**Abstract**

In Europe, the last 20 years have seen a spectacular increase in accidental introductions of marine species, but it has recently been suggested that both the actual number of invaders and their impacts have been seriously underestimated because of the prevalence of sibling species in marine habitats. The red alga *Polysiphonia harveyi* is regarded as an alien in the British Isles and Atlantic Europe, having appeared in various locations there during the past 170 years. Similar or conspecific populations are known from Atlantic North America and Japan. To choose between three competing hypotheses concerning the origin of *P. harveyi* in Europe, we employed rbcL sequence analysis in conjunction with karyological and interbreeding data for samples and isolates of *P. harveyi* and various congeners from the Pacific and North Atlantic Oceans. All cultured isolates of *P. harveyi* were completely interfertile, and there was no evidence of polyploidy or aneuploidy. Thus, this biological species is both morphologically and genetically variable: intraspecific rbcL divergences of up to 2.1% are high even for red algae. Seven rbcL haplotypes were identified. The four most divergent haplotypes were observed in Japanese samples from Hokkaido and south-central Honshu, which are linked by hypothetical ‘missing’ haplotypes that may be located in northern Honshu. These data are consistent with Japan being the centre of diversity and origin for *P. harveyi*. Two non-Japanese lineages were linked to Hokkaido and Honshu, respectively. A single haplotype was found in all North Atlantic and Mediterranean accessions, except for North Carolina, where the haplotype found was the same as that invading in New Zealand and California. The introduction of *P. harveyi* into New Zealand has gone unnoticed because *P. strictissima* is a morphologically indistinguishable native sibling species. The sequence divergence between them is 4–5%, greater than between some morphologically distinct red algal species. Two different types of cryptic invasions of *P. harveyi* have therefore occurred. In addition to its introduction as a cryptic sibling species in New Zealand, *P. harveyi* has been introduced at least twice into the North Atlantic from presumed different source populations. These two introductions are genetically and probably also physiologically divergent but completely interfertile.

**Keywords:** alien species, biological species, invasion, Japan, phylogeography, sibling species

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**Introduction**

Coastal marine ecosystems worldwide are threatened by invasions of nonindigenous species (Carlton & Geller 1993; Geller et al. 1994; Geller et al. 1997). In Europe, the last 20 years have seen a spectacular increase in accidental introductions of marine species (Boudouresque et al. 1994), but it has recently been suggested that both the actual number of invaders and their impacts have been seriously underestimated. Geller (1996) predicted that use of appropriate molecular techniques would reveal the repeated occurrence of cryptic invasions, which are of both scientific interest and practical importance. He suggested several reasons why invasions might remain undetected, for example, because the invading species closely resembles...
native organisms, and drew attention to the frequent occurrence of sibling species in marine habitats (Knowlton 1993). Geller et al. (1997) reported cryptic invasions of this type around the world in sibling species of the crab genus *Carcinus*. They also discussed the implications of a second, undetected, type of cryptic invasion, multiple introductions of one species from different populations. They noted that such multiple conspecific invasions are expected to result not only in genetic diversity, but also in variation in behaviour, life history and physiological tolerances. Shortly afterwards, multiple invasions of the marine polychaete *Nematocelis viridis* into the North Sea and the Baltic Sea were identified on the basis of partial 16S sequences (Bastrop et al. 1998). However, the high sequence divergences between lineages suggested that three cryptic species were concealed within *M. viridis* although possible intersterility was not evaluated. To date no molecular studies have been designed both to detect the occurrence of multiple conspecific invasions, and to test interfertility of the different invading lineages.

