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Original Research

Abundant Bacteria in the Proximal and Distal Intestine of Healthy Siberian Sturgeons (*Acipenser baerii*).

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ABSTRACT

The gut microbiota plays a key role in animal health, including immune and metabolic homeostasis. Despite the economic relevance of the sturgeon, studies addressing the gut microbiome of this species are scarce and have focused only on the hindgut. The objective of this work was to use Illumina metabarcoding technology to compare the bacterial microbiomes of two different intestinal locations, the proximal intestine (small intestine) and the distal intestine (hindgut containing the spiral valve), of healthy Siberian sturgeons (*Acipenser baerii*) reared on two different farms in Spain. Although a high degree of interindividual variability was observed, certain differences between the anatomical parts and between the geographical locations were clear. The results show that the sequences corresponding to the most abundant taxa were the basis for clustering according to anatomical region (proximal and distal intestine), whereas the less abundant taxa were the basis for clustering according to the geographical location of the fish farms. Finally, the results also indicate the existence of certain bacteria that are present in the intestinal tracts of all the fish analyzed in this study; this information may be useful in future studies aiming to establish differences based upon fish health or disease.

KEYWORDS: Sturgeon microbiomes, site-specific gut microbiota, gut bacterial communities, bacteria in healthy fish, development of probiotics in aquaculture.

1. INTRODUCTION

Several studies have demonstrated the importance of the intestinal bacterial composition for animal health due to its crucial role in protection against infectious diseases and in maintaining host immune and metabolic homeostasis (Llewellyn et al., 2014; Ringø et al., 2016). In fact, alteration of the gut microbiota (dysbiosis) has been associated with the development of many infections and inflammatory diseases in a wide spectrum of animals, including fishes (Wang et al., 2017). Therefore, many strategies based on the manipulation of intestinal bacteria have been proposed or developed in recent years to improve animal health.

Sturgeon farming has become an important industry around the world due to the high market price of caviar. At present, farming is the only way to obtain sturgeons since they have become extinct in most of their past natural habitats. There are approximately 30 different species of sturgeons, but the primary farmed species, which belong to the genus *Acipenser* (Order Acipenseriformes, Family *Acipenseridae),* are the Siberian sturgeon (*Acipenser baerii*), the Adriatic sturgeon (*Acipenser naccari*) and the Russian or Danube sturgeon (*Acipenser gueldenstaedti*); these species are optimal for farming because of their relatively short reproductive cycles (Williot et al., 2005). All these species were included within the categories of endangered and critically endangered species on the 2010 International Union for the Conservation of Nature and Natural Resources (IUCN) Red List. Infectious diseases involving high mortality rates are one of the primary problems in intensive aquaculture systems due to the high number of animals living in very close contact in the same tank. Disease outbreaks with high mortality rates (50 to 90%) caused by pathogenic viruses and bacteria have been described in farmed sturgeons (Ciulli et al., 2016; Kayiş et al., 2017; Yang et al., 2018). In aquaculture, the prevention and control of many infectious diseases is a complex, difficult and, sometimes, controversial problem that involves practical limitations, including legal restrictions on the use of prophylactic or therapeutic drugs, the limited protection provided by available vaccines and the stress often associated with their administration. In this context, the development of effective intensive production methods for sturgeon farms requires new strategies, such as the use of target-specific probiotics to prevent infectious diseases. Some preliminary studies have shown that the use of prebiotics and probiotics may be promising for sturgeon farms (Akrami et al., 2013; Pourgholam et al., 2016; Fei et al., 2018). However, the development of pre- and probiotics to improve future sturgeon aquaculture must be guided by an improved understanding of the gut microbiota of healthy sturgeons in the specific conditions where the fish live and grow (temperature, water, diet and other factors).

Despite the economic importance of sturgeon farming, the study of the intestinal microbiota of these species has been limited to the hindgut and the impact of prebiotics or probiotics on specific bacterial groups (Geraylou et al., 2012; Geraylou et al., 2013a; Geraylou et al., 2013b). In contrast, the bacterial composition in other important intestinal regions such as the small intestine (Buddington and Doroshov, 1986; Daprà et

al., 2009) has not yet been studied. We designed this study to improve our knowledge of the intestinal microbiota in healthy sturgeons and the subsequent development of pre- and probiotics in the aquaculture of this species. The objective of this study was to use Illumina technology to compare the bacterial microbiome of two different intestinal locations, the proximal intestine (or small intestine) and the distal intestine (or hindgut), of Siberian sturgeons (*A. baerii*) reared on two different farms in Spain with different diets and environmental conditions.

2. MATERIAL AND METHODS

2.1. Fish Samples.

Twelve healthy adult female Siberian sturgeons (*Acipenser baerii*) weighing 1.5 to 2 kg were randomly obtained from two different fish farms in summer (6 individuals from each farm). The farms were located in the northeast of Spain (40°08′29″N 0°48′51″W and 42°09′05″N 0°13′23″E). The fish were fed commercial feed: Aller Metabolica (Aller Aqua, Denmark) on farm 1 and Efico Sigma (BioMar, France) on farm 2.

The compositions of the diets administered to the fish in each location are shown in Table 1. The animals were held in outdoor ponds with a capacity of 50 m^3 (farm 1) and 380 m^3 (farm 2) and without recirculation of water. The water quality of each facility was checked periodically (Table 2). Routine microbiological analysis of water samples was carried out using Endo Agar plates for the detection of total coliform bacteria, tryptone bile X-glucuronide (TBX) agar plates for the detection of fecal coliform bacteria, and Slanetz and Bartley agar plates for the detection of enterococci. All media were purchased from Sigma-Aldrich (St. Louis, USA) and prepared following the instructions of the manufacturer.

The health status of the fish was verified by examination of behavioral signs (swimming and feeding responses). Prior to sampling, the animals did not experience antibiotic treatment or vaccinated and did not experience any physiological stress. The individuals were euthanized with tricaine methane-sulfonate (Tricaine Pharmaq, 1000 mg/g), and necropsies were performed. The following two samples from each animal were collected aseptically: (a) the content of the small intestine, or proximal intestine (PI), which is the portion of the gut between the pyloric caecum and the spiral valve; and (b) the content of the hindgut, or distal intestine (DI), which contains the spiral valve (Figure 1). The samples were processed immediately after collection.

2.2. DNA Extraction.

For the DNA extraction, the intestinal contents of the DI and PI samples of each animal were first centrifuged at 13,000 rpm for 10 min at 4ºC. The resulting pellets were washed with TE pH 8 (10 mM Tris-HCl, 50 mM EDTA); resuspended in 5mL of TE supplemented with lysozyme (5 mg/mL), mutanolysin (25,000 U/mL) and

lysostaphin (4,000 U/mL); and incubated at 37ºC for 90 min. Then, the samples were mechanically lysed using FastPrep-24 (MP Biomedicals, Solon, USA) and glass beads matrix tubes (4 cycles × 30 s, speed 5). After centrifugation, the protein fraction of the supernatant was eliminated with a proteinase K treatment (250 µg/mL) at 56ºC for 30 min. Finally, the DNA was extracted using the QIAamp DNA Stool Kit (Qiagen, Hilden, Germany). The extracted DNA was eluted with 22 µL of nuclease-free water and stored at -20º C until further analysis.The DNA concentration was estimated using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Inc., Rockland, USA).

2.3. PCR Amplification and Sequencing.

A dual-barcoded 2-step PCR was conducted to amplify a fragment of the V3–V4 hypervariable region of the bacterial 16S ribosomal RNA (rRNA) gene. Equimolar concentrations of the universal primers S-D- Bact-0341-b-S-17 (ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG) and S-D-Bact-0785-a-A-21 (TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC) were used as previously described (Klindworth et al., 2013), generating amplicons of approximately 464 bases from the V3-V4 hypervariable region. The primers were synthesized by Isogen Life Sciences (Castelldefels, Spain). Barcodes used for Illumina sequencing were appended to the 3′ and 5′ ends of the PCR amplicons to allow the separation of forward and reverse sequences. A bioanalyzer (2100 Bioanalyzer, Agilent) was used to determine the concentration of each sample. Barcoded PCR products from all samples were pooled at approximately equimolar DNA concentrations and subjected to preparative electrophoresis on an agarose gel. The band of the correct size was excised and purified using a QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany) and then quantified with PicoGreen (BMG Labtech, Jena, Germany). Finally, one aliquot of pooled, purified, barcoded DNA amplicons was sequenced using the Illumina MiSeq paired-end protocol (Illumina Inc., San Diego, CA, USA) at the facilities of the Parque Científico de Madrid (Spain).

2.4. Taxonomic Classification, Statistical and Bioinformatic Analysis.

The amplified fragments and results were taxonomically analyzed using the Illumina™ software according to the manufacturer's guidelines and pipelines (software version: 2.6.2.3).The resulting high-quality reads were assembled and classified taxonomically into operational taxonomic units (OTUs) by comparison with the Illumina-curated version 1.0.1 of the Greengenes taxonomic database, using a Bayesian classification method and a similarity level of at least 97%. Only OTUs representing more than 1% of the sequences in at least one of the samples were retained in the diversity analyses.

