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# EVOLUTION OF *MACROCYSTIS* SPP. (PHAEOPHYCEAE) AS DETERMINED BY ITS1 AND ITS2 SEQUENCES[1,*]

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***Macrocystis* (Lessoniaceae) displays an antitropical distribution, occurring in temperate subtidal regions along western North America in the northern hemisphere and throughout the southern hemisphere. We used the noncoding rDNA internal transcribed spacer regions (ITS1 and ITS2) to examine relatedness among (1) *Macrocystis* and several genera of Laminariales, (2) four species of *Macrocystis* (*M. integrifolia* Bory from the northern hemisphere, *M. angustifolia* Bory and *M. laevis* Hay from the southern hemisphere, and *M. pyrifera* [L.] C. Ag. from both hemispheres), and (3) multiple clones of several individuals. Of the taxa included in our phylogenetic analysis, the elk kelp, *Pelagophycus porra* (Lem.) Setch., was the sister taxon to *Macrocystis* spp. *Macrocystis* individuals from the southern hemisphere (representing three species) formed a strongly to moderately supported clade, respectively, when the ITS1 and ITS2 sequences were analyzed separately. No distinction was detected between the two species in the northern hemisphere. Thus, *Macrocystis* may be a monospecific genus (*M. pyrifera*). A northern-hemisphere-to-southern-hemisphere pattern of dispersal was inferred, because northern-hemisphere individuals were more diverse and displayed paraphyletic clades, whereas southern-hemisphere individuals were less diverse and formed a monophyletic clade. High intraindividual variation in ITS1 sequences was observed in one individual from Santa Catalina Island (CA), suggesting very recent and rapid mixing of genotypes from areas to the north and Baja California (Mexico) or introgressive hybridization with *Pelagophycus*.**

***Key index words:* *Alaria*; antitropical distribution; biogeography; *Costaria*; evolution; ITS; kelp; *Laminaria*; *Macrocystis*; *Nereocystis*; *Pelagophycus*; species concepts**

***Abbreviations:* CIA, chloroform isoamyl alcohol; CTAB, cetyltrimethyl ammonium bromide; CI/RC, consistency index/rescored consistency index; ITS, internal transcribed spacer; MPT, most parsimonius tree; $NH_4Ac$, ammonium acetate; ts/tv, transition/transversion**

Large brown algae, collectively known as kelps (Phaeophyceae, Laminariales), dominate near-shore, subtidal, rocky habitats in temperate seas throughout the world. The giant kelp, *Macrocystis*, is an ecologically and economically important genus because of its large size (up to 30 m), high abundance, and high productivity (see reviews by Foster and Schiel 1985, North 1994). First described in the 1600s from drifting fragments, 17 species of *Macrocystis* were combined into the single taxon *M. pyrifera* (L.) C. Ag. in the mid-1800s (Hooker 1847). Subsequently, *M. integrifolia* Bory and *M. angustifolia* Bory were recognized once again as separate species, distinguished from each other and from *M. pyrifera* by holdfast morphology (Howe 1914, Womersley 1954). A fourth species, *M. laevis* Hay (Hay 1986), was described on the basis of blade smoothness, although the validity of a species designation using the single character of blade morphology has been questioned (van Tussenbroek 1989).

The three widely recognized species, however, are not reproductively isolated. Crossing experiments in the laboratory demonstrated successful hybridization (production of normal and/or fertile sporophytes) between essentially all combinations of *M. pyrifera*, *M. integrifolia*, and *M. angustifolia* collected from several locations in the northern and southern hemispheres (Lewis et al. 1986, Lewis and Neushul 1994). Thus, up to four species can be recognized by the morphological species concept, but only one can be recognized by the biological species concept.

*Macrocystis* is a good colonizer. Float-bearing fronds emerge from the perennial holdfast, and both detached fronds and entire individuals can release spores while drifting (reviewed in Dayton 1985, Schiel and Foster 1986, Santelices 1990). Additionally, spores and germlings can remain competent in the water column for several days and dormant on the substratum for many months (Reed et al. 1992, tom Dieck 1993, Ladah et al. 1999). Therefore, the colonizing ability

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of *Macrocystis* is an important aspect of its geographical dispersal.

Two species of *Macrocystis* exhibit an antitropical distribution, whereas the other two are restricted to the southern hemisphere (Foster and Schiel 1985). The most abundant and widely dispersed species, *M. pyrifera*, is found off every major land mass and most oceanic islands in the southern hemisphere and between central Baja California (Mexico) and Monterey Bay (California) along western North America in the northern hemisphere. Recently, a few Alaskan populations were described (Gabrielson et al. 2000). In contrast, *M. integrifolia* occurs only along the Peruvian–northern Chilean coasts in the southern hemisphere and between Monterey Bay and southeastern Alaska in the northern hemisphere. *Macrocystis angustifolia* is found only off South Africa and south Australia; a southern California population was determined on the basis of morphology to be a subspecies of *M. pyrifera* (Neushul 1971, Brostoff 1977). Marion Island (south of South Africa) is the only location for *M. laevis*.

The antitropical distribution of *Macrocystis* has been attributed to an origin in one hemisphere, followed by a breaching of the tropical barrier during a cool period and subsequent colonization of the other hemisphere. A northern hemisphere center of origin has been hypothesized based on biogeography of extant kelps in the north Pacific, paleoclimatic records, and fossil records of certain obligate or facultative kelp-associated mollusks (Nicholson 1978, Estes and Steinberg 1988, Lüning 1990, Lüning and tom Dieck 1990). On the other hand, a southern hemisphere origin has been proposed because of the much more widespread distribution of the genus in the southern hemisphere (North 1971). A process of vicariant differentiation out of a Pacific Ocean/Southern Ocean ancestral complex also has been advanced (Chin et al. 1991), although other work concludes that vicariant events cannot explain antitropical distributions in the eastern Pacific Ocean (Lindberg 1991).

DNA sequence data commonly are used to resolve questions of relatedness in the kelps. In the Phaeophyceae, 18S rDNA sequences resolved ordinal relationships (Tan and Druehl 1996) but were too conserved to distinguish relationships between the Alariaceae, Laminariaceae, and Lessoniaceae (Saunders and Druehl 1992). In contrast, a phylogeny of species within the Alariaceae, Laminariaceae, and Lessoniaceae using 3′18S–internal transcribed spacer (ITS)1–5.8S sequences differed substantially from the classical phylogeny based on morphology and provided new insight into the taxonomic relationships (Saunders and Druehl 1993a, Druehl et al. 1997).

The present study examined ITS1 and ITS2 sequences from *Macrocystis* and other kelps. Individuals of *Macrocystis* were collected from the northern and southern hemispheres and represented the four morphological species. Phylogenetic analyses were conducted to infer 1) relationships among selected genera of the Laminariales; 2) relationships between *M. pyrifera*, *M. integrifolia*, *M. angustifolia*, and *M. laevis*; and 3) directionality and timing of interhemisphere dispersal of *Macrocystis*.

## MATERIALS AND METHODS

*Sample locations.* All samples were collected from attached (not beached or drift) individuals (Table 1, Fig. 1). Fresh meristematic samples of *M. pyrifera* were collected from Monterey Bay, Refugio Beach, Santa Catalina Island, and Anacapa Island, and fresh *M. integrifolia* was collected from Stillwater Cove. A single sample of *Pelagophycus porra* (Lem.) Setch. also was collected. For all other *Macrocystis* samples and the single sample of *Nereocystis leutkeana* (Mert.) Post. & Rupr., 2 to 3 g of fresh meristematic tissue were excised, blotted dry, sealed in plastic bags with silica crystals, and transported to the laboratory. Individuals were collected within an area of about 50 m, except for the Chilean samples, which were separated by about 200 km.

*DNA extraction and purification.* Tissue (2–3 g fresh or 0.4–0.8 g dried) was frozen with liquid nitrogen and ground to a powder with mortar and pestle. DNA was extracted from the powdered tissue using cetyltrimethyl ammonium bromide (CTAB), followed by separation with chloroform-isoamyl alcohol (CIA), standard precipitation with ethyl alcohol, and purification over a CsCl gradient as described previously (Coyer et al. 1994).

*PCR amplification, cloning, and sequencing.* Double-strand DNA amplification of the ITS1 and ITS2 regions in all individuals was conducted using 1 ng of CsCl-purified DNA. The PCR amplifications for the ITS1 region used 1 μM of forward primer ITS5 (5′ cgtttcctgcagcGGAAGTAAAAGTCGTAACAA 3′) complementary to the 18S gene and reverse primer ITS2 (5′ctctagaactag tcGCTGCGTTCTTCATCGATGC3′) complementary to the 5.8S gene (White et al. 1990). Each primer was constructed with a *Pst*I/*Spe*I linker at the 5′ end (lower case bases). Amplification of the ITS2 region used 1 μM of primer P5 (5′cgtttcctg cagcGCATCGATGAAGAACGCAG3′) complementary to the 5.8S gene (positions 530 to 548; Saunders and Druehl 1993b) and primer G4 (5′ctctagaactagtcCTTTTCCTCCGCTTATTGAT ATG3′) complementary to the large subunit (positions 42 to 64; Baroin et al. 1988). Again, each primer was constructed with a *Pst*I/*Spe*I linker at the 5′ end (lower case). Amplification reactions were performed in a thermal cycler with an initial stage of 82° C for 10 min and 94° C for 5 min (2.5 U *Taq* polymerase added during last 2 min at 94° C), followed by 35 cycles of denaturation at 94° C for 1.5 min, annealing at 37° C for 1.5 min, and extension at 72° C for 3 min.

