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Research Paper

Exploring interactions between *Blastocystis* sp., *Strongyloides* spp. and the gut microbiomes of wild chimpanzees in Senegal

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ABSTRACT

Background: Gut parasites exert an important influence on the gut microbiome, with many studies focusing on the human gut microbiome. It has, however, undergone severe richness depletion. Hygienic lifestyle, antimicrobial treatments and altered gut homeostasis (e.g., chronic inflammation) reduce gut microbiome richness and also parasite prevalence; which may confound results. Studying species closely related to humans could help overcome this problem by providing insights into the ancestral relationship between humans, their gut microbiome and their gut parasites. Chimpanzees are a particularly promising model as they have similar gut microbiomes to humans and many parasites infect both species.
Aims: We study the interaction between gut microbiome and enteric parasites in chimpanzees. Investigating what novel insights a closely related species can reveal when compared to studies on humans.
Methods: Using eighty-seven faecal samples from wild western chimpanzees (*Pan troglodytes verus*) in Senegal, we combine 16S rRNA gene amplicon sequencing for gut microbiome characterization with PCR detection of parasite taxa (*Blastocystis* sp., *Strongyloides* spp., *Giardia duodenalis*, *Cryptosporidium* spp., *Plasmodium* spp., Filariae and Trypanosomatidae). We test for differences in gut microbiota ecosystem traits and taxonomical composition between *Blastocystis* and *Strongyloides* bearing and non-bearing samples.
Results: For *Blastocystis*, twelve differentially abundant taxa (e.g., *Methanobrevibacter*), including *Prevotella* and *Ruminococcus-Methanobrevibacter* enterotype markers, replicate findings in humans. However, several richness indices are lower in *Blastocystis* carriers, contradicting human studies. This indicates *Blastocystis*, unlike *Strongyloides*, is associated to a "poor health" gut microbiome, as does the fact that *Faecalibacterium*, a bacterium with gut protective traits, is absent in *Blastocystis*-positive samples. *Strongyloides* was associated to *Alloprevotella* and five other taxonomic groups. Each parasite had its unique impact on the gut microbiota indicating parasite-specific niches. Our results suggest that studying the gut microbiomes of wild chimpanzees could help disentangle biological from artefactual associations between gut microbiomes and parasites.

1. Introduction

Ecosystems contain multitudes of interacting species. The great ape gut microbiome (GM), home to trillions of prokaryote and eukaryote cells, is no exception. Research into microbiomes is starting to reveal the complex interactions amongst different members of the GM and their interplay with hosts: intestinal eukaryotes are associated to particular GM communities (e.g., reviewed in Leung et al., 2018). *Blastocystis* and *Strongyloides*, traditionally deemed parasites (Brumpt, 1912; Campbell, 1893), have been described recently, by some, as beneficial members of the GM due to their correlation with certain microbial markers such as α diversity (e.g., reviewed by Stensvold and Van Der Giezen, 2018 and Zaiss and Harris, 2016). GM markers are assumed to reflect the ecological states of the microbial ecosystem: for

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instance, the human and the chimpanzee GM can be stratified into enterotypes representing stable-state communities with different ecological properties (Knights et al., 2014; Costea et al., 2018; Moeller and Ochman, 2013). Some markers, like high α diversity, correlate with host health status as the microbial community has increased resistance, resilience and redundancy in providing the host with microbial functions (Lozupone et al., 2012; Greenhalgh et al., 2016). This pattern appears to hold true for humans (reviewed in Mukherjee et al., 2018) and other primates (Clayton et al., 2016; Amato et al., 2016).

The stramenopile *Blastocystis* and the soil-transmitted nematode *Strongyloides* are two important parasites for humans. The former is a common member of the GM and colonizes about one billion people worldwide (Andersen and Stensvold, 2016), reaching prevalences of 100% in some populations (El Safadi et al., 2014). *Blastocystis*' pathogenicity is heavily debated (reviewed by Andersen and Stensvold, 2016), as most infections are asymptomatic (Salvador et al., 2016; Zhang et al., 2016), even if it seems to be the only explanation for observed symptoms in other cases (Andiran et al., 2006; Domínguez-Márquez et al., 2009). The genus *Strongyloides* comprises at least 50 species. *S. stercoralis* and *S. fuelleborni* are the only known infective species to humans. Both are capable of symptomatically infecting chimpanzees (File et al., 1976; Penner, 1981). It has been estimated that up to 370 million people are infected with *S. stercoralis* globally (Buonfrate et al., 2015). It is able to asymptomatically persist and replicate (autoinfection) within an immunocompetent host for decades, yet may cause a life-threatening infection (hyperinfection syndrome, disseminated strongyloidiasis) in immunocompromised hosts (Olsen et al., 2009). The significance of *Blastocystis* and *Strongyloides* in clinical settings makes it important to improve our understanding of relationships between these parasites and the GM.

