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Host diet drives gut microbiome convergence between coral reef fishes and mammals

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Abstract

Animal gut microbiomes are critical to host physiology and fitness. The gut microbiomes of fishes—the most abundant and diverse vertebrate clade—have received little attention relative to other clades. Coral reef fishes, in particular, make up a wide range of evolutionary histories and feeding ecologies that are likely associated with gut microbiome diversity. The repeated evolution of herbivory in fishes and mammals also allows us to examine microbiome similarity in relationship to diet across the entire vertebrate tree of life. Here, we generate a large coral reef fish gut microbiome dataset ($n = 499$ samples, 19 species) and combine it with a diverse aggregation of public microbiome data ($n = 447$) to show that host diet drives significant convergence between coral reef fish and mammalian gut microbiomes. We demonstrate that this similarity is largely driven by carnivory and herbivory and that herbivorous and carnivorous hosts exhibit distinct microbial compositions across fish and mammals. We also show that fish and mammal gut microbiomes share prominent microbial taxa, including *Ruminoccocus* spp. and *Akkermansia* spp., and predicted metabolic pathways. Despite the major evolutionary and ecological differences between fishes and mammals, our results reveal that their gut microbiomes undergo similar dietary selective pressures. Thus, diet, in addition to phylosymbiosis must be considered even when comparing the gut microbiomes of distantly related hosts.

KEYWORDS
comparative, coral reefs, fishes, gut microbiome, mammals

1 | INTRODUCTION

Microbes perform vital functions for their animal hosts, from nutrient uptake to protection against pathogens (Heiman & Greenway, 2016; Neish, 2009). Of growing interest is the gut microbiome, a commensal and possibly symbiotic community of microbes residing in the gut of most animals. Gut microbes of humans and other mammals, for example, digest complex sugars

(Mackie, 2002; Mountfort et al., 2002) and produce short-chain fatty acids that are essential to host metabolism (den Besten et al., 2013; Sanna et al., 2019). Gut microbes are also implicated in animal immune development (Takiishi et al., 2017), immune function (Round & Mazmanian, 2009), and animal behaviour (Johnson, 2020).

Despite the importance of gut microbiomes across the animal kingdom, how gut microbiomes are shaped is not fully understood. While host phylogeny (host evolutionary history) drives gut microbiome diversity in some hosts (Amato et al., 2018; Bik et al., 2016; Hird et al., 2015), host diet sometimes outweighs host phylogeny (Hale et al., 2018; Li et al., 2022; Miyake et al., 2015), even within distantly related hosts. In mammals, for example, host diet drives gut microbiome convergence across insectivores (Delsuc et al., 2014) and herbivores (Groussin et al., 2017; Muegge et al., 2011). However, whether such dietary-driven convergences extend beyond mammals remains unknown.

Two primary factors hamper our understanding of the drivers shaping vertebrate gut microbiome. First, most comparative gut microbiome studies focus on mammals (Colston & Jackson, 2016). Mammals represent a small fraction of the vertebrate tree of life; sampling a broader range of distantly related taxa is required to understand the overarching processes shaping vertebrate gut microbiomes. Second, few studies examine gut microbiomes in a comparative framework across a broad range of distantly related taxa, with varying ecological traits, while accounting for environment. Many comparative studies either focus on a limited scope of hosts (Miyake et al., 2015; Pollock et al., 2018) or span varied environments (i.e. captive vs. wild), introducing a range of environmental parameters with potentially idiosyncratic effects on microbiomes (Alberdi et al., 2021; Clayton et al., 2016; Gibson et al., 2019).

As the most diverse vertebrate clade representing a diversity of habitats and feeding ecologies, fishes provide an exciting perspective on the ecology, evolution and functionality of gut microbiomes. Yet, their gut microbiomes have received comparably little attention (Gallo et al., 2020), with most work focusing on aquaculture applications or host physiological processes (Ghanbari et al., 2015; Sullam et al., 2012). In particular, coral reef fishes are a paraphyletic group that exhibit a wide range of trophic groups and evolutionary histories, allowing for the comparative analysis of gut microbiomes across diverse wild hosts while controlling for confounding environmental factors. The repeated evolution of herbivory in fishes and mammals allows us to examine microbiome convergence in relationship to diet across the entire vertebrate tree of life. Moreover, multiple microbes have been identified as co-occurring in both fish and mammal guts (Escalas et al., 2021; Scott et al., 2020), indicating that convergences between the two clades is possible. However, large-scale analyses on fish and mammal gut microbiomes that examine both groups simultaneously are lacking. Here, we examine a large dataset of coral reef fish gut microbiomes ($N = 499$, Figure 1) to assess how host diet and phylogeny shape gut microbiomes of coral reef fishes relative to mammals. By comparing our results to existing data from other vertebrate hosts, we reveal strong conservatism and a striking convergence of gut microbiomes that spans the vertebrate tree of life, from fishes to mammals.

2 | METHODS

2.1 | Study design

To investigate the extent to which host ecology and evolution influence gut microbiomes, we sampled the gut microbiomes of 19 species of tropical coral reef fishes, encompassing a diverse range of lineages and feeding ecologies (Figure 1, Table S1). To ensure we captured phylogenetic diversity within dietary groups, we sampled fish from eight different families where at least two or more families were represented within a single feeding ecology. To account for environmental variation, we sampled fishes from back and fore reefs across three geographically distinct South Pacific islands: Moorea, Tetiaroa and Mangareva. Moorea and Tetiaroa both lie in the Society Archipelago while Mangareva lies 1600 km southeast in the Gambier Archipelago. Furthermore, we sampled a maximum of two fish per sampling site to ensure we captured habitat diversity around the island. When possible, we sampled 10 replicates per species of fish per island, totalling 499 samples across the three islands. All fishes were sampled via spearfishing and only adults were targeted. Fish were immediately stored on ice and transported to the lab for dissection. To compare between fish and mammal gut microbiomes, we downloaded a public comparative vertebrate gut microbiome dataset (Youngblut et al., 2019), which includes 160 mammal gut microbiome samples spanning 82 host species (Figure 1), and three broad diet categories: carnivores, herbivores and omnivores. We chose this study as the authors used similar methods to ours in preparing their samples for sequencing, which can have significant biases on results. The authors used PowerSoil extraction kits, targeted the same 16S V4 region, and used dada2 for sequence trimming. All mammalian gut microbiomes were sampled by experienced wildlife biologists with training in sterile techniques. We also included previously published data from Moorea, French Polynesia, which included 30 seawater and 40 algal microbiome samples (Degregori et al., 2021). Similar to the fish and mammal data, the seawater and algal data were processed through dada2 separately and merged for analysis. All sampling collection protocols were reviewed and approved by the Uni. California Los Angeles (UCLA) Animal Research Committee (ARC-2017-045).

