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Title

Genetic structure of *Saccharina japonica* in Japan and evaluation of a potential
mitochondrial region for identification of geographic origin

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Abstract

Conservation units are still unclear for the commercially important kelp *Saccharina japonica*. Previous analyses of population genetic structure have yielded inconsistent results regarding the number of clusters, especially in Japan. Thus, the genetic structure of *S. japonica* in Japan was studied using the mitochondrial *nad3*-16S rDNA region. We detected 88 haplotypes in 483 individuals collected from 46 localities. Unique haplotypes and one or a few shared haplotypes at a local scale were found in most localities. The observed genetic structure revealed cryptic invasions of *S. japonica* within Japan and the value of the *nad3*-16S rDNA region for identifying the geographic origin of kelp. Bayesian Analysis of Population Structure analysis and F_{ST} revealed the genetic divergence of populations in southwestern Hokkaido. The haplotype network showed a more detailed starburst pattern compared with the results of previous studies based on mitochondrial *COI* and *trnW-trnI*. Accordingly, *S. japonica* in Japan may represent one genetic group that experienced a recent expansion. Unique or locally shared haplotypes and similarity in haplotype diversity on different coasts of Hokkaido could be explained by refugia during the Last Glacial Maximum on various coasts. Furthermore, the present study demonstrated inconsistencies between the genetic structure and distribution of *S. japonica* varieties. Therefore, further investigations focused on the taxonomic validation of varieties are needed.

Keywords

Saccharina japonica, *Saccharina longissima*, phylogeography, kelp, cryptic invasion

Declarations

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54 The authors have no conflicts of interest directly relevant to the content of this article.

55

56 **Data availability**

57 The datasets generated during and/or analyzed during the current study are available on
58 the GenBank or from the corresponding author upon reasonable request.

59

60 **Authors' contributions**

61 Conceptualization and methodology: KC and SA, Funding acquisition: DF and SA,
62 Sampling: KC, CK, KI, YY, DF, and SA, Formal analysis: KC, Data curation: KC and
63 SA, Visualization: KC and SA, Writing, editing, and review: KC, CK, KI, HM, YY, TU,
64 DF, and SA, and Supervision: HM, TU, and SA.

Introduction

Saccharina japonica (Aresch.) CE Lane, C. Mayes, Druehl & GW Saunders was originally distributed on the northern coast of Japan, North Korea, and Far Eastern Russia; however, wild populations introduced via aquaculture have been maintained in China since 1927 and South Korea since the 1970s (Hwang et al. 2019). In the species, three varieties, *S. japonica* var. *diabolica* (Miyabe) Yotsukura, Kawashima, T. Kawai, T. Abe & Druehl, *S. japonica* var. *ochotensis* (Miyabe) Yotsukura, Kawashima, T. Kawai, T. Abe & Druehl, and *S. japonica* var. *religiosa* (Miyabe) Yotsukura, Kawashima, T. Kawai, T. Abe & Druehl, are taxonomically accepted (Yotsukura et al. 2008). Although these varieties were previously treated as independent species based on distinct morphological features (Miyabe 1902), they were merged based on their independent distributions but variable morphological characteristics (Yotsukura et al. 2008). Recently, genetic clusters roughly corresponding to the geographical distribution were reported (Yotsukura et al. 2016). *S. japonica* is a commercially important species particularly in Eastern Asia (Hwang et al. 2019; Hu et al. 2021). In Japan, production in 2022 was 31,691 t through aquaculture and 45,163 t from natural harvest (Japanese Fisheries Agency 2023). The wild stock is critical even in aquaculture because matured wild sporophytes are used in seedling production (Hwang et al. 2019). Thus, the conservation of wild stocks is essential to sustain both production methods.

Information on genetic structure is fundamental for determining the conservation unit of wild populations. For *S. japonica* and its varieties, genetic structure has been inferred in various studies (Zhang et al. 2015; Yotsukura et al. 2016; Shan et al. 2017; Zhang et al. 2017, 2019, 2021; Yotsukura et al. 2022). However, inconsistent results have been obtained, particularly for the genetic structure in Japan (Zhang et al.

2015; Yotsukura et al. 2016; Shan et al. 2017; Zhang et al. 2021; Yotsukura et al. 2022). Zhang et al. (2015) investigated the population structure for the first time using *COI* and *trnW-L* in mitochondrial DNA (mtDNA); in subsequent studies (Yotsukura et al. 2016; Shan et al. 2017; Zhang et al. 2019; Yotsukura et al. 2022), fine-scale genetic structure was evaluated using polymorphic microsatellite markers. One (Zhang et al. 2015, 2019), two (Shan et al. 2017; Yotsukura et al. 2022), or four (Yotsukura et al. 2016) genetic clusters were detected in Japanese populations. Despite the inconsistent results, the existence of four genetic clusters, suggested by Yotsukura et al. (2016), in Japan is generally accepted, probably because it is consistent with the allopatric distribution of *S. japonica* and its varieties and the treatment of the varieties as different brands in the seaweed market. However, Yotsukura et al. (2016) demonstrated that one out of four genetic clusters corresponded to a very small bay, Oshoro Bay (about 200 × 100 m²), where the sample size was 7-fold larger than those at other localities. Furthermore, the southern isolated population around the Joban region on the eastern coast of Honshu was not included (Fig. 1). Accordingly, the actual genetic clusters of *S. japonica* in Japan are unclear.

