Latin American Journal of Aquatic Research, 48(3): 456-479, 2020
DOI: 10.3856/vol48-issue3-fulltext-2419

Research Article

High-throughput sequencing of the 16S rRNA gene to analyze the gut microbiome in juvenile and adult tropical gar (*Atractosteus tropicus*)

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ABSTRACT. Tropical gar (*Atractosteus tropicus*) is freshwater and estuarine fish, inhabiting the Earth since the Mesozoic era and undergoing limited physiological variation ever since. Besides its recognized cultural and scientific relevance, the species has seen remarkable growth in its economic impact due to pisciculture. In this study, we present the first report of the whole taxonomic composition of microbial communities in gut contents in juveniles and adults of *A. tropicus*, by sex and origin (wild and cultivated). For this study, 508 genera were identified, with the most and least abundant being *Cetobacterium* and *Paludibacter*, respectively. Fusobacteria, Proteobacteria, Firmicutes, and Bacteroidetes phyla are the core gut microbiome of *A. tropicus* juvenile and adult by sex and origin. Deinococcus-Thermus phylum sequence was only identified in wild-type males. In the phylogenetic trees reconstruction *Lactococcus lactis* strains CAU929 and CAU6600, Cp6 and CAU9951, *Cetobacterium* strain H69, *Aeromonas hydrophila* strain P5 and WR-5-3-2, *Aeromonas sobria* strain CP DC28 and *Aeromonas hydrophila* were identified, some of them with probiotic potential within the three dominant phyla in core gut microbiome in *A. tropicus* adults, especially in wild-type organisms. *Myroides* genus was recognized in microbiota gut of the cultivated juvenile *A. tropicus*. Nevertheless, Alpha diversity indicated that the highest gut microbiota abundance and richness is found in cultivated juvenile and wild-type adult *A. tropicus* female, rather than adult wild-type males and the least gut microbiota abundance and richness is found in a cultivated adult of *A. tropicus* for both sexes.

Keywords: *Atractosteus tropicus*; gut microbiome; metagenomics; 16S rRNA profiling

Corresponding editor: José Gallardo

INTRODUCTION

Biological diversity in Latin America is one of the richest on the planet, including a great variety of its freshwater ichthyofauna (Flores-Nava & Brown, 2010). Some of the most significant challenges for aquaculture, during this decade, have been done with integral studies on pisciculture-relevant endemic species, as well as the development of technologies that may allow for controlled production of these fishes in a profitable, innocuous, and environmentally-conscious approach (Márquez-Couturier & Vázquez-Navarrete, 2015; Márquez-Couturier *et al.*, 2015). In other fish species, some studies have explored the bacterial populations in varying habitats, such as the skin, gills, eggs and gut microbiome (GMBiom), and enhanced the way that they influenced the host's general health and physiology (MacFarlane *et al.*, 1986; Cahill, 1990; Ringø *et al.*, 1995; Givens, 2012; Austin *et al.*, 2016). These studies have reported a significant variation of microbiota among different niches, and between species with the GMBiom as one of the most studied due to its high microorganism concentration. Thus, for the aquaculture industry, the effects caused by outbreaks of viral, bacterial, and fungal infections are of paramount importance, as they can cause devastating economic losses worldwide due to poor environmental conditions on farms, unbalanced feeding, the generation of toxins and genetic factors (Martínez-Cruz *et al.*, 2012).

Tropical gar (*Atractosteus tropicus)*, also known as "pejelagarto," is freshwater and an ample fossil record since the Cretaceous period of the Mesozoic Era (Wiley, 1976; Reséndez-Medina & Salvadores, 1983). The species morphology has remained mostly unaltered, with current specimens having lengths between 1.0 and 1.2 m and weighing between 1,000 and 3,000 g in the wild. In their natural habitat, this species exists in coastal wetlands of the tropical rainy areas of southeastern Mexico, Belize, Guatemala, El Salvador, Honduras, Nicaragua and Costa Rica (Wiley, 1976; Bussing, 1987; Miller *et al.*, 2005; Nelson *et al.*, 2016). It has a carnivorous habit with some tendency to omnivorism, feeding on other fish, decomposing organic matter, crustaceans and plants depending on availability. This exceptionally secluded species has seen a drastic decrease in wild populations caused by anthropogenic activities that have led to the loss of habitats and severe ecological alterations (Méndez-Marin *et al.*, 2012). Currently, *A. tropicus* is cultivated in fish farms for human consumption in Mexico (Márquez-Couturier *et al.*, 2015).

The gastrointestinal tract of *A. tropicus* is formed by the buccopharyngeal, esophagus, stomach, gut, pyloric blind, rectum and anus, which rapidly develops during the larval period (Frías-Quintana *et al.*, 2015). The gut is a tubular structure beginning at the mouth and ending at the anus (Márquez-Couturier *et al.*, 2006). The general character and even the length of the gut may change during ontogeny. For example, in fishes in which the larval stage is herbivorous, and the adult stage is carnivorous, the gut appears to shorten. In other fishes, the gut length remains relatively constant in proportion to body size throughout life (Smith, 1980). Early juveniles, 20 days after hatching, show a gut microbiota (GMBiot) like that of adults. Before colonization, microorganisms may access the GMBiot through food and water intake during the larval phase (Núñez de la Rosa, 2011). The high abundance of certain gastrointestinal bacteria groups in fish suggests that GMBiom poses as a unique niche for a specific but diverse group of bacteria (Cahill, 1990; Givens, 2012) when compared to the microbial composition of the surrounding water. It has been reported that GMBiot fish has between 107 and 1,011 bacteria per gram of feces (Nayak, 2010). GMBiot fish plays an important role and directly influences the host's nutrition and general homeostasis. Regular GMBiot fish may contain both beneficial and potentially pathogenic bacteria. The loss of the microbiota equilibrium (dysbiosis) has been reported to impact the host's physiological state, potentially compromising immunity, growth, general development as well as the overall quality of the aquaculture production due to an increase in fish morbidity and mortality (Al-Harbi & Uddin, 2005; Núñez de la Rosa, 2011).

The advent of culture-independent metagenomic studies during the mid-2000s enabled the simultaneous analysis of complex genomic information contained in hundreds of microbial species in a single niche (Nielsen *et al.*, 2014). These techniques circumvent most culturing requirements of microorganisms, avoiding collection and sampling biases, effectively representing the actual diversity of a microbial community. Under intensive production conditions for sustainable aquaculture, aquatic species are subjected to high-stress conditions, leading to an increased incidence of diseases that decrease productivity (Bondad-Reantaso *et al.*, 2005). It has been proposed that microbiota dysbiosis may be avoided through the regulation of their microbiota (Verschuere *et al.*, 2000).

Microbial studies in aquaculture focus on understanding the symbiotic or antagonistic interactions between microbes and their eukaryotic hosts such as fishes, crustaceans, and mollusks. In this sense, metagenomics can provide a deeper understanding of these relationships through information revealed by sequencing microbial DNA, extracted from specific

niches within host organisms, and, in the case of 16S profiling, taxa are representative of the medium (Suttle, 2007; Gianoulis *et al.*, 2009). The latter consists of surveying the 16S rRNA gene of all present microorganisms since this marker is found in all prokaryotes with enough mutations to discern each taxon. Formerly, some bacteria had been difficult to isolate because some of them are obligate intracellular microorganisms that could only be cultured in semi-aqueous and or cell culture media (Avila-Villa *et al.*, 2011). Current sequencing platforms and bioinformatics tools enable the research on the diversity of intracellular bacteria, but also ignore other culture requirements altogether, because the whole community DNA is sequenced as is, with majority and accessory species, to elucidate the relevant community configurations for the improvement of aquaculture techniques.

It was decided to evaluate the differences between sex since females are larger than males and have access to other types of prey. Also, nutritional and metabolic requirements are different between sex, so we assume that there should be differences in microbial communities due to the great intra- and inter-specific variations, which has been reported by Piazzon *et al.* (2019), where differences in the microbiome are associated with habitat, diet, trophic level, season, captivity, age, genetics, sex, among others. Consequently, the objective of this research was to explore the microbial composition in the gut of *A. tropicus* juvenile and adult, by analyzing the 16S rRNA gene profiles from adult male and female organisms, cultivated and wild, for biotechnological-relevant applications in the future.

MATERIALS AND METHODS

Specimen collection

Seventeen live *Atractosteus tropicus* organisms were collected for the study. Nine of them (seven adults + two juveniles) were cultivated in the Tropical Aquaculture Laboratory, Research Center for Conservation and Sustainable Use of Tropical Resources (CICART) at the Biological Sciences Academic Division (DACBiol), Juárez Autonomous University of Tabasco State (UJAT), Mexico. Eight were wild-type specimens, with an average weight and length of 5 kg and 1 m, respectively. Specimens were provided by fishermen from the municipalities of Nacajuca (18º14'50"N, 92º49'58"W; n: female, m: male) and Centla (18º20'N, 92º30'W; n: female, m: male), Tabasco, Mexico (Fig. 1).

Samples collection and preparation

All organisms were sacrificed by stunning percussion method, according to the NOM-062-ZOO-1999 protocol, on June 18, 2001, approved by The Ministry of Agriculture, Livestock, Rural Development, Fishing and Food. After the euthanasia method, all specimens were cut lengthwise under sterile environments to remove the intestine and extract its contents with scissors disinfected in absolute ethyl alcohol, for storage at -20°C in a 2 mL Eppendorf tubes. GMBiot and feces were separated under sterile conditions, considering the origin (wild or domesticated) and sex.

DNA extraction, sequencing, and sequence for metagenomic analysis

Two hundred milligrams of fresh feces were used, and 2 mL was enough to ensure the target DNA with the capacity of the Eppendorf tubes selected. A whole genomic DNA (gDNA) extraction was carried out for each sample with a QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. DNA integrity and concentration were evaluated by electrophoresis, submerged with 1.2% agarose gel, and by spectrophotometry with a GenovaNano spectrophotometer (Jenway, Stone, Staffs, UK), respectively. Universal primers 515F (5'-GTGCCAGCMGCCGCGG TAA-3') (Caporaso *et al.*, 2011) and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') (Tamaki *et al.*, 2011) were selected for the amplification of a region including the V4 and V5 hypervariable region in the 16S rRNA gene. PCR amplifications were carried out using Phusion High-Fidelity DNA Polymerase (Finnzymes OY, Espoo, Finland) (Klindworth *et al.*, 2013), and conditions were performed according to D'Auria *et al.* (2018). Library preparation and high throughput sequencing were performed through Research & Testing Labs (Lubbock, Texas, USA) services, with an Illumina MiSeq platform, using 16S rDNA profiling by amplifying the V4-V5 hypervariable regions of the bacterial gene, reagents sequencing for 2×300 bp paired-end reads.

