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High Abundance of *Candidatus* Arthromitus in Intestinal Microbiota of *Seriolella violacea* (Palm Ruff) under Reared Conditions

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Abstract: Intestinal microbiota has been involved in several processes that benefit the host, such as digestion, nutrient metabolism, resistance to pathogens colonization and immune function. In this study, we investigated the diversity, composition and functional prediction of microbiota of reared *Seriolella violacea* (palm ruff) in the same cohort sampled at different times (7-, 8- and 9-month-old). Microbial community structure analyses, using 16S rRNA amplicon sequencing, revealed that the intestinal microbiota was dominated by the phyla Firmicutes, Proteobacteria, Fusobacteria and Tenericutes. At the genus level, *Candidatus* Arthromitus was the most abundant in all sampled timepoints, representing in average 78% of the bacterial community (ranging from 18 to 98%), corresponding to segmented filamentous bacteria, which are interesting because they have been associated with the maturation of immune responses in the gut and protecting the host from bacterial infections. The comparisons of the intestinal microbiota among the three groups showed differences in abundance of bacterial taxa and also in alpha diversity indexes (Shannon and Simpson), as well as beta diversity metrics (weighted and unweighted UniFrac). Potential functions of the intestinal microbiota of palm ruff were retrieved using Philipin and Tax4fun and these analyses revealed high levels of genes for sugar metabolism. To our knowledge, this study represents the first description of the intestinal microbiota of *S. violacea*.

Keywords: microbiota; palm ruff; *Seriolella violacea*; *Candidatus* Arthromitus; microbiome; aquaculture; diversification

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1. Introduction

Seriolella violacea (palm ruff) is a pelagic fish found in subtropical waters along the northern Chilean and Peruvian coast, between 1° S and 34° S [1]. Palm ruff is an important resource for Chilean and Peruvian fisheries and is considered a target species for the diversification of aquaculture in northern Chile [2]. The interest in Palm ruff culture is based on comparative advantages that make this species attractive to cultivate, such as fast growth, spontaneous spawning in captivity, high fertility rates and high content of omega-3 [3–5]. This has led to advances in the knowledge of the influence of abiotic factors and biological aspects of the species, such as the effect of temperature on ammonia excretion rates [6] or on yield and abnormalities during early development [7], as well as a recent study by [8] describing the morpho-functional development of the larval digestive system.

Whilst progress has been made to advance knowledge in the *S. violacea* aquaculture, the information regarding microbial communities and their potential impact on rearing of this species remains unknown.

Microbiota research has established the key role of microbial communities in a wide range of biological processes that generate beneficial effects for the host, such as digestion, nutrient metabolism, resistance to pathogen colonization and immune function [9]. Knowledge of the fish microbiota offers a new vision that allows to improve nutrition and welfare in aquaculture fishes, interest in this field is reflected in the high number of studies as reviewed by [10,11]. The increase in these studies is related to the advancements in next-generation sequencing platforms, which have allowed to expand the knowledge of the microbiota of various species of interest for aquaculture, evaluating, for example, the influence of diet, abiotic factors, population density, among other. In this study, we focused in exploring the composition of the bacterial communities of *Seriolella violacea* at the juvenile stage and belonging to the same cohort, under reared conditions using the 16S rRNA gene-based approach with a next-generation sequencing, and the likely pathways in which the intestinal microbiota is involved. This juvenile stage is very important because is the previous step to the fish transportation to the marine cages.

2. Materials and Methods

2.1. Sample Collection

Seriolella violacea juveniles belonged to a single cohort were collected from an aquaculture facility at the Universidad Católica del Norte (Coquimbo, Chile; latitude 29.966 S; longitude 71.751 W) during summer 2019. A total of 950 fish were kept in tanks of 2 m^3 in an open-flow system; >80% oxygen saturation and water temperature (17 to 20 °C) were monitored twice a day, and salinity 35 psu. The fish were fed daily at 1.5–2% of the biomass with a commercial extruded diet (dry pellets, 42% crude protein and 20% crude fat, "Nutra Supreme 100", Skretting). Then, three sampling times were considered in the study of this unique cohort, 7-, 8- and 9-month-old (MO). All samples were taken from the same tank to avoid tank effects, with average observed fish density ranged from 11.8 (7 MO) to 23.3 K/m^3 (9 MO, see Supplementary Table S1). At each sampling time, fourteen individuals were randomly selected, euthanized with an overdose of the anesthetic tricaine methane sulfonate [12] and biometric measurements were taken. Intestines were dissected using sterile instruments and collected in a 1.5 mL eppendorf tube. Due to the amount of sample obtained, samples from two fish were pooled generating 7 samples per sampling time and stored at −80 °C until DNA extraction. The posterior of the intestine section was chosen to perform the study.

