Animal Microbiome

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Host genome drives the microbiota enrichment of beneficial microbes in shrimp: exploring the hologenome perspective

Fernanda Cornejo-Granados[1*†], Luigui Gallardo-Becerra[1†], Sandra Romero-Hidalgo[2], Alonso A. Lopez-Zavala[3], Andrés Cota-Huízar[5], Melany Cervantes-Echeverría[1], Rogerio R. Sotelo-Mundo[4] and Adrian Ochoa-Leyva[1*]

Abstract

Background Pacific Whiteleg shrimp (*Litopenaeus vannamei*) is an important model for breeding programs to improve global aquaculture productivity. However, the interaction between host genetics and microbiota in enhancing productivity remains poorly understood. We investigated the effect of two shrimp genetic lines, Fast-Growth (Gen1) and Disease-Resistant (Gen2), on the microbiota of *L. vannamei*.

Results Using genome-wide SNP microarray analysis, we confirmed that Gen1 and Gen2 represented distinct genetic populations. After confirming that the rearing pond did not significantly influence the microbiota composition, we determined that genetic differences explained 15.8% of the microbiota variability, with a stronger selective pressure in the hepatopancreas than in the intestine. Gen1, which exhibited better farm productivity, fostered a microbiota with greater richness, diversity, and resilience than Gen2, along with a higher abundance of beneficial microbes. Further, we demonstrated that a higher abundance of beneficial microbes was associated with healthier shrimp vs. diseased specimens, suggesting that Gen1 could improve shrimp's health and productivity by promoting beneficial microbes. Finally, we determined that the microbiota of both genetic lines was significantly different from their wild-type counterparts, suggesting farm environments and selective breeding programs strongly alter the natural microbiome.

Conclusions This study highlights the importance of exploring the hologenome perspective, where integrating host genetics and microbiome composition can enhance breeding programs and farming practices.

Keywords Microbiota-host interactions, Microbiome, Metagenomics, Breeding, Genetic line

[†]The authors Fernanda Cornejo-Granados and Luigui Gallardo-Becerra contributed equally to this work

*Correspondence:
Fernanda Cornejo-Granados
fernanda.cornejo@ibt.unam.mx
Adrian Ochoa-Leyva
adrian.ochoa@ibt.unam.mx
[1] Departamento de Microbiología Molecular, Instituto de Biotecnología (IBT), Universidad Nacional Autónoma de México (UNAM), Av. Universidad 2001, Col. Chamilpa, 62210 Cuernavaca, Morelos, México
[2] Departamento de Genómica Computacional, Instituto Nacional de Medicina Genómica, Secretaría de Salud (INMEGEN), Periférico Sur No. 4809, 14610 México, DF, México
[3] Departamento de Ciencias Químico Biológicas, Universidad de Sonora (UNISON), Blvd., Rosales y Luis Encinas, 83000 Hermosillo, Sonora, México
[4] Laboratorio de Estructura Biomolecular, Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD), Carretera Gustavo Enrique Astiazarán Rosas Num. 46. Col. La Victoria, 83304 Hermosillo, Sonora, México
[5] Camarones El Renacimiento SPR de RI, Justino Rubio No. 26, Col Ejidal, 81330 Higuera de Zaragoza, Sinaloa, México

Background

Understanding the factors influencing the microbiota's ability to colonize an organism at any developmental stage and how these communities change due to diet and growth conditions is crucial for gaining insight into diseases and metabolic disturbances [1]. However, the exploration of the host genome's influence on the microbiome has typically been studied using model systems or in laboratory settings. Recent studies in humans have identified host genetic variants associated with the composition of the gut microbiome [2] and microbiomes in other body sites [3]. Additionally, genetic variants in mice can lead to an underdeveloped immune system, affecting both microbiota colonization and disease susceptibility [4]. However, these findings in model organisms cannot be directly applied to non-model organisms such as shrimp due to fundamental differences in the microbiota structure. For instance, the gut microbiota of shrimp is primarily dominated by Proteobacteria [5, 6], whereas mammalian gut microbiota is dominated by Bacteroides and Firmicutes [7, 8].

In this context, there is growing interest in the aquaculture industry to study how various factors in nature — such as environment, diet, and host genetics—shape the microbiota and influence growth efficiency and disease resistance [9–11]. Consequently, experiments conducted in working production farms provide a more accurate representation of real-life conditions, yielding results with greater practical implications by evaluating mechanisms in realistic environments. On the other hand, laboratory settings allow for better control of variables, which is advantageous for more detailed analyses, such as those involving pathogen infections.

The study of the microbiota in aquaculture and fisheries is vital for global food production and security, human health, and animal welfare. According to the FAO, 89% of aquatic animal production in 2020 was directly used for human consumption [12], and this number is expected to increase due to the growing human population [13].

Crustaceans are the second leading aquaculture product worldwide, with the Whiteleg shrimp (*Litopenaeus vannamei*) being the most valuable species. However, shrimp farming faces significant challenges due to diseases, resulting in an estimated annual production loss of 1–4 billion dollars. The increasing demand for shrimp has led the aquaculture industry to explore strategies to enhance production while maintaining health and growth. These strategies include intensifying rearing systems, improving feed formulations, using dietary supplements, or employing microbial consortia to improve water quality. However, these strategies could disrupt the surrounding microbial balance, impacting shrimp health and growth.

The relationship between shrimp health and its microbiota has been extensively studied over the past decade, with mounting evidence indicating a close connection [5, 14–16]. Research indicates that a rich and diverse intestinal microbiota is associated with improved nutrient absorption, enhanced digestive efficiency, and a stronger immune system [17], Generally, incorporating probiotics and prebiotics into the diet can modulate the gut microbiome enhancing immune responses and promoting growth [18]. In contrast, exposure to antibiotics significantly modifies the gut microbiota, reducing its diversity and compromising the immune response [19]. Additionally, lower diversity in the intestinal microbiota is directly linked to diseases such as White Feces syndrome (WFS) [20], while decreased richness in the hepatopancreas has been observed in shrimps affected by Acute Hepatopancreatic Necrosis disease (AHPND) [5]. Effective management of the shrimp's internal microbiota and the one in its surrounding sediments and water reservoirs is crucial for achieving a sustainable source of high-quality proteins [13] with a lower carbon footprint compared to terrestrial livestock [21]. The composition of microbial communities is influenced by various factors, including diet [22, 23], pre- and probiotics [24, 25], antibiotics[14, 26], environmental conditions such as farmed or wild-type settings [23], water salinity, and the shrimp's developmental stage [27].

Currently, agriculture [28], livestock [29], and aquaculture [30] industries are exploring using probiotics to establish a healthy microbiota that improves intestinal health, enhances the immune system, and promotes the growth of plants and animals for consumption. However, there are challenges related to the effectiveness of potential probiotics. These beneficial bacteria must survive in sufficient numbers, adhere to the host's intestinal mucosa, withstand environmental conditions, coexist with the existing microbiota in their host, and multiply [30]. These factors underscore the host's role in shaping the microbiota [23, 31–34], and how probiotics effective for one animal species may not be optimal for others. Thus highlighting the need for further research in this area.

The concept of a unique microbiota composition for every species, as supported by numerous studies [14, 18, 19, 35], gained significant interest in 2008 with the introduction of the hologenome concept by Zilber-Rosenberg and Rosenberg [36]. This concept, defined as the sum of the total genetic information of the host and its microbiota, has profound implications for the evolution and adaptation of higher organisms, suggesting that the co-evolution of the host's microbial symbiotic genomes is a key factor. This means that the microorganisms residing in different organs are not just passive passengers;

they form an interconnected and co-regulated team that actively influences the host's phenotype and behavior. Besides, with differences in the host genotype, the same diet and environment can influence changes in the shrimp microbiota composition and function. Moreover, specific microorganisms in shrimp play crucial roles in lipid degradation, metabolic processes for short-chain fatty acids in the hepatopancreas, and glycan metabolism in the intestine [5].

Therefore, by investigating the hologenome, integrating the host genetic makeup and their associated microbiota can offer valuable insights for developing new aquaculture strategies to enhance shrimp production, disease resistance, feed efficiency, and immune responses.

In this study, we addressed these critical questions by evaluating the structure of the shrimp microbiota in the hepatopancreas and intestine of two genetic lines raised under actual hatchery aquaculture conditions. One genetic line was marketed by the laboratory as a Fast-Growth phenotype (Gen1), while the other was marketed by a second laboratory as a Disease-Resistance phenotype (Gen2). Our goal was to understand the connection between host genetics and microbiota in the shrimp's two essential digestive organs. To do this, we used genotyping arrays with over 6,400 SNPs to assess the genetic variation in the two shrimp breeds, and profiled the V3-V4 16S rRNA hypervariable regions. These analyses revealed that host genetics influences the selection of taxa in the shrimp's hepatopancreas and intestine even when maintaining the same aquaculture conditions, offering potential for future applications in developing pre- and probiotics to maintain this aquaculture species' health and efficient production.