Bioinformatics analysis

The sequences were subjected to the standard quality protocols, which included the sequencing adapters' removal with the Cutadapt tool v.2.1 (Martin, 2011). The sequences were analyzed by a pipeline of the DADA2 package (Callahan *et al.*, 2016), compatible with R (R Core Team, 2019). Through which the standard quality filters maxEE = c (2,5) and truncQ = c (2,5) was applied, the Paired-end were joined, adjusting to a final length of 370 bp. The amplicon sequence variant table (ASV) was constructed, and chimeric sequences were eliminated by consensus identification. The ASV was used for the taxonomic assignment, which was performed with the classifier tool of the RDP's Pipeline (Ribosomal Database Project) (Wang *et al.*, 2007) with a confidence cutoff of 40%.

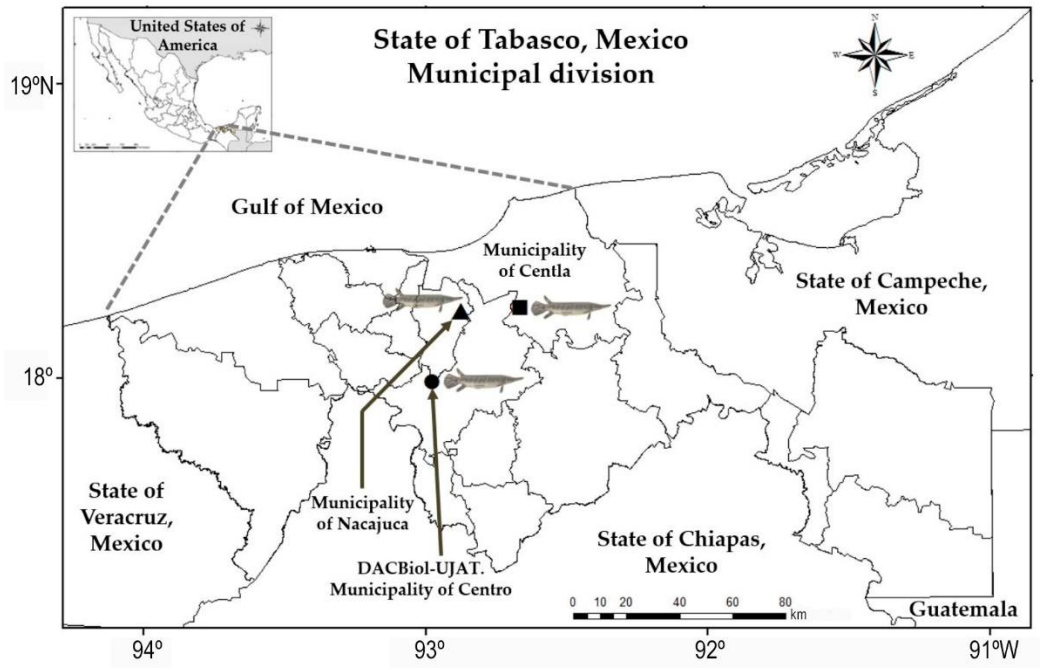

Figure 1. Tropical gars (*Atractosteus tropicus*) collection sites, wild-type adults (triangles shape identified), and cultivated juveniles/adults (circle shape identified).

Alpha diversity

The ASV table was normalized by rarefaction so that all the samples had the same number of sequences. The alpha diversity estimators of ACE, Pielou's evenness and Inverse Simpson, were calculated. Moreover, the statistical comparison of the diversity estimators among the sample groups analyzed was carried out using the nonparametric statistical tests of Kruskal-Wallis and Wilcoxon signed-rank. The value of p was adjusted to 0.05, and the FDR correction was applied to each analysis. The statistical analyses were performed in R (R Core Team, 2019).

Beta diversity

The ASV table was normalized using the CLR transformation (centered log-ratio) (Aitchison, 1982). This table was used for the construction of an Unweighted UniFrac distance matrix (Lozupone *et al.*, 2011), to compare and analyze the *A. tropicus* microbial community composition and distance matrix, respectively, under cultivated and wild-type conditions, PCoA, and a beta significance test was used by adonis nonparametric statistical method. Multiple comparison analysis paired by PERMANOVA and differential analysis of abundance was compared to abundance patterns of the taxa between the samples groups by LEfSe tool (linear discriminant analysis effect size) to identify biomarkers in clustering results (Segata *et al.*, 2011).

Phylogenetic analysis

A sequence base was constructed according to certain genera with probiotic potential in fish that we find in a literature search (Table 1); 16S rRNA gene sequences were obtained from the NCBI GenBank database. The 6,266 sequences obtained were clustered to eliminate identical sequences and generate a table of OTUs with 97% similarity, using the usearch61 algorithm of QIIME (1.9). The database was indexed (Query Sequence) to be used in a local blast with the BLAST + tool (NCBI). The local blast was performed using the sequences of the samples (subject sequences) clustered at 97%, as mentioned in the section on the processing of the sequences. The identity percentage was adjusted to 99% (-perc identity) and a coverage of 70% (-qcov_hsp_perc 70). For phylogenetic analysis, the blast resulting in Query and Subject sequences were subjec-ted to multiple alignments with the ClustalW algorithm, in MEGAX (Molecular Evolutionary Genetics Analysis), and the phylogenetic relationships were inferred by the Neighbor-joining method in MEGAx, with the predefined settings.

RESULTS

A total of 364,735 sequencing reads were generated, organized by sex and origin, according to Table 2. We can see in Table 2 that readings obtained of wild-type

Table 1. Bibliographic information obtained from certain genera with probiotic potential in fish.

Bacteria	Function	Description	Fish	Reference
Aeromonas hydrophila	Probiotic	Antagonist	*Oncorhynchus mykiss*	Irianto & Austin (2002)
Aeromonas salmonicida	Pathogen	Etiological agent of septicemic disease	*Oncorhynchus mykiss*	
Rhodococcus qingshengii	Probiotic	Antagonist	*Salevimus fontinalis*	Boutin *et al.* (2012)
Flovobacterium psychrophilum	Pathogen	Systemic infectious agent	*Salevimus fontinalis*	
Cetobacterium somerae	Probiotic	Vitamin b12 production	Various	Tsuchiya *et al.* (2008)
Vibrio fluvialis		Immune stimulation and improved survival after challenge with *Aeromonas salmonicida*	*Oncorhynchus mykiss*	Irianto & Austin (2002)
Lactococcus lactis CECT 539		Immune stimulation	*Scophthalmus maximus*	Villamil *et al.* (2002)
Lactobacillus delbrueckii CECT 287		Immune stimulation	*Sparus aurata*	Salinas *et al.* (2005)
Basillus subtilis CECT35		Immune stimulation	*Sparus aurata*	
Aeromonas sobria GC2		Immune stimulation and improved survival after challenge with *Lactococcus garvieae* and *Streptococcus iniae*	*Oncorhynchus mykiss*	Brunt & Austin (2005)
Lactobacillus rhamnosus ATCC 53103		Immune stimulation and improved survival after challenge with *Edwardsiella tarda*	*Oreochromis niloticus*	Pirarat *et al.* (2006)
Carnobacterium inhibens		Antibacterial activity against fish pathogens	*Salmo salar*	Jöborn *et al.* (1999)
Enterobacter amnigenus		Increased resistance toward *Flovobacterium psychrophilum*	*Oncorhynchus mykiss*	Irianto & Austin (2002)

Table 2. Total reads by sex and origin.

Organisms	Reads	
	Wild-type	Cultivated
Female	99,513	59,539
Male	137,792	67,891
Subtotal	237,305	127,430
Total	364,735	

male organisms are far higher than of wild-type and cultivated females, and cultivated males. Sequences were clustered into 16,503 different OTUs (97% identity), which were classified into 11 phyla, 22 class, 37 order, 86 families, and 179 genera. Table 2 shows that about twice as many reads correspond to wild males.

Microbiota composition

The microbiota in the gut of *Atractosteus tropicus* adult is composed of the Fusobacteria (42.26%), Proteobacteria (31.40%), Firmicutes (12.96%), and Bacteroidetes (11.79%) phyla (Fig. 2a, Table 3). In wild-type females, the most abundant phyla were Proteobacteria (11.15%), Firmicutes (3.87%), Fusobacteria (2.49%), and Bacteroidetes (1.23%), and in cultivated females were the Fusobacteria (8.70%), Proteobacteria (6.48%), and Bacteroidetes (3.32%). In wild-type and cultivated males, the most abundant phyla were Fusobacteria (15.36 and 15.65%, respectively), and Proteobacteria (5.06 and 3.42%, respectively) (Fig. 2a, Table 3). However, the cultivated juvenile *A. tropicus* gut microbiota is formed by the Firmicutes (5.33%), Proteobacteria (5.28%), and Bacteroidetes (2.93%), only (Fig. 2a, Table 3). At the genus level, the most abundant were *Cetobacterium* (42.24%), *Serratia* (13.02%), *Edwardsiella* (9.46%), *Paludibacter* (6.49%) and *Clostridium sensu stricto* (5.76%) in adult specimens by sex and origin, and in cultivated juveniles, *Staphylococcus* (2.62%), *Plesiocystis* (2.56%) and *Myroides* (2.36%); whereas *Edwardsiella* (6.68%) was more abundant in wild-type males' fish (Fig. 2b, Table 3). For the *Cetobacterium* (42.24%) and *Clostridium sensu stricto* (15.09%) genera, they are more abundant than Bacteroidetes and Actinobacteria in the cultivated and wild-type specimens, respectively (Fig. 2b). Likewise, the taxonomic composition of the more abundant phylogenetic groups by sex and origin in the microbiota of the *A. tropicus* gut at phylum, class, order, and family taxonomic levels (Figs. 3a-b and 4a-b), shows that the most abundant were Fusobacteria, Gammaproteobacteria, Enterobacterales, and Enterobacteriaceae, respectively (Figs. 3a-b and 4a-b). Although the primers are designed only for 16S rRNA gene amplification of bacteria, OTUs belonging to the Archaea

