Genealogical partitioning and phylogeography of *Colpomenia peregrina* (Scytosiphonaceae, Phaeophyceae), based on plastid *rbc*L and nuclear ribosomal DNA internal transcribed spacer sequences

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*Colpomenia peregrina* shows a large morphological variation, and two morphotypes have been described. We used the protein-coding plastid *rbc*L and the nuclear ribosomal internal transcribed spacer (ITS) region to investigate whether these morphotypes constitute distinct species and to explain the current distribution of the species. Here, we sequenced the *rbc*L gene from 38 specimens (32 *C. peregrina* and six putative relatives) and the ITS region from 33 specimens of *C. peregrina*, including an outgroup taxon. The *C. peregrina* specimens were variable, having up to 1.17% intraspecific divergence and nine haplotypes in the *rbc*L gene, and up to 11.01% intraspecific divergence and 21 haplotypes in the ITS region. Independent analyses of the *rbc*L and ITS data sets produced highly congruent but not identical results. *Colpomenia peregrina* is monophyletic, but is partitioned into two deeply divergent clades (‘lineage I’ and ‘lineage II’) that we interpret as different species. Lineage I consists of 27 specimens, in both *rbc*L and ITS data sets, and lineage II contains six specimens. Both lineages occur together in Australia, Korea, New Zealand and USA. Lineages I and II correspond to the epiphytic and epilithic forms, respectively, recognized by Clayton. Our *rbc*L and ITS data sets corroborate the recent anthropogenic dispersal event between the northwest Pacific and northeast Atlantic Oceans, and also suggest some natural dispersal events during the Pleistocene between the North and South Pacific Ocean.

**INTRODUCTION**

*Colpomenia peregrina* (Sauvageau) Hamel is a scytosiphon-ocean brown alga that occurs in temperate waters of both the northern and southern Pacific Ocean, as well as in the North Atlantic Ocean. It is recognized by extensive irregular sori that lack cuticles, and a thin thallus of three to four layers of colourless medullary cells (Clayton 1975). It superficially resembles *C. sinuosa* (Mertens ex Roth) DerbeÁs & Solier, but the latter has punctate sori with a cuticle and commonly four to six layers of medullary cells (Clayton 1975). The type locality of *C. sinuosa* is Cadiz in Spain, and the lectotype locality of *C. peregrina* is Morbihan in France (Yoshida 1998).

*Colpomenia peregrina* is an annual, and has a heteromorphic life history in which a parenchymatous gametophyte alternates with a pseudoparenchymatous sporophyte (Clayton 1979; Vandermeulen 1986; Kogame & Yamagishi 1997). The gametophyte is globular to irregular and 5–10 cm in diameter, whereas the sporophyte is a prostrate crust, 1–3 mm in diameter (Kogame & Yamagishi 1997). The saccate thalli of *C. peregrina* are hollow and filled with water and air, which provides buoyancy.

In the North Pacific, *C. peregrina* occurs from Korea (Cho et al. 2001) and Japan (Kogame & Yamagishi 1997), through Vladivostok in Far-East Russia (Adrianov & Kussakin 1998), to Alaska, British Columbia, Oregon and California in North America (Scagel et al. 1989), where it grows intertidally in spring (Vandermeulen 1986; Kogame & Yamagishi 1997). The species also occurs in the South Pacific in Australia and New Zealand (Blackler 1967; Clayton 1975; Parsons 1982). Although *C. peregrina* occurs from Norway to the Mediterranean in the North Atlantic, the species is considered to be introduced, possibly from the northwest Pacific (Farnham 1980; Blackler 1981; Fletcher 1987; Lüning 1990).

Clayton (1975) described two morphotypes of *C. peregrina* from southern Australia: a relatively small globose form (with thallus diameter 1–5 cm), and a larger irregular form (thallus diameter 7–10 cm) with a deeply infolded surface. The globose form is usually epiphytic and occurs in tidepools, whereas the irregular-shaped form occurs mainly on open rock surfaces in the intertidal to upper subtidal zones. The globose form is the type most commonly found in Europe (Blackler 1967), whereas the irregular form predominates in winter in southern Australia (Clayton 1975). However, Clayton (1975) suggested that variability in the two forms did not justify separate species.

