The genus *Grateloupia* C. Agardh (Halymeniaceae, Rhodophyta) in the Thau Lagoon (France, Mediterranean): a case study of marine plurispecific introductions

MARC VERLAQUE1*, PAMELA M. BRANNOCK2, TERUHISA KOMATSU3, MARTINE VILLALARD-BOHNSACK2 AND MARCIA MARSTON2

1UMR 6540, Centre d’Océanologie de Marseille, Université de la Méditerranée, Parc scientifique et Technologique de Luminy, F13288 Marseille cedex 9, France
2Department of Biology, Roger Williams University, Bristol, RI 02809, USA
3Ocean Research Institute, University of Tokyo, Tokyo 164, Japan

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Based on morphological data and molecular analyses [Nuclear ribosomal internal transcribed spacer (ITS), rbcL and mitochondrial cox2-cox3 spacer sequences] of *Grateloupia* spp. populations in the Thau Lagoon (France, Mediterranean) we demonstrated that at least five exotic species of *Grateloupia* were introduced. These include: (1) *Grateloupia asiatica*, a recently described species that was previously misidentified as *G. filicina* in Japan and *Grateloupia* sp. in the Thau Lagoon; (2) *G. lanceolata* from Japan; (3) *G. luxurians*, a Pacific species described as *G. filicina var. luxurians*; (4) *G. patens* from Japan; and (5) *G. turuturu*, a Japanese species previously misidentified as *G. doryphora* in the NE and NW Atlantic and Mediterranean Sea. These nonnative species probably were introduced in the Thau Lagoon in the 1970s along with the massive importations of Japanese oysters, *Crassostrea gigas*, into Europe for mariculture purposes. Since their introduction, they all have established large, reproductive populations with the exception of *G. patens*. The Mediterranean *Grateloupia* specimens are genetically and morphologically similar to Pacific specimens of the same species, although in the Thau Lagoon, *G. asiatica* specimens are more variable than those found in Japanese populations. This is the first report of *G. asiatica* in the Mediterranean Sea and Europe. Based on morphological data and molecular analyses (rbcL sequences) *G. subpectinata* is placed in synonymy with *G. luxurians*.

INTRODUCTION

The Thau Lagoon (France) has become one of the major hotspots of marine species introductions in the world (Verlaque 2001, 2002; Verlaque et al. 2002). The likely vector of species introductions is the importation and transport of adult oysters and spat (Hamon & Pichot 1994). With a standing stock of 25,000 t of oysters (*Crassostrea gigas* (Thunberg 1793)) and an annual production reaching 12,000–13,000 t, the Thau Lagoon is by far the leading site for mollusc shellfish aquaculture in the Mediterranean Sea (Hamon & Tournier 1990; Trousselier et al. 1991).

