Phycologia (2006) Volume 45 (6), 687–695 Published 1 November 2006

Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondria genes – origins and succession of introduced populations

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S. UWAI, W. NELSON, K. NEILL, W.D. WANG, L.E. AGUILAR-ROSAS, S.M. BOO, T. KITAYAMA AND H. KAWAI. 2006. Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondria genes – origins and succession of introduced populations. *Phycologia* 45: 687–695. DOI: 10.2216/05-66.1

To elucidate the genetic diversity of the brown alga *Undaria pinnatifida* in native and introduced populations worldwide, and to discuss the transoceanic introduction processes, we investigated the haplotype divergence of the mitochondrial loci of the coding region of *cox3* and noncoding region between *tatC* and *tLeu* genes. In its native range (Japan, Korea and China), we found 27 haplotypes, which were classified into 4 genetic and biogeographical groups: (1) Northern Japan type, distributed in Hokkaido and Pacific northern Honshu; (2) Continental type, found in Korea and China; (3) Pacific central Japan type; and (4) Sea of Japan type. Among the introduced populations, European and Mexican populations agreed with the Northern Japan type. In Australia, the Tasmanian population agreed with the Sea of Japan type, whereas the Victorian population was of the Continental type. Very high diversities were found in New Zealand: 10 haplotypes were found (including 2 only in old herbarium specimens), including both the Northern Japan type and the Continental type. The haplotype found in California agreed with a component of the Central Japan type collected at Kanagawa Prefecture. The samples from Argentina agreed with the Continental type. The alignment of the European populations with the Northern Japan type is consistent with the notion that the *Undaria* in Europe was first introduced with oyster spat. It is speculated that Californian and Mexican populations were recently introduced by shipping vectors. There have been many introduction events to New Zealand since the late 1980s, and the dominant haplotypes in the local populations appear to have changed over time. Introduction to Argentina/Australia (Victoria) could have resulted from secondary introductions from New Zealand populations, because transport within the same latitudinal range is considered to be easier than transport by shipping across the equator. Within Japan, the occurrence of both the Continental and the Northern Japan types in the Osaka Bay area is considered to be the result of recent intentional introduction for fisheries purposes.

KEY WORDS: Introduced species, *Undaria pinnatifida,* Invasion, Mitochondria gene, Biogeography

INTRODUCTION

The globalization of ship transport systems and fisheries has increased transoceanic introductions of marine organisms, posing a threat to coastal ecosystems. In particular, both the discharge from large bulk carriers of ballast water containing various planktonic and microorganisms, as well as ship hulls acting as vectors for attached organisms, have been implicated in transoceanic introductions. Globally, more than 100 seaweed species are believed to have been spread outside their native ranges by human-mediated means (Farnham & Irvine 1973; Rueness 1989; Curiel *et al.* 1998; Fletcher & Farrell 1998; Rueness & Rueness 2000; Boudouresque & Verlaque 2002; Smith *et al.* 2002; Kim *et al.* 2004). Among them, *Undaria pinnatifida* (Harvey) Suringar (Phaeophyceae, Laminariales) is regarded as one of the most urgent and aggressive threats to coastal ecosystems.

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Undaria pinnatifida was originally endemic to northeastern Asia (Japan, Korea and China; Akiyama & Kurogi 1982; Tseng 1983). The species became introduced to Europe in the 1970s associated with oysters introduced for fisheries purposes, initially to the Mediterranean coast (Etang de Thau, French Mediterranean; Perez *et al.* 1981; Boudouresque *et al.* 1985). Within a decade the species had spread to a broader area: Brittany (Castric-Fay *et al.* 1993), southern England (Fletcher & Farrell 1998) and the central Mediterranean (Cecere *et al.* 2000). In the late 1980s the species was recorded in New Zealand (Hay & Luckens 1987; Hay 1990); Tasmania, Australia (Sanderson 1990); Argentina (Casas & Piriz 1996); Victoria, Australia (Campbell & Burridge 1998); California, USA (Silva *et al.* 2002) and in Baja California, Mexico (Aguilar-Rosas *et al.* 2004). Similar to other alien species that affect invaded habitats (Carlton & Geller 1993; Travis 1993; Grosholz 2002), introduced *U. pinnatifida* is considered to cause considerable impacts to coastal ecosystems by forming dense

canopies, displacing native species, and thus reducing biodiversity (Stuart 2004). It also causes economic impacts to fishing and aquaculture (Stuart 2004).

Apart from Europe, introduction processes to other regions are not easily understood, although ballast water and hull fouling have been considered as probable dispersal vectors to other areas. Furthermore, the presence/absence of secondary introductions within the same climate ranges has not been examined. Several morphological varieties (Suringar 1873) and forms (Miyabe 1902; Yendo 1913) of *U. pinnatifida* have been described. Therefore, precise identification of specimens to the level of varieties and forms may provide clues for elucidating the origins of the introduced populations. However, because of the remarkable morphological plasticity of the species, the taxonomy of *U. pinnatifida* has been rather difficult even within its native range, and it has not been possible to clarify the origin of the introduced taxa using morphological characters.

Recently, Voisin *et al.* (2005) studied the genetic diversity of *U. pinnatifida* populations worldwide by examining mitochondrial gene sequences, and reported that the haplotypes of the introduced populations differed depending on the localities (continents), and suggested the occurrence of independent introduction events. However, due to limited sampling in the native range, the relationships between native and introduced populations were not clearly shown. More recently, Uwai *et al.* (2006) reported the detailed genetic diversity of *U. pinnatifida* in Japan using a mitochondrial gene (*cox3*), but Korean and Chinese specimens were not covered in these analyses.

