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Grateloupia turuturu (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*

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Grateloupia doryphora (Montagne) Howe, originally described from Peru, has repeatedly been reported as an invasive species in Atlantic and Mediterranean waters. Various attempts to explain this species' route of introduction have been unsatisfactory. New evidence from comparative *rbc*L sequence analysis and morphology suggests that this adventive species in the NE and NW Atlantic corresponds with *G. turuturu* Yamada, originally described from Japan. This provenance follows a well-recognized trend of invasive marine organisms that have colonized the Atlantic Ocean and Mediterranean Sea from Pacific NE Asia.

Key words: biogeography, *Grateloupia doryphora*, *Grateloupia turuturu*, Halymeniaceae, invasive alga, molecular systematics, phylogeny, *rbc*L, Rhodophyta, taxonomy

Introduction

The genus *Grateloupia* C. Agardh, with 51 currently recognized species, is the largest in the family Halymeniaceae (Kraft, 1977). Species of the genus are considered difficult to define (Ardré & Gayral, 1961; Irvine & Farnham, 1983; Cabioch *et al*., 1997), as many are very variable in gross morphology. However, recent molecular analyses are beginning to elucidate the taxonomic status of some morphologically similar species (Wang *et al*., 2000; Kawaguchi *et al*., 2001).

Grateloupia doryphora (Montagne) Howe (1914: 169–170) is a commonly reported intertidal alga throughout Pacific South America (Dawson *et al*., 1964; Santelices, 1988). The species was originally described from Callao, central Peru (as *Halymenia doryphora* Montagne) and has been reported widely along coasts of Chile, Pacific Mexico, Baja California, and the Pacific US (e.g. Abbott & Hollenberg, 1976; Ramírez & Santelices, 1991; Gonzáles-Gonzáles *et al*., 1996; Hoffman & Santelices, 1997).The species has been the focus of much recent research because it has been reported as an introduced species in the Atlantic, both in western Europe (Farnham & Irvine, 1973; Cabioch *et al*., 1997; Maggs & Stegenga, 1999) and eastern North America (Villalard-Bohnsack & Harlin, 1997), and also in the Mediterranean Sea (De Masi & Gargiulo, 1982; Tolomio, 1993). In the present paper we clarify the taxonomic position of this entity on the basis of molecular and morphological data, and provide a possible interpretation of its route of introduction.

This paper is part of a large revisionary study of the genus *Grateloupia* and other members of the Cryptonemiales (Gavio, unpublished) based on sequence analysis of chloroplast-encoded *rbc*L. This gene was selected because a large number of studies on different orders of red algae show that it is an adequate molecular marker for the determination of species boundaries (e.g. Freshwater *et al*., 1995; Hommersand *et al*., 1999; Fredericq *et al*., 1999, 2002).

Materials and methods

Morphological analyses

Specimens for morphological analyses were either fixed in 5% formalin/seawater, pressed as herbarium sheets, or were silica gel-dried, and deposited in the herbarium of the University of Louisiana at Lafayette (LAF).

Whole-mount slides, cross-sections and longitudinal sections were made by hand with a stainless steel razor blade. Photographs of unstained sections were taken on an Olympus BX60 Photomicroscope (Olympus, Melville, NY, USA) with a Polaroid DMC Ie digital camera (Polaroid, Cambridge, MA, USA). Habits of specimens were scanned using a Microtek Scanmaker III (Microtek, Redonda Beach, CA, USA). Digital images were edited and assembled on plates using Photoshop 5.0.

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Table 1. List of species used in *rbc*L analysis with GenBank accession numbers

