Gut Pathogens

BRIEF REPORT

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Investigation of gut microbiota composition in humans carrying blastocystis subtypes 1 and 2 and *Entamoeba hartmanni*

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Abstract

The composition of human gut microbiota is dominated by bacteria which have been extensively studied. The role of intestinal eukaryote microorganisms like *Blastocystis*, however, remains under investigation. Moreover, the potential impact on gut health related to *Blastocystis* presence was primarily investigated in symptomatic individuals mainly from industrialized countries, and appears to be mostly beneficial to the host microbiota. Data from surveys conducted in underdeveloped countries with higher prevalence and from asymptomatic individuals could therefore be valuable. The aim of this preliminary study was to analyze the composition of the gut microbiota in relation to the protozoa *Blastocystis* ST1 and ST2 and *Entamoeba hartmanni* carriage in asymptomatic subjects living in a semi-urban area of Côte d'Ivoire to add data into the ongoing debate on the role of *Blastocystis* in host health. The amplification of the V3 and V4 regions of bacterial 16S rDNA genes was performed to obtain the gut microbiota composition, and differential analyses on alpha and beta diversity were performed from the phylum to genus taxonomic level. The analysis revealed that individuals positive for both protozoa exhibited higher alpha and beta diversity compared to those who tested negative. Additionally, their bacterial composition showed a reduction in *Bacteroides* and an increase in *Prevotella* 9. Relative abundances of some OTUs, particularly *Faecalibacterium*, observed in individuals who tested positive for protozoa, were correlated with a good state of health of the gut microbiota. *Blastocystis* ST1 and ST2 associated with *E. hartmanni* thus appeared to be related to a state of intestinal eubiosis.

Keywords Asymptomatic carrying, Eukaryome, Côte d'Ivoire, Eubiosis, Enterotype

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Introduction

The importance of studying the gut microbiota across diverse human populations is to advance our understanding of its role in health and disease. Much of the existing knowledge about the intestinal microbiota has been derived from studies conducted in industrialized countries, where a lower infectious burden and a different lifestyle are usually observed compared to less developed regions in which fecal matter contamination is common, thus favoring the spread of intestinal parasitic pathogens [1]. Recently, the analysis of variations in the intestinal bacterial composition in populations of geographically and culturally different countries is an area of interest, given the implications for understanding the complex dynamics of the intestinal microbiota [2–4]. Additionally, particular attention has been paid to describing the variations recorded in the presence of parasites to better decipher the interaction, not yet entirely elucidated, between gut microbiota, the host, and common protozoa such as *Blastocystis* and *Entamoeba hartmanni* [5, 6].

Blastocystis is an anaerobic protist observed in various animal species. In humans, it represents the most widespread eukaryote found in over one billion people worldwide [1, 7]. In industrialized countries, its potential effect on the intestinal microbiota has been studied mainly in symptomatic subjects suffering from inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) [6, 8], and other diseases [9], although the conclusions are sometimes not unequivocal [3]. A study by Tito et al. [10] examining a Western population cohort found high gut microbiota richness in individuals carrying *Blastocystis*. Heterogeneous bacterial associations related to *Blastocystis* carriage have also been reported, and its impact appears to be predominantly beneficial to the human gut microbiota [11, 12]. Also, positive associations between *Blastocystis* and the carbohydrate-metabolizing bacteria *Ruminococcus*, *Eubacterium,* and *Cellulosibacter*, as well as the butyrate producer *Roseburia*, have been identified in French and Bangladeshi subjects [8, 13]. Recently, a meta-analysis based on a global-scale metagenomic exploration found that *Blastocystis* is widely present in healthy adults and is associated with healthier cardiometabolic profiles and plant-based diets [14].

Blastocystis subtypes also appeared to be associated with gut bacterial niches that would likely induce effects on gut colonization [15]. The contradictory conclusions reached by some studies could be linked to the different behavior of *Blastocystis* in symptomatic subjects compared to asymptomatic subjects [16, 17] or human genetic variants. Along with important factors like dietary habits, environmental conditions, and health status, it's therefore crucial to also highlight interactions dependent on *Blastocystis* subtypes in different geographical areas. Based on the small subunit (SSU) rRNA genes, at least 44 subtypes (STs) [18] meet the recommended conditions for unique subtype designation (ST1–ST17) [19], ST21, and ST23–ST32 [7, 18]. Humans have been shown to harbor at least twelve distinct genetic variants, although ST1 and ST4 are the most isolated [7]; the subtypes prevalent in African countries belong to ST1–ST3 [20–22]. However, in developing countries, investigations of this topic require greater understanding since many questions are still debated on the influence of environmental factors and pathogenicity of a ubiquitous member of the intestinal microbiota such as *Blastocystis* [3, 4, 23–25].

