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COMPOSITION OF THE MICROBIAL COMMUNITIES IN THE GASTROINTESTINAL TRACT OF PERCH (*Perca fluviatilis* L. 1758) AND CESTODES PARASITIZING THE PERCH DIGESTIVE TRACT

Running title: microbiota of fish gut and their cestodes

Kashinskaya E.N.[1], Simonov E.P.[1], Izvekova G.I.[2], Parshukov A.N.[3], Andree K.B.[4], and M.M. Solovyev[1]

[1]Institute of Systematics and Ecology of Animals of Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia;

[2]Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Nekouzskiiraion, Yaroslavskaya oblast, Russia;

[3]Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Russia;

[4]IRTA-SCR, San Carlos de la Rapita, Tarragona, Spain;

e-mail: elena.kashinskaya@inbox.ru

Abstract

Using the approach of sequencing the V3-V4 region of the 16S rRNA gene, we have analyzed the bacterial diversity associated with the distinct compartments of the gastrointestinal tract of perch (*Perca fluviatilis*), and cestodes (*Proteocephalus* sp.) parasitizing their digestive tract. The dominant microbiota associated with cestodes (*Proteocephalus* sp.) was represented by bacteria from the genera *Serratia*, *Pseudomonas*, and *Mycoplasma*. By comparing the associated microbiota of perch and cestodes a clear difference in bacterial composition and diversity were revealed between the community from the stomach content and other parts of the gastrointestinal tract of fish. Microbiota associated with cestodes was not significantly different in comparison with microbiota of different subcompartments of perch (mucosa and content of intestine andpyloric caeca) (ADONIS, $p>0.05$) excluding microbiota of stomach content (ADONIS, $p\leq0.05$). PICRUSt-based functional assessments of the microbial communities of perch and cestodes indicated they mainly linked in terms of metabolism and environmental information processing could be play an important role in the nutrition and health of host.

Key words: Perch, tapeworms, *Mycoplasma* sp., *Proteocephalus* sp.

Introduction

The microbial community of the gut is an integral and essential part of the digestive tract of all vertebrates, including fish. The microbial community plays an important role in a plethora of different physiological processes including defense against pathogenic organisms, digestion, regulation of metabolism, etc. (Nayak 2010; Ghanbari *et al.*, 2015). It has been shown that the diversity of the intestinal microbiota of fish is influenced by various internal and external factors such as feeding habits, temperature, salinity, and others (Grisez *et al.*, 1997; Šyvokienė *et al.*, 1999; Austin 2002; Sullam *et al*., 2012; Clements *et al*., 2014; Kashinskaya *et al.*, 2017, 2018). During the last decade the multiple roles of parasitic organisms in terms of physiology, behavior, energy transmission, etc. in aquatic ecosystems has been intensively studied (Cézilly *et al*., 2014; Reynolds *et al*., 2015). The definitive hosts for various taxonomic groups of parasites are known to be fish, and the gut is one of the frequently infected organs due to it providing nutrients and the possibility for parasite transmission. One such group of parasites is the tapeworm (Phylum Platyhelminths, Class Cestoda) characterized by several life cycle stages, which may infect different hosts including fish. In order to inhabit the fish gut, tapeworms possess different features that facilitate their ability to survive under specific conditions of the fish gut. Thus, cestodes are characterized by specific surface epithelium, or, tegument, associated with feeding processes and parasite protection from the host enzymes (inhibition of host proteinase activity) (Izvekova et al., 2017). It was shown that cestodes, as a component of intestinal communities, can also impact digestive processes and be an important factor that effect on the fish gut microbiota (Izvekova 2003; Izvekova and Korneva 2007).

Recent studies have shown that helminth infections can modify host metabolism, the production of antimicrobial peptides in the intestinal tract, disrupt the epithelial barrier and stimulate production of mucus that can lead to substantial shifts in the composition of the intestinal microbiota (Reynolds *et al*., 2015). The host-helminth-microbiota interactions are well-studied in mammalian systems, but most of the data available provides information regarding nematode-host interactions. Thus, experimental infection induced by the nematode *Trichuris muris* in mice lead to a reduction in a large number of metabolic products – vitamin D2/D3 derivatives, many fatty acids and related metabolites, glycophospholipids, dietary plant-derived carbohydrates, and intermediates of amino acid biosynthesis (Houlden *et al*., 2015). In the small intestine of mice infected by the nematode *Heligmosomoides polygyrus* there was observed an increased abundance of Lactobacillaceae and Enterobacteriaceae species (Walk *et al*., 2010). *Trichuris suis* infected pigs had a shift in their metabolism that resulted in reduced cofactors for carbohydrate metabolism and amino acid biosynthesis (Li *et al*., 2012).

Interactions between bacterial diversity found in host and the microbes inhabiting the helminths within host gut has been studied less extensively in fish. Most of the studies so far reported are focused on the morphological and physiological characteristics of bacteria associated with parasites inhabiting the intestine of fish (Hughes-Stamm *et al*., 1999; Izvekova 2003; Poddubnaya and Izvekova 2005; Korneva and Plotnikov 2006, 2012). There are also few studies focused on the determination of the morphological species of parasitic organisms using transmission electron microscopy (Korneva and Plotnikov 2006). Other studies have demonstrated ultrastructural features and methods of attachment of bacteria to the tegument ofcestodes (Poddubnaya 2005; Poddubnaya and Izvekova 2005; Korneva and Plotnikov 2012). Physiological studies have shown that symbiotic microbiota from helminths and bacteria from fish intestine may produce different digestive enzymes, such as proteases, that participate in the digestive processes of both the parasite and their host (Izvekova 2003; Izvekova and Plotnikov 2011). Studies of the diversity of fish intestinal microbiota and the parasites that inhabit their digestive tract are still limited. For example, using the methods of transmission and scanning electron microscopy for examining bacterial diversity, Korneva and Plotnikov (2006) demonstrated that the intestine of *Esox lucius* and the tegument of *Triaenophorus nodulosus* included microbiota not only of well-known forms of bacteria such as various cocci, bacteria from the *Vibrio* group, and rod-like bacteria, but they also documented different morphological forms of very small bacteria (0.25-0.3 nm in diameter) and spirochaetes (Korneva and Plotnikov 2006). In another study focused on determination of the gut bacterial diversity of fish in different host-parasite system such as pike *Esox lucius*, stone loach *Barbatula barbatula*, perch *Perca fluviatilis*, and Eurasian ruffe *Gymnocephalus cernuus* infected by *Triaenophorus nodulosus*, *Proteocephalus torulosus*, *P. percae*, and *P. cernuae* respectively, using culture-dependent methods. The dominant microbiota identified from those fish gut was represented by opportunistic bacteria from the genera *Aeromonas, Vibrio, Pseudomonas, Shewanella, Hafnia*, *Yersinia*, and *Carnobacterium* (Korneva and Plotnikov 2012).

Cestodes from the genus *Proteocephalus* are distributed among a wide range of potential hosts and characterized by wide boundaries of morphological variability (Anikieva *et al*., 1995, 2015). Some species of these parasites infecting the intestine of perch *P. fluviatilis* (Linnaeus, 1758) in shallow freshwater lakes and use this fish as a definitive host. This fish-parasite system is well-defined making it a good model for the study of fish-parasite interactions since the parasite´s large size makes the collection process relatively easy.

Therefore, in order to understand the relationship of host–helminth–microbiota associations, we investigated the composition and structure of microbial communities of the gastrointestinal

tract of perch *P. fluviatilis* (Linnaeus, 1758) and the cestodes (*Proteocephalus* sp.) parasitizing their digestive tract using molecular methods.

Materials and methods

Study area and sampling. Seven individuals of perch *P. fluviatilis* with total length 199.6 ± 13.1 mm and total weight 109.87±25.1 g (Table S1) parasitized by *Proteocephalus* sp. were collected in May of 2014 in the area of Malye Chany Lake in Western Siberia (Russia, $54^0$36'56.3''N, $78^0$12'5.9''E). All fish were captured using gill-nets (mesh sizes 35 and 45 mm) and transported alive to the laboratory in plastic containers (duration approximately 1 h). All fish were sacrificed and samples collected aseptically as previously described (Kashinskaya *et al*., 2015). Parasites were retrieved aseptically from the digestive tract of perch (anterior intestine). Five cestodes from each of the 7 perch were mechanically cleaned from intestinal content and placed into lysis buffer immediately after dissection without any washing in buffer solution.

A total number of 37 samples (stomach mucosa, n=6; stomach content, n=6; pyloric caeca, n=4; anterior intestinal mucosa, n=7; anterior intestinal content, n=7; parasites, n=7) were sampled. Male and female fish were identified according to gonadal development. Some samples from different sub-compartments of digestive tract of fish were excluded from further analyses due to that sequencing failed for them.

Parasitological analysis. During May of 2010, 2012-2014 years 202 individuals of perch of different sizes were collected in order to estimate the prevalence and intensity of *Proteocephalus* sp. infected fish. All fish were dissected and gut was extracted and the number of *Proteocephalus* sp. was registered. This month was chosen since this cestode was only observed occasionally in the perch gut during the rest of the year.

The prevalence and mean intensity of parasite infestation were calculated according to the definitions by Bush *et al*. (1997). The prevalence (P) of parasite infestation was calculated as:

$$P,\%=I*100/N,$$

Where *I* is number of host infected, and *N* is total number of host examined. The error of prevalence index (E) was calculated by the following formula:

$$E=\sqrt{}[P\times(100-P)/N],$$

where *P* is prevalence, and *N* is total number of host examined. Mean intensity (I) of invasion was assessed as the average of number of individuals of a particular parasite species in a single infected host:

$$I=K/n,$$

where *K* is total number of individuals of a particular parasite species, and *n* is total number of hosts infected with that parasite. Error of intensity index (SE) was calculated according to:

$$SE=SD/\sqrt{n},$$

Where *SD* is standard deviation of row of number of individuals of a particular parasite species in a single infected host, and *n* is total number of hosts infected with that parasite. To estimate the differences between parasite abundance across different sampling years Kruskal-Wallis test was applied using PAST v. 3.16 (Hammer *et al*., 2011). In the same program, to explore the correlation between parasite abundance and fish size across different sampling years, a Spearman rank correlation test was used.

Sample preparation and DNA extraction. Before DNA extraction, fresh samples (mucosa and content from different subcompartments of digestive system or cestodes) from each individual fish were collected into sterile microcentrifuge tubes with lysis buffer (300 µl) for DNA isolation and mechanically homogenized by pestle for 1 min using a hand-held homogenizer. Following the kit manufacturer protocols, DNA was extracted from 100 mg of each sample by DNA-sorb B kit (NextBio, Russia).Kit DNA sorb B is designed to extract DNA from a wide variety of clinical materials (phlegm, faeces, blood, saliva and others) and based on the lysing and nuclease-inactivating properties of the chaotropic agent guanidiniumthiocyanate. After homogenizing, the suspension was heated at 65°C for 10 min to improve lysis; 25 µlof silica particles suspension for DNA adsorption were added to each sample. The adsorption step was followed by a wash step according to kit manufacturer protocols; DNA was eluted from silica particles using TE buffer with a final sample elution volume of 50 µl. After extraction, the DNA concentration of all samples was determined spectrophotometrically (NanoVue^{TM} Plus; GE Healthcare Bio-Sciences AB, Sweden), and samples were stored at -20°C for downstream processing. A sample containing only sterile deionized water was extracted and included in PCR as a negative control.

16S rDNA metagenomic sequencing. Sequencing of the V3, V4 hypervariable regions of 16S rRNA genes was carried out on an Illumina MiSeq sequencing platform by a commercial company (Evrogen, Moscow, Russia) using the primer pair 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' (Klindworth *et al*., 2013). The amplification conditions were applied according to the original manufacture protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Forward and reverse read pairs were merged and quality filtered with Mothur 1.31.2 (Schloss *et al*., 2009). Any reads with ambiguous sites and

homopolymers of more than eight bp were removed, as well as sequences shorter that 350 or greater than 500 bp. QIIME 1.9.1 (Caporaso *et al*., 2010) was used for the further processing of the sequences. *De novo* (abundance based) chimera detection using USEARCH 6.1 (Edgar 2010) was applied to identify possible chimeric sequences ('identify_chimeric_seqs.py' with an option '-m usearch61' in QIIME). After chimera filtering, the QIIME script 'pick_open_reference_otus.py' with default options was used to perform open-reference OTU picking by UCLUST (Edgar 2010), taxonomy assignment (UCLUST), sequence alignment (PyNAST 1.2.2; Caporaso *et al*., 2010) and tree-building (FastTree 2.1.3; Price *et al*., 2010). This algorithm involves several steps of both closed-reference and open-reference OTU picking followed by taxonomy assignment, where the Greengenes core reference alignment (release 'gg_13_8'; DeSantis *et al*., 2006) was used as a reference. Chloroplast, mitochondria and non-bacterial sequences were removed from further analysis. Nucleotide sequences were deposited in the Sequence Read Archive (SRA NCBI), accession numbers SRP156215.

***Analysis of alpha and beta diversity*.** The richness (number of OTU's and Chao1 index) and diversity estimates (Shannon and Simpson index) per sample were calculated using the QIIME 1.9.1 (Caporaso et al., 2010). For estimating the differences between the richness and diversity estimates Kruskal-Wallis test with Dunn's multiple comparisons test was applied using PAST, v. 3.16 (Hammer *et al*., 2011). Then, the samples were rarified to the lowest sequencing effort (8455 sequences) using QIIME. A weighted UniFrac (Lozupone and Knight 2005) dissimilarity matrix was calculated in QIIME and Phyloseq 1.24.2 (McMurdie and Holmes 2013), and both matrices were used for downstream analyses as they known to produce slightly different results. The matrix was used to perform principle coordinates analysis (PCoA) to visualize differences among groups of samples. Permutational multivariate analysis of variance using distance matrices was used as implemented in the 'adonis' function of the vegan R package (Oksanen *et al*. 2018). Pairwise comparisons for all pairs of levels of used factors were performed using 'adonis.pair' function of the EcolUtils R package (Salazar, 2018). Analysis of multivariate homogeneity of group dispersions (variances) to test if one or more groups is more variable than the others, was performed using the 'betadisper' function of the vegan R-package. In all the aforementioned tests statistical significance was determined by 10 000 permutations.