To date, 56 nonnative macroalgal species have been identified in the Thau Lagoon (M. Verlaque, unpublished observations), including several species of *Grateloupia* C. Agardh. *G. doryphora* (Montagne) Howe, a foliose species originally described from central Peru (*Halymenia doryphora* Montagne 1839) was recorded by Riouall et al. (1985) and Ben Maiz et al. (1986). This taxon had also been reported as an introduced species in the Atlantic, both in western Europe (Farnham & Irvine 1973; Farnham 1980; Irvine & Farnham 1983; Cabioch et al. 1997; Maggs & Stegenga 1999; Quintela et al. 1999) and eastern North America (Villalard-Bohnsack & Harlin 1997) as well as in the Mediterranean Sea (De Mas & Gargiulo 1982; Tolomio 1993). Genetic analyses of ‘*G. doryphora*’ showed that the Rhode Island (USA) population was genetically similar to specimens from Brittany, France; Portsmouth, England; Tholen Island, The Netherlands; and some specimens from the Thau Lagoon (Marston & Villalard-Bohnsack 1999, 2002). In the past, foliose specimens in warm seas belonging to the genus *Grateloupia* have been regarded as conspecific with *G. doryphora* (André & Gayral 1961; Dawson et al. 1964; Irvine & Farnham 1983). On the basis of a morphological study and biogeographical considerations, Verlaque (2001) suggested that the alga previously reported as *G. doryphora* could be a misidentification of the Japanese taxon *G. turuturu* Yamada. A subsequent rbcL sequence and morphological analyses (Gavio & Fredericq 2002) confirmed that the *G. doryphora* introduced in the NE and NW Atlantic corresponds with *G. turuturu* described from Japan. Thus it is likely that *G. doryphora* from the Mediterranean Sea is also *G. turuturu*. In addition to *G. turuturu*, Verlaque (2001) also reported four other introduced species: a foliose species, *G. lanceolata* (Okamura) Kawaguchi, and three profusely branched species, *G. filicina* (J.V. Lamouroux) C. Agardh var. *luxurians* A. Gepp & E.S. Gepp, *G. patens* (Okamura) Kawaguchi & Wang (as *Prionitis patens* Okamura) and an unidentified taxon, *Grateloupia* sp. Comparative gene sequence analysis confirmed the occurrence of two distinct foliose species of *Grateloupia* in the Thau Lagoon, which were referred to as *G. doryphora* and *Grateloupia* sp. (Marston & Villalard-Bohnsack 1999, 2002). In addition to the nonnative *Grateloupia* spp., several native taxa of *Grateloupia* have been reported in the Mediterranean Sea: *G. coriacea* Kützing, *G. cos-
| Taxon | Collection site | Specimen | Voucher specimen, collection date or reference | GenBank accession no. |
|-------|-----------------|----------|-----------------------------------------------|----------------------|
| *G. acuminata* Holmes | Kanagawa, Japan | Kawaguchi et al. (2001) | | AB055480 |
| *G. asiatica* Kawaguchi & Wang | Thau Lagoon, France | Ga1 | H7135, May 1998 | **AY775393/AY775360/AY775378** |
| *G. asiatica* | Thau Lagoon, France | Ga2 | H7143, Oct. 2000 | **AY775394/AY775361/AY775376** |
| *G. asiatica* | Thau Lagoon, France | Ga3 | H7150, Oct. 2000 | **AY775395/AY775362/AY775377** |
| *G. asiatica* | Yamaguchi, Japan | Ga4 | Kawaguchi et al. (2001) | AB055486 |
| *G. catenata* Yendo | Hokkaido, Japan | Wang et al. 2000 | | AB038613 |
| *G. dichotoma* J. Agardh | Marseille, France | Gd1 | H7102a, Oct. 2001 | **AY775387/AY775363/AY775382** |
| *G. dichotoma* J. Agardh | Marseille, France | Gd2 | H7102b, Oct. 2001 | **AY775388/NA/AY775383** |
| *G. doryphora* (Montagne) M.A. Howe | Lima, Peru | Gavio & Fredericq (2002) | | AF488817/AY775368/AY775372 |
| *G. elliptica* Holmes | Kochi, Japan | Kawaguchi et al. (2001) | | AB055476 |
| *G. filicina* (J.V. Lamouroux) C. Agardh | Marseille, France | Gf1 | H7103a, Oct. 2001 | **AY775389/AY775364/AY775379** |
| *G. filicina* | Marseille, France | Gf2 | H7103b, Oct. 2001 | **AY775390/NA/AY775380** |
| *G. filicina* | Quercianella, Italy | Gf3 | Kawaguchi et al. (2001) | AB055471 |
| *G. filicina* | Livorno, Italy | Gf4 | Kawaguchi et al. (2001) | AB055472 |
| *G. imbricata* Holmes | Fukuoka, Japan | Wang et al. (2001) | | AB061377 |
| *G. kurogii* Kawaguchi | Nagasaki, Japan | Wang et al. (2001) | | AB038606 |
| *G. lanceolata* (Okamura) Kawaguchi | Thau Lagoon, France | Gl1 | H7080, Jun. 1998 | **AY775396/AF412010*/AF4149281** |
| *G. lanceolata* | Thau Lagoon, France | Gl2 | H7091, Jun. 1998 | **AY775398/AF412011*/AF4149291** |
| *G. lanceolata* | Hyogo, Japan | Gla3 | H7064, May 2000 | **AY775385/NA/AY775370** |
| *G. lanceolata* | Fukuoka, Japan | Gla4 | Kawaguchi et al. (2001) | AB055477 |
| *G. livida* (Harvey) Yamada | Kochi, Japan | Gv1 | Kawaguchi et al. (2001) | AB055482 |
| *G. livida* | Hokkaido, Japan | Gv2 | Gavio & Fredericq (2002) | AF488815 |
| *G. luxurians* (A. Gepp & E.S. Gepp) R.J. Wilkes, L.M. McIvor & Guiry | Thau Lagoon, France | Gla1 | H7151, May 1998 | **AY775392/NA/AY775374** |
| *G. luxurians* | Thau Lagoon, France | Gla2 | H7152, May 1998 | **AY775391/AY775366/AY775375** |
| *G. luxurians* | Thau Lagoon, France | Gla3 | H7153, May 1998 | **AY775384/AY775365/AY775373** |
| *G. luxurians* | Victoria, Australia | Gla4 | De Clerck et al. (2005) | AJ868489 |
| *G. luxurians* | Atlantic, Brittany, France | Gla5 | De Clerck et al. (2005) | AJ868491 |
| *G. patens* (Okamura) Kawaguchi & Wang | Hokkaido, Japan | Gp1 | Wang et al. (2001) | AB061390 |
| *G. patens* | Chiba, Japan | Gp2 | Wang et al. (2001) | AB061392 |
| *G. ramosissima* Okamura | Kochi, Japan | Wang et al. (2001) | | AB061393 |
| *Grateloupia* sp. | Thau Lagoon, France | Gt1 | H7101, May 1998 | **AY775397/AY775369/AY775381** |
| *G. sparsa* (Okamura) Chiang | Fukuishima, Japan | Kawaguchi et al. (2001) | | AB055474 |
| *G. subsectinata* Holmes | Wakayama, Japan | Gs1 | Faye et al. (2004) | AB114208 |
| *G. subsectinata* | Aichi, Japan | Gs2 | Faye et al. (2004) | AB114213 |
| *G. turuturu* Yamada | Thau Lagoon, France | Gt1 | H2826, May 1998 | NA/AF412022*/AF4149271** |
| *G. turuturu* | Thau Lagoon, France | Gt2 | H2827, May 1998 | **AY775399/AF412023*/AF4149261** |
| *G. turuturu* | Rhode Island, USA | Gt3 | Marston & Villalard-Bohnsack (2002) | **AY775386/AF412015/AF414915** |
| *G. turuturu* | Hokkaido, Japan | Gt4 | Gavio & Fredericq (2002) | AF488820/AY775367/AY775371 |
| *Halymenia floresia* (Clemente y Rubio) C. Agardh | Kedah, Malaysia | Wang et al. 2000 | | AB038603 |

1 Sequence reference, Marston & Villalard-Bohnsack (2002).
Grateloupia spp. of the Thau Lagoon. However, the status of these taxa is uncertain except for *G. dichotoma* and *G. filicina*.

In this study, we re-examined old collections of *G. dory-
Figs 2–14. *Grateloupia asiatica* Kawaguchi & Wang from the Thau lagoon.

Figs 2–4. Habit (H7135-female, H7147-female and H7149-female). Bars = 1 cm.

Fig. 5. Portion of axis showing lateral proliferations (H7135). Bar = 0.5 cm.

Fig. 6. Longitudinal section of thallus (H7112). Bar = 50 μm.

Fig. 7. Longitudinal section of cortex (H7112). Bar = 20 μm.

Fig. 8. Surface view of thallus (H7158). Bar = 20 μm.

Fig. 9. Auxiliary cell ampulla (H7135). Bar = 10 μm.

Fig. 10. Carpogonial branch ampulla (H7135). Bar = 10 μm.
phora and other Grateloupia spp. from the Thau Lagoon and performed a comparative morphological and molecular genetic study of populations of Grateloupia spp. from the Thau Lagoon, the Atlantic and Indo-Pacific. The objectives of the present study were (1) to unravel the taxonomic status of exotic Grateloupia spp. in the Thau Lagoon; (2) to confirm the misidentification of G. turuturu as G. doryphora in this coastal lagoon; and (3) to examine the possibility that the introductions of Grateloupia spp. were from Japan or Korea.