The relative abundances (%) of the most abundant bacterial sequences at different taxonomical levels in DI and PI samples from each farm were analyzed with t-test using IBM™ SPSS™ Statistics (version 25).

A bioinformatic analysis was also conducted with a combination of the R (v 3.2.3) and QIIME pipelines (v 1.8.0) (Caporaso et al., 2010). Estimates of intrasample diversity were made at a rarefaction depth of 69,000 reads per sample (size of the smallest sample). Alpha diversity was assessed with the Shannon diversity index (SDI), which considers the number and evenness of microbial species, using ANOVA. The richness distribution level (rarefaction curves) was analyzed using the Monte Carlo method. Beta diversity was studied using the principal component analysis (PCA) to visually display patterns of beta diversity through a distance matrix containing a dissimilarity value for each pairwise sample comparison. For the quantitative and qualitative analyses, the Bray-Curtis index and binary Jaccard distance were used, respectively. PERMANOVA, a multivariate statistical analysis of variance (Anderson, 2005) with 999 permutations, revealed statistically significant differences ($p<0.05$).

The linear discriminant analysis (LDA) effect size (LEfSe) algorithm (Segata et al., 2011) was performed with the online interface Galaxy (Afgan et al., 2018) to compare the relative abundance of all bacterial clades using the nonparametric Kruskal-Wallis test at a predefined α of 0.05. In this method, significantly different vectors resulting from the comparison of abundance between groups are used as input for LDA, which produces an effect size. The primary advantage of LEfSe over traditional statistical tests is that an effect size is produced in addition to a *p*-value. This addition allows the results of multiple tests to be sorted by the magnitude of the difference between groups. In the case of hierarchically organized bacterial clades, there may be a lack of correlation between p-value and effect size due to differences in the numbers of hypotheses considered at various levels, since a greater number of comparisons would be required at the genus and species levels than at the phylum and class levels.

Venn diagrams were plotted using the limma statistical package in R (Ritchie et al., 2015).

3. RESULTS

3.1. Comparison of Diet and Water Quality between Farms.

The differences in the primary nutritional components of the granulated diets used to feed the sturgeons on each farm are shown in Table 1. The two diets contained similar amounts of antioxidants, additives (vitamins A and D3), trace elements and cellulose and differed only in protein and fat percentages. The water quality was monitored twice daily on both farms, and the data obtained when the animals were collected are shown in Table 2. At the time of fish collection, the values of all tested parameters were within the ideal range for sturgeon production; however, differences were observed in the levels of dissolved oxygen, conductivity, chloride ions and nitrates. In addition, the total coliform count (which includes the species belonging to the genera *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella* and *Serratia*) was 3-fold higher on farm 2 than on farm 1 (Table 2).

3.2. Gut Bacterial Microbiome.

A total of 2,748,504 high quality-filtered sequences were obtained from the 24 samples analyzed in this study, and the number of sequences ranged from 69,826 to 274,121 per sample. The following 6 phyla were detected on both of the farms analyzed in this study: Proteobacteria, Firmicutes, Fusobacteria, Actinobacteria, Bacteroidetes and Cyanobacteria (Figure 1). The first three phyla were the most abundant in the gut samples from the two farms, independent of diet. In addition, the percentage of unclassified bacteria was higher in the animals from farm 2 (Figure 1) than in those from farm 1 (average of 1.61% in DI samples and 10.94% in PI samples, Table 3) Globally, both the diversity and the relative abundance of unclassified bacteria in the gut samples were higher among the individuals from farm 2 than among those from farm 1. The opposite trend was observed for the phylum Fusobacteria, for which the relative abundance was higher among the fish from farm 1 than among those from farm 2 (Figure 1).

On both farms, the phylum Firmicutes was represented by three classes (Clostridia, Erysipelotrichia and Bacilli), Clostridia being the dominant class in all samples except for one PI sample from farm 2 (Tables 3 and 4). In relation to the phylum Proteobacteria, the classes α- and γ-Proteobacteria were the most representative classes on both farms (Tables 3 and 4). All bacterial sequences from the phyla Fusobacteria and Actinobacteria corresponded to the classes Fusobacteria and Actinobacteria, respectively (Tables 3 and 4).

At the genus level, the most abundant and widely distributed genera were *Cetobacterium* within the phylum Fusobacteria, *Clostridium* and *Sarcina* within the phylum Firmicutes, and *Ochrobactrum* and *Mesorhizobium* within the phylum Proteobacteria (Tables 5 and 6).

Finally, the most abundant bacterial species in the PI and DI of fish from the two farms are shown in Tables 5 and 6. Among these species, *Cetobacterium somerae* was particularly abundant in most PI samples, and *Ochrobactrum thiophenivorans* and *Mesorhizobium septentrionale* were abundant in certain DI samples (Tables 5 and 6).

3.3. Comparison of Intestinal Regions and Farms.

The taxonomic composition of the gut bacterial communities differed between the two intestinal regions on both farms (Figure 1). The abundance of sequences related to the phyla Fusobacteria and Cyanobacteria was significantly higher in PI samples than in DI samples on both farms. Within the first phylum, the class Fusobacteria was significantly more abundant in PI than in DI samples (40.01±8.82 *versus* 11.13±4.98% on farm 1 and 15.84±6.52 *versus* 1.71±0.60% on farm 2 at a significance threshold of $p<0.005$) (Tables 3 and 4). The phylum Firmicutes was more abundant in DI samples than in PI samples on both farms, and the difference was especially prominent on farm

2 (26.14±6.67% in DI versus 13.22±6.7% in PI; Table 4). Sequences of the phylum Proteobacteria were also approximately two-fold more abundant in the DI than in the PI on farm 1 (Figure 1; Table 3). These DI samples had the greatest relative abundance of α-Proteobacteria (63.22±20.62 *versus* 7.65±4.07 in PI samples were statistically significant at $p<0.05$; Table 3) and contained the lowest number of sequences related to unclassified bacteria (1.61±0.72%). On farm 2, the abundance of Proteobacteria was similar in PI samples and DI samples (Table 4), although the relative abundance of α-Proteobacteria was higher in the DI than in the PI of the animals from this farm (26.48±14.69 *versus* 3.49±1.55 in PI samples; Table 4). Sequences belonging to the phyla Actinobacteria and Cyanobacteria were almost absent in the DI samples from farm 1 (Figure 1; Table 3).

At the taxonomic level of bacterial species, OTUs that were shared between the two gut locations and OTUs that were detected in only one gut location were identified (Tables 5 and 6). In farm 2, certain species belonging to the class γ-Proteobacteria (*Plesiomonas shigelloides*, *Shewanella profunda*, *Shewanella oneidensis*, *Serratia entomophila*, and *Tolumonas auensis*) exhibited a higher relative abundance in PI samples than in DI samples (Table 6). *C. somerae* was more abundant in PI samples than in DI samples from both farms; however, as stated above, the opposite trend was observed for *O. thiophenivorans* and *M. septentrionale*, which seemed to be specifically associated with the DI in both farms (Tables 5 and 6). Similarly, sequences corresponding to *Achromobacter xylosoxidans* and *Achromobacter piechaudii* were detected only in DI samples, but their frequency of detection and abundance were notably lower than those of *O. thiophenivorans* and *M. septentrionale* (Tables 5 and 6).

Within the same gut portion, certain farm-dependent differences were also detected. Briefly, sequences related to the phylum Fusobacteria, the class Fusobacteria, the genus *Cetobacterium*, and the class α-Proteobacteria were more abundant on farm 1 than on farm 2, whether in PI or DI samples (Tables 3 and 4). Sequences belonging to the classes Clostridia, Bacilli and Actinobacteria were more abundant in PI samples from farm 1 than from farm 2, while sequences corresponding to the class Erysipelotrichia were more abundant on farm 1 than on farm 2, but only in the DI samples. Sequences corresponding to the class Bacteroidia were detected only in the DI samples of two animals from farm 1 (Tables 3 and 4). In contrast, sequences of the class γ-Proteobacteria were more abundant on farm 2 than on farm 1, both in PI and in DI samples. Additionally, sequences from the classes Actinobacteria and Bacilli were more abundant in the DI samples from farm 2 than in those from farm 1, while sequences of the class Erysipelotrichia were more abundant only in the PI samples on farm 2 (Table 4). At the species level, *Sarcina ventriculi* was detected only in gut samples from farm 2, being particularly widespread (4 out of 6 animals) and abundant in the DI samples (n=4; 7.99±1.16%). *Methylobacterium goesingense* (11.27%) and *Yersinia nurmii* (15.10%) were highly abundant in the DI sample of one animal from farm 2 (Tables 5 and 6).