Species	Location	Collector, collection date	*rbc*L portion sequenced	GenBank accession number
Cryptonemia borealis Kylin	Hein Bank, Washington, USA	S. Lindstrom, 9/VIII/93	41–1467 (97·27%)	AF488812
Cryptonemia luxurians (C. Agardh) J. Agardh	Praia Rasa, Rio de Janeiro, Brazil	C.F. Gurgel, 12/XII/98	52–1467 (96·52%)	AF488813
Grateloupia americana Kawaguchi *et* Wang	Whan Park, near Sirka (Baranof Island), Alaska, USA	S. Lindstrom, 21/IV/00	38–1467 (97·37%)	AF488814
Grateloupia asiatica Kawaguchi *et* Wang	Qingdao, Shandong Province, China		107–1365 (85·82%)	*AB055488 (Kawaguchi, *et al.*, 2001)
Grateloupia dichotoma J. Agardh	Ubatuba, Brava Beach, São Paulo State, Brazil	S. Guimarães, 26/V/01	41–1467 (97·27%)	AF488824
Grateloupia dichotoma J. Agardh	Marataizes, Espiritu Santu, Brazil	S. Guimarães & M. Fujii, 15/IX/01	9–1467(99·45%)	AF488823
Grateloupia doryphora (Montagne) Howe	Playa de San Francisco, Bahia de Ancon, Ancon, Lima, Peru	P. Carbájal, 15/IX/01	9–1467 (99·45%)	AF488817
Grateloupia elliptica Holmes	Goshikinohama, Usa, Tosa, Kochi Prefecture, Japan		107–1365 (85·82%)	*AB055476 (Kawaguchi, *et al.*, 2001)
Grateloupia filiformis Kützing	Marataizes, Espiritu Santu, Brazil	S. Guimarães & M. Fujii, 15/IX/01	11–1467 (99·31%)	AF488822
Grateloupia lanceolata (Okamura) Kawaguchi	Oshoro, Hokkaido, Japan		107–1365 (85·82%)	*AB055478 (Kawaguchi *et al.*, 2001)
Grateloupia livida (Harvey) Yamada	Muroran, Hokkaido, Japan	S. Fredericq, 6/IX/93	40–1467 (97·34%)	AF488815
Grateloupia livida (Harvey) Yamada	Shinori, Hakodate, Hokkaido, Japan		107–1362 (85·61%)	*AB055481 (Kawaguchi, *et al.*, 2001)
Grateloupia ramosissima Okamura	Ho Ping Island, Keelung, North Taiwan	S. Fredericq, 12/VIII/93	40–1467 (97·34%)	AF488810
Grateloupia ramosissima Okamura	Ho Ping Dao, Keelung, North Taiwan	S. Fredericq, 5/VII/94	38–1467 (97·37%)	AF488811
Grateloupia schizophylla Kützing	Montemar, Chile	S. Fredericq, 16/I/95	393–1467 (73·27%)	AF488825
Grateloupia sp.	Sealion, E. Falkland Island	S. Fredericq, 7/I/98	41–1467 (97·27%)	AF488827
Grateloupia sp.	La Boca Navidad, Central Chile	S. Fredericq & M.E. Ramírez, 17/I/95	41–1467 (97·27%)	AF488826
Grateloupia stipitata J. Agardh	Lee Bay, Stewart Island, New Zealand (WELT A26150)	L. Phillips & W. Nelson, 18/X/01	9–1467 (99·45%)	AF488816
Grateloupia turuturu Yamada	Muroran, Hokkaido, Japan	S. Fredericq, 6/IX/93	9–1467 (99·45%)	AF488820
Grateloupia turuturu Yamada	Wolpo, East Korea	E.C. Yang & S.M. Boo, 31/I/02	9–1467 (99·45%)	AY083215
Grateloupia turuturu Yamada	Wolpo, East Korea	E.C. Yang & S.M. Boo, 31/I/02	43–1467 (97·13%)	AF488821
Grateloupia turuturu Yamada (as *G.* '*doryphora*')	Bristol, Rhode Island, USA	M. Villalard-Bohnsack, 20/VIII/00	40–1467 (97·34%)	AF488818
Grateloupia turuturu Yamada (as *G.* '*doryphora*')	Bristol, Rhode Island, USA	M. Villalard-Bohnsack, 20/VIII/00	9–1467 (99·45%)	AY100004
Grateloupia turuturu Yamada (as *G.* '*doryphora*')	Montauk Park, New York, NY, USA	E. Rolla, 10/XII/01	9–1467 (99·45%)	AF488819
Grateloupia turuturu Yamada (as *G.* '*doryphora*')	Ile Callot, Baie de Morlaix, Brittany, France	A.F. Peters, 16/XII/01	9–1467 (99·45%)	AY083216
Grateloupia turuturu[a] Yamada (as *G.* '*doryphora*')	Ile Callot, Baie de Morlaix, Brittany, France	A.F. Peters, 21/II/02	8–1467 (99·52%)	AY100003
Grateloupia turuturu Yamada (as *G.* '*doryphora*')	Langstone Harbour, The Kench, Hayling Island, Hampshire, United Kingdom	R.L. Fletcher, 28/III/02	9–1467 (99·45%)	AY100002
Grateloupia turuturu Yamada (as *G.* '*doryphora*')	Onahama, Iwaki, Fukushima Prefecture, Japan		107–1365 (85·82%)	*AB055475 (Kawaguchi, *et al.*, 2001)

*Denotes sequence downloaded from GenBank.
[a]From cultured material.

Nomenclature

We have provisionally adopted the latest nomenclatural changes merging species of *Prionitis* J. Agardh into *Grateloupia* C. Agardh (Wang *et al*., 2001) to keep this paper focused on the identity of the invasive species. A comprehensive study discussing the generic and species limits of these halymeniacean taxa will be addressed elsewhere.

Molecular analyses

Algal samples for molecular analyses were desiccated in the field in silica gel. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's instructions or by grinding a small sample (4–6 mm^2) with liquid nitrogen, and placing it in 60 μl extraction buffer, prepared with 6 μl 10 × PCR buffer (Perkin Elmer, Foster City, CA, USA), 3 μl 10 % polyvinylpyrrolidone (PVP), 0·6 μl proteinase K (at 100 μg/ml), 50·4 μl nanopure water. The samples were vortexed and placed in a waterbath at 65 °C overnight; they were then heated at 95 °C for 5 min, 140 μl nanopure water was added and the sample was centrifuged at maximum speed for 10 min. The supernatant was transferred to a new tube and used immediately for *rbc*L amplification. Silica gel-dried specimens and extracted DNA samples are deposited in the Seaweed Laboratory at the University of Louisiana at Lafayette and stored at −20 °C. Twenty-two vouchers newly selected for DNA sequence analysis are listed in Table 1 together with their GenBank accession numbers.

The gene selected was chloroplast-encoded *rbc*L; primers used for gene amplification include the following primer combinations: F7-R753, F577-R1381, and F993-R*rbc*Sstart (Freshwater & Rueness, 1994). The F7 primer (5′ AAC TCT GTA GAA CGN ACA AG 3′) was designed by Miguel Volovshek (personal communication). For *rbc*L amplification, 1–4 μl of the resulting extractions were used as templates for a 50 μl PCR consisting of 10 μl 5 M betaine, 6 μl 10 × PCR buffer (Perkin Elmer), 6 μl 25 mM $MgCl_2$ solution, 8 μl 500 mM dNTP stock, 2 μl each of the appropriate 10 mM primers and 0·3 μl Amplitaq DNA Polymerase (PE Applied Biosystems, Foster City, CA, USA). Amplification conditions consisted of 4 min at 96 °C for denaturation, followed by 35 cycles of 60 s at 94 °C, 60 s at 42 °C and 90 s at 72 °C, with a final 10 min extension cycle at 72 °C and soak cycle at 10 °C. The polymerase chain reaction was performed on a PE GenAmp PCR system 9700 or 2400 (PE Applied Biosystems). For automated gene sequencing, the amplified products were cleaned using the Prep-A-Gene DNA Purification Kit (BioRad, Hercules, CA, USA) following the manufacturer's recommendation. The concentration of the template was then estimated using either a Hoefer TKO 100 fluorometer (Hoefer Scientific Instrument, San Francisco, CA, USA), following the manufacturer's instructions, or by running 1 μl of the template on a 1 % agarose minigel and comparing the brightness of the band with known DNA concentrations. The sequences were determined over both strands using an ABI Prism 310 or 3100 Genetic Analyzer (PE Applied Biosystems) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The same primers used for gene amplification were used for sequencing, but an additional primer (R376: 5′ GCT TTA AAR CCR AAT ACR TTA CC-3′) was newly designed to sequence the beginning of the gene. The reactions were performed in 20 or 10 μl, and comprised the following: 2 μl Terminator Ready reaction mix, 2 μl X buffer, 1·6 μl 1 mM primer, 18–20 ng of template and nanopure water up to a total volume of 10 μl; for the 20 μl reactions, all the volumes were doubled. The cycle sequencing reactions were performed on a PE GenAmp PCR system 9700 or 2400 (PE Applied Biosystems) for 28 cycles (96 °C for 10 s, rapid thermal ramp to 50 °C, 50 °C for 5 s, rapid thermal ramp to 60 °C, 60 °C for 4 min, rapid thermal ramp to 10 °C). Resulting products were then purified using Centri-Sep spin columns (P/N CS-901, Princeton Separations, Adelphie, NJ, USA). For most samples, the entire *rbc*L-coding region, 1467 bp, was newly sequenced except for the first 8–10 base pairs (Table 1).

