FEMS Microbiology Letters, 2026, **373**, fnag123
https://doi.org/10.1093/femsle/fnag123
Published: 7 October 2026
Research Article – Environmental Microbiology and Microbial Ecology

Skin microbiome mirrors habitat divergence in amphibious combtooth blenny fish (Teleostei, Blenniidae)

Ewelina Rubin [1,*], Michele Felletti [2], Therese C. Miller [3,4], Bastian Bentlage [1], Diego F.B. Vaz [5], Terry J. Ord [6], Iker Irisarri [7,*]

[1]Marine Laboratory, University of Guam, Mangilao, GU 96913, United States
[2]Department of Physical Biochemistry, Max Planck Institute for Multidisciplinary Sciences, 37077 Göttingen, Germany
[3]Institute of Marine Sciences, University of Auckland, 1010 Auckland, New Zealand
[4]Cawthron Institute, 7010 Nelson, New Zealand
[5]Natural History Museum, South Kensington, SW7 5BD London, United Kingdom
[6]Evolution and Ecology Research Center, and the School of Biological, Earth and Environmental Sciences, University of New South Wales, 2033 Kensington, New South Wales, Australia
[7]Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), 28006 Madrid, Spain
*Corresponding authors. Ewelina Rubin, Marine Laboratory, UOG Station, Mangilao, 96923 Mangilao, Guam, United States. E-mail: rubine@triton.uog.edu; Iker Irisarri, Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), c/ José Gutiérrez Abascal 2, 28006 Madrid, Spain. E-mail: iker.irisarri@mncn.csic.es
Editor: Guerrino Macori

Abstract

Host–associated microbiomes play vital roles in species' ecology and evolution, yet little is known about their shift during the transition from aquatic to terrestrial habitats. We characterize the skin microbiomes of three combtooth blenny species (*Blenniella paula*, *Praealticus labrovittatus*, *Alticus arnoldorum*) that occupy distinct positions along the intertidal gradient—from fully subtidal to intertidal and supratidal environments. Using 16S rRNA sequencing, we compared skin-associated bacterial communities with those in seawater and substrate biofilms. The divergence between skin and substrate microbiomes parallels fishes' distribution along progressively higher intertidal zones. The supratidal *A. arnoldorum* had the most divergent microbiome, characterized by higher *Gammaproteobacteria* abundance and enrichment of epiphytic and mucus-associated taxa. Fish skins were enriched in 27 microbial orders that included genera commonly associated with fish (e.g. *Vibrio*, *Alteromonas*, *Cetobacterium*) but others rarely reported (e.g. *Rubritalea*, *Granulosicoccus*). Together, our findings suggest that variation in fish skin microbiomes parallels the ecological transition from subtidal (aquatic) to supratidal (terrestrial) habitats, although host and habitat effects could not be disentangled. We hypothesize that microbial symbionts may contribute to adaptations enabling amphibious lifestyles. This is the first study on fish microbiome variation across the intertidal gradient and offers a framework for predicting microbiome responses to environmental change.

Keywords 16S, *Alticus*, barcoding, Blenniidae, *Blenniella*, *Praealticus*, SSU, terrestrialization

Introduction

Microbiomes are increasingly recognized as essential players in shaping the host's function and fitness. Host and microbiome form the "holobiont", which has been proposed as the unit of evolution upon which natural selection can act (Brucker and Bordenstein 2012). In particular, skin microbiomes are essential for organisms' overall health, survival, and adaptation to environmental conditions (Ross et al. 2019, Callewaert et al. 2020, Sehnal et al. 2021). The skin acts as a physical barrier that protects the body from harmful environmental factors and potential pathogens, and skin-associated bacteria affect resistance and/or susceptibility to infections (Ross et al. 2019, Sehnal et al. 2021, Akat et al. 2022). In some fish species, the skin fulfills additional physiological roles, such as gas exchange (Martin and Lighton 1989, Glover et al. 2013). Skin microbiomes contain beneficial bacteria that enhance immunity and commensal bacteria that protect against pathogens through competitive exclusion (Llewellyn et al. 2014, Reverter et al. 2018, Sehnal et al. 2021). Often, fish skin is covered by a mucous layer that serves as a barrier and a site for microbial exchange, as it is in constant contact with the surrounding water (Esteban 2012, Llewellyn et al. 2014). Thus, skin microbiomes are often more similar to the environment than internal gut microbiomes, the latter reflecting species-specific adaptations and being likely vertically inherited (Minich et al. 2022). Skin microbiomes may be used to study environmental perturbations, whereas gut mi-

Received: 9 June 2026. **Revised:** 24 August 2026. **Accepted:** 24 September 2026
© The Author(s) 2026. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site-for further information please contact journals.permissions@oup.com

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

crobiomes are more appropriate to identify phylosymbiosis—the correspondence between host phylogeny and microbiome (Sylvain et al. 2020).

While phylosymbiosis is crucial for skin microbiome composition, the environment plays an important role in microbiome shifts and modifications (Ross et al. 2019, Lim and Bordenstein 2020, Bell et al. 2024). In aquatic animals, microbiomes are influenced by water salinity, temperature, and dissolved oxygen (Ross et al. 2019, Sehnal et al. 2021, McMurtrie et al. 2025). In commercially relevant fish, microbiomes have been extensively studied for their immediate implications in aquaculture (Gomez and Primm 2021, Debnath et al. 2023, Bell et al. 2024, McMurtrie et al. 2025), but analyses in wild settings that investigate ecological and evolutionary implications are less common, despite their importance for understanding host–microbe interactions.

One of the most extreme ecological shifts in fish is the conquest of intertidal and supratidal environments, or, more broadly, the transition from aquatic to terrestrial environments (Ord and Cooke 2016). The importance of microbiomes in the process of terrestrialization has been proposed (Cannicci et al. 2020), but only extremely fragmentary data exist to date, leaving this hypothesis largely untested. An analysis of gill-associated microbiomes in brachyuran crabs identified species-specific microbiomes, but the differences were not clearly linked to breathing adaptations (Bacci et al. 2023). Another study analyzed the microbiome of aestivating lungfish and identified granulocytes as being key for pathogen defense (Heimroth et al. 2021). Aestivation, however, is a derived evolutionary adaptation in some lungfish species that involves not only responses to terrestrial stressors but also metabolic lethargy and cocoon secretion. These additional factors can confound analyses of the microbiome's role in adaptation to out-of-water conditions.

Among fish, the family Blenniidae includes species with some of the most extreme amphibious adaptations to the supratidal environment (Ord and Cooke 2016). Combtooth blennies are benthic fish that inhabit shallow coastal waters, including the intertidal and supratidal zones (Hundt et al. 2014). They lack scales and secrete thick mucus layers, two features that could have been adaptive for species that venture out of water. For example, they could facilitate cutaneous respiration and immune function in high–oxygen environments (Lemopoulos and Montoya-Burgos 2021), provide protection against pathogens and desiccation, or reduce friction for locomotion out of water (Esteban 2012, Akat et al. 2022). Importantly, the lack of scales and the presence of a thick mucus layer could be associated with differences in the skin-associated microbiomes.

To date, there are no comparative studies of the skin microbiomes of combtooth blennies. However, three studies on the gut and gill microbiomes of blennies provide some insight into the environmental drivers of their microbiomes. Hundt et al. (2017) analyzed the gut microbiota of two species with divergent diets: *Chasmodes saburrae*, which feeds on benthic or epiphytic crustaceans, and *Scartella cristata*, which feeds on plants and detritus. The increased abundance of *Vibrionaceae*, *Methylococcaceae*, and *Fusobacteriaceae* in *S. cristata* was linked to differences in their diets. Yoshida et al. (2022) compared the gut microbiomes of *Andamia tetradactylus*, a highly amphibious species that feeds on the epilithic algal matrix, and *Entomacrodus stellifer*, which feeds on algae and invertebrates. This study also found critical diet-related differences, with a high prevalence of *Pseudomonadota* but an enrichment of *Spirochaetes* and *Tenericutes* in the gut microbiome of the strict herbivore. In addition, the absence of *Firmicutes* and *Actinobacteria* differentiated algae-eating blennies from the rest of the fish (Yoshida et al. 2022). Pratte et al. (2018) analyzed the gill and gut microbiomes of 15 reef fish families, including the blenny *Istiblennius* sp. Overall, the gill and gut microbiomes differed clearly within each species. However, the microbiomes were more similar between organs of the same species than between organs across different species. This pattern suggests that host-specific factors, potentially interacting with environmental exposure, play a stronger role than organ type alone in shaping bacterial communities in these fish.

Despite extensive work on phylogenetic patterns (Hundt et al. 2014, Muñoz-Sánchez et al. 2026), morphological and physiological adaptations (Hsieh 2010, Egan et al. 2021), predation pressures (Ord et al. 2017), and abiotic conditions in rock pools (Ord et al. 2024), the role of the skin microbiome in the terrestrialization of combtooth blennies remains unexplored. Here, we study the skin microbiomes of three combtooth blenny species that occupy distinct habitats along the intertidal gradient on the Pacific island of Guam. *Blenniella paula* resides in the subtidal zone below the lowest tide line (Egan et al. 2021) representing the ancestral aquatic condition of combtooth blennies (Ord and Cooke 2016). *Praealticus labrovittatus* prefers the intertidal zone and is often found in rock pools above the tide waterline, as well as frequently out of the water entirely, and is regarded as amphibious (Platt et al. 2016). *Alticus arnoldorum* is considered terrestrial as an adult and inhabits its rocks in the supratidal zone above the high tide line, which are constantly splashed but not submerged (Ord and Cooke 2016, Platt et al. 2016). These three species exhibit various degrees of dependence on water, and thus could be considered as representatives of evolutionary "snapshots" of a hypothetical evolutionary path of increasingly amphibious lifestyles (Ord and Cooke 2016).

The intertidal and supratidal zones are characterized by highly variable and extreme conditions, including high water velocities and turbulence, fluctuating salinity and/or oxygen levels, and intermittent air exposure that can lead to desiccation; all factors that exert intense selective pressures on fish morphology and physiology (Hsieh 2010, Egan et al. 2021). By contrast, subtidal habitats offer greater stability and structural complexity, allowing for niche partitioning and ecological specialization that may be reflected in skin microbiome compositions (Chiarello et al. 2018). Thus, we hypothesized that the skin microbiome of species inhabiting subtidal, intertidal, and supratidal habitats should reflect adaptations to these divergent environments. This is the first study to examine microbiome shifts in species occupying habitats along the water–land gradient, providing new insight into selective pressures on skin microbiomes associated with amphibious behaviors. We found marked differences along the intertidal gradient, providing a new perspective on the relationships among habitat, skin microbiome composition, and fish adaptation to terrestrial conditions. These findings have broader implications for understanding evolutionary transitions from aquatic to terrestrial life, suggesting that microbial communities may have played a role in such adaptations. Moreover, by linking habitat characteristics to microbiome composition, this research offers a framework for predicting microbiome responses to future environmental changes in coastal ecosystems.

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

Materials and methods

Sampling

Ten individuals each of *A. arnoldorum*, *P. labrovittatus*, and *B. paula,* were caught in Pago Bay, Guam (13.42764, 144.7991) in a radius of about 10 m where no freshwater river enters the immediate sampling area. The sampling was conducted on May 18 and 19, 2023. Individuals were caught in the intertidal zone using handheld nets and transferred into new zip-lock bags, each containing 50 ml (*A. arnoldorum* and *P. labrovittatus*) or 100 ml (*B. paula*) of autoclaved seawater. Fish were left in the bags for 30 s to rinse off external surfaces that could have been contaminated during fishing and handling. Water was removed, and fish were swabbed along the entire body (both ventral and dorsal areas, head to tail) to sample the mucus microbiomes. After sampling, fish were released in their native habitat. To assess the relative contribution of the environment to skin microbiomes, we sampled the biofilms on the substrates where the three species were found: subtidal rock holes for *B. paula* (n = 10), rock pools for *P. labrovittatus* (n = 7), and supratidal rocks for *A. arnoldorum* (n = 5). In the case of *B. paula*, we sampled the exact substrate on which each fish was residing; thus, the number of fish skin samples equals the number of fish substrates. For the other two fish species, which are more mobile, we sampled rocks or rock pools where the fish were found, but the numbers of skin and substrate samples do not exactly correspond. For comparison, we also collected 3 l of seawater samples from our general sampling area. Specifically, we sampled nearshore surface water next to the habitat of *A. arnoldorum* and *P. labrovittattus* and again at ∼15 m offshore, corresponding to the *B. paula* habitat. All seawater samples were collected in triplicates on two different days: May 18 (n = 6) and May 20 (n = 6), 2023. Seawater samples were filtered using a vacuum pump (GAST) through Whatman filters of either a 0.45 μm (water samples 1–4; sampled 18/05/2023) or 1.2 μm (all remaining samples). For the latter samples, a larger pore size was used to facilitate filtration due to the high resistance of the 0.45 μm filters. Fish skin swabs and filters were placed into 2 ml tubes and stored at −80°C until further processing.