In the present study, to elucidate the genetic diversity and haplotype divergence of the whole native range as well as the worldwide introduced regions of *U. pinnatifida* populations, and to discuss the origins and primary (and secondary) introduction processes, we examined the haplotype divergences of *U. pinnatifida* covering all known distributional ranges of the species (Japan, Korea, China, Europe, Pacific North America, Australia, New Zealand and Argentina) using mitochondrial gene sequences of *cox3* and *tatC-tLeu* regions. In addition, using old voucher specimens of *U. pinnatifida* collected from various localities in New Zealand since the time of early introduction, we examined the succession of the dominant haplotypes in populations in New Zealand.

MATERIAL AND METHODS

Specimens of *U. pinnatifida* used for the present study are listed in Tables 1 and 2. Small fragments of clean blade were rapidly dried in silica gel and used for DNA extractions. Specimens used for the present study are housed at Kobe University Research Center for Inland Seas, and New Zealand voucher specimens are lodged at WELT (the herbarium of the Museum of New Zealand Te Papa Tongarewa).

For molecular studies, total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and then purified using a Geneclean Kit (Bio 101, Vista, CA, USA). The purified DNA was used as template DNA in a polymerase chain reaction (PCR) to amplify two regions encoded in the mitochondrial genome: the partial *cox3* gene and the *tatC-tLeu* region (from the 3′ end of the *tatC* gene to the 5′ end of the *tLeu* gene). The primers used were CAF4A (5′-ATGTTTACTTGGTGRAGRGA-3′) and CAR4A (5′-CCCCA-CCARTAWATNGTNAG-3′) for the *cox*3 gene (Kogame *et al.* 2005) and tatCEF (5′-AAATAATATATTGAGATTTTAAGTC-TATTCAT-3′) and tLeuR (5′-AACCTAAACACCGCGTGT-ATACC-3′) for the *tatC-tLeu* region. PCR was carried out using Dice TP600 (Takara Bio, Otsu, Japan) and iCycler (BioRad Laboratories, Hercules, CA, USA) as follows: an initial denaturation step of 96°C for 2 minutes, 45 cycles (*cox3*)/35 cycles (*tatC-tLeu*) of 96°C denaturation for 30 seconds, 50°C (*cox3*)/55°C (*tatC-tLeu*) annealing for 30 seconds, a 72°C extension for 45 seconds, and a final extension of 72°C for 2 minutes. Sequencing was performed using the ABI PRISM BigDye terminator Cycle sequencing Ready Reaction kit v.3.1 (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM 310 Genetic Analyzer (Applied Biosystems), and the CE DTCS quick start kit (Beckman Coulter, Fullerton, CA, USA) and CEQ8000 DNA analysis system (Beckman Coulter) according to the manufacturer's instructions.

Four hundred seventy base pairs (bp) of the *cox3* gene of 189 specimens and 438 bp of the *tatC-tLeu* region (intergenic spacer regions of the *tTrp, tIle and tGln* genes) of 260 specimens were determined in this study. Phylogenetic relationships among haplotypes determined on the basis of a combined data set of the *cox3* and *tatC-tLeu* region was inferred using maximum likelihood (ML) and quartet-puzzling (10,000 replicates) methods, as implemented in PAUP*4.0b10 (Swofford 2002). For both methods, Modeltest v.3.06 (Posada & Crandall 1998) was used to estimate a suitable substitution models for our data sets. Because traditional phylogenetic methods assuming a bifurcating tree could not properly infer the intraspecific phylogeny (Posada & Crandall 2001), statistical parsimony network (SPN) and minimum spanning network (MSN) trees among combined (*cox3* and *tatC-tLeu*) haplotypes were inferred using TCS v.1.18 (Clement *et al.* 2000) and Arlequin v.2.0 (Schneider *et al.* 2000), respectively. In SPN and MSN tree constructions, each continuous gap was treated as a single event. Haplotype diversity and nucleotide diversity of whole native and introduced populations were calculated using Arlequin v.2.0 (Schneider *et al.* 2000).

RESULTS

Genetic diversity in northeastern Asia (native range)

Most specimens from China and Korea had a single *cox3* haplotype (470 bp), corresponding to one of the *cox3* haplotypes found in northern Japan. DNA database accession numbers of the *cox3* and *tatC-tLeu* region and sample numbers of the representative of each haplotype are shown in Table 3. Both substitutions and insertions/deletions (indels), many of which were found in the intergenic spacer region between the *tTrp* and *tIle* genes, were observed among haplotypes.

Northern Japanese and the continental (Korea and China) haplotypes differed by a long indel (an indel length of 20 bp) in the intergenic spacer region between *tTrp* and *tIle*; all northern Japanese haplotypes had a 20-bp deletion.

The combined sequence data of *cox3* and *tatC-tLeu* on the basis of specimens collected from Japan, Korea and China gave 27 haplotypes (Table 1; haplotypes -1 to -6, -10 to -13

Table 1. Sampling point and number of samples.