Functional analysis. The PICRUSt software package (Langille *et al*., 2013) was used to predict metagenome functional content of microbial communities. We generated the KEGG pathways (Kyoto encyclopedia of genes and genomes) and categorized functions to different gene categories at levels 1, 2, and 3. The categorized functions for different levels (frequency of occurrences of every group of genes in genomes) then were transformed to percentages from total

quantity of genes obtained and the differences between groups of samples were calculated by using ANOSIM, at $p \leq 0.05$. The matrix with percentages (level 1) was used to perform PCoA to understand the differences in metabolic roles for the studied microbiota (stomach mucosa, stomach content, intestinal mucosa, intestinal content, pyloric caeca, andcestodes).It should be noted that the authors take into account the main limitation of this method; that being the current reference tree supported by PICRUSt (Greengenes version13_5) is already five years old. Keeping this limitation in mind, results of the predicted metagenome functional content of the microbial communities using the PICRUST software package cannot directly identify metabolic or other functional capabilities of the microorganisms, and can only be used for discussion of possible metagenome functional content of the analyzed microbial communities.

Results

The prevalence and mean intensity of parasite infestation. Parasitological analysis of 202 individuals of perch observed at different years in May (2010, 2012–2014) in Malye Chany Lake has revealed that the prevalence of *Proteocephalus* sp. infestation ranged from 4.1 to 17.4% (Figure S1). The mean (±SE) of intensity of parasite infection was 1.0±0.0; 1.6±0.31; 1.5±0.5 and 1.6±0.22, correspondingly. According to Kruskal-Wallis test results, an abundance of *Proteocephalus* sp. in perch intestine in May across different years was not significantly different ($p>0.05$). According to a Spearman rank test the correlation was not significant (Spearman's rho: 0.188, $p=0.357$). Thus, we didn't use the fish age correction for our dataset.

The richness and diversity estimates of microbial communities. The estimation of richness of microbial communities (number of OTU's and Chao1 value) from perch gut and their cestodes has shown that the highest richness was observed in the stomach content, while the lowest were detected in intestine and pyloric caeca (Figure 1). The number of observed OTU's in the microbial community of stomach content were significantly higher than in pyloric caeca and intestinal mucosa ($H=17.16$; $p \leq 0.01$). Significant differences were also observed in Shannon index values between stomach content and intestinal mucosa ($H=13.66$; $p \leq 0.02$). Simpson index values were significantly different between stomach content, intestinal mucosa and intestinal content ($H=15.43$; $p \leq 0.02$). No significant differences in Chao1 index values were found between microbiota associated with helminths and microbiota of intestine and stomach.

When sex was considered as a factor that could affect the microbial community of the gastrointestinal tract of perch, the results of Kruskal-Wallis test showed no significant differences in richness (number of OTU's and Chao1 value) and diversity (Shannon and Simpson) estimates between males and females ($p>0.05$).

Microbiota of gastrointestinal tract of perch and their parasites. The microbial community of perch and cestodes parasitizing their digestive tract was dominated by Proteobacteria, Tenericutes, Firmicutes, Fusobacteria, and Bacteroidetes (Figure 2).

On a lower taxonomic level, microbiota of mucosa and content of stomach and pyloric caeca were varied among individual fish, and were presented by bacteria from the genera *Serratia*, *Pseudomonas*, *Candidatus Hepatoplasma*, *Mycoplasma* and unclassified taxa from the families Aeromonadaceae, Enterobacteriaceae and Comamonadaceae. In the intestinal mucosa and content of perch the dominant groups of bacteria were also presented by *Mycoplasma, Serratia,* and *Pseudomonas*. The microbiota associated with helminths was dominated by bacteria from the genera *Serratia, Pseudomonas, Mycoplasma,* and an unclassified genus of Fusobacteriaceae designated *u114* (Figure 3). It should be noted that the considerable variability in the microbiota of intestinal mucosa and content in comparison to the other compartments and the cestodes were observed.

The relative abundances of the main dominant bacteria associated with the perch gut and cestodes are shown in Table 1. Abundance of *Pseudomonas* sp. was significantly higher in the microbial community of pyloric caeca in comparison with microbiota associated with the stomach and intestinal content of perch (Dunn's post hoc at $p \leq 0.05$). *Pseudomonas* in the stomach content community was also significantly higher in comparison with microbiota associated with cestodes. Relative abundance of *Serratia* was statistically different only between microbiota of cestodes and the stomach content of perch using Dunn's post hoc at ($p \leq 0.05$). No significant differences were observed for *Mycoplasma* and unclassified clade *u114* from the family Fusobacteriaceae in the microbial community of fish and cestodes. Unique bacterial taxa associated with the cestode community are shown in Table 2.

The principal coordinates analysis (PCoA) showed in Figure 4. According to the results obtained by the ADONIS test based on matrices calculated in QIIME (Figure 4a) and Phyloseq (Figure 4b and Figure 4c), microbiota of stomach content was significantly different from microbiota associated with other parts of the digestive tract (at $p \leq 0.05$) (one exception was for Phyloseq matrix 1). Based on QIIME matrix, the microbiota associated with cestodes was not significantly different than microbiota of stomach and intestinal contents, and stomach and intestinal mucosa as well (Table 3). In the same time, the microbiota associated with helminths was significantly different than microbiota of intestinal content (matrix 1 and 2) and intestinal mucosa (matrix 2). But due to the low R2 value (0.224) for the last comparison we may conclude that there is no significant effect. The microbiota associated with pyloric caeca was significantly

different than microbiota of intestinal content (matrix 1 and 2) and intestinal mucosa (matrix 1) as well (Table 3).

Predicted functional metagenomes of the microbiota from fish gut and cestodes using PICRUSt. Metagenome data was analyzed using PICRUSt to estimate the microbiome functions, which showed that fish gut and Cestodes exhibited similar profiles of gene functions at level 1, including: 1) metabolism, 44.8±0.8% (mean±SE); 2) environmental information processing 16.5±0.5%; 3) genetic information processing, 18.4±0.3%; 4) unclassified gene functions, 14.8±0.4%; and 5) cellular processes, 3.8±0.3% (Figure S2). Significant differences at this level were only detected between bacterial community of fish and cestodes in genes associated with predicted functional potential for metabolism and environment information processing (one-way ANOSIM at $p \leq 0.05$). The relative abundance of these two metabolic pathways at level 2 and 3 which differed significantly between cestodes and different subcompartments of perch gut are shown below.

Among groups of genes associated with environmental information processing at level 2 for perch and cestodes inhabiting their digestive tract, a significant difference was shown for membrane transport (13.96±0.22%), and signaling molecules and interactions (2.33±0.13%) (ANOSIM, $p \leq 0.05$). Significant difference at $p \leq 0.05$ among groups of genes associated with metabolism pathways at level 2 for the predicted functional metagenome of the studied bacterial communities were obtained for 7 groups, including: amino acid metabolism (8.89±0.22%); energy metabolism (5.4±0.13%); metabolism of cofactors and vitamins (3.83±0.07%); lipid metabolism (3.16±0.17%); metabolism of other amino acids (1.73±0.03%); metabolism of terpenoids and polyketides (1.44±0.09%); biosynthesis of other secondary metabolites (0.65±0.02%) (Figure S3).

Predicted functional metagenomic pathways of the microbial community of perch and cestodes at level 3 within metabolism and environmental information processing pathways were represented by 328 different gene categories. Significant differences were only obtained for 41 genes and presented in the graph (ANOSIM, $p \leq 0.05$) (Figure 5). The first three dominant gene categories at level 3 in all analyzed communities were Transporters (6.4±0.22%), ABC transporters (3.77±0.10%), and Secretion system (2.23±0.10%). According to the ANOSIM test results, the relative abundance of the functional pathways at level 3 for the microbial community associated with cestodes were significantly higher in comparison with microbiota of intestinal content (Table S2). These gene categories were associated with phosphotransferase system (PTS); valine, leucine and isoleucine biosynthesis; beta-lactam resistance; penicillin and cephalosporin biosynthesis; streptomycin biosynthesis, methane metabolism; oxidative phosphorylation; one carbon pool by folate; pantothenate and CoA biosynthesis; and carotenoid biosynthesis. No

significant differences were observed in predicted functional pathways between cestodes and different subcompartments of perch gut with the exception of stomach content communities in which relative abundance of genes at level 3were also statistically different than other microbiota.

A scatter plot based on PCoA scores (the input in % of every gene category in total for level 3) showing that functional pathways are strongly divided on bacteria that associated with stomach content and all others studied compartments of fish gut (Figure 6). In both pyloric caeca and Cestode groups of bacteria, the predicted functional potential of communities was similar. No clear grouping in the functional pathways of intestinal mucosa and intestinal content were observed. According to one-way ANOSIM (at $p \leq 0.05$) microbiota associated with cestodes were significantly different than microbial communities of the gastrointestinal tract of perch excluding pyloric caeca. No significant differences were observed in predicted gene categories between other different compartments of the gastrointestinal tract of perch and cestodes ($p > 0.05$).

Discussion

The digestive tract of vertebrates, including fish, is a complex system performing a number of physiological functions that create a diverse, nutrient rich habitat, and as such it is inhabited by various micro-organisms (Austin 2002; Han *et al*., 2010; Ghanbari *et al*., 2015). The gut microbial community plays an important role in the processes of digestion, homeostasis, regulation of intestinal immune response and defense against pathogenic organisms (Hooper *et al*., 1998; Han *et al*., 2010; Xing *et al*., 2013). Parasites are an additional organismal component of the fish gut microbiota whose existence can have impacts on the host. It is known that infestations by intestinal parasites represent a serious threat for aquaculture and can lead to a direct or indirect mortality of the host through depression of immune reaction or increasing the susceptibility to opportunistic pathogens and morbidity of fish (Sitja-Bobadilla *et al*., 2016).

To study the microbial community of fish gut and cestodes parasitizing in their digestive tract we used animals from wild populations. The prevalence of invasion of *Proteocephalus* sp. in perch from Chany Lake was 12.7% that correlates with early investigations where it was shown that prevalence of infestation of *P. cernuae* in perch from Chany Lake was 13.2% (Sous and Rostovsev 2006). As the infection rate of perch was sufficiently high, we were able to collect the representative number of parasites for statistical analysis of fish and their parasite´s microbiota. For the first time the associated microbiota of the *Proteocephalus* sp. inhabiting the gastrointestinal tract of perch was analyzed using next generation sequencing (NGS) techniques. Our data has shown that the cestodes were inhabited by a diverse microbial community.

By comparing data on bacterial diversity in perch gut with microbiota data from other fish we can conclude that the microbial composition at the phylum level in the perch intestine conforms to the available literature. Thus, the dominant microbiota in the intestine of freshwater fish was represented by Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, Tenericutes and Fusobacteria (Li *et al*., 2014; Silva *et al*., 2014; Ye *et al*., 2014; Baldo *et al*., 2015; Kashinskaya *et al*., 2015; Liu *et al*., 2016; Zha *et al*., 2018). Other metagenomic studies have shown that the microbiota of the perch *P. fluviatilis* was dominated by the phyla Tenerecutes, Proteobacteria, Fusobacteria and Firmicutes. At the genus level microbiota of healthy perch from Sweden were represented by *Myroides*, *Prochlorococcus*, *Anabaenopsis*, *Cetobacterium* and an unknown Fusobacteriaceae (Zha *et al*., 2018).

Bacteria associated with parasites isolated from fish have been most extensively studied, and are known to belong to a wide range of microbial taxa (Hughes-Stamm *et al*., 1999; Korneva and Plotnikov 2006; Llewellyn *et al*., 2017 and others). Parasite associated microbiota has been described for Monogenea, Trematoda, Cestoda, Nematoda and Crustacea (Table S3 and Table S4). Thus, early studies using the methods of transmission and scanning electron microscopy (Table S3) demonstrated the bacterial diversity associated with the intestine *Esox lucius* and the tegument of *Triaenophorus nodulosus* were represented by not only well-known forms of bacteria such as cocci, bacteria from the *Vibrios* group, and rod-like bacteria, but also by different morphological forms of very small bacteria with 0.25-0.30 nm in diameter and spirochaetes (Korneva and Plotnikov 2006). Hughes-Stamm and co-authors identified 7 microbial morphotypes, including *Eubacteria*, *Spirochaetes*, associated with the tegument of *Gyliauchenn ahaensis* isolated from *Siganus doliatus*, *S. orallines*, *S. puellus* and *S. lineatus* (Hughes-Stamm *et al*., 1999). It also should be noted that no negative effects on the cestode biology could be inferred from the presence of these bacteria. More likely, the authors speculate, that there were possible mutuality interactions between cestodes and the bacteria inhabiting the gastrointestinal tract of fish (Korneva and Plotnikov 2006).

It is also known that helminths may act as vectors for pathogenic bacteria that can cause serious diseases in many invertebrates and vertebrates. For example, the studies regarding the microbiota associated with parasites have shown that the most well-known bacteria found within Trematoda and Nematoda are members of the genera *Neorickettsia* and *Wolbachia*, respectively (Kang *et al*., 2014; Madigan *et al*., 2000); in tick – *Anaplasma*, *Ehrlichia*, *Candidatus* Midichloriaceae, and *Rickettsia* (Kang *et al*., 2014). These are genera distinctly known from the literature relating to their pathogenic members. In particular, a well represented example of parasite-microbiota interaction is the liver fluke *Opisthorchis viverrini*, associated with

hepatobiliary diseases and cholangiocarcinoma. In this case, *O. viverrini* modifies its host intestinal microbiome and promotes *Helicobacter pylori* infection in the liver (Watanapa and Watanapa 2002). Another well represented example are ectoparasites (eg.: *Gyrodactylus* sp.) that may act as vectors for pathogenic aeromonads and, being almost ubiquitous on salmonid fish may be a prominent reservoir for *Aeromonas* infections (Cusack and Cone 1985). The study regarding the diversity and composition of *Salmo salar* microbiota parasitized on their skin by the copepod *Lepeophtheirus salmonis* have revealed the association of multiple, potentially pathogenic bacterial genera such as *Vibrio, Flavobacterium*, *Tenacibaculum*, and *Pseudomonas* (Llewellyn *et al*., 2017).