Results

Experimental design under farm aquaculture conditions

Two post-larvae genetic lines of *L. vannamei* were sourced from different production laboratories in Mexico, each with distinct selection breeding programs. The first laboratory offered Gen1 as shrimps with faster growth rates with optimized feed conversion, survival rates, and short-time to harvest. The second laboratory offered Gen2 as shrimps with enhanced disease resistance to common pathogens, increasing overall survival rates. These two genetic lines reflect the trade-off between growth performance and disease resilience, which are essential to consider depending on the specific needs of farming producers. However, the objective of this study was not to focus on demonstrating these biological traits.

The post-larvae from each genetic line were raised to an average weight of 12 ± 2 g in three independent aquaculture open ponds (A, B, and C) at the shrimp farm"Camarones el Renacimiento S.P.R. de R.I"in northern Mexico (Fig. 1). All three ponds received the same aquaculture management techniques and feeding regimen throughout the 100-day experiment. Ponds A and B were located in different farming areas (different environmental conditions) and contained shrimp from the same genetic line, Gen1. By comparing the shrimp from these two ponds, we aimed to investigate how the environmental conditions of two different ponds influenced the microbiota of Gen1. Conversely, Pond C, situated next to Pond A, maintained similar environmental conditions, and housed shrimp from a different genetic line, Gen2 (Fig. 1). Therefore, by comparing the shrimp from Ponds A and C we maintained similar environmental conditions and examined the effect of different genetic lines (Gen1 vs. Gen2) on the microbiota (Fig. 1).

This study was conducted at a working shrimp hatchery, following the farm producers'policies to ensure consistent technical management across all ponds. Throughout our research, all ponds were supplied with the same water source and maintained an average depth of 1.5 m. The salinity remained at 40 parts per million (ppm), with an average temperature of 29 °C, and shrimp stocking density was set at 18 shrimp per square meter. All shrimp were fed with standard commercial feed (Provimi®) twice daily, and the amount of food provided was based on 10% of the total biomass present in each pond. No prophylactic treatments were administered during this study.

Genome-wide SNP analysis confirmed two different genetic lines

Given that the post-larvae were sourced from two different laboratories with proprietary crossbreeding programs, we could expect two significantly different genetic backgrounds. However, to confirm this hypothesis, we used the Illumina Infinium ShrimpLD-24 array. This high-throughput genotyping platform can detect 6,465 single nucleotide polymorphisms (SNPs) across the shrimp genome. A total of 27 samples were genotyped with this platform, nine from each pond.

After quality control (QC), we retained 4,476 SNPs with a high call rate of 0.998, ensuring reliable data for downstream analyses. We then conducted an ADMIXTURE analysis to identify genetic populations based on genetic variability [21]. We performed this analysis for K values (number of populations) from 1 to 4 and found that K = 2 had the lowest cross-validation error (CV), dividing the 27 samples into two distinct genetic populations corresponding to both shrimp genetic lines, Gen 1 and Gen 2 (Fig. 2A). This confirmed that these lines were genetically distinguishable (Fig. 2A).

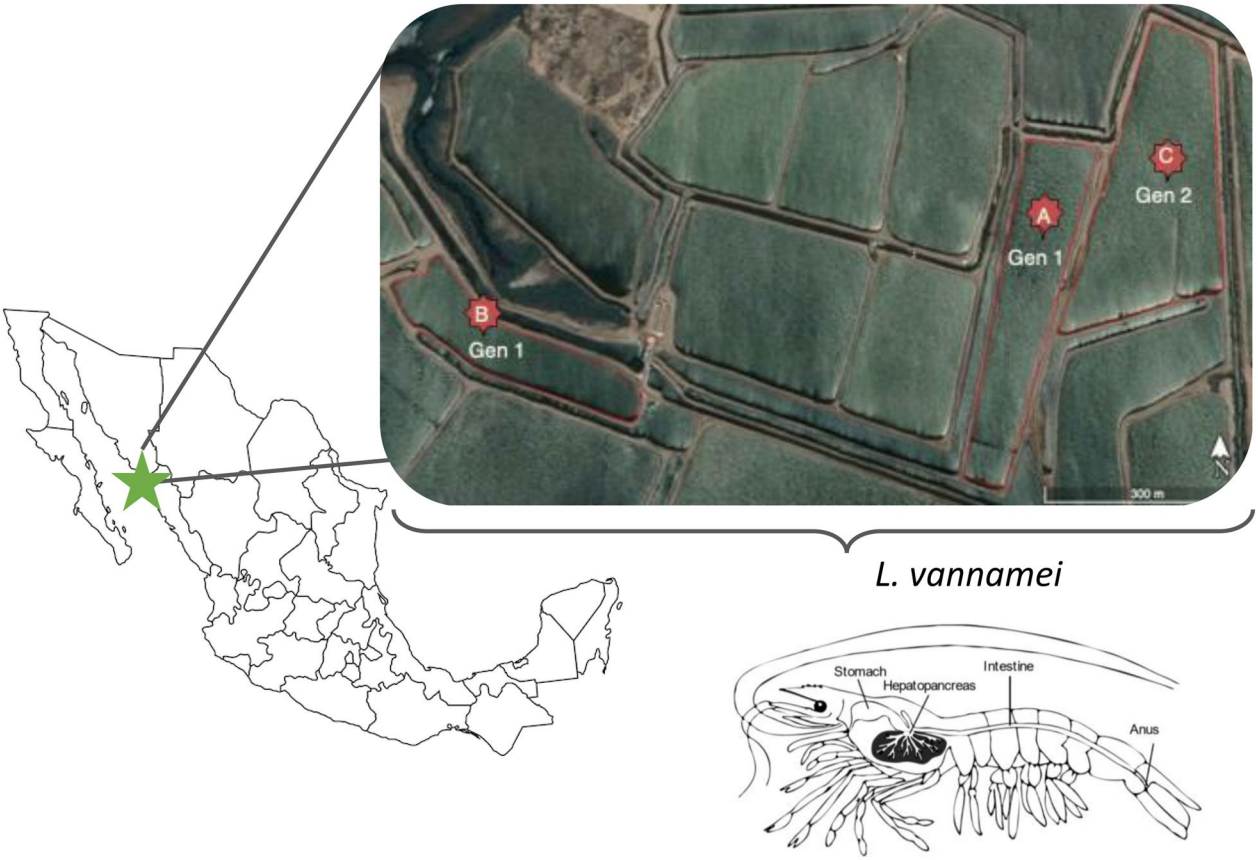

Fig. 1 Experimental design for sample collection in the shrimp farm Camarones El Renacimiento. Satellite overview of sample collection sites in Sinaloa, Mexico (CNES/Airbus©, 2020). Ponds A and B were located separated from each other and contained shrimps from the same genetic line (Gen1), while pond C was located next to pond A and contained shrimps from the second genetic line (Gen2). Below the map we show a diagram of the anatomical location of the dissected hepatopancreas and intestine

Furthermore, the multidimensional scaling analysis (MDS) revealed that samples from each genetic line clustered separately, with Gen 1 (from ponds A and B) forming one cluster and Gen 2 (from pond C) forming another cluster (Fig. 2B). The primary separation between these clusters was along the first principal component (C1), which accounted for 16.33% of the genetic variability (Fig. 2B). This clear separation indicated substantial genetic differences between the two lines, further supporting the hypothesis that they are genetically distinct.

Additionally, the estimated genetic differentiation (F_{ST}) value between the two genetic lines was 0.174 (17.4%), confirming significant genetic differences between populations. All these findings confirm that the genetic lines are markedly different and represent independent genetic groups. Consequently, microbiota variations can be associated to distinct genetic backgrounds.

The genetic line greatly influences the microbiota profiles

We sequenced the V3-V4 hypervariable regions of the 16S ribosomal RNA (rRNA) gene to identify microbial community profiles associated with each genetic line in two crucial shrimp organs: the intestine and the hepatopancreas. Thus, nine shrimps from each pond (N = 27) were selected, and microbiota profiling was conducted. Importantly, these animals were the same ones used in the genotyping analysis. We obtained 1,766,572 million clean reads (78,358 average reads per sample) after performing 16S rRNA amplicon sequencing and quality control; clustering resulted in 398 Operational Taxonomic Units (OTUs) with a frequency ≥ 0.01%. Good's coverage analysis revealed that we captured 98.7% of the total OTUs for both organs, indicating that the sequencing depth adequately represented most bacterial communities (Fig. S1). Similarly, the rarefaction curves suggested excellent resolution of bacterial communities at the sequencing depth achieved (Fig. S1). Further, the alpha

