Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Systematic and evolutionary studies of rissoellid microgastropods (Mollusca, Gastropoda, Heterobranchia)

（ガラスツボ科微小巻貝（軟体動物門・腹足綱・異鰓類）の体系学的および進化的研究）

In this study, I studied microgastropods belonging to the family Rissoellidae. The term "microgastropods" is here used to species that are less than 5 mm as adults. Rissoellidae comprises minute marine gastropods, measuring approximately 1 mm in shell length. Those microgastropods have a smooth, transparent/translucent or sometimes whitish shell. They also possess an operculum with unique morphology, which is one of the defining characteristic of the family. The operculum is yellowish, transparent, with a sharp ridge on the internal side along the columellar ridge, from which a vertical, blunt peg arises, and a short, rounded ridge passes across a portion (less than half) of the operculum. The head bears a pair of oral lobes arising from the snout, and a pair of longer cephalic tentacles. The soft body is colorful, especially around the hypobranchial gland. Other characteristics of rissoellids include a variable radula, with a central tooth, a lateral tooth, and 0–2 marginal teeth per row. Ctenidium, esophageal glands, and crystalline styles are lacking, similary to other basal heterobranchs. Rissoellids are hermaphroditic and undergo direct development, having a simple penis and a simultaneous hermaphrodite gonad. Rissoellids have a worldwide distribution and can be found in different habitats such as soft, sandy bottoms or hard substrata with algae, but they mostly associate with algae in shallow waters, where they rasp the algal surface and are believed to feed on microalgae and detritus matter. Rissoellidae remains one of the least studied taxon despite its worldwide distribution and common presence in the intertidal zone. Furthermore, although the presence of rissoellids in Japanese waters has been reported, most of them remain undescribed. Thus, the aims of this study are to unveil the species diversity of rissoellids based on morphological and molecular (barcode) studies from Hokkaido, Japan (Chapter 1) and to discuss evolutionary characters of one of the most important organs, i.e. radula (Chapter 2) based on multigene phylogenetic data in rissoellids collected in Japan and New Zealand.

Chapter 1 deals with the species diversity of microgastropods of the family Rissoellidae in Japan, and the description of a new species is included. *Rissoella elatior* (Golikov, Gulbin & Sirenko, 1987), *Rissoella golikovi* (Gulbin, 1979), *Rissoella japonica* n. sp., and *Rissoella* sp. 1 were collected in different locations around Hokkaido, Japan. To species identification, I applied the most traditional morphological characters (body color pattern, shell, and radular morphology) in the taxonomy of rissoellids. This information was complemented with the amplification of a region of the mitochondrial cytochrome c oxidase subunit I (COI) gene. *Rissoella elatior* is morphologically characterized by a highly asymmetrical radula with a deep notch encircled by 10–13 minute secondary cusps on the left dorsal margin of the central tooth. *Rissoella golikovi* is characterized by a skeneiform shell and possession of three teeth per row on the radula. *Rissoella japonica* n. sp. has *i*) five teeth per row on the radula; *ii*) a central tooth that is higher than wide; *iii*) lateral and marginal teeth that are narrow, with an outer lateral projection at the base; and *iv*) every tooth of the radula presenting numerous small cusps on the cutting edge. *Rissoella* sp. 1 is distinguished from *R. japonica* n. sp. in having i) very short oral lobes, ii) a mantle with a large, black patch and whitish blotches inside, and iii) different color patterns associated with the visceral mass. Although *Rissoella* sp. 1 probably represents another new species, additional specimens are needed to complete its morphological description. This study represents the first insight into the genetic diversity of the family Rissoellidae. While four morphospecies were recognized, the addition of COI data raised the count to eight potential species, suggesting the existence of cryptic species among rissoellids. Admittedly, I was not able to distinguish hidden lineages using those morphological traits alone. To distinguish those "possible" cryptic species might require, perhaps, detailed study of the internal anatomy (e.g. genitalia). This is the first time that molecular techniques have been applied in the taxonomy of this family.

Chapter 2 provides evolutionary hypotheses of the rissoellid radular morphology based on a multigene phylogenetic approach using mitochondrial (COI and 16S rDNA) as well as nuclear (18S rDNA and 28S rDNA) sequences. The radula is one of the most important morphological characters in the taxonomy of rissoellids because it shows a great interspecific variation. For that reason, it is important to explain the evolutionary transformation of this (the radula) taxonomic character which might help to understand species diversification, specialization. I analyzed specimens assigned to nine different *Rissoella* species collected in Hokkaido, Japan, and the South Island of New Zealand, using molecular markers, combined with radular morphology. A vast interspecific variation was found in the rissoellid radular morphology; three major groups, however, are recognized based on the number of teeth per row (three, five, and seven teeth). The phylogenetic tree showed that species with seven teeth per row formed a clade, while those with five teeth did not; only one species with three teeth was included in the analysis. The molecular analyses also suggest that the major event that has characterized the rissoellid radular evolution would involve the development of the marginal teeth. The analyses also revealed that the plate-like outer marginal teeth did not represent vestigial teeth, but a derived state. My results suggest that a factor that could influence the radular morphology in the Rissoellidae is the diet rather than the substrate. The inclusion of other species in future studies is necessary to provide a better understanding about the evolutionary radular transformation in the Rissoellidae.