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First records of *Macrobrachium grandimanus* (Randall, 1840) and *M. lepidactyloides* (De Man, 1892) (Crustacea, Decapoda, Palaemonidae) from Honshu Island, Japan

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Abstract

Although the Pacific coast of Honshu Island, Japan, is a temperate zone, tropical aquatic species have been known to occur in this area because of the influence of the Kuroshio Current. The distributional records of the southern species provide interesting information for studying the expansion of their distributional ranges owing to climate change. In the present study, we report the first records of two tropical palaemonid shrimp species, *Macrobrachium grandimanus* and *M. lepidactyloides*, from Honshu Island, Japan, with molecular identification using mitochondrial 16S rDNA sequences. These records update the northern limits of the two species.

Key words: amphidromous; DNA barcoding; Kuroshio Current; northernmost record; 16S rDNA

Introduction

The Pacific Coast of Honshu Island, Japan, is strongly influenced by the Kuroshio Current. Tropical aquatic animals, which are mainly found in low-latitude areas, such as the Ryukyu Islands and Southeast Asia, sometimes occur in this temperate area owing to transport by the current (Onikura 2013). These occurrences are thought to be abortive migrations because they die off owing to low water temperatures in winter and do not contribute directly to range expansion. However, with the recent increase in seawater temperatures, it has been suggested that these aquatic animals may overwinter and expand their distribution (Yamakawa et al. 2018; Inui et al. 2019; Itsukushima 2023). Therefore, recording the occurrence of such species provides fundamental information for predicting the impacts of climate change on the fauna of this area.

Among such records, amphidromous caridean shrimps are one of the taxa whose occurrence and abundance have been studied in this area (e.g., Imai and Oonuki 2013; Maruyama 2017). Shrimp recorded from the coast of Honshu Island include tropical species such as *Macrobrachium lar*, *M. australe*, *M. ustulatum*, *M. latimanus*, *Caridina grandirostris*, *C. laoagensis*, and *Atyopsis spinipes* (Maruyama 2018; Minagawa and Fuke 2024; Fuke and Maruyama 2024). In the present study, we report the first records of two tropical *Macrobrachium* species, *M. grandimanus* and *M. lepidactyloides*, from Honshu Island. These data represent the northernmost records of each species.

Materials and Methods

Animals

Field sampling on the Kii and Boso peninsulas was conducted in October 2018 (Fig. 1). Shrimp sampling was conducted by two or three people using hand nets (mesh size 1–3 mm).

A specimen of *M. grandimanus* was collected from the Kumanomiya River in Mie Prefecture on the Kii Peninsula (Fig. 1B). The collection site was located in a tidal area approximately 350 m upstream of the river mouth (Fig. 1D). After photography, the shrimp was fixed in 70 % ethanol.