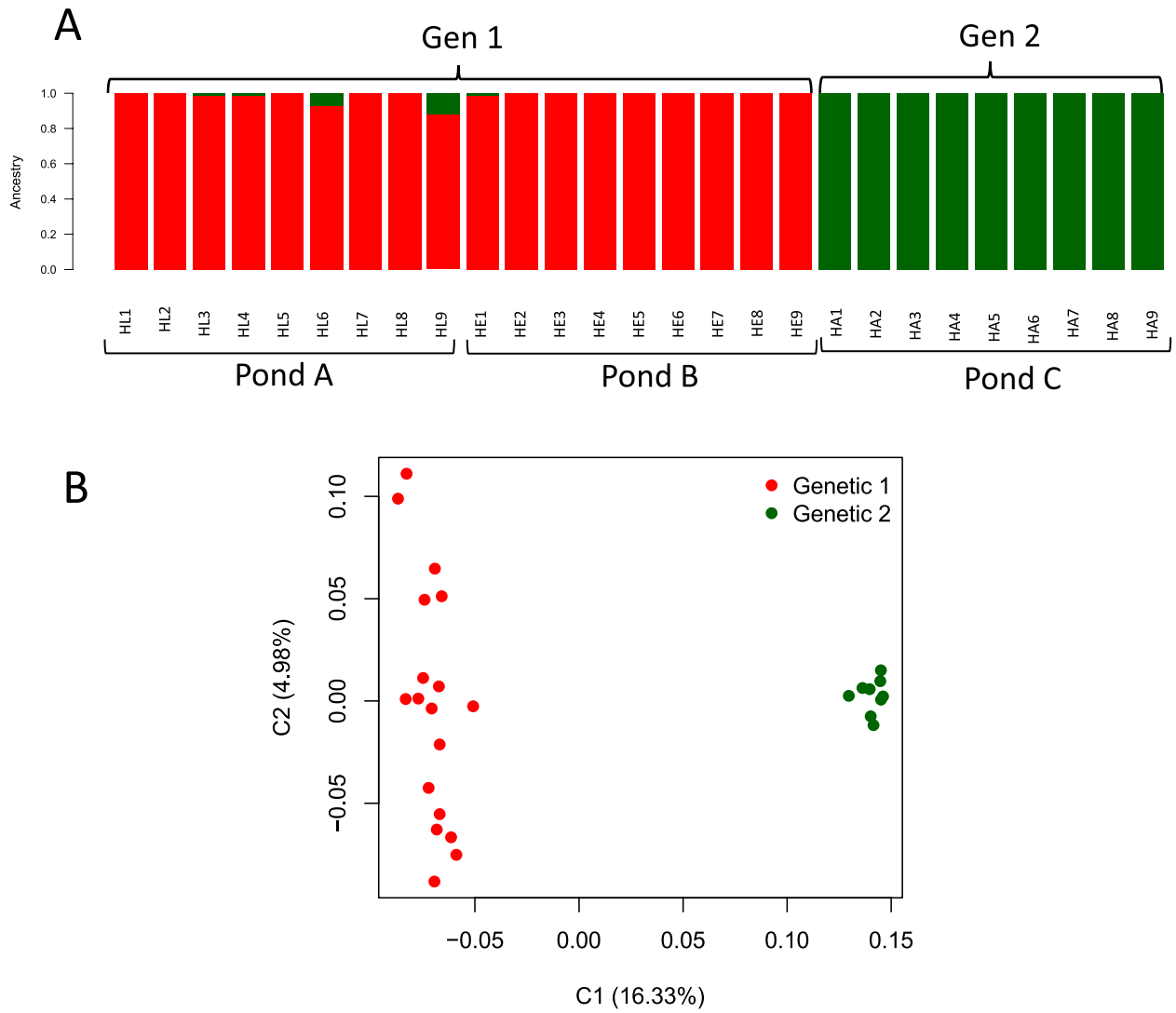

Fig. 2 Genetic variability between both genetic lines. **A** ADMIXTURE analysis (K = 2) of nine shrimp samples from each pond. The colors red and green indicate the two genetic populations represented in the samples. Samples from Ponds A and B correspond to the shrimps from Gen1, while samples from Pond C correspond to shrimps from Gen2. **B** Multidimensional scaling analysis (MDS) with samples tagged by genetic line

and beta diversity metrics were calculated and averaged after 10,000 iterations at a sequencing depth of 4,382 reads for each sample. This fixed depth represents 75% of the reads from the sample with the lowest sequencing depth.

Initially, we tested whether the environmental conditions of each pond affected the microbiota while maintaining the same genetic line (Gen1). We conducted a β-diversity (intersample) analysis using UniFrac distances on the shrimp samples from ponds A and B (Fig. 1). The Principal Coordinates Analysis (PCoA) revealed no distinct clusters between ponds A and B (Fig. 3A). Additionally, the ANOSIM ($R = 0.074$, $p = 0.084$) and PERMANOVA ($F = 1.834$, $p = 0.069$) analyses indicated that the rearing pond did not significantly influence the microbiota composition. These results suggest that the environmental conditions of distant rearing ponds did not considerably affect the microbiota composition in shrimps from the same genetic line (Gen1) under the described hatchery conditions.

Besides previously demonstrated that the pond did not significantly impact the shrimp microbiota structure, we decided to exclude the microbiota samples of pond B from all subsequent analyses. Thus, the microbiota of samples from Ponds A and C, which contained different genetic lines, was used for further investigation. Initially,

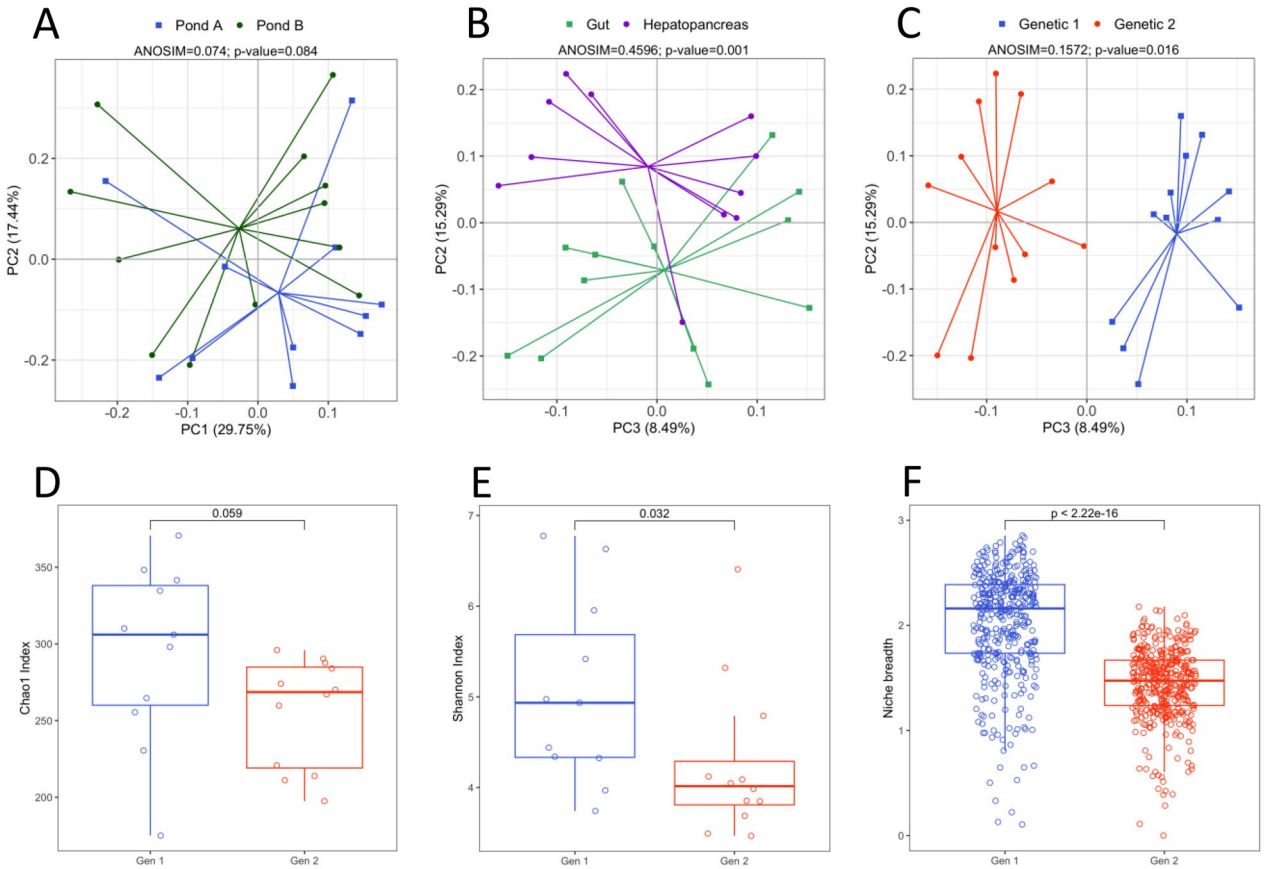

Fig. 3 Beta and alpha diversity and niche breadth analyses of microbiota composition. Unweighted principal coordinate analysis (PCoA) of UniFrac distances representing the microbiota variability in samples tagged by **A** pond, **B** organ, and **C** genetic line. The ANOSIM R and *p* values are indicated above each graph. Boxplots showing the distribution for **D** Chao1, **E** Shannon index, and **F** Niche breadth estimation for the microbiota in both genetic lines. All graphs consider both organs from pond A and C. Statistical differences between groups were evaluated with a Mann–Whitney test using a 95% confidence level of $p < 0.05$

we conducted a ß-diversity analysis using UniFrac distances, including all samples from these two ponds. The PCoA with samples tagged by organ revealed distinct clustering of microbiota samples primarily separating them along the Y-axis into two clusters corresponding to the hepatopancreas and intestine (Fig. 3B). Additionally, the genetic lines showed a clear separation, with samples from Gen1 and Gen2 forming two distinct clusters separated along the X-axis (Fig. 3C). To quantify the effect of organ and genetic line on the microbiota composition, we conducted ANOSIM and PERMANOVA analyses. The results yielded ANOSIM values of R = 0.460, $p = 0.001$ and PERMANOVA values of F = 4.68, $p = 0.001$ for organ, alongside ANOSIM: R = 0.158, $p = 0.016$; and PERMANOVA: F = 2.29, $p = 0.01$ for the genetic line. These results indicated that the organ (R = 0.460) accounted for 46.0% of the microbiota variation, while the genetic line (R = 0.158) accounted for 15.8% of the variation. Thus, our findings suggest that the organ (Fig. 3B) was the most significant factor influencing shrimp microbiota, followed by the genetic line (Fig. 3C).

