



UNIVERSIDADE DE LISBOA
Faculdade de Medicina Veterinária

GASTROINTESTINAL SYMBIONTS OF WILD CHIMPANZEES AND SYMPATRIC
COLOBUS MONKEYS LIVING IN CLOSE PROXIMITY OF HUMANS IN LIBERIA
AND UGANDA

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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“... and the vessel was not full, his intellect was not satisfied,
his soul was not at peace, his heart was not still.”

Hermann Hesse in *Siddhartha*

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RESUMO

Simbiontes gastrointestinais de chimpanzés e colobos selvagens simpátricos que vivem na proximidade de humanos na Libéria e no Uganda

Esta dissertação teve como objetivo principal caracterizar a fauna de simbiontes de chimpanzés (*Pan troglodytes*), e colobos pretos e brancos (*Colobus* sp.) de diferentes localidades Africanas (Parque Nacional de Sapo, na Libéria e em Bulindi, no Uganda), comparar resultados entre eles e estudos prévios e teorizar sobre as possíveis implicações zoonóticas dos simbiontes encontrados.

Recorrendo a técnicas de coprologia como sedimentação MIF modificada e a flutuação de Sheather, e um teste de imunofluorescência direta para detecção de oocistos de *Cryptosporidium* e cistos de *Giardia*, 47 amostras fecais preservadas de chimpanzé e 15 de colobos pretos e brancos foram examinadas.

Os protozoários detetados incluíram *Troglodytella abrassarti*, *Entamoeba* spp., *Blastocystis* sp. e *Iodamoeba buetschlii*; e nematodes, *Trichuris* sp, *Colobenterobius* sp., strongylideos e várias larvas de nematodes. Todas as amostras foram negativas no teste de imunofluorescência directa.

Em geral, o espectro de simbiontes encontrado no presente estudo está de acordo com o que é regularmente descrito em estudos que abordam chimpanzés e colobos pretos e brancos de vida selvagem. Contudo, pequenos microrganismos aquáticos denominados Rotifera ou rotíferos foram observados pela primeira vez em amostras fecais de chimpanzé e colobos pretos e brancos.

Considerando o contato próximo entre os primatas estudados e seres humanos, é importante realizar futuras pesquisas recorrendo a técnicas moleculares nos simbiontes assinalados por este trabalho e avaliar o seu real potencial zoonótico.

Palavras-chave: chimpanzé, colobos pretos e brancos, primatas, simbiontes, transmissão zoonótica.

ABSTRACT

Gastrointestinal symbionts of wild chimpanzees and sympatric colobus monkeys living in close proximity of humans in Liberia and Uganda

This dissertation aimed to characterize the symbiont fauna of chimpanzees (*Pan troglodytes*) and black and white colobus (*Colobus* sp.) from different African localities, (Sapo National Park in Liberia and Bulindi in Uganda), compare results between them and previous studies, and theorize about possible zoonotic implications of the found symbionts.

Using coprologic techniques as modified MIF sedimentation and Sheather's flotation, and a direct immunofluorescence test for detection of *Cryptosporidium* oocysts and *Giardia* cysts, 47 chimpanzee and 15 black and white colobus preserved fecal samples were examined.

Detected protozoans included *Troglodytella abrassarti*, *Entamoeba* spp., *Blastocystis* sp. and *Iodamoeba buetschlii*; and nematodes, *Trichuris* sp., *Colobenterobius* sp., strongylids and various nematode larvae. All samples were negative for the direct immunofluorescence test. Overall, the spectrum of symbionts found in the present study is in accordance with what is regularly described in studies addressing the free ranging chimpanzees and black and white colobus. However, small aquatic microorganisms denominated Rotifera or rotifers were observed for the first time in chimpanzee and in black and white colobus faecal samples. Considering the close contact between the studied primates and humans, it is important to conduct further research using molecular techniques in the found symbionts to assess the real zoonotic potential.

Key-words: chimpanzee, black and white colobus, primates, symbionts, zoonotic transmission.

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LIST OF ABBREVIATIONS

CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora

EIDs – Emerging zoonotic diseases

IFA – Immunofluorescent Antibody Detection

IMF – direct Immunofluorescence

IUCN – International Union for Conservation of Nature

L – Litre

MIF – Merthiolate-Iodine-Formalin

min – minute

mL – millilitre

PCR – Polymerase Chain Reaction

SNP – Sapo National Park

ULisboa – Universidade de Lisboa

UVPS Brno – University of Veterinary and Pharmaceutical Sciences, Brno

LIST OF SYMBOLS

% – percentage

< – inferior

> – superior

± – plus-minus

1. DESCRIPTION OF INTERNSHIP ACTIVITIES

The curricular internship took place between the 3rd of October until the 22nd of December of 2016, in the department of Pathology and Parasitology, Faculty of Veterinary Medicine from the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

The thesis project was the main activity of the student's time spent in Brno. This included analyses of a set of faecal samples from free ranging chimpanzees and sympatric black and white colobus monkeys, from Liberia and Uganda.

As an intern, besides this project, the author had the chance to be part of the daily basis life of the department, namely, attending and participating in VAPEX (Parasitoses of wildlife and exotic animals); Clinical Parasitology of dog and cat; Parasitology classes; coprologic analyses and immunofluorescence techniques in wildlife and zoological animal samples, with emphasis on primates; getting to know and learn other techniques used in Ph D projects.

In addition to this, the author had other great opportunities, such as being involved in a collection of baboon biological samples (faeces) from Liberec Zoo (CZ); attending to the first elephant foot workshop in Usti Nad Labem Zoo (CZ); some hours in a small animal practice to get acquaintance and gain insight into the local clinical practice.

To complete the author's formation and internship, an intense complementary internship in small animal medicine was held at the Hospital Veterinário do Oeste, Lourinhã, from the 27th of February until the 31st of March 2017.

2. INTRODUCTION and AIMS

Chimpanzees, as the closest relatives to humans, represent an extremely interesting study model for a broad range of researches. Due to that relatedness to humans, they also share with us a range of pathogens, including parasites.

Despite growing amount of parasitological studies, parasites of chimpanzees and other primates remain poorly studied across their natural distribution ranges. However, ongoing dramatic changes in primate's habitat, population decline observed in most of the primate species and dynamically changing interface between primates and man, stress urgent need for research on infectious disease of primates, including studies addressing the parasites. To be able to determine the threats for free-ranging primates and to mitigate their impact, we first need to evaluate what is normal, what is pathogenic and what is "suspicious".

Throughout their natural range, chimpanzees share their habitats with other primates, including colobus monkeys, as black and white colobus. Regardless their diversity and broad distribution, parasitological studies in black and white colobus are few.

Considering that infectious diseases are more potential shared between species that are closely related (as humans and chimpanzees) and inhabit the same geographical region (as black and white colobus), makes this two species relevant object of study.

The major goal of this dissertation was to contribute to understanding of diversity of parasitic, commensal and mutualistic organisms (termed all together as symbionts) of chimpanzees in two African localities and to provide baseline data for further research on symbionts of primates at both sides. The partial aims of this study can be described as follows:

- (1) To investigate the diversity of gastrointestinal symbionts in two populations of common chimpanzees (*Pan troglodytes verus* and *Pan troglodytes schweinfurthii*) that live in close proximity to rural human population in Liberia and Uganda;
- (2) To analyse faecal samples from black and white colobus (*Colobus guereza occidentalis*) living sympatrically with chimpanzees in Ugandan field site;
- (3) To theorize about the zoonotic potential of the found symbionts and analyse risk of possible transmission between common chimpanzee and black and white colobus.

3. BIBLIOGRAPHIC REVIEW

3.1. CHIMPANZEE

3.1.1 TAXONOMY AND DISTRIBUTION

Common chimpanzee (*Pan troglodytes*)

(Blumenbach, 1799)

Kingdom – Animalia

Phylum – Chordata

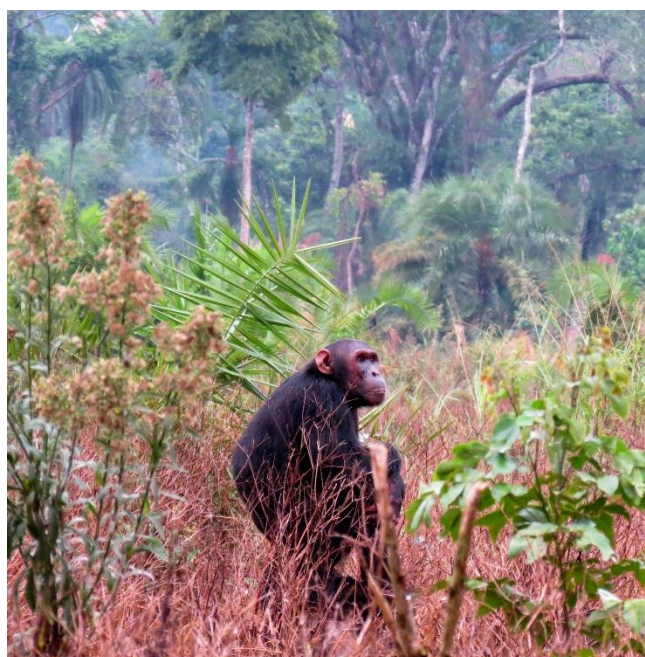
Class – Mammalia

Oder – Primates

Family – Hominidae

Genus – *Pan*

Figure 1 – Chimpanzee from Bulindi (Matt McLennan, authorized)



The word chimpanzee is believed to come from a word with a similar sound spoken by Angolans that means mock-man.

The genus *Pan* is shared between common chimpanzee (*Pan troglodytes*) and bonobo or pigmy chimpanzee (*Pan paniscus*). The latter one, in general less studied, lives exclusively in Democratic Republic of Congo and, due to the long-term instability in the country, they were and are hard to study in the wild (Nackoney et al., 2014). On the outside bonobos are quite similar to a common chimpanzee (from now on always referred only as chimpanzee), but their bigger differences are behavioural like for example, the female is the dominant led and they use intense sexual behaviour as a problem solver and to reduce tension within the group (Prüfer et al., 2012).

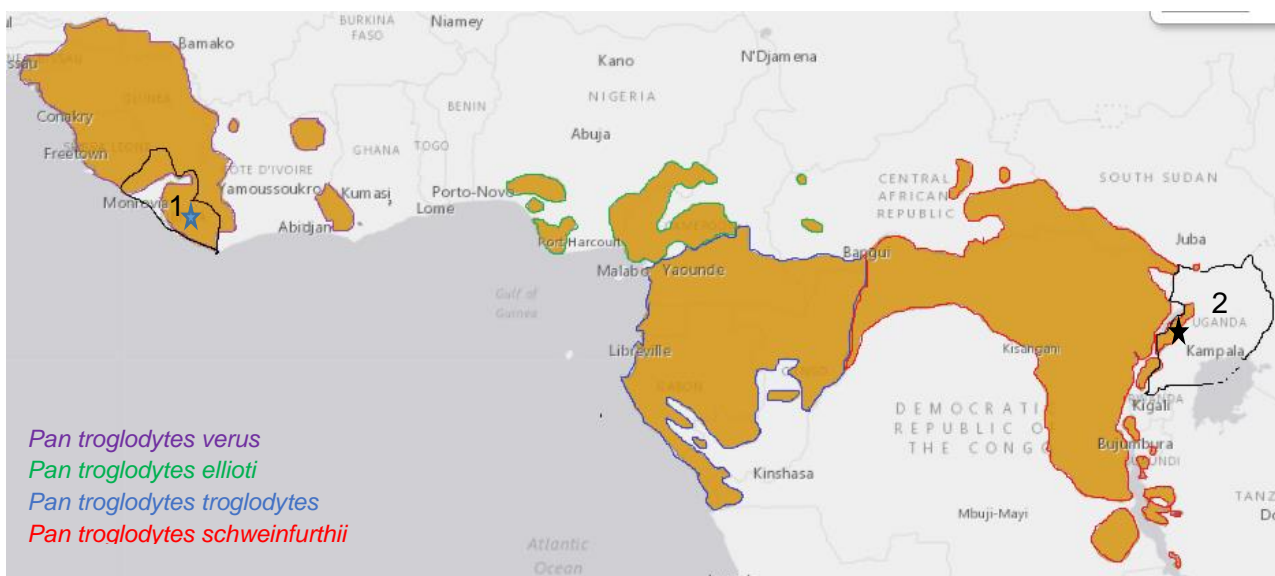
Together with bonobos, chimpanzees are our closest living relative and a great referential model (Diogo, Molnar & Wood, 2017). In fact, Varki & Altheide (2005) reported a difference between human and chimpanzee genome to be around 4%.