Country	Population code	Sampling localities	No. of specimens	Haplotypes[1]
Korea	Eastern Korea	Songjeong beach, Pusan	1	11
		Cheongsapo, Pusan	1	11
		Ayajin, Gosung, Gangown Province	2	10 (1), 12 (1)
		Anin, Gangreung, Gangown Province	1	1
		Haegeumgang, Geojedo, Gyeongnam Province	1	13
		Suryeomri, Gyeongju, Gyeongbuk Province	1	10
	Jeju Island	Ilchulbong, Seongsan, Jeju	1	1
	Western Korea	Namyangman, Gyeonggi Province	1	10
		Oeyeondo, Boryeong, Chungnam Province	1	1
		Padri, Taean, Chungnam Province	1	10
		Daecheon, Boryeong, Chungnam Province	1	10
		Jeongdori, Jindo, Jeonnam Province	1	10
		Hoedong, Jindo, Jeonnam Province	1	10
China	Kuko Island	Zoushan, Kuko Island	7	10 (6), 1 (1)
Japan	Rishiri Island	Rishiri Island, Hokkaido	6	1
	Oshoro	Oshoro, Hokkaido	4	1 (3), 3 (1)
	Moheji	Moheji, Kamiiso, Hokkaido	5	1 (3), 2 (2)
	Muroran	Muroran, Hokkaido	7	1
	Tappi	Tappi, Aomori	6	15
	Ohma	Ohma, Aomori	11	1 (7), 4 (4)
	Yamagata	Atsumi, Yamagata	3	16
	Tsuyazaki	Tsuyazaki, Fukuoka	5	16 (2), 17 (1), 30 (1), 31 (1)
	Nagasaki	Saikai-Bashi, Nagasaki	1	18
	Oh-ita	Tsurumi, Oh-ita	3	19
	Mukai-shima	Mukai-shima, Hiroshima	3	16
	Naruto	Naruto, Tokushima	2	1 (1), 6 (1)
	Yura	Sumoto, Awaji Island, Hyogo	7	26 (1), 27 (1), 28 (4), 29 (1)
	Oh-iso	Oh-iso, Awaji Island, Hyogo	4	25
	Ashiya	Ashiya, Hyogo	5	6 (1), 16 (4)
	Mie	Hamashima, Mie	4	20
	Shimoda	Shimoda, Shizuoka	3	21 (1), 22 (2)
	Miura	Kan-non-zaki, Yokosuka, Kanagawa	2	24
		Shinjuku, Zushi, Kanagawa	1	24
		Moroiso, Misaki, Miura, Kanagawa	2	24
	Chiba	Kominato, Chiba	5	23
	Fukushima	Onahama, Fukushima	3	1 (2), 6 (1)
	Miyagi	Oga, Miyagi	8	1 (5), 5 (3)
USA	California	Monterey Marina, Monterey, California	4	24
		Cabrillo, San Pedro, California	5	24
		Santa Barbara, California	2	24
Mexico	Baja California	Todos Santos Island, Baja California	5	1
New Zealand	Auckland	Viaduct Basin, Auckland	2	10
		Western boat ramp, Auckland	2	10
	Nelson	Akersten Street boat ramp, Nelson	1	10
		Wakefield Quay, Nelson	2	10
	Picton	Picton Marina, Picton	5	10
	Wellington	Chaffers Marina, Wellington	5	10
		Scorching Bay, Wellington	5	10
	Gisborne	Gisborne Port, Gisborne	6	10
	Napier	Port of Napier, Napier	7	10
	Kaikoura	New Wharf, Kaikoura	3	10
	Christchurch	Lyttelton Marina, Christchurch	4	11 (3), 1 (1)
		Taylor's Mistake, Christchurch	4	7 (3), 11 (1)
	Timaru	Slipway, Timaru	3	6 (2), 11 (1)
		North Mole, Timaru	3	11
	Oamaru	Oamaru	6	11 (4), 13 (1), 14 (1)
	Moeraki	Moeraki	3	11 (2), 7 (1)
	Dunedin	Deborah Bay, Dunedin	3	10
		Port Chalmers, Back Beach	3	10
	Stewart Island	Halfmoon Bay, Stewart Island	3	10
		Big Glory Bay, Stewart Island	1	11
	Bluff	Below Foreshore Road, Bluff	4	8
Australia	Melbourne	Williamstown, Melbourne, Australia	6	10
	Tasmania	George's Bay, St. Helens, Tasmania	1	16
		North Bay, Forestier, Tasmania	1	16
		Mercury Passage/St. Helens, Tasmania	3	16
		Triabunna, Tasmania	3	16
Argentina	Buhia Bustamente	Buhia Bustamente, Chubut, Argentina	1	10
		Golfo, Nuero, Argentina	1	10
France	Brittany	Brest, Brittany, France	2	1
		Roscoff, Brittany, France	2	9

[1] The number of each haplotype appears in parentheses if multiple haplotypes were found.

Table 2. Herbarium specimens from New Zealand examined in the present study and their haplotypes.

Region	Locality	Date	No. of specimens	Haplotypes[1]
Wellington	Wellington Harbor	17 Nov. 1987	2	10
Wellington	On hull of a Korean trawler	Dec. 1987	3	10 (1), 11 (2)
Wellington	Aotea Quay	17 Feb. 1988	2	10
Wellington	Island Bay	8 Mar. 1996	1	10
Wellington	Evans Bay	19 Feb. 2002	1	10
Picton	Picton	13 Feb. 2002	1	10
Christchurch	Lyttelton, Naval Point	29 Jan. 2002	1	32
Christchurch	Akaroa Harbor	30 Jan. 2002	1	11
Timaru	Timaru Harbor	10 Oct. 1988	1	1
Timaru	Timaru Harbor, yacht club	2 Jun. 1991	1	6
Timaru	Timaru Harbor	18 Apr. 1993	1	8
Oamaru	Vessel hull	11 Oct. 1988	1	1
Oamaru	Oamaru Harbor	18 Apr. 1993	2	1 (1), 11 (1)
Otago	Otago Harbor, Carey's Bay	1 Dec. 1993	1	10
Otago	Otago Harbor, Carey's Bay	26, 30 May 1994	2	8 (1), 10 (1)
Otago	Otago Harbor, Deborah Bay	25 Mar. 1995	1	10
Otago	Otago Harbor, Aramoana	11 Dec. 1997	1	10
Otago	Otago Harbor, Aramoana	6 Mar. 1998	1	10
Chatham Island	Hanson Bay; from sunken hull	15 Dec. 2000	1	11
Bluff	Bluff Harbor	Nov. 1998	1	33
Stewart Island	Big Glory Bay	3 Jan. 1998	1	11
Stewart Island	Big Glory Bay	9 Jan. 1999	1	11

[1] The number of each haplotype is shown in parentheses if multiple haplotypes were found.

and -15 to -31). Their geographical distributions in northeastern Asia are shown Fig. 1a.

