

Hemolymph metagenome of endemic amphipod *Eulimnogammarus verrucosus* from Lake Baikal



Ekaterina Shchapova¹, Anton Gurkov¹, Renat Adelshin^{1,2}, Natalia Belkova³, Maxim Timofeyev¹

¹Irkutsk State University, Irkutsk, Russia

²Irkutsk Anti-Plague Research Institute of Siberia and Far East, Irkutsk, Russia

³Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk

shchapova.katerina@gmail.com

Abstract

The aquatic environment contains a diverse and rich bacterial flora and, because of this fact, the presence of microbiota in the hemolymph is mostly reported for aquatic invertebrates. Moreover, some microorganisms not only survive in the animal hemolymph, but also can be beneficial for the host. Certain local microorganisms can compete with incoming ones and stimulate the host immune system, but in some cases, they can also become potential pathogens. The hemolymph microbiota, therefore, is a factor that must be considered when predicting the impact of negative environmental conditions on invertebrate populations. Crustaceans, in particular diverse endemic amphipods (Amphipoda), are one of the main ecosystem components in pristine Lake Baikal, but their hemolymph microbiomes have never been investigated using modern sequencing techniques.



Fig. 1 Lake Baikal. Photos Google maps



Fig. 2 The outflow of the Angara River. A – port Baikal; B - Listvyanka village. Photos Google maps

AIM

The aim of the study was to characterize the hemolymph microbiome of one of the most abundant littoral amphipods in Lake Baikal, *Eulimnogammarus verrucosus* (Gerstfeldt, 1858), by 16S rDNA amplicon meta-sequencing.

METHODS

DNA was isolated from pooled hemolymph of adult amphipods caught on the Baikal shoreline from two locations separated by the Angara River outflow. The first group of animals was collected in the port Baikal in June of 2016 (2 prepared hemolymph samples, 3 individuals per pool) and the second group was caught in Listvyanka village in September of 2016 (2 samples, 4 animals per pool). Amphipods were acclimated to laboratory conditions in aquaria for about a week. The surface of amphipod chitin was sterilized with ethanol before hemolymph extraction. The DNA samples were analyzed using a 454 GS Junior sequencer (Roche) after prior amplification of V4-V6 variable regions of 16S rDNA (500F primer: CCAGCAGCYGCGGTAAN; 1064R primer CGACRRCCATGCANACCT). The IDTAXA program with the GTDB database was used for taxonomic classification of the obtained amplicon libraries.

