



First records of *Phyllorhiza punctata* von Lendenfeld, 1884 (Cnidaria, Scyphozoa, Mastigiidae) from Nagasaki and Kagoshima Prefectures, Japan

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Abstract. *Phyllorhiza punctata* von Lendenfeld, 1884 originates from Australia and Coral Triangle, but its occurrence has been reported in the Atlantic Ocean and Mediterranean Sea, so it is necessary to better understand its geographic range. Two specimens were collected in Nagasaki and Kagoshima Prefectures, Japan, and morphological and molecular studies suggest that they are *P. punctata*. These are the first records of the species in Japan, and it is possible that individuals have been transported to Japan by the Kuroshio Current.

Key words. Expansion of distribution, invasion, jellyfish, medusa, new records

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INTRODUCTION

The White-spotted Jellyfish, *Phyllorhiza punctata* von Lendenfeld, 1884 (Cnidaria, Scyphozoa, Rhizostomeae), is characterized by finely granular white spots scattered over the exumbrella surface and white tips on the appendages extending from the end of the oral arms (Graham et al. 2003; Bolton and Graham 2004). *Phyllorhiza punctata* is distributed in the tropical and subtropical regions of the western Pacific Ocean from Australia, where it was originally described, to Southeast Asia such as the Philippines and Thailand (Kramp 1965; Heeger et al. 1992; Figure 1A). On the other hand, *P. punctata* occurs in many ecoregions of the world (Bayha and Graham 2014, Figure 1A): California (Larson and Arneson 1990; Rosales-Catalan et al. 2021), the Gulf of Mexico (Graham et al. 2003), the southeastern Brazil (Haddad and Nogueira 2006), the Mediterranean Sea (Galil et al. 1990; Abed-Navandi and Kikinger 2007; Gueroun et al. 2015; Deidun et al. 2017; Rizgalla and Crocetta 2020), the southwest Atlantic coast of Spain (Enrique-Navarro and Prieto 2020), and Sri Lanka (Karunaratne and De Croos 2022). In the Gulf of Mexico, mass occurrences of medusae of *P. punctata* have raised concerns about negative economic effects related to fishing (Graham et al. 2003). Many marine species can expand their habitats not only through their potential, but also through anthropogenic factors such as the discharge of ballast water of ship and aquaculture (Salimi et al. 2021). Jellyfish have environmental adaptive potential and can survive in environments different from their native habitat (Courtney et al. 2016; Rato et al. 2021), so understanding their occurrence is necessary to prevent their outbreaks.

The aim of this study is to report the first occurrence of *P. punctata* in Japan by analyzing two specimens of a White-spotted Jellyfish collected in Nagasaki and Kagoshima prefectures based on morphological and molecular analyses.

METHODS

On October 14, 2009, a single specimen of a White-spotted Jellyfish was collected at the water surface in Sasebo, Nagasaki Prefecture (33°10'3"N, 129°40'13"E; Figure 1B), and its bell diameter (37.0 cm) was