Phylogenetic relationships of the genetic types

The SPN and MSN trees based on the combined sequence data resulted in similar topologies, and the SPN tree is shown in Fig. 1b. The haplotypes were connected with each other by a branch or branches of four to five steps except for the haplotypes found in northern Japan, Korea and China. Considering the tree topology of the SPN tree and their general geographical distributions, the haplotypes can be grouped into the following four local types: (1) Continental type; (2) Northern Japan type; (3) Pacific central Japan type; and (4) the Sea of Japan type (Fig. 1b).

Exceptionally, the haplotypes found in the Bay of Osaka area showed high haplotype divergences and included all of the local types: Continental type in Oh-iso, Northern Japan type in Naruto and Ashiya, and the Sea of Japan type in Ashiya, in addition to the local Pacific central Japan type in Yura (Fig. 1a).

Genetic diversity in the introduced populations

Cox3 and *tatC-tLeu* sequences of 111 samples from introduced populations around the world were compared and 11 haplotypes were found from introduced populations. Only a single haplotype was found within each population in California (haplotype-24, 3 sites), Mexico (haplotype-1, 1 site), Argentina (haplotype-10, 2 sites), Melbourne (haplotype-10, 1 site), Tasmania (haplotype-16, 4 sites) and northern New Zealand (haplotype-10, 10 sites) (Fig. 1c, d). In contrast, two haplotypes were found in the French population (Brittany; haplotypes -1 and -9), and eight haplotypes in the southern New Zealand populations (haplotypes -1, -6, -7, -8, -10, -11, -13 and -14; Fig. 1c, d).

Regarding the relationships between the haplotypes in the native range and introduced populations, the haplotype-1 found in Mexican and European populations was common to the one dominant in northern Japan. One of the haplotypes found in Europe (haplotype-9) has not been found in the native range. The haplotype of the populations in Melbourne (Australia), Argentina and northern New Zealand (Kaikoura, Nelson, Picton and the North Island) was common in Korea and China (haplotype-10). This haplotype was also found in southern New Zealand populations, although seven other haplotypes were found, of which three haplotypes have not been found in the native range to date (haplotypes -7, -8 and -14). The Tasmanian population had a haplotype that was found in the Sea of Japan coast of Honshu (haplotype-16). The sequence of a haplotype found in California (haplotype-24) was identical to that collected in Miura, Japan.

Old specimens from New Zealand

Twenty-eight herbarium specimens collected in New Zealand from 1987 to 2003 were analyzed (Fig. 2; Table 2). Geographical distributions of the haplotypes generally agreed with those of the present distributions, although there were some differences. Two of the seven haplotypes identified from historical specimens have not been found from present day specimens (haplotype-32 from Christchurch, 2002 and type-33 from Bluff, 1998). Two of the three specimens collected from the hulls of Korean trawler ships in Wellington in 1987 were haplotype-11, which was not found in present day specimens from the same location. The haplotype found in Bluff in 2004 (haplotype-8) was found in Timaru (1993) and Otago (1994). Similarly, haplotype-1, found in Christchurch in 2004, was found in Timaru (1988) and Oamaru (1988 and 1993). Specimens from the hull of a vessel that sank off the Chatham Islands (800 km east of Christchurch) were haplotype-11.

Table 3. Combinations of Cox3 and *tatC-tLeu* regions of each haplotype. DNA database accession number of each sequence and sample number of representative of each sequence.

Haplotype	*Cox3* gene	*TatC-tLeu* region	Sample number[1]
1	AB213030	AB240644	1950
2	AB213031	AB240644	1945
3	AB213030	AB240645	1939
4	AB213030	AB240646	1952
5	AB213030	AB240647	1947
6	AB213030	AB240648	1946
7	AB213030	AB240649	1925
8	AB213030	AB240650	1924
9	AB213030	AB240651	443
10	AB213030	AB240652	1926
11	AB213030	AB240653	1927
12	AB213030	AB240654	1928
13	AB240669	AB240652	1929
14	AB213030	AB240655	1923
15	AB213032	AB240656	1942
16	AB213032	AB240657	1933
17	AB213033	AB240657	1944
18	AB213035	AB240658	1940
19	AB213038	AB240659	1938
20	AB213031	AB240660	1931
21	AB213027	AB240661	1948
22	AB213031	AB240661	1932
23	AB213034	AB240662	1949
24	AB240670	AB240663	1930
25	AB213036	AB240652	1941
26	AB240671	AB240659	1937
27	AB240672	AB240664	1936
28	AB240672	AB240659	1935
29	AB240672	AB240665	1934
30	AB213032	AB240666	1943
31	AB213032	AB240667	1951
32	AB240669	AB240668	456
33	AB240673	AB240653	471

[1] Silica-dried samples were housed in the Kobe University Research Center for Inland Seas under these numbers.

Comparison of genetic diversity between the native and introduced populations

Nearly half of the haplotypes were recorded from the introduced regions (11 haplotypes from 111 samples) in comparison to the native populations (27 haplotypes from 121 samples). Nucleotide diversity in the introduced populations (0.003391 ± 0.001975) was nearly half that found in the native range (0.006231 ± 0.003342). Haplotype diversity of native (0.8687 ± 0.025) and introduced (0.7178 ± 0.0408) populations was not largely different. This unexpectedly large diversity in introduced populations was due to the large number of haplotypes found in southern New Zealand. Haplotype diversity in southern New Zealand is larger (0.7883 ± 0.0455) than all introduced populations; other introduced populations analyzed showed haplotype diversity of 0.00 except for European populations (0.6667 ± 0.2041).

