2019-03-01

Genetic analysis of a recently established Undaria pinnatifida (Laminariales: Alariaceae) population in the northern Wadden Sea reveals close proximity between drifting thalli and the attached population

Shan, T

10.1080/09670262.2018.1532116
European Journal of Phycology
Taylor & Francis

- This is the author's accepted manuscript. The final published version of this work (the version of record) is published by Taylor and Francis in the European Journal of Phycology on 21 July 2018 available at: https://doi.org/10.1080/09670262.2018.1532116

Genetic analysis of a recently established *Undaria pinnatifida* (Laminariales: Alariaceae) population in the northern Wadden Sea reveals close proximity between drifting thalli and the attached population

Tifeng Shan[1,2*],Shaojun Pang[1,2*]Xuemei Wang[1,2,3], Jing Li[1,2], Li Su[1,2,3], Jessica Schiller[4], Dagmar Lackschewitz[5], Jason M. Hall-Spencer[6,7], Kai Bischof[4 *]

[1]Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Rd., Qingdao 266071, China
[2]Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, 7 Nanhai Rd., Qingdao 266071, China
[3]University of Chinese Academy of Science, Beijing 100049, China
[4]Bremen Marine Ecology (BreMarE), Marine Botany, University of Bremen, Leobener Str. 3, 28359 Bremen, Germany
[5]Alfred Wegener Institute, Wadden Sea Station, 25992 List/Sylt, Germany
[6]University of Plymouth, School of Biological & Marine Sciences, Plymouth, UK
[7]University of Tsukuba, Shimoda Centre of Marine Science, Shizuoka, Japan

Abstract

Undaria pinnatifida, a kelp species native to East Asia, has become cosmopolitan and drawn increasing attention due to its worldwide spread in recent decades. Floating fragments of this alga were found washed ashore on Sylt in 2016, the first record of this species in Germany. Thalli attached to local oyster reefs were detected in 2017. The genetic relationship between the floating and attached thalli on Sylt, as well as their relevance to the populations from northern Europe and native regions, was hitherto unknown. Here, 10 microsatellite markers were used to assess relationships between the recently established population on Sylt and five other northern European populations in France (Brittany, West English Channel), the Netherlands and England (Plymouth, West English Channel) plus three natural populations in China. Almost no genetic differentiation was detected between the floating and attached populations on Sylt, but they were genetically distinct from all the other studied northern European populations. The very low genetic diversity revealed in the new founder populations of Sylt suggests that they came from genetically similar parents. The marked reduction in both the number of alleles and heterozygosity in the northern European populations, as compared with the Chinese ones, is typical of founder effects in recently populated regions. Prominent genetic divergence was found between most of the northern European populations except those within Brittany and Sylt. Further studies will focus on identifying the putative source populations that might be found on shellfish farms, in local marinas or the benthic habitats around Sylt Island.

Key words
Genetic diversity, invasive seaweed, kelp, microsatellite, North Sea, Wakame

Introduction

The brown alga *Undaria pinnatifida*, an edible kelp indigenous to the Northwest Pacific, has become cosmopolitan and drawn increasing attention because of its worldwide spread in recent decades (De Leij et al. 2017; James and Shears 2016). The first record of its spread to Europe was an accidental introduction to Thau lagoon (French Mediterranean) in 1971, probably accompanying the transport of Pacific oysters (*Magallana gigas*) from Japan for mariculture (Floc'h et al. 1991). In 1983, this alga was intentionally introduced to Brittany on the French Atlantic coast for commercial cultivation and soon spread into the wild (Grulois et al. 2011). Its later spread in Spain is also mainly attributed to aquaculture activity (Peteiro 2008). Likely promoted by maritime traffic, it spread to Italy, the United Kingdom, Portugal, Belgium and the Netherlands (Minchin et al. 2017; Heiser et al. 2014). The most northerly distribution of *U. pinnatifida* in Europe so far recorded was in July 2016 in Kilmore Quay, Republic of Ireland (Kraan 2016).