The generated sequence data were compiled and aligned with Sequencher (Gene Codes, Ann Arbor, MI, USA) and exported for phylogenetic analysis in PAUP and MacClade (Madison & Madison, 2000). Phylogenetic analyses were performed using the Maximum Parsimony, Neighbor Joining and Maximum Likelihood algorithms available in the computer program PAUP (v. 4.0b10; Swofford, 2002). For Maximum Likelihood the aligned sequences were first analysed with the software Modeltest v. 3.0 (Posada & Crandall, 1998), which compared different models of DNA substitutions in a hierarchical hypothesis-testing framework to select a base substitution model that best fitted the sequence data. The optimal model found was a TrN+I+G evolutionary model (Tamura–Nei model + Invariable sites + Gamma distribution). The parameters were as follows: assumed nucleotide frequencies A = 0·3077, C = 0·1613, G = 0·2162, T = 0·3148; substitution rate matrix with A–C substitutions = 1·0000, A–G = 5·4975, A–T = 1·0000, C–G = 1·0000, C–T = 13·2146, G–T = 1·0000; proportion of sites assumed to be invariable = 0·3699; rates for variable sites assumed to follow a gamma distribution with shape parameter = 0·3332. These values were imported into a Maximum Likelihood analysis using heuristic search (PAUP).

Support for nodes was determined by calculating bootstrap proportion values (Felsenstein, 1985) using neighbor joining (5000 bootstrap resamplings), maximum parsimony (5000 bootstrap resamplings) and maximum likelihood methods (100 bootstrap resamplings).

Five additional *rbc*L sequences included in the analyses were obtained from GenBank (Table 1). Two species of *Cryptonemia* (Halymeniaceae) were used as the outgroup.

Results

Twenty-three samples representing 13 species of *Grateloupia* and two outgroup species of the Halymeniaceae (Halymeniales) were newly sequenced for inclusion in a phylogenetic tree (Fig. 1). Intraspecific *rbc*L sequence difference within the genus *Grateloupia* was typically very low. For example, *Grateloupia dichotoma* J. Agardh from two localities in Brazil, *G. ramosissima* Okamura from two localities in Taiwan, *G. livida* (Harvey) Yamada from two localities in Japan, and *G.*