DISCUSSION

Haplotype distributions in Japan and continental Asia

On the basis of combined *cox3* and *tatC-tLeu* mitochondrial sequence data, we have characterized the genetic diversity of local populations in northeastern Asia including Japan, Korea and China (Fig. 1a). The basic geographic distributional pattern of the haplotypes within Japan was similar to that only on the basis of *cox3* sequence data (Uwai *et al.* 2006). However, by adding the *tatC-tLeu* sequence data, 23 haplotypes were recognized from Japanese populations, while the number of haplotypes recognized by Uwai *et al.* (2006) was 9. Korean and Chinese populations had 5 haplotypes, 4 of which (haplotypes -10, -11, -12 and -13) were unique to continental Asia, and the other one (haplotype-1) was commonly found in northern Japan.

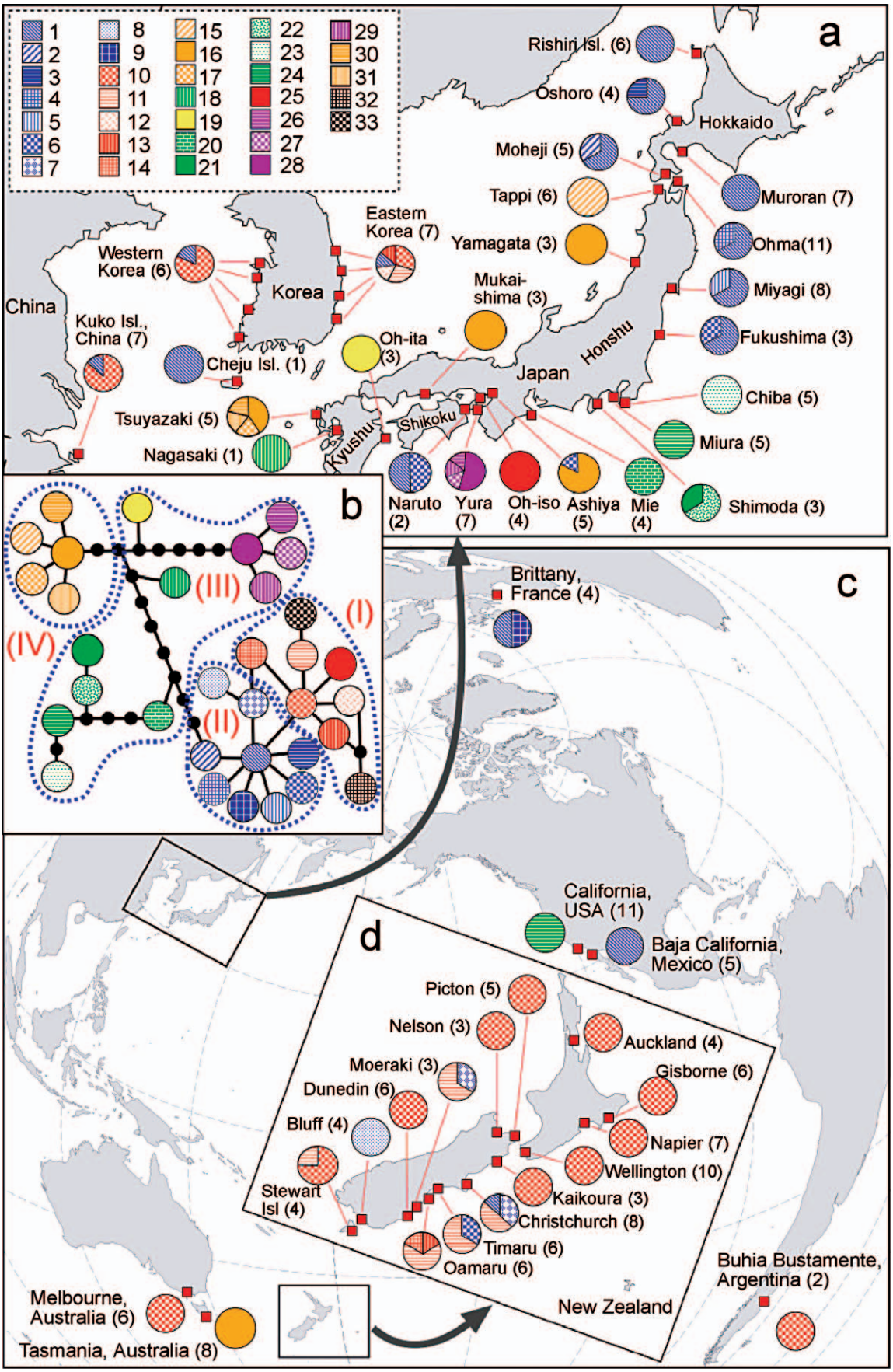
In Japan, haplotypes from the Osaka Bay area (Naruto, Yura, Oh-iso and Ashiya) showed greater divergence than those from other regions, although this region is located in the middle of the Pacific central Japan type region. On the basis of general geographical distributional patterns shown in this study and the haplotypes found in the Pacific central region, the following haplotypes are considered to have been introduced by artificial transfer for the mariculture of *Undaria* (Ohno *et al.* 1999): haplotypes -1 and -6 at Naruto (common to northern Japan populations) and haplotype-25, genetically closest to haplotype-10 (common in Korea and China) (Fig. 1b). In contrast, the occurrence of haplotypes -16, -26, -27, -28 and -29 could be the result of artificial introductions or natural distributions, because those haplotypes are relatively close to each other considering the SPN tree (Fig. 1b); considering geographically structured genetic diversity observed in the Japanese *U. pinnatifida,* haplotypes with phylogenetically close relationships could be expected to have distribution ranges with a geographical proximity. Continuous distribution of haplotype-16 and the phylogenetic distinctness and uniqueness of haplotypes -26, -27, -28 and -29 make it difficult to infer their origin.

