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Distribution patterns and introduction pathways of the cosmopolitan brown alga *Colpomenia peregrina* using mt *cox*3 and *atp*6 sequences

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Abstract *Colpomenia peregrina* is an annual brown macroalga found in temperate waters worldwide. To understand population differentiation and to reconstruct pathways of colonization/introduction, we analyzed variation in two mitochondrial protein-coding genes, cytochrome c oxidase subunit III (*cox*3) and ATP synthase F0 subunit 6 (*atp*6), and cp RuBisCO spacer. A total of 359 *cox*3, 342 *atp*6, and 38 RuBisCO spacer sequences from *Colpomenia peregrina* were obtained for samples collected at 28 sites from 12 countries. The combined *cox*3+*atp*6 sequences (1,231 bp) revealed 99 polymorphic sites and 69 haplotypes. An mt haplotype network revealed four distinct groups, separated by 7 to 26 mutation steps. NW Pacific populations were present in each group (but dominant in one), whereas SW Pacific and the Atlantic populations each were present in one group. The network and phylogenetic analyses, along with patterns of genetic diversity, suggested a NW Pacific center of origin, expanding first to the SW Pacific, then the NE Pacific, and most recently to the north Atlantic. A generalized skyline plot revealed a dramatic population expansion of the species ca. 20 kya.

Keywords *Colpomenia peregrina* · Haplotype network · Marine invasion · Mitochondrial genes · Phaeophyceae · RuBisCO spacer · Phylogeography

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Introduction

Phylogeographic studies of benthic marine species with cosmopolitan or worldwide distributions can offer insights into ecological and evolutionary processes. At one end of the continuum, an apparently widespread species may actually comprise one or more "cryptic" species that can be detected only with molecular markers. At the other end of the continuum, the species may possess efficient and effective mechanisms for long-distance dispersal and colonization, thereby promoting extensive gene flow among widespread locations. Both endpoints may be reinforced by anthropogenic introductions: single introductions may lead to a genetic bottleneck, differentiation via genetic drift, and, ultimately, speciation, whereas repeated introductions may promote gene flow and admixture of genotypes worldwide.

The focus of the present study is the brown macroalga *Colpomenia peregrina* Sauvageau (Scytosiphonaceae), a cosmopolitan species ranging from Korea, Japan, China, and Russia in the NW Pacific (Kogame and Yamagishi 1997; Cho et al. 2005; Kozhenkova 2009; Boo 2010) to Alaska, USA, and Baja California, Mexico (Pedroche et al. 2008; Lindeberg and Lindstrom 2010) in the NE Pacific and New Zealand and Australia in the SW Pacific (Clayton 1979; Parsons 1982). In the north Atlantic Ocean, *C. peregrina* occurs from Norway to Portugal (Minchin 1991) and

from Labrador/Newfoundland to the Gulf of Maine (Kennedy et al. 2010).

The annual *C. peregrina* exhibits a biphasic, heteromorphic life history consisting of saccate (5–10 cm) gametophytes and crustose sporophytes (Clayton 1979; Kogame and Yamagishi 1997). Reproduction occurs sexually, as well as by parthenogenesis of asexual zoospores. Thus, long-distance dispersal likely occurs by floating thalli that release either gametes or asexual spores and/or by reproductive individuals that are epiphytic on other species of floating algae.

Human activity, especially intercontinental shipping and oyster mariculture, has increased the frequency of species exchange among continents and oceans and thereby the probability of marine algae crossing ecogeographic barriers. A single or a few founder individuals may initiate macroalgal invasions, or the patterns may be more complex with invasive populations composed of multiple genetic lineages resulting from multiple founder events (Mineur et al. 2010). Pathways of introduction have been investigated for several species of macroalgae using molecular markers: *Ascophyllum nodosum* (Olsen et al. 2010), *Caulerpa taxifolia* (Stam et al. 2006), *Codium fragile* (Provan et al. 2005a), *Fucus distichus* (Coyer et al. 2011b), *F. serratus* (Hoarau et al. 2007), *F. spiralis* and *F. vesiculosus* (Coyer et al. 2011a), *Gracilaria vermiculophylla* (Kim et al. 2010), and *Undaria pinnatifida* (Voisin et al. 2005).

Members of the Scytosiphonaceae have a long history of transport around the globe through human activities. For example, five genera are regarded as human-mediated introductions in New Zealand and elsewhere: *Chnoospora*, *Colpomenia*, *Hydroclathrus*, *Rosenvingea*, and *Scytosiphon* (Johnson and Dromgoole 1977; Parsons 1982; Nelson and Duffy 1991, Cho et al. 2007; Nelson and Wilcox 2010). Of the 12 *Colpomenia* species, *Colpomenia bullosa*, *Colpomenia claytoniae*, and *Colpomenia peregrina* are considered invasive (Farnham 1980; Kain (Jones) et al. 2010; Boo et al. 2011b) in areas other than the NW Pacific. However, *Colpomenia expansa* and *Colpomenia tuberculata*, which are widely distributed in the NE Pacific (Pedroche et al. 2008), were reported recently in Korea (Lee 2008).

Despite their widespread distributions, phylogeographic studies of *Colpomenia* spp. have not been attempted. In view of the cosmopolitan distribution of *C. peregrina* and the potential for long-distance dispersal via natural or anthropogenic means, our goals were to examine phylogeography and to identify pathways of introductions. We used two mitochondrial markers, the commonly used cytochrome c oxidase subunit III (*cox*3) and the rarely used (in algae) ATP synthase F0 subunit 6 (*atp*6) genes, both singly and concatenated. A subset of individuals from each population was further analyzed with chloroplast RuBisCO spacer region to serve as an independent assessment of introduction pathways.

Materials and methods

Specimens of *Colpomenia peregrina* (Fig. 1a–d) were obtained from 28 locations throughout the range of the species: NW Pacific (10 from Korea, two from Japan, and one from Russia), NE Pacific (one from Mexico and three from western USA), SW Pacific (two each from Australia and New Zealand), and the north Atlantic (one each from France, Ireland, Norway, Spain, and UK, and two from eastern USA). Thalli were collected at intervals of 1 m along a horizontal intertidal line for a total of 359 individuals from all populations. All collected individuals were stored in silica gel and later cleaned of epiphytes under a dissecting microscope in the laboratory. Voucher specimens were deposited in the herbarium of Chungnam National University, Daejeon, Korea and the herbarium of the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand.

DNA extraction, amplification, and sequence alignment

A total of 359 individuals were analyzed for mitochondrial *cox*3 and 342 for *atp*6. We also selected 38 individuals (at least one from each of the 28 locations) for plastid RuBisCO spacer. DNA was extracted from pulverized thalli using NucleoSpin® Plant II (Macherey-Nagel GmbH & Co, Germany) according to the manufacturer's instructions. For amplification of *cox*3 gene, we used primers F49 and R20 (Boo et al. 2010, 2011a). Because *atp*6 (ATP synthase F0 subunit 6 gene) has not been used in marine algae, we designed primers F25P (5′-CCH TTA GAA CAA TTT BAA ATA CTY CC-3′) and R754P (5′-GCR TCR TTT ATR TAR ATR CAA CTT A-3′) based on published mitochondrial genome data from various brown algae: NC007684 for *Desmarestia viridis*, NC007685 for *Dictyota dichotoma*, NC007683 for *F. vesiculosus*, NC004024 for *Laminaria digitata*, NC003055 for *Pylaiella littoralis*, and NC93476 for *Saccharina japonica.* Thus, our *atp*6 primer set may be useful for other species of brown algae. The RuBisCO spacer region was amplified using primers RS1 and RS2 (Yoon and Boo 1999). PCR amplification, purification, and cycle sequencing were performed following Boo et al. (2011b). The sequences of forward and reverse strands were determined commercially (Genotech, Korea). Private haplotypes (haplotypes unique to a single population represented by a single mutation) were amplified twice and sequenced from a subset of samples to exclude the possibility of PCR errors. All gene sequences were aligned manually using Se-Al v.2.0a11 (Rambaut 2002). Sequences were deposited in the NCBI GenBank with accession numbers JX027338-75 for *cox*3, JX027298-337 for *atp*6, and JX843461-70 for RuBisCO spacer.

