Supporting Information

Host Dependence of Zooplankton-associated Microbes and Their Ecological Implications in Freshwater Lakes

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SUPPORTING MATERIALS and METHODS

DNA was extracted using the ALFA-SEQ Advanced Water DNA Kit. DNA concentration and purity were measured by Thermo NanoDrop One. The primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify V4 hypervariable regions of prokaryotic 16S rRNA genes. Polymerase chain reaction (PCR) system consisted of 2 × Premix Taq, 1 μL (10 μM) of each primer, 50 ng of DNA and finally added nuclease-free water constituting a total sample of 50 μL. The reactive conditions of PCR were as follows: (1) 94℃ for 5min; (2) 30 cycles of: 94℃ for 30 s, 52℃ for 30 s; (3) 72℃ for 30 s; (4) hold for 4℃. The PCR products of the same sample (3 replicates) were mixed, and the PCR instrument used was BioRad S1000 (Bio-Rad Laboratory, CA). The length and concentration of PCR products were detected by 1% agarose gel electrophoresis. Sample length in the normal range (290-310 bp) can be used for further experiments. Genetools analysis software (version 4.03.05.0, synGene) was used to compare the concentrations of PCR products. The used volume of each sample was calculated according to the equal mass principle, and the PCR products were then mixed. E.Z.N.A ® Gel Extraction and gel recovery kit (Omega, USA) was used to recover PCR mixture, and the target DNA fragment was eluted by TE buffer. The database was built according to the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The amplified library was sequenced by Illumina Nova 6000 (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China).

SUPPORTING TABLES

Table S1. Description of bacterial and environmental samples. FL, free-living bacteria; ZA, zooplankton-associated bacteria; WD, water depth; DO, dissolved oxygen; SD, Secchi depth; TN, total nitrogen; TP, total phosphorus; Lake Xiaoxingkai (XXK), Xihulu (XHL), Qianliujia (QLJ), Qingken (QK), Chagan (CG).

Lake	Moina ZA replicates	Calanoid ZA replicates	FL replicates	WD (m)	pH	DO (mg/L)	SD (cm)	TN (mg/L)	TP (mg/L)	Chl *a* (μg/L)
XXK	2	3	1	2.6	8.94	9.93	16	0.95	0.05	12.2
XHL	2	2	1	1.7	9.09	8.67	22	1.28	0.07	20.7
QLJ	2	2	1	0.8	9.36	7.57	5	2.62	0.34	27.0
QK	2	1	1	2.0	9.21	9.35	15	2.22	0.13	22.5
CG	2	2	1	2.1	9.43	7.50	25	1.69	0.10	31.9

Table S2. Taxonomic information of the top 10 OTUs in the *Moina*-, Calanoid-associated microbes and bacterioplankton sorted by their relative abundance or total reads of samples.

Objects	OTU	Phylum	Abundance	Relative abundance
Bacterioplankton	*Micromonospora*	Actinobacteria	23517	15.10%
	Brevundimonas	Proteobacteria	14445	9.42%
	Myxococcus	Proteobacteria	13151	8.58%
	Acinetobacter	Proteobacteria	6146	4.00%
	Streptomyces	Actinobacteria	5453	3.56%
	Exiguobacterium	Firmicutes	5315	3.47%
	Micromonospora	Actinobacteria	4721	3.08%
	Micrococcus	Actinobacteria	3960	2.58%
	Paracoccus	Proteobacteria	3606	2.35%
	Brevibacillus	Firmicutes	3063	2.00%
Moina	*Brevibacterium*	Actinobacteria	151731	20.36%
	Pseudomonadaceae	Proteobacteria	132854	17.83%
	Acinetobacter	Proteobacteria	71750	9.63%
	Exiguobacterium	Firmicutes	62734	8.42%
	Bacillales	Firmicutes	59271	7.95%