As with *Blastocystis*, *Entamoeba* spp. detection is widely associated with poor sanitation [26]. The morphological similarities among *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, and *Entamoeba hartmanni* make it challenging to differentiate them based solely on microscopic examination. However, molecular methods like polymerase chain reaction with higher sensitivity and specificity can now easily identify these species [27]. *E. hartmanni* is characterized by non-invasive stages and trophozoite without red blood cell ingestion. Cysts and trophozoites are smaller compared to *E. histolytica* [28]. Studying the microbial diversity in the intestinal microbiota of individuals living in sub-Saharan Africa can offer valuable information about the eukaryotic component's potential benefits in maintaining health, leveraging for this purpose the higher occurrence of non-pathogenic intestinal protozoa in these areas [29, 30]. Although our previous studies in Côte d'Ivoire reported associations between *Blastocystis* and *Entamoeba* spp. with changes in the abundance of specific bacterial taxa in the gut microbiota [5, 11], no genetic data on *Blastocystis* and the potential pathogenic effects of its subtypes were investigated. As recently highlighted [14], it would be valuable to include genetic data from underrepresented countries to further investigate *Blastocystis*'s interactions with other microorganisms and its impact on gut health.

The present study aimed to investigate for the first time in Côte d'Ivoire the possible influences on the gut microbiota plasticity related to most prevalent *Blastocystis* subtypes 1 and 2 and *Entamoeba hartmanni* carriage in asymptomatic subjects.

Materials and methods

Sample selection

A selection of data was derived from two previous studies carried out in Bonoua, a semi-urban setting in the Department of Grand-Bassam, in the south of Côte d'Ivoire [11, 31]. A more detailed description of the participant is available in Di Cristanziano et al. [11]. To mitigate bias and align with the objectives of the present

study, participants were selected to be over 3 years old as suggested by Yatsunenko et al. [32] and Korpela and deVos [33]. A total of 21 gut pathogen-free and asymptomatic subjects were found to comply with the aim of the study and were selected, including 7 women and 14 men aged between 3 and 30 years old. To describe and compare the gut microbiota composition related to *Blastocystis* subtypes, all selected subjects were categorized into A, C/C1, D/D1, and D/D2 groups as indicated in Table 1.

Eight samples (group A) were negative for any pathogen tested with xTAG Gastrointestinal Pathogen Panel and also for *Entamoeba* spp. and *Blastocystis*; three individuals were positive only for *Blastocystis* (Group C); ten patients were positive for *Blastocystis* and *E. hartmanni* (Group D).

As for *Blastocystis* subtypes, all 3 subjects positive only for *Blastocystis* resulted ST1 subtype (Group C1); among the samples coinfected with *E. hartmanni*, 7 were colonized with *Blastocystis* ST1 (Group D1) and 3 with ST2 (Group D2). None of the samples tested positive only for *Blastocystis* ST2.

Table 1 Description of participants' categorization

Sample ID	AGE	SEX	*E. hartmanni*	*Blastocystis* ST (GenBank Accession n)	Group
M108	17	M	Negative	Negative	A
M11	9	M	Negative	Negative	A
M13	30	M	Negative	Negative	A
M28	9	M	Negative	Negative	A
M31	21	M	Negative	Negative	A
M63	4	F	Negative	Negative	A
M64	3	M	Negative	Negative	A
M70	5	M	Negative	Negative	A
M25	11	M	Negative	**ST1** (MF184945)	C/C1
M65*	12	F	Negative	**ST1** (MF184957)	C/C1
M68*	15	F	Negative	**ST1** (MF184960)	C/C1
M69	14	M	**Positive**	**ST1** (MF184961)	D/D1
M71	11	F	**Positive**	**ST1** (MF184962)	D/D1
M93	18	M	**Positive**	**ST1** (MF184979)	D/D1
M94	9	M	**Positive**	**ST1** (MF184980)	D/D1
M105	11	F	**Positive**	**ST1** (MF184987)	D/D1
M109	19	M	**Positive**	**ST1** (MF184988)	D/D1
M112	12	M	**Positive**	**ST1** (MF184991)	D/D1
M66	18	M	**Positive**	**ST2** (MF184958)	D/D2
M90	13	F	**Positive**	**ST2** (MF184977)	D/D2
M104	10	F	**Positive**	**ST2** (MF184986)	D/D2

Group A indicates the control (negative for all investigated pathogens, *Blastocystis*, and *E. hartmanni*); Group C includes *Blastocystis* positive subjects; Group D includes *Blastocystis* and *E. hartmanni* positive subjects. Within Groups C and D, C1 and D1 specify the presence of *Blastocystis* ST1, and D2 the presence of *Blastocystis* ST2

*Subjects reported abdominal pain

To test for statistical differences respectively in age and sex between the four groups (Table 2) the ANOVA analysis with Tukey's post hoc test and the pairwise Fisher exact test, with the Benjamini–Hochberg p-value adjustment method, was performed using R (Supplementary Table S1). Since the results indicated that sex and age did not affect the gut microbiota, they were not considered as covariates in further analysis in this study. Furthermore, the associations between gut microbiota composition and age, as well as sex, were aligned with those reported for the complete cohort by Di Cristanziano et al. [11].