The objectives of the present study were to test Clayton’s (1975) hypothesis that two morphs can be distinguished in *C. peregrina*, using the large subunit of the Rubisco spacer (*rbc*L) and the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region, and to explain the current distribution of the species. We determined the *rbc*L sequences of biogeographically representative specimens of *C. peregrina* and its putative relatives. The *rbc*L gene is useful for identifying species because it is generally highly conserved at the species level (Draisma et al. 2001), although intraspecific variation has been
observed (Kawai et al. 2001). We also analysed the ITS region from 33 specimens including C. sinuosa as outgroup. The ITS region is useful for comparing local populations within scytosiphonacean taxa (e.g. van Oppen et al. 1993; Peters et al. 1997; Stache-Crain et al. 1997; Sasaki et al. 2001; Kim & Kawai 2002). However, we did not include other Colpomenia (En- dicher) Derbes & Solier species because ITS sequences were too divergent to be aligned in our study.

**MATERIAL AND METHODS**

**Taxon sampling**

Thirty-two individuals of C. peregrina from 29 localities in the Pacific and Atlantic Oceans were sampled (Fig. 1; Table 1). For the rbcL analysis, six putative relatives (one C. bulbosa, one C. phaeodactyla and four C. sinuosa) were also included. Additional sequences of Colpomenia spp. were downloaded from GenBank. Scytosiphon lomentaria was used as outgroup for the rbcL data set (Kogame et al. 1999; Cho et al. 2001, 2003). We examined the ITS region from C. sinuosa as the outgroup taxon as well as 32 C. peregrina specimens. Collection sites and GenBank accession numbers are listed in Table 1. All samples were air-dried and preserved with silica gel prior to extraction of genomic DNA. Voucher specimens were deposited at the Research Center of Chungnam National University, Daejon, Korea.

**Deoxyribonucleic acid extraction, amplification and sequencing**

Dried samples were ground to fine powder in liquid nitrogen. Approximately 0.01 g of the powder was used for genomic DNA extraction using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. Extracted crude DNA was stored at −20°C and used for polymerase chain reaction (PCR) amplification of rbcL and ITS.

The rbcL region was amplified and sequenced using the primers PRB-F0, F2, F3, R1A, R2, R3A (Kogame et al. 1999), and RS1 and RS2 (Yoon & Boo 1999). The ITS 1 and 2, parts of the 3′-terminus of the 18S and the 5′-terminus of the 26S ribosomal RNA genes, and the complete 5.8S gene were amplified using the LB1 and LB2 primers of Yoon et al. (2001). For the ITS region, the primer pairs LB1/BC2 (Saunders & Druelh 1993) and YB1 (Yoon et al. 2001)/LB2 were used in the sequencing reactions. PCR and sequencing reaction concentrations followed Kogame et al. (1999). The PCR products for both regions were purified using the High Pure PCR Product Purification Kit (Roche, Indianapolis, IN, USA), according to the manufacturer’s protocol. The sequences of the forward and reverse strands were determined for all taxa, using an ABI PRISM 377 DNA Sequencer (Applied Biosystem, Foster City, CA, USA). The electropherogram outputs for each sample were checked using Sequence Navigator v. 1.0.1 software (Applied Biosystems). The rbcL sequences were collated using the multisequence editing program

![Map of localities sampled for Colpomenia peregrina. Specimen codes are as in Table 1, and haplotypes are indicated (rbcL/ITS). Black arrowheads represent specimens of lineage I, and white arrowheads those of lineage II.](image-url)
Table 1. Specimens (*epilithic, **epiphytic) and sequences of the taxa included in this study.

