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The microbiome of a Pacific moon jellyfish *Aurelia coerulea*

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Abstract

The impact of microbiome in animal physiology is well appreciated, but characterization of animal-microbe symbiosis in marine environments remains a growing need. This study characterizes the microbial communities associated with the moon jellyfish *Aurelia coerulea*, first isolated from the East Pacific Ocean and has since been utilized as an experimental system. We find that the microbiome of this Pacific *Aurelia* culture is dominated by two taxa, a Mollicutes and Rickettsiales. The microbiome is stable across life stages, although composition varies. Mining the host sequencing data, we assembled the bacterial metagenome-assembled genomes (MAGs). The bacterial MAGs are highly reduced, and predict a high metabolic dependence on the host. Analysis using multiple metrics suggest that both bacteria are likely new species. We therefore propose the names *Ca. Mariplasma lunae* (Mollicutes) and *Ca. Marinirickettsia aquamalans* (Rickettsiales). Finally, comparison with studies of *Aurelia* from other geographical populations suggests the association with *Ca. Mariplasma lunae* occurs in *Aurelia* from multiple geographical locations. The low-diversity microbiome of *Aurelia* provides a relatively simple system to study host-microbe interactions.

Introduction

Jellyfish are increasingly recognized as an important player in ecosystem functions and biogeochemical cycles [1–4]. A recent estimate suggests that gelatinous zooplanktons facilitate transfer, annually, of 0.4–2.1 gigatons of carbon to the seafloor [5]—about 20% on average of the total 5–6 gigatons of carbon deposited annually to the seafloor [6]. Carbon deposition to the seafloor is an important mechanism for absorbing carbon from the world’s atmosphere, and buffering the effects of the increasing carbon dioxide emission from human activities [7]. The effects of jellyfish on the marine ecosystem and biogeochemical cycles are magnified during bloom events. During a bloom, the jellyfish population rapidly increases within a short period of time, in some instances by as much as 5000% [1]. The rapid increase in biomass impacts the

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food web dynamics [8] and alters carbon, phosphorus, and nitrogen cycles [9]. Nitrogen release by jellyfish during a bloom can be as high as supporting more than 100% of nitrogen required for daily primary production by phytoplankton [2, 10, 11]. Jellyfish blooms also have an economic impact, having disrupted the fishery and tourism industries on multiple occasions [12, 13]. There is evidence that jellyfish blooms may increase in frequency and amplitude in the future because of anthropogenic processes [14, 15], highlighting the need to better understand the biology of jellyfish.

Jellyfish from the genus *Aurelia* (Cnidaria: Scyphozoa) are highly abundant and widely distributed across the world [16]. *Aurelia* can thrive in wide-ranging environments, from tropical seas to subarctic regions, from open oceans to brackish estuaries [17, 18]. In addition to its prevalence, *Aurelia* is also increasingly utilized as a laboratory model for studying multiple biological processes [19], including metamorphosis [20], biomechanics [21], regeneration [22], and neuroscience [23]. As an early branching metazoan, *Aurelia* provides an evolutionary lens with which to probe the early forms of biological processes in animals. The genome of *Aurelia coerulea* was recently sequenced [24], further empowering the use of *Aurelia* as a comparative model. Finally, being one of the most energetically efficient propulsors on the planet [25], *Aurelia* is a biological model for engineering muscular pumps [26], robotic swimmers [26] and biohybrid ocean sensors [27].

In this study, we characterize the microbiome of *Aurelia coerulea*, originally collected from the Eastern Pacific Ocean, and has been cultured in the lab for ten years. Microbiome impacts many aspects of animal biology, including traits previously thought to be solely dependent on the host genotype, such as development and behavior [28]. Indeed, in *Aurelia* polyps, modulation of microbiome impacts asexual reproduction, feeding rate, and growth [29]. There have been several studies sequencing the microbiome of *Aurelia* from different geographical locations [30–36]. These studies revealed that although the microbial communities of *Aurelia* are regionally different, there are interesting overlaps in the bacterial taxa, which motivate the need for more comparative studies of microbiomes from different geographical populations. In this study, we analyzed the microbial communities of *Aurelia* originating from the Pacific population, the origin of the strain whose genome has recently been sequenced [24]. Our study contributes to the existing literature by expanding the comparative analysis of *Aurelia* across geographical populations as well as characterizing an *Aurelia* strain that is increasingly being used as a laboratory model.

Materials and methods

Jellyfish culture

Polyp cultures were established from polyps originally collected by the Cabrillo Marine Aquarium (San Pedro, CA) from Long Beach (33°46'04.2"N 118°07'44.2"W, GPS: 33.7678376–118.1289559). We refer to this population of *Aurelia coerulea* as *Aurelia* AcGM. Polyps were maintained in artificial sea water (ASW) at 22°C on a 12:12 light-dark cycle and fed 48 hr old *Artemia* brine shrimp enriched with RGComplete (Reed Mariculture, USA) every two days. Polyps were induced to strobilate using 5-methoxy-2-methyl-indole (Sigma-Aldrich, USA) at 25 mM in sea water [37]. Polyps were exposed to the inducer overnight, and rinsed three times with ASW prior to transfer to 1 liter Imhoff cones (Nalgene, USA) with aeration. Induced animals were fed with *Artemia* every 48 hrs leading up to strobilation. To obtain the medusa stages, ephyrae were grown in an Imhoff cone and fed rotifers every day until animals were approximately 1 cm in diameter.

Amplicon sequencing. Individual animals were collected and rinsed three times with 0.22 µM sterilized artificial sea water. To minimize contamination from their diet, animals

were starved for 24 hrs prior to sampling. For ephyrae, animals that were less than 4 days old post-strobilation were collected. For medusae, 1 cm diameter animals were collected. Excess water was removed by blotting animals on parafilm using forceps. For each sample, 3–5 individuals were pooled. For each stage (polyp, ephyra, and medusa), 5–6 total samples were prepped. Animals were flash frozen in liquid nitrogen and stored at -80°C until further processing. Four sea water controls were prepared by filtering 500 ml of ASW through a 4.7 mm diameter 0.45 μM cellulose nitrate membrane filter (Whatman, United Kingdom). Half of the membrane was cut from the disc and used for the DNA extraction. DNA from flash frozen animals and controls were extracted using the Qiagen DNeasy Powersoil kit (Qiagen, Germany) following the manufacturer's protocol. Four kit controls were prepared by performing the extraction procedure with a set of blank extraction columns. Amplification of the V4 region of the 16S rRNA gene was performed using a modified 515F–806R primer set [38]. We chose to target the V4 region because it has been shown to be effective in studies of cnidarian-associated microbiomes [39, 40]. We find the modified primer sets performed better than the standard 515F–806R set in reducing amplification of host mitochondrial DNA. PCR amplification was performed with the NEB Q5 High-fidelity master mix (New England Biolabs, USA). Amplification was performed in triplicate for each sample. Replicate reactions were combined and amplification was confirmed on a 1% agarose gel. Samples were purified using the Qiagen QIAquick PCR Purification kit (Qiagen, Germany). Purified amplicon concentrations were quantified using the Qubit dsDNA HS Assay kit (cat. Q32851). Purified samples were submitted to Genewiz or the Georgia Genomics and Bioinformatics Core for 300-bp and 250-bp paired-end sequencing, respectively, on the Illumina MiSeq. 16S rRNA gene amplicon sequencing data have been submitted to NCBI under BioProject PRJNA975886.

Amplicon sequencing analysis. Quality of sequenced reads were checked with FastQC (ver. 0.11.9; <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Reads were analyzed with the DADA2 function of Qiime2 (ver. 2022.2) [41]. Reads were trimmed for quality and chimeric reads were removed with the *denoise-paired* function of dada2 with the following parameters:—p-trunc-len-f 177—p-trunc-len-r 170—p-trim-left-f 5—p-trim-left-r 10. Trimming parameters were selected using Figaro [42]. Taxonomic assignment of denoised, paired reads was performed using the Qiime2-formatted classifier (<http://docs.qiime2.org/2023.9/data-resources/>) pre-trained with V4 region sequences (515–806 bp) extracted from the Silva 16S rRNA database (version 138_99) [43]. Reads were then filtered using the *filter-samples* and the *filter-features* function of qiime2 to remove low-count sequences and singletons. Only samples with a minimum of 3500 reads were retained. Of the remaining samples, features were minimally required to be present in 2 samples and totaling 100 reads. Features that did not meet these requirements were removed prior to downstream analysis. Rarefaction curves were generated with the *diversity alpha-rarefaction* function of Qiime2 to ensure adequate coverage and depth of sequencing. 12,000 reads were sub-sampled, which is approximate to the lowest read depth of all jellyfish samples (S1 Fig in S9 File). To further determine whether we have identified genuine microbiome members of *Aurelia* AcGM, we used the R package decontam [44]. Decontam compares control and real samples and employs statistical methods to identify likely DNA contamination. Phylogenetic analysis of the retained features was performed with the *align-to-tree-mafft-fasttree* function of qiime2 using standard settings.