The red alga *Polysiphonia harveyi* occurs on North American coasts of the North Atlantic from Newfoundland to South Carolina. In the British Isles and Atlantic Europe it is regarded as a widespread alien (Maggs & Hommersand 1993; Maggs & Stegenga 1999). It displays many of the characteristics typical of algal invaders (Ribera & Boudouresque 1995), being eurythermal, weedy, and commonly occurring as a fouling species on artificial substrata associated with boating and aquaculture activities (Maggs & Stegenga 1999). There is good evidence that *P. harveyi* was not yet established in the British Isles by the mid-nineteenth century. It is absent from W.H. Harvey’s monumental *Physiologia Britannica* (1846–1851), even though Harvey was familiar with this species, having observed it to be a very common seaweed in New England in 1846 (Harvey 1853). There are at least three possible origins of *P. harveyi* in the British Isles: (i) the first definite collection in Britain was in 1908, so Maggs & Hommersand (1993) postulated that this species might be a transatlantic immigrant to Britain and Ireland from its native range in North America; (ii) because the morphological feature unique to *P. harveyi* in the British Isles, a transparent, glassy appearance due to the absence of plastids from outer cell walls, occurs in many Japanese members of this genus, there is a link to Japan. A cascade of introductions could have originated in Japan or nearby in the north-western Pacific, leading first to the naturalization of *P. harveyi* in North America, and then to its migration to Europe; and (iii) probable specimens of *P. harveyi* were collected in Bretagne, France, in 1832 (as *P. insidiosa*) and the diagnostic plastid character was reported in material from this area by Thuret & Bornet (1878), so *P. harveyi* could have been introduced independently from the Pacific to eastern and western coasts of the North Atlantic, spreading in Europe from France to the British Isles and more recently as far as Norway and Spain (Maggs & Stegenga 1999). The aim of this study was to employ molecular data in conjunction with morphological and interbreeding data to choose between these three competing hypotheses concerning the origin of *P. harveyi* in Europe. Most phylogeographic studies in plants have used cpDNA restriction analysis, although Schaal et al. (1998) noted that sequencing would be desirable, if cpDNA loci could be found that were useful at the intraspecific level. The gene for the large subunit of rubisco, rbcL, is suitable for phylogeographic studies in green plants only at the tribal and family levels (Xiang et al. 1998; Meenow et al. 1999), but in red algae it shows some intrapopulational as well as extensive interspecific variability (Nam et al. 2000).

The rbcL gene was, therefore, selected for sequencing in order simultaneously to investigate both intraspecific and supraspecific phylogeography. For the three scenarios, the predicted rbcL and breeding results are as follows: (i) *P. harveyi* in Europe cannot be distinguished genetically or in breeding studies from this species in Atlantic North America but it differs from, and is not interfertile with, *P. harveyi*-like Japanese species; (ii) *P. harveyi* in Europe is indistinguishable from this species in North America and both are related to, and interfertile with, *P. harveyi* in Japan; and (iii) *P. harveyi* populations in Europe and Atlantic North America are, respectively, more closely related to particular Japanese populations than they are to each other; both are interfertile with Japanese strains and probably with each other.

**Materials and methods**

**Sample collection, culture methods and karyology**

Samples of *Polysiphonia harveyi* were obtained from Atlantic and Mediterranean Europe, the northern and southern parts of its range on Atlantic coasts of North America, and, on Pacific coasts, from New Zealand, California and several locations in Japan, under various synonyms (Fig. 1, Table 1). Three other taxa, *P. strictissima*, *P. simplex* and *P. forfex*, that share the plastid character state with *P. harveyi*, were also sampled. *P. forfex*, although collected in France (Table 1), is treated here as a Pacific species as it apparently represents a recent introduction from the North Pacific, where it occurs in Japan and Korea (Yoon 1986). Outgroup North Atlantic *Polysiphonia* taxa were collected in Britain and Ireland. Samples were either dried in silica gel or transported live back to the laboratory in sterilized seawater. Cultures were isolated from haploid or diploid spores of fertile individuals and maintained as described by Maggs (1998). For breeding experiments, males and females were grown separately for at least 3 weeks to ensure that there
was no parthenogenetic development of females. A normal *Polysiphonia*-type life history (Bold & Wynne 1985) was observed, except that males produced specialized reproductive structures that grew directly into normal males. Fertile male tips were mixed with three female tips in culture medium in small Petri dishes and checked weekly for fertilization. Developing diploidized structures (cystocarps) were noted, and monitored at least until release of viable diploid spores. For all pairs of isolates (except Italy 232 for which only fertile males were obtained) reciprocal male and female crosses were attempted.

Chromosome number was determined, as described by Maggs (1989), for three representative samples/isolates (Table 1), from North Carolina, Ireland and Japan (Oshoro 231).

**DNA extraction, polymerase chain reaction amplification and sequencing**

For most samples of *P. harveyi*, DNA was extracted from 1 to 5 g of fresh or silica gel-dried algal material by a modified phenol-chloroform method, followed either by isolation of plastid DNA on caesium chloride buoyant density gradients (Maggs & Ward 1996) or by purification with the DNA-binding column of Qiagen DNeasy Plant Mini Kits (Qiagen GmbH, Hilden, Germany). For all other samples, total genomic DNA was extracted from ± 0.05 g material using the DNeasy Plant Mini Kit, according to the manufacturer’s instructions.