Despite the interindividual differences observed in this study, the bioinformatic analysis of the data revealed that the microbial species richness and the alpha diversity were significantly higher among sturgeons from farm 2 than among those from farm 1 (p=0.043) (Figure 2). A principal coordinate analysis (PCoA) of the Bray-Curtis distance matrix revealed that most of the samples clustered according to intestinal section (PI and DI) (Figure 3), and the subsequent analysis of similarity (PERMANOVA) revealed that the difference between the two groups was statistically significant (p= 0.02). In contrast, a two-dimensional (2D-) PCoA of the Binnary-Jaccard distance matrix revealed that the samples clustered according to the farm (Figure 4). In this case, the analysis of similarity (Permanova) also revealed that the difference between groups was statistically significant (p=0.021). In other words, the most abundant taxa were the basis for clustering according to anatomical region (part of the gut), whereas the less abundant taxa were the basis for clustering according to geographical location (farm).

The LEfSe comparison between farm 1 and farm 2 indicated that the farm 1 microbiome was characterized by a predominance of the phylum Proteobacteria, whereas the microbiome of farm 2 was characterized by a predominance of *Sphingomonadaceae* and *Nocardioidaceae* (Figure 5). On the other hand, the LEfSe comparison between the sequences retrieved from DI and PI samples showed that the DI region was characterized by a predominance of *Staphylococcaceae*, *Bacillales* and *Stenotrophomonas,* while the PI region was characterized by the abundance of *Salinibacterium* sequences (Figure 6).

Venn diagrams illustrate the components in our samples that were shared or not shared by the different anatomical and/or geographical locations (Figure 7). When only the sequences with a total relative abundance >1% were taken into account, the common gut bacteriome of the fish analyzed in this study was composed of 19 taxonomical units (Figure 7b), and, at the species (OTU) level, the following species were included: *Turicibacter sanguinis*, *Clostridium cavendishii*, *Clostridium chartatabidum*, *Clostridium sardiniense*, *Cetobacterium ceti*, *Cetobacterium somerae*, *Tolumonas auensis*, *Enterobacter soli* and *Plesiomonas shigelloides* (Figure 7b). In addition, *Ochrobactrum thiophenivorans* and *Mesorhizobium septentrionale* (labeled with * in the figure) were shared by DI samples from both farms, while *Sarcina ventriculi* (labeled with ** in the figure) was shared by PI and DI samples from farm 2 (Figure 7b).

4. DISCUSSION

Gut microbes play a key role in host metabolism and the priming and development the immune system. Although most studies performed to date have focused on the mammalian microbiome, particularly the human microbiome, knowledge on the acquisition, composition and modulation of the intestinal microbiota of fish will

be central to defining fish physiology, nutrition and pathology in the future (Dehler et al., 2017).

In this study, the analysis of samples collected from the small intestine (PI) and the hindgut (DI) of healthy sturgeons bred in two different geographical locations revealed a high degree of interindividual variability, a finding that has already been reported for other fish species that shared the same environment and diet (Stephens et al., 2016; Bledsoe et al., 2016). This observation is not strange considering the many factors that may affect the gut microbiota of fish, including the host, the environmental conditions and microbial factors related to the diet and environment (Prakash et al., 2011; Navarrete et al., 2012; Sullam et al., 2012; Li et al., 2014; Bolnick et al., 2014a; Bolnick et al., 2014b; Li et al., 2015, Li et al., 2016; Stephens et al., 2016; Ringø et al., 2016; Dehler et al., 2017). Variability in the gut microbiota between individuals has been suggested as a potential source of variation exhibited in other research on the biology and culture of commercially valuable fish or during attempts to manipulate the microbiota on farms (Bledsoe et al., 2016).

Despite such individual fish variability, statistically significant differences between the anatomical locations and between the farms were detected. Differences in the gut bacteriomes between farms may be the result of the influence exerted by the water conditions and diet, two factors that are considered among the major drivers shaping the fish microbiota (Nayak, 2010; Ringø et al., 2016). In relation to water, the presence of similar bacterial taxa between intestinal and water samples has been found in many fish species, mainly in the early stages of life (Austin, 2006; Navarrete et al., 2009; Wu et al., 2012; Bledsoe et al., 2016; Dehler et al., 2017). Diet and feeding habits can also affect the quantity and diversity of bacterial composition in the gut microbiota community of adult fish (Campbell and Buswell, 1983; Nayak, 2010; Brunvold et al., 2007; Muegge et al., 2011; Sullam et al., 2012; Xia et al., 2014; Ye et al., 2014). In this study, the differences observed between the two farms in the relative abundance of Proteobacteria, Bacteroidetes and Firmicutes sequences detected in the PI and DI regions may be due, at least in part, to the differences in the diets. Compared to the diet used on farm 1, the one used on farm 2 had an increased fat content and a decreased protein content (Table 1), which may be responsible for the increased abundance of Firmicutes and reduced abundance of Bacteroidetes in the microbiome of the DI in comparison to the PI in the animals from this farm (Figure 1, Table 4). The PI has different metabolic and physiological functions from the DI, or hindgut. In sturgeons, the main site for fat digestion is the PI which contains the liver and pancreatic enzymes that catabolize dietary lipids. In contrast, the site for the absorption of energy nutrients, dry matter and crude protein is the region of the spiral valve of the DI (Venero et al., 2015). Regarding the relationship between diet and the Firmicutes:Bacteroidetes ratio, one study has shown an increase in the abundance of Firmicutes in the intestinal microbiota of mice after consumption of a high-fat diet (Zhang et al., 2012). Although the impact of diet on the intestinal bacterial microbiota of the sturgeon is unknown, a

recent study in zebrafish showed that Firmicutes improve the ability of enterocytes to absorb fatty acids (Semova et al., 2012); thus, an increase in the abundance of these bacteria in the DI could promote fat absorption.

Despite the influence of water and diet, there are also specific gut bacteria in fish that are not influenced by these factors but rather correspond to the species of fish (Roeselers et al., 2011; Sullam et al., 2012; Abid et al., 2013). Previous studies comparing gut microbiota and environmental microbes have revealed that the fish gut is maintained as a niche habitat, separate from the overall microbial communities present in the diets and water (Bledsoe et al., 2016; Dehler et al., 2017). In this context, the presence of coliform bacteria in the water of the farms included in this study (Table 2) was not reflected in the presence of coliform bacterial species in the gut samples.

It has been repeatedly observed that, both qualitatively and quantitatively, the microbial composition can vary significantly among different gut regions within the same fish (Zhou et al., 2009). Gut bacterial communities probably differ in each fish species concurrently with differences in the digestive tract, depending on pH, redox potential and oxygen concentration (Li et al., 2013; Llewellyn et al., 2014). Regarding the bacterial composition of the gastrointestinal tract of the Siberian sturgeon, one preliminary study revealed that the density and diversity of cultivable bacteria was significantly higher in the hindgut (containing the spiral valve) than in the small intestine and caecum (Geraylou et al., 2012). For this reason, subsequent studies on the gut microbiota of this species were exclusively focused on the hindgut (Geraylou et al., 2013a; Geraylou et al., 2013b). The spiral valve is a specific area in the hindgut that increases the effective length and, hence, the absorptive capacity of the sturgeon's intestine (Buddington and Doroshov, 1986; Venero et al., 2015). The present study is the first to examine the small intestine microbiome and its composition relative to that of the hindgut using Illumina technology. The separation of the sturgeon's gut into these two parts appears to correspond to different functionalities that likely require specialized, site-specific microbiomes.

In our study, Proteobacteria, Fusobacteria, Firmicutes and Actinobacteria were the most abundant phyla detected in the gut samples (Tables 3 and 4). The dominance of the phyla Fusobacteria, Firmicutes and Proteobacteria in the hindgut of Siberian sturgeons has previously been detected by applying PCR- denaturing gradient gel electrophoresis (DGGE) and pyrosequencing techniques (Geraylou et al., 2013a; Geraylou et al., 2013b). For the first time, our study describes the existence of members of the phyla Actinobacteria and Bacteroidetes in the gut of sturgeons. In this study, certain bacterial species were present in all samples regardless of the gut portion or the geographical location (albeit at different proportional abundances), indicating that, although microbe acquisition may occur differently in the two habitats, selective forces can act within the host, offering specific niches to specific bacterial taxa. In fact, on the basis of data from Tables 5 and 6 as well as data from Geraylou's works, *Cetobacterium somerae*, *Clostridium sardinense* and *Plesiomonas shigelloides* are bacterial species

found in all surgeon gut samples, not exclusively in the hindgut of juvenile animals as studied by Geraylou. These bacterial species are also found in the small intestine, which has been studied for the first time in our work.

The concept of a core set of microbial species fulfilling the minimal symbiotic functionality in a specific ecological niche has been suggested previously (Roeselers et al., 2011; Star et al., 2013). Although a core gut microbiota has already been proposed for certain fish species, including Atlantic cod (Star et al., 2013), zebrafish (Roeselers et al., 2011), rainbow trout (Wong et al., 2013), and Atlantic salmon parr (Dehler et al., 2017), establishing a core microbiota of the sturgeon's gut is beyond the scope of this study because of the intragroup variability and the complexity of the sturgeon intestinal microbiota. Future studies with greatly increased numbers of samples and geographical locations are required to establish the core microbiome of the sturgeon's gut. This study faces the limitation of a relatively low number of animals (n=12), which makes it difficult to obtain statistically significant results; in fairness, however, healthy Siberian sturgeons are expensive and difficult to obtain. In fact, previous studies used even smaller numbers of animals than the present study.