Phylogenetic analyses

Phylogenies of *cox*3, *atp*6, the combined *cox*3+*atp*6, and RuBisCO spacer datasets were reconstructed using maximum likelihood (ML). MODELTEST 3.7 (Posada and Crandall 1998) indicated different models for different datasets: TrN+G for *cox*3, GTR+G for *atp*6 and mt combined *cox*3+*atp*6, and TrN+I for RuBisCO spacer. Because GTR is the most common and general model for real-world DNA analysis (Stamatakis 2006), we also used the GTR+G+I nucleotide model as implemented in RAxML v.7.0.4. However, because topologies of trees reconstructed by different models are similar to those of the GTR model, we employed the GTR model instead of different models for each gene. We used 200 independent tree inferences with the "number of run" option with default optimized SPR rearrangement and 25 distinct rate categories to identify the best tree. Statistical support for each branch was obtained from 1,000 bootstrap replications using the same substitution model and RAxML program settings.

Maximum-parsimony (MP) trees were constructed for each data set with PAUP* v.4.0b.10 (Swofford 2002) using a heuristic search algorithm with the following settings: 1,000 random sequence additions, tree bisection-reconnection (TBR) branch swapping, MulTrees, all characters unordered and unweighted, and branches with a maximum length of zero collapsed. Bootstrap values for the resulting nodes were assessed using 1,000 bootstrapping replicates with 10 random sequence additions, TBR, and MulTrees. Trees were visualized using the FigTree v.1.1.2 program, available at http://tree.bio.ed.ac.uk/software/figtree/. Eight *Colpomenia* species were used as outgroups in the mitochondrial analysis and two for the plastid gene, based on their sister–group relationship to *Colpomenia peregrina*.

Population genetic analyses

Haplotype (*Hd*) and nucleotide (π) diversities were measured for each population using DNASP V.5 (Librado and Rozas 2009). Relationships and geographic distributions of haplotypes were analyzed in a network constructed using TCS V.1.21 (Clement et al. 2000), which implements the statistical parsimony procedure with a 95 % connection limit.

The hierarchical distribution of genetic variation among populations was tested using analysis of molecular variance (AMOVA) based on the number of pairwise nucleotide differences of the two mitochondrial loci (RuBisCO was not analyzed because of the much smaller dataset) using ARLEQUIN V.3.5 (Excoffier and Lischer 2010). We eliminated populations with less than ten samples to make population sizes more homogeneous. The null hypothesis of neutral evolution of the mt DNA was tested using Tajima's *D* (Tajima 1989) and Fu's *F*s test (Fu 1997) with the program ARLEQUIN V.3.5. Significant *D* values can be due to factors such as selection, population expansion, and bottlenecking (Tajima 1989).

Historical demographic expansions were investigated by examining the frequency distributions of pairwise differences between concatenated (*cox*3+*atp*6) sequences (mismatch distribution; Rogers and Harpending 1992). Mismatch distributions are used to test hypotheses on population demographic history and selection (Rogers and Harpending 1992). The distribution is usually multimodal in samples drawn from populations at demographic

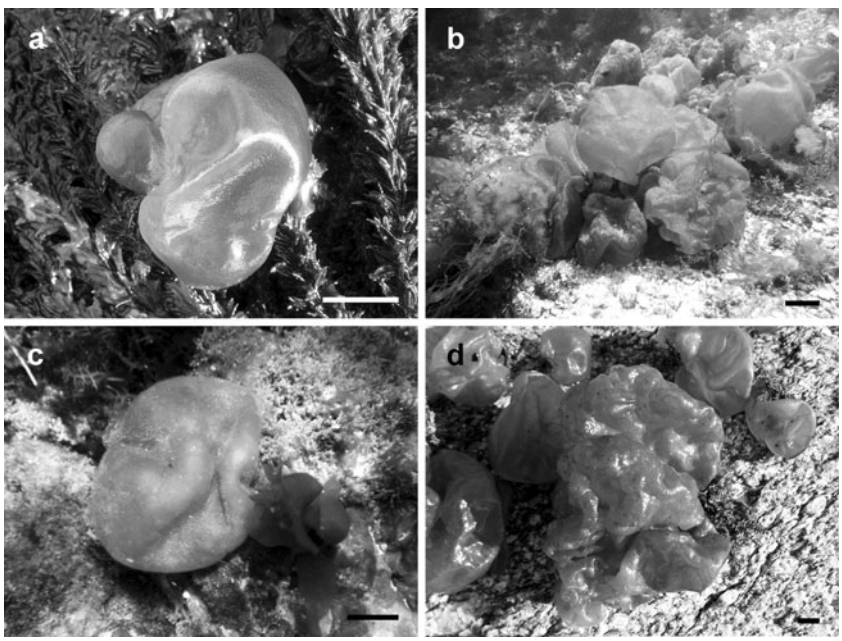


Fig. 1 Representative specimens of *Colpomenia peregrina* collected in a Sanjokam, Goseong, Korea (12 January 2005), b Ile de Batz, Roscoff, France (13 June 2010), c Santa Catalina Island, California, USA (17 July 2011), and d Appledore Island, Maine, USA (2 August 2011). *Scale bars* are 1 cm

equilibrium, but unimodal in populations following recent population demographic expansion and population range expansion (Slatkin and Hudson 1991).

Historical demographic changes of NW Pacific populations, which is the origin center of *C. peregrina* (based on *cox*3 sequences because of availability of mutation rate data in the present study), were inferred using a generalized skyline plot (Strimmer and Pybus 2001), since the shape of a genealogy depends on the demographic history. The first step of this analysis is to generate a phylogenetic tree with branch lengths proportional to time. An ML tree using TrN+G model was estimated in PAUP v.4.0b10 (Swofford 2002). Next, a generalized skyline plot was generated from the ML tree using GENIE v.3.0 (Pybus and Rambaut 2002) with a smoothing algorithm to reduce the noise in the data while simultaneously preserving the demographic signal. The smoothing parameter (ε) was estimated using the "maximize optimization" option.

Results

Characteristics of mtDNA and cp RuBisCO spacer sequences

A total of 359 *cox*3 sequences were obtained (Table 1) for a 620-bp alignment with 204 variable positions (32.9 %) and 129 parsimoniously informative positions (20.8 %). Sequences of *C. peregrina* differed by up to 3.7 %, while the average sequence divergence within the genus was 11.2 %, ranging from 4.4 % (27 bp difference between *Colpomenia peregrina* and *Colpomenia claytoniae*) to 17.9 % (111 bp difference between *Colpomenia phaeodactyla* and *Colpomenia tuberculata*).

A total of 342 *atp*6 sequences (Table 1) were generated for a 611-bp alignment with 200 variable positions (34.4 %) and 122 parsimoniously informative positions (21.0 %). Within *C. peregrina*, the sequences differed by up to 2.4 % pairwise divergence and within the genus *Colpomenia* by 4.8 %, ranging from 28 bp differences between *C. peregrina* and *C. claytoniae* to 18.6 % (108 bp differences between *Colpomenia durvillei* and *C. tuberculata*) with 11.9 % average divergence.