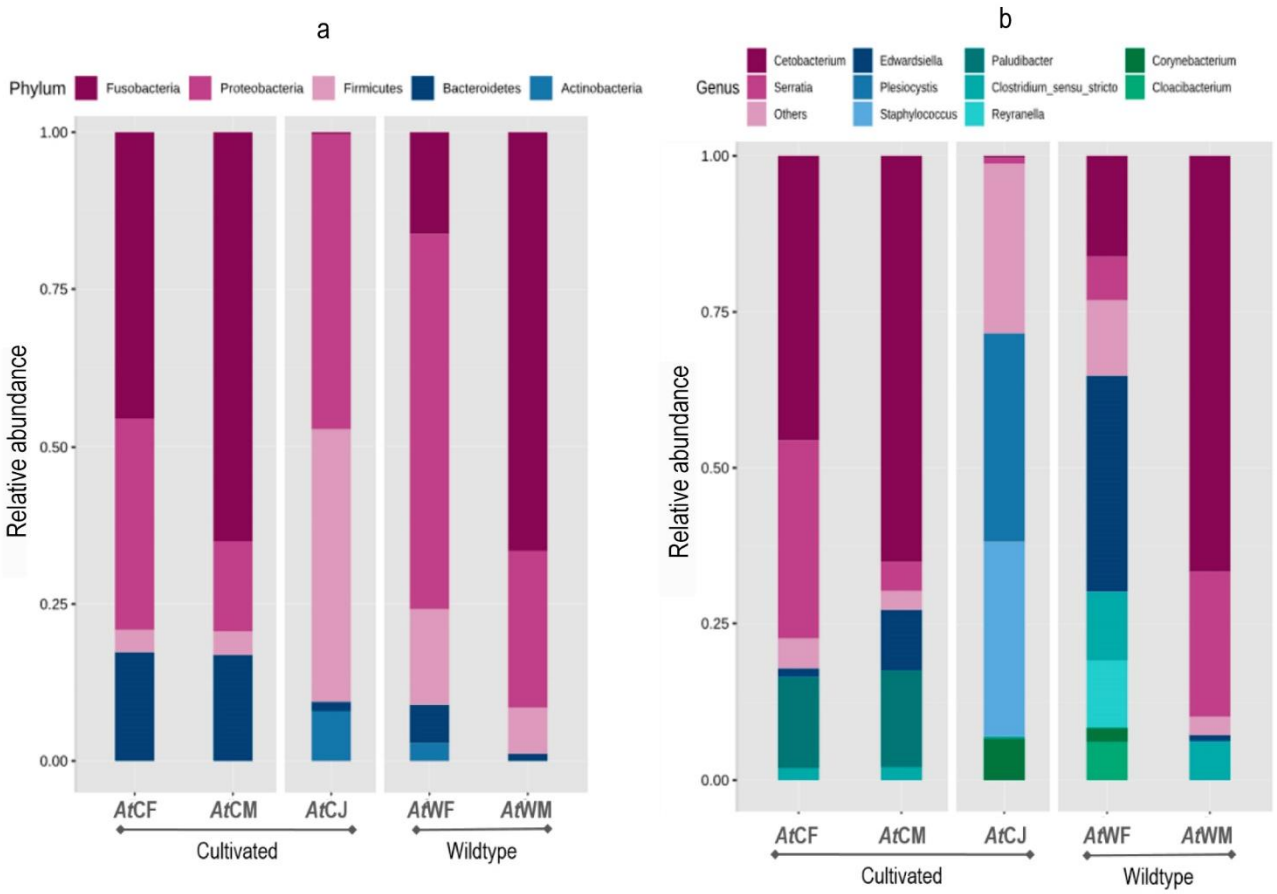

Figure 2. Core microbiome in *Atractosteus tropicus* gut, cultivated juvenile/adult and wild-type adult only, at taxonomic class-level of a) phylum and b) genus. Only taxa were plotted with abundance ≥1%. AtFCF: cultivated adult females; AtCM: cultivated adult males; AtCJ: cultivated juveniles were collected in farm; AtWF: wild-type adult females; AtWM: wild-type adult male.

domain were also identified, represented by the genera *Salinirubrum*, *Salinigranum*, and *Methano-culleus*, which together represent about 0.01% of the total abundance.

Alpha diversity

In this research, diversity, dominance, and richness indicators were measured by the ACE index, using the same OTUs number (Table 4). The ACE index indicates the microbial types in *A. tropicus* are not distributed as uniformly and thus may be more diverse in cultivated juveniles (320.82 ± 16.14) and wild-type females (103.57 ± 3.97) and cultivated (72.38 ± 6.12) than in the cultivated (48.62 ± 4.63) and wild-type (47.92 ± 3.18) male individuals (Table 4). Significant differences were detected in the microbiotic diversity component, between juveniles and adults by sex and origin, only a few samples were not statistically significant. The cultivated juveniles, and wild-type and cultivated females turned out to be the most diverse, but by origin, only the cultivated juveniles and wild-type females were the most diverse. In a total of 50 taxa, significant differences can be observed between cultivated and wild conditions.

Beta diversity

Bacterial community's beta diversity associated with the gut in juvenile and adult *A. tropicus* on origin conditions were measured through the ordination analysis from Principal Coordinates Analysis (PCoA), using Unweighted Unifrac Distances (UniFrac). The analysis produced an ordination of the dissimilarities, where similar individuals are close to one another, and dissimilar ones are distant (Fig. 5). All ordination analyses showed a clear separation between wild-type and cultivated samples. The statistically significant differences by the origin and even more between the cultivated juvenile fish and the wild type ones (Fig. 5) were found by the ANOSIM statistic ($R = 0.60933$, $P < 0.002$).

Table 3. Composition and taxonomic structure of the adult and juvenile tropical gars (*Atractosteus tropicus*) gut samples at the phylum and genus levels, classified by their global abundance, by origin and sex/origin.

GENERAL		Phylum	Fusobacteria 42.26%	Proteobacteria 31.40%	Firmicutes 12.96%	Bacteroidetes 11.79%				
		Genus	*Cetobacterium 42.24%*	*Serratia 13.02%*	*Edwardsiella 9.46%*	*Paludibacter 6.49%*	*Clostridium sensu stricto 5.76%*			
ORIGIN	C	Phylum	Fusobacteria 24.35%	Proteobacteria 9.90%		Bacteroidetes 7.39%				
		Genus	*Cetobacterium 73.06%*	*Serratia 21.48%*	*Edwardsiella 7.74%*	*Paludibacter 19.47%*	*Clostridium sensu stricto 2.58%*			
	CJ	Phylum		Proteobacteria 5.28%	Firmicutes 5.33%	Bacteroidetes 2.93%				
		Genus						*Staphylococcus 2.62%*	*Plesiocystis 2.56%*	*Myroides 2.36%*
	W	Phylum	Fusobacteria 17.86%	Proteobacteria 16.21%	Firmicutes 5.98%					
		Genus	*Cetobacterium 17.86%*	*Serratia 5.78%*	*Edwardsiella 6.88%*		*Clostridium sensu stricto 4.84%*			
SEX/ORIGIN	AtCF	Phylum	Fusobacteria 8.70%	Proteobacteria 6.48%		Bacteroidetes 3.32%				
		Genus	*Cetobacterium 8.70%*	*Serratia 6.05%*		*Paludibacter 2.74%*				
	AtCM	Phylum	Fusobacteria 15.65%	Proteobacteria 3.42%		Bacteroidetes 4.06%				
		Genus	*Cetobacterium 15.65%*		*Edwardsiella 2.31%*	*Paludibacter 3.74%*				
	AtCJ	Phylum		Proteobacteria 5.28%	Firmicutes 5.33%	Bacteroidetes 2.93%				
		Genus						*Staphylococcus 2.62%*	*Plesiocystis 2.56%*	*Myroides 2.36%*
	AtWF	Phylum	Fusobacteria 2.49%	Proteobacteria 11.15%	Firmicutes 3.87%	Bacteroidetes 1.23%				
		Genus	*Cetobacterium 2.49%*		*Edwardsiella 6.68%*		*Clostridium sensu stricto 2.96%*			
	AtWM	Phylum	Fusobacteria 15.36%	Proteobacteria 5.06%	Firmicutes 2.10%					
		Genus	*Cetobacterium 15.36%*	*Serratia 4.66%*			*Clostridium sensu stricto 1.87%*			

C = Cultivated
CJ = Cultivated juveniles
W = Wildtype

AtCF = Adult *A. tropicus* cultivated females
AtCM = Adult *A. tropicus* cultivated males
AtCJ = Juveniles *A. tropicus* cultivated

AtWF = Adult *A. tropicus* wildtype females
AtWM = Adult *A. tropicus* wildtype males

Beta diversity significance. The multiple pairwise comparison tests (MPC) were used for evaluating relationships between all family (group) permutations. The obtained MPC results of adonis tests showed all pairwise differences were statistically significant at an $\alpha = 0.05$ (*i.e.*, between cultivated adults and cultivated juveniles, between cultivated adults and WT adults, and between cultivated juvenile and WT adults; Table 5).

Significant microbial components associated with origin and sex

Eight genera were identified in wild organisms, and these are *Methanoculleus*, *Flavobacterium*, *Psychrobacter*, *Acinetobacter*, *Pseudomonas*, *Paracoccus*, *Massilia*, and *Shewanella*. Likewise, 13 genera were identified in cultivated organisms, which are *Paludibacter*, *Intestinibacter*, *Cellulosilyticum*, *Odoribacter*, *Turicibacter*, *Defluviitalea*, *Vallitalea*, *Acetivibrio*, *Terrisporobacter*, *Bacteroides*, *Acidaminobacter*, *Sporacetigenium*, and *Macellibacteroides*.