There are four recognized subspecies of chimpanzee based on differences in appearance and geographic distribution: the western (*P. t. verus*), the nigerian-cameroonian (*P. t. ellioti*), the central (*P. t. troglodytes*) and the eastern (*P. t. schweinfurthii*) chimpanzees (Fünfstück et al., 2015). They inhabit various ecosystems, ranging from closed canopy rainforest (Boesch & Boesch-Achermann, 1991), woodland (Nishida & Uehara, 1983; Goodall, 1986) and grassland (McGrew, Baldwin & Tutin, 1981). The IUCN (International Union for Conservation of Nature)

red list positions the species in the endangered (EN) level, being one of the subspecies (*P. t. verus*) critically endangered (CR), it is listed on Appendix I of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and, as Class A under the African Convention on the Conservation of Nature and Natural Resources (Humle, Maisels, Oates, Plumptre & Williamson, 2016).

Among great apes, chimpanzees have the widest geographic range and appear more resistant to anthropogenic impacts (McLennan & Asiimwe, 2016). They have discontinuous distribution from southern Senegal across the forested belt north of the Congo River to western Tanzania and western Uganda, being the four subspecies uneven distributed (Humle et al., 2016) (figure 2). Due to high levels of poaching for bushmeat and trade, infectious diseases, and habitat loss and quality caused by expanding human activities, this species is estimated to have experienced a significant population reduction in the past years (Humle et al., 2016). In 2003, Butynski estimated the chimpanzee population size between 172,700 and 299,700 individuals, being *P. t. schweinfurthii* the major contributor and *P. t. ellioti* the minor.

Figure 2 – Distribution map of the four Chimpanzee subspecies.
Adapted from *Pan troglodytes*. The IUCN RED List of Threatened Species. Version 2017-2



(1) Liberia, (blue star) Sapo National Park; (2) Uganda, (black star) Bulindi.

3.1.2. GROUP SIZE, DIET, RANGING AND SOCIAL BEHAVIOUR

The ontogenetic development of a chimpanzee is not very different from humans. They are totally dependent on their mothers until age three, but their infancy lasts until age five, followed by childhood and puberty until age thirteen (Goodall, 2010). Chimps start to look old at 45 years old. Although the known life span in the wild is 45–50 years, it can be much longer in captivity. In fact, the oldest chimpanzee died recently in her late 70's (Little Mama, Lion Country Safari: <https://www.lioncountry safari.com/oldest-living-chimpanzee-little-mama-passes-away-at-lion-country-safari/>).

Field observations show that chimpanzees reside in multimale-multifemale, fission-fusion communities, averaging 35 members of all ages, with a fixed home range and led by a dominant male. All community members know each other but feed, travel and sleep in much smaller groups. Community members associate in temporary parties that vary in size, composition and duration for territory patrol and hunting purposes. The presence of oestrus females and food availability influence chimpanzee party size (larger at these moments) (Mitani & Watts, 2005). The daily travel distance is proportional to the food available.

Chimpanzees live in a complex society, being the alpha male the one that keeps the order within the group, usually through aggression. Many aspects of their behaviour, social relation, expression of emotions and needs are similar to those in humans (Goodall, 2010). They have a rich repertoire of calls and use a panoply of non-verbal communication as kissing, hugging, pats on the back, play tickling, swaggering, punching, etc.

Chimpanzees build their own nest to sleep during the night and rest during the day (Plumptre & Reynolds, 1997). A new nest, made out of leaves, is built every night (and some also during the day), or in case they use an old one, at least bend some new branches over it (Brownlow, Plumptre, Reynolds & Ward, 2001). Each chimpanzee has its own nest, except infants that sleep with their mothers until age five or until the next new baby is born (Goodall, 2010).

Chimpanzees have the ability to craft and use tools to solve a great range of problems, using grass stems, sticks, leaves, rocks and branches. This behavior varies between populations and infants must observe adults to learn it (Goodall, 2010).

Feeding behaviour in chimpanzees varies seasonally and is significantly influenced by food availability and habitat type/subspecies. Chimpanzees have a diverse diet, ranging from fruit, bark, leaves, stems, seeds and flowers, they hunt a variety of mammals (bushpigs, monkeys as colobines, antelopes) and invertebrates (ants, termites, caterpillars) and search also for bird eggs, nuts and honey (Goodall, 2010). When living in close proximity to human agricultural

land, particularly in the low forest fruiting season, they can crop-raid (e.g. Tweheyo, Hill & Obua, 2005).

For example, chimpanzees of the Budongo Forest Reserve (Uganda) spend 80% of their daytime feeding (followed by resting (18%) and other activities), which fruits are the dominant part of their diet (71%) (Tweheyo, Lye & Weladji, 2004), while in chimpanzees from the Tai Forest (Côte d'Ivoire) meat is a great part of their diet especially during the wet season (Boesch & Boesch-Ancher mann, 2000). Meat eating behavior is also a generator of social activity and prolonged associations between individuals (Boesch & Boesch-Ancher mann, 2000).

Besides being commonly considered an abnormal behavior in captive chimpanzees, wild chimpanzee's populations can also (rarely) engage in coprophagy behaviors, either autocoprophagy (eating one's own faeces) and allocoprophagy (eating faeces from others). The cause of this behavior is unknown and can be attributed to various hypothesis, but the more plausible seems to be an adaptive feeding strategy for hard seeds during periods of food scarcity (Payne, Webster & Hunt, 2008; Sakamaki, 2010).

Numerous studies demonstrated self-medication behaviour across chimpanzee populations. Chimpanzees have been described to swallow leaves of specific plants that are folded and swallowed without chewing and defecated undigested. They seem to increase gut motility, expelling this way adult nematode worms trapped inside leaf folds or on its surface (Huffman, Gotoh, Turner, Hamai & Yoshida, 1997).

3.2. BLACK AND WHITE COLOBUS

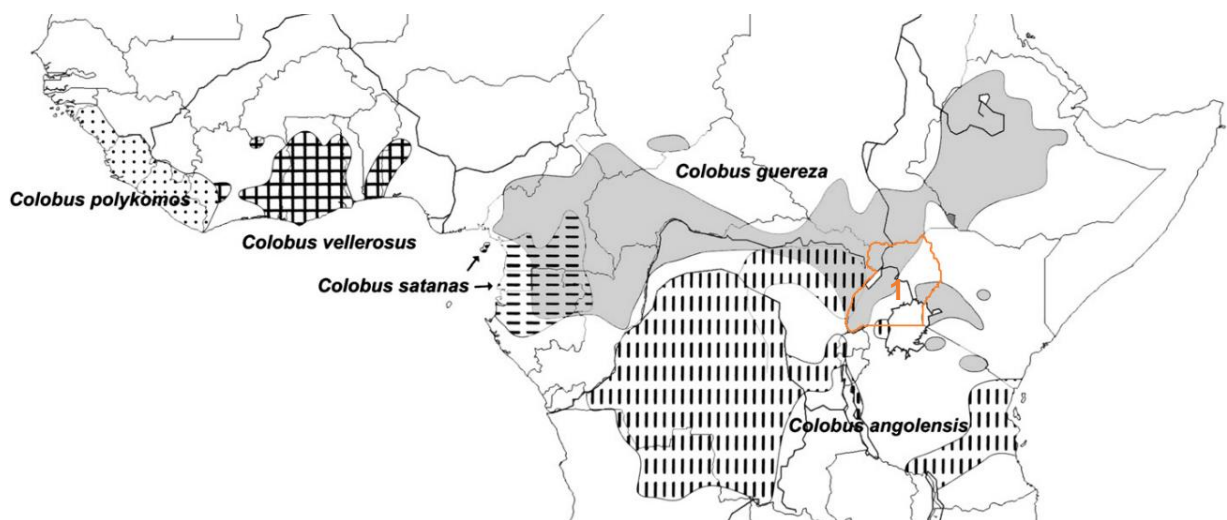
3.2.1 TAXONOMY AND DISTRIBUTION

Colobus monkeys are Old World monkeys found in Africa. The name “colobus” is derived from the Greek word for “mutilated,” because they have a small nub where the thumb should be.

In general, the African colobines are represented by three distinct genera: black-and-white colobus (*Colobus* spp.), olive colobus (*Procolobos* (*Procolobus*) *verus*) and red colobus (*Procolobus* (*Piliocolobus*) spp.) (Ting, 2008).

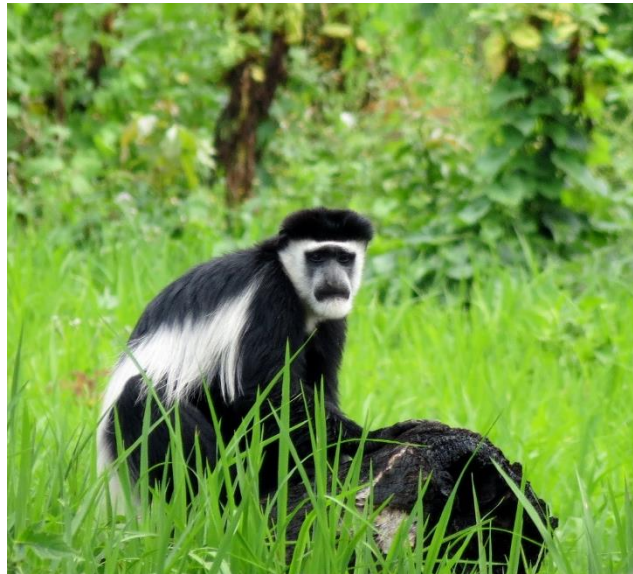
The black and white colobus species, have a disperse distribution and inhabit the woodlands and tropical forests throughout equatorial Africa (Oates 1977; Oates & Trocco, 1983) (figure 3). With some disagreements within the scientific community, there are five recognized species (*Colobus polykomos*, *C. guereza*, *C. angolensis*, *C. satanas* and *C. vellerosus*) with more subspecies related to each (Oates & Trocco, 1983; Grubb et al., 2003).

Figure 3 – Distribution map of Black-and-White colobus (*Colobus*) species. (1) Uganda.
(Adapted from Ting, 2008)



The studied colobines were *Colobus guereza occidentalis* (fig. 4). This subspecies is listed as Least Concern (LC) in the IUCN red list, on Appendix II of CITES and on Class B of the African Convention on the Conservation of Nature and Natural Resources (Kingdon, Struhsaker, Oates, Hart & Groves, 2008).

Figure 4 - Black and white colobus (*Colobus guereza occidentalis*) from Bulindi
(Matt McLennan, authorized)



3.2.2. GROUP SIZE, DIET, RANGING AND SOCIAL BEHAVIOUR

Colobus are arboreal monkeys, rarely descending to the ground. Their mantle hair and tails are believed to act as a parachute during the long leaps between branches.

Black and white colobus are sexually dimorphic. Oates (1977), in agreement with older studies, described group sizes with a range of 3–15 individuals, with at least one fully adult and one large sub-adult males, 2–5 adult females and their young offspring. A typical group has a highly cohesive structure, where the animals have a close spatial relationships engaging in affiliative interactions, as grooming (Oates, 1977). Relations between other groups are generally tense and hostile, but mixed-species associations with other primates have been described (Chapman & Chapman, 2000).

Adult males black and white colobus produce characteristic vocalizations such as the “roaring”, which is audible from a great distant, and the “snort” for short distances only (Marler, 1972). These vocal behaviours are most of the times related and vary between predator encounters (Schel, Tranquilli & Zuberbühler, 2009).

These folivorous monkeys are adapted to a leaf eating diet, facilitated by a multi-chambered ruminant-like stomach. Besides that, they have a flexible diet that might include fruits and seeds (Dasilva, 1992) that can be obtained through raiding agricultural crops (Chapman, Speirs, Gillespie, Holland & Austad, 2006).

