The Earliest Japanese records of the invasive European ascidian *Ascidiella aspersa* (Müller, 1776) (Urochordata: Ascidiidae) from Mutsu and Ago Bays, with a brief discussion of its invasion processes

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(Received August 26, 2018; Accepted November 8, 2018; Published Online January 1, 2019)

Abstract

Study of the ascidian collection at the National Museum of Nature and Science, Tsukuba, revealed some Japanese specimens of the non-native ascidian, *Ascidiella aspersa* (Müller, 1776), collected in 2007 from both mid-temperate Oominato, Mutsu Bay, in the northernmost part of Honshū, and from warm-temperate Ago Bay, Kii Peninsula, middle Honshū. These specimens were collected one year earlier than the previous earliest Japanese record from cool-temperate Funka Bay, Hokkaido. Mutsu Bay has an international port, which can be assumed to be the invasion gateway for this ascidian from abroad. On the other hand, this ascidian may have arrived at Ago Bay by domestic transport because all of the bay’s ports are strictly for domestic use under governmental regulations. A comparison of the publicly available sequences for the 18S rRNA gene among this species and its allies suggested the possibility that it inhabited Korean waters as far back as the late 1990s, and it entered Japanese waters from Korea through an as yet unknown international port(s).

Keywords: *Ascidiella aspersa*, alien species, ascidian, aquaculture, pathway of invasion

Introduction

The European ascidian *Ascidiella aspersa* (Müller, 1776) is notoriously known as the first invasive ascidian to severely damage aquaculture in Japanese waters (Nishikawa et al., 2014; for other alien ascidians in Japan see Nishikawa, 2017). In Japan, the species has been recorded since 2008 from Funka Bay, on the Pacific coast of south-western Hokkaido, and subsequently from the Sea of Japan coastline of Hokkaido, Mutsu Bay, and the Pacific coast of Tohoku District (Kanamori et al., 2014). Both these localities are situated in cool- and mid-temperate zones in Japan.

Using molecular data, Nishikawa et al. (2014) confirmed the morphological differentiation (distinguishable morphologically by comparing the number of branchial tentacles with that of inner longitudinal vessels on each side) of *Ascidiella aspersa* from *A. scabra* (Müller, 1776), which had been previously doubted by some taxonomists. Therefore, the specific identification and differentiation of species of the genus is now possible, even for formalin-preserved immature specimens. Thus, our examination of specimens deposited in the ascidian collection of the National Museum of Nature and Science, Tsukuba, Japan (coded as NSMT-Pc) has revealed the occurrence of *A. aspersa* in mid-temperate Mutsu Bay and also in warm-temperate Ago Bay. These records date from 2007, which is one year earlier than the previous first Japanese record from cool-temperate Funka Bay (in 2008). In South Korea, the species was recognized firstly in 2010 from cool-, mid-, and warm-temperate zones by Pyo et al. (2012). However, we suggest the possibility in this paper that the Korean coastline was actually already inhabited by this species in the 1990s. Taking all the available information into account, the colonization processes of Japanese local populations is briefly discussed.

Materials and methods

Details of the material examined are given in the section headed “Taxonomic Accounts” below. These specimens are registered as NSMT-Pc 2535 to 2040 and NSMT-Pc 2557. Specimens were judged as mature when sperm and/or eggs were found in the genital tracts. Cohort analyses of the Pc 2538 individuals, conspicuous in number (76), were performed using FiSAT II (Gayanilo et al., 2005), a computer program provided by the FAO (http://www.fao.org/fishery/topic/16072/en, searched on 29 June 2018). We believe that the sample size (76 individuals analysed), which is substantially greater than in any other sample, is sufficiently high.
for this cohort analysis. For a comparison of the 18S rRNA gene among the registered, publicly available sequences of Ascidiella aspersa and Ascidia zara on GenBank (AJ250777, JN573231, and AB610838 for the former, and AF165820 for the latter), together with other three species of the genus Ascidia, that is A. ahodori Oka, 1927 (AB104871), A. ceratoedes (Huntsman, 1912) (L12378), and A. sydneiensis Stimpson, 1855 (registered as A. sydneiensis samea, AF165819) for validation of AF165820, they were aligned using the multiple sequence alignment program CLUSTAL W version 2.1 provided by the DDBJ (http://clustalw.ddbj.nig.ac.jp/index.php?lang=en, searched on 3 March 2018).