The data clearly show that in these animals, *C. somerae* was more abundant in the small intestine than in the hindgut. In the small intestine, in addition to *C. somerae,* there are other notably abundant bacterial species: *Tolumonas auensis, Plesiomonas shigelloides, Calothrix parietina* and *Serratia entomophila.* Among Fusobacteria, *C. somerae* appears to be the most abundant species in the PI. In fact, our results revealed a significant decrease in the abundance of this Fusobacteria species from the proximal to the distal intestine in all the samples, a finding that suggests a physiological role for these microbes in the small intestine. This microaerotolerant microorganism was first isolated in 2003 (Finegold et al., 2003) from the stools of children undergoing treatment with vancomycin; later, this microbe was found in the intestinal tract of several fish species, such as cat fish, goldfish, carp, tilapia and zebra fish (Sugita et al., 2017; Tsuchiya et al., 2008; Bledsoe et al., 2016).. This bacterial species has also been described as the dominant species in the intestinal microbiota of some freshwater fish species, at densities of up to 1.7×10^9 CFU/g (Sugita et al., 2017). *C. somerae* has also been found to be the most abundant bacterial species in the hindgut of Siberian sturgeons, but its abundance varied after the administration of probiotics, and the biological significance of this change remains unknown (Geraylou et al., 2013b). Other bacteria previously detected in the hindgut, such as *Clostridium* sp. and *Plesiomonas* sp. (Geraylou et al., 2013b) were found to have a similar relative abundance in the PI or small intestine (Tables 3 and 4).

The results of this study also revealed the existence of a relatively high proportion of uncultured bacteria in the analyzed regions, as has been shown for other fish species (Nayak 2010). High numbers of unclassified bacteria have also been previously described in the hindgut (DI) of Siberian sturgeons by pyrosequencing (Geraylou et al., 2013b). These results suggest that the bacterial composition of the gut

microbiota of these fish species is quite complex and that data derived from culture-based approaches usually reveal only a limited range of the microbial diversity.

Another novel aspect of this study is the description of sequences from *O. thiophenivorans* and *M. septentrionale* in the bacteriome of sturgeon. Bacteria belonging to the genus *Ochrobactrum* have previously been documented in fish (Nayak, 2010) and in the human gastric niche (Kulkarni et al., 2017), but until the present, *O. thiophenivorans* had been detected only in the hindgut of the forest cockchafer (Alonso-Pernás et al., 2017). Likewise, *Bradyrhizobium* and *Rhizobium*-related species have been found in the gut microbiota of sea bass (Carda-Dieguez et al., 2014; Gatesoupe et al., 2016) and Nile tilapia (Xia et al., 2018). Such bacterial groups are interesting because of their relative abundance in DI samples and because they are associated with the production of a variety of enzymes with cellulolytic and pectolytic activities. Likewise, the presence of *Shewanella* sp. in animals from farm 2 is very interesting result, since different *Shewanella* species have shown good probiotic properties in farmed fish such as Senegalese sole (Jurado et al., 2018), gilthead seabream (Cordero et al., 2016) and abalone (Jiang et al., 2013). Some preliminary studies have shown that the use of some probiotics can enhance the innate immunity and growth performance of sturgeons (Pourgholam et al., 2016; Fei et al., 2018), but the use of *Shewanella* species in this fish species has not been investigated to date.This study represents the first application of the Illumina next-generation sequencing (NGS) approach to studying the microbiome of the sturgeon's gut and expands previous knowledge acquired through classical cultivation techniques, PCR-DGGE and pyrosequencing (Akrami et al., 2013; Geraylou et al., 2012; Geraylou et al., 2013a; Geraylou et al., 2013b). In this fish species, previous molecular approaches have focused on the effect of prebiotics on bacterial groups such as lactic acid bacteria (Akrami et al., 2013), which are relevant in the microbiome of monogastric mammals (particularly the human gut microbiome) but whose relevance in fish appears much lower or is poorly understood.

The large-scale success of sturgeon farming is heavily restricted by the paucity of information related to sturgeon diseases and the scarcity of methods to control such diseases. Intensive culture exposes fish to several sources of stress, such as high stocking densities and manipulations that predispose animals to many infectious diseases associated with viral or bacterial pathogens (Georgiadis et al., 2000; Ciulli et al., 2016). In the future, a better understanding of the intestinal microbiota and host-microbiota interactions in this cultured fish species will facilitate the development of efficient microbiota-targeted intervention strategies to improve health and growth performance.

5. CONCLUSION

The gut bacterial community of healthy Siberian sturgeon is variable between individual fish but was dominated by the phyla Proteobacteria, Fusobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Many OTUs were detected in all

intestinal regions, however, some OTUs showed regional localization. Our results revealed a significant decrease in the abundance of Fusobacteria (mainly belonging to the genera *Cetobacterium*) from the proximal to the distal region in all gut samples, a finding that suggests a physiological role for these microbes in the small intestine. Another novel aspect of this study is the description of a relative abundance of *O. thiophenivorans* and *M. septentrionale* sequences in the bacterial microbiota of DI samples.

AVAILABILITY OF DATA AND MATERIALS

The data generated and analyzed for this study are available in the Sequence Read Archive (SRA) database (accession SRP159487) and the BioSample database of the National Center for Biotechnology Information (BioProject ID PRJNA489170), with the accession numbers SAMN09949831 to SAMN09949854.

ETHICS STATEMENT

We obtained gut samples as a bioproduct of conventional business practice. All specimens were caught for commercial purposes and euthanized by the local veterinarian, and samples were taken postmortem. No scientific experiments were performed on live animals. As previously established, the animals came from two different farms. These livestock farms are required to comply with the European Union (EU) regulations on the protection of animals, including farmed fish, at the time of slaughter; these regulations stipulate that "the fish shall be spared any avoidable pain, distress or suffering during their killing and related operations" (Council Regulation (EC) 1099/2009, p. 9). The key principle of humane slaughter is that death should either be instantaneous or, if insensibility is induced gradually, then it should be without fear or pain (Farm Animal Welfare Council, 1996).

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CONFLICT OF INTEREST STATEMENT

The authors declare that this work was performed in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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FIGURE LEGENDS

Figure 1. Schematic representation of the two anatomical regions (proximal intestine or PI; distal intestine, or DI) from which samples were c*ollected and analyz*ed. The relative phylum-level a*b*undance of sequences obtained from each anatomical part and from each of the two farms that provided the sturgeons are also shown.

Figure 2. Comparison of alpha diversity between the OTUs obtained from farm 1 and farm 2 samples as assessed using the Shannon diversity index (SDI); **p*=0.043.

Figure 3. Two-dimensional principal coordinate analysis (2D-PCoA) of the Bray-Curtis distance matrix. Analysis of similarity (PERMANOVA) revealed a statistically significant difference (p= 0.02) between distal intestine (DI) (red circles) and proximal intestine (PI) (blue squares) samples.

Figure 4. Two-dimensional principal coordinate analysis (2D-PCoA) of the binary-Jaccard distance matrix. Analysis of similarity (PERMANOVA) revealed a statistically significant difference (p= 0.021) between farm1 (blue circles) and farm 2 (red squares).

Figure 5. LEfSe comparison between sequences obtained from farm1 (red) and farm 2 (green).

Figure 6. LEfSe comparison between sequences obtained from distal intestine (DI) (red) and the proxima1 intestine (PI) (green).

Figure 7. Determination of the core microbiome using Venn diagrams, which classified our data into 4 groups: farm 1-distal intestine (Farm1_DI), farm 2-distal intestine (Farm2_DI), farm 1-proximal intestine (Farm1_PI), and farm 2-proximal intestine (Farm2_PI). (A) Diagrams obtained when all the OTUs were taken into account; (B) Diagram obtained when only those OTUs with a relative abundance >1% were taken into account.

Table 1. Composition of the commercial sturgeon feed on the two farms.

Ingredient	**Farm 1**	**Farm 2**
Crude protein (%)	52	43
Crude lipids (%)	15	23
Cellulose (%)	2	2.8
Ash (%)	7	7.8
Total P (%)	1.2	1.05
Total Na (%)	0.3	0.37
Total Ca (%)	1	1.73
Vitamin A (IU/kg)	1000	1500
Vitamin D3 (IU/kg)	1000	800
Antioxidants (mg/kg)	140	150

Table 2. Comparison of the water parameters used to assess water quality on the two farms.