Gastrointestinal pathogen, *Blastocystis*, and commensal *Entamoeba* spp. detections

Briefly, from each participant, one fecal sample was collected, and an anonymous code was assigned before starting the different procedures. Recent or ongoing antibiotic therapy constituted an exclusion principle. Each sample was split into two aliquots; the first aliquot was used for DNA extraction by the automated platform VERSANT kPCR Molecular Systemfor analysis by the xTAG Gastrointestinal Pathogen Panel (xTAG GPP) (Luminex Molecular Diagnostics, Toronto, ON, Canada) able to detect fifteen human enteric pathogens among viruses, bacteria, and parasites [34]. The second aliquot was used for DNA extraction by QIAmp Stool Mini Kit (QIAGEN, Hilden, Germany) [11] for the detection, identification, and subtyping of *Blastocystis* and commensal *Entamoeba* spp. since these microeukaryotes are not included in the Luminex panels. To isolate *Blastocystis* and *Entamoeba* spp., end-point PCR analysis followed by sequencing of the amplified fragments was used as described in detail in Di Cristanziano et al. [11].

Analysis of gut microbiota composition in selected samples

To describe gut microbiota composition, employing the same second aliquot used to isolate *Blastocystis* and *Entamoeba* spp., the amplification of the V3 and V4 regions of bacterial 16S rDNA genes was performed by PCR reactions using primers selected by Klindworth et al. [35]. Sequencing data of 16S rRNA amplicon were

Table 2 Age and sex variables in the groups of participants

GROUP*	AGE Mean ± SD	SEX
A	12.25 ± 9.57	7 M/1F
C/C1	12.67 ± 2.08	1 M/2F
D/D1	13.43 ± 3.78	5 M/2F
D/D2	13.67 ± 4.04	1 M/2F

*None of the comparisons resulted in a significant difference with a p-value threshold of 0.05, (Supplementary Table S1)

processed using the QIIME DADA2 plugin [36] and taxonomic classification was performed and analyzed as expounded in Di Cristanziano et al. [11]. On the subject of the sample size, given the exploratory nature of the project and since we used existing data it was not determined by statistical methods.

To reveal a possible role played by *Blastocystis* and *E. hartmanni* in modulating bacteria abundances, all selected individuals were categorized as follows: (i) individuals negative for each pathogen based on xTAG Gastrointestinal Pathogen Panel results and negative for *Entamoeba* spp. and *Blastocystis*; (ii) individuals colonized only by *Blastocystis*; (iii) individuals colonized by both *Blastocystis* and *E. hartmanni*.

For each selected sample, the 16S-rRNA amplicon data were rarefied at a sequencing depth of 4000 sequences per sample. Statistical analyses were carried out using R for Statistical Computing (version 4.2.3, R Foundation for Statistical Computing, Vienna, Austria) [37] and Microbiomeanalyst (Version 2.0) [38]. The qza data were imported and diversity scores were calculated using the phyloseq R package [37, 39].

Alpha and beta diversity have been tested for all taxonomic levels reporting significant differences only at OTUs and genus level. The alpha diversity indices Observed, ACE, Shannon, Fisher, and Chao1 have been calculated using phyloseq R package [33]. Data normality has been tested by Shapiro–Wilk test, continous variables presented as mean and standard deviation (SD) and tested with ANOVA. In the beta diversity, the Bray, Jaccard, weighted, and unweighted UniFrac distances between the samples were visualized using principal coordinate analysis (PCoA) and group effects were tested by a permutational multivariate analysis of variance (pairwaiseAdonis).

