

Doctoral Thesis

**Morphological and genetic variations of the *Sebastes inermis*
complex along coastal waters of Japan**

(Summary)

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Sebastes is a diverse group of rockfishes usually found in temperate waters and of economic importance for several countries. Diversification of *Sebastes* rockfishes is mainly driven by ecological constraints such as depth and resource availability. The high diversity of this genus is reflected through (1) the existence of many subgeneric categories containing closely related species with subtle morphological differences but occurring in broad sympatry; and (2) the convergence of morphologies across geographically and phylogenetically distant species groups. The *Sebastes inermis* complex encompasses three species (1) *Sebastes cheni* Barsukov 1988, (2) viz *Sebastes inermis* Cuvier 1829, and (3) *Sebastes ventricosus* Temminck & Schlegel 1843. These species occur sympatrically along coastal waters of Japan, and their phylogenetic relationships remain unresolved possibly due to introgression and/or incomplete lineage sorting. The implementation of management and conservation plans for these rockfishes in the future requires tools for their clear discrimination and knowledge about their population structures. Hence, this thesis studies the morphological and genetic variations between and within species of the *Sebastes inermis* complex.

Chapter II is focused on the diversification of body colouration in the *Sebastes* genus. This chapter is motivated because in rockfishes undergoing speciation one of the outcomes is the development of dissimilar colourations, which are often linked to differences in their depth distributions. For this chapter, photographs of 103 species and phylogenetic comparative analyses were used to cover the remarkable diversity of colours exhibited by rockfishes. These analyses not only verified the segregation of brightly- and darkly-coloured rockfishes based on depth but also confirmed that body colouration is a component of the ongoing correlational selection within the genus. Closely related species analyses confirmed that the primary divergence between species with dominant bright and dark body colours is linked to the divergence of fundamental ecological morphotypes. Depth was identified as the initial driver of colour differentiation, while gene drift and mutation contribute to further colour divergence between species.

Chapter III examines the ecomorphological distinctions among species within the *Sebastes inermis* complex, focusing on populations coexisting in the Seto Inland Sea. The chapter aims to understand how closely related rockfish species adapt to mitigate competition. Seven datasets related to depth distribution, trophic niche, prey-predation interactions, and communication systems were analysed. These datasets encompass (1) colouration, (2) meristic variables, (3) body proportions related to foraging, defensive response, and swimming performance, (4) landmarks in the body, (5) landmarks in the head, (6) univariate descriptors of otolith morphology, and (7) descriptors of otolith shape. The narrower body shape and golden-brown colouration in *S. cheni* suggest a more pelagic lifestyle than the other two species. Larger eye and otolith relative sizes, deeper body shape and red colouration suggest a deeper distribution for *S. inermis*, in alignment with longer pectoral fins and dorsal spines to cope with increased foraging difficulty and predation risk because of its colouration. Meanwhile, smaller upper jaws, higher number of gill rakers, and black colouration of *S. ventricosus* indicate a shallower distribution than *S. inermis* and preference for planktonic preys. Differences in colouration, eye size, and otolith descriptors support species-specific communication systems that are crucial to maintaining reproductive isolation. Hence, the morphological divergences among these rockfishes reflect specific ecological divergences that enable their coexistence in sympatry and can facilitate their morphological identification.

Chapter IV is focused on the genetic divergences and hybridization within the species complex. This chapter was motivated by the presence of putative morphological hybrids (PMH) with plausibly higher fitness than the parent species. The contrast between genetic divergence and hybridization within the complex offers the opportunity to explore the ongoing process of speciation-with-gene-flow, which can possibly lead to the emergence of new incipient species and/ or to reinforce the species boundaries of the parents. Genetic information from 10 microsatellite loci, and sequences from both the mitochondrial control region (D-loop) and the intron-free rhodopsin (RH1) gene, were utilized. In concordance with the three species, the analyses unveiled the presence of three distinct genetic clusters, large genetic distances using D-loop sequences, and distinctive mutations within the RH1 gene. Two microsatellite loci displayed signatures of divergent selection, indicating their association with genomic regions that are crucial for speciation. Furthermore, nonsynonymous mutations within the RH1 gene detected in *S. cheni* and “Kumano” (a PMH) suggest divergent adaptations for visual perception in dim-light environments. The presence of individuals with admixed ancestry between two species confirmed hybridization. The presence of nonsynonymous mutations within the RH1 gene and the admixed ancestry of the “Kumano” morphotype underscore the potential role of hybridization in generating novelties within the species complex. The chapter discusses hybridization considering hybrid fitness and assortative mating, concluding that genetic divergence is maintained despite hybridization, as expected in a scenario of speciation-with-gene-flow.

Chapter V is centred on the morphological and genetic variations within each species of the complex. Data of body and otolith shape, microsatellite loci, and D-loop from populations of each species was analysed. For these datasets, divergences in body and otolith shape, and genetics, and estimates of genetic diversity and effective population sizes were calculated. Intraspecific differences were found in body and otolith shapes. Populations from the same place but from different species exhibit convergent deformations suggesting a strong influence of the environment despite their different genetic background. Genetic homogeneity between distant populations of *S. cheni* (>1,500 km) contrasts the significant genetic differences between populations of each *S. inermis* and *S. ventricosus* that are geographically much closer. This indicates that the genetic structure of each is moulded by oceanographic conditions rather than the geographic separation of populations. The dissimilar patterns of genetic structure allow me to propose two different management alternatives: establishing management units within *S. inermis* based on significant genetic differences in D-loop or creating evolutionary significant units within each species considering the observed morphological and genetic differences. While populations of the three species exhibited high genetic diversities, some had low effective population sizes, raising concerns about how these populations would mitigate inbreeding depression in future generations.

To conclude, findings in this thesis shed light on the morphological and genetic features differing between the three species of the *S. inermis* complex, and within each respective species. These results can be used for understanding the speciation process in this complex and to improve management policies for their sustainable harvesting.