As shown earlier in this study, the microbiota associated with *Proteocephalus* sp. extracted from perch in Chany Lake was dominated by bacteria from the genera *Serratia*, *Pseudomonas*, *Mycoplasma*, unidentified bacteria from the family *Enterobacteriaceae*, *Bacillus,* and unclassified clade u*114* from the family *Fusobacteriaceae*. This study did not demonstrate a transmission of the secondary bacterial infections to perch during the *Proteocephalus* sp. infestation, but it is interesting to note that bacterial genera from communities associated with gastrointestinal tracts of perch and tapeworms share several members including opportunistically pathogenic species. The dominant bacteria associated with the cestodes were *Serratia* spp. from the phylum Proteobacteria, family *Enterobacteriaceae*. Several members of this genus are known as pathogens (Baya *et al*., 1992; Buller 2004). As examples, pathogenic *S. marcescens* is a causative agent of mortality in striped bass fingerlings, and *S. liquefaciens* causes a redness and swelling around the anus, accumulation of ascites, and hemorrhaging of multiple internal organs in arctic char (*Salvelinus alpines*); while in turbot (*Scophthalmus maximus*) infection leads to swollen kidney and spleen, with yellow nodules and foci of necrotic liquefaction (Buller 2004). As a part of the normal microbiota *Serratia* spp. was registered early on in fish from different families (Trust 1974; Al-Harbi and Uddin 2004; Kashinskaya *et al*., 2015; Belkova *et al*., 2017 and others). Another well represented bacteria associated with cestodes inhabiting the gastrointestinal tract of perch were *Pseudomonas* spp. These bacteria constitute a part of the normal fish microbiota (Sugita *et al*., 1988; Hansen and Olafsen 1999; Al-Harbi and Uddin 2004; Wang *et al*., 2012; Wu *et al*., 2012; Gajardo *et al*., 2016), but there are opportunistic species that may become pathogenic in stressed fish (Ardura *et al*., 2013) such as *P. fluorescens* that causes a red skin disease in grass carp *Ctenopharyngodon idellus* (Tran *et al*., 2017).

Relative abundance of *Mycoplasma* (phylum Tenericutes) observed from perch and the cestodes in Chany Lake was varied among the different types of subcompartments of the digestive system or Cestode samples (Table 1). The highest diversity of mycoplasmas was revealed in the

communities of the intestinal mucosa and intestinal content (41.5±16.4 and 26.7±13.4%, correspondingly) of studied perch. The presence of *Mycoplasma* was also found as a part of the normal microbiota of many fish (from genera *Thymallus, Coregonus*, *Brachymystax, Gillichthys*, and *Salmo*) (Holben *et al*., 2002; Bano *et al*., 2007; Lyons et *al*., 2017). In addition, some Mycoplasmas are known to be pathogens, but many species appear to be simply part of the natural microbiota and have no harmful effects on the hosts (Roediger and Macfarlane 2002; Bano *et al*., 2007). However, evidence suggests the *Mycoplasma* species could utilize cytoplasmic secretions from their wild salmon host and produce lactic and acetic acids which are subsequently utilized by other bacteria (Li *et al*., 2016). Mycoplasma-helminth association in trematodes *Diclidophora merlangi* parasitizing the gills of whiting (*Merlangius merlangus*) have also been described (Morris and Halton 1975).

Another important aspect of host-parasite-bacteria interactions is to understand how bacteria are associated to cestodes, and where is the distinction between the microbiota of the host and the microbial community of parasites. At the present time there are no universal methodological approaches to explore "true" microbiota of helminths, but several authors have made attempts to explore bacteria associated with parasites. Hahn and Dheilly demonstrated the existence of a unique cestodes microbiome where none was thought to exist (Hahn et al. 2018). An interesting method regarding desorption of bacteria from tegument of cestodes was described by Izvekova and co-authors (Izvekova and Lapteva 2004). The method described is based on serial washing in a series of buffer solutions, via shaking, to recover bacterial cells from the tegument of parasites or part of the digestive tract of fish. The different fractions of material collected separately from this method then were inoculated onto nutrient medium. The resulting cultures corresponded to different types of bacterial associations to the mucosal surfaces of fish and tegument of parasites. Difficulties with the study of bacteria associated with parasites are complicated by the fact that the cestodes do not possess a digestive system and digest and absorb different nutrients via membranes and active transport. In this case, it is very difficult to conclude what degree of association exists between the bacteria of tapeworms and mucosal surfaces of fish. Future laboratory studies using different methods of bacterial removal from cestode surfaces which were collected from the host can allow us to understand specificity of the bacterial community associated with parasites. In our work we did not wash cestodes in a buffer solution or treat them by chemical reagents, but used only mechanical cleaning of parasites from chime of fish.

A similarity was observed between the microbiota of stomach and intestinal mucosa and cestodes parasitizing the perch intestine. A possible explanation for this similarity is the specific microenvironment with low pH levels that are probably formed around the tapeworm due to its

metabolic activity. Such microenvironments permit bacterial survival of those species that are moved from the stomach to the anterior intestine (from acid to neutral pH media). Indeed, it is known that cestodes may secret some organic acids (Izvekova 2001) and, hypothetically, the pH values may be shifted to the acid side and this can be a selective barrier for some bacterial groups. On the one hand, the pH level in fish intestine ranged from slightly acid (6.2–6.5) to moderately alkaline (8.0–8.5) values. Such pH range was registered for different species of fish with different feeding habits (Solovyev, Izvekova, Kashinskaya, & Gisbert, 2017). On the other hand, the tapeworms may produce acetic acid as a final product of metabolism; thus, in theory, it may decrease pH value below normal values, for example, to shift pH in the intestine to the acid side. Similarity of the microbial community associated with parasites and the microbiota of pyloric caeca, also confirmed by data, that the cestodes in the beginning of development can attach their scoleces to the epithelium of pyloric caeca with the strobilae growing and lying within the intestinal lumen (Scholz 1999). While tapeworm-specific bacterial groups were found, this cestode mimics its host not only from a morphological point of view (microvilli by microtrichii), but from the perspective of the microbial community of the host as well.

It is also should be noted that the dissimilarity between cestodes and different subcompartments of gastrointestinal tract of perch was observed only for stomach content community. These dissimilarity may be explained by that fact that this part of the gut is the first to be contacted continuously (after oesophagus) with environmental constituents and prey. Some of the OTUs founded in the stomach content may reflect a high proportion of dietary derived bacterial fragments. Thus, it was shown that food-associated microbes drive gut microbial community diversity to a greater extent than water-associated microbes (Bolnick et al., 2014; Smith et al., 2015).

The functional role of the bacterial community of the fish gut has already been investigated in a few studies (Liu *et al*., 2016; Lyons *et al*., 2017). The PICRUSt results at level 1 suggested that the gut microbiota of perch and cestodes are primarily linked to metabolism and environmental information processing and to a lesser extent to genetic information processing and cellular processes. This is somewhat similar to studies which showed that the majority of the functional pathways of rainbow trout, *Oncorhynchus mykiss* were associated with metabolism, environmental information processing, genetic information processing and cellular processes (Lyons *et al*., 2017).The PICRUSt results at level 2 suggested that physiological functions of the gut microbiota of perch and cestodes are linked to carbohydrate and amino acid metabolism, which is consistent with other studies from grass carp (Ni *et al*., 2014) and rainbow trout (Lyons *et al*., 2017). In our study a high level of the predicted functional pathways which were belonging to amino acid

metabolism could be linked to the feeding habits of perch. The diet of the perch from Chany Lake, as previously shown, is based largely on fish fry from the Cyprinid family, benthic organisms and zooplanktonic organisms (Kashinskaya *et al*., 2018). The higher level of gene pathways responsible for carbohydrate metabolism in the microbial community of the gastrointestinal tract of perch and cestodes may also play an important role in bacterial nutrition. It is known that the activity of proteases is higher in piscivorous compared to herbivorous fish, while the activity of enzymes metabolizing carbohydrates contrariwise, is lower in piscivorous and higher in herbivorous species of fish (Kuz'mina 2001). Thus, in a recent paper it was shown that activity of α-amylase in the intestine of perch was relatively high, if compared with other picsivorous fish, which allows perch to hydrolyze carbohydrates from food items successfully (Solovyev *et al.*, 2014). *Pseudomonas* sp. and *Mycoplasma* sp., which dominated in the perch gastrointestinal tract and on the tegument of cestodes, are protease (Staats *et al*., 2007; Ray *et al*., 2012) and amylase-producing bacteria (Ray *et al*., 2012) that can possibly participate in amino acid and carbohydrate metabolism.

As recently reported, genes corresponding to membrane transport at level 2 were dominated by ABC transporters and secretion system. ABC transporters (ATP-binding cassette)are essential in all eukaryotic and prokaryotic species, including parasites. These pathways are involved in diverse cellular processes such as maintenance of osmotic homeostasis, nutrient uptake, resistance to xenotoxins, antigen processing, cell division, bacterial immunity, pathogenesis, sporulation, and cholesterol and lipid trafficking. It is also reported that ABC transporters are involved in multidrug resistance in parasites (Jones and George 2005) and can be prospective targets for study as molecular markers of resistance to antihelminthics through suppression of the transporters themselves with specific inhibitors (Mordvinov *et al*., 2017). The presented results show that these pathways are important physiological processes, revealing that the function of the microbiome can play an important role in the nutrition and health of their host.

In summary, for the first time the microbial community of the gastrointestinal tract of perch and their cestodes from the genus *Proteocephalus* was analyzed using a next-generation sequencing approach. Our data has shown that the cestodes were inhabited by a diverse microbial community. The occurrence of the same bacterial taxa in the perch intestine as well on the parasite's tegument is confirmed by the data on the establishment of primary microbiota in the fish gut. This process takes place following the first feeding and comes in stages, resulting in an "adult microflora" weeks to months later (Hansen and Olafsen 1999). Fish tapeworms have a complex life cycle using multiple intermediate hosts. The parasites appearance in the fish intestine occurs after the latter already has been populated by a primary microbiota and interacts with it. It is also

possible that the parasite becomes colonized with a certain number of bacterial taxa "on the way" to the definitive host. However, to understand the role of these bacteria in the relationship between parasite and host further research is needed. The results from analysis of the microbial community of perch and cestodes using PICRUSt demonstrate that the predicted functional pathways likely play an active role in perch nutrition and health. The next-generation sequencing data implies what the functional capacities of the microbiota may be (via PICRUSt) that are intrinsic to the perch gut and its cestode parasites confirming the earlier perceptions, based on the methods of traditional microbiology and physiology, about the role of bacteria in various physiological processes (Izvekova and Lapteva 2004; Izvekova 2006). These earlier studies challenged the hygiene hypothesis by demonstrating the important role of these bacteria in aiding digestion, and providing essential vitamins and amino acids.

Notably, when parasite samples are analyzed for their microbial diversity separately from the gut, the source for unique bacterial taxa and their relative abundance in the parasite is made clearer. For this reason, we also recommend taking into account the presence of endo-parasites and their bacterial load in fish microbiome studies to improve understanding of the host-parasite-microbiota relationship. What role the microbiota of endo-parasites such as tapeworms play in adapting them to their hosts is unknown. Many interesting questions remain in this novel area of microbiome studies.

References

Al-Harbi, A. H., & Uddin, N. M. (2004). Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus*×*Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture*, **229**, 37–44. https://doi.org/10.1016/S0044-8486(03)00388-0

Ardura, A., Linde, A. R., & Garcia-Vazquez, E. (2013). Genetic detection of *Pseudomonas* spp. in commercial amazonian fish. *International Journal of Environmental Research and Public Health*, **10**, 3954–3966. https://doi.org/10.3390/ijerph10093954

Anikieva, L. V. (1995). Variability of a perch's parasite *Proteocephalus percae* in the areal of the host. *Parazitologiya*, **29(4)**, 279–289.

Anikieva, L. V., Ieshko, E. P., & Lebedeva, D.I. (2015). Morphological variability of Proteocephalus percae (Müller 1780) parasitizing coregonid fishes. *Parazitologiya*, **49(3)**, 160–170

Austin, B. (2002). The bacterial microflora of fish. *The Scientific World Journal*, **2**, 558– 572. https://doi.org/10.1100/tsw.2002.137 530

Baldo, L., Riera, J. L., Tooming-Klunderud, A., Albà, M. M., & Salzburger, W. (2015). Gut microbiota dynamics during dietary shift in eastern African cichlid fishes. *PLoS ONE*, **10(5)**, 1–23. https://doi.org/10.1371/journal.pone.0127462 533

Bano, N., DeRae Smith, A., Bennett, W., Vasquez, L., & Hollibaugh, J. T. (2007). Dominance of *Mycoplasma* in the guts of the long-jawed mudsucker, *Gillichthys mirabilis*, from five California salt marshes. *Environmental Microbiology*, **9(10)**, 2636–2641. https://doi.org/10.1111/j.1462-2920.2007.01381.x 537

Baya, A., Toranzo, A., Lupiani, B., Santos, Y.; & Hetrick, F. M. (1992). *Serratia marcescens*: a potential pathogen for fish. *Journal Fish Diseases*, **15(1)**, 15–26. https://doi.org/10.1111/j.1365-2761.1992.tb00632.x

Belkova, N. L., Sidorova, T. V., Glyzina, O.Y., Yakchnenko, V. M., Sapozhnikova, Y. P., Bukin Y.S., ... Sukhanova L. V. (2017). Gut microbiome of juvenile coregonid fishes: comparison of sympatric species and their F1 hybrids. *Fundamental and Applied Limnology*, **189(3)**, 279–290. https://doi.org/10.1127/fal/2016/0804

Bolnick, D. I., Snowberg, L. K., Hirsch, P.E., Lauber, C. L., Org, E., Parks, B., … Svanbäck R. (2014). Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nature Communications*, 5(4500), 1–13. https://doi.org/10.1038/ncomms5500

Buller, N. B. (2004). Bacteria from fish and other aquatic animals: a practical identification manual. Oxfordshire: CABI.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, **83(4)**, 575–583.

Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L., & Knight, R. (2010). PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*, **26**, 266–267. https://doi.org/10.1093/bioinformatics/btp636

Cézilly, F., Perrot-Minnot, M.-J., & Rigaud, T. (2014). Cooperation and conflict in host manipulation: interactions among macro-parasites and micro-organisms. *Frontiers in Microbiology*, **5(248)**, 144–153. https://doi.org/10.3389/fmicb.2014.00248 557

Clements, K. D., Angert, E. R., Montgomery, W., & Choat, J.H. (2014). Intestinal microbiota in fishes: what's known and what's not. *Molecular Ecology*, **23**, 1891–1898. https://doi.org/10.1111/mec.12699

Cusack, R., & Cone, D. K. (1985). A report of bacterial microcolonies on the surface of *Gyrodactylus* (Monogenea). *Journal of Fish Diseases*, **8**, 125–127. https://doi.org/10.1111/j.1365-562 2761.1985.tb01193.x

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., … Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, **72(7)**, 5069–5072. https://doi.org/10.1128/AEM.03006-05

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, **26(19)**, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Gajardo, K., Rodiles, A., Kortner, T. M., Krogdahl, A., Bakke, A. M., Merrifield, D. L., & Sørum, H. (2016). A high-resolution map of the gut microbiota in Atlantic salmon (*Salmo salar*): A basis for comparative gut microbial research. Scientific Reports, 6, 1–10. https://doi.org/10.1038/srep30893 573

584 Ghanbari, M., Kneifel, W., & Domig, K.J. (2015). A new view of the fish gut microbiome:
585 Advances from next-generation sequencing. *Aquaculture*, **448**, 464–475.
586 https://doi.org/10.1016/j.aquaculture.2015.06.033 576

587 Grisez, L., Reyniers, J., Verdonck, L., Swings, J., & Ollevier, F. (1997). Dominant intestinal
588 microflora of sea bream and sea bass larvae, from two hatcheries, during larval development.
589 *Aquaculture*, **155**, 387–399. https://doi.org/10.1016/S0044-8486(97)00113-0

590 Hammer, Ø., Harper, D. A. T, & Ryan, P. D. (2011). PAST: paleontological statistics software
591 package for education and data analysis. *Palaeontologia Electronica*, **4**, 9. http://palaeo
592 electronica.org/2001_1/past/issue1_01.htm

593 Hahn, M., & Dheilly, N. (2018). Not so sterile after all: The endomicrobiome of plerocercoids of
594 the cestode parasite *Schistocephalus solidus* and changes to the microbiome of its Threespine
595 Stickleback host. *Biorxiv*, 1-19. doi: https://doi.org/10.1101/290015

596 Han, S., Liu, Y., Zhou, Z., He, S., Cao, Y., Shi P., ... Ringø, E. (2010). Analysis of bacterial
597 diversity in the intestine of grass carp (*Ctenopharyngodon idellus*) based on 16S rDNA gene
598 sequences. *Aquaculture Research*, **42**, 47–56. https://doi.org/10.1111/j.1365-2109.2010.02543.x

599 Hansen, G. H., & Olafsen, J. A. (1999). Bacterial interactions in early life stages of marine cold
600 water fish. *Microbial Ecology*, **38**, 1–26. http://link.springer-
601 ny.com/link/service/journals/00248/bibs/38n1p1.html

602 Hooper, L., Bry, L., Falk, P., & Gordon, J. I. (1998). Host-microbial symbiosis in the mammalian
603 intestine: Exploring an internal ecosystem. *BioEssays*, **20(4)**, 336–343.
604 https://doi.org/10.1002/(SICI)1521-1878(199804)20:43.0.CO;2-3

605 Holben, W. E., Williams, P., Gilbert, M., Saarinen, M., Särkilahti, L. K., & Apajalahti, J. H.
606 (2002). Phylogenetic analysis of intestinal microflora indicates a novel *Mycoplasma* phylotype in
607 farmed and wild salmon. *Microbial Ecology*, **44**, 175–185. https://doi.org/10.1007/s00248-002-
608 1011-6

609 Houlden, A., Hayes, K. S., Bancroft, A. J., Worthington, J. J., Wang, P., Grencis, R. K., & Roberts,
610 I. S. (2015). Chronic *Trichuris muris* infection in C57BL/6 mice causes significant changes in host
611 microbiota and metabolome: effects reversed by pathogen clearance. *PLoS ONE*, **10**, e0125945.
612 https://doi.org/10.1371/journal.pone.0125945

613 Hughes-Stamm, S. R., Cribb, T. H., & Jonest, M. K. (1999). Structure of the tegument and 604
614 ectocommensal microorganisms of *Gyliauchen nahaensis* (Digenea: Gyliachenidaea), in
615 inhabitant of herbivorous fish of the great barrier reef, Australia. *The Journal of Parasitology*,
616 **85(6)**, 1047–1052.

617 Izvekova, G. I. (2006). Hydrolytic activity of enzymes produced by symbiotic microflora and its
618 role in digestion processes of bream and its intestinal parasite *Caryophyllaeus laticeps* (Cestoda,
619 Caryophyllidea). *Biology Bulletin*, **33(3)**, 287–292.

620 Izvekova, G. I. (2003). Proteolytic activity of microflora associated with the digestive transport
621 surfaces of intestine of the burbot *Lota lota* and of *Eubothrium rugosum* parasitized in it. *Journal*
622 *of Evolutionary Biochemistry and Physiology*, **39**, 529–536.
623 https://doi.org/10.1023/B:JOEY.0000015960.83715.8a

624 Izvekova, G. I. (2001). *Triaenophorus nodulosus* (Cestoda, Pseudophyllidea): final stages of
625 carbohydrate metabolism. *Helminthologia*, **38(1)**, 23–27.

626 Izvekova G. I., Frolova T. V., & Izvekov E. I. (2017). Inactivation of proteolytic enzymes by
627 Eubothrium rugosum (Cestoda) from the gut of burbot Lota lota. Folia Parasitologica, 64, 1-8.
628 https://doi.org/10.14411/fp.2017.016

629 Izvekova, G. I., Korneva, Z. V. (2007). The Localization of symbiont digestion processes on
630 digestive-transport structures of fish and Cestodes. *Biology Bulletin Reviews*, **127(5)**, 502–13.

631 Izvekova, G. I., Lapteva, N. A. Microflora Associated with the Digestive-Transport Surfaces of
632 Fish and Their Parasitic Cestodes. *Russian Journal of Ecology* 2004; 35(3):176–80.

633 Izvekova, G. I., Plotnikov, A. O. (2011). Hydrolytic activity of symbiotic microflora enzymes in
634 pike (*Eso xlucius* L.) intestine. *Inland Water Biology*, **4(1)**, 72–77.
635 https://doi.org/10.1134/S1995082911010081

636 Jones, P. M., & George, A. M. (2005). Multidrug resistance in parasites: ABC transporters, P-
637 glycoproteins and molecular modelling. *International Journal for Parasitology*, 35(5), 555-566.
638 https://doi.org/10.1016/j.ijpara.2005.01.012

639 Kang, Y.-J., Diao, X.-N., Zhao, G.-Y., Chen, M. H., Xiong, Y., Shi, M...., Zhang, Y.Z. (2014).
640 Extensive diversity of Rickettsiales bacteria in two species of ticks from China and the evolution
641 of the Rickettsiales. BMC Evolutionary Biology, 14(1), 1-12. https://doi.org/10.1186/s12862-014-
642 0167-2

643 Kashinskaya, E. N., Andree, K. B., Simonov, E. P., & Solovyev, M. M. (2017). DNA extraction
644 protocols may influence biodiversity detected in the intestinal microbiome: a case study from wild
645 Prussian carp, *Carassius gibelio*. *FEMS Microbiology Ecology*, **93**, 1–14.
646 https://doi.org/10.1093/femsec/fiw240

647 Kashinskaya, E. N., Belkova, N. L., Izvekova, G. I., Simonov, E. P., Andree, K. B., Glupov, …
648 Solovyev, M. M. (2015). A comparative study on microbiota from the gut of Prussian carp
649 (*Carassius gibelio*) and their aquatic environmental compartments, using different molecular
650 methods. *Journal Applied Microbiology*, **119**, 948–961. https://doi.org/10.1111/jam.12904

651 Kashinskaya, E. N., Simonov, E. P., Kabilov, M. R., Izvekova, G. I., Andree, K. B., & Solovyev,
652 M. M. (2018). Diet and other environmental factors shape the bacterial communities of fish gut in
653 an eutrophic lake. *Journal Applied Microbiology*, **125(6)**, 1626-1641.
654 https://doi.org/10.1111/jam.14064

655 Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O.
656 (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-
657 generation sequencing-based diversity studies. *Nucleic Acids Research*, **41(1)**, 1–11.
658 https://doi.org/10.1093/nar/gks808

659 Korneva, Z. V., & Plotnikov, A. O. (2006). Symbiont microflora colonizing the tegument of
660 *Triaenophorus nodulosus* (Cestoda) and intestine of its Host, Pike. *Parazitologiya*, **40(6)**, 535–
661 546.

Korneva, Z. V., & Plotnikov, A. O. (2012). Ultrastructure adaptations of symbiotic bacteria associated with freshwater fish and their Cestode parasites. *Inland Water Biology*, **5(2)**, 178–183. https://doi.org/10.1134/S1995082912020071

Kuz'mina, V. V. (2001). Physiological Adaptations (By the Example of the Exotrophy Process in Fish). *Journal of Evolutionary Biochemistry and Physiology*, **37(3)**, 285–299. https://doi.org/ 10.1023/A:1012627625563

Langille, M. G. I., Zaneveld, J., Caporaso, G. J., McDonald, D., Knights, D., Reyes, J. A., … Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, **31**, 814–821. https://doi.org/10.1038/nbt.2676

Li, X., Yan, Q., Ringø, E., Wu, X., He, Y., & Yang, D. (2016). The influence of weight and gender on intestinal bacterial community of wild largemouth bronze gudgeon (*Coreius guichenoti*, 1874). *BMC Microbiology*, **16(191)**, 1–8. https://doi.org/10.1186/s12866-016-0809-1

Li, J., Ni, J., Li, J., Li, X., Wu, S., Zhang, T., … Yan, Q. (2014). Comparative study on gastrointestinal microbiota of eight fish species with different feeding habits. *Journal of Applied Microbiology*, **117**, 750–760. https://doi.org/10.1111/jam.12663

Li, R. W., Wu, S., Li, W., Navarro, K., Couch, R. D., Hill, D., & Urban, J. F. Jr. (2012). Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. *Infection and Immunity*, **80**, 2150–2157. https://doi.org/10.1128/IAI.00141-12

Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan F. & Wang W. (2016). The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Scientific Reports*, **6**, 24340. https://doi.org/ 10.1038/srep24340

Llewellyn, M. S., Leadbeater, S., Garcia, C., Sylvain, F. E., Custodio, M., Ang, K. P. … Derome, N. (2017). Parasitism perturbs the mucosal microbiome of Atlantic Salmon. *Scientific Reports*, 7, 43465. https://doi.org/10.1038/srep43465

Lyons, P. P., Turnbull, J. F., Dawson, K. A., & Crumlish, M. (2017). Phylogenetic and functional characterization of the distal intestinal microbiome of rainbow trout *Oncorhynchus mykiss* from both farm and aquarium settings. *Journal of Applied Microbiology*, **122(2)**, 347–363. https://doi.org/10.1111/jam.13347

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, **71(12)**, 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Madigan, J. E., Pusterla, N., Johnson, E., Chae, J. S., Pusterla, J. B., Derock, E., & Lawler, S. P. (2000). Transmission of *Ehrlichia risticii*, the agent of Potomac horse fever, using naturally infected aquatic insects and helminth vectors: preliminary report. *Equine Veterinary Journal*, **32**, 275–279.

McMurdie, P. J., Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, **8(4)**, 1–11.

Mordvinov, V. A., Ershov, N. I., Pirozhkova, D. S., Pakharukov, Y. V., & Pakharukova, M. Y. (2017). ABC transporters in the liver fluke *Opisthorchis felineus*. *Molecular and Biochemical Parasitology*, **216**, 60–68. https://doi.org/10.1016/j.molbiopara.2017.07.001

Morris, G. P., & Halton, D. W. (1975). The Occurrence of bacteria and Mycoplasma-like organisms in a monogenean parasite *Declidophora merlangi*. *International Journal for Parasitology*, **5**, 495–498. https://doi.org/10.1016/0020-7519(75)90039-9

Nayak, S. K. (2010). Role of gastrointestinal microbiota in fish. *Aquaculture Research*, **41**, 1553–1573. https://doi.org/10.1111/j.1365-2109.2010.02546.x

Ni, J., Yan, Q., Yu, Y., & Zhang, T. (2014). Factors influencing the grass carp gut microbiome and its effect on metabolism. *FEMS Microbiology Ecology*, **87**, 704–714. https://doi.org/10.1111/1574-6941.12256

Oksanen, J., Blanchet, F. G., Friendly, M., *et al*. (2018). vegan: Community Ecology Package. R package version 2.5-3. https://CRAN.R-project.org/package=vegan.

Poddubnaya, L. G. (2005). Electron microscope investigation of bacteria associated with the tegument of the tape worm species *Eubothrium rugosum*, a parasite of the intestine of Burbot. *Parazitologiya*, **39(4)**, 293–298.

Poddubnaya, L. G., & Izvekova, G. I. (2005). Detection of bacteria associated with the tegument of Caryophyllidean Cestodes. *Helminthologia*, **42(1)**, 9–14.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2--approximately maximum likelihood trees for large alignments. *PLoS ONE*, **5(3)**, e9490. https://doi.org/10.1371/journal.pone.0009490

Ray, A. K., Ghosh, K., & Ringø, E. (2012). Enzyme- producing bacteria isolated from fish gut: a review. *Aquaculture Nutrition*, **18(5)**, 465–492. https://doi.org/ 10.1111/j.1365-2095.2012.00943.x

Reynolds, L. A., Finlay, B. B., & Maizels, R. M. (2015). Cohabitation in the intestine: Interactions among helminth parasites, bacterial microbiota, and host immunity. *Journal of Immunology*, **195**, 4059–4066. https://doi.org/10.4049/jimmunol.1501432

Roediger, W. E., & Macfarlane, G. T. (2002). A role for intestinal mycoplasmas in the aetiology of Crohn's disease? *Journal of Applied of Microbiology*, **92(3)**, 377–381.

Salazar, G. (2018). EcolUtils: Utilities for community ecologyanalysis. R package version 0.1. https://github.com/GuillemSalazar/EcolUtils.

Sitja-Bobadilla, A., Estensoro, I., Perez-Sanchez J. (2016). Immunity to gastrointestinal microparasites of fish. Development and Comparative Immunology, **64**, 187–201.

Scholz, T. (1999). Life cycles of species of *Proteocephalus*, parasites of fishes in the Palearctic region: a review. *Journal of Helminthology*, **73(1)**, 1–19.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: open-source, platform-independent, community supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, **75(23)**, 7537–7541. https://doi.org/10.1128/AEM.01541-09

Silva, F. C. P., Nicoli, R., Zambonino-Infante, J. L., Kaushik, S., & Gatesoupe, F. J. (2011). Influence of the diet on microbial diversity of fecal and gastrointestinal contents in gilthead sea bream (*Sparus aurata*) and intestinal contents in goldfish (*Carassius auratus*). *FEMS Microbiology Ecology*, **78(2)**, 285–296. https://doi.org/10.1111/j.1574-6941.2011.01155.x

741 Smith, C. C. R., Snowberg, L. K., Caporaso, G. J., Knight, R., Bolnick, D. I. (2015). Dietary input
742 of microbes and host genetic variation shape among-population differences in stickleback gut
743 microbiota. *The ISME Journal*, 9(11), 2515-2526. https://doi.org/10.1038/ismej.2015.64

744 Solovyev, M. M., Kashinskaya, E., Izvekova, G., Gisbert, E., & Glupov, V. V. (2014). Feeding
745 habits and ontogenic changes in digestive enzyme patterns in five freshwater teleosts. *Journal of*
746 *Fish Biology*, **85(5)**, 1–18. https://doi.org/10.1111/jfb.12489

747 Sous, S.M., & Rostovtsev, A. A. (2006). *Parasites of fishes of the Novosibirsk region: in two parts*.
748 Tyumen: State Fishery Center. In Russian.