	Rhodococcus	Actinobacteria	59202	7.95%
	Aeromonadaceae	Proteobacteria	25453	3.41%
	Exiguobacterium	Firmicutes	11982	1.61%
	Bacillales	Firmicutes	10461	1.40%
	Pseudoalteromonadaceae	Proteobacteria	9606	1.29%
Calanoids	Pseudomonadaceae	Proteobacteria	89173	12.29%
	Exiguobacterium	unassigned	78769	10.86%
	Acinetobacter	Firmicutes	62834	8.66%
	Comamonadaceae	Proteobacteria	59980	8.27%
	Aeromonadaceae	Proteobacteria	30249	4.17%
	Methylobacteriaceae	Proteobacteria	23220	3.20%
	Pseudoalteromonadaceae	Proteobacteria	18260	2.52%
	Enterobacteriaceae	Proteobacteria	18119	2.50%
	Rhizobiales	Proteobacteria	17065	2.35%
	Rhizobiales	Proteobacteria	15841	2.18%

Table S3. Co-occurence network attributes of *Moina*- and Calanoid-associated microbes. FL, free-living bacteria; ZA, zooplankton-associated bacteria

Objects	Average degree	Average path length	Average clustering coefficient
FL	7.224	3.071	0.755
Moina ZA	61.379	1.754	0.831
Calanoid ZA	86.378	1.820	0.736

Table S4. Species information of zooplanton in five freshwater lakes.

Lakes	*Moina*	Calanoids
Qianliujia	*Moina micrura*	*Sinocalanus dorrii*, *Sinodiaptomus sarsi*
Qingken	*Moina chankensis*	*Sinocalanus dorrii*
Xihulu	*Moina chankensis*	*Sinocalanus dorrii*
Xiaoxingkai	*Moina chankensis*	*Sinocalanus dorrii*
Chagan	*Moina rectirostris*	*Sinocalanus dorrii*

Table S5. Top 4 potential bacterial functions of zooplankton-associated bacteria.

Potential bacterial functions	Relative abundance
RNA polymerase	0.41%
iron complex outermembrane recepter	0.40%
enoyl-CoA hydratase	0.40%
methyl-accepting chemotaxis protein	0.32%

SUPPORTING FIGURES

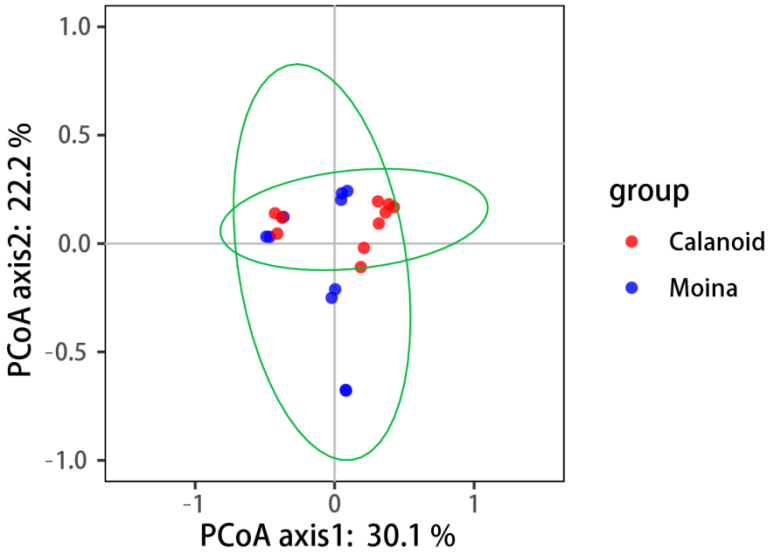

Figure S1. Divergence in two bacterial communities were presented by principal co-ordinates analysis (PCoA).

