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

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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 the 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; colour 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).

Littorina horikawai inhabits the intertidal zones of rocky shores. It is distributed along the western coast of Kyushu, Japan, and its adjacent islands including Tsushima Island, Iki Island, Hiradojima Island, Amakusa-shimoshima Island, the Kusagaki Islands, Satsuma-Kuroshima Island, Tanegashima Island, Yakushima Island, and Jeju Island of South Korea (Reid 1996; Higo et al. 1999; Yata and Shiosaki 2001; Kuwazuru et al. 2004; Hasegawa 2017; Lee et al. 2018; Yamazaki 2019). Because the dispersal ability of *L. horikawai* is recognised as low level (Erlandsson 2002; Johannesson 2003), it may facilitate differentiation among local populations such as morphological variation. On Hiradojima Island, the type locality of *L. horikawai*, a dark to pale marbled shell colour polymorphism occurs continuously (Reid 1996). However, little is known about the range of shell variation and the local morphological differentiation of *L. horikawai* (Johannesson 2003), because there

have been very few literature records after its description.

Several *Littorina* species were collected at Tsushima Island in 1971 and 1974, which were recorded as undescribed species (Yakuwa et al. 2009; Hasumi and Honma 2019; Kumamoto Prefecture 2008). The specimens collected in August 1971 include two shell colour morphs (red and black), which are deposited in the Yamagata Prefectural Museum (Collection number: 3Ga000325; Yamagata Prefectural Museum 2017; Hasumi and Homma 2019). In 1979, *L. horikawai* was formally described based on specimens from Hiradojima Island. Thereafter, the populations of Tsushima Island, including the red and black shell morphs, have been treated as *L. horikawai* and it is known that the number of red morph individuals is small (Reid 1996). However, it has not been confirmed whether colour dimorphism is intraspecific variations.

In the present study, we illustrate both shell colour morphs of *L. horikawai* on Tsushima Island and report the number of individuals of each colour morph. First, field observations and material investigations were conducted to obtain information on the colour dimorphism of each shell. Thereafter, molecular analysis was performed to test whether this shell colour dimorphism occurs within a single species.

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 snails to determine their sex. Fisher's exact test was used to determine whether there was a difference in the sex ratio of the colour morphs because it is known that some groups of caenogastropods (e.g. cyclophoroideans and truncatelloideans) show sexual dimorphism in shell colouration (H. F. in preparation).

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.

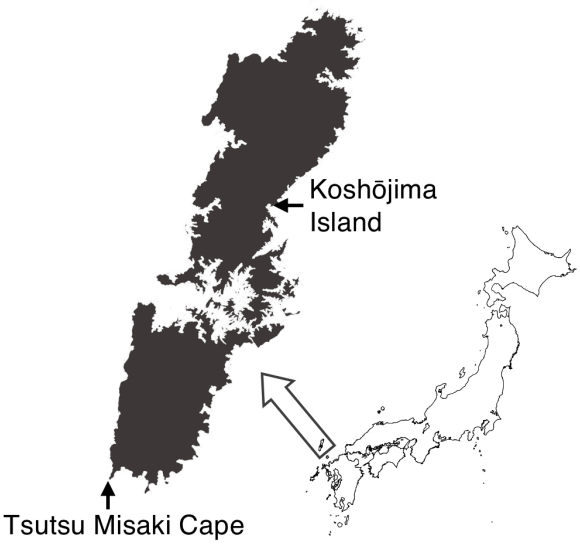

Fig. 1. Map of survey localities in Tsushima Island.

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 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 using the PCR primers with the BigDye™ 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).

Fig. 2. Shell colour dimorphism (red and black) observed in *Littorina horikawai* on Tsushima Island.

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 the males and females (Table 1; $P > 0.05$). 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 length of the *Cytb* sequence alignment was 426 base pairs. All four individuals, including both red and black shell morphs, share a single haplotype (GenBank accession number: LC640085–LC640088).