Studies exploring the interaction between GM eukaryotes and prokaryotes in modern humans may become confounded by the many disturbances human gut environments are exposed to. Therapies like extensive antibiotic usage and anthelminthic treatments may, in some cases, eliminate prokaryotes and eukaryotes alike (Freeman et al., 1997; Jenkins et al., 2018; Rosa et al., 2018). A hygienic lifestyle, including an urban lifestyle and the ingestion of processed foods (Sonnenburg et al., 2016), may reduce colonization opportunities for both GM members and parasites with faecal-oral transmission. Host health disturbances, especially intestinal disorders (Beghini et al., 2017) or poly-parasitism (Iebba et al., 2016), may also affect eukaryote carriage and prokaryote diversity by inducing prolonged pro-inflammatory gut environments, which anaerobic taxa are unlikely to resist (Parija and Jeremiah, 2013; Le Chatelier et al., 2013). Further, varying geographic or socioeconomic origins (Rosa et al., 2018; Forsell et al., 2017) may comprise some or all of these confounding factors. The extent to which these factors confound results remains unassessed. Focusing on wild chimpanzees may provide an opportunity to minimize the potential confounding factors present in modern humans while offering a closer understanding of the impact of intestinal parasites on the early human gut microbiota.

To do this, we analysed the GM of wild western chimpanzees (*Pan troglodytes verus*), the composition of which is similar to humans' (Ellis et al., 2013; reviewed in Nishida and Ochman, 2019), in the presence and absence of *Blastocystis* and *Strongyloides*. This allowed us to observe what GM traits and taxa are associated with carriage, whether these are novel or have previously been identified in humans. To the best of our knowledge, this has not been done before in either wild or captive chimpanzees, and only once in the western lowland gorilla where Vlčková et al. (2018) found an impact of *Blastocystis* and *Strongyloides* on the GM.

2. Material and methods

2.1. Study site

We collected samples from four neighbouring chimpanzee communities in the Community Nature Reserve of Dindéfélo (RNCD) and from the Bandafassi outgroup (~50 km north), south-eastern Senegal. There, wild chimpanzees roam freely in patches of forest found amongst villages and human crops; hence, there is extensive overlap between their home ranges and the forests villagers use to hunt and gather. Both fruits from key plant species and water holes are extensively used by chimpanzees, other wild fauna, and humans and their livestock (personal observation). For more information on the potential conflict extending from this overlap, see Ramon et al. (2017).

2.2. Sample collection and DNA extraction

We collected eighty-seven fresh (< 24 h old) wild chimpanzee faecal samples living in sympatry with humans; ≥15 from each of the four communities in the RNCD and 30 from Bandafassi. We got help from local Jane Goodall Institute Spain (IJGE) field guides, experienced in chimpanzee tracking, to opportunistically sample during the months of November through December 2015. We collected 5 g from the centre of faecal droppings, diluted them in 5 mL saline solution (NaCl 0.9%) and immediately transferred them in triplicates to Whatman™ WB120205 FTA™ Classic Cards (GE Healthcare Life Sciences, MA, USA) for conservation and transportation to the lab. We performed negative controls in the field to capture environmental contaminants. For each sample, we collected metadata on seed and insect contents in the faeces as a proxy for diet, estimated time since defecation, noted consistency (i.e., diarrhoea or formed) and chimpanzee community affiliation. For each community, we used data collected by IJGE on anthropic pressure, camping area, chimpanzee density and number of members per community (Pancorbo, 2017) After transfer to the laboratory, we extracted DNA using the PowerSoil DNA Extraction Kit (MO BIO Laboratories, Carlsbad, CA, USA).

2.3. Molecular detection of Blastocystis sp.

We achieved identification of the heterokont *Blastocystis* sp. (named *Blastocystis* hereafter) by a direct PCR protocol targeting a ~600 bp fragment of the *ssu* rRNA gene (Scicluna et al., 2006). PCR mixtures (25 μL) comprised 5 μL DNA sample, 0.5 μM of the primer pair RD5/BhRDr (see Additional file 1: Table S1), 2.5 units of MyTAQ™ DNA polymerase (Bioline GmbH, Luckenwalde, Germany), and 5 × MyTAQ™ Reaction Buffer containing 5 mM dNTPs and 15 mM $MgCl_2$. We routinely used laboratory-confirmed positive and negative DNA extracts for each parasite species as controls and included them in each round of PCR. We visualized PCR amplicons on 2% D5 agarose gels (Conda, Madrid, Spain) stained with Pronasafe nucleic acid staining solution (Conda, Madrid, Spain). We direct-sequenced positive-PCR products in both directions using the appropriate internal primer set (Additional file 1: Table S1). We conducted DNA sequencing by capillary electrophoresis using the BigDye® Terminator chemistry (Applied Biosystems, CA, USA) on an ABI 3730xl automated DNA sequencer.

2.4. Blastocystis sp. sequence analyses

We viewed raw sequencing data in both forward and reverse directions using the Chromas Lite version 2.1 sequence analysis program (http://chromaslite.software.informer.com/2.1/). We used the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare nucleotide sequences with sequences retrieved from the NCBI GenBank database. We aligned generated DNA consensus sequences to appropriate reference sequences using the MEGA 6 free software (Tamura et al., 2013). We submitted *Blastocystis* sequences at the *Blastocystis* 18S database (http://pubmlst.org/blastocystis/) for sub-type confirmation and allele identification.

