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Short Note

Unique morphological characteristics of Japanese sand lances, genus *Ammodytes*, possibly endemic along the northern Sanriku Coast

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Abstract — Herein, we report a unique morphological characteristic of Japanese sand lances, genus *Ammodytes*, inhabiting the northern Sanriku Coast. Japanese sand lances are distributed widely in Japanese coastal waters and possess vivid spots on the both sides of their head, which reflect environmental light and can be visually observed. However, the population in Otsuchi Bay, Iwate completely lacks these spots, which is mutual in the sympatric species *A. japonicus* and *A. heian*. This difference in the local population in the Otsuchi Bay relative to those in other localities has been likely overlooked because the spots quickly disappear after the fish dies; therefore, they are not recognizable in preserved specimens. Furthermore, the population in Sendai Bay, Miyagi consists of individuals with these spots present (75%) and absent (25%), suggesting an immigration of the latter from the northern Sanriku Coast. Microscopic observation revealed that the spots are iridophore-like cells because of their oval shape and the absence of pigments and are probably connected to the nervous system. The present study should encourage future research to clarify the taxonomy and population structure of Japanese sand lances, whose stock has been decreasing and for which a management plan is urgently needed.

Key words: *Ammodytes*, iridophore, Japanese sand lance, morphology, Otsuchi Bay, Sanriku Coast

Introduction

Sand lances, genus *Ammodytes*, play an important role in the food-web of the coastal ecosystem by linking nutritional levels between zooplankton and larger predatory organisms. Indeed, sand lances are consumed by numerous species in various taxa, such as fishes (>45 species), sea birds (>40 species), and sea mammals and whales (>12 species) (O'Connell and Fives 2004). In addition, they are an important fishery resource and have been utilized as food and aquaculture feed. However, the stock of Japanese sand lances has been decreasing rapidly, and urgent resource management is required (Nakamura et al. 2017).

Recent taxonomic revision of *Ammodytes* revealed that *A. japonicus* is distributed widely from the Seto Inland Sea to Soya Strait, whereas the peaceful sand lance *A. heian* is distributed in a limited area in northern Japan (Orr et al. 2015, Aoyama et al. 2017). The latter species, *A. heian*, has been cryptic and placed under *A. japonicus* (*A. personatus* until revised by Orr et al. 2015), because the morphological characteristics between *A. japonicus* and *A. heian* almost completely overlap (Orr et al. 2015, Tanaka et al. 2016). Furthermore, it has been reported that *Ammodytes* in the Pacific Ocean and Sea of Japan consists of two genetically distinct southern and northern populations, each involving the aforementioned two species (Han et al. 2012, 2018). *A. japonicus* and *A. heian* are strictly distinguished by genetic traits in the mitochondrial genome (Orr et al. 2015, Tanaka et al. 2016, Aoyama et al. 2017), but the genetic difference between them is possibly derived from unusual evolutionary events, such as introgression and secondary contact (Harrison and Larson 2014). Accordingly, the taxonomy and population structure of Japanese sand lances are still controversial and require further examination.

While observing live *Ammodytes* from various localities for rearing experiments, we occasionally noted that the population in Otsuchi Bay, Iwate, Japan may present a significant difference in its morphological characteristics from those in other localities. The difference was obvious that the *Ammodytes* in the Seto Inland Sea possesses spots on both sides of their head, which reflect environmental light and can be visu-

ally observed. However, the population in Otsuchi Bay completely lacks these spots, suggesting a possible morphological difference between the species/local populations, which is recognizable only in live fish. Accordingly, in this study, we investigated the presence and absence of the spots in populations from different localities to garner information on the taxonomy and population structure of Japanese sand lances.

Materials and methods

We observed live Japanese sand lances, genus *Ammodytes*, from four localities in Japan. The fish in Seto Inland Sea (Itsuki Nada, off Matsuyama City, Ehime; 33°55′N, 132°44′E) were collected by commercial fishermen in May 2016, transferred immediately to Kitasato University, Kanagawa, and reared in aquarium tanks. The Sendai Bay population, collected in April 2017 (38°8′N, 141°0′E), was maintained at the Hakatajima branch of National Research Institute of Fisheries and Environment of Inland Sea (Fig. 1). The Otsuchi Bay population was collected in May 2017 (39°20′N, 141°56′E) and reared at the International Coastal Research Center, Atmosphere and Ocean Research Institute, the University of Tokyo (Fig. 1). The population in Uchiura Bay, Hokkaido (Osatsube; 41°54′N, 141°1′E) was kindly provided by Professor Kazushi Miyashita of Hokkaido University and were transferred alive to Kitasato University in June 2018 (Fig. 1).