This study followed the recommendations of Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Committee on the Ethics of Animal Experiments of the AquaPacífico approved the protocol.

2.2. DNA Extraction and PCR Amplicon Sequencing

DNA was extracted using a QIAamp PowerFecal®DNA Isolation Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol, including prior treatment with lysozyme (SIGMA, Darmstadt, Germany) at 0.8 mg/mL at 37 °C for 60 min and then with proteinase K (Invitrogen) 0.1 mg/mL at 37 °C for 60 min. DNA concentrations were measured with Qubit® dsDNA HS Assay kit (Life Technologies, Carlsbad, CA, USA). Subsequently, the V4 region of 16S rRNA gene was amplified with the primer pair 515F (5′-GTG CCA GCM GCC GCG GTA A -3′) and 806R (5′- GGA CTA CHV GGG TWT CTA AT -3′) described by [13]. Each 30 µL PCR reaction contained 1.5 U (5 U/µL) GoTaq® G2 Flexi DNA Polymerase (Promega, Madison, WI, USA), 6 µL of buffer (5×), 1.5 mM $MgCl_2$, each primer at 0.25 pM, 0.5 mM dNTPs and 20 ng of DNA. Reaction negative controls without DNA were included. All PCR reactions and amplicon purifications were performed as described by [12] with the QIAquick® PCR Purification kit (Qiagen). The amplicon concentration of each sample was measured using the Qubit®dsDNA HS Assay

kit (Life Technologies). Libraries were sequenced on an Illumina MiSeq platform via 2 × 250 bp.

2.3. Bioinformatics Analysis

The raw sequences have been deposited in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information under Bioproject accession PJRNA6592926. Amplicon sequence variants (ASVs) were generated using the DADA2 (v1.8.0) package in R [14]. Briefly, forward and reverse reads were trimmed at 240 bp and 210 bp, respectively, using the *filterAndTrim* function with standard parameters (maxN = 0, truncQ = 2, and maxEE = 2). Then, sample inference was performed using the inferred error model and chimeric sequences were removed using the *removeBimeraDenovo* function. Taxonomic assignment was performed using SILVA version 1.3.2. as reference with a minimum bootstrap confidence of 80. Subsequently, using the R package Phyloseq [15], unassigned ASVs at the phylum level were discarded. Due to variation of sequence depths among samples, the dataset was normalized according to the sample with the least number of sequences (i.e., 20,928 reads per sample). The microbial diversity of each sample (alpha diversity) was assessed using species richness (Chao1) and the Shannon and Simpson diversity indexes. Beta Diversity was estimated by computing the unweighted and weighted UniFrac distances and was visualized using PCoA. To test for homogeneity of multivariate dispersion among age groups, permutation multivariate analysis of dispersion was performed with the betadisper and permutest functions of the Vegan package [16].

To investigate the possible functional pathways of the intestinal microbiota, we used Piphillin [17] and Tax4Fun [18], based on the Silva database version 1.3.2 and 1.2.3, respectively. First, we normalized our data by 16S rRNA gene copy number and then inferred the metagenomic contents. For the analysis, a sequence identity cut-off of 97% was implemented, and the inferred metagenomic functions were assigned using the Kyoto Encyclopedia of Genes and Genomes database (KEGG). To assess the quality of the predictions performed with Tax4Fun, the Fraction of Taxonomic units Unexplained (FTU) scores were obtained. The FTU measures the fraction of sequences assigned to taxonomic units that cannot be mapped to KEGG organism using the Tax4Fun association matrix.

