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Využití velkokapacitního sekvenování k identifikaci druhů a linií zástupců *Strongyloides* v prostředí s výskytem více hostitelských druhů

Diplomová práce

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Abstrakt

Tato diplomová práce se zabývá diverzitou a distribucí hlístic rodu *Strongyloides* v prostředí s výskytem více hostitelských druhů v Národním parku Cantanhez v Guinea-Bissau. Cílem práce bylo charakterizovat genetické linie rodu *Strongyloides* u člověka a šesti druhů nehumánních primátů (NHP) a posoudit jejich potenciál pro mezidruhový přenos. Fekální vzorky byly analyzovány pomocí qPCR a pozitivní vzorky následně podrobeny velkokapacitnímu sekvenování cílenému na oblast HVR-IV genu 18S rRNA. Celkem bylo identifikováno 16 ASV (variant sekvencí amplikonu), přičemž dominantním a nejvíce diverzifikovaným taxonem u NHP byl *Strongyloides fuelleborni*, zatímco *Strongyloides stercoralis* byl převážně detekován u člověka. Zoonotický haplotyp A druhu *S. stercoralis* byl zjištěn jak u člověka, tak u šimpanzů, což naznačuje možnost mezidruhového přenosu. Výsledky ukazují, že společenstva rodu *Strongyloides* jsou formována jak hostitelskou specifitou, tak sdílenými cestami přenosu. Práce zdůrazňuje význam molekulárních metod pro studium parazitární diverzity a přispívá k pochopení zoonotického přenosu v rámci konceptu One Health.

Abstract

This thesis focuses on the diversity and distribution of *Strongyloides* nematodes in a multi-host system in the Cantanhez National Park in Guinea-Bissau. The aim of the study was to characterize genetic lineages of *Strongyloides* in humans and six species of non-human primates (NHPs) and to assess their potential for cross-host transmission. Faecal samples were screened using qPCR and positive samples were analysed by high-throughput sequencing targeting the HVR-IV region of the 18S rRNA gene. A total of 16 amplicon sequence variants were identified, with *Strongyloides fuelleborni* representing the dominant and most diverse taxon in NHPs, while *Strongyloides stercoralis* was detected in both humans and chimpanzees, indicating potential cross-species transmission. The results suggest that *Strongyloides* communities are structured by both host specificity and shared transmission pathways. This study highlights the importance of molecular approaches for understanding parasite diversity and contributes to the knowledge of zoonotic transmission in multi-host systems within the One Health framework.

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Název práce anglicky:	High-throughput Sequencing for Species and Lineage Identification of Strongyloides in Mixed-Species Habitats
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Oficiální zadání:

Strongyloides is a genus of soil-transmitted nematodes that infects a wide range of hosts. Among these, Strongyloides stercoralis is the most prominent, infecting an estimated 600 million people worldwide. Other hosts include non-human primates (NHPs) and dogs, with zoonotic transmission potential, especially in populations sharing close habitats. Despite its significant public health impact, effective diagnostic and treatment methods are still lacking. Even with advancements in diagnostics and treatments, identifying all potential reservoir hosts remains crucial to controlling these infections in humans. This master's thesis aims to implement a novel diagnostic approach using high-throughput sequencing (HTS) to identify individual Strongyloides haplotypes and evaluate cross-species transmission. Samples from humans (n=17) and non-human primates [Western chimpanzee (Pan troglodytes verus), n=77; Green monkey (Chlorocebus sabaeus), n=16; Guinea baboon (Papio papio), n=19; King colobus (Colobus polykomos), n=6; Western red colobus (Piliocolobus badius), n=7; Lesser spot-nosed monkey (Cercopithecus petaurista), n=13)] sharing habitats in Cantanhez National Park, Guinea-Bissau, which were previously identified as Strongyloides-positive using qPCR in a bachelor's thesis, will be analysed using HTS. This thesis has the potential to inform public health policy by advancing our understanding of Strongyloides transmission pathways, thereby contributing to the development of more targeted and effective infection control strategies.

Aims: 1. Master high-throughput sequencing methodologies, including Illumina library preparation. 2. Develop skills in high-throughput data processing and statistical evaluation. 3. Identify the species diversity within Strongyloides, including distinct lineages and haplotypes. 4. Investigate lineage/haplotype sharing across host species, particularly among humans and NHPs.

Hypotheses: 1. Lineages/haplotypes of Strongyloides stercoralis will be shared among NHPs and humans, while Strongyloides fuelleborni fuelleborni will only be detected in NHPs. 2. Strongyloides f. fuelleborni infections will be more prevalent in NHPs than humans. 3. Strongyloides stercoralis infections will be more common in humans than in NHPs.

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Prohlášení

Prohlašuji, že jsem svou diplomovou práci vypracoval samostatně pod vedením vedoucí práce a s využitím informačních zdrojů uvedených v této práci. Dále prohlašuji, že jsem při přípravě práce využil nástroj AI (ChatGPT) za účelem jazykové úpravy a také na výpomoc se statistickým zpracováním dat. Po použití nástroje AI jsem provedl kontrolu obsahu a přebírám za něj plnou zodpovědnost.

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Glossary

ANOSIM	–	analysis of similarities
ASVs	–	amplicon sequence variants
bPCR	–	bridge polymerase chain reaction
CAR	–	Central African Republic
CNP	–	Cantanhez National Park
cox1	–	cytochrome c oxidase subunit I
cPCR	–	conventional polymerase chain reaction
C_q	–	quantification cycle
ELISA	–	enzyme-linked immunosorbent assay
ePCR	–	emulsion polymerase chain reaction
HTS	–	high-throughput sequencing
HVR	–	hypervariable region
iL3	–	infective third stage larvae
ITS	–	internal transcribed spacer region
L1	–	first stage larvae
LAMP	–	loop-mediated isothermal amplification
MDA	–	mass drug administration
MSP	–	major sperm protein
NHPs	–	non-human primates
NZDs	–	neglected zoonotic diseases
PCR	–	polymerase chain reaction
PERMANOVA	–	parmutational multivariate analysis of variance
qPCR	–	quantitative real-time polymerase chain reaction
SARS	–	severe acute respiratory syndrome
WGS	–	whole genome sequencing
WHO	–	World Health Organization

1 Introduction

Strongyloides nematodes represent an important group of soil-transmitted helminths with a global distribution, particularly in tropical and subtropical regions (Puthiyakunnon et al., 2014). Among them, *Strongyloides stercoralis* and *S. fuelleborni* are recognized as the primary species infecting humans and causing strongyloidiasis, a disease with a wide spectrum of clinical manifestations ranging from asymptomatic infections to severe, potentially fatal hyperinfection in immunocompromised individuals. In addition to humans, these parasites commonly infect a range of other hosts, particularly non-human primates (NHPs), forming complex multi-host systems in which transmission dynamics are shaped by interactions among hosts and their shared environment (Nosková et al., 2023a).

In recent decades, increasing anthropogenic pressure on natural ecosystems has led to habitat fragmentation, environmental change, and intensified contact between humans and wildlife. In tropical regions, such as western Africa, humans and NHPs frequently share overlapping habitats, including forest fragments, agricultural areas, and village surroundings. Such conditions facilitate both direct and indirect interactions, creating opportunities for cross-species transmission of pathogens (Estrada et al., 2017; Hill, 2018). In the case of environmentally transmitted parasites such as *Strongyloides*, transmission does not require direct host contact but may occur through contaminated soil, further emphasizing the importance of environmental reservoirs in shaping parasite circulation (White et al., 2019). These dynamics highlight the relevance of the One Health framework, which integrates human, animal, and environmental health to better understand and manage zoonotic diseases (Deiana et al., 2024).

Despite increasing recognition of the zoonotic potential of *Strongyloides*, the structure of parasite populations and the extent of cross-host transmission in natural multi-host systems remain incompletely understood. Traditional diagnostic approaches, including microscopy and targeted PCR, provide limited resolution for

distinguishing between genetic lineages and detecting mixed infections (Nosková et al., 2023a; Wong et al., 2014). In contrast, high-throughput sequencing enables the identification of multiple genetic variants within individual samples, offering new insights into parasite diversity and transmission pathways in complex ecological settings (Metzker, 2010).

The present study aims to characterize the genetic diversity and distribution of *Strongyloides* spp. in a multi-host system, focusing on humans and six species of NHPs inhabiting the Cantanhez National Park in Guinea-Bissau. Using a metabarcoding approach targeting the HVR-IV region of the 18S rRNA gene, this study seeks to identify distinct genetic haplotypes and evaluate their distribution across host species.

The main hypotheses of this study are: (a) *Strongyloides stercoralis* will be shared among NHPs and humans, while *Strongyloides fuelleborni* will only be detected in NHPs; (b) *S. fuelleborni* will be more prevalent and genetically diverse in NHPs compared to humans; (c) *Strongyloides stercoralis* infections will be more common in humans than in NHPs. By integrating molecular data with ecological context, this study aims to contribute to a better understanding of *Strongyloides* transmission dynamics in natural multi-host systems and to provide insights relevant for both public health and wildlife conservation within a One Health framework.

2 Current state of knowledge

2.1 Emerging and neglected zoonotic diseases

Zoonotic diseases are defined by the World Health Organization (2020) as diseases caused by bacteria, viruses, fungi, parasites, or unconventional agents that are transmissible between vertebrate animals and humans. They represent a growing global public health threat. It is estimated that around 75% of emerging infectious diseases are zoonotic in origin and primarily arise from wildlife reservoirs (Rabozzi et al., 2012), with a substantial proportion, presumably 23%, involving arthropod vectors (Wikel, 2024). In recent decades, outbreaks of previously unknown or poorly understood pathogens have highlighted the increasing relevance of zoonoses in global health. Notable examples include viral pathogens such as SARS (severe acute respiratory syndrome), avian influenza virus, Ebola virus, and hepatitis E virus (Rabozzi et al., 2012), bacterial infections such as Lyme disease (caused by *Borrelia burgdorferi*) and Q fever (caused by *Coxiella burnetii*) (Blancou et al., 2005), and parasitic diseases including for example leishmaniosis or strongyloidiasis (Barratt et al., 2019; Gómez et al., 2024; Nosková et al., 2026).

The emergence and re-emergence of zoonotic diseases are driven by a combination of ecological, technological, and socio-economic factors. Anthropogenic activities such as urbanization, deforestation, climate change, tourism, zoos, and wildlife exploitation and trade increasingly alter natural ecosystems and promote interactions among humans, domestic animals, and wildlife (Esposito et al., 2023; Rabozzi et al., 2012). These changes reshape host distribution and create novel ecological conditions that may facilitate pathogen spread across species boundaries.

While some zoonoses such as salmonellosis or avian influenza receive considerable attention in public health, others remain underrecognized. These are referred to as neglected zoonotic diseases (NZDs) (Maudlin et al., 2009). Due to inadequate surveillance, limited diagnostic capacity, and under-reporting, the true burden of NZDs is largely underestimated. Many of the NZDs are endemic diseases that

disproportionately affect low-income populations in developing countries, often impacting both human health and economic livelihoods (Kalambhe & Wavhal, 2024). Affected populations often live near livestock or domestic animals (e.g., domestic cattle in Uganda, which acts as a reservoir for *Trypanosoma brucei rhodesiense*), increasing the risk of zoonotic spillover while having poor access to healthcare services (Welburn et al., 2015).

Examples of NZDs include, among others, parasitic and bacterial infections, such as anthrax, brucellosis, bovine tuberculosis, cystic echinococcosis, leishmaniasis and rabies (Di Bari et al., 2023). These diseases remain a major public health concern due to persistent gaps in their control and surveillance. Although cost-effective control strategies such as vaccination, deworming, and meat inspection are available, their implementation is often inconsistent or limited in practise. As a result, NZDs continue to impose a substantial health and socio-economic burden, particularly in endemic regions (Welburn et al., 2015; World Health Organization, 2024). Importantly, NZDs can be effectively addressed using the One Health approach (Molyneux et al., 2011), as they are often multi-host pathogens; therefore, successful control requires comprehensive knowledge of all involved hosts, their life cycles, and the environmental factors shaping circulation dynamics.

2.2 Parasitic zoonotic diseases

Parasitic zoonotic diseases, caused by protozoa, helminths, and arthropods, remain a major health concern, particularly in low-income countries and regions where close contact between humans and animals, poor hygiene, and insufficient health infrastructure facilitate parasite spread (Kaminsky & Mäser, 2025; Torgerson & Macpherson, 2011). Despite significant scientific advances, these diseases remain insufficiently detected and poorly monitored in terms of global funding and policy attention and are therefore classified among NZDs (Welburn et al., 2015).

Zoonotic protozoa, such as *Toxoplasma gondii*, *Cryptosporidium* spp., *Giardia duodenalis* and *Leishmania* spp., represent important parasites in multi-host

transmission systems involving humans, domestic animals, and wildlife (Kaminsky & Mäser, 2025). Their transmission is commonly mediated through environmental pathways, including contaminated food and water, or via vectors and direct contact between hosts (see below). *Toxoplasma gondii* is typically transmitted through ingestion of contaminated food or water, with domestic and wild animals acting as reservoirs (Marín-García et al., 2022). *Cryptosporidium* spp. and *G. duodenalis* are primarily spread via the feacal-oral route and are frequently associated with waterborne transmission, enabling circulation between humans and animals sharing the same environment (Putignani & Menichella, 2010). *Giardia duodenalis* comprises several genetic assemblages (A–H), of which assemblages A and B exhibit a broad host range and infect both humans and animals, indicating their zoonotic potential (Shu et al., 2022). In contrast, *Leishmania* spp. are transmitted by sandfly vectors, with zoonotic cycles involving domestic dogs and wildlife reservoirs, particularly in case of *L. infantum* (Lombardi et al., 2014).

Besides protozoa, helminth zoonoses constitute another major group of parasitic infections with significant zoonotic potential. They pose a persistent threat due to their complex life cycles, wide host ranges, and ecological adaptability. *Echinococcus*, *Trichinella*, *Toxocara* and hookworms (*Ancylostoma* and *Necator*) are among the most prominent zoonotic helminths. Their transmission often involves domestic and wild animals, contaminated environments with infective stages, or the consumption of undercooked or raw contaminated meat (Thompson, 2023). Among helminths, strongyloidiasis, caused by nematodes of the genus *Strongyloides*, represents a particularly relevant example of a neglected zoonotic parasitic disease with global distribution, especially in tropical and subtropical areas.

Despite the global burden of parasitic infections, affecting over one billion people worldwide (Centers for Disease Control and Prevention, 2024; Pritt, 2015), their diagnosis remains challenging. This is largely due to the vast diversity of parasitic species, their complex life cycles, interactions with hosts, and the existence of external reservoirs (Morchón et al., 2025). These challenges are further amplified in systems

where multiple host species coexist and interact within shared environments, creating complex circulation networks that are difficult to disentangle. Addressing such complexity requires an integrative perspective that considers host–environment relationships, as conceptualized within the One Health framework (Zinsstag et al., 2005).

2.3 One Health and human–wildlife interface

The One Health concept recognizes the interconnectedness of humans, animals, and environmental health and aims to optimize the health of these domains simultaneously (Deiana et al., 2024). It promotes interdisciplinary collaboration among veterinarians, physicians, and public health experts to improve surveillance, early detection, and response strategies (Elsohaby & Villa, 2023; Sharan et al., 2023, Zinnstag et al., 2005). This framework is increasingly applied to address complex health challenges, including zoonoses, neglected tropical diseases, vector-borne diseases, antimicrobial resistance, food safety, and environmental contamination (Centers for Disease Control and Prevention, 2025). In addition to its public health relevance, the One Health approach has been associated with substantial economic benefits, with projected global savings exceeding 300 billion dollars over the next century (Shaheen, 2022).