Assembly of bacterial MAGs from host genome sequencing data. Sequences generated for the assembly of the *A. coerulea* genome were retrieved from the National Center for Biotechnology Information (NCBI) [45] (Accession PRJNA490123). Initially, barcodes were removed from the reads and quality filtered using Trimmomatic-0.39 with standard parameters [46]. The trimmed reads were assembled with MegaHit (ver. 1.2.9), which incorporates DNA composition and abundance of unique sequences of DNA (k-mers) to group reads

during the assembly process [47]. The assembly was performed with the following settings:—k-min 25—k-max 115—k-step 10—min-contig-len 300 -m 0.4. Assembled contigs were binned into MAGs using MetaBAT (version 2.12.1) [48], Vamb (ver. 4.1.3) [49], and Rosella (ver. 0.4.2) (<https://rhysnewell.github.io/rosella/>). We chose to include Rosella because it incorporates long read information into the binning process. Consensus bins were generated using DAS Tool (ver. 1.1.6) (S1 Table in [S10 File](#)) [50]. We performed a BLAST search of the five longest sequences against the NCBI nt database to determine whether the binned MAGs were comprised of host or bacterial sequences. Binned contigs were then scaffolded using sspace-standard (ver. 3) or sspace-longread [51, 52]. Paired-end read libraries (SRR7889280, SRR7866920) and mate-paired libraries (4000 bp; SRR7834587, 8000 bp; SRR7866321) were used as input for sspace-standard. Pacbio reads (SRR7866923) were used as input for sspace-longread. Resulting scaffolded genomes with greater length and fewer contigs were chosen for subsequent processing and analysis. Gaps were closed with LR_Gapcloser [53]. Polishing of the scaffolded MAGs was performed using Pilon [54]. Briefly, paired-end libraries were aligned to the scaffolded genomes using BWA (ver. 0.7.12-r1039) [55] to generate bam format files and sorted using Samtools (ver. 1.15.1) [56]. Host sequences were identified from the MAGs using BLASTx against the nr database. Gene prediction was performed with the Rapid Annotation using Subsystem Technology tool kit (RASTtk) pipeline [57, 58]. The MAGs have been submitted to NCBI under BioProject PRJNA975886.

Quality of the MAGs was assessed using multiple metrics. Genome completeness and contamination was quantified using CheckM, as part of the Protologger galaxy web application (ver. 0.99) [59, 60]. A homology based method employed in the MiGA Online web server was additionally used to confirm the CheckM results [61]. CheckM performs the quality assessment using a taxonomically relevant set of marker genes. MiGA utilizes a set of essential genes defined in Dupont et al. [62] for a homology search performed by HMMER [63]. Taxonomic identification of the binned contigs was performed by comparing the 16S rRNA gene and the whole genome to existing databases. Full length 16S rRNA gene sequences were retrieved using Bedtools (ver. 2.26.0) [64] for *Aurelia* Mollicutes and *Aurelia* Rickettsiales to perform a homology search against the NCBI rRNA/ITS database with BLASTn, with uncultured/environmental sample sequences excluded [65, 66]. Relatedness to existing bacterial genomes was computed with average nucleotide identity (ANI) values using GTDB-Tk [67] as part of the Protologger pipeline [59], MiGA Online [61], as well as using the Type Strain Genome Server (TYGS) [68]. In addition to the Mollicutes and Rickettsiales MAGs, a third bacterial bin of roughly 4 Mb was recovered. However, the third bin is of low quality, possibly coming from lower-abundance symbionts. Further analysis of this third MAG therefore needs more directed sequencing.

Comparative analysis of the assembled MAGs. To assess the degree of genome reduction, we compared protein coding sequence content and genome length of the *Aurelia* Mollicutes MAG and *Aurelia* Rickettsiales MAG with those of existing genomes. Representative genomes, defined by the NCBI according to a set of predefined criteria (www.ncbi.nlm.nih.gov/refseq/about/prokaryotes/#representativegenome) were retrieved from NCBI RefSeq. A total of 128 representative genomes were retrieved for the Mollicutes (See [S7 File](#) for RefSeq accessions). An additional genome of *Ca. Spiroplasma holothuricola* was included (GCA_002135175.2) [69] in the analysis. A total of 82 representative Rickettsiales genomes were retrieved from NCBI (See [S8 File](#) for RefSeq accession). *Ca. Aquarickettsia rohweri* was also included in the analysis (GCF_003953955.1) [70]. Coding sequence and genome size were plotted with ggplot2 (ver. 3.4.0) in R (ver. 4.1.0).

Metabolic analysis with NetCooperate. The degree of metabolic reliance by the assembled genomes on the jellyfish host was quantified using NetCooperate [71]. Genome-scale

metabolic modeling of *A. coerulea* as well as the *Aurelia* Mollicutes and *Aurelia* Rickettsiales MAGs was performed with PRIAM using the predicted coding sequences as input [72]. The resulting sbml file was converted to a network format with a custom perl script prior to the analysis with NetCooperate. Draft metabolic models generated from the MAGs for each bacterial taxon were paired with a draft metabolic reconstruction of the *Aurelia* genome to calculate the biosynthetic support scores (BSS). Enzymatic reaction equations were parsed for its constituent metabolites, with products and substrates connected by edges. Completeness of metabolic pathways were manually confirmed against the KEGG database using KofamKoala [73]. Genomic data for *Aurelia* associated bacteria and the script used for NetCooperate data processing have been uploaded to the open repository CaltechDATA (<https://doi.org/10.22002/371wp-5nb51>).

Phylogenetic tree construction. Reconstruction of the *Aurelia* Mollicutes and *Aurelia* Rickettsiales phylogeny was performed using both a concatenated set of single-copy orthologs and the ribosomal 16S rRNA gene. Single-copy orthologs were identified using OrthoFinder (ver. 2.5.4) [74]. A subset of genomes for each taxa were used as input for the orthogroup analysis due to computational constraints. Identified single-copy orthologs were retrieved from the protein predictions and manually confirmed to be single copy for all genomes included in the analysis. A total of 11 single-copy orthologs were identified for the Mollicutes and 56 single-copy orthologs for Rickettsiales. Mafft (ver. 7.4.29) [75] was used for alignment of both single-copy ortholog sequences and 16S rRNA gene sequences with the accuracy-oriented method (L-INS-i) with 1000 cycles of iterative refinement. Aligned sequences were trimmed with BMGE (ver. 1.12) [76] with default parameters. For the single-copy ortholog tree, aligned sequences were concatenated using catsequences (ver. 1.4) (<https://zenodo.org/record/7956648>). Maximum-likelihood phylogenetic reconstruction with ultrafast bootstrapping was performed with IQ-TREE (ver. 2.03) [77, 78] with the following parameters: -alrt 1000 -bb 2000 -bnni -m MFP. Model selection and partition finding was performed with ModelFinder [79] and PartitionFinder. The resulting tree was visualized and modified in iTOL [80].

Results

The microbiome of *Aurelia coerulea* AcGM is dominated by two taxa

Our lab population of *Aurelia coerulea*, which we refer to as *Aurelia* AcGM, was originally collected from the East Pacific Ocean (the GPS coordinates given in Methods). It was classified as *Aurelia aurita* sp. 1, and recently revised to *Aurelia coerulea* [81]. To determine the bacterial composition of the *Aurelia* microbiome, we performed 16S amplicon sequencing (Fig 1A; S2 Table in S10 File provides the complete sequencing results). We recovered a total of 197 amplicon sequence variants, 95% of which could be identified to at least the phylum level and 78% of which to the genus level. To verify that the bacterial sequences represent animal-associated microbes and not a carryover from the environmental microbiome, we sequenced and verified that the ambient artificial sea water and the kit reagents contain distinct bacterial compositions (S2 Fig in S9 File, S3 Table in S10 File), confirming that the bacterial sequences obtained from *Aurelia* are genuine members of the *Aurelia* microbiome.