Concatenation of *cox*3 and *atp*6 (1,231 bp) resulted in 69 haplotypes with 404 variable positions (32.8 %) and 251 positions (20.4 %) parsimoniously informative. Of the 69 haplotypes, 53 were privates, represented by a mutation of a single sequence and unique to a single population. Within *C. peregrina*, the sequences differed by up to 2.9 % pairwise divergence and within the genus *Colpomenia* by 4.6 % (55 bp differences between *C. peregrina* and *C. claytoniae*) to 18.2 % (218 bp differences between *C. phaeodactyla* and *C. tuberculata*) with 11.4 % average divergence.

A total of 10 haplotypes of RuBisCO spacer (549 bp) were generated from 38 specimens (Table 1). Consequently, fewer positions were variable (14.8 %) and parsimoniously informative (4.4 %), with up to 1.0 % pairwise divergence within *C. peregrina*. The pairwise divergence ranged from 8.2 % (43 bp difference between *C. peregrina* and *C. sinuosa*) to 10.4 % (55 bp difference between *C. peregrina* and *C. bullosa*) with 9.6 % average divergence.

Genetic diversity

Genetic diversity was considerably lower in the north Atlantic than in all other areas (Table 2). For example, haplotype diversity (*H*d) of *cox*3+*atp*6 sequences ranged from 0.92 in southern Korea to 0.00 in Spain and the eastern USA, whereas nucleotide diversity varied from 0.85 (northern Japan) to 0.00 (Spain and the eastern USA). Overall haplotype diversity was 0.93±0.07, and nucleotide diversity (π) was 0.86±0.03.

Haplotype network and divergence

The concatenated haplotype network revealed four groups (Figs. 2 and 3) identical to the ML analyses of haplotypes (Fig. 4). Group 1 formed a star-like network with the core haplotype (*h*1) shared by 56 individuals among nine populations in the NW Pacific and one population in the NE Pacific (see Fig. S1 for haplotype networks of *cox*3 and *atp*6 singly). Group 1 haplotypes (*h*1 to *h*42) occurred in the NW and NE Pacific: haplotypes *h*27 to *h*35 were private to the NE Pacific (Santa Catalina Islands, USA and Baja California, Mexico), with *h*27 as the core. Group 2 consisted of haplotypes *h*43 to *h*46 found in the NW Pacific (southern Korea and northern Japan) and the NE Pacific (Alaska and Oregon, USA). Nine haplotypes (*h*47 to *h*55) formed group 3 and were present in the NW Pacific (Korea, northern Japan, and Russia) and the North Atlantic. Group 4 consisted of 14 haplotypes (*h*56 to *h*69) from Australia, New Zealand, and western Korea in a star-like network with *h*56 at the center.

Haplotype networks for *cox*3 and *atp*6 were similar to one another, and each revealed the same four groups identified with the combined analysis (Fig. S1). A two-group pattern was revealed within the RuBisCO spacer network (Fig. S1). Groups 2 (NW and NE Pacific) and 4 (NW and SW Pacific) were resolved with the RuBisCO spacer network, but not groups 1 and 3, undoubtedly because an order of magnitude fewer individuals were used for the analysis.

Significant genetic concordance with biogeographic regions was found in *C. peregrina* (AMOVA, Table 3), and high

Table 1 Collection sites and haplotypes of mt *cox*3 and *atp*6 and cp RuBisCO spacer for *Colpomenia peregrina*

Sample	Collection location	*n cox*3	*cox*3 haplotypes	*n atp*6	*atp*6 haplotypes	*n cox*3+*atp*6	*cox*3+*atp*6 haplotypes	*n* RuBisCO spacer	RuBisCO spacer haplotypes
NW Pacific									
AninKR	Anin, Gangneung, Korea	30	c1(20), c2(1), c9(4), c21(2), c22(1), c25(2)	22	a1(11), a10(2), a25(1), a36(6), a39(2)	22	h1(10), h2(1), h20(2), h39(1), h40(4), h41(1), h44(1), h52(1), h54(1)	1	r9
ChonKR	Chongjin3ri, Pohang, Korea	5	c1(4), c26(1)	5	a1(4), a36(1)	5	h1(3), h42(1), h48(1)	1	r5
SuryKR	Suryeomri, Gyeongju, Korea	6	c1(3), c11(1), c13(2)	5	a2(3), a25(2)	5	h1(2), h3(1), h14(3)	1	r1
EuihKR	Euihangri, Taean, Korea	5	c1(1), c8(1), c9(3)	6	a9(3), a11(1), a12(1), a40(1)	5	h19(2), h23(1), h24(1), h55(1)	1	r4
DaecKR	Daecheon harbor, Boryeong, Korea	4	c22(2), c28(2)	2	a30(1), a40(1)	2	h56(1), h62(1)	1	r9
GyeoKR	Gyeokpo, Buan, Korea	21	c8(7), c9(4), c22(10)	21	a9(3), a11(1), a12(5), a13(1), a30(1), a36(6), a40(4)	21	h19(4), h22(1), h23(1), h24(5), h47(6), h55(4)	1	r4
SangKR	Sangjokam, Goseong, Korea	32	c1(4), c7(18), c12(9), c22(1)	32	a1(11), a3(2), a21(1), a23(15), a24(1), a36(1)	32	h1(3), h5(11), h6(1), h9(1), h23(1), h36(13), h38(1), h47(1)	2	r4, r6
DolsKR	Dolsando, Yeosu, Korea	24	c1(10), c3(1), c11(11), c12(2)	22	a1(12), a5(8), a6(1), a7(1)	22	h1(8), h3(9), h4(1), h5(2), h7(1), h8(1)	2	r1, r2
JeonKR	Jeongdori, Wando, Korea	13	c1(2), c6(2), c8(3), c9(4), c11(2)	12	a1(3), a9(3), a11(2), a13(2), a22(2)	12	h1(2), h3(2), h18(1), h19(3), h21(1), h23(1), h37(2)	1	r3
HaenKR	Haengwonri, Jeju, Korea	39	c1(29), c4(1), c5(4), c10(4), c11(1)	40	a1(29), a4(1), a8(1), a13(1), a14(7), a15(1)	39	h1(22), h3(1), h10(1), h11(1), h12(4), h13(1), h15(2), h16(1), h17(1), h25(1), h26(4)	3	r2
ShimJP	Shimane, Honshu, Japan	1	c1(1)	1	a1(1)	1	h1(1)	1	r2
HakoJP	Hakodate, Hokkaido, Japan	17	c1(4), c13(5), c14(7), c24(1)	16	a1(4), a25(3), a27(6), a36(3)	16	h1(4), h45(2), h46(3), h49(7)	1	r8
VladRU	Vladivostok, Russia	2	c24(1), c27(1)	1	a36(1)	1	h49(1)	1	r1
Subtotal		199		185		183		17	
SW Pacific									
BateAU	Batemans Bay, New South Wales, Australia	6	c28(3), c29(1), c30(1), c31(1)	6	a30(4), a31(1), a35(1)	6	h56(3), h60(1), h61(1), h66(1)	3	r9
MckeAU	Mckenzie Bay, Sydney, Australia	7	c28(4), c32(1), c34(1), c35(1)	7	a28(1), a29(4), a30(1), a32(1)	7	h56(3), h62(1), h67(1), h68(1), h69(1)	3	r9, r10
WellNZ	Wellington, New Zealand	11	c28(8), c36(1), c37(1), c38(1)	11	a30(6), a33(5)	11	h56(5), h57(4), h58(1), h59(1)	2	r9
OtagNZ	Otakou, Otago harbour, New Zealand	10	c28(6), c33(4)	10	a30(9), a34(1)	10	h56(5), h64(1), h65(4)	1	r9
Subtotal		34		34		34		9	
NE Pacific									
KruzUS	Kruzof Island, AK, USA	3	c13(3)	3	a25(3)	3	h44(3)	1	r7
SunsUS	Sunset Bay, OR, USA	1	c13(1)	1	a26(1)	1	h46(1)		
CataUS	Santa Catalina Island, CA, USA	24	c1(3), c15(16), c16(1), c17(2), c18(1), c20(1)	24	a1(18), a16(1), a18(1), a19(3), a20(1)	24	h1(2), h27(13), h28(1), h29(2), h30(1), h31(3), h34(1), h35(1)	4	r3, r6
SonoMX	Sonora, Mexico	3	c15(2), c19(1)	3	a17(3)	3	h32(1), h33(2)	2	r1, r3