Polymerase chain reaction (PCR) amplification and direct sequencing were performed as described by Nam et al. (2000), with rbcLFC as the forward external primer and rbcLRD as the reverse external primer, except for *P. simplex* (sample 476). For this the external forward and reverse primers were, respectively, Ant1 and Ant4, which were designed using GenBank sequence X54532 for *Antithamnionella spirographidis* (Kostrzewa et al. 1990; as *Antithamnion* sp.). Ant1 (5′ CAC AAC CAG GTG TTG ATC CAA TTG AAG C 3′) anneals to positions 143–171 and Ant4 (5′ CTA CGA AAG TCA GCT GTA TCT GTA GAA GTA TA 3′) to positions 1504–1536 in that sequence. After sequencing and aligning 1245 bp of the *rbcL* gene for samples from a wide geographical area, a *P. harveyi*-specific primer pair (harvF, 5′ CAG GAA TTG TTG TAG AAC GTG AAC G 3′; harvR, 5′ CCT TTC ACT TCT CCA GTT GAG 3′) was designed to amplify a 253-bp fragment from DNA samples that were contaminated with other rhodomelacean red
Table 1  

Polysiphonia samples, indicating original identification of *P. harveyi* collections, collection data, length of rbcL sequences obtained, and whether the field-collected sample was isolated into unialgal culture for interbreeding, karyological and/or molecular studies

<table>
<thead>
<tr>
<th>Sample identification</th>
<th>Code</th>
<th>Location of collection</th>
<th>Date and collector</th>
<th>Length (bp)</th>
<th>GenBank accession</th>
<th>Cultured?</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Polysiphonia harveyi</em> Bailey</td>
<td>34</td>
<td>Portstewart, Antrim, N. Ireland</td>
<td>20 Jan 1992, CAM</td>
<td>207</td>
<td>AF342917</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>64</td>
<td>Helen’s Bay, Down, N. Ireland</td>
<td>15 Nov 1993, CAM</td>
<td>—</td>
<td>AF342918</td>
<td>No*</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>102</td>
<td>Bradwell Marina, Essex, England</td>
<td>27 July 1992, CAM</td>
<td>207</td>
<td>AF342919</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>111</td>
<td>Maghery, W. Donegal, Ireland</td>
<td>2 Aug 1992, CAM</td>
<td>1245</td>
<td>AF342997</td>
<td>Yes</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>114</td>
<td>Beaumaris, Anglesey, Wales</td>
<td>12 Aug 1992, CAM</td>
<td>207</td>
<td>AF342919</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>122</td>
<td>La Roche, Jersey, Channel Is.</td>
<td>13 Aug 1992, CAM</td>
<td>207</td>
<td>AF342920</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>138</td>
<td>Skerries, Dublin, Ireland</td>
<td>30 Aug 1992, CAM</td>
<td>1245</td>
<td>AF342998</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. insulosa</em> (J. Agardh) P &amp; H Crouan</td>
<td>146</td>
<td>St Malo, Bretagne, France</td>
<td>22 Aug 1992, CAM</td>
<td>207</td>
<td>AF342921</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>175</td>
<td>Hayling I., Hampshire, England</td>
<td>4 Oct 1992, CAM</td>
<td>1245</td>
<td>AF342900</td>
<td>Yes</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>180</td>
<td>Kimmeridge, Dorset, England</td>
<td>13 Oct 1992, CAM</td>
<td>207</td>
<td>AF342922</td>
<td>Yes</td>
</tr>
<tr>
<td>as <em>P. abies</em>-formis Sega</td>
<td>230</td>
<td>Akkeshi, Hokkaido, Japan</td>
<td>24 June 1993, K Kogame</td>
<td>1245</td>
<td>AF342901</td>
<td>Yes</td>
</tr>
<tr>
<td>as <em>P. japonica</em> Harvey</td>
<td>231</td>
<td>Oshoro, Hokkaido, Japan</td>
<td>1 July 1993, T Abe</td>
<td>1245</td>
<td>AF342902</td>
<td>Yes*</td>
</tr>
<tr>
<td>as <em>P. mutri</em> Lauret</td>
<td>232</td>
<td>Lerici, nr La Spezia, Italy</td>
<td>16 July 1993, CAM</td>
<td>207</td>
<td>AF342923</td>
<td>Male only</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>246</td>
<td>Mahone Bay, Nova Scotia, Canada</td>
<td>5 Nov 1992, M Madenowe via CJ Bird</td>
<td>207</td>
<td>AF342924</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. japonica</em></td>
<td>284</td>
<td>Shimoda, Honshu, Japan</td>
<td>5 Sept 1993, CAM</td>
<td>1245</td>
<td>AF342903</td>
<td>Yes</td>
</tr>
<tr>
<td>as <em>P. japonica</em></td>
<td>286</td>
<td>Choshi Harbour, Honshu, Japan</td>
<td>9 Sept 1993, CAM</td>
<td>986</td>
<td>AF342904</td>
<td>Yes</td>
</tr>
<tr>
<td>as <em>P. acuminate</em> Gardner</td>
<td>321</td>
<td>Monterey, California, USA</td>
<td>21 July 1994, CAM</td>
<td>1245</td>
<td>AF342905</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>249</td>
<td>Morehead City, North Carolina, USA</td>
<td>5 June 1994, M Deal via DW Freshwater</td>
<td>—</td>
<td>AF342906</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>358</td>
<td>Dale, Pembrokeshire, Wales</td>
<td>10 Oct 1996, CAM</td>
<td>1245</td>
<td>AF342909</td>
<td>No</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>448</td>
<td>Wilmington, North Carolina, USA</td>
<td>1 June 1998, DW Freshwater</td>
<td>1245</td>
<td>AF342900</td>
<td>Yes</td>
</tr>
<tr>
<td>as <em>P. strictissima</em> JD Hooker &amp; Harvey</td>
<td>460</td>
<td>Wellington, New Zealand</td>
<td>1998, W Nelson</td>
<td>1245</td>
<td>AF342907</td>
<td>Yes, by M-S Kim</td>
</tr>
<tr>
<td>as <em>P. harveyi</em></td>
<td>1047</td>
<td>Marble Hill, N Donegal, Ireland</td>
<td>July 1998, CAM</td>
<td>207</td>
<td>AF342925</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Other taxa in *P. harveyi* clade