Along the German coastline, *U. pinnatifida* was first found washed ashore on intertidal sandflats on the east side of the island of Sylt in the northern Wadden Sea in August 2016 during a routine monitoring survey (Schiller et al., submitted). At that time, more than 100 stranded sporophytes were found ashore. Subsequently, a total of 91 attached *U. pinnatifida* individuals were found growing in tide pools on the western (coastward) side of the island in June 2017. The origin of the floating and attached populations was unknown (Schiller et al., submitted). Therefore, analysis of the genetic relationship between these founders and populations from other regions in Europe was carried out to provide insights into their origin and the vector of spread.

Microsatellites have been the marker of choice for genetic structure analysis due to their merits such as codominance, high polymorphism and even genomic distribution (Liu and Cordes 2004). There used to be 20 microsatellites available for *U. pinnatifida* and they were used to analyze the genetic polymorphism and structure on both intercontinental and regional scales, in which only French populations were included (Daguin et al. 2005; Grulois et al. 2011). However, genetic structure of *U. pinnatifida* populations from different European countries has never been investigated by using microsatellite markers. In order to provide more alternatives for population genetic analysis, thirty trinucleotide microsatellites have been developed *de novo* by next-generation sequencing, which proved to be highly informative (Shan et al., submitted).

In this study the genetic structure of representative populations of *U. pinnatifida* from Europe was investigated by using microsatellite markers to infer the genetic relationship between specimens on the island of Sylt and populations in other European countries. Three representative populations from China were analyzed and used as the calibrator to evaluate the genetic diversity of the introduced populations in Europe.

Materials and methods

Sampling and DNA isolation

Drift and attached populations (DEU1 and DEU2) of *U. pinnatifida* were collected on the island of Sylt in the northern Wadden Sea in 2016 and 2017, respectively (Fig.1 and Table S1). Five sites near the newly detected populations on Sylt, were sampled from three countries, including two (NLD1 and NLD2) from marinas in Vlissingen and Terschelling, the Netherlands, two (FRA1 and FRA2) from the Marina of the Moulin Blanc and Castle Marina in Brest, France as well as one (GBR) from Plymouth, Great Britain. Three populations were sampled in Dalian (DL), Qingdao (QD) and Gouqi Island (GQ) in China in 2016 and used to compare with the specimens from Europe. Thirty individuals (except GQ, with 29 individuals) were randomly chosen from each population. One piece of thallus from each individual was cleaned with sterilized seawater, dried and preserved in silica gel for DNA extraction. Genomic DNA was extracted by using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the instructions.

Microsatellite genotyping

Ten markers (UPN130, 161, 1143, 1528, 3177, 3197, 3205, 3530, 6327 and 9919) were randomly selected from the 30 trinucleotide microsatellites that have been newly developed (Shan et al., submitted) and used for genetic diversity and structure analysis. PCR was carried out in 20 μL volume containing AmpliTaq Gold 360 Master Mix (Applied Biosystems, USA), 0.5 μM fluorescent-labeled (forward) and unlabeled (reverse) primers and 5 ng of genomic DNA by using a T-gradient thermocycler (Biometra, Göttingen, Germany). The PCR program comprised an initial denaturation at 95 °C for 4 min, followed by 30 cycles of 94 °C for 30 s, annealing at 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 7 min. Microsatellite genotyping was performed on an ABI 3730XL automated sequencer (Applied Biosystems, Carlsbad, CA) and allele sizes were determined with GeneMapper version 4.0.

Data analysis

Number of alleles (N_a), observed and expected heterozygosity (H_o and H_e), inbreeding coefficient F_{is} as well as Nei's standard genetic distance (Nei 1972) were calculated by using GenAlEx 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012). The probability for Hardy-Weinberg equilibrium (HWE) for each locus was tested by using ARLEQUIN version 3.11 (Excoffier et al. 2005). Genetic distance between populations was used to construct a dendrogram by POPTREE software (Takezaki et al. 2010) using the neighbor-joining (NJ) clustering approach. During bootstrapping 1,000 permutations were performed to evaluate the robustness of the clusters. Pairwise population genetic differentiation (F_{st} value) was assessed by using ARLEQUIN version 3.11 with 1,000 permutations.