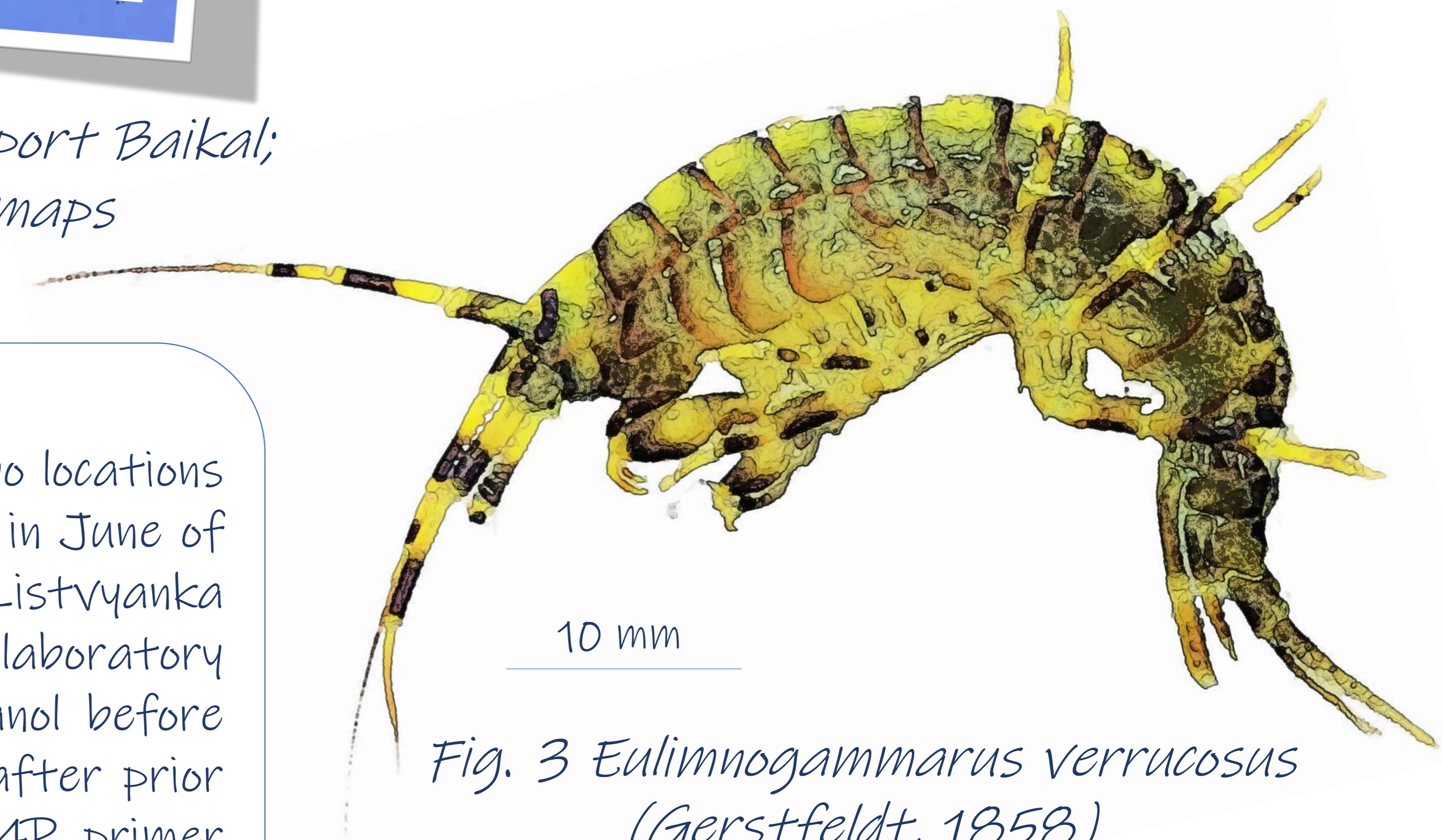
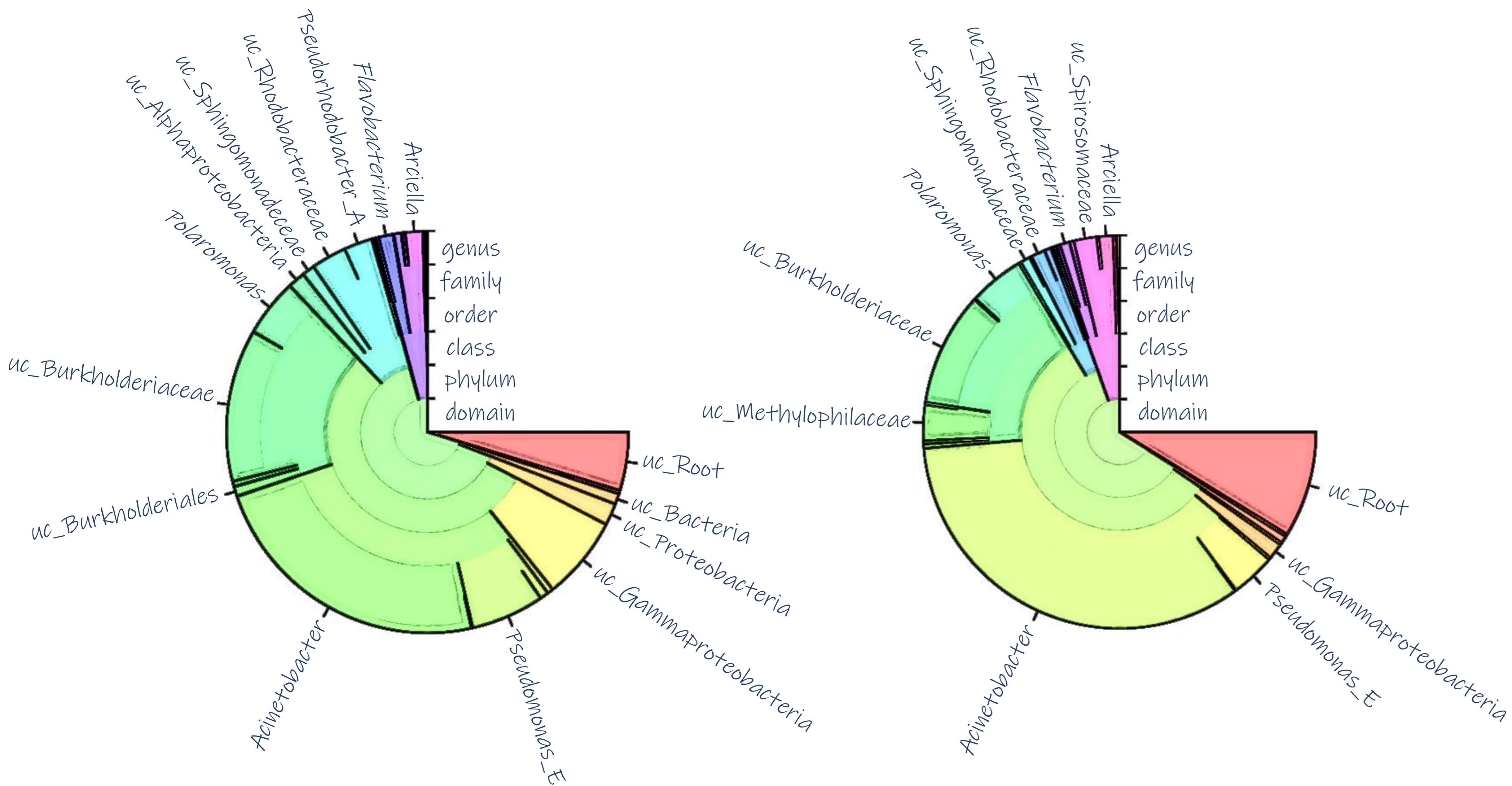
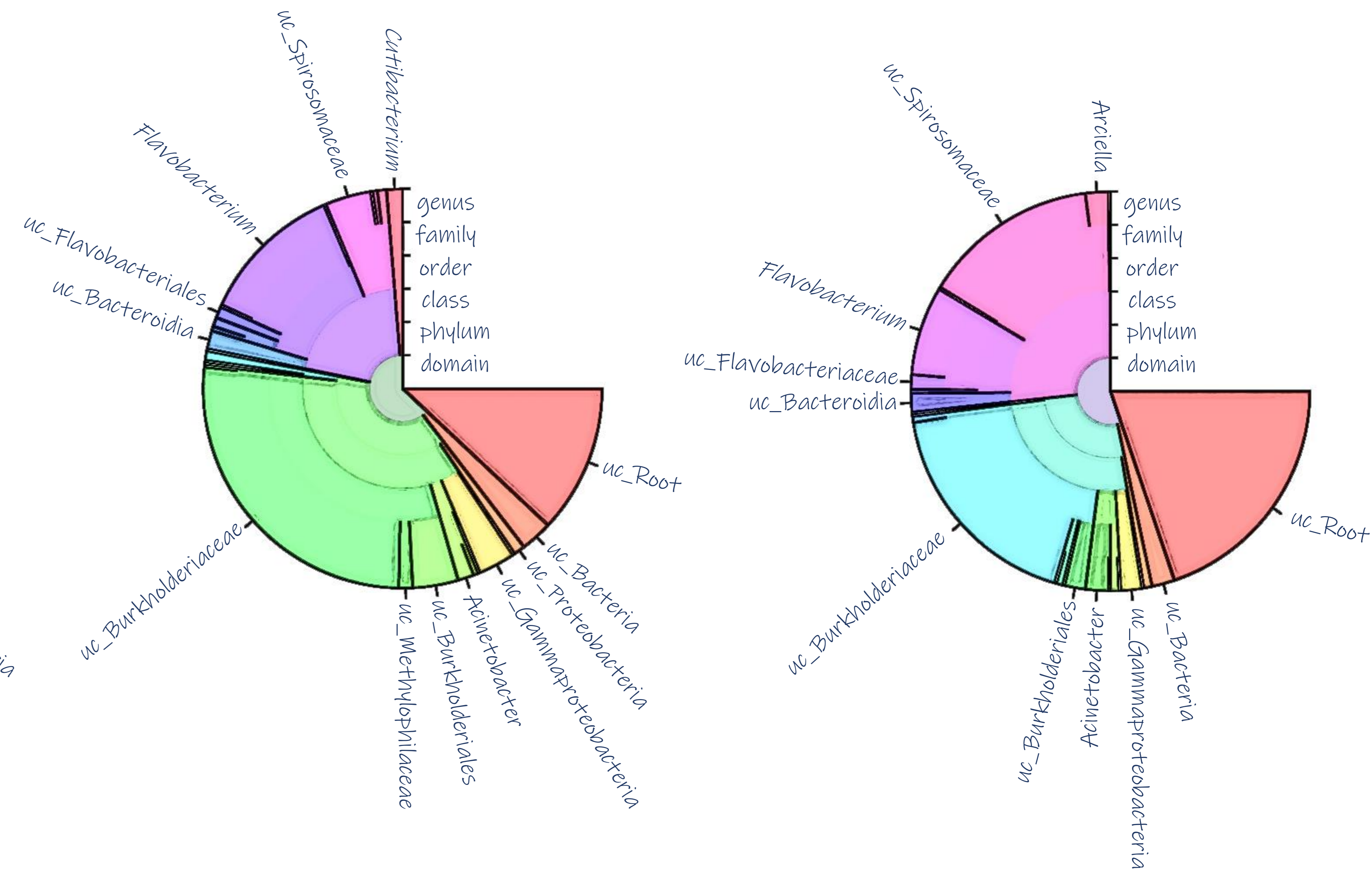


Fig. 3 *Eulimnogammarus verrucosus* (Gerstfeldt, 1858)

Individual variation in each of the samples.
Port Baikal



Individual variation in each of the samples.
Listvyanka village



RESULTS

Microbiomes of the analyzed hemolymph samples of *E. verrucosus* were generally similar within each of the locations but varied in composition between the locations. In particular, we identified in total 29 bacterial genera, but only five of them were present in all samples: *Acinetobacter*, *Flavobacterium*, *Arcicella*, *Polaromonas*, and *Pseudomonas*. As the core components of *E. verrucosus* hemolymph metagenome these genera may deserve the main attention during further research on host-symbiont relationships for amphipods of Lake Baikal.

ACKNOWLEDGMENT: The study was funded by the RFBR grant #19-34-90137