Amplification products were precipitated with 0.2 M $NH_4Ac$/ethanol and digested with the restriction enzymes *Pst*I and *Spe*I according to the manufacturer's instructions (New England Biolabs, Beverly, MA). Restriction products were purified from a 1.5% agarose gel (Heery et al. 1990), precipitated with 0.2 M $NH_4Ac$/ethanol, ligated to pBluescript II KS (+/−) phagemid vector (Stratagene, La Jolla, CA) previously digested with *Pst*I/*Spe*I, and transformed into the SURE strain of *Escherichia coli* (Stratagene) via electroporation. Some samples were cloned using the pGEM-T vector system according to the manufacturer's instructions (Promega, Madison, WI). Insert-containing plasmids were isolated from overnight bacterial cultures (Quantum Prep, BioRad, Hercules, CA).

Nucleotide sequences were determined (both directions) by an ABI 310 or 374 automated sequencer (PE Biosystems, Foster City, CA). Sequencing dyes (fluorescent dideoxynucleotides) were incorporated by adding 1 ng of purified ITS1 or ITS2 DNA to a 20-μL PCR reaction containing T-mix (PE Biosystems) and 1 μM of either T3 primer (forward direction) or T7 primer (reverse direction) according to the manufacturers protocol (PE Biosystems).

*Alignment and properties of sequences.* ITS boundaries were delineated according to Saunders and Druehl (1993b). An AGT triplet found at the 3′ end of ITS1 was unique to southern hemisphere individuals and assumed to be inserted at the end of ITS1 rather than the beginning of the 5.8S subunit. Forward and reverse sequences of each sample were aligned and edited

with Sequence Navigator (PE Biosystems). Additionally, three to four clones were analyzed from each of five *Macrocystis* individuals (two from Santa Catalina and one each from Bahia Tortugas, Marion Island, and Tasmania) to determine the degree of intraindividual sequence variation.

Sequences of all individuals were assembled and initially aligned using the Clustal method (MegAlign, DNASTAR, Madison, WI). Final alignment required adjustments by eye. Properties of the alignment such as lengths, base composition, sequence divergences, and transition/transversion (ts/tv) ratios were computed using MEGA v1.01 (Kumar et al. 1993). Alignments of the data sets are available in GenBank by retrieving any component species.

*Outgroup selection.* Within the Laminariales, considerable disparity exists between traditional morphological classification and more recently determined molecular divergences (Saunders and Druehl 1993a, Druehl et al. 1997). Consequently, we chose several genera for analysis in an attempt to determine an appropriate sister taxon to *Macrocystis*. In addition to the sequences of *Nereocystis* and *Pelagophycus* reported here, we used published sequences for *Alaria marginata* Post. & Rupr. (Saunders and Druehl 1993b; GenBank no. AF362997), *Costaria costaria* (Turner) Saunders (Yotsukura et al. 1999), and *Laminaria saccharina* (L.) Lamouroux (Peters 1998; GenBank no. AF362996).

*Analysis of aligned sequences.* The ITS1 and ITS2 sequences were treated independently because each was cloned separately from a single individual (as opposed to cloning the intact 5′18S–ITS1–5.8S–ITS2–5′ 23S region) and because each may evolve at different rates (Hershovitz and Lewis 1996). The sequence alignment of the six laminarian genera included areas of ambiguity that were excluded from the analyses (positions 65–89 and 167–179 of the 308 base ITS1 alignment; positions 16–127 and 302–425 of the 448 base ITS2 alignment). Maximum parsimony analysis was conducted using the branch and bound search option of PAUP 4.0b3 (Swofford 2000), gaps were treated as missing data, and 1000 replicates were used in bootstrap analyses.

Analyses of *Macrocystis*-only taxa were conducted using *Pelagophycus* as an outgroup. The entire data set (*Pelagophycus* plus all *Macrocystis* samples including the multiple clones of five individuals) for each ITS region was analyzed with gaps excluded (= most conservative treatment) and with gaps included (gaps counted as single events regardless of gap length = intermediate treatment) (Peters et al. 1997), using maximum parsimony and a heuristic search with the Goloboff fit criterion (k = 2) (PAUP 4.0b3; Swofford 2000). Because the number of near-identical sequences in the ITS2 alignment precluded complete analysis in a reasonable amount of time, the maximum tree option was set at 2000. A subsequent bootstrap analysis (1000 rep-

Table 1. Collection details and accession numbers of each sample.

| Species | Location | Date collected/collector | Coordinates | Id. no. | GenBank accession No. (ITS1/ITS2) |
|---|---|---|---|---|---|
| *Macrocystis pyrifera* | Monterey Bay, CA, USA | June 93, September 92/J. Coyer | 36°36′N, 121°56′W | 1 | AF352089/AF352125 |
| | | | | 2 | AF352090/AF352126 |
| *Macrocystis integrifolia* | Stillwater Cove, CA, USA | December 98/M. Edwards | 36°34′N, 121°56′W | 1 | AF352091/AF352127 |
| | | | | 2 | AF352092/AF352128 |
| *M. pyrifera* | Refugio Beach, CA, USA | May 92/J. Coyer | 34°28′N, 129°13′W | | AF352093/AF352129 |
| *M. pyrifera* | Anacapa Island, CA, USA | July 96/J. Coyer | 34°00′N, 119°25′W | 4 | AF352094/AF352130 |
| | | | | 7 | AF352095/AF352131 |
| *M. pyrifera* | Santa Catalina Island, CA, USA | May 96/J. Coyer | 33°28′N, 118°29′W | 3a | AF352096/AF352132 |
| | | | | 3b | AF352097/AF352133 |
| | | | | 3c | AF352098/AF352134 |
| | | | | 3d | AF352099/AF352135 |
| | | | | 6 | AF352100/AF352136 |
| | | November 97/J. Coyer | | 11a | AF352101/AF352137 |
| | | | | 11b | AF352102/AF352138 |
| | | | | 11c | AF352103/AF352139 |
| *M. pyrifera* | Bahia Tortugas, Baja California, Mexico | July 96/A. Terry | 27°40′N, 114°56′W | 7a | AF352104/AF352140 |
| | | | | 7b | AF352105/AF352141 |
| | | | | 7c | AF352106/AF352142 |
| | | | | 8 | AF352107/AF352143 |
| | | | | 9 | AF352108/AF352144 |
| *M. pyrifera* | Punta Pucatrihue, Chile | September 97/A. Buschmann | 40°33′S, 73°48′W | 1 | AF352109/AF352145 |
| *M. pyrifera* | Metri Bay, Chile | September 97/A. Buschmann | 41°36′S, 72°43′W | 4 | AF352110/AF352146 |
| *Macrocystis angustifolia* | Cape Town, South Africa | October 97/G. Branch | 33°56′S, 18°28′E | 1 | AF352111/AF352147 |
| | | | | 2 | AF352112/AF352148 |
| *Macrosystis laevis* | Marion Island (South Africa) | April 99/S. Lawrie | 46°55′S, 37°50′E | 2a | AF352113/AF352149 |
| | | | | 2b | AF352114/AF352150 |
| | | | | 2c | AF352115/AF352151 |
| | | | | 4 | AF352116/AF352152 |
| *M. angustifolia* | Melbourne, Australia | December 97/R. Wetherbee | 37°45′S, 144°58′E | 1 | AF352117/AF352153 |
| | | | | 4 | AF352118/AF352154 |
| *M. pyrifera* | Kingston, Tasmania | August 97/R. King | 42°55′S, 147° 20′E | A | AF352119/AF352155 |
| | | | | B | AF352120/AF352156 |
| | | | | C | AF352121/AF352157 |
| *M. pyrifera* | Dunedin, New Zealand | September 97/A. & C. Hepburn | 45°49′S, 170°37′E | 1 | AF352122/AF352158 |
| | | | | 2 | AF352123/AF352159 |
| *Pelagophycus porra* | Santa Catalina Island, CA, USA | June 93/J. Coyer | 33°28′N, 118°29′W | | AF352088/AF352124 |
| *Nereocystis leutkeana* | Friday Harbor, WA, USA | March 98/M. Dethier | 48°33′N, 123°04′W | | AF357843/AF358262 |

licates) was performed for both ITS1 and ITS2 using the "fast" stepwise addition method of PAUP, again because the number of nearly identical sequences precluded a bootstrap analysis with a heuristic search in a reasonable amount of time. Based on this analysis, selected individuals of each species of *Macrocystis* and from each area were subjected to a more comprehensive analysis.

The data set consisting of the selected sequences also was analyzed with gaps excluded and included using maximum parsimony and a heuristic search (Goloboff fit with k = 2). Again, the maximum tree option was set at 2000 for the ITS2 analysis. Bootstrapping (1000 replicates) used a heuristic search option.