2.4. Statistical Analyses

Statistical analyses were performed using R packages Phyloseq [15] and Vegan [16]. The normality of the alpha diversity indexes was tested through Shapiro–Wilk test [19]. The non-parametric Wilcoxon test was used for comparison between two age groups, whereas a Friedman test was used in case of three groups comparison. We tested the effects time on community dissimilarity using the permutational multivariate ANOVA (PERMANOVA) using the *Adonis* function in the R package Vegan with 999 permutations, p values < 0.05 were considered as significant. Differential abundance of taxa was performed using LEfSe [20] with default parameters (KW = 0.05; Wilcoxon = 0.05; LDA score threshold = 2.0). LEfSe was also used to identify the KEGG pathways significantly associated with each age group (KW = 0.05; Wilcoxon = 0.05; LDA score threshold = 3.0), for the results performed by Tax4Fun. In the case of the KEGG pathways inferred with Piphillin, the significant differences between the age groups were performed using DESeq2 [21] with default parameters, after floored fractional counts to the nearest integer. p values < 0.05 were considered as significant. All p-values were corrected with the Benjamini–Hochberg false discovery rate method.

3. Results

3.1. Fish Growth

The mean weight of juvenile *Seriolella violacea* increased from 26.00 ± 6.34 g at the first sampling (7 MO) to 36.4 ± 8.79 (8 MO) and then to 49.30 ± 12.02 g at the last sampling time (9 MO). There were significant differences in weight among age groups (ANOVA, $p < 0.001$). Biometric measurements were taken and showed in Supplementary Table S1.

3.2. Diversity of Intestinal Microbiota

We analyzed the bacterial communities of juvenile *Seriolella violacea* using next-generation sequencing of the 16S rRNA gene. All fish sampled in this study were apparently healthy at the time of sampling. Rarefaction curves reached the saturation phase plateau (Supplementary Figure S1), indicating that the sequencing depth per sample was adequate to represent the bacterial communities. We compared the alpha diversity indexes across different sampling time using metrics the richness estimator (Chao1) and diversity indexes Shannon and Simpson (Supplementary Figure S2). Statistical testing showed no difference for Chao1 ($p = 0.222$; Friedman test); in the case of Shannon and Simpson indexes, these showed significant increment over time ($p = 0.008$, $p = 0.008$, respectively; Friedman test). The comparisons between the different pair times are shown in Table 1.

Table 1. Summary of alpha diversity for intestinal microbiota from *Seriolella violacea*.

Indexes	7MO	8MO	9MO
Chao1	52.80 ± 24.94^{a}	71.10 ± 23.64^{a}	59.40 ± 21.50^{a}
Shannon	3.34 ± 0.01^{a}	3.41 ± 0.10^{a}	3.67 ± 0.18^{b}
Simpson	0.96 ± 0.00^{a}	0.96 ± 0.00^{a}	0.97 ± 0.01^{b}

Row values with different superscript letters (a and b) indicate significant differences between sampling time using Wilcoxon test, with $p < 0.05$.

Differential effect of time on the bacterial community composition were visualized by PCoA (Supplementary Figure S3). The PERMANOVA analysis results, based on unweighted and weighted UniFrac metrics, revealed a significant effect of age groups (unweighted: $R^2 = 0.230$, $p = 0.016$; weighted: $R^2 = 0.605$, $p = 0.001$). Betadisper indicated that microbiota community dispersion between individuals did not vary between age groups using the unweighted distance ($p = 0.925$), reinforcing the effect of age obtained from the PERMANOVA results. This was in contrast to that obtained using the weighted distance ($p = 0.001$). The comparisons between different times are shown in Supplementary Table S2.

3.3. Microbial Composition of the Intestinal Microbiota

Palm ruff microbiota was dominated by bacteria assigned to the phyla Firmicutes, Proteobacteria, Fusobacteria and Tenericutes, which jointly accounted for over 99% of the total bacterial communities across all the samples. The most dominant phylum was Firmicutes, which showed a decreasing trend along the sampling period, with an average abundance of 93.15%, 84.54% and 57.79% at 7 MO, 8 MO and 9 MO, respectively. In contrast, phylum Tenericutes presented an average abundance < 1% at 7 MO and 8 MO, which increased to 32.11% at 9 MO (Figure 1A).