Differential analysis of abundance

Specific taxa distributed differentially between wild and cultivated fish were identified through the LefSe tool; this allows obtaining statistical differences for each taxon. Figures 6-8, shows a bar graph of linear discriminant analysis (LDA) by LefSe Biomarkers identification between cultivated juveniles and adults specimens were identified, which correspond to 98 taxa with differential abundance. Wild-type adults and cultivated juveniles showed 61 taxa with differential abundance, and wild-type and cultivated adults had 10 taxa with differential abundance.

Phylogenetic reconstruction analysis

The sequences-base of the rRNA 16S gene constructed of some organisms found in the literature with probiotic potential in fish, according to Table 1, allowed us to find and identify possible organisms with probiotic potential in the microbiome gut of adult *A. tropicus*, adjusted to 99% identity and 70% coverage. In Figure 9, we obtained a phylogenetic tree based on the identified sequences of the most abundant phyla per sample, such as Fusobacteria, Proteobacteria, and Firmicutes. Besides, we used *Methanosarcina thermophila* and *Archaeoglobus profundus* sequences data of thermophilic methanogens and sulfate-reducing archaea, respectively, as outgroups for the root identification, considering that they may be related only distantly with the identified sequences in our work.

In the phylogenetic tree reconstruction (Fig. 9), we determined that the evolutionary conclusion of these relationships is that the two species of Achaea or outgroups selected are ancestors of the nine species or ingroups identified with blue squares. Likewise, they belong to the three most dominant phyla groups that

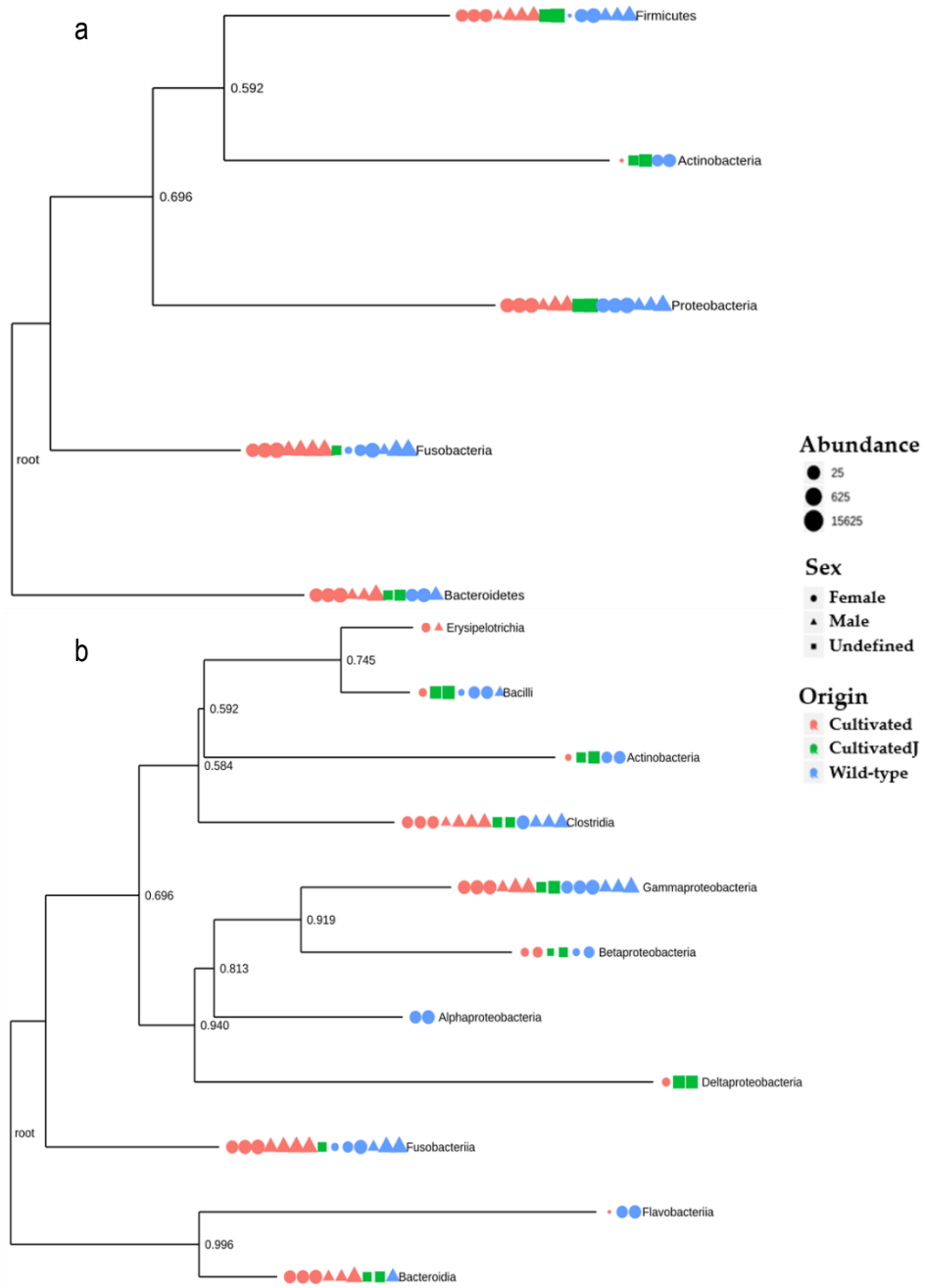

Figure 3. Taxonomic description by most abundant phylogenetic groups: a) phylum and b) class in the central microbiome of the gut tropical gars (*Atractosteus tropicus*), juveniles and adults, wild-type and cultivated.

integrate the core microbiome gut in adult *A. tropicus*. On the other hand, these nine species, identified by blue squares, are those that have probiotic potential.

DISCUSSION

We identified and compared the core microbiome and microbial diversity, respectively, in the gut of juveniles and adult *Atractosteus tropicus* by sex and origin. Previous studies have shown that the fish gut hosts an estimated 107 to 1,011 bacteria per gram of intestinal content (Nayak, 2010). The bacterial colonizers in fishes gut include Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Clostridia, Bacilli, and Verrucomicrobia (Ringø *et al.*, 2006; Desai *et al.*, 2012; Li *et al.*, 2013; Carda-Diéguez *et al.*, 2014; Ingerslev *et al.*, 2014a,b) with the first four being the most abundant, depending on environmental conditions

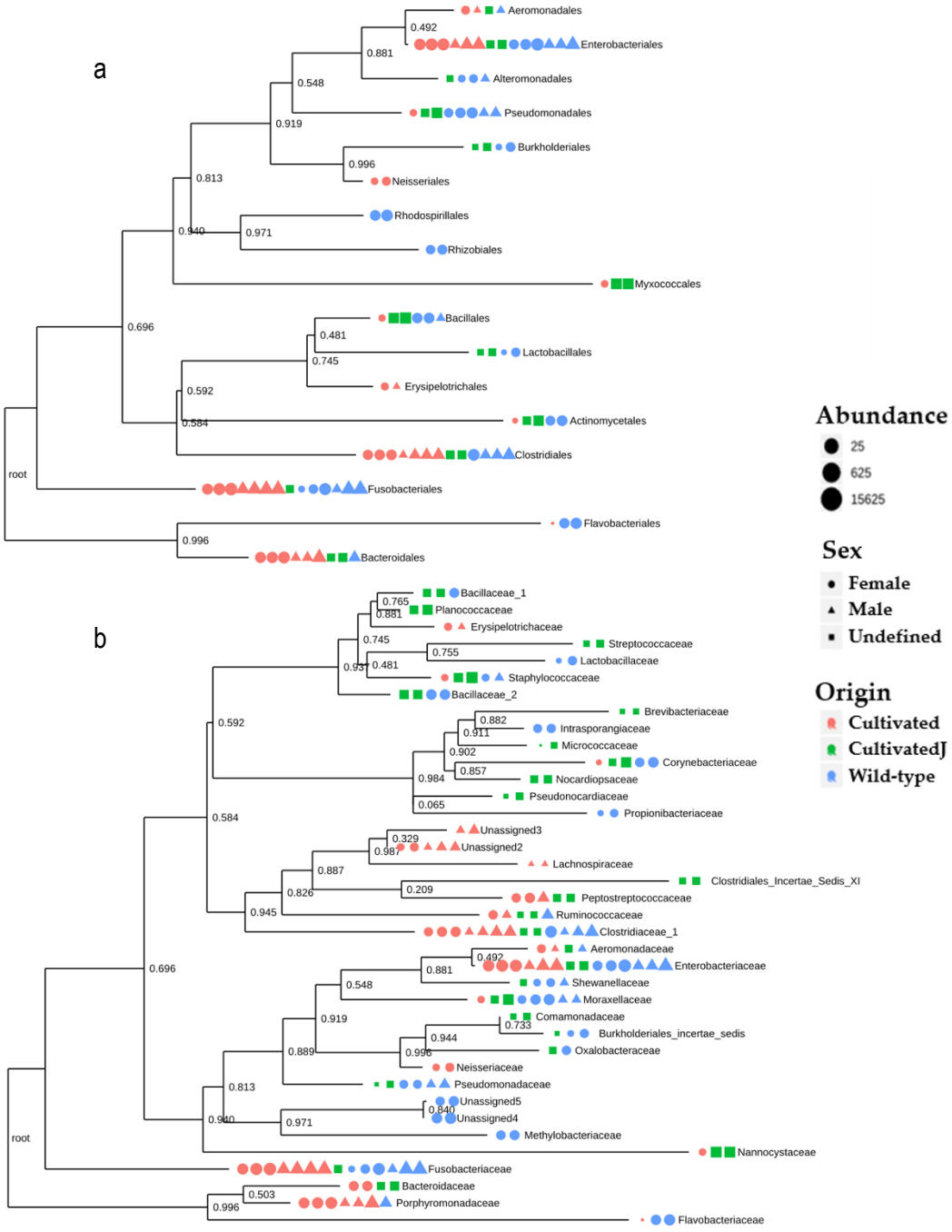

Figure 4. Taxonomic description by most abundant phylogenetic groups: a) order and b) family in the central microbiome of the gut tropical gars (*Atractosteus tropicus*), juveniles and adults, wild-type and cultivated.

and the host's diet (Wang *et al.*, 2018). Almost all Fusobacteria, Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria 16S rRNA sequences that we detect in GMBiot of the juvenile and adult *A. tropicus* (Fig. 2a, Table 3) belonged to the *Cetobacterium*, *Serratia*, *Edwardsiella*, *Paludibacter*, *Clostridium sensu stricto*, *Staphylococcus*, *Plesiocystis*, and *Myroides* genres (Fig. 2b, Table 2). The observed bacterial profiles in the gut of *A. tropicus* adult by sex and origin may reflect specific central microbiota, beyond the differences most likely attributable to feeding behavior. At the phylum level, almost 90% of the total bacterial abundance was classified into a total of four phyla. Among these phyla, Fusobacteria and Proteobacteria were dominant in the 17 *A. tropicus* samples by sex and origin (Figs. 2a-3a). In contrast, Firmicutes, Proteobacteria, and

Table 4. Comparative statistical analysis of alpha diversity estimators by origin and sex. ASVs: Amplicon sequence variants, ACE: Abundance-based coverage estimators, MPC[1]: Multiple pairwise comparison between groups of samples, MPC[2]: Multiple pairwise comparison between the origin of sampling, *J'*: Pielou's evenness index, D^2: Inverse Simpson index.

