

## The Diversity of the Endobiotic Bacterial Communities in the Four Jellyfish Species

QING LIU<sup>1</sup>, XINTONG CHEN<sup>2,3</sup> , XIAOYA LI<sup>1,2</sup>, JIANPING HONG<sup>3</sup>, GUIXIAN JIANG<sup>4</sup>, HONGYU LIANG<sup>2,5</sup>,  
WENWEN LIU<sup>2,5</sup>, ZHENG XU<sup>6</sup>, JING ZHANG<sup>5</sup>, WEI WANG<sup>7\*</sup> and LIANG XIAO<sup>2\*</sup>

<sup>1</sup> College of Animal Science and Veterinary Medicine; ShanXi Agricultural University, TaiGu, ShanXi, China

<sup>2</sup> Faculty of Naval Medicine, Second Military Medical University (Naval Medical University), Shanghai, China

<sup>3</sup> College of Resources and Environment; ShanXi Agricultural University, ShanXi, TaiGu, China

<sup>4</sup> Clinical Medicine, Grade 2015, Second Military Medical University (Naval Medical University), Shanghai, China

<sup>5</sup> College of Traditional Chinese Medicine; Jilin Agricultural University, Changchun, Jilin, China

<sup>6</sup> Administration Office for Scientific Research, Second Military Medical University (Naval Medical University),  
Shanghai, China

<sup>7</sup> Department of Otorhinolaryngology-Head and Neck Surgery, Changhai Hospital, Second Military Medical University  
(Naval Medical University), Shanghai, China

Submitted 30 July 2019, revised 4 September 2019, accepted 19 September 2019

### Abstract

The associated microbiota plays an essential role in the life process of jellyfish. The endobiotic bacterial communities from four common jellyfish *Phyllorhiza punctata*, *Cyanea capillata*, *Chrysaora melanaster*, and *Aurelia coerulea* were comparatively analyzed by 16S rDNA sequencing in this study. Several 1049 OTUs were harvested from a total of 130 183 reads. Tenericutes (68.4%) and Firmicutes (82.1%) are the most abundant phyla in *P. punctata* and *C. melanaster*, whereas *C. capillata* and *A. coerulea* share the same top phylum Proteobacteria (76.9% vs. 78.3%). The classified OTUs and bacterial abundance greatly decrease from the phylum to genus level. The top 20 matched genera only account for 9.03% of the total community in *P. punctata*, 48.9% in *C. capillata*, 83.05% in *C. melanaster*, and 58.1% in *A. coerulea*, respectively. The heatmap of the top 50 genera shows that the relative abundances in *A. coerulea* and *C. capillata* are far richer than that in *P. punctata* and *C. melanaster*. Moreover, a total of 41 predictive functional categories at KEGG level 2 were identified. Our study indicates the independent diversity of the bacterial communities in the four common Scyphomedusae, which might involve in the metabolism and environmental information processing of the hosts.

**Key words:** jellyfish, endobiotic bacteria, diversity, 16S rDNA

### Introduction

Microorganisms are considered to be the most diverse and abundant organisms on Earth (Gans et al. 2005; Shanmugam et al. 2017). Microorganisms are constantly facing changing environmental conditions at the microscale, and a variety of survival strategies e.g. secondary metabolites secretion are well-developed to establish long-term relationships with their hosts. Consequently, it is important to consider that the evolution of animals and plants has occurred and will continue

to occur in the presence of microflora, forming parasitic, commensal, mutualistic, or even pathogenic relationships with their hosts (Sevellec et al. 2018). These resident microbes influence host fitness and ecological traits, ultimately forming a symbiotic organism that consists of a multicellular host and a community of associated microorganisms (Bosch 2013). The composition as well as the associations between hosts and microorganisms profoundly affects the development, maturation and almost all the biological processes of the hosted organisms (Stephens et al. 2016).

\* Corresponding author: L. Xiao, Faculty of Naval Medicine, Second Military Medical University (Naval Medical University), Shanghai, China; e-mail: [hormat830713@hotmail.com](mailto:hormat830713@hotmail.com)

W. Wang, Department of Otorhinolaryngology-Head and Neck Surgery, Changhai Hospital, Second Military Medical University (Naval Medical University), Shanghai, China; e-mail: [wangw0503@163.com](mailto:wangw0503@163.com)

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Marine animals provide a unique habitat for attachment and colonization of microorganisms, and each organism hosts a specific microbial community (Weiland-Bräuer et al. 2015; Paharik and Horswill 2016). Jellyfish are marine free-swimming with high water content (>95%) and possess a rich diversity of symbiotic microorganisms. They are generalist predators of planktonic prey, such as protists, fish eggs, and polychaeta larvae and also act as prey for a range of different animals, including other jellyfish, fish, birds, and turtles (Cleary et al. 2016). In recent decades, the frequency and duration of noteworthy jellyfish outbreaks appear to have increased at a global scale. These blooms are reported to be linked to overfishing, climate change, and eutrophication, leading to the damage to the marine ecosystems by affecting the planktonic food web (Viver et al. 2017). Meanwhile, with the considerable increase in jellyfish swarms in coastal areas, the number of victims stung by jellyfish, including swimmers, fishermen and divers, has consequently been increasing (Cleary et al. 2016; Lee et al. 2018).

Despite the great concerns raised regarding their potential harm to both the marine ecosystem and human health, little is known about the associated microbiota of jellyfish (Cortés-Lara et al. 2015). To date, cost-effective and powerful high-throughput sequencing techniques have been developed to identify microbial phylotypes and to detect rare taxa in samples. It is reasonable to speculate that the endobiotic microorganisms play a vital role in the growth and development of jellyfish with the effect of either 'harm' or 'health'. Understanding the diversity and effect of the endogenous colonies is crucial to the homeostasis and health of jellyfish, and also useful for the comprehension of the feasible microbial infection and guidance of the medication during the jellyfish envenomation. In this study, the endobiotic bacterial communities were screened by 16S rDNA sequencing in the four common species of jellyfish including *Phyllorhiza punctata*, *Cyanea capillata*, *Chrysaora melanaster* and *Aurelia coerulea*, to evaluate the diversity and richness as well as their potential functions involving the life of the four different jellyfish species.

## Experimental

### Materials and Methods

**Jellyfish samples.** Individuals of four jellyfish species (*P. punctata*, *C. capillata*, *C. melanaster*, and *A. coerulea*) were collected alive from an aquafarm in Shanghai, China. The jellyfish *P. punctata* and *A. coerulea* were fed on shrimp eggs with different temperatures 24–28°C and 18–25°C, while *C. capillata* and *C. melanaster* were both

cultured on shrimp eggs and *A. coerulea* at the same temperature 10–18°C. The jellyfish fasted for one day before sampling, and then transported to the laboratory in a 3-liter plastic bag filled with seawater to prevent damage from sloshing. All jellyfish used in this research were approved by the Faculty of Naval Medicine, Second Military Medical University (Faculty of Naval Medicine, Naval Medical University).

**DNA extraction.** Total bacterial genomic DNA was extracted from the jellyfish of four species using the FastDNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions and was stored at –20°C before further analysis. The quantity and quality of the extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

**16S rDNA amplicon pyrosequencing.** PCR amplification of the V3-V4 regions of the bacterial 16S rRNA genes was performed using the forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components included 5 µl of Q5 reaction buffer (5×), 5 µl of Q5 High-Fidelity GC buffer (5×), 0.25 µl of Q5 High-Fidelity DNA Polymerase (5 U/µl), 2 µl (2.5 mM) of dNTPs, 1 µl (10 µM) of each forward and reverse primer, 2 µl of DNA template, and 8.75 µl of ddH<sub>2</sub>O. Thermal cycling consisted of initial denaturation at 98°C for 2 min, followed by 25 cycles consisting of denaturation at 98°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, with a final extension of 5 min at 72°C. PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2 × 300 bp sequencing was performed using the Illumina MiSeq platform with the MiSeq Reagent Kit v3 at Personal Biotechnology Co. Ltd (Shanghai, China).