Table 1 (continued)

Sample	Collection location	*n cox*3	*cox*3 haplotypes	*n atp6*	*atp6* haplotypes	*n cox*3+*atp6*	*cox*3+*atp6* haplotypes	*n* RuBisCO spacer	RuBisCO spacer haplotypes
Total		31		31		31		7	
Atlantic									
BatzFR	Ile de Batz, Roscoff, France	22	c22(2), c24(20)	23	a36(20), a38(3)	22	h47(2), h49(17), h50(3)	1	r1
OnaNO	Ona, More og Romsdal, Norway	5	c22(4), c25(1)	5	a36(5)	5	h47(4), h52(1)	1	r1
KinvIR	Kinvarra, Co.Clare, Ireland	2	c24(2)	2	a38(2)	2	h50(2)		
PlymUK	Plymouth, Devon, United Kingdom	3	c23(1), c24(2)	2	a36(2)	2	h49(1), h51(1)	1	r4
CastSP	Castillo San Antonio (La Coruna), Spain	16	c24(16)	17	a36(17)	16	h49(16)	1	r3
BootUS	East Booth Bay, ME, USA	20	c25(20)	20	a36(20)	20	h52(20)	1	r1
ApplUS	Appledore Island, ME, USA	27	c25(27)	23	a3621)(, a37(2)	23	h52(21), h53(2)		
Subtotal		95		92		90		5	
Total		359		342		338		38	

n number of individuals examined

genetic structure was detected among sites in the combined data (F_{ST}=0.772, p<0.0001), suggesting the occurrence of different genetic entities or taxonomic units. A significant level of genetic structure among regions was also detected (F_{CT}= 0.562, p<0.0001).

Phylogeny of *Colpomenia peregrina*

The ML tree of the 69 concatenated mt DNA haplotypes resolved four major groups within *C. peregrina*, with the NW and NE Pacific (group 2) basal to the other three (Fig. 4). The individual *cox*3 and *atp*6 phylogeny (Fig. S2) generally agreed with those of four major groupings. *C. peregrina* likely originated in the NW Pacific Ocean, as populations from the NW Pacific occurred in each of the other groups. The NE Pacific populations occurred in two groups (groups 1 and 2), and the north Atlantic and SW Pacific populations each occurred in only one group, but were mixed with NW Pacific populations (groups 3 and 4, respectively) (Figs. 2 and 3).

Historical demography

The mismatch distribution was multimodal for the NW Pacific, reflecting a low frequency of highly divergent haplotypes, but unimodal for the NE and SW Pacific (Fig. 5). The mismatch distributions for the SW Pacific closely aligned with the expected pattern under the sudden expansion model, as both. Tajima's *D* and Fu's *F*s were significantly negative (P<0.05), indicating a recent population demographic expansion.

The generalized skyline plot suggested a growth expansion of NW Pacific populations (the origin center of *C. peregrina*) beginning ca. 50 kya, several glacial/interglacial cycles prior to the Last Glacial Maximum (LGM), 30–22 kya (Bradwell et al. 2008), with a dramatic acceleration around 20 kya (Fig. 6).

Discussion

Genetic diversity and historical demography

The NW Pacific was the center of mtDNA haplotype diversity for *Colpomenia peregrina*, and the haplotype network strongly also suggested that the area was the center of origin with subsequent pre-LGM introductions to the SW Pacific, NE Pacific, and the north Atlantic over a wide span of time. Haplotype data also revealed that NE Atlantic populations were the source of a post-LGM introduction to the NW Atlantic. Thus, *C. peregrina* ranks with *U. pinnatifida* (introduced to SW Pacific, South and North America, and Europe; Voisin et al. 2005; Uwai et al. 2006) and

Table 2 Diversity measures of *cox*3, *atp*6, and combined datasets for *Colpomenia peregrina*. Populations with <10 samples were eliminated to make population size more homogeneous

Region	*cox*3					*atp*6					*cox*3+*atp*6				
	n	*h*	*S*	*H*d	π	*n*	*h*	*S*	*H*d	π	*n*	*h*	*S*	*H*d	π
NW Pacific															
AninKR	22	6	12	0.54±0.12	0.37±0.10	22	5	12	0.69±0.08	0.63±0.07	22	9	24	0.78±0.08	0.50±0.08
GyeoKR	21	3	7	0.66±0.06	0.53±0.03	21	7	10	0.85±0.05	0.62±0.04	21	6	17	0.85±0.05	0.57±0.03
SangKR	32	4	13	0.64±0.07	0.55±0.06	32	6	18	0.68±0.06	0.72±0.07	32	8	31	0.81±0.06	0.63±0.06
DolsKR	22	4	3	0.65±0.06	0.13±0.02	22	4	4	0.59±0.07	0.13±0.03	22	6	7	0.87±0.04	0.13±0.02
JeonKR	12	5	8	0.85±0.07	0.50±0.09	12	5	8	0.83±0.07	0.43±0.01	12	7	16	0.92±0.05	0.47±0.10
HaenKR	39	5	4	0.44±0.09	0.08±0.02	39	6	5	0.47±0.09	0.09±0.02	39	11	9	0.62±0.09	0.09±0.02
HakoJP	16	4	20	0.74±0.06	1.01±0.25	16	4	9	0.78±0.06	0.60±0.09	16	4	29	0.79±0.06	0.85±0.15
Subtotal	164	17	33	0.82±0.03	0.78±0.08	164	22	29	0.81±0.03	0.69±0.05	164	42	64	0.89±0.02	0.68±0.05
SW Pacific															
WellNZ	11	4	5	0.49±0.18	0.15±0.07	11	2	1	0.55±0.07	0.09±0.01	11	4	6	0.67±0.12	0.12±0.05
OtagNZ	10	2	1	0.53±0.10	0.09±0.02	10	2	2	0.20±0.15	0.07±0.05	10	3	2	0.64±0.10	0.06±0.01
Subtotal	21	5	6	0.54±0.11	0.13±0.04	21	3	2	0.45±0.11	0.09±0.02	21	6	8	0.73±0.08	0.11±0.03
NE Pacific															
CataUS	23	6	6	0.52±0.12	0.13±0.04	24	5	6	0.44±0.12	0.13±0.04	24	8	12	0.70±0.10	0.13±0.03
Atlantic															
BatzFR	22	2	2	0.17±0.10	0.06±0.03	23	2	1	0.28±0.11	0.12±0.08	21	3	3	0.41±0.12	0.05±0.02
CastSP	16	1	0	0	0	17	1	0	0	0	16	1	0	0	0
BootUS	20	1	0	0	0	20	1	0	0	0	20	1	0	0	0
ApplUS	27	1	0	0	0	23	2	1	0.17±0.10	0.03±0.02	23	2	1	0.09±0.08	0
Subtotal	85	4	5	0.57±0.02	0.24±0.01	83	3	2	0.15±0.05	0.02±0.01	80	6	7	0.63±0.03	0.13±0.01
Total	359	38	56	0.90±0.01 (mean)	0.99±0.04 (mean)	342	40	45	0.82±0.02 (mean)	0.74±0.02 (mean)	338	69	99	0.93±0.07 (mean)	0.86±0.03 (mean)

n number of individuals, *h* number of haplotypes, *S* number of polymorphic sites, *H*d haplotype diversity, π nucleotide diversity

Sargassum muticum (introduced to Europe and NE Pacific; Cheang et al. 2010; Bae et al. 2013) as among the most invasive of brown macroalgae.