Parameters	**Farm 1**	**Farm 2**
Temperature (ºC)	18.3	12.8
Dissolved O_2 (mg/L)	7.76	9.14
O_2 saturation (%)	77	84.9
Chemical oxygen demand (mg/L)	4.07	6.59
MES (mg/L)	2.0	2.0
pH	7.11	7.56
Conductivity (mS/cm)	1185	331
Nitrates (mg/L)	11.8	2.05
Nitrites (mg/L)	0.02	0.01
Ammonium (mg/L)	0.03	0.04
Chlorides (mg/L)	37.3	7.40
Phosphates (mg/L)	<0.01	<0.01
Cu (mg/L)	0.02	ND
Total coliforms (per 100 ml)	500	1400
Total fecal coliforms (per 100 ml)	<10	10
Total fecal enterococci (per 100 ml)	10	10

ND: Not detected

Table 3. Relative abundances (%) of the most abundant bacterial sequences at the phylum and class levels in the DI and PI samples of *Acipenser baerii* from farm 1.

Phylum	Class
Unclassified PI: 10.94±5.11 (6) DI: 1.61±0.72 (6)	Unclassified PI: 12.58±4.75 (6) DI: 2.97±1.32 (6)
Fusobacteria PI: 33.44±9.74 (6) DI: 11.13±4.98 (6)	Fusobacteria PI: **40.01±8.82 (5)**[a] DI: **11.13±4.98 (6)**[b]
Firmicutes PI: 20.29±12.83 (6) DI: 25.85±13.04 (6)	Clostridia PI: 20.95±15.02 (5) DI: 26.01±13.38 (5)
	Erysipelotrichia PI: 0.22 (1) DI: 7.75±4.51 (2)
	Bacilli PI: 2.33±0.27 (5) DI: 1,29±0.59 (6)
Proteobacteria PI: 25.29±5.58 (6) DI: 58.68±18.31 (6)	ϒ-Proteobacteria PI: 13.89±4.66 (6) DI: 10.74±7.05 (6)
	β-Proteobacteria PI: 4.63±2.18 (4) DI: 8.15±0.23 (3)
	α-Proteobacteria PI: **7.65±4.07 (5)**[a] DI: **63.22±20.62 (4)**[b]
Cyanobacteria PI: **2.96±1.36 (6)**[a] DI: **0.06±0.01 (6)**[b]	Nostocophycideae PI: 4.98 (1) DI: 0.07 (1)
Actinobacteria PI: 5.63±2.99 (3) DI: 0.31±0.14 (5)	Actinobacteria PI: **11.01±3.94 (3)**[a] DI: **0.34±0.21 (4)**[b]
Bacteroidetes PI: 1.60±0.61 (6) DI: 1.50±0.89 (6)	Sphingobacteria PI: 2.41±0.53 (3)
	Bacteroidia DI: 3.63±0.01 (2)

Values are presented as the mean ± standard error (n)
Values (PI *vs* DI) with different superscripts in the same phylum or class are significantly different ($p<0.005$).

Table 4. Relative abundances (%) of the most abundant bacterial sequences at the phylum and class levels in the DI and PI samples of *Acipenser baerii* from farm 2.

Phylum	Class
Unclassified PI: 15.76±7.46 (6) DI: 21.39±9.44 (6)	Unclassified PI: 16.47±7.34 (6) DI: 21.92±9.36 (6)
Fusobacteria PI: 15.84±6.52 (5) DI: 1.47±0.42 (3)	Fusobacteria PI: 15.84±6.52 (5) DI: 1.71±0.60 (2)
Firmicutes PI: 13.22±6.69 (6) DI: 26.14±6.67 (6)	Clostridia PI: 9.39±3.94 (5) DI: 21.12±7.75 (6)
	Erysipelotrichia PI: 11.19±9.39 (2) DI: 1.41 (1)
	Bacilli PI: 0.74±0.30 (4) DI: 5.65±3.10 (5)
Proteobacteria PI: 44.79±13.50 (6) DI: 41.22±13.47 (6)	ϒ-Proteobacteria PI: 39.45±14.52 (6) DI: 20.10±13.95 (4)
	β-Proteobacteria PI: 2.33±0.88 (3) DI: 5.95±2.16 (5)
	α-Proteobacteria PI: 3.49±1.55 (6) DI: 26.48±14.69 (5)
Cyanobacteria PI: 3.30±3.06 (3) DI:1.82±0.84 (5)	Nostocophycideae PI: 8.58 (1) DI: 2.78 (1)
Actinobacteria PI: 4.77±1.98 (6) DI: 6.42±3.80 (6)	Actinobacteria PI: 5.42±1.95 (5) DI: 6.41±3.79 (6)
Bacteroidetes PI: 4.09±3.12 (5) DI: 2.09±1.11 (6)	Sphingobacteria PI: 8.28±8.20 (2) DI: 3.90±1.67 (3)
Spirochaetes PI: 15.35 (1)	Spirochaetes PI: 15.31 (1)

Values are presented as the mean ± standard error (n).

Table 5. Relative abundances (%) of the most abundant bacterial sequences (> 1%) at the species levels in the DI and PI samples of *Acipenser baerii* from farm 1.

Species	Proximal intestine (PI)		Distal intestine (DI)		Shared by the same sturgeon
	Means±SE	n	Means±SE	n	
Cetobacterium somerae	**22.81±6.45[a]**	6	**6.68±2.86[b]**	6	Yes (6)
Cetobacterium ceti	**2.69±0.68[a]**	5	**0.82±0.38[b]**	6	Yes (5)
Propionigenium modestum	2.24±1.08	5	1.03±0.54	6	Yes (5)
Clostridium chartatabidum	6.95±3.85	2	7.15±5.27	3	Yes (2)
Clostridium cavendishii	6.66±6.10	3	7.57±4.18	3	Yes (3)
Clostridium sardiniense	5.37±2.53	2	4.25±2.22	2	Yes (1)
Clostridium barati	2.84±1.47	2	3.02±1.68	2	Yes (1)
Clostridium bovipellis	1.05±0.30	2	0.47±0.16	2	Yes (1)
Clostridium saccharoperbutylacetonicum	0.99±0.46	2	1.27±0.84	2	Yes (1)
Turicibacter sanguinis	0.22	1	4.61±2.78	2	No
Carnobacterium maltaromaticum	1.84	1	0.01	1	No
Plesiomonas shigelloides	5.37±2.80	3	7.16±6.57	2	Yes (1)
Serratia entomophila	1.85±1.05	4	3.19±2.84	2	Yes (1)
Tolumonas auensis	1.34±0.14	4	0.75±0.64	3	Yes (2)
Pseudomonas lutea	2.31	1			
Stenotrophomonas retroflexus			3.86±0.75	3	
Chromobacterium piscinae	2.19±1.24	2	0.05±0.04	3	Yes (2)
Cupriavidus metallidurans	1.16±0.36	4	0.22±0.19	2	Yes (1)
Janthinobacterium agaricidamnosum	1.65	1			
Achromobacter piechaudii			3.63±0.13	3	
Achromobacter xylosoxidans			1.28±0.03	3	
Rhizobium alamii	4.37	1			
Sphingomonas wittichii	2.53	1			
Roseospira thiosulfatophila	1.21	1			
Ochrobactrum thiophenivorans			61.01±2.90	3	
Mesorhizobium septentrionale			15.71±1.21	3	
Bradyrhizobium pachyrhizi			3.33±0.39	3	
Calothrix parietina	2.21±1.15	3	0.06±0.02	2	Yes (1)
Corynebacterium tuberculostearicum	1.53	1	0.02	1	No
Corynebacterium imitans	5.52	1			
Corynebacterium ureicelerivorans	1.17	1			
Aeromicrobium marinum	3.06	1			
Nocardioides islandensis	2.37	1			
Rhodococcus qingshengii	1.95	1			
Salinibacterium xinjiangense	1.23	1			
Pedobacter kwangyangensis	1.64±1.42	2			
Parabacteroides goldsteinii			1.16±0.13	2	

Values are presented as the mean ± standard error (n)
Species-level values (PI *vs* DI) with different superscripts in the same row are significantly different ($p<0.005$).

Table 6. Relative abundances (%) of the most abundant bacterial sequences at the species levels (> 0.5) in the DI and PI samples of *Acipenser baerii* from farm 2.