Maximum likelihood analyzes were performed on the aligned ITS sequences (gaps excluded) of the selected individuals and outgroup. The aligned sequences first were analyzed with ModelTest 2.1 (Posada and Crandall 1998), which compared different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fit the sequence data. The optimal model for both ITS1 and ITS2 was a general time reversal model, which accounted for regional rate differences, invariable sites, and skewed base composition. The estimated values of each variable were imported into a maximum likelihood analysis using a heuristic search (PAUP).

## RESULTS

Thirty-five ITS1 and ITS2 sequences were determined for 24 *Macrocystis* individuals: *M. pyrifera* (16), *M. angustifolia* (4), *M. integrifolia* (2), and *M. laevis* (2). Additionally, three to four clones were sequenced from each of five individuals: Catalina-3 (4), Catalina-11 (3), B. Tortugas-7 (3), Marion Island-2 (3), and Tasmania (3). The ITS1 and ITS2 regions averaged 289 and 378 bases in length, respectively, and base composition was balanced with a mean GC content of 54.1% for ITS1 and 63.5% for ITS2 (Table 2).

*Sequence divergence.* Sequence divergences (%) among the 35 ITS1 sequences ranged from 0 (= identical sequences) among 10 sequences in the northern hemisphere (Monterey Bay-2; Refugio; Anacapa-4, 7; Catalina-3d, 6, 11a-c; B.Tortugas-8) and among nine sequences in the southern hemisphere (Chile 1, 4; South Africa 1, 2; Marion Island 2a-c, 4; New Zealand-2) to a maximum of 6.8% between Australia-4 and Catalina-3c (data not shown). For ITS2 sequences, divergence ranged from 0 among five sequences in the northern hemisphere (Monterey Bay-2; Stillwater-1, 2; Anacapa-7; Catalina-3a) and seven sequences in the southern hemisphere (South Africa-1, 2; Marion Island-2a, b, 4; New Zealand-1, 2) to a maximum of 3.3% between Catalina-6 and B.Tortugas-7c.

Intraindividual variability in ITS1 sequences ranged from no differences among the clones for Catalina-11 and Marion Island-2 to a mean divergence of 2.6% among the four clones of Catalina-3 (Table 3). For ITS2 sequences, divergences ranged from a mean of 0.2% among three clones of Catalina-11 and Marion-2 to 1.0 % among the four clones of Catalina-3.

Sequence divergence for all *Macrocystis* individuals was nearly twice as high for ITS1 (2.2%) than for ITS2 (1.4%), suggesting greater diversity and a faster rate of evolution in the ITS1 region. The trend also was observed when comparing sequence divergence for ITS1 and ITS2 among all northern hemisphere individuals (1.7% vs. 1.2%), among southern hemisphere individuals (0.8% vs. 0.6%), and between northern and southern hemisphere individuals (3.0% vs. 1.8%) (data not shown).

For all pairwise comparisons of ITS sequences in *Macrocystis,* ts/tv ratios were calculated to determine the likelihood of saturation. Saturation is indicated by ratios $< 1.0$; slight saturation was apparent for ITS1 (0.86) but not for ITS2 (2.99).

*Phylogenetic analysis.* Maximum parsimony analyses of all kelp genera (rooted with *Alaria*) revealed that the sister taxon to *Macrocystis* was *Pelagophycus porra* (Fig. 2), an endemic species found adjacent to *Macrocystis* populations in coastal regions between southern California (United States) and central Baja California (Mexico) (Miller and Dorr 1994). Consequently, analyses with *Macrocystis* taxa were rooted with *Pelagophycus* (Figs. 3–5).

The three analyses (maximum parsimony analysis with gaps excluded, maximum parsimony analysis with gaps included, and maximum likelihood analysis) of the ITS1 sequences for selected *Macrocystis* sequences (from

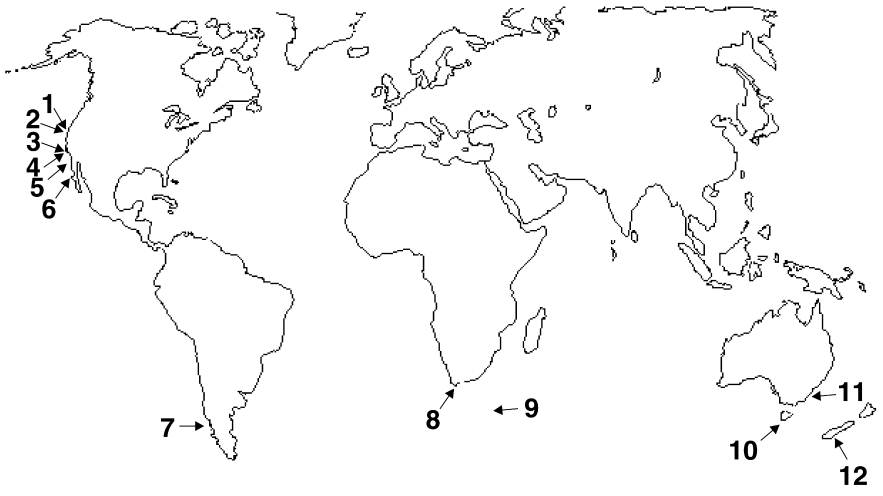


FIG. 1. Location of sampling areas (see Table 1 for coordinates). Site 1: Monterey Bay, California, United States *(Macrocystis pyrifera)*; 2: Stillwater Cove, California, United States (*Macrocystis integrifolia*); 3: Refugio Beach, California, United States (*M. pyrifera*); 4: Anacapa Island, California, United States (*M. pyrifera*); 5: Santa Catalina Island, California, United States (*M. pyrifera*); 6: Bahia Tortugas, Baja California, Mexico (*M. pyrifera*); 7: Metri Bay and Punta Pucatrihue, Chile (*M. pyrifera*); 8: Cape Town, South Africa (*Macrocystis angustifolia*); 9: Marion Island, South Africa (*Macrocystis laevis*); 10: Kingston, Tasmania (*M. pyrifera*); 11: Melbourne, Australia (*M. angustifolia*); 12: Dunedin, New Zealand (*M. pyrifera*).

TABLE 2. Comparative summary of ITS analyses among individuals of *Macrocystis* spp.

| | All *Macrocystis* | | Subset | |
|---|---|---|---|---|
| | ITS1 | ITS2 | ITS1 | ITS2 |
| Variation | | | | |
| Average length | 289 | 378 | — | — |
| Range of lengths | 285–293 | 372–384 | — | — |
| Length of alignment | | | | |
| Gaps excluded | 297 | 403 | — | — |
| Gaps included | 304 | 414 | | |
| Invariable positions | | | | |
| Gaps excluded | 248 | 342 | 252 | 351 |
| Gaps included | 248 | 342 | 252 | 351 |
| Variable positions | | | | |
| Gaps excluded | 49 | 62 | 45 | 53 |
| Gaps included | 56 | 72 | 52 | 63 |
| Informative positions (%) | | | | |
| Gaps excluded | 16 (33) | 19 (31) | 13 (29) | 12 (23) |
| Gaps included | 23 (41) | 29 (40) | 20 (38) | 19 (30) |
| Trees | | | | |
| Number of MPTs | | | | |
| Gaps excluded | 8 | 2000 | 2 | 2000 |
| Gaps included | 8 | 2000 | 2 | 2000 |
| Length | | | | |
| Gaps excluded | 52 | 71 | 46 | 58 |
| Gaps included | 60 | 89 | 54 | 73 |
| CI/RC | | | | |
| Gaps excluded | 0.962/0.947 | 0.944/0.904 | 0.978/0.960 | 0.948/0.879 |
| Gaps included | 0.950/0.935 | 0.890/0.781 | 0.963/0.929 | 0.890/0.799 |
| Base Composition (mean %) | | | | |
| A | 22.4 | 16.4 | — | — |
| T | 23.5 | 20.1 | — | — |
| C | 30.2 | 33.2 | — | — |
| G | 23.9 | 30.3 | — | — |

Subset refers to individuals selected after the "fast" step-wise addition bootstrap search (see Fig. 3); maxtree option for ITS2 was set at 2000 (see text).

MPT, most parsimonious tree; CI/RC, consistency index/rescored consistency index.

Fig. 3) produced identical tree topologies; thus, we present a strict consensus and phylogram tree from maximum parsimony analysis with the gaps included (Fig. 4). The southern hemisphere clade was strongly supported, which in turn was closely aligned with a Bahia Tortugas clade, and both were well embedded within a strongly resolved northern hemisphere clade.

For selected ITS2 sequences (from Fig. 3), the three analyses also produced identical tree topologies, and we present the maximum parsimony analysis with gaps included (Fig. 5). The southern hemisphere clade was moderately supported and the Bahia Tortugas clade was strongly supported, but both were equivalent clades to the moderately resolved northern hemisphere clade.