Origin	Sex	Group	NºSeqs	NºASVs	Alpha diversity index comparison							
					ACE	MPC[1]	MPC[2]	*J'*	MPC[1]	MPC[2]	D^2	MPC[1]	MPC[2]
Cultivated	Female	AtCF	32,315	49	72.38 ± 6.12	***	***	0.41 ± 0.01	***	***	0.05 ± 0.01	***	***
	Male	AtCM	32,315	105	48.62 ± 4.63	***	***	0.37 ± 0.01	***	***	0.05 ± 0.01	***	***
	Undefined	AtCJ	32,315	50	320.82 ± 16.14	***	***	0.69 ± 0.01	***	***	0.06 ± 0.01	***	***
Wild-type		AtWF	32,315	73	103.57 ± 3.97	***	***	0.65 ± 0.01	***	***	0.09 ± 0.01	***	***
		AtWM	32,315	327	47.92 ± 3.18	***	***	0.59 ± 0.01	***	***	0.14 ± 0.01	***	***

Bacteroidetes were identified as dominant phyla in the gut microbiota of the cultivated *A. tropicus* juvenile (Fig. 2a, Table 2). The inferred physiological roles of these dominant prokaryotes are related to the metabolism of carbohydrates and nitrogenous compounds (Kormas *et al.*, 2014). Unlike the dominant microbiota of marine fish is facultative anaerobes, including *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Corynebacterium*, *Alteromonas*, *Flavobacterium* and *Micrococcus* (Onarheim *et al.*, 1994; Blanch *et al.*, 1997; Verner-Jeffreys *et al.*, 2003).

Alpha diversity results indicate the highest gut microbiota abundance and richness is found in cultivated juvenile and wild-type adult *A. tropicus* female, rather than adult wild-type males and the least gut microbiota abundance and richness is found in adult *A. tropicus*, cultivated females or males (Table 3). Ley *et al.* (2008) concluded that the gut microbiota of herbivorous mammals has an enormous richness and phylogenetic diversity, and both richness and phylogenetic diversity decreased among omnivores and decreased further among carnivores. We found the lowest richness and phylogenetic diversity (Table 3) in gut microbiomes from adults *A. tropicus*, which is defined as top piscivores (carnivores, *e.g.*, cultivated female and male *A. tropicus*). Indeed, MacFarlane *et al.* (1986) observed that farm-raised fish had a simpler gut microbiota than their wild counterparts.

Likewise, our coefficients of UniFrac similarity or dissimilarity by PCoA ordination analysis, indicate gut microbiota of cultivated juvenile, and wild-type and cultivated *A. tropicus* adult are only similar to two wild-type adult samples and one cultivated adult. Also, dissimilarity by most of the wild-type and cultivated samples, but the cultivated juvenile is more significant than all the other organisms, particularly (Fig. 4). The PCoA revealed that gut bacterial communities from adult *A. tropicus* by origin formed different clusters. Cultivated and wild organisms formed distinct clusters in the PCoA space (Fig. 4), suggesting that the enrichment and diversity of gut microbiota are affected by the origin. This result is similar to the research of Ni *et al.* (2012) in that the origin and host phylogeny are entirely related to the composition of adult *A. tropicus* gut bacteria. Moreover, previous studies have shown that the microbiotic diversity content in all intestinal sections depends not only on the fish size but also on their age (Moran *et al.*, 2005; Cantas *et al.*, 2012; Bolnick *et al.*, 2014; Clements *et al.*, 2014), such as the case of cultivated *A. tropicus* juvenile (sexually immature fish).

Numerous studies have been built on these results, demonstrating that many species of herbivores and omnivores fishes contain diverse intestinal communities

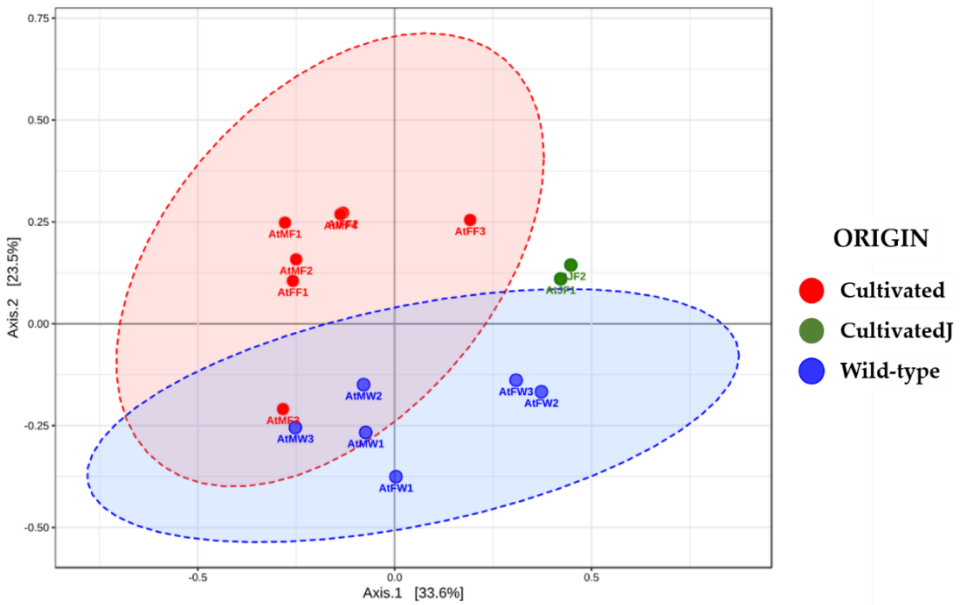

Figure 5. Similarity or dissimilarity coefficients of Unweighted UniFrac distances (UniFrac) by PCoA ordination analysis of the microbiota in *Atractosteus tropicus*. The green circles represent the cultivated juvenile, and blue and red circles correspond to wild and cultivated adult, respectively.

Table 5. Multiple pairwise comparison adonis test (*post-hoc*) of the different families (groups) of *Atractosteus tropicus*) specimens.

Family	Group		Sample size	Permutations	pseudo-F	*P*-value	*q*-value
	1	2					
1	Cultivated adults	Cultivated juveniles	9	999	4.31	0.02	0.03
2	Cultivated adults	Wild-type	13	999	2.32	0.02	0.03
3	Cultivated juveniles	Wild-type	8	999	2.34	0.04	0.04

nities (Rimmer & Wiebe, 1987; Clements *et al.*, 1989; Clements, 1991, 1997; Martínez-Díaz & Pérez-España, 1999; Ray *et al.*, 2012;) and that herbivorous and detritivorous fish species harbor distinctive microbial populations (Clements *et al.*, 2014). Feeding habits are also an essential factor that generally influences the microbial diversity in the fish core gut microbiome (CGMBiom), displaying a higher diversity in the following order: carnivores > omnivores > herbivores (Ward *et al.*, 2009; Larsen *et al.*, 2014; Li *et al.*, 2014a,b; Miyake *et al.*, 2015; Liu *et al.*, 2016). He *et al.* (2013) revealed that the herbivorous carp (*Ctenopharyngodon idellus*) reported a wider variety of bacterial species than the dark carnivorous carps and Gibel (*Carassius gibelio*), which are exclusively omnivorous, and also the sea bream, under the same culture conditions. We also identify this same trend widely in the CGMBiom in *A. tropicus* juvenile and adult, by sex and origin, because although these are omnivores, they prefer carnivorous habits (Méndez-Marin *et al.*, 2012) (Fig. 5a). At the sex level, we observed that the core microbiome is more diverse in female organisms, and particularly in wild-type organisms (Figs. 5b, 6a-b). In this sense, we can suggest that the feeding type of juveniles cultivated at the farmed and used in this work is more omnivore or herbivore than a carnivore.

More recent sequence-based approaches show that fish hindgut microbial communities much more closely resemble those of mammals than environmental microbial communities (Fidopiastis *et al.*, 2006; Sullam *et al.*, 2012), especially in the prevalence of Proteobacteria, Firmicutes and Bacteroidetes (Clements *et al.*, 2007; Smriga *et al.*, 2010; Sullam *et al.*, 2012; Ye *et al.*, 2014). These findings indicate that fish, like other vertebrates, harbor specific gastrointestinal communities (Clements *et al.*, 2014). We identified Proteobacteria as the most dominant phylum and the second dominant phylum in CGMBiom of the adult and cultivated *A. tropicus* juvenile, respectively (Fig. 2a).