Microbiome profiles

DNA from 64 samples (30 fish skin swabs, 22 substrate swabs, and 12 seawater filters) was extracted using the ZymoBIOMICS DNA extraction kit (Zymo Research) following the manufacturer's protocol. The V4 region of the 16S rRNA gene was amplified using modified forward 515F (Parada et al. 2016) and reverse 806R (Apprill et al. 2015) primers. The product from the first PCR was used in the second PCR, which was designed to complete the addition of Illumina adapters and the 6-mer dual indices as previously described by Kozich et al. (2013). Both PCRs were performed using Takara PCR buffer and Hot Start polymerase (Takara). The first PCR was carried out in 30 μl reactions with the following final concentrations: dNTPs at 0.8 mM, each primer at 0.5 μM, and 30–100 ng of total DNA. The first PCR cycling conditions were as follows: a hot-start activation step for 2 min at 95°C, followed by 30 cycles of 95°C for 40 s, 58°C for 2 min, and 72°C for 1 min, with a final extension of 5 min at 72°C. The PCR was purified using GeneJet (Thermo Fisher Scientific). The second PCR was performed in a 20-μl reaction volume using the same components and final concentrations as above, with 30 ng of the first PCR product serving as the template. The second PCR cycling conditions were as follows: a hot-start activation at 95°C for 2 min, followed by five cycles of 95°C for 40 s, 59°C for 2 min, and 72°C for 1 min, and concluded with a final extension at 72°C for 7 min. All samples were pooled and gel-purified using the Qiagen gel purification kit.

Negative controls containing no template DNA were included in both PCR amplification steps; however, field/swab blanks and DNA extraction blanks were not included. The V4 16S rRNA gene library pool, comprising 64 biological samples and one negative control from the second PCR amplification, was sent to the Michigan State University Research Technology Support Facility Genomics Core for Illumina MiSeq sequencing using a 2 × 250-bp sequencing kit. Data quality of the raw sequencing reads for each sample was evaluated using FastQC v.0.12.1 (Brown et al. 2017). The sequenced PCR negative control yielded 1778 raw reads, of which 678 remained following DADA2 filtering, paired-read merging, and chimera removal. Alternative sequence variants (ASVs) recovered from the negative control were also detected in biological samples, including fish skin, substrate, and seawater samples, and no additional ASVs unique to the negative control were identified; therefore, no ASVs were removed solely on the basis of the negative-control sequencing. Because field/swab and extraction blanks were not included, potential contamination introduced during field sampling or DNA extraction could not be directly assessed, and interpretations of very low-abundance taxa are therefore treated cautiously. Three V4 amplicon samples, including two seawater samples and one fish skin swab, yielded very low read numbers (100–300 reads) and were excluded from further analyses. The number of reads retained for each sample throughout sequence processing is provided in Supplementary Table S9.

Subsequent analyses of the remaining 61 samples were conducted in R v.4.5.1 using multiple packages, including DADA2 v.1.34 (Callahan et al. 2016), phyloseq (McMurdie and Holmes 2013), microbiome (Lahti and Sudarshan 2012-2019), microViz (Barnett et al. 2021), vegan (Oksanen et al. 2026), and ggplot2 (Wickham 2016) . Following quality inspection, paired-end reads were filtered and trimmed using DADA2. Twenty-four nucleotides were removed from both the 5′ and 3′ ends of forward and reverse reads (trimLeft = 24, trimRight = 24). Reads were truncated at the first nucleotide with a Phred quality score ≤ 10 (truncQ = 10), reads containing ambiguous bases were discarded (maxN = 0), and reads shorter than 50 bp following trimming and quality truncation were removed (minLen = 50). PhiX sequences were removed (rm.phix = TRUE). No fixed truncation length or maximum expected-error (maxEE) threshold was specified. Error rates were learned separately for forward and reverse reads, followed by dereplication and ASV inference using DADA2. Denoised forward and reverse reads were merged using mergePairs() with default parameters. Chimeric ASVs were subsequently identified and removed using removeBimeraDenovo() with the consensus method.

Taxonomy was assigned using the SILVA (small subunit ribosomal RNA) SSU rRNA database v.138.2 (Quast et al. 2013). ASVs classified as mitochondria, chloroplasts, eukaryotes, or an unknown kingdom were removed. Low-abundance ASVs were subsequently filtered by retaining those with a mean abundance higher than five reads across samples, resulting in a final count table of 2821 ASVs. Following common practice in bacteriology, all scientific names, including those above the genus level, are italicized (Thines et al. 2020). Count data were transformed into relative abundances for community composition analyses and visualization. Relative

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

abundances were visualized using stacked bar charts generated with Fantaxtic (Teunisse 2022) ; other plots were generated using ggplot2.

Bacterial community composition was evaluated using Bray–Curtis dissimilarities. Principal coordinates analysis (PCoA) was performed using dist_calc() and ord_calc(method = "PCoA") in microViz, and ordinations were visualized using ord_plot(). Differences in community composition were assessed using permutational multivariate analysis of variance (PERMANOVA) with dist_permanova() in microViz, with 999 permutations and a fixed random seed of 1234. Three PERMANOVA models were evaluated: Bray–Curtis dissimilarity ∼ environment for the full dataset, where environment comprised fish skin, substrate, and seawater; Bray–Curtis dissimilarity ∼ species for substrate samples only; and Bray–Curtis dissimilarity ∼ species for fish skin samples only. Pairwise PERMANOVA comparisons among host species were performed for the substrate and fish skin datasets using pairwise.adonis2() with 999 permutations and Benjamini–Hochberg (BH) correction for multiple testing. Homogeneity of multivariate dispersion was assessed using betadisper() in vegan, with significance evaluated using permutest() with 999 permutations. PERMANOVA results were interpreted alongside beta-dispersion tests because significant differences in dispersion can contribute to apparent differences among groups.

To directly quantify skin–substrate differentiation among host species, Bray–Curtis dissimilarity was calculated between each individual fish skin sample and all substrate samples associated with the corresponding host habitat. Because individual fish–substrate pairing was not available for all three species, no paired structure was imposed. Instead, the mean of these distances was calculated for each fish and used as the fish-level measure of skin–substrate dissimilarity. Differences among the three host species were evaluated using a Kruskal–Wallis rank-sum test, followed by pairwise Wilcoxon rank-sum tests with BH correction for multiple comparisons.

Skin–substrate ASV overlap was additionally quantified as the percentage of ASVs detected on each individual fish that were also detected in substrate samples from the corresponding habitat. Because substrate sampling effort differed among host species (10, 7, and 5 substrate samples for *B. paula*, *P. labrovittatus*, and *A. arnoldorum*, respectively), sampling effort was standardized by randomly subsampling five substrate samples per habitat without replacement over 1000 iterations. For each fish, the percentage of shared ASVs was calculated for each iteration and then averaged across iterations, retaining individual fish as the unit of biological replication. Differences in standardized ASV overlap among species were evaluated using a Kruskal–Wallis rank-sum test followed by pairwise Wilcoxon rank-sum tests with BH correction.

Alpha diversity was calculated using the alpha() function in the microbiome R package. Differences in Shannon diversity among environmental sample types (fish skin, substrate, and seawater) were evaluated using a Kruskal–Wallis rank-sum test, followed by pairwise Wilcoxon rank-sum tests with BH correction for multiple comparisons. Shannon diversity among the skin microbiomes of the three host species was evaluated separately using a Kruskal–Wallis rank-sum test.

Differential abundance analysis was performed using the corncob R package (Martin et al. 2020) on the original, untransformed count data; relative-abundance-transformed data were not used for differential abundance testing. Prior to analysis, ASV counts were taxonomically agglomerated at the class, order, or genus level, as appropriate for each comparison. Corncob differential-abundance models were fitted using the differentialTest() function with beta-binomial regression. For comparisons among sample environments (fish skin, substrate, and seawater), environment was included as the predictor of taxon abundance (formula = ∼ environment) and dispersion (phi.formula = ∼ environment), with an intercept-only null model for abundance (formula_null = ∼ 1) and environment retained in the null dispersion model (phi.formula_null = ∼ environment). Fish skin was specified as the reference environment. For comparisons among host species, analyses were restricted to fish skin samples, and species was included as the predictor of both abundance (formula = ∼ species) and dispersion (phi.formula = ∼ species), with an intercept-only null model for abundance (formula_null = ∼ 1) and species retained in the null dispersion model (phi.formula_null = ∼ species). *Alticus arnoldorum* was specified as the reference host species. Differential abundance was evaluated using Wald tests without bootstrapping (test = "Wald", boot = FALSE), and statistical significance was assessed at a false discovery rate (FDR) threshold of 0.05.

Functional profiles of all ASVs derived from the fish skin samples (2559 ASVs) were inferred using PICRUSt2 v2.5.2 (Douglas et al. 2020). Six ASVs that failed the minimum alignment criterion (–min_align 0.8) were excluded from phylogenetic placement. Of the 2553 successfully placed ASVs, five with nearest-sequenced taxon index (NSTI) values > 2 were excluded from metagenomic prediction, resulting in 2548 ASVs used for functional prediction. Prediction reliability was assessed using abundance-weighted NSTI values generated by PICRUSt2 for each sample. Predicted KEGG (Kyoto Encyclopedia of Genes and Genomes) Ortholog (KO) abundances were mapped to the KEGG BRITE hierarchy using the three-tier classification system: levels A–C (Kanehisa and Goto 2000).

To evaluate differences in predicted metabolic potential among host species, KO abundances from each sample were aggregated into KEGG BRITE level C metabolic categories. The relative representation of each BRITE C category was calculated within each sample, resulting in 164 metabolic categories represented across the 29 fish skin samples. Overall differences in predicted metabolic profiles among host species were evaluated using Bray–Curtis dissimilarities and permutational multivariate analysis of variance (PERMANOVA; adonis2 function, vegan package) with the model Bray–Curtis dissimilarity ∼ species and 999 permutations. Homogeneity of multivariate dispersion was evaluated using the betadisper() and permutest() functions in vegan with 999 permutations. Differences in the relative representation of individual BRITE C metabolic categories among host species were evaluated using Kruskal–Wallis rank-sum tests. *P*-values from the 164 category-level tests were adjusted for multiple comparisons using the BH false discovery rate procedure.

To quantify the relative contribution of bacterial genera to predicted metabolic functions, KO abundances were subsequently merged with taxonomic assignments obtained from the SILVA database. KO abundances were aggregated by genus and KEGG BRITE level C category by summing normalized taxon–function contributions (norm_taxon_function_contrib). For each KEGG BRITE level C category, the proportional contribution of each genus was calculated as a percentage of the total predicted functional abundance for that category. These genus-level functional

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

contributions were visualized using heatmaps generated with the ComplexHeatmap R package (Gu et al. 2016). Because PICRUSt2 infers functional potential based on phylogenetic placement relative to reference genomes, these predictions represent putative functional potential and do not demonstrate the presence of complete metabolic pathways or their expression or activity in the fish skin microbiome.

All R code used in our analyses, including figure generation, is available in the GitHub repository: https://github.com/ewrubin/combtooth-blenny-microbiome.

Results

Skin–environment microbial divergence differs across host species and habitats

In this study, we generated bacterial microbial profiles from the skin of three combtooth blenny fish species that inhabit increasingly higher zones of the intertidal gradient: *B. paula* in the subtidal areas, *P. labrovittatus* in intertidal rock pools, and *A. arnoldorum* on supratidal rocks. The skin microbial profiles of all three species were markedly different from the microbial communities found in their surrounding environment, whether in seawater or on the substrate they inhabited (Fig. 1A; Permutational Multivariate Analysis of Variance or PERMANOVA, $F = 7.37$, $P < 0.01$). However, beta-dispersion analyses also detected significant differences in within-group variability among environments (Fig. 1A, permutest, $F = 22.51$, $P = 0.001$), with seawater communities exhibiting lower dispersion than both fish skin- and substrate-associated communities. Among environmental samples, seawater bacterioplankton was the most distinct, clearly separating from both the skin and rock biofilm microbial profiles (Fig. 1A).

Direct quantification of skin–substrate dissimilarities revealed an ordered increase across the three host species. Mean Bray–Curtis dissimilarity increased from 0.768 ± 0.106 in the subtidal *B. paula* to 0.847 ± 0.036 in the intertidal *P. labrovittatus,* and 0.898 ± 0.032 in the supratidal *A. arnoldorum*. Skin–substrate dissimilarity differed significantly among host species (Kruskal–Wallis $\chi^2 = 11.35$, df $= 2$, $P = 0.003$). Pairwise comparisons indicated significantly greater dissimilarity in *A. arnoldorum* than in *B. paula* and *P. labrovittatus* (BH-adjusted Wilcoxon $P = 0.015$ and 0.014, respectively), whereas the difference between *B. paula* and *P. labrovittatus* was not significant ($P = 0.111$). Consistent with these compositional differences, skin communities also differed in their overlap with substrate-associated ASVs. After standardizing substrate sampling effort to five samples per habitat, the mean proportion of skin ASVs also detected in the corresponding substrate community was 79.2% in *B. paula*, 73.5% in *P. labrovittatus,* and 73.7% in *A. arnoldorum* (Kruskal–Wallis $\chi^2 = 7.58$, df $= 2$, $P = 0.023$). Pairwise comparisons detected significantly greater ASV overlap in *B. paula* than in *A. arnoldorum* (BH-adjusted $P = 0.038$), whereas the other comparisons were not significant ($P \geq 0.056$). These quantitative patterns were consistent with the greater separation of skin and substrate communities observed in the species-specific ordinations (Fig. S1).

