

Large-scale metagenetic community analysis of epipelagic and mesopelagic copepods in the Pacific

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Planktonic copepods are among the most important zooplankton in marine food webs and biogeochemical cycles, and their community structures have a pronounced effect on global marine ecosystems. Marine planktonic copepods are ubiquitous and diverse with more than 2,500 described species, and there are many undescribed or cryptic species in the ocean. Because morphological identifications of copepods are time-consuming and require sophisticated expertise, few studies have focused on large-scale community structures of copepods especially in the Pacific Ocean. We investigated the large-scale community structures of epipelagic and mesopelagic copepods in the Pacific using metagenetic analysis of nuclear large subunit ribosomal DNA (28S). This method is rapid and taxonomically comprehensive, revealing community structure of copepods based on massive sequence data without depending on morphological classification. VMPS net was used to collect zooplankton samples at both epipelagic (0-200 m) and mesopelagic (200-500 and 500-1000 m) layers in each sampling site. Total >100 community samples covering 40°S-68°N and 138°E-95°W were collected during 2011-2015. After DNA extraction and PCR amplification of 28S D2 region, massive sequence data were obtained using high-throughput sequencers Illumina MiSeq. All sequence reads were quality-filtered, and approximately 18,000 sequence reads in each sample were clustered into Molecular Operational Taxonomic Units (MOTUs) for community analysis. Community structures of copepods were distinct between cold and warm waters both at epipelagic and mesopelagic layers, and community boundary existed between subtropical gyre and transition zones. In the epipelagic layer, copepod communities were almost corresponded with water masses including arctic, subarctic, transition, subtropical and equatorial zones. Within warm water regions in the low latitude, chlorophyll *a* concentration highly correlated with copepod communities, and communities in the Kuroshio region were more similar to those in the equatorial regions than to those in the subtropical regions. We also observed several groups of copepod communities even within the subtropical gyres. In addition to epipelagic layer, mesopelagic layer in the low latitude also showed different communities of copepods in the tropical, subtropical, and Kuroshio regions both at 200-500 and 500-1000 m depth. Therefore, small-scale community structure existed even within warm water regions in the epipelagic regions, which might affect community structures in the mesopelagic layer within relatively stable water environments. In the analysis of copepod diversity, latitudinal gradients were evident in the epipelagic layer. High diversity was observed in the tropical and subtropical areas, with a peak in the North Pacific subtropical gyre. This pattern was also observed in the mesopelagic layer, suggesting the influence of epipelagic ecosystems on mesopelagic communities. Although diversity was higher in the mesopelagic layer than in the epipelagic layer, the epipelagic community was composed of various taxonomic groups with high genetic diversity. Specific taxonomic groups were diverse in the mesopelagic layer, indicating that different mechanisms maintain diversity within each layer. Distribution patterns were clearly different between low-latitude and high-latitude groups. Phylogenetic analysis revealed recent divergence of the high-latitude group, suggesting that a relatively short evolutionary history is a factor underlying the low diversity at high latitudes. These results indicate that both environmental and evolutionary factors have determined the current diversity and biogeography of planktonic copepods.