2.5. Molecular detection of Strongyloides spp.

We carried out qPCR to detect the presence of helminths of the genus *Strongyloides* by amplification of a ~101 bp fragment of the *ssu* rRNA gene (Verweij et al., 2009). We adapted this protocol to use

SybrGreen reagents (Invitrogen, San Diego CA, USA) as described elsewhere (Saugar et al., 2015). qPCR reactions (25 µL) included 1× Quantimix EasyMaster Mix (Biotools B&M Laboratories, Madrid, Spain), 0.2 µM of each primer, and 0.5 µL of 50× SybrGreen (Invitrogen). We assayed DNA samples (5 µL) in duplicates. We used purified genomic DNA from *Strongyloides venezuelensis* L3 as a positive control. We also included 10 ng of *S. venezuelensis* DNA in each reaction as internal inhibition control as well as negative and no template controls in each run. We performed detection of parasitic DNA on a Corbett Rotor Gene™ 6000 real-time PCR system (Qiagen, Hilden, Germany) and we analysed the data with Rotor Gene 6000 Series software version 1.7. Primer sequences and cycling conditions are given in Additional file 1: Table S1. We assessed specificity of amplified products by melting curve analysis as described by Saugar et al. (2015).

2.6. Molecular detection of other parasites

Additionally, we also searched for the presence of other enteric protozoan (*Giardia duodenalis*, *Cryptosporidium* spp.), malarial (*Plasmodium* spp.), filarial, and trypanosomatid (*Trypanosoma* spp. and *Leishmania* spp.) parasites (see Additional file 1: Tables S1–S4) using the same faecal samples. All direct and nested PCR protocols were conducted on a 2720 thermal cycler (Applied Biosystems, CA, USA) using the primer pairs and cycling conditions described in Additional file 1: Table S1. For detailed methods see supplementary materials.

2.7. 16S rRNA gene amplicon sequencing

To amplify the 16S rRNA variable region V3-V4 (expected amplicon size ~460 bp) we used the primer pair described in the MiSeq rRNA Amplicon Sequencing protocol (Illumina, 2013) with the included Illumina adapter (16S_F 5′-TCG TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG **CCT ACG GGN GGC WGC AG**-3′, 16S_R 5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G**GA CTA CHV GGG TAT CTA ATC C**-3′).

We generated amplicons in 25 µL reactions, consisting of 2.5 µL DNA template, 5 µL of each primer at 1 µM and 12.5 µL of KAPA HiFi HotStart Ready Mix (KAPA Biosystems Inc., Wilmington, MA, USA). For thermal cycling conditions, we denatured for 3 mins at 95 °C as an initial denaturation step, then conducted 30 cycles of 30 s denaturation at 95 °C, 30 s annealing at 55 °C and 30 s extension at 72 °C, with a final extension step of 10 mins at 72 °C. We cleaned DNA templates for non-DNA molecules and attached Illumina sequencing adapters and dual indices using Nextera XT Index Kit (Illumina Inc.) We followed with the PCR amplification program described in the MiSeq 16S rRNA Amplicon Sequencing protocol (Illumina, 2013). We performed a second clean-up and quantified using Quant-iTTM PicoGreen® dsDNA Assay Kit (Invitrogen, Carlsbad, MA, USA) before diluting in equimolar concentrations for pooling. We sent amplicons for 300 bp paired-end sequencing on an Illumina MiSeq™ platform (Illumina Inc.) at the genomics core facility in Germans Trias i Pujol research campus, in Badalona, Spain.

2.8. Sequencing data QC and pre-processing

We treated a total of 6.477.920 (15.244–210.442 per sample) paired-end reads in eighty-seven samples with the DADA2 pipeline v1.6.0. (Callahan et al., 2016) in R v3.4.2 (Core Team, 2017). We used default parameters except in the filtering step when we set maxEE to 2, 10 to account for the lower quality of reverse reads inherent in Illumina technology. We removed chimeric reads using the *removeBimeraDenovo* function in the same package. We kept a total of 3.250.502 high-quality reads for further analysis (8.322–99.739 reads/sample). We performed taxonomic assignment of clusters using the *assignTaxonomy* function in DADA2 against the *silva_nr_v123_train_set.fa* file (Quast et al., 2013). We used phyloseq v1.22.3 (McMurdie and Holmes, 2013) for downstream analysis. We removed all Amplicon Sequence Variants (ASVs) assigned to neither Bacteria nor Archaea and all ASVs found in controls (mostly *Rhizobium*) from the data set. Before doing so for the latter, we confirmed the relative abundance ratio between controls and samples was above 0.2 for every ASV to avoid removing biologically-relevant taxa (as in Hu et al., 2017). We removed six outlier samples that clustered with human samples (not included in this study) because we suspected that they were in fact of human origin. We deposited all clean raw data under the NCBI SRA accession number PRJNA523828.

After visual inspection of the rarefaction plots (Additional file 2: Sup. Fig. S1) we down-sampled to 11.600 reads per sample in a reproducible fashion using the *rarefy_even_depth* function in the phyloseq R package (McMurdie and Holmes, 2013). Two samples that did not meet the rarefaction requirements were excluded from the richness and β diversity calculations and the respective statistical analyses.