Skin microbiomes of the three fish species were species-specific (Fig. 1B; PERMANOVA, $F = 5.79$, $P < 0.01$), with the supratidal *A. arnoldorum* displaying the most dissimilar profile (Fig. 1B). No significant differences in beta dispersion were detected among host skin microbiomes (Fig. 1B, permutest, $F = 2.98$, $P = 0.07$), suggesting that the observed PERMANOVA result primarily reflected differences in community composition rather than within-group variability. In addition, substrate-associated bacterial communities exhibited substantial overlap among host species in ordination space (Fig. 1B), but PERMANOVA detected a significant effect of species identity on community composition ($F = 2.85$, $P = 0.001$). Again, the beta-dispersion analyses also indicated significant differences in within-group dispersion (permutest, $F = 3.97$, $P = 0.04$), suggesting that the observed PERMANOVA result may partially reflect differences in community variability among host-associated substrates.

Alpha diversity was significantly lower in fish skin microbial communities than in the surrounding environmental communities (Fig. 2). Shannon diversity differed significantly among fish skin, seawater, and substrate communities (Kruskal–Wallis, $\chi^2 = 31.11$, df $= 2$, $P < 0.001$), with mean ($\pm$ SD) values of 4.47 ± 0.682, 5.65 ± 0.417, and 5.75 ± 0.552, respectively (Fig. 2A). Pairwise Wilcoxon rank-sum tests with BH correction showed significantly lower Shannon diversity in fish skin than in seawater and substrate communities (both adjusted $P < 0.001$), whereas seawater and substrate communities did not differ significantly (adjusted $P = 0.452$). Observed ASV richness showed a similar pattern (Fig. 2B), differing significantly among environments (Kruskal–Wallis, $\chi^2 = 29.96$, df $= 2$, $P < 0.001$). Fish skin communities had significantly lower ASV richness than seawater and substrate communities (both adjusted $P < 0.001$), whereas seawater and substrate communities did not differ significantly (adjusted $P = 0.210$). Neither Shannon diversity nor observed ASV richness differed significantly among the skin microbiomes of the three fish species (Kruskal–Wallis: Shannon, $\chi^2 = 5.83$, df $= 2$, $P = 0.054$; richness, $\chi^2 = 2.93$, df $= 2$, $P = 0.231$).

Differential bacterial abundance at the class, order, and genus levels

Gammaproteobacteria were the most abundant class in fish skin microbiomes, accounting for 19%–68% of the total bacterial relative abundance (Fig. 3). Their abundance was significantly higher on fish skin than in either seawater or substrate microbiomes (Fig. 3, Table S1; Wald test, FDR < 0.05). Other classes enriched on fish skin relative to both seawater and substrate included *Fusobacteriia*, *Verrucomicrobiia*, and *Bacilli*, which contributed 7%–34%, 3–11%, and 2%–5% of the total relative abundance, respectively (Fig. 3, Table S1; Wald test, FDR < 0.05). In contrast, *Bacteroidia*, *Cyanobacteriia*, *Desulfobulbia*, and *Deinococci* were enriched in both seawater and substrate relative to fish skin (Fig. 3, Table S1; Wald test, FDR < 0.05). Classes with an average relative abundance below 0.3% across the dataset were grouped as "Other" in Fig. 3; therefore, not all differentially abundant classes are displayed. *Alphaproteobacteria* were also abundant across all sample types, contributing 3%–43% of the fish skin microbiome, 19%–43% of seawater communities, and 16%–31% of substrate communities. *Alphaproteobacteria* were not differentially abundant among the three environments (Table S1; Wald test, FDR > 0.05) but differed among host species, with lower relative abundance in *A. arnoldo-*

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

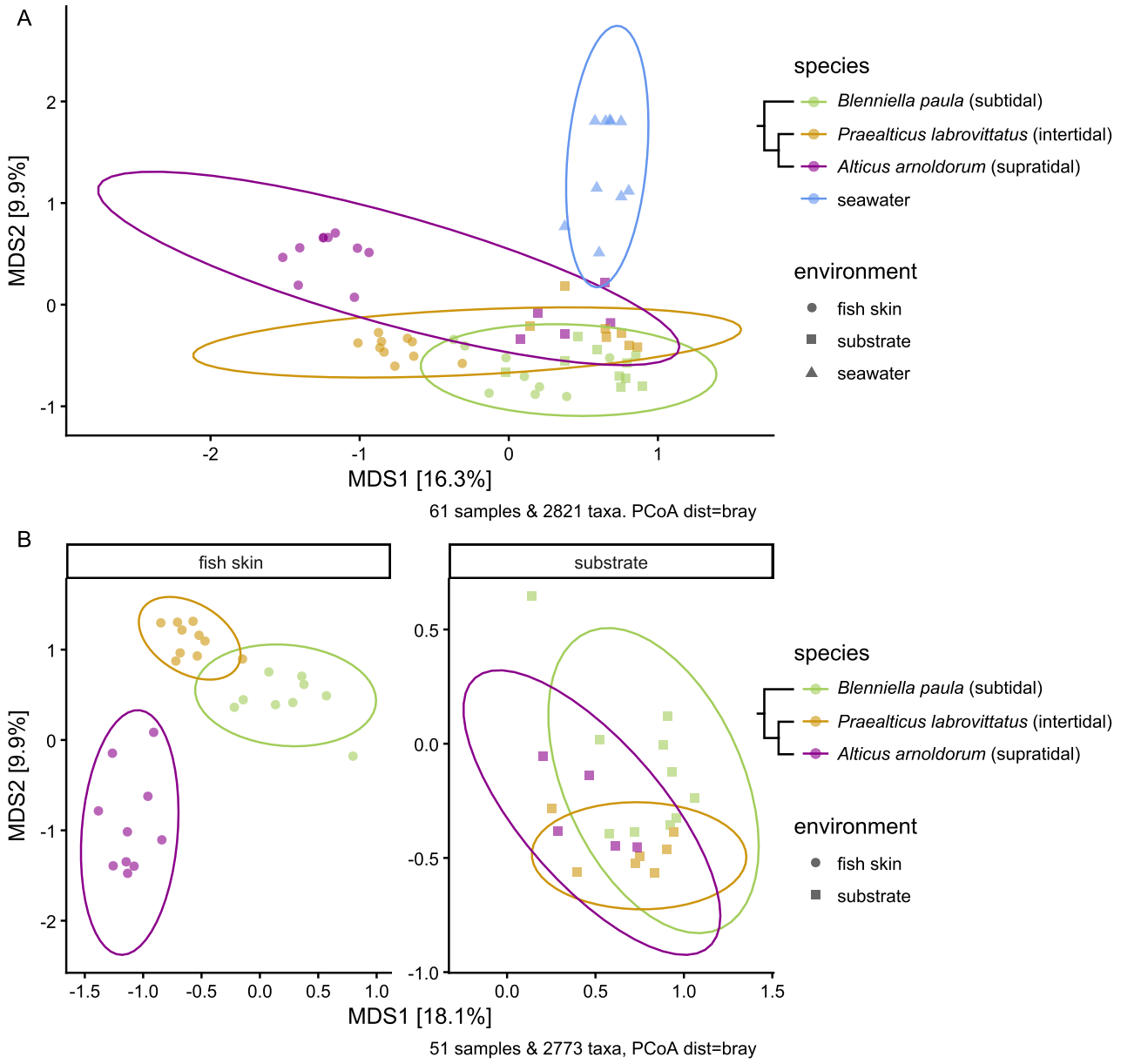


Figure 1 Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarities of bacterial communities associated with seawater, fish skin, and rock substrate samples. (A) Ordination of all samples. (B) Fish skin and substrate biofilm communities associated with the three combtooth blenny species. Ellipses indicate 95% confidence intervals around group centroids. Phylogenetic relationships and habitat of host fish are indicated.

rum (7%) than in *B. paula* (23%) and *P. labrovittatus* (23%) (Table S2; Wald test, FDR < 0.05).

Out of the 128 order-level groupings, 79 (62%) were differentially abundant among the three environments: seawater, substrate, and fish skin (Table S3; Wald test, FDR < 0.05). Of these, 48 were enriched on fish skin relative to at least one of the two environmental sample types, and 27 were enriched on fish skin relative to both seawater and substrate (Table S3; Wald test, FDR < 0.05). Among these, the strongest increases were observed in *Kordiimonadales*, *Cardiobacteriales*, *Spirochaetales*, and *Burkholderiales* (Table S3). Conversely, 31 orders were enriched in both seawater and substrate relative to fish skin (Table S3; Wald test, FDR < 0.05).

Among skin microbiomes of the three fish species, 43 orders were differentially abundant (Table S4; Wald test, FDR < 0.05). Ten orders were enriched in the supratidal *A. arnoldorum* relative to both *B. paula* and *P. labrovittatus*, including *Arenicellales*, *Beggiatoales*, *Cardiobacteriales*, *Flavobacteriales*, *Granulosicoccales*, *Phormidesmiales*, *Synechococcales*, and *Verrucomicrobiales*, as well as unclassified orders within Gammaproteobacteria and the SAR324 clade (Table S4; Wald test, FDR < 0.05). By contrast, 27 orders were enriched in both *B. paula* and *P. labrovittatus* relative to *A. arnoldorum*, including *Kordiimonadales*, *Pseudomonadales*, and *Balneolales* (Table S4; Wald test, FDR < 0.05).

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

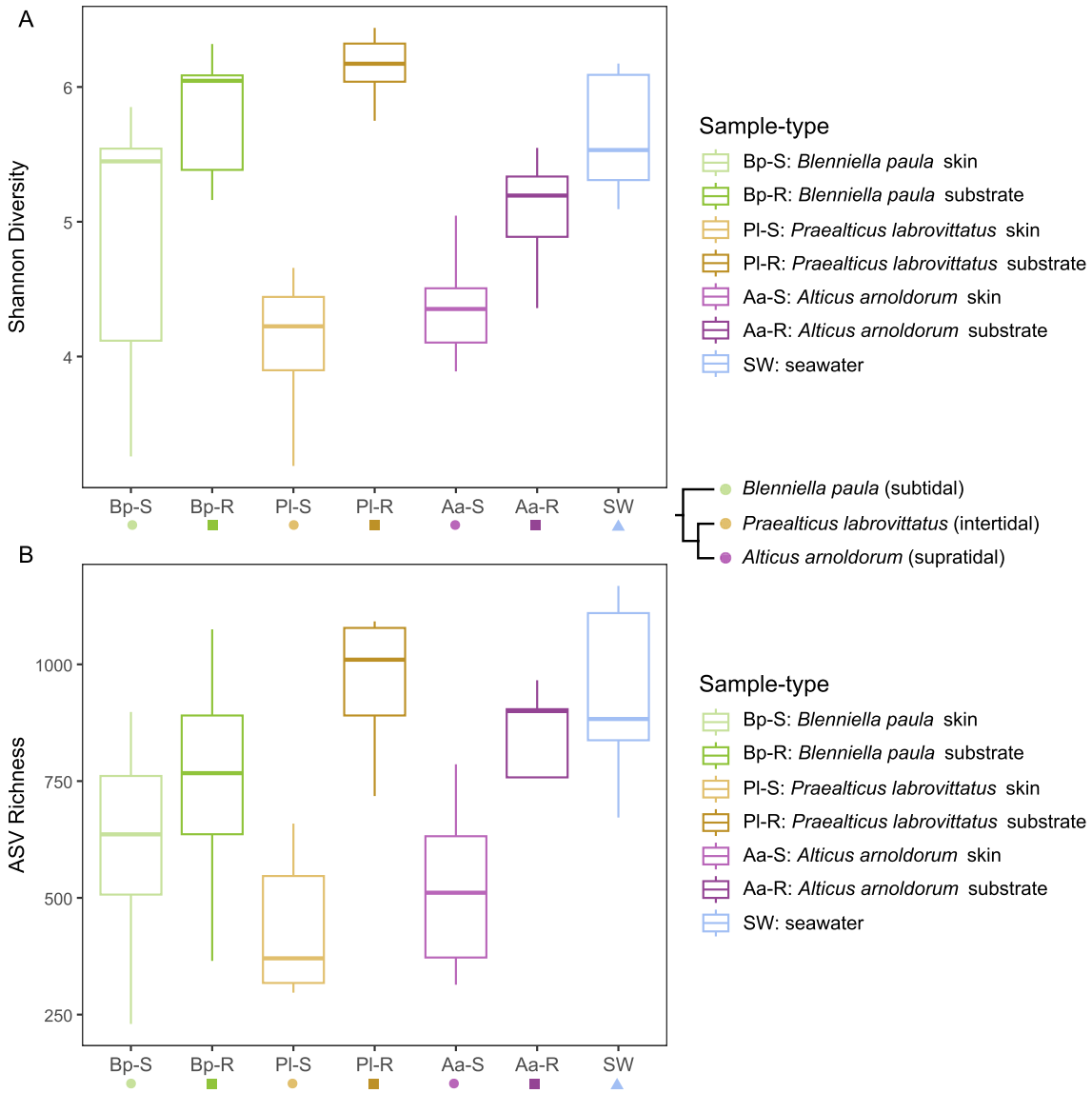


Figure 2 Alpha diversity indices of microbial communities from fish skin, rock substrate, and seawater samples. Shannon diversity (A) and ASV richness (B) are shown for skin (S) and substrate (R) microbiomes associated with *Blenniella paula* (Bp), *Praealticus labrovittatus* (Pl), and *Alticus arnoldorum* (Aa), and seawater (SW).