The One Health concept has been successfully implemented in the study and control of a wide range of pathogens, including viruses such as hepatitis E virus, rotavirus, influenza A viruses, Ebola virus, and rabies virus (Shaheen, 2022), and parasites such as *Taenia solium*, *Giardia* spp. (Krecek et al., 2020), *Toxoplasma gondii* (Innes et al., 2020), *Trypanosoma cruzi*, *Echinococcus multilocularis*, *Trichinella* (Thompson, 2013), *Strongyloides* spp. (Nosková et al., 2026), strongylid nematodes (Ilík et al., 2023), and *Entamoeba* spp. (Vlčková et al., 2018).

To understand the relevance of the One Health approach in the context of zoonotic diseases, it is essential to consider the ecological and demographic drivers that shape interactions among hosts, pathogens, and their environments. Emerging infectious diseases are often associated with changes in host density, mobility, and behaviour, as

well as environmental alterations that influence pathogen transmission (Pedersen & Davies, 2009). In tropical and subtropical regions in particular, anthropogenic environmental change has led to increasing spatial overlap between humans and wildlife, creating conditions that facilitate pathogen exchange. One of the most important consequences of these changes is the alteration of natural habitats. Expansion of agriculture, deforestation, urbanization, and infrastructure development have transformed continuous ecosystems into mosaic landscapes composed of forest fragments, agricultural areas, human settlements, and transportation networks (Estrada et al., 2017; Hill, 2018; Wolfe et al., 2005). As a result, wildlife, livestock, and human populations increasingly occupy shared spaces, leading to more frequent encounters and altered patterns of resource use. Many wildlife species are therefore increasingly exposed to human-dominated environments and may occupy forest edges, agricultural areas, and even village surroundings (Estrada et al., 2017) (Fig. 1). These processes affect both protected and non-protected areas, although the nature and intensity of human–wildlife interactions differ between them.

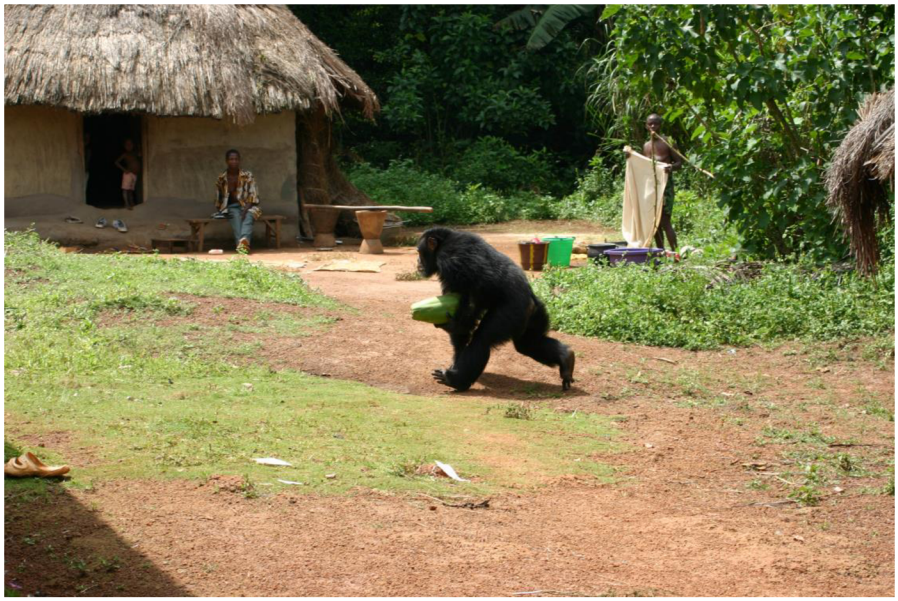

Fig. 1: Adult male chimpanzee observed in a village environment in Bossou (Guinea) carrying a papaya fruit. Such behaviour reflects the increasing overlap between human settlements and wildlife habitats, facilitating human–non-human primate interactions. Adapted from: Meijaard et al., 2023.

Protected areas play a key role in biodiversity conservation and in maintaining relatively intact ecosystems. However, the level of protection and human access varies among different categories of protected landscapes: (a) national parks permit no resource extraction, but tourism and scientific research still involve regular human presence in wildlife habitats, (b) game reserves allow tourist hunting, and (c) forest reserves permit some human activities as for example selective logging (Estrada et al., 2017; Stoner et al., 2007). These activities increase the frequency of encounters between humans and wildlife and may promote cross-species pathogen exchange (Gillespie & Chapman, 2008; Woodford et al., 2002).

Outside protected areas, habitat fragmentation and land-use change frequently force wildlife to utilize human-modified environments. In such landscapes, animals may exploit agricultural crops as a reliable and energy-rich food source, leading to crop-foraging behaviour and conflicts with local communities. In addition to direct interactions, indirect contact through shared environmental resources may occur when humans and wildlife use the same water sources, agricultural fields, or forest fragments. Such environments may act as a reservoir for environmentally transmitted pathogens, including soil-transmitted helminths (Herrera et al., 2020; McLennan et al., 2018; Nunn & Altizer, 2006).

Phylogenetic relatedness between host species also plays an important role in cross-species pathogen transmission (Davies & Pedersen, 2008). Closely related host species often share similar physiological traits and immune responses, which may enable pathogen host switching. This relationship is particularly evident among primates, where the close evolutionary relationship between humans and NHP, particularly great apes, increases the likelihood that pathogens may infect multiple host species (Wolfe et al., 2005).

Within this broader context, interactions between humans and NHPs represent a particularly important and well-documented interface for zoonotic pathogen transmission (Davies & Pedersen, 2008). In many regions, these interactions are not

limited to passive coexistence but often involve direct conflict situations, including aggressive encounters, crop-foraging responses, or even predation of livestock by certain NHP species, such as chimpanzees and baboons (Humle & Hill, 2016). Such conflicts may result in physical contact, injuries, or handling of animals, all of which increase the likelihood of pathogen transfer. In addition, NHPs are frequently hunted for bushmeat, live pet trade, traditional medicine, ornamental purposes, or rituals (Humle & Hill, 2016) (Fig. 2). Moreover, illegal wildlife trade and primate trafficking represent additional high-risk interfaces, as animals are captured, transported, and handled under conditions that facilitate close contact between humans and NHPs (Karesh et al., 2005).

These processes are often associated with physiological stress and immunosuppression in animals, which may increase vulnerability to diseases, as well as recrudescence of latent and normally innocuous pathogens (Hing et al., 2016). Bushmeat hunting and subsequent handling of infected wildlife carcasses, particularly those of apes, have been linked to the emergence of major infectious diseases, including HIV and Ebola virus (Muehlenbein, 2012). Indirect transmission pathways also play a crucial role in human–NHP interfaces. Shared environments contaminated with faecal material represent important sources of infection, particularly for environmentally transmitted pathogens.

Fig. 2: Examples of bushmeat derived from non-human primates – (a) skull, (b) hand, (c) skull and torso, (d) arm. The hunting, and trade of wildlife products increase the likelihood of direct contact between humans and animal tissues, thereby facilitating potential zoonotic pathogen transmission. Adapted from: Smith et al., 2012.

Activities such as ecotourism, wildlife research, and primate rehabilitation further intensify human–NHP contact, often leading to habituation of animals to human presence (Gillespie & Chapman, 2008). This loss of natural avoidance behaviour may increase the frequency of close encounters, as a result of reduced fear of humans, including crop foraging, use of anthropogenic food sources such as garbage pits, and contact with human waste sites, while also making NHPs more vulnerable to poaching (Muehlenbein, 2012). These conditions create opportunities for bidirectional pathogen exchange, including the spread of soil-transmitted helminths.

Given the high phylogenetic proximity between humans and great apes, pathogens adapted to one host may more easily establish infection in the other (Wolfe et al., 2005), emphasizing the need to study parasite transmission within human–NHP interfaces from a One Health perspective. These interaction patterns also point to the

importance of considering pathogen-specific transmission dynamics when studying zoonotic diseases within human–wildlife interfaces.

The likelihood of cross-species transmission is further shaped by pathogen-specific traits. Viruses, particularly RNA viruses, are frequently associated with host shifts and emerging zoonotic diseases because of their high mutation rates and rapid evolutionary dynamics (Cleaveland et al., 2001; Pedersen et al., 2005). In contrast, many helminths exhibit higher levels of host specificity, although transmission between closely related host species may still occur under suitable ecological conditions (Pedersen et al., 2005). These ecological interactions create conditions that facilitate pathogen transmission across species boundaries. In parasitology, numerous examples illustrate how the One Health framework can help explain the dynamics of zoonotic parasites.

2.3.1 Parasitic infections at the human–non-human primate interface

The One Health concept has been widely applied in parasitology to better understand the circulation patterns of parasites between humans, animals, and the environment. In multi-host systems, such as those involving humans and NHPs, the integration of ecological, molecular, and epidemiological approaches is essential for identifying transmission pathways and assessing zoonotic potential. This section focuses on selected parasitic systems that illustrate key transmission mechanisms within human–NHP interfaces.

One of the most well-documented examples of such interactions is represented by the genus *Plasmodium*, the causative agent of malaria. Several *Plasmodium* species, including *P. knowlesi*, naturally circulate in NHP populations, particularly in macaques, and may be transmitted to humans via *Anopheles* mosquito vectors that feed on both host groups (Lempang et al., 2022; Phang et al., 2025). Unlike *P. malariae*, infections with *P. knowlesi* can lead to severe or even fatal disease in humans (Lee et al., 2011). The increasing overlap between humans, NHPs, and vectors, driven by environmental changes such as deforestation and land-use transformation, has been associated with

a rising incidence of zoonotic malaria, particularly infections caused by *P. knowlesi*, which is naturally maintained in macaque populations but increasingly infects humans in Southeast Asia (Divis et al., 2017). Moreover, evolutionary studies have shown that *P. falciparum*, the most virulent human malaria parasite, originated from gorilla parasites following a cross-species transmission event, highlighting the potential for host shifts between humans and NHPs (Liu et al., 2010). Environmental factors, including temperature, precipitation, and seasonality, influence vector abundance and transmission intensity (Masse et al., 2025). In regions where humans and NHPs coexist, NHP-associated *Plasmodium* species may represent a significant challenge for malaria control, as NHPs act as reservoirs (Lee et al., 2011) that are not targeted by conventional interventions. These systems exemplify the interconnected roles of hosts, vectors, and ecological conditions in shaping disease transmission.

A different mode of transmission is observed in intestinal protozoa, such as *Giardia duodenalis*, which is primarily transmitted via the faecal–oral route through contaminated food and water (Shu et al., 2022). Within a One Health framework, this mode of transmission highlights the importance of environmental reservoirs and hygiene conditions in mediating parasite exchange between humans and animals (Li et al., 2021). Assemblages A and B infect both humans and NHPs, indicating their zoonotic potential (Shu et al., 2022). The prevalence and transmission of *Giardia* are further influenced by environmental and anthropogenic factors, including hygiene conditions, climate, and host interactions between humans and NHPs (Kuthyar et al., 2022). Evidence from human-managed environments, such as zoological gardens, has further demonstrated the occurrence of *G. duodenalis* in both NHPs and their caregivers, highlighting the potential for cross-species transmission in close-contact settings (Brožová et al., 2025).

Soil-transmitted helminths represent another group of parasites in human–NHP systems. Excellent example are strongylid nematodes that infect a wide range of mammalian hosts, including humans and NHPs. In humans, the most significant strongylids are hookworms such as *Necator americanus*, *Ancylostoma duodenale*, and

A. ceylanicum, collectively infecting over 400 million people worldwide, while in great apes, hookworm *Necator* spp. and other representative of strongylids, *Oesophagostomum* spp., are among the most prevalent helminths (Ilík et al., 2023). These parasites inhabit primarily the gastrointestinal tract, where they feed on host tissues or blood (Anderson, 2000; Zajac, 2006), and can cause intestinal inflammation, lesions, weight loss, and, in severe cases, mortality (Roeber et al., 2013). Their spread depends on infective larvae developing in contaminated soil, making factors such as temperature, humidity, and vegetation key determinants of parasite survival and spread, emphasizing the importance of the environment in the One Health framework (Mason et al., 2024). Molecular studies have revealed complex patterns of host specificity and cross-species transmission in strongylid communities. For example, *N. gorillae* haplotypes have been found to be shared between humans and gorillas, while *N. americanus* appears to be more restricted to humans, however, also present in great apes.

The degree of parasite sharing is influenced primarily by habitat overlap rather than direct contact, as both habituated and unhabituated NHPs share strongylids with humans (Pafčo et al., 2019). Also, human populations with greater exposure to forest environments exhibit more similar strongylid communities to those of NHPs. On the other hand, NHPs carried strongylids that were host specific and not shared with others. These findings indicate that strongylid transmission in human–NHP systems is shaped by interactions between host ecology and environmental conditions, and that both zoonotic and host-specific lineages may coexist within the same system. Understanding these dynamics requires integrating molecular data with ecological context to better assess transmission pathways and zoonotic risk. Among these systems, *Strongyloides* spp. represent a particularly relevant and similar model due to their complex life cycle, environmental transmission, and potential for multi-host circulation.

2.3.2 One Health and *Strongyloides stercoralis*

Strongyloidiasis, caused by *Strongyloides* nematodes, is considered as one of the most neglected tropical diseases, affecting hundreds of millions of people worldwide (Buonfrate et al., 2023). *Strongyloides stercoralis*, the predominant causative agent of strongyloidiasis, is capable of causing severe disease due to its ability to undergo autoinfection, which may culminate in hyperinfection. These heavy infections can lead to fatal outcomes in extreme cases (Nosková et al., 2023a). Its zoonotic potential is increasingly recognized, as infections have been documented not only in humans but also in dogs (e.g., Gorgani-Firouzjaee et al., 2022; Jaleta et al., 2017; Nosková et al., 2026) and NHPs (Bradbury et al., 2021; Nosková et al., 2026), underscoring the potential for cross-species spread.

Environmental studies show that larvae can persist in water, fruits, vegetables, and in soil (under favourable humidity and soil porosity) (White et al., 2019). In Ethiopia, a systematic review linked poor environmental health to higher prevalence rates, reinforcing the need to integrate environmental and social determinants into strongyloidiasis control (Terefe et al., 2019). Recent review from Lyu et al. (2025) emphasizes that effective control requires regular parasitological testing and monitoring also on dogs, mass drug administration (MDA), environmental surveillance and public awareness.

Historically, strongyloidiasis was overshadowed by other soil-transmitted helminthiases in global health agendas. However, due to the key scientific and policy changes along with the requests from endemic countries, the WHO has recently shifted its focus to include *Strongyloides*. In 2024, WHO published the guideline for public health control of strongyloidiasis, recommending annual MDA with single dose ivermectin in all age groups from 5 years in endemic areas, where prevalence reaches or exceeds 5%, measured either with Bearmann or agar plate culture from stool samples (Lo et al., 2025). Furthermore, *S. stercoralis* is now officially included in the WHO Roadmap for Neglected Tropical Diseases 2021–2030, as it was not included in the targets for 2020. At that time, there used to be included only *Ascaris lumbricoides*,

Trichuris trichiura and hookworms – *Necator americanus* and *Ancylostoma duodenale* as targets for soil-transmitted helminthiases (World Health Organization, 2020).

Although the WHO does not explicitly label their strategy as "One Health" when it comes to strongyloidiasis, the approach is reflected in practise. Strongyloidiasis control requires: (a) human health interventions: improved case detection, ivermectin-based MDA, and clinical management of high-risk populations (Lo et al., 2025; World Health Organization, 2020); (b) animal health interventions: evidence of animal reservoirs in several regions calls for veterinary engagement in control strategies (Gorgani-Firouzjaee et al., 2022; Jaleta et al., 2017); and (c) environmental interventions: addressing soil contamination, sanitation, and hygiene to reduce larval persistence (White et al., 2019).

