

# Gut microbiota signatures in *Schistosoma japonicum* infection-induced liver cirrhosis patients

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## Research

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# Abstract

**Background:** One of the serious complications induced by *Schistosoma japonicum* infection is the development of liver cirrhosis. Several studies have assessed the role of gut microbiota in cirrhosis of different etiologies, but none has done so in the context of *S. japonicum* infection in humans. Here, to explore the possible role of gut microbiota in *S. japonicum* infection-induced liver cirrhosis, we conducted an observational clinical study.

**Methods:** Twenty-four patients with *S. japonicum* infection-induced liver cirrhosis and 25 age- and gender-matched controls from Zhejiang Province, China, were enrolled in the study. Fecal samples were collected and used for 16S rRNA gene hypervariable V4 region Illumina MiSeq sequencing.

**Results:** Eight hundred seven operational taxonomic units (OTUs) were detected, of which 491 were common in the two groups, while 123 and 193 were unique for the control and cirrhosis groups, respectively. Overall, no significant differences in both alpha and beta diversities and overall microbiota structure were found in the two groups. However, *Bacilli* (Class) and *Lactobacillales* (Order) were significantly higher in patients with *S. japonicum* infection-induced liver cirrhosis than in healthy individuals.

**Conclusions:** Altogether, our results suggest that the gut microbiota of *S. japonicum* infection-induced liver cirrhosis patients is similar to that of healthy individuals.

## Background

Schistosomiasis is one of the most prevalent parasitic diseases in the world [1]. An estimated 240 million people are affected by this disease, together with 800 million more at risk of infection [2]. Worryingly, schistosomiasis alone is associated with a high disease burden of as much as 3.3 million disability-adjusted life years (DALYs), accounting for more than 10% of the global burden of Neglected Tropical Diseases (NTDs) [3, 4]. There are 5 known etiologic agents of schistosomiasis, all of which are trematode parasites of the *Schistosoma* genus: *S. japonicum*, *S. mansoni*, *S. haematobium*, *S. intercalatum*, and *S. mekongi* [5]. Only *S. japonicum* exists in China, and it is predominant in 12 provinces south of the Yangtze River [6]. In the 1950s, an estimated 11.6 million people were infected, while more than 100 million more were at risk of infection [7]. From the mid-1950s, the number of infected cases has reduced by over 99%; however, schistosomiasis is still endemic in 140 counties in China [1].

Liver cirrhosis is a common (end-stage) consequence of several liver diseases and a major cause of mortality worldwide [8]. Previous studies have reported an association between gut microbiota alterations and chronic liver disease [9, 10], including cirrhosis secondary to hepatitis B or C virus infections [11, 12], alcohol over-consumption [13, 14], nonalcoholic fatty liver disease [13, 15, 16], and even primary biliary cirrhosis [17]. Furthermore, cirrhosis complications, including hepatic encephalopathy, spontaneous bacterial peritonitis, and sepsis, were linked to gut microbiota alterations [18]. Some studies on the gut-

liver axis have even demonstrated that the gut microbiota (and bacterial translocation) plays an important role in the pathogenesis of cirrhosis and its complications [13, 19].

Recent studies using not only animal models but also human clinical samples have demonstrated a clear relationship between gut microbiota alterations and *Schistosoma* spp. infection [20, 21, 22, 23, 24, 25]. However, no study has explored the relationship between gut microbiota and *S. japonicum* infection-induced liver cirrhosis; the potential role of gut microbiota alterations in disease onset and progression is still unclear.

Here, to explore the possible association between gut microbiota and *S. japonicum* infection-induced liver cirrhosis, we conducted an observational clinical study. Fecal samples collected from 24 patients and 25 matched healthy individuals from the Zhejiang Province, China, were used for 16S rRNA Illumina MiSeq sequencing. The main objectives of this study were: (1) to characterize the relevant gut microbiota alterations associated with *S. japonicum* infection-induced liver cirrhosis; and (2) to identify potential bacterial taxa to be used as non-invasive biomarkers for the early diagnosis of liver cirrhosis secondary to schistosomiasis.

## Methods

### Ethics Statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. All experiments were approved by the First Affiliated Hospital Clinical Research Ethics Committee, School of Medicine, Zhejiang University (ref: 2017411-1), and the Jiaxing Wangdian People's Hospital Ethics Committee (ref: 2017002). All participants provided written informed consent.

### Exclusion criteria and subject enrolment

Eligibility was defined by the application of the following exclusion criteria: (I) history of digestive disorders, such as other causes of liver disease, irritable bowel syndrome, inflammatory bowel disease, and chronic diarrhea; (II) history of previous gastrointestinal surgery; (III) history of probiotics, prebiotics, synbiotics, antibiotics, acid suppressors, metformin, and gastrointestinal motility targeting-drugs use, less than eight weeks before enrollment; (IV) history of bowel preparation, less than four weeks before enrollment; (V) diagnosis of major diseases such as malignant tumors, cardiovascular disease, respiratory, autoimmune and allergic diseases, neurological disorders, renal insufficiency, diabetes, uncontrolled hypertension (blood pressure  $\geq 150 / 90$  mmHg), dyslipidemia, depression, mania, and bipolar affective disorder; and (VI) hepatitis B virus, hepatitis C virus and HIV infection.

From December 2017 to November 2019, 24 patients with *S. japonicum* infection-induced liver cirrhosis and 26 age- and gender-matched healthy individuals were enrolled from Hangzhou and Jiaxing, northern cities of the Zhejiang Province, China, an *S. japonicum* infection endemic area. Since the stool specimen from one healthy individual was unsatisfactory, the healthy controls group was ultimately composed of

25 individuals. All participant demographics information, laboratory tests, computed tomography (CT), and B ultrasonic scans were collected from the hospital electronic medical records system. Additionally, questionnaires were filled by all subjects. Schistosomiasis diagnosis was performed using the Kato-Katz method to detect *S. japonicum* eggs in stool samples. Liver cirrhosis was diagnosed according to clinical and biochemical data and imaging exams (CT or B ultrasonic scans) [26, 27]. Liver function was evaluated with the Child-Pugh classification system [28].

## Fecal sample collection

The participants were trained by a researcher to obtain a complete stool collection method. Fresh stool samples (self-collected from patients and controls) were collected into anaerobic bags (Mitsubishi Gas Chemical). Samples were divided into five 200 mg aliquots, in the first 30 min after collection, and immediately stored at  $-80^{\circ}\text{C}$  for posterior analysis.

## Genomic DNA extraction

Total genomic DNA was extracted per sample using the MagPure Stool DNA KF kit B (Magen, Guangzhou, China) according to the manufacturer's instructions. DNAs were quantified in the Qubit Fluorometer (Invitrogen, Qubit 2.0) using the Qubit® dsDNA BR Assay kit (Invitrogen, CA, USA). DNA quality was assessed using gel electrophoresis (1% agarose gels). Negative controls containing the elution buffer only were included during DNA extraction and quantification to monitor possible contamination.