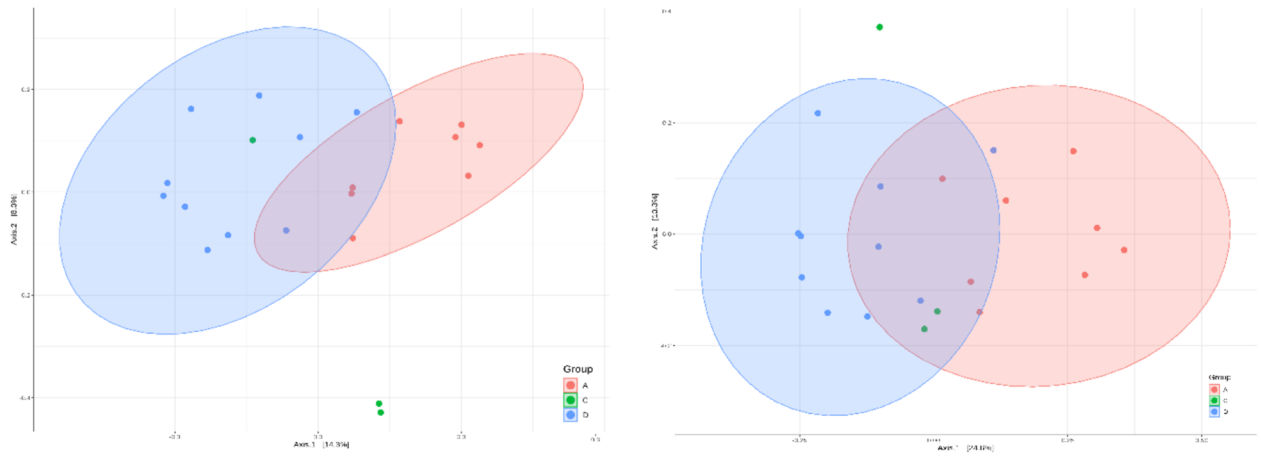
Among several methods that can be used for performing differential relative abundance analysis, significant taxa variations between groups were detected using linear discriminant analysis (LDA) effective size (LefSe) [40]. To perform a multigroup analysis, we utilized a general framework with bias correction 2 (ANCOM BC-2) [41]. We performed the heat tree analysis (Metacoder R package) [42] with non-parametric Wilcoxon Rank Sum Test, to explore the hierarchical structure of taxonomic differences between the groups. All statistical tests were two-tailed, and a Benjamini–Hochberg adjusted p-value < 0.05 was considered statistically significant.

Results

Gut microbiota comparison in *Blastocystis* and *Entamoeba* carriage

For a more in-depth study of the effects linked to the presence of *Blastocystis* and *Entamoeba*, based on the results obtained by Di Cristanziano et al. [11], the composition of the gut microbiota was compared by different categorizations of the selected samples. Firstly, all twenty-one samples categorized into 3 groups, A, C, and D were compared (Table 2), (Supplementary Table S1).

The Observed, ACE, Shannon, Fisher, and Chao1 indices at OTUs level ($p < 0.01$) showed greater alpha diversity in the D group than in the control A group (Supplementary Fig.S1). In detail, by unweighted UniFrac, the OTUs of three groups appeared distant (D *versus* A, $p < 0.01$, D *versus* C, and C *versus* A, $p < 0.05$) (Fig. 1A). At genus



A **B**

Fig. 1 PCoA unweighted UniFrac with 95% confidence ellipses showing the significant distances among the control group A and *Blastocystis* positive groups C and D; **A**: OTUs level; **B**: genus level. Samples M65 and M68 are located in the lower right quadrant of the diagram. (**A**); sample M25 is situated in the upper left quadrant (**B**)

level, only D *versus* A appeared significantly distant by unweighted UniFrac ($p < 0.01$) (Fig. 1B). To note, in group C samples M65 and M68 were from the only two individuals reporting mild abdominal pain and showed distant placement from asymptomatic individual M25.

Alpha-diversity and beta-diversity related to Blastocystis and E. hartmanni carriage

To analyze the possible gut microbiota variation related to the different *Blastocystis* subtypes, with or without *E. hartmanni*, the group C, including the samples carrying only *Blastocystis* ST1, was renamed C1; the samples from group D, carrying *E. hartmanni* and *Blastocystis* ST1 or ST2, were divided into groups D1 (with *Blastocystis* ST1) and D2 (with *Blastocystis* ST2) (Table 1) By comparison, the alpha diversity indices showed a significant increase only among positive samples D1 and D2 related to control samples (A) in Observed, Chao1, ACE, Shannon, and Fisher metrics at OTUs level ($p < 0.05$) (Fig. 2).

In the comparison between D2 and C1 groups, the abundances of Observed and the Fisher indices were significantly different only at the family taxonomic level ($p < 0.05$). Only by unweighted UniFrac distances, the beta-diversity of the bacterial component showed significant differences in D1 and D2 *Blastocystis* and *E. hartmanni*-carrier groups compared to *Blastocystis*-free subjects (A groups). In detail, between D1 (ST1 *Blastocystis*-carrier) and A groups, significant distances were at genus taxonomic levels ($p < 0.05$). Between D2 (ST2 *Blastocystis*-carrier) and A groups, significant distances were