MATERIAL AND METHODS

Specimen collection and herbariums

Observations and sampling in the Thau Lagoon (7000 ha, mean depth 3.8–4.5 m, maximum depth 10 m; Fig. 1) were performed from September 1994 to April 2003. Specimens were hand-collected in shallow water (0 to – 1 m mean low water) and preserved in buffered 4% formaldehyde–seawater. Previous collections from 1984, 1986, 1988, 1990, 1993, 1997 and 1998 (coll. N. Ben Maiz, M. Gerbal, H. Nedelec and M. Pellegrini) were also re-examined. Fixed material and dried specimens were studied under the light microscope. Materials were sectioned manually with a razor blade. Transverse sections were stained in 1% aqueous Aniline Blue, washed, and then acidified with a drop of 1 N HCl. Photomicrographs were made using a Nikon Optiphot-2 (Nikon, Tokyo, Japan).

The following samples of Grateloupia spp. were studied (see Fig. 1 for details of locations in the Thau Lagoon).

Grateloupia asiatica Kawaguchi & Wang. Thau Lagoon: Mèze (N. Ben Maiz, December 1984, H7107); Bouzigues (M. Verlaque, October 1994, H2597); Bouzigues and Mèze (M. Verlaque, September 1995, H2871 and H7110–H7119); Le Moure Blanc (M. Verlaque, June 1996, H7157–H7166); Bouzigues and Mèze (M. Verlaque, June 1996, H7167–H7174); Bouzigues and Mèze (M. Verlaque, October 1997, H7175–H7178 and H7120); Le Moure Blanc (M. Verlaque, October 1997, H7122–H7133); Bouzigues (M. Verlaque, May 1998, H7134–H7136 and H7179–H7182); Le Moure Blanc (M. Verlaque, June 1998, H7139–H7142); Bouzigues (M. Pellegrini, September 1998, H7183–H7184); Le Moure Blanc (M. Verlaque, October 2000, H7143–H7151).

Grateloupia dichotoma J. Agardh. Mediterranean Sea: Toulon, France (M. Verlaque, July 1979, H3895) midlittoral; Cassis, France (M. Verlaque, July 1980, H3896) midlittoral; Carry-le-Rouet, France (M. Verlaque, November 1987, H3898) midlittoral; Marseille, France (M. Verlaque, October 2001, H7102a–H7102b) midlittoral.

Grateloupia doryphora (Montagne) Howe. Middle Chile: Montemar, Pacific (G. Collantes, December 2000, H7058).

Grateloupia filicina (J.V. Lamouroux) C. Agardh. Mediterranean Sea: Marseille, France (M. Verlaque, June 1978, H3901) midlittoral; Marseille, France (M. Verlaque, October 2001, H7103a–H7103b) midlittoral; Toulon, France (M. Verlaque, June 1979, H7104) midlittoral; Saintes-Marie-de-la-Mer, France (M. Verlaque, May 1979, H7105) midlittoral.

Grateloupia lanceolata (Okamura) Kawaguchi. Thau Lagoon: Parcs B (N. Ben Maiz, December 1986, H2629–H2630); Parcs A (M. Gerbal, June 1993, H2631–H2632); Balaruc (M. Verlaque, October 1994, H7065–H7066); Bouzigues (M. Verlaque, October 1994, H7067–H7070); Mèze (M. Verlaque, September 1995, H7071–H7073); Parcs C (M. Verlaque, June 1996, H2666–H2674 and H7074); Mèze (M. Pellegrini, March 1997, H7075–H7077); Le Moure Blanc (M. Verlaque, October 1997, H7078); Parcs A (M. Verlaque, June 1998, H2870 and H7079–H7095). Japan: Shimoda, Izu Peninsula, Shizuoka Prefecture, Pacific (T. Komatsu, April 2000, H7063) 1 m depth; Kobe, Hyogo Prefecture, Inland Sea (T. Komatsu, May 2000, H7064) 1 m depth.

Grateloupia patens (Okamura) Kawaguchi & Wang. Thau Lagoon: Bouzigues (M. Verlaque, October 1994, H2598) 2 m depth, on oyster pockets; Bouzigues and Mèze (M. Verlaque, September 1995, H2599–H2616, F1367–F1368) 0–1 m depth.

Grateloupia sp. Thau Lagoon: Marseillan (M. Verlaque, May 1998, H7101 and H7156).

Grateloupia turuturu Yamada. Thau Lagoon: Mèze (N. Ben Maiz, December 1984, H2636–H2641); (H. Nedelec, November 1988, H2642); (M. Pellegrini, September 1990, H2633–H2635); Bouzigues and Mèze (M. Verlaque, September 1995, H2620–H2624); Bouzigues and Mèze (M. Verlaque, June 1996, H2660–H2665); Bouzigues and Mèze (M. Verlaque, October 1997, H7096–H7099); Bouzigues and Mèze (M. Verlaque, May 1998, H2825–H2829); Le Moure Blanc (M. Verlaque, October 2000, H7100). USA: Newport, Rhode Island, northwestern Atlantic (M. Villalard-Bohnsack, November 1997, H7185); Newport, Rhode Island, northwestern Atlantic (M. Villalard-Bohnsack, September 1996, H7186–H7187); Newport, Rhode Island, northwestern Atlantic (M. Villalard-Bohnsack, July 1998, H7188–H7189).

The above material has been deposited in the Herbarium Verlaque, COM, Marseille, France. Specimens used in the molecular analyses were preserved in silica gel prior to DNA isolation (Table 1).

Voucher material was compared with the following exsiccata from Japan distributed as ‘The Marine Algae of the Seto Inland Sea’, Kobe University Research Center for Inland Seas, coll. S. Enomoto, det. S. Kawaguchi.
Figs 15–27. Grateloupia lanceolata (Okamura) Kawaguchi from the Thau lagoon.

Figs 15–17. Habit (H7091-tetrasporophyte, H7086-tetrasporophyte, and H7080-female). Bars = 1 cm.

Fig. 18. Longitudinal section of thallus (H2632). Bar = 50 μm.