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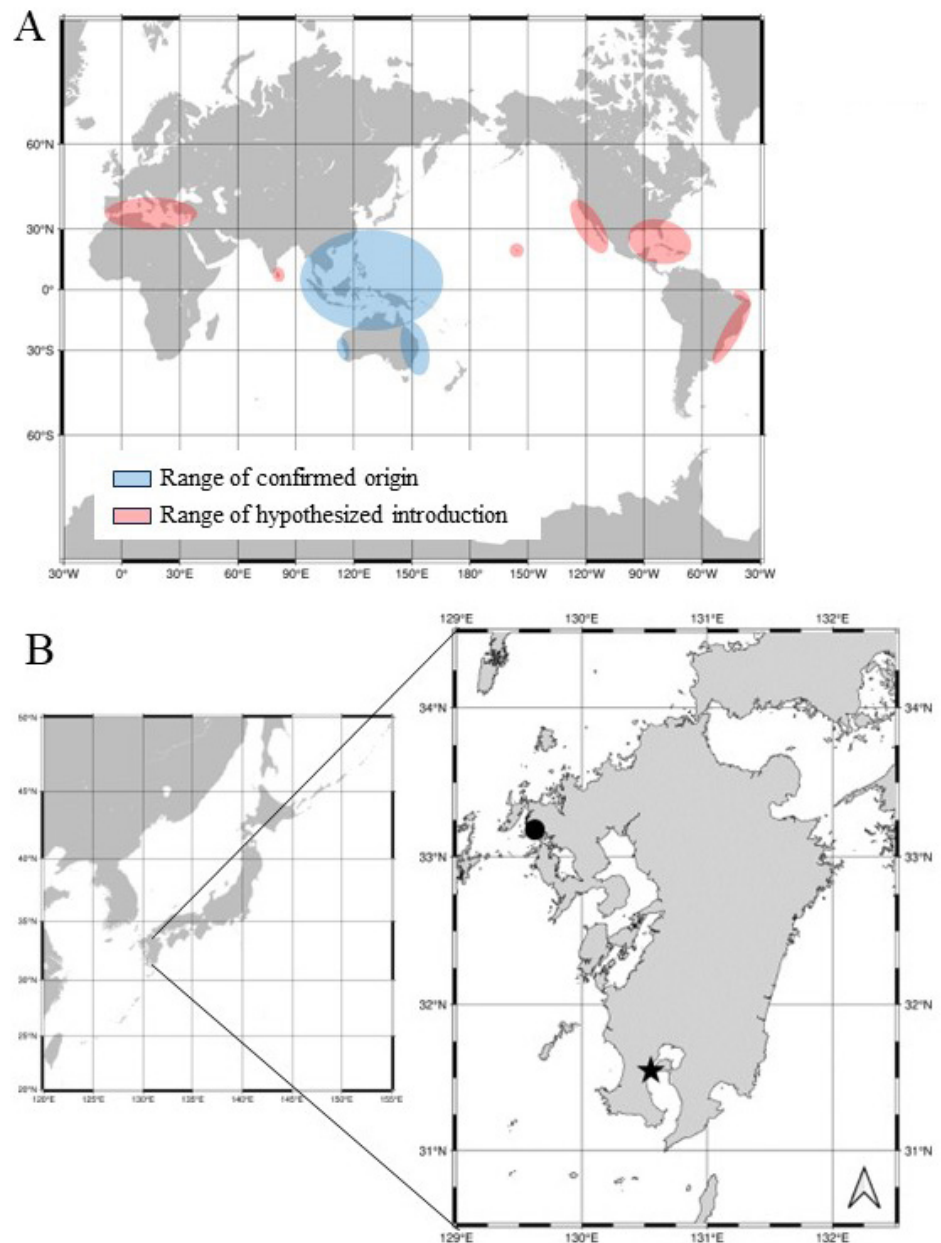


Figure 1. Previously known range and first record of *Phyllorhiza punctata* in Japan.

A. Distribution of *P. punctata*. **B.** First record in Japan, (●) Sasebo-shi, Nagasaki Prefecture, (★) Kagoshima-shi, Kagoshima Prefecture.

measured using a ruler (minimum scale of 0.1 cm). The morphology of each body part was observed by eye following Bolton and Graham (2004).

On August 15, 2021, another specimen was collected by skin diving in the canal at Yojirogahama, Kagoshima Prefecture (31°33'50"N, 130°33'55"E; Figure 1B). The bell diameter (11.0 cm) of the collected specimen was measured using a ruler (minimum scale of 0.1 cm), and a portion of oral arm cut to approximately 1 cm was preserved in 99.5 % ethanol for DNA analysis. The jellyfish was kept at the Kagoshima Aquarium. The tissue sample was subjected to total DNA extraction using the DNeasy Blood and Tissue Kit (QIAGEN), and the cytochrome c oxidase subunit I (COI) region of mitochondrial DNA (mtDNA) was amplified by polymerase chain reaction (PCR) using a thermal cycler (Bio-Rad Laboratories, Japan). The primers used were LCOjf (Dawson 2005) and HCO2198 (Folmer et al. 1994). After thermal denaturation at 95 °C for 4 min, 35 cycles of 95 °C for 30 s, 51 °C for 30 s, and 72 °C for 60 s were performed and incubated at 72 °C for 5 min. After confirming the amplification of the target region by agarose gel electrophoresis, the PCR products were purified by using ExoSAP-IT (Thermo Fisher), and sequencing reactions were performed using Big dye Terminator v. 3.1 (Applied Biosystem). After purification by ethanol precipitation, sequencing was performed using an automated capillary DNA sequencer (ABI-3130XL, Applied Biosystem). The sequence data obtained were aligned using MEGA X (Kumar et al. 2018) and were used to confirm homology with known sequences using the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/nucleotide>). In addition, a molecular phylogenetic tree was generated by performing 1000-iteration bootstrap analysis with known sequences using the maximum likelihood method (Table 1).

