First record of the tropical holothurian *Stichopus naso* Semper, 1867 (Echinodermata: Holothuroidea: Synallactida) from the temperate coast of Kyushu mainland, Japan, in relation to ocean warming

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Abstract: In 2011, a tropical holothurian was found off the temperate coast of Kagoshima, Kyushu, Japan. This holothurian was identified as *Stichopus naso* Semper, 1867, which was first described in the Philippines, and is known to be distributed within the tropical Indo-West Pacific region. Herein, we examined its morphological characteristics and compared it with pre-described examples and discuss its origin from the perspective of climate change and artificial transfer. We considered that both perspectives are plausible because sea water temperature has recently risen around Japan, creating the possibility of eggs and larval transfer from southern regions and the consequent establishment of adult *S. naso* populations. Accidental introduction via ballast water from vessels and other pathways may not be implausible. In any case, ocean warming caused by global climate change is considered as a key factor in the survival and settlement of adult *S. naso* populations.

Key words: global warming, holothurian, new record, pole-ward expansion

Because of recent climate change and rise in seawater temperature, many marine species have expanded their distribution pole–ward in many regions of the world, and have caused community composition shifts, displacing indigenous species and changing ecosystem function (Firth and Hawkins 2011, Vergès et al. 2016, Wernberg et al. 2016). Around the coastal waters of Japan, issues of pole–ward expansion of species from warmer waters also have been reported in northern sites where they had not been previously identified, especially in the 21st century (Kiyomoto 2004, Kohtsuka 2006, Nojima and Okamoto 2008, Tanaka et al. 2012, Terada et al. 2016, Yamakawa et al. 2018, Nakano et al. 2019). Among them, observations of juveniles and young individuals restricted during the summer season have been considered as invalid dispersion since they have died off during winter. However tropical organisms, especially echinoderms, have been reported along the coasts north of Kyushu Island, where the influence of the Kuroshio or Tsushima Current is significant (Kiyomoto 2004, Kohtsuka 2006, Nakano et al. 2019) and some species have passed winters and settled. Until the last century, when unfamiliar species were found, we first attempt to describe them as new species; but today it is important to determine whether they were species originating from warmer waters. Because taxonomic studies of shallow coastal water species have a long history, we should often search for literature from the 19th to early 20th century, especially for exotic tropical organisms. Therefore re–description and detailed documentation of already known species in new localities has become necessary for subsequent monitoring of ecosystem shifts due to climate change.

The family Stichopodidae (Echinodermata: Holothuroidea) consists of diverse and commercially important species (Byrne et al. 2010, Purcell et al. 2012), especially those in the Indo-West Pacific region (Purcell et al. 2012). Because the Japanese archipelago is elongated from north to south, the holothurian fauna in the northern region is characteristic of cold temperate climate, whereas the southern region is characteristic of warm temperate climate. The holothurian fauna around the southwestern Amami Islands and Ryukyu Islands is almost the same as that for the tropical Indo-West Pacific fauna (Imaoka 1995, Yamana et al. 2019). Kiyomoto (2004) and Kohtsuka (2006) reported the ‘so called’ *Stichopus oshimae* from Nagasaki and the Sea of Japan, respectively. Although this species has unresolved taxonomic problems, it is obviously a warmer water species. Byrne et al. (2010) investigated the
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molecular phylogeny of the Stichopodidae in the tropical Indo West–Pacific and found that *Stichopus naso* and *S. monotuber culatus* have been often misidentified as *S. horrens*, which is fished and traded in the world markets and stocked even in museum collections. Their results suggest that this group is hard to identify even in their distribution center, however, molecular identification techniques are not always available, especially at the marginal or novel habitats caused by anthropogenic introduction or climate change. Therefore, it is worth describing morphological keys of known species from novel habitat ranges or sites to monitor ecosystem changes such as the 1,000 Monitoring Sites of the Ministry of Environment of Japan, which includes a great number of individuals of the general public. In this paper, we i) report the first occurrence of a tropical holothurian *Stichopus naso* from the mainland of Kyushu, Japan, ii) provide identification keys of species that they closely resemble, and iii) discuss its origin in the context of global climate change.