**Sequence analysis.** The QIIME (Quantitative Insights Into Microbial Ecology, v1.8.0, <http://qiime.org/>) pipeline was employed to process the sequencing data as previously described (Caporaso et al. 2010). Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. The low-quality sequences were filtered according to the following criteria: sequences that had a length of < 150 bp, had average Phred scores of < 20, contained ambiguous bases, and mononucleotide repeats of > 8 bp were removed. Paired-end reads were assembled using FLASH (v1.2.7, <http://ccb.jhu.edu/software/FLASH/>) (Magoč and

Salzberg 2011). After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity with UCLUST (Edgar 2010). A representative sequence was selected from each OTU using default parameters. OTU taxonomic classification was conducted with BLAST by comparing the set of representative sequences against those in the Greengenes Database (release 13.8, <http://greengenes.secondgenome.com/>) using the best hit (DeSantis et al. 2006). An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of these OTUs. OTUs containing less than 0.001% of the total sequences across all samples were discarded. To minimize the difference in sequencing depth across samples, an averaged, rounded, rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under 90% of the minimum sequencing depth for further analysis.

**Bioinformatics and statistical analysis.** Sequence data analyses were mainly performed using QIIME and R packages (v3.2.0). OTU-level alpha diversity indices, such as the Chao1 richness estimator, ACE metric (abundance-based coverage estimator), Shannon diversity index, and Simpson index, were calculated using the OTU table in QIIME. OTU-level ranked abundance curves were generated to compare the richness and evenness of OTUs among samples. Beta diversity analysis was performed to evaluate the structural variation in the microbial communities across samples using UniFrac distance metrics and visualized via principal coordinate analysis (PCoA), nonmetric multidimensional scaling (NMDS) and unweighted pair-group method with arithmetic means (UPGMA) hierarchical clustering (Ramette 2007). Differences in the UniFrac distances for pairwise comparisons among groups were determined using Student's t-test and the Monte Carlo permutation test with 1000 permutations and visualized with box-and-whisker plots. Principal component analysis (PCA) was also conducted based on the genus-level compositional profiles (Ramette 2007). The taxonomic compositions and abundances were visualized using MEGAN (Segata et al. 2011) and GraPhlAn (Lesueur et al. 2015). A Venn diagram was generated to visualize the shared and unique OTUs among samples or groups using the R package "VennDiagram" ([https://en.wikipedia.org/wiki/Venn\\_diagram](https://en.wikipedia.org/wiki/Venn_diagram)) based on the occurrence of OTUs across samples/groups regardless of their relative abundance. Taxon abundances at the phylum, class, order, family, genus, and species levels were statistically compared among samples or groups with Metastats (<http://metastats.cbcb.umd.edu/>) (White et al. 2009) and visualized with scatter plots. Co-occurrence analysis was performed by calculating Spearman's rank correlations between the dominant taxa. Correlations with  $|\text{RHO}| > 0.6$  and  $P < 0.01$  were visualized

as co-occurrence networks using Cytoscape (<http://www.cytoscape.org/>) (Shannon et al. 2003). Microbial functions were predicted by PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) based on high-quality sequences (Langille et al. 2013) (KEGG PATHWAY Database <http://www.genome.jp/kegg/pathway.html>).

## Results

**Diversity of the bacterial communities of the four jellyfish.** According to the sequencing results, a total of 130 183 reads was obtained with the general length of 420–452 bp, of which the top three sequences occupying an overall ratio of 92.26% have the DNA lengths 425 bp (27 413 reads), 450 bp (59 837 reads) and 451 bp (32 852 reads), respectively (Fig. 1.1). For each jellyfish, *P. punctata*, *C. capillata*, *C. melanaster*, and *A. coerulea* had the PCR counts of 29 662 (22.78%), 34 232 (26.30%), 33 031 (25.37%) and 32 581 (25.03%), respectively. After removing the rare OTUs that are less than 0.001% of the total counts, a total of 1049 operational taxonomic units (OTUs) from the preliminarily divided 3813 OTUs, according to the standard of 97% sequence similarity, were finally screened, where 242 (23.07%), 561 (53.48%), 193 (18.40%), and 629 (59.96%) OTUs were distributed in the jellyfish *P. punctata*, *C. capillata*, *C. melanaster* and *A. coerulea* samples, respectively (Fig. 1.2). Consistent with the number of OTUs, the indexes including Chao1, ACE, Simpson, and Shannon were much higher in the jellyfish *C. capillata* and *A. coerulea*, indicating a higher richness and evenness of a diversity than in the jellyfish *P. punctata* and *C. melanaster* (Table I). As for the  $\beta$  diversity analyzed by NMDS (Nonmetric Multidimensional Scaling), the big distances on the graph indicate the obvious difference of the bacterial community structure in the four jellyfish (Fig. 1.3).

We then constructed the Venn Diagram by using the screened 1049 OTUs. The number of OTU unions of all the four jellyfish is 931 (88.75%) while the OTU intersection is 79 (7.53%) (Fig. 1.4). The number of unique OTUs in *A. coerulea*, *C. capillata*, *C. melanaster*,

Table I  
Summary of  $\alpha$ -diversity indices of the bacterial communities in the four jellyfish species.

Species	Chao1	ACE	Simpson	Shannon
<i>Phyp</i>	242	242	0.5	2.01
<i>Cyac</i>	561.93	570.29	0.96	5.96
<i>Chrm</i>	193.04	194.13	0.39	1.73
<i>Aura</i>	629.4	634.45	0.95	6.14

Notes: *Chrm* – *C. melanaster*; *Aura* – *A. coerulea*; *Phyp* – *P. punctata*; *Cyac* – *C. capillata*

