**Shell colour dimorphism in *Littorina horikawai* on Tsushima Island, Japan**

Daishi Yamazaki1\*, Takumi Saito2, Hiroshi Fukuda3

1Center for Northeast Asian Studies, Tohoku University, 41 Kawauchi, Aoba-ku, Sendai, Miyagi, 980-8576, Japan. 2Department of Biology, Faculty of Science, Toho University, Funabashi, Chiba, Japan. 3Conservation of Aquatic Biodiversity, Faculty of Agriculture, Okayama University, Tsushima-naka 1-1-1, Kita-ku, Okayama 700-8530, Japan.

\*Corresponding author, e-mail: zaki.daishi@gmail.com, Tel: +81-22-795-7560.

**Abstract**

The marine snail genus *Littorina* has been treated as a model group for ecological and evolutionary studies. It includes a morphologically and ecologically enigmatic species in East Asia, *Littorina horikawai*. Here, we illustrate shell colour dimorphism of *L. horikawai* collected in Tsushima Island in Nagasaki Prefecture, Japan. Molecular analysis indicated that shell colour dimorphism is intra-specific variation. Because the morphological characteristics, including shell colour variation, of *L. horikawai* have not been sufficiently studied in the entire distribution area, further investigation is necessary to understand the morphological diversities of *L. horikawai*.

**Key words:** Gastropoda; Littorinidae; *Littorina horikawai*; Japan Sea; Tsushima Island; color dimorphism; intraspecific variation; intertidal

**Introduction**

The marine snail genus *Littorina* Férussac, 1822 is one of the most important intertidal organisms for ecological and evolutionary studies because of its high levels of morphological, ecological, and genetic variations (Rolán-alvarez et al. 2015; Johannesson 2016). Particularly in the European coastal areas, *Littorina* species have long been treated as a model study taxon (e.g. *L. saxatilis* (Olivi, 1792)). In contrast, the East Asian *Littorina* includes an enigmatic species whose ecology and morphology are not sufficiently known: *Littorina horikawai* Matsubayashi and Habe in Habe, 1979 (Reid 1996; Johannesson 2003).

**Materials and Methods**

**Field survey and material investigation**

In October 2018, a field survey and sampling in Tsutsu Misaki Cape, at the south western corner of Tsushima Island, was conducted by searching for *Littorina horikawai* for about 30−60 min along a 15–20 m width of shoreline (Fig. 1: 34°06′10.5″ N; 129°10′06.8″ E). We also qualitatively checked the substrate to which *L. horikawai* was attached. In the laboratory, we dissected adult snailsto determine their sex. Fisher’s exact test was used to determine whether there was a difference between sexes in the colour morphs. The water temperature measured was 25 ºC.

In addition, we examined specimens of *L. horikawai* collected from Koshōjima Island, at the central part of Tsushima Island, in August 1974 (Fig. 1: 34°27′08.3′′ N; 129°22′41.5′′ E). This was done by one of the present authors (H.F.). The sexes of these individuals were not examined.

**Molecular experiment**

A portion of muscle tissue from two individuals each of the red and black shell morph of *Littorina horikawai* was cut and stored in 99.5 % ethanol (20 ml) for subsequent molecular experiments. Total DNA was extracted using the method described by Hirano et al. (2015). Fragments of the cytochrome b (*Cytb*) gene were amplified according to the method outlined by Azuma et al. (2017). The obtained PCR products were purified using ExoSAP-IT (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Cycle sequencing was performed at 50°C using the PCR primers with the BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). The products were directly sequenced from both directions using an ABI 3130xl automated sequencer (Applied Biosystems). We checked the validity by inspecting the quality scores of each base using the software package 4Peaks (Griekspoor and Groothuis 2004) and the sequences were aligned using MUSCLE v3.8 (Edgar 2004).

**Results**

On Tsushima Island, we observed shell colour dimorphism in *Littorina horikawai* (Fig. 2). Amongst those collected in 2018, the two colour morphs of *L. horikawai* were attached to rock at the upper part of the intertidal zone in Tsutsu Misaki Cape (Fig. 3). The black morph was more abundant than the red morph (black: 24 individuals; red: 7 individuals). There was no significant difference in the number of red and black morph individuals between male and female (Table 1). On Koshōjima Island, amongst the individuals collected in 1974, there was no clear difference in the number of individuals in the red and black morphs (black:119; red: 104). The difference was 10 %.

The length of the *Cytb* sequences determined was 426 bp, and all sequences of four individuals, including both red and black shell morphs, were identical one another (GenBank accession number: LC334556–LC334600).

**Discussion**

The present study is the first to show a colour-printed illustration of the shell colour dimorphism of *Littorina horikawai* on Tsushima Island. Genetic analysis revealed that shell colour dimorphism occurs within a single species. The colour morph is consistently the same colour from the shell apex to the aperture, and both of them were observed to attach to the same type of substrate. In addition, this colour dimorphism was observed in both the 1974 and 2018 specimens.

**Acknowledgements**

We appreciate the suggestive and helpful comments on the present study by four anonymous reviewers.

**References**

Azuma, N., Zaslavskaya, N. I., Yamazaki, T., Nobetsu, T., Chiba, S. (2017). Phylogeography of *Littorina sitkana* in the northwestern Pacific Ocean: Evidence of eastward trans-Pacific colonization after the last glacial maximum. Genetica 145: 139–149.

Bond, A. B. (2007). The evolution of color polymorphism: crypticity, searching images, andapostatic selection. Ann. Rev. Ecol. Evol. Syst. 38: 489–514.

Griekspoor, A., Groothuis, T. (2005). 4peaks. Ver. 1.7.1. Available at: http://nucleobytes.com/4peaks/.

Hasegawa, K. (2017). Family Littorinidae. In: Okutani, T. (Ed.), Marine Mollusks in Japan, the Second Edition. Tokai University Press, Hiratsuka, pp. 112–114.

Higo, S., Callomon, P., Goto, Y. (1999). Catalogue and Bibliography of the Marine Shell-bearing Mollusca of Japan. Elle Scientific Publications, Yao, Ōsaka.

Hirano, T., Saito, T., Chiba, S. (2015). Phylogeny of freshwater viviparid snails in Japan. J. Mollusc. Stud. 81: 435– 441. (In Japanese with English abstract).

WoRMS (2022). C*helarctus* Holthuis, 2002. https://www.marinespecies.org/aphia.php?p=taxdetails&id=382. (accessed on 20 July 2022).

Figure legends

Fig. 1. Size composition of collected in Tsushima Islands.