The fish were observed in aquarium tanks and the presence/absence of spots on both sides of their heads was directly confirmed under normal light conditions. Subsequently, detailed structure around the spots was observed under a binocular microscope. To identify species from Sendai, Otsuchi, and Uchiura Bays, genetic analysis was conducted according to our previous studies (Tanaka et al. 2016, Aoyama et al. 2017). A partial fragment of the cytochrome *c* oxidase subunit 1 gene of mitochondrial DNA was amplified by PCR. Subsequently, the DNA nucleotide sequence was determined, and the species was then determined using a database search.

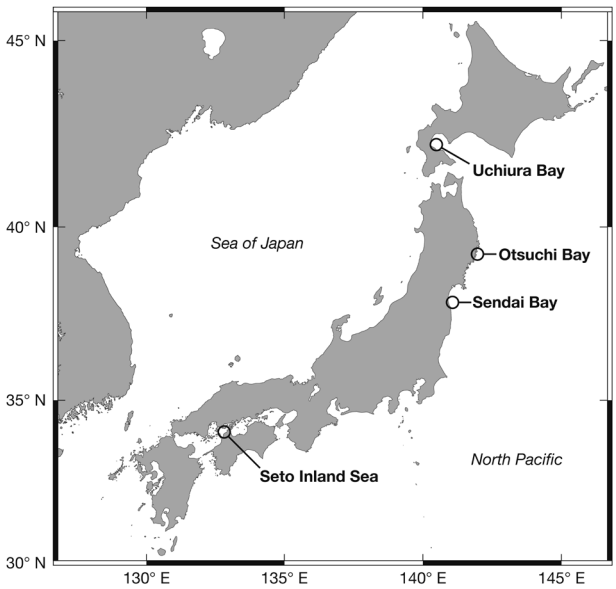

Fig. 1. Map showing the collection sites of Ammodytes specimens observed in the study.

Results and discussion

The specimens observed in this study were clearly divided into two types based on the presence or absence of the spots on the sides of their head. There was no individual with only a single spot on either side (Fig. 2). The populations in Seto Inland Sea and Uchiura Bay totally consisted of the spot-present individuals (*n*=over 100 each; Fig. 2A, C, E). Conversely, there was no individual with the spots in the Otsuchi Bay population (*n*=over 200; Fig. 2B, D, G). Interestingly, there were two types in the Sendai Bay population (present, 75%; absent, 25%; *n*=over 80; photographs not available). Based on these results, it was inferred that the spot-absent type was unique to Otsuchi Bay and possibly in adjacent areas along the northern Sanriku Coast. *A. japonicus* and *A. heian* were included in the populations from Sendai, Otsuchi, and Uchiura Bays at approximately 9 : 1, 5 : 5, and 4 : 6, respectively. Furthermore, in Sendai Bay where both spot-present and spot-absent individuals are coexisted, these differences did not correspond to a particular species. Thus, it was found that the presence/absence of the spots observed in the present study was locality specific and common in the two species at each locality. The individuals without the spots in Sendai Bay might have been immigrated from the northern Sanriku Coast. In support of this, Hashimoto and Kawasaki (1981) reported that the population in Sendai Bay consisted of two groups with a different mode for their vertebral number, which has been used to discriminate the northern and southern populations along the Sanriku Coast.

In the two populations from Seto Inland Sea and Uchiura Bay, the position of the spots was similar, located at the edge of the brain (Fig. 2F, H). Although our observations were limited, the spots were more likely visible in individuals larger than 40–50 mm. Microscopic observations were made by removing the skin and parietal bone above the brain area. The spots were iridophore-like cells because of their oval shape and absence of pigments (Fujii 1993). There was a gap between the iridophore-like cell and the brain, suggesting that the cells do not likely function as photoreceptors. However, we observed that the spots disappeared within a few minutes under anesthetic conditions, supporting a connection between the iridophore-like cells and the nervous system. Unlike other chromatophores, such as melanophores and leucophores, which actively function to change the fish