2.4 Biology and diversity of *Strongyloides* in primates

The genus *Strongyloides* comprises soil-transmitted parasitic nematodes within the order Rhabditida. Members of this genus infect the gastrointestinal tract of a wide range of terrestrial vertebrates, including mammals, birds, reptiles and amphibians (Dorris et al., 2002; Hunt et al., 2016). These nematodes are uniquely characterized by a complex life cycle that alternates between parasitic and free-living generations. More than 50 species have been described to date (Viney & Lok, 2015), although their true diversity is likely underestimated due to the morphological similarity among species and the increasing recognition of cryptic lineages through molecular studies.

In the context of primate infections and human health, four major taxa are recognised: *S. stercoralis*, *S. fuelleborni* (comprising two subspecies: *S. f. fuelleborni* and *S. f. kellyi*) (Bradbury et al., 2021) and the Neotropical species *S. cebus* (Mati et al., 2013). Among these species, only two are considered established human pathogens: *S. stercoralis* and *S. fuelleborni* (Nosková et al., 2023a). Recent estimates of the global burden of human strongyloidiasis have shifted dramatically. While data from the WHO estimated 30–100 million infections, more recent epidemiological modelling suggested a burden exceeding 600 million people worldwide (Buonfrate et al., 2020).

This discrepancy is largely attributed to the low sensitivity of traditional diagnostic methods and the high prevalence in remote, underprivileged regions where soil-transmitted helminths are endemic (World Health Organization, 2025).

2.4.1 Life cycle and general biology

A defining characteristic of the genus *Strongyloides* is its complex life cycle, which alternates between free-living and parasitic generations (Hunt et al., 2016). The parasitic generation consists exclusively of parthenogenetic females, whereas the free-living generation includes both males and females inhabiting organic soil (Viney & Lok, 2015). Entry into a parasitic stage is obligatory and is influenced by conditions such as temperature, pH, quantity of food, and class of fatty acids available. Development into infective filariform larvae in the soil occurs more frequently than development into free-living adults (Yamada et al., 1991).

Infections of new host occurs primarily through transdermal penetration of infective larvae, although infection may also occur through ingestion of larvae or rarely through organ transplantation (Page et al., 2018). Infective third-stage larvae (iL3) penetrate the host's skin and migrate via the bloodstream to the right side of the heart and subsequently to the lungs. From there, the larvae ascend the trachea, are swallowed, and reach the small intestine where they mature into adult females. The blood-pulmonary pathway is not the only route by which *Strongyloides* larvae may reach the gut, but they may also randomly migrate through viscera and other tissue directly to the duodenum. Adult females reproduce parthenogenetically and produce eggs (majority of *Strongyloides* species including *S. fuelleborni* and *S. cebus*) or first-stage larvae (L1) (*S. stercoralis*), which are shed in faeces. Depending on environmental conditions, L1 larvae may either develop directly into infective iL3 or differentiate into free-living adults that reproduce sexually, producing a subsequent generation of infective larvae (Hunt et al., 2016; Page et al., 2018) (Fig. 3).

A unique feature of *S. stercoralis* is its capacity for hyperinfection. Larvae produced by parthenogenetic females do not necessarily leave the host with faeces but

can remain within the host and reinfect it. Larvae in non-disseminated hyperinfection remain in organs involved in the autoinfective cycle but are increased in numbers. This mechanism enables persistent, potentially lifelong infections and may lead to disseminated strongyloidiasis, which refers to migration of larvae to organs beyond autoinfective cycle. Mortality in severe disseminated cases may reach up to 85% (Nutman, 2017).

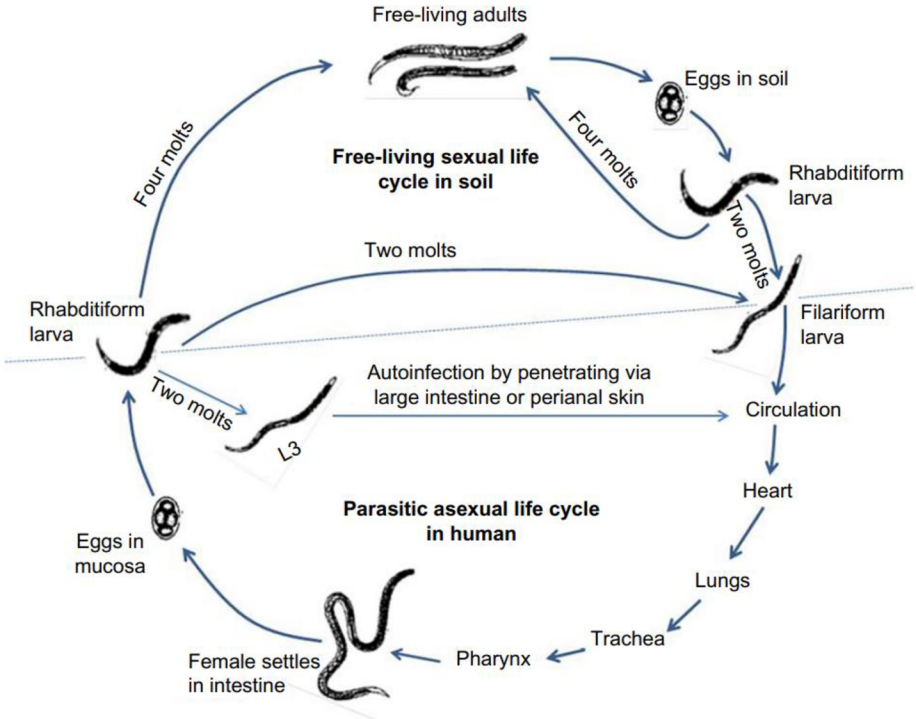

Fig. 3: Life cycle of *Strongyloides stercoralis* alternating between free-living and parasitic cycles. Adapted from: Varatharajalu & Rao, 2016.

2.4.2 Key species and host range in primates

Strongyloides stercoralis is the most clinically significant species and the primary causative agent of human strongyloidiasis. It infects a broad range of hosts, including humans, dogs, NHPs, and occasionally cats, and is therefore considered a key species in multi-host transmission systems (Nutman, 2017; Peregrine & Carnevale, 2023).

In NHPs, *S. stercoralis* has been reported in both captive and wild populations. In captive environments, infections have been documented in chimpanzees in San Diego Zoo (Penner, 1981) and orangutans in Czech and Slovak zoos, where close contact with humans may facilitate transmission (Nosková et al., 2023b). In wild populations, molecular studies have confirmed infections in western lowland gorillas in Central African Republic (Nosková et al., 2026) and chimpanzees in Tanzania (Hasegawa et al., 2016). These findings indicate that *S. stercoralis* is not restricted to anthropogenic environments but can persist in natural ecosystems where hosts share contaminated habitats.

The species is distinguished from other primate-infecting *Strongyloides* species by being ovoviviparous with first stage larvae (L1) being passed in faeces (Fig. 4), in contrast to the egg-shedding pattern observed in other species (Buonfrate et al., 2023; Nosková et al., 2023a). Infections with *S. stercoralis* shows a wide clinical spectrum, ranging from asymptomatic infection to life-threatening disease. This variability is largely due to its unique ability to undergo autoinfection, allowing the parasite to persist in the host for decades (Czeresnia & Weiss, 2022). Acute infection occurs after skin penetration of larvae and is usually mild, presenting with localized skin irritation, followed by pulmonary symptoms such as cough and dyspnoea during larval migration. Gastrointestinal symptoms, including abdominal pain and diarrhoea, may also occur (Czeresnia & Weiss, 2022; Raj et al., 2020). Chronic strongyloidiasis is most often asymptomatic but can cause intermittent gastrointestinal symptoms and respiratory manifestations, including asthma-like symptoms (Czeresnia & Weiss, 2022). In immunocompromised individuals, particularly those receiving corticosteroids or co-infected with human T cell lymphotropic virus type 1, hyperinfection syndrome may develop (Czeresnia & Weiss, 2022; Ye et al., 2022). This severe form is characterized by uncontrolled parasite multiplication, leading to gastrointestinal and pulmonary involvement and high mortality, which may reach 85–100% in untreated cases (Czeresnia & Weiss, 2022). Dissemination to other organs,

including the liver, central nervous system, and skin, has also been reported (Ye et al., 2022).

Fig. 4: Rhabditiform larva (L1) of *Strongyloides stercoralis* recovered from a stool sample. Adapted from: Nadhem et al., 2014.

Numerous fatal cases in NHPs were described, and they have been exclusively caused by *S. stercoralis* or *S. cebus* (Nosková et al., 2023a) and reported especially in young or immunocompromised individuals. Lethal infections with *S. stercoralis* have been documented for example in young chimpanzees and gorillas at San Diego Zoo, where massive parasite burdens were observed at autopsy (Penner, 1981), as well as in a juvenile Sumatran orangutan, with disseminated infection (Kleinschmidt et al., 2018). Although orangutans are considered highly susceptible, especially juveniles under five years of age, no clinical signs were observed in infected individuals in Czech zoos (Nosková et al., 2023b).

Strongyloides fuelleborni fuelleborni is the predominant *Strongyloides* species infecting wild NHPs in Africa and Asia (Nosková et al., 2023a). It has been reported in

a wide range of Old World primates, including western chimpanzees, western lowland gorillas (Hasegawa et al., 2016), mountain gorillas, olive baboons (Bukamba et al., 2026), vervet monkeys (Juhász et al., 2025), and orangutans (Labes et al., 2011). It has also been documented, typically at lower prevalence, in captive and pet primates (Pourrut et al., 2011).

In contrast to *S. stercoralis*, *S. f. fuelleborni* is more strongly associated with NHP hosts, although human infections have also been reported, particularly in regions where humans are in close contact with NHP populations (Nosková et al., 2023a), such as tropical forest ecosystems in Gabon (Zhao et al., 2025) and the Democratic Republic of Congo (Potters et al., 2020).

Infections with *S. f. fuelleborni* is typically asymptomatic in NHPs and generally asymptomatic or mild in humans. When clinical signs occur, they are usually limited to gastrointestinal symptoms such as diarrhoea and abdominal discomfort, occasionally accompanied by dermatological manifestations or mild respiratory symptoms (Nosková et al., 2023a). Severe or disseminated infections have not been reported in association with *S. f. fuelleborni*, which is likely related to the absence of autoinfection in its life cycle (Nosková et al., 2023a).

The subspecies *S. fuelleborni kellyi* has historically been described as a geographically restricted taxon occurring in Papua New Guinea and associated with human infections. It has been particularly linked to severe infections in infants and young children, including swollen belly syndrome, characterized by abdominal distension, diarrhoea, respiratory distress, and potentially fatal outcomes (Bradbury, 2021). However, recent study by Zhao et al. (2025) provides molecular evidence that challenges the validity of this subspecies. High-throughput sequencing shown that infections previously attributed to *S. fuelleborni kellyi* are in fact genetically consistent with the Asia-Pacific clade of *S. fuelleborni fuelleborni*, rather than representing a distinct subspecies. In addition, this analysis suggests the possible presence of an undescribed *Strongyloides* species infecting humans in Papua New Guinea, which may have been misidentified as *S. f. kellyi* in earlier studies. Unlike *S. f. fuelleborni*, which is

primarily associated with NHPs, no confirmed animal reservoir has been identified for these infections in Papua New Guinea, likely due to the absence of native NHP hosts (Bradbury, 2021; Zhao et al., 2025).

Strongyloides cebus is a species primarily associated with Neotropical primates and has not been reported in humans (Nosková et al., 2023a). It has been documented in several New World monkey species, including gray wooly monkey (*Lagothrix cana*) (Mati et al., 2013) and yellow-tailed wooly monkey (*Lagothrix flavicauda*) (Guzmán et al., 2020). Occasional infections have also been reported in captive NHPs outside this group, such as ring-tailed lemurs (*Lemur catta*) in United States zoo, likely representing spillover events rather than natural host associations (Hiebert et al., 2023). Phylogenetic analyses based on mitochondrial genome sequences indicate that *S. cebus* is distantly related to both *S. stercoralis* and *S. fuelleborni.* In contrast to these species, *S. cebus* diverged early from the ancestral lineages of the genus and does not cluster within either the stercoralis/procyonis group or the papillosus group (including *S. fuelleborni*), suggesting an independent evolutionary origin of primate parasitism in this lineage (Ko et al., 2023).

Infections caused by *S. cebus* in New World monkeys can range from mild to severe clinical forms. The most commonly reported symptom is diarrhoea, often accompanied by dehydration and appetite loss. In more severe cases, animals may require hospotalization, and fatal outcomes have been documented in captive setting. Pathological findings include enteritis with inflammatory changes, and in severe cases ulcerative enteritis (Mati et al., 2013). These observations indicate that, although often overlooked, *S. cebus* infections can represent a significant health problem in NHPs, particularly under captive conditions.

2.4.3 Genetic diversity and multi-host transmission

Recent molecular studies have demonstrated that the diversity of *Strongyloides* in primates is substantially underestimated, with multiple cryptic lineages that cannot be distinguished morphologically. Analyses based on 18S rRNA, cytochrome c oxidase

subunit I (*cox1*), and 28S genes in primates from regions such as Borneo (Frias et al., 2018), Mexico (Sólorzano-García & de León, 2017), Central African Republic (CAR) (Nosková et al., 2026), and a research centre in the USA (Anderson et al., 2012) have identified sequences that do not cluster with known species, sometimes forming unresolved or sister clades with other mammalian *Strongyloides* species. Such findings may reflect host-specific cryptic speciation, local adaptation, or historical host switching events.

A key advance in understanding transmission dynamics has been the identification of host-associated and host-shared haplotypes. For *S. stercoralis*, analyses targeting the hypervariable region (HVR) IV of the 18S rRNA gene and the mitochondrial *cox1* gene have identified distinct lineages/haplotypes, with haplotype A occurring in humans, dogs, and NHPs, suggesting zoonotic transmission, whereas haplotype B appears to be restricted to dogs (Bradbury et al., 2021; Jaleta et al., 2017; Nosková et al., 2026). Similarly, studies on *S. fuelleborni* have revealed a diversity of HVR-I (haplotypes XII–XIV) and HVR-IV (haplotypes K–T) variants, some of which are shared across host species, while others appear to be host-specific (Barratt et al., 2019). These findings indicate that *Strongyloides* populations may consist of a mixture of generalist and host-adapted lineages. Previous studies (e.g., Hasegawa et al., 2016; Nosková et al., 2026) further suggest that HVR-IV haplotypes often correspond well with broader transmission patterns also detected using *cox1*, indicating that the HVR-IV marker alone can provide informative resolution for studying host associations and cross-host transmission. Although mitochondrial *cox1* is more variable and can reveal finer within-species diversity, short *cox1* amplicons commonly used in HTS studies often generate large numbers of highly variable sequence types that are difficult to classify and interpret consistently. In contrast, the HVR-IV region provides a more conservative and readily interpretable marker for detecting shared haplotypes among host species (Jaleta et al., 2017; Nosková et al., 2026).

Direct evidence of cross-species transmission has been demonstrated in natural multi-host systems. A notable example is the study by Nosková et al. (2026) conducted

in the Dzanga-Sangha Protected Area (CAR), where humans, great apes, and domestic dogs coexist in close ecological contact. In this system, identical haplotypes of both *S. stercoralis* and *S. fuelleborni* were detected across host species, providing strong molecular evidence for ongoing transmission. In particular, *S. stercoralis* haplotype A was found in both humans and gorillas, while *S. fuelleborni* haplotypes L and P were detected in humans, gorillas, chimpanzees, and dogs, indicating active circulation within a shared transmission network (Fig. 5).