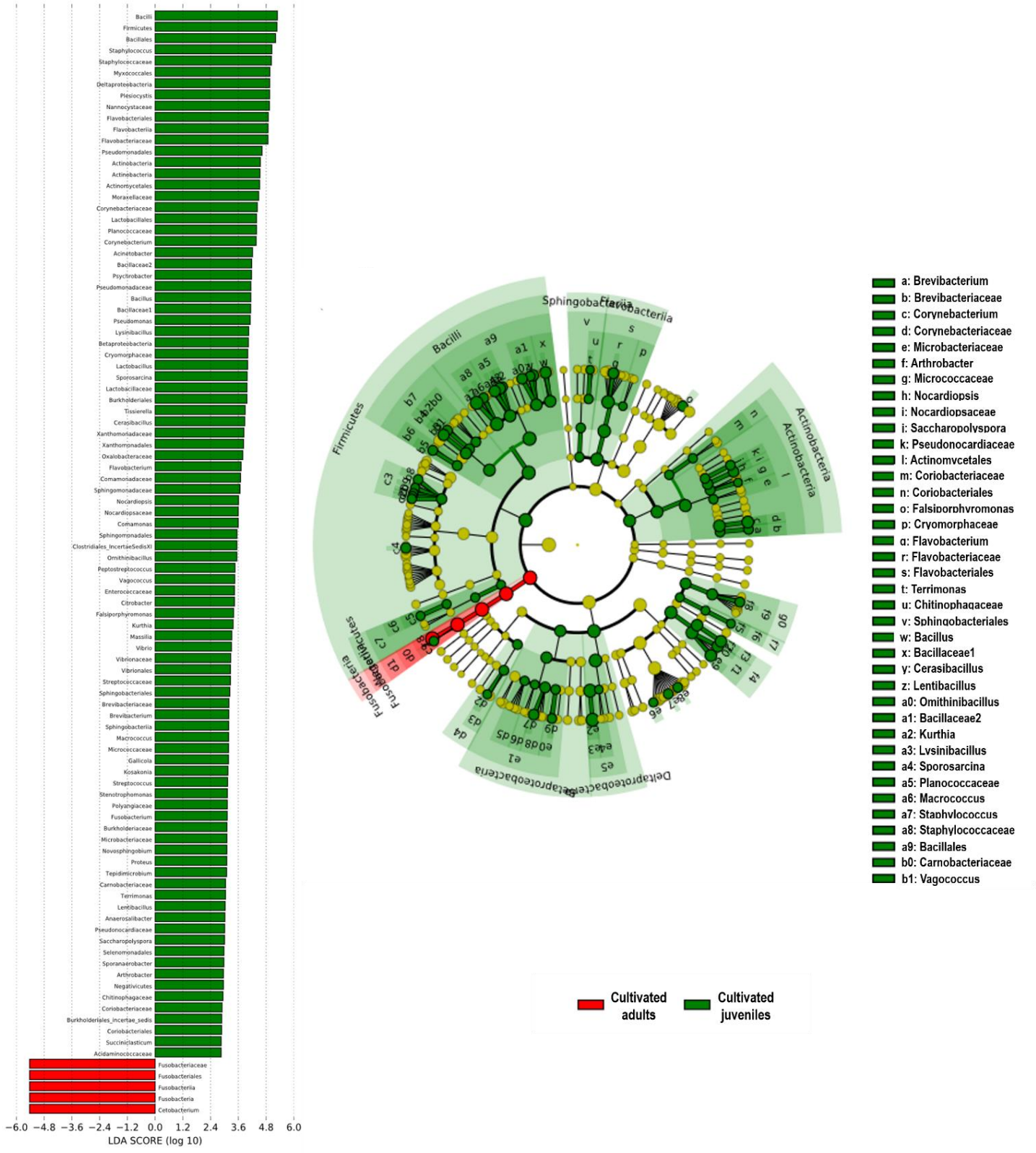

Figure 6. LEfSe Biomarkers identification analysis with LDA score histogram of the microbiota composition in *Atractosteus tropicus*. Comparison among cultivated juvenile and adult organisms. Highlighting that, at the phylum level, Firmicutes are dominant in cultivated juvenile organisms, whereas Fusobacteria is dominant in cultivated adult organisms. Red and green colors indicate cultivated juveniles and adult organisms, respectively.

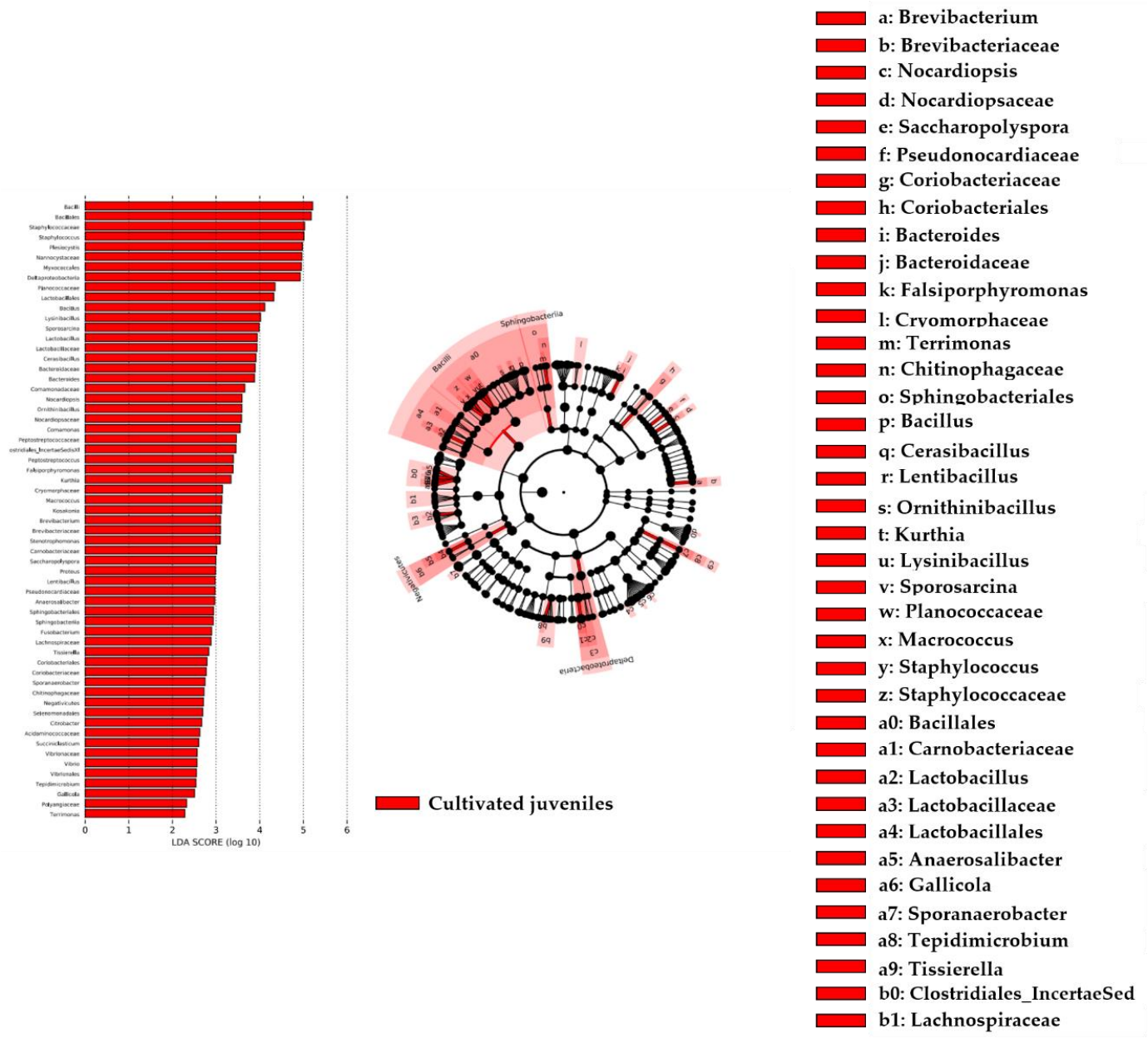

Figure 7. LEfSe Biomarkers identification analysis with LDA score histogram of microbiota in *Atractosteus tropicus* composition in cultivated juvenile organisms. We show statistically of gut microbiota abundance in *A. tropicus* gut of the cultivated juvenile organisms. Red color indicates cultivated juveniles.

According to Rudi *et al.* (2018), Proteobacteria phylum is very characteristic in wild-type fish and denotes of a high-fat diet.

We identified Fusobacteria as the most dominant phylum and the second dominant phylum in the gut of the cultivated *A. tropicus* juvenile and male adult (wild and cultivated), respectively (Fig. 2a). A few studies have shown Fusobacteria as dominant members of the gut microbiota of freshwater fishes (Van Kessel *et al.*, 2011; Di Maiuta *et al.*, 2013). Fusobacteria is anaerobic, Gram-negative bacilli that produce butyrate (Bennett & Eley, 1993), a short-chain fatty acid that is often the end product of the fermentation of carbohydrates including those found in mucins (Titus & Ahearn, 1988; Von Engelhardt *et al.*, 1998). In mammals, butyrate provides many benefits to the host, including providing a majority of the energy supply to gastrointestinal cells (Von Engelhardt *et al.*, 1998; Collinder *et al.*, 2003), enhancing mucus production, acting as an anti-carcinogen and anti-inflammatory, as well as playing a role in satiation (McBain *et al.*, 1997; Von Engelhardt *et al.*, 1998; Andoh *et al.*, 1999; Hamer