Although a molecular clock for marine algae remains elusive, evolutionary rates of the *cox*1 gene for a variety of organisms (1.5–3.5 % per My; Papadopoulou et al. 2010) suggest that the high pairwise divergence of *cox*3 (3.1 %) within *C. peregrina* likely evolved before the Pleistocene (>2.5 Ma). Furthermore, application of a brown algal chronogram constructed by Silberfeld et al. (2010) suggested that *C. peregrina* diverged 7.4–8.6 Ma during the Tortonian Age of the late Miocene (7.2–11.6 Ma). The 10 mutation steps separating NW Pacific haplotypes *h*1 and *h*36/*h*41 within group 1 and the 24, 16, and 27 mutation steps separating *h*1 from NW Pacific haplotypes *h*44 (group 2), *h*47 (group 3), and *h*56 (group 4) collectively suggested a pre-LGM expansion within the NW Pacific, supported also by the multimodal mismatch distribution and the skyline plot (ca. 20 kya). A pre-LGM expansion has also been demonstrated for other marine algae: *Palmaria palmata* (Provan et al. 2005b), *F. serratus* (Hoarau et al. 2007), and *F. vesiculosus* (Coyer et al. 2011a).

NW Pacific

The high haplotype diversity, numerous private haplotypes, and star-shaped haplotype network all suggest a rapid colonization sweep of *C. peregrina* in the NW Pacific. More specifically, the center of diversity for *C. peregrina* may lie along the west and south coasts of Korea, where extensive sampling of several populations revealed numerous private haplotypes. Many offshore islands (created as sea levels rose during the transition from glacial to interglacial periods) along the west and south coasts create a complex oceanographic environment and, concomitantly, diverse habitats that may have fostered the observed genetic diversification. Other intertidal algae, e.g., *Gelidium elegans* and *Ishige okamurae*, also have been shown to exhibit high genetic diversity (*cox*1 and *cox*3, respectively) on the west and south coasts (Kim et al. 2012;

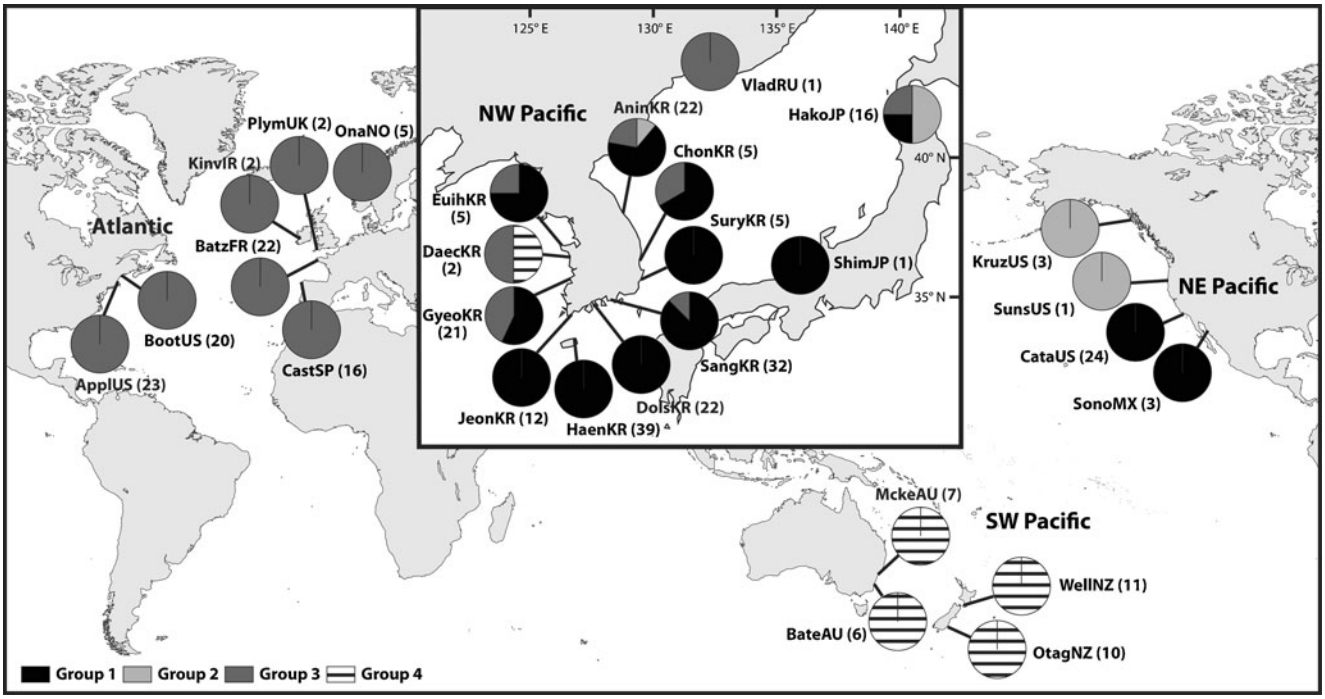


Fig. 2 Geographic distributions combined mtDNA haplotypes for *Colpomenia peregrina* analyzed in the present study. Each *color* represents different groups

Lee et al. 2012). Although areas outside the NW Pacific also displayed high haplotype diversity for *C. peregrina* (Table 2), phylogenetic analysis (Fig. 4) revealed that all four groups included populations from the NW Pacific, again suggesting this area as a putative center of origin for the species.

NE Pacific

The grouping of the NE Pacific populations with the NW Pacific in groups 1 and 2 and shared haplotypes (*cox*3, c1; *atp*6, a1) with NW Pacific populations (Fig. 2; Table 1) suggested multiple introductions to the NE Pacific from Korea and/or Japan. In addition, *h*1 from Santa Catalina Island (California, USA) and *h*44 from Alaska (USA) were found in Korea and Japan (Table 1). A total of 29 species of marine algae, including *C. peregrina* in the present study, have been introduced to the southern California/Baja California, Mexico region, many within the last century (reviewed in Miller et al. 2011), and *Sargassum filicinum* has been rapidly extending southward (Riosmena-Rodríguez et al. 2012).

The relatively high haplotype diversity in NE Pacific (*H*d=0.70; see Table 2) may result from multiple introductions from the NW Pacific. The long-held hypothesis of low genetic diversity in introduced populations due to founder effects and bottlenecks has been modified by the recovery of high genetic diversity in introduced populations due to multiple introductions (Dlugosch and Parker 2008; Wilson et al. 2009; Pérez-Portela et al. 2012). For example, for *Gracilaria vermiculophylla*, a species native to the NW Pacific, a large number of haplotypes were reported in an introduced population in the NW Atlantic (Virginia coast, USA) (Gulbransen et al. 2012).