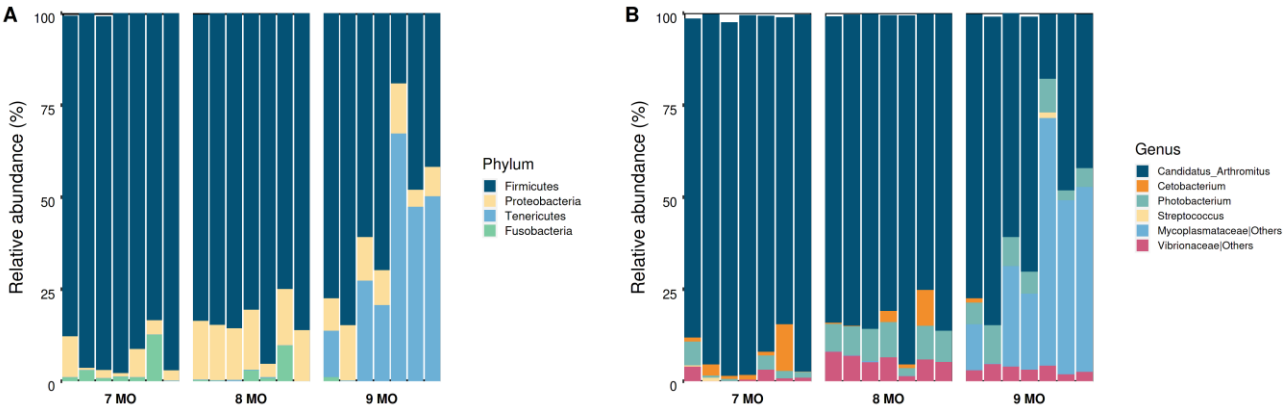

Figure 1. Relative abundance distributions of bacterial phyla (**A**) and genera (**B**) assignable to amplicon sequence variants (ASVs) from intestinal microbiota of *Seriolella violacea*. Those components with relative abundance > 0.5% in at least one sample were considered.

At the genus level, *Candidatus* Arthromitus, *Photobacterium* and *Cetobacterium* presented greater abundance. *Sequences* of the ASV assigned to *Candidatus* Arthromitus were compared to sequence from databases to check assignation and they all clustered together (Supplementary Figure S4). Highlighting *Candidatus* Arthromitus the finding of as the most abundant genus in all the sampled timepoints (n = 21); at 7 MO accounted for 92.90% of abundance, but its abundance decreased to 84.47% at 8 MO, and a further decrease to 57.45% was registered at 9 MO (Figure 1B). By contrast, the abundance of Photobacterium increased over time, with a 2.62% at 7 MO, and 11.22% and 9.49% at 8 MO and 9 MO, respectively. Interestingly, the sequences affiliated to family Mycoplasmataceae were detected an average abundance < 1% in 7 MO and 8 MO, increased to 32.11% at 9 MO; however, the resolution taxonomic not associated with any genus within this family (Figure 1B; Supplementary Figure S5).

Differences in abundance of the bacterial taxa between sampling times were determined through a LEfSe analysis. A total of 22 taxa at different classification levels were found to have significant differences among age groups (Figure 2). At 7 MO the genus *Cetobacterium* were significantly increased (p = 0.0096), as well as the higher taxonomic levels of which this genus is part (Fusobacteriaceae family, Fusobacteriales Order, Fusobacteriia Class and Fusobacteria Phylum). By contrast, at 8 MO phyla Proteobacteria (p = 0.0029) and Firmicutes (p = 0.0214), and genera *Candidatus* Arthromitus, *Photobacterium* and *Candidatus* Xenohaliotis were significantly increased, among other taxa. Meanwhile, Tenericutes phylum (p = 0.0004) were significantly increased at 9 MO, but no significant genera were revealed at this time; however, at family level, Mycoplasmataceae (p = 0.0004) was registered significant.