The genetic structure of a wild population can also be useful for PCR-based identification (Gopi et al. 2019) of the geographic origin of a food product. Among DNA-based methods used in the food and aquaculture industries, nucleotide sequencing-based DNA barcoding is preferable because it requires only a query against a public database, unlike microsatellite and SNP genotyping. Indeed, candidate markers in mitochondrial genes have been identified (Shimizu et al. 2004, 2010; Yotsukura et al. 2010), including 16S rDNA, *trnI*, *trnM*, *rps19*, *ORF41*, and *atp8-16S*, to delimitate *S. japonica* and its varieties using PCR-based identification. However, further analyses,

including the validation and practical application of these markers, are lacking. In *Ecklonia*, a genus belonging to the same family (Arthrothamnaceae), a clear geographically related genetic structure was shown using *atp8*-16S rDNA (Akita et al. 2020). Therefore, this mitochondrial region is a candidate DNA marker to elucidate the genetic structure and to identify geographic origins. The purpose of this study was to reveal the genetic structure of *S. japonica* in Japan using the *nad3*-16S rDNA region, which includes *atp8*-16S rDNA. The value of the region as a genetic marker was evaluated using publicly available sequences.

Materials and Methods

Sample collection

Sporophytes of *S. japonica* were collected at 46 localities from 2022 to 2023, covering various regions of its distribution (Fig. 1, Table 1). Two to twenty-three thalli were collected at each site. The samples were transported to the laboratory under cool conditions and rinsed with sterilized fresh water. A clean part of the thallus was excised and stored at -30 °C until DNA extraction. All specimens were identified to the variety level based on the distribution described in Yotsukura et al. (2016). With respect to the southern isolated population on the eastern coast of Honshu, which was not included in previous studies, two morphological types, *S. japonica* and *S. japonica* var. *religiosa*, were reported (Kawashima 2012). However, in the present study, we provisionally treated the specimens from the area as *S. japonica* var. *religiosa*, because this was geographically closest population, distributed in northeastern Honshu according to Yotsukura et al. (2016). In addition, we distinguished *S. japonica* var. *religiosa* in Hokkaido and Honshu as *S. japonica* var. *religiosa* 1 and *S. japonica* var. *religiosa* 2,

respectively (Fig. 1, Table 1).

DNA extraction, PCR, and sequencing analysis

In total, 483 individuals were used to detect genetic clusters. A freeze-dried small fragment from each sample (ca. $2 \times 2 \text{ cm}^2$) was cut out and ground with metal beads using Shakeman 6 (Bio Medical Science Inc., Tokyo, Japan). DNA was extracted using a HEPES/CTAB method described by Shepherd and McLay (2011), which was developed for polysaccharide-rich terrestrial plants. PCR amplifications of the mitochondrial *nad3*-16S rDNA region (total 2,019 bp), which includes partial *nad3* (110 bp), *rps14* (291 bp), *atp8* (162 bp), *trnS* (88 bp), *trnD* (73 bp), *rps10* (333 bp), *trnS* (85 bp), and *rpl31* (212 bp) and a non-coding spacer (669 bp), were performed using the KOD One™ PCR Master Mix (Toyobo, Osaka, Japan), primer sets designed in this study (*nad3*-16s_F32863: ATCCGTATGAAGATGCTCGTA, *nad3*-16s_R35073: CTTATAGCCTTCACTCTGAGC), and the GeneAtlas K02 (Astec, Fukuoka, Japan) under the following conditions: pre-denaturation at 98 °C for 5 min, followed by 5 cycles of denaturation at 98 °C for 10 s, annealing at 60 °C for 10 s, and elongation at 68 °C for 1 min, 35 cycles of denaturation at 68 °C for 10 s, annealing at 55 °C for 10 s, and elongation at 68 °C for 1 min, and a final elongation at 68 °C for 5 min. The PCR mix was prepared using KOD One™ PCR Master Mix, following the manufacturer's protocol. After purification using ExoSAP-IT (Thermo Fisher Scientific Inc., Waltham, MA, USA), PCR products were used as templates for the cycle sequencing reaction using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and 3130xl DNA Analyzers (Thermo Fisher Scientific). For cycle sequencing, another primer designed in this study (*nad3*-16s_R34517: TGCCATAAACCCTCGAC) was

also used to cover the middle region of the amplified fragment. The obtained sequences were aligned manually using AliView ver. 1.28 (Larsson 2014).

Detection of genetic diversity and structure

To acquire genetic characteristics for each locality, the number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (Pi), and haplotype distribution were detected using ARLEQUIN ver 3.5.1.3 (Excoffier and Lischer 2010). A statistical parsimony network was inferred using TCS ver.1.21 (Clement et al. 2000). In addition, population structure was assessed using Bayesian Analysis of Population Structure (BAPS) ver. 6 (Corander et al. 2008) with the “clustering for linked loci” method, which determines the most likely number of genetic clusters. In the analysis, ten replicates were run for each *K*-value of 2–20. The result with the highest log marginal likelihood was selected among the runs.

To evaluate population demography, Tajima's D (Tajima 1989) and Fu's *F_s* (Fu 1997) neutrality tests were performed. Significance of each value was assessed using 10,000 coalescence simulations, implemented in ARLEQUIN v3.5 (Excoffier and Lischer 2010).

Evaluation of the *nad3*-16S rDNA region as a molecular marker

To evaluate the effectiveness of the *nad3*-16S rDNA region for identification, we determined the haplotype of *S. japonica* from Japan deposited in GenBank. The results were compared with those for previously established markers, *COI* and *trnW-L* (Zhang et al. 2015, 2019). The region was manually retrieved from five complete mtDNA sequences for *S. japonica* (*S. japonica*: AP011493, *S. japonica* var. *religiosa*:

AP011494, *S. japonica* var. *ochotensis*: AP011495, *S. japonica* var. *diabolica*:
AP011496, and *S. longipedalis* [currently synonymized to *S. japonica* var. *diabolica*]:
AP011497). We queried the five sequences in our dataset.

S. japonica var. *diabolica* (AP011496) was collected at the same locality in the
present study, which was Aikappu (locality code: 2). The others were collected at sites
neighboring our sampling sites. *S. japonica* (AP011493), *S. japonica* var. *religiosa*
(AP011494), *S. japonica* var. *ochotensis* (AP011495), and *S. longipedalis* (AP011497)
were collected at sites close to locality codes 24, 10, 7, and 2 in the present study,
respectively.