2.2 | Microbiome sample processing and sequencing

We removed the intestines of each fish using sterile techniques (Givens et al., 2015). Fish were cut ventrally from the anus to the

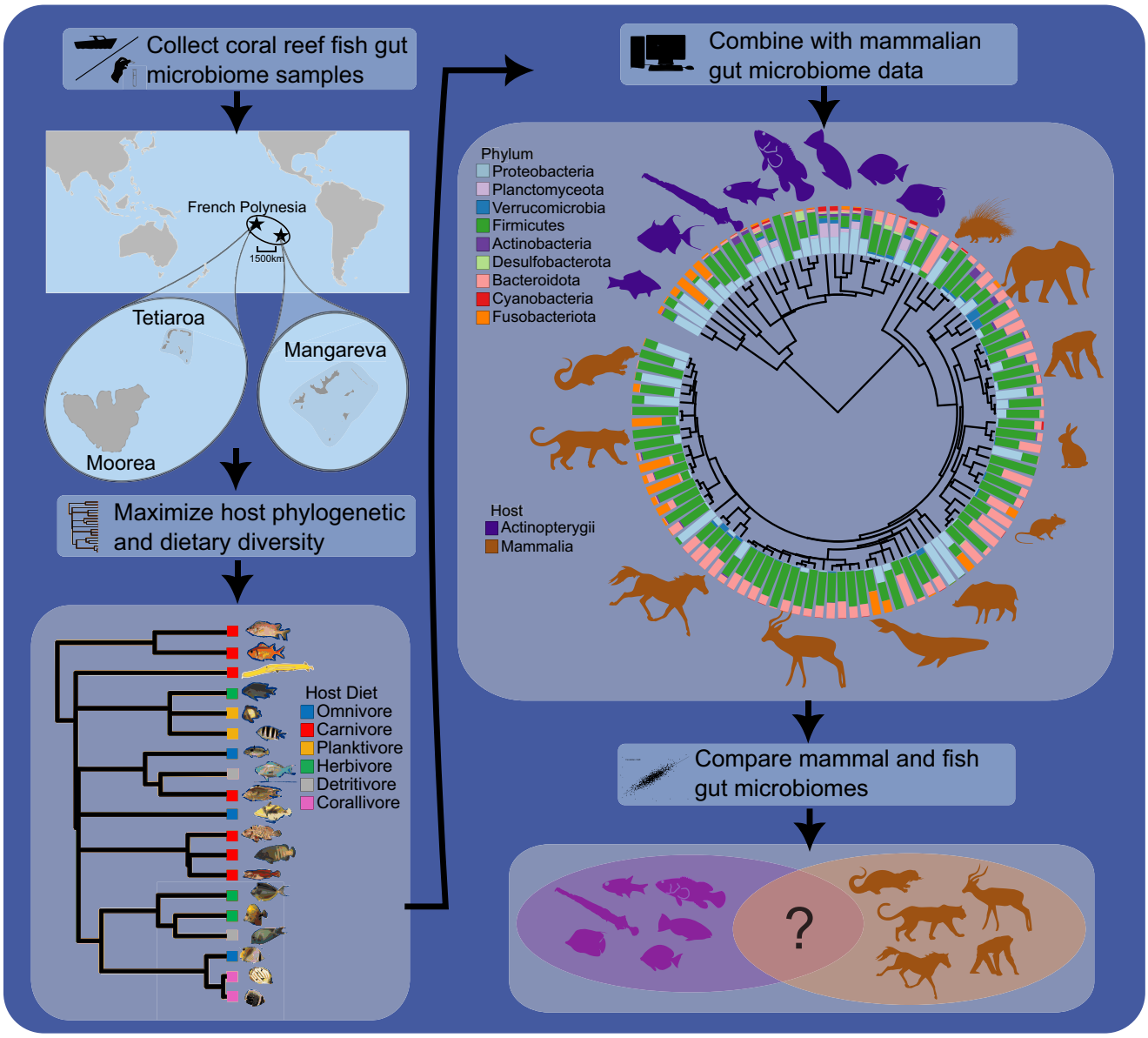

FIGURE 1 Workflow of study design and aims.

gills with a scalpel that was sterilized with bleach and rinsed with sterile water. Fish intestines were removed by snipping the anus and esophagus with sterile scissors. Digesta from the hindgut was then squeezed into sterile 2 mL tubes using sterile forceps and stored in a −80°C freezer.

To isolate bacterial DNA, we used Qiagen PowerSoil Extraction kits following the manufacturer's instructions to extract DNA from fish digesta samples. We also extracted three negative controls to test whether our extraction process suffered from contamination, and one positive control to ensure we were targeting microbial DNA. We then amplified the V4 16S rRNA gene region using 515F and 806R primers following the Earth Microbiome Project protocol (Caporaso et al., 2011). We conducted PCR in 25 μL reactions (triplicate) using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) with the following thermocycler conditions: 1 cycle of 94°C for 3 min; 35 cycles of 94°C for 45 s, 50°C for 60 s and 72°C for 90 s; and 1 cycle of 72°C for 10 min. Each PCR batch included a negative control. We confirmed successful PCR through electrophoresis on an agarose gel then pooled triplicate reactions, including negatives, prior to cleaning using Agencourt AMPure magnetic beads (Beckman Coulter, Indianapolis).

To prepare the sequencing library, we dual-indexed the pooled PCR products using the Nextera XT Index Kit (Illumina, San Diego) with the following thermocycler conditions: 1 cycle of 95°C for 3 min; 10 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s; and 1 cycle of 72°C for 5 min. We then conducted a second round of bead cleaning. Next, we quantified all pooled PCR products using a Qubit dsDNA BR kit (Thermo Fisher Scientific, Waltham). Finally, we pooled indexed samples in equimolar ratios for sequencing on an Illumina Miseq v3 (2 × 300 paired-end; 20% PhiX) at the Technology Center for Genomics and Bioinformatics core at the University of California at Los Angeles.

2.3 | Bioinformatic processing of sequence data

The sequencing of 499 fish gut microbiomes (representing 19 host species and 6 distinct feeding ecologies) yielded 32,976,488 total reads. Sequence depth ranged from 11,491 to 405,266 reads per sample, with a mean of 74,953 and median of 74,985 reads per sample. PCR and extraction blanks had a maximum of 18 reads with a majority only having one or two reads. After denoising, filtering and merging with publicly available microbiome datasets, 25,236,927 total reads and 129,273 amplicon sequence variants (ASVs) remained across a combined 946 samples. Of these samples, mammal and fish gut microbiomes comprised 4,559,955 reads and 59,841 ASVs across 716 samples after filtering. Sequencing coverage across all samples are visualized in Figure S1.