*P. forfex* Harvey | 1034 | Biarritz, Aquitaine, SW France | 15 July 1999, CAM | 1245 | AF342910 | No |

*P. simplex* Holenberg | 476 | Shelter I., San Diego, California | 23 July 1996, CAM | 1245 | AF342909 | Yes |

*P. strictissima* | 292 | Titahi Bay, Wellington, New Zealand | 3 October 1995, W Nelson | 1245 | AF342908 | No |

Outgroup taxa

*P. boudieri* (Dillwyn) Harvey | 438 | Portaferry, Down, N Ireland | 20 Mar 1998, CAM | 1245 | AF342916 | No |

*P. denudata* (Dillwyn) Sprengel | 479 | Plymouth, Devon, England | 30 Sep 1998, F Bunker | 1245 | AF342914 | No |

*P. elongata* (Dillwyn) Harvey | 442 | Fanad, N. Donegal, Ireland | 17 May 1998, CAM | 1245 | AF342911 | No |

*P. elongata* Harvey | 468 | Pwllheli, Cardigan, Wales | 20 Aug 1998, CAM | 1245 | AF342913 | No |

*P. inflata* (Dillwyn) Harvey | 257 | Marble Hill, N. Donegal, Ireland | 5 Aug 1993, CAM | 1245 | AF342915 | No |

*P. fibrillosa* (Dillwyn) Sprengel | 255 | Marble Hill, N. Donegal, Ireland | 5 Aug 1993, CAM | 1245 | AF342912 | No |

*Chromosome no. determined.*
trees were rooted with 100 times. The transition:transversion ratio of 2:1, and this was bootstrapped.

In all parsimony analyses the input order was randomized 10 times. The tree with the highest likelihood (ML) was determined using an expected input order was randomized 10 times. The tree with the highest likelihood (ML) was determined using an expected likelihood algorithm, and data were analysed by maximum likelihood (ML) methods with PAUP* (Swofford 1999). All samples of *P. harveyi* tested were fully inter-fertile (Fig. 1), and in all attempted crosses, at least two out of three female tips produced viable diploid spores. For some crosses representing isolates from different geographical areas (Donegal 111 female × Akkeshi 230 male; Donegal 111 female × Italy 232 male; Oshoro 231 female × Donegal 111 male; Oshoro 231 female × Down male), development of diploids (sporophytes) was followed through to meiosis and the release of viable haploid spores. Of these crosses, two (Oshoro female × Down male; Donegal female × Choshi male) were taken through a full second life history. Haploid spores released after meiosis in the hybrid sporophytes grew into male and female gametes that underwent successful fertilization and gave rise to viable diploid spores. No reduction of viability or growth rate was observed in hybrids relative to parental isolates. For all three strains of *P. harveyi* in which chromosomes were examined, n = 29.