Results

Genetic diversity

We detected 88 haplotypes (GenBank accession numbers: LC820751–
LC820838, corresponding to haplotype numbers H01–H88) in the 483 individuals of *S.*
japonica. H22, H51, and H53 contained a deletion of 28 bp in the upstream region of
16S rRNA in comparisons with the other haplotypes. Among the 88 haplotypes, 19 (i.e.,
H03, H04, H05, H06, H10, H22, H23, H30, H32, H35, H50, H55, H57, H58, H65, H68,
H75, H77, and H78) were shared haplotypes. H04 and H05 showed extensive
distributions, and the other haplotypes were shared at neighboring or regional scales (for
example, H03 in northeastern Hokkaido, H22 in southern Hokkaido, and H30 in locality
codes 12 and 13). The remaining 69 haplotypes were unique to each locality (Fig. 1,
Table 1). The most frequent haplotype was H05, found in 168 individuals, and dominant
in southern Hokkaido to Honshu, representing 34.7% of the total sample. The second
most frequent haplotype was H04, which accounted for 8.9% of the total sample; it was

observed in northern and eastern Hokkaido (locality codes: 01, 02, 03, 06, 08, and 09), except in Miyako, Iwate Pref. (locality code: 30) (Fig. 1, Table 1). Disjunct haplotype distributions were found for H04, H05, H10, H22, H50, H58, and H65. For example, H22 was the dominant haplotype in southern Hokkaido, but it was also detected in western Hokkaido in locality code 9.

The number of haplotypes (*h*) in each locality ranged from one to seven, haplotype diversity (*H_d*) ranged from 0 (locality codes: 01, 31, 36, 39, 42, 43, 45, and 46) to 1.000 ± 0.272 (locality code: 33), and nucleotide diversity (*P_i*) ranged from 0 (locality codes: 01, 31, 36, 39, 42, 43, 45, and 46) to 0.00824 ± 0.00442 (locality code: 21). Unique haplotypes were found in only 2 (localities codes: 40–41) out of 7 (localities codes: 40–46) localities in Joban (*H_d*: 0.302 ± 0.009), while 7 out of 14 localities (locality codes: 25–39) had unique haplotypes in the other region in Honshu (*H_d*: 0.780 ± 0.037). In Hokkaido (locality codes: 1–24), unique haplotypes were found in 20 out of 25 localities (*H_d*: 0.914 ± 0.077).

Genetic divergence was higher in spacer regions (*H_d* = 0.774 ± 0.018 , *P_i* = 0.00621 ± 0.00342) than in coding regions (*H_d* = 0.392 ± 0.029 , *P_i* = 0.00036 ± 0.00034) in *nad3*-16S rDNA. Diversity (*H_d* = 0.404 ± 0.028 , *P_i* = 0.01456 ± 0.00837) was highest in the spacer after *rpl31*, among the genes and spacers (Table S1).

Genetic structure

A statistical parsimony method detected a starburst type network centered on H05. Thirty-three haplotypes showed a single-nucleotide difference from H05. In addition to the large starburst from H05, several small starbursts centered on H03, H04, H22, H23, H30, H32, H35, H41 and H65 were obtained (Fig. 2).

BAPS inferred that the most likely number of genetic clusters in Japan was $K = 3$ (log marginal likelihood of optimal partition = -2062.6665, probabilities of two clusters = 0.14179 and three clusters = 0.85821, Fig. 3a, b). The haplotypes included in cluster 1 were dominant in most of the localities. For clusters 2 and 3, by contrast, the dominance was limited to the localities 4–6, 10–13, 15, 25, 26, 32, 35, and 40 (Fig. 3b). Although the haplotypes in cluster 2 were detected in various localities, haplotypes in cluster 3 were found specifically within the distribution of *S. japonica* var. *religiosa* 1. A list of haplotypes for each cluster is shown in Table S2. F_{ST} values for all pairs of localities indicated relatively strong genetic divergence between localities 10–15 and the others and between 21–25 and the others (Fig. 4).

Neutrality tests

We obtained negative Tajima's D values for 30 localities and all individuals, including significantly negative values for three localities (codes: 14, 18 and 21) and all individuals. Fu's F_s was negative for 26 localities and all individuals. Values were significantly negative for ten localities (codes: 4, 5, 12–15, 17, 18, 40, and 41) and all individuals (Table S3).

Evaluation of the *nad3*-16S rDNA region as a molecular marker

The haplotypes in the *nad3*-16S rDNA region differed among the five samples. The haplotypes of AP011496, AP011493, and AP011494 were matched to H04, H47, and H23 in the present study. The other haplotypes obtained from AP011495 and AP011497 were not matched; however, these two new haplotypes had a single bp difference from H04. For *COI*, AP011493-6 had the same sequence as those of

KT963115, KT963119, and KT963135, and AP011497 matched MK227363. For *trnW-trnL*, AP011493 matched KT963107, and AP011494, -6, and -5 were identical to KT963094. No match was obtained for AP011495; however, the haplotype differed by 1 bp from KT963094 (Table 2).

Discussion

We revealed the population genetic structure of *S. japonica* based on the *nad3*-16S rDNA region in mtDNA. The key to this type of study is the adoption of an informative marker (Yow et al. 2013; Chan et al. 2014). In the present study, unique haplotypes and one or a few shared haplotypes on a local scale were found in most localities. This fine genetic structure was not detected in previous phylogeographic studies of *S. japonica* using mtDNA regions, *COI* and *trnW-trnL* (Zhang et al. 2015, 2019). Furthermore, similar to a previous study using microsatellite markers (Yotsukura et al. 2016), individuals with genotypes distributed in northern Hokkaido (H04) were detected in the same locality, Miyako, Iwate Pref. (locality code: 30). These findings suggest that the marker employed in this study provided enough information to discuss the genetic structure of *S. japonica*. In the genus *Ecklonia*, which also belongs to the family Arthrothamnaceae, a highly similar resolution in population structure was observed based on the *nad3*-16S rDNA region and microsatellite markers (Akita et al. 2020). This mtDNA region is a potential marker for studies of population structure in the family using a DNA sequencing-based strategy.