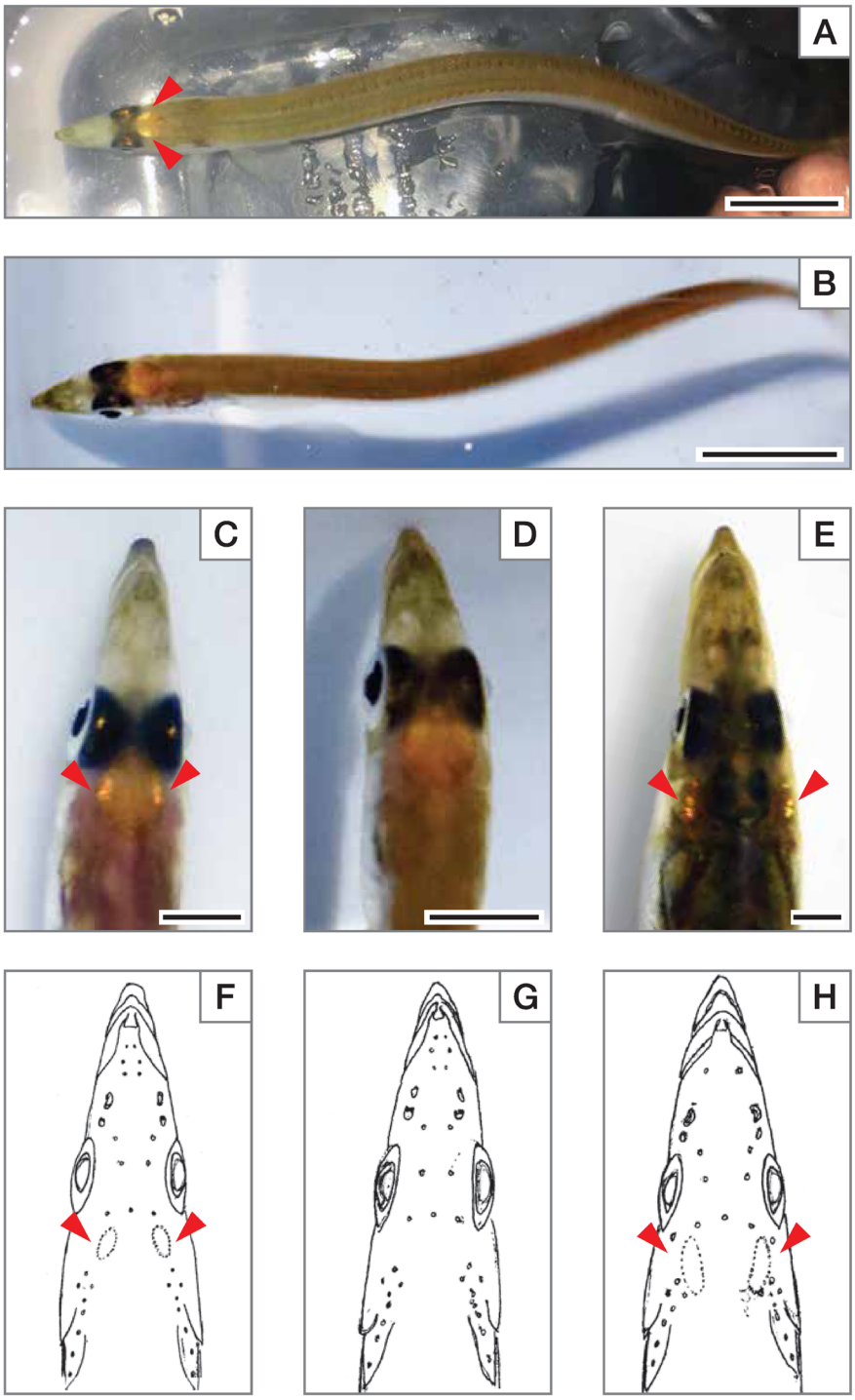

Fig. 2. Photographs showing the presence of spots on the head of *Ammodytes japonicus* from Seto Inland Sea (red arrows, **A**) and absence of spots in the same species from Otsuchi Bay (**B**). Photographs and sketches of the head region are shown for *A. japonicus* from Seto Inland Sea (**C**, **F**), Otsuchi Bay (**D**, **G**), and Uchiura Bay(**E**, **H**). Scale bars, 10 mm (**A**, **B**) and 5 mm (**C**–**E**).

skin color by aggregating and spreading pigment granules, the iridophore passively reflected environmental light through its main component, guanin, and presented a green-yellow color. However, some types of iridophores are known to actively regulate fish skin color (Fujii et al. 1989), and the iridophore-like cells observed in the present study could have a similar function. Therefore, further examination to elucidate the function of iridophore-like cells in Japanese sand lances is needed, as well as studies on the taxonomic and geo-phylogenetic relationships among spot-present and spot-absent populations.

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