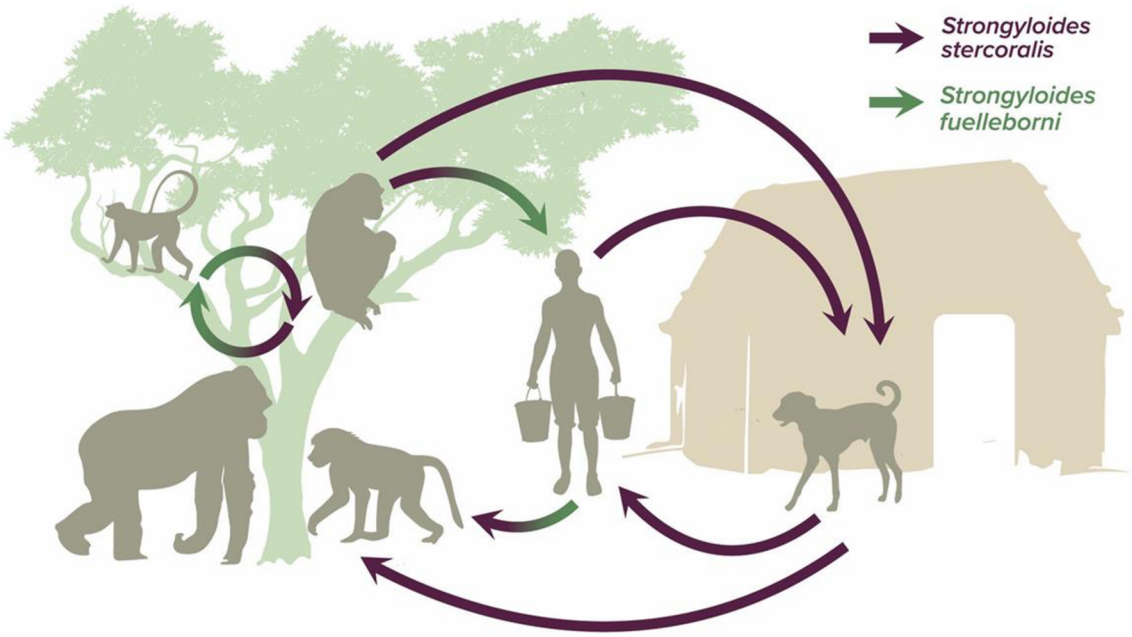

Fig. 5: Potential transmission pathways of *Strongyloides* spp. in a multi-host system, highlighting interactions among humans, non-human primates, and domestic animals. Adapted from: Bukamba et al., 2026.

Zoonotic transmission of *S. fuelleborni* to humans has also been documented. Human infections have been reported in individuals living in close proximity to NHPs in rainforest ethnic communities with frequent environmental exposure. The highest prevalence was found in hunters–gatherers in the CAR (Pampiglione & Ricciardi, 1974; Pampiglione et al., 1979). Haplotypes K, L, and O were found in villagers living near NHP habitats in Gabon (Zhao et al., 2025). In addition, cases of infection have been described in individuals working with primates, such as a researcher working with

chimpanzees in Tanzania (Hasegawa et al., 2010) or a student collecting faecal and urine samples of bonobos in the Democratic Republic of the Congo (Potters et al., 2020), where molecular analyses confirmed the zoonotic origin of the parasite. These observations demonstrate that *S. fuelleborni*, although primarily associated with NHPs, can be transmitted to humans under conditions of close contact. Although, study conducted in Dzanga-Sangha Protected Area (CAR) showed based on phylogenetic analysis of *cox1* and comparison of HVR-IV sequences that *S. fuelleborni* populations in humans differ from those in NHPs (Hasegawa et al., 2016).

Evidence for *S. stercoralis* infection in NHPs alone does not necessarily indicate ongoing cross-species transmission. Molecular studies provide a more nuanced picture. For example, analyses of *S. stercoralis* populations in humans and macaques in Asia revealed distinct host-associated genetic clusters, suggesting limited gene flow between host species despite ecological overlap (Hasegawa et al., 2016). This indicates that, even in systems where the parasite occurs in multiple hosts, transmission between host species may be infrequent. Similarly, whole-genome sequencing of *S. stercoralis* from humans and dogs demonstrated clear genetic differentiation between host-associated populations, indicating largely independent transmission cycles with only occasional host-switching events (Liu et al., 2025). Studies from West Africa have also shown that parasite communities may differ significantly between humans and NHPs in sympatric populations, further supporting the existence of host-structured transmission systems (Mensah et al., 2026).

These findings indicate that cross-species transmission of *Strongyloides* is not universal. While extensive haplotype sharing in some systems provides clear evidence of transmission, other studies demonstrate strong host-associated structuring indicating that parasite populations may remain largely independent despite ecological overlap. Overall, *Strongyloides* populations in multi-host systems are structured by a balance between host specificity and ecological connectivity. While molecular data clearly demonstrate that cross-species transmission occurs, it is not universal and varies across ecological contexts, highlighting the importance of

integrating genetic, ecological, and epidemiological data when interpreting transmission patterns.

2.5 Diagnostic methods for *Strongyloides*

The diagnosis of *Strongyloides* spp. has long presented a challenge in both clinical and epidemiological contexts. It is challenging due to its low parasite load, intermittent eggs/larval excretion, and autoinfective cycle, often leading to underdiagnosis when traditional methods are used (Luvira et al., 2016; Requena-Méndez et al., 2013). For diagnosis of *Strongyloides* spp., there are three main methods used: (a) microscopy-based methods, (b) immunological methods, and (c) molecular methods (Costa et al., 2021).

2.5.1 Microscopy-based methods

In endemic regions, diagnosis often relies on direct smear methods applied to faecal samples. However, the sensitivity of these tests is highly dependent on the number of parasite stages excreted, sample handling, and trained personnel (Luvira et al., 2016; Wong et al., 2014).

The direct faecal smear is the simplest diagnostic method. A small portion of fresh stool (approximately 2 mg) is mixed with saline or iodine solution, smeared onto the glass slide and directly examined microscopically for the presence of eggs/larvae (Al-Mekhlafi et al., 2019; de Kaminsky, 1993). The method is inexpensive, requiring minimal equipment, but it detects less than 30% of chronic infections (Requena-Méndez et al., 2014). Given the typically low parasite burden of *S. stercoralis*, direct smears often yield false negatives (Luvira et al., 2016). Moreover, differentiation from other nematode larvae (e.g., hookworms) requires experienced microscopists (Wong et al., 2014). To enhance the sensitivity of direct faecal smear, albendazole was used in one study to stimulate the secretion of *Strongyloides* larvae. This approach increased detection by approximately 50% in previously negative samples (Anamnart et al., 2010).

To increase sensitivity, various stool concentration techniques have been developed. The formalin-ether sedimentation and zinc sulphate or sugar flotation methods are commonly applied to detect protozoan cysts and trophozoites, as well as helminth eggs or larvae, including *Strongyloides* larvae. For heavy parasite eggs, the concentration technique based on sedimentation is useful, as they concentrate in sediment, whereas the flotation is used to recover rather protozoa cysts because of their low density (Inês et al., 2016). However, for detection of *Strongyloides*, it has been showed that zinc sulphate flotation is able to recover *Strongyloides* eggs (Inês et al., 2016; Pouillevet et al., 2017), but not heavy *S. stercoralis* larvae. Anamnart et al. (2013) modified formalin-ether concentration technique to the point, where its efficacy has become comparable to agar plate culture for *S. stercoralis* targeting. He used two layers of wire meshes instead of gauze to avoid the trapping of larvae in the gauze during filtration, and he also reduced the exposure time of larvae to formalin to avoid the density reduction of larvae caused by long exposure to it.

The Bearmann method remains one of the most effective parasitological techniques for detecting *Strongyloides* larvae. It exploits the thermotactic and hydrotactic behaviour of rhabditiform larvae, which migrate from faecal material suspended in warm water (37°C) for at least two hours (for species producing eggs in the faeces up to 48 hours), toward the bottom of a funnel. Although this technique has some limitations, e.g., requires fresh stool, is labour intensive and time-consuming, it significantly outperforms direct smear and concentration techniques (sedimentation, flotation), and is easy to implement in the basic laboratory in endemic countries because of its simplicity and low cost (Camacho-Alvarez et al., 2022; Hernández-Chavarría & Avendaño, 2001; Steinmann et al., 2007).

The agar plate culture method represents one of the most sensitive, traditional tools for *Strongyloides* detection and is considered the gold standard for diagnosis (Kaewrat et al., 2020). In this method, a small portion of faeces (about three grams) is placed on the centre of a nutrient agar-plate in petri dish, which is then sealed and incubated for 48 hours at 26°C. Motile larvae and adult worms leave visible tracks on

the agar surface, which can be observed with dissection microscope or with naked eyes. Formalin solution is then added to the agar surface, after a few minutes the formalin suspension is transferred to a tube and centrifuged. The sediment is then examined by a microscope (Hailu et al., 2021). Since this method has low sensitivity in the case of light infections, it can be modified by changing temperature, pH, nutrition and salinity (Kaewrat et al., 2020).

In summary, conventional microscopy-based techniques remain fundamental in *Strongyloides* detection, especially in resource-limited endemic areas. However, their sensitivity and specificity vary widely, and none can distinguish between species or detect low-intensity infection reliably. These limitations have led to the development of immunological and molecular diagnostic methods, which complement and, in some cases, surpass traditional approaches.

2.5.2 Immunological methods

Immunological methods play a crucial role in the detection of *Strongyloides* infections. Unlike direct parasitological techniques that rely on visualization of larvae, immunological assays are based on the detection of different classes of antibodies (IgG, IgM, IgA and IgE) produced by the host in response to infection (Costa et al., 2021). These methods are generally more sensitive than parasitological approaches and are particularly valuable for screening in endemic communities and among migrants. However, their performance is limited by reduced sensitivity during the early stages of infection and in immunocompromised patients, as well as by limited specificity due to cross-reactivity with other helminth infections (Hall et al., 2024).

The most used antibody for serodiagnosis of strongyloidiasis is specific IgG, with its subclasses IgG1 and IgG4 showing improved specificity (Rodrigues et al., 2004). A recent study by Ahmad et al. (2021) also suggested a potential role of IgE in the early detection of *Strongyloides* infections. IgG (including its subclasses) and IgE are commonly used antibodies in enzyme-linked immunosorbent assay (ELISA) (e.g., Ahmad et al., 2021; Gonzaga et al., 2018; Ortiz-Martínez et al., 2020; Toledo et al., 2019)

and immunoblotting techniques (e.g., de Carvalho et al., 2015; Rodrigues et al., 2004). Since IgG ELISA may yield false positive results, and remain positive for several months after successful treatment, Balachandra et al. (2021) developed a *Strongyloides* antigen detection ELISA (SsAg-ELISA), which is based on detection of immune complexes formed by circulating NIE antigens bound to *Strongyloides*-specific IgG antibodies.

Due to the difficulty of obtaining sufficient quantities of *S. stercoralis* larvae for the preparation of homologous antigen extracts (mainly because of low parasite load and irregular larval shedding), larvae from *S. venezuelensis*, and *S. ratti* are often used as alternative antigen sources. These larvae hatch from eggs released by experimentally infected rats and serve as a practical substitute for immunodiagnostic assay development (de Carvalho et al., 2015; Rodrigues et al., 2004). However, because invasive samples such as blood cannot usually be collected from wild NHPs, serology has not been used for *Strongyloides* detection in NHPs (nor in wild, nor in captive NHPs) (Nosková et al., 2023a). And therefore, serological assays have not yet been validated for the detection of *Strongyloides* species infecting NHPs, particularly for *S. fuelleborni*, which has zoonotic potential, and *S. cebus.*

Although immunological methods provide high sensitivity, they do not allow species- and lineage-level identification, which can be achieved using molecular methods, mainly by high-throughput sequencing (HTS).

2.5.3 Basic molecular methods

Molecular methods, based on detection of parasite DNA, have demonstrated high sensitivity and specificity in the diagnosis of *Strongyloides* infections (Paula et al., 2018). Polymerase chain reaction (PCR) based on the amplification of *Strongyloides* DNA, has shown particularly promising diagnostic results (Costa et al., 2021). Several studies have compared the diagnostic efficacy of conventional PCR (cPCR) and quantitative real-time PCR (qPCR) (e.g., de Paula et al., 2015; Formenti et al., 2019; Gryschek et al., 2023; Paula et al., 2018; Robertson et al., 2017), consistently demonstrating higher sensitivity of qPCR assays. PCR products obtained by cPCR are

loaded on an agarose gel, stained with fluorescent dye (e.g., SYBR Safe), and separated by electrophoresis. In contrast, qPCR enables real-time detection of amplification, with sample positivity determined based on the quantification cycle (C_q), which is defined at the beginning of the experiment (Gryschek et al., 2023).

Most molecular assays target DNA regions within the small subunit of the nuclear ribosomal RNA (18S) gene using specific primers (Robertson et al., 2017), most commonly hypervariable regions HVR-I and HVR-IV (Nosková et al., 2023a). These regions are amplified using either cPCR or qPCR (Gryschek et al., 2023) and have demonstrated higher sensitivity compared to assays targeting the *cox1* gene or species-specific repeated sequences (Verweij et al., 2009). They also exhibit sufficient sequence variability among *Strongyloides* species, making them suitable markers to distinguish between different genotypes in both human (Beknazarova et al., 2019) and NHP infections (Richins et al., 2023). Other DNA-based techniques, such as loop-mediated isothermal amplification (LAMP), have also been developed, typically targeting the 28S rRNA gene (28S rDNA) of *Strongyloides* (Watts et al., 2014). Additional molecular targets include the internal transcribed spacer regions (ITS1 and ITS2) and the major sperm protein (MSP) gene (Chan & Thaenkham, 2023; Nosková et al., 2023a).

For molecular identification, phylogenetic analyses, and the assessment of genetic diversity, subsequent sequencing of PCR amplicons is required. For isolated single *Strongyloides* larvae (or eggs), Sanger sequencing following cPCR is sufficient, however, this approach is inadequate for the identification of *Strongyloides* species from total DNA extracted from complex samples, such as faeces, which may contain DNA from multiple *Strongyloides* species or genotypes (Nosková et al., 2023a,b). In such cases, HTS technologies provide a more suitable approach (Chan & Thaenkham, 2023).

2.5.4 High-throughput sequencing

HTS refers to sequencing technologies that enable the simultaneous sequencing of millions of DNA fragments in parallel. Compared to conventional Sanger sequencing, HTS is faster and cheaper (von Bubnoff, 2008) and allows the detection of multiple sequence variants within a single sample (Metzker, 2010). Such capability is especially important for parasites such as *Strongyloides*, where infections may involve multiple species and genetic lineages (Barratt et al., 2019; Bedoya-Urrego et al., 2026).

The application of HTS has significantly advanced the study of *Strongyloides* in complex biological samples, particularly through amplicon-based metabarcoding approaches. These methods typically target HVR regions of the 18S rRNA gene, such as HVR-I and HVR-IV, allowing high-resolution identification of amplicon sequence variants corresponding to distinct genetic haplotypes. In contrast to single-locus sequencing, HTS enables the simultaneous detection of multiple *Strongyloides* lineages within a single sample, making it particularly suitable for studying genetically diverse parasite populations, mixed infections and multi-host systems. These approaches have revealed extensive intra-host diversity and the coexistence of multiple lineages/haplotypes within individual hosts, as well as sharing them across host species (Antonopoulos et al., 2024; Barratt et al., 2019; Metzker, 2010; Nosková et al., 2023a).

While amplicon-based HTS approaches provide high-resolution information on selected genetic markers, they remain limited to predefined loci and therefore capture only a fraction of the total genetic variation. Whole-genome sequencing (WGS) represents a complementary approach that enables the analysis of genome-wide genetic variations. Unlike targeted sequencing of specific loci, WGS provides insights into population structure, evolutionary relationships, host-associated genetic differentiation, and the presence of drug resistance alleles (Afshan et al., 2024; Doyle et al., 2019). Studies on *S. stercoralis* have revealed substantial genetic differences between parasite populations infecting different host species, suggesting that transmission cycles may be partially host-specific, despite the presence of shared

haplotypes identified using marker-based approaches (Jaleta et al., 2017). In addition, WGS has revealed that individual hosts may harbour multiple genetically distinct parasite lineages, further emphasizing the complexity of pathogen circulation in natural systems (Bradbury et al., 2021). However, this application remains limited in non-invasive samples due to low parasite biomass and susceptibility to contamination (Doyle et al., 2019).