Given that the evaluation of genetic distance and differentiation was conducted on a priori population level, a Bayesian model-based clustering analysis was performed by

using STRUCTURE 2.3.4 software to estimate the most likely number of genetic clusters (Pritchard et al. 2000). This clustering approach was used to identify genetically distinct subpopulations on the basis of allele frequencies. The admixture model was applied and the number of clusters (*K* value) was set from 2 to 10 with 10 independent runs for each fixed number *K*. Each run included a burn-in length of 100,000 followed by 1,000,000 MCMC (Monte Carlo Markov Chain) repetitions. The most likely value of *K* was determined based on the method described in Evanno et al. (2005) by submitting all results files of $K = 2$ to 10 to STRUCTURE HARVESTER (Earl and von Holdt 2011). Among the 10 independent runs, the one with the highest Ln Pr ($X|K$) value (log probability) was selected and represented as bar plots by using the software DISTRUCT 1.1 (Rosenberg 2004).

Results

Monomorphism was detected at least at one locus in all European populations, with the number of homozygous loci being 6, 5, 2, 7, 1, 1 and 2 in DEU1, DEU2, NLD1, NLD2, GBR, FRA1 and FRA2, respectively (Table 1). The average N_a, H_o and H_e were all at minimum in NLD2, being 1.4, 0.110 and 0.093, respectively. Within European populations, the maximum N_a (3.3) was found in the two French populations, while the maximum H_e (0.469) was found in GBR. The average N_a, H_o and H_e of DEU1 and DEU2 were a little higher than those of NLD2 but lower than those of other populations. The average N_a, H_o and H_e in all the three Chinese populations were much higher than those in the European ones (Fig.2). Private alleles were detected at 7, 6 and 5 loci in DL, QD and GQ with an average number of 1.5, 1.0 and 1.0 at each locus, respectively. Among the 100 population-loci cases (10 populations×10 loci), 7 cases were estimated to be significantly deviated from HWE ($P<0.05$) after sequential Bonferroni's correction for multiple tests (Rice 1989).

The genetic distance between DEU1 and DEU2 was lowest (0.005) and no significant differentiation ($F_{st}=0.016$, $P>0.01$) was detected between them (Table 2). The genetic distance (0.075) and F_{st} (0.076) between the two French populations were relatively low, although the genetic differentiation among them was estimated to be significant ($P<0.01$). The genetic distance-based dendrogram grouped all the populations into three major clusters (Fig.3). Chinese populations and all European populations but NLD1 were grouped as two major clusters, respectively. NLD1 was revealed to be different from the others. Although NLD2 was found to be most closely related to DEU1 and DEU2 according to the values of genetic distance, the F_{st} values between NLD2 and them were very high (0.519 and 0.470).

The most likely number of *K* was determined to be 8 by using STRUCTURE HARVESTER based on the ΔK value (Fig.4). Individuals from German (DEU1 and DEU2) and French populations (FRA1 and FRA2) were assigned to two individual clusters (green and red) with high proportions of membership ($Q>0.95$ and $Q>0.90$ on average), respectively. Individuals from other populations were grouped to six different individual clusters (brown, white, yellow, pink, orange and purple), respectively, with the proportion of membership all being higher than 0.90. No prominent admixture was

found in any population.

Discussion

Both genetic distance and Bayesian model-based analyses showed there was no genetic differentiation between the two founding populations (DEU1 and DEU2) recently detected on Sylt Island. However, they were genetically distinct from all the other populations tested, which are thus not the source of these new founders. The fact that the attached individuals were detected only one year after the finding of fertile floating ones in a site nearby suggests that the former is the offspring rather than the parents of the latter. In addition, the growth pattern on the oyster reef strongly supports this, as was previously discussed (Schiller et al., submitted). The nearest known population to Sylt is in Terschelling, The Netherlands, over 240 km by air and over 300 km along the coast from Sylt. This large distance is unlikely to be covered by the limited dispersal ability known for kelp species (Sundene 1962).