We grouped the bacterial amplicon sequences by their lowest taxonomic classification (Fig 1A). We found that the *Aurelia* AcGM microbiome is dominated by a bacterium from the class Mollicutes (phylum Firmicutes, recently renamed as Mycoplasmatota) and a bacterium from the order Rickettsiales (phylum Proteobacteria, also called Pseudomonadota). Mollicutes and Rickettsiales make up on average 89% of the amplicon reads across the life stages (Fig 1B). The next five most abundant taxa each comprises 1.5% or less of the total bacterial abundance

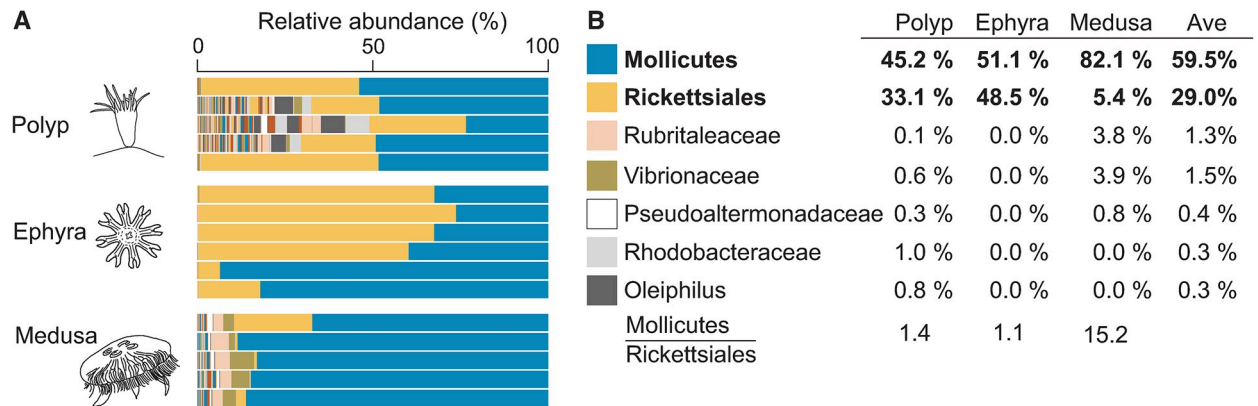


Fig 1. The bacterial microbiome of *Aurelia* AcGM is dominated by two taxa. (A) To characterize the jellyfish-associated microbiome associated with *Aurelia* AcGM, we performed next-generation amplicon sequencing of the V4 region of the 16S rRNA gene. Each biological replicate comes from 3–5 animals. The top ten taxa, color-coded, are listed in Fig 1B. (B) The top ten most abundant taxa present in *Aurelia* AcGM across life stages. Percent abundance shown is the average across biological replicates. The last column shows average ('Ave') across life stages. In the last row, the ratios of Mollicutes and Rickettsiales abundance are computed across life stages.

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(Fig 1B). We conclude that the microbiome of *Aurelia* AcGM in our population is dominated by Mollicutes and Rickettsiales.

The relative composition of Mollicutes and Rickettsiales varies across life stages. Like other jellyfish, *Aurelia*'s life cycle consists of two post-larval forms, the sessile polyps and free-swimming medusae (Fig 1A). In *Aurelia* and many scyphozoans, the transition from polyps to medusae involves an ephyra stage, a hardy stage that facilitates dispersal. In *Aurelia* AcGM polyps and ephyrae, Mollicutes and Rickettsiales are found at approximately equal ratios (1.4:1 in polyp and 1:1 in ephyra; Fig 1A and 1B). Strikingly, the medusa stage is characterized by a dramatic increase in Mollicutes, at 15-fold greater abundance than Rickettsiales (Fig 1A and 1B).

Association with Mollicutes recurs in several geographical populations

Next, we surveyed existing reports to assess how the microbiome of *Aurelia* AcGM compares to those of other *Aurelia* populations. Microbiome composition varies across *Aurelia* from different parts of the world (S4 Table in S10 File lists the taxa found across *Aurelia* populations) [30–36]. However, we notice recurring patterns. First, across the geographical populations analyzed so far, the *Aurelia* microbiome tends to be of a low-diversity, consisting of 2–5 dominant taxa. There are no clear differences between microbiome characterized from lab vs wild specimens, i.e., microbiome of lab specimens is not necessarily less complex or show distinct bacterial communities. Second, several bacterial families recur, as summarized in Fig 2B. Highest in frequency, although ranging in abundance, is an association with a Mollicutes, found in six out of the twelve populations analyzed so far (Table 1). Third, most of the Mollicutes, remarkably, appear to be closely related (S4 Table in S10 File). *Aurelia* from the Atlantic, Baltic, China, and Pacific, each associate with a Mollicutes that shows >97% sequence similarity to the Mollicutes found in *Aurelia* AcGM, as analyzed by BLAST analysis of the 16S amplicon sequences, suggesting that the Mollicutes found in these different geographical populations are likely the same species.

Metagenome-assembled genomes of *Aurelia* AcGM-associated microbes

To characterize the *Aurelia* Mollicutes and Rickettsiales, we mined the publicly available host genome sequencing data [24] to assemble draft bacterial genomes. For Rickettsiales, we

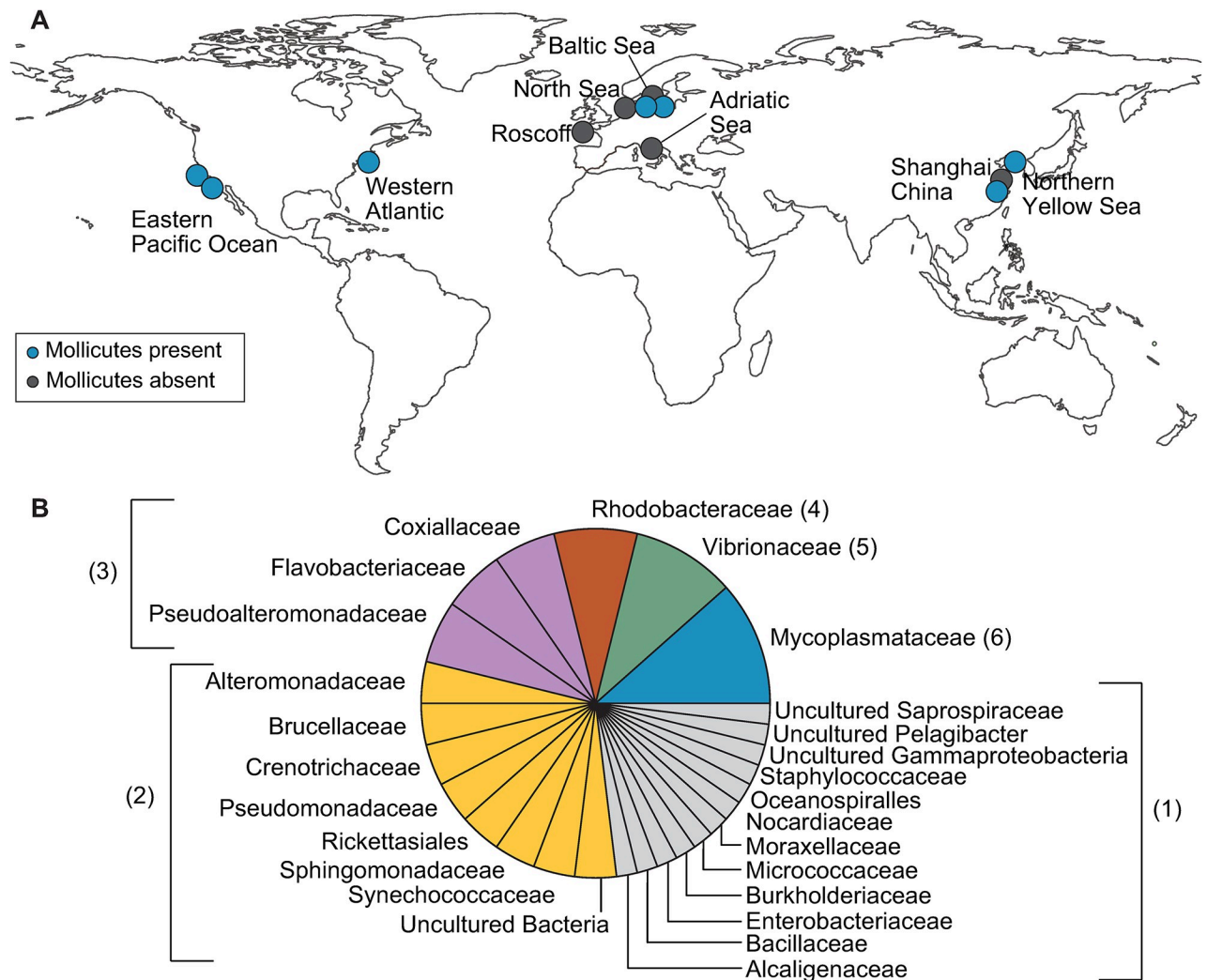


Fig 2. Microbiome composition of *Aurelia* varies across geographical populations. (A) Geographic locations of *Aurelia* populations that have been analyzed so far for their microbiome composition. (B) Bacterial families found to associate with *Aurelia* studied so far. Families included in this pie chart make up at least 5% of the relative abundance. We plotted the family level because this is the lowest common taxonomic classification reported in all of the studies. The numbers in parentheses indicate the number of geographical locations in which the families were found to associate with an *Aurelia* species. The colors simply group the families based on the number of geographical occurrences. See S4 Table in [S10 File](#) for the detailed survey of the existing *Aurelia* microbiome studies.