The 29 most abundant genera, defined as those reaching >1% relative abundance in the fish skin microbiome of at least one host species, are shown in Fig. 4. The dominant genera across fish skin samples were *Vibrio* (mean relative abundance 7.1%), *Cetobacterium* (5.5%), and *Alteromonas* (4.9%) (Fig. 4). Among these 29 genera, 18 were differentially abundant among the three host species (Fig. 4A, Table S5; Wald test, FDR < 0.05), whereas the remaining 11 did not differ significantly among host species (Fig. 4B). Eight of the 18 differentially abundant genera—*Arenicella*, *Gammaproteobacteria* UG (undescribed genus), *Granulosicoccus*, *Lewinella*, *Maribacter*, *Phormidesmis*, *Rubritalea*, and *Schizothrix*—were enriched on the skin of *A. arnoldorum* relative to both other host species. *Cetobacterium* and *Kordiimonas* were enriched on *P. labrovittatus*, whereas *Acinetobacter* was enriched on *B. paula* (Fig. 4, Table S5; Wald test, FDR < 0.05). The undescribed genus *Burkholderiales* UG was enriched on the skin of both *B. paula* and *A. arnoldorum* relative to *P. labrovittatus* (Fig. 4, Table S5; Wald test, FDR < 0.05). Additional lower-abundance genera were also differentially abundant among host species (Table S5; Wald test, FDR < 0.05).

Functional potential of bacteria associated with fish host skins

Functional predictions were restricted to the 2548 filtered ASVs detected in fish skin samples. Prediction reliability was assessed using abundance-weighted NSTI values, which across the 29 fish skin samples had a median of 0.151 (IQR 0.138–0.166; range 0.082–0.183), indicating moderate phylogenetic distances between the sampled ASVs and available reference genomes. The relative con-

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

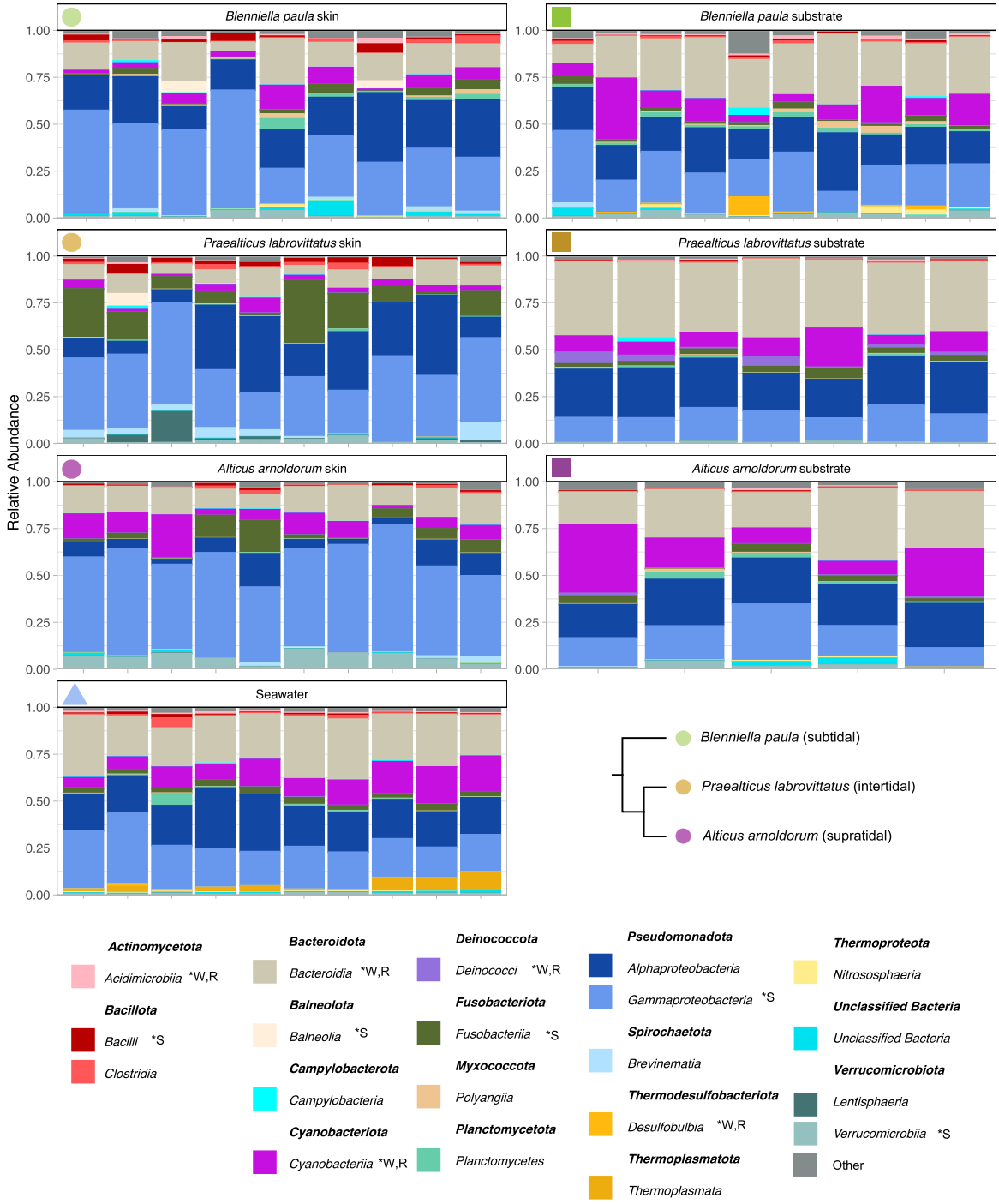


Figure 3 Relative abundance of the 20 most abundant microbial classes across fish skin microbiomes, associated rock substrate biofilms, and seawater samples from the three host species studied. Letter designations indicate classes that were significantly enriched either in fish skin relative to seawater or substrate (∗S), or in both seawater and substrate relative to fish skin (∗W, R), as determined by beta-binomial regression and Wald tests (FDR < 0.01).

tribution of microbial taxa to functional potential was assessed by calculating the proportional contribution of all 549 bacterial genera detected on fish skin to 164 KEGG level C metabolic categories (Table S6). For visualization, we focused on genera that were ecologically meaningful contributors by selecting those that reached at least 1% relative abundance in the skin microbiome of at least one host species, including those 18 that were differentially abundant among the host species (Fig. 4A, Table S6) and those that did not differ in relative abundance (Fig. 4B). Because functional predictions by PICRUSt2 are based on 16S similarity to reference genomes, proposed functions should be interpreted as hypothetical rather than demonstrated metabolic activity, which would require additional data.

Several metabolic categories were dominated by a small number of differentially abundant genera, revealing strong taxon-specific functional signatures across host species. Of particular in-

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

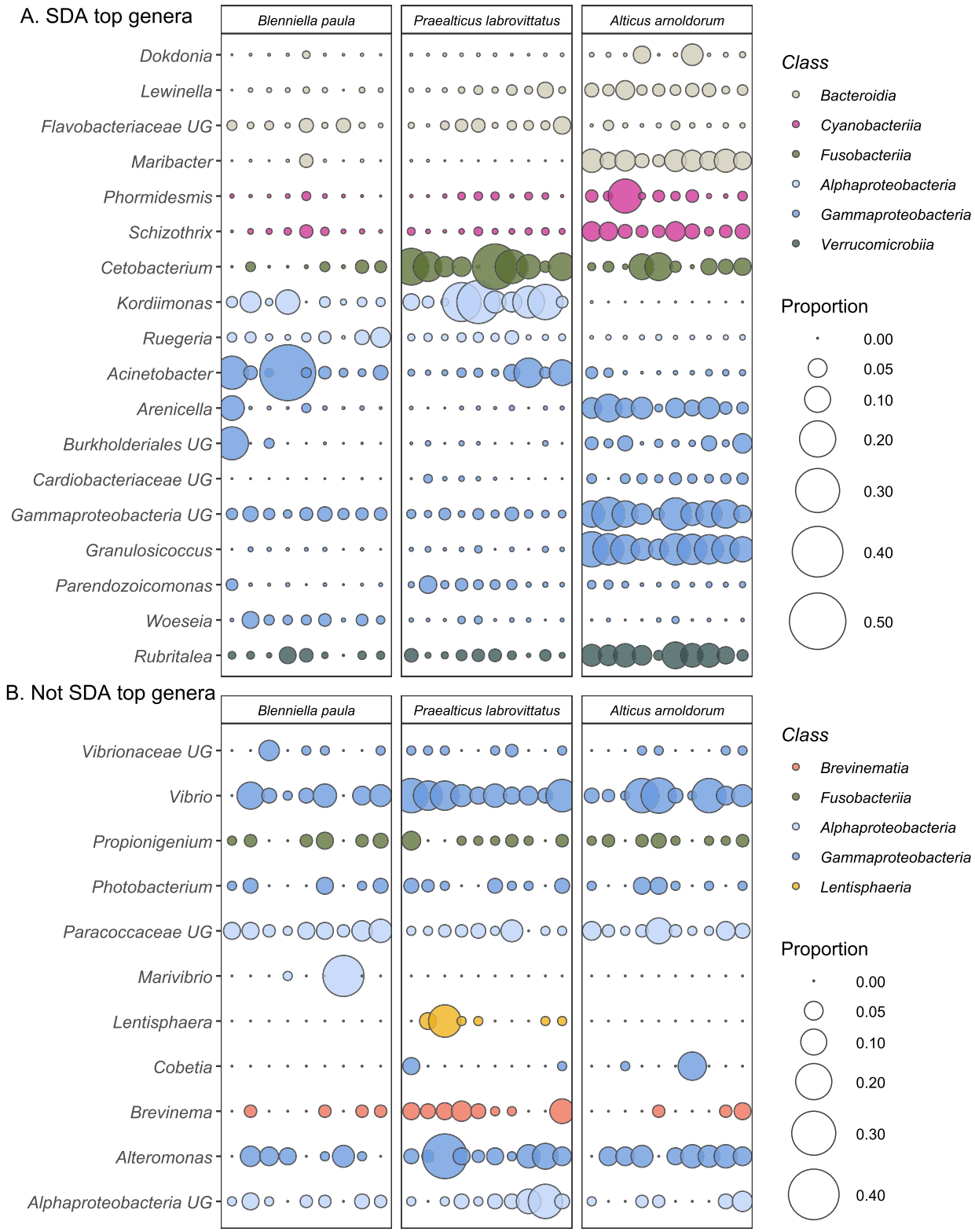


Figure 4 Relative abundance of the 29 most abundant genera on skin microbiomes (mean relative abundance $\geq$ 1% in at least one host species), colored by class and separated into (A) 18 statistically differentially abundant (SDA) genera (Wald test, FDR $<$ 0.05) and (B) 11 non-differentially abundant genera across the three host species. UG: undescribed genera.

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

terest were *Cetobacterium*, *Kordiimonas*, and *Parendozoicomonas*, which were enriched on the skin of *P. labrovittatus* and were predicted to contribute mostly to a few xenobiotic degradation pathways (Fig. 5). The skin microbiome of *B. paula* was characterized by strong functional contributions from *Acinetobacter,* which was predicted as a dominant contributor to multiple xenobiotic biodegradation pathways (Fig. 5).

Energy metabolism was largely driven by cyanobacteria enriched on the skin of *A. arnoldorum.* The genera *Phormidesmis* and *Schizothrix*, enriched in the skin of *A. arnoldorum,* were the primary contributors to photosynthesis-related pathways (Fig. 5). In addition, *Granulosicoccus* and *Rubritalea*, also enriched on *A. arnoldorum,* are expected to contribute substantially to glycosaminoglycan metabolism and flavonoid degradation. Several other genera were also predicted to contribute disproportionately to specialized metabolite pathways, highlighting the role of less abundant but functionally specialized taxa in shaping the metabolic landscape of the skin microbiome.

Several of the most prevalent genera were not differentially abundant among host species but are predicted to make substantial functional contributions (Fig. S2). For example, a genus-level taxon within *Paracoccaceae* (139 ASVs and 46 reference genomes) may be involved in xenobiotic degradation, while *Vibrio* and *Alteromonas* are expected to contribute to sulfoquinovose metabolism and flavonoid degradation, respectively. Interestingly, our functional analysis suggests that some shared core taxa might contribute substantially to glycan degradation and galactose metabolism (Fig. S2).

Predicted metabolic profiles differed among host species (PERMANOVA, $F = 5.19$, $R^2 = 0.285$, $P = 0.001$). However, multivariate dispersion also differed among species (permutest, $F = 4.60$, $P = 0.018$), suggesting that differences in within-species variability may partially contribute to the observed PERMANOVA result. Sample-level comparisons further indicated significant differences among host species in the relative representation of 103 of the 164 predicted KEGG BRITE C metabolic categories examined (Kruskal–Wallis tests, BH-adjusted $P < 0.05$; Table S8).