Maternal inheritance of mitochondria has been described in Laminariales (reviewed by Motomura et al. 2010). As an exceptional inheritance of mitochondria, apomixis is observed in many species under laboratory conditions, including *Alaria*

crassifolia Kjellman (Nakahara and Nakamura 1973), *Lessonia berteriana* Montagne (Müller et al. 2019), *Laminaria pallida* Greville (Liboureau et al. 2024), and *S. japonica* (Yue et al. 2024). However, few sporophytes are found in the wild thalli (Oppliger et al. 2007; Klochkov et al. 2017). Accordingly, the unusual inheritance of mitochondria can be ignored for the genetic structure obtained here.

Previous studies indicated the existence of one (Zhang et al. 2015, 2019), two (Shan et al. 2017; Yotsukura et al. 2022), or four (Yotsukura et al. 2016) genetic clusters for *S. japonica* in Japan. These studies mainly used thalli from Hokkaido. In the present study, we included a number of samples from populations in Honshu, Japan. Unique or locally shared haplotypes were detected, and a distinct genetic cluster was detected for locality codes 10–15 based on BAPS and pairwise F_{ST} . However, the haplotype network showed a more detailed starburst pattern compared with previous results (Zhang et al. 2015, 2019). This pattern of network strongly suggests that this kelp underwent a recent expansion. Neutrality tests also supported this expansion. Combining these results, a reasonable phylogeographical interpretation of Japanese *S. japonica* is that a single population underwent a recent expansion. Although the genetic structure differs among studies (Zhang et al. 2015; Shan et al. 2017; Zhang et al. 2019; Yotsukura et al. 2022; this study), genetic clusters do not correspond to the distribution of varieties, excepting in Yotsukura et al. (2016). Accordingly, it is presumable that the genetic clusters do not match to the current distribution of varieties. Further investigation is needed for the taxonomic validation of varieties, including analyses of gene flow based on nuclear markers. In the order Laminariales, an inconsistency between morphological traits and genetic clusters has been described in various genera (*Ecklonia*: Rothman et al. 2015;

Akita et al. 2020, *Macrocystis*: Coyer et al. 2001; Demes et al. 2009, *Undaria*: Uwai et al. 2007, 2023).

Several haplotypes with disjunct distributions, such as H04 at locality code 30, were detected. In kelp species, the dispersal distance is usually a few meters (Coyer et al. 1997; Kusumo et al. 2006; Alberto et al. 2011; Parada et al. 2016; Mooney et al. 2018; Suzuki et al. 2024). Thus, genetic divergence is generally observed on a regional scale (e.g., Breton et al. 2018; Neiva et al. 2020; Chimura et al. 2022). Considering this nature of dispersal in kelp, haplotypes with disjunct distributions could represent intraspecific cryptic invasions, which are invasions of another lineage within a species into an area where the species already exists (Morais and Reichard 2018). Examples of intraspecific cryptic invasion have been reported in various kinds of organisms (Morais and Reichard 2018). We found the unexpected distributions of H04 at locality code 30, H05 at locality codes 4, 5, and 7, H10 at locality code 4, H22 at locality code 9, H50 and H58 at locality code 40, and H65 at locality code 44. As an exception, for H04, locality codes 6, 8, and 9 are probably within the original distribution of the haplotype because a number of sister haplotypes (H17–19, H20–21, and H24) were detected at locality codes 8 and 9. The vector of the invasion is indeterminable; however oyster aquaculture (Fletcher and Manfredi 1995; Miller et al. 2011), aquaculture of invaded species (Miller et al. 2011; Hwang et al. 2019), and boats (Voisin et al. 2005) are candidates, in line with previous studies on invasive species. In Japan, this is the second reported case of cryptic invasion in seaweed species, following the genera *Undaria* (Uwai et al. 2006, 2024).

The isolated population in Joban was first mentioned in the literature in the 1890s (Suyama 1890; Okamura 1896). Thereafter, Kawashima (2012) reported that this

population has been maintained only inside of fishing ports and recognized *S. japonica* and *S. japonica* var. *religiosa* morphotypes in this region (Kawashima 2012). In the present study, we found evidence of cryptic invasion at locality codes 40 and 44 in the Joban region. In addition, Hd was clearly lower in Joban ($Hd: 0.302 \pm 0.009$) than at other sites in Honshu ($Hd: 0.780 \pm 0.037$) and Hokkaido ($Hd: 0.914 \pm 0.077$). Such low genetic diversity is usually seen areas with recently established populations (Kwai et al. 2016; Hanyuda et al. 2016). Indeed, aquaculture of the kelp using seedlings purchased from Hirota Bay (neighboring locality code 32) and Shiriya (neighboring locality code 26) is recorded by fisheries cooperatives of this area (D. Fujita personal communication). This aquaculture dates back to the 1700s. Initially, the grandson of the Edo shogun tried aquaculture using sporophytes from southern Hokkaido (Inagaki 1943). Based on the haplotype at locality code 40, seedlings from southern Hokkaido were also likely used. Thus, on this coast, the population was likely established by an introduction via aquaculture from various regions of Japan. This type of introduction is also observed in the kelp growing in China and Korea (Hwang et al. 2019).

