

**Genetic divergence between two sympatric coastal fish species,
Girella punctata and *Girella leonina***

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Introduction

Girella punctata and *Girella leonina* being regarded as sister species occur allopatrically despite having a sympatric distribution in the shallow rocky reefs south of the Japanese Islands in the Pacific Ocean. The taxonomic confusion about *Girella* had been caused in East Asia. The genetic relationships and behavior of *G. punctata* and *G. leonina* are interesting, particularly within a phylogenetic context.

Materials and Methods

A total of 113 specimens belonging to the genus *Girella* were collected at various coast in Japan. The morphological characters of *Girella* specimens were examined, and sequencing analysis for partial mitochondrial DNA (mtDNA) amplified by PCR was carried out. Subsequently, to estimate the divergence time between two species, Bayesian analysis was conducted using a data set comprised concatenated nucleotide sequences from 12S and 16S ribosomal RNA (rRNA) genes of the three Girellids and nine other fish species including an outgroup.

Results and Discussion

The differences in external morphological characters such as number of pored lateral line scales, color of opercular flap and shape of caudal fin between *G. punctata* and *G. leonina* corresponded with genetic characters derived from partial mtDNA sequences. Nucleotide identity in the 3.3 kbp region of partial mtDNA containing the D-loop region, 12S and 16S rRNA, and transfer RNA genes between the two species was 95%. Using the elopomorpha-clupeocephala split proposed by previous report as a calibration point (265 million years ago), divergence time between *G. punctata* and *G. leonina* was estimated as occurring 6.0 ± 1.4 million years ago. We suggest that the speciation between two species might have been caused by geographical isolation associated with the formation of the Japanese Islands, which resulted in disjunction of *Girella* habitat.