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recovered a 1 Mb uncircularized metagenome-assembled genome (MAG; [Fig 3A and 3B](#)). The Rickettsiales MAG was 95.5% complete with 0.2% contamination. The Rickettsiales MAG contains 91.5% of essential bacterial genes. Finally, the assembled Rickettsiales MAG is within the expected size for the Rickettsiales group ([Fig 3D](#)), containing 926 protein coding genes. The genome contains 38 tRNA cognates for all 20 essential amino acids. These metrics suggest that the draft Rickettsiales MAG, although uncircularized, provides a good coverage.

For Mollicutes, we successfully assembled a complete, circularized genome of 0.4 Mb in size ([Fig 3A and 3B](#)), with a 2.6% contamination. The Mollicutes MAG was assembled with a read depth of 2800X, giving us confidence that we have recovered the full genome. Mollicutes are known for having small genome sizes, having undergone genome reduction. The *Aurelia* Mollicutes MAG consists of 391 protein coding genes—and is one of the smallest genomes described to date ([Fig 3C and 3E](#)). Gene completeness analysis shows that the *Aurelia*

Table 1. *Aurelia* from several parts of the world associate with a Mollicutes likely of the same species. We surveyed existing studies that characterize bacteria that associate with *Aurelia* for Mollicutes association. S4 Table in [S10 File](#) provides the detailed taxa composition recovered from each study.

	Geographical origin	Specimen	Mollicutes abundance	Life stage analyzed	References	Similarity to AcGM Mollicutes
1	Atlantic Ocean, US	Wild	20%	Medusa	30	97%
2	Pacific Ocean, US	Lab culture	45–82%	Polyp, ephyra, medusa	This study	100%
3	Pacific Ocean, US	Lab culture (San Diego strain)	Abundant in host sequencing data	Polyp, ephyra, medusa	This study; mining data from ref. 24	100%
4	Roscoff, France	Lab culture	Absent	Polyp, strobila, ephyra, medusa	31	N/A
5	Baltic Sea	Lab culture	Absent	Polyp	31	N/A
6	Baltic Sea	Wild	42–90%	Medusa	31	75%
7	Baltic Sea	Wild	9%	Medusa	32	98%
8	North Sea	Lab culture	Absent	Polyp	31	N/A
9	Adriatic Sea	Wild	Absent	Medusa	33	N/A
10	Shanghai, China	Aquafarm	Absent	Medusa	34	N/A
11	Shanghai, China	Aquafarm	9–17%	Medusa	35	97%
12	Northern Yellow Sea,	Wild	1%	Medusa	36	97%

<https://doi.org/10.1371/journal.pone.0298002.t001>

Mollicutes MAG contains only 80% of known Mollicutes marker genes and 72% of essential bacterial genes, suggesting that it is a reduced genome. Of the representative Mollicutes genomes available in the NCBI database ([Fig 3C](#)), the most closely related to *Aurelia* Mollicutes is the one associated with sea cucumber, *Ca. Spiroplasma holothuricola* (the phylogenetic relationship will be analyzed in [Fig 4](#)), which has a similar genome size to the *Aurelia* Mollicutes ([Fig 3C](#), [69]).

***Aurelia* AcGM-associated microbes rely on host for nutrients**

The small genomes suggest that Rickettsiales and Mollicutes rely on the *Aurelia* host for nutrients. To assess the degree of metabolic coupling, we performed a pairwise NetCooperate analysis between the draft metabolic reconstructions of each bacterium and the host *Aurelia* [71]. NetCooperate calculates a Biosynthetic Support Scores (BSS), which reflects the degree to which genome 1 relies on genome 2 for metabolites. A BSS score of 1 reflects a complete reliance. The BSS score of *Aurelia* Rickettsiales is 0.84, and the BSS score of *Aurelia* Mollicutes is 0.91—suggesting a high degree of metabolic reliance on the host.

Indeed, directly assessing the genomes for absence/presence of metabolic pathways suggest reduced metabolic capacities (S6 and S7 Tables in [S10 File](#)). *Aurelia* Rickettsiales has a complete TCA pathway, but lacks the glycolysis pathway that supports the input to the TCA pathway, as well as most of the pentose phosphate pathway (PPP). The lack of glycolysis appears to be prevalent in Rickettsiales, as analysis of Rickettsiales genomes from the NCBI database confirms widespread loss of glycolytic enzymes (S6 Table in [S10 File](#)). Lacking glycolysis to generate pyruvate, Rickettsiales likely employ alternative methods to fuel the TCA and anabolic pathways. Pyruvate can be synthesized from intermediates of the PPP, intermediates of Entner-Doudoroff pathway, or from lactate and alanine. However, *Aurelia* Rickettsiales does not have the necessary enzymes to do so. Therefore, it is likely that *Aurelia* Rickettsiales uptake pyruvate from the host. Finally, *Aurelia* Rickettsiales are missing about half of the amino acid biosynthesis pathways, and therefore likely acquire multiple amino acids from the host, as evidenced by amino acid transporters in the genome (S7 Table in [S10 File](#)). Other species of Rickettsiales are also known to uptake amino acids [82–84]. Amino acids can also fuel the TCA

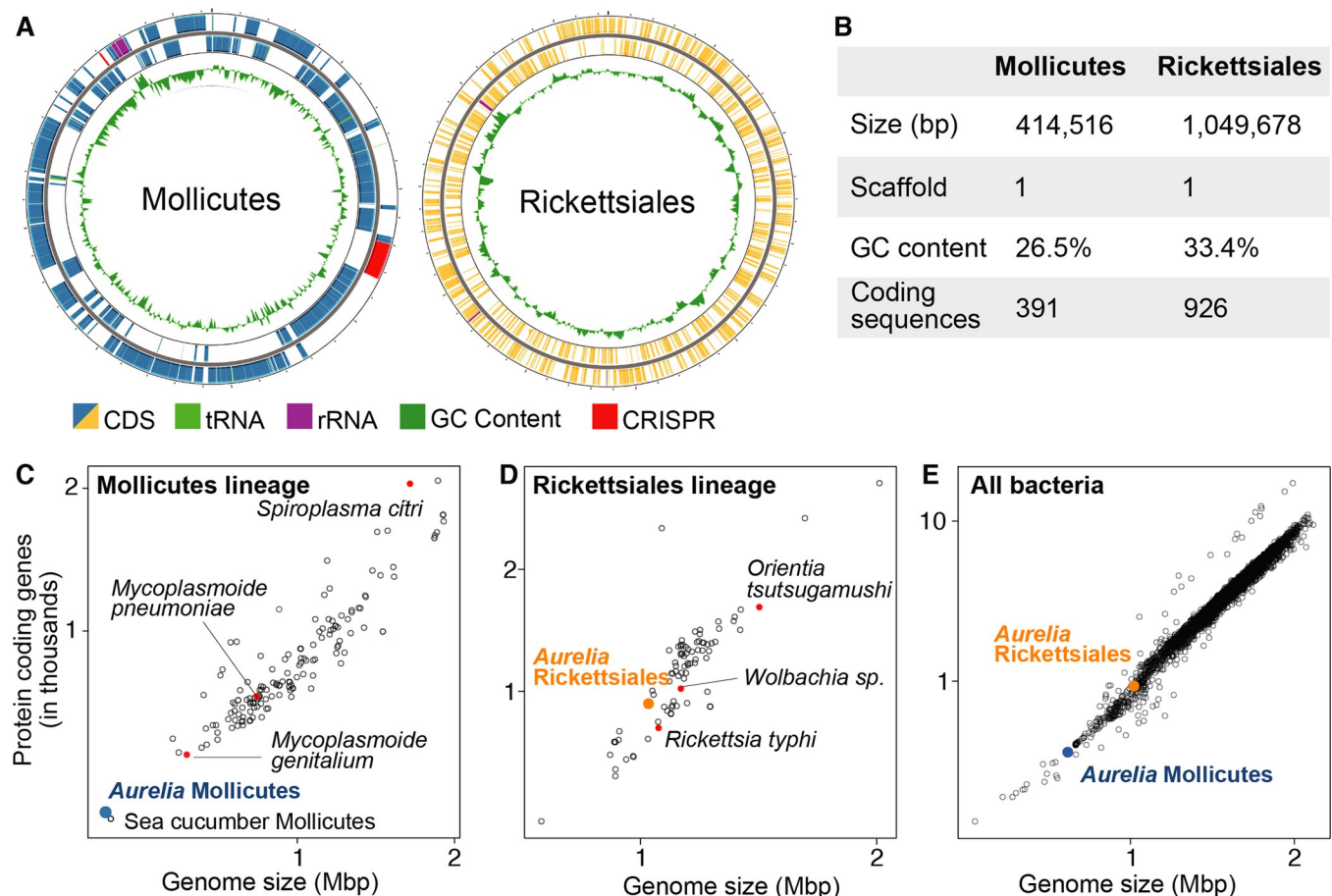


Fig 3. Metagenome-assembled genomes (MAGs) of the *Aurelia* AcGM-associated Mollicutes and Rickettsiales. The MAGs were recovered from the host genomic sequencing reads [24]. (A) Circos plots of *Aurelia* Mollicutes and *Aurelia* Rickettsiales MAGs. (B) Assembly statistics for the *Aurelia* Mollicutes and *Aurelia* Rickettsiales MAGs. More detailed statistics are described in S5 Table in S10 File. (C-E) Representative genomes of bacterial species were retrieved from the NCBI RefSeq database. Genome size and number of protein coding sequences were plotted for Mollicutes (C), Rickettsiales (D) and representative genomes of all accepted bacterial species (E). Genome size and number of protein coding sequences are log₁₀ transformed for visualization.