2.9. Richness and β diversity measures and associations

We calculated Chao1 and ACE richness indices, which incorporate measures of variance, and observed richness, all using the phyloseq *estimate_richness* function (McMurdie and Holmes, 2013) on the rarefied dataset, after clustering to genus level.

We performed an ANOVA to test for differences in richness between *Blastocystis* and *Strongyloides* carriers and non-carriers after confirming no deviations from normality and homoscedasticity were found using Shapiro-Wilk test (Royston, 1982) and Breusch-Pagan test (Breusch and Pagan, 1979) on the dataset excluding three diarrhoeic samples.

Using the vegan R package v2.5-2 (Oksanen et al., 2018) we calculated a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957). We used this to perform Analysis of Similarities and PERMANOVA tests (using Anosim (Clarke, 1993) and ADONIS (Anderson, 2001), respectively, both with 9999 permutations) to test for significant differences in microbial communities as a function of seed number, community affiliation, and *Blastocystis* and *Strongyloides* carriage. We also conducted a PERMANOVA test for a combination of all of the above in the following order: *Strongyloides* carriage, *Blastocystis* carriage, seed number and community affiliation. Anthropic pressure, camping area, chimpanzee density and number of members per community were removed from the models as they did not contribute significantly to explaining richness or β diversity.

2.10. Differential taxa abundance between parasite carriers and non-carriers

After removing taxa with < 10 reads from the unrarefied data, we used DAtest v2.7.9 (Russel et al., 2018) to test for differential abundance of prokaryote taxa between parasite-positive and -negative samples at all taxonomic levels from genus through order using Benjamini and Hochberg (1995) false discovery rate correction (fdr). We chose a best-fit model based on the *testDA* function results and only proceeded when the training false discovery rate was below 0.05. Following these criteria, we chose the statistical tests presented in Additional file 2: Table S5. Using the same approach, we tested which taxa correlated with prokaryote richness using a LIMMA log linear regression (Phipson et al., 2016). In order to control for the time between defecation and sample collection, we run DAtest (LIMMA log linear regression) and PERMANOVA (Anderson, 2001) as internal controls to show that it did not affect the microbial community (p.adj > 0.1; p > .1, respectively).

3. Results

3.1. Parasite prevalence and diversity

We detected *Blastocystis* in 40.7% (33/81) of samples (81.8% ST1, 3% ST2/3, 3% ST3 and 12.1% could not be typed), *Strongyloides* in 12.8% (10/78), *Tetratrichomonas* spp. in 3.7% (3/81) and *Giardia duodenalis* in a single sample (1/81). While *Cryptosporidium* spp., *Plasmodium* spp., Filariae, and the genera *Trypanosoma* and *Leishmania* all went undetected.

3.2. Western chimpanzee gut microbiome composition

We found Firmicutes (39.3%), Bacteroidetes (23.1%) and Actinobacteria (22.5%) to be the most abundant phyla in the western chimpanzee GM, followed by Lentisphaerae (4%) and Proteobacteria (3.1%). Results which generally agree with previous work (e.g., Gogarten et al., 2018; Ellis et al., 2013). The genera that correlated positively with GM richness were *Oxalobacter*, Lachnospiraceae_AC2044_group and Ruminococcaceae_UCG-003, while negative associations were found with Coriobacteriaceae_UCG-003, Prevotellaceae_UCG-00, *Solobacterium*, *Dialister*, [*Eubacterium*]_*coprostanoligenes*_group, *Oribacterium*, *Ruminiclostridium*_5, Rikenellaceae_RC9_gut_group, *Bifidobacterium*, *Dorea*, Lachnospiraceae_NK3A20_group, *Prevotella*_7, *Prevotella*_9, *Anaerovibrio*, *Olsenella*, *Lachnoclostridium*, Erysipelotrichaceae_UCG-004, Erysipelotrichaceae_UCG-001 (Fig. 1).

3.3. Blastocystis carriage affects microbiome composition and richness

Blastocystis was associated with decreased richness for all measured metrics (observed richness: F-stat = 3.994, p = .0494; Chao1: F-stat = 3.993, p = .0495; and ACE: F-stat = 4.122, p = .0462), confirming the pattern observed in Fig. 2 of parasite presence predominantly in poorer communities.

β diversity tests (Table 1) showed that parasite carriage, as well as diet diversity and community affiliation, had significant effects on chimpanzee gut microbial communities, together explaining 17.7% of the total variance. *Blastocystis* carriage showed a pronounced effect on microbial communities (Figs. 2A–B and 3) and was significantly associated to abundance changes in twelve taxa (Fig. 3). The families Enterobacteriaceae and Methanobacteriaceae; and the genera *Prevotella*_1, *Peptococcus*, *Methanobrevibacter* and *Holdemanella* were more abundant in *Blastocystis* carriers compared to non-carriers. In contrast, the orders Bifidobacteriales, Opitutae_vadinHA64, Rhodospirillales, Selenomonadales and Coriobacteriales; and the genus *Faecalibacterium*, showed the opposite trend.