Discussion

Fish skin as an ecological filter on environmental microbiomes

The skin microbiomes were significantly different and less diverse than the bacterioplankton in seawater and the biofilms on the substrates on which fish lived. This suggests that fish skin acts as an ecological filter for the bacterial species available in the environment. Our findings contrast with those of Pratte et al. (2018), which found higher alpha diversity in the gill and gut microbiomes of fish than in the environment. However, the species studied, geographic location, and tissues differed from those in our study. The ecological filter is further corroborated by the marked differences between fish skin microbiomes and the substrates on which they live. This pattern was consistent across all three studied fish . Moreover, skin microbiomes increasingly diverged among species inhabiting increasingly higher areas along the intertidal gradient, while substrate microbiomes were largely overlapping. Substrate and skin microbiomes were most similar for the subtidal *B. paula,* which lives in a tight association with the substrate in rock holes and crevices in the reef flat. Substrate and skin microbiomes diverged for *P. labrovittatus,* which inhabits intertidal rock pools, and were most dissimilar between substrate and skin of *A. arnoldorum,* which typically inhabits supratidal rocks (Ord and Hsieh 2011). This suggests that the intertidal gradient imposes a second ecological filter on the bacterial species that occur on fish skin, a filter that increases with the increasingly terrestrial lifestyles of fish hosts.

Habitat as a determinant of skin microbiome in combtooth blennies

The three fish species inhabiting the subtidal, intertidal, and supratidal zones differed markedly in their skin microbiomes, with these differences broadly corresponding to their contrasting habitat use along the intertidal gradient. Host-specific profiles indicate that skin microbiomes are shaped by intrinsic host factors, in agreement with previous research (Gomez and Primm 2021, Sehnal et al. 2021). Microbiomes can also display a phylosymbiotic signal, whereby differences in microbial community composition parallel the evolutionary divergence of their hosts. For example, skin microbiomes were more similar within than between fish host families in a study of seven wild fish species from four families (*Labridae*, *Sebastidae*, *Sparidae*, and *Rajidae*) off the southern coast of Korea (Han et al. 2024). Other studies have shown that environmental factors such as habitat and diet can significantly alter this phylosymbiotic signal (Bell et al. 2024). In our study, the skin microbiome profiles of *B. paula* and *P. labrovittatus* were more similar to each other than either was to that of *A. arnoldorum*. This pattern does not correspond to phylogenetic affinities, as *P. labrovittatus* is more closely related to *A. arnoldorum* than to *B. paula* (Hundt et al. 2014, Muñoz-Sánchez et al. 2026). Instead, the observed pattern is consistent with habitat similarity along the intertidal gradient: *B. paula* and *P. labrovittatus* remain largely submerged, respectively, in the subtidal zone and in intertidal rock pools, whereas *A. arnoldorum* has a terrestrial lifestyle on rocks above the waterline, consistent with its more divergent microbiome profile. This pattern suggests that habitat-associated environmental conditions can weaken or override potential phylosymbiotic signals in microbiome composition.

Adults of *A. arnoldorum* live out of water, and their activity typically peaks around mid-tide and at moderate temperatures, with individuals retreating to shelter in rock holes and crevices at low tides (Ord and Hsieh 2011, Ord and Cooke 2016). Thus, *A. arnoldorum* likely experiences markedly different environmental conditions in the supratidal zone, including higher light and UV exposure, more variable temperatures, increased osmotic stress and desiccation risk, and distinct food resources and pathogen communities. Differences between the skin microbiomes of *B. paula* and *P. labrovittatus* may likewise be associated with environmental conditions in their respective habitats. Unlike subtidal waters, tidal pools such as those inhabited by *P. labrovittatus* can experience large fluctuations in temperature, dissolved oxygen, and other abiotic factors (Ord et al. 2024), which could affect skin microbiome composition. Despite the correspondence between habitat use and microbiome divergence, other species-specific traits—including skin physiology, mucus composition, body size, diet, and behavior—could act synergistically with habitat or contribute substantially to the observed differences in microbiome

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

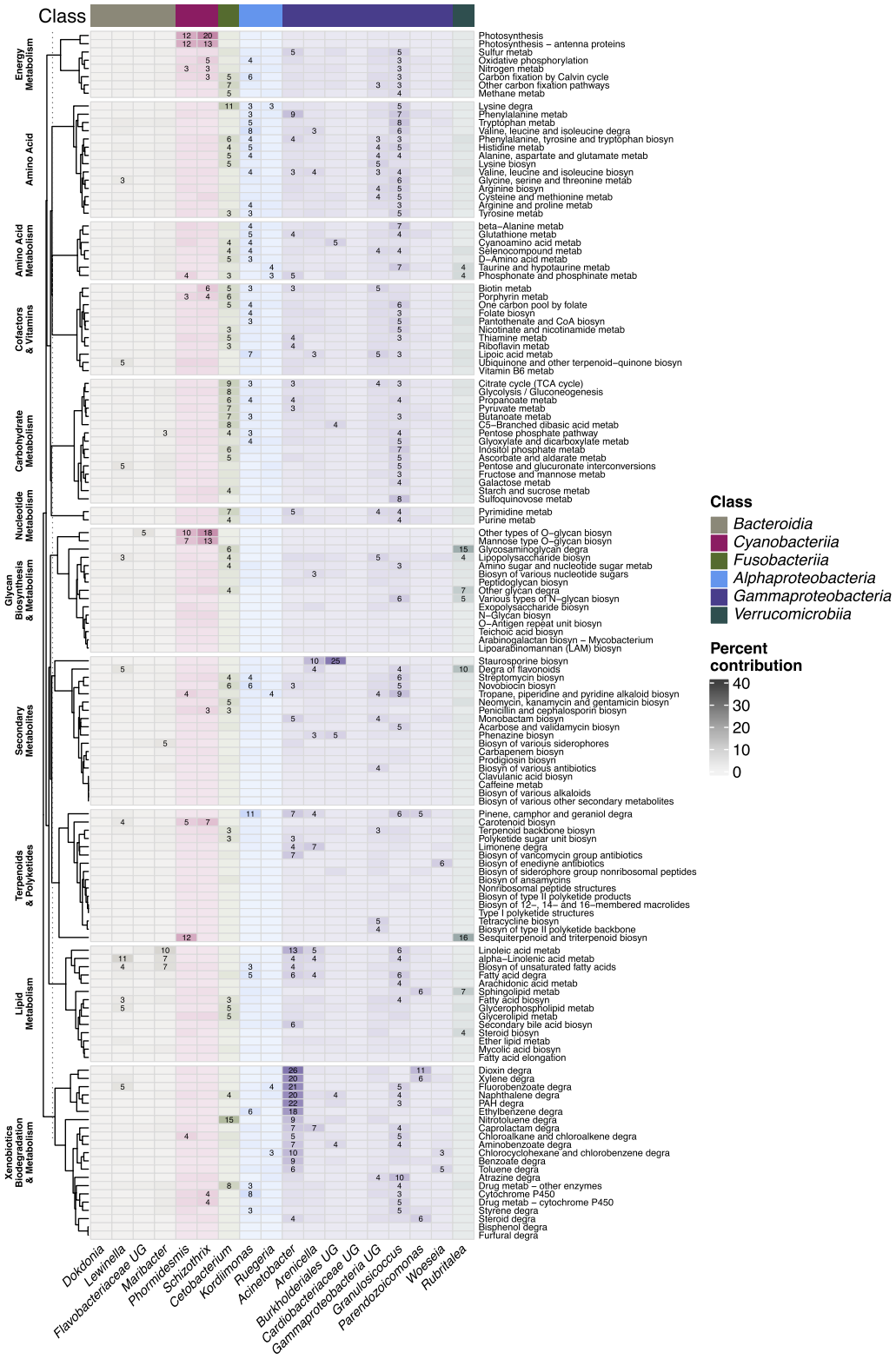


Figure 5 Predicted functional metabolic profiles of the 18 most abundant (mean relative abundance $\geq$ 1% in at least one host species) skin-associated genera that were also found to be differentially abundant across the three host species (Table S5, Wald test, FDR < 0.05). Genera are grouped and color-coded by bacterial class. Functional profiles were inferred using PICRUSt2 and are shown as the percentage contribution of each genus to the Kyoto Encyclopedia of Genes and Genomes) briteC-level pathways. UG: undescribed genus.

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

composition along the intertidal gradient. Because each habitat along the gradient was represented by a single host species, the present sampling design does not allow the effects of host species identity, habitat, and degree of terrestriality to be statistically disentangled.

Gammaproteobacteria and *Alphaproteobacteria* dominate fish skin microbiomes

This study identified six bacterial classes and 27 orders that were more abundant on the skin of the three blenny species than in seawater or the substrate where they lived. Among these, *Gammaproteobacteria* were the most abundant across all three species (Fig. 3), with a statistically higher relative abundance in *A. arnoldorum* than in the other two hosts. This class also showed statistically higher relative abundances in skin (43%–68%) than in substrate (10%–39%) and seawater (16%–38%). *Gammaproteobacteria* are the dominant group in the skin microbiomes of many marine fish (Larsen et al. 2013, Gomez and Primm 2021, Bell et al. 2024). The relative abundance of *Alphaproteobacteria* was similar in seawater (19%–33%), substrate (16%–31%), and fish skin (3%–43%), but varied significantly among hosts, with lower abundance in *A. arnoldorum* (7%) than in the other two species (23% in both). Thus, the skin microbiome of the highly amphibious *A. arnoldorum* is characterized by higher proportions of *Gammaproteobacteria* and lower proportions *of Alphaproteobacteria*.

Within *Gammaproteobacteria*, fish skins were enriched in bacterial orders *Enterobacterales, Burkholderiales, Francisellales, Arenicellales, Cardiobacteriales*, and *Granulosicoccales*. Members of the first three orders have been reported in fish microbiomes (Debnath et al. 2023, Bell et al. 2024, Jacobsen et al. 2025) whereas the latter three have not and may be unique to our species or location. Notably, *Granulosicoccus*, a genus found in Antarctic seawater (Baek et al. 2014) or as epiphytes on marine algae (Kurilenko et al. 2010, Park et al. 2014), was among the top five most abundant genera across all three host species and particularly enriched in *A. arnoldorum.* The predicted functional profile of *Granulosicoccus* suggests that it could contribute to several processes, some of the most important being specialized metabolism, xenobiotic degradation, and primary (carbon and amino acid) metabolism. Interestingly, it may contribute to the highest proportion of sulfoquinovose metabolism, a sulfated monosaccharide produced by photosynthetic organisms and an important source of carbon and sulfur for many environmental bacteria.

The dominant *Enterobacterales* (2.8%–48.5%) genera were *Vibrio* (0.4%–19%), *Alteromonas* (0.2%–31%), and *Photobacterium* (0.1%–3.6%), whose abundances did not differ among fish hosts. These genera commonly dominate the microbiomes of marine fish, yet their functional roles remain poorly understood (Chiarello et al. 2018, Bell et al. 2024, Han et al. 2024, Jacobsen et al. 2025). Recently, Yoshida et al. (2022) found a high prevalence of *Vibrio* species in the guts of two other combtooth blenny species. They isolated and sequenced the genomes of two *Vibrio* strains that encode multiple digestive enzymes, including alginate lyases, amylases, chitinases, and mannosidases, which could help degrade algal polysaccharides in fish guts. In our study, *Vibrio's* highest functional contributions were to carbohydrate metabolism, whereas *Photobacterium's* were to glycan and carbohydrate metabolism, and *Alteromonas'* to specialized metabolism. Together with the findings of Yoshida et al. (2022), this might suggest that a consistent portion of *Enterobacterales* might be involved in catabolizing compounds such as sulfoquinovose and flavonoids produced by algae and cyanobacteria or glycosaminoglycans (GAGs) from the fish skin mucus (Scholz et al. 2021, Fernando et al. 2022, Chen et al. 2026). A key limitation of our barcoding approach is that 16S-V4 lacks sufficient resolution to distinguish among *Vibrio* species or strains and other *Enterobacterales*. This is important because some *Vibrio* strains are pathogenic and can cause skin lesions under stress (Manchanayake et al. 2023) and there exist pathogenic, mutualistic, and commensal strains of *Photobacterium* (Labella et al. 2017, Moi et al. 2017). Strain-level resolution and experimental testing are therefore necessary to clarify the functions of these bacterial genera in greater detail. Nonetheless, their consistently high abundance in healthy fish suggests that many species are commensal or beneficial, although the possibility of them being opportunistic pathogens cannot be ruled out.

Additional *Gammaproteobacteria* genera with predicted interesting functional profiles include *Arenicella*, *Acinetobacter,* and *Woeseia,* and an undescribed genus (UG) within *Burkholderiales*. *Arenicella* had a higher relative abundance in the *A. arnoldorum* skin microbiome, and *Burkholderiales UG* was enriched in *A. arnoldorum* and *B. paula* compared to *P. labrovittatus*. Our functional analysis suggests that both *Arenicella* and *Burkholderiales UG* may be able to perform staurosporine biosynthesis (Fig. 5). This KEGG module comprises a number of tryptophane-derived antimicrobial and antifungal specialized metabolites that could protect their host from fungal infection (Hammer et al. 1999, Hwang et al. 2002). A metagenomic analysis might confirm the presence of this metabolic function in the microbiome of these fish. *Acinetobacter* had the highest relative abundance on the skin of the subtidal *B. paula* in comparison to *P. labrovittatus* and *A. arnoldorum.* The most important predicted functional roles were associated with degradation of xenobiotics, among which are polycyclic aromatic hydrocarbons (PAHs) that tend to accumulate in marine sediments (Ghosal et al. 2016) and are found in high levels in some Guam locations (Denton et al. 2006).