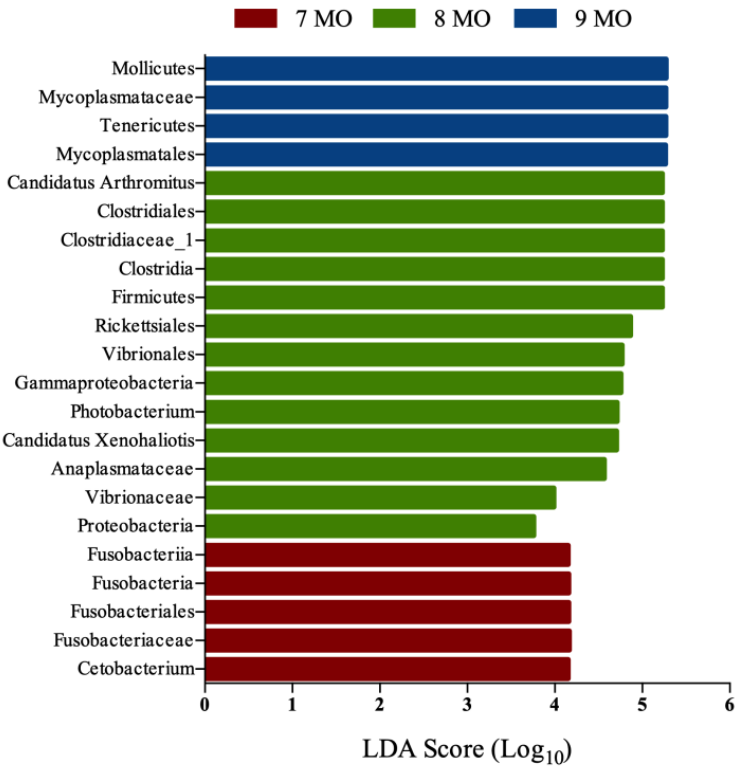

Figure 2. LEfSe identified the differentially abundant taxa among sampling time. The histogram shows the linear discriminant analysis (LDA) scores computed for different level taxa, based on their relative abundance in the intestinal microbiota of *Seriolella violacea*.

3.4. Microscopic Visualization of Filamentous Bacteria in Palm Ruff Intestine

Candidatus Arthromitus has been described as a segmented filamentous bacteria (SFB) belonging to the family Clostridiaceae. Members of this group display a unique life cycle

which involves binding to epithelial cells and elongate to form long filaments of up to 100 µm in length. In order confirm the presence of such peculiar bacterial morphology, palm ruff intestines were examined using scanning electron microscopy (SEM). The presence of SFB, probably *Candidatus* Arthromitus, attached to villi was observed by SEM as shown in Figure 3.

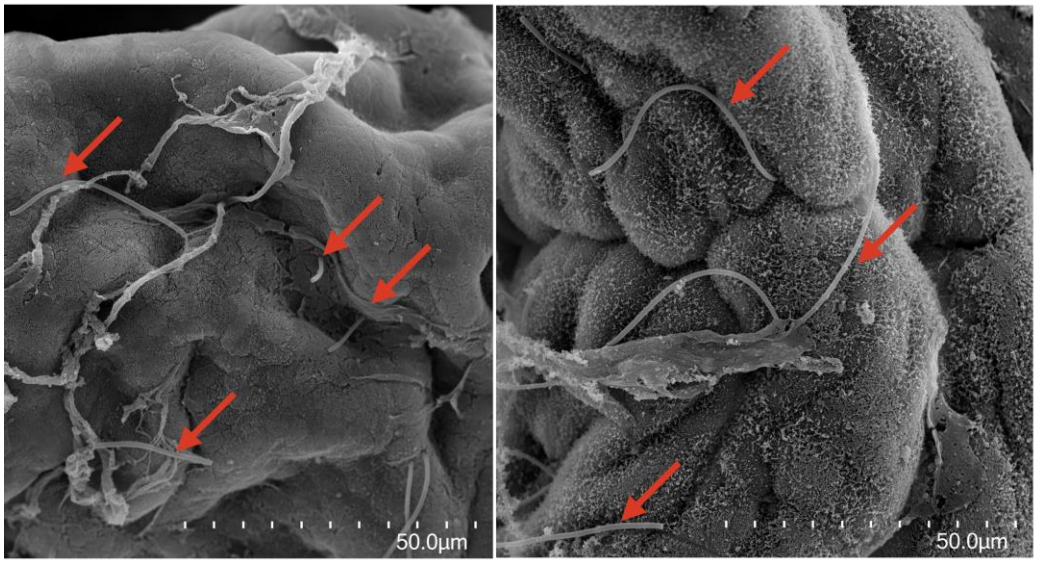

Figure 3. Scanning electron microscopy (SEM) showing the presence of segmented filamentous bacteria (SFB, in red arrows), probably *Candidatus* Arthromitus, associated to the epithelial cells in palm ruff intestines.