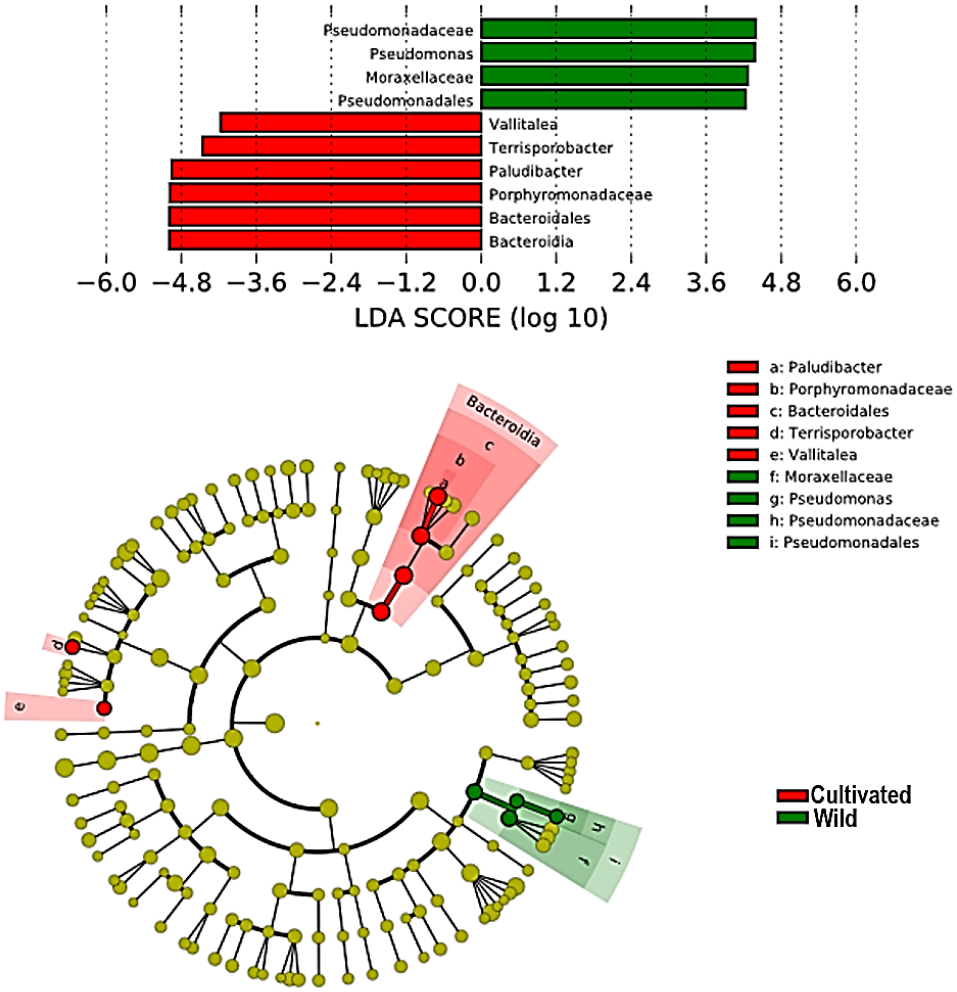

Figure 8. LEfSe Biomarkers identification analysis with LDA score histogram of microbiota in adults *Atractosteus tropicus* composition. Comparison between wild-type and cultivated organisms. Both show statistically significant differences depending on the origin. Red and green colors indicate cultivated and wild-type adult organisms.

et al., 2009;). This fatty acid has been found in the gut of herbivorous and omnivorous fishes only (Clements *et al.*, 1994; Clements & Choat, 1995). Nuez-Ortin *et al.* (2012) demonstrated that the ability of butyric acid to inhibit potential freshwater fish pathogens and sodium butyrate is currently sold as a food additive to promote fish health and growth. However, trials using blends of sodium butyrate and other additives have not proven beneficial (Owen *et al.*, 2006; Gao *et al.*, 2011).

OTUs sequences of the *Cetobacterium* genus were identified as dominant mainly in the gut microbiome of *A. tropicus* adult, wild and cultivated male fish. *Cetobacterium* genus is widely recognized in freshwater and warm water fish species (Tsuchiya *et al.*, 2008; Larsen *et al.*, 2014; Li *et al.*, 2017). *Cetobacterium* genus members' presence can perform fermentative metabolism of peptides and carbohydrates and produce vitamin B12 (cobalamin) (Larsen *et al.*, 2014). *Cetobacterium* and *Bacteroides* were reported as significant producers of the vitamin B12 in the intestine (Tsuchiya *et al.*, 2008; Vogiatzoglou *et al.*, 2009) and they were the dominant genera in grass carp's intestine, with the abundance of more than 50% (Li *et al.*, 2015a). Animals, plants, and fungi are incapable of cobalamin production, and it is the only vitamin that is exclusively produced by microorganisms, particularly by anaerobes (Roth *et al.*, 1996; Martens *et al.*, 2002; Smith *et al.*, 2007). Qi *et al.* (2017) showed that different concentrations of ammonia would affect the abundance of *Bacteroides* and *Cetobacterium* in gut fish, and the higher the concentration, the lower the abundance.

The majority of the most abundant OTUs present in CGMBiom of the cultivated *A. tropicus* juvenile samples were from the *Staphylococcus* (2.62%),

Plesiocystis (2.56%) and *Myroides* (2.36%) genera. *Staphylococcus* is the only genus exclusive for the distal gut digesta of fish fed a median carbohydrate and protein diet (Villasante *et al.*, 2019) or is associated with the gut immune system, which is known as gut-associated lymphoid tissues (GALT, Nayak, 2010). *Plesiocystis* is a genus of Myxobacteria and a monotypic taxon containing only its type species, *Plesiocystis pacifica.* Both the genus and the species were first described in 2003, based on two strains isolated from samples collected from the Pacific coast of Japan (Iizuka *et al.*, 2003). They have unique cellular metabolism featuring, among other characteristics, partially saturated menaquinone [MK-8(H2)] or vitamin K2, polyunsaturated fatty acid production, and an absence of hydroxy fatty acids. Like typical myxobacteria, they have high GC content (Iizuka *et al.*, 2003). In nature, Myxobacteria appears to be ubiquitous and diverse, and are not just terrestrial but found in marine, estuarine, and a variety of other saline ecosystems. Metagenomic analyses indicate that the marine myxobacteria identified to date occupy only a small portion of the potential *Nannocystineae* phylogenetic tree (García *et al.*, 2018). *Myroides* strains have been identified in clinical samples, marine environments, freshwater fish and insect gut (Holmes *et al.*, 1977; Gonzalez *et al.*, 2000; Spiteller *et al.*, 2000; Yoon *et al.*, 2006; Zhang *et al.*, 2008), but are not components of the normal human microbioma (Maraki *et al.*, 2012). *Myroides* genus is a member of the Bacteroidetes and Flavobacteriaceae, phylum and family, respectively. JS-08T strain belonging to the genus *Myroides* contains other menaquinone-6 (MK-6) type or vitamin K2, as the predominant menaquinone, and dominant fatty acids, such as iso-C15: 0, iso-C17: 0 3-OH and a summarized characteristic that consists of iso-C15: 0 2 -OH or C16: 1v7c. The G + C DNA content of this strain is 34.2 mol % (Cho *et al.*, 2011). Vitamin K plays a vital role in blood coagulation and bone mineralization in fish, but the suggested minimum requirement varies considerably depending on the vitamin K source used. Vitamin K deficiency is characterized by mortality, anemia, increased blood clotting time, and histopathological changes in liver and gills (Krossøy *et al.*, 2011). In the case of *M. odoratimimus* can be classified as a "strong biofilm-producer" (Stepanović *et al.*, 2007) and their use as a probiotic potential in certain juvenile fish turns out to be interesting (Villamil-Diaz & Esguerra-Rodríguez, 2017), this suggests that a vast diversity of species is unexplored.

We detected OTUs of Deinococcus-Thermus bacterial phyla to be non-dominant (0.003 ± 0.006 SD) in the gut microbiome of *A. tropicus* adult, wild-type males only. However, Deinococcus-Thermus species are known for their resistance to extreme stresses, such as radiation, oxidation, desiccation, and high temperature (Li *et al.*, 2015b). The deeply branching Deinococcus-Thermus lineage is recognized as one of the most extremophilic phyla of bacteria (Theodorakopoulos *et al.*, 2013). Sequence information from Deinococcus-Thermus phylum is presently available for only a limited number of species. However, the sequenced genomes include species from both the leading families (*i.e.*, Deinococcaceae and Thermaceae) within this phylum (Griffiths & Gupta, 2007). In recent years, researchers have begun using *Deinococcus* spp. in biotechnologies and bioremediation due to their specific ability to grow and express novel engineered functions. More recently, the sequencing of several *Deinococcus* spp.

Furthermore, comparative genomic analysis has provided new insight into the potential of this genus. Features such as the accumulation of genes encoding cell cleaning systems that eliminate organic and inorganic cell toxic components are widespread among *Deinococcus* spp. Other features, such as the ability to degrade and metabolize sugars and polymeric sugars, make *Deinococcus* spp. an attractive alternative for use in industrial biotechnology (Gerber *et al.*, 2015). That is why their functional role in the gut of *A. tropicus* adults deserves further research.

Bacteroidetes phylum has a low relative abundance in the gut microbiota of *A. tropicus* adult, cultivated and wild-type, and very low relative abundance in the gut of cultivated *A. tropicus* juvenile (Fig. 2a, Table 2). Despite, this phylum is composed of three large classes of Gram-negative, non-spore-forming, anaerobic or aerobic, and rod-shaped bacteria that are widely distributed in the environment, including in soil, sediments, and seawater, as well as in the guts and on the skin of animals (Ley *et al.*, 2008). Likewise, a large part of the proteins synthesized by the genome of *Bacteroides*, a genus of Bacteroidetes, can break down polysaccharides and metabolize their sugars, playing a fundamental role in the degradation of complex molecules in the gut of the host. Their ability to harvest alternative energy sources from food could allow *Bacteroides* to be more competitive than other bacteria in CGMBiom of fish during starvation stage (Xu *et al.*, 2003; Xia *et al.*, 2014).