| Species, collection sites and date; voucher | Code | rbcL haplotype | ITS haplotype | GenBank accession number |
|--------------------------------------------|------|----------------|---------------|-------------------------|
| **Colpomenia peregrina** (Sauvageau) Hamel  |
| Korea: east coast                           |
| Anin1, Gangreung, 20 Apr. 2001; **PE017    | KE1  | 1              | 1             | AY398434                | AY398471 |
| Anin3, Gangreung, 12 Jan. 2002; *PE018     | KE2  | 6              | 17            | AY398435                | AY398472 |
| Gangpo, Geonjui, 19 Apr. 2001; **PE019     | KE3  | 2              | 2             | AY398436                | AY398473 |
| Hupo, Uijin, 19 Apr. 2001; *PE020          | KE4  | 7              | 18            | AY398437                | AY398474 |
| Sacheon, Gangreung, 23 Feb. 1999; **PE021  | KE5  | 1              | 3             | AY398438                | AY398475 |
| Sinnam, Weonbuk, 19 Apr. 2001; **PE022     | KE6  | 3              | 4             | AY398439                | AY398476 |
| Korea: south coast                          |
| Hoedong, Jindo, 9 Mar. 2001; **PE023       | KE1  | 4              | 5             | AY398440                | AY398477 |
| Jeongdori, Wando, 15 Dec. 1998; **PE024    | KE2  | 5              | 6             | AY398441                | AY398478 |
| Seosang1, Namhaedo, 23 Mar. 2001; **PE025  | KE3  | 1              | 1             | AY398442                | AY398479 |
| Seosang2, Namhaedo, 23 Mar. 2001; **PE026  | KE4  | 1              | 7             | AY398443                | AY398480 |
| Songjeong, Busan, 18 Apr. 2001; **PE027    | KE5  | 1              | 8             | AY398444                | AY398481 |
| Korea: west coast                           |
| Daeedoryedo, Sinan, 27 Jun. 2001; **PE028  | KE1  | 7              | 13            | AY398445                | AY398482 |
| Dongbakejong, Seocheon, 3 Dec. 1998; **PE029 | KE2  | 5             | 9             | AY398446                | AY398483 |
| Gyeokpo, Buan, 10 Feb. 2001; **PE030       | KE3  | 5              | 9             | AY398447                | AY398484 |
| Korea: Jejudo                              |
| Hansuri, Hanrim, 24 Mar. 2000; **PE031     | KEJ1 | 10             | 1             | AY398448                | AY398485 |
| Ilchulbong, Seongsan, 23 Mar. 2000; **PE032 | KEJ2 | 11             | 11            | AY398449                | AY398486 |
| Japan                                      |
| Oshoro, Hokkaido                           |
| AU1  | 8              | 19            | AY398450                | AY398487 |
| AU2  | 12             | 1            | AY398451                | AY398488 |
| AU3  | 12             | 1            | AY398452                | AY398489 |
| France                                    |
| Ille de Batz, Roscoff, 8 Apr. 2000; **PE036 | KE1  | 1              | 1             | AY398453                | AY398490 |
| New Zealand                                |
| Brighton Beach, Dunedin, 5 Aug. 2001; **PE037 | NZ1  | 13             | 1             | AY398454                | AY398491 |