To evaluate the influence of the genetic line on the microbiota of each organ, we conducted ANOSIM and PERMANOVA analyses separating the microbiota samples of each corresponding organ. The ANOSIM for unweighted UniFrac distances revealed a significant impact of the genetic line on the microbiota composition of the hepatopancreas (R = 0.30, $p = 0.026$), indicating 30.0% of the variations in microbiota composition. This result was reinforced by the PERMANOVA analysis (F = 1.999, $p = 0.044$) also showing statistically significant differences. Conversely, ANOSIM (R = 0.122, $p = 0.108$) and PERMANOVA (F = 1.521, $p = 0.097$) revealed no significant effect of the genetic line on the intestine.

We assessed how the host genetics Gen1 and Gen2 affected the richness and diversity of the microbiota. Our analysis revealed that Gen1 had significantly higher richness (Fig. 3D) and diversity (Fig. 3E) compared to Gen2

when considering the samples from both organs. Furthermore, when examining each organ separately, the intestine of Gen1 exhibited higher richness and diversity, although this difference was not statistically significant (Fig. S2). In contrast, there were no differences in richness and diversity between Gen1 and Gen2 in the hepatopancreas (Fig. S2).

Next, we assessed how the host genetics affected the microbiota niche breadth, and found that the microbiota of Gen1 exhibited a significantly broader niche breadth compared to Gen2 ($p < 0.0001$) (Fig. 3F). This difference was evident whether the organs were analyzed together (Fig. 3F) or separately (Fig. S2). A wider niche breadth in Gen1 indicates that its microbiota may be more adaptable and resilient at utilizing the available resources than Gen2. These findings highlight the critical role of host's genetic in shaping the microbiota's composition and their functional capacity.

Additionally, we analyzed the shared and unique OTUs between the two genetic groups to identify taxonomical differences. The results showed that Gen1 had more unique OTUs than Gen2 in the hepatopancreas and intestine (Fig. S3). Specifically, Gen1 had 9.5% unique OTUs in the hepatopancreas, while Gen2 had only 1%. Gen1 exhibited 1.5% unique OTUs in the intestine, whereas Gen2 had none (Fig. S3). This reinforces the notion that host genetics play a significant role in shaping the microbiota composition, with a more marked effect in the hepatopancreas compared to the intestine.

The genetic line influences the abundance of beneficial microbes in the microbiota

We performed a LEfSe (Linear Discriminant Analysis Effect Size) analysis to identify significantly enriched taxas in each genetic line. The results showed 12 significantly enriched taxa for Gen1 and 19 for Gen2 in the hepatopancreas (Fig. 4A). In contrast, the intestine had 43 enriched taxa for Gen1 and 13 for Gen2 (Fig. 4B). Notably, Gen1 showed an enrichment of probiotic bacteria such as *Enterobacter, Ruegeria, Loktanella,* and *Brevibacteria*, while Gen2 presented an enrichment of some cyanobacteria such as *Arthrospira platensis, Nostocaceae, Lyngya,* and *Phormidium.*

The previous analysis indicated an enrichment of taxa with probiotic potential in Gen1, prompting us to conduct a focused analysis of beneficial microbes. To this end, we examined the abundance of 80 species known for their probiotic or health-enhancing properties in shrimp [24]. These beneficial microbes were identified using a previously established method in which a systematic search yielded 80 bacterial species with experimental results linking them to positive effects on shrimp health [24]. Furthermore, we determined that most of these bacteria were present in the Silva 132

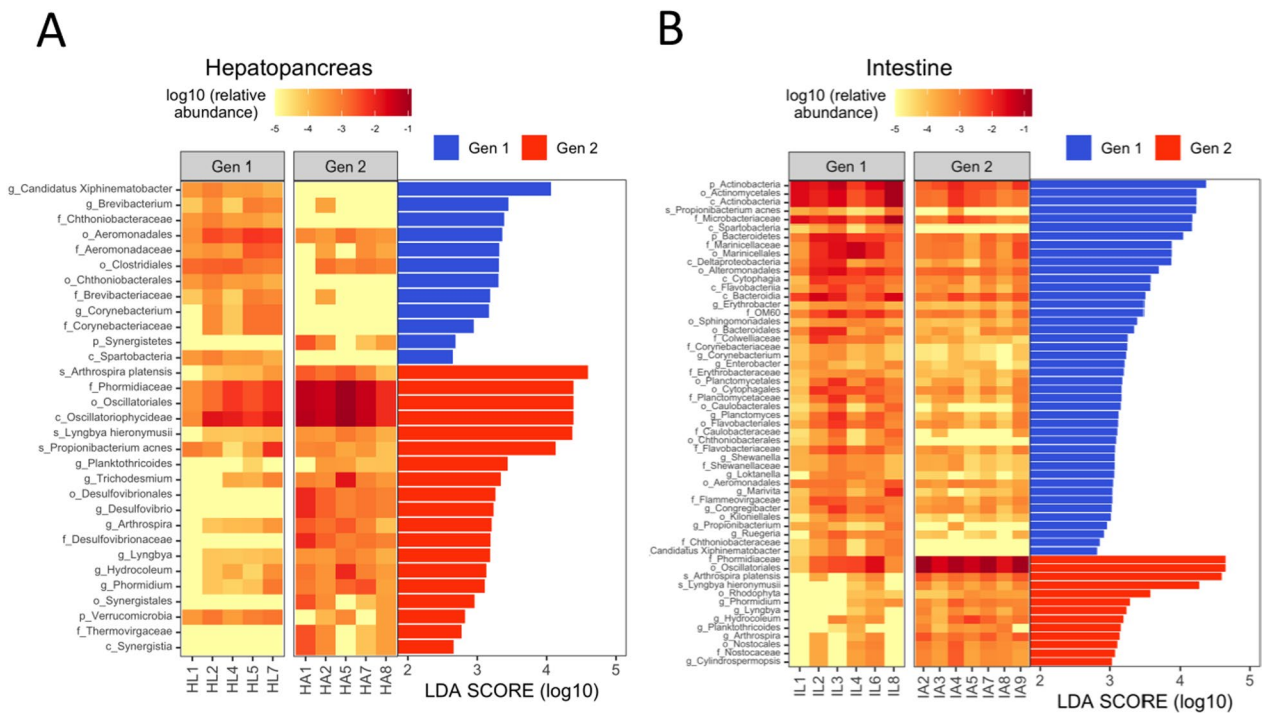

Fig. 4 Significantly enriched taxas in each genetic line using LEfSe analysis. **A** hepatopancreas and **B** intestine. The heat maps represent the relative abundance of each bacteria in all samples

database, so we used it as our reference to analyze our sequences considering all species within the ribosomal databases, eliminating bias in assigning sequences solely to the beneficial bacteria on our list.

Our findings revealed that Gen1 contained sequences for 19 species, while Gen2 contained sequences for 17 species. Upon analyzing the abundance of each species, Gen1 exhibited a significant ($p < 0.05$) enrichment of *Bacillus cereus* in the hepatopancreas. Notably, *B. cereus* is known to produce toxins harmful for humans and other animals [37]. However, particular strains are acknowledged for their probiotic and health-promoting benefits in aquaculture conditions. Strains such as *B. cereus* NP5 have improved reproductive performance and increased larvae survival rates in fish like *Clarias gariepinus* and improve water quality in hatchery ponds [39]. Additionally, *B. cereus* has shown the ability to enhance water quality in hatchery ponds [39]. This suggests that some *B. cereus* strains have a promising role as a probiotic that could benefit aquaculture; however, careful strain selection is crucial to mitigate potential risks.