Haplotype distributions of worldwide introduced populations

Genetic diversity and relationships among haplotypes shown in this study principally agreed with the results reported by Voisin *et al.* (2005); the haplotype divergence in *Undaria* populations was limited in most regions where it has been introduced (France, California, Mexico, Australia, Tasmania and Argentina), but was relatively high in New Zealand.

Origins of European populations

Voisin *et al.* (2005) reported 9 haplotypes from Europe (25 haplotypes worldwide), although all of these haplotypes were generally close to each other considering their DNA sequences. The haplotype-9 found in Brittany, France, in the present study has not been found from the Pacific coast of northern Honshu, although the oysters introduced to Etang de Thau in the French Mediterranean coast originated from this region. However, haplotype-1, one of the haplotypes found in Brittany, is common in northern Japan, and also both the haplotypes found in Brittany (haplotypes -1 and -9) are genetically close to those found from Pacific coast of Honshu (haplotypes -2, -3, -4, -5 and -6). Therefore, it seems likely that the present European populations originated from Japan. Korean and Chinese populations also had the haplotype-1, although this haplotype was in a minority in these populations.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Tasmania, Australia (8)

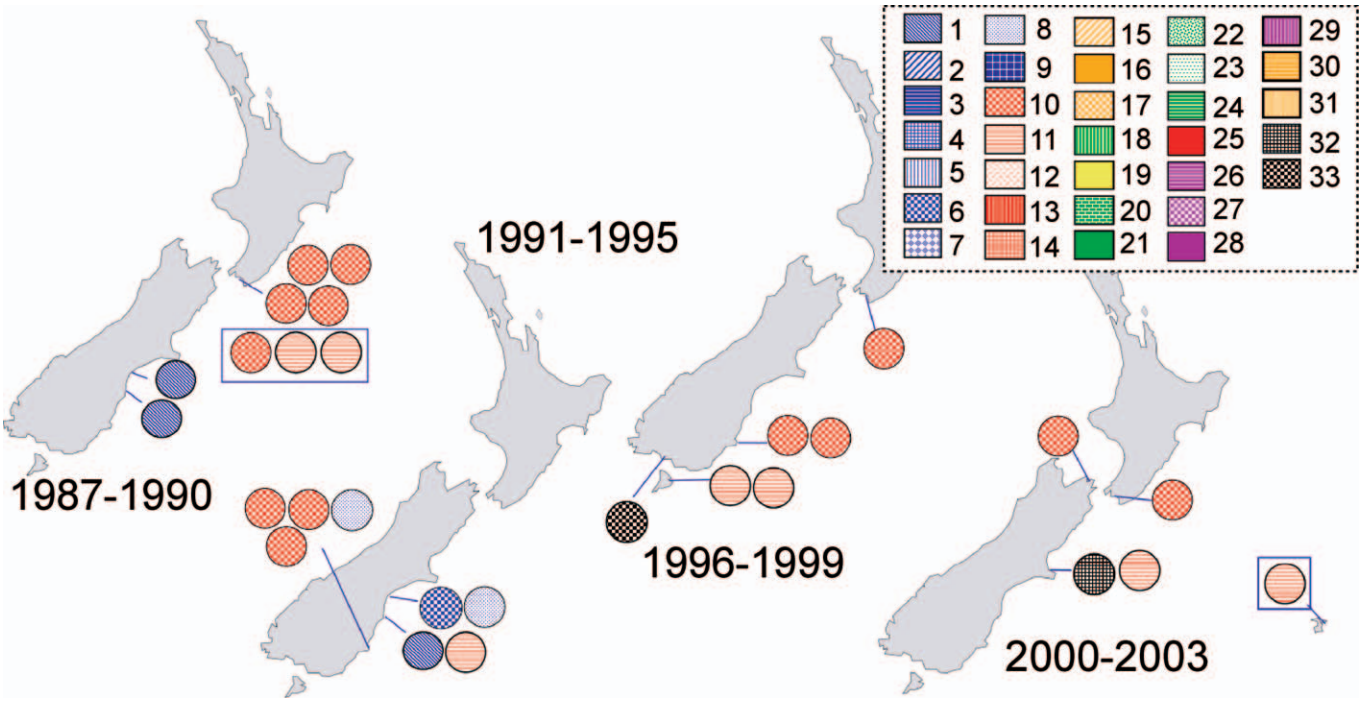

Fig. 2. Change in haplotype distribution in New Zealand over time. Combinations of colour and pattern represent different haplotypes, which correspond to those in Fig. 1. The number of haplotype symbols represents the number of samples in each region in each period. Haplotypes within blue line were found from samples on ship hulls.

Origins of southern hemisphere populations

In the southern hemisphere, the first introductions of *Undaria* were reported from two ports in New Zealand (Wellington in 1987 and Timaru in 1988), followed by Tasmania (1990). Introductions were reported from Melbourne (Australia) and Argentina about 10 years later than the initial report in New Zealand, and may be regarded as secondary introductions into the southern hemisphere.

In New Zealand, only a single haplotype was found from all localities on the North Island and northernmost part of the South Island. In contrast, an additional seven haplotypes were found on the South Island, and the localities differed in haplotype composition. All haplotypes present in New Zealand corresponded to the Continental and Northern Japan types.

Examination of herbarium specimens collected in the early stage of *Undaria* introduction to New Zealand provides evidence that the North Island had received material of continental Asian origin, whereas material with an origin of northern Japan had been introduced to the South Island. Currently, one haplotype occurs throughout the North Island populations that were sampled. It is probable that translocation occurred from the populations in Wellington to other areas in the North Island by means of commercial and recreation vessels, and aquaculture activities. In contrast, the South Island has apparently received multiple transoceanic introductions from the native range. Although the number of specimens examined in the present study was limited, there is evidence of a shift in the dominant haplotypes of the populations sampled over time.