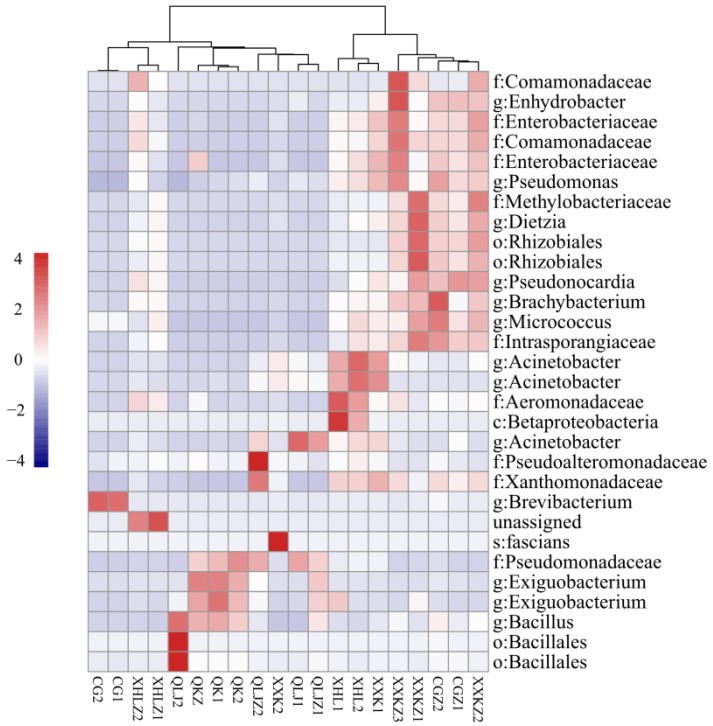

Figure S2. Heat map of top 30 OTUs among microbes associated with *Moina* spp. and Calanoids in five freshwater lakes. *Moina* samples are listed as XXK, QK, XHL, QLJ, CG and a following "Z" represents Calanoids. The highest taxonomy level was listed in the right.

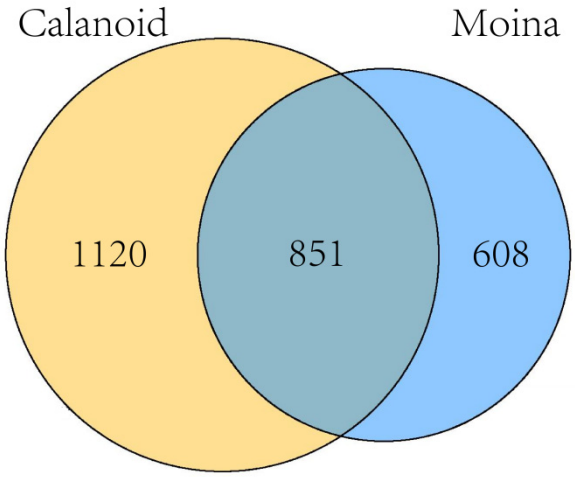

Figure S3. Shared OTUs between microbial groups associated with *Moina* and Calanoids in five freshwater lakes.

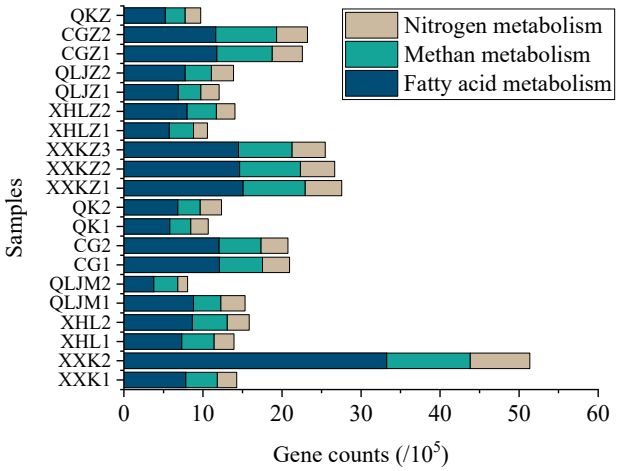

Figure S4. Nitrogen, methan and fatty metabolism gene counts across all samples associated with *Moina* (top panel) and Calanoids (lower panel) from five freshwater lakes are respectively shown. Lakes are listed as XXK, QK, XHL, QLJ, CG and a following "Z" represents Calanoids.