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cycle. *Aurelia* Rickettsiales has the genes that facilitate conversion of glutamine to glutamate and ultimately to alpha-ketoglutarate, a TCA intermediate. *Aurelia* Rickettsiales also has the enzymes for converting proline to glutamate and aspartate to oxaloacetate, another TCA intermediate. Finally, Rickettsiales are known to acquire vitamins and cofactors from their host [85]. Indeed, *Aurelia* Rickettsiales appears incapable of synthesizing vitamins, therefore likely acquiring them from the host. *Aurelia* Rickettsiales is capable of synthesizing heme A, but likely acquire other cofactors from the host.

Compared to Rickettsiales, the even smaller Mollicutes genome suggests more reliance on host (S7 Table in S10 File). Key energy-generation pathways—glycolysis, TCA, and pentose phosphate pathways—are all missing from the Mollicutes genome. As an alternative energy-generating pathway, the Mollicutes genome encodes the arginine deiminase (ADI) pathway, which generates ATP by metabolizing arginine. The ADI pathway has indeed been utilized in other Mollicutes species [86]. As Mollicutes lacks the ability to synthesize most essential amino acids, Mollicutes likely acquire amino acids from the host. We verify the presence of an ABC transporter that can transport multiple metabolites, as well as the arginine-ornithine antiporter required for the ADI pathway (S7 Table in S10 File). Despite the uptake requirements of the

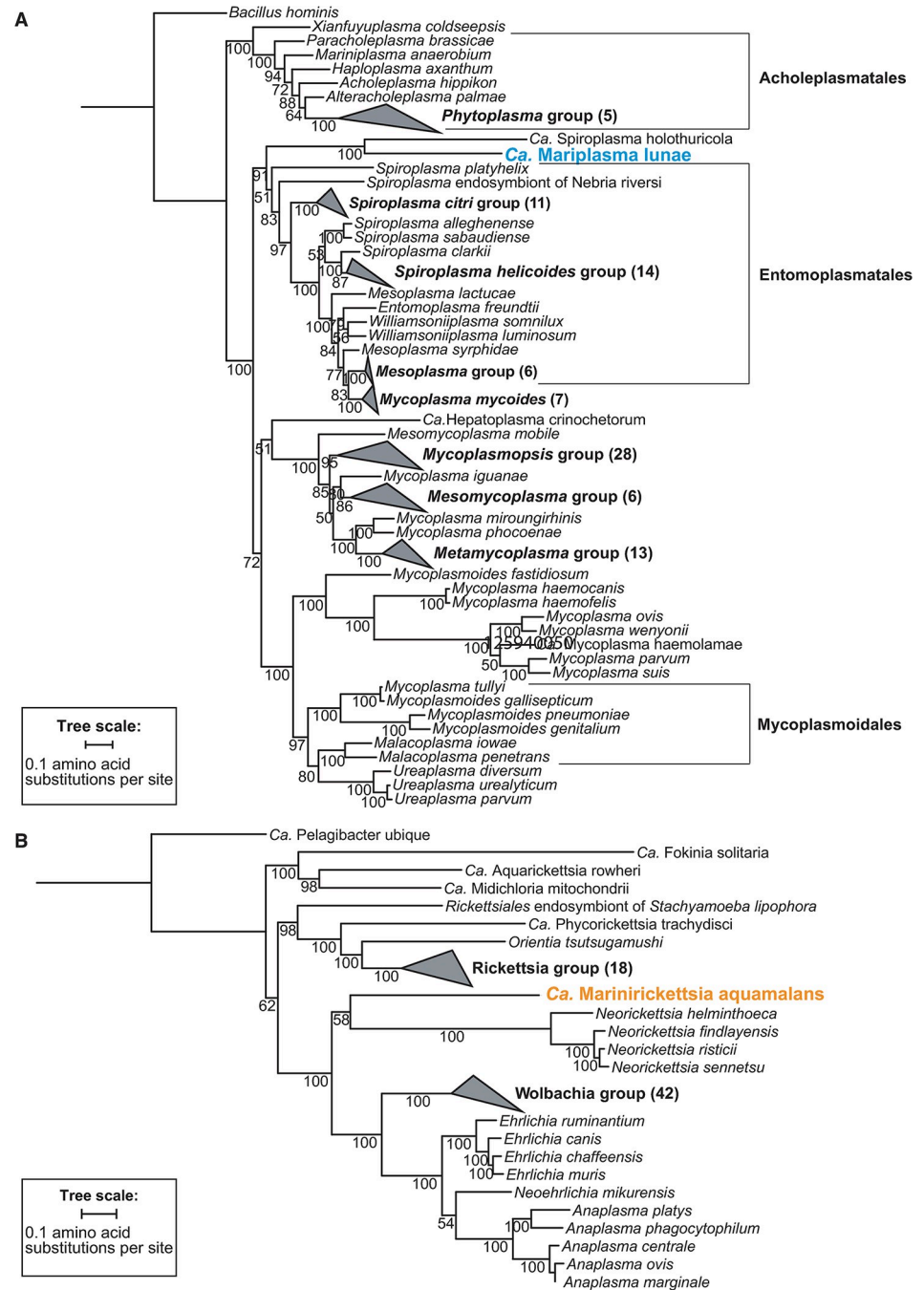


Fig 4. Bacteria that associate with *Aurelia* AcGM are likely new species. A concatenated maximum-likelihood phylogenetic tree was constructed using IQ-TREE with ultra-fast bootstrapping (n = 2000). Ultrafast bootstrap support values are shown for each branch point. The number of species comprising collapsed branches are shown in parentheses. (A) Phylogenetic reconstruction of the *Aurelia* Mollicutes. Eleven single-copy orthologs from 128 Mollicutes species were analyzed. (B) Phylogenetic reconstruction of the *Aurelia* Rickettsiales. Fifty-six single-copy orthologs from 82 Rickettsiales species were analyzed.

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Aurelia Mollicutes genome, only two genes were annotated as transporters. This suggests *Aurelia* Mollicutes transporters are capable of up-taking a broad set of metabolites, or unannotated genes have unidentified transporter functions. Finally, genes responsible for vitamin and

cofactor biosynthesis are completely absent from the *Aurelia* Mollicutes genome, suggesting the bacterium is totally reliant on the host for these molecules.

***Aurelia* Mollicutes and Rickettsiales are likely new species**

Using the bacterial genomes, we performed taxonomic classification with the Genome Taxonomy Database toolkit (GTDB-Tk; [67]). Taxonomic classification is assigned by calculating a relative evolutionary divergence (RED) value and the average nucleotide identity relative to reference genomes in the GTDB database [67]. We failed to determine an average nucleotide identity (ANI) values with high confidence for both genomes (S5 Table in S10 File), due to their dissimilarities to known genomes within the respective lineages. The GTDB-Tk analysis suggests the *Aurelia* Mollicutes and Rickettsiales likely belong to novel genera of bacteria: Mollicutes potentially in the family Spiroplasmataceae, and Rickettsiales in the family Anaplasmataceae. To further corroborate this finding, we performed a digital DNA-DNA hybridization analysis (dDDH; S5 Table in S10 File). The dDDH analysis fails to find any genome similar to the Mollicutes or Rickettsiales MAG with a score higher than 30%, verifying that the two bacteria associated with *Aurelia* AcGM are likely novel taxa.

We next performed homology analysis using the 16S rRNA genes, whose full sequences were recovered from the Mollicutes and Rickettsiales MAGs. We find that the Mollicutes 16S rRNA gene is most similar to *Spiroplasma platyhelix*, with an 80% sequence similarity (S1 File). The Rickettsiales 16S rRNA gene is most similar to *Ehrlichia chaffeensis*, with a sequence similarity of 85.2% (S2 File). Species-level matches typically fall in the range of >97% similarity. The homology analysis to find good homologies for the 16S rRNA genes further supports the findings from the average nucleotide identity and digital DNA-DNA hybridization analyses that the *Aurelia* AcGM-associated microbes are likely new species.