Species	Proximal intestine (PI)		Distal intestine (DI)		Shared by the same sturgeon
	Means±SE	n	Means±SE	n	
Cetobacterium somerae	11.38±5.08	5	0.98±0.53	3	Yes (3)
Cetobacterium ceti	0.74±0.26	5	0.12±0.07	3	Yes (3)
Propionigenium modestum	0.63±0.24	5	0.05±0.02	2	Yes (2)
Sarcina maxima	2.25±2.23	2	0.06±0.01	3	Yes (1)
Sarcina ventriculi	**0.93±0.83**[a]	3	**7.99±1.16**[b]	4	Yes (3)
Clostridium cavendishii	1.05±0.74	5	2.62±0.98	5	Yes (5)
Clostridium sardiniense	0.33±0.20	3	0.88±0.58	4	Yes (3)
Clostridium chartatabidum	0.33±0.20	3	0.90±0.59	4	Yes (3)
Clostridium barati	0.28±0.18	3	0.77±0.51	4	Yes (3)
Clostridium bovipellis	0.03±0.02	2	0.13±0.03	4	Yes (2)
Turicibacter sanguinis	8.03±6.74	2	0.93	1	Yes (1)
Streptococcus parauberis	0.34±0.34	2	1.43±0.56	2	Yes (1)
Plesiomonas shigelloides	6.36±5.95	3	0.14±0.02	2	Yes (1)
Shewanella profunda	6.22±0.36	2	0.87	1	Yes (1)
Shewanella oneidensis	6.02±0.41	2	1.05	1	Yes (1)
Tolumonas auensis	6.16±5.57	5	0.71±0.18	2	Yes (2)
Serratia entomophila	2.66±2.37	3	0.07±0.05	2	Yes (1)
Yersinia frederiksenii	1.58±1.50	3	0.42	1	Yes (1)
Yersinia nurmii	**0.41±0.34**[a]	3	**15.10**[b]	1	Yes (1)
Enterobacter soli	0.54±0.32	4	17.82±17.68	2	No
Stenotrophomonas retroflexus			3.95	1	
Janthinobacterium agaricidamnosum	1.01±0.74	3	0.91±0.79	2	Yes (1)
Cupriavidus metallidurans	0.67±0.38	2	0.85±0.55	3	Yes (1)
Achromobacter piechaudii			3.02	1	
Achromobacter xylosoxidans			0.99	1	
Methylobacterium goesingense	2.00	1	11.27	1	Yes (1)
Ochrobactrum thiophenivorans	0.01	1	13.89±13.62	4	Yes (1)
Mesorhizobium septentrionale			20.03	1	
Bradyrhizobium pachyrhizi			3.12	1	
Calothrix parietina	4.31±4.11	2	2.37±0.37	2	Yes (1)
Curtobacterium flaccumfaciens	2.00	1	2.11±0.84	2	Yes (1)
Salinibacterium xinjiangense	1.40±0.70	4	0.27±0.27	2	Yes (1)
Nocardioides plantarum	1.32	1	0.17	1	No
Nocardioides islandensis	1.06	1	0.33±0.21	3	No
Corynebacterium tuberculostearicum	0.71	1	3.97	1	Yes (1)
Frankia alni	0.59±0.35	3	0.31±0.13	4	Yes (3)
Pedobacter kwangyangensis	0.13±0.08	3	1.00±0.84	5	Yes (3)
Treponema porcinum	4.12	1			

Values are presented as the mean ± standard error (n). Species-level values (PI *vs* DI) with different superscripts in the same row are significantly different ($p<0.005$).

1.- The gut bacterial community of healthy Siberian sturgeon is dominated by the phyla Proteobacteria, Fusobacteria, Firmicutes, Actinobacteria and Bacteroidetes.

2.- The bacterial composition of healthy Siberian sturgeons changes along the GI tract, with major abundance of populations of *Cetobacterium* species in the small intestine.

3.- There was a relative abundance of *O. thiophenivorans* and *M. septentrionale* sequences in the bacterial microbiota of the hindgut.

4.- *Shewanella* species which have shown good probiotic properties in farmed fish, have been detected in some animals, suggesting that Illumina-based sequence analysis can be used to guide probiotic candidate selection and isolation.

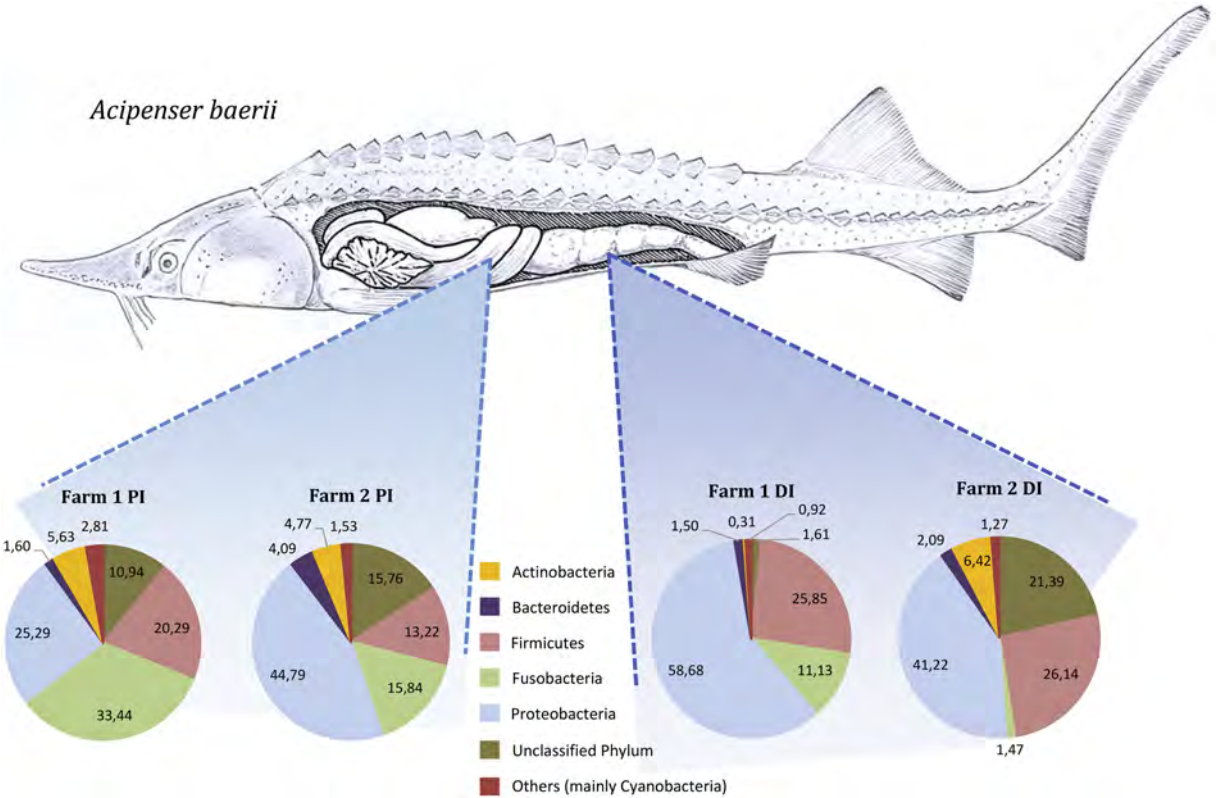

Acipenser baerii
Farm 1 PI
1,60
5,63
2,81
10,94
20,29
25,29
33,44
Farm 2 PI
4,77
1,53
4,09
15,76
13,22
44,79
15,84
Actinobacteria
Bacteroidetes
Firmicutes
Fusobacteria
Proteobacteria
Unclassified Phylum
Others (mainly Cyanobacteria)
Farm 1 DI
1,50
0,31
0,92
1,61
25,85
58,68
11,13
Farm 2 DI
2,09
1,27
6,42
21,39
41,22
26,14
1,47

