Speciation in the *Patelloida saccharina* species complex across the Japanese Archipelago

PARAN, Faith Jessica Moron

Abstract

INTRODUCTION

The process of speciation among closely-related species and the underlying dynamics have long been challenging subjects in the field of evolutionary biology. Cytoplasmic DNA markers, which are commonly employed in molecular barcoding, have often proven insufficient in resolving phylogenies and other related subjects. However, the advent of next-generation sequencing technologies and reduced genome representation techniques have resulted in a significant improvement to resolve phylogenies of closely related species with greater detail. Furthermore, these approaches have enabled a much better understanding of the divergence and speciation patterns and processes. This study examined the extent of speciation in the *Patelloida saccharina* species complex using a combination of single-locus sequencing and single nucleotide polymorphism (SNP) discovery technology.

METHODOLOGY

The mitochondrial cytochrome oxidase I gene (COI), the 16S RNA (16S), and the nuclear histone3 gene (H3), as well as SNP markers, were sequenced from 165 specimens collected across 37 localities in the Japanese Archipelago. In total, 28 unique COI haplotypes, 16 16S haplotypes, 11 H3 haplotypes, and 13847 unlinked SNPs were obtained. The phylogenetic trees and genetic assignment analyses revealed three genetically distinct lineages: *Patelloida saccharina saccharina* (Linnæus, 1758), *Patelloida saccharina lanx* (Reeve, 1855), and an unknown *Patelloida* sp. From Shionomisaki, Kushimoto, Wakayama Prefecture.

RESULTS

Divergence time analysis estimated that the split between *P. saccharina saccharina* and *P. saccharina lanx* occurred ~45,000 years ago, which is too recent to accumulate morphological differences that would make it difficult for taxonomic identification. Demographic history analyses suggest that continuous gene exchange occurred after the initial split, allowing for the introgression of the *P. saccharina lanx* genome and the proliferation of intermediate individuals. The eventual reproductive isolation that followed after the initial split with gene flow led to speciation despite the existence of a

contact zone in the Ryukyu Islands. Using environmental niche modeling (ENM), I demonstrated that contemporary bioclimatic conditions were differentiated in the contact zone, where the habitats of *P. saccharina*, *P. lanx*, and admixed lineages overlap. These environmental parameters could be one of the factors influencing the distribution and maintenance of different lineages within a narrow range.

DISCUSSION

The results strongly support the separation of *P. saccharina saccharina* and *P. saccharina lanx* into two distinct species which should be taxonomically treated as *P. saccharina* and *P. lanx*. This warrants a revision to the current taxonomic descriptions of both *P. saccharina* and *P. lanx* based on their phylogeny, demographic history, and species distribution. As *P. lanx* is the more dominant species in the Japanese archipelago and nearby waters, I described the patterns of genetic distribution and population connectivity across its species range in Japan. Using mtDNA COI and SNP markers, I identified a north-south genetic differentiation between populations in the Ryukyu Islands and mainland Japan, as well as an east-west division between populations in the Sea of Japan and the Pacific seaboard. These population structuring patterns are common in marine species in the archipelago, given the major oceanographic currents that highly influence species distribution. However, the use of SNP markers enabled a fine-scale resolution that revealed sub-structuring between populations within the contact zone and between populations within the same biogeographic basin, as well as subtle genetic connectivity between basins. The genetic differentiation among samples along the same water routes could be attributed to local adaptations, while admixtures correspond to minor oceanographic patterns that sustain genetic connectivity. This investigation also expounds upon the morphological characteristics and molecular phylogeny of an undescribed species, while scrutinizing variations vis-à-vis *P. saccharina* and *P. lanx*. With the use of the 16S molecular marker, the samples procured from Wakayama and Kochi exhibit a monophyletic group with the antecedent samples with 16S and SNP sequences obtained from Wakayama, thereby corroborating the presence of *Patelloida* sp. in Kochi Prefecture.

CONCLUSION

The amalgamation of a multi-locus approach featuring both traditional markers and reduced genome representation technology, not only enhances our comprehension of the geographical distribution patterns of the *Patelloida saccharina* species complex and its populations but also expounds upon the speciation status of the species complex. Furthermore, this strategy elucidates the contemporary population genetic connectivity of

P. lanx with regard to contemporary oceanographic pathways. Lastly, the utilization of environmental niche comparison facilitates the identification of bioclimatic factors that affect the persistence of *P. lanx* beyond its recognized habitat.