| Portobello, Dunedin, 6 Aug. 2001; *PE038   | NZ2  | 10             | 20            | AY398455                | AY398492 |
| Weller’s Rock1, Dunedin, 6 Aug. 2001; *PE039 | NZ3  | 20             | 9             | AY398456                | AY398493 |
| Weller’s Rock2, Dunedin, 6 Aug. 2001; **PE040 | NZ4  | 13             | 1             | AY398457                | AY398494 |
| Cook Strait, Wellington, 3 Aug. 2001; **PE041 | NZ5  | 13             | 1             | AY398458                | AY398495 |
| Kau Bay, Wellington, 3 Aug. 2001; **PE042  | NZ6  | 14             | 1             | AY398459                | AY398496 |
| Scorching Bay, Wellington, 3 Aug. 2001; *PE043 | NZ7  | 15             | 1             | AY398460                | AY398497 |
| Russia                                     |
| Nakhodka, Vladivostok, 23 May 2002; **PE044 | RU1  | 1              | 1             | AY398461                | AY398498 |
| UK                                         |
| Port Erin Bay, Isle of Man, 9 Jul. 2000; **PE045 | UK1  | 1              | 1             | AY398462                | AY398499 |
| USA                                        |
| Dana Point, California, 3 Dec. 1999; *PE046 | US1  | 7              | 21            | AY398463                | AY398500 |
| Monterey, California, 11 Dec. 1999; **PE047 | US2  | 2              | 16            | AY398464                | AY398501 |
| Sunset Bay, Oregon, 15 May 2001; **PE048   | US3  | 2              | 16            | AY398465                | AY398502 |
| **Colpomenia bullosa** (Saunders) Yamada in Yamada & Kinoshita  |
| Muroran, Hokkaido, Japan                   |
| JP2  | 1              | —             | AB0222363     | —                      |
| Wellers Rock, Dunedin, New Zealand, 6 Aug. 2001; PE049 | NZ8  | 1          | —             | AY398466                | —        |
| **Colpomenia phaeodactyla** M.J. Wynne & J.N. Norris  |
| Hoedong, Jindo, Korea, 9 Mar. 2001; PE050  | KE5  | 1              | —             | AY398467                | —        |
| Tsuyazaki, Fukuoka, Japan                  |
| JP3  | 1              | —             | AB0222373     | —                      |
| **Colpomenia sinuosa** (Mertens ex Roth) Derbès & Solier  |
| Guryongpo, Pohang, Korea; PE012             |
| KE7  | 1              | —             | AY623653      | —                      |
| Hanrim, Jejudo, Korea, 4 Dec. 2002; PE051   |
| KE3  | 1              | —             | AY398468      | —                      |
| Kasumi, Hyogo Pref., Japan                 |
| JP4  | 2              | —             | AB0222343     | —                      |
| Zushi, Kanagawa, Japan, 25 Jul. 2002; PE052 |
| JP5  | 1              | —             | AY398469      | —                      |
| Tamarama Beach, Sydney, Australia, 11 Aug. 2001; PE053 | AU4  | 1          | —             | AY398470                | —        |
| Port Hutt, Chatham Island, New Zealand, 23 Apr. 2004; PE327 | NZ1  | —             | —             | —                      | AY706206 |
| **Scytosiphon lomentaria** (Lyngbye) Link   |
| Oshoro, Hokkaido, Japan                    |
| AB0222383                                  | —        | —             | —                      |