Red colobus are known to eat charcoal to help with their digestion, since charcoal eliminates toxic compounds that are present in some of the ingested leaves (Struhsaker, Cooney & Siex, 1997).

3.3. HUMAN IMPACT

With the increase of human population and population density, speeding destruction and fragmentation of the wildlife habitats, the growing contact seems inevitable. Logging, agriculture, establishment of new human settlements lead to habitat fragmentation (Chapman, Gillespie & Goldberg, 2005). Habitat fragmentation and creation of edges, with different conditions than the forest core, forces the animals to concentrate into smaller areas (fragments) increasing animal density affecting their behaviour and their possible infectious diseases (Benitez-Malvido & Arroyo-Rodríguez, 2008).

The population decline across all chimpanzee subspecies can be directly or indirectly linked to human activities. Among the demonstrated stressors, the infectious diseases play a significant role. The so called emerging infectious diseases (EIDs), some of them zoonotic, pose a serious threat not only for human health, but also for endangered animals. This is especially true for apes, whose close phylogenetic relationship with humans increases the risk of zoonotic transmission (Davies & Pedersen, 2008).

Colobines are forest-dependent and are intensely threatened by human activities that cause decrease in the forest cover (Gillespie, Greiner & Chapman, 2005). Besides that, they have great aptitude for ecological flexibility.

Furthermore, all these phenomena support growing demand for wild animal meat (so called bushmeat) and pet-owning, which significantly contributes to local extinction or diversity of vertebrate species and to spreading of infectious diseases. The meat of wild animals represents not only an important part of the local's diet, but also a source of their income. Due to changes in hunting patterns (fire arms used), habitat fragmentation, logging, agriculture and so on, it is easier to kill either for bushmeat or as pet to collect these animals from nature, helping spread their parasites and other infectious diseases (LeBreton et al., 2006; Pourrut et al., 2011).

Although not always targeted, chimpanzees are accidentally trapped in snares and may suffer from severe and permanent mutilation, which can influence the symbiont prevalence in these affected animals (Yersin, Asiimwe, Voordouw & Zuberbühler, 2016).

Specific phenomenon related mainly to the great apes is the habituation, which consists in repeated neutral contacts between primates and humans that lead to a reduction of fear, with the purpose of the animal ignoring an observer (Williamson & Feistner, 2003). Regardless the habituation for tourism or research purposes, habituated apes always have close contact with humans, contributing to the pathogen exchange. The demonstrated cases involve mainly the respiratory viral diseases (e.g. Köndgen et al., 2006; Zommers, Macdonald, Johnson & Gillespie, 2013), however, increased presence of humans in habitats of great apes might contribute also to the risk of transmission of parasitic diseases.

3.4. GASTROINTESTINAL PARASITES/ SYMBIONTS OF PRIMATES

Parasitism is a type of relationship between organisms, compulsory or not, with benefits for the parasite and different level of harm for the host. However, part of the organisms that inhabit the gastrointestinal system of vertebrates are difficult to be classified as “real” parasites, as their pathogenic effect was never proved or is questionable. To avoid misleading classification, the term symbiont is used, as it is broader and includes also the parasites as such. In general the symbionts can be (i) mutualists (reciprocally positive interactions between pairs); (ii) commensals (neither benefit nor harm their facilitators); and (iii) parasites (Bronstein, 2009; Sá et al., 2013).

Close phylogenetic and evolutionary relationship between humans and great apes results in a partial overlap of symbiont fauna and potential risk of pathogen transmission (Kalousová, 2010).

Environmental changes and land use transformation are worldwide affecting the dynamics of disease transmission between wild populations of primates and humans (Gottdenker, Streicker, Faust & Carroll, 2014), making a priority to study and assess the real zoonotic potential of parasites.

It should be also taken into consideration that parasite infection intensities in primates can be affected by several factors including season, as well as social factors, age, sex, or dominance rank of the animals (Huffman et al., 1997)

Although parasites can coexist with their hosts without apparent damage, it is important to consider the long-term effects they may have for general health, survival, and reproduction of the host.

This chapter summarizes the typical gastrointestinal symbiont fauna of chimpanzees and black and white colobus with emphasis on the studies on free-ranging animals.

3.4.1. PROTISTS

The gastrointestinal system of primates is, similar to that of other mammals, colonized by a range of protozoans. Whilst some of them are obviously harmless commensals, several others are considered true parasites with negative impact on their hosts. In the following overview, it is summarized the basic data on major protozoan groups detectable in primate faeces. Recently, it was proven that faeces can also be used for the detection of DNA of vector-borne protozoans (like *Plasmodium* (e.g. Mapua et al., 2016) and *Trypanosoma* (e.g. Jirků et al., 2015) spp.) however, this text focus only on those detectable by light microscopy and immunofluorescence technique.

Among the parasitic protozoans, the genera *Giardia* and *Cryptosporidium*, attract particular interest. They are able to infect humans, livestock and wild animals, representing an important cause for diarrheal disease in humans. They both have a direct life cycle, being transmitted faecal-oral way (Smith, Cacciò, Cook, Nichols & Tait, 2007; Debenham, Atencia, Midtgaard & Robertson, 2015).

Giardia duodenalis is a species complex with seven distinct groups called assemblages, the assemblages A and B are considered having major zoonotic potential (Sprong, Cacciò & van der Giessen, 2009).

Cryptosporidium is a genus of opportunistic protists infecting a wide range of hosts, notable for cross-species transmission due to the low host specificity of some of the species/genotypes (Parsons et al., 2015).

Giardia and *Cryptosporidium* are reported in free ranging and captive chimpanzees with different prevalence and probably underestimated since infected animals can be low or intermittent shedders, which points the problem with one shot sampling and diagnostics (Kváč, 2015). Both *Giardia* and *Cryptosporidium* were also detected using microscopic and molecular techniques in free ranging black and white colobus (Salyer, Gillespie, Rwego, Chapman & Goldberg, 2012; Kouassi et al., 2015).

Protozoans of the genus *Blastocystis* are ubiquitous among the vertebrates and invertebrates and the opinions about their pathogenicity varies greatly. Some studies suggest that they may be harmless commensals that become pathogenic in immunocompromised individuals (Doležalová, 2015a). However, the pathogenic role of *Blastocystis* is still unclear as asymptomatic shedding is highly common in immunocompromised as well as immunocompetent individuals (Drakulovski et al., 2014).

The cysts detectable in faeces represent the infectious stage and are responsible for faecal-oral transmission or contamination of water and food.

Molecular studies have addressed the genetic diversity of *Blastocystis* isolates, with up to 13 distinct subtypes (ST1–ST13) (see e.g. Petrášová et al., 2011). As the PCR based diagnostics was repeatedly proved as more sensitive, the coproscopy-based data about the occurrence and prevalence should be treated with caution.

Blastocystis was first reported in black and white colobus in 2009 (Teichroeb, Kutz, Parkar, Andrew Thompson & Sicotte, 2009), but is a common report in chimpanzees (e.g.: Kalousová, 2010; Drakulovski et al., 2014; McLennan et al., 2017).

Entamoeba spp. are parasites or commensals commonly found in mammals including primates and humans (Hooshyar, Rostamkhan & Rezaeian, 2015).

To this day, seven *Entamoeba* species (*Entamoeba histolytica*, *E. dispar*, *E. nuttalli*, *E. moshkovskii*, *E. coli*, *E. poleckii* and *E. hartmanni*) have been described in non-human primates (Vlčkova, 2017). Entamoebas represent a common finding in both chimpanzees and black and white colobus (e.g.: Okanga, Muchemi, Maingi, Mogoia & Munene, 2006; McLennan et al., 2017).

Entamoebid amoebas can be divided in groups based on the number of nuclei inside the cysts: uni-, tetra- and octonucleated (Jirků-Pomajbíková, 2015).

Entamoebas have a simple life cycle, in which the trophozoite (vegetative state) lives in the large intestine or tissue of the host and the cyst (transmissible stage) allows survival outside the host and transmission (Vlčkova, 2017). Faecal-oral transmission and environment contamination by resistant mature cysts perpetuates the infection.

Most entamoebas are considered harmless commensals with the exception of the pathogenic ones (*E. histolytica* and *E. nuttalli*) belonging to the tetranucleated group. Most infections are asymptomatic but if clinical signs are present (amoebiasis), they consist of bloody-mucoid diarrhea, vomitus, weakness, general malaise, crouched posture, anorexia and weight loss (European Association of Zoo and Wildlife Veterinarians, 2003). Amoebiasis, an infection by pathogenic amoeba *E. histolytica* is listed as third leading parasitic disease causing human mortality (Nath, Ghosh, Singha & Paul, 2015), but is rarely reported fatal in chimpanzees (Fremming, Vogel, Benson & Young, 1995) and in black and white colobus (Ulrich et al., 2010). Molecular methods are crucial for differential diagnosis, especially to distinguishing commensal from pathogenic species (Verweij et al., 2003; Tachibana et al., 2007; Levecké et al., 2010; Dong et al., 2017).

Among other amoebas, *Iodamoeba buetschlii* is considered to be a commensal amoeba commonly found in humans, pigs and other mammals' intestines. It has been described diarrheal illness in immuno-compromised humans (Tanyuksel & Petri Jr., 2003).

It is reported in a low prevalence in chimpanzees (e.g.: Howells, Pruetz & Gillespie, 2011; Sá et al., 2013; McLennan et al., 2017). It was reported in a medium prevalence in black and white colobus from Taï forest (Koassi et al., 2015).

Entamoebas are not the only typical commensal in the apes' intestine. Colon of chimpanzees (and other African great apes) is inhabited by a diversity of entodiniomorphid ciliates, as *Troglodytella abrassarti* and *Troglocorys cava*. Entodiniomorphids are typically non-pathogenic and, leastwise *T. abrassarti* is involved in hindgut fermentation of fibre (Profousová et al., 2011a).

These ciliates do not form cysts and the transmission between hosts is facilitated by trophozoites. Since their survival outside the host is limited (trophozoite fragmentation starts at 30hr after defecation), close contact between animals is necessary for transmission (Profousová, Petrželková, Pomajbíková & Modrý, 2011b).

Routine faecal flotation and sedimentation are good techniques to easily detect the infective stage, the trophozoite. However, as prevalence varies between different studies for various reasons, Kaur, Singh & Lindsay (2010) recommends that at least three stool samples from different collection days from an individual chimpanzee, should be found to be negative before it can be considered truly negative for trophozoites of *T. abrassarti*.

Balantioides coli, a facultative pathogenic ciliate, is frequently reported in captive great apes but rarely found in free-ranging African great apes. It is also the only known ciliate to infect humans (Schuster & Ramirez- Avila, 2008).

Increased contact with reservoir hosts (e.g. pigs and humans) and diet, influences presence and population of ciliates in the intestine. Regarding the diet of captive chimpanzees, low in fiber and starch rich, seems to be responsible for high intensities of infection and might predispose captive animals to clinically balantidiasis (Shovancová et al., 2013), however, in many cases the infection is asymptomatic (Pomajbíková, Petrželková, Profousová, Petrášová & Modrý, 2010b).

3.4.2. HELMINTHS

Following the common classification schemes, the helminth parasites are divided into three major groups: trematodes (Digenea), cestodes (Cestoda) and nematodes (Nematoda).

Trematodes are rarely recorded in wild chimpanzees (e.g. Gillespie et al., 2010; McLennan et al., 2017) and in black and white colobus (Gillespie et al., 2005; Teichroeb et al., 2009). *Concinnum* sp. and *Dicrocoelium* sp. have been reported in chimpanzees (Healy & Myeres, 1973). *Schistosoma mansoni*, one of the most important trematodes of man, is actually shared quite heavily between the staff and the chimpanzee population of Ngamba Island, Lake Vitoria in Uganda (Standley et al., 2011).

Sedimentation is the advised technique since the eggs are rather heavy but should be complemented with molecular and serological techniques for a more accurate diagnose (Standley et al., 2011). Life cycle is indirect (requires a mollusk as intermediate host), which possible can be related to the low frequency of occurrence.