We processed the sequences, both from our fish gut microbiome samples and the supplemental samples from publicly available datasets, through QIIME2 (v. 2019.7) using the microbiome data science platform (Bolyen et al., 2019) for quality control, ASV taxonomy assignment and community diversity analyses. We demultiplexed and denoised the fish and mammal sequencing data, separately, using dada2 (Callahan et al., 2016) and merged the resulting output into a feature table for subsequent analysis. We assigned taxonomy to ASVs, using a naïve Bayes taxonomy classifier trained on the SILVA database (Quast et al., 2013), conducting reference sequence clustering at 99% similarity. To avoid unwanted reads, we removed ASVs with less than two reads as well as ASVs occurring in less than 3% of the samples (Karstens et al., 2019). To ensure that microbiomes only included microbial sequences, we removed any ASVs assigned to eukaryotes or chloroplasts. Because certain cyanobacteria taxa can persist in the gut (Jančula et al., 2008) and potentially even colonize the gut (Hu & Rzymski, 2022), we included cyanobacteria in downstream analyses. To control for variation in sequencing depth across treatments, we rarefied sequence reads to 1000 reads, which allowed us to retain 80% of samples while also retaining sample diversity. Certain fish gut microbiome samples, particularly carnivores and planktivores, began with low biomass extractions, resulting in low read counts under 1000. Thus, while the mammalian data from Youngblut et al. (2019) was rarefied at 5000 reads we decided to opt for a lower rarefaction limit to preserve as many samples as possible. However, to account for rarefaction biases in microbiome diversity analyses, we performed alpha diversity analyses with and without rarefying (Table S6). We found no statistical differences between analyses before and after rarefaction, so we only report analyses performed after rarefaction.

2.4 | Host data

We used TimeTree (timetree.org) to construct a phylogeny of all sampled hosts and the Interactive Tree of Life online tool (https://itol.embl.de/) to annotate the host phylogeny. Diet categories (carnivore, herbivore, omnivore, planktivore, detritivore and corallivore) were assigned to hosts following previous published work on mammals (Youngblut et al., 2019) and fishes (Casey et al., 2019). We relied on Casey et al. (2019), in particular, as the authors assigned diet to the same taxa of fish we sampled using diet metabarcoding techniques. Because the mammalian dataset contained less samples but more host species, we generated randomly subsampled datasets with more comparable phylogenetic diversities and sample sizes for certain analyses discussed further below. See Table S1 for sample sizes across diet categories for both fish and mammals and Table S2 for further sample size info across fish host species.

2.5 | Statistical analyses

2.5.1 | Beta-diversity metric

To quantify and visualize beta diversity across samples, we constructed an unweighted UniFrac distance matrix (Lozupone & Knight, 2005) and visualized the matrix through a principal coordinate analysis (PCoA). We focused on the UniFrac metric of beta-diversity since this metric captures microbial diversity at multiple taxonomic scales (Lozupone & Knight, 2005), and host diet acts on various microbial taxonomic scales (Groussin et al., 2017). We report Jaccard and Bray–Curtis metric results as well, but focused on the UniFrac metric for analyses and visualizations as this metric is often used in broad comparative analyses of vertebrate gut microbiomes (Callahan et al., 2016; Youngblut et al., 2019).

2.5.2 | Measuring compositional similarity across dietary guilds

To explore potential fish and mammal gut microbiome similarity, we used Bayesian multi-level modelling (Bürkner et al., 2018), using the *brms* (v2.21.0) package (Bürkner, 2017) in R, to test whether the similarities between fish and mammal gut microbiomes were driven by diet. We used the unweighted 1-UniFrac distance values between 0 and 1 to represent gut microbiome similarity, with 1 being the most similar and 0 being the most dissimilar. We then averaged similarity per host species pair, yielding 176,715 data points. Each species was assigned to a diet category (fish herbivore, fish omnivore, fish carnivore, mammal herbivore, mammal omnivore, and mammal carnivore), so each species pair had one assigned diet category pair out of 28 diet category pairs. We fitted a Bayesian linear mixed model with a student-*t* error distribution to predict similarity as follows:

$$mu = (a + a_j),$$

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where *mu* is the average predicted value, *sd* is the standard deviation, *a* is the global intercept of the regression and a_j is the effect of a diet combination of two species on microbiome similarity. We then visualize the results in a barplot where *mu* values are plotted from 0

to 1 where 1.0 signifies the highest possible predicted similarity between two diet groups and 0 signifies the lowest predicted similarity. We opted for student's *t*-distribution to build a robust regression, as our data includes outliers (Motulsky & Brown, 2006). We used uninformative priors and ran the model with four chains, 2000 iterations per chain and a warmup of 1000 iterations. To ensure a good model fit, we inspected posterior predictive plots, Rhat and the Bayesian R^2. We solely report the similarities between herbivores and carnivores because fish and mammal omnivores did not show any notable similarities in our beta-diversity analyses.

In addition to the Bayesian analysis and to quantify and analyse the distance between clusters, we employed a Permutational Multivariate Analysis of Variance (PERMANOVA) analysis (Anderson, 2017) for each diet comparison between fish and mammal gut microbiomes (*n* randomizations = 999). Because we did not have equal sample sizes across fish and mammal gut microbiome samples, we also ran an iterative PERMANOVA analysis on randomly subsampled datasets to account for pseudo-replication. For each subset, one fish and one mammal gut microbiome sample were randomly chosen from each host species from each diet group. 999 random subsets were generated, totalling 6678 pairwise comparisons across fish and mammal carnivore and herbivore gut microbiomes. Statistics are reported in Table S3 and *F* values visualized in Figure S3. The ASV tables and distance matrices were produced with the packages *phyloseq* (v1.30.0) and *vegan* (v2.5-7) using the statistical software R (v3.6.1).

2.5.3 | Distribution of most abundant bacterial taxa

To generate a heatmap of the most abundant microbial genera across host diet and sample type, we collapsed our ASV table to the genus level. We chose microbial genus over species to show the degree at which samples shared microbial taxa without moving too far up in taxonomic rank and losing unclassified species. We then merged samples by diet (across fish and mammals) or sample type (algae and seawater) and rarefied the merged samples to 10,000 reads. To target the most abundant microbial taxa, we then filtered all reads under counts of 750. We generated a log-scaled heatmap using qiime2's heatmap plugin. The cluster analysis utilized Euclidian distances and took the average distance between clusters to generate dendrograms for samples and microbial genera.