Figure 1

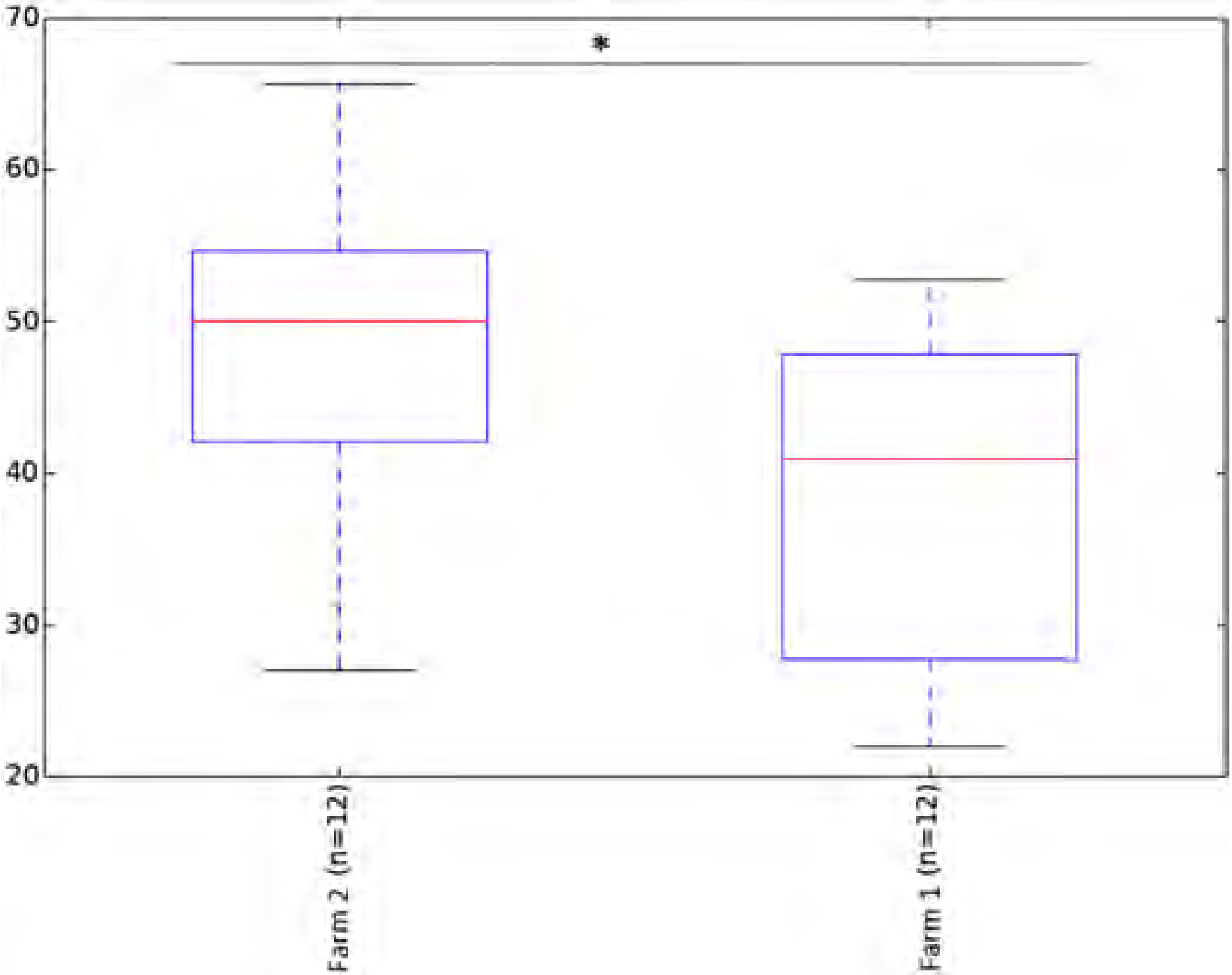


Figure 2

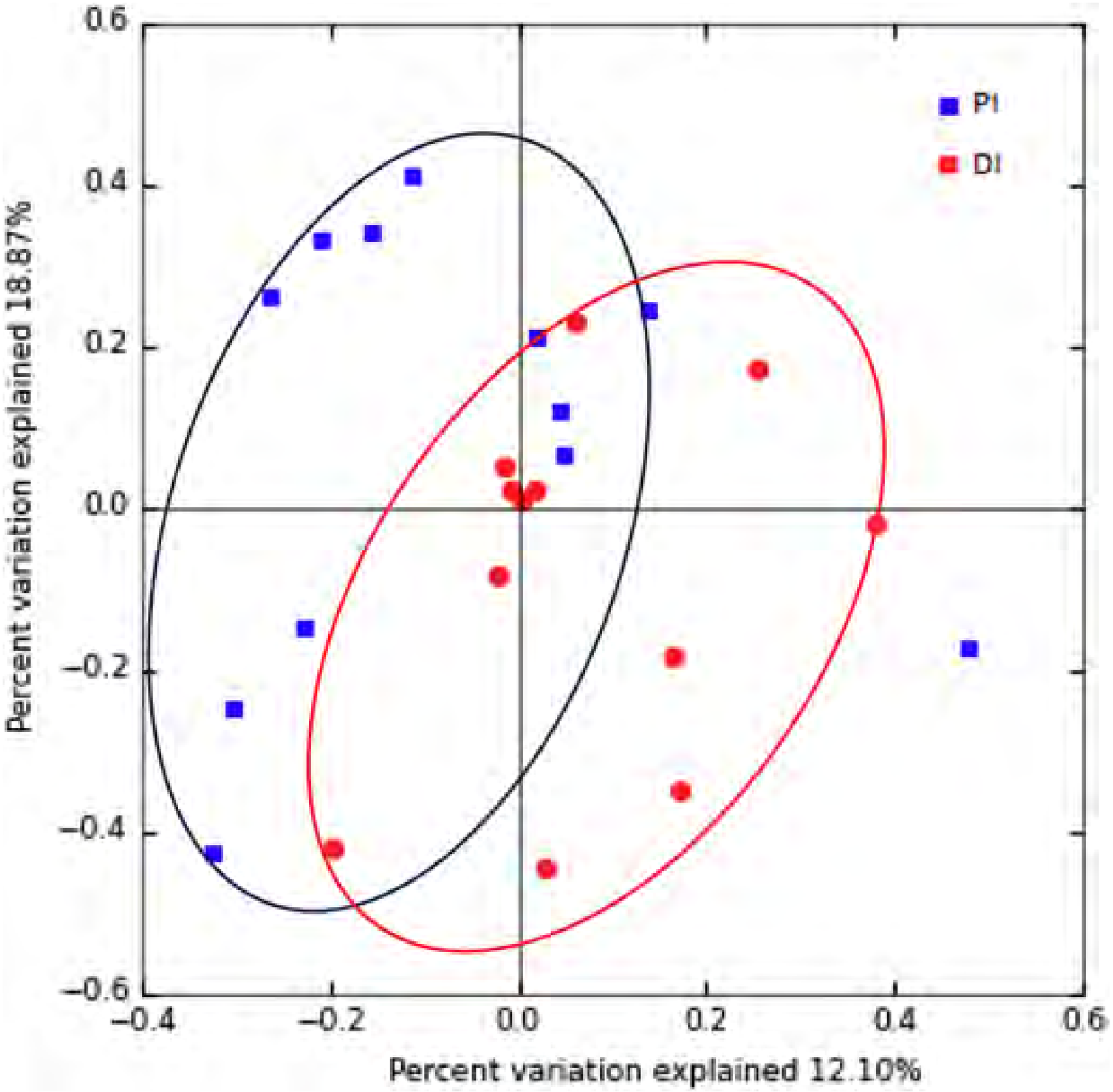

PI
DI
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
Percent variation explained 18.87%
Percent variation explained 12.10%

Figure 3

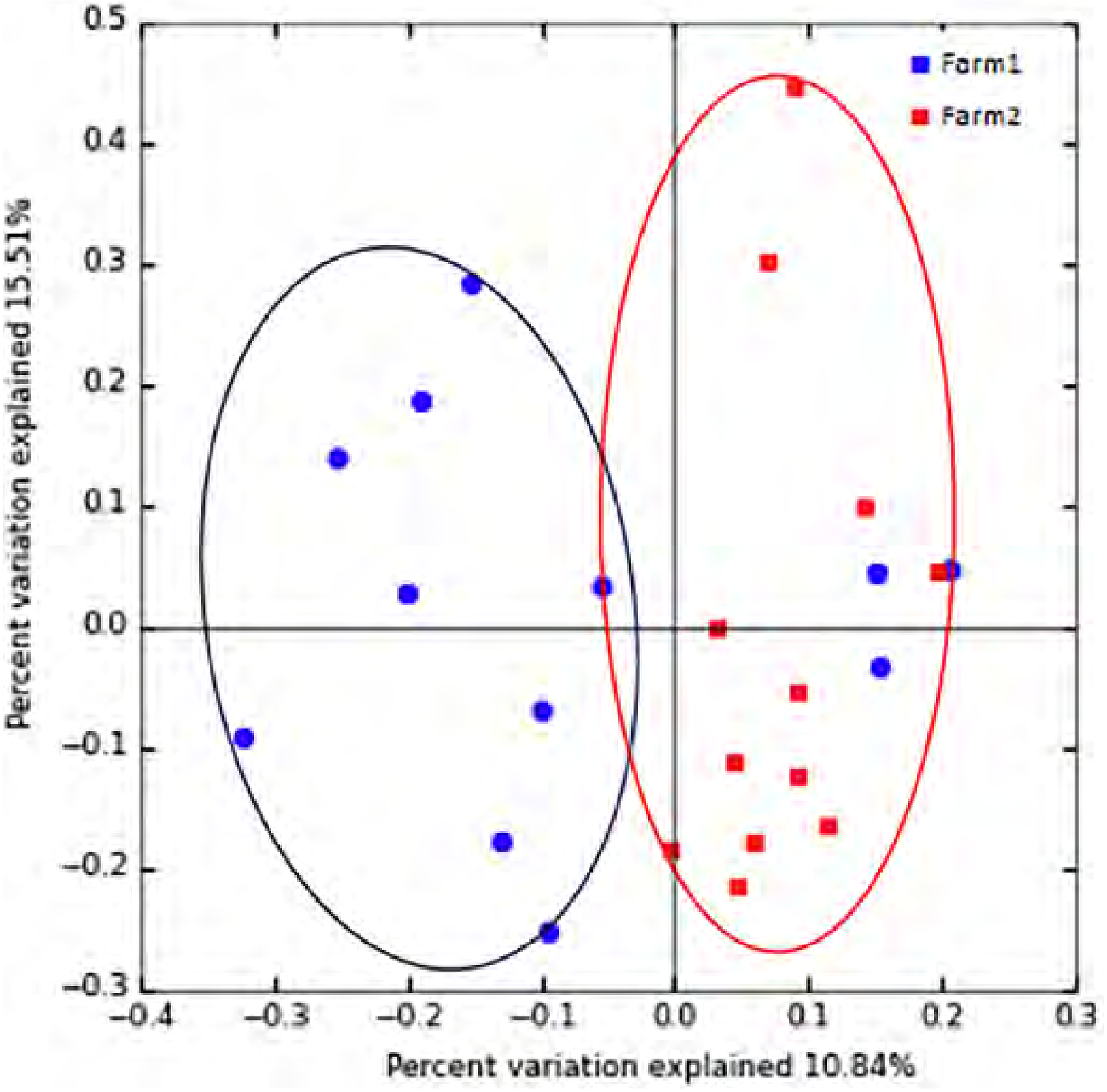

Farm1
Farm2
Percent variation explained 15.51%
Percent variation explained 10.84%
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3