SW Pacific

Colpomenia peregrina was reported in Australia in 1967 and in New Zealand in 1982 (Womersley 1967; Parsons 1982), but its native or non-native status has not been evaluated prior to this study. Our analyses suggest that the SW Pacific populations stemmed from the NW Pacific. Specifically, the haplotype network for the combined genes revealed a similar pattern for the SW Pacific (group 4) and NW Pacific/NE Pacific (group 1) in that both were primarily star-shaped, yet 26 mutational steps separated the two groups. In the cp RuBisCO spacer, haplotype *r*9 was shared between the SW and NW Pacific, with a private *r*10 haplotype in the SW Pacific. The most parsimonious explanation is that the SW Pacific represents a very old introduction or expansion from the NW Pacific, with a subsequent rapid colonizing sweep throughout the region. The alternative explanation of the SW Pacific being the center of origin with subsequent spread to other areas is unlikely, given the derived position of the SW Pacific populations in the phylogenetic analysis, unimodal mismatch distribution, and generally lower haplotype and nucleotide diversities

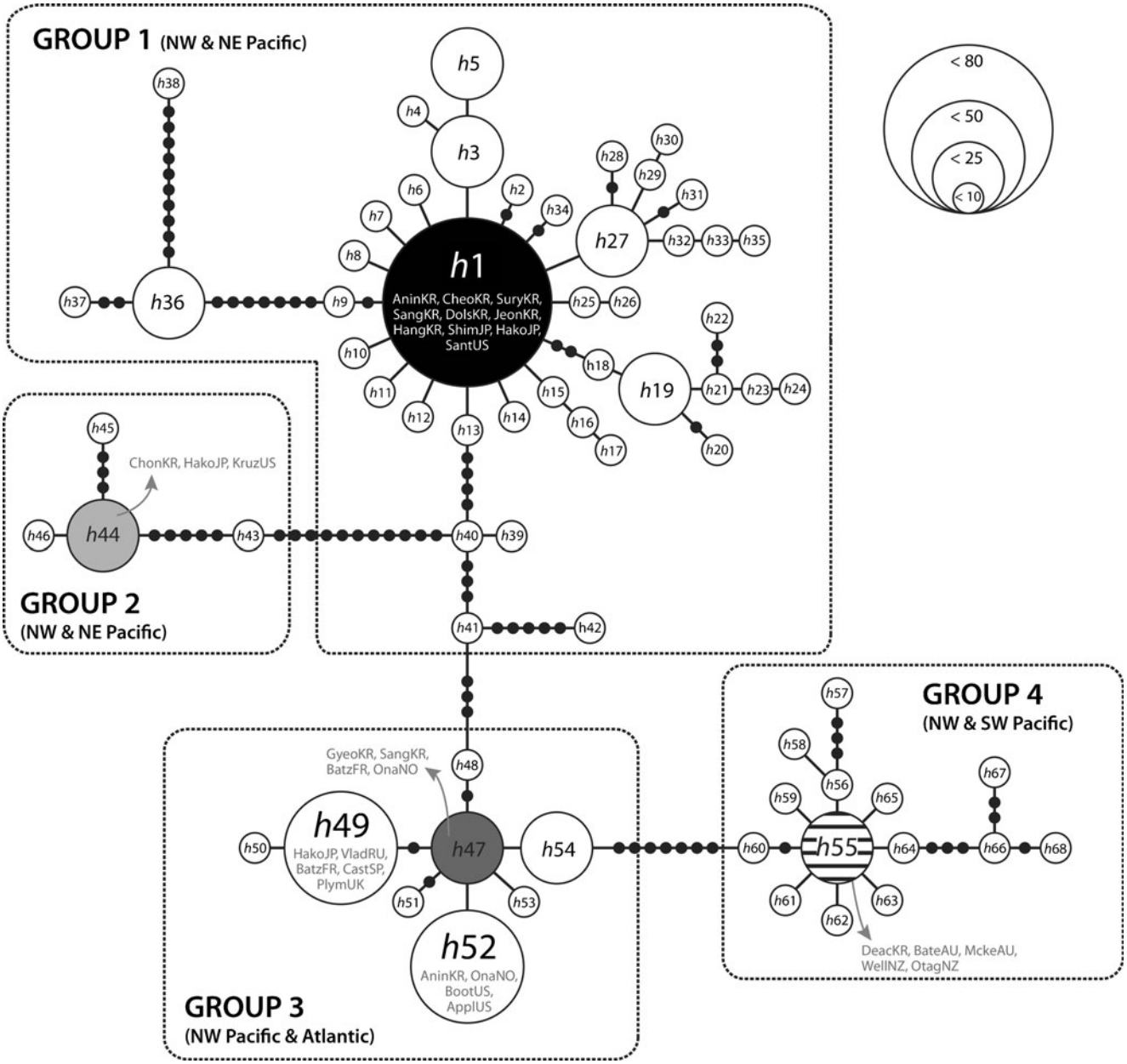
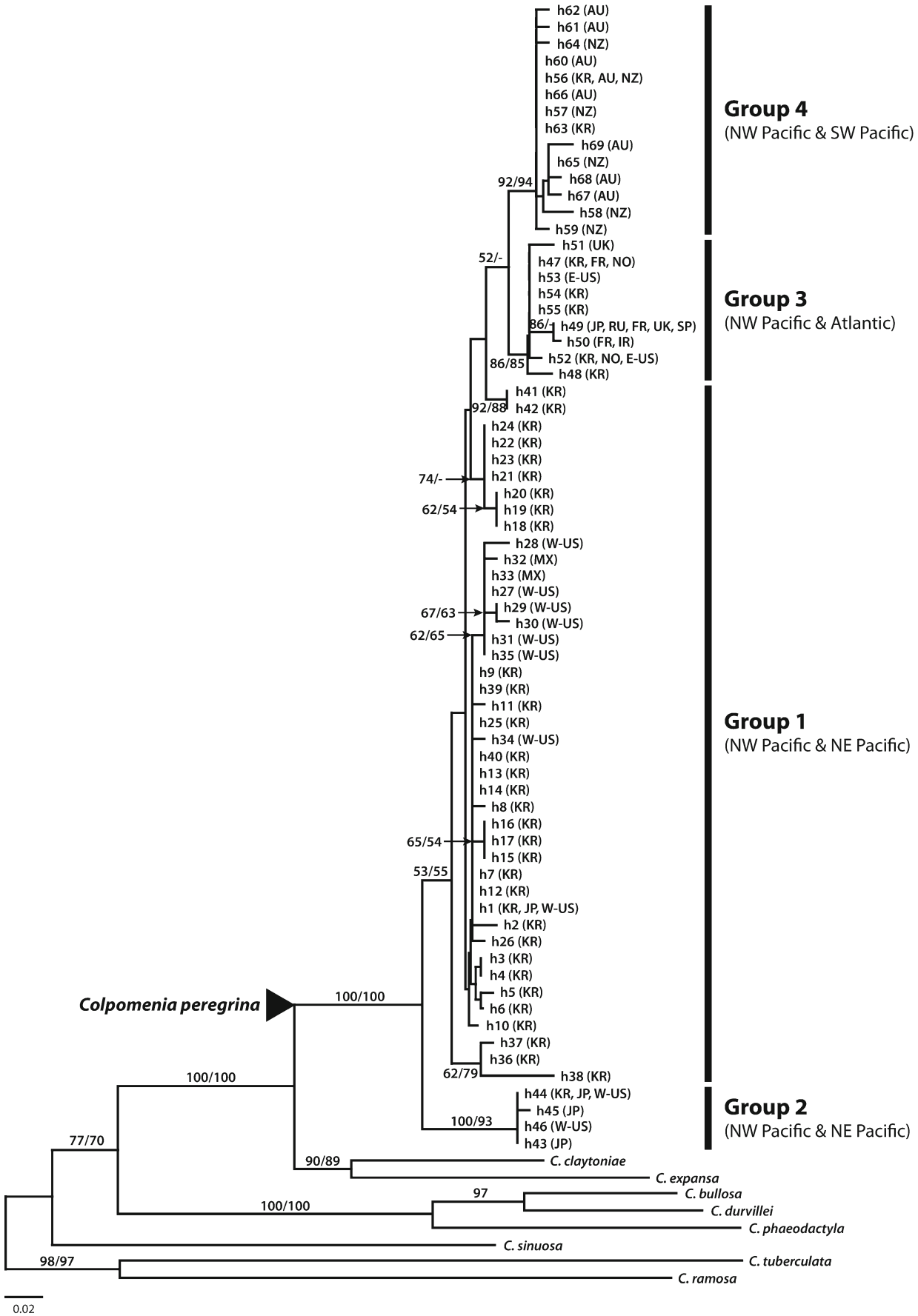