The *S. japonica* population expanded after the Last Glacial Maximum (LGM) from putative refugia on the coast of southern Hokkaido and northern Honshu (Zhang et al. 2019), which corresponds to locality codes 11–39 in this study. In the area, haplotype diversity was 0.572 ± 0.264 , on average. Similar diversity was also detected in northern and eastern Hokkaido, such as locality codes 2 ($Hd: 0.756 \pm 0.130$), 6 ($Hd: 0.625 \pm 0.069$), and 10 ($Hd: 0.524 \pm 0.209$), even excluding the locality with cryptic invasion. Invasion usually increases genetic diversity by providing new haplotypes (Morais and Reichard 2018). The haplotype network also included several small starbursts (e.g., centered on H03 or H04, which were dominant haplotypes in northern and/or eastern

Hokkaido). These findings suggest that refugia of *S. japonica* in the LGM were established on various coasts on Hokkaido. In the Sea of Japan, Zhang et al. (2019) hypothesized a post-LGM expansion of the population originating from southern Hokkaido northward to Sakhalin. However, the dominant haplotypes differed clearly among locality codes 8–9, 10–11, and 12–13. This pattern is inconsistent with a signal of recent population expansion, in which shared or related haplotypes are generally found (Hoarau et al. 2007; Hu et al. 2011; Neiva et al. 2014). It is possible that the population on the coast of the Sea of Japan persisted in each region since at least the LGM.

The *nad3*-16s rDNA region is a potential marker to identify the geographic origin of *S. japonica*, as unique and shared haplotypes at a regional scale were detected. Using sequences deposited in GenBank, we determined the geographic origins of AP011493 and AP11494. However, we could not identify the geographic origin of AP011495-7 because of emerging new haplotypes or a ubiquitous haplotype (H04). The enrichment of the *nad3*-16s rDNA database would improve the identification accuracy, except in the case of ubiquitous haplotypes (H04 and H05). The potential of this genetic region was also suggested in previous studies (Shimizu et al. 2004, 2010; Yotsukura et al. 2010). Indeed, the power of identification using *COI* and *trnW-trnL* was very weak because four of five individuals and three of five individuals had the same *COI* and *trnW-trnL* sequences, respectively. Of note, we found a critical misidentification of a deposited sequence. A complete mitochondrial genome deposited as *Saccharina longissima* (Miyabe) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (JN099684: Zhang et al. 2013) showed 100% identity to H22 in this study. The sequence is derived from the Culture Collection of Seaweed at the Ocean University of China. Although H22

includes a deletion of 28 bp in the region upstream of 16S rRNA, the same deletion was also recognized in a sample from a site close to locality no. 22 (Shimizu et al. 2004). The *nad3*-16S rDNA sequence of *S. longissima* (LC834026, newly determined in the present study) differs by 134–158 bp from the corresponding *S. japonica* sequences.

In the present study, we inferred the genetic structure of *S. japonica* on the coast of Japan. The results suggested that one genetic cluster underwent a recent expansion, with various refugia during the LGM and cryptic invasions on several coasts. Further, *nad3*-16s rDNA was identified as a candidate marker. Unlike previous phylogenetic studies (Zhang et al. 2015, 2019), fine-scale regional genetic variation in the kelp species was detected. Conservation efforts on each coast are needed to preserve locally unique genes, and further cryptic invasions via human activity should be avoided. Furthermore, studies aimed at the taxonomic validation of the varieties are needed because the distribution of varieties and genetic clusters were inconsistent. To solve this issue, gene flow among varieties should be investigated using highly polymorphic markers.

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572

Figure Captions

Fig. 1. Distribution and ratio of haplotypes (H01–H88) at each location. Shared haplotypes are shown with color, and unique haplotypes to each location are shown in white. The locality codes indicated in parentheses correspond to the numbers shown in Table 1. The distribution of varieties of *Saccharina japonica* described by Yotsukura et al. (2016) is shown on the map.

Fig. 2. Haplotype network of *Saccharina japonica* distributed in Japan. The colors of haplotypes are identical to those in Fig. 1.

Fig. 3. Genetic structure of *Saccharina japonica* inferred by BAPS. (a) The genetic cluster for each individual is indicated with a vertical bar, and (b) the ratio of clusters at each locality are shown in a map. The locality codes indicated in parentheses correspond to the numbers shown in Table 1.