We identified *Clostridium sensu stricto* genus sequences in the GMBiom of *A. tropicus* adult, wild-type males only. This genus has also been identified in GMBiom of carp fish (Li *et al.*, 2015a). The members of the genus *Clostridium sensu stricto* are dominant in the intestinal microbiota of grass carp (*Ctenopharyngodon idellus*) and are also versatile in their ability

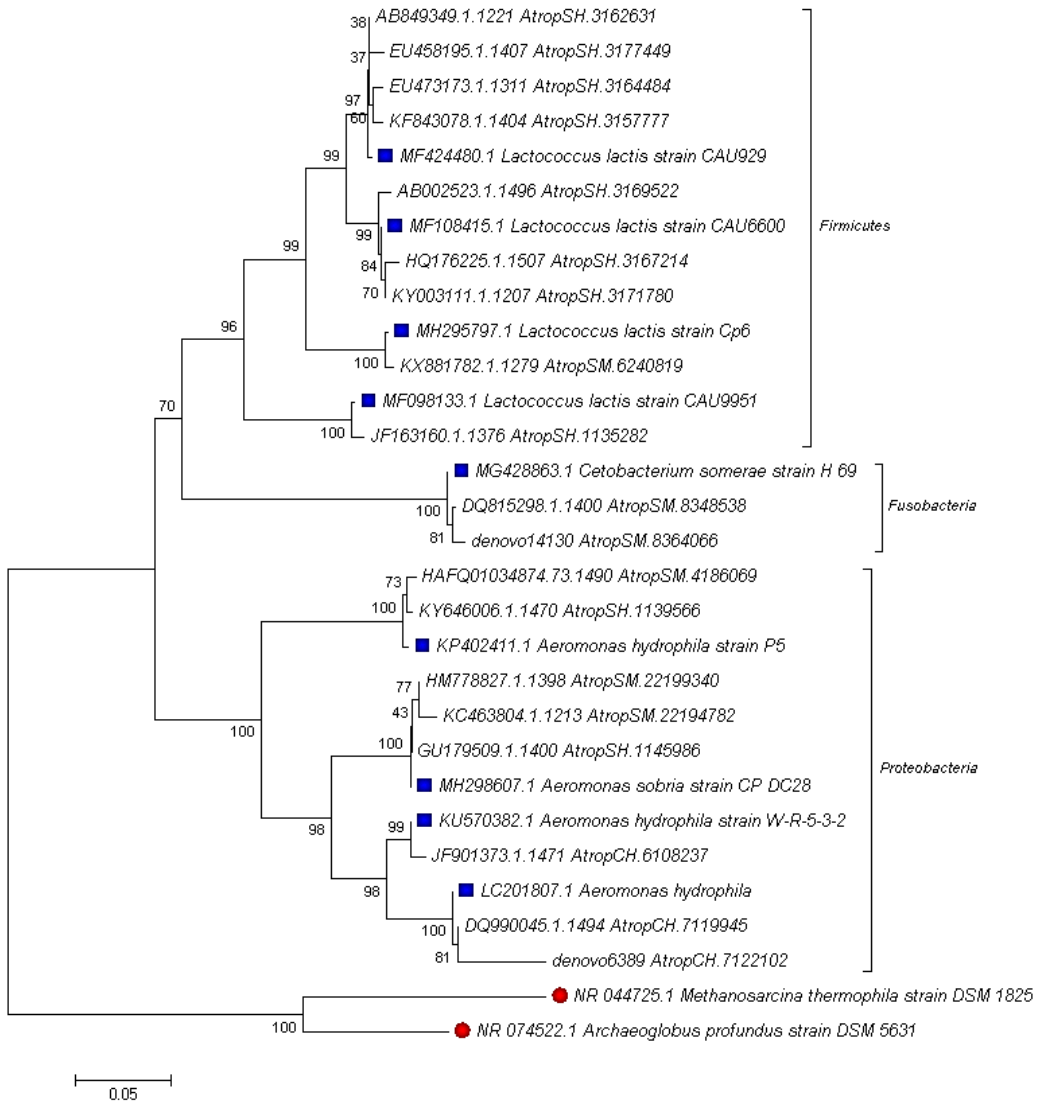

Figure 9. Phylogenetic tree from the rRNA 16S gene constructed of some organisms found in the literature with probiotic potential in fish (see Table 1). Red circles and blue squares represent outgroup and reference sequences, respectively.

to utilize various polysaccharides, such as cellulose, xylan, and hemicelluloses, which constitute the significant part of vegetal fibers (Uffen, 1997; Uz & Ogram, 2006; Li *et al.*, 2015a). Others also include not only species with saccharolytic and fiber-fermenting activities but also proteolytic species (Lubbs *et al.*, 2009; Pikuta *et al.*, 2009; Li *et al.*, 2015a).

Serratia, *Edwardsiella*, *Plesiomonas*, and *Reyranella* are genera belonging to the Enterobacteriaceae family and the Proteobacteria phylum. These isolated bacterial species are facultative pathogens for fish and humans and may be isolated from fish without apparent symptoms of the disease (Walczak *et al.*, 2017). *Serratia* produces serrawettin that acts as a wetting agent to reduce the surface tension of the environment (Chan *et al.*, 2013). *Edwardsiella* has been isolated from tortoises (Iveson, 1971), crocodiles (Iveson, 1971), aquarium water (Bartlett & Lior, 1977), and seagull roosting areas (Berg & Anderson, 1972). *Edwardsiella* was isolated on several occasions during the examination of dressed catfish for *Salmonella* (Wyatt *et al.*, 1979). This report provides information on the isolation, identification, and incidence of *Edwardsiella* in freshwater catfish and their environment. There are many unanswered questions regarding their importance in freshwater fish. Liu *et al.* (2015) reported the pathogenicity of *Plesiomonas shigelloides* to fish. *P. shigelloides* can occur as natural intestinal flora of fish, but in case of stress conditions, the following symptoms are observed: darkening of the

body, hemorrhaging, fin rotting, ascitic fluid in the abdominal cavity, and lesions in internal organs. Phylogenetically, the *Reyranella* genus has an evolutionary lineage within the family Rhodospirillaceae in the class Alphaproteobacteria. The type species *Reyranella massiliensis* was initially identified by Pagnier *et al.* (2011). Subsequently, Kim *et al.* (2013) amended several characteristics (*e.g.*, nitrate reduction, respiratory quinone information) into the genus description, and more recently, Cui *et al.* (2017) found that reduction of nitrate to nitrite is variable, the predominant isoprenoid quinone is ubiquinone-10 (Q-10), major polar lipids are PME, DPG, PG, PE, and one unknown amino lipid. We detected these genera mainly in the gut microbiome of *A. tropicus* adult, wild-type females, and cultivated females and male fish (Figs. 2b, 3b). We consider that, although commonly known for their ability to cause deadly infectious diseases, this is a population of bacteria (identified as normal flora of adult *A. tropicus*), which despite symbiotically living on and between wild-type females and cultivated females and males of *A. tropicus* adult, really has a positive impact on host survival (Figs. 2b, 3b).

The *Paludibacter* genus has been found exclusively in African microbiota, probably due to their increased fitness to grow on polysaccharides abundant in xylan or cellulose diets (De Filippo *et al.*, 2010; Thomas *et al.*, 2011). We identified OTUs of the *Paludibacter* genus in the core gut microbiome of *A. tropicus* adult, in females and males cultivated fish, only (Fig. 2b).

In phylogenetic trees reconstruction (Fig. 9) and according to Table 1, we identified the species *Lactococcus lactis* strains CAU929 and CAU6600, Cp6 and CAU9951, *Cetobacterium* strain H69, *Aeromonas hydrophila* strains P5 and WR-5-3- 2, *Aeromonas sobria* strain CP DC28 and *Aeromonas hydrophila* with probiotic potential within the three dominant phyla in core gut microbiome of *A. tropicus* adult.

Considering the above, fish CGMBiom can influence nutrition, growth, reproduction, general population dynamics, and the host's vulnerability to diseases, thus supporting a crucial role in aquaculture practice (Ghanbari *et al.*, 2015). Current DNA sequencing technologies and bioinformatic analysis have contributed towards a deeper understanding of the complex microbial communities associated to diverse habitats, including CGMBiom of fish in response to a variety of factors affecting the host, including temperature variations, salinity, growth stage, digestive physiology and feeding strategy (Cahill, 1990; Jammal *et al.*, 2017). The concept' core microbiota' referred to a set of abundant microbial lineages that are shared by all individuals from the same species (Wong *et al.*, 2013). The concept of CGMBiom has been explored both in mammalian host's context and in freshwater fish (Turnbaugh *et al.*, 2009; Nam *et al.*, 2011; Roeselers *et al.*, 2011; Wu *et al.*, 2012; Wong *et al.*, 2013.

CONCLUSIONS

Greater diversity and richness in *Atractosteus tropicus* adult gut microbiome of wild than cultivated and *A. tropicus* gut microbiome of juvenile and adult wild females were higher than wild and cultivated males' adults, at the genus level, respectively. The core gut microbiome of *A. tropicus* juvenile and adult are constituted by Proteobacteria, Fusobacteria, Firmicutes, and Bacteroidetes phyla. Further, *A. tropicus* adults have adapted to the environment and diet due to their high saccharolytic capacity, fiber fermentation, and starvation activities. Also, it has been identified in *A. tropicus* juvenile gut microbiome the *Staphylococcus*, *Plesiocystis*, and *Myroides* genera. Likewise, Deinococcus-Thermus bacterial phyla and *Clostridium sensu stricto* genus were only identified in *A. tropicus* adult gut microbiome of the wild-type males, of great interest at biotechnology. In phylogenetic trees reconstruction, *Lactococcus lactis strains* CAU929, CAU6600, Cp6 and CAU9951, *Cetobacterium* strain H69, *Aeromonas hydrophila* strains P5 and WR-5-3-2, *Aeromonas sobria* strain CP DC28 and *Aeromonas hydrophila* were identified, which have the probiotic potential within the three dominant phyla in core gut microbiome of *A. tropicus* adult. The CGMBiom of *A. tropicus*, due to essential roles in the immune system modulation, the intestinal epithelium proliferation, and the regulation of the dietary energy intake, is increasingly regarded as an integral component of the host. Understanding the factors that influence the composition of these microbial communities is essential to health management, and the application to aquatic animals still requires more investigation.

ACKNOWLEDGMENTS

The authors thank the Mexican National Council of Science and Technology for the postgraduate studies thesis scholarship and the mixed scholarship granted to carry out the research internship at the University of Valencia (Spain). This research received external funding from the Mexican National Council of Science and Technology "Strengthening of the Master's Degree in Environmental Sciences for its Permanence in the National Register of Quality Graduates of CONACYT" Reg. No. TAB-2014-C29-245836 and the Project "Estudio de la fisiología digestiva en larvas y juveniles de pejelagarto (*Atractosteus tropicus*) con base en técnicas histológicas, bioquímicas y moleculares" Reg. No. CB-2016-01-282765.

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Received: 14 August 2019; Accepted: 7 April 2020