The presence of only a single haplotype in the North Island and northern South Island (northern region of Kaikoura) of New Zealand suggests that only a single successful introduction has occurred in this region and that no coastal secondary introductions from southern populations have occurred. Alternatively, it is possible that some ecological or physiological factors (or both) have prevented the establishment of strains of other haplotypes. Comparisons of both ecophysiological factors and human activities between northern (the North Island and northern region of the South Island above Kaikoura) and southern New Zealand (southern region below Christchurch) are warranted because the boundary of haplotype distribution coincides with biogeographical boundaries reported in marine invertebrate species in New Zealand (Apte & Gardner 2002; Waters & Roy 2004).

Because *Undaria* gametophytes are relatively tolerant to high temperatures (up to about 30°C; tom Dieck 1993), it is possible that the gametophytes have been transported alive across the tropics, either attached to hulls or in the ballast water tanks of large ships. It is likely that the initial introduction to New Zealand ports occurred in this way. The collec-

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Fig. 1. Geographical distributions of mitochondrial DNA haplotypes (*cox3* and *tatC-tLeu* regions) of *U. pinnatifida* in native and introduced populations, and the SPN tree. Combinations of colour and pattern represent different haplotypes. Numerals in parentheses represent the number of samples in each population. a. Native range; b. SPN tree of mitochondrial DNA sequences. Small black circles represent haplotypes that have not been found. Each line connecting haplotypes corresponds to one base mutation; c. Worldwide introduced populations except New Zealand; d. New Zealand.

tions of *Undaria* sporophytes of two Continental type haplotypes from the hull of a Korean trawler in 1987 strongly suggest that these fishing boats acted as introduction vectors. However, considering the expansion of *Undaria* populations in New Zealand since the 1990s, especially in the major port areas, and that the survival of *Undaria* gametophytes (and fertile sporophytes) through ship operations is considered to be much higher within temperate regions than those crossing equators, it is possible that they were carried from New Zealand to Melbourne or Argentina. In contrast, the haplotype found in Tasmania, Australia, is unique in the introduced populations, and because the report of its introduction is relatively early (1990), its presence is more likely to be the result of a direct introduction from Japan.

Origins of Pacific North American populations

The haplotype of the *Undaria* population sampled in California (USA) was the same as that found at Miura, in Pacific central Japan, and has not been found elsewhere in introduced populations, and thus is considered to be a result of ship transport from Japan. In contrast, the Baja California (Mexico) population was relatively closely situated to California, but the haplotype of its *Undaria* population was of the Northern Japan type. Therefore, the Californian and Mexican populations have different origins.

ACKNOWLEDGEMENTS

We thank Dr Akira Peters and Kazuhiro Kogame for their help in sampling French specimens; Sheryl Miller (NIWA), Lou Hunt and Sean Cooper (NZ Department of Conservation) for assistance in New Zealand collections and Dr Leslie H. Harris (Natural History Museum of Los Angeles County) for assistance in San Pedro collections. This work was supported by the Global Environment Research Fund (D-4) by the Ministry of Environment, Japan, to H. Kawai; JSPS Research Fellowships for Young Scientists to S. Uwai and by a grant from the Marine and Extreme Genome Research Center Program, Ministry of Maritime Affairs & Fisheries of Korea, to S.M. Boo. The New Zealand components of this study were funded by MAF – Biosecurity New Zealand (ZBS200312).

REFERENCES

AGUILAR-ROSAS R., AGUILAR-ROSAS L.E., AVILA-SERRANO G. & MARCOS-RAMIREZ R. 2004. First record of *Undaria pinnatifida* (Harvey) Suringar (Laminariales, Phaeophyta) on the Pacific coast of Mexico. *Botanica Marina* 47: 255–258.

AKIYAMA K. & KUROGI M. 1982. Cultivation of *Undaria pinnatifida* (Harvey) Suringar, the decrease in crops from natural plants following crop increase from cultivation. *The Bulletin of Tohoku Regional Fisheries Research Laboratory* 44: 91–100.

APTE S. & GARDNER J.P.A. 2002. Population genetic subdivision in the New Zealand greenshell mussel (*Perna canaliculus*) inferred from single-strand conformation polymorphism analysis of mitochondrial DNA. *Molecular Ecology* 11: 1617–1628.

BOUDOURESQUE C.F. & VERLAQUE M. 2002. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin* 44: 32–38.

BOUDOURESQUE C.F., GERBAL M. & KNOEPFFLER-PEGUY M. 1985. L'algue japonnaise *Undaria pinnatifida* (Phaeophyta, Laminariales) en Mediterranee. *Phycologia* 24: 364–366.

CAMPBELL S.J. & BURRIDGE T.R. 1998. Occurrence of *Undaria pinnatifida* (Phaeophyta: Laminariales) in Port Philip Bay, Victoria, Australia. *Marine and Fresh Water Research* 49: 379–381.

CARLTON J.T. & GELLER J.B. 1993. Ecological roulette: global transport of nonindigenous marine organism. *Science* 261: 78–82.

CASAS G.N. & PIRIZ M.L. 1996. Survey on *Undaria pinnatifida* (Laminariales, Phaeophyta) in Golfo Nuevo, Argentina. *Hydorobiologia* 326/327: 213–215.

CASTRIC-FAY A., GIRARD A. & L'HARDY-HALOS M.T. 1993. The distribution of *Undaria pinnatifida* (Phaeophyceae, Laminariales) on the coast of St. Malo (Brittany France). *Botanica Marina* 36: 351–358.

CECERE E., PETROCELLI A. & SARACINO O.D. 2000. *Undaria pinnatifida* (Fucophyceae, Laminariales) spread in the central Mediterranean: its occurrence in the Mar Piccolo of Taranto (Ionian Sea, southern Italy). *Cryptogamie, Algologie* 21: 305–309.

