

Systematics and Diversification Patterns of the Extant Freshwater Snail Genus *Semisulcospira* (Semisulcospiridae: Caenogastropoda: Mollusca) in the Ancient Lake Biwa

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Introduction

Ancient lakes are hotspots of speciation. Lake endemics have played crucial roles in the elucidation of diversification process of organisms. The freshwater snail genus *Semisulcospira* has extremely diverged in the water system of the ancient Lake Biwa, Japan with a history of approximately four million years.

Establishment of taxonomic accounts as well as clarification of phylogenetic relationships and natural history of lake endemics are essential for understanding their diversification process. Species richness of the lacustrine *Semisulcospira* has been assumed to be well understood by the past taxonomic studies based on shell morphology and karyotype. However, neither genetic relationships among *Semisulcospira* species nor type specimens of older species have not been investigated by the previous taxonomic studies. Furthermore, knowledge of morphological diversification and niche differentiation among the species has been fragmented. Information on the intraspecific variation of their diagnoses, which are important for the evaluation of morphological differences, has also been lacked.

In the present thesis, the type specimens of all the 1800's species were examined, and morphological and genetic analyses were conducted to revisit taxonomic accounts of *Semisulcospira*. In addition, morphological differentiation and segregation among non-rupicolous species were investigated. The growth-related changes of shell morphological characters were also evaluated.

Materials and Methods

The name-bearing types of 12 nominal taxa and newly collected living snails of *S. niponica*, *S. decipiens*, *S. reticulata*, and their relatives were used for systematic revision. Their teleoconch, and protoconch, radula, and reproductive organ of the newly collected specimens were examined.

Shell morphological analyses were conducted employing measurements of traditional morphological characters and/or geometric morphometrics based on 2D landmarks. The growth-related changes of *S. niponica* were also explored using the same methods.

Genetic relationships among newly collected specimens were analysed using Multiplexed ISSR Genotyping by sequencing (MIG-seq). Population genetic structures and phylogenetic relationships were estimated based on the obtained SNPs. For ecological assessments, semisulcospirid fauna of coastal non-rupicolous habitats was assessed at three lake sites using a quadrat method. Additionally, information on the habitat preference of the lacustrine *Semisulcospira* were obtained from the newly collected snails and bibliographic records.

Results

Systematic revision

Taxonomic accounts of 13 described species were arranged and seven new species were established. In addition, the two species flocks were taxonomically defined as the *S. niponica*- and *S. nakasekoe*-groups and they were concluded to include ten and nine extant endemics in the Lake Biwa system, respectively.

Niche differentiation and morphological evolution

The conditions that whether rupicolous substrates or not, and vertical zonation on non-rupicolous bottoms were estimated to shape distribution of the lacustrine *Semisulcospira* in coastal areas. Non-rupicolous species of both species groups comprised three layers and geographical differences of species composition were observed only in the *S. nakasekoe*-group. Interspecific morphological

diversification was detected in teleoconch, protoconch, and radula parallelly between both species groups.

Growth-related change of shell morphological characters

Both traditional and geometric morphometrics have detected sexual dimorphism and allometric growth in *S. niponica*. Females possess larger and wider teleoconchs than males. Different growth patterns between males and females, and the correlations between measurements of teleoconch and protoconch were observed.

Discussion

Taxonomic accounts of the 20 nominal species/subspecies of the lacustrine *Semisulcospira*, including all species described in the 1800s, were arranged. Patterns of niche differentiation and morphological diversification of the genus were also elucidated. In addition, appropriate sample conditions for morphological examination were clarified by the analyses of growth-related morphological changes.

The observed geographical differences in species composition between the two species flocks suggest their unique diversification processes in different periods of the lake. Similar morphological features of teleoconch, protoconch, and radula detected parallelly among the *S. niponica*- and *S. nakasekoe*-group species in similar habitats indicate multiple adaptation to inorganic conditions of habitats and parallel evolution of its reproductive strategies and trophic morphology occurred in the genus.

The present taxonomic arrangements and clarification of morphological and ecological differentiation are essential to understand the diversification patterns and process of *Semisulcospira* in Lake Biwa. The observed preferences for different substrates and depths among radiated members reinforced the previous estimation that niche differentiation played a major role in early stages of their speciation. Knowledge accumulated by this study can be a foundation for further integrated

studies, including analyses of genome and karyotype, and will lead to elucidation of the fundamental mechanisms of speciation and maintenance of diversity in ancient lakes.