Aquaculture and maritime traffic are regarded as the two major vectors contributing to the worldwide spread of *U. pinnatifida* (Voisin et al. 2005). However, given the fact that no individuals of *U. pinnatifida* were found in any marina on Sylt or the neighboring island of Föhr, shipping (via hull fouling or ballast water) is probably not the vector of introduction. These is widespread commercial farming of mussels and oysters around Sylt, and so aquaculture is likely to be the vector leading to the introduction of this alga, as was the case in Brittany. This is supported by the observation of individuals that were attached to oyster shells in the stranded floating population. It is also possible that there exists an unknown benthic population near Sylt which serves as the source of the floating mature individuals. This kelp is thought to spread in Tasmania and New Zealand at a rate of 1-10 km a year (Sliwa et al., 2006). However, after the floating individuals were first found on Sylt, dredge sampling of mussel reefs was performed in the area east of the wash zone in March 2017, but no *U. pinnatifida* were observed. On Sylt the floating sporophytes were significantly larger than the attached ones (Schiller et al., submitted). However, the results of the present study clearly prove this to be phenotypic, rather than genetic variation.

The founding populations of Sylt had very low genetic variation, with a high percentage of homozygous (monomorphic) loci and very few alleles averaged at each locus. This implies that the new founders on Sylt were derived from a limited number of genetically close parents from a single source. Many investigations have been conducted on solving the paradox that introduced populations with low genetic diversity successfully become invasive (Roman and Darling 2007). Possible solutions include being asexual or self-fertilizing, having high reproductive rates, or having high migration rates where multiple introductions occurred to ameliorate founder effects and inbreeding (Frankham 2005). *U. pinnatifida* has versatile reproduction including out- and self-fertilizing, parthenogenesis as monoecious gametophytes (Nakahara 1984; Shan et al. 2013; Li et al. 2017). Its reproductive capacity is also high, with hundreds of millions of zoospores released from one mature sporophyte. Sporophytes are often able to persist all year round, with multiple generations coexisting at the same period

(James et al. 2015). All these characteristics contribute to the invasive success of this kelp. Besides these inherent advantages, multiple introductions are closely associated with an increase in genetic diversity of *U. pinnatifida* in Australasia (Voisin et al. 2005). The new founders on Sylt thus allow investigations into the dynamics of genetic diversity in the future.

Contrary to the previous results revealed by mitochondrial inter-genic sequences, much lower genetic diversity, demonstrated by fewer N_a and private alleles as well as lower H_e, was found within the introduced populations in Europe as compared to that in Chinese waters (Voisin et al. 2005). The two French populations, which were from the primary introduced region of Europe, showed highest genetic diversity within European populations. The values of N_a and H_e were very similar to those in the bay of St-Malo (Grulois et al. 2011). The population of Terschelling, which was first detected in 2009, had the lowest in genetic diversity. Reduction in both allelic richness and heterozygosity in these introduced populations are most likely caused by founder effects (Dlugosch and Parker 2008). The genetic diversity showed a descending order from the earlier to the later founding populations (Fig.S2). This may be explained by more introduction events happening in the earlier founding populations, which mitigated the founder effects and increased genetic diversity. Almost all the investigated European populations were genetically distinct from each other except those within France and Germany. This significant divergence may be for two reasons. Firstly, populations from different countries were introduced from different sources, which were genetically distinct and the migration (gene flow) among them was low. Secondly, even though the French populations served as the primary source to spread satellite populations in other countries, genetic drift might occur in these secondary populations due to the limited number of founding individuals.

In summary, the genetic relationship between the founders of *U. pinnatifida* newly detected on the island of Sylt and the representative populations in other European countries was elucidated for the first time by informative microsatellite markers. The source of these founders still remains unknown. Tracking the origin of introduced species with worldwide distribution is very difficult because comprehensive sampling efforts to include all the putative sources are required (Rius et al. 2014). Further studies will focus on identifying the respective donor population, expansion routes and transportation vectors.

