

Rhopilema asamushi Uchida (Cnidaria, Scyphozoa, Rhizostomidae) newly occurred in Tanabe Bay, Wakayama Prefecture, Japan

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Abstract. A blue jellyfish body fragment of *Rhopilema asamushi* Uchida, that is identifiable by mitochondrial gene analysis (COI gene sequences: 644 bps), is washed ashore at Hatakejima Island in Tanabe Bay, Wakayama Prefecture, Japan. This occurrence is the first record in the Pacific side of southern Japan.

Key words: *Rhopilema asamushi*, *Rhopilema esculentum*, COI, medusa, stranding, Hatakejima.

Introduction

Rhopilema asamushi Uchida was originally described based on the samples collected from Asamushi, Mutsu Bay, Aomori Prefecture by Uchida (1927). Omori and Kitamura (2004) reexamined morphological characters of three species of the genus *Rhopilema*, *R. esculentum* Kishinouye, *R. hispidum* (Vanhöffen), *R. asamushi* Uchida in the family Rhizostomidae using newly collected samples, and reported that *R. esculentum* and *R. asamushi* cannot be identified morphologically each other and *R. asamushi* is the synonym of *R. esculentum*. However, Tezuka et al. (2006) reported that *R. esculentum* and *R. asamushi* are genetically differentiated at different species level in the family Rhizostomidae.

A blue body fragment of jellyfish probably belonging to the family Rhizostomidae was stranded on the seashore at Hatakejima, Tanabe Bay, Wakayama Prefecture, Japan on January 5, 2015. Here we iden-

tified the jellyfish at molecular level and discussed its geographical distribution.

Materials and Methods

A tissue sample of the jellyfish preserved in 99% ethanol was homogenized in DNA extraction buffer (Asahida et al., 1996) containing proteinase K and digested at 37°C. Total DNA was extracted using a standard phenol-chloroform method, precipitated using an equal volume of 99.7% isopropanol, dissolved in a buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and stored at -20 °C. Using universal primer HCO2198 (Folmer et al., 1994) and original primer CnLCOXI (5'-gtttcaactaaycayaaag-3'), mitochondrial COI gene was amplified. The gene sequence was determined using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Using Clustal X (Thompson et al., 1997), the sequence was aligned with those of 4 species in the family Rhizostomidae and p-distances were calculated between the aligned sequences. The species-identification was conducted based on the distance data.

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Results and Discussion

A blue jellyfish fragment (Fig. 1), that is washed ashore on the seashore at Hatakejima Island, Tanabe Bay, Wakayama Prefecture on January 5, 2015, almost consists of only bell portion (approximately 15 cm in diameter), and parts of body representing morphological characters for species-identification, such as oral arms or tentacles have already lost. We accordingly conducted molecular identification of this fragment based on mitochondrial COI gene sequences. The partial gene sequence (644 bps) was detected from the jellyfish tissue and was further comparatively analyzed with those of *Rhopilema esculentum* and *R. hispidum* collected from Ariake Sea, Saga Prefecture, *R. asamushi* collected from Asamushi, Aomori Prefecture, Tsuruoka, Yamagata Prefecture and Enoshima, Kanagawa Prefecture, and *Nemopilema nomurai* collected from Tsuruoka, Yamagata Prefecture.

The mean p-distance is the smallest between the present jellyfish and *Rhopilema asamushi*, and the sequence similarity is 99.4 % between them. On the other hand, that is 89.8 %, 80.3 %, 82.5 %, respectively between the jellyfish and *R. esculentum*, *R. hispidum*, and *Nemopilema nomurai* (Table 1). From these data, the jellyfish found in Tanabe Bay is apparently identified as *R. asamushi*.

On the other hand, coloration of *R. asamushi* is very variable, and blue, gray, light brown and white individuals are actually observed in this species (Hanzawa et al., unpublished). The coloration of the jellyfish occurred in Tanabe Bay agrees well with the

blue observed in *R. asamushi*.