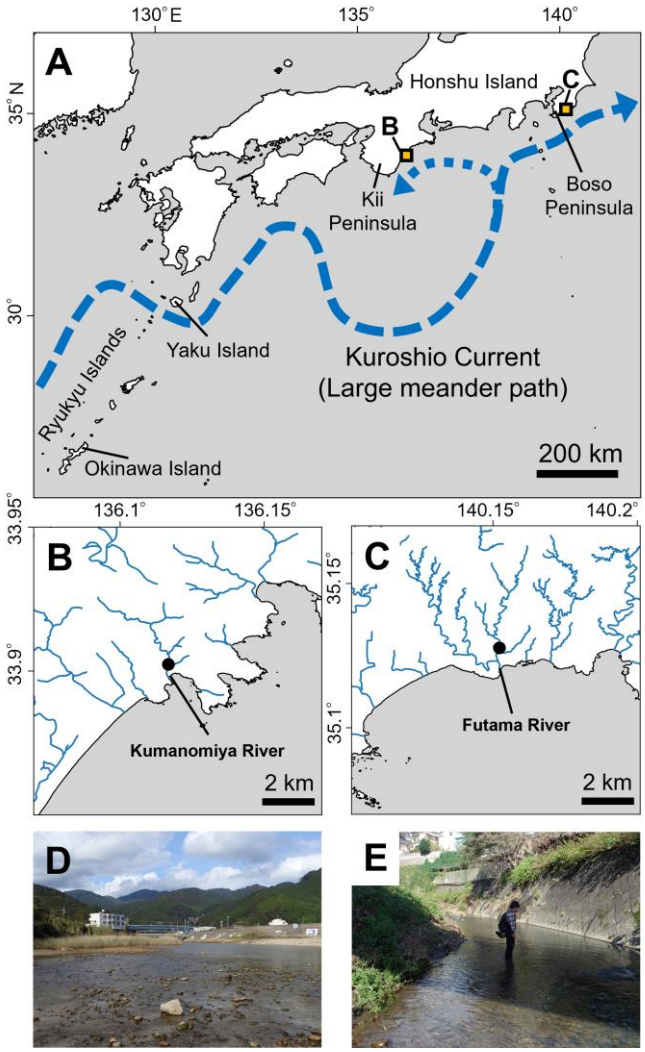

Fig. 1. Collection sites in Honshu Island, Japan. A. Location of the Kii and Boso peninsulas in Japan. The previous northernmost records of the focal species are shown in the figure: Yaku Island for *Macrobrachium grandimanus* and Okinawa Island for *M. lepidactyloides*. The Kuroshio Current followed a large meandering path in 2018 (Sugimoto et al. 2020). B. Collection site in the Kii Peninsula (black circle). C. Collection site in the Boso Peninsula (black circle). These maps are edited and processed by the authors based on the National Land Numerical Information (Coastline and River Data, http://nlftp.mlit.go.jp/ksj/). D. Collection site at Kumanomiya River. E. Collection site at Futama River.

A specimen of *M. lepidactyloides* was collected from the Futama River in Chiba Prefecture on the Boso Peninsula (Fig. 1C). The collection site was located in the middle reaches, approximately 750 m upstream of the river mouth (Fig. 1E). After photography, the shrimp was fixed in 70 % ethanol.

For each specimen, carapace length (CL) was measured using a digital caliper. The rostral formula (RF) was defined as the 'number of dorsal rostral teeth above the carapace + number of dorsal rostral teeth above the rostrum / number of ventral rostral teeth'. The position of the tooth was determined by its anterior root, and the boundary between the carapace and rostrum was defined as the posterior margin of the orbit.

The specimens were deposited in the Kanagawa Prefectural Museum of Natural History (registration numbers: KPM-NH 5117, 5118). Note that owing to zero padding, a seven-digit number is used for the catalogue number in the invertebrate specimen collections of the museum because of the convenience of the database software; however, zero suppression is adopted for the expression of the essential numbers here.

Species identifications

Because the preserved specimens were in poor condition and juveniles, morphological identification was difficult. Therefore, we performed molecular identification using DNA barcoding based on mitochondrial DNA.

Total DNA was extracted from muscle tissues using the Monarch Genomic DNA Purification Kit (New England Biolabs). A partial mitochondrial gene for 16S ribosomal RNA (16S rDNA) was amplified using PCR with the primer pairs 16S-F1 (5′-GTA CCT TTT GTA TCA GGG-3′) and 16S-R1 (5′-CGG TYT GAA CTC AAA TCA TG-3′) (Fuke and Maruyama 2023). The reaction mixture of 12.5 µL contained the following: 0.8 µL of template DNA, 0.25 µL of PrimeSTAR GXL DNA Polymerase (TaKaRa), 2.5 µL of 5 × PrimeSTAR GXL buffer, 1.0 µL of dNTP mixture, 0.75 µL of each 5 µM primer, and 6.45 µL of ultrapure water. The PCR amplification conditions were as follows: initial denaturation (98°C, 30 s); 30 cycles of denaturation (98°C, 10 s), annealing (55°C, 15 s), and extension (68°C, 45 s); and a final extension (68°C, 30 s). The PCR products were