Acknowledgements

This research was supported by projects from the National Natural Science Foundation of China (No. 41676128, 41476141 and 31702365), the Sino-German Science Center (GZ 1080), the Deutsche Forschungsgemeinschaft (Bi 772/14-1), the National Key Technology Support Program (2015BAD13B05), the National Infrastructure of Fishery Germplasm Resource (2017DKA30470), China Agriculture Research System (CARS-50), the Taishan Scholar Program of Shandong Province and the Foundation for Huiquan Scholar of Institute of Oceanology, Chinese Academy of Sciences.

References

Daguin C, Voisin M, Engel C, Viard F (2005) Microsatellites isolation and polymorphism in introduced populations of the cultivated seaweed *Undaria pinnatifida* (Phaeophyceae, Laminariales). Conserv Genet 6 (4):647-650. doi:10.1007/s10592-005-9020-x

De Leij R, Epstein G, Brown MP, Smale DA (2017) The influence of native macroalgal canopies on the distribution and abundance of the non-native kelp Undaria pinnatifida in natural reef habitats. Mar Biol 164 (7). doi:10.1007/s00227-017-3183-0

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17 (1):431-449. doi:10.1111/j.1365-294X.2007.03538.x

Earl DA, von Holdt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4 (2):359-361. doi:10.1007/s12686-011-9548-7

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14 (8):2611-2620

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics online 1:47

Floc'h J, Pajot R, Wallentinus I (1991) The Japanese brown alga *Undaria pinnatifida* on the coast of France and its possible establishment in European waters. Journal du Conseil: ICES Journal of Marine Science 47 (3):379-390

Frankham R (2005) Resolving the genetic paradox in invasive species. Heredity (Edinb) 94 (4):385. doi:10.1038/sj.hdy.6800634

Grulois D, Lévêque L, Viard F, Frangoudes K, Valero M (2011) Mosaic genetic structure and sustainable establishment of the invasive kelp *Undaria pinnatifida* within a bay (Bay of St-Malo, Brittany). CBM-Cahiers de Biologie Marine 52 (4):485

Heiser S, Hall-Spencer JM, Hiscock K (2014) Assessing the extent of establishment of *Undaria pinnatifida* in Plymouth Sound Special Area of Conservation, UK. *Marine Biodiversity Records* 7, e93.

James K, Kibele J, Shears NT (2015) Using satellite-derived sea surface temperature to predict the potential global range and phenology of the invasive kelp Undaria pinnatifida. Biol Invasions. doi:10.1007/s10530-015-0965-5

James K, Shears NT (2016) Proliferation of the invasive kelp Undaria pinnatifida at aquaculture sites promotes spread to coastal reefs. Mar Biol 163 (2). doi:10.1007/s00227-015-2811-9

Kraan S (2016) Undaria marching on; late arrival in the Republic of Ireland. J Appl Phycol. doi:10.1007/s10811-016-0985-2

Li J, Pang S, Shan T (2017) Existence of an intact male life cycle offers a novel way in pure-line crossbreeding in the brown alga *Undaria pinnatifida*. J Appl Phycol