In order to better resolve the phylogenetic position of *Aurelia* associated bacteria to known Mollicutes and Rickettsiales, we built phylogenetic trees using single-copy orthologs (Fig 4, S3 File). The *Aurelia* Mollicutes is most closely related (sister) to another Mollicutes associated with a marine invertebrate, *Ca. Spiroplasma holothuricola*, a Mollicutes associated with sea cucumbers. It is also interesting that both Mollicutes also have similar genome sizes (both on the small end; Fig 2). Although the two species group together relative to all other Mollicutes, they themselves appear to have undergone some divergence, as indicated by the long branch lengths from the split. Altogether, these results support the placement of *Aurelia* Mollicutes as a unique genus within the Mollicutes, sister to the Entomoplasmatales. To further verify the single-copy ortholog tree, we performed phylogenetic reconstruction with the 16S rRNA gene (S3 Fig in S9 File, S4 File), and obtained a similar taxonomic placement of *Aurelia* Mollicutes. We therefore propose the name *Ca. Mariplasma lunae*, to signify a new marine genus related to *Spiroplasma* that dwells in moon jellyfish.

Phylogenetic reconstruction of the Rickettsiales order places the *Aurelia* Rickettsiales with high confidence as a sister clade to the Wolbachia/Anaplasma/Ehrlichia group (Fig 4B, S5 File). The support score at the branch point between *Aurelia* Rickettsiales and *Neorickettsia*, the position of the *Aurelia* AcGM-associated bacteria is unclear. However, consistent with the single-copy ortholog analysis, analysis with the 16S rRNA gene consistently places the *Aurelia* Rickettsiales as sister to *Neorickettsia*, and with greater support (S4 Fig in S9 File, S6 File). Despite the 16S rRNA gene tree, greater taxonomic sampling is necessary within the *Aurelia* Rickettsiales-*Neorickettsia* clade to fully resolve the relationship. Regardless of the precise placement, both phylogenetic inference and genome similarity metrics strongly support the classification of the *Aurelia* Rickettsiales as a new genus. We therefore propose the name *Ca. Marinirickettsia aquamalans* for a new genus and species of Rickettsiales that associates with jellyfish.

Discussion

In this study, we characterized the bacterial microbiome of the moon jellyfish *Aurelia coerulea*, originally collected from the Eastern Pacific Ocean and has been cultured in the lab for ten years, which we call *Aurelia coerulea* AcGM. We find that *Aurelia coerulea* AcGM has a low-diversity bacterial microbiome, dominated by two taxa. A low-diversity microbiome appears to be a stable feature of *Aurelia*, as the microbiome of *Aurelia* from other geographical locations, lab and wild specimens, consistently reveal 2–5 dominant taxa [30–36].

The Pacific *Aurelia* AcGM that we analyzed associates with bacteria from two taxa, a Mollicutes and a Rickettsiales. The diversity of the *Aurelia* microbiome is stable throughout its life stages, but the relative abundance of the Mollicutes and Rickettsiales changes. In particular, Mollicutes are significantly enriched in the medusa stage. Analysis using multiple metrics (average nucleotide identity, 16S rRNA homology, multi-gene phylogenetic analysis, and 16S rRNA taxonomy) suggests that *Aurelia* Mollicutes, as well as *Aurelia* Rickettsiales, are likely new genera. We therefore propose the names *Ca. Mariplasma lunae* (Mollicutes) and *Ca. Marinickettsia aquamalans* (Rickettsiales).

Association with a Mollicutes appears to extend beyond our Pacific strains, as it has also been found in *Aurelia* from multiple other geographical populations, both in lab or wild specimens [30, 31, 34]. Remarkably, the 16S amplicon sequences of the *Aurelia*-associated Mollicutes from these previous studies show 97–100% sequence similarity to the Mollicutes we identify in *Aurelia* AcGM (Table 1 and S4 Table in S10 File). This suggests that association of *Aurelia* and *Ca. Mariplasma lunae* may be convergent across biogeographies and environmental conditions, potentially pointing to the existence of an *Aurelia* Mollicutes. On the other hand, association with Rickettsiales has been observed in only one other study [30], suggesting a more opportunistic relationship.

Mollicutes are known for having small genome sizes [86]. *Mycoplasma genitalium* with 0.58 Mb genome and 470 protein coding sequences [87] is among the smallest free-living forms of life, and a model system in minimal genome research. The *Aurelia* Mollicutes has 0.4 Mb and almost 100 fewer coding sequences than *M. genitalium*. Together with the sea cucumber associated Mollicutes, these are the smallest Mollicutes identified so far (Fig 3C) and among the smallest known bacterial genomes known so far (Fig 3E). Marine Mollicutes are much less studied than their land counterparts. Some of the most well-known Mollicutes are parasites studied for their impact on crop plants (e.g., *Spiroplasma citri*) and human health (e.g., *Mycoplasma pneumoniae*, *Mycoplasma genitalium*). The finding of a new marine species of Mollicutes in an animal host that can be studied in the lab provides an opportunity to add to our understanding of marine Mollicutes biology. At the same time, *Aurelia* is one of the most widespread jellyfish with increasing ecological impact due to their population dynamics and resilience in wide-ranging environments. It will be interesting to understand next how association with Mollicutes impacts *Aurelia* biology, and why association with Mollicutes appears to be recurrent across geographical populations of *Aurelia*.

Supporting information

S1 File.
(XLSX)

S2 File.
(XLSX)

S3 File.
(TXT)

S4 File.

(TXT)

S5 File.

(TXT)

S6 File.

(TXT)

S7 File.

(CSV)

S8 File.

(CSV)

S9 File.

(DOCX)

S10 File.

(XLSX)

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References

1. Pitt KA, Kingsford MJ, Rissik D, Koop K. Jellyfish modify the response of planktonic assemblages to nutrient pulses. *Marine Ecology Progress Series*. 2007; 351:1–13. <https://doi.org/10.3354/meps07298>
2. Pitt KA, Koop K, Rissik D. Contrasting contributions to inorganic nutrient recycling by the co-occurring jellyfishes, *Catostylus mosaicus* and *Phyllorhiza punctata* (Scyphozoa, Rhizostomeae). *Journal of Experimental Marine Biology and Ecology*. 2005; 315(1):71–86. <https://doi.org/10.1016/j.jembe.2004.09.007>
3. Hays GC, Doyle TK, Houghton JDR. A Paradigm Shift in the Trophic Importance of Jellyfish? *Trends in Ecology & Evolution*. 2018; 33(11):874–84. <https://doi.org/10.1016/j.tree.2018.09.001> PMID: 30245075
4. Tinta T, Kogovšek T, Turk V, Shiganova TA, Mikaelyan AS, Malej A. Microbial transformation of jellyfish organic matter affects the nitrogen cycle in the marine water column—A Black Sea case study. *Journal of Experimental Marine Biology and Ecology*. 2016; 475:19–30. <https://doi.org/10.1016/j.jembe.2015.10.018>