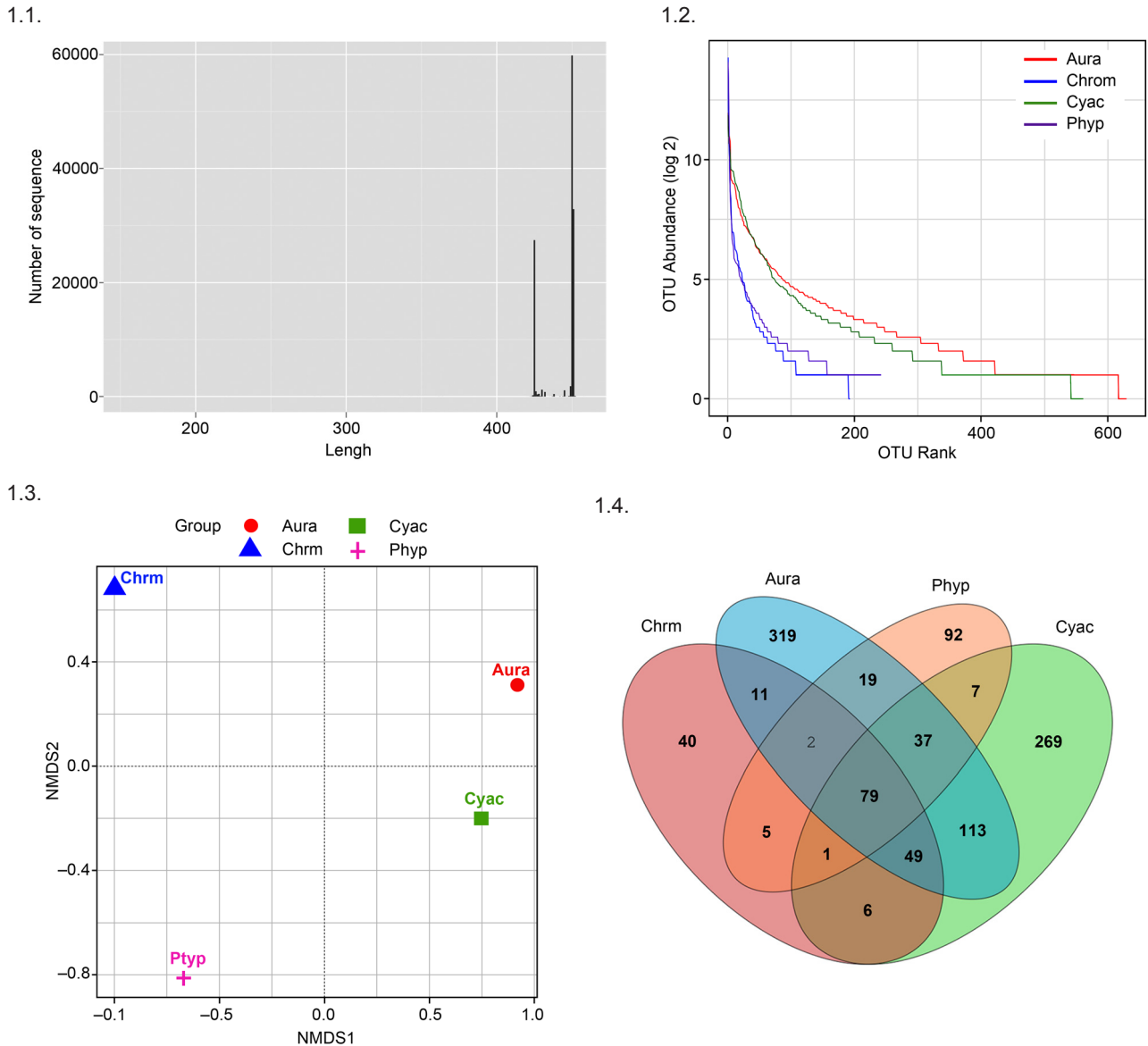


Fig. 1. Diversity of the bacterial communities of the four jellyfish species at OTU level. 1.1. Sequence length distribution of bacteria in the four jellyfish. 1.2. Rank abundance curve of the four jellyfish species. 1.3. Unweighted UniFrac NMDS plot of the bacterial communities associated with the four jellyfish species. 1.4. Venn diagram representing the shared operational taxonomic units (OTUs) among jellyfish species. Chrm, *C. melanaster*; Aura, *A. coerulea*; Phyp, *P. punctata*; Cyac, *C. capillata*.

and *P. punctata* was 319 (50.72%), 269 (47.95%), 92 (47.67%), and 40 (16.53%), respectively. The maximal and minimal numbers of overlapped OTUs between two jellyfish were 278 and 87 in the *A. coerulea* vs. *C. capillata* and *C. melanaster* vs. *P. punctata*, and the maximal and minimal numbers among three jellyfish were 128 and 80 in *A. coerulea* vs. *C. melanaster* vs. *C. capillata*, and *C. melanaster* vs. *C. capillata* vs. *P. punctata*. Also, the core microbiota (OTU intersection) mainly consisted of Firmicutes and Proteobacteria, which accounted for 11.4% (9 OTUs) and 82.3% (65 OTUs) of the total intersection (Table II). The bacterial phyla [Thermi], Actinobacteria and Planctomycetes had only one OTU, and Bacteroidetes contained two OTUs. (Table II). Among the phylum Proteobacteria,

Moraxellaceae (35 OTUs), and Pseudomonadaceae (17 OTUs) the two major families in the dominant order Pseudomonadales of the class Gammaproteobacteria were observed.

**Composition of the bacterial communities from phylum to family.** The assessment of different taxonomic levels is equivalent to viewing community composition structures at different resolutions, thus, the differences in the bacterial community associated with the four jellyfish species were firstly explored from the phylum to family according to the alignment of 16S rDNA sequences. A roughly equal bacterial numbers at different classification levels were obtained for *P. punctata* and *C. melanaster*, where the bacterial numbers of phylum, class, order, and family were 10 (234)

Table II  
Core microbiotas (OTU intersection) of the four jellyfish species.

Phylum	Class	Order	Family	Genus	Matched OTUs	
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	<i>Staphylococcus</i>	1528	
			Bacillaceae	<i>Bacillus</i>	1198, 1208	
				Unclassified_ Bacillaceae	238, 3199	
				<i>Geobacillus</i>	1362	
		Lactobacillales	Streptococcaceae	<i>Lactococcus</i>	1589, 2873	
			Carnobacteriaceae	<i>Carnobacterium</i>	1719	
Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	<i>Ochrobactrum</i>	1108	
			Methylobacteriaceae	<i>Methylobacterium</i>	3041	
			Methylobacteriaceae	Unclassified_ Methylobacteriaceae	2810	
			Phyllobacteriaceae	<i>Aminobacter</i>	2641	
			Sphingomonadaceae	<i>Sphingomonas</i>	2987, 3014	
		Sphingomonadales	Sphingomonadaceae	Unclassified_ Sphingomonadaceae	2284	
		Betaproteobacteria	Burkholderiales	Comamonadaceae	Unclassified_ Comamonadaceae	861
				Oxalobacteraceae	<i>Cupriavidus</i>	366
	Unclassified_ Burkholderiales			Unclassified_ Burkholderiales	476	
	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Unclassified_ Moraxellaceae	2146, 1618, 2256, 1522, 2988, 303, 2486, 303, 141, 2317, 929, 3334, 75, 1212, 2345, 1235, 3227, 1057, 2091, 3331, 3766, 2425, 2535, 389, 3630, 3372, 3440, 565, 1954, 1508, 3325, 872, 268, 1805, 2942	
			Pseudomonadaceae	<i>Pseudomonas</i>	1241, 3991, 3058, 2494, 1363, 2255, 1633, 2730, 1743, 306, 1348, 2740, 899, 3160, 909, 3051, 3320	
			Pseudomonadaceae	Unclassified_ Pseudomonadaceae	692	
			Xanthomonadales	Xanthomonadaceae	Unclassified_ Xanthomonadaceae	3013
			Vibrionales	Vibrionaceae	<i>Vibrio</i>	2553
			Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae
[Thermi]	Deinococci	Thermales	Thermaceae	<i>Thermus</i>	3649	
Bacteroidetes	[Saprosirae]	[Saprosirales]	Chitinophagaceae	<i>Sediminibacterium</i>	3146, 1112	
Planctomycetes	Phycisphaerae	Phycisphaerales	Unclassified_ Phycisphaerales	Unclassified_ Phycisphaerales	3775	

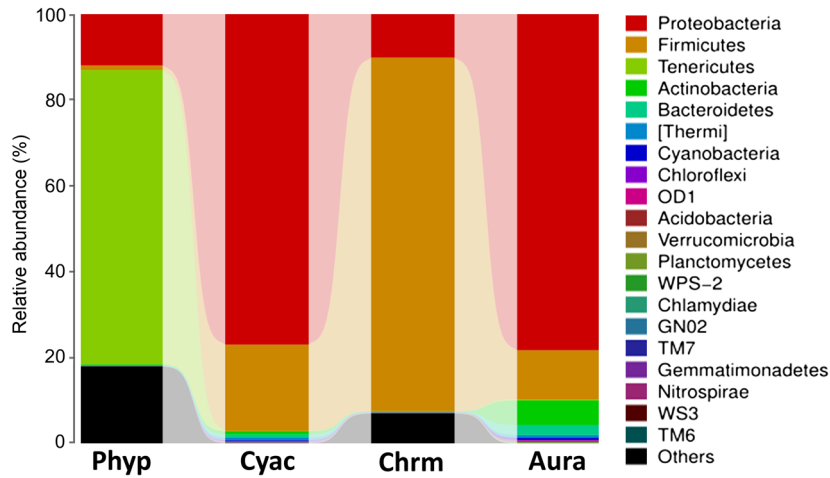
Square brackets indicate that the nomenclature requires updating.

vs. 12 (189), 15 (234) vs. 17 (188), 26 (228) vs. 26 (181), and 40 (218) vs. 33 (171), respectively. By comparison, the total number at different classification levels are much higher in *C. capillata* and *A. coerulea*, and the numbers of classification were 22 (558) vs. 18 (628), 39 (556) vs. 37 (622), 52 (534) vs. 56 (606), and 77 (424) vs. 95 (555), respectively (Table III).