749 Staats, C. C., Boldo, J., & Broetto, L. (2007). Comparative genome analysis of proteases,
750 oligopeptide uptake and secretion systems in *Mycoplasma* spp. *Genetics and Molecular Biology*,
751 **30(1)**, 225–229. https://doi.org/10.1590/S1415-47572007000200009

752 Sugita, H., Tsunohara, M., Ohkoshi, T., & Deguchi, Y. (1988). The establishment of an intestinal
753 microflora in developing goldfish (*Carassius auritus*) of culture ponds. *Microbial Ecology*, **15**,
754 333–344. https://doi.org/10.1007/BF02012646

755 Sullam, K. E., Essinger, S. D., Lozupone, C. A., O'Connor, M. P., Rosen, G. L., Knight, R., ... S.,
756 Russell, J. A. (2012). Environmental and ecological factors that shape the gut bacterial
757 communities of fish: a meta-analysis. *Molecular Ecology*, **21**, 3363–3378.
758 https://doi.org/10.1111/j.1365-294X.2012.05552.x

759 Syvokiene, J., Mickeniene, L., & Bubinas, A. (1999). Characteristics of microflora of the digestive
760 tract of commercial fish depending on fish feeding. *Ekologija*, **4**, 46–54.

761 Tran, N. T., Wang, G.-T., & Wu, S.-G. (2017). A review of intestinal microbes in grass carp
762 *Ctenopharyngodon idellus* (Valenciennes). *Aquaculture Research*, **48(7)**, 3287–3297.
763 https://doi.org/10.1111/are.13367

764 Trust, T. J., & Sparrow, R. A. H. (1974). The bacterial flora in the alimentary tract of freshwater
765 salmonid fish. *Canadian Journal of Microbiology*, **20**, 1219–1228.

766 Walk, S. T., Blum, A. M., Ewing, S. A., Weinstock, J. V., & Young, V. B. (2010). Alteration of
767 the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides*
768 *polygyrus*. *Inflammatory Bowel Diseases*, **16**, 1841–1849. https://doi.org/10.1002/ibd.21299

769 Wang, A. R., Ran, C., Ringø, E., & Zhou Z. G. (2017). Progress in fish gastrointestinal microbiota
770 research. *Reviews in Aquaculture*, **0**, 1–15. https://doi.org/10.1111/raq.12191

771 Watanapa, P., & Watanapa, W. B. (2002). Liver fuke-associated cholangiocarcinoma. *The British*
772 *Journal of Surgery*, **89**, 962–970. https://doi.org/10.1046/j.1365-2168.2002.02143.x

773 Wu, S., Wang, G., Angert, E. R., Wang, W., Li, W., & Zou, H. (2012). Composition, diversity,
774 and origin of the bacterial community in grass carp intestine. *PLoS ONE*, **7(2)**, 1–11.
775 https://doi.org/10.1371/journal.pone.0030440

776 Xing, M., Hou, Z., Yuan, J., Liu, Y., Qu, Y., & Liu, B. (2013). Taxonomic and functional
777 metagenomic profiling of gastrointestinal tract microbiome of the farmed adult turbot
778 (*Scophthalmus maximus*). *FEMS Microbiology Ecology*, **86(3)**, 432–443.
779 https://doi.org/10.1111/1574-6941.12174

780 Ye, L., Amberg, J., Chapman, D., Gaikowski, M., Liu, W. T. (2014). Fish gut microbiota analysis
781 differentiates physiology and behavior of invasive Asian carp and indigenous American fish. *The*
782 *ISME Journal*, **8**, 541–551. https://doi.org/10.1038/ismej.2013.181

783 Zha, Y., Eiler, A., Johansson, F., & Svanbäck, R. (2018). Effects of predation stress and food
784 ration on perch gut microbiota. *Microbiome,* **6(28)**, 1–12. https://doi.org/10.1186/s40168-018- 766
785 0400-0

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788 **Conflict of interest.** None declared.

Table 1. Relative abundance of dominant bacterial genera (% of V3-V4 region of the 16S rRNA gene sequences) in gastrointestinal tract of perch and their cestode parasites.

Different subcompartments	***Serratia***	***Pseudomonas***	***Mycoplasma***	***u114***
Stomach mucosa (n = 6)	31.84±6.40^{ab}	18.13±3.30^{ab}	11.70±10.44	0.19±0.18
Stomach content (n = 6)	**6.86±3.92^{a}**	**4.66±2.49^{a}**	0.13±0.05	0.19±0.17
Pyloric caeca (n = 4)	**44.82±2.85^{b}**	**27.74±2.17^{bc}**	10.59±5.82	0.01±0.00
Intestinal mucosa (n = 7)	24.22±6.73^{ab}	13.40±3.61^{ab}	40.49±16.28	4.54±4.53
Intestinal content (n = 7)	10.31±5.55^{ab}	**6.47±3.33^{abd}**	25.60±13.25	14.76±13.49
Cestodes (n = 7)	**41.79±2.62^{b}**	**23.36±1.38^{b}**	7.31±3.96	4.23±4.19

Upper-case and extra bold indicates significance at $p \leq 0.05$after Dunn's post hoc test.

794 Table 2. Relative abundance of unique bacterial taxa associated with cestodes

Phylum	Genus	*Range of the relative abundance, %	Occurrence of unique taxa, %
Actinobacteria	Unclassified genus from *Dietziaceae* family	0.013-0.155	25
Actinobacteria	*Williamsia*	0.012-0.034	37.5
Proteobacteria	*Psychrobacter*	0.006-0.050	25.0
Firmicutes	*Paenibacillus*	0.050	12.5
Bacteroidetes	*Sporocytophaga*	0.050	12.5
Firmicutes	*Tissierella*	0.043	12.5
Bacteroidetes	*Hymenobacter*	0.034	12.5
Actinobacteria	*Pimelobacter*	0.023	12.5
Firmicutes	*Allobaculum*	0.020	12.5
Bacteroidetes	*Segetibacter*	0.014	12.5
Firmicutes	*Planomicrobium*	0.014	12.5
Aquificae	Unclassified genus from *Aquificaceae* family	0.007	12.5
Proteobacteria	*Ewingella*	0.0025-0.029	25

795 * - among samples where these taxa were detected
796

Table 3. Comparison of microbiota associated with different subcompartments of digestive tract of perch and cestodes calculated in QIIME and Phyloseq (ADONIS test)

Combination	QIIME		Phyloseq			
			Matrix 1		Matrix 2	
	R2	Corrected P-value (FDR)	R2	Corrected P-value (FDR)	R2	Corrected P-value (FDR)
Cs *vs.* IC	0.142	0.120	0.443	**0.010**	0.300	**0.016**
Cs vs. IM	0.200	0.153	0.170	0.076	0.244	**0.049**
Cs vs. PC	0.087	0.519	0.181	0.145	0.101	0.353
Cs vs. SC	0.660	**0.006**	0.493	**0.010**	0.501	**0.015**
Cs vs. SM	0.061	0.714	0.099	0.259	0.164	0.134
IC vs. IM	0.065	0.519	0.189	0.145	0.089	0.353
IC vs. PC	0.122	0.428	0.534	**0.032**	0.284	**0.049**
IC vs. SC	0.393	**0.006**	0.157	0.187	0.180	**0.036**
IC vs. SM	0.080	0.519	0.309	0.076	0.171	0.058
IM vs. PC	0.122	0.428	0.403	**0.025**	0.319	0.058
IM vs. SC	0.550	**0.006**	0.320	**0.025**	0.282	**0.041**
IM vs. SM	0.133	0.348	0.069	0.494	0.058	0.506
PC vs. SC	0.684	**0.017**	0.635	**0.025**	0.498	**0.036**
PC vs. SM	0.071	0.755	0.269	0.117	0.252	0.134
SC vs. SM	0.459	**0.010**	0.356	**0.025**	0.317	**0.037**

SM – stomach mucosa; SC – stomach content; PC – pyloric caeca; Cs – cestodes; IM – intestinal mucosa; IC – intestinal content. The bolded data means significance at $p \leq 0.05$. R2 value is percent of explained dispersion.

804 Table S1. Sample information

Number of fish	Sex	Total length. mm	Standard length. mm	Total weight. g	Eviscerated weight. g
1	Male	193.6	162.4	-	-
2	Female	247.8	210.2	206.8	184.8
3	Female	139.0	116.0	27.0	24.6
4	Male	214.4	180.5	104.8	96.2
5	-	178.1	148.4	67.0	60.6
6	Male	203.4	173.5	119.0	102.8
7	Female	221.0	127.5	134.6	119.0
Mean ± SE		199.6±13.1	159.8±12.2	109.87±25.1	98.00±22.2

805

Table S2. The analysis of similarity (ANOSIM) of relative abundance of genes (KEGG pathway) showing the predicted functional of microbial community of perch and cestodes inhabiting their digestive tract at level 3 according to PICRUST

Level 2	Level 3	SM	SC	PC	IM	IC	Cs
		Mean±SE					
Membrane Transport	ABC transporters	3.87±0.04^{ABC}	**3.33±0.09^{AB}**	3.96±0.07^{ABC}	3.82±0.09^{ABC}	**3.66±0.03^{AB}**	**3.97±0.02^{AC}**
	Bacterialsecretionsystem	0.86± 0.06^{A}	**0.85±0.02^{AB}**	0.94±0.01^{A}	0.98±0.04^{A}	0.90±0.05^{A}	**0.89±0.01^{AC}**
	Phosphotransferasesystem (PTS)	0.80±0.07^{ABC}	**0.28±0.11^{AB}**	0.74±0.01^{ABC}	0.79±0.02^{ABC}	**0.65±0.07^{AB}**	**0.76±0.02^{AC}**
	Secretionsystem	2.24±0.19^{A}	**2.05±0.02^{AB}**	2.53±0.6^{A}	2.11±0.16^{A}	**1.91±0.15^{AC}**	2.51±0.05^{A}
	Transporters	6.75±0.14^{A}	**5.39±0.28^{AB}**	6.72±0.11^{A}	6.54±0.14^{A}	6.21±0.09^{A}	**6.78±0.04^{AC}**
Signaling Molecules and Interaction	Ionchannels	0.04±0.00^{A}	**0.02±0.00^{AB}**	0.04±0.00^{A}	0.02±0.01^{A}	0.03±0.01^{A}	**0.04±0.00^{AC}**
Amino Acid Metabolism	Alanine. aspartate and glutamate metabolism	0.75±0.08^{A}	**0.86±0.00^{AB}**	0.72±0.03^{A}	**0.53±0.11^{AC}**	0.68±0.10^{A}	**0.75±0.02^{AC}**
	Histidinemetabolism	**0.47±0.05^{A}**	**0.57±0.00^{B}**	0.43±0.02^{AB}	**0.34±0.05^{A}**	**0.41±0.05^{A}**	**0.46±0.01^{A}**
	Phenylalanine. tyrosine and tryptophan biosynthesis	0.51±0.06^{A}	**0.66±0.01^{AB}**	**0.52±0.03^{AC}**	0.35±0.09^{A}	0.46±0.09^{A}	**0.54±0.02^{AC}**
	Tryptophanmetabolism	0.43±0.05^{A}	**0.64±0.04^{AB}**	0.49±0.02^{A}	0.36±0.06^{A}	0.38±0.05^{A}	**0.49±0.02^{AC}**
	Valine. leucine and isoleucine biosynthesis	0.59±0.01^{A}	**0.66±0.01^{AB}**	0.57±0.01^{A}	0.62±0.02^{A}	**0.66±0.02^{AB}**	**0.57±0.01 ^{AC}**
	Valine. leucine and isoleucine degradation	0.59±0.06^{A}	**0.95±0.08^{AB}**	0.66±0.02^{A}	0.52±0.07^{A}	0.56±0.06^{A}	**0.65±0.02^{AC}**
Biosynthesis of Other Secondary Metabolites	beta-Lactamresistance	0.02±0.00^{A}	**0.03±0.00^{AB}**	0.02±0.00^{A}	0.01±0.00^{A}	**0.02±0.01^{AC}**	0.02±0.00^{A}
	Novobiocinbiosynthesis	0.11±0.01^{A}	**0.13±0.00^{AB}**	0.11±0.01^{A}	**0.07±0.02^{AC}**	0.10±0.02^{A}	**0.12±0.00^{AC}**
	Penicillinandcephalosporinbiosynthesis	0.03±0.00^{A}	**0.06±0.00^{AB}**	0.03±0.00^{A}	0.02±0.01^{A}	**0.03±0.01^{AB}**	**0.03±0.00^{AC}**
	Phenylpropanoidbiosynthesis	0.05±0.01^{A}	**0.07±0.00^{AB}**	0.04±0.00^{A}	**0.03±0.0**1^{AC}	0.05±0.01^{A}	**0.05±0.00^{AC}**
	Streptomycinbiosynthesis	0.16±0.00^{A}	**0.22±0.01^{AB}**	**0.16±0.00^{AC}**	**0.16±0.00^{AC}**	**0.21±0.02^{AD}**	**0.16±0.00^{ACF}**
Energy Metabolism	Carbon fixation pathways in prokaryotes	0.85±0.05^{A}	**1.04±0.03^{AB}**	**0.83±0.02^{AC}**	0.70±0.08^{A}	0.85±0.09^{A}	**0.86±0.02^{AC}**
	Methanemetabolism	0.92±0.05^{A}	**0.97±0.01^{AB}**	0.84±0.02^{A}	0.96±0.05^{A}	**1.00±0.04^{AB}**	**0.85±0.01^{AC}**
	Oxidativephosphorylation	1.10±0.07^{A}	**1.31±0.05^{AB}**	1.08±0.04^{A}	1.28±0.11^{A}	**1.30±0.08^{AB}**	**1.05±0.02^{AC}**
Lipid Metabolism	Fattyacidbiosynthesis	0.40±0.05^{A}	**0.53±0.01^{AB}**	0.39±0.02^{A}	0.27±0.07^{A}	0.35±0.06^{A}	**0.41±0.02^{AC}**
	Fattyacidmetabolism	0.50±0.07^{A}	**0.78±0.06^{AB}**	0.57±0.03^{A}	0.37±0.01^{A}	0.42±0.09^{A}	**0.58±0.02^{AC}**
	Glycerolipidmetabolism	0.42±0.02^{A}	**0.32±0.02^{AB}**	0.40±0.01^{A}	0.46±0.03^{A}	0.45±0.03^{A}	**0.40±0.01^{AC}**
	Linoleicacidmetabolism	0.03±0.00^{A}	**0.06±0.00^{AB}**	0.03±0.00^{A}	0.02±0.01^{A}	0.03±0.01^{A}	**0.03±0.00^{AC}**
	Lipidbiosynthesisproteins	0.61±0.03^{A}	**0.78±0.03^{AB}**	0.63±0.01^{A}	0.56±0.04^{A}	0.60±0.04^{A}	**0.63±0.01^{AC}**
	Steroidhormonebiosynthesis	0.01±0.00^{A}	**0.02±0.00^{AB}**	0.01±0.00^{A}	0.01±0.00^{A}	0.02±0.0^{A}	**0.01±0.00^{AC}**
	Synthesis and degradation of ketone bodies	0.08±0.01^{A}	**0.19±0.02^{AB}**	0.09±0.01^{A}	0.06±0.02^{A}	0.08±0.02^{A}	**0.09±0.01^{AC}**