**Results**

**Interfertility of Polysiphonia harveyi isolates**

All isolates of *Polysiphonia harveyi* tested were fully interfertile (Fig. 1), and in all attempted crosses, at least two out of three female tips produced viable diploid spores. For some crosses representing isolates from different geographical areas (Donegal 111 female × Akkeshi 230 male; Donegal 111 female × Italy 232 male; Oshoro 231 female × Donegal 111 male; Oshoro 231 female × Down male), development of diploids (sporophytes) was followed through to meiosis and the release of viable haploid spores. Of these crosses, two (Oshoro female × Down male; Donegal female × Choshi male) were taken through a full second life history. Haploid spores released after meiosis in the hybrid sporophytes grew into male and female gametes that underwent successful fertilization and gave rise to viable diploid spores. No reduction of viability or growth rate was observed in hybrids relative to parental isolates. For all three strains of *P. harveyi* in which chromosomes were examined, n = 29.

**Infraspecific phylogeography**

The compressed alignment of *rhl* sequences for *P. harveyi* (Fig. 2) identifies six haplotypes, A to F, as shown in the minimum spanning tree (Fig. 3). Their geographical distribution is shown in Fig. 1. The four most divergent haplotypes B, C, D and E, were observed in Japanese samples, which are linked by hypothetical ‘missing’ haplotypes X, Y and Z. Of the two non-Japanese haplotypes A and F, haplotype F (NZ 460, NC 448, Monterey 321) is linked in the minimum spanning tree (Fig. 3) to haplotype A by two of the hypothetical Japanese haplotypes. Haplotype A has one unique synapomorphy relative to F (position 1159), which is a synonymous substitution, and was found in all North Atlantic and Mediterranean samples except NC 448 (Fig. 1). Although for nine of these samples only 207 bp were sequenced (Table 1), they shared a G at position 351, which separated them clearly from the other non-Japanese clade of *P. harveyi*, consisting of NC 448, NZ 460 and CA 321 (Fig. 4), which has a synapomorphic A in this position.

The two greatest sequence divergences were between Japanese samples, Choshi 286 vs. Oshoro 231 (2.13%), and Oshoro 231 vs. Shimoda 284 (1.79%), with all other divergences ≤ 1.3%. All samples of *P. harveyi* form a clade in which the position of *P. japonica* Shimoda 284 is unresolved although the ML analysis places it basally (Fig. 4). The remaining samples constitute two sister clades. The first, which is strongly supported (BP = 95–97%), consists of British Isles + Hokkaido, in which the Hokkaido samples share two nucleotide synapomorphies (positions 872, 1024; Fig. 2). The second, less robust subclade (BP = 67–81%) is composed of Honshu and a widely distributed group of identical samples (*P. harveyi* CA 321, *P. harveyi* NZ 460 and *P. harveyi* NC 448).
Supraspecific phylogeography

P. strictissima was clearly more divergent from P. harveyi samples (4.05–5.08% vs. P. harveyi) than they were from each other. Together, P. harveyi and P. strictissima form a strongly supported monophyletic clade (BP = 94–98%), P. strictissima being placed basally within this clade with 99–100% bootstrap support (Fig. 4). The North Pacific clade has 100% bootstrap support in all analyses (Fig. 4). Within this, the most divergent taxa were P. simplex and P. forfex, with divergences ranging from 6.46% (P. forfex vs. P. harveyi NC 448) to 9.21% (P. simplex vs. P. harveyi Oshoro 231). Although the relative positions of the two basal taxa, P. simplex and P. forfex, cannot be resolved in some analyses, the ML tree places P. forfex basally (Fig. 4). The highest sequence divergences (10.25–13.10%) were between members of the Pacific clade and the North Atlantic outgroup taxa.