3.5. Microbial Functional Prediction

A total of 294 inferred functional pathways were associated with the intestinal microbiota of *S. violacea* by Piphillin. Thus, the most abundant functional pathways were those corresponding to metabolic pathways (ko01100; mean proportion ranged from 14.67 to 15.17%), biosynthesis of secondary metabolites (ko01110; 6.09–6.39%), microbial metabolism in diverse environments (ko01120; 4.61–5.57%) and biosynthesis of antibiotics (ko01130; 4.27–4.54%), among others (Supplementary Figure S6A). The statistical comparison the KEGG pathways in relation to the different sampling time, revealed only one pathway corresponding a Pertussis (ko05133) as significant between 8- and 9-month-old fish ($p = 0.004$). However, the average proportion of this pathway was low (0.026% and 0.021% at 8 MO and 9 MO, respectively). In contrast, 275 KEGG pathways were inferred using Tax4Fun, of which the most abundant were those corresponding to ABC transporters (ko02010; mean proportion ranged from 9.71 to 12.80%), two-component system (ko02020; 6.10–9.13%), purine metabolism (ko00230; 3.16–3.78%) and bacterial secretion system (ko03070; 1.65–2.51%), among others (Supplementary Figure S6B). A total of 11 pathways resulted significantly different among different sampling time groups by LEfSE (Figure 4), highlighting the histidine metabolism associated with 7 MO fish and starch and sucrose metabolism on 8MO fish. The FTU scores, as a measure of the quality of the predictions, were high for all the samples, ranging between 0.57 and 0.97 (Table 2).

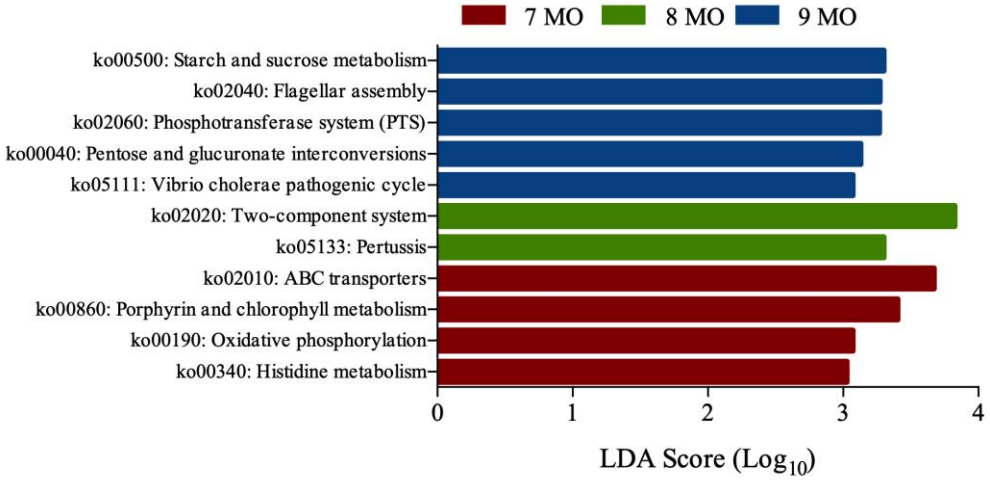

Figure 4. LEfSe identified the differentially abundant metabolic pathways among sampling time. The histogram shows the linear discriminant analysis (LDA) scores in different colors, according to age group.

Table 2. The Fraction Taxonomic Units Unexplained (FTU) scores of Tax4Fun metagenome prediction for *Seriolella violacea* microbiota, according to sampling time.