29 (2):993-999. doi:10.1007/s10811-016-1005-2
Liu ZJ, Cordes JF (2004) DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238 (1-4):1-37. doi:10.1016/j.aquaculture.2004.05.027
Minchin D, Nunn J, Murphy J, Edwards H, Downie A (2017) Monitoring temporal changes in the early phase of an invasion: Undaria pinnatifida (Harvey) Suringar using the abundance and distribution range method. Management of Biological Invasions 8 (1):53-60. doi:10.3391/mbi.2017.8.1.05
Nakahara H (1984) Alternation of generations of some brown algae in unialgal and axenic cultures. 北海道大學理學部海藻研究所歐文報告= Scientific papers of the Institute of Algological Research, Faculty of Science, Hokkaido University 7 (2):77-194
Nei M (1972) Genetic distance between populations. Am Nat:283-292
Peakall R, Smouse PE (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6 (1):288-295. doi:10.1111/j.1471-8286.2005.01155.x
Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28 (19):2537-2539. doi:10.1093/bioinformatics/bts460
Peteiro C (2008) A new record of the introduced seaweed *Undaria pinnatifida* (Laminariales, Phaeophyceae) from the Cantabrian Sea (northern Spain) with comments on its establishment. Aquatic Invasions 3 (4):413-415. doi:10.3391/ai.2008.3.4.6
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155 (2):945-959
Rice WR (1989) Analyzing tables of statistical tests. Evolution 43 (1):223-225
Rius M, Turon X, Bernardi G, Volckaert FAM, Viard F (2014) Marine invasion genetics: from spatio-temporal patterns to evolutionary outcomes. Biol Invasions 17 (3):869-885. doi:10.1007/s10530-014-0792-0
Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22 (9):454-464. doi:10.1016/j.tree.2007.07.002
Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4:137-138
Schiller J, Lackschewitz D, Buschbaum C, Reise K, Pang SJ, Bischof K (submitted) Heading northward to Scandinavia – *Undaria pinnatifida* in the northern Wadden Sea. Bot Mar
Shan TF, Pang SJ, Gao SQ (2013) Novel means for variety breeding and sporeling production in the brown seaweedUndaria pinnatifida(Phaeophyceae): Crossing female gametophytes from parthenosporophytes with male gametophyte clones. Phycol Res 61 (2):154-161. doi:10.1111/pre.12014
Sliwa C, Johnson C, Hewitt C (2006) Mesoscale dispersal of the introduced kelp *Undaria pinnatifida* attached to unstable substrata. Bot Mar 49: 396-405
Sundene O (1962) The implications of transplant and culture experiments on the growth and distribution of *Alaria esculenta*. Nytt Mag Bot 9:155–174

Takezaki N, Nei M, Tamura K (2010) POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. Mol Biol Evol 27 (4):747-752. doi:10.1093/molbev/msp312

Voisin M, Engel CR, Viard F (2005) Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. Proc Natl Acad Sci U S A 102 (15):5432-5437. doi:10.1073/pnas.0501754102

Figure captions

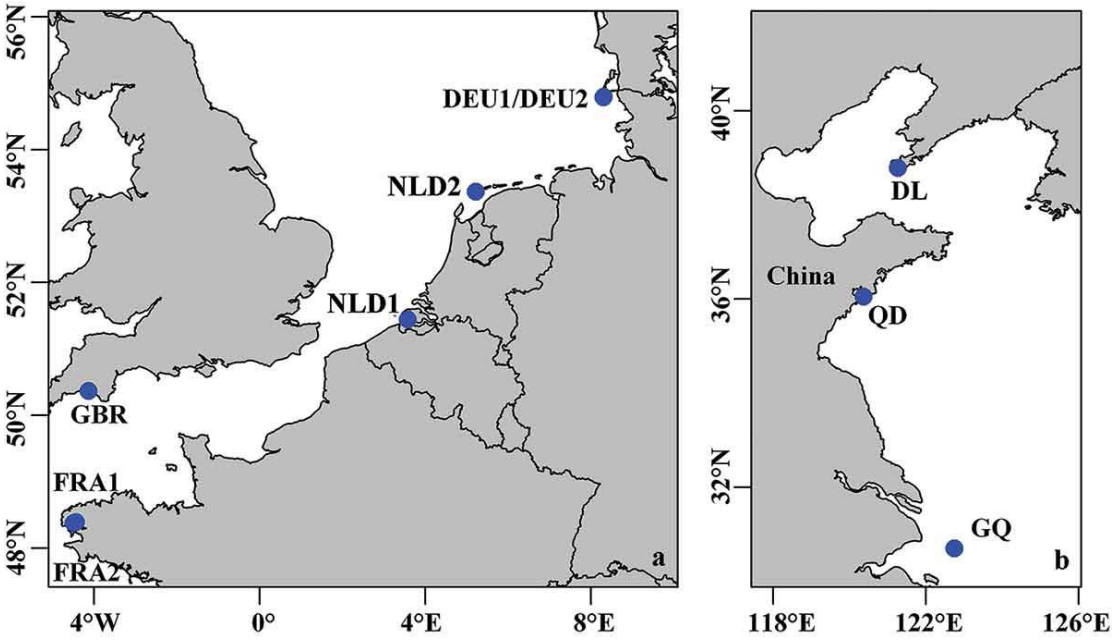

Fig.1 Sampling map of *Undaria pinnatifida* in Europe and China.