2.5.4 | Comparing the relative impact of host phylogeny and diet on gut microbiota beta-diversity

To determine the relative impact of host factors in shaping fish and mammal gut microbiomes, we conducted a PERMANOVA (adonis) test on host phylogeny, host diet and host habitat. To match the relatively broad categories used for host diet (6 categories for fish and 3 for mammals), we opted to use host Order as a proxy for host phylogeny (11 orders in fish and 20 in mammals). We also conducted adonis tests on subsetted mammalian datasets with 11 randomly selected orders to match the fish phylogenetic variation to ensure such variability did not bias the resulting R^2 values. Because adonis only accepts categorical data, we also ran a multiple regression on matrices (MRM) analysis (Breiman, 2001) using host relatedness values between host species to further compare the impact of host phylogeny between fish and mammal gut microbiomes. Host relatedness matrices for mammals and fishes were constructed by transforming the phylogenetic trees into distance matrices with the package *ecodist* (v2.0.7). We had geographical locations for our fish samples but not for the publicly sourced mammal dataset, so we include host habitat (island) as a factor only for the fish gut microbiomes in the adonis analysis.

2.5.5 | Differentially abundant microbes across hosts

To analyse differentially abundant microbial taxa between host diet groups and between mammals and fishes, we conducted a combination of Venn Diagram analyses with the *limma* package (v3.14) and ALDEx2 analyses (Fernandes et al., 2014) to identify the most shared and differentially abundant ASVs within each group. Shared ASVs were considered as either ASVs that fell into the same species identification or unidentified species that were 97% similar. The number of fish and mammal samples were normalized to 112 samples each to ensure sample size did not bias these analyses. We then employed the ALDEx2 analysis to ensure all taxa identified by the Venn Diagram analysis were significantly differentially abundant. This ensured that we identified biologically meaningful microbial taxa while avoiding rare microbes that may erroneously show up in differential abundance analyses (Lin & Peddada, 2020). For visualization, we report the raw abundances of each ASV after rarefaction and repeated these analyses to ensure the results did not change significantly. Because these read numbers do not represent true relative abundance, we also reported the relative abundance of these reads at the phyla level to supplement our analyses using read counts. When reporting differential abundance results, we refer to 'top shared' taxa between fish and mammals as microbial taxa that have the highest relative abundances in both clades. In contrast, the 'top differential' taxa do not necessarily have the most shared reads but show the greatest discrepancy in relative abundance between two groups of hosts.

2.5.6 | Predicting microbial functions

To predict potential microbial functions across host factors, we utilized the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States PICRUSt2 (Douglas et al., 2019) and employed a Random Forest model (Breiman, 2001) to determine likely functional pathways. We generated functional pathways by correcting ASVs by their predicted 16S rRNA gene copy

number (Douglas et al., 2019; Louca & Doebeli, 2018) then inferring function based on the Kyoto Encyclopedia of Genes and Genomes orthologs and Enzyme Commission numbers.

3 | RESULTS

3.1 | Fish and mammals with similar diets share similar gut microbiomes

Despite markedly different evolutionary histories and residing in drastically different environments, reef fish and mammal gut microbiomes were similar, especially within herbivorous and carnivorous hosts (Figure 2). Bayesian linear modelling suggests that fish and mammal carnivores were the most similar in composition (0.167; 95% CI: 0.154, 0.157; Figure 3; Table S4), followed by fish and mammal herbivores (0.132; 95% CI: 0.131, 0.134). The most dissimilar gut microbiomes were fish carnivores and mammal herbivores, followed by fish herbivore and mammal carnivores (Figure 3; Table S4). A PERMANOVA analysis comparing fish and mammal carnivore and herbivore gut microbiomes (treated as four separate groups; see ellipses in Figure 2) confirms the Bayesian analysis. Fish and mammal carnivore gut microbiomes were most similar of all possible comparisons (i.e. the corresponding model has relatively low F-values: $N = 158$, $F_{PERMANOVA} = 15.293$, $p < 0.001$ Figures 2 and 3; Table S3), while fish and mammal herbivores were the third most similar ($N = 182$, $F_{PERMANOVA} = 33.556$, $p < 0.001$, Table S3).

3.2 | Host diet and host phylogeny shape fish and mammal gut microbiomes in differing magnitudes

To compare the relative impacts of host phylogeny and host diet on vertebrate gut microbiomes, we employed a PERMANOVA analysis, highlighting that host diet explained a significant amount of variation in fish (14.3%, $F_{PERMANOVA} = 12.105$, $p < 0.001$; Figure 4; Table S5) and less so in mammal gut microbiomes (2.9%, $F_{PERMANOVA} = 2.548$, $p < 0.001$). Compared to diet, host phylogeny (measured at the Order level) explained a similar portion of variation in fish (14.8%, $F_{PERMANOVA} = 8.774$, $p < 0.0001$), while explaining a majority of the variation in mammal gut microbiomes (29.4%, $F_{PERMANOVA} = 3.453$, $p < 0.0001$). In turn, host habitat explained a minimal, yet significant, amount of variation in fish gut microbiomes (1.0%, $F_{PERMANOVA} = 2.726$, $p < 0.0001$; Table S5), with more variation explained by the interactions between host habitat and diet (3.0%, $F_{PERMANOVA} = 1.662$, $p < 0.0001$) as well as host habitat and phylogeny (5.2%, $F_{PERMANOVA} = 1.438$, $p < 0.0001$). Overall, when combining fish and mammals together, host diet explained 7.7% ($F_{PERMANOVA} = 10.864$, $p < 0.0001$) of the variation in fish and mammal gut microbiomes while host phylogeny explained 27.7% ($F_{PERMANOVA} = 6.980$, $p < 0.0001$).

To further investigate the effects of host phylogeny, we conducted a MRM analysis, using host relatedness values as an input, and showed that host phylogeny explained a smaller but significant amount of variation in mammals (5.1%, $P_{MRM} < 0.0001$) but not at all in fishes (0.0%, $P_{MRM} = 0.785$).