The four species of *Macrocystis* were not resolved in either the ITS1 or the ITS2 analyses, primarily because of the polyphyletic nature of *M. pyrifera* and *M. angustifolia* (Figs. 3–5). Neighboring individuals (<50 m) of *M. pyrifera* collected from Bahia Tortugas were less related than either individual of *M. pyrifera* from Chile and either individual of *M. angustifolia* from South Africa (<7500 km). Similarly, neighboring individuals (<50 m) of *M. pyrifera* collected from Santa Catalina Island were less related than either individual of *M. pyrifera* from Chile and *M. laevis* from Marion Island (<7500 km). *Macrocystis angustifolia* from Australia and *M. pyrifera* from Tasmania (ca. 200 km) were more closely related in our analyses than the neighboring individuals of *M. pyrifera* from Bahia Tortugas

TABLE 3. Sequence divergence (%) among clones (a–d) of individuals of *Macrocystis*.

| | a | b | c | d |
|---|---|---|---|---|
| | | Catalina-3 (*M. pyrifera*) | | |
| a | — | 0.27 | 1.08 | 1.63 |
| b | 3.16 | — | 0.82 | 1.36 |
| c | 4.63 | 2.80 | — | 0.53 |
| d | 2.46 | 0.69 | 2.10 | — |
| | | Catalina-11 (*M. pyrifera*) | | |
| a | — | 0.00 | 0.27 | |
| b | 0.00 | — | 0.27 | |
| c | 0.00 | 0.00 | — | |
| | | Bahia Tortugas-7 (*M. pyrifera*) | | |
| a | — | 0.80 | 0.27 | |
| b | 1.04 | — | 1.07 | |
| c | 0.00 | 1.04 | — | |
| | | Tasmania (*M. pyrifera*) | | |
| a | — | 0.52 | 0.52 | |
| b | 0.35 | — | 0.52 | |
| c | 0.69 | 1.04 | — | |
| | | Marion-2 (*M. laevis*) | | |
| a | — | 0.00 | 0.26 | |
| b | 0.00 | — | 0.26 | |
| c | 0.00 | 0.00 | — | |

Values to left and right of diagonal represent ITS1 and ITS2, respectively.

or from Santa Catalina Island. Additionally, greater differences were observed within the four ITS1 and ITS2 clones from one individual of *M. pyrifera* (Catalina-3) than between *M. pyrifera* from Chile and *M. pyrifera* from New Zealand (or *M. angustifolia* from Australia or *M. laevis* from Marion Island).

The southern hemisphere taxa formed a monophyletic group in all analyses. The Australian/Tasmanian taxa always formed a clade within the southern hemisphere group, but no phylogenetic resolution was found for the remaining southern hemisphere taxa (Figs. 3–5). The northern hemisphere taxa were more diverse in terms of sequence divergence.

## DISCUSSION

Our analysis of both ITS1 and ITS2 demonstrated that *Pelagophycus* is the sister taxon to *Macrocystis* and that *Pelagophycus* is less closely related to the morphologically similar *Nereocystis* than it is to the morphologically different *Macrocystis*. Bootstrap support for the *Pelagophycus–Macrocystis* relationship was strongest in the ITS1 analyses. The phylogenetic relationships of these three genera, as inferred from our analyses, mirror the patterns of hybridization observed in the laboratory and field. Whereas *Pelagophycus* × *Macrocystis* hybrids frequently have been produced in the laboratory and naturally occurring hybrids have been collected at numerous field locations, laboratory crosses between *Nereocystis* and either *Macrocystis* or *Pelagophycus* have been largely unsuccessful, and no *Nereocystis* × *Macrocystis* hybrids have been reported from the field (Sanbonsuga and Neushul 1978, Coyer and Zaugg-Haglund 1982, Coyer et al. 1992, Lewis and Neushul 1995, J. A. Coyer, unpublished data). No field occurrences of *Nereocystis* × *Pelagophycus* hybrids have been reported, but because the species display geographically disjunct distributions, the absence of this potential cross is expected.

*Intrageneric relationships.* Analyses of the ITS sequences showed *M. integrifolia* and *M. laevis* to be monophyletic with weak bootstrap support. More importantly, *M. pyrifera* was polyphyletic with respect to the other three morphological species, and *M. angustifolia* from South Africa and Australia failed to form a monophyletic group. Consequently, our analyses do not support recognition of the four morphological species and are consistent with crossing experiments showing extensive hybridization among and within species of *Macrocystis* from both hemispheres (Lewis et al. 1986, Lewis and Neushul 1994). The crossing experiments suggest only one biological species (e.g. *M. pyrifera*) using the definition proposed by Meier and Willmann (2000); however, geographical isolation usually prevents "natural" hybridization and, therefore, two or more "biological" species might be recognized using the definition of Mayr (2000).

An inability to distinguish intrageneric relationships in *Macrocystis* also is evident in chloroplast DNA sequences. For example, Fain (1992) measured considerably less divergence in chloroplast DNA sequence between British Columbian *M. integrifolia* and Californian *M. pyrifera* (0.08%) than between Californian and Chilean *M. integrifolia* (0.3%).

One may argue that the four *Macrocystis* species should be combined to one species based on the "molecular" (use of molecular data to infer species identities, *sensu* Andersen 1992) and biological species concepts and the results of genetic crossings. However, when one considers definitions of genera using the same criteria, the fertile hybrids of *Pelagophycus* × *Macrocystis* (Coyer et al. 1992) could be interpreted as

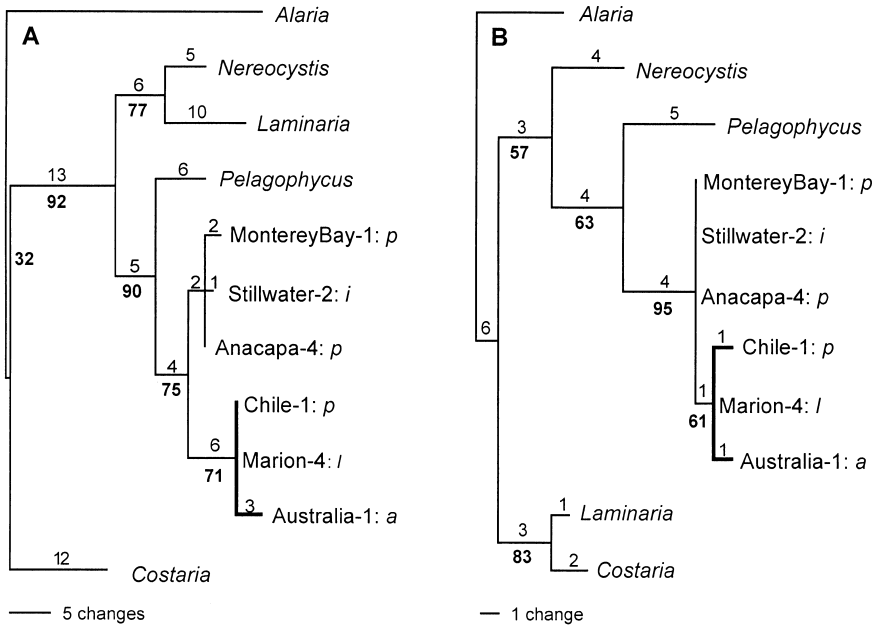


FIG. 2. Rooted parsimony analysis of the ITS1 (A) and ITS2 (B) data set for six genera of Laminariales, including six individuals of *Macrocystis*. Gaps (continuous gaps counted as a single event regardless of gap length) were included in the analysis. *Alaria* was the outgroup, and a branch and bound search option was used. For A and B, respectively: length of alignment = 271, 211; invariable positions = 192, 167; variable positions = 78, 25; informative positions (%) = 33 (42), 13 (52); number of most parsimonious trees = 2, 1; CI/RC = 0.888/0.704, 0.996/0.768. Upper numbers are steps (= nucleotide changes); lower numbers (boldface) are bootstrap percentages (1000 replicates); southern hemisphere clade of *Macrocystis* is delineated with bold lines. *p*, *Macrocystis pyrifera*; *i*, *Macrocystis integrifolia*; *a*, *Macrocystis angustifolia*; *l*, *Macrocystis laevis*.

members of a single species. It is unlikely that our data convincingly resolve the problems of species concepts within *Macrocystis*, but our data do further question the validity of the four morphological species currently recognized.

*Directionality of dispersal.* A northern to southern hemisphere dispersal route has been suggested by others on the basis of biogeography of extant kelps in the north Pacific, paleoclimatic records, and fossil records of certain obligate or facultative kelp-associated mollusks (Nicholson 1978, Estes and Steinberg 1988, Lüning 1990, Lüning and tom Dieck 1990). Our analyses of ITS1 and ITS2 sequences suggest that *Macrocystis* dispersal between the hemispheres involved populations in the Baja California area of Mexico (northern hemisphere) and/or Santa Catalina Island (United States) as the intermediate or bridging organisms. The direction of dispersal cannot be determined unambiguously from our data. However, one might infer a northern hemisphere to the southern hemisphere dispersal based on (1) the paraphyletic relationship of northern hemisphere individuals relative to the well-supported monophyletic origin of southern hemisphere individuals and (2) the greater sequence diversity within the northern hemisphere individuals.

Southern hemisphere individuals displayed very little divergence in ITS sequences, despite the vast distances between sampling localities, which suggest high gene flow between most areas and/or a very recent dispersal from the initial southern hemisphere population. If one assumes a northern hemisphere to southern hemisphere track, then South America is likely to bear the first southern hemisphere population. Once *Macrocystis* colonized the coastal area of western South America, subsequent dispersal throughout the southern hemisphere could have occurred via dislodged rafts of kelp that were transported eastward by the Antarctic Convergence Current (West Wind Drift), a primarily unidirectional current with a velocity of 0.75 to 1.3 $km \cdot h^{-1}$ (United States Defense Mapping Agency 1988, Pickard and Emery 1990).