Bertiella sp. is the only cestode recorded regularly in wild chimpanzees, but in a rather low prevalence (Kawabata & Nishida, 1991; Ashford et al., 2000; Howells et al., 2011; McLennan et al., 2017). It has an indirect life cycle with oribatid mites involved as intermediate host.

There are reported associations between leaf swallowing behaviour and *Bertiella* sp. because the leaves probably stimulate the shedding of the gravid segment, a natural part of the cestode life cycle (McLennan et al., 2017). There are several species of *Bertiella* known from primates, however, species distinguishing is almost impossible based on the egg morphology. A recent study employing molecular data proved a rather complex situation, with unexpected genetic diversity suggesting the existence of several *Bertiella* species in primates and humans (Doležalová et al., 2015a). So far, finding in chimpanzee are referred to as *Bertiella studeri* (Doležalová, 2015b). *Bertiella* sp. was described also in black and white colobus (Gillespie et al., 2005), however, without detailed data.

Nematodes are a large group of helminths, found in all kinds of habitats. Very adapted from free-living existence through commensalism to a facultative and ultimate obligate parasitism. Such broad group of helminths, as parasitic nematodes are, shows a large range of adaptations, including the transmission to a new host. Primates can get infected by ingestion of infective stages (egg or third stage larvae L3) from the environment or by larval penetration of the skin (Healy & Myeres, 1973). In case of nematodes with indirect life cycle, the ingestion of arthropod intermediate host (e.g. spirurids) or inoculative transmission by blood-feeding arthropod (filaroids) are common ways through which the nematodes reach their final host.

Ascaris spp. (the roundworms) are potential parasites of chimpanzees but rarely reported in free ranging African apes (Landsound-Soukate, Tutin & Fernandez, 1995). On the contrary, human *Ascaris lumbricoides* and pig *A. suum*, seem to be involved in the common infection of captive chimpanzees (Nejsum et al., 2006).

Ascaridic nematodes are only rarely reported in black and white colobus in a low prevalence (Gillespie et al., 2005; Teichroeb et al., 2009).

Strongyloides spp., namely *Strongylides stercoralis* and *S. fulleborni*, have been reported and characterized in chimpanzees (Hasegawa, Hayashida, Ikeda & Sato, 2009). Eggs and larvae are quite similar, however, both species can be tentatively distinguished based on stages detected in fresh samples: *S. stercoralis* eggs hatch shortly after deposition, so larvae are usually present in faeces in case of infection by this species, while presence of the eggs is characteristic for *S. fulleborni* (Speare, 1989).

Most common strongylids in chimpanzees are members of genera *Oesophagostomum*, *Necator*, *Ancylostoma*, and/or *Ternidens*.

Eight species of *Oesophagostomum* have been recognized so far to occur in non-human primates (Blotkamp et al., 1993). Among them, *Oesophagostomum bifurcum*, *O. stephanostomum* and *O. aculeatum* are also reported in humans (Polderman & Blotkamp, 1995).

Although multi-nodular lesions are commonly associated with this parasite infection, wild chimpanzees seem to tolerate *Oesophagostomum* related injuries without clinical signs (McLennan & Asiimwe 2016), with the exception of some chimpanzees in Mahale (Huffman et al., 1997) and Gombe (Terio et al., 2016). Self-medication behaviour as rough leaf swallowing in wild chimpanzees can be triggered by repeated *Oesophagostomum* spp. infection (Huffman et al., 1997).

Necator americanus and *Ancylostoma duodenale* are the main hookworm species infecting humans (Hasegawa et al., 2014) and common in free-ranging great apes including chimpanzees (Kalousová & Hasegawa, 2015).

Taxonomy of this group of nematodes is largely based on adult worm's morphology and the identification from faeces is always complicated. Whilst the morphology of L3 larvae (obtained by coprocultures) can help to identify the strongylids at least at generic level, the species identification based on larvae and eggs requires molecular techniques (e.g. Ghai, Chapman, Omeja, Davies & Goldberg, 2014; Ota et al., 2015). *Strongyloides* and strongylid nematodes are probably the most prevalent parasites in wild chimpanzees (e.g. Ashford et al., 2000; Muehlenbein, 2005; Gillespie et al., 2010; McLennan et al., 2017), with the exception for Savanna-dwelling chimpanzees (Kalousová, 2010).

Strongyloides and strongylids nematodes are also common in colobus (Gillespie et al., 2005; Teichroeb et al., 2009).

Whipworms from genus *Trichuris* (Enoplida) have been recorded in several primates including wild chimpanzees and colobines (e.g. Gillespie et al., 2005; Sá et al., 2013; Ebbert, McGrew & Marchant, 2015).

Trichuris trichiura, originally a *Homo sapiens* pathogen, is traditionally considered having a broad host range and expected to be the only species that occurs in primates. Recent studies using molecular tools (e.g. Nissen et al. 2012; Cutillas, de Rojas, Zurita, Oliveros & Callejón, 2014; Doležalová et al., 2015b) suggest a more complex scenario. *T. trichiura* might in fact consist in a group of several species, some being found only in non-human primates and existence of several new species, as e.g. *Trichuris colobae* described recently from *Colobus guereza kikuyensis* (Cutillas et al., 2014).

Species determination based exclusively on egg morphology is practically impossible and adult worms are hard to obtain from wild primates (Doležalová et al., 2015b). Molecular analyses are a powerful approach and should be used to evaluate the diversity of trichurid species in chimpanzees and colobus.

Pinworms (Oxyurida) occur in the majority of primates. They are rarely reported in the wild probably due to the fact that routine coproscopic examination is fairly suboptimal method for their detection. Chimpanzees are parasitized by their own pinworm species, *Enterobius anthropopithecii*, however captive chimpanzees can be infected with the human originating *E. vermicularis*, which is rarely fatal (e.g. Murata, Hasegawa, Nakano, Noda & Yanai, 2002).

Eggs can be found in faecal examination by sedimentation techniques, but it represents a very incomplete way to enquire prevalence. As female pinworms deposit eggs around perianal skin of primates, they are more reliably diagnosed by examination of the perianal skin or by necropsy (Ashford et al., 2000), barely applicable in the field. Gauze washing or the Baerman method is a way to obtain adults.

Colobenterobius is the genus of pinworms in colobus monkeys, however individual species can only be recognized by the adult worms' morphology (Hasegawa, Greiner & Gillespie, 2008). *Probstmayria* spp. (*Probstmayria gombensis* and *P. vesiculata*) are viviparous nematodes phylogenetically close to oxyurids that are probably common throughout the distribution range (File, McGrew & Tutin, 1976). However, as the detection of these nematodes depends on finding adults or rather large larvae, the proper detection requires sieving of the faecal material followed by the thorough stereomicroscope examination (Hasegawa, 2015a).

Among the nematodes with heteroxenous life cycles, the spirurid nematodes (Spirurida) are commonly reported from most of the chimpanzee populations, though in low prevalence and in low infection intensities (e.g. McLennan et al., 2017). The savanna chimpanzees tend to have higher prevalence's of spirurids, probably due to larger amount of insects in their diet (Kalousová, 2010). Based on the reported egg morphology, it is obvious that several species are involved in the infection of great apes. However, it seems that chimpanzees (and gorillas) are more or less accidental, or occasional hosts, for the species that occur in other animals. The spirurids detected in chimpanzees are most commonly referred to as members of the genera *Protospirura* or *Streptopharagus*. However, this identification is rather tentative, considering limited value of egg morphology for the determination (Hasegawa, 2015b). *Chitwoodspirura* eggs (Spirurida) were reported in red colobus (Kooryama et al., 2012), but there is no information of spirurid nematodes in black and white colobus.

4. MATERIAL AND METHODS

4.1. STUDY SITE

4.1.1. LIBERIA

Sapo National Park (SNP) is part of the Sapo-Grebo-Taï complex, which represents one of the largest and most important forest complex in West Africa. It's located in Liberia, containing some of the most intact ecosystems of the country covering 180 365 ha (hectares), composed predominately of lowland rainforest, swamps and riparian forest, a river and some streams.

There are several sympatric primates species inhabiting SNP, diurnal species as chimpanzee (*Pan troglodytes verus*), red colobus (*Procolobus badius*), black and white colobus (*Colobus polykomos*), olive colobus (*Procolobus verus*), sooty mangaby (*Cercocebus torquatus*), diana monkey (*Cercopithecus diana*), lesser spot-nosed monkey (*Cercopithecus petaurista*) and mona monkey (*Cercopithecus mona*); and some nocturnal species as bushbabies (Galagonidae) and pottos (*Perodicticus potto*) (David Modrý, personal communication, January 28, 2018).

Human settlement and activities are forbidden inside the park, however, there are 70 communities surrounding the park and throughout the years there has been some illegal activities threatening the park.

Regarding the weather, it is considered a tropical climate with temperatures ranging between 22–28 °C, with two marked seasons: dry, from November to April and wet, from May to October.

4.1.2. UGANDA

Bulindi is located in Hoima District in western Uganda, between Budongo and Bugoma forest reserves, linking these two together. These reserves are separated by approximately 50 km (McLennan & Ganzhorn, 2017). Since Bulindi is not a reserve or a National Park, the landscape is densely populated by humans and dominated by subsistence and commercial agriculture surrounding small unprotected riverine forest fragments.

This area is inhabited by sympatric primate species, as chimpanzee (*Pan troglodytes schweinfurthii*), black and white colobus (*Colobus guereza occidentalis*), tantalus monkey (*Chlorocebus tantalus budetti*), blue monkey (*Cercopithecus mitis stuhlmanni*) and olive baboon (*Papio anubis*) (McLennan & Plumptre, 2012).

There is a rainfall pattern: wet, from March – May and August – November and dry, from December to February, while June-July are transient months. The average annual temperature in Bulindi is 22.3 °C.

4.2. STUDY POPULATION

4.2.1. SNP

Western chimpanzees are still common inhabitants of SNP and surrounding community forests. They are usually not hunted and frequently visit the immediate vicinity of villages at the perimeter of SNP. The enormous size of Sapo forest, difficult accessibility of many parts and rather mobile chimpanzee groups, makes the detailed data about the structure and size of the chimpanzee population not available.

A 1983 study (Anderson, Williamson & Carter, 1983) estimated the SNP chimpanzee's density in 0.24 individuals per square kilometre (km²).

4.2.2. BULINDI

4.2.2.1. CHIMPANZEES

A genetic census from 2015 reported that 256–319 chimpanzees from at least nine distinct groups/ communities live in this fragmented forest landscape (McCarthy et al., 2015). The Bulindi chimpanzee community is one of these, with a home range of 20 Km² but usually spend most of their time in a 'core area' of less than 5 km² (McLennan & Asiimwe, 2016).

These chimpanzees were first studied in 2006 (McLennan & Hill, 2010). In late 2016, the community was formed by 22 individuals, including 6 adult and 1 subadult females, 2 adults and 1 subadult males, 1 juvenile female, 3 juvenile males, 4 infant females and 4 infant males (infants: 0–3 years; juveniles: 4–8 years; subadults: females: 8–first birth / males: 8–12 years; adults: females: > first birth/ males: >12 years) (Matt McLennan, personal communication, December 29, 2017).

4.2.2.2. BLACK AND WHITE COLOBUS

The most recent information about black and white colobus is a master's thesis from 2014 (Lorenti, 2014), that evaluated the black and white colobus abundance in several forest fragments from Bulindi. The highest concentration was 13.41 ± 0.24 individuals per ha and the lowest 3.45 ± 0.38 individuals per ha (abundance \pm Standard Error)

4.3. SAMPLING AND SAMPLE PRESERVATION

In order to assess the gastrointestinal symbiont fauna of SNP and Bulindi chimpanzees, forty-seven (seventeen and thirty respectively) faecal samples were identified and collected from the field. Fifteen black and white colobus faecal samples were also collected in Bulindi.

Seventeen faecal samples from chimpanzees were collected by David Modrý and his team in SNP, Liberia during September 2016 (17 sampling days). Due to adverse weather conditions the tracking of the animals was very difficult and the faeces collection delayed (estimated as 12–24 hours after defecation). Closer identification of animals was not possible as these chimpanzees are unhabituated.