## Library construction and sequencing

Bacterial 16S rRNA gene hypervariable region V4 was amplified using degenerate polymerase chain reaction (PCR) and the primers 515Fw (5'-GTGCCAGCMGCCGCGGTAA-3') and 806Rv (5'-GGACTACHVGGGTWTCTAAT-3'), which contained the Illumina adapter sequence, a pad, and a linker. Fifty microliters reaction volumes, containing 30 ng of DNA templates together with the primers (10  $\mu\text{M}$ ) and the PCR master mix, were used. Amplification was performed under the following conditions: 95  $^{\circ}\text{C}$  for 3 minutes; 30 cycles of 95  $^{\circ}\text{C}$  for 45 seconds, 56  $^{\circ}\text{C}$  for 45 seconds, and 72  $^{\circ}\text{C}$  for 45 seconds; and a final extension step at 72  $^{\circ}\text{C}$  for 10 minutes. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, CA, USA) according to the manufacturer's instructions. Library quality was evaluated using the 2100 Bioanalyzer (HT DNA 1K Labchip 760517) instrument (Agilent Technologies, CA, USA). Sequencing was performed using the Illumina HiSeq 2500 platform (BGI, Shenzhen, China) following the Illumina standard pipelines to get  $2 \times 250$  bp paired-end reads.

## Bioinformatics analysis

Raw reads were filtered to remove adaptors and low-quality and/or ambiguous bases, and paired-end reads were merged using the Fast Length Adjustment of Short reads (FLASH, v1.2.11) software [29]. Sequences were clustered into operational taxonomic units (OTUs) with a cutoff value of 97% using the UPARSE software (v7 .0.1090) [30]. Chimeric sequences were detected (compared with the Gold database) using the UCHIME software (v4.2.40) [31] and removed from the analysis. Sequences of representative OTUs were then taxonomically classified using the Ribosomal Database Project Classifier

(v.2.2) with a minimum confidence threshold of 0.6 and trained on the Greengenes database (v201305) using the QIIME pipeline (v1.8.0) [32]. The USEARCH\_global command [33] was used to quantify OTUs abundance in each sample.

## Bacterial diversity analysis

Alpha and beta diversities were estimated using the MOTHUR software (v1.31.2) [34] and the QIIME pipeline (v1.8.0) [32], respectively. Sample clustering was conducted using the QIIME pipeline (v1.8.0) [32] and the unweighted pairwise grouping method with averaging (UPGMA). Barplots and heatmaps of different classification levels were plotted with the R package (v3.4.1) and the R package “gplots” tool, respectively. Venn diagrams and accumulation curves were obtained using the R package “VennDiagram” (v 3.1.1). A principal component analysis (PCA) was performed using the R package “ade4” tool. Taxonomic characterization was performed using a Linear Discriminant Analysis (LDA) with the calculation of the LDA effect size (LEfSe).

## Statistical analysis

Due to the lack of data on effect size, a power calculation might be difficult. However, a recent similar study on *S. japonicum* infection in which each group consisted of 24 animal subjects might have helped with power calculations [23]. Continuous variables are presented as mean  $\pm$  standard deviation (SD), and categorical variables are presented as percentages (%). The student's *t*-test or Wilcoxon test and the  $\chi^2$  test were used to evaluate differences between the two groups for continuous and categorical variables, respectively. Whenever applicable, the false discovery rate (FDR) was used to correct the calculated *p*-values. Statistical analyses were performed using the SPSS software (v 22.0; SPSS, IL, USA). Statistical significance was given by  $p < 0.05$ ,  $FDR < 0.05$ , or LDA scores  $> 2$ .

## Results

### Participants' characterization

The study population included a total of 24 patients with *S. japonicum* infection-induced liver cirrhosis (12 males and 12 females), and 25 age- and gender-matched healthy individuals (12 males and 13 females). The average ages of cirrhosis and control groups were comparable:  $82.7 \pm 7.3$  years old and  $82.3 \pm 7.1$  years old, respectively (Table 1). Furthermore, no significant differences were observed concerning gender, body mass index (BMI), or smoking and drinking habits between the two groups. In addition, the platelet counts and the serum levels of total bilirubin (TB), direct bilirubin (DB), and alanine aminotransferase (ALT) were comparable between the groups. On the other hand, while the serum levels of aspartate aminotransferase (AST) and glutamyl transpeptidase (GGT), together with the prothrombin time (PT) were significantly increased in the *S. japonicum* infection-induced liver cirrhosis group, the total protein, and globulin serum levels were significantly decreased in the same group, always in comparison with the control group (Table 1; at least  $p < 0.05$ ). Regarding disease severity stratification, performed only

for the cirrhosis group, 13 patients (54.2%) were classified as Child-Pugh A, 7 (29.2%) as Child-Pugh B, and 4 (16.7%) as Child-Pugh C (Table 1).

## **Gut microbial diversity is comparable in individuals with *S. japonicum* infection-induced liver cirrhosis and healthy controls**

To characterize the gut microbiota in the context of *S. japonicum* infection-induced liver cirrhosis, we sequenced bacterial 16S rRNAs extracted from fecal samples and employed an OTU-based bioinformatics analysis to estimate alpha and beta diversities. Importantly, the obtained accumulation curve reached a plateau, indicating that most of the gut microbial populations have been detected at the used sequencing depth (Fig. 1). A total of 807 OTUs were obtained, of which 491 were equally detected in samples from cirrhosis individuals and healthy controls (Fig. 2). Notably, as represented in the Venn diagram, 123 and 193 OTUs were uniquely detected in healthy individuals and patients with *S. japonicum* infection-induced liver cirrhosis, respectively (Fig. 2).

Concerning alpha diversity, we used 6 different metrics for the analysis: the Observed species, Chao, ACE, Shannon, Simpson, and Good's coverage indexes were calculated. Overall, we did not detect any significant changes when healthy individuals and patients with *S. japonicum* infection-induced liver cirrhosis were compared. However, a slight tendency towards a lower bacterial diversity was observed in the cirrhosis group (particularly looking at Observed species, Chao, ACE, and Shannon indexes) (Fig. 3). In addition, considering the small number of cases showing the disease severity in the cirrhosis group (13 Child-Pugh A, 7 Child-Pugh B, and 4 Child-Pugh C cases), we did not discuss the variance in microbiota data further by disease severity.

We further analyzed beta diversity (based on weighted UniFrac distances), to evaluate differences between samples. Overall, and once more, no major differences were observed when healthy individuals and patients with *S. japonicum* infection-induced liver cirrhosis were compared (Fig. 4A). PCA data (Fig. 4B) confirmed this similarity. Samples from each group showed heterogeneous distributions that were not species-specific, indicating that the structure of gut bacterial communities is similar in healthy individuals and patients with *S. japonicum* infection-induced liver cirrhosis (Fig. 4B).