Fig. 19. Longitudinal section of cortex (H2667). Bar = 20 μm.

Fig. 20. Surface view of thallus (H2667). Bar = 20 μm.

Fig. 21. Auxiliary cell ampulla (H7080). Bar = 20 μm.

Fig. 22. Carpogonial branch ampulla (H2632). Bar = 20 μm.

Fig. 23. Ostiole of cystocarp.

Figs 24, 25. Cystocarp (H2632, H2673). Bars = 50 μm.

Figs 26, 27. Cruciately and irregularly divided tetrasporangia (t) (H2666). Bars = 20 μm.
**Grateloupia asiatica** Kawaguchi & Wang. No 052 (collection no 0400), as *G. filicina* (J.V. Lamouroux) C. Agardh, loc. Kuotsu-saki, Kunisaki, Ōita Prefecture (33°32.5’N, 131°45.5’E), Inland Sea, April, 1988.

**Grateloupia lanceolata** (Okamura) Kawaguchi. No 042-1 (collection no 3077), loc. Kamaguchi, Higashiura, Awaji-shima, Hyōgo Prefecture (34°28.9’N, 134°57.5’E), Inland Sea, May 1993; no 042-2 (collection no 0575), loc. Kitaki-jima, Okayama Prefecture (34°21.8’N, 133°33.6’E), Inland Sea, April 1989; no 042-3 (collection no 0417), loc. Myōjin, Mimasai-cho, Shimane Prefecture (34°9.8’N, 132°07.4’E), Inland Sea, April 1988.

**Grateloupia turuturu** Yamada. No 143 (collection no 0506), loc. Tanoshiro, Iwaya, Awaji-shima, Hyōgo Prefecture (33°34.8’N, 135°01.5’E), Inland Sea, September 1988.

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

Total DNA was isolated from 18 specimens representing eight species of *Grateloupia* using a hexadecyltrimethyl ammonium bromide (CTAB) method (J.J. Doyle & J.L. Doyle 1987) as previously described by Marston & Villallard-Bohnsack (2002) (Table 1). Chloroplast-encoded *rbcL* sequences were amplified using the primer pair F8-R1150 (Wang et al. 2000). The internal transcribed spacer ITS1, 5.8S ribosomal DNA (rDNA), and ITS2 regions were amplified via polymerase chain reaction (PCR) using the primer pair TW81-AB28 (Goff et al. 1994). A noncoding region between the Cytochrome Oxidase subunit 2 and subunit 3 genes in the mitochondrial genome (*cox2-3* spacer) was amplified using two degenerate primers: *cox2-4or* and *cox3-rev* (Zuccarello et al. 1999). Amplifications were carried out in 25 or 50 μl volumes as previously described (Marston & Villallard-Bohnsack 2002).

For each set of reactions, a control sample containing all reagents but lacking template DNA was included. Identical cycling parameters were used to amplify chloroplast *rbcL*, ITS and mitochondrial *cox2-3* spacer sequences and included 4 min denaturation at 95°C, followed by 45 s at 95°C, 45 s at 55°C, and 1 min at 72°C for 32 cycles and a final extension of 3 min at 72°C. Products of all PCR reactions were visualized on a 1% agarose gel stained with ethidium bromide. The products from two or three different PCR reactions all containing the same primer and template combination were pooled prior to cloning. Products were cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) following the vendor’s instructions. Plasmid DNA was isolated using a Qia-gen plasmid mini kit (Qiagen Inc, Valencia, CA, USA). For each individual and region, two to five clones were sequenced using an ABI Prism 377 automated sequencer (Applied Biosystems, Foster City, CA, USA). M13 forward and reverse primers were used in the sequencing reactions. In a few cases, single nucleotide differences were observed between clones from the same individual. In these instances, consensus sequences were used. All sequences have been deposited in GenBank (Table 1).

**Sequence alignment and phylogenetic analyses**

The *rbcL*, ITS and *cox2-3* spacer sequences were aligned for phylogenetic analyses using the Clustal X version 1.83 computer program (Thompson et al. 1994). Additional sequences included in the alignments and phylogenetic analyses were obtained from GenBank (Table 1). The *rbcL* alignment was 1030 bp representing positions 120 to 1149 of the full *rbcL* gene and contained no gaps. The ITS alignment was 816 bp and contained many insertions and (or) deletions (indels) due to species-specific length variations of the ITS1 and ITS2 regions (i.e. the ITS1, 5.8S rDNA, and ITS2 regions in these species ranged from 645 bp in *G. doryphora* to 725 bp in *G. asiatica*). The *cox2-3* spacer sequence alignment of 349 bp included only one gap of 1 bp. Primer sequences were not included in the alignments or in any of the analyses. The final sequence alignment for each region was used to calculate uncorrected pairwise distances.

The PAUP software package (Version 4.0b10, Swofford 2002) was used to reconstruct phylogenetic gene trees from the aligned data sets of *rbcL*, ITS (including the 5.8S rDNA) and *cox2-3* spacer sequences. Maximum parsimony (MP) and maximum likelihood (ML) methods were used to estimate phylogenetic relationships for each data set. *Halymenia floresia* (Clemente y Rubio) C. Agardh (Wang et al. 2000) was used as an outgroup for rooting the *rbcL* trees. Based on its basal position in the *rbcL* tree topology, *G. filicina* was used to root the ITS and *cox2-3* trees. In the parsimony analysis, trees were constructed using the heuristic search option with 500 random sequence additions. Tree bisection-reconnection branch swapping was performed, and all characters and character state transformations were unweighted. To compare relative support of the branches, 1000 bootstrap replications of full heuristic searches (Felsenstein 1985) were performed. For the ML analyses, the computer program Modeltest 3.06 (Posada & Crandall 1998) was used to estimate parameters and find the model of sequence evolution that best fit each data set. This program uses a hierarchical hypothesis-testing framework. The optimal model selected for the *rbcL* dataset was a General Time Reversible model with a gamma distribution (GTR + G). For the ITS and mitochondrial *cox2-3* data sets the optimal model was Hasegawa-Kishino-Yano (HKY) (Hasegawa et al. 1985) with a gamma distribution (HKY85 + G). The model of sequence evolution and estimated parameters were then imported into PAUP to estimate phylogenetic relationships using heuristic ML searches (10 random additions). For the ITS and *cox2-3* spacer sequences, bootstrap resampling support for ML was based on 500 iterations. Due to computational limitations, a ML bootstrap analysis of the *rbcL* data set was limited to 250 iterations.