The eastward dispersal pattern is consistent with observations of others who view the Antarctic and sub-Antarctic as a single biogeographical province for algae and hypothesize long distance dispersal from sources in southern South America via the Antarctic Convergence Current (Papenfuss 1965, Lawson 1988, Lüning 1990, John et al. 1994). Indeed, Helmuth et al. (1994) measured a patchy distribution of kelp rafts (ca.1.5 rafts$\cdot km^{-2}$), each about 4 $m^3$ in size and comprising 100 to 200 individuals of *M. pyrifera*, between South America, the Falkland Islands, and South Georgia (total distance ca. 2000 km). Thus, the relatively rapid eastward dispersal (and putative gene flow) con-

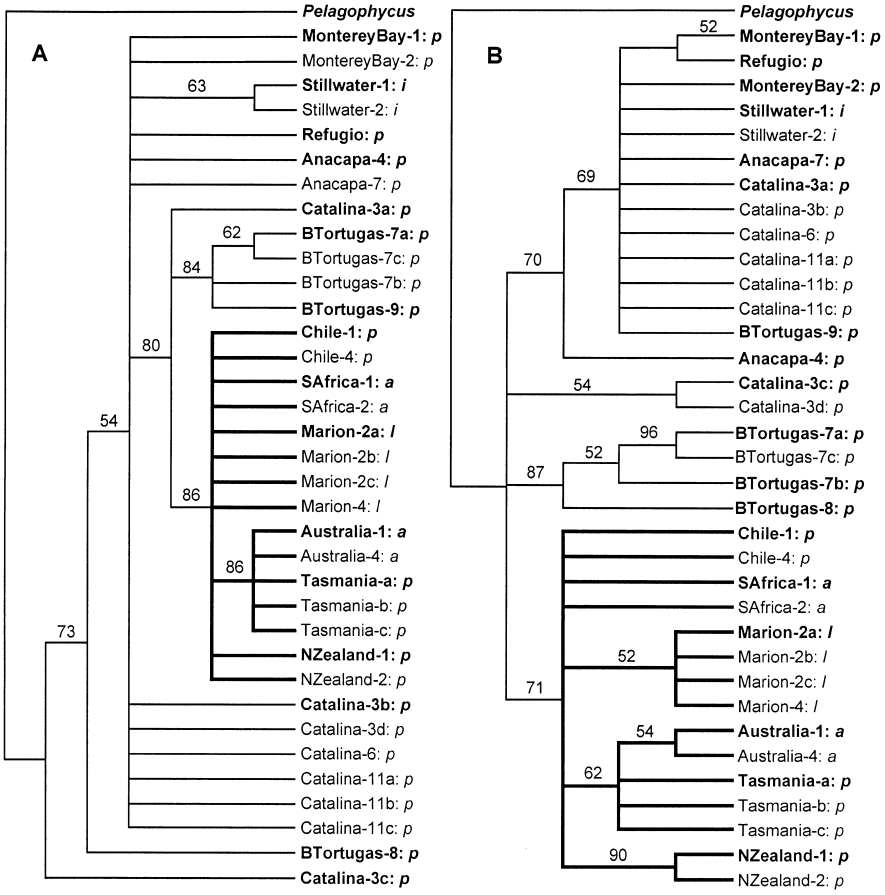


FIG. 3. Bootstrap consensus tree of ITS1 (A) and ITS2 (B) for all individuals. Gaps are included (as a single event regardless of length) in the analysis; bootstrap percentages (1000 replicates) were generated with the "fast" step-wise addition search option of PAUP. Individuals in boldface were selected for further analysis. The southern hemisphere clade is delineated with bold lines. *p, Macrocystis pyrifera*; *i, Macrocystis integrifolia*; *a, Macrocystis angustifolia*; *l, Macrocystis laevis.*

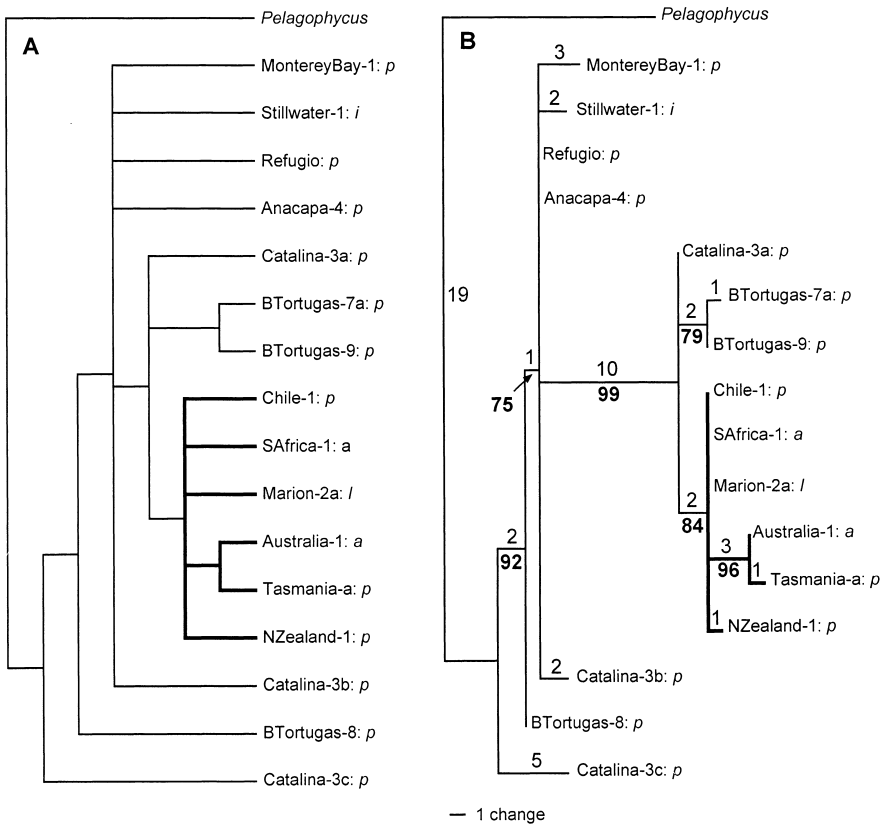


FIG. 4. Rooted parsimony analysis of the ITS1 regions of individuals in Figure 3 that were selected for analysis. Gaps were included (as a single event regardless of length) and a heuristic search was used. See Table 2 for details of trees. (A) Strict consensus tree (most parsimonious tree = 2). (B) Phylogram; upper numbers are steps (=nucleotide changes), lower numbers (boldface) are bootstrap percentages (1000 replicates). The southern hemisphere clade is delineated with bold lines in both trees. *p*, *Macrocystis pyrifera*; *i*, *Macrocystis integrifolia*; *a*, *Macrocystis angustifolia*; *l*, *Macrocystis laevis*.

tinues today and may account for the lack of sequence divergence observed in *Macrocystis* from Chile, South Africa, and New Zealand.

The strongly resolved Australia/Tasmania clade within the monophyletic southern hemisphere ITS1 clade, however, suggests less gene flow between Australia/Tasmania and other areas of the southern hemisphere. Edgar (1987) reported that drifting *Macrocystis* individuals never were found more than 50 km offshore of the southern Australian coast or in the Tasman Sea and suggested that the Tasman Sea was an imposing barrier to drifting *Macrocystis* because nitrate levels are an order of magnitude below levels necessary for growth and presumably also for maintenance of buoyancy. Reduced gene flow between Australia/Tasmania and New Zealand populations of *Macrocystis* also is supported by the large numbers of benthic invertebrate and algal species from Tasmania that, although experimentally were able to survive extended periods adrift within kelp holdfasts, remained restricted to Australian coasts (Edgar 1987). Thus, the Australia/Tasmania clade may represent a founder effect and warrants further investigation.

*Timing of dispersal.* ITS sequences suggest a very recent dispersal of *Macrocystis* from the northern to the southern hemisphere. The short branch lengths overall, lack of sequence diversity among southern hemisphere individuals, and the high ts/tv ratio for the ITS2 sequences (2.99) suggest a recent origin of the genus and a recent breaching of the tropical barrier. The ts/tv ratios generally decrease with increasing divergence time between lineages, and ratios $> 2.0$ are characteristic of recently diverged sequences (Brown and Simpson 1982, DeSalle et al. 1987, Mindell and Honeycutt 1990).

In the absence of a molecular clock, the specific timing of *Macrocystis* dispersal into the southern hemisphere must remain speculative. It is reasonable, however, to hypothesize that kelps first breached the eastern tropical barrier between 0.01 and 3.1 Ma, based on evidence from other organisms. A diverse array of marine invertebrates, vertebrates, and other algal species also displays antitropical distributions and available geological and paleontological evidence suggests that these species crossed the tropics in the eastern Pacific on several occasions, rather than during a single event (reviewed in Lindberg 1991). Worldwide, the only fossil record for kelps is the genus *Julescrania* from California Miocene deposits (Parker and Dawson 1965). Molluscan fossils from the Middle Miocene (15 Ma) and earlier suggest that climatic conditions in the north Pacific were unsuitable for kelps until the Middle to Late Miocene, and on a worldwide basis, kelp-associated limpets appear only in the last 3 million years (Parker and Dawson 1965, Estes and Steinberg 1988). Thus, direct and indirect fossil evidence indicates that kelps appeared recently and radiated from the north Pacific following the Middle (15 Ma) to Late Miocene (Estes and Steinberg 1988, 1989).