Additionally, we analyzed the abundance of all beneficial microbes in the microbiota of two genetic lines (see Materials and Methods). Our results indicated no significant difference in the abundance of all beneficial microbes in the hepatopancreas between the two genetic lines ($p = 0.29$) (Fig. 5A). However, we found that beneficial microbes were significantly enriched ($p = 0.0074$) in the intestine of Gen1 (Fig. 5B). Furthermore, we analyzed the abundance of beneficial microbes, categorizing them at the genus level to assess the discrepancies that could arise from not achieving species-level resolution using V3-V4 of the 16S rRNA gene. This genus-level analysis confirmed the trends we observed at the species level. Specifically, the abundance of beneficial microbial genera was significantly higher in both the hepatopancreas and intestine of Gen1 compared to Gen2, with p-values of 0.024 and 0.006, respectively.

The previous findings indicated a greater presence of beneficial microbes in Gen1 compared to Gen2. To explore the potential association between the higher abundance of beneficial microbes and the overall health of the shrimp, we analyzed the microbiota of intestines of a cohort of healthy and diseased (early mortality syndrome (EMS)) shrimps previously collected by our laboratory [5], (see Materials and Methods). Notably, healthy shrimp exhibited a significantly higher abundance of beneficial microbes than their diseased counterparts ($p < 0.05$) (Fig. 5C). This trend persisted when we compared the abundance of beneficial microbial genera; healthy shrimp showed a significantly greater abundance, with a p-value of 0.004. This suggest that a higher abundance of beneficial microbes can be associatted with a healthy status of shrimps.

The microbiota of both genetic lines differs from the wild-type shrimps

Previous observations in shrimp [5, 27] and other organisms [40, 41], including humans [42], suggest the native microbiota can be associated with improved host fitness. To investigate whether the microbiota of Gen1 and Gen2 resembled that of wild-type shrimps, we collected nine intestine samples of wild-type shrimps. First, three of these samples were used for genotyping to investigate their genetic background. The ADMIXTURE analysis confirmed that wild-type samples had a distinct genetic structure from the two genetic lines (Fig. S4). Additionally, the MDS analysis revealed three distinct sample clusters: one corresponding to the wild-type shrimps and the other two corresponding to Gen1 and Gen2 shrimps (Fig. 6A). This clear separation indicates substantial genetic differences between the two genetic lines and wild-type samples, supporting the idea that aquaculture shrimps (Gen1 and Gen2) were genetically different to their wild counterparts.

After confirming a different genetic background between wild-type and both genetic lines samples, we compared their microbiota using an unweighted PCoA analysis of UniFrac distances (Fig. 6B). This analysis revealed two distinct clusters of samples along the X-axis, accounting for 24.64% of the variability (Fig. 6B). One cluster corresponded to the shrimps from aquaculture lines (Gen1 and Gen2), while the other grouped wild-type shrimp. The ANOSIM analysis also revealed a significant influence of sample origin on the microbiota composition (R=0.548; p=0.001), indicating that approximately 54.8% of the variation in the microbiota composition was due to whether the shrimp originated from aquaculture or the wild environment. These findings suggest that the intestinal microbiota of wild-type shrimp differs from that of both genetic lines. We acknowledge that the limited sample size restricts our ability to evaluate the wild-type microbiota's variability. However, as an initial exploration, the MDS and Beta diversity analyses revealed significant genetic and microbiota differences between aquaculture and wild-type shrimp, highlighting the substantial impact of breeding programs on shaping the native genetic structure and its associated microbiota.

Overall, these results emphasize the substantial impact of breeding programs on shaping the native genetic structure and its associated microbiota. They also highlight the importance of adopting a hologenome perspective, which integrates host genetics and microbiome composition to enhance breeding programs and improve farming management practices.

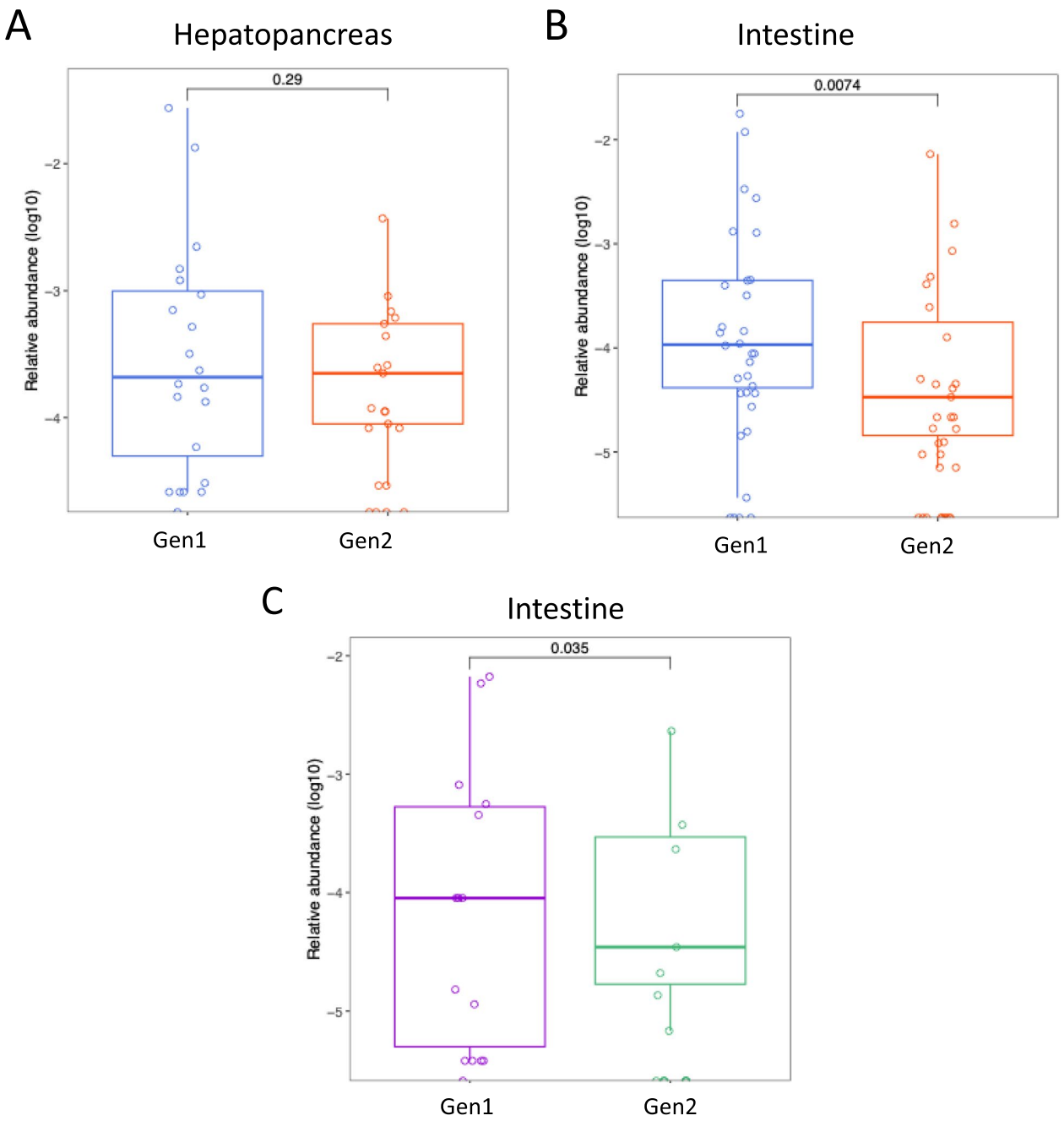

Fig. 5 Distribution of the relative abundance of beneficial microbes for each organ, genetic line and health status. The boxplots compare the relative abundance (log10) of beneficial microbes at species level between Gen1 and Gen2 in **A** hepatopancreas, **B** intestine, and **C** between the intestine of healthy and diseased shrimps. The statistical differences were determined with a Wilcoxon test ($p < 0.05$)

Discussion

The microbiota plays a pivotal role in maintaining health and promoting the development of any organism [43]. It aids in the absorption of nutrients, the regulation of metabolic processes, and the modulation of immune responses [44, 45]. In shrimp, the dynamics of the microbiota are influenced by a variety of factors, both external and internal to the host, such as water quality [46], feeding formulations [23], diet probiotics [24], developmental stage [23], and diseases [5, 16]. Furthermore, genetic variations have been shown to impact these microbial communities'composition,