Group	FTU Scores Range (Average ± SD)
7MO	0.78–0.97 (0.87 ± 0.08)
8MO	0.57–0.91 (0.70 ± 0.10)
9MO	0.68–0.89 (0.77 ± 0.07)

4. Discussion

To our knowledge, this study represents the first description of the intestinal microbiota of *Seriolella violacea*. The intestinal microbiota of juvenile palm ruff was represented by few phyla, mostly by the phylum *Firmicutes*. The dominance of this phylum in microbiota of fishes under aquaculture conditions has been supported by research studies in different species, such as *Paralichthys adspersus* [22], *Oncorhynchus mykiss* [23] and *Genypterus chilensis* [24]. These authors also described the phyla *Proteobacteria*, *Fusobacteria* and *Tenericutes* as components of the microbiota of these fish species, in accordance with the observed in the present study.

Interestingly, our results indicated that *Cantidatus* Arthromitus, which belongs to the family *Clostridiaceae* (phylum *Firmicutes*), represents the most abundant genus in the intestinal microbiota of juvenile *S. violacea*. The observation of dominant taxa has been reported in other marine fish such as *Seriola lalandi*, *Paralichthys adspersus* and *Paralichthys olivaceus* [22,24–27].

The present study is the first to register *C.* Arthromitus as dominant in the microbiota from healthy fish. Previously, this genus has been described in *O. mykiss* associated with the disease called Rainbow Trout Gastroenteritis (RTGE) [28]. Nevertheless, *C.* Arthromitus has also been described as part of the microbiota of different fishes such as *Acipenser baerii* [29], *Piaractus mesopotamicus* [30] and *Oreochromis aureus* [31].

C. Arthromitus has been described as a microbiota component of many animals such as horses, cattle, mice, rats, pigs, insects, turkey and even humans [32]. This genus has been proposed as a group of segmented filamentous bacteria (SFB) commonly found attached to the intestinal walls of many animals [33]. Segmented filamentous bacteria (SFB) are host-adapted, intestinal symbionts that influence the adaptive and innate immune responses of their host. SFB are Gram-positive, spore-forming, microaerophilic bacteria characterized by a distinctive filamentous morphology, long filaments of up to 100 µm in length. Amongst intestinal bacteria, SFB are unique because they penetrate the intestinal

mucus layer and intimately associate with host cells without invading the host [32]. These SFB tightly adhere to small intestinal epithelial cells, influencing the immune responses, specifically, SFB showed the unique ability to drive the accumulation of Th17 cells in the small intestinal lamina propria of mice [34–36]. These cells play vital roles in protecting the host from bacterial and fungal infections, especially at the mucosal surface [37]. In addition, a large amount of research that has demonstrated the positive role that SFB play in the maturation of the host gut immune barrier, inducing both innate and adaptive immune responses, for example in the mouse, gut is important during the postnatal period [38]. Microbiome research has revealed the presence of *C.* Arthromitus in higher proportions in higher-performing flocks as compared to matched lower- performing flocks, suggesting that SFB may help to improve gut health and protect commercial turkeys from pathogens that negatively impact productive parameters [32]. The high abundance of *C.* Arthromitus in juvenile *S. violacea* may be important to gut mucosa maturation and protection against diseases, however, given the limited evidence of *C.* Arthromitus and SFB in fishes, further studies by metatranscriptomic analysis are required to determine the influence of this genus on the immune parameters of juvenile *S. violacea*.

Our study has revealed that the intestinal microbiota of *Seriolella violacea* juveniles at both alpha (Shannon and Simpson indexes) and beta diversity levels were influenced for age groups or different sampling times. Due to the fact that in this study the type of administered feed remained constant along the sampling period, the shifts in the microbiota could be associated to host-specific intrinsic factors, such as age, as described previously [39]. Yin et al. [40] performed screening of SBF on human fecal samples from subjects ages 0 months to 75 years old (Supplementary Figure S7), they revealed that 25% of individuals carry SFB in their gut from ages 0–6 months, 75% carry SFB from ages 7–12 months, and only 6.2% carry SFB from ages 3–75 [40]. Liao et al. [41] reported that, in chickens, SFB colonization peaked at approximately 2 weeks of age and decreased as they aged to 6 weeks and that reduced numbers of SBF were inversely proportional to the amount IgA present in the of intestine.