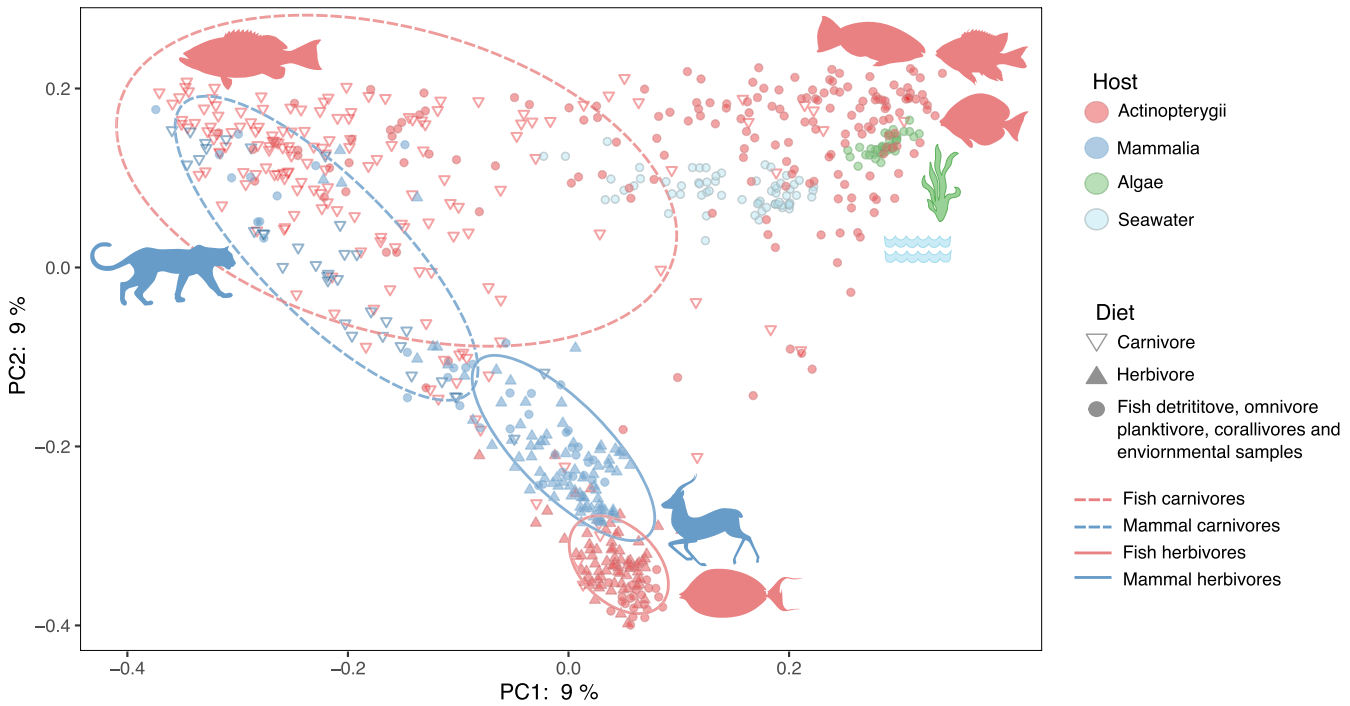

FIGURE 2 Unweighted UniFrac principal coordinate analysis (PCoA) plot of coral reef fish gut microbiomes ($N = 499$) and other gut microbiome data ($N = 447$). Colours denote microbiome hosts or source. Host silhouettes depict representative host taxa for each diet category. Ellipses are drawn with 95% confidence. Coral, seawater and algal microbiome data are also included.

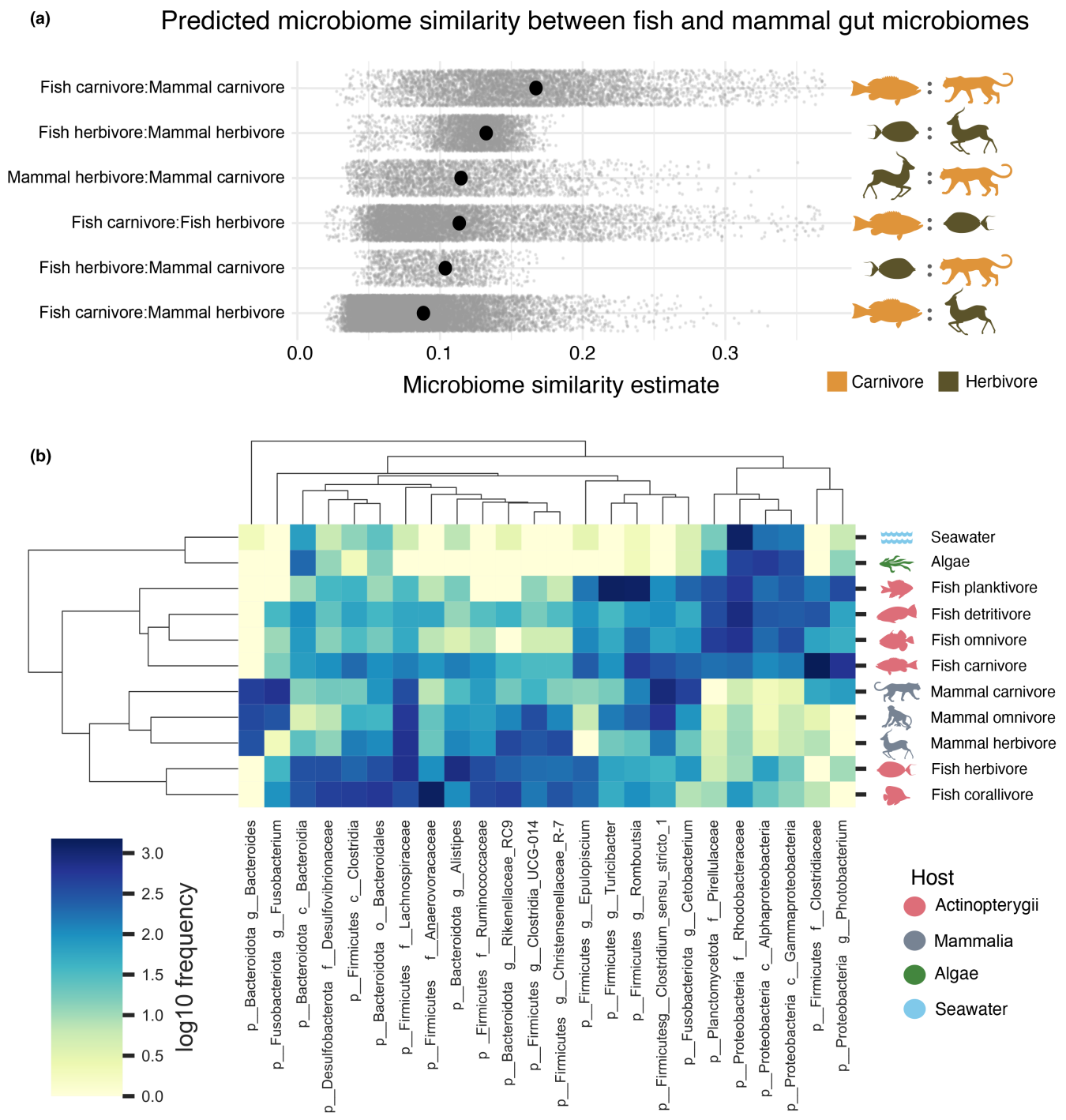

FIGURE 3 Microbiome similarity between fish and mammal gut microbiomes based on a Bayesian multi-level model and a heatmap visualization of abundant microbes across all samples. (a) The grey points represent observed similarity between two respective gut microbiome samples, and the black points represent the predicted mean of each comparison within two diet groups of interest. Credible intervals are too small to appear on the graph but are reported in Table S4. Similarity estimates are calculated by taking the sum of the global regression intercept and the change in that intercept for each diet combination (i.e. fish herbivore vs. mammal herbivore)—where 1 represent the most similar and 0 represents the least similar. Sample distances were calculated using unweighted UniFrac distance values. (b) Heatmap of the top 24 most abundant microbes across all samples. Samples were merged by host diet or sample type, rarefied to counts of 10 k and collapsed to the genus level. Colours correspond to log-transformed read counts. Samples and microbes also underwent a cluster analysis based on Euclidian distances and denoted by the dendrogram connections.

