

Population density and genetic diversity of sympagic copepods in the Southern Ocean

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The Southern Ocean has an annual cycle of maximum sea ice extent in winter and minimum in summer (Parkinson and Cavalieri, 2012). Sea ice contains bacteria and protists and their predators (e.g., protozoa and metazoans) originally incorporated from seawater. Steady sea ice fixed to land or ice shelves is called fast ice, while sea ice that moves with the wind or currents is called drift ice (Toyota, 2018). The three copepod species, *Stephos longipes*, *Paralabidocera antarctica*, and *Drescheriella glacialis*, are known to occur in high density in drift ice of the Southern Ocean (Makabe et al., 2022). After sea ice transported to offshore and melting, the released copepods are rapidly incorporated into the food web by predation. In the Southern Ocean, 80% of the area of ice melts, and the amount of drift ice in the Southern Ocean is massive, these copepods have a significant impact on the ecosystem (Makabe et al., 2022). However, previous studies collected drift ice from limited sites without genetic information. To obtain the regional distribution and intraspecific genetic polymorphism and the biomass of copepods in drift ice, it is necessary to analyze a large number of sea ice samples collected from a wide range of observation sites. In this study, we focused on the three copepod species, *S. longipes*, *P. antarctica*, and *D. glacialis*, to clarify the distribution, population density, and genetic diversity within each species.

A total 18 pieces of drift ice were collected in three areas of the Indian Sector of the Southern Ocean (Lützow-Holm Bay, off Cape Darnley, and off Totten Glacier) by the 61st Antarctic Research Expedition from December 2019 to March 2020. *S. longipes*, *P. antarctica*, and *D. glacialis* were selected from Lugol's iodine-fixed sea-ice meltwater under a stereomicroscope, and the population densities at each life stage (nauplii, copepodite, and adult) were determined. Five to 10 individuals of each species were randomly selected per area, and DNA was extracted from each individual using a modified Lysis buffer method (Kobayashi et al., 2022). Using this DNA as a template, the mitochondrial cytochrome b gene (*mt-cytb*) and 18S rRNA gene (*18SrRNA*) were amplified by PCR. An evolutionary phylogenetic tree (maximum likelihood method) was created based on the nucleotide sequences of the PCR products.

In this study, *P. antarctica* was not detected from any sea ice samples. The average population densities of *S. longipes* in sea ice were approximately 1.6×10^4 ind/m³ and 0.6×10^4 ind/m³ for larvae (nauplii and copepodites) and adults, respectively, while those of *D. glacialis* were 0.4×10^4 ind/m³ and 0.2×10^4 ind/m³, respectively. The population density of *S. longipes* nauplii was higher than those of copepodites and adults. The population density was highest off Totten Glacier, followed by Lützow-Holm Bay and off Cape Darnley. Evolutionary phylogenetic analysis showed that *S. longipes* was monophyletic in both *mt-cytb*- and *18SrRNA*-based evolutionary phylogenetic trees, and no intraspecific genetic polymorphisms were identified. On the other hand, *D. glacialis* was monophyletic on the *18SrRNA*-based phylogenetic tree and divided into two lineages on the *mt-cytb*-based phylogenetic tree.

The different population densities at each growth stage were thought to reflect the diverse life cycles of the three copepod species. *S. longipes*, dominated by nauplii in sea ice, migrates to seawater with copepodites (Tamimura et al., 1996). On the other hand, *D. glacialis*, which showed no significant difference in population density among nauplii, copepodite, and adult, has a life cycle in sea ice throughout the year (Tanimura et al., 1996). *P. antarctica* survives in fall and winter sea ice as a nauplius and a copepodite stage (Tanimura et al., 2002). This species migrates to seawater in early summer following the maturation (Tanimura et al., 2002). Therefore, it was probably not found from summer ice in this study.

Molecular phylogenetic analysis revealed the presence of two haplotypes of *mt-cytb* in *D. glacialis*. The *18SrRNA* sequences of these individuals were nearly identical, suggesting that the two haplotypes are intraspecific genetic polymorphisms. These findings provide new insights into the ecology and genetic diversity of the sympagic copepods in the Southern Ocean.

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