Additionally, shifts in bacterial composition of *S. violacea* were observed, showing a decrease of *Firmicutes* (*Clostridiaceae* family) and an increase in *Tenericutes* (*Mycoplasmataceae* family) as a consequence of the increase in months of age of juvenile *S. violacea*. Previous works have reported changes in gut microbiota within a same experimental group during time, suggesting that these differences were associated with age-related physiological changes during the experiment [42,43]. According to this observation, age has been reported to be a factor affecting beta diversity of gut microbiota in Chinook salmon, reared either in freshwater or saltwater environment [44]. Furthermore, several research in *S. salar* have showed that the relative abundance of genera belonging to *Mycoplasmataceae* family increased consistently with of host age [45–47].

Palm ruff is a carnivorous species, whose natural feed is rich in protein (>40%) and low in carbohydrates (<1%) [48], and thus natural selection pressure has shaped their capacity to better deal with changes in dietary protein source rather than changes in digestible carbohydrates. However, artificial feed contains important levels of carbohydrates and fibers, promoting adaptation in the microbiota composition and explain the enrichment of pathways related to sugars and starch metabolism, which were more associated to the last sampling time. This is based on fish from different trophic levels exhibit differences in metabolic capacity of their gut microbiota. Liu et al. [49] observed cellulose-degrading bacteria as the dominant bacterial group in herbivorous fish, while protease-producing bacteria were dominant in carnivorous species. Another interesting metabolic pathway was flagellar assembly, and this is coincident to the sequenced genome of mouse SFB showed that this bacterium might encode more than 40 putative chemotaxis- and flagella-related proteins [50,51].

Molecular characterization of microbiota is a good alternative to cultures-based methods, which can be applied to many fields using 16S rRNA sequencing [52]. However, accurate taxonomic classification of metagenomic data relies on reference sequence databases,

and their associated taxonomy [53]. Hence, for understudied environments such as the new species microbiome many sequences could be derived from novel or uncultured microbes that are not present in reference databases. These limitations also affect the accuracy in the predicted metabolic pathways derived from the taxonomic classification [54]. Therefore, some authors indicate that whole metagenomic sequencing (WMS) should be required to validate findings. To expand our knowledge about microbiota impact on fish, it will be necessary to complement targeted metagenomics and WMS.

5. Conclusions

In conclusion, the microbial composition of juvenile *Seriolella violacea* showed significant differences with sampling time (age). These differences are also observed when the predicted metabolic capacities of the bacterial populations are compared. Furthermore, microbiota of *S. violacea* is dominated by *Candidatus* Arthromitus in all the sampling points. To our knowledge, this study represents the first description of the intestinal microbiota of *S. violacea*, establishing a reference point of future studies on the microbiota of this species in order to advance in a better understanding of the potential role of the bacterial composition on health status and growth performance of this species in aquaculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8020109/s1, Figure S1: Rarefaction curves for sequence depth in different groups of age; Figure S2: Alpha diversity indexes during time, 7MO; 8MO and 9 MO; Figure S3: Principal coordinate analysis unweighted (A) and weighted (B) UniFrac distance; Figure S4: Phylogenetic tree comparing *Candidatus* Arthromitus sequences. Blue diamonds indicate ASV from palm ruff assigned to *Candidatus* Arthromitus, branches without diamonds indicates sequences of *Candidatus* Arthromitus retrieved from the RDPII database. There was a total of 274 positions in the dataset. Analyses were conducted in MEGA X. https://www.megasoftware.net/; Figure S5: Relative abundance of taxa at Family taxonomic level; Figure S6: Relative proportion of the 20 most abundant KEGG pathways associated with intestinal microbiota of *Seriolella violacea* in different sampling time, inferred with Piphillin (A) and Tax4Fun (B); Figure S7: Presence of SFB in human fecal samples. Data adapted from Yin et al 2012; Table S1: Biometric data (mean ± SD) of fish in different groups of age; Table S2: Permanova (*Adonis*) results for comparison between groups of age based on unweighted and weighted UniFrac distance.

Author Contributions: N.C. performed DNA extraction, data collection and data analysis; C.R. data analysis and draft preparation. M.O. and H.F. performed fish experiments and collected/preparing the samples; M.S.R. prepared intestinal samples and performed SEM. analysis; C.D.M. critical review and editing; R.R. and J.R. conceptualization of the study, writing—review and editing, provided funding acquisition, project administration, and resources. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be available on request.

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Conflicts of Interest: The authors declare no conflict of interest.

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