The samples were collected under each recently used nest (previous evening) and only one stool was collected in order to avoid repeated sampling of the same individual. Chimpanzees predictably defecate and urinate out of their nests on to the ground below, before leaving them in the morning (McGrew, Ensminger, Marchant, Pruetz & Vigilant, 2004). Nests are usually used only by one individual, except when there is an infant sleeping with its mother, where there is a clear difference on faeces size.

In Bulindi, Uganda, Klára J. Petrželková and her team collected thirty faecal samples from chimpanzees and fifteen from black and white colobus during October 2016 (12 sampling days).

The studied chimpanzee group was habituated and allowed a close tracking of the animals and also a very fresh faeces collection (within minutes after defecation), however, considering the general low amount of samples and the uneven number of samples per animal (some had three, some only one), the samples were treated as individual samples. Regarding black and white colobus, the team followed the group and founded/ collected the faeces from the trails (no more than 12 hours after defecation).

Before collecting a sample, its consistency was inspected, presence of blood, mucus, tapeworm proglottids, fly larvae and larger nematodes were noted, if any. Following basic personal safety, using gloves and optionally a protective mask and with help of sterile spatulas, samples were collected from inside the faecal bolus and homogenized; then individually stored in 10% formalin solution (dilution 1:9 in treated/drinkable tap water). The tubes were labelled, air-tightly closed and sealed with Parafilm M® and later shipped by plane under the necessary legal permits to the Department of Pathology and Parasitology of the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic (UVPS Brno). The samples were analysed at the same department, as part of the author's internship chores, between October and December of 2016.

4.4. COPROSCOPIC METHODS

Coproscopic examination is a set of methods and techniques to diagnose symbionts stages in faeces. In order to achieve accurate results it is recommended to apply several techniques at the same time.

Before parasitological analyses, each faecal sample was labelled, homogenized and strained through 2 sheets of gauze to a 50 millilitres (mL) conical Falcon tube in order to decant the formalin; tap water was added to make up 50 mL and centrifuged (5–10 minutes (min) at 466 xg). Supernatant was discarded and the remaining sediment was fixed with 10% formalin (4% formaldehyde) to make up 10 mL.

4.4.1. MODIFIED MERTHIOLATE – IODINE - FORMALIN (MIF) SEDIMENTATION (Blagg, Schloegel, Mansour & Khalaf, 1995)

MIF sedimentation method is used in fresh samples as the solution used has formaldehyde, a fixative. For preserved (fixed) samples, like the studied ones, there is an alternative protocol where the MIF solution is replaced by formalin.

Two mL of faecal suspension were mixed with 5 mL of 10% formalin and 6 ml of ether in a 15 mL tube and centrifuged (2 min at 262 xg). Supernatant was poured off, the remaining sediment was transferred with a Pasteur pipette onto a microscopic slide and a drop of Lugol's iodine was added, then, sample was cover-glassed and examined by light microscopy under 100x, 400x and 1000x magnification.

4.4.2. SHEATHER'S FLOTATION WITH MODIFIED SUGAR SOLUTION (1.33) (Sheather, 1923)

To prepare the modified Sheather's sugar solution, 1563 grams of sugar were mixed with 1 litre (L) of boiling water and, at the end, 9 mL of phenol, as preservative, was added.

Two ml of faecal suspension (re-suspended faecal sediment) was mixed with tap water in a flotation tube and centrifuged (3 min at 466 xg). Supernatant was removed and the sediment was mixed with the sugar solution and centrifuged again (3 min at 466 xg). The surface film from the top was transferred with a horizontal culture loop onto 2 microscopic slides and cover-glassed. Each slide was examined by light microscopy under 100x and 400x magnification.

4.4.3. DIRECT IMMUNOFLUORESCENCE (IMF) (MERIFLUOR®)

This test is an in vitro direct immunofluorescent detection procedure for the simultaneous detection of *Giardia* sp. cysts and *Cryptosporidium* sp. oocysts in faecal material. The detection reagent contains a mixture of monoclonal antibodies directed against cell wall antigens of *Giardia* sp. cysts and *Cryptosporidium* sp. oocysts.

One drop of sediment (recovered after the first centrifugation step in the flotation method) was saved in the fridge until the execution of this test.

The procedure followed the manufacture instructions that went as follows: using a transfer loop, one drop of the faecal material was transferred to a treated slide well and spread over the same, without scratching. Using a different loop for each, one drop of positive and one of negative control was dropped into two different wells, of the same slide as the sample, and spread over without scratching the surface of the slide. The slide was allowed to dry completely at room temperature (more or less 30 min). One drop of Reagent Detection and Counterstain were placed in each well, mixed and spread. The slide was incubated in a humidified chamber for 30 minutes, so it could be protected from light. The slide was rinsed with a gentle stream of wash buffer until excess of the last added reagents were removed. The excess of buffer was removed with help of a clean paper towel. One drop of montage oil was added and the cover slip applied. The slide (wells) was scanned on a florescent microscope using the 100–200x magnification.

If the sample is positive for *Giardia* sp. cysts and *Cryptosporidium* sp. oocysts, it should be found apple green color and characteristic oval to round shapes while the background material should counterstain in a dull orange to red.

4.5. EQUIPMENT

Centrifuge: HERMLE Z 200A

Microscope: OLYMPUS BX53

Microscope camera: OLYMPUS DP73

Incubator: LABOTECT Inkubator C16

4.6. DATA ANALYSES AND STATISTICS

Data was analysed using The R Project for Statistical Computing version 3.3.3.

Due to the nature of the samples, the preformed statistics are more descriptive. It is reported sample symbiont prevalence (percent of samples with a given taxa) and Chi-square test of independence to examine the relation between chimpanzee symbionts and the localities (SNP, Bulindi).

5. RESULTS

Forty-seven chimpanzees and fifteen black and white colobus faecal samples were examined by modified MIF sedimentation, Sheather's flotation and MERIFLUOR® *Cryptosporidium/Giardia* direct immunofluorescence detection assay in order to determine parasites/commensals. Spectrum of detected taxa in faeces of both species of chimpanzee includes several, protists, namely: *Troglodytella abrassarti*, *Entamoeba* spp., *Blastocystis* sp. and *Iodamoeba buetschlii*; and nematodes: *Trichuris* sp., strongylids and various nematodes larvae. Examination of black and white colobus revealed, protist: *Entamoeba* sp.; nematodes: *Trichuris* sp., strongylids, *Colobenterobius* sp. and various nematodes larvae.

Stages of rotifers, a microorganism commonly found in freshwater environments, were found in faecal samples from tested animals.

5.1. DESCRIPTION OF OBSERVED STAGES

Intestinal amoebas

Entamoeba cysts (Amoebozoa: Entamoebidae) (figs. 5, 6 and 7) were observed using sedimentation techniques, with and without Lugol's iodine staining. Diagnostic is based mainly on the morphology of nuclei and, their number, presence of glycogen vacuole and cyst size. Observed *Entamoeba* cysts were almost spherical with obvious nuclei with centrally located karyosome and distinct peripheral chromatin around. Measured cysts of *Entamoeba* in examined samples had sizes comprehending 6–17.5 µm for Bulindi chimpanzees and 8–12 µm for black and white colobus.

Iodamoeba buetschlii (Amoebozoa: Mastigamoebae) cysts were typically ellipsoidal and having only one nucleus and one glycogen vacuole that stained darker with lugol's iodine. Observed cysts from SNP chimpanzees ranged between 12.5 and 20 µm long.

Blastocystis (Heterokontophyta: Blastocystida)

Found with sedimentation techniques, cysts (fig. 7) have a thick multi-layered wall, spherical to subspherical form containing a large central body with a narrow rim of cytoplasm with nuclei inside. The diameter of the found forms in Bulindi chimpanzees was 5–6.5 µm. It usually exhibits a green shine halo with a peripheral nuclei.

Troglodytella abrassarti (Entodiniomorphida: Troglodytellidae)

This ciliate is more commonly seen on flotation, body is ovoid to fusiform with truncated anterior end and a short rounded posterior protuberance (figs. 8, 9 and 10). Trophozoites typically show four somatic bands of non-retractable cirri and an adoral membranelle zone around the cytostome; size and number of contractile vacuoles varied based on the levels the trophozoites

were feed to. Measured trophozoites were 135–195 µm long and, width 86–120 µm wide. In SNP samples, most of the trophozoites had decomposed ciliae (fig. 10) and body, making the trophozoite smaller, attributable to older stage of collected samples.

Strongylid (Nematoda: Strongylida)

Found with sedimentation and flotation, strongylid eggs are thin-walled, ellipsoidal and colourless containing blastomeres inside (fig. 11). In later stage of development, L1 larvae were observed. Size of strongylid eggs found in samples varied from 55–95 µm in chimpanzees and 60–70 µm length in black and white colobus. As the egg morphology is insufficient for proper species or genus identification, findings were grouped under the collective term “strongylids”. Beside the eggs in different stage of development, nematode larvae (figs. 12 and 13) were observed in many samples. As they can represent either hatched larvae of GI strongylids or free-living nematodes (as contamination), it was impossible to perform their proper distinguishing. For this reason, they are listed as separate category “nematode larvae” (Table 1).

Trichuris sp. (Nematoda: Enoplida)

Observed eggs were brownish with a typical lemon or barrel shape and mucoid polar plugs at each end (fig. 14 and 15). Eggs found in chimpanzee samples measured 49–55 in length and 22–24 µm in width (N = 2) and in black and white colobus samples 63–68 x 29–34 µm. Egg morphology is insufficient to determine the species

Colobenterobius sp. (Nematoda: Oxyurida)

Eggs were found in black and white colobus samples using flotation method (besides not being the favourite method). The morphological features of adult worms are necessary for species determination. Eggs were ellipsoidal asymmetrical flattened one side with thick colourless wall, partially embryonated (fig. 16). Size ranged from 62–67.5 x 30 µm (N = 2)

Rotifers

The measured rotifers had their sizes comprehending 47–80 x 35–55 µm (figs. 17, 18 and 19).

5.2. PREVALENCE OF FOUND SYMBIONTS

All chimpanzee faecal samples, except for three (including positives only to rotifers) were positive for at least one symbiont taxa (Table 1), with *Troglodytella abrassarti* and nematode larvae being the most prevalent in case of Bulindi and in SNP samples, respectively. All samples from black and white colobus were positive for at least one symbiont taxa with *Trichuris* sp. and *Entamoeba* sp. being the most prevalent (Table 1).

Various Chi-square (X^2) tests of independence were performed to examine the relation between chimpanzee symbionts and the localities (SNP, Bulindi). The relation between these variables was significant for larvae: $X^2(1, N=47) = 16,46$, $p = 4,967 \times 10^{-5}$; *Troglodytella*: $X^2(1, N=47) = 25,28$, $p = 4,693 \times 10^{-7}$; and *Blastocystis*: $X^2(1, N=47) = 4,66$, $p = 0.03086$, as $p < 0.05$. Meaning that there was a relation between the parasite positivity and the localities. Samples from Bulindi were more positive for *T. abrassarti* and *Blastocystis* sp., probably because of the fresh faeces collection, opposed to SNP samples that are richer in nematode larvae, indicating older faeces when collected.

5.2.1. IMF (MERIFLUOR®) DETECTION OF *GIARDIA* AND *CRYPTOSPORIDIUM*

The IMF technique was performed in all samples, and all of them came out negative for *Giardia* and *Cryptosporidium* (stool specimens with no apple green fluoresce). This is a very specific and sensitive test and was performed twice at different moments in time.