Table 1. GenBank data used in this study.

Species	GenBank accession number	Country
<i>Phyllorhiza punctata</i>	KU900938	Australia
	KU900939	Australia
	EU363342	Australia
	KY611060	Mexico
	KY611061	Mexico
	KY611062	Mexico
<i>Phyllorhiza pacifica</i>	MN395673	Indonesia
	JN202999	Malaysia
	JN203007	Malaysia
<i>Mastigias papua</i>	KU900920	Papua New Guinea
	KU900931	Papua New Guinea
	KU900935	Papua New Guinea
	KU901444	Palau
	KU901452	Palau
	LC594632	Japan
<i>Mastigias albipunctatus</i>	MH460554	Philippines
	MH460556	Philippines
<i>Versuriga anadyomene</i>	KX904853	China
<i>Cassiopea ornata</i>	KF683387	USA

RESULTS

Phylum Cnidaria
 Class Scyphozoa Goette, 1887
 Order Rhizostomeae Cuvier, 1800
 Family Mastigiidae Stiasny, 1920
 Genus *Phyllorhiza* Agassiz, 1862

Phyllorhiza punctata von Lendenfeld, 1884

First report. JAPAN – NAGASAKI • Sasebo; 33°10'3"N, 129°40'13"E; 14.X.2009; Akiyama H. leg.; collected in bucket. – KAGOSHIMA • Yojirogahama Long Canal; 31°33'50"N, 130°33'55"E; 15.VIII.2021; Takauchi S., Miyake H., Watabe M., Chikuchishin M. leg.; collected in plastic bag by skin diving.

Identification. The specimen collected in Nagasaki Prefecture had an exumbrella with numerous small circular white spots and the brown oral arms (Figure 2A, Table 2). The bell diameter measured 37 cm. The specimen collected in Kagoshima Prefecture had a finely granulated exumbrella surface with numerous circular white spots (Figure 2B). The bell diameter measured 11.0 cm. The edge of the umbrella had numerous incisions. The terminal appendages were long with distal swelling, and the tips were white. The exumbrella was generally light brown; the base of the oral arm was colorless, and the oral arms were brown. The width of the oral arm was about the same size as the bell diameter (Figure 2B, Table 2). A lot of individuals of *Mastigias papua* (Lesson, 1830) occurred at the same site (Figure 2C), but only one of *Phyllorhiza punctata* was found.

DNA analysis indicated 100 % similarity with the sequence of *P. punctata* using a BLAST search of 556 bp of the COI region. Moreover, the molecular phylogenetic tree showed that this species was included in the same clade as *P. punctata* listed in GenBank (Figure 3).

DISCUSSION

Phyllorhiza punctata has been reported around the world, but some reports have been based only on photographs or morphological data. The morphology of jellyfish has the high intraspecific morphological variation (Morandini et al. 2006; Anthony et al. 2022; Daglio et al. 2022), and species identification has been studied using a combination of morphological and molecular analyses. In *Aurelia* spp., even morphologically similar species have been identified by molecular analyses (Lawley et al. 2021). In this study, based