Fig. 3 Statistical parsimony network for 69 combined mtDNA haplotypes for *Colpomenia peregrina.* Each *circle* represents a haplotype, and circle size is proportional to haplotype frequency. *Dashed lines* delineate the four main lineage clusters. *Lines* between haplotypes are single mutational steps; *small closed circles* indicate missing haplotypes (either extinct or not sampled)

than that found in NW Pacific. Thus, the "native" or "non-native" status of *C. peregrina* in the SW Pacific may depend upon the temporal definition. Both *C. bullosa* and *C. claytoniae* are regarded as introduced species in the SW Pacific (Kain et al. 2010; Boo et al. 2011b).

NE Atlantic

The shared core haplotype between the NW Pacific and the NE Atlantic (*h*47 in group 3), as well as the unimodal mismatch distribution and generally low genetic diversities in the NE Atlantic, strongly suggested that *C. peregrina* was introduced to the NE Atlantic from the NW Pacific. Populations have since expanded throughout the NE Atlantic, ranging from Ireland, Norway, and Sweden to Portugal (Minchin 1991), at a rate of 58 km y^{-1} (Mineur et al. 2010). Introduction and a rapid sweep throughout Europe within the last 100 years are reflected in the star-shaped network of group 3 composed of few haplotypes separated by only one or two mutational steps. Multiple trans-Arctic introductions from Asia to Europe are also


h62 (AU)
h61 (AU)
h64 (NZ)
h60 (AU)
h56 (KR, AU, NZ)
h66 (AU)
h57 (NZ)
h63 (KR)
h69 (AU)
h65 (NZ)
h68 (AU)
h67 (AU)
h58 (NZ)
h59 (NZ)
92/94
Group 4
(NW Pacific & SW Pacific)
h51 (UK)
52/-
h47 (KR, FR, NO)
h53 (E-US)
h54 (KR)
h55 (KR)
86/-
h49 (JP, RU, FR, UK, SP)
h50 (FR, IR)
86/85
h52 (KR, NO, E-US)
h48 (KR)
Group 3
(NW Pacific & Atlantic)
h41 (KR)
92/88
h42 (KR)
h24 (KR)
h22 (KR)
h23 (KR)
74/-
h21 (KR)
h20 (KR)
62/54
h19 (KR)
h18 (KR)
h28 (W-US)
h32 (MX)
h33 (MX)
h27 (W-US)
67/63
h29 (W-US)
h30 (W-US)
62/65
h31 (W-US)
h35 (W-US)
h9 (KR)
h39 (KR)
h11 (KR)
h25 (KR)
h34 (W-US)
h40 (KR)
h13 (KR)
h14 (KR)
h8 (KR)
h16 (KR)
65/54
h17 (KR)
h15 (KR)
53/55
h7 (KR)
h12 (KR)
h1 (KR, JP, W-US)
h2 (KR)
h26 (KR)
h3 (KR)
h4 (KR)
h5 (KR)
h6 (KR)
h10 (KR)
h37 (KR)
h36 (KR)
62/79
h38 (KR)
Group 1
(NW Pacific & NE Pacific)
Colpomenia peregrina
100/100
100/100
h44 (KR, JP, W-US)
h45 (JP)
100/93
h46 (W-US)
h43 (JP)
Group 2
(NW Pacific & NE Pacific)
77/70
90/89
C. claytoniae
C. expansa
97
C. bullosa
100/100
C. durvillei
C. phaeodactyla
C. sinuosa
98/97
C. tuberculata
C. ramosa
0.02

◀ **Fig. 4** Maximum likelihood tree of mtDNA haplotypes based on combined (*cox*3+*atp*6) data rooted with eight *Colpomenia* species. Each clade is labeled as groups 1–4

reported for *Codium fragile* (Provan et al. 2005a), *F. distichus* (Coyer et al. 2011b), *Gracilaria vermiculophylla* (Kim et al. 2010), *Grateloupia turuturu* (Gavio and Fredericq 2002), *Heterosiphonia japonica* (Sjøtun et al. 2008), *Neosiphonia harveyi* (McIvor et al. 2001), and *U. pinnatifida* (Uwai et al. 2006).

NW Atlantic

The first report of *C. peregrina* in the NW Atlantic was from Nova Scotia in 1960 (Blackler 1964; Bird and Edelstein 1978), and the species subsequently expanded south to the Gulf of Maine and north to the Strait of Belle Isle, Labrador/Newfoundland (Kennedy et al. 2010). An earlier hypothesis of a European origin (Blackler 1964; Bird and Edelstein 1978; Mathieson et al. 2008) was supported by our network analysis, which suggested that the introduction stemmed from northern Europe, because the NW Atlantic haplotypes were one mutation step from the core *h*47 haplotype found in NW Pacific and NE Atlantic (Fig. 3).

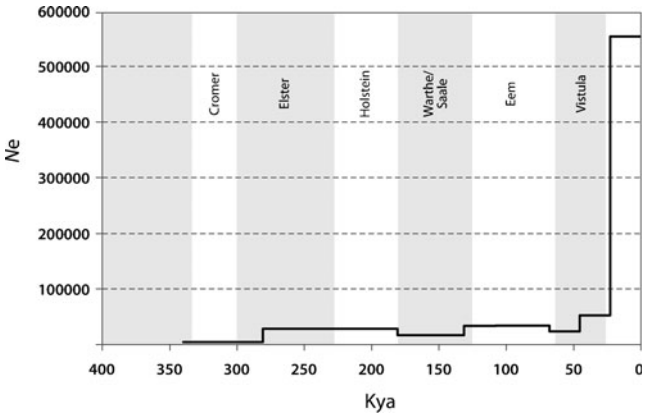


Fig. 6 Generalized skyline plot of population growth through time for NW Pacific populations, the putative center of origin for *Colpomenia peregrina*, based on *cox*3 sequences. The *x*-axis represents time before present (thousand years ago) and the *y*-axis (log) the estimated effective population size (*Ne*). Glacial (gray) and interglacial (white) periods are indicated