CLEMENT M., POSADA D. & CRANDALL K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1660.

CURIEL D., BELLANO O., MARZZOCCHI M., SCATTOLIN M. & PARISI G. 1998. Distribution of introduced Japanese macroalgae *Undaria pinnatifida, Sargassum muticum,* (Phaeophyta) and *Antithamnion pectinatum* (Rhodophyta) in the Lagoon of Venice. *Hydorobiologia* 385: 17–22.

FARNHAM W.F. & IRVINE L.M. 1973. The addition of foliose species of *Grateloupia* to the British marine flora. *British Phycological Journal* 8: 208–209.

FLETCHER R.L. & FARRELL P. 1998. Introduced brown algae in the North Atlantic, with particular respect to *Undaria pinnatifida* (Harvey) Suringar. *Helgolander Meeresuntersuchungen* 52: 259–275.

GROSHOLZ E. 2002. Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology and Evolution* 17: 22–27.

HAY C.H. 1990. The dispersal of sporophytes of *Undaria pinnatifida* by coastal shipping in New Zealand and implications for further dispersal of *Undaria* in France. *British Phycological Journal* 25: 301–313.

HAY C.H. & LUCKENS P.A. 1987. The Asian kelp *Undaria pinnatifida* (Phaeophyta; Laminariales) found in a New Zealand harbor. *New Zealand Journal of Botany* 25: 329–332.

KIM M.-S., YANG E.C., MANSILLA A. & BOO S.M. 2004. Recent introduction of *Polysiphonia morrowii* (Ceramiales, Rhodophyta) to Punta Arenas, Chile. *Botanica Marina* 47: 389–394.

KOGAME K., UWAI S., SHIMADA S. & MASUDA M. 2005. A study of sexual and asexual populations of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyceae) in Hokkaido, northern Japan, using molecular markers. *European Journal of Phycology* 40: 313–322.

MIYABE K. 1902. *Konbu. Hokkaido Suisan Hokoku,* vol. 3 (in Japanese).

OHNO M., MATSUOKA M., DAN N., PAND S. & WU C. 1999. Morphological characteristics of the wild *Undaria pinnatifida* strain from Qindao, China grown in Naruto, Japan, as compared with wild plants in Naruto. *Suisan Zousyoku* 47: 61–64 (in Japanese with English summary).

PEREZ P., LEE J.Y. & JUGE C. 1981. Observations sur la biologie de l'algue japoniase *Undaria pinnatifida* (Harvey) Suringar introduite accidentellement dans l'etang de Thau. *Science et Peche* 315: 1–12.

POSADA D. & CRANDALL K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

POSADA D. & CRANDALL K.A. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution* 16: 37–45.

RUENESS J. 1989. *Sargassum muticum* and other introduced Japanese macroalgae: biological pollution of European coasts. *Marine Pollution Bulletin* 20: 173–176.

RUENESS J. & RUENESS E.K. 2000. *Caulacanthus ustulatus* (Gigartinales, Rhodophyta) from Brittany (France) is an introduction from the Pacific Ocean. *Cryptogamie Algologie* 21: 355–363.

SANDERSON J.C. 1990. A preliminary survey of the distribution of the introduced macroalga *Undaria pinnatifida* (Harvey) Suringar on the coast of Tasmania, Australia. *Botanica Marina* 33: 153–157.

SCHNEIDER S., ROESSLI D. & EXCOFFIER L. 2000. Arlequin: a software for population genetics data analysis. Ver. 2.000. Genetics and Biometry Lab, Department of Anthropology, University of Geneva.

SILVA P.C., WOODFIELD R.A., COHEN A.N., HARRIS L.H. & GODDARD J.H.R. 2002. First report of the Asian kelp *Undaria pinnatifida* in the northeastern Pacific Ocean. *Biological Invasions* 4: 333–338.

SMITH J.E., HUNTER C.L. & SMITH C.M. 2002. Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Island. *Pacific Science* 56: 299–315.

STUART M.D. 2004. Review of research on *Undaria pinnatifida* in New Zealand and its potential impacts on the eastern coast of the South Island. DOC Science Internal Series 166. Department of Conservation. Wellington, New Zealand. 40 pp.

SURINGAR W.F.R. 1873. Illustrationes des algues du Japon. *Musée Botanique de Leide* 1: 77–90, Pls 26–33.

SWOFFORD D.L. 2002. *PAUP*: phylogenetic analysis using parsimony (and other methods).* Version 4.0, beta release version 10. Sinauer Associates, Sunderland, MA, USA.

TOM DIECK I. 1993. Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. *Marine Ecological Progress Series* 100: 253–264.

TRAVIS J. 1993. Invader threatens Black, Azov Seas. *Science* 262: 1366–1367.

TSENG C.K. 1983. *Common Seaweed in China.* Science Press, Beijing. x + 316 pp.

UWAI S., YOTSUKURA N., SERISAWA Y., MURAOKA D., HIRAOKA M. & KOGAME K. 2006. Intraspecific genetic diversity of *Undaria pinnatifida* in Japan, based on the mitochondrial *cox3* gene and the ITS1 of nrDNA. *Hydrobiologia* 553: 345–356.

VOISIN M., ENGEL C.R. & VIARD F. 2005. Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. *Proceedings of National Academy of Sciences of the United States of America* 102: 5432–5437.

WATERS J.M. & ROY M.S. 2004. Phylogeography of a high-dispersal New Zealand sea-star: does upwelling block gene-flow? *Molecular Ecology* 13: 2797–2806.

YENDO K. 1913. *Kaisan-Syokubutsugaku.* Hakubunkan, Tokyo. 748 pp.

Received 13 December 2005; accepted 19 May 2006
Associate editor: J. Brodie