Orders within *Alphaproteobacteria* that occurred at higher relative abundances on the skin of the more submerged blennies included *Kiloniellales*, *Kordiimonadales*, and *Rhodospirillales*. Of these, *Kordiimonas* and *Ruegeria* were most prevalent in submerged *B. paula* and P. *labrovittatus* than in the supratidal *A. arnoldorum*. *Kordiimonas* was predicted to be involved in several metabolic functions, including the degradation of some terpenoids and xenobiotics (Fig. 5). *Ruegeria* (*Rhodobacteraceae*) is a widely abundant bacterial symbiont of corals (Huggett and Apprill 2019) and was found enriched on the skin of the two more aquatic fish species (*B. paula* and *P. labrovittatus*).

Other bacterial classes enriched in fish skin microbiome

Fusobacteriia were a third bacterial class enriched in the blennies' skin microbiomes. In some cases, it constitutes a significant proportion of the microbiome (7%–34%), showing a statistically higher relative abundance than in seawater (2%–4%) or on sub-

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

strates (0%–6%). *Fusobacteriia* were significantly more prevalent in *P. labrovittatus* (13%) than in *A. arnoldorum* (5%) and *B. paula* (2%). They were represented by a single order, *Fusobacteriales*, and four genera: *Cetobacterium* (0.74%–11.59%), *Propionigenium* (1.23%–1.59%), *Fusobacterium* (0%–0.46%), and *Psychrilyobacter* (0.01%–0.08%). *Cetobacterium* is one of the best-studied fish bacterial symbionts (Tsuchiya et al. 2008, Xie et al. 2022, Tsuchiya and Sugita 2023, Zhao et al. 2023, Wang et al. 2025a, Wang et al. 2025b) commonly found in the gut and skin of many freshwater fish (Ramírez et al. 2018, Arias et al. 2019, Krotman et al. 2020, Coetzer et al. 2021) and occasionally on marine fish skin (Han et al. 2024). *Cetobacterium* isolates from the guts of aquatic animals can synthesize vitamin B12, helping their hosts meet vitamin requirements (Tsuchiya et al. 2008, Suzuki A et al. 2022) and supplementing fish diets with *Cetobacterium* improves growth and overall health and enhances resistance to bacterial infections (Xie et al. 2022, Zhou et al. 2022, Wang et al. 2025, Wang et al. 2025). In the KEGG classification, vitamin B12 production was grouped with porphyrin metabolism, and *Cetobacterium* was predicted to be among the top-contributing genera to this functional category (Fig. 5). Interestingly, *Cetobacterium* was also predicted to contribute the highest proportion to butanoate metabolism, a short-chain fatty acid found in the guts of omnivorous and herbivorous fish that has been shown to inhibit pathogens and provide health benefits to aquaculture fish (Yin et al. 2025). *Propionigenium* (*Fusobacteriales*), one of the most abundant genera in all three host species, is reported to be strictly anaerobic, succinate-fermenting, and to grow in marine sediments (von Hoyningen-Huene et al. 2022).

Verrucomicrobiia was another bacterial class with significantly higher relative abundance on fish skin (3%–11%) than in seawater (0%–1%) and substrate (0%–4%). It was also significantly differentially abundant between the three fish hosts, comprising on average, 1.4% in *B. paula,* 1.6% in *P. labrovittatus,* and 6.4% in *A. arnoldorum. Verrucomicrobiia* has been only occasionally reported in fish skin, e.g. as a seasonal component in diadromous salmonids (Hamilton et al. 2023) and in lumpfish (Jacobsen et al. 2025). In our study, *Verrucomicrobiia* was represented by 62 ASVs, more than half (35) belonging to *Rubritalea*. Among the top 18 most abundant bacterial genera in the fish skin microbiome, *Rubritalea* accounted for an average of 2.8% total abundance. It is a key commensal on the skin of healthy European seabass and its abundance decreases following *Vibrio* infection (Cámara-Ruiz et al. 2021), which suggests *Rubritalea* may help maintain a healthy microbiome. Among the seven described *Rubritalea* species, five have been isolated from marine animals (Scheuermayer et al. 2006, Yoon et al. 2007, Yoon et al. 2011) and in sponges it provides antioxidant protection by producing carotenoids and squalene (Shindo and Misawa 2014), but its role in fish has not been studied yet. Our functional prediction suggested a role in the degradation of glycosaminoglycans (GAGs). Since GAGs are major components of skin mucus (Tiralongo et al. 2020), we speculate that *Rubritalea* might be an important contributor to the maintenance and recycling of the fish mucosal surface. *Rubritalea* is significantly enriched in *A. arnoldorum* compared to the other two fish species, which may be related to the specific composition or thickness of its mucus coating. Two other genera within *Verrucomicrobiia* were enriched in fish skin: *Haloferula,* which is sensitive to salinity changes and reduces its abundance at lower salinities (Lai et al. 2022), and *Roseibacillus,* found on lumpfish skin (Jacobsen et al. 2025). Multiple *Roseibacillus* species have been isolated from algae, indicating that fish skin microbiomes can be temporarily colonized by epiphytic bacteria, particularly in species that spend time in algal-covered habitats.

The *Spirochaetia* class was also enriched in the fish skin microbiomes, represented by 10 ASVs classified as *Treponema*. These are often parasites in a variety of animal hosts, including humans, but non-pathogenic species are also found in the oral cavities and intestinal tracts of humans and other animals (Miller et al. 1992, Edwards et al. 2003). *Treponema* has been detected in the gut of marine herbivorous fish living on rocky reefs in northern New Zealand and temperate Australia (Pisaniello et al. 2023). Interestingly, the presence of *Treponema* was restricted to mucosal surfaces in the gut (Pisaniello et al. 2023), suggesting that the mucus-covered skin of combtooth blennies may also provide a suitable habitat.

Within the class *Brevinematia, Brevinema* was among the most abundant genera in skin microbiomes. It is more commonly found in animal guts, including Atlantic salmon (Li et al. 2021) and European seabass (Alfonso et al. 2023), but again, the thick mucus layer of combtooth blennies might provide a suitable habitat for what are normally intestinal microaerophilic bacteria, thanks to the presence of low-oxygen micro-niches.

Four additional bacterial classes enriched on fish skin occurred at relatively low abundances: *Bacilli* (mean relative abundance 1.7%), *Balneolia* (0.6%), *Actinobacteria* (0.4%), and *Deferribacteres* (0.1%). Several low-abundance genera within *Bacilli* and *Actinobacteria* were also enriched in fish skin samples, including *Staphylococcus*, *Turicibacter*, *Streptococcus*, *Mycoplasma*, *Lactobacillus*, *Cutibacterium*, *Corynebacterium*, *Mycobacterium*, *Bifidobacterium*, and *Actinomyces*. Low-abundance taxa can contribute to microbiome function despite their limited numerical representation (Lynch and Neufeld 2015); however, their ecological significance in the blenny skin microbiome remains uncertain. Because field and extraction blanks were not included in this study, the occurrence of very low-abundance taxa should be interpreted with caution, and their association with fish skin will require confirmation in future studies.

The skin of supratidal *A. arnoldorum* hosts more photosynthetic cyanobacteria

Differential abundance analysis of predicted functional categories identified several biochemical pathways that differed among the three fish host species. Specifically, photosynthesis was identified as an important metabolic pathway in the skin microbiome of the supratidal *A. arnoldorum*, likely due to the cyanobacterial genera *Phormidesmis* and *Schizothrix*, which were at higher relative abundances. This could be explained by the greater light availability on supratidal rocks, which likely offers better conditions for oxygenic photosynthesis than the below-water environments. A particularity of *Alticus* is its ability to perform gas exchange not only in the gills but also in the skin, with approximately two-fold rates of oxygen and carbon dioxide exchange when emerged (Martin and Lighton 1989). This led us to hypothesize that cyanobacteria inhabiting its skin mucus could be providing additional oxygen for fish respiration. Moreover, cyanobacteria could be respon-

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

sible for the biosynthesis of carotenoids and other terpenoids (Fig. 5) that might help protect the emerged *A. arnoldorum* from prolonged light and UV exposure and oxidative stress (reactive oxygen species). This would suggest true mutualistic interactions between these cyanobacteria and the host. These hypotheses, however suggestive, would require formal testing using additional data (e.g. microscopy, metatranscriptomes, or metabolomics) and experimental verification.

The role of specialized metabolism and xenobiotic degradation

Many of the identified bacterial taxa enriched in fish skin microbiomes could potentially be beneficial for the host by producing specialized metabolites and vitamins (e.g. *Alteromonas, Cetobacterium, Granulosicoccus, Paracoccaceae* UG*, Phormidesmis, Rubritalea, Schizothrix*). Furthermore, various genera (*Acinetobacter*, *Paracoccaceae* UG, *Granulosicoccus*, *Alteromonas*, *Kordiimonas*, *Cetobacterium*, *Arenicella*, *Burkholderiales* UG) could have the metabolic potential to synthesize antimicrobial metabolites (Fig. 5) that may help maintain healthy skin microbiomes. Several other bacterial taxa enriched in fish skin microbiomes appear capable of degrading xenobiotics. These included *Acinetobacter*, *Paracoccaceae* UG, *Granulosicoccus, Alphaproteobacteria UG, Cetobacterium,* and *Parendozoicomonas*. Xenobiotics such as bisphenol and PAHs are persistent pollutants that accumulate in marine sediments and are thus particularly toxic to bottom-dwelling organisms. In marine environments, abiotic degradation of these xenobiotics is limited and microbial metabolism is the primary removal pathway. The higher importance of these pathways in submerged (subtidal and intertidal) fish may reflect their continuous exposure to these pollutants compared to the supratidal *A. arnoldorum.* In addition, some of these chemicals, including xylene, can be degraded by sunlight, which is more intense out of water, and thus might also partly explain the absence of xylene-degrading bacteria on *A. arnoldorum* skin.

Bacterial motility in the mucus layer of combtooth blenny skins

Several bacterial taxa enriched in fish skins appear to be adapted to live in the mucus layer of combtooth blennies. This layer is secreted by superficial epithelial and goblet cells and consists of sulfated acid glycoproteins and mucus (Whitear and Mittal 1984). This mucus not only provides a viscous environment but also probably low-oxygen micro-niches for microaerophilic bacteria typical of the gut. Some bacteria enriched in fish skin (*Cetobacterium*, *Rubritalea*, *Photobacterium*) have the potential to degrade GAGs, which are constituent components of mucus and the extracellular matrix. The suggested contribution to amino sugar and galactose metabolism observed in *Vibrio* and *Photobacterium*, and the enrichment in amino acid metabolism in *Cetobacterium*, could also correlate with their capacity to degrade and utilize mucus and extracellular matrix components. Notably, most of the genera shown in Fig. 4 (i.e. all except *Dokdonia*, *Phormidesmis*, *Cetobacterium*, *Arenicella*, *Rubritalea*) contain motile species, an important ability to navigate a viscous environment such as the blenny skin mucus layer. Moreover, some bacteria exhibit cell morphologies that are vibrioid (in *Vibrio* and *Marivibrio*; members of *Gammaproteobacteria, Enterobacterales*) and/or spiral forms (*Marivibrio* and *Brevinema*), which are well-known adaptations for motility in viscous environments and might be important for virulence in pathogenic bacteria (Bartlett et al. 2017, Xu et al. 2020).

Conclusions

The skin microbiomes of three combtooth blenny species were studied in the context of increasingly amphibious lifestyles along the intertidal gradient. Skin-associated communities were distinct from those in surrounding seawater and rock substrates, indicating that fish skin acts as an important ecological filter. The magnitude of skin–substrate microbiome divergence differed among the three host species and increased along the subtidal-to-supratidal gradient, with the greatest divergence observed in the supratidal species *Alticus arnoldorum*. In addition, the skin microbiome of *A. arnoldorum* was more distinct from those of the predominantly submerged *Blenniella paula* and *Praealticus labrovittatus*. Together, these patterns are consistent with habitat-associated environmental conditions contributing to microbiome differentiation across increasingly terrestrial lifestyles, although species-specific host traits may also contribute to these differences.

Pseudomonadota (*Alpha-* and *Gammaproteobacteria*) dominated the skin microbiome, and their relative representation differed among hosts: *Alphaproteobacteria* were more abundant in the subtidal *B. paula* and intertidal *P. labrovittatus*, whereas *Gammaproteobacteria* were enriched in the supratidal *A. arnoldorum*. Skin communities also showed enrichment of epiphytic-associated taxa, consistent with the benthic lifestyles of these fishes and their frequent contact with algae-covered substrates. PICRUSt2 predictions further suggested differences in the functional potential of the skin microbiomes among host species, including differences in energy metabolism, specialized metabolism, and xenobiotic degradation. Because these predictions are inferred from 16S rRNA gene data, they should be considered hypotheses about potential metabolic capabilities and metagenomic, metatranscriptomic, and culture-based approaches will be required to determine whether these predicted differences contribute to host–microbiome interactions across the intertidal gradient.