Discussion

All cultured isolates of Polysiphonia harveyi were completely interfertile, and there was no evidence of polyploidy or aneuploidy. This biological species is thus both morphologically and genetically variable: intraspecific rbcL divergences of up to 2.1% are high even for red algae. Although similar values (0–1.8% intraspecific divergence) have been reported in the Gelidiales (Freshwater & Rueness 1994), some intergeneric rbcL divergences in the Gracilariales and Gigartinaceae were only 3% (Hommersand et al. 1994; Bailey & Freshwater 1997), and were even lower in other algal groups, such as the heterokont algae (e.g. 1.6% sequence divergence between Eustigmatos magna and Visheria helvetica, Eustigmatophyta; Daugbjerg & Andersen 1997).

The highly divergent haplotypes associated with Japanese accessions are consistent with Japan being the centre of
diversity of the species. Two related haplotypes, B and C, found in Hokkaido, were distantly related to another pair of haplotypes, D and E, from the south-central coast of Honshu (Figs 1–3). It is, therefore, entirely possible that the ‘intermediate’ haplotypes (X, Y, Z) represented by intervening mutations do exist, perhaps in northern Honshu, but were simply not sampled. High levels of genetic diversity in P. harveyi in Japan can also be inferred from an extensive breeding study (Kudo & Masuda 1986) showing a partial sterility barrier between two breeding groups. The two groups were nearly allopatric, one in Hokkaido, the other in Honshu, with the exception that an Oshoro isolate was part of the Hokkaido breeding group, probably because of northward gene flow along the Japanese coast with the Tsushima current, a northwards extension of the Kurishio current (Lüning 1990). Our crossability data suggest that we failed to sample the Hokkaido breeding group, perhaps because the Honshu breeding group has recently extended its distribution in Hokkaido into the range of the Honshu group (e.g. at Akkeshi). This implies that, if samples of the second breeding group were sequenced, even greater genetic diversity might be detected in Japanese P. harveyi. Such genetic diversity is not unexpected because Japan has an exceptionally rich seaweed flora, attributed to stability of annual sea temperature regimes over long periods (Pleistocene glaciations reduced temperatures by less than 5 °C) and the extensive rocky shores (Lüning 1990). In Codium fragile, for example, great morphological heterogeneity occurs in Japan, such that almost all subspecies worldwide can be matched by one or more Japanese collections (Silva 1955).

From the minimum spanning tree, it is obvious that there are at least two separate invasive lineages in P. harveyi, haplotypes A and F. Although these haplotypes were not observed in Japan, we suggest they are Oshoro isolates of part of the Hokkaido breeding group, probably because of northward gene flow along the Japanese coast. The wide distribution of a single haplotype over much of the North Atlantic and in the Mediterranean, in conjunction with the high genetic variability displayed by P. harveyi in Japan over a much smaller latitude range, suggests that all of the northern North Atlantic was colonized from one source in or near Hokkaido. It is not possible to determine whether Nova Scotian populations were introduced from Europe, or vice versa, but during the last two centuries several conspicuous European algae appeared in eastern Canada (e.g. Fucus serratus, Taylor 1957; C. fragile ssp. tomentosoides, Chapman 1999). The first known dates for P. harveyi in each area are consistent with its having been introduced first into France or Iberia (perhaps with the Portuguese oyster, Crassostrea angulata, which may have been brought from Asia to Europe in early historical times, O’Foighil et al. 1998), then having spread in Europe, slowly initially, and later rapidly.

P. harveyi was first noted in the Mediterranean in 1959 in the Etang de Thau, an important oystericultural basin (Lauret 1967; as P. mottei), so it appears to have been secondarily introduced from Atlantic Europe, perhaps with oysters being relaid from Atlantic France.

Haplotype F (California, North Carolina, New Zealand) is more closely linked to Honshu than to Hokkaido sequences, both in the minimum spanning tree and by phylogenetic analysis. The presence of the same haplotypes in these three geographically distant regions is good evidence that P. harveyi has been a recent introduction into at least two of these areas. The marine flora of New Zealand now includes large numbers of species associated with ports and believed to have been introduced by shipping; five introduced Polysiphonia species have already been reported (Nelson & Maggs 1996). P. harveyi was first noticed in California in 1927 (Gardner 1927; as P. acuminata). The marine floras of Japan and California have many species in common (Lüning 1990), and P. harveyi could have spread to California naturally.