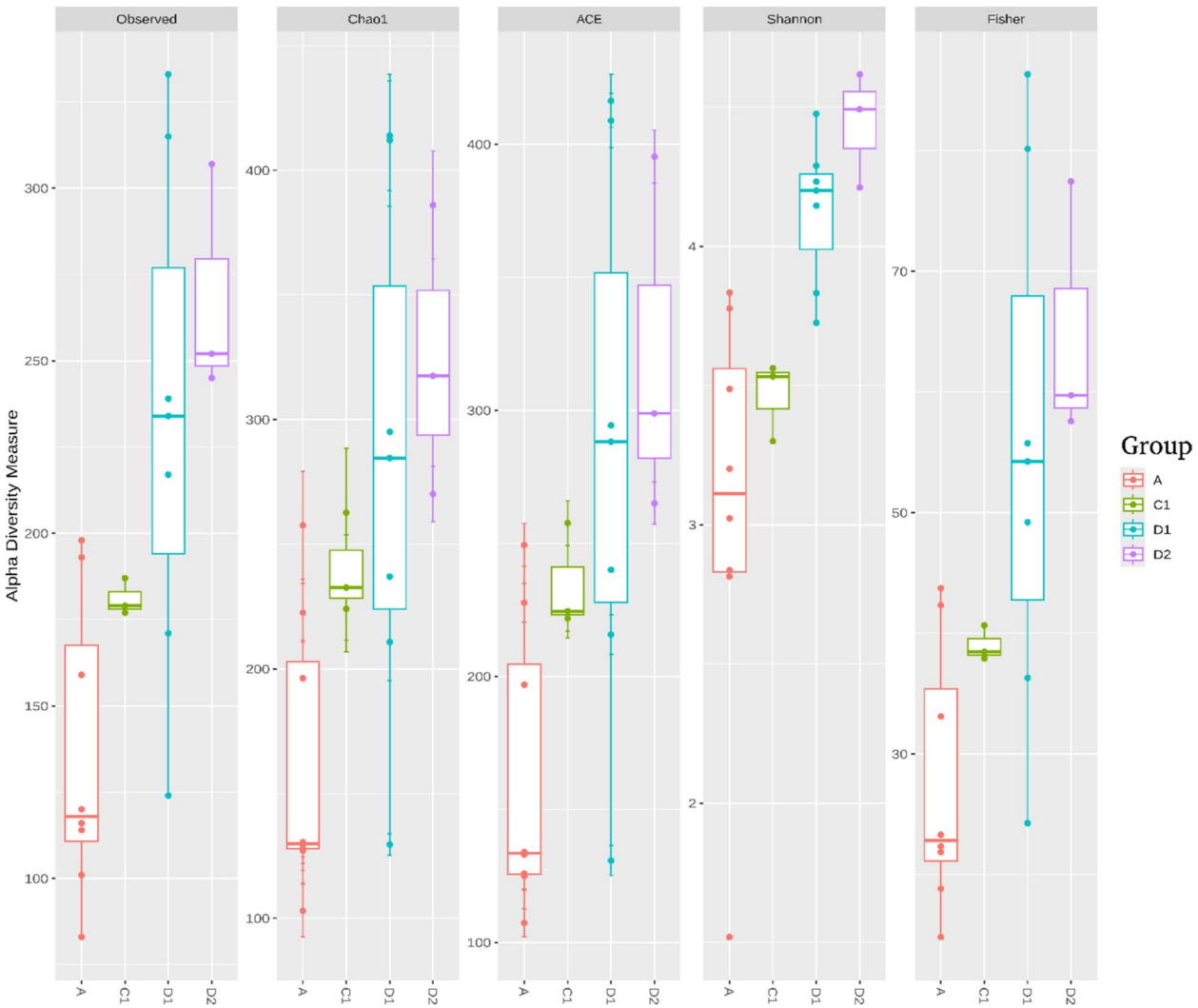


Fig. 2 Alpha diversity indices at the OTUs level among *Blastocystis* ST1 and ST2 carriers (C1, D1, D2) and the control group (A). Only differences between D1 and D2 *versus* A in Observed, Chao1, ACE, Shannon, and Fisher indices were statistically significant ($p < 0.05$)

at OTUs and genus taxonomic levels ($p < 0.05$). Firmicutes/Bacteroidetes (F/B) ratio did not show significant differences among the groups of *Blastocystis* carriers and the group of *Blastocystis*-free samples.

Bacterial abundance analysis related to Blastocystis and E. hartmanni carriage

In the descriptive analysis of the Top 20 most abundant genera (Fig. 3), greater variability is highlighted in the D1 and D2 groups in concordance with alpha-diversity analysis and then confirmed by the abundance of taxonomic clades of LDA score (log 10) from the LefSe analysis and ANCOM BC-2 analysis. By LefSe analysis (Supplementary Fig. S2), the following genera included in the Top 20, are differentially enriched: *Dialister*, *Ruminococcaceae* UCG-02, *Richenellaceae*, *Subdoligranulum*, [Eubacterium] *coprostanoligenes* group more abundant in D2 group ($p < 0.05$, $p < 0.01$); *Succinivibrio*, *Alloprevotella*, and *Ruminococcaceae* UCG-010 more abundant in D1 group ($p < 0.05$, $p < 0.01$); only *Prevotella* 9 was more abundant in C1 group ($p < 0.05$). LefSe analysis detected some differences among the 4 groups for other minority taxa, not included in the Bar-plot analysis of the Top 20 genus, such as *Catenibacterium*, *Holdemanella*, *Oribacterium* with the greater abundance in D2, *Butyrivibrio* in D1, *Coprococcus* 2 and *Desulfovibrio* in C1, *Blautia* and *Ruminoclostridium* 5 in A.