Results and discussion
1. Taxonomic Accounts

Ascidiella aspersa (Müller, 1776)

Material Examined: NSMT-Pc 2535, 1 individual, from an artificial fish-attracting reef under pearl culturing rafts set off Nishiyama in the town of Ago, Shima City, Ago Bay, Mie Pref., SCUBA, 9 m deep, 19 June 2007, M. Otani coll.; NSMT-Pc 2536, 3 individuals, from the rafts off Tatagami, Agocho, Shima City, Ago Bay, Mie Pref., 34°17.5′ N and 135°50′ E, 1 m, 21 July 2007, M. Otani coll.; Pc 2537, 2 individuals, from buoys and ropes on the rafts off Tatagami, Agocho, Shima City, Ago Bay, Mie Pref., 0–1 m, 22 July 2007, T. Nishikawa coll.; NSMT-Pc 2538, 76 individuals, attached to benthic shells of the oyster Crassostrea gigas (Thunberg, 1793) under the rafts off Komushiro Islet, Katada, Shimacho, Shima City, Ago Bay, Mie Pref., SCUBA, 9.5 m, 25 June 2008, Y. Murata, A. Yasuda, M. Otani, and T. Nishikawa coll.; NSMT-Pc 2539, 1 individual, from a culture raft off Hasama, Minami-ise-cho, Gokasho Bay, Mie Pref., 0-1m, 15 April 2011, M. Otani coll.; NSMT-Pc 2540, 3 individuals, from the rafts in front of the National Research Institute of Aquaculture, Nakatsuhama-ura, Nansei-cho, Gokasho Bay, Mie Pref., 9°40′N and 136°41′E, 0–1 m, 15 April 2011, M. Otani coll.; Pc 2557, 1 individual, Oominato City, Mutsu Bay, Aomori Pref., dredged by epibenthic sled, depth unknown, June 2007, M. Otani coll.

Description: Body cylindrical or oval in outline, attached to substratum by whole left side, 16.0–60.0 mm long. Tunic thin, cartilaginous, white, semitransparent; the surface nearly smooth or furnished sparsely with low prominences. Mantle pigmented reddish orange wholly on right, and anteriorly on left when alive; right musculature composed centrally of a meshwork of thick bundles, which become thinner peripherally and run transversely (at dorsal and ventral margins) or longitudinally (at posterior margin). Number of branchial tentacles (including minute ones) and that of inner longitudinal vessels on each side given in Table 1. Ciliated groove simple, C-shaped, opening anteriorly, located adjacent posteriorly to dorsal ganglion, followed posteriorly by a narrow groove two or three times as long as dorsal tubercle. Prebranchial zone narrow and smooth-surfaced. Longitudinal vessels without secondary papillae. Edge of dorsal lamina serrated at tips of ribs with some undulations in between them. Visceral mass situated posteriorly, roughly at two-thirds of mantle body, with its anterior margin at, or slightly lower than, anterior base of atrial siphon. Viscera usually covered densely with dark brown renal vesicles. Stomach globular, sometimes with a few inner plications. First and second intestinal loops very deep, with their axes almost longitudinal; axis of second loop passing through middle of esophagus. Tunica vessels issuing from mantle along ventral side of mid-intestine. Gonad maturity in NSMT-Pc 2539 and 2540 specimens collected in April.

Remarks: The specimens examined can be confidently

Table 1. Measurements of Japanese specimens of Ascidiella aspersa (Müller, 1776) in the ascidian collection of National Museum of Nature and Science, Tsukuba, Japan (NSMT-Pc), collected in 2007–2011

