

Body-wall microbial diversity of elpidiid holothurians (Echinodermata: Elasipodida) in the Japan and Kuril–Kamchatka Trenches

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Marine invertebrates have been found to host diverse microbial symbionts, which have been reported to have vital roles for hosts, including disease resistance, digestion and tolerance to abiotic environmental conditions. These hosts and their microbes are often regarded as a single integrated unit, i.e. holobiont. Among deep-sea benthic invertebrates, holothurians or sea cucumbers (Echinodermata: Holothuroidea) are dominant in terms of abundance and biomass; therefore, their microbiome may play important roles in the ecosystem. Some previous studies have focused on the analysis of their intestinal bacteria, employing genomic or metabarcoding approach. These bacteria have been believed to protect the host from invasive viruses and harmful bacteria, and/or to have roles on metabolism. On the other hand, although bacterial symbionts of echinoderms are prevalent on the body surface and show high host specificity, very little is known about the body-wall microbiome of deep-sea holothurians.

In this study, we investigated the body-wall microbial compositions of abyssal and hadal holothurians of the family Elpidiidae (Elasipodida). Specimens were captured by 24 trawl hauls in the Japan and Kuril-Kamchatka Trenches (3,879–7,654 m) during the R/V Hakuho-maru cruises KH-22-8 and KH-23-5. The study elpidiids included 15 individuals of *Elpidia*, 19 of *Peniagone* and 19 of *Scotoplanes*. A piece of the body wall was cut off from each specimen and preserved in pure ethanol for molecular analysis. Total DNA was extracted using a Lab-Aid824s DNA Extraction Kit (ZEESAN), and then amplicon sequencing targeting the V3-V4 hypervariable region of bacterial 16S rDNA was conducted using an Illumina NextSeq 1000 platform (2 x 300 bp; Illumina) by an outsourcing company. Subsequent data analysis was performed with QIIME 2. The impacts of the generic position (*Elpidia*, *Peniagone*, or *Scotoplanes*), collection depth and locality on the microbial composition of the elpidiids' body wall were tested using statistical approaches. In addition, the DNA solutions were also subjected to amplification and sequencing of the mitochondrial 16S rDNA of the host holothurians for species identification and phylogenetic analysis.

All specimens of *Elpidia* were identified as *E. kurilensis*, while *Scotoplanes* specimens were found to comprise two species, which corresponded to the phylogenetic lineages, *S. hansenii* and *S. kurilensis*. Specimens of *Peniagone* were assigned to four (*P. mus*, *P. cf. coccinea*, and two unidentified) species belonging to two lineages. In 25 elpidiid samples, *Alphaproteobacteria* (phylum Proteobacteria), chloroplast (assigned to phylum Cyanobacteria in this analysis), unknown classes (phylum "OD1"), or a totally unassigned lineage were detected as predominant microbial groups (exceeding than 80% in class level). Furthermore, 14 ASVs was found to be dominant, which occupy more than 20% of the dominant microbial groups. In *Elpidia*'s body wall, two ASVs belonging to unknown classes (phylum "OD1") were dominant. BLAST searches revealed that these ASVs are related to the family *Candidatus* Oceanoplasmataceae, (phylum Mycoplasmatota) which consists of symbiotic bacteria of marine invertebrates. The result of statistical approaches indicated that the generic position affected to the microbial composition of elpidiid body wall significantly ($P < 0.001$), whereas the collection depth and locality did not. These results suggested phylogenetic constraint of the microbial composition in Elpidiidae and the presence of a dominant and important bacterial lineage in the abyssal and hadal

holothurians.

Keywords: Abyssal zone, Hadal zone, Holothuroidea, Microbial diversity, Symbiosis