HTS platforms differ in read length, number of DNA molecules they sequence in parallel and cost (von Bubnoff, 2008). Second-generation sequencing platforms include technologies such as 454 Roche, Illumina HiSeq, MiSeq and NextSeq, MGI or Aviti, whereas third-generation platforms including PacBio and Oxford Nanopore Technologies, are characterized by the ability to sequence single DNA molecules without prior PCR amplification and providing longer reads (Illumina, 2026; Mandlik et al., 2024). These third-generation technologies can also be deployed in field conditions using portable laboratory systems, such as Bento Lab and the Oxford Nanopore MinIon sequencer, with reported diagnostic accuracies for *Strongyloides* ranging from 96 to 100% (Knot et al., 2020). The general workflow of HTS, including template preparation, sequencing, imaging, and data analysis, is largely consistent across platforms, although specific protocols may vary.

The first step, template preparation, includes two interconnected parts: library preparation, and library amplification. During library preparation, DNA is processed to create sequencing-ready fragments. In whole-genome or metagenomic approaches, genomic DNA is typically fragmented into smaller pieces, whereas in amplicon-based approaches, specific target regions are first amplified by PCR using amplicon-specific primers. Subsequently, platform-specific adaptor sequences and sample-specific indices are attached to the amplified fragments, enabling their identification, multiplexing, and sequencing. Library preparation methods may vary and can include ligation-based, transposase-based, or tagging approaches (Li et al., 2015; Mandlik et al., 2024).

Following library preparation, the DNA fragments are clonally amplified to generate sufficient signal for sequencing. Amplification is typically achieved using PCR-based methods, such as emulsion PCR (ePCR) on microbeads, or bridge PCR (bPCR) on a flow cell. This step results in clusters of identical DNA fragments, which can be separately captured by the targeted adaptor primers.

The next phase involves sequencing and imaging. Most HTS platforms employ sequencing-by-synthesis approach, in which nucleotides are incorporated sequentially into a growing DNA strand complementary to the template. Each incorporation event generates a detectable signal. For example, Illumina platforms use fluorescently labelled reversible terminators, while Ion Torrent systems detect pH changes associated with nucleotide incorporation (Li et al., 2015; Mandlik et al., 2024).

The Aviti platform (Element Biosciences), used in this study, also builds on a sequencing-by-synthesis principle but utilizes avidity sequencing chemistry. In this approach, the processes of nucleotide incorporation and base identification are separated and independently optimized. Prior to sequencing, library molecules are circularized and subsequently amplified on the flowcell into clonal concatemers (polonies). During the detection step, fluorescently labelled multivalent nucleotide ligands (avidites) bind to the template DNA via polymerase-mediated base pairing. Due to their multivalent nature, avidites can simultaneously interact with multiple copies of DNA within a polony, resulting in increased binding stability and prolonged signal detection. Importantly, avidites are not incorporated into the DNA strand but are removed after imaging. Subsequently, an unlabelled nucleotide is incorporated, and the cycle is repeated. This strategy results in improved signal stability and high sequencing accuracy, and has been suggested to achieve higher accuracy than Illumina platforms in a recent study (John et al., 2024). In contrast, third-generation platforms such as PacBio utilize single-molecule real-time sequencing. In all cases, the generated signals are recorded in real time and translated into nucleotide sequences (Mandlik et al., 2024).

The final step of the workflow is data analysis, which involves processing and interpreting the large volumes of sequence data generated. Initial steps include removal of adaptor sequences and trimming of low-quality reads. If multiple samples are sequenced simultaneously, demultiplexing is performed to assign reads to individual samples. Subsequently, sequence reads are either aligned to a reference genome or assembled de novo (Li et al., 2015; Mandlik et al., 2024).

Despite its advantages, HTS also presents several challenges that may limit its widespread use. These include the computational demands of data processing and analysis, the need for substantial data storage capacity, relatively short read lengths in some platforms, and the complexity of bioinformatic pipelines required for accurate interpretation of results (Mandlik et al., 2024). A major limitation is the lack of comprehensive reference sequence databases. Many parasite lineages remain uncharacterized, which complicates taxonomic assignment and may result in a high proportion of unclassified or ambiguously assigned sequences (Lane et al., 2023; Wylezich et al., 2020).

HTS provide new opportunities for investigating parasite diversity. These approaches allow the detection of multiple genetic lineages within individual hosts and enable the analysis of complex samples derived from environments where different host species interact. Despite these advances, the application of HTS to *Strongyloides* populations in natural multi-host systems remains limited. Therefore, a deeper understanding of the genetic diversity of *Strongyloides* and host distribution of *Strongyloides* is essential for elucidating pathways of parasite spread and assessing the zoonotic potential within multi-host systems, particularly in the context of human-NHP interfaces. In this context, the present study aims to apply HTS approach to characterize *Strongyloides* populations in a system, where multiple host species coexist, with a particular focus on identifying genetic lineages and evaluating their distribution across host species.

3 Aims of the study

The main aim of this study was to characterize the genetic diversity and host-associated patterns of *Strongyloides* spp. in a multi-host system in the Cantanhez National Park in Guinea-Bissau using high-throughput sequencing (HTS) of the HVR-IV region of the 18S rRNA gene. By applying a metabarcoding approach, this study aimed to provide a high-resolution view of parasite communities and to detect multiple genetic haplotypes within individual hosts. HTS was chosen as the primary method because it allows the detection of multiple genetic variants within a single sample, including low-abundance haplotypes that may remain undetected using conventional methods. This approach is particularly suitable for studying genetically diverse parasite populations and mixed infections, which are common in *Strongyloides* spp.

Specifically, the study focused on (i) assessing the diversity of *Strongyloides* haplotypes in humans and non-human primates (NHPs), (ii) evaluating the extent of cross-host transmission and sharing of haplotypes among host species, and (iii) identifying host-associated patterns in parasite community structure. Originally, the study design also included sampling of domestic dogs to assess their potential role as bridge hosts in parasite transmission. Ethical approval and permits for dog sampling were obtained, however, due to changes in the political situation in Guinea-Bissau, field sampling could not be completed. As a result, the present study focuses on humans and NHPs.

4 Material and methods

4.1 Study site

The study was conducted in Guinea-Bissau, West Africa, specifically in Cantanhez National Park (CNP) and the Bijagòs Archipelago. CNP is located in the southern Tombali region (Cubucaré sector) and covers approximately 1,068 km^2 (Fig. 6). The area is characterized by a tropical humid climate with a pronounced rainy season (June–October) and dry season (November–May), average temperatures ranging from 28 to 31°C, and annual rainfall of around 2,000 mm (Sá et al., 2013).

The landscape of CNP is highly heterogeneous, comprising sub-humid and secondary forests, savannas, mangroves, and riverine systems, interspersed with agricultural land and human settlements. Importantly, there are no clear boundaries between natural and anthropogenically modified habitats, resulting in a mosaic environment (Parathian et al., 2018; Sousa et al., 2017). Although designated as a protected area, CNP is inhabited by local communities and includes villages and agricultural activities, which contribute to frequent interactions between humans and wildlife (Estrada et al., 2017).

The Bijagòs Archipelago is located off the coast of Guinea-Bissau and consists of approximately 88 islands characterized by diverse ecosystems, including mangroves and forest habitats (Durrans et al., 2019; Rainho & Palmeirim, 2017). These islands are partially connected to the mainland by intertidal mudflats, creating unique ecological conditions and influencing host distribution and contact patterns.

Both study areas are inhabited by populations of humans and non-human primates (NHPs), which often utilize overlapping habitats. Such environmental and ecological conditions create opportunities for direct and indirect contact through shared resources, and thus facilitate the transmission of environmentally transmitted parasites, such as soil-transmitted helminths.

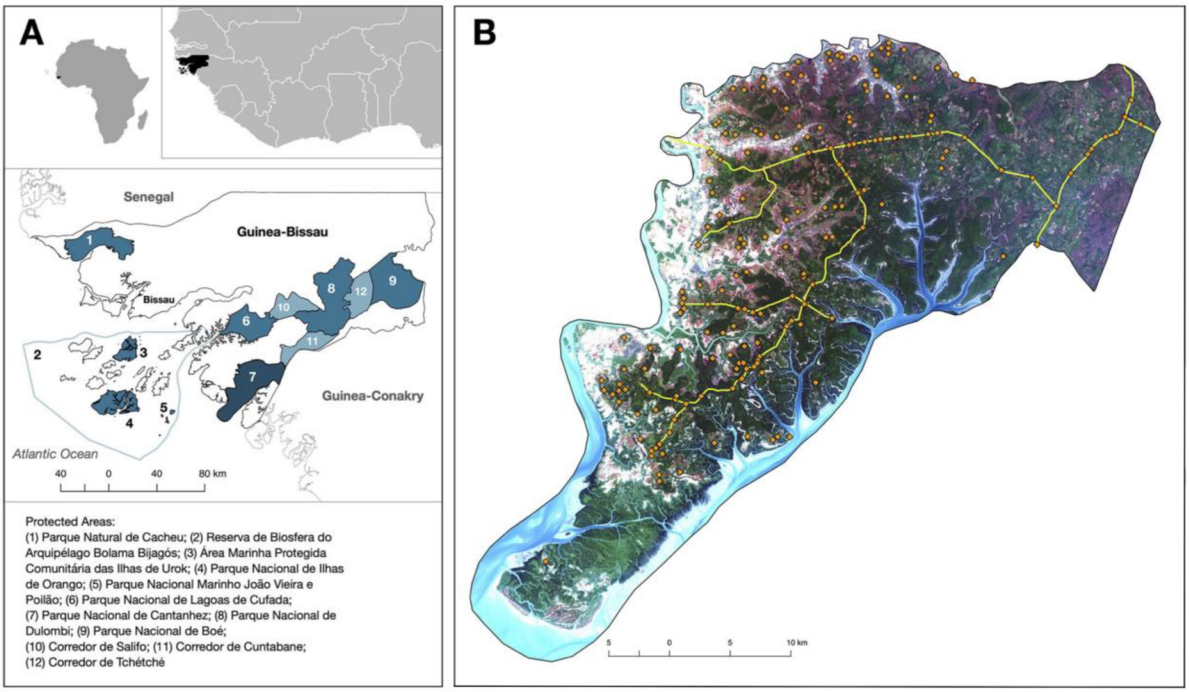

Fig. 6: (A) Location of Guinea-Bissau in West Africa, with the network of protected areas; Cantanhez National Park is indicated as area 7 and the Bijagòs Archipelago as area 2. (B) Map of Cantanhez National Park in the Tombali region. Adapted from: Bersacola et al., 2021.

4.2 Samples collection and selection for HTS analysis

A total of 426 faecal samples from humans and six species of NHPs were included in the study. Samples were collected non-invasively in Guinea-Bissau, primarily in CNP and the Bijagòs Archipelago. The dataset comprised samples from the following host species: Guinea baboons (*Papio papio*, n = 50), green monkeys (*Chlorocebus sabaeus*, n = 25), king colobus (*Colobus polykomos*, n = 40), western red colobus (*Piliocolobus badius*, n = 38), lesser spot-nosed monkeys (*Cercopithecus petaurista*, n = 53), western chimpanzees (*Pan troglodytes verus*, n = 114), and humans (*Homo sapiens*, n = 81). Samples from all host species were collected non-invasively in CNP, with the exception of lesser spot-nosed monkeys, whose samples were obtained exclusively from the Bijagòs Archipelago.

All samples were previously analysed by qPCR in a bachelor's thesis (Audrlický, 2024).

Samples that tested positive by qPCR during the bachelor's thesis were subsequently included in this diploma thesis. In brief, a total DNA was extracted by DNeasy PowerSoil Pro Kits for Soil DNA Extraction (Qiagen, Germany) from faecal material and screened for *Strongyloides* spp. using an 18S rDNA-targeting qPCR assay (Verweij et al., 2009). All reactions were performed in duplicate and included appropriate positive and negative controls. Samples with $C_q < 35$ in both replicates were considered positive (Becker et al., 2015; Zueter et al., 2014) and selected for downstream HTS analysis. In total, 157 samples were analysed by HTS. These included the following host species: Guinea baboon (*Papio papio*, n = 19), green monkey (*Chlorocebus sabaeus*, n = 16), king colobus (*Colobus polykomos*, n = 6), western red colobus (*Piliocolobus badius*, n = 7), lesser spot-nosed monkey (*Cercopithecus petaurista*, n = 13), western chimpanzee (*Pan troglodytes verus,* n = 79), and humans (*Homo sapiens*, n = 17). Human samples were collected within CNP, although detailed metadata on sampling locations and demographic characteristics (age and sex) were not available for this study.

All samples were collected non-invasively in accordance with the American Society of Primatologists – Principles for the Ethical Treatment of Non-Human Primates (American Society of Primatologists, 2021) and transported under particular permits. Human samples were collected following approval by the ethics committee of Guinea-Bissau.

4.3 Library preparation and HTS sequencing

High-throughput sequencing (HTS) was performed using a three-step PCR approach (Nosková et al., 2026). All samples were analysed in PCR duplicates, PCR clear water was used as a negative control. Primer sequences and adapter design used in this study are summarized in Table 1.

In the first step, a nested PCR targeting the HVR-IV region of the 18S rRNA gene was performed. Each reaction had a final volume of 10 µl, consisting of 8 µl of reaction mixture and 2 µl of template DNA. The reaction mixture contained KAPA 2 G Robust Hot Start polymerase (Kapa Biosystems, USA), forward and reverse primers specific for the HVR-IV region (Nested HVRIV 775F and Nested HVRIV 1.575R), and nuclease-free water. Thermal cycling conditions were as follows: 95°C for 3 min, (95°C 15s, 65°C 15s, 72°C 30s) x 20, and 72°C for 1 min.

The second PCR amplified the HVR-IV region of the 18S rRNA gene. The reaction volume and composition were identical to those in the first step, except that a different set of primers containing Nextera adapters for third PCR (NGS HVRIV F, NGS HVRIV R, NGS HVRIV F2 and NGS HVRIV R2) was used, with the product of the first PCR serving as the template. Thermal cycling conditions were: 95°C for 3 min, (95°C 15s, 60°C 15s, 72°C 15s) x 30, and 72°C for 1 min.

In the third step, indexing PCR using Nextera design was performed to attach sample-specific barcodes and sequencing adapters. Each reaction had a final volume of 20 µl, consisting of 18 µl of reaction mixture and 2 µl of diluted PCR product from the second PCR. The reaction mixture was the same as in the previous step, except for the use of an inner locus-specific primer mix (primers with unique barcodes for each technical PCR replicate). Thermal cycling conditions were: 95°C for 3 min, (95°C 15s, 55°C 30s, 72°C 30s) x 16, and 72°C for 3 min.

Amplicons were subsequently pooled according to sample groups based on their DNA quantities and volumes and purified using a magnetic bead-based cleanup (Agencourt AMPureXP beads, Beckman Coulter Life sciences) to remove primers and short fragments. Purified pools were quantified using an automated electrophoresis system (TapeStation, Altium International s.r.o) to assess fragment size distribution and a fluorometric method (Qubit, Thermo Fisher Scientific) to determine DNA concentration.

Based on these measurements, samples were normalized and combined in equimolar ratios to create the final sequencing library. The final library was prepared

according to standard protocols and sequenced on an Aviti platform (2x300 bp) using paired-end reads allowing accurate identification of *Strongyloides* species and genetic variants.

Tab. 1: Primer set used for amplification of qPCR positive samples from NHPs and humans from Guinea-Bissau. Target-specific primer sequences are in black colour, Nextera sequences are in orange colour and green colour is for additional base to increase reding heterogeneity during the sequencing.