1 Kogame et al. (1999).
Sequencing (Gilbert 1995), and aligned visually with those published previously (Kogame et al. 1999). A total of 43 rbcL sequences, which included five previously published sequences, were used for the phylogenetic analyses. All 33 new ITS sequences, including that of C. sinuosa, were aligned by eye.

**Data analysis**

We used only haplotypes of both rbcL and ITS sequences for phylogenetic reconstructions. Analysis of phylogenetic relationships between haplotypes of each data set was conducted using PAUP* 4.0b10 (Swofford 2002). For the rbcL data, a maximum likelihood (ML) analysis was conducted using the TrN + G model, which was selected by ModelTest (v. 3.06; Posada & Crandall 1998). The parameters of the model were as follows: estimated substitution rates: A ↔ G = 5.7302614; C ↔ T = 11.192832; A ↔ C = A ↔ T = C ↔ G = G ↔ T = 1, and shape parameter: 0.014413. Tree likelihoods were estimated using a heuristic search with 100 random sequence-addition replicates, and tree bisection–reconnection (TBR) branch swapping. Maximum parsimony (MP) analysis was done using a heuristic search algorithm with the following settings: 100 random sequence-addition replicates, TBR branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed to yield polytomies.

For the ITS data, the optimal model was the TrN + I + G model. The parameters used were as follows: estimated substitution rates: A ↔ G = 2.1800647; C ↔ T = 4.6409321; A ↔ C = A ↔ T = C ↔ G = G ↔ T = 1, proportion of invariant sites: 0.518586, and shape parameter: 0.607407. The MP analysis followed the same method as was used for the rbcL data set. For both rbcL and ITS trees, nonparametric bootstrap values for nodes in ML and MP phylograms were calculated based on 100 and 1000 resamplings, respectively. The neighbour-joining analyses for both rbcL and ITS sequence data were done with the same parameters used for the ML analyses but, because results were congruent with the ML and MP analyses, they are not shown.

**RESULTS**

**rbcL sequences**

The rbcL alignment contained 1467 nucleotides for the 43 rbcL sequences of C. peregrina and putative relatives without gaps. Of these, 117 positions were variable (8%), and 89 (6.1%) were parsimony-informative. Within C. peregrina, 25 positions (1.7%) were variable and 17 (1.2%) were parsimony-informative (Fig. 2).

In C. peregrina specimens, all substitutions except one were at the third codon position and silent. However, in the Gelibrand specimen (AU1), at position 751, which is a first codon position, adenine was changed to guanine, and thus valine was replaced by methionine.

We found nine haplotypes among the 33 rbcL sequences of C. peregrina, including one previously published sequence (Figs 1, 2; Table 1). Haplotype 1 was found in 19 specimens from Korea, Japan, Russia, Australia, New Zealand, France and the UK. Haplotypes 2 and 7 were observed in both Korea and USA. Haplotype 8 occurred only in Australia, and haplotype 9 only in New Zealand. Haplotypes 1 and 6 occurred together at Anin, Korea, and haplotypes 1 and 9 were found at Weller’s Rock, New Zealand.

Colpomenia bullosa specimens from Japan and New Zealand had identical rbcL sequences, as did those of C. phaeodactyla from Korea and Japan. Among five specimens of C. sinuosa, two haplotypes, differing by a single base pair, were found: one from Korea, Japan, Australia and the other from Japan.

The pairwise divergence between C. peregrina haplotypes ranged from 0.07% to 1.17%, the latter between haplotypes 4 (KS1) and 8 (AU1). The interspecific divergence was 3.65–3.87% between C. peregrina and C. bullosa, 3.80–3.94% between C. peregrina and C. phaeodactyla, and 3.58–3.87% between C. peregrina and C. sinuosa. However, the divergence was low (0.34%) between C. bullosa and C. phaeodactyla.

The tree produced by the ML analysis based on the rbcL haplotypes is illustrated in Fig. 3, and is identical with the single most parsimonious tree [Tree length = 139, Consistency index (CI) = 0.855, and Retention index (RI) = 0.916]. The trees consistently showed a monophyletic clade of all 33 C. peregrina specimens, which were separated into two major assemblages designated “lineage I” and “lineage II”. Each of the two lineages was strongly supported by bootstrap values. Lineage I contained specimens from Korea, Australia, France, Japan, New Zealand, Russia, the UK and the United States. Within lineage I, haplotypes 1, 3 and 5 formed a weakly supported clade [bootstrap support (BS) 61% for both ML and MP]. Lineage II contained the remaining specimens from Korea, Australia, New Zealand and USA. However, lineage II consisted of two subclades; one subclade contained haplotypes 6 (KE2) and 8 (AU1) (BS 86% for ML and 93% for MP), and the other consisted of haplotypes 7 (KE4 and US1) and 9 (NZ2 and 3) (BS 95% for ML and 98% for MP).

Colpomenia sinuosa was consistently the sister clade to C. peregrina with strong support. Colpomenia bullosa and C. phaeodactyla formed a single clade with maximum support.