At the phylum level, *Tenericutes* (68.4%) and *Proteobacteria* (12.1%) were the most abundant in *P. punctata*, while *Firmicutes* (82.1%) and *Proteobacteria* (10.3%) occupied the top two phyla in *C. melanaster*. A similar phylum distribution was found in *C. capillata* and *A. coerulea*, where the major phyla were *Proteobacteria* (76.9% vs. 78.3%), followed by *Firmicutes* (20.0%



2.1.



2.2.

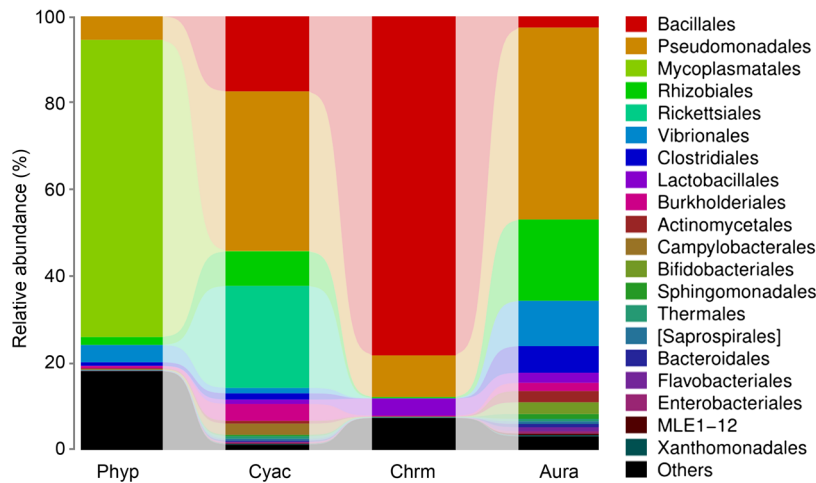


Fig. 2. Comparative analysis of the composition of the bacterial communities in the four jellyfish species across different classification levels. 2.1. Relative abundances of the representative phyla. 2.2. Relative abundances of the representative classes

vs. 11.4%) (Fig. 2.1). At the class level, Mollicutes (68.4%) from the phylum Tenericutes was the most abundant in *P. punctata*, which was followed by Gammaproteobacteria (9.5%) from Proteobacteria. In *C. melanaster*, Bacilli (82.1%) from Firmicutes and Gammaproteobacteria (9.6%) from Proteobacteria were the most abundant. By comparison, *C. capillata* and *A. coerulea* had a more dispersed distribution, where Gammaproteobacteria (38.5%) and Alphaproteobacteria (31.9%) from Proteobacteria, Bacilli (18.5%) from Firmicutes were the main classes in *C. capillata*, while Gammaproteobacteria

(55.8%) and Alphaproteobacteria (20.3%) from Proteobacteria were the top in *A. coerulea* (Fig. 2.2).

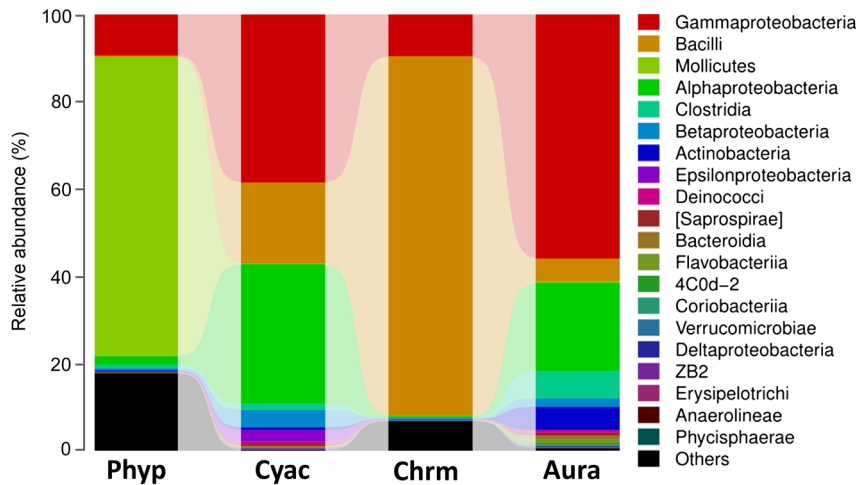
At the order level, Mycoplasmales (68.3%) from the class Mollicutes, Pseudomonadales (5.5%), and Vibrionales (4.0%) from Gammaproteobacteria were the top three orders in *P. punctata*. Bacillales (78.2%) from Bacilli and Pseudomonadales (9.6%) from Gammaproteobacteria constituted the main orders in *C. melanaster*. The order distributions of *C. capillata* and *A. coerulea* were more dispersed. Bacillales (17.5%) from Bacilli, Pseudomonadales (36.7%)

Table III  
A classification table of the OTUs and bacteria of the four jellyfish at different levels.

	The jellyfish species	Phylum	Class	Order	Family	Genus
Bacteria (OTUs)	Phyp	10 (234)	16 (234)	26 (228)	40 (218)	36 (98)
	Cyac	22 (558)	39 (556)	52 (534)	77 (424)	74 (226)
	Chrm	12 (189)	17 (188)	26 (181)	33 (171)	25 (81)
	Aura	18 (628)	37 (622)	56 (606)	95 (555)	120 (308)

Notes: Chrm - *C. melanaster*; Aura - *A. coerulea*; Phyp - *P. punctata*; Cyac - *C. capillata*

2.3.



2.4.