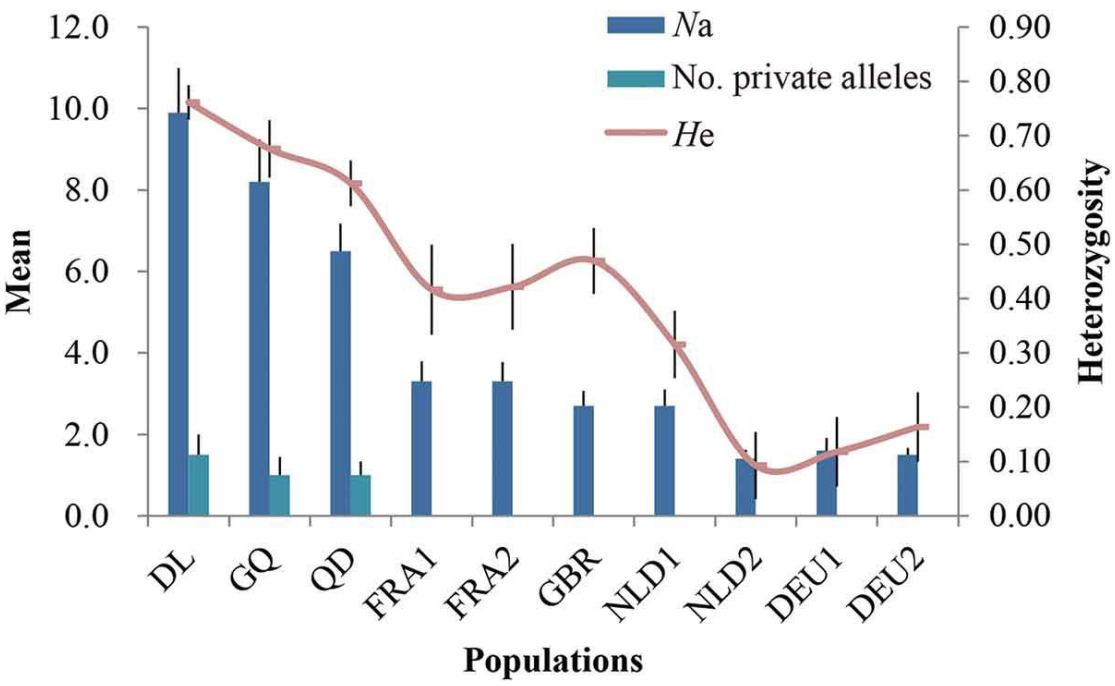

Fig.2 **The number of alleles (*Na*), private alleles and expected heterozygosity (*He*) of *Undaria pinnatifida* from northern Europe and China..**

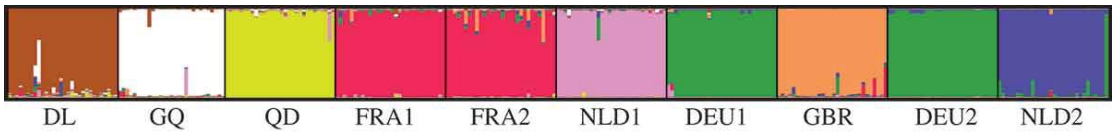

Fig.3 **Estimated genetic structure resulting from the Bayesian model-based analysis by using STRUCTURE 2.3.4 for populations of *Undaria pinnatifida* from northern Europe and China. Each individual is indicated by a vertical coloured bar, and the proportion of the colour in each bar represents the probability of membership in the relevant cluster.**

