526284.1	Tanzania (Mahale)
S. fuelleborni	*Pan troglodytes schweinfurthii*	ChimpTanz7_	AB526285.1	Tanzania (Mahale)
S. fuelleborni	*Pan troglodytes schweinfurthii*	ChimpTanz9_	AB526286.1	Tanzania (Mahale)
S. fuelleborni	*Pan troglodytes verus*	ChimpGab1_	AB526287.1	Gabon
S. fuelleborni	*Pan troglodytes verus*	ChimpGab2_	AB526288.1	Gabon
S. fuelleborni	*Gorilla gorilla*	GorillaGab4_	AB526289.1	Gabon
S. fuelleborni	*Macaca fuscata fuscata*	MacaqueJPO1_	AB526290.1	Japan (Oita)
S. fuelleborni	*Macaca fuscata fuscata*	MacaqueJPS1_	AB526291.1	Japan (Shodoshima)
S. fuelleborni	*Macaca fuscata fuscata*	MacaqueJPS2_	AB526292.1	Japan (Shodoshima)
S. fuelleborni	*Macaca fuscata fuscata*	MacaqueYam1_	AB526293.1	Japan (Yamaguchi)
S. fuelleborni	*Macaca fuscata fuscata*	MacaqueYak1_	AB526294.1	Japan (Yaku)
S. fuelleborni	*Papio cynocephalus*	YelBabTanz1_	AB526306.1	Tanzania (Mahale)
S. stercoralis	*Homo sapiens*	HumTanz1_	AB526297.1	Tanzania (Mahale)
S. stercoralis	*Homo sapiens*	HumJPTok1_	AB526298.1	Japan (Tokyo)
S. stercoralis	*Homo sapiens*	HumJPOk1_	AB526299.1	Japan (Okinawa)
S. stercoralis	*Homo sapiens*	HumJPOk2_	AB526300.1	Japan (Okinawa)
S. stercoralis	*Homo sapiens*	HumJPHam1_	AB526301.1	Japan (Hamamatsu)
S. stercoralis	*Canis familiaris*	DogJP2_	AB526303.1	Japan
S. stercoralis	*Pan troglodytes* (captive)	ChimpCapt1_	AB526304.1	Japan
S. stercoralis	*Pan troglodytes schweinfurthii*	ChimpTanz2_	AB526305.1	Tanzania
S. stercoralis	not specified	unknownUSA_	AJ558163.1	USA
S. planiceps	*Nyctereutes procyonoides*	RaccoondogW1_	AB526295.1	Japan (Wakayama)
S. planiceps	*Nyctereutes procyonoides*	RaccoondogY1_	AB526296.1	Japan (Yaku)
[***N. americanus*	*Homo sapiens*	OUT_N.am_	**AJ417719 .1**	China]

** outgroup

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Selbständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen Anspruch genommen habe.

Ottobrunn, den 30.01.2014 Sonja Metzger