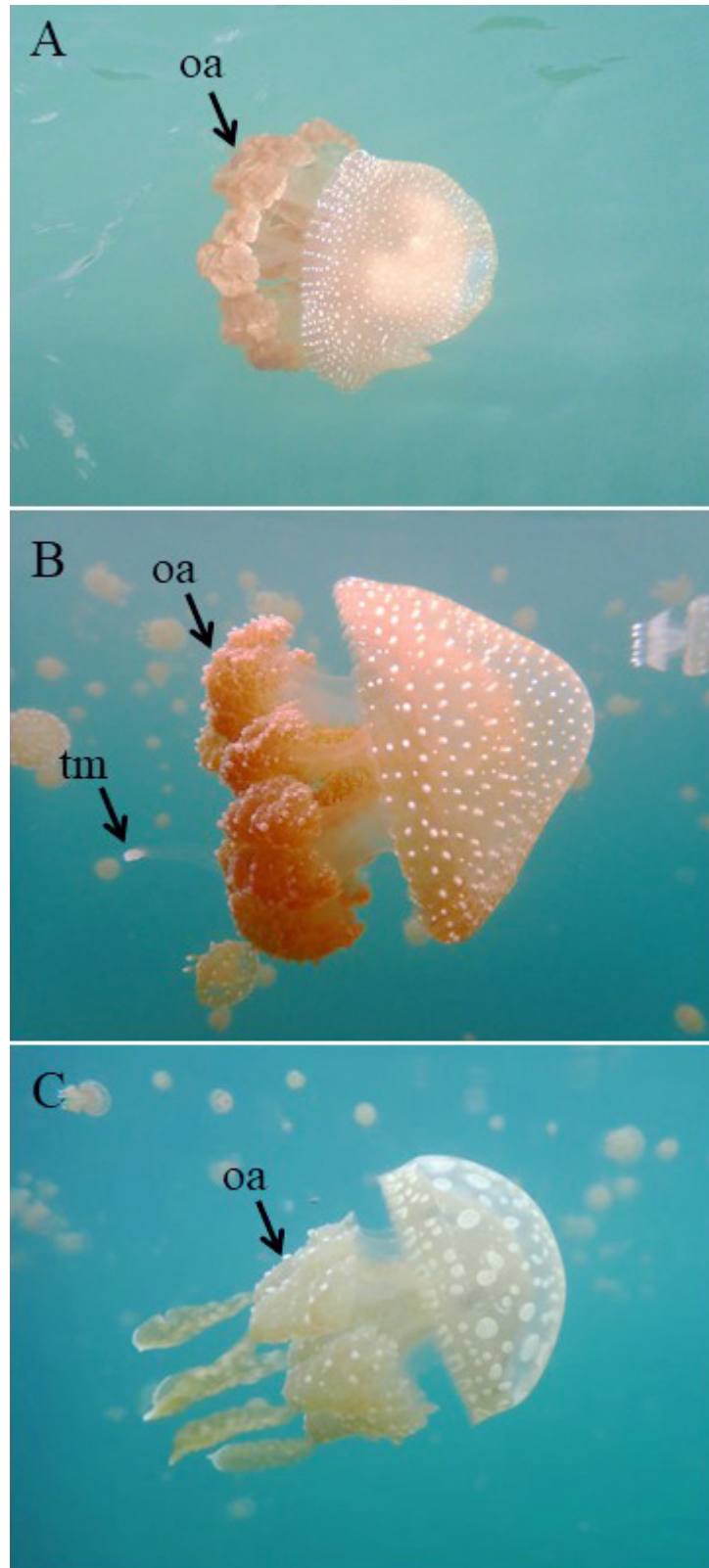


Figure 2. The white-spotted jellyfish, *Phyllorhiza punctata*, in the habitat in Nagasaki (A) and Kagoshima (B) prefectures, and *Mastigias papua* in Kagoshima prefectures (C). Abbreviations: (oa) oral arms, (tm) terminal appendage.

on the results of morphological and molecular analyses, it was determined that the specimen collected in Japan is *P. punctata*.

Phyllorhiza trifolium Haeckel, 1880 (Cnidaria, Scyphozoa, Rhizostomeae), has been recorded in Japan, but this species was described by Haeckel (1880) and only one poorly preserved specimen was collected from the Japanese coast; therefore, it is considered a doubtful species (Uchida 1936; Uchida 1954; Jarms and Morandini 2019). In addition, Abed-Navandi and Kikinger (2007) described the distribution range of

Table 2. Morphological characteristics of species of Mastigiidae.