Dispersal

Colpomenia peregrina has broad physiological tolerance for desiccation, salinity (15–30 pfu), temperature (13–20 °C), and light (both long and short day) (Oates 1985; Vandermeulen 1986). Consequently, *C. peregrina* has the

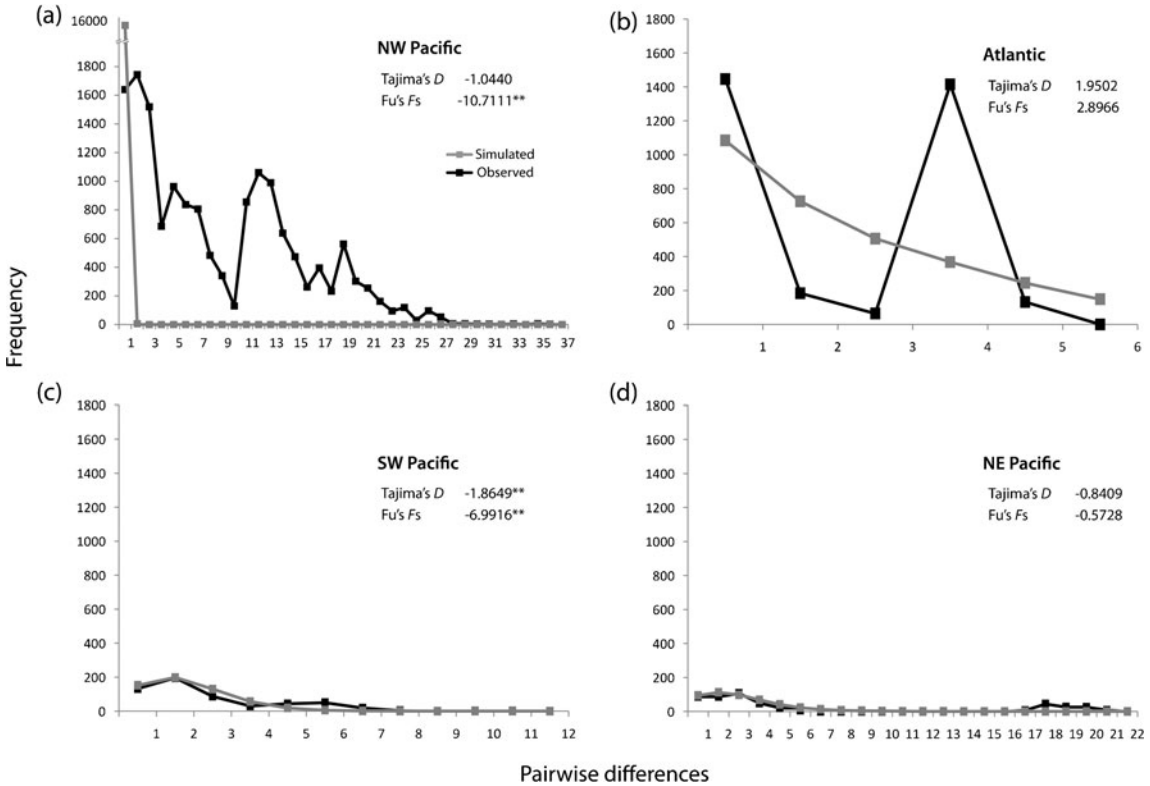


Fig. 5 The observed pairwise difference (*gray lines*) and the expected mismatch distributions under the sudden expansion model (*black lines*) of concatenated (*cox*3+*atp*6) dataset for *Colpomenia peregrina*

capacity to survive an introduction to areas that differ markedly from the source location.

Perhaps related to the physiological plasticity, *C. peregrina* displays considerable variation in morphology and habitat utilization throughout its worldwide distribution, neither of which correlated with mtDNA sequence variability in our study. For example, *C. peregrina* from the UK and New Zealand is epiphytic or rarely epilithic (Parsons 1982; Fletcher 1987), whereas in Australia, it is attached directly to intertidal or shallow subtidal substrates (Boo et al. 2011b). Morphology in Nova Scotia (NW Atlantic) resembles that of European specimens, and individuals are mostly epiphytic in the subtidal zone and very large (up to 21 cm diameter) (Bird and Edelstein 1978; Boo et al. 2011b). In France, epiphytic *C. peregrina* is characterized by larger thalli (up to 35 cm diameter) (Hamel 1937) than the epiphytic forms in Nova Scotia (up to 21 cm diameter) and Korea/Japan (up to 9 cm diameter) (Yoshida 1998; Boo 2010). Indeed, morphology and ecology of the European specimens (NE Atlantic; Fig. 1b) are more similar to *C. claytoniae* (Boo et al. 2011b), although the latter is primarily epilithic in deeper subtidal habitats off Korea (Boo et al. 2011b). In the Gulf of Maine (NW Atlantic), however, intertidal forms are epiphytic on turf algae and small in size (up to 10 cm diameter) (Fig. 1d). The relationships among genetic differentiation, physiological/ecological plasticity, and native vs. introduced are intriguing and warrant more detailed study.

Epiphytic characteristics may be an important factor in dispersal, since the saccate thalli of *C. peregrina* are often epiphytic on floating or drift macroalgae such as *Sargassum muticum* and many other brown and red algae (Parsons 1982; Boo 2010). Storms or other disturbances occurring when epiphytic *C. peregrina* is reproductive could potentially produce large quantities of drift algae and a "pulse" of *C. peregrina* gametes/spores into new regions. Consequently, dispersal could occur over vast areas in a matter of years via a "stepping stone" model.

An important anthropogenic vector for macroalgae, as well as invertebrates, is the transport of brood stock or shells for oyster mariculture (Haydar and Wolff 2011). Oyster shells have been implicated in the introduction of *C. peregrina* to France (Blackler 1964) via either attached saccate gametophytes or crustose sporophytes from Japan and/or British Columbia (Farnham 1980; Fletcher 1987). Historically, *C. peregrina* has been reported in Vannes, France in 1905 (Lund 1945), Britain in 1908 (Cotton 1908), and Ireland in 1934 (Lund 1945). The secondary introduction from Europe (NE Atlantic) to Nova Scotia (NW Atlantic) also was likely mediated by oyster mariculture (Blackler 1964; Mathieson et al. 2008). Several other algal species have been introduced via oyster mariculture from Japan to the NE Pacific, including *Grateloupia lanceolata*, *Sargassum muticum*, *Sargassum filicinum*, and *Cutleria cylindrica* (see Miller et al. 2011).

Because there are no reports of the transport of macroalgae on oyster shells to Australia and New Zealand (Parsons 1982; Uwai et al. 2006), fishing vessels may be important vectors. For example, Korean fishing vessels were identified as the likely vector for multiple introductions of *U. pinnatifida* to New Zealand (Uwai et al. 2006). Vessel hulls may harbor small sporophytes of *C. peregrina*, as well as attached crustose gametophytes, as documented for other introduced macroalgae by Hewitt et al. (2007).

Conclusions

Our study of two protein-coding mitochondrial genes suggested that the NW Pacific is the center of origin for *C. peregrina.* From here, it expanded several ice ages ago to the SW Pacific, later and perhaps more than once to the NE Pacific, and then to the NE Atlantic, which served as a source for an anthropogenic introduction to the NW Atlantic within the past century. Thus, *C. peregrina* joins the ever-growing list of brown algae that have dispersed well beyond their native range through anthropogenic means (see Cheang et al. 2010; Miller et al. 2011). The cosmopolitan distribution of *C. peregrina* further affords numerous opportunities to examine specific relationships between ecological and evolutionary processes, given the widely different times and means of introduction to each area.

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