## **Fecal microbial communities of healthy individuals and patients with *S. japonicum* infection-induced liver cirrhosis are slightly different**

Next, we employed a qualitative approach to perform a more in-depth characterization of the intestinal bacterial communities present in the stool samples collected from patients and controls. Briefly, we evaluated bacterial relative abundance at both phylum and genus levels (Fig. 5A and B, respectively). The

analysis of the most abundant bacterial phyla in the fecal samples collected from the two different groups of individuals revealed a similar picture: *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were the three most relevant phyla in both groups, accounting for 97.1% and 94.4% frequencies in healthy controls and cirrhosis patients, respectively (Fig. 5A). On the other hand, some different patterns were observed at the genus level, being *Bacteroides*, *Prevotella*, and *Faecalibacterium*, the three most prevalent genera in the gut of healthy individuals, while in cirrhosis patients, the third most prevalent genus was *Escherichia* (Fig. 5B).

Additionally, to explore statistical relevance, we performed a LEfSe analysis:  $p < 0.05$  (Wilcoxon test) and an LDA scores  $> 2$  were considered statistically significant (Figs. 6 and 7). Of note, we also considered FDR values in this analysis to minimize the number of false-positive results. Overall, we have found that only the *Bacilli* Class and the *Lactobacillales* Order were significantly increased in cirrhosis patients compared to healthy individuals (Fig. 8).

## Discussion

One of the serious complications induced by *S. japonicum* infection is the development of liver cirrhosis [35]. Most egg-laying adult *Schistosoma* trematodes have liver tropism, and the local (hepatic) host immune responses to the deposited eggs result in granuloma formation, which eventually evolves to progressive liver fibrosis and portal hypertension [36]. Both *in vivo* and clinical studies focusing on *S. japonicum* infections revealed that hepatic stellate cells (HSCs) are the key drivers of hepatic fibrosis [35, 37], via collagen production and deposition. Importantly, liver fibrosis presentations have the potential to evolve into liver cirrhosis or liver cancer in the future [38]. Several studies have assessed the role of gut microbiota in cirrhosis of different etiologies [11, 16, 39, 40, 41]. Microbiota dysbiosis was often reported in cirrhosis patients: (I) autochthonous/non-autochthonous taxa ratio reduction; (II) *Firmicutes*/*Bacteroidetes* ratio inversion; or (III) increased prevalence of potentially pathogenic bacteria [42, 43, 44]. Interestingly, the liver-gut axis was involved in the physiopathology of cirrhosis: overgrown intestinal bacteria have the potential to translocate across the leaky gut barrier and reach the liver, leading to cirrhosis development or progression [45]. However, few studies explored the gut microbiota in the context of *S. japonicum* infection-induced liver cirrhosis.

In fact, to our knowledge, this is the first study exploring the possible association between gut microbiota and *S. japonicum* infection-induced liver cirrhosis in humans. Surprisingly, here, we found that the microbiota of *S. japonicum* infection-induced liver cirrhosis patients was as rich and diverse as that of matched healthy individuals. Overall, these results are not consistent with findings reported for *in vivo* studies. For instance, in a mouse model of *S. japonicum* Ova-induced granulomas, a significantly higher beta diversity was detected versus controls [23]. Similarly, an overall reduction in alpha and a significant increase in beta diversities were found in the gut of *S. mansoni* infected mice compared to uninfected controls [21]. However, some studies align with our findings. For instance, Schneeberger *et al.* suggested that *S. mansoni* infection (in children) does not affect the gut microbial composition [25].

Furthermore, the taxonomic composition at the phylum level was similar in cirrhosis patients and healthy individuals (prevalence of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*), aligning with *S. japonicum* *in vivo* infections' data [23]. Additionally, even at the genus level, no major changes were detected, indicating that in our context, there is no gut dysbiosis in *S. japonicum* infection-induced liver cirrhosis patients. This result is somehow unexpected and different from other studies that have reported gut dysbiosis in the context of human schistosomiasis (e.g., those caused by *S. mansoni* and *S. haematobium*) [20, 21, 22, 23, 24]. Additionally, *in vivo* studies have reported *Schistosoma* infection-related alterations in gut microbial composition. For instance, *S. japonicum* infection in mice was associated with significant changes in the gut prevalence of *Firmicutes* (relative decrease), *Bacteroidetes*, and *Proteobacteria* (relative increase) [23].

However, we did find some composition alterations in the gut microbiota of *S. japonicum* infection-induced liver cirrhosis patients. *Bacilli* (at the class level) and *Lactobacillales* (at the order level) were significantly increased in patients with *S. japonicum* infection-induced liver cirrhosis compared to healthy individuals. Importantly, our results are consistent with those of a previous study, which may suggest that the increased prevalence of *Lactobacillales* in gut microbiota is a hallmark of human liver cirrhosis [46]. Additionally, a different study showed that *Lactobacillales* and *Bacilli* were the key contributors to gut dysbiosis in primary liver cancer and proposed them as potential diagnostic markers [47]. Altogether, these findings suggest a strong association between gut *Bacilli* and *Lactobacillales* and liver disease that deserves to be further explored in different pathological contexts.

Overall, our data do not support the idea that *S. japonicum* infection-induced cirrhosis is associated with significant alterations of gut microbiota profiles, opposing hypotheses proposed by others concerning schistosomiasis. Some reasons may justify our opposing report. Different studies focused on different *Schistosoma* spp. that have different organ tropisms: e.g., *S. mansoni* and *S. japonicum* target the gut and liver, while *S. haematobium* targets the bladder and the urogenital system [20, 21, 22, 24]. Importantly, the notion that *S. haematobium* infection was equally associated with human gut dysbiosis suggests that potential alterations of intestinal microbial communities in the context of schistosomiasis are probably a consequence of infection-induced systemic immune responses (and not of local events). In fact, some studies suggest this [20, 21, 22]. For instance, mesenteric lymph node cells isolated from mice infected with *S. mansoni* (dysbiotic) responded differently compared to the ones isolated from controls: higher interleukin (IL)-4, IL-10, and IL-17 secretion, together with lower interferon- $\gamma$  production [22]. Relevantly, aging is accompanied by a deterioration of immune responses, recently named as immunosenescence or immune-aging [48]. In our study, the enrolled patients were quite old (average of  $82.7 \pm 7.3$  years old), which may justify the absence of phenotype: their potentially weaker immune responses to *S. japonicum* infection were not strong enough to impact their gut microbial communities.

Nevertheless, our geographic target-region conditioned the age range of our study population, which is a limitation of our study. Since 1995, due to the application of effective Schistosomiasis control measures, there are almost no newly reported cases in Zhejiang [1]. Therefore, patients with *S. japonicum* infection-induced liver cirrhosis are not many and tend to be of advanced ages [27]. Therefore, we cannot exclude a

possible age selection bias that, however, reflects the reality of the population studied: among the cirrhosis patients, 17 and 7 individuals were 80 or 90 years of age or older, respectively, and none of the others was less than 60 years old. We can, however, also hypothesize that our population has a survival bias. Living patients may be the ones with better outcomes due to stable gut microbiota profiles, while the fatalities may have been a consequence of gut microbiota disorders. This is, however, only a speculative exercise. Therefore, further studies focusing on much larger and heterogeneous populations (different geographies, with a wider age range) are needed to completely understand if *S. japonicum* infection-induced liver cirrhosis is accompanied by gut microbiota alterations. In addition, the effective Schistosomiasis control in Zhejiang and advanced age of participants might affect our conclusion because it was generally almost impossible to include a cohort of age-matched acute *S. japonicum* infected patients without liver cirrhosis.