	<i>Mastigias papua</i> (Lesson, 1830)	<i>Phyllorhiza punctata</i> von Lendenfeld, 1884	<i>Phyllorhiza pacifica</i> (Light, 1921)	<i>Phyllorhiza luzomi</i> Mayer, 1915	<i>Phyllorhiza punctata</i> (this study, Nagasaki Prefecture)	<i>Phyllorhiza punctata</i> (this study, Kagoshi- ma Prefecture)
Umbrella	60 to 80 mm wide, hemispherical	Up to 500 mm wide, hemispherical, about half as high as broad	Up to 400 mm wide, flat-hemispherical, about half as high as broad	Up to 60 mm wide, fairly flat	370 mm wide, hemispherical	110 mm wide, hemispherical
Exumbrella	With very fine nematocyst warts	Surface finely granulated and with some elevated warts	Surface finely granulated and marked by transparent or white spots, which become more irregular, numerous and larger towards the margin	Surface finely granulated	Surface with numerous small circular white spots	Surface finely granulated and with numerous small circular white spots
Marginal lappets	Per octant eight rounded velar lappets and two narrower rhopalial ones	Up to 112: per octant up to 14 velar lappets, all connected by a thin membrane, two sickle- shaped rhopalial lappets per octant	80 per octant eight broad rounded velar rhopalial lappets	88 per octant nine rounded velar lappets and two sickle-shaped rhopalial ones; these as long as velar ones	Not observed	Not observed
Oral arm	As long as bell radius, numerous club-shaped vesicles between mouthlets, the simple upper portion nearly as long as the three- winged lower portion	Eight three-winged oral arms with window- like openings on lateral membranes, two third the length of the umbrella's diameter	Eight oral arms with window-like openings in the lateral membranes, proximal portion of arms lacking appendages and is 1/4 of the length of the distal portion, which has numerous small pedunculated	Eight three-winged slender oral arms, strongly compressed, naked, upper part slightly longer than lower 3-winged portion	Not observed	The width of oral arm was about the same size as the bell diameter
Terminal appendages	Oral arm terminating in a club-like filament, triangular in cross- section	Very long and with a distal swelling, tips were white	As long as oral arm, centre of arm disc with numerous slender filaments	No appendages on arms or arm disc	Not observed	Long and with a distal swelling, tips were white
Subgenital cavity	No description	4 large oval subgenital ostia	Large oval subgenital ostia	Four wide subgenital ostia	Not observed	Not observed
Colour	Exumbrella bluish- green, olive to brownish-green rhopalial canals, rusty marginal lappets and gonads, mouth arms blue-green with brown spots, mouthlets and terminal appendages blue or green, terminally sometimes changing into brown- red.	Exumbrella transparent bluish or yellow- brown (caused by zooxanthellae)	Exumbrella bluish with brownish mosaic of zooxanthellae and white spot, canal network blue, blue band goes form clefts to middle of lappets, terminal appendages proximally transparent, but band goes from clefts to middle of lappets, terminal appendages proximally transparent, but terminal club white and green	Greenish with greyish- white spots	Exumbrella was light brown, the oral arms were brown	Exumbrella was generally light brown, and the base of the oral arm was colorless, the oral arms were brown

Reference: *World Atlas of Jellyfish* (Jarms and Morandini 2019).

the *P. punctata* from Australia to Japan in accordance with the study of Heeger et al. (1992), but Heeger et al. (1992) does not report the occurrence of *P. punctata* in Japan. Therefore, this is the first record of *P. punctata* confirmed in Japan.

In this study, only one specimen was collected at each site, so it is considered that the collected individuals were derived from ephyra or medusa that had migrated to this area and grown there. The Kuroshio Current flows into this area, flowing north from off the coast of the Philippines (Qu and Lukas 2003). The Kuroshio Current is known to transport zooplanktons, and it has been suggested that *Eutiara decorata* Berberian, Michenet and Goy, 2021, observed in Palau, the Philippines, and Japan, was transported to each region by the Kuroshio Current and the North Equatorial Current (Watabe et al. 2022). *P. punctata* has been