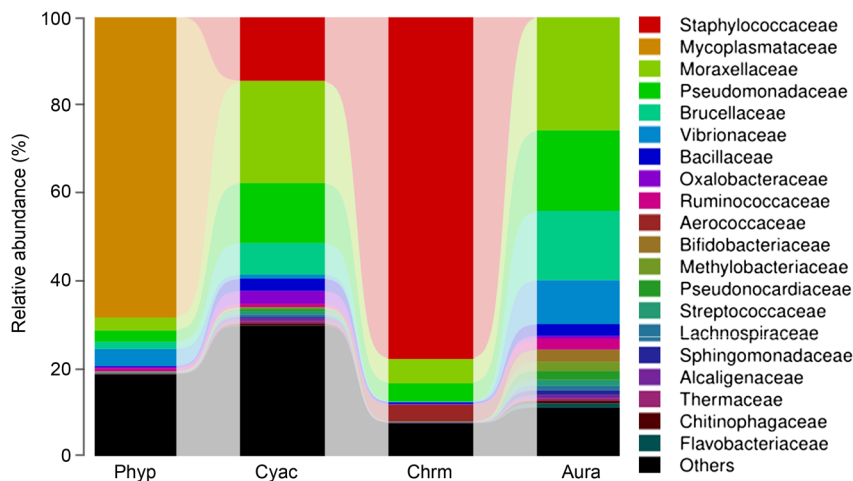


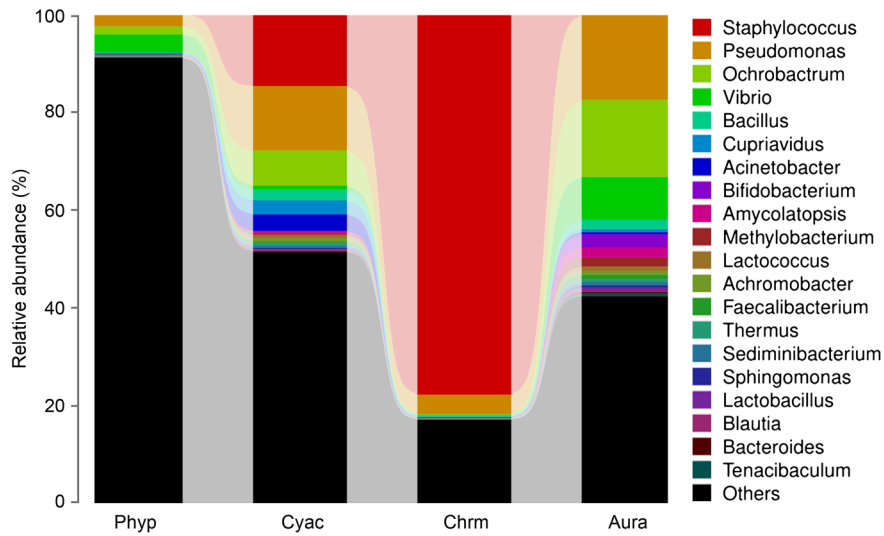
Fig. 2. Comparative analysis of the composition of the bacterial communities in the four jellyfish species across different classification levels. 2.3. Relative abundances of the representative order. 2.4. Relative abundances of the representative families.

from Gammaproteobacteria, Rhizobiales (8.0%) from Alphaproteobacteria, Rickettsiales (23.4%) from Alphaproteobacteria were the main bacterial classes in *C. capillata*, while Pseudomonadales (44.1%) from Gammaproteobacteria, Rhizobiales (18.7%) from Alphaproteobacteria, Vibrionales (10.4%) from Gammaproteobacteria, Clostridiales (6.1%) from Clostridia in *A. coerulea* (Fig. 2.3). At the family level, Mycoplasmataceae (68.3%) from the order Mycoplasmatales was the dominant in *P. punctata*, and the most abundant family in *C. melanaster* was Staphylococcaceae (77.7%) from Bacillales. Staphylococcaceae (14.7%) from Bacillales, Moraxellaceae (23.1%) from Pseudomonadales, and Brucellaceae (7.2%) from Rhizobiales were the major families in *C. capillata*. The jellyfish *A. Coerulea* has 11 families with >1% proportion, where Moraxellaceae (25.8%) from Pseudomonadales, Pseudomonadaceae (18.3%) from Pseudomonadales, and Brucellaceae (15.7%) and Vibrionaceae (9.9%) from Vibrionales were the top four components (Fig. 2.4).

**Composition of the bacterial communities at the genus level.** The obvious feature at the genus level is

the great increases in the unclassified OTUs and low-abundant genera among all four jellyfish species. There were 36 genera from 98 OTUs, 74 from 226, 25 from 81, and 120 from 308 that were detected in *P. punctata*, *C. capillata*, *C. melanaster*, and *A. coerulea*, respectively (Table III). The top 20 matched genera account for 9.03% of the total community in *P. punctata*, 48.9% in *C. capillata*, 83.05% in *C. melanaster*, and 58.1% in *A. coerulea* (Fig. 3.1). The top three genera in *P. punctata* were *Pseudomonas* (2.5%) from the family Pseudomonadaceae, *Vibrio* (3.7%) from Vibrionaceae, and *Ochrobactrum* (1.6%) from Brucellaceae. *Staphylococcus* from Staphylococcaceae (77.7%) was the dominant genus in *C. melanaster*, followed by *Pseudomonas* from Pseudomonadaceae with a much smaller proportion of 4.0%. The genus diversity was much richer in *C. capillata* and *A. coerulea*. There were six genera with the proportion >1% in *C. capillata*, *Staphylococcus* (14.7%), *Pseudomonas* (13.2%), and *Ochrobactrum* (7.2%) from Brucellaceae were the top three. *Pseudomonas* (17.5%), *Ochrobactrum* (15.7%), *Vibrio* (8.7%), *Bacillus* (1.9%), *Bifidobacterium* (2.7%) from Bifidobacteriaceae,

3.1.



3.2.

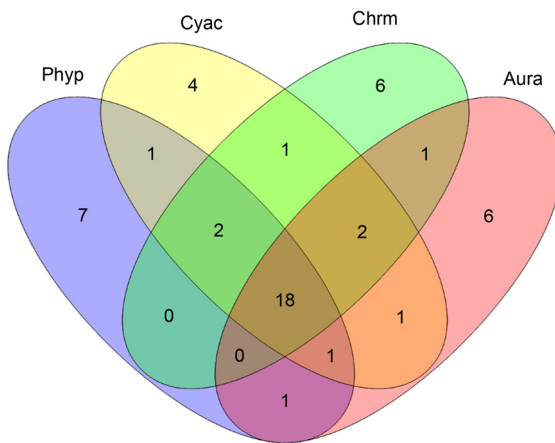


Fig. 3. Analysis of the differences in the composition of the bacterial communities associated with the four jellyfish species across the genus levels. 3.1. Relative abundances of the representative genus found in the four jellyfish species. 3.2. The Venn diagram representing the shared top 30 genera of the bacterial communities in the jellyfish species.

*Amycolatopsis* (2.0%) from Pseudonocardiaceae, and *Methylobacterium* (1.9%) from Methylobacteriaceae were the >1% genera (Fig. 3.1).

We then constructed the Venn diagram using the top 30 bacterial genera of the four jellyfish species, where the number of genera union is 51 and 18 genera were found in all four jellyfish species (Fig. 3.2). The numbers of unique genera were seven, four, six, and six in *P. punctata*, *C. capillata*, *C. melanaster*, and *A. coerulea*, respectively, while the number of overlapped genera between two jellyfish was 20~23, and the number of overlapped genera among three jellyfish was 18~20. The large proportions of both the overlapped and unique genera indicated the coexistence of the stabilized genera distribution and rich genera diversity of the four jellyfish species.

The direct impression of the heatmap graph with the top 50 bacterial genera was that the relative abundance in *A. coerulea* is much higher than in the other three jellyfish species. Most of the bacterial genera in *A. coerulea* were very numerous, and only nine genera were less numerous than the average in the four jellyfish species. *C. capillata* displayed as the second abun-

dant, and six bacterial genera were the most numerous, including *Salmonella*, *Geobacillus*, *Janthinobacterium*, *Cupriavidus*, *Acinetobacter*, and *Clostridium*. *P. punctata* and *C. melanaster* exhibit the lowest relative abundance, where *Alvinella* was found to be the most abundant in *P. punctata*, and *Staphylococcus* and *Rubritalea* are the most abundant in *C. melanaster* (Fig. 3.3).

**Functional annotation of the microbiotas of the jellyfish species.** The putative microbial functions associated with the four jellyfish species were predicted by assignment of the predicted metagenome using PICRUSt. KEGG (Kyoto Encyclopedia of Genes and Genomes) was utilized to map the pathways of the identified microbial functions. According to the Venn diagram, the number of the microbial functions is 5833 and the function intersection is 4936, thus, it accounted for a big proportion of 84.6% of the total functional groups, indicating the functional similarity of the bacteria among the four jellyfish species (Fig. 4.1). The number of unique functions in *A. coerulea*, *C. capillata*, *C. melanaster*, and *P. punctata* were only 127 (2.2%), 39 (0.7%), 25 (0.4%) and 1 (0.0%), respectively. The quantities of overlapped functions between two jelly-



3.3.