| Registration Number | Locality       | Collection date | Body Length (mm) | Number of tentacles | Number of inner longitudinal vessels on the left | Number of inner longitudinal vessels on the right | Maturation |
|---------------------|----------------|-----------------|------------------|---------------------|-----------------------------------------------|-----------------------------------------------|------------|
| NSMT-Pc 2535        | Ago Bay        | 19 June 2007    | 31.0             | 16                  | 33                                             | 42                                             | No         |
| NSMT-Pc 2536        | Ago Bay        | 21 July 2007    | 16.0             | 22                  | 27                                             | 31                                             | No         |
| NSMT-Pc 2537        | Ago Bay        | 21 July 2007    | 21.0             | ca. 20              | 30                                             | 37                                             | No         |
| NSMT-Pc 2538        | Ago Bay        | 25 June 2008    | 25.0             | 24                  | 39                                             | 42                                             | No         |
| NSMT-Pc 2539        | Gokasho Bay    | 15 Apr. 2011    | 60.0             | 28                  | 36                                             | 42                                             | Yes        |
| NSMT-Pc 2540        | Gokasho Bay    | 15 Apr. 2011    | 33.0             | 20                  | 32                                             | 41                                             | Yes        |
| NSMT-Pc 2541        | Gokasho Bay    | 15 Apr. 2011    | 41.0             | 20                  | 33                                             | 41                                             | Yes        |
| NSMT-Pc 2542        | Gokasho Bay    | 15 Apr. 2011    | 55.0             | 21                  | 31                                             | 36                                             | Yes        |
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identifiable as *A. aspersa* in the sense of Nishikawa et al. (2014), as distinct from the closely related *A. scabra*, based on the lower number of branchial tentacles relative to that of inner longitudinal vessels on each side in the former (see Table 1).

2. Ecological Notes

The specimens of *Ascidiella aspersa* living on the ropes at Ago Bay (NSMT-Pc2537) were collected together with the following nine other species of ascidians: *Diplosoma mitsu-kurii* Oka, 1892, *Ciona robusta* Hoshino and Tokioka, 1967 (previously referred to as *C. intestinalis* complex sp. A), *C. savignyi* Herdman, 1882, *Aiscidia sydneiensis* Stimpson, 1855, *A. ahodori* Oka, 1927, *A. zara* Oka, 1935, *Styela canopus* Savigny, 1816, *S. plicata* (Lesueur, 1823), and *Botrylloides* sp., all of which are well-known fouling ascidians in Japan. *Ascidiella aspersa* was the rarest of all the ascidians present on this occasion.

Figure 2 shows the body length composition of all the 76 immature specimens of *A. aspersa* of NSMT-Pc 2538, 16.2–49.0 mm long, collected in June from Ago Bay, 9.5 m deep. FiSAT II analyses yielded equal support for both the single cohort model (29.64 ± 5.69 mm long) and the two cohort model (23.08 ± 2.92 mm long with 31 individuals, and 32.98 ± 4.57 mm long with 46 ones). The present material includes specimens collected in April, June and July, among which only the April specimens (NSMT-Pc 2539 and 2540, collected in 2011), 33.0–60.0 mm long, were mature from Gokasho Bay adjacent eastward to Ago Bay. Consequently, April can be regarded within the breeding period there, and the whole (in the former analysis) or the younger (in the latter) cohort may probably have settled about this month. The present study regarded within the breeding period there, and the whole (in the former analysis) or the younger (in the latter) cohort may probably have settled about this month. The present study

3. Possible Pathways of Invasion

3-1. A. aspersa in the Far East

Nishikawa et al. (2014) suggested that the previous records of *“Ascidiella aspersa”*, including alien populations, might sometimes be unreliable, because of the lack of morphological or molecular information to distinguish *A. aspersa* from *A. scabra*, both of which are known as colonizers (for the latter species see Nishikawa and Otani, 2004). In the Pacific Ocean, Brewin’s (1946) description of *A. aspersa* in New Zealand is informative, from which its introduction to the southern half of the Pacific Ocean about 1945 seems definitely to be the earliest.

In the northern Pacific Ocean, the specimen-based present study has revealed the earliest occurrence of *A. aspersa* in Japan was in 2007, where no earlier occurrence has yet been recorded (see Nishikawa (1980) for Ago and Gokasho Bays, and Nishikawa (1990, 1991, 1992) for Mutsu Bay). Figure 1 shows the known localities of *A. aspersa* with the year of first occurrence in the Far East region, clearly showing its ability to survive in cool-, mid-, and even warm-temperate zones. In South Korea, the undoubted occurrence of *A. aspersa* has been confirmed by comparing Pyo et al.’s (2012) COI data with that of Nishikawa et al.’s (2014). Pyo et al.’s (2012) first specimens were collected in October 2010 at eight out of 26 harbors or ports along the entire South Korean coast during their survey (Fig. 1), which started in August 2009. Therefore, the first documented occurrence of *A. aspersa* in South Korea is almost three years later than that in Japan. However, its Korean occurrence may possibly go back to the 1990s.