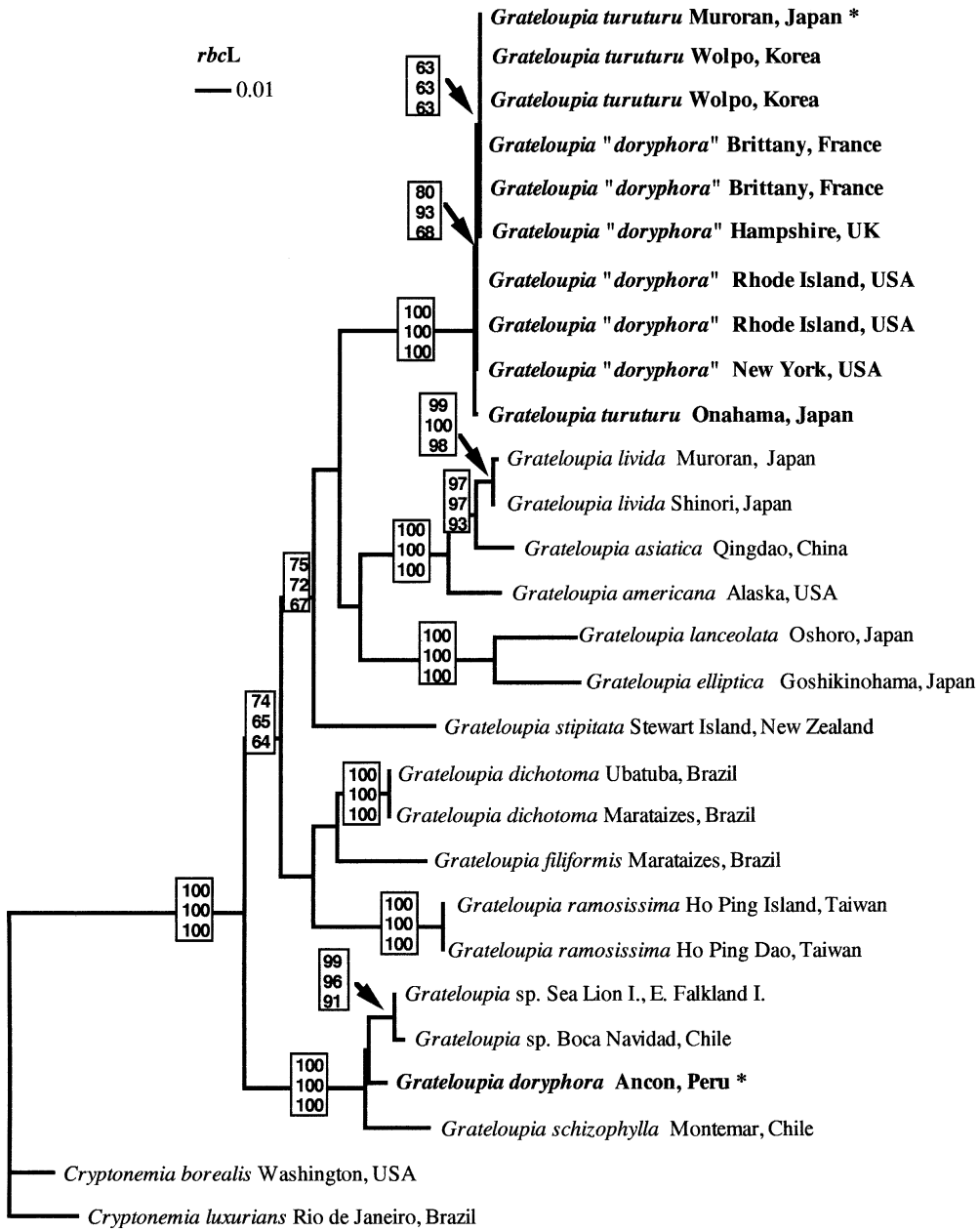


Fig. 1. Maximum likelihood tree for *rbc*L sequences showing the distant positions of *Grateloupia doryphora* and *G. turuturu* within the genus using *Cryptonemia* spp. as the outgroup. *Refers to topotype material. Tree length = 540 steps, CI = 0·58, RI = 0·81, informative characters = 254 out of 1436 included sites (= 18%). Bootstrap proportion values (> 50%) for maximum parsimony (top, 5000 replicates), neighbor joining (middle, 5000 replicates) and maximum likelihood (bottom, 100 replicates) are shown at the nodes. Branch lengths are proportional to the amount of sequence change.

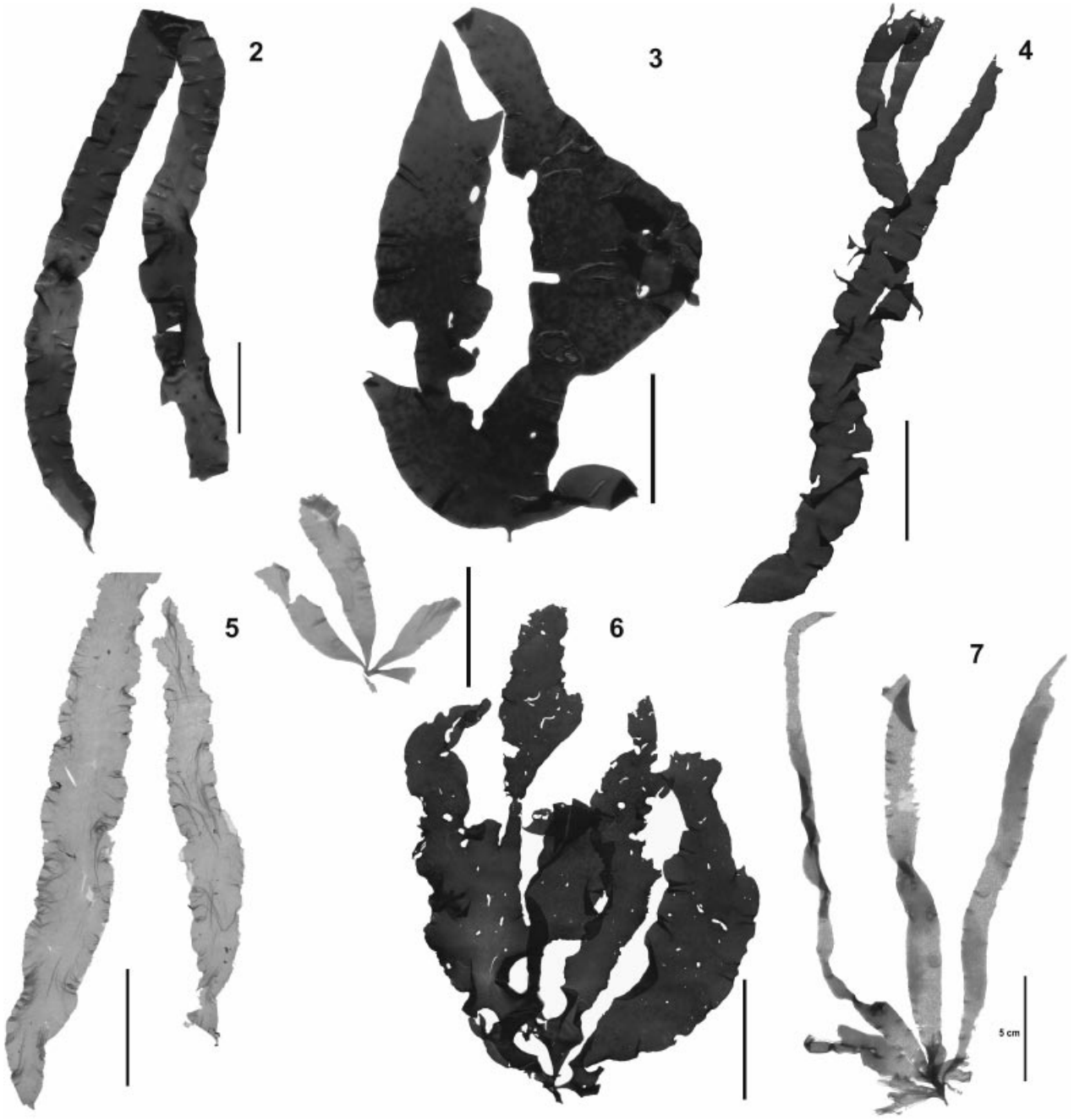
turuturu from six separate localities (Fig. 1) showed 0–2 base pair differences (99·9–100% sequence identity). By contrast, interspecific *rbc*L sequence difference among *Grateloupia* species varied from 1·5% to 10·6% (Table 2).

In the analysis we included material of *Grateloupia doryphora* collected from Ancon, Peru, about 35 km north of the type locality, Callao, Peru (Fig. 2). The *rbc*L sequences of two western Atlantic populations of algae also going under the name of *G. doryphora* – one from Rhode Island, USA, and one from New York State, USA (Fig. 3) – and two eastern Atlantic populations from Brittany, France (Fig. 4) and Hampshire, UK were 100% identical, but differed from the Peruvian sample by 118 base pairs, equivalent to 8·2% sequence difference (Table 2).