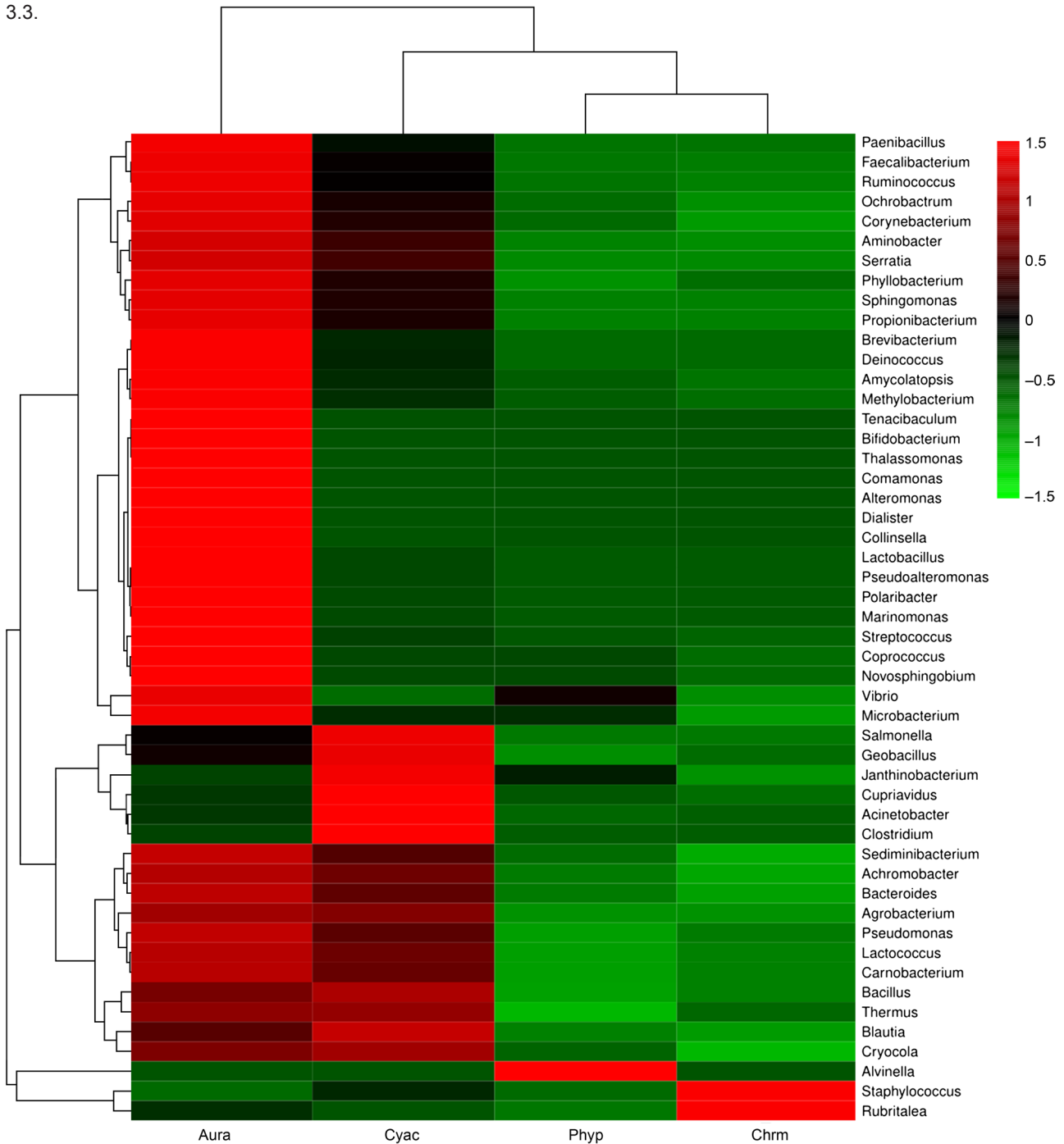


Fig. 3. Analysis of the differences in the composition of the bacterial communities associated with the four jellyfish species across the genus levels. 3.3. Heat map of the top 50 genera of the bacterial communities in the four species of jellyfish. Red represents the genera with high abundance in the corresponding jellyfish species, while green represents genera with low abundance. “Others” indicates the other bacterial genera in each jellyfish species except the top 20 genera with the highest abundance. *Chrm*, *C. melanaster*; *Aura*, *A. coerulea*; *Phyp*, *P. punctata*; *Cyac*, *C. capillata*.

fish were from 4942 in *C. melanaster* vs. *P. punctata* to 5600 in *A. coerulea* vs. *C. capillata*, and the maximal and minimal numbers among three jellyfish were 4936 in *P. punctata* vs. *C. melanaster* vs. *C. capillata* and 5111 in *C. melanaster* vs. *C. capillata* vs. *A. coerulea* (Fig. 4.1).

A total of 41 predictive categories in the KEGG level 2 functional modules were identified in the microbiota of the four jellyfish species. The relative abundances

of the functional categories among the four jellyfish were quite similar, and only tiny variations were seen in each category (Fig. 4.2). Membrane transport, amino acid metabolism, and carbohydrate metabolism were the three functional groups with the highest relative abundance. Metabolism and environmental information processing were the modules where the bacterial functions were concentrated (Fig. 4.2).

4.1.