3.4. Strongyloides carriage affects microbiome composition, but not richness

While GM richness was unaffected by *Strongyloides* presence (0.03-fold decrease in observed richness, F-stat = 1.075, p = .304), we detected significant effects on β diversity when combined with the diet proxy and community affiliation data, the latter explaining the largest variation (Table 1, Fig. 2E–F). Carriage explained nearly 2% of the variance in microbial community composition (Table 1, Fig. 4), and we detected significant associations to six taxonomic groups (Fig. 5). The order Mollicutes_RF9; the families Desulfovibrionaceae, Clostridiaceae_1 and Bacteroidaceae; and the genera *Alloprevotella* and Lachnospiraceae_ND3007_group, were all more abundant in the presence of *Strongyloides*.

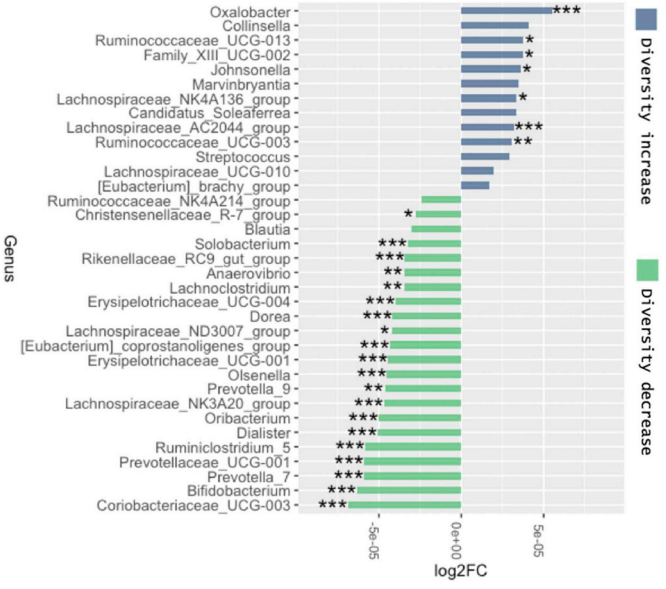

Fig. 1. Gut microbial taxa $\log_2$ fold-change as a function of observed richness. We plotted genera with unadjusted p-values below 0.05. Coloration indicates direction of association trend. Significance levels are indicated with asterisks: p.adj < 0.1 (*), p.adj < 0.05 (**), and p.adj < 0.01 (***).

4. Discussion

4.1. Blastocystis associates with a lower richness in contrast to humans, but most taxonomic patterns mirror human studies

We investigated interactions between GM and enteric parasites in wild chimpanzees and here compare our results with previous knowledge from human studies. We found chimpanzees carrying *Blastocystis* had a lower GM richness than non-carriers. This contradicts several studies in humans that either found a positive association between α diversity and *Blastocystis* carriage (Andersen et al., 2018; Audebert et al., 2016; Nash et al., 2017; Forsell et al., 2017; Tito et al., 2018) or failed to find an association (Beghini et al., 2017).

For specific taxa, however, most results mirror studies on humans. Notably, *Methanobrevibacter* was significantly more abundant in carriers, replicating a pattern Beghini et al. (2017) found in eight independent datasets; and Tito et al. (2018) found associated to *Blastocystis* subtype 4. This, together with an increase of *Prevotella*_1 and a non-significant decrease of *Bacteroides* (Fig. 3; p.val = 0.035, p.adj = 0.186), indicate a well replicated pattern (Forsell et al., 2017; Beghini et al., 2017; Andersen et al., 2018; O'Brien Andersen et al., 2016; Tito et al., 2018): *Blastocystis* is found in *Prevotella*- and *Ruminococcus-Methanobrevibacter*-dominated enterotypes, but not in the *Bacteroides*-dominated enterotypes. Stensvold and Van Der Giezen (2018) suggested that this pattern may be due to a decrease of butyrate-producing microbes in the *Bacteroides*-dominated enterotype, which leads to a decrease in oxygen metabolism by the epithelial colonocytes (Donohoe et al., 2012) and therefore a less suitable environment for the strict anaerobe *Blastocystis* (Parija and Jeremiah, 2013). However, Nieves-Ramírez et al. (2018) found decreased gut butyrate concentrations in asymptomatic *Blastocystis* carriers compared to non-carrying humans. We found an increase of the facultative anaerobic Enterobacteriaceae family (Donnenberg, 2015), and an absence of butyrate-producing *Faecalibacterium* in *Blastocystis* carriers (Lopez-siles et al., 2012), which also challenges Stensvold and Van Der Giezen (2018)'s hypothesis. The *Bacteroides*-dominated enterotype correlates with low-grade inflammation (Le Chatelier et al., 2013) and higher levels of C-reactive protein (Costea et al., 2018). An alternative explanation for the absence of *Blastocystis* in the *Bacteroides*-dominated enterotype may thus be that it is unable to survive in a sustained inflammatory environment.