Nested_HVRIV_775F	ACAAACGGGGGCATTCGTAT
Nested_HVRIV_1.575R	TATTGCTCAGTTCCGGGCAG
NGS_HVRIV_F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGGCCGGACACTATAAGG
NGS_HVRIV_F_2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACGGGCCGGACACTATAAGG
NGS_HVRIV_R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCTCTAAACAGGAACATAATGATCACTAC
NGS_HVRIV_R2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNATCTCTAAACAGGAACATAATGATCACTAC

4.4 Bioinformatic processing

Raw sequencing data were processed using a standard bioinformatics pipeline adapted from a recent study on *Strongyloides* metabarcoding (Nosková et al., 2026). First, raw paired-end reads were demultiplexed, and primer sequences were removed using skewer (v0.2.2) (Jiang et al., 2014). Subsequently, reads were quality filtered, trimmed and merged.

Sequence processing, including dereplication, denoising and inference of amplicon sequence variants (ASVs) was performed in R (version 4.2.2) using the DADA2 package (Callahan et al., 2016). This approach allows high resolution identification of ASVs while minimizing sequencing errors. After processing, the final amplicon length was 255 bp for the 18S rRNA region. Chimeric sequences and low-quality reads were removed prior to downstream analysis. Only high-quality sequences were retained. ASVs not consistently detected across technical PCR duplicates were considered potential artifacts and excluded from further analyses.

Taxonomic assignment was performed by comparison against reference sequences available in NCBI database. Sequences with low identity or coverage (< 85 % identity and < 90 % coverage) were excluded to ensure reliable identification (Chamberlain & Szöcs, 2013). The resulting dataset of ASVs was used to identify *Strongyloides* species and genetic haplotypes present in individual samples (Barratt et al., 2019). Species-level assignment of *Strongyloides* was performed in DADA2 based on BLAST closest hits, whereas haplotype-level identification was conducted manually using BLAST searches, as haplotype designations are not included in annotations of *Strongyloides* sequence placed in NCBI.

4.5 Statistical analysis

All statistical analyses were performed in R (version 2026.01.1) using RStudio (https://www.rstudio.com). The phyloseq package was used for data handling and visualization. Sequencing depth per sample was calculated as the total number of reads assigned to each sample after quality filtering. Samples with zero reads were excluded from sequencing depth calculations. A bar plot was generated to visualize the relative abundance of *Strongyloides* HVR-IV-18S rRNA haplotypes in each sample for better resolution. Alpha diversity was evaluated as the haplotype richness (number of ASVs) per sample based on HVR-IV ASVs. In addition, Shannon and Simpson diversity indices were calculated to account for both richness and evenness. Differences among host species were tested using the Kruskal–Wallis rank sum test. Post hoc pairwise

comparisons were performed using Dunn's test with p-value correction (Benjamini–Hochberg). Beta diversity was assessed using both Bray–Curtis dissimilarity and Jaccard index. While Bray–Curtis dissimilarity accounts for differences in relative abundance of ASVs, the Jaccard index is based on presence/absence data and reflects differences in community composition independent of abundance. Differences in community composition among host species were visualized using principal coordinate analysis (PCoA). Hierarchical clustering based on Bray–Curtis distance was performed using the average linkage method to explore similarities among samples. The effect of host species on community composition was statistically tested using permutational multivariate analysis of variance (PERMANOVA) implemented in the vegan package with 999 permutations. To verify the assumption of homogeneity of multivariate dispersion, PERMDISP was performed using the betadisper function. Additionally, analysis of similarities (ANOSIM) was conducted as a complementary non-parametric test to assess differences in community composition among host species.

5 Results

5.1 *Strongyloides* HVR-IV-18S rRNA diversity

A total of 426 faecal samples were screened for the presence of *Strongyloides* spp. using qPCR (Audrlický, 2024), of which 157 samples (36.9%) tested positive and were subsequently analysed by high-throughput sequencing (HTS). Prevalence varied among host species. Specifically, positivity rates were: 38% (19/50) in Guinea baboons, 64% (16/25) in green monkeys, 15% (6/40) in king colobus, 18% (7/38) in western red colobus, and 25% (13/53) in lesser spot-nosed monkeys. Among chimpanzees, prevalence reached 69% (79/114), while in humans it was 21% (17/81).

Of the 157 qPCR-positive samples, 89 yielded sufficient sequencing reads and were retained for downstream HTS analyses. These included 45 western chimpanzees, 12 Guinea baboons, 11 green monkeys, 9 lesser spot-nosed monkeys, 8 humans, and 2 samples each from king colobus and western red colobus. Samples that did not yield sufficient sequencing reads generally had higher qPCR C_q values, mostly above approximately 30 cycles.

Genotyping identified a total of 5,230,765 high-quality HVR-IV-18S rDNA reads, with a median sequencing depth of 42,381 reads per sample (range: 322 to 342,228). A total of 16 ASVs assigned to the genus *Strongyloides* were identified across all analysed samples and merged to particular and ideally previously described *Strongyloides* species and haplotypes (Table 2).

Tab. 2: Distribution of *Strongyloides* HVR-IV 18S rRNA haplotypes across host species assessed from qPCR-positive samples from Cantanhez National Park, Guinea-Bissau. Hum – human, Chim – western chimpanzee, Bab – Guinea baboon, GM – green monkey, KC – king colobus, RC – western red colobus, SNM – lesser spot-nosed monkey.

	No. (%)						
Haplotype	**Hum (n = 8)**	**Chim (n = 45)**	**Bab (n = 12)**	**GM (n = 11)**	**KC (n = 2)**	**RC (n = 2)**	**SNM (n = 9)**
S. stercoralis A	8 (100)	16 (35.6)	0	0	0	0	0
S. fuelleborni K	0	1 (2.2)	2 (16.7)	0	0	0	0
S. fuelleborni L	0	6 (13.3)	6 (50)	4 (36.4)	0	0	3 (33.3)
S. fuelleborni M	0	18 (40)	7 (58.3)	8 (72.7)	0	0	6 (66.7)
S. fuelleborni P	0	12 (26.7)	9 (75)	5 (45.5)	1 (50)	1 (50)	2 (22.2)
S. stercoralis unclassified	0	2 (4.4)	0	0	0	0	0
S. fuelleborni unclassified	0	11 (24.4)	5 (41.7)	1 (9.1)	2 (100)	1 (50)	0
Strongyloides unclassified	0	2 (4.4)	0	1 (9.1)	0	0	0

Overall, *S. fuelleborni* was the dominant species in NHPs, whereas humans were exclusively infected with *S. stercoralis*. ASVs assigned to *S. stercoralis* and unclassified *Strongyloides* occurred at lower relative abundance and were less widely distributed across host species.

Multiple ASVs corresponded to previously described haplotypes. Within *S. fuelleborni*, haplotypes K, L, M and P were identified, whereas within *S. stercoralis*, one ASV corresponded to the potentially zoonotic haplotype A and another represented a previously unclassified ASV that differed from the zoonotic haplotype by a single SNP at position 144 (A -> T substitution). In humans, infections were exclusively represented by *S. stercoralis* haplotype A. In NHPs, a broader diversity of *S. fuelleborni* haplotypes was observed, including K, L, M and P, as well as unclassified ASVs (Figure 7).

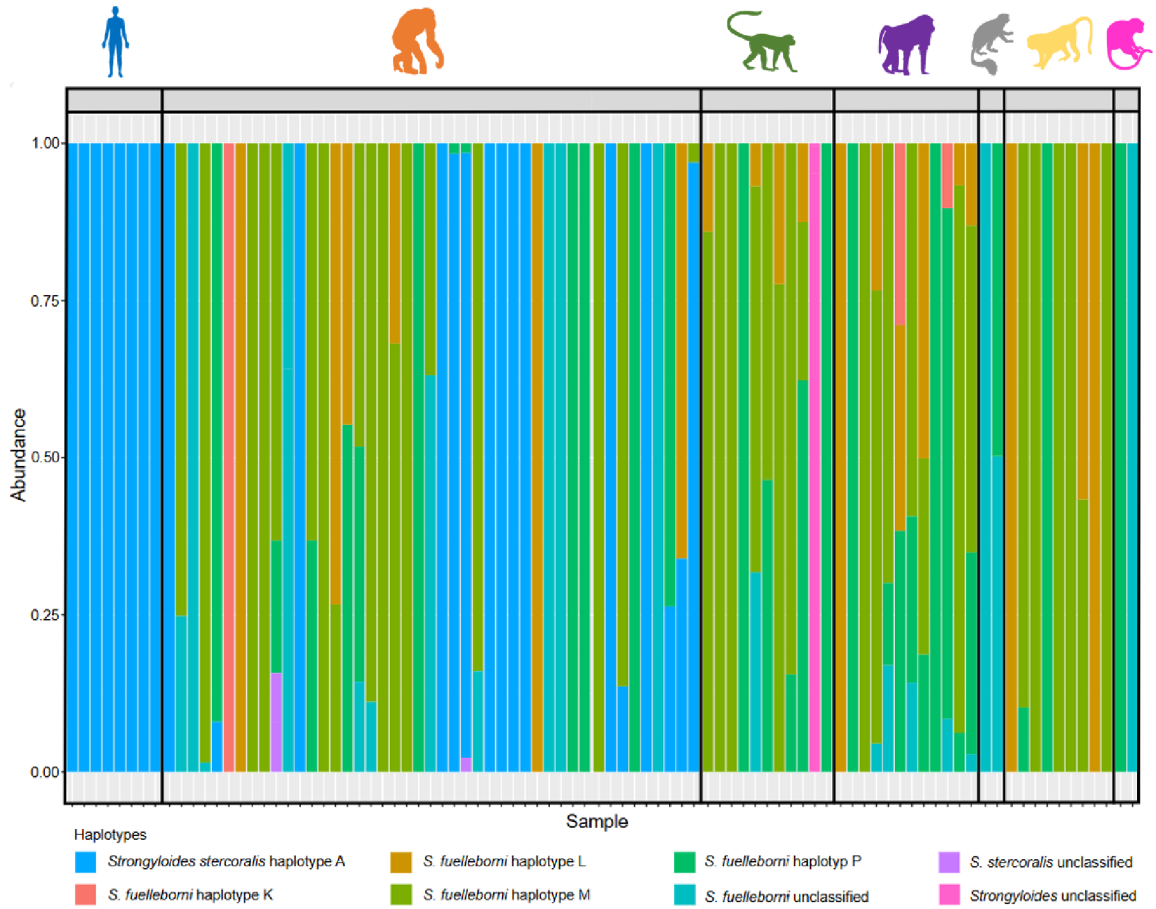

Fig. 7: Relative abundance of *Strongyloides* HVR-IV 18S rRNA haplotypes across individual samples. Each column represents a single sample, and colours indicate individual *Strongyloides* haplotypes. Samples are grouped by host species, indicated by silhouettes above the plot. Silhouette colours represent host species: blue – human; orange – chimpanzee; green – green monkey; purple – Guinea baboon; grey – king colobus; yellow – lesser spot-nosed monkey; pink – western red colobus.

5.2 Zoonotic potential of *Strongyloides* on the basis of HVR-IV-18S rRNA

The zoonotic potential of *Strongyloides* was assessed based on the sharing of HVR-IV-18S rRNA ASVs (haplotypes) among host species. In humans, *Strongyloides* infections were exclusively represented by *S. stercoralis* haplotype A, which was detected in

100% (8/8) of samples. This haplotype was also detected in western chimpanzees, where it occurred in 35.6% (16/45) of samples, representing the only ASV shared between humans and NHPs, accounting for 6% of all detected ASVs. In contrast, *S. fuelleborni* haplotypes were restricted to NHPs and were not detected in humans. Multiple haplotypes (K, L, M and P) were identified across NHP species, indicating a higher level of genetic diversity within this host group. Among these, haplotype P showed the widest host distribution, being detected across all studied NHP species, whereas haplotype M reached the highest prevalence within individual host species, particularly in green monkeys (72.7%) and lesser spot-nosed monkeys (66.7%). Unclassified *Strongyloides* ASVs were detected in 28.1% (25/89) of analysed samples and occurred exclusively in NHPs, indicating the presence of previously undescribed genetic diversity (Table 2).

Overall, these results indicate a limited zoonotic signal restricted to *S. stercoralis* haplotype A, whereas the diversity of *S. fuelleborni* appears to be largely maintained within NHP populations.

5.3 Differences in *Strongyloides* HVR-IV-18S rRNA communities

The number of ASV occurrences (i.e., presence of ASVs across samples) varied among host species. The highest ASV richness was observed in chimpanzees, followed by Guinea baboons and green monkeys, while humans exhibited lower values and colobus monkeys the lowest. Overall, these results indicate higher *Strongyloides* diversity in NHPs compared to humans (Figure 7). The number of haplotypes per sample was generally low across all host species, with most samples containing one or two haplotypes. However, when alpha diversity was evaluated using indices accounting for both richness and evenness, significant differences among host species were detected. Shannon diversity differed significantly among host species (Kruskal–Wallis test, χ^2 = 16.91, df = 7, p = 0.018) (Fig. 8, Panel A), as did Simpson diversity (Kruskal–Wallis test, χ^2 = 15.66, df = 7, p = 0.028). These results indicate that although the number of

haplotypes per sample was generally low, their relative distribution differed among host species.

Post hoc pairwise comparison using Dunn's test with Benjamini–Hochberg correction revealed that these differences were primarily driven by a significant difference between Guinea baboons and humans (Shannon: $p = 0.018$; Simpson: $p = 0.034$), while no other pairwise comparisons were statistically significant. This suggests that differences in alpha diversity among host species are limited and largely reflect lower diversity in humans compared to Guinea baboons.

Differences in community composition were assessed using Bray–Curtis dissimilarity and visualized by principal coordinate analysis. The ordination showed partial clustering according to host species, although substantial overlap among groups was observed (Figure 8, Panel B). PERMANOVA analysis confirmed a significant effect of host species on *Strongyloides* community composition (Bray–Curtis: $F = 2.288$, $R^2 = 0.165$, $p = 0.001$). Despite this statistical significance, host species accounted for only 16.5% of the total variation. Similar results were obtained using the Jaccard index, indicating that differences among host species were consistent when considering both abundance–based and presence/absence–based community structure (Jaccard: $F = 2.271$, $R^2 = 0.164$, $p = 0.001$). These findings suggest that host species differ not only in the relative abundance of haplotypes but also in their overall composition.

However, PERMDISP analysis revealed significant differences in dispersion among host species for both Bray–Curtis ($F = 2.956$, $p = 0.011$) and Jaccard distances ($F = 2.809$, $p = 0.015$), indicating that the observed differences in community composition may be partially influenced by heterogeneity in within-group variability. Consistent with this, ANOSIM did not detect significant differences among host species (Bray–Curtis: $R = 0.047$, $p = 0.11$; Jaccard: $R = 0.048$, $p = 0.079$), suggesting a high degree of overlap in community composition across host species. Hierarchical clustering further showed that samples were interspersed across clusters rather than strictly grouped by host species, suggesting partial sharing of *Strongyloides* ASVs among NHP species, but limited sharing between humans and NHPs.