5. Luo JY, Condon RH, Stock CA, Duarte CM, Lucas CH, Pitt KA, et al. Gelatinous zooplankton-mediated carbon flows in the global oceans: A data-driven modeling study. *Global Biogeochemical Cycles*. 2020; 34:e2020GB006704. <https://doi.org/10.1029/2020GB006704>
6. Henson SA, Laufkötter C, Leung S, Giering SLC, Palevsky HI, Cavan EL. Uncertain response of ocean biological carbon export in a changing world. *Nature Geoscience*. 2022; 15(4):248–54. <https://doi.org/10.1038/s41561-022-00927-0>
7. DeVries T. The ocean carbon cycle. *Annual Review of Environment and Resources*. 2022; 47. <https://doi.org/10.1146/annurev-environ-120920-111307>
8. Jellyfish Blooms: Causes, Consequences, and Recent Advances: Springer Dordrecht; 2009.
9. Pitt KA, Welsh DT, Condon RH. Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. *Hydrobiologia*. 2008; 616(1):133–49. <https://doi.org/10.1007/s10750-008-9584-9>
10. Hubot ND, Giering SLC, Füssel J, Robidart J, Birchill A, Stinchcombe M, et al. Evidence of nitrification associated with globally distributed pelagic jellyfish. *Limnology and Oceanography*. 2021; 66(6):2159–73. <https://doi.org/10.1002/lno.11736>
11. West EJ, Pitt KA, Welsh DT, Koop K, Rissik D. Top-down and bottom-up influences of jellyfish on primary productivity and planktonic assemblages. *Limnology and Oceanography*. 2009; 54(6):2058–71. <https://doi.org/10.4319/lo.2009.54.6.2058>
12. Richardson AJ, Bakun A, Hays GC, Gibbons MJ. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology and Evolution*. 2009; 24(6):312–22. <https://doi.org/10.1016/j.tree.2009.01.010> PMID: 19324452
13. Bosch-Belmar M, Milisenda G, Basso L, Doyle TK, Leone A, Piraino S. Jellyfish impacts on marine aquaculture and fisheries. *Reviews in Fisheries Science & Aquaculture*. 2020; 29(2):242–59. <https://doi.org/10.1080/23308249.2020.1806201>
14. Condon RH, Graham WM, Duarte CM, Pitt A, Lucas CH, Haddock SHD, et al. Questioning the rise of gelatinous zooplankton in the world's oceans. *BioScience*. 2012; 62(2):160–9. <https://doi.org/10.1525/bio.2012.62.2.9>
15. Boero F, Brotz L, Gibbons MJ, Piraino S, Zampardi S. Ocean Warming 3.10 Impacts and effects of ocean warming on jellyfish. In: Laffoley D, Baxter JM, editors. *Explaining Ocean Warming: Causes, scale, effects and consequences*. Gland, Switzerland: IUCN; 2016. p. 213–37
16. Lawley JW, Gamero-Mora E, Maronna MM, Chiaverano LM, Stampar SN, Hopcroft RR, et al. The importance of molecular characters when morphological variability hinders diagnosability: systematics of the moon jellyfish genus *Aurelia* (Cnidaria: Scyphozoa). *PeerJ*. 2021; 9:e11954. <https://doi.org/10.7717/peerj.11954> PMID: 34589293
17. Lucas CH. Reproduction and life history strategies of the common jellyfish, *Aurelia aurita*, in relation to its ambient environment. *Hydrobiologia*. 2001; 451:229–46. <https://doi.org/10.1023/A:1011836326717>
18. Schroth W, Jarms G, Streit B, Schierwater B. Speciation and phylogeography in the cosmopolitan marine moon jelly, *Aurelia* sp. *BMC Evolutionary Biology*. 2002; 2:1. <https://doi.org/10.1186/1471-2148-2-1> PMID: 11801181
19. Widmer CL. *How to Keep Jellyfish in Aquariums: An introductory guide for maintaining healthy jellies*. Tucson, AZ, USA: Wheatmark; 2008.
20. Kraus JE, Fredman D, Wang W, Khalturin K, Technau U. Adoption of conserved developmental genes in development and origin of the medusa body plan. *EvoDevo*. 2015; 6:23. <https://doi.org/10.1186/s13227-015-0017-3> PMID: 26075050
21. Abrams MJ, Basinger T, Yuan W, Guo CL, Goentoro L. Self-repairing symmetry in jellyfish through mechanically driven reorganization. *Proceedings of the National Academy of Science*. 2015; 112(26):E3365–73. <https://doi.org/10.1073/pnas.1502497112> PMID: 26080418
22. Abrams MJ, Tan FH, Basinger T, Heithe ML, Li Y, Raffiee M, et al. A conserved strategy for inducing appendage regeneration. *eLife*. 2021; 10:e65092. <https://doi.org/10.1101/2020.11.21.392720>
23. Pallasdies F, Goedeke S, Braun W, Memmesheimer R-M. From single neurons to behavior in the jellyfish *Aurelia aurita*. *eLife*. 2019; 8:e50084. <https://doi.org/10.7554/eLife.50084> PMID: 31868586
24. Gold DA, Katsuki T, Li Y, Yan X, Regulski M, Ibberson D, et al. The genome of the jellyfish *Aurelia* and the evolution of animal complexity. *Nature Ecology & Evolution*. 2019; 3:96–104. <https://doi.org/10.1038/s41559-018-0719-8> PMID: 30510179
25. Gemmell BJ, Costello JH, Colin SP, Stewart CJ, Dabiri JO, Tafti D, et al. Passive energy recapture in jellyfish contributes to propulsive advantage over other metazoans. *Proceedings of the National Academy of Science*. 2013; 110(44):17904–9. <https://doi.org/10.1073/pnas.1306983110> PMID: 24101461

26. Nawroth JC, Lee H, Feinberg AW, Ripplinger CM, McCain ML, Grosberg A, et al. A tissue-engineered jellyfish with biomimetic propulsion. *Nature Biotechnology*. 2012; 30(8):792–7. <https://doi.org/10.1038/nbt.2269> PMID: 22820316
27. Xu NW, Dabiri JO. Low-power microelectronics embedded in live jellyfish enhance propulsion. *Science Advances*. 2020; 6(5):1–10. <https://doi.org/10.1126/sciadv.aaz3194> PMID: 32064355
28. McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Loso T, Douglas AE, et al. Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Science*. 2013; 110(9):3229–36. <https://doi.org/10.1073/pnas.1218525110> PMID: 23391737
29. Weiland-Bräuer N, Pinnow N, Langfeldt D, Roik A, Güllert S, Chibani CM, et al. The native microbiome is crucial for offspring generation and fitness of *Aurelia aurita*. *mBio*. 2020; 11(6):e02336–20. <https://doi.org/10.1128/mBio.02336-20> PMID: 33203753
30. Daley MC, Urban-Rich J, Moisaner PH. Bacterial associations with the hydromedusa *Nemopsis bachei* and scyphomedusa *Aurelia aurita* from the North Atlantic Ocean. *Marine Biology Research*. 2016; 12(10):1088–100. <https://doi.org/10.1080/17451000.2016.1228974>
31. Weiland-Brauer N, Neulinger SC, Pinnow N, Kunzel S, Baines JF, Schmitz RA. composition of bacterial communities associated with *Aurelia aurita* changes with compartment, life stage, and population. *Applied and Environmental Microbiology*. 2015; 81(17):6038–52. <https://doi.org/10.1128/AEM.01601-15> PMID: 26116680
32. Jaspers C, Weiland-Bräuer N, Rühlemann MC, Baines JF, Schmitz RA, Reusch TBH. Differences in the microbiota of native and non-indigenous gelatinous zooplankton organisms in a low saline environment. *Science of The Total Environment*. 2020; 734:139471. <https://doi.org/10.1016/j.scitotenv.2020.139471> PMID: 32464382
33. Kos Kramar M, Tinta T, Lučić D, Malej A, Turk V. Bacteria associated with moon jellyfish during bloom and post-bloom periods in the Gulf of Trieste (northern Adriatic). *Plos One*. 2019; 14(1):e0198056. <https://doi.org/10.1371/journal.pone.0198056> PMID: 30645606
34. Liu Q, Chen X, Li X, Hong J, Jiang G, Liang H, et al. The diversity of the endobiotic bacterial communities in the four jellyfish species. *Polish Journal of Microbiology*. 2019; 68(4):465–76. <https://doi.org/10.33073/pjm-2019-046> PMID: 31880891
35. Chen X, Li X, Xu Z, Liu Q, Peng Z, Zhu Y, et al. The distinct microbial community in *Aurelia coerulea* polyps versus medusae and its dynamics after exposure to 60Co- γ radiation. *Environmental Research*. 2020; 188:109843. <https://doi.org/10.1016/j.envres.2020.109843> PMID: 32846637
36. Peng S, Hao W, Li Y, Wang L, Sun T, Zhao J, et al. Bacterial communities associated with four blooming scyphozoan jellyfish: potential species-specific consequences for marine organisms and humans health. *Frontiers in Microbiology*. 2021; 12:647089. <https://doi.org/10.3389/fmicb.2021.647089> PMID: 34025606
37. Fuchs B, Wang W, Graspentner S, Li Y, Insua S, Herbst EM, et al. Regulation of polyp-to-jellyfish transition in *Aurelia aurita*. *Current biology: CB*. 2014; 24(3):263–73. <https://doi.org/10.1016/j.cub.2013.12.003> PMID: 24440392
38. Barlow JT, Bogatyrev SR, Ismagilov RF. A quantitative sequencing framework for absolute abundance measurements of mucosal and luminal microbial communities. *Nature Communications*. 2020; 11:2590. <https://doi.org/10.1038/s41467-020-16224-6> PMID: 32444602
39. Pollock FJ, McMinds R, Smith S, Bourne DG, Willis BL, Medina M, et al. Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nature Communications*. 2018; 9(1):4921. <https://doi.org/10.1038/s41467-018-07275-x> PMID: 30467310; PubMed Central PMCID: PMC6250698.
40. Li Y, Hao W, Peng S, Sun T, Zhao J, Dong Z. Composition and potential functions of bacterial communities associated with *aurelia* polyps. *Frontiers in Marine Science*. 2022;9. <https://doi.org/10.3389/fmars.2022.864872>
41. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*. 2016; 13(7):581–3. <https://doi.org/10.1038/nmeth.3869> PMID: 27214047
42. Weinstein MM, Prem A, Jin M, Tang S, Bhasin JM. FIGARO: An efficient and objective tool for optimizing microbiome rRNA gene trimming parameters. *bioRxiv*. 2019;610394. <https://doi.org/10.1101/610394>
43. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*. 2018; 6(1). <https://doi.org/10.1186/s40168-018-0470-z> PMID: 29773078
44. Davis NM, Proctor DM, Holmes SP, Felman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*. 2018; 6(1). <https://doi.org/10.1186/s40168-018-0605-2> PMID: 30558668

45. Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau Donald C, et al. Database resources of the national center for biotechnology information. *Nucleic Acids Research*. 2022; 50(D1):D20–D6. <https://doi.org/10.1093/nar/gkab1112> PMID: 34850941
46. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014; 30(15):2114–20. <https://doi.org/10.1093/bioinformatics/btu170> PMID: 24695404
47. Li D, Luo R, Liu C-M, Leung C-M, Ting H-F, Sadakane K, et al. MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. *Methods*. 2016; 102:3–11. <https://doi.org/10.1016/j.ymeth.2016.02.020> PMID: 27012178
48. Kang DD, Li F, Kirton E, Thomas A, Egan R, An H, et al. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ*. 2019; 7:e7359. <https://doi.org/10.7717/peerj.7359> PMID: 31388474
49. Nissen JN, Johansen J, Allesøe RL, Sønderby CK, Armenteros JJA, Grønbech CH, et al. Improved metagenome binning and assembly using deep variational autoencoders. *Nature Biotechnology*. 2021; 39(5):555–60. <https://doi.org/10.1038/s41587-020-00777-4> PMID: 33398153
50. Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, et al. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. *Nature Microbiology*. 2018; 3(7):836–43. <https://doi.org/10.1038/s41564-018-0171-1> PMID: 29807988
51. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics*. 2011; 27(4):578–9. <https://doi.org/10.1093/bioinformatics/btq683> PMID: 21149342
52. Boetzer M, Pirovano W. SSPACE-Longread: scaffolding bacterial draft genomes using long read sequence information. *BMC Bioinformatics*. 2014; 15(211):1–9. <https://doi.org/10.1186/1471-2105-15-211> PMID: 24950923
53. Xu G-C, Xu T-J, Zhu R, Zhang Y, Li S-Q, Wang H-W, et al. LR_Gapcloser: a tiling path-based gap closer that uses long reads to complete genome assembly. *GigaScience*. 2018; 8(1):1–14. <https://doi.org/10.1093/gigascience/giy157> PMID: 30576505
54. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE*. 2014; 9(11):e112963. <https://doi.org/10.1371/journal.pone.0112963> PMID: 25409509
55. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009; 25(14):1754–60. <https://doi.org/10.1093/bioinformatics/btp324> PMID: 19451168
56. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. *GigaScience*. 2021; 10(2):1–4. <https://doi.org/10.1093/gigascience/giab008> PMID: 33590861
57. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics*. 2008; 9:75. <https://doi.org/10.1186/1471-2164-9-75> PMID: 18261238
58. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, et al. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*. 2015; 5:8365. <https://doi.org/10.1038/srep08365> PMID: 25666585
59. Hitch TCA, Riedel T, Oren A, Overmann J, Lawley TD, Clavel T. Automated analysis of genomic sequences facilitates high-throughput and comprehensive description of bacteria. *ISME Communications*. 2021; 1:16. <https://doi.org/10.1038/s43705-021-00017-z> PMID: 36732617
60. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research*. 2015; 25(7):1043–55. <https://doi.org/10.1101/gr.186072.114> PMID: 25977477
61. Rodriguez-R LM, Harvey WT, Rosselló-Mora R, Tiedje JM, Cole JR, Konstantinidis KT, et al. Classifying prokaryotic genomes using the Microbial Genomes Atlas (MiGA) webserver. In: Whitman WB, editor. *In Bergey's Manual of Systematics of Archaea and Bacteria* 2020. 6p. 1–11.
62. Dupont CL, Rusch DB, Yooseph S, Lombardo M-J, Alexander Richter R, Valas R, et al. Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. *The ISME Journal*. 2012; 6(6):1186–99. <https://doi.org/10.1038/ismej.2011.189> PMID: 22170421
63. Eddy SR. Accelerated Profile HMM Searches. *PLoS Computational Biology*. 2011; 7(10):e1002195. <https://doi.org/10.1371/journal.pcbi.1002195> PMID: 22039361
64. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*. 2010; 26(6):841–2. <https://doi.org/10.1093/bioinformatics/btq033> PMID: 20110278
65. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *Nucleic Acids Research*. 1990; 215:403–10. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2) PMID: 2231712

66. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. *BMC Bioinformatics*. 2009; 10:421. <https://doi.org/10.1186/1471-2105-10-421> PMID: 20003500
67. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH, Hancock J. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics*. 2019; 36(6):1925–7. <https://doi.org/10.1093/bioinformatics/btz848> PMID: 31730192
68. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nature Communications*. 2019;10(1). <https://doi.org/10.1038/s41467-019-10210-3> PMID: 31097708
69. He L-S, Zhang P-W, Huang J-M, Zhu F-C, Danchin A, Wang Y. The enigmatic genome of an obligate ancient *Spiroplasma* symbiont in a hadal holothurian. *Applied and Environmental Microbiology*. 2018; 84:e01965–17.
70. Klings JG, Rosales SM, McMinds R, Shaver EC, Shantz AA, Peters EC, et al. Phylogenetic, genomic, and biogeographic characterization of a novel and ubiquitous marine invertebrate-associated Rickettsiales parasite, *Candidatus Aquarickettsia rohveri*, gen. nov., sp. nov. *The ISME Journal*. 2019; 13(12):2938–53. <https://doi.org/10.1038/s41396-019-0482-0> PMID: 31384012
71. Levy R, Carr R, Kreimer A, Freilich S, Borenstein E. NetCooperate: a network-based tool for inferring host-microbe and microbe-microbe cooperation. *BMC Bioinformatics*. 2015; 16:164. <https://doi.org/10.1186/s12859-015-0588-y> PMID: 25980407
72. Claudel-Renard C. Enzyme-specific profiles for genome annotation: PRIAM. *Nucleic Acids Research*. 2003; 31(22):6633–9. <https://doi.org/10.1093/nar/gkg847> PMID: 14602924
73. Aramaki T, Blanc-Mathieu R, Endo H, Ohkubo K, Kanehisa M, Goto S, et al. KofamKOALA: KEGG ortholog assignment based on profile HMM and adaptive score threshold. *Bioinformatics*. 2020; 36(7):2251–2. <https://doi.org/10.1093/bioinformatics/btz859> PMID: 31742321
74. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biology*. 2015; 16:157. <https://doi.org/10.1186/s13059-015-0721-2> PMID: 26243257
75. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*. 2013; 30(4):772–80. <https://doi.org/10.1093/molbev/mst010> PMID: 23329690
76. Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evolutionary Biology*. 2010; 10:210. <https://doi.org/10.1186/1471-2148-10-210> PMID: 20626897
77. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*. 2018; 35(2):518–22. <https://doi.org/10.1093/molbev/msx281> PMID: 29077904
78. Lanfear R, von Haeseler A, Woodhams MD, Schrempf D, Chernomor O, Schmidt HA, et al. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*. 2020; 37(5):1530–4. <https://doi.org/10.1093/molbev/msaa015> PMID: 32011700
79. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*. 2017; 14(6):587–9. <https://doi.org/10.1038/nmeth.4285> PMID: 28481363
80. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research*. 2019; 47(W1):W256–W9. <https://doi.org/10.1093/nar/gkz239> PMID: 30931475
81. Scorrano S, Aglieri G, Boero F, Dawson MN, Piraino S. Unmasking *Aurelia* species in the Mediterranean Sea: an integrative morphometric and molecular approach. *Zoological Journal of the Linnean Society*. 2016:243–67. <https://doi.org/10.1111/zoj.12494>
82. Weiss E, Williams JC, Dasch GA, Kang Y-H. Energy metabolism of monocytic *Ehrlichia*. *Proceedings of the National Academy of Science*. 1989; 86:1674–8. <https://doi.org/10.1073/pnas.86.5.1674> PMID: 2922404
83. Huang H, Wang X, Kikuchi T, Kumagai Y, Rikihisa Y. Porin activity of *Anaplasma phagocytophilum* outer membrane fraction and purified P44. *Journal of Bacteriology*. 2007; 189(5):1998–2006. <https://doi.org/10.1128/jb.01548-06> PMID: 17172334
84. Cheng Z, Lin M, Rikihisa Y, Swanson MS. *Ehrlichia chaffeensis* proliferation begins with NtrY/NtrX and PutA/GlnA upregulation and CtrA degradation induced by proline and glutamine uptake. *mBio*. 2014; 5(6):e02141–14. <https://doi.org/10.1128/mBio.02141-14> PMID: 25425236
85. Driscoll TP, Verhoeve VI, Guillotte ML, Lehman SS, Rennoll SA, Beier-Sexton M, et al. Wholly *Rickettsia*: reconstructed metabolic profile of the quintessential bacterial parasite of eukaryotic cells. *mBio*. 2017; 8(5):e00859–17. <https://doi.org/10.1128/mBio.00859-17> PMID: 28951473

86. Razin S. Molecular biology and genetics of Mycoplasmas (Mollicutes). *Microbiological Reviews*. 1985; 49(4):419–55. <https://doi.org/10.1128/mr.49.4.419-455.1985> PMID: 3912655
87. Fraser CM, Gocayne JD, White O, Adams MD, Clayton RA, Felishcman RD, et al. The minimal gene complement of *Mycoplasma genitalium*. *Science*. 1995; 270:397–403. <https://doi.org/10.1126/science.270.5235.397> PMID: 7569993