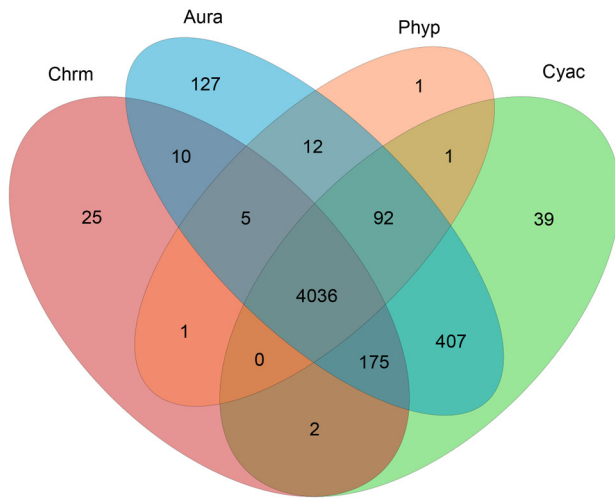
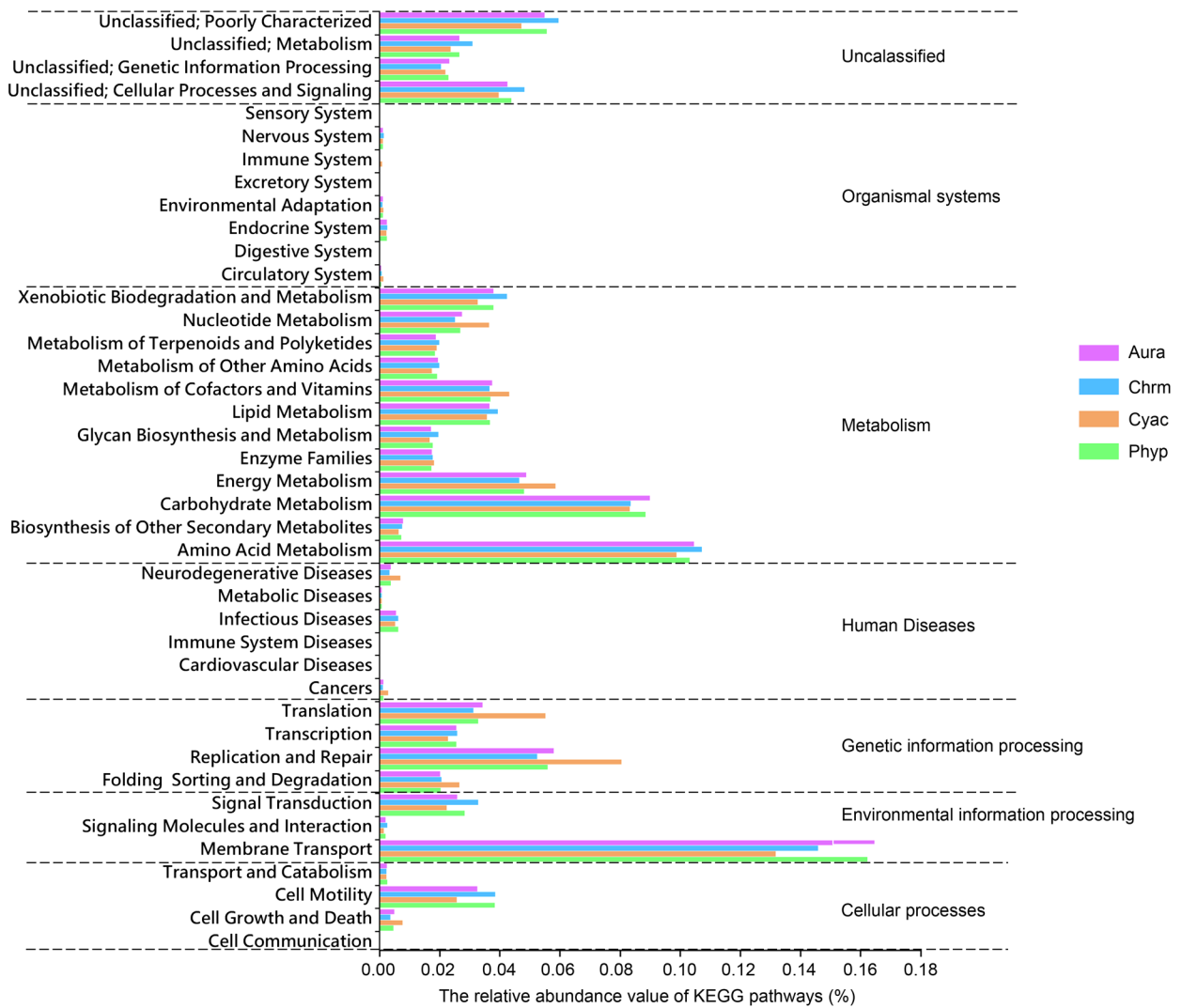


Fig. 4. The function prediction and KEGG pathway analysis of bacteria in four jellyfish species. 4.1. The Venn diagram analysis of common bacterial functional groups in the four jellyfish species. 4.2. The relative abundance of each predicted functional category given in the KEGG pathways (level 2).

4.2.



### Discussion

The associated microbiota of jellyfish plays an essential role in the jellyfish life processes, and the information on the bacterial community is of great importance

to jellyfish homeostasis and its health. In this study, all the four jellyfish species belonging to Scyphozoa were raised under artificial culture conditions and the phylum Proteobacteria (76.9–78.3%, especially Moraxellaceae, *Pseudomonas*, and *Vibrio*) dominated in *C. capil-*

*lata* and *A. coerulea*, while Firmicutes (82.1%, mostly *Staphylococcus* and Aerococcaceae) and Tenericutes (68.4%, mainly Mycoplasmataceae) in *C. melanaster* and *P. punctata*, indicating that the bacterial diversity is host-specific. Meanwhile, the symbiotic microbes in the same or close jellyfish species are possibly diversified with their geographic distributions or breeding settings due to the variation of environmental parameters, such as temperature, salinity, and cleanness (Tinta et al. 2019). Daley et al. (2016) showed that the bacterial communities associated with *A. aurita* are mainly composed of Mycoplasmatales (Tenericutes, Mollicutes) that, in this study, is rarely found in *A. coerulea*, which is another moon jellyfish very close to *Aurelia aurita*. Similarly, the endobiotic bacteria *Pseudoalteromonas* of the tentacles of *C. capillata* (Schuett and Doepke 2010) were not detected in *C. capillata* in this study. Here, the jellyfish *P. punctata* and *A. coerulea* were fed with the same shrimp eggs under similar temperatures 18–28°C, while *C. capillata* and *C. melanaster* were both cultured on shrimp eggs and *A. coerulea* at the same temperature 10–18°C. However, the number of OTUs shared between *P. punctata* and *A. coerulea*, and between *C. capillata* and *C. melanaster* with similar breeding settings were 137 and 135 respectively. It is much less than 178 between *C. capillata* and *A. coerulea* that is the highest number, but still much higher than 87 between *C. melanaster* and *P. punctata* with different breeding conditions, which was the lowest number observed in this study. We therefore cautiously concluded that the microbiota of the four jellyfish is more dependent on the jellyfish species although we do not neglect the impact of the different breeding environments.

The host-specificity of the symbiotic bacteria is not surprising when considering that the different jellyfish species can represent distinct morphological and biological features, and, therefore, providing distinctive microniches for bacteria (Lee et al. 2018). Usually, the symbiotic bacterial communities should satisfy certain distinct host necessities, and these requirements likely help to drive corresponding differences in the structures of the associated bacterial communities. Moreover, some types of bacteria might be commonly shared because of the common phylogeny and/or ecological characteristics of their hosts. For example, three jellyfish species, including *P. punctata*, *C. capillata*, and *A. coerulea*, were found to host a small number of Cyanobacteria that have been mainly studied as the model organisms of plant-like photosynthesis or carbon and nitrogen fixation (Mulkidjanian et al. 2006; Schuergers et al. 2017), and therefore possibly photosynthesize to provide nutrients for both themselves and their hosts. Interestingly, a small number of *Vibrio* were detected in all four jellyfish species. When the inhibitory factors are removed e.g. the defensive mechanism

of jellyfish is compromised or the water temperature rises at the end of the reproductive period, the *Vibrio* will quickly increase in number to release the *Vibrio* toxic virulence factors, and their viability, resistance to antimicrobial compounds, hemolysis and cytotoxicity would significantly increase, and could finally play a dominant role in the biomass degradation of jellyfish (Shanmugam et al. 2017; Tinta et al. 2019).

Membrane transport, amino acid metabolism, and carbohydrate metabolism are the most abundant among the 41 matched KEGG pathways, supporting the conventional function of symbiotic microorganisms that involve the communication between the hosts and external environments and metabolic processes of their hosts. The host provides an ideal habitat for the microorganisms (van de Water et al. 2018). Mutually, these microorganisms play an important role in the health and adaptive response of the hosts to the environment instead of impairing their hosts (Rosenberg et al. 2007, van de Water et al. 2018). Moreover, the big proportion (84.6%) of the matched OTUs, as well as little variation of relative abundance of KEGG pathways among the four jellyfish species suggest that these microbial groups perform similar functions to meet the necessities of their hosts even when the dominant symbiotic bacteria are diversified. In conclusion, we first detected and comparatively analyzed the endobiotic bacterial community by 16S rDNA sequencing in the four common Scyphomedusae, *P. punctata*, *C. capillata*, *C. melanaster*, and *A. coerulea*. A few 1049 OTUs were harvested from a total of 130 183 reads. The number of OTU unions of all the four jellyfish species was 931 while the OTU intersection was 79. The classified OTUs and bacterial abundance greatly decrease from the phylum to genus level. The top 20 genera account for 9.03%, 48.9%, 83.1%, and 58.1% of the total community in *P. punctata*, *C. capillata*, *C. melanaster*, and *A. coerulea*, respectively. The relative abundances of top 50 genera in *A. coerulea* and *C. capillata* are far richer than that in *P. punctata* and *C. melanaster*. Moreover, 41 predictive functional categories at KEGG level 2 were identified. Our study indicates the independent diversity of the bacterial communities in the four jellyfish species that might be involved in the metabolism and environmental information processing in the hosts.

