

# Evolution and adaptation of the epizoism in Patellogastropoda

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## Intorduction

Epizoism is a lifestyle of organisms characterized by living on the surfaces of other animals. The relationships between epizoites and hosts differ in each case, and adaptive significances of epizoites are also different. Understanding epizoism in marine invertebrates would be a key to clarifying the symbiotic relationship in marine ecosystems. Patellogastropoda is one of the common animals in marine ecosystems. Most of the species inhabit hard platforms such as rocky shores, however, several species showed epizoism and are usually found on mollusks shells. However, studies focused on the epizoism of Patellogastropoda are poor. *Lottia tenuisculpta* is a tiny lottiid limpet distributed in Japan and also an epizoic species usually found on tegulid and muricid snails. In this thesis, I demonstrate the evolution and adaptive significance of epizoism for Patellogastropoda by investigating *L. tenuisculpta* through the aspects of phylogenetic position, host preference, life-history, and larval settlement.

## Materials & Methods

### *Phyhlogenetic analysis*

To reveal the phylogenetic positions of *L. tenuisculpta*, 113 specimens of *L. tenuisculpta* and 42 specimens of *L. lindbergi*, a sister species of *L. tenuisculpta*, were collected from both whole

distribution areas. Molecular phylogenetic analyses using COI, 16S and Histone H3 were conducted.

Haplotype network analysis of *L. tenuisculpta* and *L. lindbergi* was also conducted based on COI gene.

#### *Host preference*

To clarify the host preference of *L. tenuisculpta*, specimen survey based on samples of limpet collected from their entire distribution area along with substrate organisms, and field observations focusing substrate utilization rates of the limpet in Shirahama, Wakayama Prefecture were conducted.

#### *Life-history*

The life-history of *L. tenuisculpta* was investigated through 13 months of periodic sampling in the rocky coasts of Shirahama, Wakayama Prefecture. Over one hundred individuals of the primary two hosts, *Tegula nigerrima* and *Reishia clavigera*, were collected every month from September 2013 to September 2014, and limpet on them were measured and counted. Limpets more than 4 mm in length were marked with paint marker for tracking.

#### *Larval settlement*

The settlement preference of the larvae of *L. tenuisculpta* was investigated using planktonic larvae. About 2,600 individuals of planktonic larvae were prepared in each 500 mL beaker which filled with filtered sea water. Four small plastic plates covered with benthic diatoms were used as settlement substrates. Three of them were pasted different mollusks mucus: tegulid host (*T.*

*nigerrima*), muricid host (*R. clavigera*), and conspecific adult. The remaining one was gently smeared with filtered sea water as a control. After 24 hours, the settled and metamorphosed larvae on each plate were counted.

## Results

### *Phylogenetic analysis*

Molecular phylogenetic analysis clarified that *Lottia* comprises four distinct clades, and *L. tenuisculpta* and *L. lindbergi* were placed within the North Pacific lineage. Haplotype network of *L. tenuisculpta* and *L. lindbergi* exhibits different shape, suggesting expansion of both species occurred at different times.

### *Host preference*

*Lottia tenuisculpta* was attached to wide variety of marine invertebrates, including 33 species of mollusks and 2 species of crustaceans (Balanomoropha), but predominantly utilized the molluscan family Tegulidae and Muricidae throughout its geographic range.

### *Life-history*

The result showed that numerous juveniles appear on hosts in early spring, but their numbers decrease as their shell length increases, and in autumn, they move from the hosts to the rocky shores. Additionally, it was found that the shell length of the limpet on hosts depends on the species and size of the host, as well as the number of other conspecifics attached to the same host.

### *Larval settlement*

The number of settled larvae was significantly higher in the mucus of the tegulid host than in the control.

### **Discussions**

The phylogenetic position of *L. tenuisculpta* demonstrated that epizoism is limited to the family Lottiidae and Patelliidae within Patellogastropoda., suggesting the epizoism has evolved at least twice in Patellogastropoda.

The host preference of *L. tenuisculpta* suggests that this limpet is a host-general species which has a preference for certain families, and host utilization is determined by the size and abundance of the potential substrate animals.

The investigation of the life-history of *L. tenuisculpta* reveals that morphological and ecological constraints of the host influence the epizoite in terms of growth, survival, lifestyle, and intraspecific competition.

The larval settlement preference of *L. tenuisculpta* suggests attachment to the host likely occurs at a very early stage of its life-history.

These results indicate that the epizoic ecology of *Lottia tenuisculpta* is advantageous in environments with limited resources or high competition. It is adaptive for reducing interspecific and intraspecific competition, decreasing physical stress, and increasing access to available resources.