Won et al. (1999: P. 360) sequenced the partial 18S rRNA gene of *“Ascidia zara”* derived from a specimen “from the southeastern parts of Korea between 1996 and 1998”, with the accession number of AJ250777. A comparison with the AF165820 sequence of *Ascidia zara* (probably from Japan)
has shown a marked difference at 14 sites (2.19%) in the aligned 638 sites. Pair-wise 18S rRNA sequence comparisons in those sites among the four species of the genus *Ascidia*, *A. zara* (AF165820), *A. ahodori* (AB104871), *A. sydneiensis* (AF165819), and *A. ceratodes* (L12378), showed that the number of different sites ranged from 6 (between *A. ahodori* and *A. ceratodes*) to 15 (between *A. zara* and *A. sydneiensis*), with *A. zara* having that number of 8 (from *A. ceratodes*), 9 (from *A. ahodori*), or 15 (from *A. sydneiensis*); these four species were different from *Ascidia aspersa* (see below) at 9 (for *A. sydneiensis*) to 16 (for *A. ahodori*) sites. Consequently, the AF165820 sequence is more similar to those of *Ascidia ceratodes* and *Ascidia ahodori* than the AJ250777, which may demonstrate the reliability that AF165820, instead of AJ250777, was supposed derivable from the “true” *Ascidia zara*. Instead, the AJ250777 data are almost the same as the JN573231 (from Korea) and AB610838 (from Spain) sequences of *Ascidiella aspersa*, differing from one another only at a single (0.15%) out of the aligned 637 sites; the 18S rRNA gene of *Ascidiella scabra*, which is possibly very similar to that of *A. aspersa*, has not yet been registered in GenBank. For precise identification, we tried to obtain the specimen responsible for AJ250777 to undertake a morphological comparison, but it was unavailable (Dr. Jun Hye Won, personal communication). However, it seems that Won et al.’s (1999) “Ascidia zara” (shown by AJ250777) may probably represent *Ascidia aspersa*, which would have been living in South Korean waters in the late 1990s, thus far predating the confirmed Japanese occurrences in 2007. It might even be
possible that the Japanese population came originally from South Korea; Kumagai (2011) confirmed that the edible ascidian *Halocynthia roretzi* (Drasche, 1884) species complex was imported in 2004 or 2006 for aquaculture from South Korea to Miyagi Prefecture, Japan, which may be a possible route of invasion. 

3-2. *A. aspersa* in mid-temperate Mutsu Bay and Tohoku district

The present study has revealed the occurrence of *A. aspersa* in the benthos in Oominato, at the north-eastern end of Mutsu Bay in 2007. According to the annual reports published by the Fisheries Research Institute of Aomori Prefectural Industrial Technology Research Center, the species was rarely found attached to cultured scallops since 2009 in the eastern part of the bay (Kudo et al., 2011; Kotani et al., 2012; etc.), so far fortunately without severe damages to aquaculture. This might possibly show a time lag of at least two years between the benthic introduction and the fouling of cultured scallops set in rafts. If this is applicable to Funka Bay, the species might have colonized the sea floor there in 2006 (that is two years prior to the sudden appearance in 2008) at the latest. Mutsu Bay has an international port, Aomori Port, that is possibly responsible for the invasion gateway directly from abroad, like Funka Bay with its international Muroran Port. Once established there, *Ascidella aspersa* could have spread to Oominato.

Chida et al. (2011) reported the results of a questionnaire to fishermen working for aquaculture in Miyagi Prefecture, showing that *A. aspersa* and/or *Ascidia zara* had been recognized since in 1996 on oyster- and scallop-culturing rafts in Kesen’numa Bay (38°51′N and 141°35′E) and it began to spread along neighboring bays around 2003, so far usually with low densities. Therefore, the possibility cannot be excluded that *A. aspersa* appeared in the bay in 1996. For reference, Nishikawa’s unpublished data shows that the ascidian fauna fouling aquaculture rafts in Otsuchi Bay (39°20′N and 141°55′E), Iwate Prefecture, annually from 1990 to 1996 and again in 1999, included *Ascidia zara* frequently, but neither *A. aspersa* nor *A. scabra*. In any event, Kesen’numa is located near an international Sendai-Shiogama Port, which might possibly show a time lag of at least two years between the benthic introduction and the fouling of cultured scallops set in rafts. Miyagi Prefecture, Tsu, 39 pp.

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