Table 1 - Overall presence of intestinal symbionts in chimpanzees (*Pan Troglodytes*) and black and white colobus (*Colobus guereza occidentalis*) from SNP and Bulindi

SYMBIONT TAXA	PREVALENCE (%)			
	<i>Pan troglodytes</i>			<i>Colobus guereza occidentalis</i> (Bulindi) N=15
	<i>P.t.verus</i> (SNP) N=17	<i>P.t. schweinfurthii</i> (Bulindi) N=30	TOTAL N=47	
<i>Trichuris</i> sp.	11.76	0	4.26	100
strongylid eggs	41.18	66.67	57.45	40
nematode larvae	88.24	26.67	48.94	6.67
<i>Colobenterobius</i> sp.	/	/	/	6.67
<i>Troglodytella abrassarti</i>	11.76	86.67	59.57	/
<i>Entamoeba</i> spp.	0	26.67	17.02	100
<i>Blastocystis</i> sp.	0	23.33	14.89	0
<i>Iodamoeba buetschlii</i>	5.88	0	2.13	0
Rotifers*	76.47	70	72.34	53.33

* - not a symbiont; / - not expected

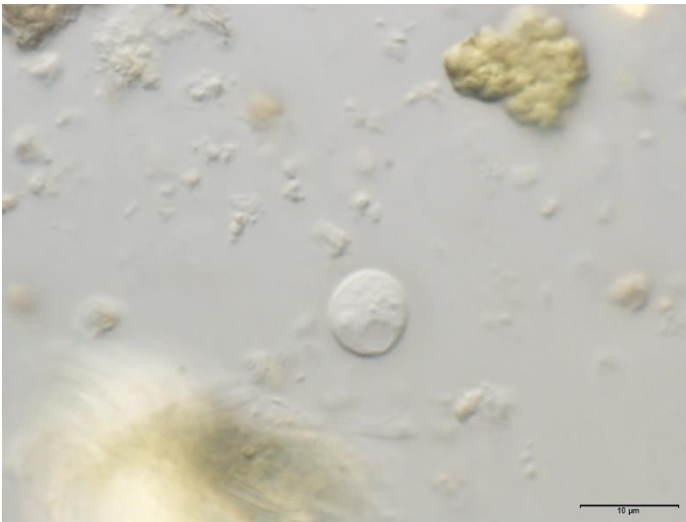


Figure 5 -- *Entamoeba* spp. cyst, chimpanzee, Bulindi. (1000x) Bar = 10 µm (Original)

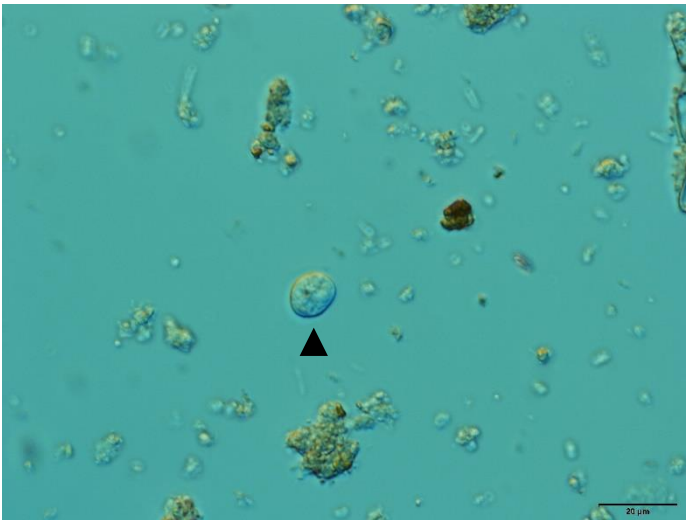


Figure 6 – *Entamoeba* spp. cyst (arrow), black and white colobus, Bulindi. (Nomarski interference contrast: 600x) Bar = 20 µm (Original)

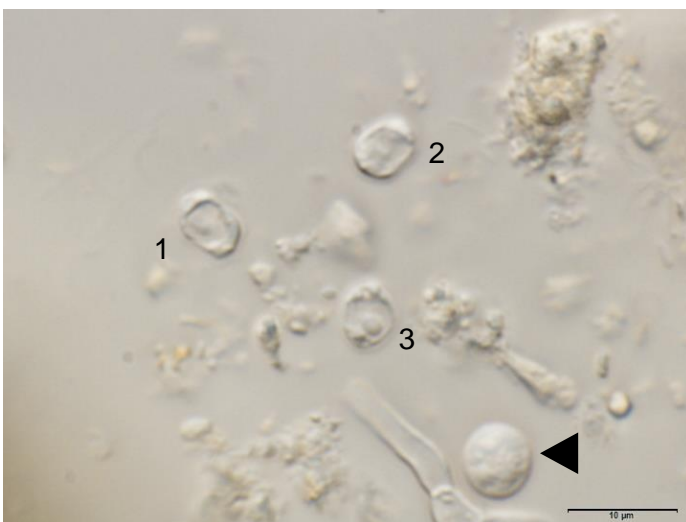


Figure 7 – *Blastocystis* sp. cysts (1, 2, and 3) and *Entamoeba* spp. cyst (arrow), chimpanzee, Bulindi. (1000x) Bar = 10 µm (Original)

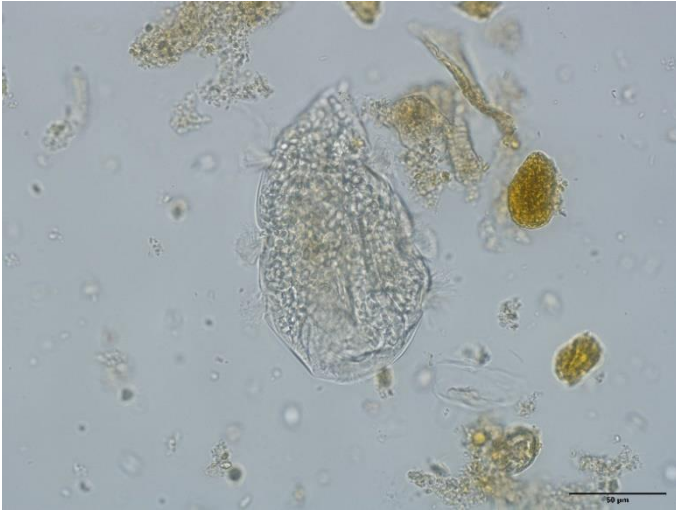


Figure 8 – *Troglodytella abrassarti*, chimpanzee, Bulindi.
(200x) Bar = 50 μm
(Original)



Figure 9 – *Troglodytella abrassarti*, chimpanzee, Bulindi.
(400x) Bar = 20 μm
(Original)

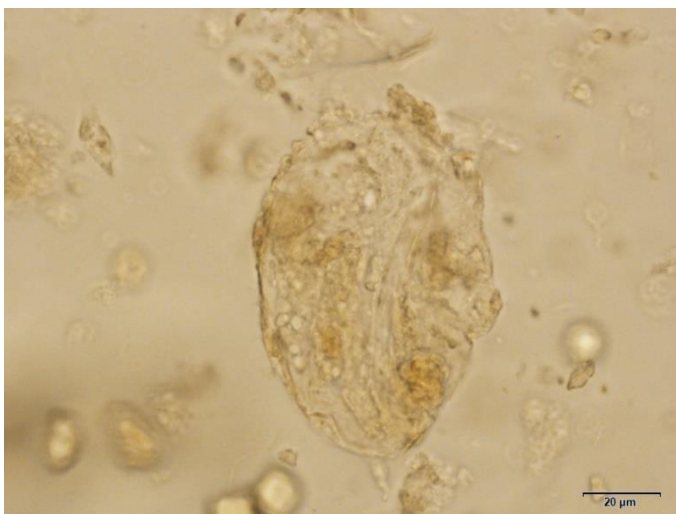


Figure 10 – *Troglodytella abrassarti*, chimpanzee, SNP
(notice the absence of ciliae)
(400x) Bar = 20 μm
(Original)



Figure 11 – Typical strongylid egg, chimpanzee, Bulindi.
(400x) Bar = 20 μm
(Original)



Figure 12 – Anterior end of a nematode larva, chimpanzee, SNP.
(400x) Bar = 20 μm
(Original)



Figure 13 – *Necator*-like larva, chimpanzee, SNP.
(200x) Bar = 50 μm
(Original)



Figure 14 – *Trichuris* sp. egg, chimpanzee, SNP. (400x) Bar = 20 μ m (Original)



Figure 15 – *Trichuris* sp. eggs, black and white colobus, Bulindi. (400x) Bar = 20 μ m (Original)



Figure 16 – *Colobenterobius* sp. egg, black and white colobus, Bulindi. (400x) Bar = 20 μ m (Original)



Figure 17 – Rotifer
(400x) Bar = 20 µm
(Original)



Figure 18 – Rotifer (*Lecane arcuata*)
(400x) Bar = 20 µm
(Original)

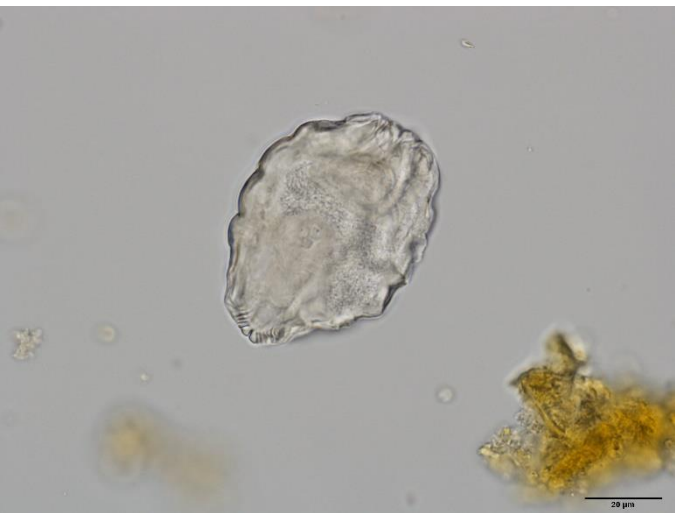


Figure 19 – Rotifer
(400x) Bar = 20 µm
(Original)

6. DISCUSSION

On-going ecological and demographic changes drastically influence the human-wildlife interface, transmission and impact of infectious diseases. As humans overpopulate, there is an increase in use of primates' natural habitat for housing, farm landing and logging, which facilitates circulation of agents, as parasites.

Continued interaction with livestock may result in altered transmission rates and virulence of gastrointestinal parasites (Wheeler, 2010).

On the other hand, primates are very flexible and able to adapt, as example animals that crop raid (as chimpanzees and black and white colobus) are in better physical condition as result of feeding from crops and that can influence their ability to resist parasite infections (Chapman, Speirs, Gillespie, Holland & Austad, 2006). For more examples see Hockings et al. (2015).

With this in mind, it seems relevant to investigate the parasite/symbiont gastrointestinal fauna of primates that live very close to human populations, as the ones studied in this work.

In this study, using coproscopic techniques, light microscopy and MERIFLUOR® *Giardia/Cryptosporidium* direct immunofluorescence detection assay, 47 faecal samples were examined from free ranging chimpanzees and seven distinguishable taxa of symbionts were found. As comparative material, there was a chance to use the same methodology and examine 15 faecal samples from black and white colobus, this investigation revealed five distinct taxa. Diversity of detected taxa of symbionts always depends on methodology and research goal. Thus, the diversity and prevalence of symbionts from different sites and primate hosts may be influenced by differences in sample size, duration of research, microscopic techniques and also by actual differences in symbiont faunas (McLennan et al., 2017).

Two different subspecies of chimpanzees from different geographical areas were object of study, namely *Pan troglodytes verus* (western chimpanzee) from Sapo National Park, Liberia and *Pan troglodytes schweinfurthii* (eastern chimpanzee) from Bulindi, Uganda. Studied populations have different habitat characteristics, feeding habits, and level of contact with humans. Regarding gastrointestinal symbionts, *P. t. verus* it is less studied (McGrew et al., 1989; Howells et al., 2011; Sá et al., 2013; Metzger, 2014; Ebbert et al., 2015) comparing to *P. t. schweinfurthii* (File et al., 1976; Krief et al., 2005; Muehlenbein, 2005; Gillespie et al., 2010; Zommers et al., 2013; Kalousová, 2010; McLennan et al., 2017). This discrepancy is caused by traditional interest in eastern chimpanzee (as demonstrated well for e.g. by the work of Jane Goodall) and accessibility of several populations of eastern chimpanzee resulting from their habituation and political stability of areas they inhabit.