We found and collected *Stichopus naso* from Uchinoura Bay, (next to the south of Shibushi Bay), along the east coast of Kagoshima Prefecture, and on the southeastern coast of Kyushu Island (Fig. 1). Uchinoura Bay is open to the Pacific Ocean with the Kuroshio current flowing along the coast; therefore, the lowest annual sea-water temperature was over 15°C (Fig. 2) at 10 m depth. The coastal seabed of the sampling site consists of coarse sand and boulders. In 2011, the holothurian was first noticed during a SCUBA study by Yoshimura. Samples collected during that study degraded due to mishandling, and new samples were collected in 2017 with plastic sampling bags and careful handling. Underwater photos of living specimens were also taken with a flood light and a guide to provide measurements for an accurate description (Fig. 3). Just after lifting them up to the boat, they were anesthetized in 5% magnesium chloride for 2 hours and fixed using 99.5% ethyl alcohol. Fixed specimens are housed in the Invertebrate Collection (INV) of the Wakayama Prefectural Museum of Natural History (WMNH) in Kainan, Wakayama, Japan. In the laboratory, these specimens were dissected, and the outer and inner morphology of the specimens were observed in detail. Small sections from several parts, including the body wall, tube feet, tentacles, and tips of dorsal papillae, were taken to observe the ossicles. The sections were treated with a commercial bleach,
and the ossicles were observed using a scanning electronic microscope (JEOL, NeoScope™, JCM-6000).

*Stichopus naso* was found only on coarse sand bottom; not on hard substrata, interstice of rocks, nor under cobbles. The density was not examined quantitatively, but the number estimated by field photographs was 3 to 5 individuals m⁻² on the shallow coast of Uchinoura Bay. In late November 2017, we found some individuals undergoing asexual fission, being constricted in the center of the body and exposing their intestine that connected the anterior and posterior parts (Fig. 4). Several individuals that finished fission were also found, and most specimens were bearing the marks of their recent fission, i.e., missing anterior/posterior body parts (Table A1).

Most of the present specimens had 20 tentacles (Table A1), although 18 tentacles were previously reported by most researchers (e.g., Semper 1867; Massin 2007). Regardless of this contradiction, we decided that the present species was *Stichopus naso* based on all the other previous reports for the external morphology and ossicle morphology (Massin 2007; Yamana et al. 2019).

Congeneric tropical holothurians that overlap geographical distribution with *S. naso* in Amami and Ryukyu Islands are *S. chloronotus*, *S. hermanni*, and *S. horrens*. Among them, *S. horrens* has the most similar external morphology, however, this species is distinguishable by its dorsal color patterns, habitat (*S. horrens* is a hard bottom dweller), active time (*S. horrens* nocturnal), and large table ossicles from the dorsal papillae (*S. horrens* has large tack like tables with merging pillars and no spike) (Massin et al. 2002, Byrne et al. 2010, Purcell et al. 2012). Abundant long C-shaped ossicles from dorsal body wall of *S. naso* is also a diagnostic feature. These characteristics are easy to observe by eye and optical microscope, so it is convenient for the general public that participate in monitoring biological changes in the ocean environment.

Massin (2007) re-described *Stichopus naso* based on the specimens from Madagascar, Papua New Guinea, Thailand, and Okinawa. He also examined various literature; from original description by Semper (1867) to modern reports, such as that of Kohtsuka (2006), assembling several names as synonyms of *S. naso*. Among the synonyms, Massin (2007) wrote that *S. ohshimae* (considered as a miss-spelling of *S. ohshima*) of Japan mainlands (known as a Japanese name AKAONI-NAMAKO) identified by Kohtsuka (2006) remained doubtful. However, the AKAONI-NAMAKO reported by Kiyomoto (2004) and Kohtsuka (2006), and visualized photos in many identification guidebooks published in Japan such as Imaoka (1995), are quite different from *S. naso* in coloration, outer morphology, adult size, and habitat. Since Massin (2007) did not examine specimens of AKAONI-NAMAKO, he might have only examined the original description of *S. ohshima* by Mitsuiri (1912) and considered it to be synonymous to *S. naso*. The strict taxonomy of *S. ohshima* with relation to the other Stichopodidae from Japan is still vague. It is quite possible *S. ohshima* is the same species as *S. naso* (this is an issue that must be solved by observing the type materials in the study), however, we considered that AKAONI-NAMAKO is not *S. naso*, because of several critical differences we observed between AKAONI-NAMAKO and *S. naso* (pers. comm. by Yamana, preparations for publishing a report are carried on). Massin (2007) also assumed that the *S. horrens* reported by Kohtsuka et al. (2005) was *S. naso*. This case was a report of fission that occurred on the soft bottom during the daytime. As pointed out by Byrne et al. (2010), *S. naso* and *S. monotuberculatus* have been frequently misidentified as *S. horrens*, and they doubted the asexual reproduction of *S. horrens*. Among these three species, *S. naso* is the only diurnally active soft substratum dweller, whereas the other two are nocturnal and prefer hard substrate. We assumed Massin’s identification to be right when we observed the photos of Kohtsuka et al. (2005). The specimen appeared to be quite similar to *S. naso*; based on its body color, outer morphology, habitat, and observation date of fission (14 December 2004). As reported by Byrne et al. (2010) and Dombatov (2014), *S. naso* is an asexual reproducer in tropical shallow waters; contrary to Dombatov’s (2014) hypothesis, that assumed fission of holothurians occurs during the summer in the northern hemisphere while the fission of *S. naso* in Japan occurred in early winter.