An additional factor that should be considered in future studies is the timing of infection, particularly if the study is designed to find biomarkers for cirrhosis diagnosis and/or prognosis. Indeed, the infection status may also condition the results. Human studies have mainly targeted adolescents [20] or children [24], and consequently, the early phase of infection. Additionally, the most commonly used mouse model of schistosomiasis in microbiome studies evaluated acute infection – less than 8 weeks post-challenge according to the model characterization [49, 50, 51]. The *in vivo* published data refers to stool samples collected at 28 days [21], 42 days [23], and 8 weeks [22] after infection. We cannot exclude that our different findings are just a consequence of our study context. Cirrhosis is the end-stage of chronic schistosomiasis, quite far time-wise from the early phase of infection. Both systemic immune responses and *Schistosoma* infection were associated with gut microbiota alterations [20]. However, in *S. japonicum* infection-induced liver cirrhosis, both are expected to decrease in magnitude, which may partially explain our findings.

## Conclusion

In conclusion, overall, we showed that gut microbial taxonomic profiles and diversity in *S. japonicum* infection-induced liver cirrhosis patients appear to be similar to the ones from age-matched healthy people. Therefore, in our context, patients with *S. japonicum* infection-induced liver cirrhosis present a “healthy” gut structure, which may partially explain the better prognosis of cirrhosis secondary to this infection, compared to cirrhosis of different etiology in China [26, 27]. Future studies with much larger sample sizes across wider age ranges are, however, needed to further explore the relationship between *S. japonicum* infection-induced liver cirrhosis and gut microbiota and potentially disclose the possible mechanisms by which gut microbiota alterations influence cirrhosis and/or *vice versa*. Importantly, these studies should also evaluate immunity and inflammation to finally address if the gut-liver axis is implicated in *S. japonicum* infection-induced liver cirrhosis development and progression. Such studies should be further complemented with *in vivo* works with transplantation of human gut microbiota samples to ultimately establish (or exclude) a causal relationship between dysbiosis and cirrhosis secondary to schistosomiasis.

# Declarations

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## Authors' contributions

Conceptualization: Y.M.Y. and H.L.J. Data curation: Q.F.G. and F.Z. Formal analysis: Q.F.G., C.X. and J.X. Funding acquisition: Q.F.G., C.X. and Q.Z. Investigation: F.Z. Methodology: H.F.L. Supervision: Q.Z. Writing - original draft: Q.F.G. Writing - review & editing: Y.M.Y. and H.L.J. All authors read and approved the final manuscript.

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## Availability of data and materials

The data supporting the conclusions of this article are included within the article. The datasets analysed during the present study are available from the corresponding author upon reasonable request.

## Ethics approval and consent to participate

All participants were approved by the First Affiliated Hospital Clinical Research Ethics Committee, School of Medicine, Zhejiang University (ref: 2017411-1), and the Jiaying Wangdian People's Hospital Ethics Committee (ref: 2017002). All participants provided written informed consent in Chinese. Participation was entirely voluntary and only participants with signed informed consent forms were enrolled into the study. and the rights of these subjects were protected.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

# Abbreviations

OTUs: operational taxonomic units; DALYs: disability-adjusted life years; NTDs: Neglected Tropical Diseases; CT: computed tomography; PCA: principal component analysis; LDA: Linear Discriminant Analysis; LEfSe: LDA effect size; SD: standard deviation; FDR: false discovery rate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; DB: direct bilirubin; GGT: glutamyl transpeptidase; PT: prothrombin time; SLC: *S. japonicum* infection-induced liver cirrhosis; TB: total bilirubin; HSCs: hepatic stellate cells; IL: interleukin.

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## Tables

**Table 1. Demographic and clinical characteristics of the study population per group.**

	SLC group	Control group	<i>P-value</i>
n	24	25	
Gender (male/female)	12/12	12/13	0.889
Age (years)	82.7 ± 7.3	82.3 ± 7.1	0.852
Active smoker (%)	0%	12%	0.248
Active drinker (%)	0%	20%	0.066
BMI (Kg/m <sup>2</sup> )	22.4 ± 1.8	23.3 ± 2.3	0.915
Platelets (10 <sup>9</sup> /L)	152.3 ± 108.3	179.6 ± 59.5	0.275
PT (s)	19.4 ± 8.1	15.3 ± 1.6	0.003
Total protein (g/L)	59.5 ± 8.3	74.0 ± 4.1	< 0.001
Albumin (g/L)	31.4 ± 5.1	29.6 ± 4.4	0.186
Globulin (g/L)	27.7 ± 6.0	44.5 ± 3.8	< 0.001
TB (µmol/L)	19.8 ± 19.3	15.3 ± 5.1	0.711
DB (µmol/L)	7.9 ± 9.9	4.2 ± 1.5	0.509
AST (U/L)	32.2 ± 17.5	17.6 ± 6.6	< 0.001
ALT (U/L)	22.0 ± 16.0	22.2 ± 5.4	0.114
GGT (U/L)	61.0 ± 51.6	33.6 ± 21.5	0.024
Child-Pugh			
A	13	/	NA
B	7	/	NA
C	4	/	NA

Note: The continuous and categorical variables are listed as mean ± SD and percentage (%), respectively. Statistical differences were determined using the Student's t-test (parametric data) or the Wilcoxon test (non-parametric data) and the  $\chi^2$  test for continuous and categorical variables, respectively. Abbreviations: ALT - alanine aminotransferase; AST - aspartate aminotransferase; BMI - body mass index; DB - direct bilirubin; GGT - glutamyl transpeptidase; PT - prothrombin time; SLC - *S. japonicum* infection-induced liver cirrhosis; and TB - total bilirubin.