Metabolism of Cofactors and Vitamins	Folatebiosynthesis	0.37±0.03^{A}	**0.43±0.00^{AB}**	0.37±0.01^{A}	0.30±0.04^{A}	0.35±0.04^{A}	**0.38±0.01^{AC}**
	One carbon pool by folate	0.45±0.02^{A}	**0.46±0.00^{AB}**	0.42±0.01^{A}	0.47±0.03^{A}	**0.49±0.03^{AB}**	**0.41±0.01^{AC}**
	PantothenateandCoA biosynthesis	0.47±0.02^{A}	**0.52±0.00^{AB}**	0.44±0.00^{A}	**0.44±0.01^{AC}**	**0.49±0.02^{AD}**	**0.45±0.00^{ACE}**
	Riboflavinmetabolism	0.28±0.01^{A}	**0.26±0.00^{AB}**	0.29±0.00^{A}	**0.28±0.00^{AC}**	0.27±0.00^{A}	**0.28±0.00^{AC}**
	Thiaminemetabolism	**0.32±0.03^{AC}**	**0.34±0.00^{AB}**	0.27±0.00^{A}	**0.29±0.00^{AC}**	0.33±0.01^{A}	**0.28±0.00^{AC}**
Metabolism of Other Amino Acids	D-Glutamine and D-glutamate metabolism	0.10±0.02^{A}	**0.11±0.00^{AB}**	0.09±0.01^{A}	0.06±0.02^{A}	0.09±0.02^{A}	**0.10±0.00^{AC}**
Metabolism of Terpenoids and Polyketides	Biosynthesis of siderophore group nonribosomal peptides	0.06±0.01^{A}	**0.04±0.00^{AB}**	0.08±0.01^{A}	0.05±0.01^{A}	0.05±0.01^{A}	**0.08±0.00^{AC}**
	Biosynthesis of vancomycin group antibiotics	0.02±0.00^{A}	**0.04±0.00^{AB}**	0.02±0.00^{A}	0.01±0.00^{A}	0.03±0.01^{A}	**0.02±0.00^{AC}**
	Carotenoidbiosynthesis	0.01±0.00^{A}	**0.04±0.01^{AB}**	0.01±0.00^{A}	0.01±0.00^{A}	**0.03±0.01^{AB}**	**0.01±0.00^{AC}**
	Polyketidesugarunitbiosynthesis	0.07±0.01^{A}	**0.13±0.01^{AB}**	0.07±0.00^{A}	**0.05±0.01^{AC}**	0.09±0.02^{A}	**0.07±0.00^{AC}**
	Prenyltransferases	0.19±0.03^{A}	**0.27±0.01^{AB}**	0.17±0.01^{A}	**0.12±0.03^{AC}**	0.19±0.04^{A}	**0.19±0.01^{AC}**
	Terpenoidbackbonebiosynthesis	0.34±0.05^{A}	**0.45±0.01^{AB}**	0.29±0.02^{A}	**0.21±0.06^{AC}**	0.31±0.06^{A}	**0.31±0.01^{AC}**
	Tetracyclinebiosynthesis	0.12±0.02^{A}	**0.15±0.00 ^{AB}**	0.11±0.01^{A}	0.08±0.02^{A}	0.11±0.02^{A}	**0.12±0.00^{AC}**
	Zeatinbiosynthesis	0.02±0.00^{A}	**0.03±0.00 ^{AB}**	0.02±0.00^{A}	**0.01±0.00^{AC}**	0.02±0.00^{A}	**0.02±0.00^{AC}**

809 Upper-case and extra bold indicates significance at $p \leq 0.05$.

Table S3. The occurrence of bacteria associated with different fish host and parasites using the methods of transmission and scanning electron microscopy

Fish	Parasite	Localization of parasites	Helminth microbiota	Fish microbiota	References
Whiting *Merlangius merlangus*	*Diclidophora merlangi* (Monogenea: Diclidophoridae)	Gills	Mycoplasmas and mycoplasma-like bacteria	Not observed	Morris and Halton 1975
Blackspotted stickleback *Gasterosteus wheatlandi*	*Gyrodactylus avalonia* (Monogenea. Gyrodactylidae)	Fin and skin	Rod-shaped bacteria	Not observed	Cusack and Cone 1985
Rabbit fish *Siganus doliatus*. *Siganus corallinus*. *Siganus puellus* and *Siganus lineatus*	*Gyliauchenna haensis* (Trematoda. Gyliauchenidaea)	Hindgut	Spirochaetes and different morphological forms of very small bacteria with 0.25-0.3 nm in diameter	Not observed	Hughes-Stamm et al. 1999
Bream *Abramis brama*	*Caryophyllaeus laticeps* (Cestoda: Caryophyllidea)	Intestine	Chains of vibrio-like cells	Not observed	Poddubnaya and Izvekova 2005
Khramulya *Varicorhinus capoeta sevangi*	*Khawia armeniaca* (Cestoda: Caryophyllidea)	Intestine	Chains of vibrio-like cells	Not observed	Poddubnaya and Izvekova 2005
Burbot *Lota lota*	*Eubothrium rugosum* (Cestoda: Pseudophyllidea)	Pyloric caeca	Gram-positive and gram-negative bacteria: rod-shaped cells with pointed ends, chains of rod-shaped cells and cocci	Not observed	Poddubnaya 2005
Pike *Esox lucius*	*Triaenophorus nodulosus* (Cestoda. Triaenophoridae)	Intestine	Rodlike bacteria, Coccoid bacteria, Spirochetes, Bacteria of uncommon form	Not observed	Korneva and Plotnikov 2006
Pike *Esox lucius*	*Triaenophorus nodulosus* (Cestoda. Triaenophoridae)	Intestine	Bacilli, cocci	Aeromonas. Vibrio. Pseudomonas. Shewanella. Hafnia. and Yersinia. Carnobacterium	Korneva and Plotnikov. 2012
Stone loach *Barbatulabarbatula*	*Proteocephalus torulosus (Cestoda. Proteocephalidae)*				
Perch *Perca fluviatilis*	*P. percae (Cestoda. Proteocephalidae)*				
Ruffe *Gymnocephalus cernuus*	*P. cernuae (Cestoda. Proteocephalidae)*				

814 Table S4. The occurrence of bacteria associated with different fish host and parasites using
815 molecular methods

Fish	Parasite	Localization of parasites	Helminth microbiota	Fish microbiota	Method	References
Brown-marbled groupers *Epinephelus fuscoguttatus*	Multiple species of ecto- and endo-parasites	Intestinal content	Not observed	Gammaproteobacteria, Fusobacteria, Clostridia, and Betaproteobacteria	16S rRNA V4 region	Hennersdorf et al. 2016
Sixbar groupers *Epinephelus sexfasciatus*	Multiple species of ecto- and endo-parasites	Intestinal content	Not observed	Betaproteobacteria, Clostridia, Gammaproteobacteria, and Alphaproteobacteria	16S rRNA V4 region	Hennersdorf et al. 2016
Yellowtales cads *Atule mate*	Multiple species of ecto- and endo-parasites	Intestinal content	Not observed	Betaproteobacteria, Alphaproteobacteria	16S rRNA V4 region	Hennersdorf et al. 2016
Atlantic Salmon *Salmo salar*	*Lepeophtheirus salmonis* (Crustacea. Caligidae)	Skin	*Vibrio*, NS10 marine group (family Cryomorphacae). *Arcobacter*, Rhizobiales, *Tenacibaculum*, *Pseudomona*, *Aeromonas*	In skin: *Tenacibaculum*, *Pseudomonas*, *Lewinella*, *Vibrio*, *Flavobacterium*	16S rRNA V4 region	Llewellyn et al. 2017

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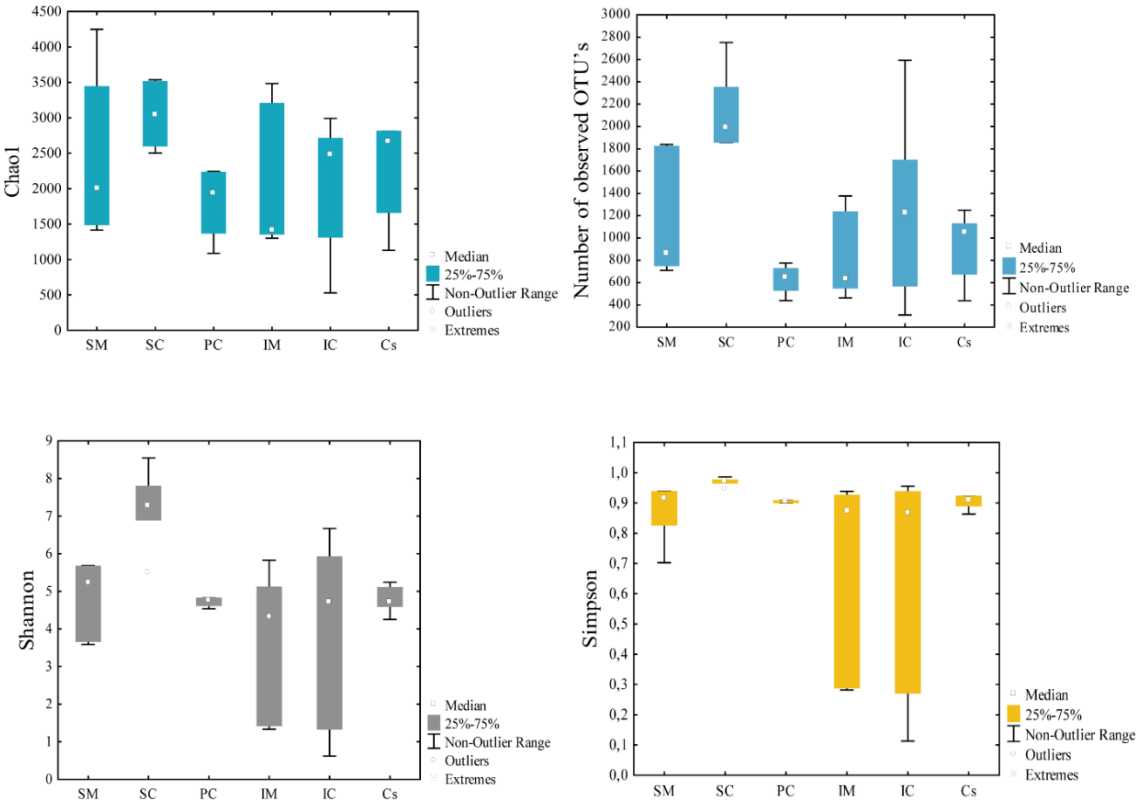


Figure 1. Diversity analysis of microbial community in the gastrointestinal tract of perch and cestodes parasitizing their digestive tract. SM – stomach mucosa; SC – stomach content; PC – pyloric caeca; Cs – cestodes; IM – intestinal mucosa; IC – intestinal content. The lower case character indicates significance at $p \leq 0.05$.

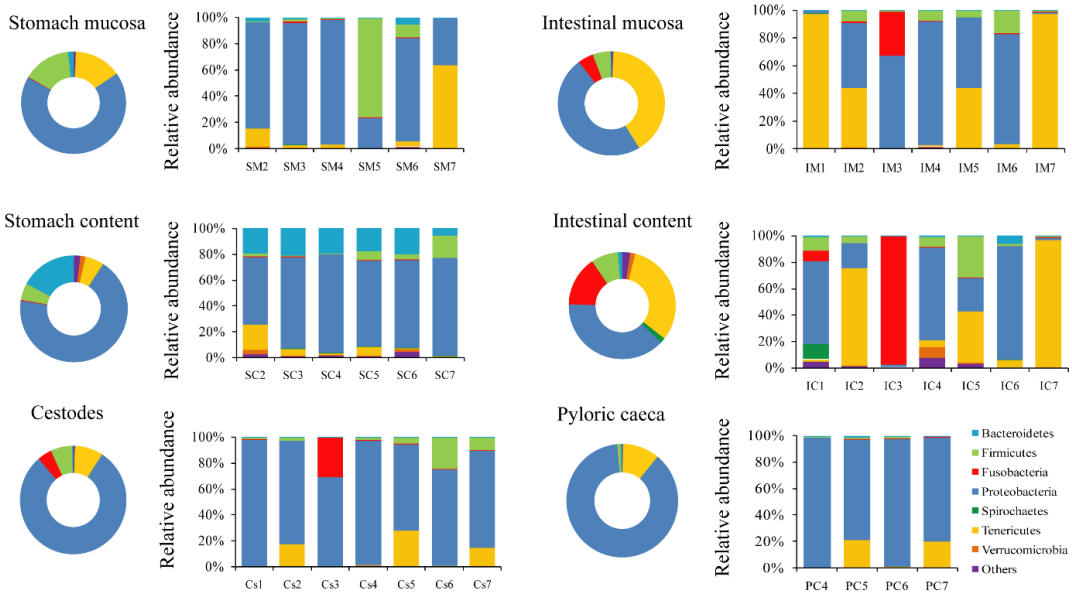


Figure 2. Phylum composition of microbiota from gastrointestinal tract of perch and cestodes parasitizing their digestive tract.