Because Kuroshio is the dominant warm current along the Japanese southern coast, eggs, larvae, or juveniles of tropical marine species have relocated and adults have frequently been found on the coast of the Pacific Ocean, especially during warm seasons. However, they normally die off during winter and may be considered as invalid dispersion. From the last quarter of the 20th century, many tropical or warm-temperate marine species tended to expand their distribution northward around Japan and settled year-round, because the water temperature around Japan has had a tendency to rise (Japan Meteorological Agency 2019). Tanaka et al. (2012) examined seawater temperature and seaweed composition and distribution and found that temperate canopy forming seaweeds disappeared or were reduced in range, and tropical seaweeds occupied the formers’ niche along the coast of the Kochi Prefecture. On the coast of Shirahama, Wakayama Prefecture, Nakano et al. (2019) reported a tropical sea urchin *Echinometra* sp. C whose original distribution was only in Okinawa and southward. In this case, all individuals of this urchin species died during a colder winter in 2018; however, tropical species may reach and settle there again in the future. Other possibilities for the occurrence of *S. naso* include the anthropogenic transfer of larvae by ballast water, and contamination through ornamental trade of ‘live rocks’ that may contain larvae and juveniles of various taxa (Padilla and Williams 2004). These cases are plausible but difficult to differentiate; however, considering the invasion of the southeast Asian lionfish (*Pterois* spp.) to Atlantic coasts by tank escape (Côté and Smith 2018), they are not exclusive. Even in these cases, a rise in seawater temperature is essential for the presence of *S. naso* along Kyushu mainland.

After the first identification of *S. naso* in 2011, its population has been stable until 2019 and it was recently found in another location to the north, adjacent Shibushi Bay (K.
Watanabe pers. comm.). Therefore, the population of *S. naso* has been established, is stable, and may further expand poleward in the future. However, none of the individual specimens had matured gonads, but had traces of recent fission; thus, the range expansion may have not been caused by larval transfer from Uchinoura Bay. Regarding the primary factors for asexual reproduction of shallow-dwelling holothurians, Chao et al. (1994) revealed that environmental inappropriateness can introduce asexual reproduction in the tropical holothurian *Holothuria (Halodeima) atra* Jaeger, 1833; thus, there is a possibility that asexual reproduction of *S. naso* may take priority over sexual reproduction in Uchinoura Bay, where environmental stresses (especially the low water temperature) are considered to limit the survival of *S. naso*. Massin (2007) also noticed that gonads were absent in all specimens he studied, even samples from various seasons. Thus, asexual fission might be primary mode of reproduction of *S. naso*, however, we do not deny that this species can also reproduce sexually. After all, the habitat of southern Japan, i.e., Amami Islands (Yamana et al. 2019) and Ryukyu Islands (Kohtsuka et al. 2005), may be the source supplying mainland Kyushu.

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Appendix

Description

Order Synallactida (MILLER, KERR, PAULAY, REICH, WILSON, CARVAJAL & ROUSE, 2017)
Family Stichopodidae HAECKEL, 1896
Genus Stichopus BRANDT, 1835
Stichopus naso Semper, 1867

Material examined. 7 specimens, WMNH-INV-2018-29–35 (length 64–98 mm) (Fig. A1, Table A1).

Description: Medium holothuroid, approximately 100 to 150 mm length in live specimens and approximately 60 to 100 mm in alcohol. Body cross section, trapezium in contracted specimens with ventral wider than dorsal, whereas relaxed specimens were circular or oval. Both ends of animals were slightly narrower than the central part of the body. Both body color and shape were not seriously affected by fixation/preservation. Dorsal body color uniformly beige; except tips of podia and papillae that appeared light brown. No color pattern observed. Ventral sole color paler than dorsal color (Fig. A1). Body color changed quickly because of handling from original to yellowish brown, to dark brown, and finally black, but was maintained with careful treatment and anesthetizing. Ventral mouth surrounded by 20 tentacles (also 18 tentacles detected in one specimen) (Table A1), and collar-like papillae surrounded the tentacles. Anus terminal without papillae. Dorsal papillae appear to have obscure concentric rings of shallow wrinkles. Linear deep wrinkles found on dorsal surface, dividing the dorsal side (bivium) into two to four rows of papillae. Large papillae were generally single, whereas some medium papillae assembled mostly at anterior and posterior ends. A row of prominent papillae was well defined along the vent-lateral edge of the sole. On the ventral side (trivium), podia were assembled as three bands, comprising ambulacrum zones. Narrow inter-ambulacra of trivium. Tips of ventral podia and dorsal papillae and podia light brown, colorations were lost after specimens were fixed with alcohol.