**nrDNA ITS sequences**

The length of the ITS region (ITS 1 + 5.8S + ITS 2) of C. peregrina specimens analysed in the present study was from

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**Fig. 2. Compressed alignment of the rbcL gene of Colpomenia peregrina showing different haplotypes (see Fig. 1; Table 1). Numbers above the sequences indicate sites, and dots indicate the same nucleotide as haplotype 1.**
analyses. Lineage I was supported by 73% BS for ML and 100% BS for MP, and lineage II was supported by 64% BS for ML and 100% BS for MP. In lineage I, most of the branch-es between haplotypes were not well resolved. Lineage II con-sisted of two subclades: the first included haplotypes 19 (AU1), 20 (NZ2 and 3), and 21 (US1), and the second con-tained haplotypes 17 (KE2) and 18 (KE4). One subclade was supported by 62% BS for ML and 93% BS for MP, and the other by 59% BS for ML and 97% for MP.

DISCUSSION

The *rbcL* tree highlights a single evolutionary origin for *C. peregrina*. Both the *rbcL* and ITS trees reveal partitioning of *C. peregrina* into two groups (lineages) with the same con-stituent specimens. Each lineage is supported by high boot-strap values for both markers. In the *rbcL* data, the two groups of *C. peregrina* have greater divergence (0.89–1.17%, 13–17 bp) than that between North and South Pacific populations of *C. sinuosa* (0.07%, 1 bp), and between *C. bullosa* and *C. phaeodactyla* (0.34%, 5 bp). These levels of divergence within *C. peregrina* are equal to or surpass those between other spe-cies of scytosiphonacean algae (0.5–5.9%; Kogame et al. 1999).

Similar high divergence values (9.20–11.01%) are found between the two ITS lineages of *C. peregrina*. This genetic divergence within samples of *C. peregrina* is in marked con-trast to the lower levels of variability recorded between spec-imens of *Petalonia binghamiae* (J. Agardh) Vinogradova from Korea and USA (0.33%; Cho et al. 2002) and between arctic–antarctic populations of *Desmarestia viridis* (O.F. Müller) Lamouroux – *D. willii* Reinsch (0.09%; van Oppen et al. 1993). The divergence value within *C. peregrina* is higher than those (0.5–6.4%) among five species of *Fucus* Linnaeus (Leclerc et al. 1998), and higher than or equal to those (3.8–17.4%) among species of *Desmarestia* Lamouroux (Peters et al. 2000).

It is therefore clear that *C. peregrina* consists of two deeply divergent and evolutionarily distinct lineages, which we inter-pret as two different species. A full taxonomic and nomen-clatural revision of the species will appear elsewhere. Here, we will only briefly discuss some morphological and ecolog-ical characteristics. In our samples from Korea and elsewhere, we observed features discriminating the two lineages of *C. peregrina*, as described by Clayton (1975). Lineage I corre-sponds to her epiphytic group and lineage II to her epilithic group (see Table 1). Lineage II specimens were irregular in shape, usually found subtidally, and always epilithic, whereas the lineage I samples were often but not always globose, usually intertidal, and always epiphytic. Other morphological characters such as thallus diameter need further study before diagnosing the lineages.

*Colpomenia sinuosa* was consistently the sister taxon to *C. peregrina* in our *rbcL* tree. These results are congruent with similarities of both species in morphology and life history: both have pluri- and unilocular zoidangia on prostrate thalli (Kogame et al. 1999) and form plurilocular zoidangia in long-day culture conditions, but mostly unilocular zoidangia under short-day conditions (Kogame 1997). Biogeographically, *C. peregrina* and *C. sinuosa* overlap in the North and South Pa-
Fig. 4. Compressed alignment of the ITS region (ITS 1: 1–553, 5.8S: 662–673, ITS 2: 743–952) of *Colpomenia peregrina* showing different haplotypes (see Fig. 1; Table 1). Numbers above the sequences indicate sites, and dots denote the same nucleotide as haplotype 1; dashes represent alignment gaps.
Our \textit{rbcL} and ITS data are considered to reflect dispersal events of the \textit{C. peregrina} taxa. In lineage I, the occurrence of identical \textit{rbcL} haplotypes but different ITS types between Korea and USA and between Korea and Australia–New Zealand leads us to infer natural dispersal events in the Pacific Ocean. The continuous distribution of \textit{C. peregrina} from Korea, Far-East Russia and northern Japan, through Alaska and British Columbia to Oregon and California, USA, indicates that this species might have spread naturally. The species is speculated to float on the warm Kuroshio Current from the west to the east side of the Pacific Ocean or on the North Equatorial Current from the east side to the west side. Between the North and South Pacific Oceans, the species is inferred to have crossed a land bridge between Australia and Indonesia during the Pleistocene, when seawater temperatures in this area were 6–8°C lower than they are today (Clayton 1984; Raven \textit{et al.} 2002). This is consistent with the views of van den Hoek (1982), who considered that brown algal species, such as \textit{S. lomentaria} and \textit{Petalonia fascia} (O.F. Müller) Kuntze, may have crossed the tropics during cool periods of the Pleistocene. In contrast, there is no evidence that \textit{C. peregrina} migrated along the east coast of the Pacific Ocean at this time because its absence in Chile (Ramírez & Rojas 1991).