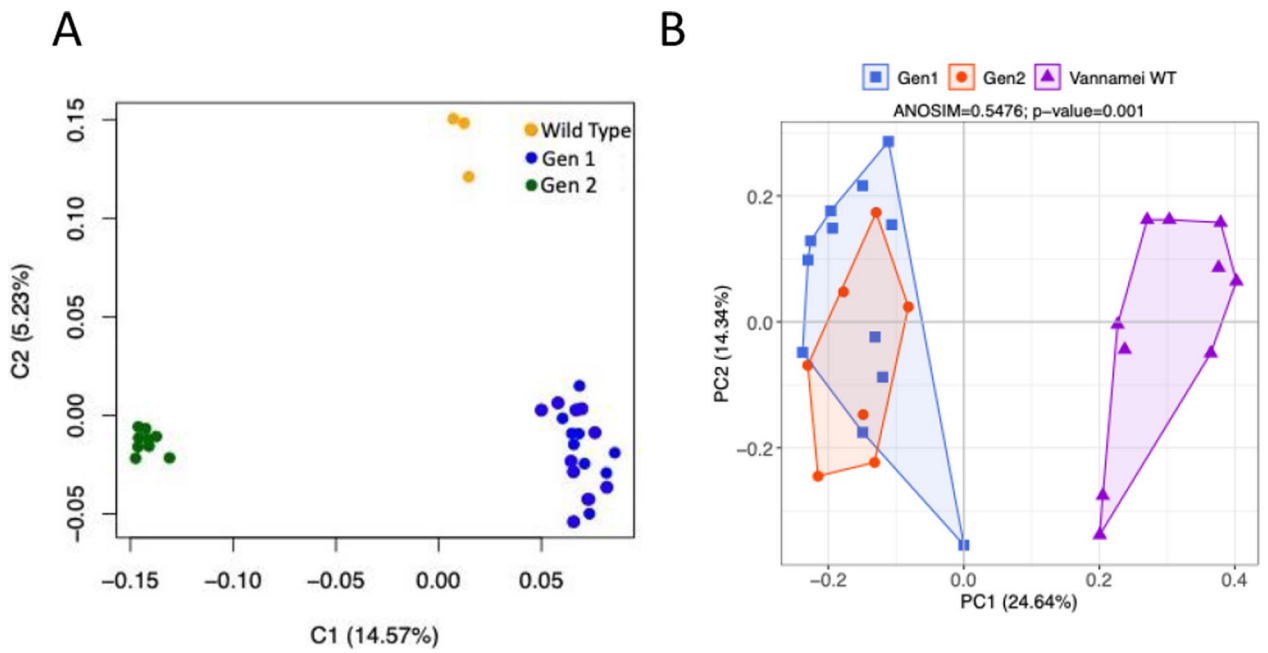

Fig. 6 Genetic and microbiota variability between both genetic lines and wild-type shrimps. **A** Multidimensional scaling analysis (MDS) representing the genetic variability in samples tagged by genetic line and wild-type origin. **B** Unweighted principal coordinate analysis (PCoA) of UniFrac distances representing the microbiota variability with samples tagged by genetic line and wild-type origin. The ANOSIM R and *p* values are indicated above the graph

highlighting the host's crucial role in shaping the microbiota [47, 48].

The selection of genetic traits is crucial in all intensive agricultural activities, including animal husbandry. Recognizing the importance of genetic traits is also a cornerstone of the shrimp aquaculture industry. Many shrimp larvae production laboratories have developed crossbreeding programs to offer breeds with desirable characteristics for high-quality production, such as fast growth or disease resistance. However, combining all these traits in one "ideal larvae breed" has proven challenging. In this sense, understanding how the host genetics influences their microbiota profiles could help us develop or complement these desirable traits.

To enhance our understanding, we analyzed whether a unique microbiota was related to two different genetic lines within the hepatopancreas and intestine of the same shrimp species (*L. vannamei*) under aquaculture hatchery conditions. We sought to investigate if this microbiota could indicate a superior fitness for one of the genetic lines. This findings could have far-reaching implications for aquaculture and genetic lines' fitness.

The post-larvae used in the study were sourced from two independent larval laboratories in Mexico, each employing unique crossbreeding strategies. One genetic line was marketed as having a Fast-Growth, while the other was marketed as a Disease-Resistant phenotype, two critical traits in aquaculture production. Our objective was to confirm that the genetic variability of both lines was sufficient to consider them as independent genetic lines, a claim validated by the genotyping array. As a result, we focused on analyzing and comparing their microbiota instead of verifying the fast-growing or disease-resistant traits claimed by the larval laboratories.

To confirm the genetic variability, we conducted a comprehensive and detailed genome microarray analysis. The admixture analysis indicated that the lowest cross-validation error occurred when $K = 2$, and the F_{ST} value was 17.4%. In population studies of plants of the same species F_{ST} values greater than 15% indicate significant differentiation between populations, while values around 5% are considered insignificant [49]. These results support the notion that genetic profiling in our study revealed two independent genetic backgrounds.

Extensive studies have shown that environmental conditions such as temperature, pH, salinity, and sediment organic compounds can impact the shrimp's microbiota [50, 51]. Thus, we maintained consistent hatchery conditions by applying the same technical management, feeding regime, and stocking density in the three studied ponds. Additionally, by raising the same genetic line in two different ponds (ponds A and B), ß-diversity analysis ruled out any influence of the pond on the microbiota composition.

Consistent with previous studies [23], our ANOSIM and Permanova analysis confirmed that the organ significantly shaped the microbiota composition, with the genetic line also playing a substantial role. Our data revealed that the genetic line had a more pronounced impact on the selection of the hepatopancreas microbiota, accounting for 30% of the variability, compared to the intestine's 12%. These findings were supported by the LefSe analysis and the OTUs comparison in the Venn diagrams, which suggest that the hepatopancreas provides a more selective environment for microbial communities. This is likely due to the physiological function of the hepatopancreas as the main organ for enzyme activity, nutrient absorption, and immunity in crustaceans with less influence on the environment than the intestine [52], thereby imposing a stronger selective pressure on the colonizing bacteria. In contrast, the intestine appears to host a more adaptable microbiota that can respond to external influences, as seen in other studies [53].

The alpha diversity analysis revealed that Gen1 had higher microbial richness and significantly greater diversity than Gen2. This finding suggests potential implications for shrimp growth and ecosystem stability, which could greatly interest our field. In humans, a reduced diversity in the intestinal microbiota has been linked to diseases such as Crohn's disease [54], irritable bowel syndrome [55] and colorectal cancer [56]. Particularly for shrimp, a study by Huang et al. (2020) found dramatically decreased microbial richness and diversity in the gut microbiota of shrimps with White feces syndrome [20]. Similarly, lower richness in the hepatopancreas microbiota was associated with shrimp suffering from Acute Hepatopancreatic Necrosis Disease (AHPND) [5]. On the other hand, a microbial community with high richness and diversity is considered more stable [57], resilient [58] and resistant to pathogen invasion [59, 60].

Additionally, the niche breadth estimation indicated a significantly wider niche breadth for Gen1 compared to Gen2. Previous studies in shrimp and other animals suggest that a wider niche breadth corresponds to a generalist microbiota [61–63], whereas a microbiota with a narrow niche breadth is referred to as a specialist [61–63]. Therefore, the microbiota associated with Gen1 may exploit more diverse resources and tolerate a more variable environment than Gen2. Overall, the alpha diversity and niche breadth may point to a more complex bacterial ecosystem capable of more efficient nutrient and energy assimilation [64], which could contribute to better growth in the Fast-Growth shrimp strain.

Our research has revealed a significant difference in the enriched taxa between Gen1 and Gen2. In Gen1, we identified taxa considered beneficial for their host's metabolic health, such as the genus *Brevibacteria*, which produces short-chain fatty acids [65], and has been shown to enhance shrimp's immune response by reducing pathogenic Vibrio strains in the gut [66]. Additionally the genus Corynebacterium and class Bacteroida, have been reported to boost the immune system and metabolize dietary fibers in humans [67, 68], while demonstrating antiviral activity [69] and facilitating nutrient absorption in fish [70] However, in Gen2, we observed an enrichment of diverse cyanobacteria, such as *Arthrospira platensis* [71], the family Nostocaceae and genera Lyngya and Phormidium [72–74]. While these taxa have been associated with beneficial effects on human health, it is essential to note that some cyanobacteria, like *Nodular spumigena,* can pose a risk to shrimp aquaculture due to their potentially harmful impact on water quality and feed conversion ratios [75]. Furthermore, research has demonstrated that cyanotoxins can reduce feeding and growth rates in fish [76]. As previously stated, this study aimed to characterize the microbiota and examine the genetic variability among different lines. However, our objective was not to confirm the phenotypes reported by the larvae laboratories. In this context, the presence of these bacteria does not explain the disease-resistant phenotype indicated for Gen 2. Instead, it suggests that cyanobacteria may be linked to reduced growth rates and the lower shrimp production observed at the end of the production cycle compared to Gen1.

Along these lines, the abundance analysis of microbes particularly beneficial for shrimp showed an increased abundance in Gen1, which could be associated with this genetics' better growth performance. Additionally, there was a higher abundance of beneficial microbes in the microbiota of healthy than in EMS-diseased shrimps, suggesting that the abundance of beneficial bacteria are associated with a better shrimp health status. Importantly, the diseased shrimps were previously collected at the same shrimp farm from a pond that presented mortality along with clinical and pathological symptoms of AHPND/EMS.