We did not detect *Faecalibacterium* in any of the *Blastocystis* carriers while nine non-carriers (~19%) bore a considerable amount of this genus (> 0.1% of reads). This is in agreement with Nourrisson et al. (2014), who found a *F. prausnitzii* decrease in *Blastocystis*-positive men, yet contradicting Iebba et al. (2016), who through qPCR found a lower *F. prausnitzii-Escherichia coli* ratio in carriers. In chimpanzees, the absence of "gut-protective" *F. prausnitzii* (Martín et al., 2015; reviewed in Lopez-Siles et al., 2017), the lower α diversity (reviewed in Guinane and Cotter, 2013), and an increase of Enterobacteriaceae in carriers hint towards *Blastocystis* inhabiting a "low health" GM.

The presence of the parasite may trigger changes in immune system and/or gut homeostasis. For instance, *Peptococcus* (Gérard, 2014), *Holdemanella* (Martin et al., 2018), and members of the Bifidobacteriales (Gérard, 2014), all involved in secondary bile acid metabolism, were differentially abundant in *Blastocystis* carrying chimpanzees

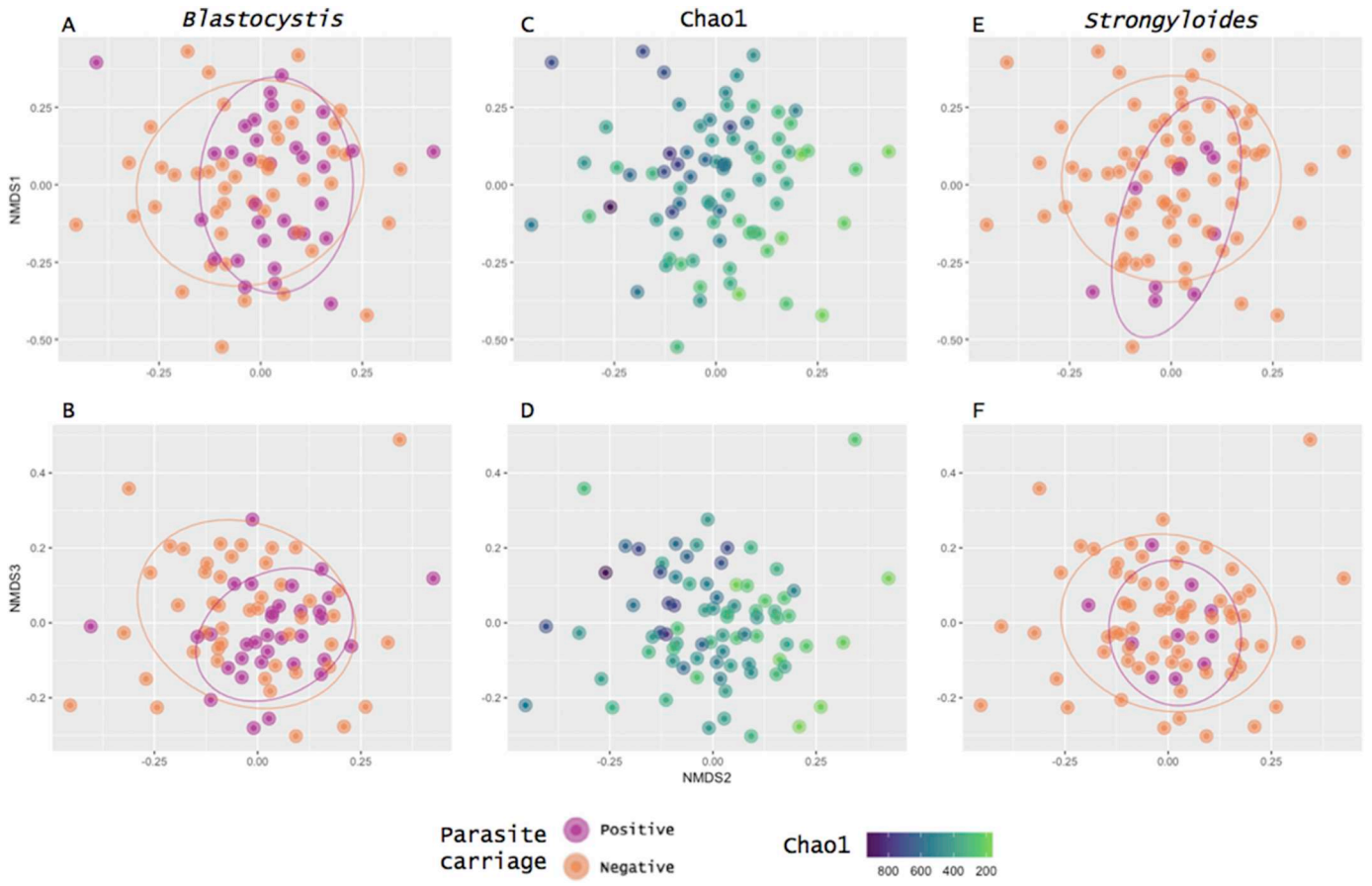

Fig. 2. NMDS plots with three axes (stress = 16.0%) colored according to *Blastocystis*-carriage (A–B), Chao1 α diversity (C–D), and *Strongyloides*-carriage (E–F). Confidence intervals are plotted at 80%. All plots have the same data points in the same positions for each axis, facilitating the comparison between richness and parasite-carriage; particularly in the overlap between *Blastocystis* and low α diversity.

compared to non-carriers (Fig. 3). Nieves-Ramírez et al. (2018) detected differences in bile acid metabolism between asymptomatic *Blastocystis* carriers and non-carriers using PICRUSt (Langille et al., 2013). The *Methanobrevibacter* increase suggests other research avenues: it has been associated to gut passage time increase and a decrease in stool consistency (Vandeputte et al., 2016). Conversely, parasite colonization may be precluded by the presence of certain prokaryote taxa, their metabolites or through immune cross-reactivity as examples reviewed by Zaiss and Harris (2016) elucidate. Both causal directions are not mutually exclusive, and we cannot discern between them in associational studies such as this one.