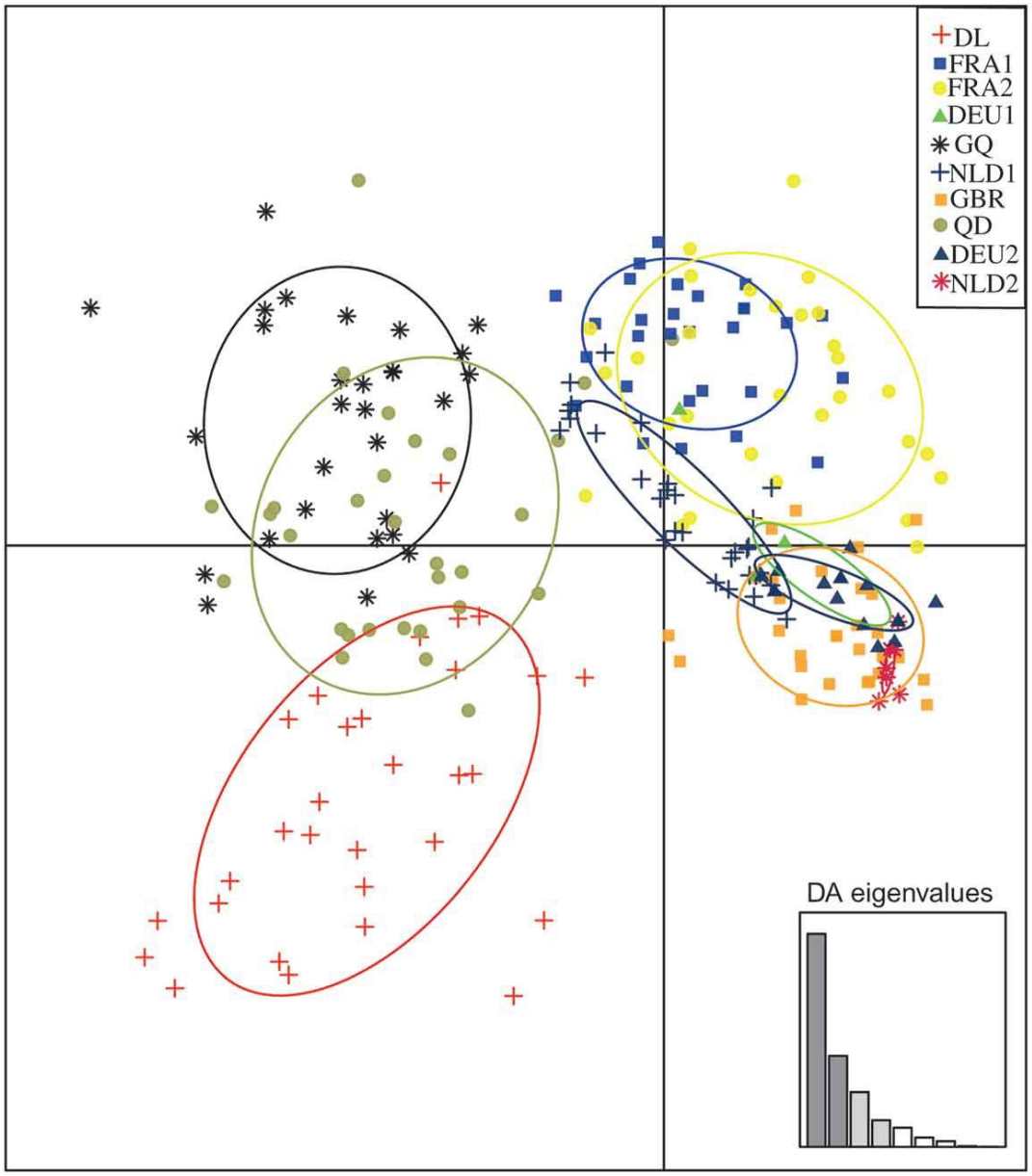

Fig.4 **Discriminant analyses of principal components for populations of *Undaria pinnatifida* from northern Europe and China.**