Our study highlights the distinctive nature of the scaleless, mucus-covered skin of combtooth blennies. The presence of anaerobic or microaerophilic taxa, including those commonly associated with the gut, such as *Cetobacterium*, suggests that the thick mucus layer provides low-oxygen microhabitats. Future work directly measuring physicochemical conditions within the mucus would be needed to test this hypothesis. The presence of motile bacteria and taxa with vibrioid or spiral cell morphologies also offers avenues for investigating microbial adaptation to the viscous mucus environment.

This study provides the first comparative assessment of skin microbiomes among combtooth blennies occupying increasingly terrestrial habitats and highlights the intertidal gradient as a useful natural system for investigating microbiome assembly under contrasting environmental conditions. Future work should resolve microbial taxa at the strain level and experimentally test functional mechanisms—including photosynthesis, vitamin and specialized metabolism, antioxidant production, mucin/glycan

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

utilization, and xenobiotic degradation—to determine how skin-associated micro-organisms interact with their hosts across an intertidal gradient.

Acknowledgements

We are grateful to Elizabeth A. Surovic, Héctor Torrado, and David Combosch for their support during fieldwork, to Sarah Lemer and Héloïse L. Rouzé for facilitating laboratory work, and to Mireia Mestre and Cristian Villena-Alemany for insightful discussions. We thank Jeffrey Quitugua, Brent Tibbatts, William Paulino II, and the remaining staff from Government of Guam Department of Agriculture, Division of Aquatic & Wildlife Resources (DAWR) for collection permits (SCR-23–006) and for their continuous support during fieldwork in Guam. We also thank the University of Guam UOG-IACUC committee and the University of New South Wales Animal Care and Ethics Committee for ethical permits. We are grateful to Laurie Raymundo for support in obtaining sampling permits and endorsing our stay at the University of Guam Marine Laboratory. We thank Angela Duenas, the Biorepository team, and the rest of the University of Guam Marine Laboratory Staff for supporting our visit.

Supplementary material

Supplementary material are available at *FEMSLE Journal* online.

Conflicts of interest

None declared.

Funding

This project was funded by the Spanish State Research Agency (AEI) MICIU/AEI/10.13039/501100011033, ESF +, and ERDF/EU (Grants RyC2022-038245-I, PID2023-152168NB-I00, and CNS2025-165952) and the Leibniz Institute for the Analysis of Biodiversity Change (LIB), Germany.

Data availability

Raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1459627. Associated metadata and processed data are available at https://github.com/ewrubin/combtooth-blenny-microbiome.

References

Akat E, Yenmiş M, Pombal MA *et al.* Comparison of vertebrate skin structure at class level: a review. *Anat Rec* 2022;**305**:3543–608. https://doi.org/10.1002/ar.24908.

Alfonso S, Mente E, Fiocchi E *et al.* Growth performance, gut microbiota composition, health and welfare of European sea bass (*Dicentrarchus labrax*) fed an environmentally and economically sustainable low marine protein diet in sea cages. *Sci Rep* 2023;**13**:21269. https://doi.org/10.1038/s41598-023-48533-3.

Apprill A, McNally S, Parsons R *et al.* Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol* 2015;**75**:129–37. https://doi.org/10.3354/ame01753.

Arias CR, Ray L, Cai W *et al.* Fish are not alone: characterization of the gut and skin microbiomes of Largemouth Bass (*Micropterus salmoides*), Bluegill (*Lepomis macrochirus*), and Spotted Gar (*Lepisosteus oculatus*). *SDRP-JAFFS* 2019;**2**:1–15. https://doi.org/10.25177/JAFFS.2.2.RA.459.

Bacci G, Meriggi N, Cheng CLY *et al.* Species-specific gill's microbiome of eight crab species with different breathing adaptations. *Sci Rep* 2023;**13**:21033. https://doi.org/10.1038/s41598-023-48308-w.

Baek K, Choi A, Kang I *et al. Granulosicoccus marinus* sp. nov., isolated from Antarctic seawater, and emended description of the genus *Granulosicoccus*. *Int J Syst Evol Microbiol* 2014;**64**:4103–8. https://doi.org/10.1099/ijs.0.070045-0.

Barnett DJM, Arts ICW, Penders J. MicroViz: an R package for microbiome data visualization and statistics. *JOSS* 2021;**6**:3201. https://doi.org/10.21105/joss.03201.

Bartlett TM, Bratton BP, Duvshani A *et al.* A periplasmic polymer curves *Vibrio cholerae* and promotes pathogenesis. *Cell* 2017;**168**:172–185.e15. https://doi.org/10.1016/j.cell.2016.12.019.

Bell AG, McMurtrie J, Bolaños LM *et al.* Influence of host phylogeny and water physicochemistry on microbial assemblages of the fish skin microbiome. *FEMS Microbiol Ecol* 2024;**100**:fiae021. https://doi.org/10.1093/femsec/fiae021.

Brown J, Pirrung M, McCue LA. FQC Dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. *Bioinformatics* 2017;**33**:3137–9. https://doi.org/10.1093/bioinformatics/btx373.

Brucker RM, Bordenstein SR Speciation by symbiosis. *Trends Ecol Evol* 2012;**27**:443–51. https://doi.org/10.1016/j.tree.2012.03.011.

Callahan BJ, McMurdie PJ, Rosen MJ *et al.* DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3. https://doi.org/10.1038/nmeth.3869.

Callewaert C, Ravard Helffer K, Lebaron P. Skin Microbiome and its Interplay with the Environment. *Am J Clin Dermatol* 2020;**21**:4–11. https://doi.org/10.1007/s40257-020-00551-x.

Cámara-Ruiz M, Cerezo IM, Guardiola FA *et al.* Alteration of the immune response and the microbiota of the skin during a natural infection by *Vibrio harveyi* in European seabass (*Dicentrarchus labrax*). *Microorganisms* 2021;**9**:964. https://doi.org/10.3390/microorganisms9050964.

Cannicci S, Fratini S, Meriggi N *et al.* To the land and beyond: crab microbiomes as a paradigm for the evolution of terrestrialization. *Front Microbiol* 2020;**11**:575372. https://doi.org/10.3389/fmicb.2020.575372.

Chen Y, Liu D, Chu R *et al.* Advances in microbial sulfoquinovose catabolism. *Front Microbiol* 2026;**17**:1758990. https://doi.org/10.3389/fmicb.2026.1758990.

Chiarello M, Auguet J-C, Bettarel Y *et al.* Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. *Microbiome* 2018;**6**:147. https://doi.org/10.1186/s40168-018-0530-4.

Coetzer WG, Coetzee LM, Cason ED *et al.* A preliminary assessment of skin microbiome diversity of zebrafish (*Danio rerio*): south

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

African pet shop fish. *Indian J Microbiol* 2021;**61**:81–84. https://doi.org/10.1007/s12088-020-00900-8.

Debnath SC, McMurtrie J, Temperton B *et al.* Tilapia aquaculture, emerging diseases, and the roles of the skin microbiomes in health and disease. *Aquacult Int* 2023;**31**:2945–76. https://doi.org/10.1007/s10499-023-01117-4.

Denton GRW, Concepcion LP, Wood HR *et al.* Polycyclic aromatic hydrocarbons (PAHs) in small island coastal environments: a case study from harbours in Guam, Micronesia. *Mar Pollut Bull* 2006;**52**:1090–8. https://doi.org/10.1016/j.marpolbul.2006.05.002.

Douglas GM, Maffei VJ, Zaneveld JR *et al.* PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 2020;**38**:685–8. https://doi.org/10.1038/s41587-020-0548-6.

Edwards AM, Dymock D, Woodward MJ *et al.* Genetic relatedness and phenotypic characteristics of *Treponema* associated with human periodontal tissues and ruminant foot disease. *Microbiology* 2003;**149**:1083–93. https://doi.org/10.1099/mic.0.26111-0.

Egan JP, Buser TJ, Burns MD *et al.* Patterns of body shape diversity and evolution in intertidal and subtidal lineages of combtooth blennies (Blenniidae). *Integr Org Biol* 2021;**3**:obab004. https://doi.org/10.1093/iob/obab004.

Esteban MA. An overview of the immunological defenses in fish skin. *ISRN Immunol* 2012;**2012**:1–29. https://doi.org/10.5402/2012/853470.

Fernando IPS, Lee W, Ahn G. Marine algal flavonoids and phlorotannins; an intriguing frontier of biofunctional secondary metabolites. *Crit Rev Biotechnol* 2022;**42**:23–45. https://doi.org/10.1080/07388551.2021.1922351.

Ghosal D, Ghosh S, Dutta TK *et al.* Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. *Front Microbiol* 2016;**7**:1369.

Glover CN, Bucking C, Wood CM. The skin of fish as a transport epithelium: a review. *J Comp Physiol B* 2013;**183**:877–91. https://doi.org/10.1007/s00360-013-0761-4.

Gomez JA, Primm TP. A slimy business: the future of fish skin microbiome studies. *Microb Ecol* 2021;**82**:275–87. https://doi.org/10.1007/s00248-020-01648-w.

Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016;**32**:2847–9. https://doi.org/10.1093/bioinformatics/btw313.

Hamilton EF, Juurakko CL, Engel K *et al.* Environmental impacts on skin microbiomes of sympatric high Arctic salmonids. *Fishes* 2023;**8**:214. https://doi.org/10.3390/fishes8040214.

Hammer PE, Burd W, Hill DS *et al.* Conservation of the pyrrolnitrin biosynthetic gene cluster among six pyrrolnitrin-producing strains. *FEMS Microbiol Lett* 1999;**180**:39–44. https://doi.org/10.1111/j.1574-6968.1999.tb08775.x.

Han GH, Yu J, Kang MJ *et al.* Phylosymbiosis in seven wild fish species collected off the southern coast of Korea: skin microbiome most strongly reflects evolutionary pressures. *Microb Ecol* 2024;**87**:153. https://doi.org/10.1007/s00248-024-02467-z.

Heimroth RD, Casadei E, Benedicenti O *et al.* The lungfish cocoon is a living tissue with antimicrobial functions. *Sci Adv* 2021;**7**:eabj0829. https://doi.org/10.1126/sciadv.abj0829.

Hsieh S-TT. A locomotor innovation enables water-land transition in a marine fish. *PLoS One* 2010;**5**:e11197. https://doi.org/10.1371/journal.pone.0011197.

Huggett MJ, Apprill A. Coral microbiome database: integration of sequences reveals high diversity and relatedness of coral-associated microbes. *Environ Microbiol Rep* 2019;**11**:372–85,. https://doi.org/10.1111/1758-2229.12686.

Hundt PJ, Hundt MJ, Staley C *et al.* The diet and gut microbial communities of two closely related combtooth blennies, *Chasmodes saburrae* and *Scartella cristata*. *Ichthyol Herpetol* 2017;**105**:249–56.

Hundt PJ, Iglésias SP, Hoey AS *et al.* A multilocus molecular phylogeny of combtooth blennies (Percomorpha: blennioidei: blenniidae): multiple invasions of intertidal habitats. *Mol Phylogenet Evol* 2014;**70**:47–56. https://doi.org/10.1016/j.ympev.2013.09.001.

Hwang J, Chilton WS, Benson DM. Pyrrolnitrin production by *Burkholderia cepacia* and biocontrol of *Rhizoctonia* stem rot of poinsettia. *Biol Control* 2002;**25**:56–63. https://doi.org/10.1016/S1049-9644(02)00044-0.

Jacobsen Á, Mols Mortensen A, Eliasen K *et al.* Effect of plastic and seaweed shelters on the skin microbiome of lumpfish *Cyclopterus lumpus* used as cleaner fish in aquaculture pens. *PLoS One* 2025;**20**:e0322261. https://doi.org/10.1371/journal.pone.0322261.

Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2000;**28**:27–30. https://doi.org/10.1093/nar/28.1.27.

Kozich JJ, Westcott SL, Baxter NT *et al.* Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microb* 2013;**79**:5112–20. https://doi.org/10.1128/AEM.01043-13.

Krotman Y, Yergaliyev TM, Alexander Shani R *et al.* Dissecting the factors shaping fish skin microbiomes in a heterogeneous inland water system. *Microbiome* 2020;**8**:9. https://doi.org/10.1186/s40168-020-0784-5.

Kurilenko VV, Christen R, Zhukova NV *et al. Granulosicoccus coccoides* sp. nov., isolated from leaves of seagrass (*Zostera marina*). *Int J Syst Evol Microbiol* 2010;**60**:972–6. https://doi.org/10.1099/ijs.0.013516-0.

Labella A, Arahal DR, Castro D *et al.* Revisiting the genus *Photobacterium*: taxonomy, ecology and pathogenesis. *Int Microbiol* 2017;**20**:1–10.

Lahti L, Sudarshan S. *Microbiome R package. Version 3.23*. 2012–2019. https://doi.org/10.18129/B9.bioc.microbiome.