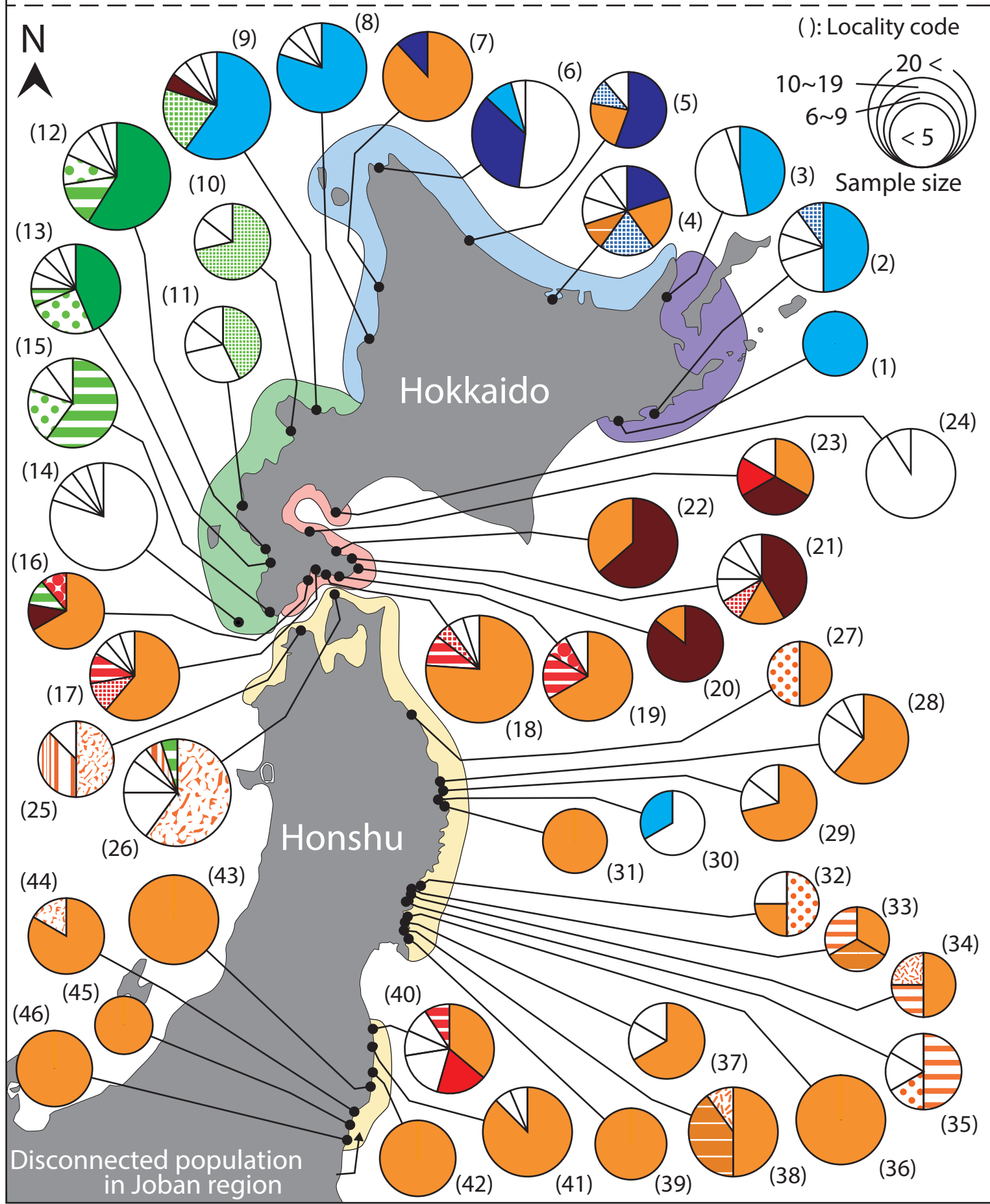
Fig. 4. F_{ST} values among all pairs of localities. Cross marks indicate no significance. Bars of different colors show the varieties distributed at