The external administration of probiotics is a widely recommended strategy for controling diseases [14, 77]. However, their application in shrimp-rearing conditions has shown variable results and limited success [78–80]. One potential explanation is that the applied probiotics cannot properly colonize and maintain viable populations [15, 81]. In this sense, identifying beneficial microbes that naturally reside in the shrimp microbiota could guide the design and administration of prebiotics to promote the proliferation of beneficial bacteria in the host. Furthermore, the finding that a specific genetic line of shrimp can increase the probiotics under aquaculture conditions opens the opportunity to be a selectable trait in the search for better shrimp larvae for production. Further,

when considering that all shrimp received the same technical management, it is worth noting that ponds with Gen1 had better productivity on the farm than Gen2. Specifically, at the end of the rearing cycle, Gen1 had a total production of 2.9 tons/ha, while Gen2 had a total production of 2.3 tons/ha. Interestingly, these results align with our findings that Gen1 had a microbiota with all the characteristics of being richer, more diverse, more resilient, and enriched in beneficial microbes.

Previous studies have shown that several factors impact the shrimp microbiota [23], with the surrounding sediment having the strongest influence [51], followed by water [82]. Research on juvenile shrimp suggests that water can contribute as little as 8% and as much as 29.69% [82–84] to the shrimp microbiota composition. The sediment, on the other hand, is the major contributor, accounting for 30% to 60% [85, 86]. While further research is needed to quantify these contributions fully, our study suggests that genetic lineage contributes to approximately 17% of the microbiota variation.

It is important to mention that both genetic lines (Gen1 and Gen2) were raised in separate ponds, each approximately of 10 hectares in size. Due to logistical constraints and the need to conduct our research under actual shrimp production conditions, we were unable to include additional ponds in the study. While we demonstrated that the pond environment did not significantly affect the microbiota structure, it remains possible that differences in their respective environments contributed to the observed variations in the microbiome.

The two shrimp postlarvae lineages were sourced from different commercial production laboratories, each employing unique management techniques, including variations in the holding tank microbiota and feeding practices. Due to logistical challenges, as detailed in our methodology, we could only collect samples after the organisms were placed in the rearing ponds However, prior research indicates that shrimp larvae exhibits notable variability and lower microbiota diversity compared to juveniles and adults [23, 87]. Furthermore, it has been established that the environmental dispersal significantly influences the succession of intestinal microbiota more than the host during the larval and postlarval phases [88]. This suggests that the microbiota in larvae is quite transient and stabilizes as the shrimp develops. Therefore, our findings on microbiota profiles may closely reflect the genetic line selective pressure encountered in farming environments.

Our research is a crucial initial step in exploring whether the genetic lineage influences the microbiota composition of two shrimp genetic lines. We recognize some limitations, including a limited sample size and that the study focused on only a one-time point. Nevertheless, our findings offer significant insights into how host genetics may influence the microbiota composition in shrimp within actual real aquaculture environments instead of laboratory-controlled conditions. Further, we focused on juvenile shrimp samples, because this developmental stage has shown increased microbiota stability [82]. Future research should involve multiple time points and larger sample sizes to deepen our understanding of how the genetic background influences the microbiota composition at various shrimp developmental stages. Despite these limitations, our results indicate that the genetic lines significantly impact the microbiota, paving the way for further studies conducted under farming conditions.

This study provides a preliminary investigation into the role of genetic lineage in shaping the microbiota composition and its possible link to shrimp production. We recognize that our sample size restricts our research, hindering our ability to perform a metagenome-wide association study (mGWAS) that would correlate specific genetic traits (genes/genotypes) with distinct microbes. Nevertheless, bridging this gap continues to be a primary goal for our future research efforts.

Conclusions

Our research confirms the co-evolutionary relationship between the shrimp and its microbiota. It enhances our understanding of genetics as a factor influencing microbial communities and demonstrates its potential as a tool for guided microbiome manipulation in aquaculture. It is evident that adopting a holobiome perspective is crucial for integrating characteristics such as genetics, development, environment, and microbiota into breeding and management programs, ultimately leading to improved growth efficiency, enhanced tolerance to environmental stress, and strengthened immune responses (Fig. 7). Our next objective is to gather a more extensive dataset that will allow us to conduct genome-wide association studies (GWAS) to identify host genes associated with different microbiota profiles.

Materials and methods

Pond distribution and sample collection

The post-larvae of (*Litopenaeus vannamei)* from the Fast-Growth genetic line (Gen 1) were obtained from a de-identified reproduction laboratory in the South of Sinaloa, Mexico, and post-larvae from the Disease-Resistant genetic line (Gen 2) were obtained from a de-identified reproduction laboratory located in the North of Sinaloa, Mexico. All post-larvae were grown under the same conditions in the shrimp hatchery Camarones El Renacimiento located in the Northwest Mexican Pacific area of Sinaloa, Mexico (25°58′02.7″ N,

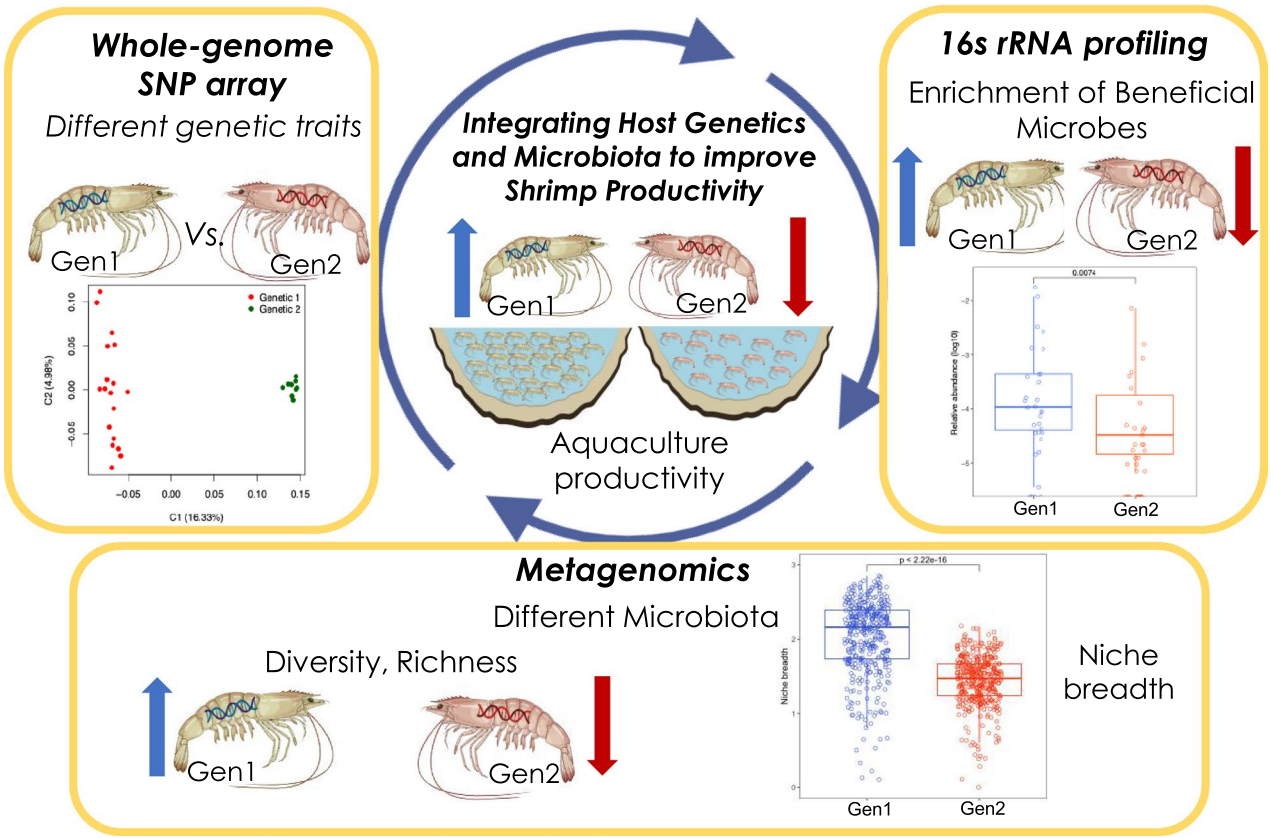

Fig. 7 Graphic representation of the insights obtained in this study. Some image elements were created in BioRender [89] https://BioRender.com/vivea2r

109°18′11.6″ W) (Fig. 1). The organisms were distributed in three ponds as follows: ponds A (26°01′49.3"N 109°23′21.9"W) and B (26°01′44.5"N 109°23′51.9"W) were located separately from each other and contained shrimps from Gen1 (Fig. 1), while pond C (26°01′55.4"N 109°23′12.5"W) was located next to pond A and contained shrimp from Gen2 (Fig. 1). All ponds received identical technical management (same water source, same feeding regime, same diet, same maintenance personnel, etc.). All ponds maintained similar conditions: an average water depth of 1.5 m, water salinity of approximately 40 ppm, average temperature of 29 °C, and a shrimp stocking density of 12 shrimps per square meter. All ponds were fed twice daily with standard commercial feed.

After approximately three months of rearing, an average of six shrimp weighing 12 ± 2 g were collected from each pond. The hepatopancreas and intestine were aseptically dissected in situ, submerged individually in RNAlater® for 24 h at 4 °C (as recommended), and stored at −80°C until DNA extraction. In total, 35 samples (16 hepatopancreas and 19 intestines) were collected for this study.