# Figures

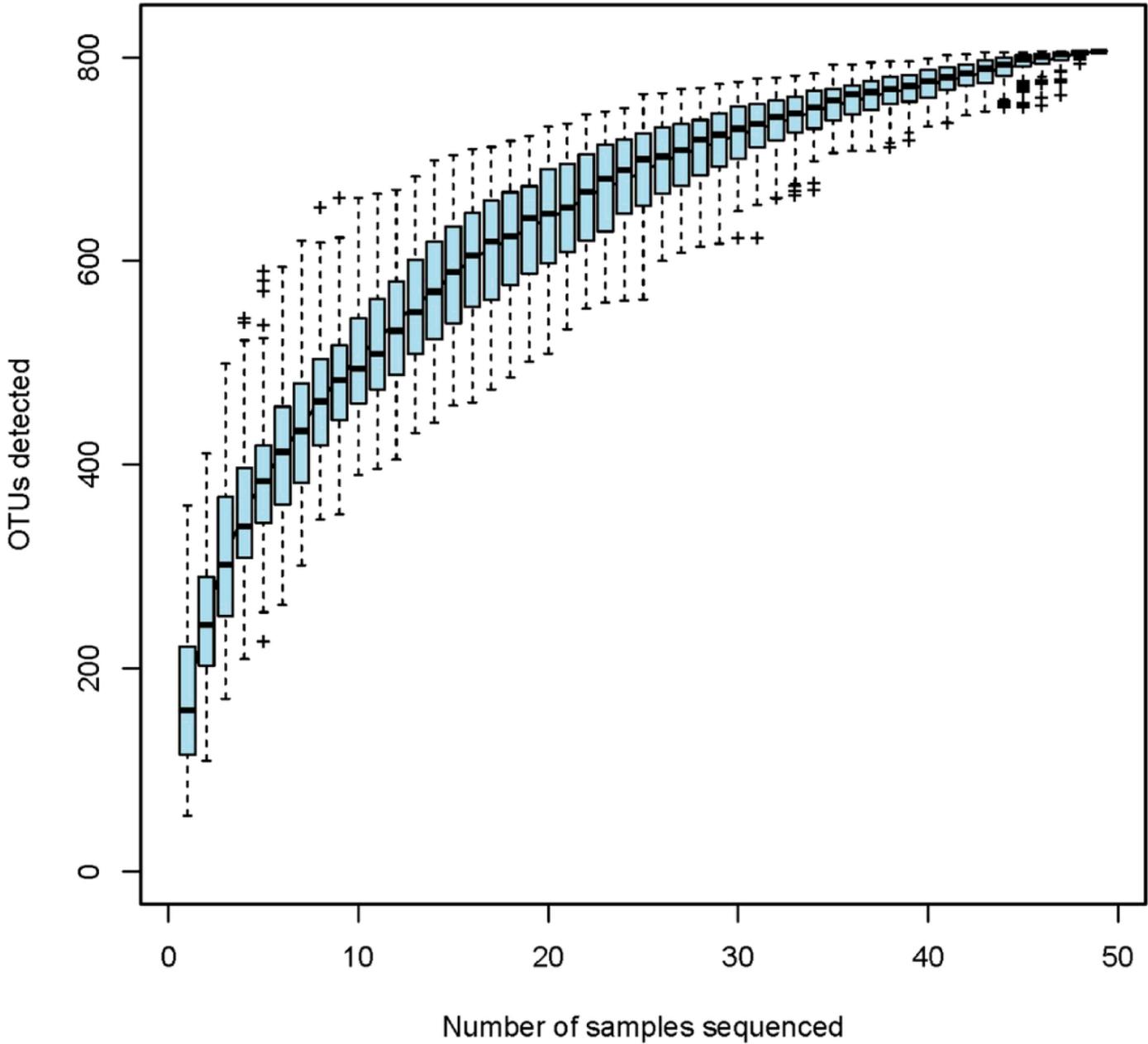
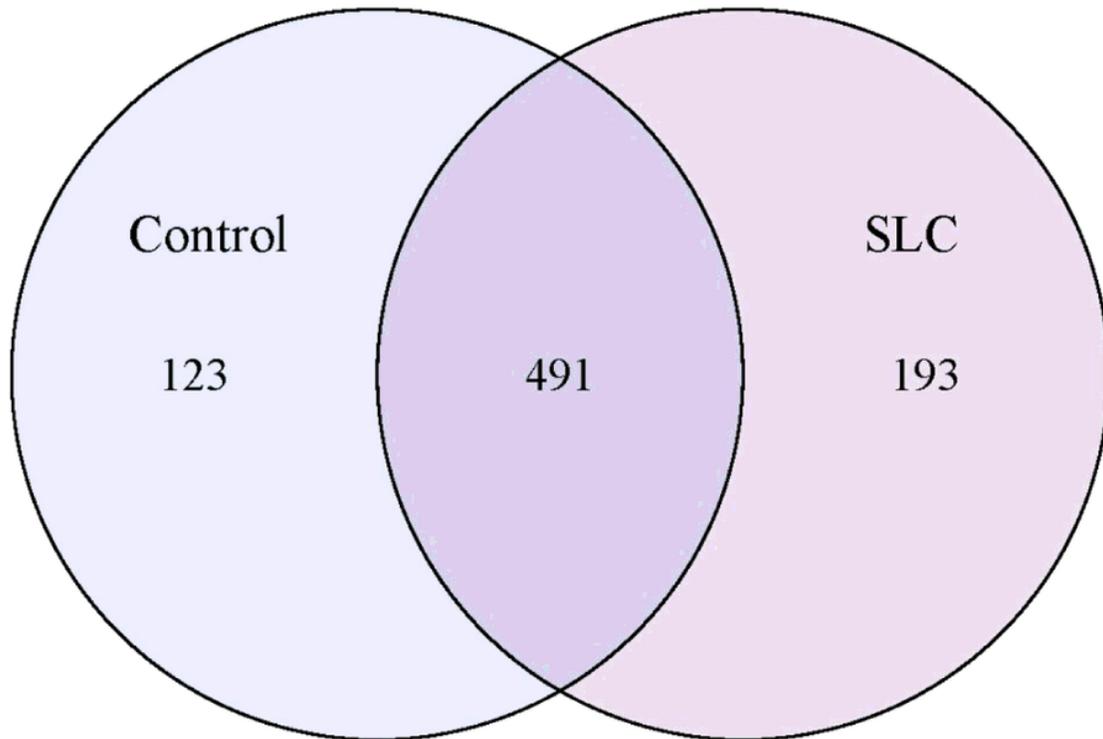