Lindberg (1991) summarized fossil histories of many mollusk species associated with rocky shores in the east-

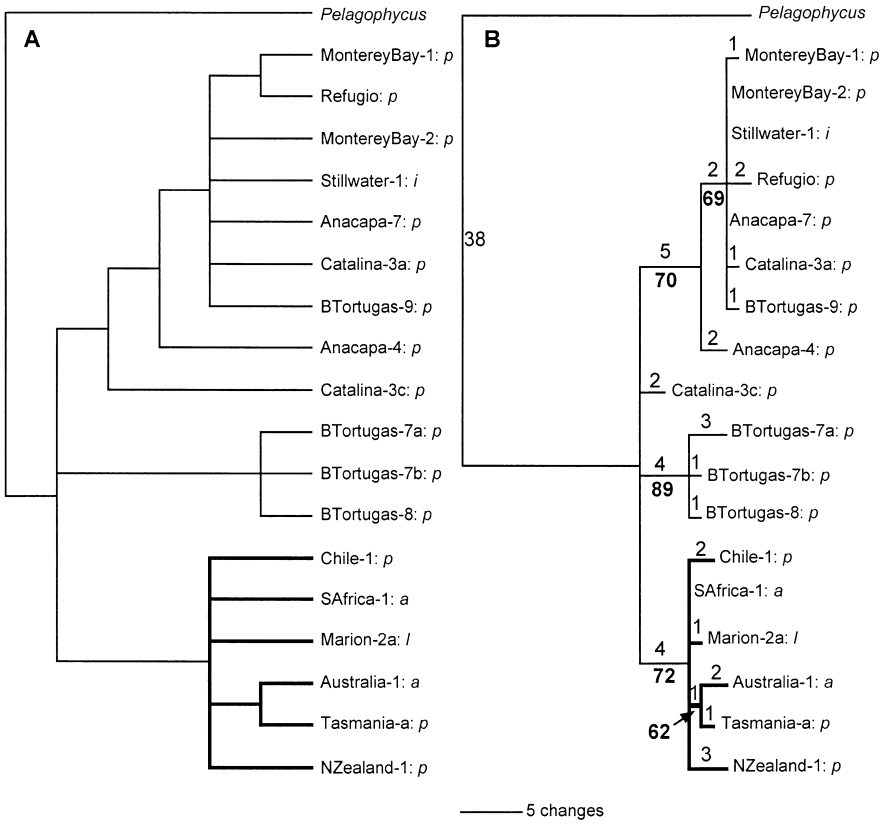


FIG. 5. Rooted parsimony analysis of the ITS2 regions of individuals in Figure 3 that were selected for analysis. See Figure 4 legend for details. Maxtree option of PAUP was set to 2000 for the strict consensus tree.

ern Pacific Ocean that display antitropical distributions. Lindberg's analysis suggested that biotic interchange between the hemispheres occurred in both directions during at least two major periods: emergence of the Panama Isthmus (3.1 Ma) and the Pleistocene glaciations (0.01–0.17 Ma). Weaver (1990) proposed that before closure of the Panama portal, the now south-flowing California current flowed north, thereby suppressing coastal upwelling and producing a milder climate in the Baja California and southern California region than what currently exists. Emergence of the Panama Isthmus and concomitant closure of the Panama portal resulted in a reversal of the California current to its present southerly direction, thereby intensifying coastal upwelling and producing the extant oceanographic climate (Weaver 1990). In the eastern Pacific, therefore, a coastal marine environment conducive for temperate water kelps probably existed only after emergence of the Panama Isthmus. Hemispheric exchanges during the Pleistocene glaciations may have been via a series of upwelling cells along the coast, which served as refuge and stepping stones for population expansion (reviewed in Lindberg 1991).

Van Oppen et al. (1993) examined ITS sequences in *Acrosiphonia arcta* (Dillwyn) J.G. Agardh (Chlorophyta) and *Desmarestia viridis/willii* (Phaeophyta), both found in Arctic and Antarctic boreal and subboreal marine floras. As virtually no sequence divergence (<0.5%) was found in each of the species collected from both hemispheres, van Oppen et al. hypothesized that the hemispheric disjunctions occurred as recently as the last Pleistocene glacial maximum (18,000 years ago). Working with species of Fucaceae, Serrão et al. (1999) found sequence divergences of 4% to 21% among northern hemisphere Fucaceae and 36% to 45% between these species and the southern hemisphere genus *Xiphorora*, suggesting a hemispheric divergence starting during the Oligocene/Miocene (38–7 Ma). Assuming equal rates of evolution and a correlation between sequence divergence and divergence time, our finding of 2% to 3% sequence divergences between northern and southern hemisphere individuals of *Macrocystis* is consistent with dispersal from north to south 0.01 to 3 Ma.

*Intraindividual variability.* At Santa Catalina Island, one individual of *Macrocystis* (Catalina-3) displayed a high degree of ITS1 variation, in which some ITS1 clones aligned with individuals from more northern areas and others either were intermediate between northern and southern hemisphere individuals or were aligned with Baja California/southern hemisphere individuals. In contrast, intraindividual variability in the Bahia Tortugas and southern hemisphere individuals (Tasmania, Marion Island) was minimal to nonexistent, respectively. Two hypotheses may explain the intraindividual variation observed at Santa Catalina Island: 1) a history of extensive, rapid, and recent mixing of genotypes from areas to the north and the south, resulting in gene mixing at a rate exceeding the homogenization rate by concerted evolution, and/or 2) introgressive hybridization with *Pelagophycus*.

An equivalent degree of intraindividual variation in ITS sequences was found in *Fucus vesiculosus* L. (Fucaceae), leading Serrão et al. (1999) to hypothesize a recent and rapid radiation that exceeded homogenization rates. Thus, the extensive intraindividual variation observed in some individuals at Santa Catalina Island may result from recent "hybridization" of genotypes representative of more northern and southern areas.

It is quite plausible that Santa Catalina Island could simultaneously harbor genotypes of *M. pyrifera* from northern and southern areas. The island is located in the middle of the species' distribution and well south of Pt. Conception (California), an area that has experienced significant fluctuations of *Macrocystis* populations since the early 1900s (reviewed in Foster and Schiel 1985). Although several factors (grazing, sewage/thermal effluents, intense storms) are responsible for local-level fluctuations in this area, oceanographic conditions such as the large-scale low frequency El Niño–La Niña events and the Pacific Decadal Oscillations (e.g., 1977–1993) are much more widespread and dramatic in their effect (Foster and Schiel 1985, Tegner et al. 1996, 1997, Edwards 1998).

El Niño events depress the thermocline, thereby increasing the extent of warm and nutrient-poor surface water along coastal areas of western North America, particularly south of Pt. Conception, and subsequently reducing or eliminating *Macrocystis* populations (Tegner and Dayton 1987, Dayton et al. 1992, 1998, Foster and Schiel 1992, Tegner et al. 1996, 1997). In the past 98 years 23 El Niños have been recorded, and two recent events (1982–1983, 1997–1998) have been notably severe. During the 1997–1998 El Niño, for example, *M. pyrifera* was eliminated from the southernmost limit of the species at Bahia Tortugas and its distribution receded northward by about 500 km (Ladah et al. 1999). Additionally, a recent study of 90 sites (spanning the range of *M. pyrifera* along the continental United States and Mexico) just before, immediately after, and several months after the 1997–1998 El Niño revealed that Pt. Conception was a critical boundary. Populations to the north were relatively unaffected, but populations to the south were depleted severely with highly variable rates of recovery (Edwards 1998).

La Niña events essentially are the reverse of El Niño. Colder than normal, nutrient-rich water flows south along the coast of North America during a La Niña, creating optimal growth conditions and rapid recolonization for *M. pyrifera* (Tegner et al. 1997). Between the extremes of major El Niño and La Niña events, intermediate oceanographic conditions exist for variable periods of time.

*Macrocystis* populations adapt to local oceanographic conditions in both the northern and southern limits of its range off North America. For example, Santa Catalina individuals are more tolerant of low nitrate concentrations than individuals from Monterey Bay and populations at the southern edge of the distribution (Bahia Tortugas) are much more tolerant of high temperatures (North 1971, 1972, Kopczak et al. 1991). If the observed ecotypic variation among the various *Macrocystis* populations has a genetic basis, population-specific genotypes may be present among and within individuals inhabiting areas that can receive individuals from many different populations.