**RESULTS**

**Description of Thau Lagoon specimens**

**Grateloupia asiatica** Kawaguchi & Wang

Figs 2–14

Other figures: Ben Maíz et al. [1986, fig. 1, as *G. filicina* (Wulfen) C. Agardh], Verlaque (2001, fig. 9, as *Grateloupia* sp.).

Reference: Kawaguchi et al. (2001), pp. 433±442, figs 1, 2a–l.

Type locality: Tsuyazaki, Fukuoka Prefecture, northern Kyushu, Sea of Japan.

Thalli exhibit substantial morphological plasticity in texture, number, and branching patterns of axes and lateral branchlets; upright...
Figs 28–40. *Grateloupia luxurians* (A. Gepp & E.S. Gepp) R.J. Wilkes, L.M. McIvor & Guiry from the Thau lagoon.

Figs 28, 29. Habit (H7152-tetrasporophyte, H2697-female). Bars = 1 cm.

Fig. 30. Portion of axis showing lateral proliferations (H2701). Bar = 5 mm.

Fig. 31. Longitudinal section of thallus (H7151). Bar = 50 μm.

Figs 32, 33. Longitudinal section of young and old cortex (H7151). Bar = 20 μm.

Fig. 34. Surface view of thallus (H7151). Bar = 20 μm.

Fig. 35. Auxiliary cell ampulla (H2703). Bar = 10 μm.

Fig. 36. Carpogonial branch ampulla (H2703). Bar = 10 μm.

Fig. 37. Cross-section through a cystocarp (H2697). Bar = 50 μm.
axes, 12–15 cm long, are dark red in colour and gelatinous to cartilaginous in texture; percurent axis are compressed to flattened, 1–5 mm wide, up to 620–630 μm thick, tapering above; simple or dichotomously branched once to three times, set with numerous lateral branchlets with pinnate arrangement and occasionally on the surface; branchlets are simple or dichotomously branched with pinnate proliferations along the margins and occasionally on the surface (Figs 2–4); marginal proliferations, 0.2–13.0 cm long and 1–2 mm wide, are compressed to oval in section and usually with second-order proliferations (Fig. 5); thallus is multiaxial and consists of a compact cellular cortex and a loose filamentous medulla (Fig. 6); medullary filaments are 5–8 μm in diameter; cortex is 7–9 cells thick (outer cortex 4–5 cells thick); outer cortical cells, 3–5 μm in diameter by 3–9 μm in length, are ovoid to slightly cylindrical (Figs 7, 8); medulla is lax but not hollow; gametophytes produce reproductive structures over the entire thallus except for the basal portion; auxiliary cell ampullae are initiated in the inner cortex; they are small and composed of 3–4 simple ampullary filaments, 5–10 cells long; mature auxiliary cells are oval in shape, slightly larger than other ampullary cells (Fig. 9); carpogonial ampullae are with 2–3 simple secondary filaments and a two-celled carpogonial branch (Fig. 10); cystocarps are spherical, not protruding, 150–200 μm wide, and scattered over the entire thallus except for the basal portion, arising laterally from cortical cells in the fourth to fifth cortical cell layers from surface; cortical cells tending to become elongated as paraphyse cells (Figs 13, 14); spermatangial thalli were not observed.

Grateloupia lanceolata (Okamura) Kawaguchi

Figs 15–27

OTHER FIGURES: Possibly Riouall et al. [1985, figs 4, 5, as G. doryphora (Montagne) Howe], Verlaque (2001, fig. 10).

BASIONYM: Aedes lanceolata Okamura.

REFERENCE: Okamura (1935), pp. 42–43, pl. 322.

TYPE LOCALITY: Kiit Province, Enoshima, Tateyama, Kazusa, Pacific.

SYNONYM: Pachymeniopsis lanceolata (K. Okamura) Y. Yamada ex S. Kawabata.

Upright blades, 10–70 cm long, dull rose-reddish to brownish in colour, membranaceous, lubricous in texture, are attached to substrate by means of discoidal holdfast of 7–20 mm diameter; blades, lanceolate foliose, 500–700 μm thick, 3–15 cm broad, are stipitate, simple, branched dichotomously to palmately and com- planate; margin entire or sometimes finely serrate in old fronds (Figs 15–17); thallus is multiaxial and consists of a compact cellular cortex and a filamentous medulla (Fig. 18); medullary filaments, 3–8 μm in diameter, are compactly interlaced; cortex is 9–13 cells thick (outer cortex 5–6 cells thick); outer cortical cells, 2–3 μm in diameter by 6–10 μm in length, are cylindrical elongated, (Figs 19, 20); gametophytes produce reproductive structures over the entire thallus except for the basal portion; auxiliary cell ampullae are initiated in the inner cortex; they are small, conical and composed of 2–3 simple or once branched ampullary filaments, 5–11 cells long; mature auxiliary cells are oval in shape, conspicuously larger than other ampullary cells (Fig. 35); carpogonial ampullae are small with 2–3 simple secondary filaments and a two-celled carpogonial branch (Fig. 36); cystocarps are spherical, not protruding, 225–250 μm broad including pericarp; enveloping filaments are largely derived from ampullary cells; ostiole is not protruding to slightly de- pressed (Figs 37–39); tetrasporangia are ellipsoidal, 24–40 μm long and 15–20 μm wide, and scattered over the entire thallus except for the basal portion; auxiliary cell ampullae are initiated in the inner cortex; they are small, conical and composed of 2–3 simple or once branched ampullary filaments, 5–11 cells long; mature auxiliary cells are oval in shape, conspicuously larger than other ampullary cells (Fig. 35); carpogonial ampullae are small with 2–3 simple secondary filaments and a two-celled carpogonial branch (Fig. 36); cystocarps are spherical, not protruding, 225–250 μm broad including pericarp; enveloping filaments are largely derived from ampullary cells; ostiole is not protruding to slightly de- pressed (Figs 37–39); tetrasporangia are ellipsoidal, 24–40 μm long and 11–22 μm wide, and scattered over the entire thallus except for the basal portion, arising laterally from cortical cells in the fourth to fifth cortical cell layers from surface without any modification of the cortex (Fig. 40); spermatangial thalli were not observed.