#### ORCID

Xintong Chen 0000-0002-4657-5833

#### Acknowledgments

This work was supported by the excellent youth talent program from Shanghai Municipal Health Commission (2017YQ007), Military Youth Training Program – Talent Project (18QNP016), and the general program from the National Natural Science Foundation of China (81600791, 81770329). The authors thank Li Xinshu for providing four different jellyfish samples.

### Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

### Literature

- Asnicar F, Weingart G, Tickle TL, Huttenhower C, Segata N. Compact graphical representation of phylogenetic data and meta-data with GraPhlAn. *PeerJ*. 2015 Jun 18;3:e1029. <https://doi.org/10.7717/peerj.1029>
- Bosch TCG. Cnidarian-microbe interactions and the origin of innate immunity in metazoans. *Annu Rev Microbiol*. 2013 Sep 08;67(1):499–518. <https://doi.org/10.1146/annurev-micro-092412-155626>
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010 May;7(5):335–336. <https://doi.org/10.1038/nmeth.f.303>
- Cleary DFR, Becking LE, Polónia ARM, Freitas RM, Gomes NCM. Jellyfish-associated bacterial communities and bacterioplankton in Indonesian Marine lakes. *FEMS Microbiol Ecol*. 2016 May 01;92(5):fiw064. <https://doi.org/10.1093/femsec/fiw064>
- Cortés-Lara S, Urdiain M, Mora-Ruiz M, Prieto L, Rosselló-Móra R. Prokaryotic microbiota in the digestive cavity of the jellyfish *Cotylorhiza tuberculata*. *Syst Appl Microbiol*. 2015 Oct;38(7): 494–500. <https://doi.org/10.1016/j.syapm.2015.07.001>
- Daley MC, Urban-Rich J, Moisaner PH. Bacterial associations with the hydromedusa *Nemopsis bachei* and scyphomedusa *Aurelia aurita* from the North Atlantic Ocean. *Mar Biol Res*. 2016 Nov 25; 12 (10):1088–1100. <https://doi.org/10.1080/17451000.2016.1228974>
- DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol*. 2006 Jul 01;72(7):5069–5072. <https://doi.org/10.1128/AEM.03006-05>
- Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*. 2010 Oct 1;26(19):2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>
- Gans J, Wolinsky M, Dunbar J. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science*. 2005 Aug 26;309(5739):1387–1390. <https://doi.org/10.1126/science.1112665>
- Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkpile DE, Vega Thurber RL, Knight R, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol*. 2013 Sep;31(9):814–821. <https://doi.org/10.1038/nbt.2676>
- Lee MD, Kling JD, Araya R, Ceh J. Jellyfish life stages shape associated microbial communities, while a core microbiome is maintained across all. *Front Microbiol*. 2018 Jul 12;9:1534. <https://doi.org/10.3389/fmicb.2018.01534>
- Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. 2011 Nov 01; 27(21):2957–2963. <https://doi.org/10.1093/bioinformatics/btr507>
- Mulkidjanian AY, Koonin EV, Makarova KS, Mekhedov SL, Sorokin A, Wolf YI, Dufresne A, Partensky F, Burd H, Kaznadzey D, et al. The cyanobacterial genome core and the origin of photosynthesis. *Proc Natl Acad Sci USA*. 2006 Aug 29;103(35): 13126–13131. <https://doi.org/10.1073/pnas.0605709103>
- Paharik AE, Horswill AR. The staphylococcal biofilm: Adhesins, regulation, and host response. *Microbiol Spectr*. 2016 Apr; 4(2): 126–135. <https://doi.org/10.1128/microbiolspec.VMBF-0022-2015>
- Ramette A. Multivariate analyses in microbial ecology. *FEMS Microbiol Ecol*. 2007 Nov;62(2):142–160. <https://doi.org/10.1111/j.1574-6941.2007.00375.x>
- Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol*. 2007 May;5(5):355–362. <https://doi.org/10.1038/nrmicro1635>
- Schuerger N, Mullineaux CW, Wilde A. Cyanobacteria in motion. *Curr Opin Plant Biol*. 2017 Jun;37:109–115. <https://doi.org/10.1016/j.pbi.2017.03.018>
- Schuett C, Doepke H. Endobiotic bacteria and their pathogenic potential in cnidarian tentacles. *Helgol Mar Res*. 2010 Sep;64(3): 205–212. <https://doi.org/10.1007/s10152-009-0179-2>
- Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. *Genome Biol*. 2011;12(6):R60. <https://doi.org/10.1186/gb-2011-12-6-r60>
- Sevellec M, Derome N, Bernatchez L. Holobionts and ecological speciation: the intestinal microbiota of lake whitefish species pairs. *Microbiome*. 2018 Dec;6(1):47. <https://doi.org/10.1186/s40168-018-0427-2>
- Shanmugam SG, Magbanua ZV, Williams MA, Jangid K, Whitman WB, Peterson DG, Kingery WL. Bacterial diversity patterns differ in soils developing in sub-tropical and cool-temperate ecosystems. *Microb Ecol*. 2017 Apr;73(3):556–569. <https://doi.org/10.1007/s00248-016-0884-8>
- Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003 Nov 01;13(11):2498–2504. <https://doi.org/10.1101/gr.1239303>
- Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannan BJM. The composition of the zebrafish intestinal microbial community varies across development. *ISME J*. 2016 Mar;10(3):644–654. <https://doi.org/10.1038/ismej.2015.140>
- Tinta T, Kogovšek T, Klun K, Malej A, Herndl GJ, Turk V. Jellyfish-associated microbiome in the marine environment: exploring its biotechnological potential. *Mar Drugs*. 2019 Feb 01; 17(2):94. <https://doi.org/10.3390/md17020094>
- van de Water JAJM, Allemand D, Ferrier-Pagès C. Host-microbe interactions in octocoral holobionts – recent advances and perspectives. *Microbiome*. 2018 Dec;6(1):64. <https://doi.org/10.1186/s40168-018-0431-6>
- Viver T, Orellana LH, Hatt JK, Urdiain M, Díaz S, Richter M, Antón J, Avian M, Amann R, Konstantinidis KT, et al. The low diverse gastric microbiome of the jellyfish *Cotylorhiza tuberculata* is dominated by four novel taxa. *Environ Microbiol*. 2017 Aug; 19(8):3039–3058. <https://doi.org/10.1111/1462-2920.13763>
- Weiland-Bräuer N, Neulinger SC, Pinnow N, Künzel S, Baines JF, Schmitz RA. Composition of bacterial communities associated with *Aurelia aurita* changes with compartment, life stage, and population. *Appl Environ Microbiol*. 2015 Sep 01;81(17):6038–6052. <https://doi.org/10.1128/AEM.01601-15>
- White JR, Nagarajan N, Pop M. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLOS Comput Biol*. 2009 Apr 10;5(4):e1000352. <https://doi.org/10.1371/journal.pcbi.1000352>
- Yin M, Liu D, Xu F, Xiao L, Wang Q, Wang B, Chang Y, Zheng J, Tao X, Liu G, et al. A specific antimicrobial protein CAP-1 from *Pseudomonas* sp. isolated from the jellyfish *Cyanea capillata*. *Int J Biol Macromol*. 2016 Jan;82:488–496. <https://doi.org/10.1016/j.ijbiomac.2015.10.056>