Low-frequency and alternating El Niño–La Niña events, interspersed with longer and intermediate oceanographic regimes, could result in mixing of the northern and southern ecotypes (genotypes) of *Macrocystis* in spatially intermediate areas such as Santa Catalina Island. Potential mechanisms of gene flow to the intermediate areas include (1) drifting individuals/spores from northern populations (via the southerly flowing California Current) during La Niña conditions, (2) drifting individuals/spores from Baja California during El Niño conditions, (3) cold water-adapted macroscopic individuals surviving El Niño conditions or warm-water macroscopic individuals surviving La Niña conditions, and (4) "seedbanks" of spores and/or gametophytes surviving both conditions. The ability of detached individuals and spores to drift for weeks to months in the water column, as well as months-long survival of spores and/or gametophytes on the substratum, has been demonstrated (Reed et al. 1992, Ladah et al. 1999, Hobday 2000). Furthermore, laboratory investigations suggest that spores/gametophytes can become semidormant and tolerate more stressful conditions than macroscopic individuals (tom Dieck 1993). Thus, one can hypothesize a scenario in which southern genotypes "hybridize" with northern genotypes in intermediate areas such as Santa Catalina Island, thereby leading to the high degree of intraindividual variation revealed by ITS sequences in some individuals. The hypothesis remains to be tested by expanded sampling in the field and description of ITS (or other gene) sequence dynamics in laboratory crosses between the northern and southern ecotypes.

Alternatively, some of the intraindividual variability observed in one Santa Catalina individual (Catalina-3) may be due to introgressive hybridization with *Pelagophycus*. Examining ITS2 sequences of *M. pyrifera*, *Pelagophycus*, and putative *Pelagophycus* × *Macrocystis* hybrids collected from the field, Miller (1999) found that the parental species and hybrids shared multiple alleles of ITS2, suggesting both introgressive hybridization and a lack of homogenization in the area. Additional molecular studies are needed of the genetic relationships between *Macrocystis* and *Pelagophycus* in areas where both occur.

In conclusion, our analysis of ITS sequences in *Macrocystis* suggests that the genus (1) dispersed from the northern to the southern hemisphere, (2) is monospecific, and (3) is subjected to environmental perturbations that can be evaluated with molecular biological methods. The latter point provides a new approach to population and evolutionary questions in kelp species. For example, a survey of ITS (or other gene) sequence variability among kelp populations before and after

various El Niño–La Niña events should further our understanding of how minor and major oceanographic events influence genetic structure. The survey would be particularly important at the distributional limits of *Macrocystis*, such as Bahia Tortugas. Additionally, examination of ITS (or other gene) sequence variability among individuals along a region of the Peruvian/Chilean coast comparable with the region between Santa Catalina Island and Bahia Tortugas in the northern hemisphere (both areas strongly influenced by major El Niño events) may reveal the relative importance of El Niño events to population genetic structure in the two hemispheres. Although the Chilean samples of *Macrocystis* in our study displayed no differences despite a separation of ca. 200 km, both individuals were collected from areas too far south to experience oceanographic changes related to any El Niño events (A. Buschmann, personal communication). We conclude that ITS sequences in kelps can reveal local-scale patterns of population changes due to environmental changes, as well as global-scale patterns of speciation and biogeography.

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Andersen, R. A. 1992. Diversity of eukaryotic algae. *Biodivers. Conserv.* 1:267–92.

Baroin, A., Perasso, R., Qu, L.-H., Brugerolle, G., Bachellerie, J. P. & Adouette, A. 1988. Partial phylogeny of the unicellular eukaryotes based on rapid sequencing of a portion of 28S ribosomal RNA. *Proc. Nat. Acad. Sci. USA* 85:3474–8.

Brostoff, W. N. 1977. *A Taxonomic Revision of the Giant Kelp* Macrocystis *in Southern California Based on Morphometric and Transplant Studies.* M.Sc. Thesis, San Diego State University, San Diego, California, 75 pp.

Brown, G. & Simpson, M. 1982. Novel features of animal mtDA evolution as shown by sequences of two rat cytochrome oxidase subunit II genes. *Proc. Nat. Acad. Sci. USA* 79:3246–50.

Chin, N. K. M., Brown, M. T. & Heads, M. J. 1991. The biogeography of Lessoniaceae, with special reference to *Macrocystis* C. Agardh (Phaeophyta: Laminariales). *Hydrobiologia* 215:1–11.

Coyer, J. A., Engle, J. M. & Zimmerman, R. C. 1992. Discovery of a fertile, *Pelagophycus* × *Macrocystis* (Phaeophyta) putative hybrid and subsequent production of $F_2$ sporophytes in the laboratory. *J. Phycol.* 28:127–30.

Coyer, J. A., Robertson, D. L. & Alberte, R. S. 1994. Genetic variability within a population and between diploid/haploid tissue of *Macrocystis pyrifera* (Phaeophyceae). *J. Phycol.* 30:545–52.

Coyer, J. A. & Zaugg-Haglund, A. C. 1982. A demographic study of the elk kelp, *Pelagophycus porra* (Laminariales, Lessoniaceae), with notes on *Pelagophycus* × *Macrocystis* hybrids. *Phycologia* 21:399–407.

Dayton, P. K., Tegner, J. J., Parnell, P. E. & Edwards, P. B. 1992. Temporal and spatial patterns of disturbance and recovery in a kelp forest community. *Ecol. Monogr.* 62:421–45.

Dayton, P. K. 1985. Ecology of kelp communities. *Annu. Rev. Ecol. Syst.* 16:215–45.

Dayton, P. K., Tegner, M. J., Edwards, P. B. & Riser, K. L. 1998. Sliding baselines, ghosts, and reduced expectations in kelp forest communities. *Ecol. Appl.* 8:309–22.

DeSalle, R., Freedman, T., Prager, E. M. & Wilson, A. C. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. *J. Mol. Evol.* 26:157–64.

Druehl, L. D., Mayes, C. T., Tan, I. H. & Saunders, G. W. 1997. Molecular and morphological phylogenies of kelp and associated brown algae. *Plant Syst. Evol.* 11:221–35.

Edgar, G. J. 1987. Dispersal of faunal and floral propagules associated with drifting *Macrocystis pyrifera* plants. *Mar. Biol.* 95:599–610.

Edwards, M. S. 1998. Disaster and recovery in kelp forests in the Northeast Pacific. Abstract, 79th Annual Meeting, Western Society of Naturalists, San Diego, California.

Estes, J. A. & Steinberg, P. D. 1988. Predation, herbivory, and kelp evolution. *Paleobiology* 14:19–36.

Estes, J. A. & Steinberg, P. D. 1989. Response to Domning. *Paleobiology* 15:57–60.

Fain, S. R. 1992. Interpopulation chloroplast DNA sequence variation in the giant kelp, *Macrocystis*. *J. Phycol.* 28(Suppl):16.

Foster, M. S. & Schiel, D. R. 1985. *The Ecology of Giant Kelp Forests in California: A Community Profile.* U.S. Fish and Wildlife Serv., Biol. Rep. 85 (7.2), Slidell (LA), 152 pp.

Foster, M. S. & Schiel, D. R. 1992. Zonation, El Niño disturbance, and the dynamics of subtidal vegetation along a 30 m depth gradient in two giant kelp forests. *In* Battershill, C. N., Schiel, D. R., Jones, G. P., Creese, R. G. & MacDiarmid, A. B. [Eds.] *Proceedings of the Second International Temperate Reef Symposium*, 7–10 January 1992. Auckland, New Zealand. NIWA Marine, Wellington, pp. 152–62.

Gabrielson, P. W., Widdowson, T. B., Lindstrom, S. C., Hawkes, M. W. & Scagel, R. F. 2000. *Keys to the Benthic Marine Algae and Seagrasses of British Columbia, Southeast Alaska, Washington and Oregon.* Phycological Contribution No. 5, University of British Columbia, Botany, 189 pp.

Hay, C. H. 1986. A new species of *Macrocystis* C.Ag. (Phaeophyta) from Marion Island, southern Indian Ocean. *Phycologia* 25: 241–52.

Heery, D. M., Gannon, F. & Powel, R. 1990. A simple method for subcloning DNA fragments from gel slices. *Trends Genet.* 6:171.

Helmuth, B., Veit, R. R. & Holberton, R. 1994. Long-distance dispersal of a subantarctic brooding bivalve (*Gaimardia trapesina*) by kelp-rafting. *Mar. Biol.* 120:421–6.

Hershovitz, M. A. & Lewis, L. A. 1996. Deep-level diagnostic value of the rDNA-ITS region. *Mol. Biol. Evol.* 13:1276–95.

Hobday, A. J. 2000. Age of drifting *Macrocystis pyrifera* (L.) C. Agardh rafts in the southern California Bight. *J. Exp. Mar. Biol. Ecol.* 253:97–114.

Hooker, J. D. 1847. *The Botany of the Antarctic Voyage of H.M. Discovery Ships* Erebus *and* Terror. Vol. 1. *Flora Antarctica.* Part 2. *Algae.* Reeve Bros., London, pp. 209–574.

Howe, M. A. 1914. The marine algae of Peru. *Mem. Torrey Bot. Club.* 15:1–185.

John, D. M., Tittley, I., Lawson, G. W. & Pugh, P. J. A. 1994. Distribution of seaweed floras in the Southern Ocean. *Bot. Mar.* 37:235–9.

Kopczak, C. D., Zimmerman, R. C. & Kremer, J. N. 1991. Variation in nitrogen physiology and growth among geographically isolated populations of the giant kelp, *Macrocystis pyrifera* (Phaeophyta). *J. Phycol.* 27:149–58.