The *rbc*L sequence of the western Atlantic *G.* '*doryphora*' differed only by one base pair (position 1075, non-synonymous substitution, G instead of A) from that of topotype material of *Grateloupia turuturu* Yamada (1941 : 205, pl. 46) from Muroran, Hokkaido, Japan (Fig. 5). The sequence of *G.* '*doryphora*' from the eastern Atlantic was identical to the *G. turuturu* topotype, as well as to the material from Wolpo, East Korea (Fig. 6). A sequence of *G. turuturu*, from Onahama, Fukushima Prefecture, Japan, downloaded from GenBank (Kawaguchi *et*

Table 2. Uncorrected p distances (percentage) in *rbc*L sequences among the species of *Cryptonemia* and *Grateloupia* used in this study

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	*Cryptonemia borealis*	–																										
2	*C. luxurians*	2·90	–																									
3	*G. livida* (Muroran)	9·41	9·53	–																								
4	*G. livida* (Shinori)	10·11	10·27	0·08	–																							
5	*G. stipitata*	9·27	9·32	6·04	6·05	–																						
6	*G. doryphora*	8·29	8·40	7·30	7·72	7·09	–																					
7	*G.* sp. (Falkland I.)	8·29	8·62	7·16	7·48	7·37	1·26	–																				
8	*G.* sp. (Chile)	8·36	8·55	7·16	7·56	7·44	1·55	0·28	–																			
9	*G. schizophylla*	9·67	9·95	8·37	8·76	8·00	2·33	2·33	2·61	–																		
10	*G. turuturu* (Muroran)	8·50	8·83	6·39	6·69	6·81	8·29	8·15	8·15	10·05	–																	
11	*G. turuturu* (Korea)	8·50	8·83	6·39	6·69	6·81	8·29	8·15	8·15	10·05	0·00	–																
12	*G. turuturu* (Korea)	8·50	8·83	6·39	6·69	6·81	8·29	8·15	8·15	10·05	0·00	0·00	–															
13	*G.* '*doryphora*' (France)	8·50	8·83	6·39	6·69	6·81	8·29	8·15	8·15	10·05	0·00	0·00	0·00	–														
14	*G.* '*doryphora*' (France)	8·50	8·83	6·39	6·69	6·81	8·29	8·15	8·15	10·05	0·00	0·00	0·00	0·00	–													
15	*G.* '*doryphora*' (RI)	8·43	8·76	6·32	6·61	6·74	8·22	8·08	8·08	9·95	0·07	0·07	0·07	0·07	0·07	–												
16	*G.* '*doryphora*' (RI)	8·43	8·76	6·32	6·61	6·74	8·22	8·08	8·08	9·95	0·07	0·07	0·07	0·07	0·07	0·00	–											
17	*G.* '*doryphora*' (NY)	8·43	8·76	6·32	6·61	6·74	8·22	8·08	8·08	9·95	0·07	0·07	0·07	0·07	0·07	0·00	0·00	–										
18	*G. turuturu* (Onahama)	9·06	9·45	6·67	6·53	7·07	8·98	8·74	8·66	10·59	0·24	0·24	0·24	0·24	0·24	0·16	0·16	0·16	–									
19	*G. asiatica*	9·69	9·85	1·59	1·43	6·35	7·78	7·55	7·63	8·94	6·83	6·83	6·83	6·83	6·83	6·75	6·75	6·75	6·59	–								
20	*G. lanceolata*	10·56	10·88	7·23	7·09	7·39	9·45	9·37	9·45	10·48	7·86	7·86	7·86	7·86	7·86	7·78	7·78	7·78	7·71	6·91	–							
21	*G. elliptica*	10·49	10·41	7·78	7·64	7·55	9·53	9·45	9·53	10·07	7·86	7·86	7·86	7·86	7·86	7·78	7·78	7·78	7·71	7·47	4·37	–						
22	*G. dichotoma* (Ubatuba)	7·65	8·33	6·81	7·09	5·83	6·39	6·53	6·60	7·91	6·18	6·18	6·18	6·18	6·18	6·11	6·11	6·11	6·35	6·83	7·86	7·63	–					
23	*G. dichotoma* (Marataizes)	7·65	8·33	6·81	7·09	5·83	6·39	6·53	6·60	7·91	6·18	6·18	6·18	6·18	6·18	6·11	6·11	6·11	6·35	6·83	7·86	7·63	0·00	–				
24	*G. filiformis*	7·58	7·91	6·39	6·61	6·25	6·67	6·67	6·81	8·09	5·76	5·76	5·76	5·76	5·76	5·69	5·69	5·69	5·88	6·51	7·71	7·71	3·51	3·51	–			
25	*G. ramosissima* (H.P.D.)	8·01	8·19	7·94	8·20	7·16	7·16	7·02	7·02	8·28	7·09	7·09	7·09	7·09	7·09	7·02	7·02	7·02	7·47	8·18	8·90	8·34	4·49	4·49	5·62	–		
26	*G. ramosissima* (H.P.I.)	8·01	8·19	7·94	8·20	7·16	7·16	7·02	7·02	8·28	7·09	7·09	7·09	7·09	7·09	7·02	7·02	7·02	7·47	8·18	8·90	8·34	4·49	4·49	5·62	0·00	–	
27	*G. americana*	9·69	9·46	2·53	2·39	6·46	7·23	7·09	7·23	8·93	6·46	6·46	6·46	6·46	6·46	6·39	6·39	6·39	6·67	3·02	6·91	7·63	6·74	6·74	6·32	7·65	7·65	–