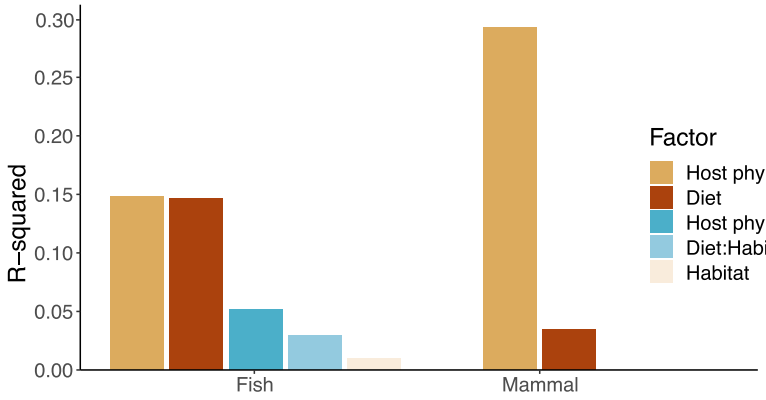

FIGURE 4 Adonis analysis demonstrating the relative impact of host phylogeny and host diet on fish and mammal gut microbiomes. The effect of habitat, and its interaction with host phylogeny and diet, on fish gut microbiomes is also reported. Vertical bars denote R^2 values from the adonis analysis.

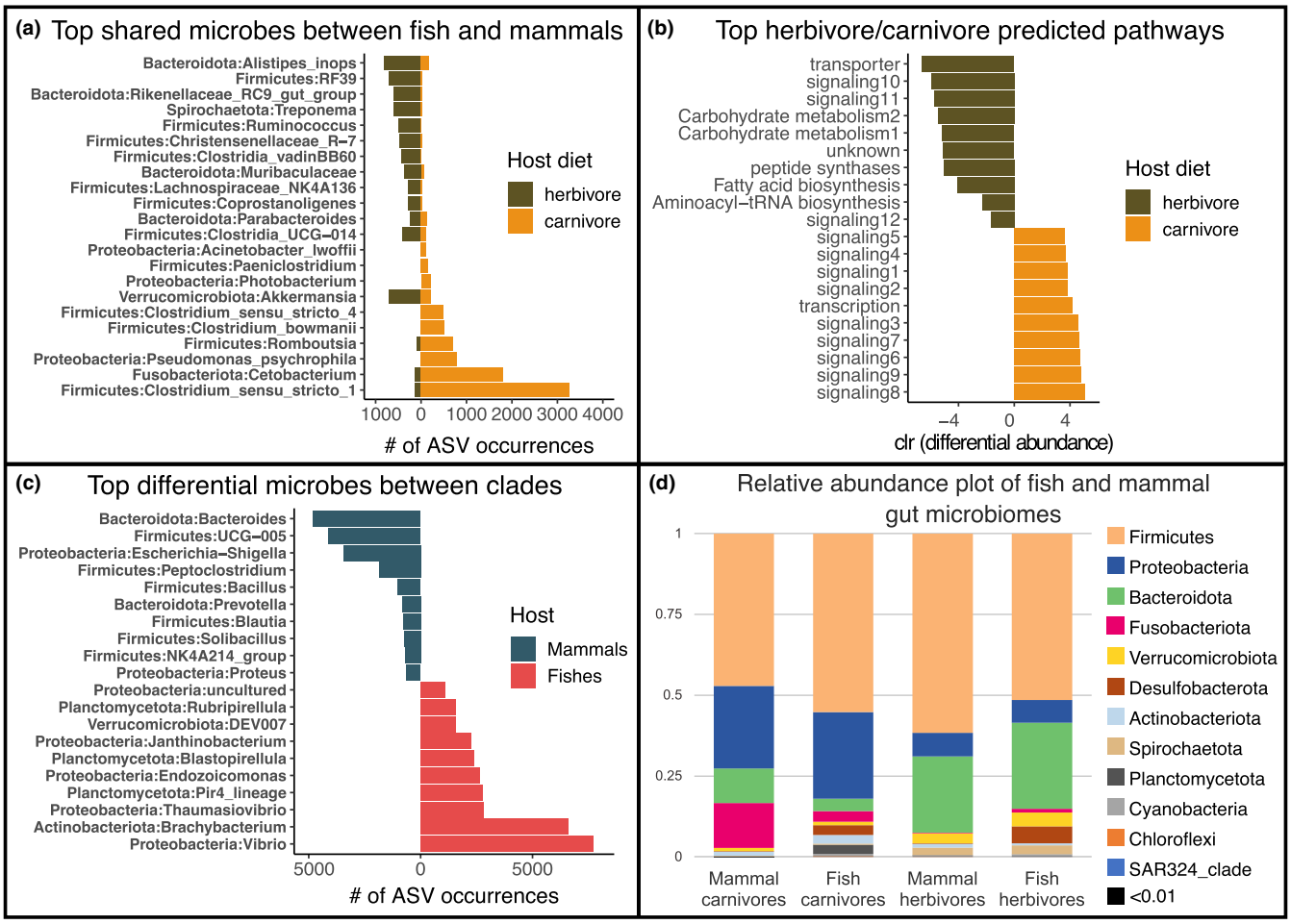

FIGURE 5 Shared amplicon sequence variants (ASVs) and predicted pathways between fish and mammal gut microbiomes. (a) The top (ASVs) between fish and mammal gut microbiomes coloured by host diet. ASVs are written as Phyla:Species or Phyla:Genus depending on resolution. (b) Top differentially abundant pathways identified by PICRUST2 fish and mammal gut microbiomes. ALDEX2 clr values are shown with positive values denoting pathways more abundant in carnivore hosts (orange) and negative values denoting pathways more abundant in herbivore hosts (purple). (c) Top differentially abundant ASVs between fish and mammal gut microbiomes. (d) Relative abundance plot of microbial phyla composition for each diet group.