Black and white colobus are abundant arboreal primates commonly occurring in sympatry with chimpanzees, sharing their habitat and possibly symbionts and pathogens. Interestingly chimpanzees are known by their hunting abilities and predation on various species of colobine monkeys, however red colobus (*Procolobus* spp.) are a more frequent prey (Watts & Mitani, 2015). All these aspects make black and white colobus from localities inhabited by chimpanzees an interesting animal to study.

Free ranging primates are uneasy animals to study, they are extremely mobile, often avoiding the contact with humans, difficult to handle and generally strictly protected. Due to these reasons, most of the parasitological studies rely on examination of faecal samples, which is the approach adopted also in this study. Undoubtedly, direct examination of faecal samples using range of coproscopic techniques remains a paramount of parasitological studies addressing the gastrointestinal parasites of free ranging primates (e.g. Zommers et al., 2013; Ebbert et al., 2015). However, microscopic techniques used alone have several limitations, since most symbionts cannot be described to species level only based on the morphology of detectable stages that are shed in faeces. Generally, identification lower than genus level is complicated or even impossible using microscopy alone. Ignoring this fact can lead (and in many published papers really leads) to premature conclusions regarding the host specificity, zoonotic potential and diversity of observed parasites/commensals. Similarly, also ecological studies can be deeply biased by low sensitivity of microscopic detection of many protozoans. On the contrary, range of molecular methods is being applied to study primates symbionts, used either as primary method of investigation (e.g. Jirků-Pomajbíková et al., 2016), or as a complementary approach to microscopic detection (e.g. McLennan et al., 2017). In most cases, if properly applied, these methods allow differentiation of symbiont species, which is a prerequisite for evaluation of host specificity and assessing the real zoonotic potential connected to some pathogens.

Overall, the spectrum of symbionts found in the present study is in accordance with what is regularly described in previous studies addressing the free ranging chimpanzees and black and white colobus (as e.g. the ones used on Table 2, 3 and 4), with some exceptions that will be talked about ahead. It is important to say that all samples were treated as individual due to the general low amount of samples, which can generate a bias in the described prevalence. However, it gives an idea of what type of symbionts are present in the studied populations. Microscopy revealed that the infection prevalence and the symbiont load were significantly higher in the Bulindi chimpanzees than in the SNP ones. As said before, this is possibly due to the fact that SNP samples were not as fresh as recommended.

Black and white colobus were subject of parasitological studies far less than chimpanzees. In general, several studies suggest that these primates are more heavily parasitized when compared to other colobines. The reason may be multifactorial (complex interactions among environmental, demographic, genetic, and behavioural factors) and hard to define (Gillespie et al., 2005).

Considering all the limitations described above, it is rather uneasy to compare the parasite richness between chimpanzees from two study sites as well as between the chimpanzees and colobus monkeys.

In following lines, it is provide detailed discussion on symbiont taxa found in examined samples.

6.1. INDIVIDUAL PARASITES/COMMENSAL DETECTED

This part comprehends a comparison of the found coproscopic results with more recent studies using the same methodology.

Amoebas

The most common protozoan in examined Bulindi chimpanzees, as well as black and white colobus samples, were the cysts of *Entamoeba* spp.

This study followed the approach prevailing in recent publications using microscopic techniques to detect the amoebas (e.g. Muehlenbein, 2005; Gillespie et al., 2008; Kalousová, 2010; Sá et al., 2013; Kouassi et al., 2015). This suboptimal (though widely used) method has its limits in sensitivity and specificity which may impact on observed level of *Entamoeba* prevalence.

Entamoeba was absent from SNP chimpanzees, present in 26.67% of Bulindi chimpanzees and 100% in black and white colobus.

Members of the subfamily Colobinae have specialised sacculated stomachs, an adaption to their leaf-eating lifestyle. It is suggested that the normal neutral pH in these compartments provides favourable conditions for ingested *Entamoeba histolytica* cyst excystation and trophozoite tissue invasion (Ulrich et al., 2010). These may explain why black and white colobus seem to have high prevalence of *Entamoeba* spp. However, molecular identification of *Entamoeba* is necessary as final prove of occurrence of “true” *E. histolytica* in any of the colobine population in Africa.

Entamoebas known from primates are usually classified either as commensals or as pathogenic species. The tetranucleated group is the most important since it comprises two pathogenic species (*Entamoeba histolytica* and *E. nuttalli*) among the commensals (*E. dispar* and *E. moshkovskii*). Unfortunately, faecal microscopy is insufficient to distinguish the

pathogenic entamoebas from other four nuclei non-pathogenic ones as the cysts are morphological indistinguishable (Kebede, Verweij, Petros & Polderman, 2004). Differential diagnosis is achieved using molecular methods (as amplification of 18S rDNA followed by sequencing) (Verweij et al., 2003; Tachibana et al., 2007; Levecke et al., 2010; Dong et al., 2017), however, these are rarely applied to wild chimpanzee samples (Jirků-Pomajbíková et al., 2016).

Cysts of *Entamoeba* spp. are easily transmissible via contaminated food or water, especially in situation of close spatial coexistence of their hosts. As Jirků-Pomajbíková et al. (2016) demonstrated, even wild chimpanzees that do not live in close proximity to human populations harbour several *Entamoeba* species closely related to those occurring in humans. Consequently, there is a need to investigate the presence pathogenic *Entamoeba* using proper molecular tools, to then assess the risk of zoonotic transmission.

Beside the *Entamoeba* spp., *Iodamoeba* cysts were also detected. They were only present in SNP chimpanzee samples and in a low prevalence (5.9%).

This symbiont is a regular finding in African primates, however, usually detected in a low prevalence. Study investigating Tai monkeys (Kouassi et al., 2015), including a species of black and white colobus, *C. polykomos*, reported *Iodamoeba* in relatively high prevalence, namely 24.8 % (N=125).

Blastocystis

Blastocystis sp. was only detected in chimpanzee samples from Bulindi (23.33%). Regarding SNP chimpanzees, *Blastocystis* was absent from the sample set, however in a study with the same chimpanzee subspecies (Sá et al., 2013) it was found 49% (N=132) prevalence for the same symbiont (table 4).

Petrášová et al. (2011) proved this symbiont prevalence is actually underestimated using microscopy detection after MIF technique, as PCR diagnostic based methods (ssrDNA region was extracted and amplified) revealed very different values (*Pan troglodytes* MIF: 32.5% vs. PCR: 71.4% (N=206) and *Colobus guereza* MIF: 61.2% vs PCR: 83.7% (N=49)). In the same study (Petrášová et al., 2011), primate and human (researchers) faeces were analysed. Although the researchers had daily contact with the sampled primate faeces, the sequences obtained from the human isolates were not identical with any primate-derived sequences of that study. This work did not demonstrated zoonotic transmission and showed some high host specificity of *Blastocystis* genus.

Giardia* and *Cryptosporidium

Cysts/oocysts of these two opportunistic protists are uneasy to detect, specifically in situation when the infection/excretion intensities are low. This is why several methods detecting either the copro-antigen, or visualizing the cysts by fluorescein-labelled antibodies, were developed and are broadly used in human and veterinary medicine to detect these protists in faeces (Weitzel, Dittrich, Möhl, Adusu & Jelinek, 2006). Application of these methods is usually straightforward and not excessively expensive. The microscopy-based coproscopic techniques used in the present study did not reveal any cysts/oocysts of *Giardia/Cryptosporidium* in examined samples. To extend the range of used methods and to prove the absence of these opportunistic pathogens in the set of samples, MERIFLUOR® *Giardia/Cryptosporidium* direct immunofluorescence detection assay was used. This is a very sensitive method that contains a mixture of monoclonal antibodies directed against cell wall antigens of *Giardia* cysts and *Cryptosporidium* oocysts and did not prove the presence of the target protists. To the author's knowledge, the use of this test in faeces from chimpanzees and black and white colobus is not published. However, a 2007 study from Western Uganda (Salzer, Rwego, Kuhlenschmidt & Gillespie, 2007), resorting to immunofluorescent antibody detection (IFA), reported absence of *Giardia* and *Cryptosporidium* in black and white colobus. Interestingly, the Bulindi chimpanzees studied in the period of 2012–2013 (McLennan et al., 2017) showed a low prevalence of *Giardia* (1.6%) using a simple sedimentation technique.

Entodiniomorphid ciliates

These non-pathogenic ciliates are present among the populations of wild chimpanzees, bonobos and gorillas. Also, the captive African great apes often carry these symbionts, sometimes even in significantly greater number than the wild ones (Modrý et al., 2009; Pomajbíková et al., 2010a).

The absence of ciliates in the black and white colobus faecal samples is in agreement with other studies. Since these ciliates were never reported in other primates, they seem to be exclusive inhabitants of the intestine of African great apes.

Troglodytella abrasarti was the most prevalent symbiont in Bulindi samples (86.67%) comparing to a low prevalence in SNP samples (11.76%). Whilst the prevalence in Bulindi corresponds well to similar studies from other free ranging chimpanzee populations (e.g.: 97% - Muehlenbein et al., 2005, 92% - Kaur et al., 2010, 79.6% - McLennan et al., 2017), low prevalence in SNP is probably attributable to the quality of the samples.

Entodiniomorphid ciliates do not form cysts and their survival in faeces is rather limited. Natural environment contributes to a faster decomposition of the ciliates due to higher and fluctuation in the temperature and gradual desiccation (Profousová et al., 2011b). The laboratory studies investigating the survival and detectability of *Troglodytella* trophozoites in samples from captive chimpanzees reported changes in the trophozoites thought out time: visible cilia and

motile trophozoites less than 10hr after defecation; absence of ciliature from 15hr after defecation until 55–60 hr; trophozoite fragmentation starts at 30hr after defecation (Profousová et al., 2011b). The sampling conditions in SNP were more challenging and the faeces collection delayed so that probably influenced the ciliate prevalence.

Recently, another entodiniomorphid ciliate species named *Troglocorys cava* was described from chimpanzee (Tokiwa et al., 2010), reported usually in a high prevalence together with *T. abrassarti* (as e.g. see Kooryama et al., 2012 or McLennan et al., 2017), but seems to be absent in captive apes (Pomajbíková et al., 2011). However this species was missing from this sample set.

Besides the entodiniomorphids, *Balantioides coli* is a potentially harmful ciliate of apes, capable of causing clinical balantidiasis, reported mainly in the captive ones (Pomajbíková et al., 2010b). This ciliate is detectable in faeces either as trophozoites or cysts, its absence in both sample set from chimpanzees is in line with most studies on free-ranging chimpanzees (e.g.: Muehlenbein, 2005; Kalousová, 2010; Sá et al., 2013), since its rarely described (Lilly et al., 2002; Zommers et al., 2013; McLennan et al., 2017). The absence in black and white colobus it was also expected since it was never reported in these hosts.

The discrepancy in *Balantioides* occurrence between captive and free-ranging apes is noteworthy. Limited experiments with captive chimpanzees (Schovancová et al., 2013) proved the dependence of *Balantioides* population in the amount of starch present in their diet, showing the need for re-evaluation of nutrition plans for captive apes.

Strongylid nematodes

The most prevalent nematodes in chimpanzees from both SNP and Bulindi and second most prevalent in black and white colobus were the strongylids, as determined based on the presence of typical thin-walled eggs. Similarly to other large herbivorous mammals, strongylids in African great apes occur in communities that consist of several species. Taxonomy of this group of nematodes is largely based on adult worm's morphology. Morphology of L3 larvae obtained by coprocultures from fresh faecal material, is a way to reach the determination at the genus level (Polderman, Krepel, Baeta, Blotkamp, Gigase, 1991; Blotkamp et al., 1993), however preserved faecal material, like the studied samples, is unsuitable for this approach. Molecular techniques (like PCR assay for the specific amplification of the ITS-2 rDNA) represent an ultimate tool for determination of the species (Gasser, 2006; Ghai et al., 2014; Makouloutou et al., 2014).

Considering the most common and prevalent strongylids in chimpanzees, it is assumed that the studied ones can be any members of genera *Oesophagostomum*, *Necator*, *Ancylostoma*, and/or *Ternidens*.

Despite the fact that light microscopy has very limited power to differentiate among the strongylid genera/species, is still broadly used in studies (Gillespie et al., 2005; Teichroeb et al., 2009; Kalousová, 2010; Sá et al., 2013). Recent studies are resorting to complementary molecular techniques (e.g. Ghai et al., 2014; McLennan et al., 2017).