Grateloupia patens (Okamura) Kawaguchi & Wang

Figs 41–51

OTHER FIGURE: Verlaque (2001, fig. 14, as Prioniopsis Okamura).

BASIONYM: Prioniopsis patens Okamura.

REFERENCE: Okamura (1899), p. 3, pl. 1, figs 18–20.

TYPE LOCALITY: Mikawa, Aichi Prefecture, Pacific.

Upright axes, up to 20 cm long, are purplish red to dull brownish red in colour, and cartilaginous and becoming firmer in texture when dried; percurent axis are compressed, up to 5 mm wide, up to 490–575 μm thick, tapering above; di- or trichotomously branched, set with numerous lateral branchlets produced from the margin in a pinnate manner; branchlets, simple or dichotomous, linear-lanceolate or oblong-lanceolate, some of them continuing to grow and produce lateral proliferations, as the main branches (Figs 41, 42);
Figs 41–51. *Grateloupia patens* (Okamura) Kawaguchi & Wang from the Thau lagoon.  
Fig. 41. Habit (H2607-tetrasporophyte). Bar = 1 cm.  
Fig. 42. Portion of axis showing fertile lateral proliferations (H2607). Bar = 0.5 cm.  
Fig. 43. Longitudinal section of thallus (F1367). Bar = 50 μm.  
Fig. 44. Longitudinal section of cortex (F1367). Bar = 20 μm.  
Fig. 45. Surface view of thallus (H2607). Bar = 20 μm.
In this study, the rbcL gene from 16 specimens, the ITS region from 10 specimens, and the mitochondrial cox2-cox3 spacer region of 14 specimens were sequenced to use in phylogenetic analyses along with sequences obtained from GenBank (Table 1). Grateloupia patens was not included in the phylogenetic analyses because thalli have not been found since September 1995 and no material was available for DNA extraction. For each of the three regions (rbcL, ITS, and cox2-cox3 spacer), the overall topologies of the phylogenetic trees obtained from the MP and ML analyses were congruent. Phylogenetic analyses of the chloroplast rbcL, nuclear ITS sequences, and mitochondrial cox2-cox3 spacer sequences confirmed the identity of four nonnative Grateloupia spp. in the Thau Lagoon: G. asiatica, G. lanceolata, G. luxurians and G. turuturu.

The Japanese and Thau lagoon G. asiatica specimens form a well-supported clade in the rbcL tree (Fig. 62). The rbcL sequence divergence between the G. asiatica specimen from Japan and specimens from the Thau Lagoon was 0.2%. The three G. asiatica specimens from the Thau Lagoon had identical rbcL and identical mitochondrial cox2-cox3 spacer sequences, whereas the sequence divergence of the ITS region ranged from 0.0% to 0.1% (Figs 62–64).

In the rbcL and cox2-cox3 gene trees, the G. lanceolata specimens from Japan and the Thau Lagoon fell into a well-supported clade (Figs 62, 64). The two G. lanceolata specimens from the Thau Lagoon had rbcL sequences that were identical to one another and differed from the two Japanese G. lanceolata specimens by 0.0%–0.1%. The cox2-cox3 spacer sequences of Thau Lagoon G. lanceolata and Japanese G. lanceolata were identical. The ITS sequences of two Thau Lagoon specimens were identical (Fig. 63).

The Thau Lagoon, Atlantic France, and Australian specimens of G. luxurians and the Japanese specimens of G. subpectinata grouped together in the rbcL gene tree (Fig. 62). The Thau Lagoon, Atlantic France, and Australian specimens had identical rbcL sequences that differed from the Japanese specimens by 0.7%. The G. luxurians specimens from Thau Lagoon had identical mitochondrial cox2-cox3 spacer sequences and ITS sequences that differed by 0.4% (Figs 63, 64).

Specimens of G. turuturu from Japan, Thau, and the USA formed a well-supported clade in all three gene trees (Figs 62–64). The rbcL and ITS sequences of Thau Lagoon G. turuturu specimens were identical to those of the Japanese G. turuturu specimen. Intraspecific variation in mitochondrial cox2-cox3 spacer sequences, ranging up to 0.6%, was observed among the G. turuturu specimens.

The native G. filicina specimens form a robust clade in the rbcL and cox2-cox3 gene trees that is clearly distinct from the other clades of Grateloupia spp. (Figs 62, 64). The G. filicina rbcL sequences differ from all other rbcL sequences used in the analyses by 4.5–10.0%. The ITS and cox2-cox3 sequences of G. filicina differ from the sequences of all other species by 11.4–21.8% and 9.7–13.5%, respectively.

Phylogenetic analyses

In this study, the rbcL gene from 16 specimens, the ITS region from 10 specimens, and the mitochondrial cox2-cox3 spacer
The other Mediterranean species, *G. dichotoma*, also forms a distinct clade in *rbcL* and *cox2-cpx3* trees (Figs 62, 64). The *rbcL* sequence divergence between specimens in the *G. dichotoma* clade and all other species in the analysis is 7.1–11.0%. Interestingly, in all three trees, there is an undetermined isolate (*Grateloupia* sp.) from the Thau Lagoon that falls within the *G. dichotoma* clade (Figs 62–64) but that differs from the open sea *G. dichotoma* specimens by *rbcL*, ITS
Fig. 62. Phylogenetic relationships of Grateloupia spp. inferred from partial rbcL gene sequences (1030 bp). One of four equally most-parsimonious trees is shown (546 steps, consistency index = 0.590, retention index = 0.843). Halymenia floresia was used as the outgroup. Branch lengths are drawn proportional to the amount of sequence change. The numbers at internal nodes are the MP and ML bootstrap values: only values about 50% bootstrap support are shown.
Tetrasporangia 13±16
Medullary structure laxly constructed laxly constructed loosely or densely constructed
Thickness of cortex (outer cortex) 5±8 cells thick (2±3 cells) 5±6 cells thick (3±4 cells) 9±12 cells thick (3±5 cells)
Surface proliferations occasional to common occasional to common common
Marginal proliferations numerous, constricted at the texture soft and mucilaginous soft and mucilaginous mucilaginous, gelatinous to
Texture
Marginal proliferations numerous, constricted at the base, simple occasional to common
Surface proliferations
Thickness of cortex (outer cortex) 5–8 cells thick (2–3 cells) laxly constructed 5–6 cells thick (3–4 cells) laxly constructed
Medullary structure
Tetrasporangia 13–16 × 30–40 μm 11–22 × 24–40 μm
Mature auxiliary cell oval, larger than ampullary cells oval, larger than ampullary cells
Mature cystocarp 120 μm to over 300 μm in diameter 225–250 μm in diameter
Native regions Australia, Tasmania — Japan