Figure 4

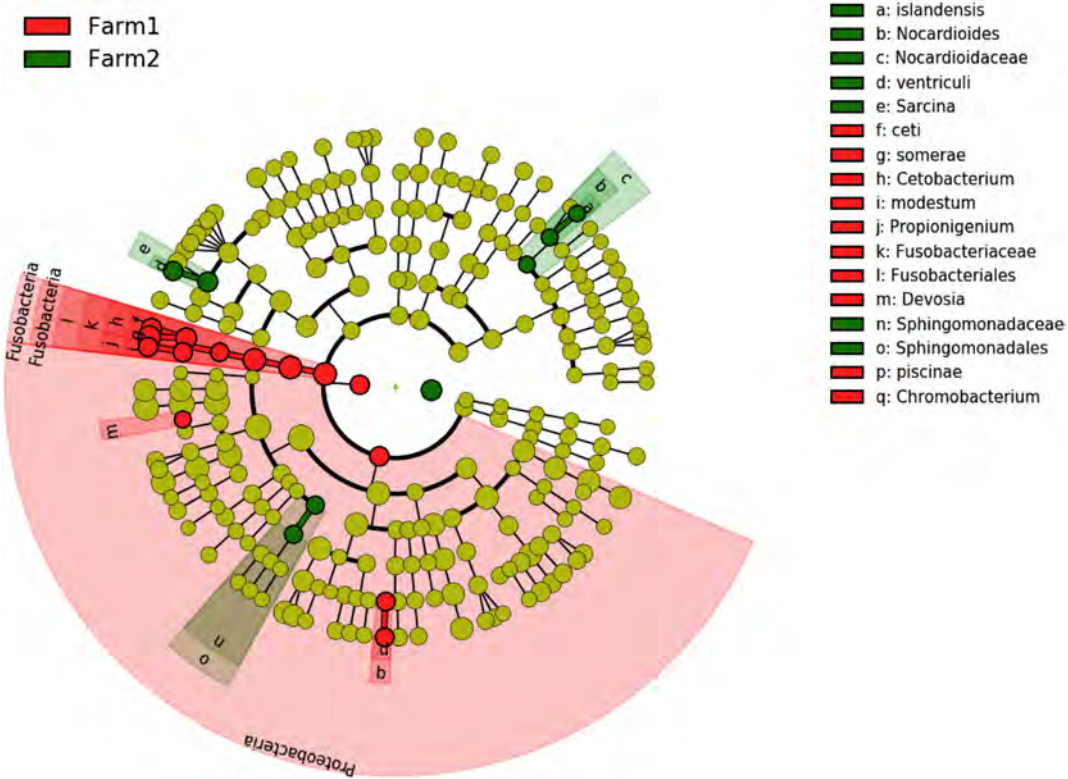

Farm1
Farm2
a: islandensis
b: Nocardioides
c: Nocardioidaceae
d: ventriculi
e: Sarcina
f: ceti
g: somerae
h: Cetobacterium
i: modestum
j: Propionigenium
k: Fusobacteriaceae
l: Fusobacteriales
m: Devosia
n: Sphingomonadaceae
o: Sphingomonadales
p: piscinae
q: Chromobacterium
Fusobacteria
Fusobacteria
Proteobacteria

Figure 5

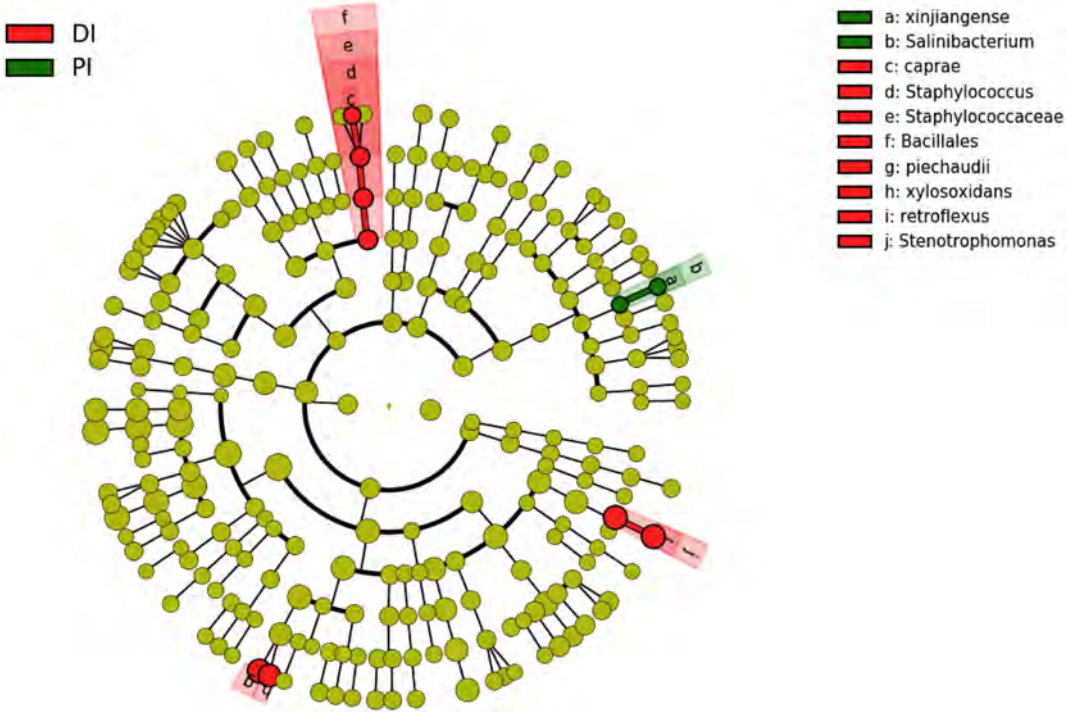
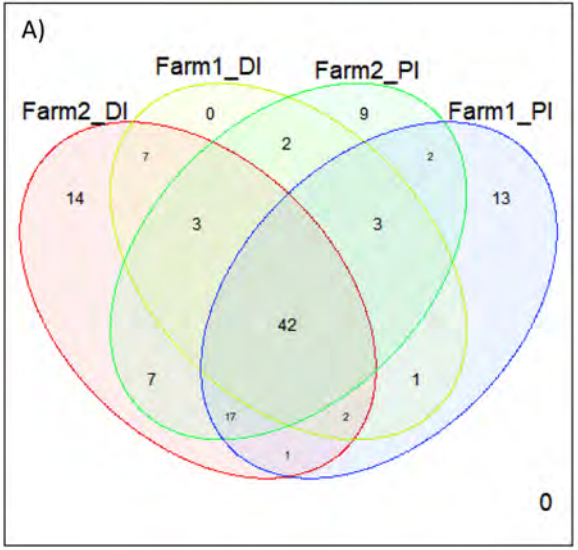

DI
PI
f
e
d
c
a: xinjiangense
b: Salinibacterium
c: caprae
d: Staphylococcus
e: Staphylococcaceae
f: Bacillales
g: piechaudii
h: xylosoxidans
i: retroflexus
j: Stenotrophomonas

Figure 6


A)
Farm1_DI
Farm2_PI
Farm2_DI
Farm1_PI
0
9
2
7
2
14
3
3
13
42
7
1
17
2
1
0

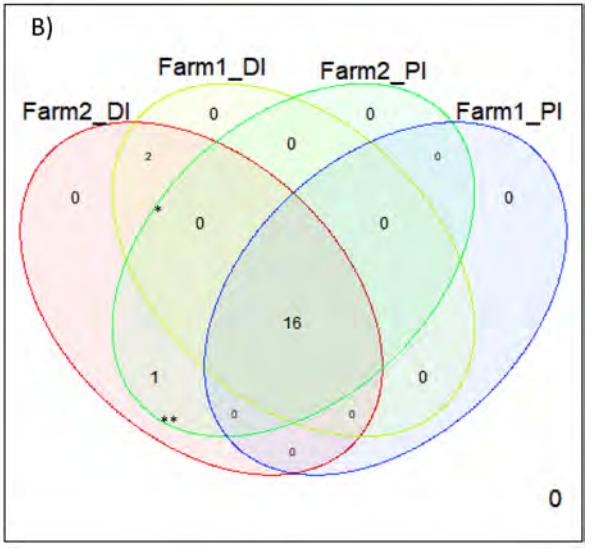

B)
Farm1_DI
Farm2_PI
Farm2_DI
Farm1_PI
0
0
0
2
0
0
0
*
0
0
0
16
1
0
**
0
0
0
0

Figure 7