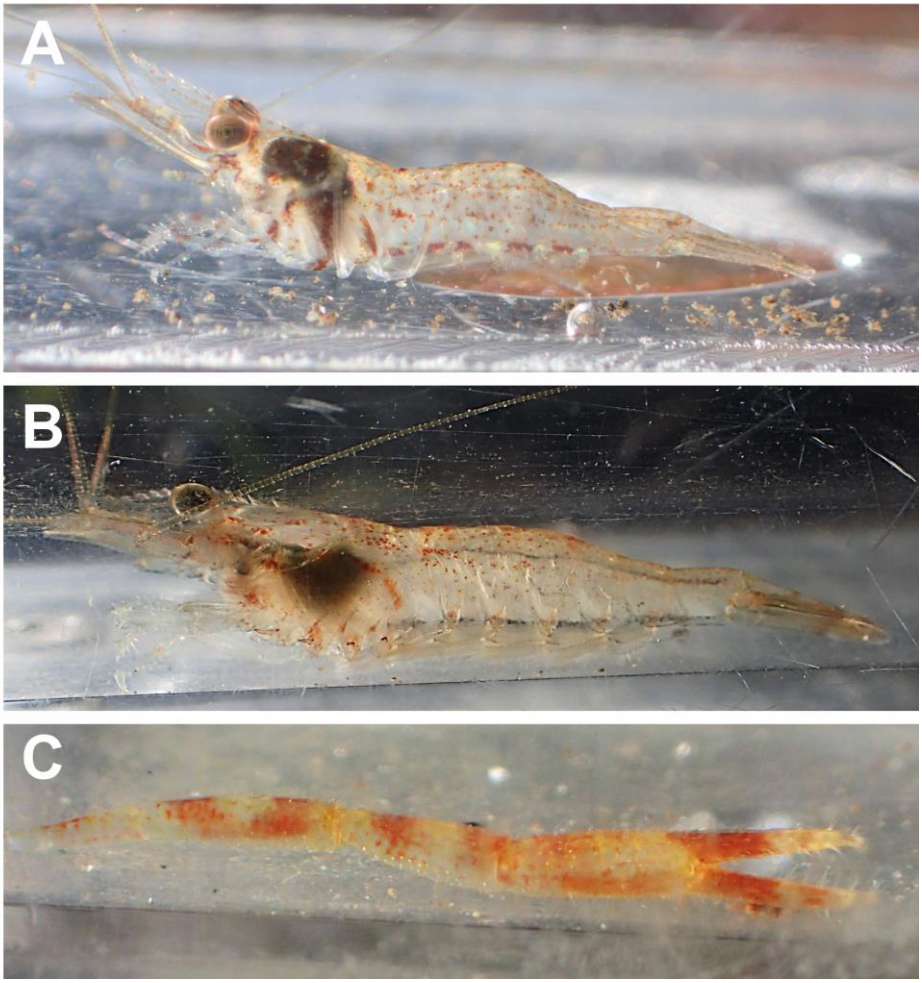

Fig. 2. Live specimen of *Macrobrachium grandimanus* KPM-NH 5117 (A) and *M. lepidactyloides* KPM-NH 5118 (B: whole body, C: second pereopod).

purified using ExoSAP-IT Express (Thermo Fisher Scientific) and outsourced to Eurofins Genomics for Sanger sequencing.

The sequences obtained were manually checked for quality and editing errors. To conduct DNA barcoding, similarity searches of nucleotide sequences were performed using BLASTn (Altschul et al. 1990; Johnson et al. 2008) with default settings. The sequences were deposited in the International Nucleotide Sequence Database via DDBJ (accession numbers: LC849818 and LC849819).

Results and Discussion

Order Decapoda

Family Palaemonidae Rafinesque, 1815

Genus *Macrobrachium* Spence Bate, 1868

Macrobrachium grandimanus (Randall, 1840)

(Fig. 2A)

Material examined

KPM-NH 5117; CL 3.5 mm; RF 4+10/4; 33°54′2.4″ N, 136°6′58.5″ E; Kumanomiya River; 14.X.2018; Collected by Tomoaki Maruyama; Accession No.: LC849818.

Molecular identification

A similarity search of 896 bp of 16S rDNA from our specimen using BLAST showed 99.78–100 % identity with the sequences from specimens identified as *M. grandimanus* from Ryukyu Island, Japan, collected in previous studies (Table 1).

Table 1. BLAST search results for molecular identification.