Some studies suggest that regional variation in GM is so big it precludes its use as indicator of parasite carriage (He et al., 2018). However, our finds replicate remarkably well previous studies in humans, suggesting that some patterns may be useful and hold true even across different ape species in vastly different environments. Wild apes may be appropriate to further the understanding of the human gut microbiome and circumvent confounders in human populations. Taken together, these highlight the value of studying and especially preserving our closest relatives.

4.2. *Strongyloides* associates with certain GM taxa, echoing the effects of other helminths on the human GM

In humans, *Strongyloides* carriage (Jenkins et al., 2018), and the presence of helminths in general (Giacomin et al., 2015; Lee et al., 2014), is associated to a GM α diversity increase. While this is in contrast with our findings, it may be due to a limited number of *Strongyloides*-positive samples.

We found an *Alloprevotella* increase in *Strongyloides* carriers which has not been previously reported. However, this basal genus to the *Prevotella* clade shares the ability to synthesize succinate and acetate in the human gut with the closely related *Paraprevotella* (Buhl et al., 2016), which has been associated to carriage of the helminth *Trichuris trichura* in humans (Lee et al., 2014). The increased abundance of the Mollicute order RF9 is consistent with an increased Mollicutes abundance in helminth-carriers (Lee et al., 2014). Similarly, the increase of Bacteroidaceae in carriers resonates with the increased non-Prevotellaceae Bacteroidales abundance in helminth carrying humans (Lee et al., 2014). Unlike others (e.g., Wu et al., 2012), we did not find any GM patterns correlating with the abundance of *Strongyloides* in faecal samples (not shown), yet our sample size may not have been large enough to allow detection.

Table 1
β diversity testing using Analysis of Similarities and PERMANOVA for each factor individually as well as PERMANOVA for all four factors combined.

	Analysis of Similarities	PERMANOVA	Combined PERMANOVA
Blastocystis	$p = .015$, $R = 6.0\%$	$p = .027$, $R^2 = 2.0\%$	$p = .005$, $R^2 = 2.3\%$
Strongyloides	$p > .1$	$p = .09$, $R^2 = 1.8\%$	$p = .044$, $R^2 = 1.9\%$
Number of seeds	$p = .020$, $R = 11.1\%$	$p = .018$, $R^2 = 2.2\%$	$p = .003$, $R^2 = 2.3\%$
Community affiliation	$p < .001$, $R = 19.7\%$	$p < .001$, $R^2 = 12.1\%$	$p < .001$, $R^2 = 11.1\%$ Total $R^2 = 17.7\%$

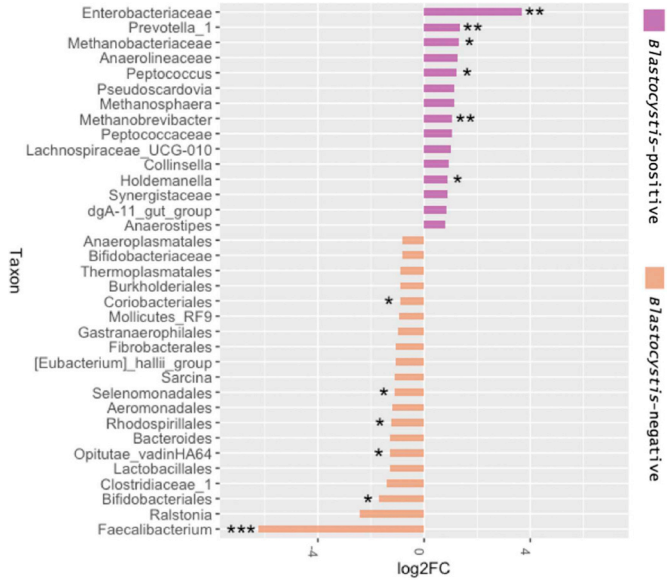

Fig. 3. Gut microbial taxa $\log_2$ fold-change of microbial abundance between *Blastocystis* carriers and non-carriers. We plotted taxa at genus, family and order levels above 0.75 $\log_2$ fold-change and below 0.5 adjusted p. value (p.adj). Coloration indicates direction of association trend. Significance levels are indicated with asterisks: p.adj < 0.1 (*), p.adj < 0.05 (**), and p.adj < 0.01 (***).

