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A *Mollicutes* Metagenome-Assembled Genome from the Gut of the Pteropod *Limacina rangii*

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ABSTRACT A nearly complete genome of an uncultured *Mollicutes* sp. was obtained from the metagenome of the gut of *Limacina rangii* (open-ocean snail), an important grazer and prey for higher trophic animals along the rapidly warming region of the western Antarctic Peninsula.

A recent metabarcoding study of *Limacina rangii*, a dominant grazer among zooplankton and an important prey along the western Antarctic Peninsula (WAP), revealed that *Mollicutes* bacteria are a cosmopolitan and dominant component of the gut microbiome (1, 2). Here, we report a nearly complete metagenome-assembled genome (MAG) of the class *Mollicutes* from the *L. rangii* gut obtained along the WAP, contributing to the growing number of genomic resources available for host-associated marine *Mollicutes* (3–8).

L. rangii organisms were collected at selected stations along the WAP (600.200, 300.200, and 100.040) in January 2017 (9). Gut samples were dissected onboard and immediately frozen at -80°C . Total DNA was extracted from three gut samples per station (9), using the Qiagen DNeasy blood and tissue kit, and then pooled to create a single extract for sequencing. Libraries were prepared with 30 to 50 ng DNA per sample using the Nextera DNA sample preparation kit (Illumina).

Sequencing was performed at a read length of 2×150 bp by MR DNA (Molecular Research LP) on the Illumina HiSeq 2500 platform. Raw read quality was visualized with FASTQC version 0.111.14 (10). A total of 31,260,228 read pairs across three samples underwent quality filtering with Trimmomatic version 0.38 (11) with the following parameters: minimum length of 90 bp, 4-bp sliding window with an average quality score of 15, and leading/trailing bases with quality scores less than 3 were removed. Quality-filtered reads were coassembled using default parameters with MEGAHIT version 1.1.1 (12). The coassembly was indexed with bowtie2 version 2.2.9, and the quality-filtered reads from each sample were mapped to the coassembly (13). SAM files were converted to BAM format and ordered using SAMtools release 1.5 (14). MetaBAT2 version 2.12.1 (15) was used to bin the coassembled contigs with default parameters. The *lineage_wf* function in CheckM version 1.0.5 (16) estimated completeness and contamination. Gene prediction and annotation were completed with PGAP (17). Conserved single-copy genes (CSCGs) were identified through analysis of bidirectional best BLAST hits between the MAG, *Mycoplasma* reference genomes, and outgroup *Firmicutes* genomes. Each CSCG cluster was aligned with MUSCLE version 3.8.3, and a phylogenetic reconstruction was performed with RAxML version 8.2.10 using the JTT substitution model and the GAMMA model of rate heterogeneity as previously described (3).

In total, 637,502 contigs were coassembled, but only one MAG, taxonomically assigned to the class *Mollicutes*, was obtained with high estimated completeness. The mean depth of coverage was highest at the most northern site (600.200) at $22.9\times$, while it was less than $2\times$ at the other sites (300.200 and 100.040). The *Mollicutes* MAG contained 85 contigs and a

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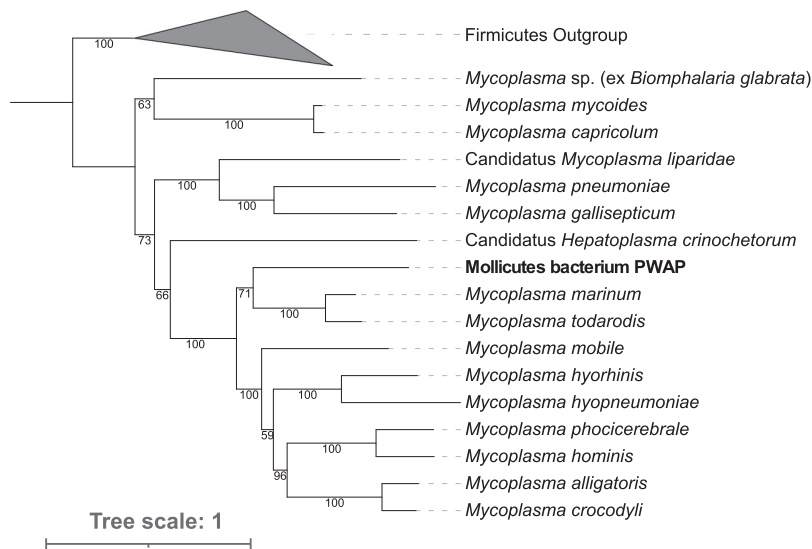