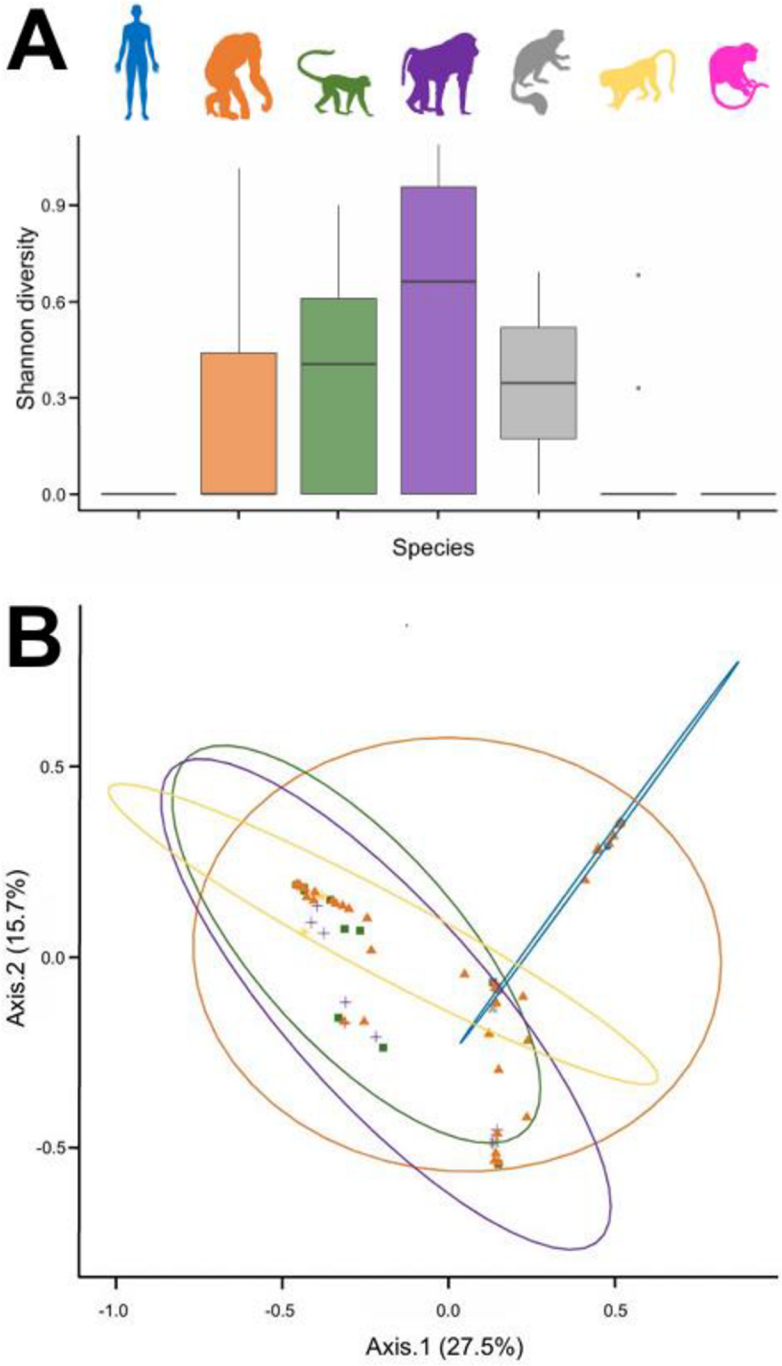

Fig. 8: Diversity of *Strongyloides* HVR-IV 18S rRNA haplotype communities across NHPs and humans from Guinea-Bissau. (A) Alpha diversity measured using the Shannon index. (B) Beta diversity based on Bray–Curtis dissimilarity, visualized by principal coordinate analysis. Colours indicate host species: blue – human; orange – chimpanzee; green – green monkey; purple – Guinea baboon; grey – king colobus; yellow – lesser spot-nosed monkey; pink – western red colobus.

6 Discussion

Strongyloides represents an important group of soil-transmitted helminths with significant implications for both human and animal health. Its ability to infect multiple host species, including humans and non-human primates (NHPs), makes it particularly relevant in the context of zoonotic transmission and multi-host parasite systems (Bradbury et al., 2021; Nosková et al., 2023a). Despite increasing evidence of genetic diversity and host sharing within this genus (e.g., Barratt et al., 2019; Nosková et al., 2026), the structure of *Strongyloides* populations in natural multi-host environments remains insufficiently understood, particularly at the level of intraspecific variation.

In this study, high-throughput sequencing (HTS) of the HVR-IV region of the 18S rRNA gene was used to characterize the diversity and host distribution of *Strongyloides* spp. in humans and six NHP species inhabiting Guinea-Bissau. By applying a metabarcoding approach, this study provides a high-resolution insight into parasite communities and enables the detection of multiple genetic haplotypes within individual hosts, which is often not achievable using conventional diagnostic methods (Nosková et al., 2023a).

Based on previous studies, three hypotheses were formulated: (i) *Strongyloides stercoralis* would be shared between humans and NHPs, whereas *S. fuelleborni* would be restricted to NHPs, (ii) *S. fuelleborni* infections would be more prevalent in NHPs than in humans, and (iii) *S. stercoralis* would be more common in humans than in NHPs. The following discussion interprets the results in the context of these hypotheses and place them within the framework of existing literature.

6.1 Genetic diversity and host-associated patterns of *Strongyloides*

The discrepancy between the number of qPCR-positive samples and those successfully sequenced by HTS represents an important methodological consideration. While qPCR screening identified 157 positive samples, only 89 yielded sufficient reads for downstream analyses. This difference is most likely explained by variation in parasite

DNA concentration, as samples with higher quantification cycle values (above ~30 cycles) were less likely to produce sufficient amplicons for sequencing. Although successful sequencing was still achieved in some cases within this range, suggesting that additional factors such as degradation of DNA (qPCR targets much shorter fragment), stochastic effects during PCR amplification, library preparation efficiency, and sequencing depth may also influence HTS success. Overall, these findings indicate that metabarcoding approaches may underestimate prevalence, particularly in low-intensity infections, even when qPCR detects the presence of parasite DNA.

Despite these limitations, the results provide insight into the genetic diversity and host-associated distribution of *Strongyloides*. A total of 16 ASVs were identified, which clustered into 8 haplotypes, reflecting relatively low overall diversity compared to studies using multilocus approaches (e.g., Barratt et al., 2019; Hasegawa et al., 2016; Jaleta et al., 2017; Nosková et al., 2026). In NHPs, *S. fuelleborni* represented the dominant and most diverse taxon, whereas human infections were exclusively associated with *S. stercoralis* haplotype A. The composition of haplotypes detected in this study is consistent with previous studies. Human infections were represented by *S. stercoralis* haplotype A, which has been repeatedly reported as the dominant haplotype in humans and is considered to have zoonotic potential (Barratt et al., 2019; Jaleta et al., 2017; Nosková et al., 2026). In contrast, *S. fuelleborni* haplotypes were associated with NHPs, supporting their role as primary hosts of this species. At the same time, the absence of *S. fuelleborni* in humans should be interpreted cautiously, as cross-host transmission of this species has been reported in other settings and may be context-dependent (Bradbury et al., 2021; Nosková et al., 2026).

In addition to the classified haplotypes, 6 ASVs could not be confidently assigned to described *Strongyloides* species and were therefore categorized as unclassified. Two identical ASVs were classified as *S. fuelleborni* unclassified based on their closest similarity to *S. fuelleborni* haplotypes. Similarly, two identical ASVs were assigned as *S. stercoralis* unclassified. Notably these sequences differed from the zoonotic *S. stercoralis* haplotype A by only a single SNP, suggesting the presence of closely related

genetic variants that may represent microdiversity within these haplotypes or locally circulating variants. In addition, two entries initially represented as separate ASVs were found to contain identical sequences, likely reflecting inconsistent trimming of the HVR-IV primer region. These sequences were classified as *Strongyloides* unclassified and showed approximately 97% similarity to *S. robustus.* This relatively low similarity indicates that these variants may represent more divergent or potentially undescribed haplotypes within the genus. The occurrence of such unclassified variants highlights the limitations of current reference databases and the HVR-IV marker in resolving the full extent of *Strongyloides* diversity. At the same time, it suggests the presence of previously uncharacterized genetic diversity within this multi-host system, as has been reported in other molecular studies on *Strongyloides* populations (Barratt et al., 2019).

Previous molecular studies provide a more detailed context for interpreting the relatively low diversity observed in this study. For example, Hasegawa et al. (2016) investigated *Strongyloides* infections in humans and sympatric great apes and demonstrated that NHPs were predominantly infected with *S. fuelleborni*, which was represented by multiple genetically distinct lineages based on *cox1*. In addition, mixed infections involving both *S. fuelleborni* and *S. stercoralis* were detected in some hosts, indicating the co-occurrence of several parasite lineages within the same population. In contrast, human infections were primarily associated with *S. stercoralis*, although *S. fuelleborni* was also occasionally detected. Importantly, genetic analyses revealed that *S. fuelleborni* populations in humans differed from those infecting sympatric NHPs, suggesting the presence of host-associated lineages/haplotypes despite ecological overlap, while some genotypes were shared across hosts.

A more recent metabarcoding study from Dzanga-Sangha Protected Areas (CAR), the same locality previously described by Hasegawa et al. (2016), further demonstrated the extent of *Strongyloides* diversity in a multi-host system. While the earlier study detected *S. stercoralis* only in humans and distinct *S. fuelleborni* haplotypes in gorillas and chimpanzees, Nosková et al. (2026) reported a high number

of *Strongyloides* haplotypes across humans, NHPs and dogs, including multiple *S. fuelleborni* lineages/haplotypes and the widespread occurrence of *S. stercoralis* haplotype A across host species. This pattern suggested frequent cross-host transmission and strong epidemiological connectivity within the system.

Compared to these findings, the present study detected a lower overall diversity and no evidence of *S. fuelleborni* infection in humans, suggesting that transmission patterns in this system may be more restricted or context dependent. In the present study, *S. stercoralis* haplotype A was detected in both humans and chimpanzees, supporting its zoonotic potential. This haplotype was represented by two ASVs, both of which were detected in both host species. The sharing of identical ASVs between humans and chimpanzees provide stronger evidence for cross-host transmission than haplotype-level similarity alone, as ASVs represent highly resolved sequence variants. This pattern suggests either recent transmission events or exposure to a shared environmental source of infection.

The lower diversity observed in humans compared to NHPs may reflect both biological and methodological factors. Studies incorporating mitochondrial markers, such as *cox1*, have revealed substantial hidden genetic diversity within *S. stercoralis* infections, even when a single haplotype is detected using 18S-based approaches (Nosková et al., 2026; Thanchomnang et al., 2017). However, interpretation of *cox1*-based diversity is not always straightforward, as this marker is highly variable and may include a large number of haplotypes, while short amplicons can limit reliable haplotype assignment and complicate comparisons across studies (Barratt et al., 2019). These limitations highlight that, while multilocus approaches can improve phylogenetic resolution in some contexts, the choice of genetic markers should reflect the specific analytical goal. In *Strongyloides*, hypervariable nuclear regions such as HVR-IV may already provide sufficient resolution for assessing genetic diversity and host-associated patterns. In contrast, short mitochondrial fragments such as *cox1* (215 bp) can be challenging to interpret due to limited phylogenetically informative sites, particularly in closely related (Nosková et al., 2026) or cryptic taxa (Frias et al., 2018).

Similar limitations have been reported previously, where short *cox1* sequences were insufficient for resolving cryptic *Strongyloides* lineages in dogs (Beknazarova et al., 2019).

Differences in parasite diversity between host species may be explained by variation in ecological exposure. Previous work in Cantanhez National Park (CNP) has shown that habitat fragmentation and increased contact between humans and NHPs can influence parasite transmission dynamics and prevalence (Sá et al., 2013), which is consistent with the heterogeneous environmental conditions observed in this study. Hosts with higher prevalence also tended to harbour greater haplotype diversity, suggesting that increased exposure to infective stages may promote both prevalence and genetic diversity of *Strongyloides* populations. For example, chimpanzees exhibited relatively high prevalence and diversity in this study (*S. fuelleborni* 42% and *S. stercoralis* 14%). This pattern is consistent with previous findings from CNP, where *Strongyloides* spp. were detected in chimpanzees, with *S. fuelleborni* and *S. stercoralis* reported at 22% and 6% respectively, based on coproscopical methods (Sá et al., 2013). However, direct comparisons should be interpreted with caution, as microscopy-based approaches generally underestimate prevalence compared to molecular methods, particularly in low-intensity or mixed infections.

Although statistical analyses detected significant differences in alpha and beta diversity among host species, these results should be interpreted with caution. The overall number of haplotypes identified in this study was relatively low, which limits the resolution of diversity analyses and reduces statistical power. The observed differences in alpha diversity were primarily driven by comparisons between humans and Guinea baboons, while other pairwise differences were not significant. Similarly, beta diversity analyses indicated substantial overlap among host groups, with low explained variance and inconsistent result across methods. These patterns suggest that, although host species contributes to variation in *Strongyloides* communities, the low overall diversity constrains the ability to detect strong or consistent structuring.

Taken together, these findings indicate that *Strongyloides* populations in this system are shaped by a combination of host-associated factors, environmental exposure, and methodological constraints. While some evidence of host-associated patterns and limited cross-host sharing was observed, the relatively low diversity detected in this study highlights the need for cautious interpretation and supports the use of multilocus approaches for a more comprehensive understanding of parasite population structure.

6.2 Cross-host transmission in a multi-host system

Cross-host transmission in this system is primarily reflected by the presence of the zoonotic *S. stercoralis* haplotype A, detected in both humans and chimpanzees. This finding is consistent with previous molecular studies demonstrating that this haplotype can infect multiple host species (e.g., Barratt et al., 2019; Nosková et al., 2026). However, its low relative abundance in chimpanzees suggests that such transmission is limited.

The extent of cross-host transmission observed in this study appears lower than in some previously described systems. For example, metabarcoding studies from the Central African Republic (CAR) reported extensive sharing of *Strongyloides* haplotypes among humans and NHPs, including both *S. stercoralis* haplotype A and *S. fuelleborni* haplotypes K, L and P, indicating frequent cross-host transmission (Nosková et al., 2026). In particular, *S. fuelleborni* haplotype L has been described as one of the most widespread haplotypes across host species in the study from CAR. In contrast, such widespread sharing was not observed in the present study, suggesting that transmission dynamics may vary considerably across ecological settings.

A contrasting pattern was recently reported from Ghana, where humans, dogs, and NHPs were sampled in three protected areas with close contact among host groups. In this study, *Strongyloides* showed clear host-associated patterns: *S. stercoralis* was detected in humans, *S. fuelleborni* in NHPs, and a *Strongyloides* taxon closely related to *S. papillosus* in dogs. No overlap of *Strongyloides* spp. was detected

between humans and animals, although the authors emphasized that the number of *Strongyloides*-positive hosts was very small (Mensah et al., 2026). This finding is consistent with the present study in showing that close ecological proximity does not necessarily result in extensive sharing of *Strongyloides* haplotypes.

A more complex pattern of transmission has been described in other molecular studies. For example, Hasegawa et al. (2016) demonstrated that, although *Strongyloides* species may be shared among humans and great apes, *S. fuelleborni* populations often form genetically distinct lineages/haplotypes in different host species, even in sympatric populations. At the same time, some genotypes were found to be identical across hosts, indicating that cross-host transmission may occur, but is not necessarily frequent or unrestricted. Similarly, studies incorporating multilocus data have suggested that genetic structuring of *Strongyloides* populations may be influenced by both host association and geographic origin, rather than reflecting a simple pattern of unrestricted transmission (Bradbury et al., 2021).

Human infections with *S. fuelleborni* have been documented in both Africa and Asia, including cases in Tanzania (Hasegawa et al., 2010), CAR (Hasegawa et al., 2016, Nosková et al., 2026) and southeast Asia (de Ree et al., 2024). However, detailed genetic analyses suggest that *S. fuelleborni* lineages found in humans often differ from those infecting sympatric NHPs, and that genetic structuring may be more strongly associated with geographic origin than with host species identity (Bradbury et al., 2016; Hasegawa et al., 2016). This indicates that, although cross-host transmission of *S. fuelleborni* may occur, it is not necessarily frequent or unrestricted, and may depend on specific ecological and epidemiological conditions.

Further insight into transmission patterns can be obtained by considering ecological differences among host species. In this study, terrestrial NHPs (western chimpanzees, Guinea baboons, and green monkeys) exhibited both higher prevalence and greater diversity of *Strongyloides* haplotypes compared to more arboreal species (king colobus, western red colobus, and lesser spot-nosed monkeys). In particular, *S. fuelleborni* haplotypes M, L and P were consistently detected across terrestrial hosts,

often at relatively high frequencies, indicating substantial overlap in parasite communities among these species. In contrast, arboreal species exhibited lower haplotype diversity, with only *S. fuelleborni* haplotype P detected across all arboreal species. However, these patterns should be interpreted with caution due to the relatively small sample size for some arboreal hosts.