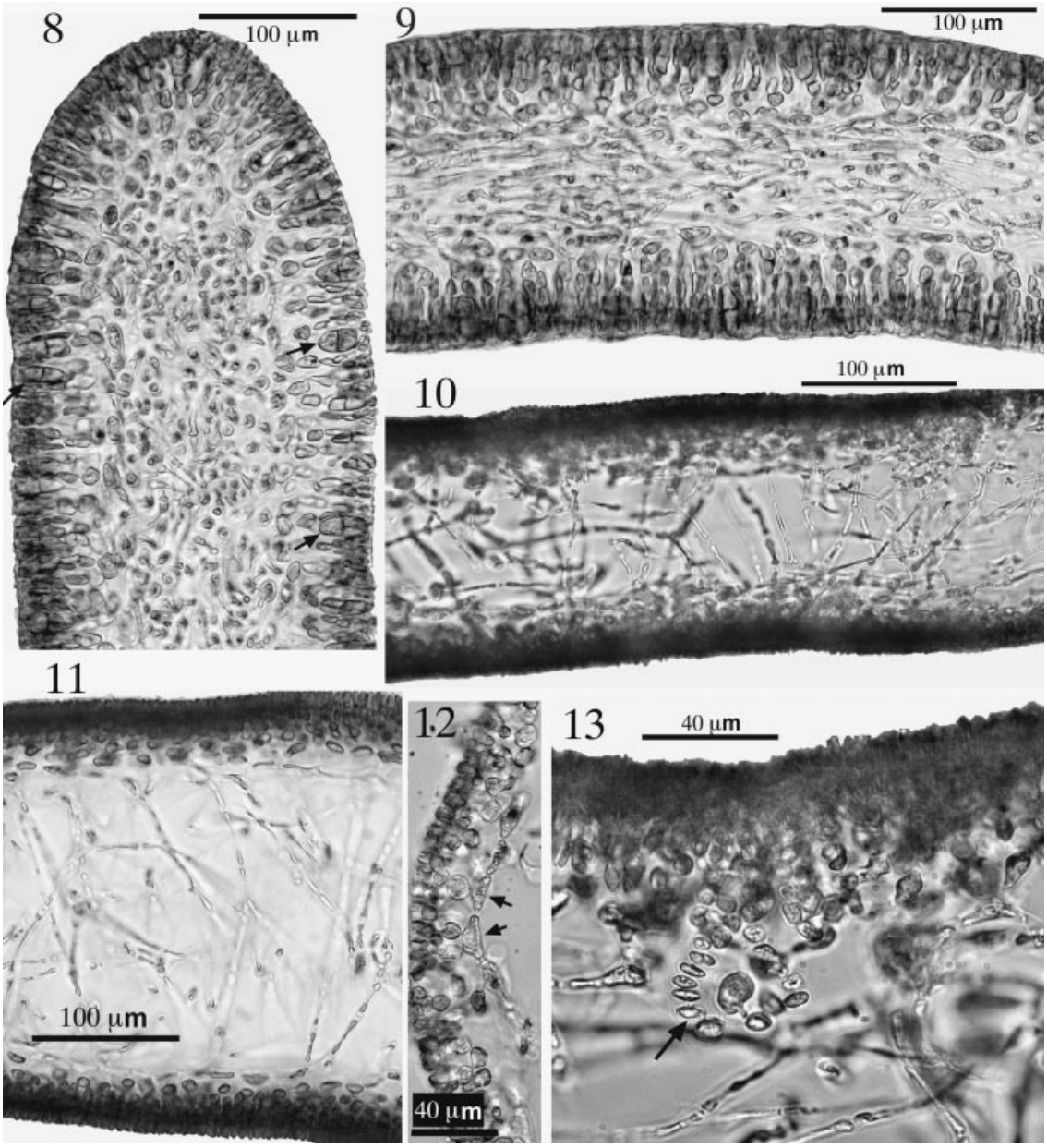
Figs 2–7. Habit of *Grateloupia doryphora* and *G. turuturu* specimens used in this study. Fig. 2. *G. doryphora*, tetrasporophyte, Bahia de Ancon, Peru. Fig. 3. *G. turuturu*, female gametophyte, Montauk Park, New York, USA. Fig. 4. *G. turuturu*, cystocarpic specimen, Ile Callot, Baie de Morlaix, Brittany, France. Fig. 5. *G. turuturu*, tetrasporophyte, Muroran (type locality), Japan. Fig. 6. *G. turuturu*, cystocarpic specimen, Wolpo, East Korea, Fig. 7. *G. schizophylla*, Montemar, Central Chile. All scale bars represent 5 cm.

al., 2001) differed by 2 base pairs from the newly sequenced *G. turuturu* from Muroran.

An unidentified *Grateloupia* species from Central Chile (Boca Navidad) differed by 4 nucleotides (0·28 % sequence difference) from a specimen from Sea Lion I., E. Falkland Islands, in the South-western Atlantic; these apparently conspecific taxa differed by 18/22 bp (1·3/1·6 %) from the Peruvian *G. doryphora*. Another species from Montemar, Valparaiso, Central Chile, identified as *G. schizophylla* Kützing (1867: 11; pl. 36), originally described from Chile (Dawson 1954: 250) (Fig. 7), differed by 25 bp (2·3 %) from the Peruvian *G. doryphora*, and is clearly a distinct entity (Table 2).

Neighbor-joining, maximum parsimony and maximum likelihood analyses were performed, with only the maximum-likelihood-based tree shown here (Fig. 1). The difference between the three analyses lies in the unsupported position of *G. stipitata* J. Agardh from New Zealand.

The three Pacific South American species centred around *G. doryphora* formed a strongly supported clade (100 % bootstrap), separate from an equally strongly supported clade (100 % bootstrap) encom-