each locality based on Yotsukura et al. (2016).

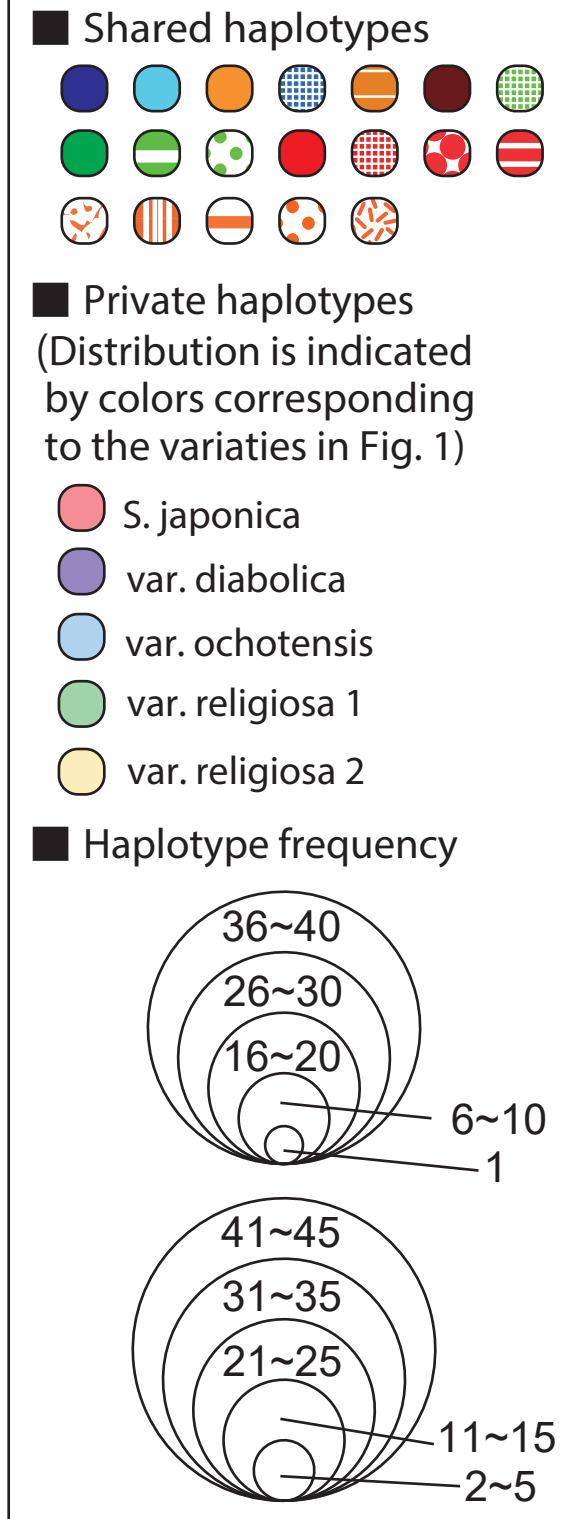
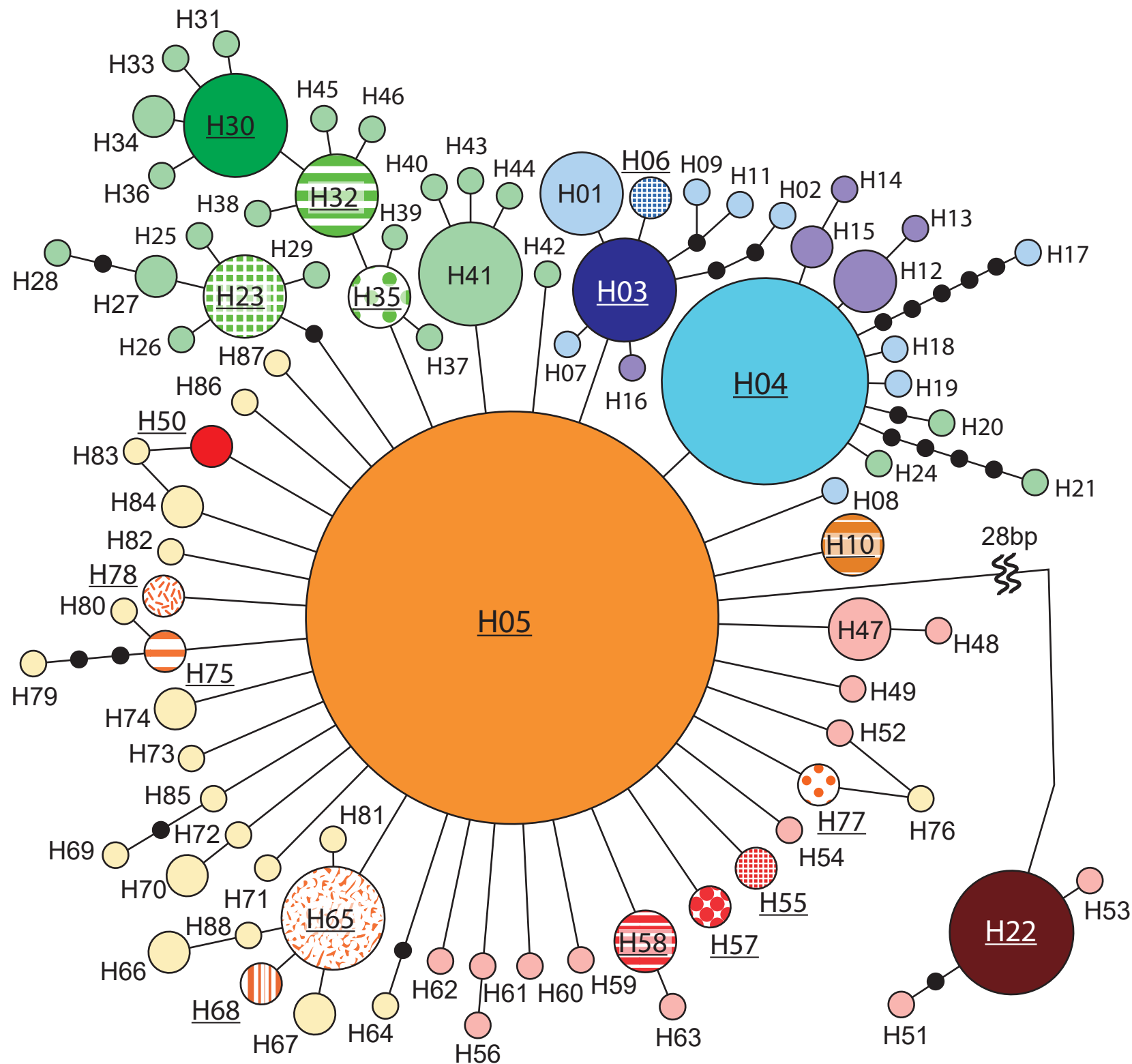
Distribution of varieties based on Yotsukura et al. (2016)