Calcareous ring short, thick, with both radial and inter-radial solid plates, rectangular radial plates with one posterior depression and three anterior depressions, and interradial plates with one sharp anterior prolongation (Fig. A2). Polian vesicles in medioventral up to three. Stone canal in mediodorsal, single, with a large madreporite up to 1.5 mm length. Two bands of longitudinal muscle observed in each radius in the body cavity; however, were frequently broken off at the anterior/posterior body end, where another half of the body part

Table A1. Counts and measurements of *Stichopus naso* Semper, 1867, housed in WMNH.

| Registration  | Body status | Gonad state | Body size (mm) | Counts |
|--------------|-------------|-------------|----------------|--------|
|              |             |             | Length | Width | Tentacles | Polian vesicles |
| WMNH-INV-2018-29 | Anterior half | immature | 74    | 29    | 20         | 1           |
| WMNH-INV-2018-30 | Posterior half | immature | 64    | 28    | 0          | 0           |
| WMNH-INV-2018-31 | Anterior half | immature | 77    | 38    | 20         | 1           |
| WMNH-INV-2018-32 | Anterior half | immature | 80    | 30    | 20         | 1           |
| WMNH-INV-2018-33 | Anterior half | immature | 82    | 30    | 18         | 3           |
| WMNH-INV-2018-34 | Posterior half | immature | 80    | 34    | 0          | 0           |
| WMNH-INV-2018-35 | Complete | immature | 98    | 35    | 20         | 1           |

Fig. A1. The specimens of *Stichopus naso* Semper, 1867 (Table A1), dorsal view of WMNH-INV-2018-29–35 and abdominal view of WMNH-INV-2018-32.

Fig. A2. Elements of calcareous rings, body-left of WMNH-INV-2018-33 viewed from the body cavity, anterior upper. Abbreviations: md, mid-dorsal part; mv, mid-ventral part; -ir, inter radial element; -r radial element.
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A tropical holothurian expanding to temperate coasts in Japan was lacking and occurring with regenerating tissues present. Mature gonads not observed in any specimen (Table A1).

Ossicles of dorsal body-wall comprised of tables, rosettes, C-shaped rods, and S-shaped rods (Fig. A3, C–F). Usual table possessing circular disc ranging 30–40 $\mu$m in width, with 4 central holes, up to 12 peripheral holes, possessing a spire ranging 25–40 $\mu$m in height with four pillars (Fig. A3, F). Tips of pillars split into two short branches. Rosettes flat, 15–26 $\mu$m long, exhibiting arabeque patterns (Fig. A3, D). Two types of C-shaped rods: abundant long ones, 150–200 $\mu$m, and fewer short ones, 66–85 $\mu$m (Fig. A3, C). Width of long C-shaped rods 7–9 $\mu$m, that of short ones 4–5 $\mu$m at the center of rods. S-shaped rods 200–220 $\mu$m long and very rare (Fig. A3, E). Ventral body-wall containing tables, similar in size and shape as dorsal ones, large perforated rosettes (86–270 $\mu$m long and 58–70 $\mu$m width), C-shaped rods (fewer and smaller than those of dorsal ones), and spiny rods (280–360 $\mu$m). On the surface of spiny rods, many minute prickles observed, being dense and conspicuous at both ends of rods. Both or one end of some rods forked. Ossicles of tentacles, predominantly spiny rods, appearing as a dorsal body-wall (Fig. A3, A), but much longer (450–740 $\mu$m), with more conspicuous prickles arranged into lines along the rods. Tentacles also having small C-shaped rods (30–50 $\mu$m long) (Fig. A3, B). Dorsal papillae contained small and large tables, rosettes, and C-shaped rods (Fig. A3, L–N). Small tables, rosettes, and C-shaped rods are similar to those of the body-wall, but with large tables (Fig. A3, N) showing characteristic features: polyporous disc with 75–115 $\mu$m diameter (mostly 90–100 $\mu$m) and a spire height of 60–105 $\mu$m, having four pillars with spikes, and connected by one to three transversal beams. Tips of pillars extend straight, not branched, but sometimes merge into one, shaped like a tack.

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Fig. A3. Scanning electron microscope image of ossicles of *Stichopus naso* Semper, 1867. A, rod ossicles of the tentacles; B, small C-shaped rods of the tentacles; C, various-sized C-shaped rods of the dorsal body-wall; D, small rosette ossicles of the dorsal body-wall; E, large S-shaped rods of the dorsal body-wall; F, regular table ossicles of the dorsal body-wall; G, regular table ossicles of the pedicel; H, small rosette ossicles of the pedicel; I, rod ossicles of the pedicel; J, plate ossicles of the pedicel; K, end plate (1/4 part) of the pedicel; L, large C-shaped rods of the dorsal papilla; M, regular table ossicles of the dorsal papilla; N, large-sized table ossicles of the dorsal papilla.