Lai KP, Zhu P, Boncan DAT *et al.* Integrated omics approaches revealed the osmotic stress-responsive genes and microbiota in gill of marine medaka. *mSystems* 2022;**7**:e0004722. https://doi.org/10.1128/msystems.00047-22.

Larsen A, Tao Z, Bullard SA *et al.* Diversity of the skin microbiota of fishes: evidence for host species specificity. *FEMS Microbiol Ecol* 2013;**85**:483–94. https://doi.org/10.1111/1574-6941.12136.

Lemopoulos A, Montoya-Burgos JI. From scales to armor: scale losses and trunk bony plate gains in ray-finned fishes. *Evol Lett* 2021;**5**:240–50. https://doi.org/10.1002/evl3.219.

Li Y, Bruni L, Jaramillo-Torres A *et al.* Differential response of digesta- and mucosa-associated intestinal microbiota to dietary insect meal during the seawater phase of Atlantic salmon. *Anim Microbiome* 2021;**3**:8. https://doi.org/10.1186/s42523-020-00071-3.

Lim SJ, Bordenstein SR. An introduction to phylosymbiosis. *Proc Biol Sci* 2020;**287**:20192900.

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

Llewellyn MS, Boutin S, Hoseinifar SH *et al.* Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Front Microbiol* 2014;**5**:207. https://doi.org/10.3389/fmicb.2014.00207.

Lynch MDJ, Neufeld JD. Ecology and exploration of the rare biosphere. *Nat Rev Micro* 2015;**13**:217–29. https://doi.org/10.1038/nrmicro3400.

Manchanayake T, Salleh A, Amal MNA *et al.* Pathology and pathogenesis of *Vibrio* infection in fish: a review. *Aquac Rep* 2023;**28**:101459. https://doi.org/10.1016/j.aqrep.2022.101459.

Martin KLM, Lighton JRB. Aerial CO_2 and O_2 exchange during terrestrial activity in an amphibious fish, *Alticus kirki* (Blenniidae). *Copeia* 1989;**1989**:723. https://doi.org/10.2307/1445501.

Martin BD, Witten D, Willis AD. Modeling microbial abundances and dysbiosis with beta-binomial regression. *Ann Appl Stat* 2020;**14**:94–115. https://doi.org/10.1214/19-AOAS1283.

McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;**8**:e61217. https://doi.org/10.1371/journal.pone.0061217.

McMurtrie J, Bell AG, Cable J *et al.* The ecology and plasticity of fish skin and gill microbiomes: seeking what matters in health and disease. *FEMS Microbiol Rev* 2025;**49**:fuaf027. https://doi.org/10.1093/femsre/fuaf027.

Miller JN, Smibert RM, Norris SJ. The genus *Treponema*. *The Prokaryotes*. New York, NY: Springer New York, 1992,3537–59. https://doi.org/10.1007/978-1-4757-2191-1 10.1007/978-1-4757-2191-1_30.

Minich JJ, Härer A, Vechinski J *et al.* Host biology, ecology and the environment influence microbial biomass and diversity in 101 marine fish species. *Nat Commun* 2022;**13**:6978. https://doi.org/10.1038/s41467-022-34557-2.

Moi IM, Roslan NN, Leow ATC *et al.* The biology and the importance of *Photobacterium* species. *Appl Microbiol Biotechnol* 2017;**101**:4371–85. https://doi.org/10.1007/s00253-017-8300-y.

Muñoz-Sánchez Á, Kulikov N, Irisarri I. Stitch or cluster? A comparison of alternative phylogenomic dataset assembly strategies for blenny fish. *Bull Soc Syst Biol* 2026;**5**. https://doi.org/10.18061/bssb.6848.

Oksanen J, Simpson GL, Blanchet FG *et al. vegan: Community Ecology Package.* https://cran.r-project.org/web/packages/vegan/index.html, 2026.

Ord TJ, Cooke GM. Repeated evolution of amphibious behavior in fish and its implications for the colonization of novel environments. *Evolution* 2016;**70**:1747–59. https://doi.org/10.1111/evo.12971.

Ord TJ, Hsieh TS. A highly social, land-dwelling fish defends territories in a constantly fluctuating environment. *Ethology* 2011;**117**:918–27. https://doi.org/10.1111/j.1439-0310.2011.01949.x.

Ord TJ, Summers TC, Noble MM *et al.* Ecological release from aquatic predation is associated with the emergence of marine blenny fishes onto land. *Am Nat* 2017;**189**:570–9. https://doi.org/10.1086/691155.

Ord TJ, Surovic EA, Vaz DFB *et al.* Abiotic factors that prompt major ecological transitions: are fish on land to escape an intolerable aquatic environment? *Functional Ecol* 2024;**38**:2648–64. https://doi.org/10.1111/1365-2435.14672.

Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol* 2016;**18**:1403–14. https://doi.org/10.1111/1462-2920.13023.

Park S, Jung Y-T, Won S-M *et al. Granulosicoccus undariae* sp. nov., a member of the family Granulosicoccaceae isolated from a brown algae reservoir and emended description of the genus *Granulosicoccus*. *Antonie Van Leeuwenhoek* 2014;**106**:845–52. https://doi.org/10.1007/s10482-014-0254-9.

Pisaniello A, Handley KM, White WL *et al.* Host individual and gut location are more important in gut microbiota community composition than temporal variation in the marine herbivorous fish *Kyphosus sydneyanus*. *BMC Microbiol* 2023;**23**:275. https://doi.org/10.1186/s12866-023-03025-2.

Platt ERM, Fowler AM, Ord TJ. Land colonisation by fish is associated with predictable changes in life history. *Oecologia* 2016;**181**:769–81. https://doi.org/10.1007/s00442-016-3593-6.

Pratte ZA, Besson M, Hollman RD *et al.* The gills of reef fish support a distinct microbiome influenced by host-specific factors. *Appl Environ Microb* 2018;**84**:e00063–18. https://doi.org/10.1128/AEM.00063-18.

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:D590–6. https://doi.org/10.1093/nar/gks1219.

Ramírez C, Coronado J, Silva A *et al. Cetobacterium* is a major component of the microbiome of giant Amazonian fish (*Arapaima gigas*) in Ecuador. *Animals* 2018;**8**:189. https://doi.org/10.3390/ani8110189.

Reverter M, Tapissier-Bontemps N, Lecchini D *et al.* Biological and ecological roles of external fish mucus: a review. *Fishes* 2018;**3**:41. https://doi.org/10.3390/fishes3040041.

Ross AA, Rodrigues Hoffmann A, Neufeld JD. The skin microbiome of vertebrates. *Microbiome* 2019;**7**:79. https://doi.org/10.1186/s40168-019-0694-6.

Scheuermayer M, Gulder TAM, Bringmann G *et al. Rubritalea marina* gen. nov., sp. nov., a marine representative of the phylum "Verrucomicrobia", isolated from a sponge (Porifera). *Int J Syst Evol Microbiol* 2006;**56**:2119–24. https://doi.org/10.1099/ijs.0.64360-0.

Scholz S, Serif M, Schleheck D *et al.* Sulfoquinovose metabolism in marine algae. *Bot Mar* 2021;**64**:301–12. https://doi.org/10.1515/bot-2020-0023.

Sehnal L, Brammer-Robbins E, Wormington AM *et al.* Microbiome composition and function in aquatic vertebrates: small organisms making big impacts on aquatic animal health. *Front Microbiol* 2021;**12**:567408. https://doi.org/10.3389/fmicb.2021.567408.

Shindo K, Misawa N. New and rare carotenoids isolated from marine bacteria and their antioxidant activities. *Mar Drugs* 2014;**12**:1690–8. https://doi.org/10.3390/md12031690.

Suzuki A, Shirakata C, Anzai H *et al.* Vitamin B12 biosynthesis of *Cetobacterium ceti* isolated from the intestinal content of captive common bottlenose dolphins (*Tursiops truncatus*). *Microbiology* 2022;**168**:1465–2080. https://doi.org/10.1099/mic.0.001244.

Sylvain F-É, Holland A, Bouslama S *et al.* Fish skin and gut microbiomes show contrasting signatures of host species and habi-

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026

tat. *Appl Environ Microb* 2020;**86**:e00789–20. https://doi.org/10.1128/AEM.00789-20.

Teunisse GM. *Fantaxtic—Nested bar plots for Phyloseq data*. https://github.com/gmteunisse/Fantaxtic. 2022.

Thines M, Aoki T, Crous PW *et al.* Setting scientific names at all taxonomic ranks in italics facilitates their quick recognition in scientific papers. *IMA Fungus* 2020;**11**:25. https://doi.org/10.1186/s43008-020-00048-6.

Tiralongo F, Messina G, Lombardo BM *et al.* Skin mucus of marine fish as a source for the development of antimicrobial agents. *Front Mar Sci* 2020;**7**:541853. https://doi.org/10.3389/fmars.2020.541853.

Tsuchiya C, Sakata T, Sugita H. Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Lett Appl Microbiol* 2008;**46**:43–8.

Tsuchiya C, Sugita H. Oxygen tolerance of *Cetobacterium* somerae isolated from the gut of freshwater fish and their environments. *Int J Aquat Biol* 2023;**11**:301–4.

von Hoyningen-Huene AJE, Schneider D, Fussmann D *et al.* DNA- and RNA-based bacterial communities and geochemical zonation under changing sediment porewater dynamics on the Aldabra Atoll. *Sci Rep* 2022;**12**:4257.

Wang S, Li X, Zhang M *et al.* Cetobacterium somerae-derived argininosuccinic acid promotes intestinal and liver ureagenesis to alleviate ammonia intoxication. *Microbiome* 2025b;**13**:163. https://doi.org/10.1186/s40168-025-02152-4.

Wang M, Tang C, Zhang Z *et al.* Effect of the gut core microbiota *Cetobacterium* on the growth, physiology, and nutritional metabolism of Nile tilapia (*Oreochromis niloticus*). *Aquac Rep* 2025a;**40**:102583. https://doi.org/10.1016/j.aqrep.2024.102583.

Whitear M, Mittal AK. Surface secretions of the skin of *Blennius (Lipophrys) pholis* L. *J Fish Biol* 1984;**25**:317–31. https://doi.org/10.1111/j.1095-8649.1984.tb04879.x.

Wickham H. *Ggplot2: Elegant graphics for data analysis*. https://ggplot2.tidyverse.org, 2016 (Date accessed 18 August 2026).

Xie M, Hao Q, Olsen RE *et al.* Growth performance, hepatic enzymes, and gut health status of common carp (*Cyprinus carpio*) in response to dietary *Cetobacterium somerae* fermentation product. *Aquac Rep* 2022;**23**:101046. https://doi.org/10.1016/j.aqrep.2022.101046.

Xu J, Koizumi N, Nakamura S. Crawling motility on the host tissue surfaces is associated with the pathogenicity of the zoonotic spirochete *Leptospira*. *Front Microbiol* 2020;**11**:1886. https://doi.org/10.3389/fmicb.2020.01886.

Yin M, Zheng Z, Xing R. *et al.* Effects of butyric acid on the inhibition of V*ibrio parahaemolyticus* and its impacts on growth performance, intestinal microbiota, and water microbiota in *Litopenaeus vannamei*. *Aquac Rep* 2025;**45**:103078. https://doi.org/10.1016/j.aqrep.2025.103078.

Yoon J, Matsuda S, Adachi K *et al. Rubritalea halochordaticola* sp. nov., a carotenoid-producing verrucomicrobial species isolated from a marine chordate. *Int J Syst Evol Microbiol* 2011;**61**:1515–20. https://doi.org/10.1099/ijs.0.025031-0.

Yoon J, Matsuo Y, Matsuda S *et al. Rubritalea spongiae* sp. nov. and *Rubritalea tangerina* sp. nov., two carotenoid- and squalene-producing marine bacteria of the family Verrucomicrobiaceae within the phylum "Verrucomicrobia", isolated from marine animals. *Int J Syst Evol Microbiol* 2007;**57**:2337–43. https://doi.org/10.1099/ijs.0.65243-0.

Yoshida M-A, Tanabe T, Akiyoshi H *et al.* Gut microbiota analysis of Blenniidae fishes including an algae-eating fish and clear boundary formation among isolated *Vibrio* strains. *Sci Rep* 2022;**12**:4642. https://doi.org/10.1038/s41598-022-08511-7.

Zhao Y, Li S, Lessing DJ *et al.* Characterization of *Cetobacterium somerae* CPU-CS01 isolated from the intestine of healthy crucian carp (*Carassius auratus*) as potential probiotics against *Aeromonas hydrophila* infection. *Microb Pathog* 2023;**180**:106148. https://doi.org/10.1016/j.micpath.2023.106148.

Zhou W, Xie M, Xie Y *et al.* Effect of dietary supplementation of *Cetobacterium somerae* XMX-1 fermentation product on gut and liver health and resistance against bacterial infection of the genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*). *Fish Shellfish Immunol* 2022;**124**:332–42. https://doi.org/10.1016/j.fsi.2022.04.019.

Received: 9 June 2026. **Revised:** 24 August 2026. **Accepted:** 24 September 2026

© The Author(s) 2026. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site-for further information please contact journals.permissions@oup.com

Downloaded from academic.oup.com/femsle/article/doi/10.1093/femsle/fnag123/8875694 by guest on 22 October 2026