

# Species diversification through parallel freshwater adaptation in

## *Rhinogobius gobies*

Yo Yamasaki

### Background

Elucidating speciation patterns and processes is important for understanding how biodiversity has been shaped. Speciation studies involve learning about the increases in phylogenetic species richness and the evolution of reproductive isolation. However, these research areas have been pursued separately, and few attempts have been made to explain their relationship. An empirical study on this subject is difficult because phylogeny usually involves speciation events associated with various speciation modes. To understand the relationship between the two subjects, it would be necessary to target a simple system consisting of only one or a few speciation modes.

*Rhinogobius* is the most specious genus of the freshwater gobies in East and Southeast Asia. Most members of this genus have an amphidromous life history, but fewer than a half are freshwater forms that can be further classified into fluvial and lentic types. The key mechanism of the evolutionary diversification of *Rhinogobius* has been postulated to be a freshwater adaptation. Studying this system would improve our knowledge about the relationship between species richness and the evolution of reproductive isolation.

This thesis aimed to illustrate the importance of habitat expansion into new niches as a cause of species diversification and investigate the relationship between speciation patterns and processes, by studying the evolution of *Rhinogobius* gobies as a model system. First, an attempt was made to reconstruct the phylogenetic relationships among Japanese *Rhinogobius* species, thereby revealing the evolutionary patterns of freshwater species. Second, the factors affecting the evolution of reproductive isolation between

freshwater and amphidromous populations were examined. Based on these two studies, the increase in freshwater *Rhinogobius* species richness is discussed and explained as a condition for the establishment of reproductive isolation of freshwater populations.

## **Materials and Methods**

In Chapter 2, a phylogenetic analysis of all 18 known *Rhinogobius* species in Japan was conducted. Six nuclear and three mtDNA loci were sequenced and used to reconstruct separate phylogenetic trees. Divergence times and ancestral life history characteristics were estimated.

In Chapter 3, to infer the origins and the factors that had affected the evolution of freshwater forms, multiple origins hypothesis of the fluvial *Rhinogobius* sp. YB populations were tested using population genetic analyses. Specimens of the *Rhinogobius* sp. YB and their migratory ancestor *Rhinogobius brunneus* were collected from seven of the Ryukyu Islands. Twenty microsatellite loci were used for population genetic analysis. Potential speciation scenarios at several geographic scales were statistically compared using the Approximate Bayesian Computation framework. The effect of ecosystem size on speciation probability was analyzed using logistic regression.

## **Results and Discussion**

Based on six nuclear loci, the phylogenetic analysis of the Japanese *Rhinogobius* species revealed that they diverged in the early to mid-Pleistocene era (Chapter 2). Speciation through freshwater adaptation occurred at least four or five times. The estimated dates were approximately 1 million years ago in four of the five speciation events and 2 to 5 million years ago in the other event. These results suggest that the frequent, recent speciation with freshwater adaptation partly accounts for the species

richness of *Rhinogobius*. Comparisons between phylogenies based on nuclear and mitochondrial DNA have revealed that large-scale interspecific hybridization involving almost all Japanese species occurred approximately 1.5 million years ago.

Analyses using microsatellite markers have revealed five independent origins of the freshwater *Rhinogobius* sp. YB populations in the Ryukyu Archipelago (Chapter 3). Gene flow accompanied the speciation events from amphidromous to freshwater populations on the islands. The repeated speciation with gene flow strongly suggests that divergent natural selection promoted the evolution of the reproductive isolation of amphidromous and freshwater forms. There was a significant correlation between island size (a proxy for ecosystem size) and the speciation probability of freshwater populations. This correlation suggests positive relationships exist between ecosystem size and both the strength of divergent natural selection and size of the open niche.

## **Conclusion**

In *Rhinogobius* gobies, phylogenetic analysis revealed recent and frequent speciation with freshwater adaptation (Chapter 2). Reproductive isolation with adaptation to fluvial and lentic environments might have evolved by divergent natural selection. Fluvial species in the *R. brunneus*–*Rhinogobius* sp. YB complex are positively correlated with ecosystem size (Chapter 3). These observations suggest that large ecosystems offered strong divergent natural selection and facilitated the persistence of freshwater populations. For the frequent establishment of freshwater species of *Rhinogobius*, I hypothesized that the historical fluctuation of the available freshwater area might have played an important role in this speciation pattern, especially on islands such as the Japanese and Ryukyu Archipelagos. This thesis examined the relationship between species diversification and the evolution of reproductive isolation using a parallel evolution system.