It had ever been reported that *R. asamushi* is distributed in the coast of the Sea of Japan from northernmost tip of Honshu to Kyushu and is not distributed in the Pacific side (Uchida, 1938a, b, 1958; Yasuda and Suzuki, 1992; Omori and Kitamura, 2004). This species is available as the edible jellyfish in the Tohoku and Kyushu Regions, but life cycle and migration of the species have been still unknown (Omori and Kitamura, 2004; Miyake and Lindsay, 2013). However, the authors actually identified jellyfishes collected from Shiogama, Miyagi Prefecture and Enoshima, Kanagawa Prefecture as *R. asamushi*



Fig. 1. A blue jellyfish body fragment of *Rhopilema asamushi*, that is washed ashore at Hatakejima Island in Tanabe Bay, Wakayama Prefecture, Japan.

Table 1 P-distances between four species in the family Rhizostomidae and the jellyfish occurred in Tanabe Bay, Wakayama Prefecture, Japan based on COI gene sequences

	<i>R. hispidum</i>	<i>N. nomurai</i>	<i>R. esculentum</i>	<i>R. asamushi</i>
<i>Rhopilema hispidum</i>				
<i>Nemopilema nomurai</i>	0.169			
<i>Rhopilema esculentum</i>	0.183	0.171		
<i>Rhopilema asamushi</i>	0.193	0.172	0.099	
Jellyfish occurred in Wakayama	0.197	0.175	0.102	0.006

based on mitochondrial gene analyses (Hanzawa et al., unpublished). The present record of *R. asamushi* in Wakayama Prefecture is the first record in the Pacific side of southern Japan, whereas it has been still unclear where and how the individual was drifted. This record of *R. asamushi* is noteworthy, because it shows the possibility that *R. asamushi* is also widely distributed in the Pacific side of southern Japan.

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References

- Asahida, T., Kobayashi, T., Saitoh, K. and Nakayama, I., 1996. Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. *Fish. Sci.*, **62**: 727-730.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.*, **3**: 294-299.
- Miyake, H. and Lindsay, D. J., 2013. Encyclopedia of jellyfish - Mysterious ecology of 110 species. 127 pp. Seibundo shinkosha, Tokyo.
- Omori, M. and Kitamura, M., 2004. Taxonomic review of three Japanese species of edible jellyfish (Scyphozoa: Rhizostomeae). *Plankton Biol. Ecol.*, **51**(1): 36-51.
- Tezuka, A., Uye S., and Hanzawa N., 2006. Genetic differentiation and a new distribution record of four species in the family Rhizostomidae. Summary of the 9th Annual Meeting of the Society of Marine Biotechnology. Tokyo University of Marine Science and Technology, Tokyo.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G., 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **25**: 4876-4882.
- Uchida, T., 1927. Report of the biological survey of Mutsu Bay. 2. Medusae of Mutsu Bay. *Sci. Rep. Tohoku Imp. Univ., Biol.*, **2**(3): 215-238.
- Uchida, T., 1938a. Report of the biological survey of Mutsu Bay. 32. Medusae from Mutsu Bay (revised report). *Sci. Rep. Tohoku Imp. Univ., Biol.*, **13**(1): 37-46.
- Uchida, T., 1938b. Medusae in the vicinity of the Amakusa Marine Biological Station. *Bull. Biogeogr. Soc. Japan.*, **8** (10): 143-149.
- Uchida, T., 1958. Hydroids and medusae from the vicinity of the Sado Marine Biological Station. *Jour. Fac. Sci. Niigata Univ., Ser. II.*, **2**(5): 163-165.
- Yasuda, T. and Suzuki, Y., 1992. Notes on an edible medusa, *Rhopilema asamushi* Uchida, caught in Wakasa Bay, Japan. *J. Plankton Soc. Japan*, **38**: 147-148.

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