* Dimensions of European specimens, which appear to be more robust than the native specimens.

and cox2-cox3 spacer sequence divergences of 1.3%, 1.6% and 3.4%, respectively.

Distribution and seasonality

In the Thau Lagoon, G. asiatica, G. lanceolata, G. luxurians and G. turuturu are common, but not invasive. They are found along the north coast of the Thau Lagoon, from Marseillan to Sète and in the aquaculture facilities (Parcs A–C). Grateloupia turuturu, however, was not observed in Parcs B and C (Fig. 1). Grateloupia patens, which has not been found since September 1995, was observed only along the north coast of the Thau Lagoon, between Bouzigues and Mèze, and in Parcs B. Thalli were observed from February to December (G. turuturu), March to December (G. asiatica and G. lanceolata), May to October (G. luxurians) and from September, October and April (G. patens). Field observations were not conducted in January. Thalli grow from 0 to −1 m Mean Low Water, attached to hard substrata: bedrock outcrops, loose stones, man-made rocky structures, aquaculture facilities and shellfishes (mussels and oysters).

DISCUSSION

Identity of the introduced Grateloupia spp. of the Thau Lagoon

Grateloupia C. Agardh, with 51 currently recognized species, is the largest genus in the family Halymeniaceae (Kraft 1977; Gavio & Fredericq 2002; Guiry & Nic Dhomna 2004). In the Thau Lagoon, the only native species of Grateloupia previously reported is G. filicina (J.V. Lamouroux) C. Agardh (Calvet 1905; Pavillard 1905; Laurent 1967). The present study demonstrated that five species of Grateloupia, originally described from the Pacific, were introduced in the Thau Lagoon: (1) G. asiatica Kawaguchi & Wang, previously identified as Grateloupia sp. (Verlaque 2001); (2) G. lanceolata (Okamura) Kawaguchi; (3) G. luxurians (A. Gepp & E.S. Gepp) R.J. Wilkes, L.M. McIvor & Guiry, previously identified as G. filicina var. luxurians A. Gepp & E.S. Gepp (Verlaque 2001); (4) G. patens (Okamura) Kawaguchi & Wang, previously identified as Prionitis patens Okamura (Verlaque 2001); and (5) G. turuturu Yamada, previously misidentified as G. doerophora. The Pacific origin of a species introduced into the Thau Lagoon has been genetically demonstrated only twice, in the case of Sphaerotrichia firma (Gepp) Zinova and Heterosiphonia japonica Yendo (Peters et al. 1993; Bjaerek & Rueness 2003, 2004; Kim et al. 2003). The present study provides four new examples: G. asiatica, G. lanceolata, G. luxurians and G. turuturu.

Mediterranean specimens of G. lanceolata, G. luxurians, G. patens and G. turuturu are similar to descriptions of Pacific specimens (Okamura 1899, 1910, 1935; Yamada 1941; Kawabata 1962, 1963; Kawaguchi 1989, 1997; H.B. Lee & I.K. Lee 1993; Womersley & Lewis 1994; De Clerck et al. 2005; Wilkes et al. 2005).

The specimen collected in 1984 and attributed to G. filicina in Ben Maiz et al. (1986) belongs to G. asiatica. This was the first collection of this species in the Mediterranean Sea. Grateloupia asiatica specimens from the Thau Lagoon are morphologically more diverse than Asiatic specimens. All the transitional forms were observed; some specimens possess a morphology similar to Japanese specimens of G. asiatica (gelatinous in texture; percurent axis flattened, simple or dichotomously branched once or twice; numerous flattened lateral branchlets with pinnate arrangement, see Kawaguchi et al. 2001), whereas other specimens possess a cartilaginous thallus, percurent axis almost cylindrical and dichotomously branched similar to other Japanese Grateloupia species (e.g. G. divaricata Okamura) (Fig. 3); the most extreme forms have a percurent axis dichotomously branched up to seven times, entirely covered with short dichotomous branchlets similar to the branchlets of the Prionitis spp. (now included in the genus

| References | G. luxurians | G. subpectinata |
|------------|-------------|---------------|
| Womersley & Lewis (1994), De Clerck et al. (2005) | present study, Thau Lagoon, France | Faye et al. (2004) |
| Branching pattern | discoid holdfast and erect axes, to 10–25 (40–70)* cm | discoid holdfast and erect axes, to 15–40 cm |
| Axes | one or twice pinnate compressed, to 5–10 mm wide and 1–3 mm thick | one or twice pinnate compressed to flattened, to 4.5–10 mm wide, up to 1.1 mm thick |
| Texture | soft and mucilaginous | soft and mucilaginous |
| Marginal proliferations | numerous, constricted at the base, simple | occasional to common |
| Surface proliferations | 5–8 cells thick (2–3 cells) laxly constructed | 5–6 cells thick (3–4 cells) laxly constructed |
| Thickness of cortex (outer cortex) | 13–16 × 30–40 μm | 11–22 × 24–40 μm |
| Medullary structure | oval, larger than ampullary cells | oval, conspicuously larger than other ampullary cells |
| Mature auxiliary cell | 120 μm to over 300 μm in diameter | 225–250 μm in diameter |
| Native regions | Australia, Tasmania | — Japan |
Grateloupia, Wang et al. 2001) (Fig. 4). Despite this morphological variation, no differences in the molecular markers considered here were observed between the different forms of G. asiatica in the Thau Lagoon. The G. asiatica specimens from the Thau Lagoon all had identical rbcL sequences that were only 0.2% divergent from the rbcL sequences of the Japanese G. asiatica specimen. This level of rbcL divergence is within the intraspecific rbcL variation observed for other Grateloupia species (Wang et al. 2000, 2001; Gavio & Fredericq 2002; Faye et al. 2004; De Clerck et al. 2005; Wilkes et al. 2005), thus confirming the identity of these specimens as G. asiatica. Although the initial introduction may have been restricted to a few individuals (small inoculate), new phenotypic variations could be the result of inbreeding, interspecific hybridization or phenotypic plasticity associated with different environmental conditions (Ellstrand & Schierenbeck 2000; Holland 2000; Lee 2002).