The differential analysis of gut bacterial niches confirmed some descriptive differences among the 4 groups observed by Top 20 abundances (Fig. 3) as indicated in Table 3.

In the comparison exclusively restricted to groups D1 and D2, the heat tree analysis (Fig. 4) revealed a decrease of the phyla Lentisphaerae in D1group driven by a

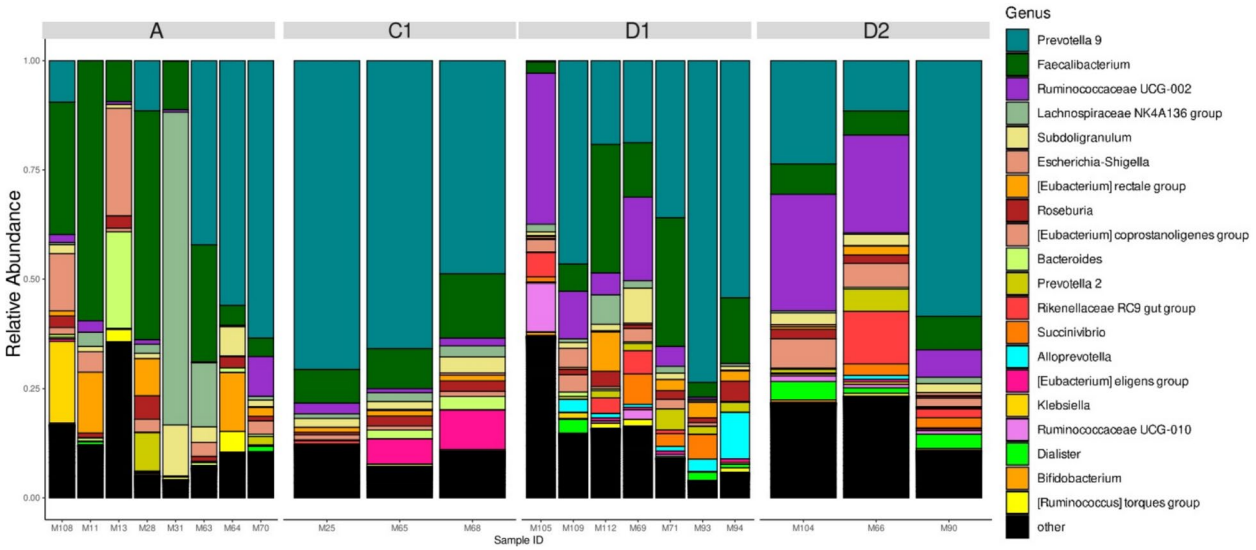


Fig. 3 Bar-plot analysis shows the genus Top 20 abundances in A, C1, D1, and D2 groups

Table 3 The results of the ANCOM BC-2 differential analysis at OTUs level

Comparison genus	A vs C1	A vs D1	A vs D2	D1 vs C1	D2 vs C1	D2 vs D1
Ruminococcaceae UCG-002	3.148	3.033	**6.044**[a]	− 0.115	2.896	3.011
(Prevotellaceae f) uncultured	**3.958**[a]	− 0.250	0.017	− **4.208**[c]	− **3.941**[d]	0.267
Faecalibacterium	− 2.805	− **2.749**[b]	− 2.917	0.055	− 0.112	− 0.168
Christensenellaceae R-7 group	0.130	2.471	**4.569**[a]	2.341	4.440	2.099
[*Ruminococcus*] torques group	− 1.055	− **4.024**[b]	− 4.170	− 2.969	− 3.115	− 0.146
Ruminococcaceae NK4A214 group	**3.121**[a]	− 0.250	1.566	− 3.371	− 1.555	1.815
(Christensenellaceae f) N/D	− 0.318	− 0,034	**3.557**[a]	0.284	3.876	**3.592**[e]
[Eubacterium] *coprostanoligenes* group	1.074	0.047	**4.295**[a]	− 1.027	3.221	**4.248**[e,]

Log_2FC and significant differences are indicated by **a–e** letters ($p < 0.05$)

[a] Means increasing of the genus in A group, [b]means decreasing of the genus in A group, [c]means decreasing of the genus in D1 group, [d]means decreasing of the genus in D2 group, and [e]means increasing of the genus in D2 group