Prevalence of strongylids in the studied sample set seems to be in agreement with other data (Table 3 and 4). SNP samples have less strongylid eggs (41.18%) compared to Bulindi (66,67%), possibly due to delayed faeces collection giving more time for egg development into larvae, which showed a high prevalence (88.24%) on the same sample set (considering some part of the larvae are strongylids and other may be free living nematodes). The fact, that the SNP samples were collected during rainy season can also contribute to fast development of strongylid eggs.

Regarding the black and white colobus, the studied samples had higher strongylid prevalence (40%) when compared to other studies (Gillespie 2005; Gillespie & Chapman, 2006), but it should be kept in mind the studied sample size was rather small (N=15). Black and white colobus seem to be less predisposed to *Oesophagostomum* sp. infection thanks to its gastrointestinal physiology (Ghai et al., 2014).

There are recent studies showing at least some species of *Oesophagostomum* and *Necator* can cross infect humans and primates (Ghai et al., 2014; Kalousová et al., 2016). Therefore, in future research, it is extremely important to properly determine found strongylids and then assess their possible zoonotic potential.

Trichuris

Although all black and white colobus were infected (100%) with *Trichuris* sp., only two chimpanzee samples from SNP were and had only one egg each. The re-examination of the positive samples did not reveal more *Trichuris* sp. eggs, which confirms very low number of eggs in the material.

Trichuris infection is very common among African small primates including the colobines. In black and white colobus, presence of *Trichuris* eggs was reported often in high prevalence (Gillespie et al., 2005 (78.99–100 %); Gillespie et al., 2008 (84–90%); Teichroeb et al., 2009 (80.7%); Kouassi et al., 2015 (75.2%)). It has been described, that when colobus density rises as a result of immigration to a fragmented area, that may contribute to nutritional stress from quick overpopulation and the prevalence of *Trichuris* rises (Chapman et al., 2006; Gillespie et al., 2008). However, as *Trichuris* spp. represent a typically soil transmitted nematode the simple increase of population density can also lead to higher environmental contamination and increased infection levels. As colobus have their own recently described species (Cutillas et al., 2014), *Trichuris colobae*, it would be very interesting to prove or exclude if this was in fact the one affecting the studied colobines.

General absence of *Trichuris* in chimpanzees deserves attention, both from methodological and evolutionary point of view. Similar to the present study, also Ashford et al. (2000) found only one *Trichuris* egg in a total of 123 chimpanzee faecal samples. Likewise, Kooryama et al. (2012) reported a 3% prevalence (N=254). Besides this, Sá et al. (2013) reported an unusual high prevalence in Guiné-Bissau chimpanzees (Table 4). The presence of low amount of eggs in few samples (only) suggest that the observed eggs may represent pseudo-parasites, resulting from accidental ingestion of *Trichuris* eggs originating from other hosts (e.g.: sympatric colobus or other monkeys). Chimpanzees typically hunt other mammals including other primates and *Trichuris* eggs observed in their faeces might be a result of a passage from eaten prey. Alternatively, the eggs can also originate from environment contaminated by eggs shed in faeces of sympatric mammals.

Molecular studies (as amplification and sequencing of COX1 gene and 18S rDNA) prove that *Trichuris suis* (originally from suids) is different species from *T. trichiura* (originally from humans), however they cannot be differentiated morphologically (Liu et al., 2012; Callejón et al., 2013), proving close relatedness and suggesting possible hybridization between the two species (Nissen et al., 2012; Meekums et al., 2015). The general absence of whipworms in chimpanzees (as the closest human relatives) suggests possibility, that human infections were historically acquired from pigs (or other suids) as Schwartz (1926) first suggested, rather than “inherited” through the co-evolution process. This intriguing aspect deserves further attention.

Pinworms

Although the egg morphology does not allow for proper determination of primate pinworms, the eggs detected in black and white colobus faeces probably belong to some species of the genus *Coloboenterobius*, which is the colobines pinworm. In a similar study, Gillespie et al. (2005) reported that from 476 faecal samples from *Colobus guereza* only 3 were positive (0.63% prevalence) for pinworm eggs.

Chimpanzees can be infected with *Enterobius anthopopithecii* (Hasegawa et al., 2005), however, no egg was recovered from both SNP and Bulindi chimpanzees fecal samples.

Sedimentation and flotation techniques are not the appropriate way to find eggs or adults of these nematodes, so other techniques as Gauze washing, Baermann method or even adhesive tape or swab of the perianal region (in case of close contact with studied animals), should be applied in future to assess the occurrence and prevalence of pinworms properly.

6.2. MISSING TAXA

Some symbionts that are more or less common findings in chimpanzees and/or in black and white colobus were absent in this sample set, as *Bertiella* sp., *Strongyloides* sp., *Troglocorys cava*, eggs of spirurid nematodes, etc.

Because they were absent from the studied samples, they were kept off the tables (2, 3 and 4). To discuss the absence of particular symbionts is quite hard, as it can be caused by a broad range of reasons, starting from the real absence of those symbionts in given samples through methodological errors and inexperience up to errors in previously published data. Longitudinal sampling throughout wet and dry season, higher numbers of samples including the resampling of the same individuals, emphasis on fresh faecal samples collection and immediate preservation and implementation of broader range of diagnostic techniques are among the dominant approaches to be used to obtain more robust data (Modrý, Petrželková, Kalousová & Hasegawa, 2015).

6.3. ROTIFERS

Interestingly, stages of rotifers were found in several of the examined samples, which is the first report of Rotifera in chimpanzees and in black and white colobus faeces. This unusual finding deserves some attention.

Rotifera is a group of primary freshwater invertebrates (Wallace & Snell, 2010). They are ubiquitous, usually occur in large densities and are important filter-feeders on algae and bacteria (Segers, 2008). Rotifera are preferentially found in freshwater, but they can also live in marine and limnoterrestrial environment and even in interstitial water of soils and sediments (Wallace & Snell, 2010). Along with Cladocera and Copepoda species, they are important food providers in natural environment and aquaculture (Bozkurt, Ülgü & Duysak, 2016). They are permanently and obligatorily connected to aquatic habitats in all active stages, only their resting stages are drought-resistant (Segers, 2008).

In fact, these microorganisms are occasionally reported in animal faeces, like geese, that browse on mosses and by eating mosses and vegetation, they also ingest this microscopic inhabitants (Diego Fontaneto, personal communication, December 16, 2016).

There are several possible causes for the presence of rotifers in primate faeces: (i) oral intake with food and/or water; (ii) they are commensal of gastrointestinal tract; (iii) contamination of the faecal samples after defecation; (iv) contamination of the water used to dilute the formalin in field (faeces preservative); (v) contamination in the lab; (vi) and finally be commonly found and ignored by other investigators.

Regarding suspicious (iv) and (v), the water used for both formalin dilution and the sugar solution is filtered and was tested separately and come out as negative. Reason (iii) is also

very unlikely since the Bulindi chimpanzee samples were collected almost immediately after defecation. Regardless it is appealing, the reason (ii) is quite improbable and the oral intake of rotifers with food or water (i) represents the most plausible scenario. Considering the high prevalence of this rotifers found in the studied samples, it is very likely they are common findings but usually ignored (vi).

This topic anyway deserves further attention and field research, addressing mainly possible presence of alive stages in fresh faeces, assessed by direct microscopy in field conditions.

Table 2 - Comparison between the present study and other literature (*Colobus* sp.)

	Gillespie et al., 2005 (<i>C.guereza</i>) (n=476)	Gillespie et al., 2005 (<i>C. angolensis</i>) (n=19)	Okanga et al., 2006 <i>C. angolensis</i> (n=72)	Gillespie & Chapman, 2008 <i>C. guereza</i> forest fragmented(n=94)	Gillespie & Chapman, 2008 <i>C. guereza</i> forest unfragmented (n=106)	Present study (n=15)
Black and white colobus						
<i>Trichuris</i> sp.	78.99%	100%	5–14%	90%	84%	100%
Unidentified strongylids	1.05 %	10.53%	0	5%	0	0
<i>Oesophagostomum</i> sp.	6.09%	0	0	4%	9%	0
Strongylid eggs	0	0	0	0	0	40%
Nematode larvae	0	0	0	0	0	6.67%
<i>Colobenterobius</i> sp.	0.63%	0	0	0	0	6.67%
<i>Entamoeba</i> spp.	0	0	0	0	0	100%
<i>Entamoeba coli</i>	7.77%	15.79%	93–100%	6%	9%	0
<i>Entamoeba histolytica</i>	7.56%	10.53%	87–90%	5%	9%	0
Rotifers*	#	#	#	#	#	53.33%

* - not a symbiont; 0 – not found or not divided in this category; # - not reported (probably ignored)

Table 4 - Comparison between the present study and other literature (*Pan troglodytes verus*)

<i>P. t. verus</i>	Howells et al., 2011 (n=132)	Sá et al., 2013 (n=132)	Present study (n=17)
<i>Trichuris</i> sp.	0.78%	15%	11.76%
Strongylid eggs	0	38%	41.18%
Nematode larvae	16.41%	0	88.24%
Hookworm egg	3.91%	0	0
Oesophagostomum sp.	0	0	0
<i>Troglodytella abrassarti</i>	64.84%	62%	11.76%
<i>Entamoeba</i> spp.	0	18%	0
<i>Entamoeba coli</i>	10.16%	0	0
<i>Blastocystis</i> sp.	0	49%	0
<i>Iodamoeba buetschlii</i>	9.38%	4%	5.88%
Rotifers*	#	#	76.47%

*- not a symbiont; 0 – not found or not divided in this category;
- not reported (probably ignored)

Table 3 - Comparison between the present study and other literature (*Pan troglodytes schweinfurthii*)

<i>P. t. schweinfurthii</i>	Kooryama et al., 2012 (n=254)	McLennan et al., 2017 (n=432)	Present study (n=30)
<i>Trichuris</i> sp.	3%	0	0
Strongylid eggs	0	0	66.67%
Nematode larvae	0	(23.1% - free living)	26.67%
Hookworm egg	0	44.2%	0
Oesophagostomum sp.	61%	48.1%	0
<i>Troglodytella abrassarti</i>	38%	79.6%	86.67%
<i>Entamoeba</i> spp.	11%	39.1%	26.67%
<i>Entamoeba coli</i>	35%	8.3%	0
<i>Blastocystis</i> sp.	0	7.4%	23.33%
<i>Iodamoeba buetschlii</i>	0	3%	0
Rotifers*	#	#	70%

*- not a symbiont; 0 – not found or not divided in this category;
- not reported (probably ignored)

7. CONCLUSION

This study comprised a research of the symbiont fauna of two chimpanzee subspecies (*Pan troglodytes verus* and *Pan troglodytes schweinfurthii*) from two different geographical locations (Sapo National Park and Bulindi) and complementary data from black and white colobus from Bulindi that sympatrically live with chimpanzees. Using coproscopic methods such as flotation and sedimentation, and immunofluorescence assay (MERIFLUOR®) to detect *Giardia* and *Cryptosporidium*, the aims were partially answered:

- (1) To characterize the symbiont fauna of both chimpanzee and (2) black and white colobus, but unfortunately with some incongruities, since most of the symbionts require molecular techniques to achieve the species level and only coproscopic methods and microscope were used. The application of MERIFLUOR® immunofluorescence assay confirmed that all examined animals were negative for both *Cryptosporidium* and *Giardia*.
- (3) Zoonotic potential and possible transmission of agents between chimpanzee and black and white colobus is neither completely ruled out nor confirmed, since the molecular techniques are required.

Although it was not a goal of this dissertation, the finding of rotifers in chimpanzee and black and white colobus faeces it is curious. Further work should be performed to fully comprehend the true origin and relation between this aquatic microorganism and the studied animals, taken its prevalence in both chimpanzees and colobus studied populations.

This dissertation contributed with more information regarding symbiont fauna of black and white colobus and *P. t. verus*, as less studied species comparing to *P. t. schweinfurthii*. Further research is needed regarding zoonotic potential/transmission of the found symbionts.

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