Although it was identified from diverse localities (Australia, Korea, New Zealand and USA), lineage II is represented by only six out of 32 samples. The subtidal habitat occupied by most populations of lineage II means that its haplotypes were under-represented in the present study. Lineage II consists of two subclades, supported by high bootstrap values. However, KE4 and US1 occur in the same subclade in the \textit{rbcL} tree, whereas the former is linked with KE2 in the ITS tree. This incongruence is difficult to explain in our data, although the phenomenon may be due to a higher substitution rate in the noncoding ITS region compared to the coding \textit{rbcL} region. The close relationship between NZ2/3 and US1 suggests the possibility of human-mediated dispersal between New Zealand and USA.

We interpret, as evidence of recent introduction, the occurrence of identical haplotypes of both \textit{rbcL} and ITS sequences between two areas. Despite the biogeographical barrier between the Pacific and Atlantic Oceans, specimens with \textit{rbcL} haplotype 1 and ITS haplotype 1, belonging to lineage I, occur in France and UK and Korea and Far-East Russia. This is consistent with previous reports that the species was introduced anthropogenically from the northwest Pacific to Europe in the early 1900s (Farnham 1980; Blackler 1981; Fletcher 1987). According to Lünig (1990), \textit{C. peregrina} was probably introduced from Japan through oyster cultures.

All specimens of \textit{C. sinuosa} from Korea, Japan and Australasia that we analysed have the same \textit{rbcL} haplotypes, except a specimen from Hyogo Prefecture, Japan (Kogame \textit{et al.} 1999), which has a single base difference. Investigations of \textit{C. sinuosa}, which has a world-wide distribution (Silva \textit{et al.} 1996), using the ITS region, will provide an interesting comparison with our results for \textit{C. peregrina}.

According to Parsons (1982), \textit{C. bullosa} was recently introduced into New Zealand on ships from either Japan or North America. In the present study, \textit{C. bullosa} from Japan and New Zealand had identical \textit{rbcL} haplotypes. This result leads us to recognize \textit{C. bullosa} in New Zealand. Although...
Ramírez & Rojas (1991) and Adams (1994) considered C. bullosa (type locality: Pacific Grove, California) as a later synonym of C. durvillei (Bory) M.E. Ramírez, a molecular study of material from Concepción, Chile, the type locality of C. durvillei, is required to confirm synonymy of the two species.

In conclusion, this study has contributed important details to our understanding of the phylogeny of a brown alga, C. peregrina, which can be seasonally conspicuous in the intertidal zone at some localities. Within the species, we have identified two distinct monophyletic groups, which have additional distinctive characteristics, and which we interpret as two different species. Failure to recognize two evolutionarily distinct groups of C. peregrina could lead to the underestimation of brown algal diversity. The rbcL and ITS data corroborate a recent anthropogenic dispersal event between the northwest Pacific and the North Atlantic Oceans, and also suggest some natural dispersal events during the Pleistocene between the North and South Pacific Ocean. Finally, a full taxonomic and nomenclatural revision of this species is clearly needed, but is beyond the scope of this paper and will appear elsewhere.

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