Table 1. The number of two shell colour morphs (black and red) in male and female of *Littorina horikawai*.

	black morph	red morph
male	11	3
female	13	4

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

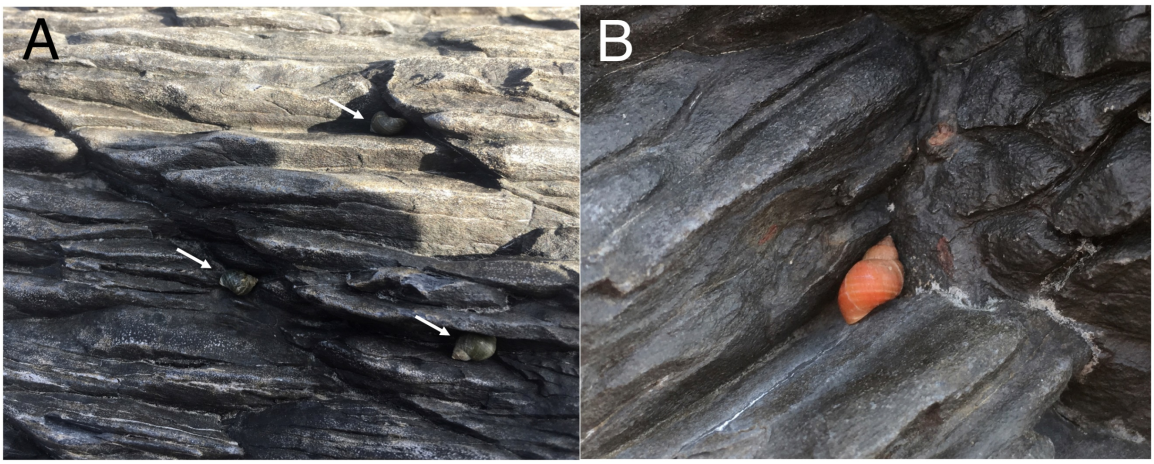

Fig. 3. Field photographs of *Littorina horikawai*. (A) Three individuals of black shell morph indicated by white arrows. (B) Red shell morph.

substrate. In addition, both the 1974 and 2018 specimens contained this colour dimorphism. This indicates that colour dimorphism is a fixed trait in the Tsushima Island population. The reason for the occurrence of colour dimorphism, including conspicuous colour morphs, is currently unknown, at least from the field observations conducted in 2018. In addition, because both the black and red morphs were attached to the same dark rock, we could not find a specific association between colour dimorphism and their preferred substrates.

For other *Littorina* species, the presence of the red colour morphs is thought to be maintained by a stochastic process, such as genetic drift and/or the founder effect (Reid 1996). Furthermore, apostatic selection (negative frequency-dependent selection) is an important mechanism for maintaining colour polymorphism within a population through visually hunting predators such as bird (sensu Johanesson and Butlin 2016; Bond 2007). However, it is not known if birds prey on *L. horikawai*, even though other *Littorina* species distributed in the Japanese Archipelago are preyed on by birds (e.g. *L. brevicula* (Philippi, 1844): Morii et al. 2021). Johannesson and Butlin (2016) have suggested that the conspicuous shell colour morph, such as red morph within the population, is maintained by a complex mechanism involving multiple processes. The colour dimorphism observed in Tsushima Island population is not known in other local populations of *L. horikawai*. Since *L. horikawai* belongs to direct larval development group (Erlandsson 2002), gene flow between populations may be restricted. Therefore, the colour dimorphism may have been maintained only in Tsushima Island. Our present results showed that the ratio of red and black individuals is spatiotemporally different on Tsushima Island. To explain why the colour dimorphism of *L. horikawai* has been maintained, it is necessary to examine the population demographics and the influence of visually hunting predators by a regular field investigation.

The genus *Littorina* has long been a popularly studied group of intertidal organisms. However, for *L. horikawai*, there is insufficient information. The present study showed an interesting colour dimorphism that occurred in the Tsushima Island

population, which differs from the morphological characteristics of the Hiradojima Island population (Reid 1996). Because *L. horikawai* inhabits offshore islands on the west coast of Kyushu, each population may have undergone its own morphological evolution. Therefore, it is necessary to deepen the understanding of this species through morphological comparison, and genetic and ecological investigations of each population.

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