S. japonica var. diabolica var. ochotensis var. religiosa 1 var. religiosa 2

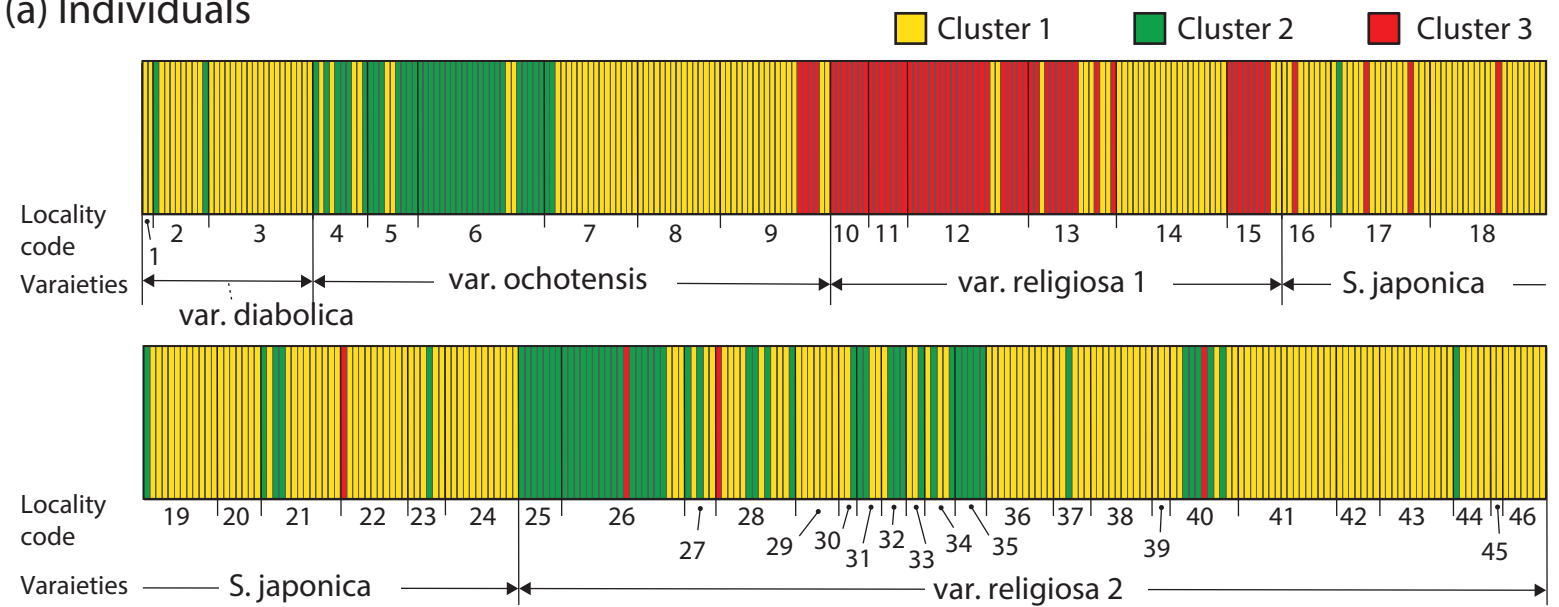
Shared haplotypes

H03 H04 H05 H06 H10 H22 H23 H30 H32 H35
H50 H55 H57 H58 H65 H68 H75 H77 H78

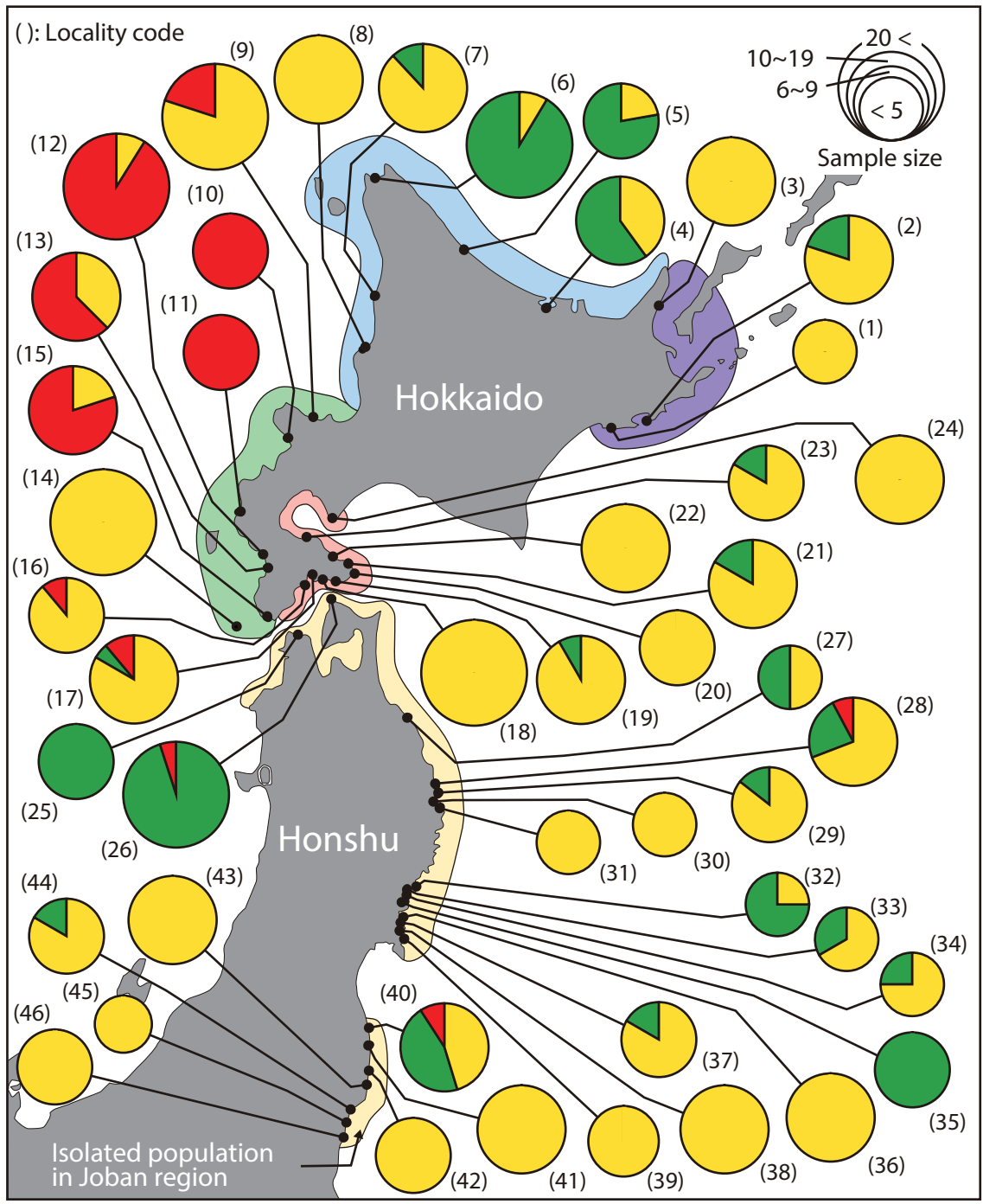


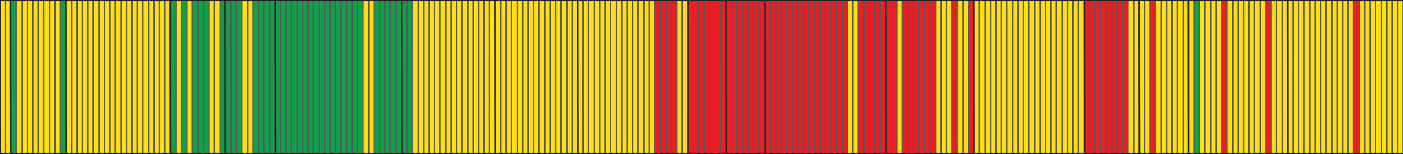


(a) Individuals



(b) Percentage





Varieties based on
Yotsukura et al. (2016)

Locality
code

var. diabolica

var. ochtensis

var. religiosa 1

S. japonica

var. religiosa 2

F_{ST}

1.0

0.8

0.6

0.4

0.2

0.0