DNA extraction and amplicon sequencing

Total DNA was extracted from each organ using the Quick DNA Fecal/Soil Microbe Miniprep kit (Zymo Research, CA, USA, Cat. D6010) following the manufacturer's recommendations. The concentration and integrity of the DNA were determined using a Qubit fluorometer (Life Technologies, CA, USA) and agarose gel electrophoresis. The 16S rRNA amplicons of the V3-V4 regions were generated as described in the 16S Metagenomics Sequencing Library Preparation user's guide from Illumina (Illumina, CA, USA). All reactions were amplified under the following conditions: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final elongation step at 72 °C for 5 min. After amplification, PCR amplicons were checked in 2% agarose gel, purified with Ampure XP beads (Beckman Coulter, Inc., CA, USA), and barcoded according to the 16S Metagenomics Sequencing Library Preparation user's guide from Illumina. The quantity and size distribution of each library were assessed using the Qubit fluorometer and the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). All libraries were mixed in equal concentrations and sequenced on the Illumina Miseq sequencing

platform using a 2 ×150 paired-end format at the National Institute of Genomic Medicine (INMEGEN) in Mexico City, México.

Data preprocessing and taxonomic identification

The amplification primers for the corresponding 16S regions and Illumina adaptors were removed with Trimmomatic (v 0.4) [90]. All trimmed sequences from each sample were joined (R1-R2) with fastq-join and filtered for quality (> Q20), where ambiguous bases were removed. The reads were clustered into 398 operational taxonomic units (OTUs) using QIIME (version 1.8) [91] and the uclust algorithm based on 3% divergence (97% sequence similarity) against the GreenGenes sequence database (version 13_5). The reverse strand matching option was enabled, and reads that did not hit against Green Genes were excluded. OUTs with an abundance of ≤0.01% were removed to exclude potential transitory microorganisms [92].

Diversity (alpha and beta) and similarity (ANOSIM and Permanova) analyses

The alpha and beta diversity metrics were calculated using QIIME v1.9 from the final OTU table. The alpha diversity metrics were calculated at a sequence depth of 4,382 reads (the minimum number of reads obtained for the sequenced samples) and averaged from 10,000 iterations. The alpha diversity comparisons were evaluated using the Mann–Whitney test (nonparametric t-test) using a 95% level of confidence ($p < 0.05$). The beta diversity was estimated by computing from the phylogenetic tree. Rarefaction curves for the alpha diversity indices were calculated at the smallest sample sequence depth (4832 reads) and 10,000 iterations.

The weighted and unweighted UniFrac distances among samples, and the UniFrac distance matrices were visualized using a PCoA analysis. The robustness of the UPGMA tree was based on 1,000 replicates. To quantitatively assess the effects of different factors (pond, organ, and genetic line) on the microbiota, a permutational multivariate analysis of variance (PERMANOVA) [93] with Adonis function was calculated on the unweighted UniFrac and Bray–Curtis distance matrices within QIIME. Additionally, the difference among groups in the distance matrices was evaluated with ANOSIM for every beta analysis.

Linear Discriminant (LEfSe), niche breadth analyses and beneficial microbes analyses

For the differential abundance analysis, OTUs between ponds and host genetics were compared using LefSe. A significance level (alpha) of 0.05 and an LDA threshold >2 were applied to identify significantly different taxas. OTUs were subjected to a differential abundance analysis with LefSe, considering a significance level (alpha) of 0.05 and an LDA threshold >2. The niche breadth estimation was calculated using the niche.width function of the spa R package; the final results were plotted with ggplot2 in R.

Further, the beneficial microbes were identified following the previously reported method [24]. Briefly, we performed a systematic search of all available studies related to shrimp or prawn where beneficial microbes for shrimp health were identified. This search resulted in 80 bacterial species with experimental results linking the bacteria to beneficial effects on shrimp health. Further we observed the Silva 132 database contained 16S sequences for most of the identified beneficial microbes, thus we used Silva132 as our reference. In this manner, the sequence analysis was done considering all species of the ribosomal databases so there is no bias to assign the sequences only to the beneficial bacteria on the list. To this end, we constructed a new BIOM table where the OTUs assignment was performed against Silva132 with an identity level of 97%. From the newly generated BIOM table, the taxonomy was assigned at the species level with Qiime 1.9.1, using the command summary_taxa_through_plots.py. The relative abundance for the beneficial microbes was taken from this taxonomy table. Finally, Wilcoxon tests were performed between the abundance of beneficial microbes for each group to determine a significant enrichment (p value ≤0.05).

Microbiota analysis of Healthy (EMS-) and Diseased (EMS +) shrimps

Samples of diseased shrimp (n =9, average weight =15.3 ±0.4 g) were collected prior to this study from a pond at the shrimp farm, where there had been reports of mortality, as well as clinical and pathological symptoms consistent with AHPND/EMS [5]. These symptoms included lethargy, an empty intestine, and a pale, watery hepatopancreas. In contrast, healthy shrimp samples (n =9, average weight =17.1 ±1.2 g) were collected from a nearby pond on the same farm, which showed no symptoms. The intestines of the selected shrimp were aseptically dissected in situ and preserved in RNA-later for 24 h at 4 °C before being stored at −80 °C until further analysis. All samples were individually screened using the AP3 diagnostic method, a single-step PCR test that targets the PirA gene [94]. All shrimp exhibiting symptoms that tested positive for the AP3 test were labeled EMS +, conversely, all healthy shrimp that tested negative were labeled EMS-. Additionally, the V3-V4 hypervariable regions of the 16S rRNA gene were sequenced for all samples using the same methodology applied to both shrimp genetic lines.

Genotyping arrays

A total of 30 genomic DNA samples (9 from each pond plus three blind samples duplicated for quality control) were genotyped using the Illumina Infinium ShrimpLD-24 v1.0 beadchip genotyping array, which included 6,465 SNPs [95]. Quality control of the genotype data involved removing SNPs with a minor allele frequency of less than 0.05 and a call rate of less than 0.95. Pairwise identity-by-descent (IBD) estimates were used to identify the cryptic relationships among samples. The genotype data from the 27 samples were used for population admixture analysis. A total of 4,476 SNPs were used to perform a multidimensional scaling analysis (MDS) based on pairwise IBD estimates using PLINK [96]. ADMIXTURE estimated individual ancestry proportions for K = 1 to K = 4. The fit of different K values was assessed using cross-validation (CV) procedures, where K = 2 showed the lowest CV error.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42523-025-00414-y.

Supplementary Material 1.

Acknowledgements

We thank M.T.I Juan Manuel Hurtado Ramírez for informatics technical support as well as Biol. Filiberto Sánchez López for experimental and technical support. M.C. Alfredo Mendoza-Vargas from Unidad de Secuenciación Masiva and M.C. Raúl Mojica Espinosa from the Unidad de Microarreglos from the National Institute of Genomic Medicine (INMEGEN). Also the authors would like to thank the"Unidad Universitaria de Secuenciación Masiva y Bioinformática"of the"Laboratorio Nacional de Apoyo Tecnológico a las Ciencias Genómicas,"UNAM, especially to Ricardo Alfredo Grande Cano and Lizeth A. Matías Valdez for the technical sequencing support. And to the shrimp farm Camarones el Renacimiento S.P.R. de R.I. for providing the infrastructure and technical support during the sample collection.

Author contributions

Experimental design and conceptualization F.C.G, L.G.B and A.O.L, sample collection A.L.Z. and A.C.H, data curation L.G.B and A.O.L, formal analysis L.G.B, M.C.E., S.R.H, F.C.G. and A.O.L, investigation and methodology F.C.G, L.G.B, R.S.M and A.O.L funding acquisition F.C.G and A.O.L, writing original draft F.C.G, R.S.M and A.O.L, writing, review and editing F.C.G, and A.O.L

Funding

This research was funded by CONAHCYT Fronteras de la Ciencia CF2019-G-263986 and DGAPA PAPIIT UNAM (IN219723). To CONACyT for the doctoral fellowships of Luigui Gallardo-Becerra (CVU 778192), and Melany Cervantes-Echeverría (CVU 887285), and the postdoctoral support by Estancias Posdoctorales por México 2022 program for Fernanda Cornejo-Granados (CVU 443238), and to the Academic Exchange program of CIC-UNAM. Also, to the shrimp farm Camarones el Renacimiento S.P.R. de R.I. for providing additional funding to support this bioassay.

Availability of data and materials

The sequencing data relevant to this publication is available at NCBI under BioProject PRJNA1153518.

Declarations

Ethics approval and consent to participate

An ethics statement was not required for the current study as locations for the specimen collection are not protected, and field studies did not include endangered or protected species. Animals were sacrificed under university protocols to avoid animal suffering.

Competing interests

All authors declare no financial or non-financial competing interests.

Received: 15 January 2025 Accepted: 18 April 2025
Published online: 22 May 2025

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