Kumar, S., Tamura, K. & Nei, M. 1993. *MEGA: Molecular Evolutionary Genetic Analysis.* The Pennsylvania State University, University Park.

Ladah, L. B., Zertuche, J. A. & Hernandez-Carmona, G. 1999. Giant kelp (*Macrocystis pyrifera*, Phaeophyceae) recruitment near its southern limit in Baja California after mass disappearance during ENSO 1997–1998. *J. Phycol.* 35:1106–12.

Lawson, G. W. 1988. Seaweed distribution patterns as revealed by ordination with reference to the Atlantic and southern Oceans. *Helgo. Wiss. Meeres.* 42:187–97.

Lewis, R J., Neushul, M. & Harger, B. W. W. 1986. Interspecific hybridization of the species of *Macrocystis* in California. *Aquaculture* 57:203–10.

Lewis, R. J. & Neushul, M. 1994. Northern and southern hemisphere hybrids of *Macrocystis* (Phaeophyceae). *J. Phycol.* 30: 346–53.

Lewis, R. J. & Neushul, M. 1995. Intergeneric hybridization among five general of the family Lessoniaceae (Phaeophyceae) and evidence for polyploidy in a fertile *Pelagophycus* × *Macrocystis* hybrid. *J. Phycol.* 31:1012–7.

Lindberg, D. R. 1991. Marine biotic interchange between the northern and southern hemispheres. *Paleobiology* 17:308–24.

Lüning, K. 1990. Seaweeds. *Their Environment, Biogeography, and Ecophysiology*. Wiley, New York, pp. 241–2.

Lüning, K. & tom Dieck, I. 1990. The distribution and evolution of the Laminariales: North Pacific-Atlantic relationships. *In* Garbary, D. J. & South, G. R. [Eds.] *Evolutionary Biogeography of the Marine Algae of the North Atlantic*. Springer-Verlag, Berlin, pp. 187–204.

Mayr, E. 2000. The biological species concept. *In* Wheeler, Q. D. & Meier, R. [Eds.] *Species Concepts and Phylogenetic Theory*. Columbia University Press, New York. pp. 17–29.

Meier, R. & Willmann, R. 2000. The Hennigian species concept. *In* Wheeler, Q. D. & Meier, R. [Eds.] *Species Concepts and Phylogenetic Theory*. Columbia University Press, New York, pp. 30–43.

Miller, K. A. & Dorr, H. W. 1994. Natural history of mainland and island populations of the deep-water elk kelp *Pelagophycus* (Laminariales, Phaeophyta): how many species? *In* Halvorson, W. L. & Maender, G. J. [Eds.] *Fourth California Islands Symposium: Update on the Status of Resources*. Santa Barbara Museum of Natural History, Santa Barbara, pp. 59–70.

Miller, K. A. 1999. Molecular evidence for hybridization and introgression in kelps. Abstract, 80th Annual Meeting, Western Society of Naturalists, Monterey, California.

Mindell, D. P. & Honeycutt, R. L. 1990. Ribosomal RNA in vertebrates: evolution and phylogenetic applications. *Annu. Rev. Ecol. Syst.* 21:541–66.

Nicholson, N. L. 1978. Evolution within *Macrocystis*: Northern and Southern Taxa. *New Zeal. Dep. Sci. Industr. Res. Inform. Ser.* 137:433–42.

Neushul, M. 1971. The species of *Macrocystis*. *Nova Hedw. Heft.* 32:123–68.

North, W. J. 1971. Introduction and background. *Nova Hedw. Heft.* 32:1–97.

North, W. J. 1972. Observations on populations of *Macrocystis*. *In* Kurogi, M. & Abbott, I. A. [Eds.] *Contributions to the Systematics of Benthic Algae of the North Pacific*. Japanese Society of Phycology, Kobe, Japan. pp. 75–82.

North, W. J. 1994. Review of *Macrocystis* biology. *In* Akatsuka, I. [Ed.] *Biology of Economic Algae*. Academic Publishing, The Hague, Netherlands, pp. 447–527.

Papenfuss, G. F. 1965. Catalogue and bibliography of Antarctic and sub-Antarctic benthic marine algae. *Antarctic Res. Ser. Wash.* 1:1–76.

Parker, B. C. & Dawson, E. Y. 1965. Non-calcareous marine algae from California Miocene deposits. *Nova Hedw. Heft.*10:273–95.

Peters, A. F., van Oppen, M. J. H., Wiencke, C., Stam, W. T. & Olsen, J. L. 1997. Phylogeny and historical ecology of the Desmarestiaceae (Phaeophyceae) support a Southern Hemisphere origin. *J. Phycol.* 33:294–309.

Peters, A. F. 1998. Ribosomal DNA sequences support taxonomic separation of the two species of *Chorda*: reinstatement of *Halosiphon tomentosus* (Lyngbe) Jaasund (Phaeophyceae, Laminariales). *Eur. J. Phycol.* 33:65–71.

Pickard, G. L. & Emery, W. J. 1990. *Descriptive Physical Oceanography*, 5th ed. Pergamon Press, Oxford.

Posada, D. & Crandall, K. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–8.

Reed, D. C., Amsler, C. D. & Ebeling, A. W. 1992. Dispersal in kelps: factors affecting spore swimming and competency. *Ecology* 73:1577–85.

Sanbonsuga, Y. & Neushul, M. 1978. Hybridization of *Macrocystis* (Phaeophyta ) with other float-bearing kelps. *J. Phycol.* 14:214–24.

Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanogr. Mar. Biol. Annu. Rev.* 28:177–276.

Saunders, G. W. & Druehl, L. D. 1992. Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta): implications for kelp evolution. *J. Phycol.* 28:544–9.

Saunders, G. W. & Druehl, L. D. 1993a. Revision of the kelp family Alariaceae and the taxonomic affinities of *Lessoniopsis* Reinke (Laminariales, Phaeophyta). *Hydrobiologia* 260/261:689–97.

Saunders, G. W. & Druehl, L. D. 1993b. Nucleotide sequences of the internal transcribed spacers and 5.8S rRNA genes from *Alaria marginata* and *Postelsia palmaeformis* (Phaeophyta: Laminariales). *Mar. Biol.* 115:347–52.

Schiel, D. R. & Foster, M. S. 1986. The structure of subtidal algal stands in temperate waters. *Oceanogr. Mar. Biol. Annu. Rev.* 24:265–307.

Serrão, E. A., Alice, L. A. & Brawley, S. H. 1999. Evolution of the Fucaceae (Phaeophyceae) inferred from nrDNA-ITS. *J. Phycol.* 35:382–94.

Swofford, D. L. 2000. PAUP (Phylogenetic Analysis Using Parsimony), Version 4.0b3. Sinauer, Sunderland, Massachuesetts.

Tan, I. H. & Druehl, L. D. 1996. A ribosomal DNA phylogeny supports the close evolutionary relationships among the Sporochnales, Desmarestiales, and Laminariales (Phaeophyceae). *J. Phycol.* 32:112–8.

Tegner, M. J. & Dayton, P. K. 1987. El Niño effects on southern California kelp forest communities. *Adv. Ecol. Res.* 17:243–79.

Tegner, M. J., Dayton, P. K., Edwards, P. B. & Riser, K. L. 1996. Is there evidence for long term climatic change in southern California kelp forests? *CalCOFI Rep.* 37:111–26.

Tegner, M. J., Dayton, P. K., Edwards, P. B. & Riser, K. L. 1997. Large-scale, low-frequency oceanographic effects on kelp forest succession: a tale of two cohorts. *Mar. Ecol. Prog. Ser.* 146: 117–34.

tom Dieck, I. 1993. Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographic implications. *Mar. Ecol. Prog. Ser.* 100:253–64.

United States Defense Mapping Agency. 1988. *Sailing Directions (Planning Guide) for the South Atlantic Ocean*, 2nd ed. Publication No. 121. Defense Mapping Agency Hydrographic/Topographic Center, Washington DC.

Van Oppen, M. J. H., Olsen, J. L., Stam, W. T., van den Hoek, C. & Wiencke, C. 1993. Arctic-Antarctic disjunctions in the benthic seaweeds *Acrosiphonia arcta* (Chlorophyta) and *Desmarestia viridis/willii* (Phaeophyta) are of recent origin. *Mar. Biol.* 115:381–6.

van Tussenbroek, B. I. 1989. Smooth-bladed *Macrocystis* (Laminariales, Phaeophyta) from the Falkland Islands. *Phycologia* 28:520–3.

Weaver, A. J. 1990. Ocean currents and climate. *Nature* 47:432.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* Innis, M. A., Gelfand, D. H., Sminsky, J. J. & White, T. J. [Eds.] *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–24.

Womersley, H. B. S. 1954. The species of *Macrocystis* with special reference to those on southern Australian coasts. *University Calif. Publ. Bot.* 27:109–32.

Yotsukura, N., Denboh, T., Motomura, T., Horiguchi, T., Coleman, A. W. & Ichimura, T. 1999. Little divergence in ribosomal DNA internal transcribed spacer 1 and 2 sequences among non-digitate species of *Laminaria* (Phaeophyceae) from Hokkaido, Japan. *Phycol. Res.* 47:71–80.