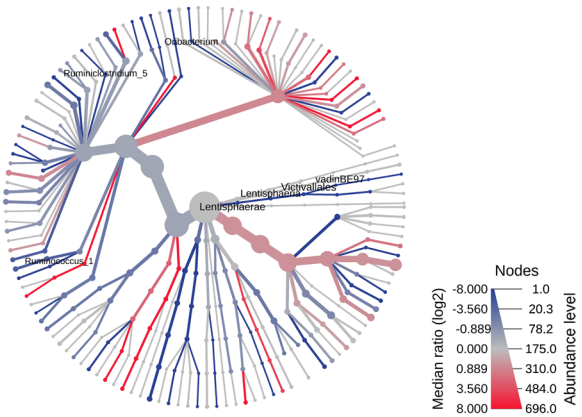


Fig. 4 Heat tree analysis depicting gut microbiota variations in comparison D1 *versus* D2. The color and size of edges and nodes are proportional to the abundance of organisms in each taxonomic category. Only significant taxa are labeled. The blue and red color of nodes and edges indicate a reduction and an increase respectively in the group D1. Unclultured taxa has not been included in the plot for better readability

reduction of vadinBE97 family ($p < 0.05$). Furthermore *Ruminococcus*_1 and *Ruminoclostridium*_5 genera from Ruminococcaceae Family and *Oribacterium* from Lachnospiraceae Family were all statistically decreased in D1 group.

Discussion

Multiple subtypes of *Blastocystis* and *Entamoeba* species were detected in individuals living in non-industrial areas [43]. Since *Blastocystis* and *E. hartmanni* represent the most prevalent microeukaryotes in the semi-rural areas of the Côte d'Ivoire [44], this study, although preliminary, will help to better analyze the role of the different subtypes of *Blastocystis* in the intestinal microbiota, especially in relation to the presence of co-infection with *E. hartmanni*.

When *Blastocystis* ST1 and ST2 co-occur with *E. hartmanni* (groups D1 and D2, respectively), the evidence of a greater distance and diversity from the control group (group A, negative patients) suggest to consider these two microeukaryotes ineffective in promoting unfavorable perturbations and rather commensal interaction can be attributed to them [3, 6]. Gut microbiota composition in groups C1 (harboring only *Blastocystis* ST1), D1, and D2, reveals a picture free of harmful bacterial groups. The Firmicutes/Bacteroidetes (F/B) ratio was calculated, as described by Guangorena-Gómez et al. [45] and in *Blastocystis*-free subjects the increase of the F/B ratio did not reach statistical significance.

Moreover, as previously shown in Andersen et al. [8] different gut microbiota enterotypes seem associated with the presence of *Blastocystis;* as observed in our study, the analysis at subtype level showed an increased abundance of the Prevotellaceae in the C1 group (carrying only *Blastocystis* ST1) and of the Rumincoccaceae in the D2 group (with *Blastocystis* ST2*).*

Indeed, the major abundance of OTUs belonging from Christensenellaceae family in the D2 groups can be considered a marker of a healthy gut. Christensenellaceae displays high heritability in many animal species and humans show a wide diffusion across all continents [46]. This family, according to studies conducted by some authors, would also have an interaction with the genetics of the host in the reduced risk of Ileal Crohn's disease or colorectal cancer [47–49]. Furthermore, according to several authors, the negative correlation between the central adiposity, serum lipids, and the relative abundance of the Christensenellaceae family would mark a good metabolic state [46].

As stated by Ma et al. [50], the stability of the microbiota could be explained by the greater abundance of beneficial taxa. In our study, some helpful taxa are abundant in the groups harboring *Blastocystis* compared to the control group (A), as occurs for *Succinivibrio* and *Butyrivibrio*, in association with *E. hartmanni* (group D1), or for *Butyrivibrio*, and *Desulfovibrio* in group C1 without *E. hartmanni*. A trend indicating a higher abundance of *Succinivibrio* in *Blastocystis* ST1 groups was consistent with Stensvold et al. [51], where however the analysis at subtype level was not performed. *Butyrivibrio* is one of colonic butyrogenic microbes that play a significant physiological importance in the dynamism of the gut microbiota in preserving the integrity of the barrier by short-chain fatty acid (SCFA) production in various conditions [52]. It is interesting to underline that [Eubacterium] *coprostanoligenes*, *Subdoligranulum*, *Coprococcus*, and *Butyrivibrio*, differentially more abundant in *Blastocystis*-carriers, are genera playing a role in the pathways related to SCFA biosynthesis and, as stated by Yamamura et al. [53], they would seem to be valid indicators of intestinal eubiosis in humans.