Figure 1

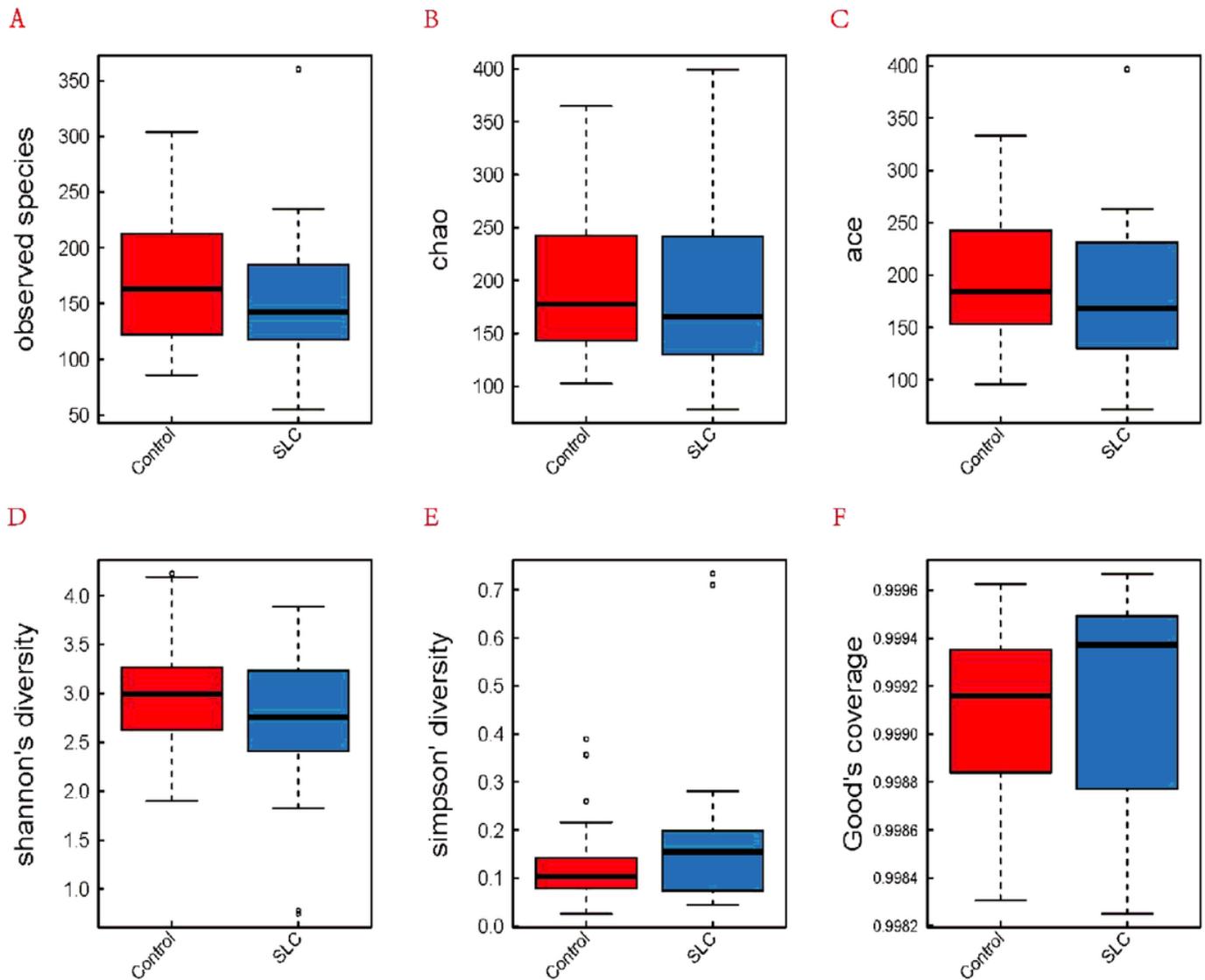
Accumulation curves attest to the sequencing depth adequacy. Accumulation curve is given by cumulative data of OTUs per sample. Each box and whiskers' plot represents OTUs quantified for the

respective number of samples sequenced (e.g., the first plot refers to one sample, while the second plot represents the cumulative data of two samples, and so forth).



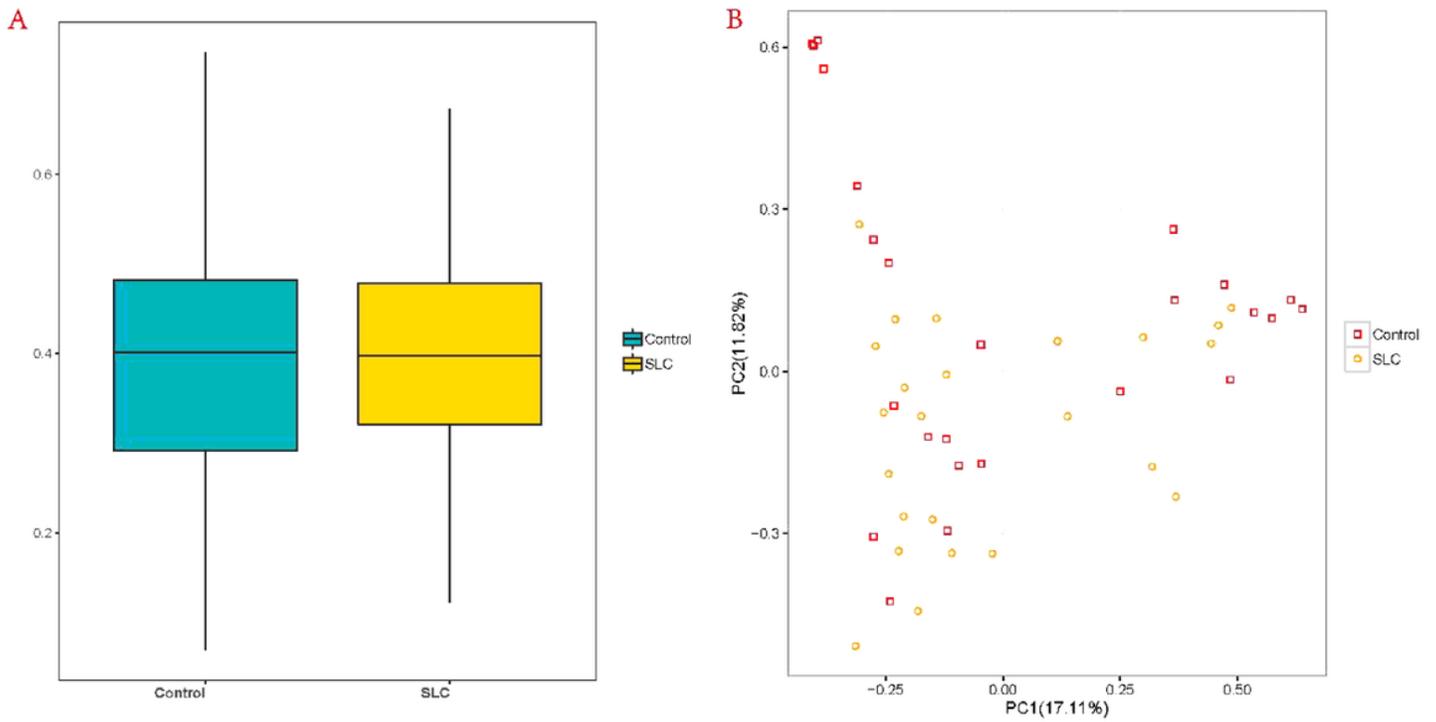
**Figure 2**

Representation of the OTUs detected per group of samples. Venn diagram depicts the number of OTUs unique for healthy individuals (blue circle) and patients with *S. japonicum* infection-induced liver cirrhosis (SLC; purple circle), as well as the ones shared by the two groups (circles' intersection).



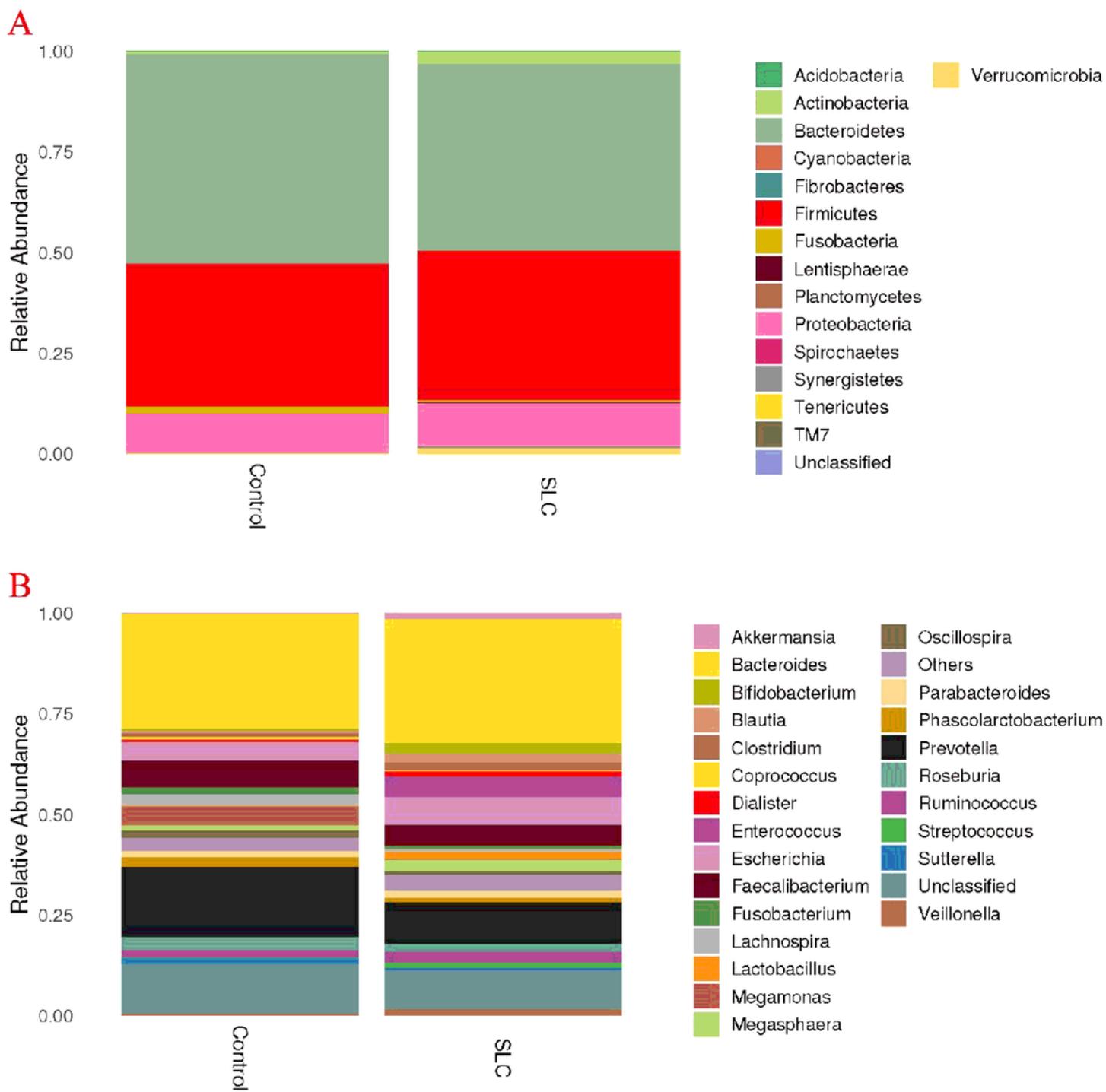
**Figure 3**

Alpha-diversity estimations are similar for control and cirrhosis groups. Intra-sample bacterial species diversity and richness were evaluated through the calculation of 6 different metrics. Results are presented as box and whiskers' plots per group (control and *S. japonicum* infection-induced liver cirrhosis – SLC - plots in red and blue, respectively) and per metric: (A) Observed species; (B) Chao index; (C) ACE index; (D) Shannon index; (E) Simpson index; and (F) Good-coverage index. No significant differences were detected between groups.



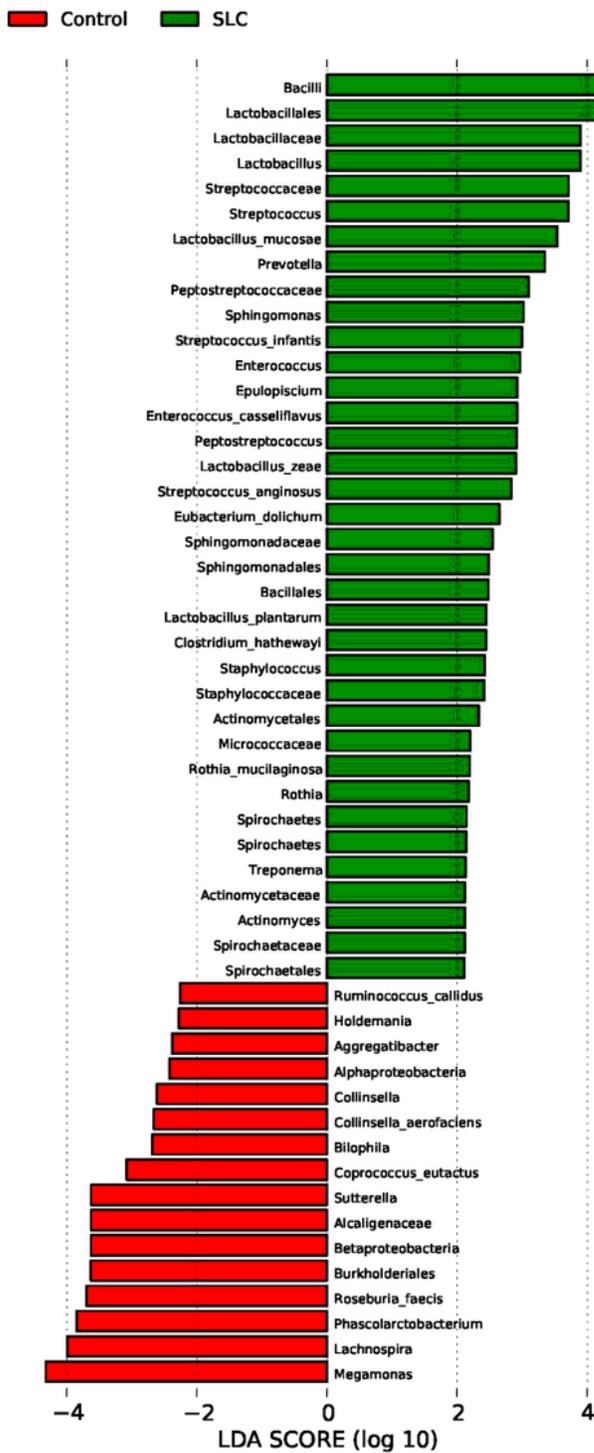
**Figure 4**

Gut microbiota diversity is not affected in patients with *S. japonicum* infection-induced liver cirrhosis (SLC). Beta diversity was calculated based on weighted UniFrac distances and is represented per group as box and whiskers' plots (A). A principal component analysis (PCA) was also performed and is represented (per sample, and group) (B). Yellow plot/symbols and blue or red plot/symbols refer to SLC and healthy control samples, respectively. No statistical differences were detected between the two groups.