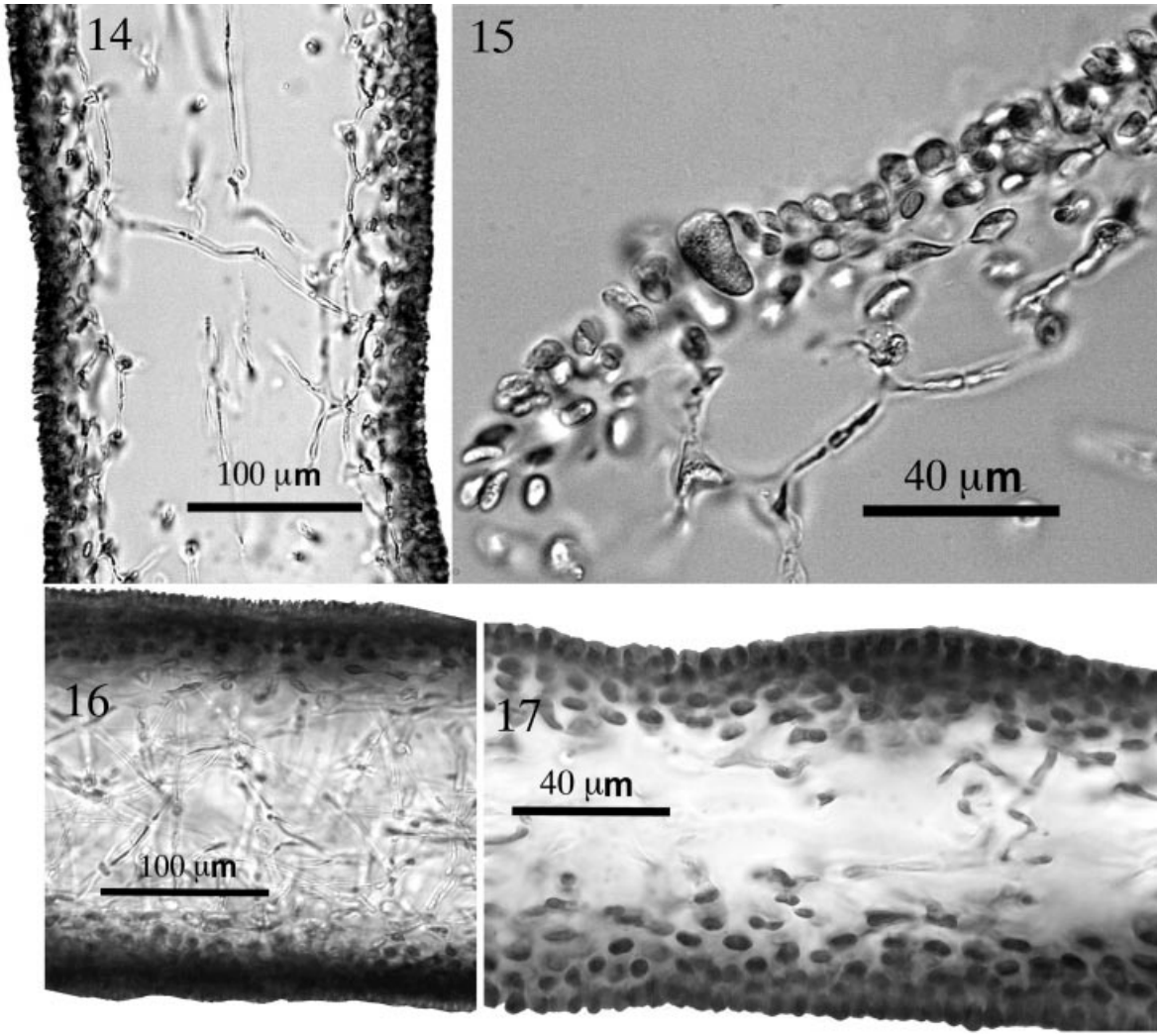


Figs 8–13. *Grateloupia doryphora* and *G. turuturu*. Vegetative and reproductive morphology. Figs 8, 9. *G. doryphora* from Bahia de Ancon, Peru. Fig. 8. Cross-section through tetrasporophytic specimen showing dense medulla of small roundish cells, and a 4- to 6-layered cortex of elliptical cells. Arrows point to tetrasporangia. Fig. 9. Longitudinal section through tetrasporophytic specimen showing predominant periclinal arrangement of medullary filaments, and gradual transition between cortex and medulla. Figs 10–13. *G. turuturu*. Figs 10, 11. Cross-section through a specimen from Montauk Park, New York, showing abrupt transition between medulla and cortex, and significant anticlinal arrangement of medullary cells in both dense (Fig. 11) and lax medulla (Fig. 10). Fig. 12. Longitudinal section showing expanded innermost cortical cells (arrows) in a specimen from Bristol, Rhode Island. Fig. 13. Cross-section showing a simple and unbranched auxiliary cell ampulla (arrow) in a female gametophyte from Montauk Park, New York.

passing the western and eastern Atlantic *G.* '*doryphora*' and the NW Pacific *G. turuturu* (Fig. 1).

Morphology

Transverse sections of *G. doryphora* from Peru show a tetrasporangial cortex of 4–6 cell layers of elliptical cells and a medulla consisting of a dense mass of small roundish cells (Fig .8). The rhizine-like medullary arrangement results from the fact that in longitudinal section (Fig. 9) the medullary filaments run rather parallel in an adaxial direction and are evenly interwoven without producing many secondary filaments linked to the opposite side. This results in the typical periclinal arrangement of medullary filaments as shown in longitudinal sections (Fig. 9), and absence of significant anticlinal filaments in transverse section (Fig. 8). The innermost cortical cells do not expand significantly, and the transition from cortex to medulla is gradual (Figs 8, 9). By contrast, in the Atlantic material, the cortex consists of fewer layers of roundish cells



Figs 14–17. *Grateloupia turuturu*. Vegetative and reproductive morphology. Figs 14, 15. Cross-sections through a tetrasporangial topotype specimen from Muroran, Japan. Fig. 14. Sparse anticlinally arranged medullary filaments, and abrupt transition between cortex and medulla. Fig. 15. Tetrasporangial initial in outer cortex. Fig. 16. Cross-section through a specimen from Wolpo, Korea showing numerous secondarily produced medullary filaments. Fig. 17. Specimen from Roscoff, France, showing sparse medullary filaments.

(Figs 10–13, 17) and the transition between medulla and cortex is abrupt. Medullary filaments in younger thallus parts are sparse (Fig. 11) and become more numerous in older regions (Fig. 10). Typically, secondarily produced medullary filaments consisting of 6–8 cells and originating from lower cortical cells frequently cross towards the opposite thallus side where they link to medullary cells via secondary pit connections, resulting in a predominant anticlinal medullary arrangement in both transverse (Figs 10, 11, 17) and longitudinal section (Figs 12, 13). The innermost cortical cells expand significantly as shown in both transverse (Fig. 11) and longitudinal sections (Fig. 12). This cortical and medullary cell arrangement corresponds to the vegetative structure in W. Pacific *Grateloupia turuturu*. In young material (Fig. 14) bearing tetrasporangial initials (Fig. 15), medullary filaments are sparse and show a significant anticlinal arrangement in transverse section, with secondarily formed medullary filaments becoming numerous in older thalli (Fig. 16).