3.3 | Shared composition between fish and mammal gut microbiomes

Fishes and mammals with similar feeding ecologies shared a significant number of gut microbial taxa. After rarefying and subsetting reads only belonging to herbivorous and carnivorous fishes and mammals, 72,485 sequences belonging to 66 out of 1448 (4.6%) bacterial genera were shared between fish and mammal gut microbiomes. For reference, 2.4% of genera were shared between fish herbivores and carnivores. Carnivory and herbivory largely explained

the shared genera between fish and mammals with ~87.1% of shared reads being shared within these two diet groups (Figure 5a) and with 74.1% of those reads belonging to carnivores and 25.9% to herbivores. The most abundant of these genera was an uncultivated *Firmicutes* clade, *Clostridium_sensu_stricto_1*, totalling 3271 reads of which 95.5% belonged to both fish and mammal carnivores, followed by a *Fusobacteria* genera, *Cetobacterium*, totalling 1806 reads with 93% belonging to only carnivores. The most abundant taxa shared between fish and mammal herbivores were *Alistipes inops*, of the phylum *Bacteroidota*, and the uncultivated genera *RF39*, belonging to *Firmicutes*, comprising 77.9% and 96.2% reads respectively. Two notable genera, *Akkermansia* and *Ruminococcus* were found in high abundance in both fish and mammal herbivore gut microbiomes. The majority of the shared predicted functions within fish and mammal carnivore gut microbiomes belonged to cell signalling, while the shared herbivore predicted functions belonged to a diverse array of functions, including carbohydrate metabolism and protein biosynthesis (Figure 5b).

Our heatmap analysis, focusing on the top 24 most abundant microbial taxa across all samples, also identified similar gut microbial taxa shared between fish and mammal herbivores and carnivores (Figure 3b). Fish and mammal carnivores shared *Clostridium_sensu_stricto_1*, *Cetobacterium*, *Clostridiaceae* and *Photobacterium* at levels greater than 1% relative abundance. Fish and mammal herbivores gut microbiomes shared an unidentified microbial genus of the *Lachnospiraceae* family at 6% relative abundance. Fish corallivores, fish herbivores, and mammal herbivores all shared a *Ruminococcaceae* genus and *Rikenellaceae_RC9* at 1% relative abundance or greater. Cluster analysis also showed fish herbivores and corallivores clustering with mammals rather than their fish counterparts. Fish carnivores, detritivores, planktivores, and omnivores clustered together and shared multiple genera with algae and seawater microbiomes notably an unidentified *Pirellulaceae* (*Planctomycetes* phylum), and an unidentified *Gamaproteobacteria*, *Alphaproteobacteria* and *Rhodobacteraceae* from the *Proteobacteria* phylum, all at levels above 1% relative abundance.

4 | DISCUSSION

Strong differences in the diversity and composition of coral reef fish gut microbiomes were highly associated with differences in feeding ecologies (e.g. carnivore vs. herbivore), a pattern previously only reported in mammals (Delsuc et al., 2014; Muegge et al., 2011). This pattern transcended vertebrate classes; gut microbiomes of mammals and fishes with shared feeding ecologies were more similar to each other than to other mammals and fishes respectively. Thus, despite the profound differences in marine and terrestrial environments and 365 million years of evolution separating fishes and mammals, their gut microbiomes appear to be shaped by similar selective pressures, particularly host diet, providing important insights into the processes shaping vertebrate gut microbiomes.

Carnivory and herbivory are the two major feeding ecologies shared between fishes and mammals (Román-Palacios et al., 2019). Gut microbiome compositions were strikingly similar within these feeding ecologies despite the drastic differences between the environments inhabited by fish and mammals and the hundreds of millions of years of evolution separating these vertebrate classes (Jones & Safi, 2011). Our analyses consistently indicate that herbivory and carnivory drive the similarities we observed in fish and mammal gut microbiomes. In contrast, fish omnivores, detritivores and planktivores formed their own clusters with environmental microbiome samples (Figure 3), further suggesting that feeding ecology and not host habitat or phylogeny, drives gut microbiome variation in herbivores and carnivores across vertebrates.

While our results are novel in regards to convergences between reef fish and mammal gut microbiomes, other convergences between distantly related vertebrates have been reported. For example, flight adaptation appears to drive bird and bat gut microbiome convergence (Song et al., 2020). In mammals, myrmecophagy (Delsuc et al., 2014) and herbivory (Muegge et al., 2011) drives gut microbiome convergence even between relatively distant hosts. Specific microbes can also provide insight into possible convergences, such as, *Ruminococcus*, a genus shared between fish and mammal herbivore gut microbiomes in this study, that also dominates the gut microbiomes of most mammalian herbivores in previous studies (Malmuthuge & Guan, 2016; Meng et al., 2018). *Ruminococcus* also occurs in the gut microbiome of the herbivorous marine iguana (Lankau et al., 2012) and other fish herbivores (Escalas et al., 2021; Scott et al., 2020), further supporting the link between diet and the gut microbiome across vertebrate classes. Thus, the convergence between fish and mammal gut microbiomes we observe in our study, while novel, is supported by other ecologically driven convergences in other vertebrate hosts.

Taxonomic congruence between fish and mammal gut microbiomes extended to the species level as well, with high abundances of *Pseudonomas psychrophila* and *Clostridium bowmanii* found in the gut microbiomes of both fishes and mammals, indicating that individual microbial species occur in the guts of both marine and terrestrial hosts. Moreover, when comparing herbivores to carnivores, the microbial taxa most commonly shared across fish and mammal hosts were also the most differentially abundant when grouped by diet. This convergence occurs across taxonomic levels, with the strongest differences in beta diversity occurring at higher microbial taxonomic levels, supporting previous findings indicating that host diet acts on higher taxonomic scales in mammalian gut microbiomes (Rojas et al., 2021; Youngblut et al., 2019). Given the vast evolutionary distance separating fish and mammals, these results strongly suggest that host diet may universally govern the composition of vertebrate gut microbiomes, across multiple taxonomic scales.