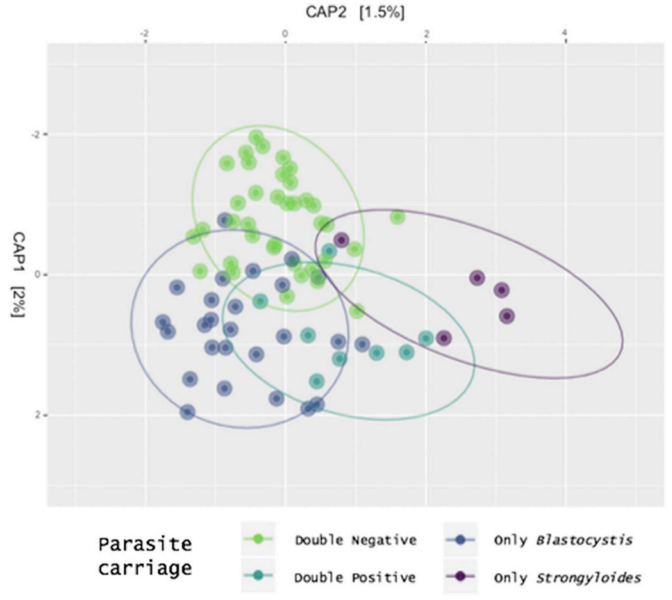

Fig. 4. Constrained ordination plot (CAP; Legendre and Anderson, 1999) shows microbial community segregation between parasite-positive and -negative samples when ordination is constrained by these variables.

We observed a large difference between the effects of *Blastocystis* and *Strongyloides* on the prokaryote communities of the western chimpanzee with no overlapping taxonomic associations or overall community traits. This discrepancy seems to indicate specific niches for each of these in the gut ecosystem, and possibly different effects on the host microbiome and immune system.

4.3. Discrepancies between effects on the human and wild chimpanzee GM: confounded associational studies?

Discrepancies between results in humans and chimpanzees, when it comes to parasite-GM associations, may be due to differences in host

Fig. 5. Gut microbial taxa $\log_2$ fold-change of microbial abundance between *Strongyloides* carriers and non-carriers. We plotted taxa at genus, family and order levels above 0.75 $\log_2$ fold-change and below 0.5 adjusted p. value (p.adj). Coloration indicates direction of association trend. Significance levels are indicated with asterisks: p.adj < 0.1 (*), p.adj < 0.05 (**), and p.adj < 0.01 (***).

biology, such as the longer small intestine in humans (Stevens and Hume, 1995) or in their GM composition, like a notable Actinobacteria depletion in humans (Moeller et al., 2014; Sonnenburg et al., 2016; reviewed in Nishida and Ochman, 2019). Discrepancies may however also be due to vast genetic – and therefore also ecological/pathogenic – differences within *Blastocystis* (Gentekaki et al., 2017) and between different helminth species. Even though we surveyed nine parasite taxa (see supplementary methods in Additional File 1) an additional confounder to our results could be colonization by other parasites that share faecal-oral transmission. For instance, a 2016 study in the Ivory Coast found that out of fourteen subjects colonized with *Blastocystis*, ten were co-colonized with either *Giardia*, *Entamoeba* spp., or both (Iebba et al., 2016).

We suggest that most studies in humans are heavily confounded by factors such as geography or lifestyle (Forsell et al., 2017; Rosa et al., 2018), host health status (Beghini et al., 2017) or poly-parasitism (Iebba et al., 2016). These factors will both affect parasite carriage and gut prokaryote diversity by influencing gut colonization rates or by inducing pro-inflammatory gut environments. A wide range of factors including chronic stress, obesity, and gut disorders such as irritable bowel syndrome or inflammatory bowel disease, are transforming the GM in so-called western societies (Yatsunenko et al., 2012; Obregon-Tito et al., 2015; Moeller et al., 2014). These produce prolonged pro-inflammatory environment with oxygenic bursts, which neither strict anaerobic *Blastocystis* nor many prokaryotes should be able to tolerate (Parija and Jeremiah, 2013). Hence, caution in interpreting associations between rich GM communities and high parasite prevalence is merited.

We therefore suggest that studying these interactions in a broad range of human conditions and closely related species might help decipher observed complex patterns. Causal relationships, particularly their direction and mechanism, will undoubtedly have to be verified in more controlled laboratory experiments. Only with both, the first as a hypothesis generator and the second as proof-of-concept, will we be able to answer whether certain GM communities avoid successful parasite colonization, whether parasite infections are causally shaping the observed GM community structure, and address the roles of the host immune system and gut homeostasis, in this ménage-a-trois (Leung et al., 2018).

Author contributions

JR-H conceived and designed the experiment, conducted data analysis, drafted the first versions of figures and the manuscript; JR-H and LP designed and conducted sampling; MN-J, MPa and RP conducted microbiome characterization; PK and DC conducted molecular detection of *Blastocystis* sp., *Cryptosporidium* spp. and *Giardia duodenalis*, ED and JS conducted molecular detection of *Strongyloides* spp.; JR conducted molecular detection of *Plasmodium* spp., filariae and Trypanosomatidae; and MPo, PK and DC contributed to discussions of data, analyses, and figures, and helped revise the manuscript. All authors contributed to writing the manuscript.

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Declaration of Competing Interest

All authors declare there are no known conflicts of interest associated with this publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.104010.

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