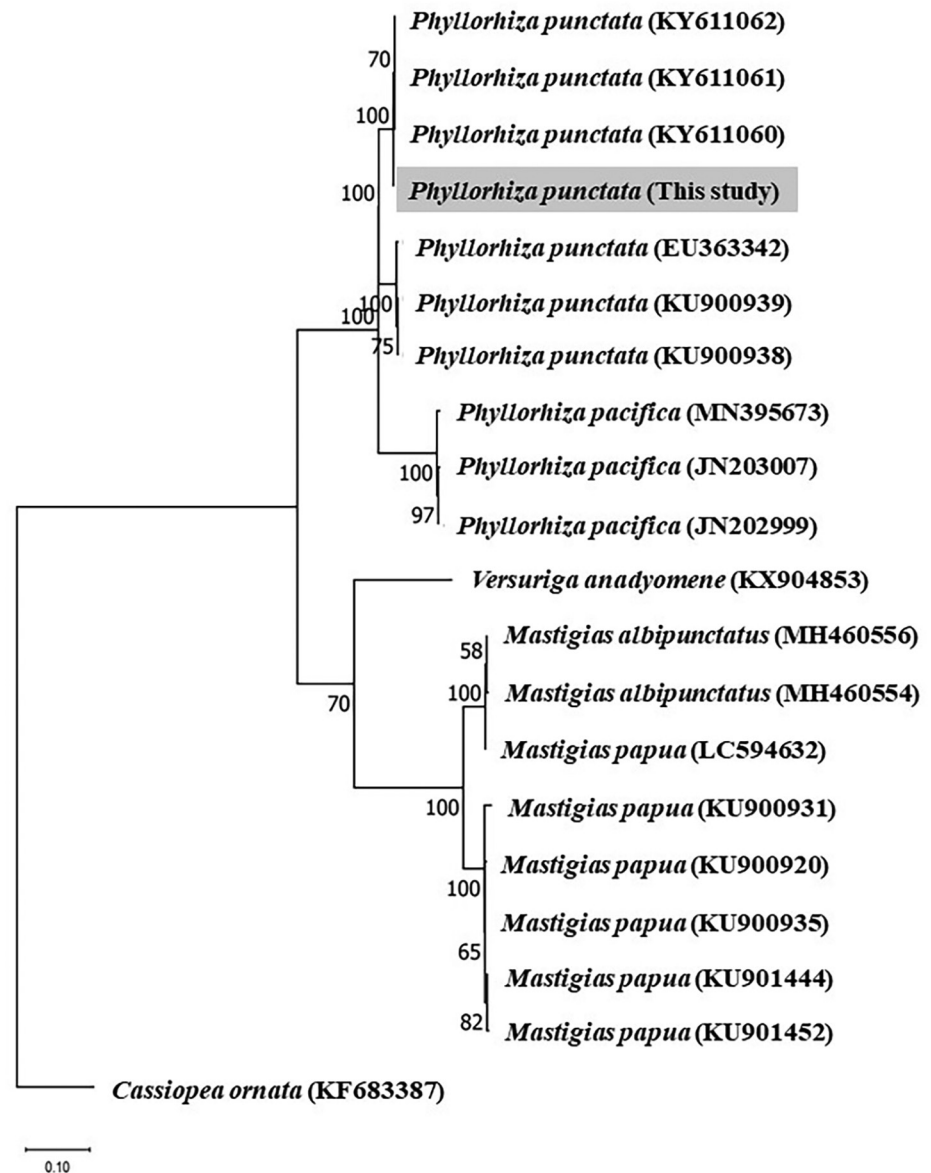


Figure 3. Phylogenetic tree based on Maximum likelihood analysis of the cytochrome c oxidase subunit I (COI) region of mitochondrial DNA of the jellyfish. Bootstrap values are shown in each branch. The specimen collected in this study is highlighted in gray.

reported to be occurred in the Philippines and Malaysia (Maghsoudlou et al. 2016; Boco and Metillo 2018), and it is possible that individuals have been transported to Japan by the Kuroshio Current. In addition, since no additional specimens have been reported to date in Japan, it is considered that the polyps of *P. punctata* are not yet inhabit in this area. On the other hand, it is suggested that rising sea temperatures due to global warming will cause subtropical species to expand their distribution farther northward (Yamano et al. 2011; Yuan et al. 2023). Scyphozoan jellyfish are able to distribute widely by adapting to environmental stresses at different stages of the life cycle, and suitable water temperatures promote population growth (Pitt et al. 2018; Fernández-Alías et al. 2024). Thus, in the future, it is possible that *P. punctata* of subtropical species also inhabit in Japan, so further investigations are required to confirm that this species has become inhabit in this area.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement


No ethical statement is reported.


Author contributions

Conceptualization: ST, HM. Data curation: ST, HM. Formal analysis: ST, HM. Investigation: ST, HM, MW, HA, MC. Methodology: ST, HM, MW, HA, MC. Resources: ST, HM, MW, HA, MC. Visualization: ST, HM. Project administration: ST, HM. Software: ST, HM. Validation: ST, HM. Writing – original draft: ST, HM. Writing – review and editing: ST, HM, MW, HA, MC.

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Data availability

All data that support the findings of this study are available in the main text.

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