Query sample	Hit species	Length (bp)	Query cover (%)	Identity (%)	Accession	References
KPM-NH 5117 (LC849818)	*Macrobrachium grandimanus*	527	95	100	DQ194926.1	Liu et al. (2007)
		462	92	100	LC569849.1	Fuke and Sasazuka (2021)
		460	86	100	LC661477.1	Fuke and Imai (unpublished)
		460	86	100	LC661480.1	Fuke and Imai (unpublished)
		460	86	100	LC661482.1	Fuke and Imai (unpublished)
		460	86	100	LC661483.1	Fuke and Imai (unpublished)
		460	86	100	LC661484.1	Fuke and Imai (unpublished)
		460	86	100	LC661485.1	Fuke and Imai (unpublished)
		462	92	99.78	LC569860.1	Fuke and Sasazuka (2021)
		462	92	99.78	LC569855.1	Fuke and Sasazuka (2021)
KPM-NH 5118 (LC849819)	*Macrobrachium lepidactyloides*	460	86	99.77	LC661509.1	Fuke and Imai (unpublished)
		460	86	99.77	LC661508.1	Fuke and Imai (unpublished)
		460	86	99.77	LC661507.1	Fuke and Imai (unpublished)
		460	86	99.77	LC661506.1	Fuke and Imai (unpublished)
		460	86	99.77	LC661505.1	Fuke and Imai (unpublished)
		460	86	99.77	LC661504.1	Fuke and Imai (unpublished)
		460	86	99.77	LC661503.1	Fuke and Imai (unpublished)
		460	86	99.77	LC661502.1	Fuke and Imai (unpublished)
		528	95	99.58	DQ194929.1	Liu et al. (2007)
		534	98	98.19	EU493138.1	Chen et al. (2009a)

Distribution

Honshu Island (this study), Ryukyu Islands (Yaku Island, Okinoerabu Island, Okinawa Island, Kume Island, Miyako Island, Ishigaki Island, Iriomote Island, Yonaguni Island) (Shokita 1979; Satou 1995; Shokita 2003; Yoshigou et al. 2005; Shokita, 2019), Guam (Fuke and Sasazuka 2021), Hawaii Islands (Holthuis 1973), Fiji (Short and Marquet 1998), New Caledonia (Short and Marquet 1998), and Tonga (Maciolek and Yamada 1981).

Remarks

The specimen is a juvenile. The rostral formula was consistent with that of *M. grandimanus* examined in previous studies (Holthuis 1950; Fuke and Sasazuka 2021). The specimen was collected from submerged plants in a pool. *Macrobrachium nipponense* was collected at the same habitat. Regarding live coloration, the entire body was translucent and three lateral lines were observed on the carapace and an indistinct yellow band was observed on the third abdominal somite (Fig. 2A). Regarding the distributional records in Japan, Hayashi (2000) and Yoshigou (2002) included Tanega Island in their distributions of this species. However, we could not identify the original references or specimens for these records and did not include Tanega Island in the distribution.

Macrobrachium lepidactyloides (De Man, 1892)
(Fig. 2B, C)

Material examined

KPM-NH 5118; CL 5.6 mm; RF 4+7/2; 35°7′36.6″ N, 140°9′7.7″ E; Futama River; 28.X.2018; Collected by Tomoaki Maruyama; Accession No.: LC849819.

Molecular identification

A similarity search of 919 bp of 16S rDNA from our specimen using BLAST showed 98.19–99.77 % identity with the sequences from specimens identified as *M. lepidactyloides* from Ryukyu Islands and Taiwan, collected in previous studies (Table 1).

Distribution

Honshu Island (this study), Ryukyu Islands

(Okinawa Island, Iriomote Island, Taiwan) (Chen et al. 2009a, b; Saeki et al. 2018; Hanai 2021), Philippines (Cai and Shokita 2006), Indonesia (Holthuis 1950; Holthuis 1952), Papua New Guinea (de Mazancourt et al. 2024), Solomon Islands (de Mazancourt et al. 2024), and Fiji (Holthuis 1950).

Remarks

The specimen is a juvenile. The rostral formula was consistent with that of *M. lepidactyloides* examined in previous studies (Holthuis 1950; Saeki et al. 2018). The specimen was collected from submerged plant litter in a gentle stream. Regarding live coloration, the entire body was translucent, and one lateral line was observed on the carapace (Fig. 2B). The second pereopod had several indistinct brown or yellow dots (Fig. 2C). Live adults of this species are known to have a distinct longitudinal line on their abdomen (Saeki et al. 2018); the live specimen did not show such a line on the abdomen (Fig. 2B).

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