The arrangement of the young auxiliary cell ampulla is simple and unbranched in *G. turuturu* female gametophytes from both the Atlantic (Fig. 12) and the northwestern Pacific.

Discussion

It is clear that the populations from Rhode Island, New York, Brittany (France) and England reported in the literature as *G.* '*doryphora*' (e.g. Cabioch *et al*., 1997; Villalard-Bohnsack & Harlin, 1997) have been misidentified. Both comparative *rbc*L sequence analysis and morphology point to significant differences between the Peruvian and Atlantic material.

The *rbc*L sequences from the two eastern Atlantic populations analysed (France, UK), were identical to topotype material of *G. turuturu* from Muroran,

Japan, as well as vouchers from Wolpo, Korea. Because the western Atlantic populations (Rhode Island and New York, USA) differed from these sequences by only one non-synonymous substitution, the northwestern and northeastern Atlantic entity should therefore be known as *G. turuturu*, originally described from Hokkaido, Japan. Several entities worldwide are currently placed under the name *Grateloupia doryphora*, so careful morphological, molecular and nomenclatural analyses are needed to clarify the identity of these samples. The same taxonomic problems are also apparent in species of *Grateloupia* such as the type *G. filicina* (Lamouroux) C. Agardh, a 'species' that encompasses a number of other species (Wang *et al*., 2000; Kawaguchi *et al*., 2001; Gavio, unpublished results).

Morphologically, *G. turuturu* can be readily distinguished from *G. doryphora sensu stricto* by the anticlinal arrangement of medullary filaments, a thinner cortex of roundish cells and an abrupt transition between cortex and medulla in the former. By contrast, *G. doryphora* has a dense medulla of small rhizine-like roundish cells in transverse section, a thicker cortex of elliptical cells, a periclinal medullary arrangement in longitudinal section and a gradual transition between cortex and medulla. This arrangement persists in both young, unfertilized and reproductive thalli, differing only by the number of secondary medullary filaments formed.

Grateloupia turuturu (as '*G. doryphora*') has been the focus of much research as it has been shown to be an introduced species on both sides of the Atlantic (Cabioch *et al*., 1997; Maggs & Stegenga, 1999; Villalard-Bohnsack & Harlin, 1997), where it is invasive and currently expanding its range (Liddle, personal communication; Simon *et al*., 2001; Villalard-Bohnsack & Harlin, 2001), and the Mediterranean Sea (DeMasi & Gargiulo, 1982; Tolomio, 1993). ITS rDNA and RAPD data for the Rhode Island *G. turuturu* (as '*G. doryphora*') population are very similar to those from populations in Brittany, England and the Mediterranean (Marston & Villalard-Bohnsack, 1999). Although we did not have the opportunity to analyse material from the Mediterranean Sea, it is very likely that *G.* '*doryphora*' from this locality is also *G. turuturu* (see Verlaque, 2001).

Common features of many invasive species typically may include a larger or different habit, or variation in life history strategies (e.g. Castric-Fey *et al*., 1993; Curiel *et al*., 1998). The Atlantic *G. turuturu* specimens differ from the Asiatic specimens in gross morphology, i.e. they are routinely larger and may possess more lateral proliferations than individuals from Japan, Korea and adjacent waters (T. O. Cho, personal communication). It is well known that many allochthonous species of Japanese origin have been introduced, voluntarily or not, into Atlantic and Mediterranean waters, mainly because of aquaculture of Japanese oysters (Ribera & Boudouresque, 1995; Cabioch *et al*., 1997; Fletcher & Farrell, 1999; McIvor *et al*., 2001). Similarly, a Japanese origin of invasion is the likely scenario for the establishment of *G. turuturu* on both sides of the Atlantic and possibly the Mediterranean, and is consistent with the general trend of numerous Asiatic invaders in the European and North American Atlantic waters as a result of aquaculture introductions (e.g. Verlaque, 1981, 2001; Ribera & Boudouresque, 1995; Maggs & Stegenga, 1999; Pigeot *et al*., 2000; Aldridge & Muller, 2001; Curiel *et al*., 2001; Garcia-Meunier *et al*., 2001; McIvor *et al*., 2001; Breton *et al*., 2002).

Since intraspecific *rbc*L difference is so low in *Grateloupia* compared with other taxa (e.g. up to 2·1 % in *Polysiphonia harveyi*: McIvor *et al*., 2001), and not enough representative populations from both sides of the Atlantic and NE Asia were investigated, it is not possible on the basis of this analysis to determine the exact Asiatic population source of introduction. The eastern Atlantic *rbc*L sequences are identical to the Hokkaido, Japan, and Korean sequences, but apparently differ by 2 bp from a sample from Fukushima Prefecture, Japan. The 1 base pair substitution between the eastern and western Atlantic taxa may be indicative of two different source populations of Asiatic *G. turuturu* into the Atlantic. For this reason, the possibility of multiple invasions in the Atlantic as well as in the Mediterranean Sea (see Marston & Villalard-Bohnsack, 1999), cannot be ruled out at the moment. This question needs to be investigated further based on precise correlation of Japanese vouchers encompassing the entire distribution range of the species and using more rapidly evolving population-level molecular markers such as nuclear ITS sequences (Famá *et al*., 2000) or RAPDs.

As Ribera & Boudouresque (1995) and Cabioch *et al*. (1997) have already speculated, it is very likely that the introduction vector of *G. turuturu* in the Eastern Atlantic and Mediterranean was the import of oysters for aquaculture. Specifically, the Pacific oyster *Crassostrea gigas* Thunberg is farmed commercially in Brittany, Etang de Thau (Mediterranean France) and the Venice Lagoon (e.g. Verlaque, 1981; Cesari & Pellizzato, 1985; Riouall, 1985; Critchley *et al*., 1990; Simon *et al*., 2001).

Instead of having a wide distribution as documented in the literature (Howe, 1914; Dawson *et al*., 1964; Abbott & Hollenberg, 1976; Santelices, 1988; Ramírez & Santelices, 1991; Gonzáles-Gonzáles *et al*., 1996; Hoffmann & Santelices, 1997) the true *G. doryphora* appears to be restricted to

Peru and perhaps Chile (Gavio, unpublished results).

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