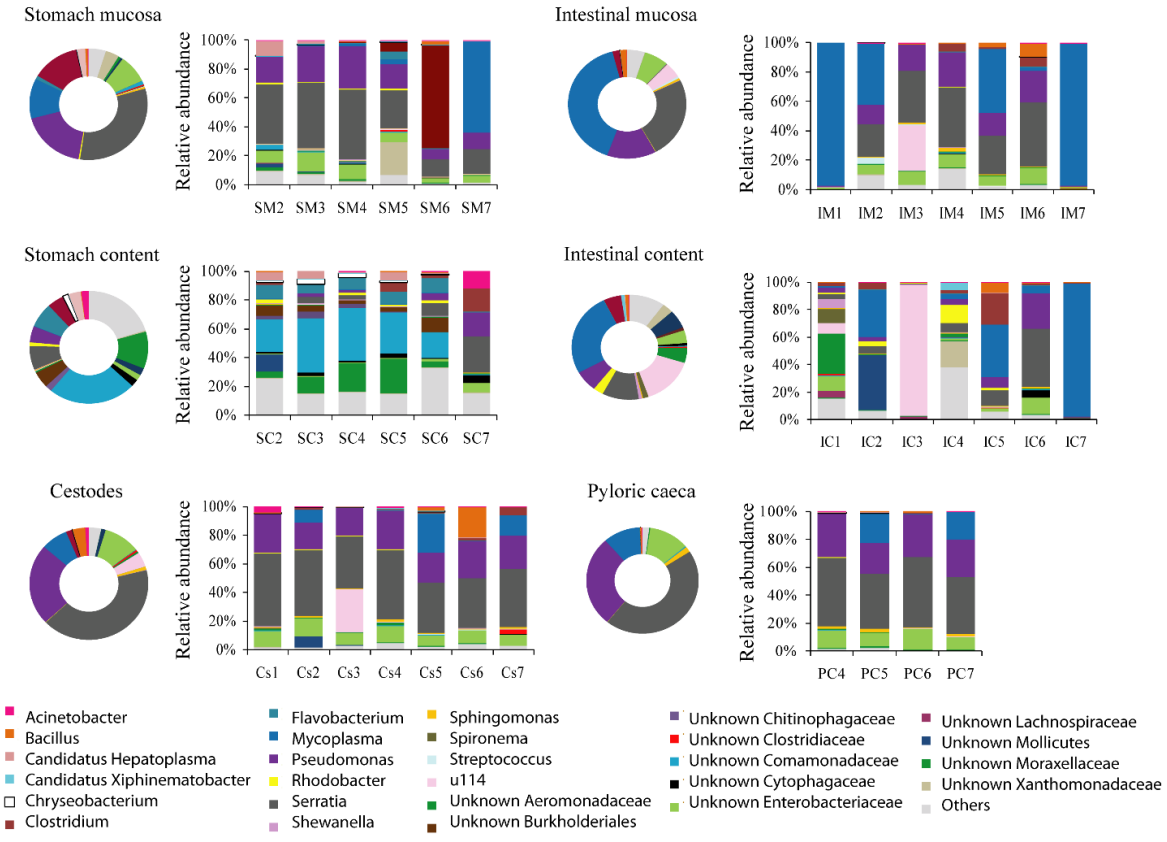
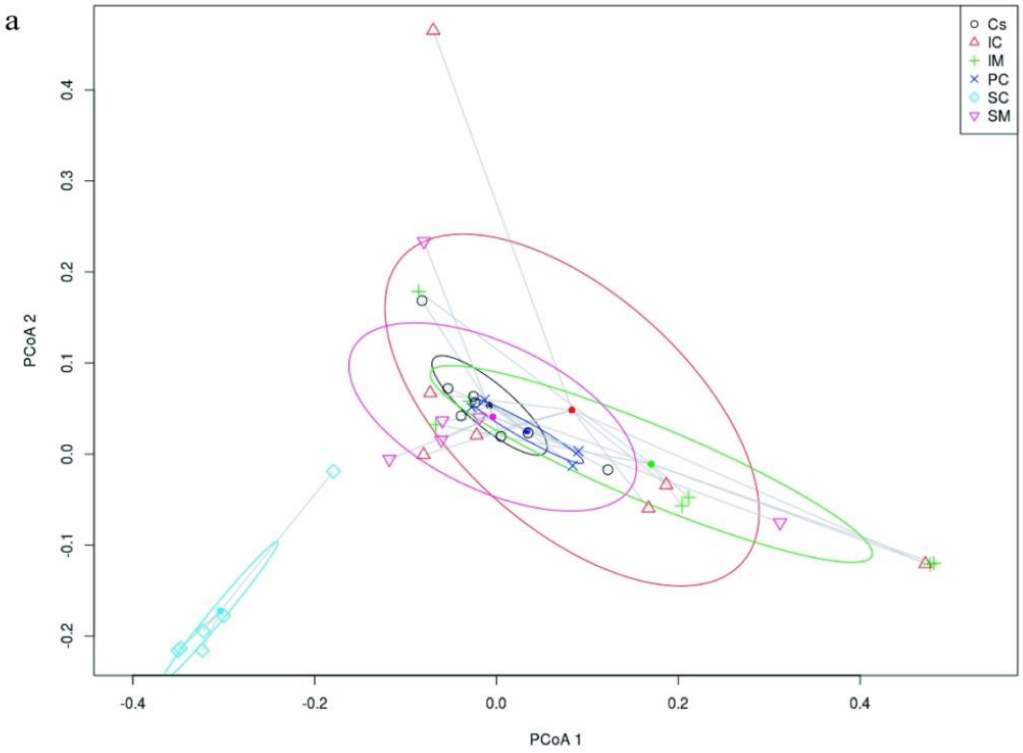


Figure 3. Genus and family proportions of microbial communities identified in different parts of the gastrointestinal tract of perch and their parasites (cestodes).


a
Cs
IC
IM
PC
SC
SM
PCoA 2
PCoA 1
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2
0.4

833

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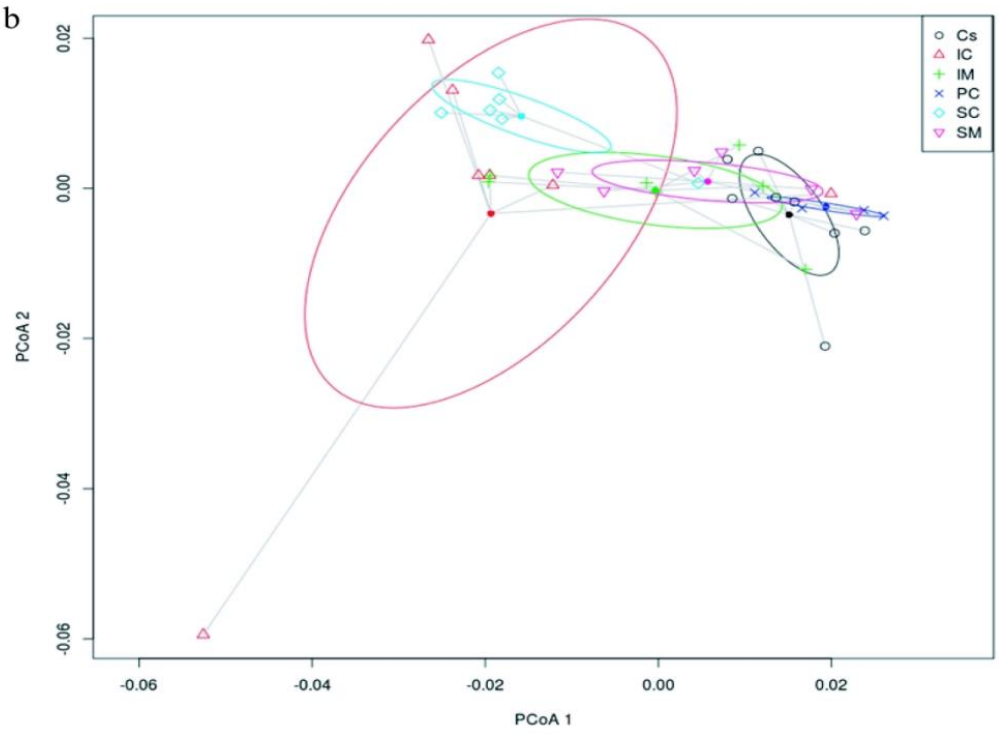
b
Cs
IC
IM
PC
SC
SM
PCoA 2
PCoA 1
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0.00
-0.02
-0.04
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-0.06
-0.04
-0.02
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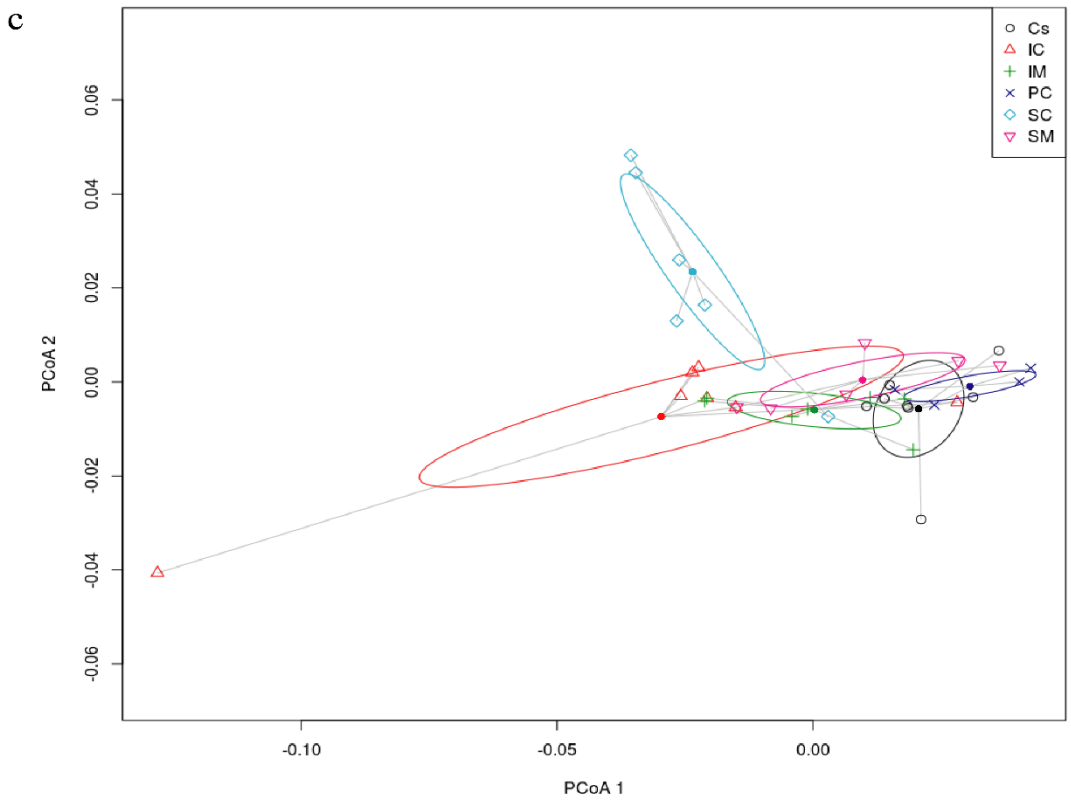


Figure 4. Principal coordinates analysis (PCoA) for microbiota associated with intestine of perch and their cestode parasites.SM – stomach mucosa; SC – stomach content; PC – pyloric caeca; Cs – cestodes; IM – intestinal mucosa; IC – intestinal content. a - QIIME matrix; b – Phyloseq matrix 1; c – Phyloseq matrix 2.