These findings suggest that cross-host transmission may be influenced by host ecology, particularly differences in environmental exposure. Because *Strongyloides* spp. are transmitted via infective larvae developing in the environment, exposure depends on contact with contaminated substrates such as soil (Centers for Disease Control and Prevention, 2019). Terrestrial species, which spend more time on the ground, are therefore more likely to encounter infective stages and facilitate the circulation of multiple parasite haplotypes. In contrast, arboreal species may experience reduced exposure due to limited contact with soil, resulting in lower diversity and fewer opportunities for transmission. Similar links between host ecology and parasite communities, where increased terrestriality has been associated with higher prevalence of soil-transmitted helminths (Kooriyama et al., 2012; Mbora & McPeek, 2009).

Despite ecological conditions that would be expected to facilitate transmission, the overall extent of cross-host sharing in this study remained limited. This finding is particularly notable given the characteristics of the study system. In CNP, humans and NHPs coexist in close spatial proximity within a mosaic landscape that includes villages, agricultural areas and forest fragments. NHPs frequently enter human-modified environments, including crop fields and village surroundings, resulting in repeated opportunities for indirect contact through shared substrates such as soil. Such conditions would be expected to promote cross-species transmission of environmentally transmitted parasites.

However, despite this high level of ecological overlap, cross-host sharing of *Strongyloides* haplotypes was limited and involved both *S. stercoralis* haplotype A, shared between humans and chimpanzees, and several *S. fuelleborni* haplotypes (K, L,

M and P) detected across host species. Given the high sequencing depth and stringent bioinformatic filtering applied in this study, it is unlikely that substantial parasite diversity remained undetected. These results therefore likely reflect biological patterns rather than methodological artefacts. The limited cross-host transmission observed may indicate the presence of additional barriers, such as host-specific adaptation, differences in immune response, or microhabitat partitioning that reduces effective contact with infective stages.

Taken together, the findings largely support the initial hypotheses. In line with hypothesis (a), *Strongyloides stercoralis* showed limited sharing between humans and NHPs, whereas *S. fuelleborni* remained restricted to NHP hosts. Hypothesis (b) was strongly supported, as *S. fuelleborni* was more prevalent and genetically diverse in NHPs, while it was not detected in humans. Although *S. stercoralis* predominated in humans, its detection in chimpanzees indicates that cross-host transmission across the human–NHP interface is possible, consistent with hypothesis (c). However, the overall extent of sharing was low despite close ecological overlap, suggesting that transmission is not necessarily frequent and is likely shaped by a combination of ecological, behavioural, and host-related factors. Further studies integrating molecular, ecological, and epidemiological data will be important for understanding the mechanisms underlying *Strongyloides* circulation in multi-host systems.

6.3 Zoonotic and One Health implications of *Strongyloides* infections

Given the soil-transmitted nature of *Strongyloides* nematodes and the coexistence of humans and NHPs within the studied system, environmental exposure represents a key challenge for disease control. The life cycle of *Strongyloides*, which includes both parasitic and free-living stages in the soil, facilitates continuous environmental contamination and transmission (Bradbury et al., 2021). Although anthelmintic treatment can be effective in reducing infection in the short term, the persistence of infective larvae in the environment, together with the ability of *S. stercoralis* to undergo

autoinfection, complicates long-term control of strongyloidiasis, particularly in tropical settings (Page et al., 2018).

In this context, the limited cross-host transmission observed in the present study does not eliminate the risk of occurrence of new transmissions or reinfection. Even in the absence of extensive haplotype sharing between humans and NHPs, both host groups may act as independent sources of environmental contamination, maintaining transmission cycles within the ecosystem. This is consistent with previous findings suggesting that the epidemiology of *Strongyloides* infections is shaped not only by direct host-to-host transmission but also by environmental persistence and indirect exposure pathways (Bradbury et al., 2021; Page et al., 2018).

Effective control strategies should therefore extend beyond treatment of infected individuals and focus on reducing exposure to infective stages in the environment. Practical preventive measures, as mentioned above, have been shown to significantly reduce the risk of infection with soil-transmitted helminths, including *Strongyloides* (Page et al., 2018; Strunz et al., 2014). Such interventions are particularly important in regions where humans and wildlife share the same habitats and where environmental contamination cannot be easily controlled.

Because direct intervention in free-ranging wildlife populations is not feasible, management of strongyloidiasis in such systems requires a One Health approach that integrates human health, animal reservoirs, and environmental factors (Elsohaby & Villa, 2023; Sharan et al., 2023; Zinsstag et al., 2005). In practise, this approach implies targeted interventions in human populations, including treatment of infected individuals and improved sanitation, combined with efforts to reduce environmental contamination and limit potential transmission to NHPs. The One Health framework emphasizes that effective control of zoonotic and parasitic diseases requires integrated strategies addressing human, animal and environmental health, with particular importance placed on environmental management and reduction of risk factors such as soil and water contamination (Page et al., 2018; Zinsstag et al., 2005).

At the same time, monitoring of infections in wildlife populations, particularly NHPs, may provide valuable insight into transmission dynamics and help assess potential spillover or spillback events. NHPs are considered important components of zoonotic systems, as they can act as reservoirs or indicators of pathogen circulation at the human–wildlife interface (Estrada et al., 2017; Wolfe et al., 2005). Surveillance of parasite infections in primate populations may therefore contribute to a better understanding of transmission pathways and help identify potential risks for both human and animal health. The zoonotic potential of *Strongyloides* spp., particularly *S. fuelleborni*, which has been suggested to be transmitted from NHPs to humans in some settings (see above), further highlights the need for such an integrated perspective.

Overall, the findings of this study underscore the importance of considering environmental exposure and multi-host dynamics when designing sustainable control strategies for strongyloidiasis. A comprehensive One Health framework, combining treatment, improved sanitation, and reduction of environmental exposure, is therefore essential for effective long-term control of *Strongyloides* infections in multi-host systems.

An additional factor that may play an important role in *Strongyloides* transmission dynamics, is the involvement of dogs, which have been identified as potential bridge hosts in multi-host systems. For example, study from CAR have demonstrated that dogs may harbour distinct *Strongyloides* haplotypes, such as *S. stercoralis* haplotype A, as well as *S. fuelleborni* haplotypes K, L and M, and facilitate parasite transmission between humans and wildlife (Nosková et al., 2026). Although dogs were not included in the present study, sampling of canine populations was originally planned. However, due to changes in the political situation in Guinea-Bissau, field sampling could not be carried out. Future studies incorporating dogs may therefore provide a more comprehensive understanding of transmission pathways and the role of bridge species in *Strongyloides* circulation.

7 Conclusion

The present study provides a comprehensive characterization of *Strongyloides* diversity and host-associated patterns in a multi-host system in Guinea-Bissau using high-throughput sequencing of the HVR-IV region of the 18S rRNA gene. The results demonstrate differences in genetic diversity among host groups, with non-human primates (NHPs) harbouring a greater diversity of haplotypes compared to humans. In contrast, human infections were represented exclusively by *S. stercoralis* haplotype A, supporting its role as the dominant haplotype in humans and confirming its zoonotic potential, as it was also found in chimpanzees.

Although cross-host transmission between humans and NHPs was detected, it was limited and restricted to a single haplotype, suggesting that such transmission is not a dominant process in this system. Rather than strong host-specific structuring, the observed patterns indicate that parasite communities are shaped by a combination of host identity, ecological exposure, and environmental conditions. These findings highlight that transmission dynamics of *Strongyloides* are context-dependent and may vary substantially across ecological settings.

The study also demonstrates the utility of metabarcoding approaches for detecting and characterizing parasite diversity in complex multi-host environments. At the same time, several limitations should be considered. Incomplete reference databases limited the taxonomic assignment of some ASVs, and variation in sample size among host species may have influenced estimates of diversity and prevalence. Future studies incorporating multilocus approaches, as well as whole-genome sequencing, will be essential to achieve higher resolution of genetic diversity and to better resolve transmission pathways and population structure of *Strongyloides* spp. Expanding the dataset to include additional host species, particularly dogs, would further improve understanding of transmission dynamics and help clarify the role of potential reservoir hosts within the system.

From a broader perspective, the findings of this study underscore the importance of considering *Strongyloides* infections within a One Health framework. The combination of environmental transmission, multi-host dynamics and the potential for zoonotic exchange highlights the need for integrated control strategies. These should address not only treatment of infected individuals, but also environmental exposure, human behaviour together with monitoring of NHPs and dogs, which would also allow for interventions if necessary. Overall, this study contributes to a better understanding of parasite diversity and transmission in natural multi-host systems and provide a foundation for future research and control efforts targeting strongyloidiasis.

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Appendix A Analysis script

```
###### --- DIPLOMA THESIS: STRONGYLOIDES HTS ANALYSIS --- ######

### 1 LOAD LIBRARIES ###
library(phyloseq)
library(ggplot2)
library(vegan)
library(dplyr)
library(FSA)

### 2 LOAD PHYLOSEQ OBJECT ###
load("PHYLOSEQ.merged.tax.R")

PHYLOSEQ <- PHYLOSEQ.merged.tax

### 3 BASIC FILTERING ###
PHYLOSEQ <- prune_samples(sample_sums(PHYLOSEQ) > 0, PHYLOSEQ)
PHYLOSEQ <- prune_taxa(taxa_sums(PHYLOSEQ) > 0, PHYLOSEQ)

### 4 BASIC STATISTICS ###
# total reads
sum(otu_table(PHYLOSEQ))

# reads per sample
reads_per_sample <- sample_sums(PHYLOSEQ)

# HISTOGRAM – reads per sample
hist(
  reads_per_sample,
  breaks = 7,
  col = "purple",
  main = "Histogram of reads_per_sample",
  xlab = "reads_per_sample",
  ylab = "Frequency"
)

# haplotype counts per sample (ASV richness)
haplotype_counts <- rowSums(otu_table(PHYLOSEQ) > 0)
```

```
# HISTOGRAM – haplotypes per sample
hist(
  haplotype_counts,
  breaks = 5,
  col = "purple",
  main = "Histogram of sample_haplotype_counts",
  xlab = "sample_haplotype_counts",
  ylab = "Frequency"
)

### 5 CREATE DATA FRAME ###
DF <- data.frame(
  haplotype_counts = haplotype_counts,
  sample_data(PHYLOSEQ)
)

### 6 MERGE CHIMPANZEE GROUPS ###
DF$Host_plot <- as.character(DF$Species)

DF$Host_plot[DF$Host_plot         %in%         c("chimpanzee",
"chimpanzee_Daris")] <- "chimpanzee"

### 7 SET ORDER OF HOST SPECIES ###
DF$Host_plot <- factor(
  DF$Host_plot,
  levels = c(
    "human",
    "chimpanzee",
    "green_monkey",
    "guinea_baboon",
    "king_colobus",
    "lesser_spot_nosed_monkey",
    "red_colobus"
  )
)

### 8 COLOR PALETTE ###
host_cols <- c(
  "human" = "#0072B2",
  "chimpanzee" = "#E87522",
```

```
      "green_monkey" = "#3B7A2A",
      "guinea_baboon" = "#6F2DA8",
      "king_colobus" = "#8C8C8C",
      "lesser_spot_nosed_monkey" = "#FFD34E",
      "red_colobus" = "#FF33B5"
    )

    ### 9 BOXPLOT – HAPLOTYPE COUNTS ###
    ggplot(DF, aes(x = Host_plot, y = haplotype_counts, fill =
Host_plot)) +
      geom_boxplot(alpha = 0.7) +
      scale_fill_manual(values = host_cols) +
      labs(x = "species", y = "HVR-IV ASV richness") +
      theme_bw() +
      theme(legend.position = "none")

    ### 10 GLM TEST (HAPLOTYPE RICHNESS) ###
    DF$Host_plot <- as.factor(DF$Host_plot)

    glm_model <- glm(haplotype_counts ~ Host_plot, family =
quasipoisson, data = DF)
    summary(glm_model)

    ### 11 ALPHA DIVERSITY ###
    div <- estimate_richness(PHYLOSEQ, measures = c("Shannon",
"Simpson"))
    div$Species <- sample_data(PHYLOSEQ)$Species

    # merge chimpanzees
    div$Host_plot <- as.character(div$Species)
    div$Host_plot[div$Host_plot %in% c("chimpanzee",
"chimpanzee_Daris")] <- "chimpanzee"

    div$Host_plot <- factor(div$Host_plot, levels =
levels(DF$Host_plot))

    ### 12 SHANNON BOXPLOT ###
    ggplot(div, aes(x = Host_plot, y = Shannon, fill = Host_plot))
+
      geom_boxplot(alpha = 0.7) +
      scale_fill_manual(values = host_cols) +
```

```
  labs(x = "species", y = "Shannon diversity") +
  theme_bw() +
  theme(legend.position = "none")

### 13 KRUSKAL–WALLIS TEST ###
kruskal.test(Shannon ~ Host_plot, data = div)
kruskal.test(Simpson ~ Host_plot, data = div)

### 14 POST-HOC TEST (DUNN) ###
dunnTest(Shannon ~ Host_plot, data = div, method = "bh")
dunnTest(Simpson ~ Host_plot, data = div, method = "bh")

### 15 BETA DIVERSITY ###
# prepare OTU table
otu <- as.data.frame(otu_table(PHYLOSEQ))
if (taxa_are_rows(PHYLOSEQ)) {
  otu <- t(otu)
}

# Bray-Curtis
Bray <- vegdist(otu, method = "bray")

# Jaccard (presence/absence)
otu_pa <- otu
otu_pa[otu_pa > 0] <- 1
Jacc <- vegdist(otu_pa, method = "jaccard", binary = TRUE)

### 16 PERMANOVA ###
adonis2(Bray ~ DF$Host_plot)
adonis2(Jacc ~ DF$Host_plot)

### 17 PERMDISP ###
bd_bray <- betadisper(Bray, DF$Host_plot)
permutest(bd_bray)

bd_jacc <- betadisper(Jacc, DF$Host_plot)
permutest(bd_jacc)

### 18 ANOSIM ###
anosim(Bray, DF$Host_plot)
anosim(Jacc, DF$Host_plot)
```

```
    ### 19 PCoA ###
    Bray.ord <- ordinate(PHYLOSEQ, distance = Bray, method = "PCoA")

    plot_ordination(PHYLOSEQ, Bray.ord, color = "Species") +
      geom_point() +
      stat_ellipse(aes(group = Species))

    ### 20 ABUNDANCE PLOT ###
    PHYLOSEQ.rel <- transform_sample_counts(PHYLOSEQ, function(x) x
/ sum(x))

    mdf <- psmelt(PHYLOSEQ.rel)

    mdf$Host_plot <- as.character(mdf$Species)
    mdf$Host_plot[mdf$Host_plot        %in%        c("chimpanzee",
"chimpanzee_Daris")] <- "chimpanzee"

    mdf$Host_plot <- factor(
      mdf$Host_plot,
      levels = c(
        "human",
        "chimpanzee",
        "green_monkey",
        "guinea_baboon",
        "king_colobus",
        "lesser_spot_nosed_monkey",
        "red_colobus"
      )
    )

    abundance_plot <- ggplot(mdf, aes(x = Sample, y = Abundance,
fill = Haplotype)) +
      geom_bar(stat = "identity", position = "stack") +
      facet_grid(. ~ Host_plot, scales = "free", space = "free") +
      theme_bw() +
      theme(
        axis.text.x = element_text(angle = -90, size = 0),
        axis.text.y = element_text(size = 8),
        axis.title = element_text(size = 12),
        strip.text = element_text(size = 10),
```

```
        legend.position = "bottom"
      ) +
      labs(
        x = "Sample",
        y = "Relative abundance",
        fill = "Haplotypes"
      )

    abundance_plot

###### --- END OF SCRIPT --- ###
```