Functional inference suggests that convergence of microbiomes by feeding ecologies across vertebrate classes is likely a result of metabolic function, particularly within herbivores. Carbohydrate degradation pathways were common across herbivore gut microbiomes, and further supported by microbial taxa we

identified across herbivore hosts. For example, *Ruminococcus*, a key fermentative microbe associated with plant digestion in mammalian herbivores (Degregori et al., 2021; Karstens et al., 2019; Quast et al., 2013), made up a significant portion of the shared herbivore microbes between fishes and mammals. *Treponema*, also abundant across herbivores, has been linked to fibre digestion in humans (Angelakis et al., 2019; Schnorr et al., 2014) and termites (Tokuda et al., 2018). On a broader taxonomic scale, six of the 10 taxa shared between herbivores belonged to the class *Clostridia*, which is linked to carbohydrate degradation (Hong et al., 2011) and short-chain fatty acid production (Levy et al., 2016). Moreover, we found that fish herbivore gut microbiomes were significantly different from the algal microbiomes—their own food source—in this study (Figure S4), further highlighting the adaptive specialization of herbivorous fish gut microbes. Herbivores rely on microbes to digest plant material (Hummel et al., 2006; Owens & Basalan, 2016), and possess elongated intestines to house such microbes (Herrel et al., 2008; Karasov & Douglas, 2013). Thus, fish and mammal gut microbiomes likely undergo similar selective pressures resulting in microbiome convergence.

While the composition and metabolic pathways of carnivore gut microbiomes are less known, our study revealed a convergence between fish and mammal carnivores that surpassed the convergence between their herbivore counterparts. In fish and mammal carnivores, *Clostridium* spp., including *C.* sensu stricto *4*, *C.* sensu stricto *1* and *C. bowmanii*, dominated the shared ASVs. While *Clostridium* is one of the most abundant taxa in human gut microbiomes—likely providing vital short-chain fatty acids from indigestible fibre (Guo et al., 2020)—*Clostridium* also metabolizes amino acids (Fonknechten et al., 2010; Neumann-Schaal et al., 2015). As such, *Clostridium* may play a central role in amino acid degradation in carnivores. However, fermentation performed by *Clostridium* spp. cannot be ruled out in the carnivore gut microbiomes we sampled. Fermentation signals have been recorded in feline guts, potentially due to the processing of cartilage, hair and bone (Depauw et al., 2012). For carnivore fishes, the challenge of obtaining nutrition from otherwise undigestible material (e.g. bones, scales or exoskeletons) is enhanced by the necessity to swallow prey whole, suggesting that gut microbiomes capable of assisting with fermentation may be crucial for fish carnivores.

While carnivore gut microbiomes have often been touted as more stochastic than herbivore gut microbiomes due to their fast digestion times, low diversity and high variability (Bolyen et al., 2019; Callahan et al., 2016; Douglas et al., 2019), our results suggest that carnivore gut microbiomes are more deeply ingrained than previously thought. However, some fish carnivore gut microbiomes also clustered with fish detritivores, planktivores and omnivores as well as coral reef algae and seawater microbiomes. In fact, these groups were more similar to each other than fish herbivore gut microbiomes were to algal microbiomes (Figure S4). Unlike fish herbivores and corallivores, these samples had higher abundances of *Proteobacteria* and *Planctomycetes*, which are prevalent marine microbiota (Degregori et al., 2021; Rocca et al., 2020). Thus, the gut microbiomes of fish carnivores may partially mimic the external environment as well. Moreover, some of the fish carnivores we sampled were nocturnal (Collins et al., 2022; Schmitz & Wainwright, 2011), which may have played a role in shaping their gut microbiomes given the reported relationship between host circadian rhythm and gut microbes (Parkar et al., 2019; Voigt et al., 2016).

While phylosymbiosis, the observation that gut microbes can be specific to host species (Brooks et al., 2016), has been explored in vertebrate hosts (Amato et al., 2018; Kartzinel et al., 2019; Nishida & Ochman, 2018), studies comparing the strength of phylosymbiosis in distantly related hosts, such as mammals and fishes, are lacking (but see 1, 2). Our results show that when accounting for host relatedness, the effect of host phylogeny is not as large as one would expect in fish gut microbiomes. Over evolutionary time, diet may overtake host phylogeny in shaping gut microbiomes (Groussin et al., 2017). Thus, as a relatively recent clade (Escalas et al., 2021), mammals may not have had the same time as coral reef fishes for dietary selective pressures to shape their gut microbiomes. We find that the strength of phylosymbiosis varies widely in fishes depending on their feeding strategy (Figure S2), further highlighting the importance of diet and its potential confounding impact on measurements of phylosymbiosis in fishes. Alternatively, unlike fishes, mammal traits may simply enable phylosymbiosis. Nearly all mammals are viviparous, produce lactate microbe-rich milk for their young, and possess complex immune systems that likely promote gut microbiome specificity (Cabrera-Rubio et al., 2012; Mallott & Amato, 2021; Sanders et al., 2014). Moreover, unlike fish, which lacked any pervasive microbial genera present in all our fish samples, mammal gut microbiomes all possessed high abundances of Bacteroides (Figure 3b), a well-known mammalian gut microbe (Wexler & Goodman, 2017) regardless of different host diets. Thus, mammalian gut microbiomes appear to have conserved aspects that are not as pronounced in fish.

Coral reef fish gut microbiomes are deeply integrated into host trophic ecology and undergo similar dietary selective pressures as mammals, despite major evolutionary and ecological differences between these vertebrate groups. While gut microbiome origins remain elusive, we highlight host diet as a driving force shaping gut microbiome diversity across the vertebrate tree of life. Future work should test whether diet-driven convergences exist beyond fish and mammal gut microbiomes and whether such convergences are specific to carnivory and herbivory or also occur across other feeding strategies.

AUTHOR CONTRIBUTIONS

SD and PB conceived the study. SD, JMC, SJB, NMDS, AM, and VP collected samples in the field. SD executed laboratory processing. SD carried out analyses and manuscript writing. NMDS conducted the Bayesian multi-level modelling and wrote the corresponding methods. PB led editing process, contributed to main text, and provided guidance on all stages of the study. All authors provided feedback on the analyses and edits for the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All code, associated metadata, additional fish biometric data and alpha rarefaction curves are available at https://github.com/samd1993/FishyMammals and raw sequences files at https://figshare.com/articles/dataset/Fish_gut_microbiome_sequences_16S_/21529092. Figures S1–S4 and Tables S1–S6 can be found in the attached Supporting Information file.

BENEFITS-SHARING STATEMENT

All research conducted in French Polynesia and Gambier Archipelago received prior approval from local authorities. We presented the aims and implications of our research at local research stations for the public. All of our results will be disseminated in their entirety to the Gump and CRIOBE research stations in the form of detailed research reports.

INCLUSION AND DIVERSITY

One or more authors self-identifies as an underrepresented minority. One or more authors has received support from funding sources designed to increase diversity in STEM. The laboratory work in this study was leveraged as a platform to engage with minority first-gen undergraduates at UCLA and provide them with valuable training for future careers in STEM. We support the need for diversity and inclusion in academia.

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