Recently G. subpectinata Holmes, a taxon described from Japan [Holmes 1912; type locality: not specified in protologue; Enoshima, Kanagawa Prefecture according to Okamura
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Fig. 64. Phylogenetic relationships of _Grateloupia_ spp. inferred from mitochondrial _cox2-cox3_ spacer sequences (349 bp). Strict consensus tree of five equally parsimonious trees is shown (169 steps, consistency index = 0.722, retention index = 0.889). _Grateloupia filicina_ was used as the outgroup. The numbers at internal nodes are the MP and ML bootstrap values: only values about 50% bootstrap support are shown.

(1936)] and placed in synonymy with _G. filicina_, was reinstated (Faye et al. 2004). This species is morphologically similar to _G. luxurians_ (Table 2). Furthermore, the _rbcL_ sequence divergence between _G. luxurians_ and _G. subpectinata_ is only 0.7% (present study), a value far lower than the interspecific divergence values reported for the genus _Grateloupia_ (i.e. reported values range from 1.5% to 10.0%, but are usually between 5.0% and 8.8%; Wang et al. 2000, 2001; Gavio & Fredericq 2002; Faye et al. 2004; De Clerck et al. 2005; Wilkes et al. 2005). The morphological comparison between the Australian, Mediterranean and Atlantic specimens of _G. luxurians_ and some Japanese specimens of _Grateloupia_ previously identified as _G. subpectinata_ (H7060–7061; Figs 65, 66) did not reveal any differences. As a result, _G. subpectinata_ and _G. luxurians_ appear to be conspecific and _G. subpectinata_ Holmes (1912) is considered as a later taxonomic synonym of _G. filicina_ var. _luxurians_ A. Gepp & E.S. Gepp (1906) (now _G. luxurians_). Consequently, the geographical distribution of _G. luxurians_ is extended to Japan.

The re-examination of the N. Ben Maiz collections confirmed the hypothesis proposed by Verlaque (2001) and Gavio & Fredericq (2002) that specimens attributed to _G. doryphora_ in Ben Maiz et al. (1986) belong to _G. turuturu_. Moreover, according to the description and the illustrations given in Riouall et al. (1985), it is probable that the first observation of _G. doryphora_ in the Thau Lagoon in 1982 was a misidentification of either _G. turuturu_ (thallus linear foliose, undulate with margin entire or provided with proliferations, see figs 2, 3, in Riouall et al. 1985) or _G. lanceolata_ (thallus foliose, palmately branched in one plane with margin entire, cortex
9–10 cells thick, see figs 4, 5, in Riouall et al. 1985). Pacific South-American *G. doryphora* differs from Japanese, N Atlantic and Mediterranean populations of *G. turuturu* by having 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. According to Gavio & Fredericq (2002), instead of having a wide distribution, the true *G. doryphora* appears to be restricted to the South Eastern Pacific. Thus it is likely that the other reports of *G. doryphora* in the Mediterranean are also misidentifications. For example, the Lagoon of Venice has been heavily impacted by species introductions from Asia (Tolomio 1993) and *G. doryphora* specimens reported there are probably *G. turuturu* or (and?) *G. lanceolata*. Grateloupia doryphora thalli recorded in the southern Mediterranean, in Sicily (De Masì & Gargiulo 1982) and in the South of Spain (Rull Lluch et al. 1991) may be *G. lanceola* (J. Agardh) J. Agardh, a species described from Atlantic coast of North Africa (Agardh 1841, as Halymenia lanceola J. Agardh) and regarded as conspecific with *G. doryphora* (Ardre & Gayral 1961; Dawson et al. 1964) on the basis of some likeness in foliose habit (see Benhissoune et al.)
2002). The identity of these populations awaits molecular determination.

Vector, date and origin of the introductions

From 1971 to 1976, there were massive importations of Japanese oysters, *C. gigas* (Thunberg), from Japan (Sendai, Miyagi Prefecture, NE Honshu) to the Thau Lagoon (Grizel & Héral 1991; Grizel 1994). The presence of the introduced species of the Thau Lagoon in Japan and the dates they were first observed in the lagoon (i.e. *G. lanceolata* and *G. turuturu*; possibly 1982; *G. asiatica*; 1984; and *G. luxurians*; 1990) are consistent with the introduction of spores, crusts, germings or fragments by oyster importations during this period. In addition to *Grateloupia* spp., many other species were introduced during the same time period including *Chrysymenia wrightii* (Harvey) Yamada, *Dasya sessilis* Yamada, *Desmarestia viridis* (O.F. Müller) J.V. Lamouroux, *Laminaria japonica* Areschoug, *Lomentaria hakodatensis* Yendo, *Sargassum muticum* (Yendo) Fensholt, *Sphaeroretichia firma* (E. Gepp) Zinova, *Ulva pertusa* Kjellman, *Undaria pinnatifida* (Harvey) Suringar (Verlaque 2001, 2002; Verlaque et al. 2002; Kim et al. 2003). In the case of *G. patens*, its discovery in 1994 and its apparent fast disappearance could be due to an introduction in the early 1990s by illicit oyster importations from Japan, as was probably the case with *Chondrus giganteus* Yendo f. *flabellatus* Mikami (Verlaque 1996; