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Bacterial communities from *Trichuris spp.* A contribution to deciphering the role of parasitic nematodes as vector of pathogens

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ABSTRACT

Microbiome taxa associated with parasitic nematodes is unknown. These invertebrate parasites could act not only as reservoirs and vectors for horizontally transferred virulence factors, but could also provide a potential pool of future emerging pathogens. *Trichuris trichiura* and *Trichuris suis* are geohelminths parasitizing the caecum of primates, including humans, and pigs, respectively. The present work is a preliminary study to evaluate the bacterial communities associated with *T. trichiura* and *T. suis*, using High Throughput Sequencing and checking the possible presence of pathogens in these nematodes, to determine whether parasitic helminths act as vectors for bacterial pathogens in human and animal hosts. Five *T. trichiura* adult specimens were obtained from the caecum of macaque (*Macaca sylvanus)* and two *T. suis* adults were collected from the caecum of swine (*Sus scrofa domestica*). The 16S rRNA gene HTS approach was employed to investigate the composition and diversity of bacterial communities in *Trichuris* spp., with special emphasis at its intestinal level.

All samples showed a rich colonization by bacteria, included, preferently, in the phyla *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Cyanobacteria* and *Verrucomicrobia*. A total of 36 phyla and more than 200 families were identified in the samples. Potential pathogen bacteria were detected in these helminths related to the genera *Bartonella, Mycobacterium, Rickettsia, Salmonella, Escherichia/Shigella, Aeromonas* and *Clostridium.* The presence of pathogenic bacteria in *Trichuris* spp. would position these species as a new threat to humans since these nematodes could spread new diseases. This study will also contribute to the understanding of the host–microbiota relation.

1. Introduction

The soil-transmitted nematode parasites, or geohelminths, are well-known because they have a direct life cycle, which involves no intermediate hosts or vectors, and are transmitted by fecal contamination of soil, foodstuffs and water supplies. They all inhabit the intestine in their adult stages but most species also have tissue-migratory juvenile stages, so the disease manifestations they cause can be both, local and systemic. The geohelminths present an enormous infection burden on humanity (Holland and Kennedy, 2002). Soil-Transmitted Helminths (STH) refer to the intestinal worms infecting humans that are transmitted through contaminated soil: *Ascaris lumbricoides* Linnaeus, 1758 (sometimes called just "*Ascaris*"), whipworm (*Trichuris trichiura* Linnaeus, 1771), and hookworm (*Anclostoma duodenale* Dubini, 1843 and *Necator americanus* Stiles, 1903). A large part of the world's population (approximately 604–795 million) is infected with whipworm (CDC, 2020).

Geohelminths are considered to have important effects on immunity to mucosal vaccines, infectious disease susceptibility, and anti-inflammatory effects in inflammatory bowel disease and asthma (Cooper, 2009). Pathogens of invertebrate and unicellular organisms represent an extensive reservoir of bacterial strains equipped with virulence factors that evolved to overcome the innate immune responses of their hosts (Waterfield et al., 2004).

Trichuris trichiura and *T. suis* Schrank, 1788 are geohelminths (nematodes) parasitizing the caecum of humans and pigs, respectively. PCR molecular techniques demonstrated that *T. trichiura* and *T. suis* from primates and swine and wild boar, respectively, are two well-defined genetically different species and can be identified by their ITS1 + ITS2 sequences (Cutillas et al., 2009).

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Recent studies of the intestinal microbiota in mice infected with *Trichuris muris* Schrank, 1788, in pigs infected with *T. suis*, and in rhesus macaques infected with *T. trichiura* have provided evidence that the presence of *Trichuris* parasites is associated with an altered microbiota (Broadhurst et al., 2012; Hayes et al., 2010; Li et al., 2011, 2012; White et al., 2018; Wu et al., 2012). This alteration has been reported even in some human studies, although not in all cases (Cooper et al., 2013; Lee et al., 2014). In addition, analyses of porcine microbiome alterations associated with *T. suis* showed that this nematode may have the potential to positively affect gut health and synergistically influence host immune responses (Myhill et al., 2018; Stolzenbach et al., 2020). Walk et al. (2010) also demonstrated that murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus* Dujardin, 1845 was also affected, although the clinical consequences of these changes are yet to be explored. This study is one of the few helminth microbiomes currently available.

Nevertheless, far less is known about the microbiome taxa associated with free-living or parasite invertebrate hosts and the role of their own intestinal microbiota. Waterfield et al. (2004) believe that invertebrate pathogens act not only as reservoirs and vectors for horizontally transferred virulence factors, but could also provide a potential pool of future emerging pathogens. Emerging evidence from invertebrate taxa has underlined the evolutionary and ecological significance of microbiome assemblages. Microbial symbionts are known from a few nematode species, such as the crucial symbiotic role of *Wolbachia* in filarial nematodes (Taylor et al., 2005), although the majority of reported associations represent isolated or anecdotal evidence (Bhadury et al., 2011; Moens et al., 2013).

Nematode species have different bacterial phylotypes, which are attached to the outer cuticle and utilized as the primary food source (Bayer et al., 2009). Recently, Dirksen et al. (2016) used High Throughput Sequencing (HTS) to characterize the native microbiome of *Caenorhabditis elegans* Maupas, 1900 isolated from soils across Europe, demonstrating that the native microbiome is highly diverse and distinct from other nematode species in the same genus. Further, different studies confirm the presence of distinct host-associated microbial assemblages in metazoan species such as nematodes and suggest species-specific microbiome patterns that may be influenced by both, evolutionary and ecological factors.

Parasitic helminths are more intimately associated with bacterial pathogens than the non-parasitic helminths. For example, they are often found in a host alongside a concomitant pathogen infection (Kau et al., 2011; Li et al., 2012). Additionally, many parasitic helminth species have a free-living stage in the environment and are in direct contact with bacterial pathogens that are excreted from infected hosts. Direct life cycle parasitic helminths are excreted with the host feces as eggs and, after a short period of approximately a few days, develop into larval helminths ready to infect a susceptible host. During this development period, the helminths are associated with bacteria in the environment. Given the long evolutionary history and sympatric distribution of gastro-intestinal bacterial pathogens and parasitic helminths both inside the host and in the environment, it would be surprising if helminths were not associated with pathogens (Waterfield et al., 2004).

To determine whether parasitic helminths act as vectors for bacterial pathogens in human and animal hosts, the first step is to investigate whether parasitic helminths carry viable pathogenic bacteria. Thus, Lacharme-Lora et al. (2009) isolated bacteria from parasitic nematodes and they concluded that bacteria of livestock can be isolated in parasitic helminths and that this suggests a mechanism by which bacteria, pathogenic or otherwise, can be transmitted between individuals. The potential for helminths to play a role as pathogen vectors poses a potential livestock and human health risk. They postulated that further work is required to assess the epidemiological impact of this finding.

The present work is an attempt to evaluate the microbiota associated with *T. trichiura* and *T. suis*, using High Throughput Sequencing and checking the possible presence of pathogens in these nematodes.

2. Material and methods

2.1. Sample collection

The samples analyzed in this work are shown in Table 1. Five *T. trichiura* adult specimens were obtained from the caecum of macaque (*Macaca sylvanus)* kept in captivity at Castellar Zoo (Cádiz, Spain). In addition, two *T. suis* adults were collected from the caecum of swine (*S. s. domestica*) slaughtered at abattoirs in different locations in Andalusia (Spain), specifically from Seville and Huelva provinces. The adults were thoroughly washed with saline solution of 0.9% sodium chloride, and frozen at −20 °C. Subsequently, whole body, intestine and intestine mixed with genitalia from five *T. trichiura* and two *T. suis* were extracted to analyze them independently (Table 1).

2.2. DNA extraction, high-throughput sequencing (HTS) and data processing of bacterial communities

Metagenomic DNA was extracted from nematode samples (T1-T7, Table 1) using E.Z.N.A. ® Stool DNA Kit D4015–01 (Omega Bio-tek, Norcross, Georgia, USA), following the manufacturer's instructions.

Bacterial communities were characterized by Illumina Sequencing for the 16S rRNA V3-V4 gene region. DNA was amplified for this hypervariable region with specific primers and further reamplified in a limited-cycle PCR reaction to add sequencing adaptor and dual indexes. First, PCR reactions were performed for each sample using 2X KAPA HiFi HotStart Ready Mix. 5 μL of template DNA and 5 μL of each PCR primer (1 mM) were added to the master mix to reach a total volume of 25 μl. The forward primer used was Bakt_341F 5′-CCTACGGGNGGCWGCAG-3′ and the reverse primer was Bakt_805R 5′-GACTACHVGGGTATCTAATCC-3′ (Herlemann et al., 2011; Klindworth et al., 2013). The PCR conditions involved 3 min of denaturation at 95 °C, followed by 35 cycles of 95 °C for 1 min, 55 °C for 30 s and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Negative controls (without DNA), which were run through the extraction kit, were also included for all amplification reactions. These PCR products were stored at 4 °C. Further, electrophoresis of the PCR products was undertaken on a 2% (w/v) agarose gel using Green Safe DNA Gel Stain (Canvax, Córdoba, Spain) and the 490 bp V3-V4 amplified fragments were purified using Mag-Bind ® TotalPure NGS (Omega Bio-tek, Norcross, Georgia, USA) according to the manufacturer instructions. The amplicons were quantified by fluorimetry with a Quantus Fluorometer ONE dsDNA quantitation kit (Invitrogen, Life Technologies) and dual indices were attached to both ends of the PCR products using Nextera XT Index Kit v2 (Illumina, San Diego, CA, USA). Equimolar concentrations of DNA per sample were pooled and on a MiSeq® sequencer (Illumina, San Diego, CA, USA). They were multiplexed automatically by the MiSeq® sequencer using the CASAVA package (Illumina, San Diego, CA, USA)

Table 1
Samples analyzed in this study.

Sample code	Host	Species and Gender	Sample description
T1	*Macaca sylvanus*	*Trichuris trichiura* ♀	Adult specimen
T2	*Macaca sylvanus*	*Trichuris trichiura* ♀	Intestine
T3	*Macaca sylvanus*	*Trichuris trichiura* ♀	Intestine
T4	*Macaca sylvanus*	*Trichuris trichiura* ♂	Intestine
T5	*Macaca sylvanus*	*Trichuris trichiura* ♂	Intestine
T6	*Sus scrofa domestica*	*Trichuris suis* ♂	Intestine mixed with genitalia
T7	*Sus scrofa domestica*	*Trichuris suis* ♀	Intestine

and quality-filtered with PRINSEQ software (Schmieder and Edwards, 2011) using the following parameters: (1) bases with average quality lower than Q25 in a window of 5 bases were trimmed, and (2) reads with less than 220 bases were discarded. The forward and reverse reads were then merged by overlapping paired-end reads using the AdapterRemoval v2.1.5 (Schubert et al., 2016) software with default parameters. The QIIME package v1.8.0 (Caporaso et al., 2010) was used for Operational Taxonomic Units (OTU) generation, taxonomic identification, sample diversity, and richness indexes' calculation. Sample IDs were assigned to the merged reads and converted to fasta format (split_libraries_fastq.py, QIIME). Chimeric merged reads were detected and removed using UCHIME (Edgar et al., 2011) against the Greengenes v13.8 database (DeSantis et al., 2006). OTUs were selected at 97% similarity threshold using the open reference strategy. First, merged reads were prefiltered by removing sequences with a similarity lower than 60% against the Greengenes v13.8 database. The remaining merged reads were then clustered at 97% similarity against the same database. Merged reads that did not cluster in the previous step were again clustered into OTU at 97% similarity. OTUs with less than two reads were removed from the OTU table. A representative sequence of each OTU was then selected for taxonomy assignment (pick_rep_set.py, assign_taxonomy.py; QIIME).

Downstream statistical data analyses were performed using the online software Calypso (Zakrzewski et al., 2017).

206,130 sequencing reads were obtained in total, which were distributed as follows: 36,863 for T1, 41,108 for T2, 26,022 for T3, 35,883 for T4, 28,107 for T5, 17,605 for T6 and 20,542 for T7. Sequence data were uploaded to the NCBI Sequence Read Archive (SRA) under Bioprojects **PRJNA777035** and **PRJNA776568** (**SRR16691106; SRR16691108** - **SRR16691113**).

3. Results

The sequencing of the V3-V4 region of the 16S rRNA gene was carried out to investigate the composition and diversity of bacterial communities in *Trichuris* spp., with special emphasis at its intestinal level. All nematode samples showed a rich colonization by bacterial communities, dominated by the phyla *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Cyanobacteria* and *Verrucomicrobia*. Taxa related to a total of 36 phyla and more than 200 families were identified in the samples. The distribution of the representatives of different families in the nematode samples is shown in Fig. 1 and the 50 most abundant taxa are summarized in Fig. 2.

A total of 192 taxa related to different bacterial families were identified on adult *T. trichiura* (sample T1), which showed the highest diversity of bacteria. The most abundant were *Pseudomonadaceae* (1.81%), *Sphingomonadaceae* (1.69%), *Enterobacteriaceae* (1.20%), *Rhodobacteraceae* (0.85%) and *Bacillaceae* (0.81%).

Regarding T2 sample, 131 taxa related to different bacterial families were identified. The most representative prokaryotic communities belong to the *Methylobacteriaceae* (3.93%), *Pseudomonadaceae* (1.26%), *Bacillaceae* (0.61%), *Bradyrhizobiaceae* (0.60%) and *Sphingomonadaceae* (0.51%) families. T3 showed a similar pattern, only with slight differences in the percentages obtained for the most commonly detected families: *Methylobacteriaceae* (5.75%), *Pseudomonadaceae* (1.83%), *Sphingomonadaceae* (0.87%), *Bradyrhizobiaceae* (1.20%) and *Bacillaceae* (0.99%)

On the other hand, sample T4 reached 141 families detected, with *Methylobacteriaceae* (5.68%) *Pseudomonadaceae* (4.34%), *Sphingomonadaceae* (1.80%), *Bradyrhizobiaceae* (1.59%) and *Enterobacteriaceae* (1.58%) as the most representatives.

Sample T5 showed a similar distribution to T4, but with *Bacillaceae* being more abundant than *Sphingomonadaceae: Methylobacteriaceae*

Fig. 1. Distribution of bacterial families retrieved from *Trichuris* sp. samples: T1, T2, T3, T4, T5, T6 and T7.

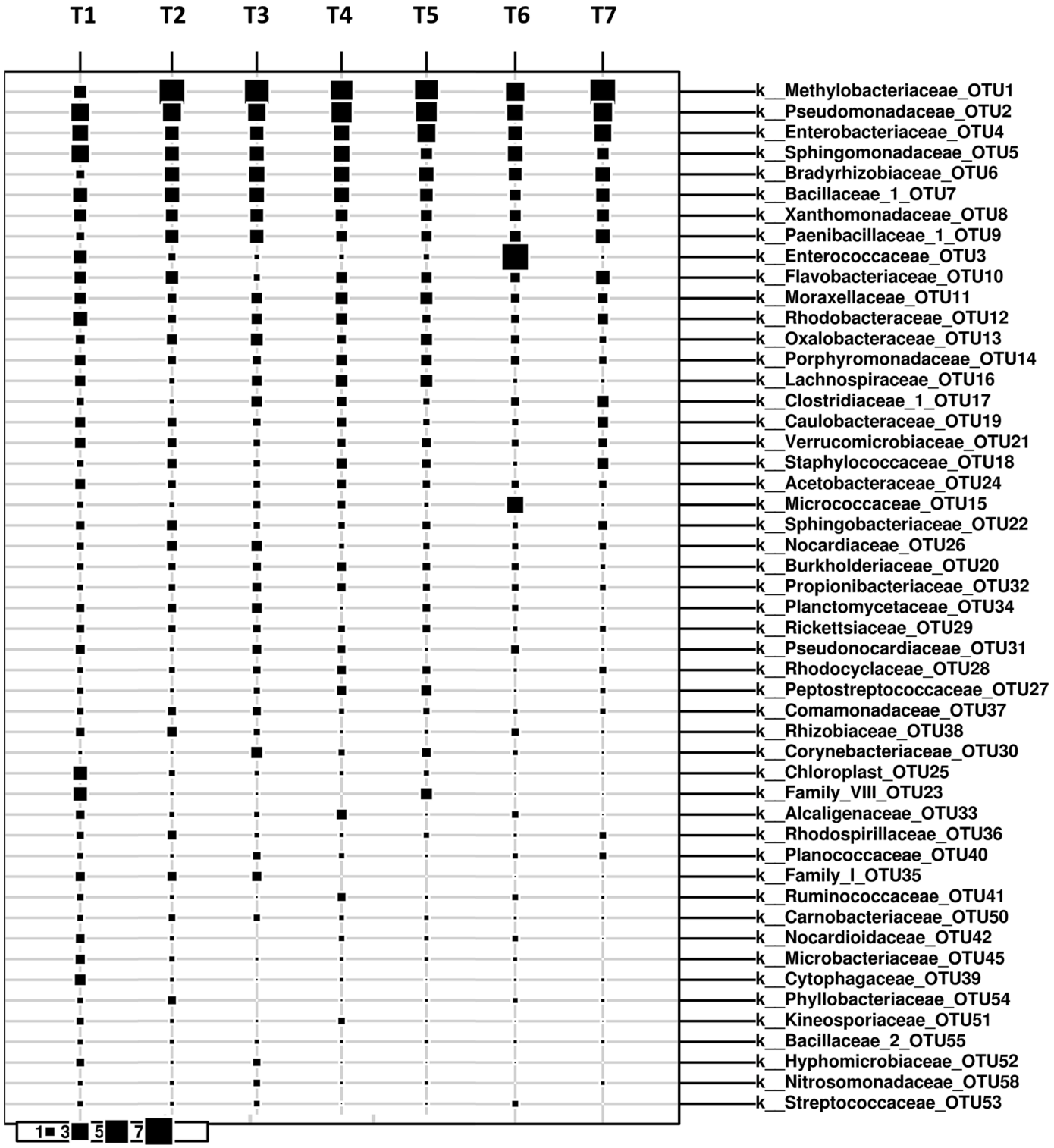

Fig. 2. Bubble plot for quantitative representation of the 50 most abundant taxa in the samples T1, T2, T3, T4, T5, T6 and T7. Each row represents an OTU, and each column, a sample. Squares indicate the total-sum scaling (TSS) - normalized relative abundances. OTU, operational taxonomic unit.

(5.45%), *Pseudomonadaceae* (3.88%), *Enterobacteriaceae* (1.31%), *Bradyrhizobiaceae* (1.07%) and *Bacillaceae* (0.69%).

In T6, 104 families were identified, being the sample with the lowest diversity of bacteria. The families with more representation were *Enterococcaceae* (16.35%), *Methylobacteriaceae* (5.0%), *Pseudomonadaceae* (2.99%), *Sphingomonadaceae* (2.26%) and *Enterobacteriaceae* (1.82%). In particular, the high presence of *Enterococcaceae* stands out in comparison to the rest of the samples.

Finally, sample T7 depicted 111 families, with *Methylobacteriaceae* (11.54%), *Pseudomonadaceae* (4.20%), *Enterobacteriaceae* (2.98%), *Bradyrhizobiaceae* (1.82%) and *Paenibacillaceae* (1.59%), *Flavobacteriaceae* (1.41%) and *Bacillaceae* (1.29%) as the most representative.

The predominant families of bacteria present on the nematode samples were also classified taking into account their host and the type of sample (Fig. 3). The dendrogram illustrates the arrangement of the clusters produced by the analyses of the sequences. Some samples

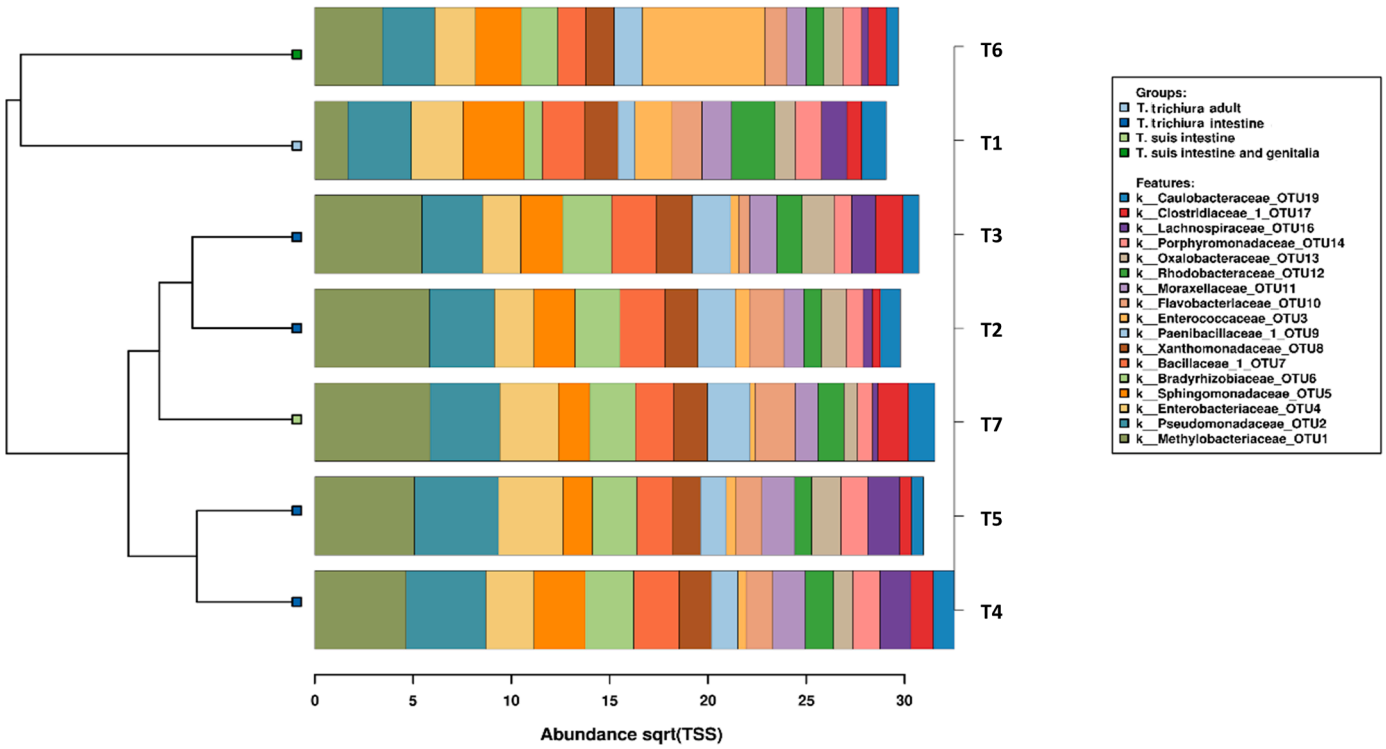

Fig. 3. Clustered-Barchart showing the predominant bacterial families in terms of relative abundance present on the nematode samples T1, T2, T3, T4, T5, T6 and T7. The groups are classified taking into account their host and the type of sample. TSS: Total-sum scaling. OTU: operational taxonomic unit.

showed a similar pattern, only varying in the relative abundance of the bacteria detected (Fig. 3). Taking this aspect into account, the nematode samples appeared distributed in 4 clusters: the first was composed by T1 (*T. trichiura* adult) and T6 (*T. suis* intestine and genitalia), the second, grouped female *T. trichiura* gut samples (T2 and T3), the third was integrated only by T7 (*T. suis* intestine), and the last one was the cluster of male *T. trichiura* gut samples (T4 and T5).

Additionally, a detailed analysis was performed at the genus level in order to characterize the relative abundance of bacteria population present on the samples. These results are summarized in Fig. 4. A total of 812 bacteria genera were identified in the *Trichuris* spp. samples. Taking into account all samples, the 10 most abundant genera were *Methylobacterium, Pseudomonas, Enterococcus, Bacillus, Bradyrhizobium, Sphingobium, Pantoea, Paenibacillus, Chryseobacterium* and *Enterobacter* (Fig. 4).

In sample T1, more than 83% of the sequences remained unidentified at the genus level. Besides this, it was possible to identify bacteria from a wide variety of genera. The most abundant genus from sample T1 was *Pseudomonas* (10.59% of total genera in this sample), followed by *Bacillus* (4.72%), *Sphingobium* (4.64%), *Sphingomonas* (4.01%) and *Enterococcus* (3.64%).

On the other hand, bacteria from the genus *Methylobacterium* were the most predominant microorganism identified in samples T2 (3.93%), T3 (5.75%), T4 (5.67%), T5 (5.43%) and T7 (11.54%), followed by the genus *Pseudomonas* (1.25%, 1.81%, 4.32%, 3.85% and 4.17% of relative abundance, respectively). *Bacillus, Bradyrhizobium* and *Paenibacillus* were other predominant genera detected in these samples. A high percentage of the prokaryotic population remained unidentified at the genus level: 88.96% in T2, 81.3% in T3, 74.46% in T4, 79.60% in T5 and 66.49% in T7.

Sample T6 was dominated by *Enterococcus,* reaching almost a 40% of the identified genera. The other genera were less representated, with *Methylobacterium, Kocuria* and *Pseudomonas* as the most representative. In the case of sample T6, more than 59% of the bacterial population did not match with any known genera.

Furthermore, the Shannon diversity index was used to evaluate the spread of bacteria distributed between the different clusters (Fig. 5A). In this case, the Shannon index showed homogeneity between the 4 groups, with values that ranged between 3 and 4.5, indicating that there were no significant differences in terms of diversity of bacterial communities, despite the presence of an important variety of species in the studied samples.

Furthermore, through analysis of variance (ANOVA), it was possible to analyze the 4 clusters, with no statistical significance observed. However, we detected some species that presented significant differences between them (Fig. 5B). Most of these species were isolated from related environments, such as activated sludge and swine/human feces, and soil, as recorded in their National Center for Biotechnology Information (NCBI) files. One remarkable species is *Acinetobacter calcoaceticus*, an opportunistic pathogen that can infect patients with underlying diseases, with a high mortality rate (Glew et al., 1977). Among these bacteria, some species were recently described, in particular *Muribaculum intestinale* isolated from the intestinal content of a mouse (Lagkouvardos et al., 2016) and *Blastocatella fastidiosa,* the first described species of Acidobacteria subdivision 4 (Foesel et al., 2013).

Finally, potential pathogen bacteria were detected in some samples related to the genera *Bartonella, Mycobacterium* and *Rickettsia*, with a low hit percentage in general. *Bartonella* was detected in the T1 sample (6 hits), *Rickettsia* in T3, T6 and T7, and *Mycobacterium* was the most widely distributed, being present in all the samples except for T4 and T7 samples. *Salmonella* was detected in all samples except for T7; *Escherichia/Shigella* was present in all samples, with an important representation in T5 (Fig. 4); *Aeromonas* was detected in T1 and T5 with only one hit, and *Clostridium* was present in all samples, with the highest representation in T4 and T5. Two species of this genera known for the production of clostridial toxins were also related to *C. septicum* in T3 and T7 and *C. sordelli* in T5. *C. septicum* is responsible of intestinal myonecrosis in adults (Forrester et al., 2016; Gegúndez Gómez et al., 2007) and *C. sordelli* is an important pathogen of humans, causing a range of diseases, including myonecrosis, sepsis and shock, with high mortality rates

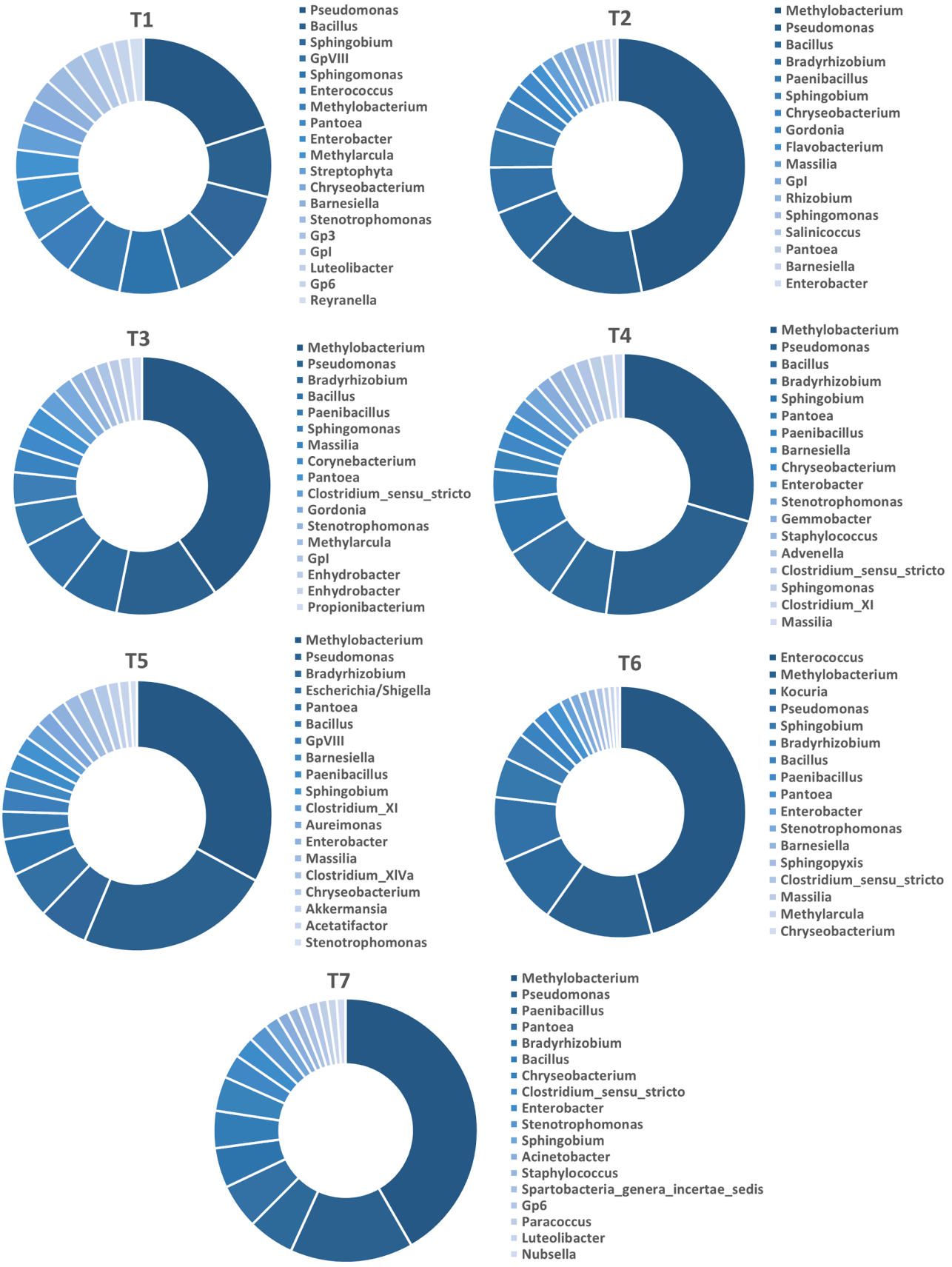

Fig. 4. Relative abundance of bacteria population present on T1, T2, T3, T4, T5, T6 and T7 *Trichuris* sp. samples identified at genus level.

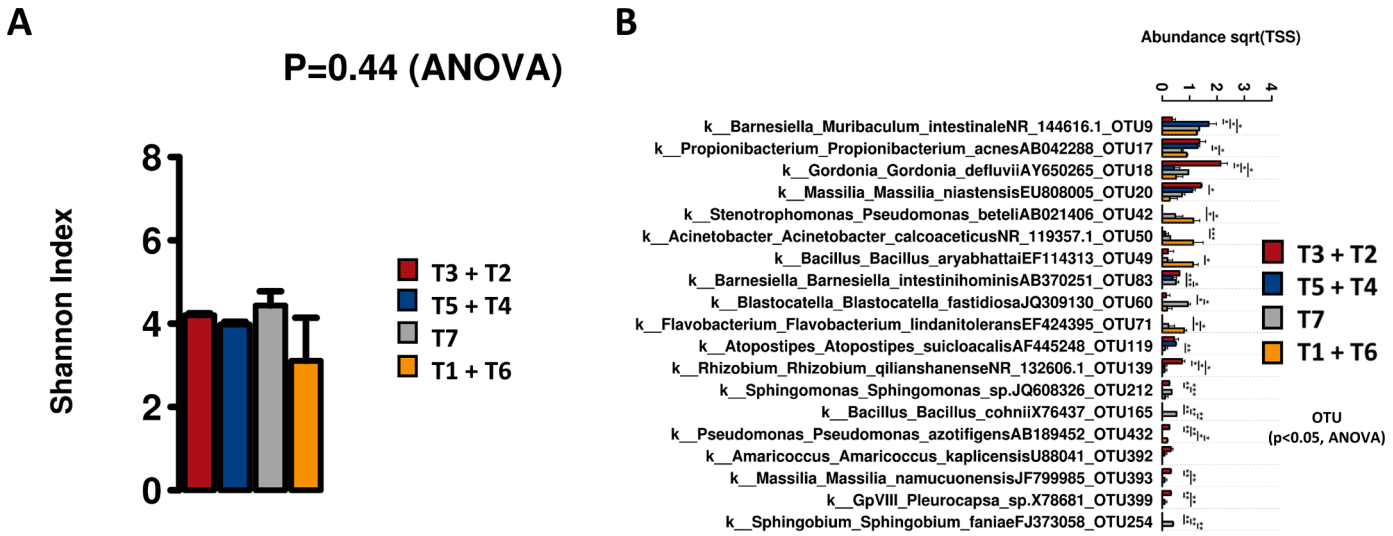

Fig. 5. A. Shannon index. There are no significant changes in diversity between the clusters. B. Bacterial species with significant differences between the clusters, obtained though ANOVA. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

(Carter et al., 2011).

4. Discussion

The *Trichuris* spp. samples analyzed in this study showed a rich intestinal microbiota. The phyla *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Cyanobacteria* and *Verrucomicrobia* are the most abundant in the samples.

A drawback when comparing this kind of data is that there are a limited number of published helminth microbiomes as of today. White et al. (2018), discovered that *T. muris* in mice acquires its own distinct microbiota from the host. These authors also identified the microbiota from *T. muris* of a murine host, which was dominated by the *Bacteroidetes* and *Firmicutes* phyla, with a notable rise in the proportion of the *Proteobacteria* phylum from respect their host. Accordingly, these three phyla are also in great presence in the nematode samples analyzed in the present work.

On the other hand, the dynamics of the gut microbiota in response to parasitic infections have only been examined recently. The gut microbiota of swine has been studied in greater depth due to the possibility of comparison with the human gut microbiota (Li et al., 2012). Wu et al. (2012), analyzed the porcine colon microbiota affected by *Trichuris suis* infection. Between the most abundant bacteria in the proximal colon microbiota of the parasite naive pigs. The most abundant bacteria in the proximal colon microbiota of the parasite naive pigs were considerably different from the taxa detected in our nematode samples, highlighting the distinction between the microbiota of the host and that of the parasite. For instance, *Prevotella, Bacteroides, Ruminococcus, Faecalibacterium* and *Fibrobacter* are important genera in the pig microbiota, while none of them were detected in our nematode samples (Fig. 4).

In addition, *Prevotella* exhibited also abundance in the surveyed macaque anal community by Chen et al. (2018), dominated by *Bacteroidetes, Firmicutes* and *Proteobacteria*.

It is important to note that there was not a clear dominance of specific bacteria in the analyzed samples. The family *Pseudomonadaceae* was detected with an important presence in all the samples. *Methylobacteriaceae* was highly common in T2, T3, T4, T5 and T7 (Intestine), but it is less represented in T1 and T6 (adult and intestine with genitalia, respectively). Among the most abundant families in the samples, the presence of fecal bacteria such as *Pseudomonas* and *Enterobacteria* is very persistent, which correlates with this kind of samples, considering the location of these nematodes in the host.

It is remarkable that some samples showed a similar pattern, only varying in the relative abundance of the bacteria detected (Fig. 3). The *T. trichiura* female gut samples (T2 and T3) have a very similar profile despite belonging to different individuals. This scenario was the same for the *T. trichiura* male gut samples (T4 and T5). These data suggest that the microbiota between individuals of the same species and sex is relatively homogeneous.

In contrast, T1 and T6 were the samples with the most different profiles (Fig. 3): compared to the other samples, both presented a greater proportion of enterobacteria, in addition to a lower presence of *Methylobacteriaceae*. These samples not only include the intestines, but also include other parts of the nematode's body (T1 sample: the whole body in *T. trichiura* and T6 sample: genitalia in *T. suis*). This fact could explain the differences observed in the abundance of the bacterial communities. Some species of the family *Methylobacteriaceae* are opportunistic human pathogens and others have been found in insect tissues. Specifically, the genera *Methylobacterium* is considered ubiquitous and have been isolated from a wide range of natural environments, earthworms, the human body and as opportunistic pathogen (Kelly et al., 2014).

Enterobacteriaceae has been detected previously in nematodes (Hu et al., 1999; Sajnaga and Kazimierczak, 2020). In fact, some studies have investigated a novel mechanism by which gastrointestinal nematodes are potentially spared from the effects of benzimidazoles thanks to the protection provided by intestinal *Enterobacteriaceae* (Whittaker et al., 2016).

Enterococci are commensal bacteria found in the intestines of humans and animals, and are capable of causing infections in humans (Hammerum, 2012). This kind of bacteria have been isolated from oral, rectal and fecal samples of pigs (Tan et al., 2018) and primates (Grassotti et al., 2018), even showing multidrug-resistant properties (Tan et al., 2018). Due to the fact that *Trichuris* spp. samples were isolated from the intestines of these animals, these bacteria could be also present in the *Trichuris* spp. guts. It is remarkable the representation of Enterococci in sample T6, with almost 40% of the identified genera. This sample includes *Trichuris* genitalia in addition to intestine. *Enterococcus* is one of the most common bacteria able to infect genitals and the urinary tract, provoking balanitis and posthitis in humans (Morris and Krieger, 2017). Streptoccocal and Enterococcal infections are common in pigs as these organisms are commensal in their gastrointestinal and reproductive tracts and environments. A multitude of diseases affecting many systems can be the result of infection by these organisms (Cheon and Chae, 1996; Torremorell et al., 1998).

Considering the high rate of sequences that could not be assigned to any known taxa (unclassified bacteria), the nematodes could also

represent an important isolation source of new species of bacteria, reinforced by the identification of recently described bacteria (Foesel et al., 2013; Lagkouvardos et al., 2016).

The detection of opportunistic and potential pathogenic bacteria, such as *Bartonella, Rickettsia, Mycobacterium, Salmonella, Escherichia/Shigella, Aeromonas* and *Clostridium,* associated with *Trichuris* spp. would suggest a potential capability as vector of diseases for its host, in addition to the development of trichuriasis. This factor is in accordance with Waterfield et al. (2004), who reported that invertebrates such as nematodes represent a potential pool of emerging pathogens. Lacharme--Lora et al. (2009) demonstrated that, regardless of whether the bacteria associated with the parasitic nematodes are pathogens or commensals, the bacteria can survive inside these helminths, protected from the environment and possibly providing additional methods of pathogen transmission between hosts. In fact, there is previous evidence of parasitic helminths acting as vectors in plants, but parasitic helminths of vertebrates represent an understudied field relying on theoretical frameworks (Perkins and Fenton, 2006).

On the other hand, Cooper et al. (2013) stated that human infections with *T. trichiura* may have no effect on fecal microbiota. However, it would be necessary to evaluate the potential influence of these pathogenic bacteria inside *Trichuris* sp. intestine on the human microbiota. In fact, Trichuriasis has been associated with severe colitis caused by *Campylobacter jejuni* in humans (Shin et al., 2004) and pigs (Mansfield et al., 2003). Cooper et al. (2013) also detected a very small number of sequences matching *C. jejuni* in samples from individuals with helminth infection, but they did not detect any other bacterial pathogens such as *Salmonella* spp., *Yersinia enterocolitica*, *Vibrio cholerae, Staphylococcus aureus* and *Aeromonas hydrophila*. In the present study, it has been possible to detect some of these genera inside *T. trichiura* and *T. suis* (Fig. 4). However, it is not possible to discriminate pathogenic species from other closely related but commensal strains through short fragments of the 16S rRNA gene, such as *Shigella* spp.-*Escherichia coli* (Cooper et al., 2013), so it would be necessary to apply complementary techniques to reach this specific goal. Ideally, presence of these putative pathogens in the samples should be verified with full length 16S rRNA gene sequences.

5. Conclusion

Next Generation Sequencing is a promising approach for monitoring the microbiota associated with parasitic nematodes. The metagenomic analysis carried out in this study provided an enhanced resolution up to the genus level and the relative abundance of the different bacterial communities on the different *Trichuris* spp. samples analyzed, showing a rich intestinal microbiota in these nematodes. The potential presence of pathogenic bacteria such as *Bartonella, Mycobacterium, Rickettsia, Salmonella, Escherichia/Shigella, Aeromonas* and *Clostridium* would position *Trichuris* spp. as a new threat to humans in case these nematodes act as a vector of new diseases. This study will also contribute to the understanding of the host–microbiota relation, especially in parasitic nematodes. Taking into account that parasitic helminth infection is quite common and the consequences to host health, further studies are needed to deepen in the knowledge of helminth vectoring.

Ethical statement

Ethical Statement: All procedures performed in studies did not involve human participants, neither animal experimentation and does not require the approval of animal ethics committee.

Credit authorship contribution statement

Cristina Cutillas: Conceptualization, Supervision, Reviewing, Data curation, Writing. Angela Maria García-Sánchez: Methodology, Investigation, Writing, Data analysis, Data curation. Ana Zelia Miller: Supervision, Reviewing. Ana Teresa Caldeira: Supervision, Data analysis, Reviewing.

Declaration of Competing Interest

The authors declare no competing interests.

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