As concern the genus *Desulfovibrio,* it has been detected as a producer of hydrogen sulfide (H_2S), an essential signal transmitter that influences several biological systems as the resistance to intestinal pathogens [54]. Although it resulted in a minority taxon, a positive association of *Desulfovibrio* and *Coprococcus* 2 with beneficial bacteria such as *Prevotella* 9 (Supplementary Fig. 2), and a negative association with *Escherichia-Shigella* in C1 group were in line with Chen et al. [55]. A negative correlation of *Desulfovibrio* with body mass index, waist size, triglyceride levels, and uric acid levels was also highlighted by Chen et al. [55]. Our study lacked anthropometric data for *Blastocystis* carriers but further studies

evaluating the BMI in *Blastocystis*-carriers and non-carriers would be recommended [56, 57].

Despite the limited number of samples, this preliminary descriptive study conducted by different analysis of bacterial abundance, may provide an attempt to highlight key signatures of the simultaneous presence of *E. hartmanni* and *Blastocystis* ST1 and ST2, with an effect on the potentially positively affecting the balance of intestinal bacteria and preserving a state of eubiosis characterized by a healthy and diverse microbial community. In line with Even et al. [3], the major abundance of *Christensenellaceae* R-7 group, *Ruminococcaceae* UCG-002, and [Eubacterium] *coprostanoligenes* group, highlighted by ANCOMB-BC2 differential analysis, and of *Dialister*, *Richenellaceae*, *Subdoligranulum*, and *Lachnospiraceae* NK3A20, evidenced by LefSe analysis, may explain a beneficial/eubiotic cooperation among host gut microbiota, *Blastocystis* ST2, and *E. hartmanni*.

The positive associations of [Eubacterium] *coprostanoligenes*, *Subdoligranulum*, *Coprococcus*, and *Butyrivibrio* in ST1/ST2 *Blastocystis*-carriers founded in this study contribute to better defining a possible role of these *Blastocystis* genetic variants in promoting or preserving the gut homeostasis and anti-inflammatory molecules production [58]. It is interesting to note that recently Bubeck et al. [59] hypothesized an association with cholesterol metabolism of some genera as *Christensenellaceae* R-7 group, *Ruminococ*caceae UCG-002 [Eubacterium] *coprostanoligenes,* here resulted more abundant in D2 group versus D1 group. Subtypes ST1 and ST2 in association with *E. hartmanni*, could reveal that *Blastocystis* ST2 and *E. hartmanni* promote a major presence of some minority taxa related to obesity [60].

Finally, despite two symptomatic individuals out of three positives for *Blastocystis* (group C), the modest effect on alpha and beta diversity seems to confirm the hypothesis, although not univocal, of the commensal habit of this protozoan [61].

In conclusion, although our results are limited by the relatively small sample size, precluding specific conclusions, they represent the first study conducted in Côte d'Ivoire on *Blastocystis* ST1 and ST2 in association with gut microbiota composition among healthy subjects living in West Africa. These findings may be considered within the ongoing debate on the relationship between the bacterial composition of the gut microbiota and commensal protozoan co-occurrences such as *Blastocystis* and *E. hartmanni*. In particular, given the evident ethnogeographical patterns in the presence of *Blastocystis* subtypes, additional genetic studies targeting underrepresented countries are warranted [14]. More extensive research is also needed to determine if specific *Blastocystis* subtypes are causative factors or merely associated with inflammatory bowel diseases such as IBS and IBD. Additionally, this study highlights the need to elucidate the impact of dietary habits, environmental factors, and lifestyle together with the presence of other commensal microeukaryotes on the plasticity of the gut microbiota.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-024-00661-5.

Additional file 1

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Author contributions
Conceptualization, L.A. and R.D.; Data curation, V.D., F.B., and R.D.; Formal analysis, L.A. and F.F.; Investigation, M.S.; Methodology, R.D.; Software, L.A.; Supervision, R.D.; Validation, L.A., F.F. and M.D.; Writing—original draft, R.D., and F.B.; Writing—review & editing, V.D., K.A.E., and M.F. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The scientific reasons and the protocol of the study were reviewed and approved by the Medical Director of the Don Orione Center in Bonoua and all Directors of each service on the date 20.01.2014. This research was conducted without any "Invasive act", affecting the physical, psychological, or moral integrity of the participants. The identity of each participant was protected by the attribution of an anonymous code to each stool sample collected. All experiments were performed following the ethical standards established by the Universal Declaration of Human Rights (1948), and the Declaration of Helsinki (1964), and its successive amendments were complied with.

Consent for publication
Participation of adults and children occurred voluntarily. A verbal agreement on the methods and purposes of the study was acquired before proceeding with sampling collection by adults and parents/guardians of children. A local dialect interpreter of dialect was used to explain the study's aims, procedures, and significance when necessary. All participants were assured that all data were analyzed anonymously, and the human biological sample would provide data exclusively on intestinal microorganisms and not on human tissues.

Competing interest
The authors declare no competing interests.

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