The occurrence of two rather distantly related haplotypes of P. harveyi in the North Atlantic Ocean shows that there have been two separate introductions from Japan into the North Atlantic, one of which has also spread across the North Atlantic. Of the two invasive lineages in Atlantic North America, haplotype A, linked to the cold-temperate island of Hokkaido, was detected near the northern limit of P. harveyi, whereas haplotype F, which we suggest originated in the warm-temperate Honshu, is present in the southern part of its American range. It is, therefore, possible that these two lineages have different temperature tolerances. However, all introduced P. harveyi strains were fully interfertile, showing that if these two lineages come into contact along American coasts, or elsewhere in the world, they will be able to interbreed.

Phylogenetic analysis confirmed that P. harveyi groups with other Pacific species, and that the diagnostic plastid character seen in P. harveyi and other North Pacific taxa is a morphological marker for this clade. Although some species with this plastid character, e.g. P. sphaerocarpa and P. feralacea, are widespread in the tropics including the Caribbean (Price & Scott 1992), we interpret these as having a North Pacific origin and having spread to the tropical Atlantic via the Panama seaway which was open for most of the Tertiary and closed only 6–4 Ma (Lüning 1990).

One of the species in our ‘Pacific clade’ is P. forfex, which is an easily recognizable alga with an unusual morphological character state (5–6 perivacial cells). The discovery of P. forfex in Biarritz, an area well studied in both the nineteenth and mid-twentieth centuries (Bornet & Thuret 1876; Dangeard 1961) strongly suggests a recent introduction from the Pacific where it occurs in Japan and Korea (Yoon et al. 1999), which may have been brought from Asia to Europe in early historical times, O’Foighil et al. 1998), then having spread in Europe, slowly initially, and later rapidly.

P. harveyi was first noted in the Mediterranean in 1959 in the Etang de Thau, an important oystericultural basin (Lauret 1967; as P. mottei), so it appears to have been secondarily introduced from Atlantic Europe, perhaps with oysters being relaid from Atlantic France.

Haplotype F (California, North Carolina, New Zealand) is more closely linked to Honshu than to Hokkaido sequences, both in the minimum spanning tree and by phylogenetic analysis. The presence of the same haplotypes in these three geographically distant regions is good evidence that P. harveyi has been a recent introduction into at least two of these areas. The marine flora of New Zealand now includes large numbers of species associated with ports and believed to have been introduced by shipping; five introduced Polysiphonia species have already been reported (Nelson & Maggs 1996). P. harveyi was first noticed in California in 1927 (Gardner 1927; as P. acuminata). The marine floras of Japan and California have many species in common (Lüning 1990), and P. harveyi could have spread to California naturally.

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Marine algae have been introduced around the world by a variety of means. Most macroalgae introduced into Europe during the last two centuries are believed to have come from Japan with oysters (Farum 1880, 1994). Another common vector is shipping, with algae being carried as fouling organisms (Carlton & Holdway 1995; Ribeira & Boudouresque 1995), and the recent spectacular spread of Caulerpa taxifolia in the Mediterranean may have resulted from aquarium escapes (Olsen et al. 1998). How P. harveyi has been introduced from Japan into Europe, Atlantic North American and New Zealand is unknown, although it is notable that it is particularly abundant on the invasive species C. fragile in Japan, Europe and Nova Scotia. One subspecies of C. fragile was introduced to Europe in the eighteenth century or earlier (Silva 1955). A second subspecies, tomentosoides, was present in 1900 in Atlantic Europe and in 1957 in the north-west Atlantic, and has since become abundant in New England and Nova Scotia (Trowbridge 1998). It also appeared in Mediterranean France in 1946 (Verlaque 1994), and more recently in California (Goff et al. 1992) and New Zealand (Trowbridge 1998). It, therefore, seems feasible that, if not originally introduced from the Pacific with C. fragile, P. harveyi has been ‘hitchhiking’ along with it.

How does the evidence presented here accord with our three proposed scenarios for the introduction of P. harveyi into Europe? It is the centre of its distribution and genetic diversity, which suggests that this species originated there. Although our results resemble those of the study by O Foighil et al. (1998) on C. taxifolia, in that we could not link introduced lineages to particular Japanese populations, we found high genetic diversity in Japanese P. harveyi that we suggest is consistent with these lineages being present in Japan also. Scenario 1, in which P. harveyi is native to Atlantic North America, appears unlikely.

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CRYPTIC INVASIONS OF POLYSIPHONIA HARVEYI