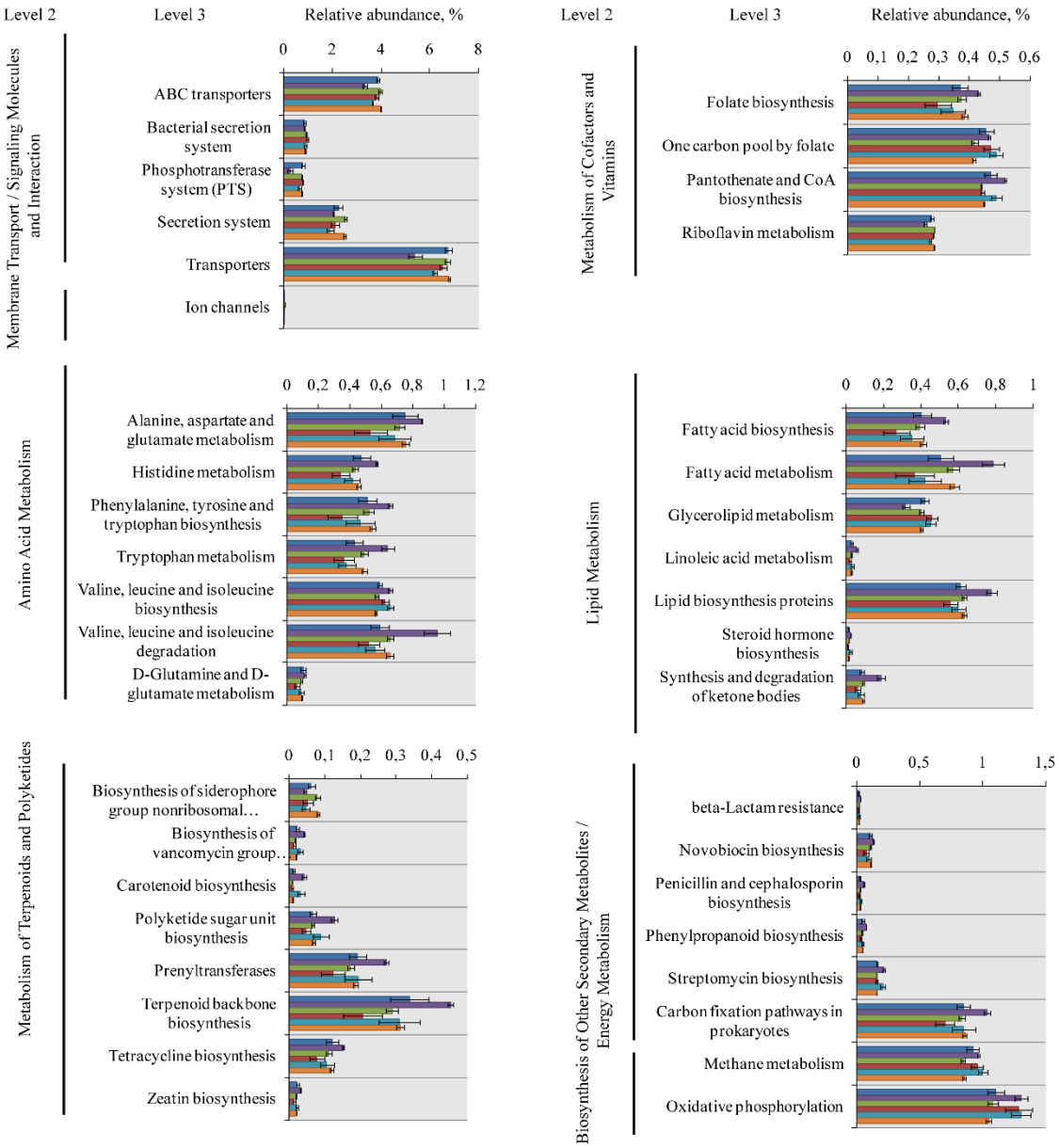
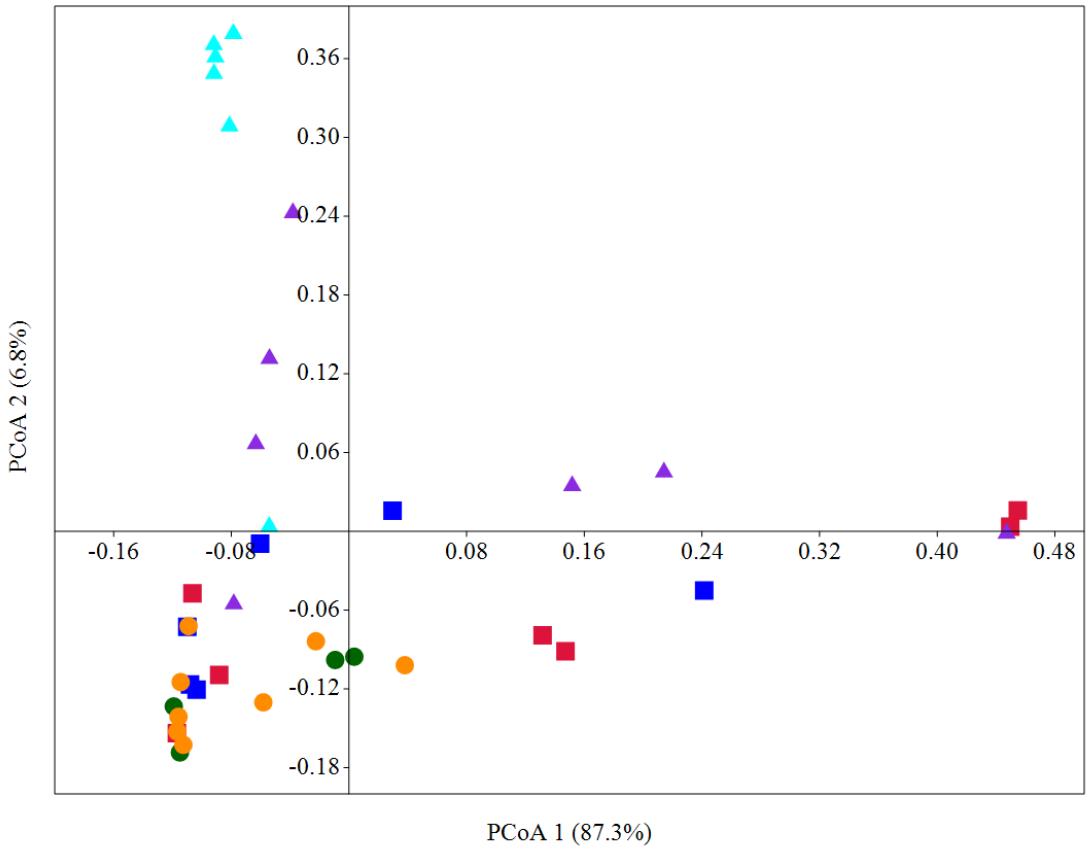


Figure 5. Predicted functional metagenomic pathways for stomach and intestinal mucosa and content of perch and cestodes at level 2 and 3. Blue – stomach mucosa; violet – stomach content; green – pyloric caeca; red – intestinal mucosa; light blue – intestinal content; orange – cestodes.



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848 Figure 6. Principle coordinates analysis (PCoA) of potential metabolic role for studied
849 microbiota. Red – intestinal mucosa. blue – stomach mucosa. violet – intestinal content. light blue
850 – stomach content. green – pyloric caeca and orange – cestodes.

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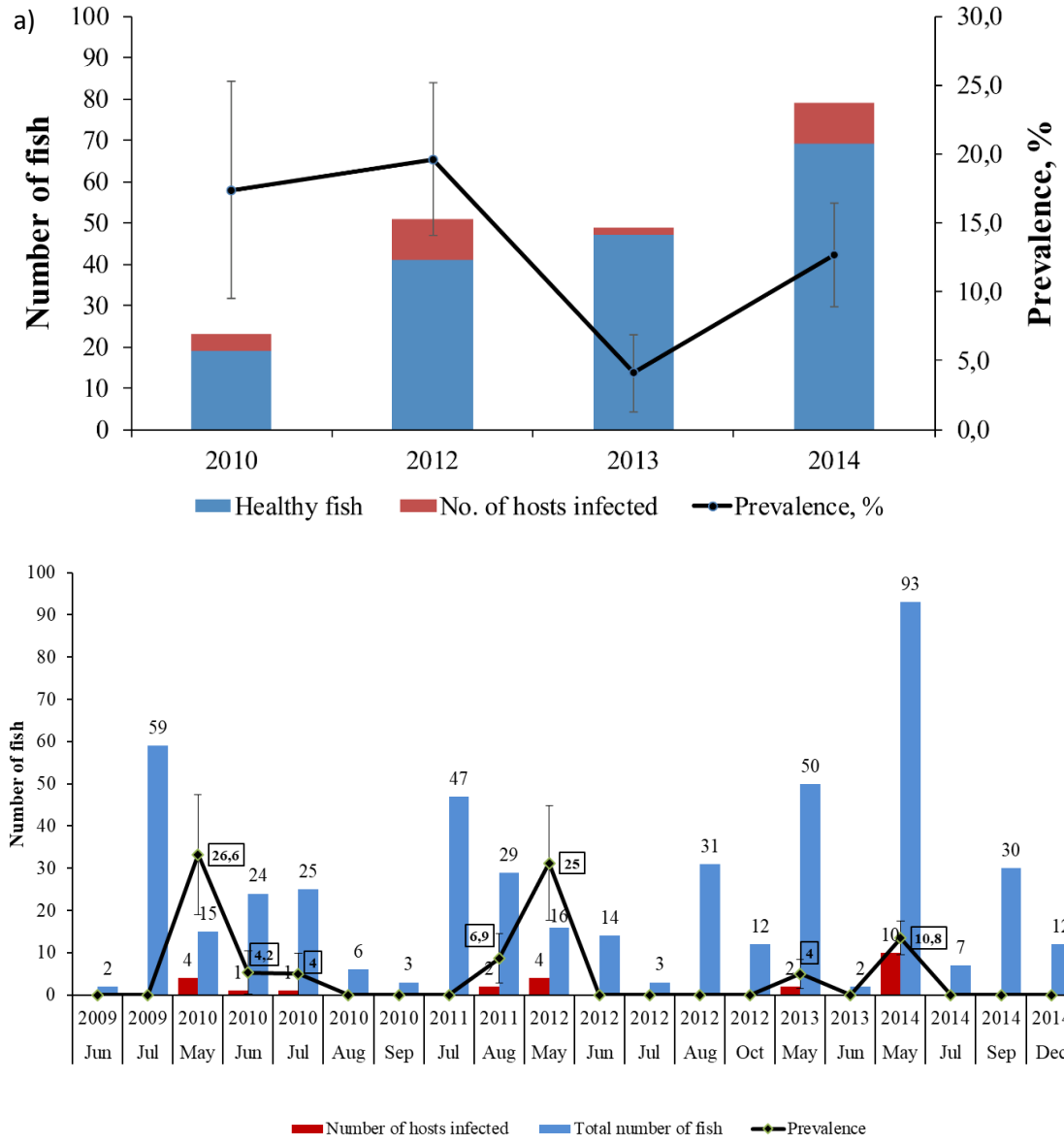

Figure S1. Prevalence of *Proteocephalus* sp. infestation on different sampling dates. a) change in prevalence during different years of May; b) change in prevalence with month of the year from 2009-2014.

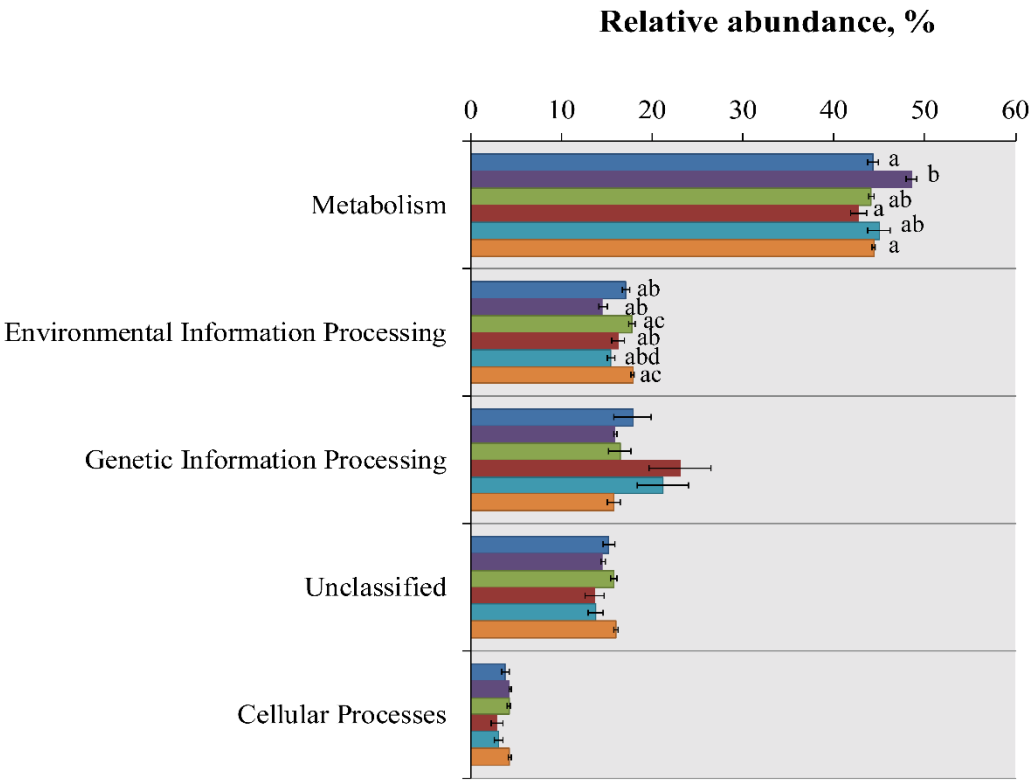

Figure S2. Predicted functional metagenomic pathways of fish gut and cestodes identified by PICRUSt at level 1. Significant differences (lower case letters) in gene categories were analyzed by ANOSIM. The differences were significant at $p \leq 0.05$. Blue – stomach mucosa; violet – stomach content; green – pyloric caeca; red – intestinal mucosa; light blue – intestinal content; orange – cestodes.

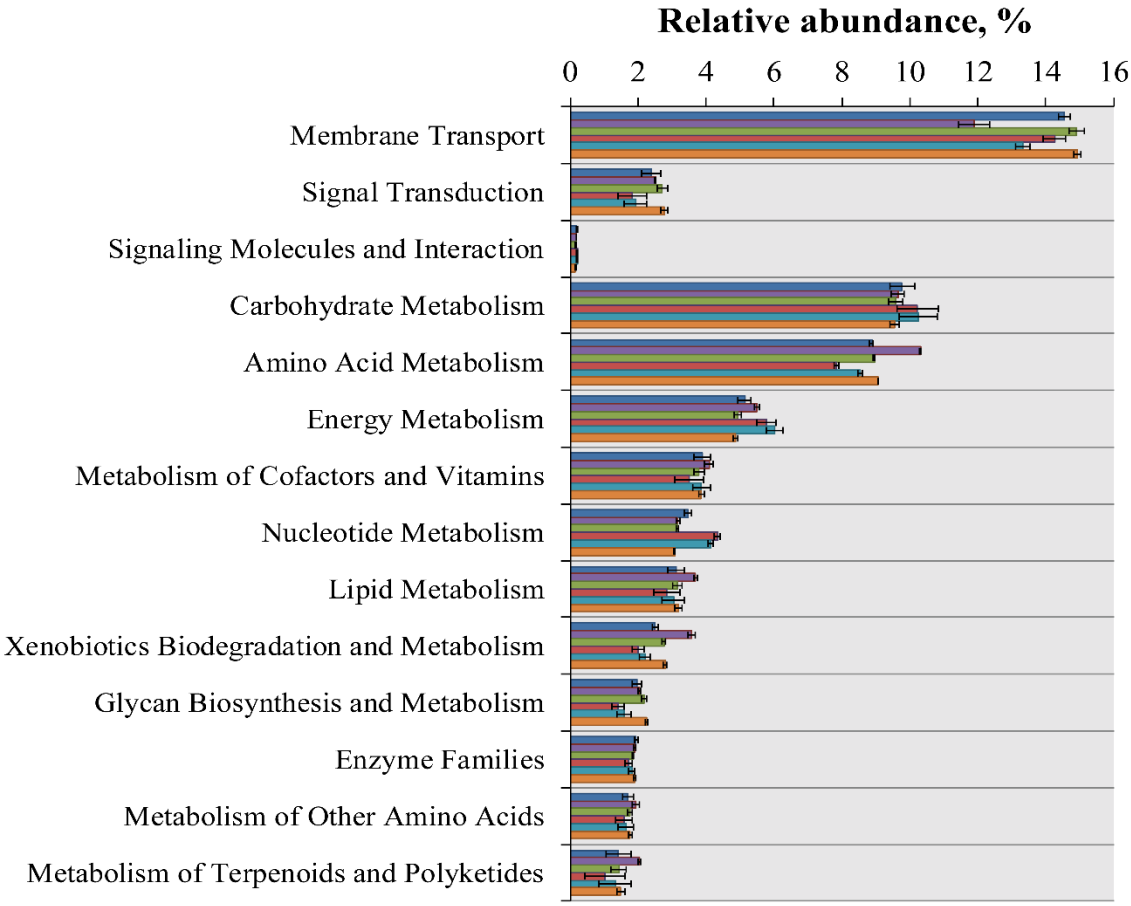

Figure S3. Predicted functional metagenomic pathways for stomach and intestinal mucosa and content of perch and cestodes at level 2. Blue – stomach mucosa; violet – stomach content; green – pyloric caeca; red – intestinal mucosa; light blue – intestinal content; orange – cestodes.