FIG 1 Maximum-likelihood phylogeny based on conserved single-copy genes between the *Mollicutes* MAG from this study (PWAP), reference *Mycoplasma* genomes, and four genomes from the *Firmicutes* used as an outgroup. One hundred iterations were used to compute bootstrap values. The GenBank accession numbers for the genomes in the phylogenetic reconstruction are included in Table 1.

genome size of 0.55 Mb, with an N_{50} of 7,347 bp, estimated completeness of 88.16%, contamination and strain heterogeneity of 0%, and GC content of 25.1%. The *Mollicutes* MAG included 527 protein-coding genes and contained multiple rRNA genes (one each of 16S, 23S, and 5S). The MAG was most closely related to *Mycoplasma marinum* and *Mycoplasma todarodis*, which were isolated from an octopus and squid, respectively, based on a phylogenetic reconstruction from 63 CSCGs (Fig. 1; Table 1).

Data availability. The raw reads were deposited in the NCBI SRA database with accession numbers [SRR12228976](https://www.ncbi.nlm.nih.gov/sra/SRR12228976), [SRR12228977](https://www.ncbi.nlm.nih.gov/sra/SRR12228977), and [SRR12228978](https://www.ncbi.nlm.nih.gov/sra/SRR12228978), and the MAG assembly is available under [ASM1966174v1](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA646234), all attached to BioProject [PRJNA646234](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA646234).

TABLE 1 NCBI accession numbers for bacterial genomes included in the phylogenetic reconstruction

| Organism | NCBI RefSeq accession no. |
|------------------------------------------------------------------|------------------------------------------------------------------------------------|
| <i>Mycoplasma pneumoniae</i> M129 | GCF_000027345.1 |
| <i>Mycoplasma gallisepticum</i> | GCF_001676495.1 |
| "Candidatus <i>Mycoplasma liparidae</i> " | GCA_009884515.1 |
| <i>Mycoplasma</i> sp. (ex <i>Biomphalaria glabrata</i>) | GCF_001484045.1 |
| <i>Mycoplasma hyopneumoniae</i> | GCF_002257505.1 |
| "Candidatus <i>Hepatoplasma crinochetorum</i> " Av | GCF_000582535.1 |
| <i>Mycoplasma mycoides</i> subsp. <i>capri</i> | GCF_900489525.1 |
| <i>Mycoplasma alligatoris</i> A21JP2 | GCF_000178375.1 |
| <i>Mycoplasma capricolum</i> subsp. <i>capripneumoniae</i> 87001 | GCF_000835085.1 |
| <i>Mycoplasma crocodyli</i> MP145 | GCF_000025845.1 |
| <i>Mycoplasma hyorhinis</i> | GCF_001705605.1 |
| <i>Mycoplasma hominis</i> | GCF_000759375.2 |
| <i>Mycoplasma phocicerebrale</i> | GCF_003383595.3 |
| <i>Mycoplasma todarodis</i> | GCF_004335995.1 |
| <i>Mycoplasma mobile</i> 163K | GCF_000008365.1 |
| <i>Mycoplasma marinum</i> | GCF_004335975.1 |
| <i>Lactobacillus plantarum</i> WCFS1 | GCF_000203855.3 |
| <i>Listeria monocytogenes</i> EGD-e | GCF_000196035.1 |
| <i>Enterococcus faecalis</i> V583 | GCF_000007785.1 |
| <i>Staphylococcus aureus</i> subsp. <i>aureus</i> NCTC 8325 | GCF_000013425.1 |

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Z.T.P. wrote the manuscript and analyzed sequence data. P.S.T. wrote the manuscript and provided sequence data. B.S. provided sequence data and revised the manuscript. Y.Z. provided analysis oversight and revised the manuscript.

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