**Figure 5**

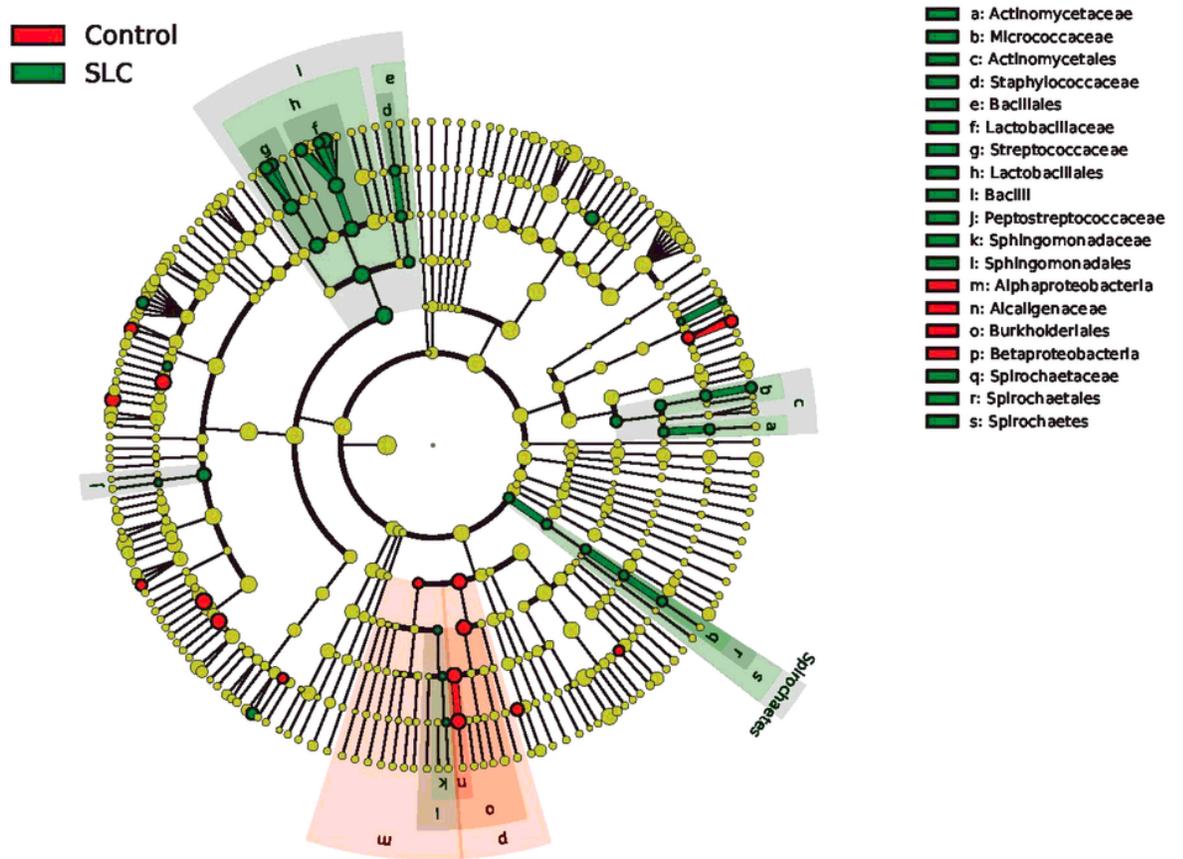
Gut bacterial composition is affected in *S. japonicum* infection-induced liver cirrhosis (SLC) patients. The relative abundance of bacterial phyla (A) and genera (B) detected in fecal samples was determined and is represented per group of samples. At the genus level, relative abundances of less than 0.5% were grouped and are represented as “others.” Phyla and genera are color-coded.



**Figure 6**

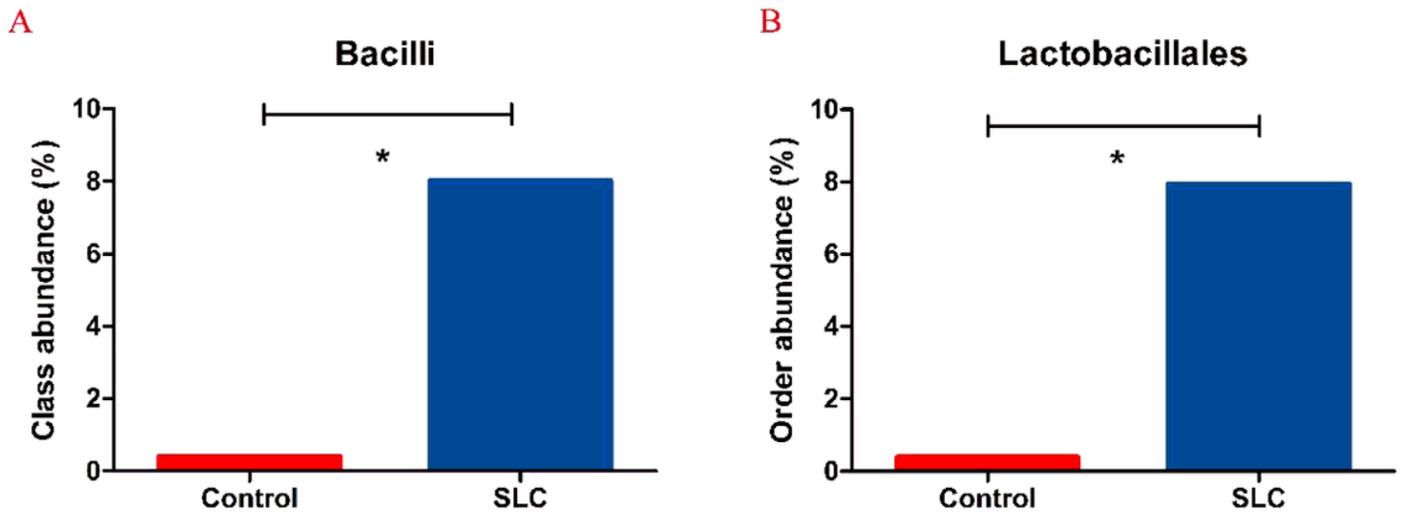
Taxonomic differences in gut microbiotas of *S. japonicum* infection-induced liver cirrhosis (SLC) patients. LDA with the calculation of LDA effect size (LEfSe) was performed to identify the differentially abundant taxa in the two groups of samples: represented in red and green for healthy controls and SLC patients, respectively. LDA scores >2 were considered statistically significant.

# Cladogram



**Figure 7**

Gut microbiota community differences between healthy individuals and *S. japonicum* infection-induced liver cirrhosis (SLC) patients. Linear discriminant analysis (LDA) was translated into a cladogram that is represented. The red and green nodes represent specific microorganisms relevant in healthy controls and cirrhosis patients, respectively. The diameter of each node is proportional to the taxon's abundance. Significant bacterial taxonomic groups are identified: genus – g; family – f; species – s; order – o).



**Figure 8**

Bacilli Class and Lactobacillales Order are increased in *S. japonicum* infection-induced liver cirrhosis (SLC) patients. The relative abundance of relevant taxa in the gut of SLC patients at the Class (A) and Order (B) levels was calculated and is represented per group: red and blue columns refer to healthy individuals and SLC patients. Statistical significance was calculated and is represented: \* FDR < 0.05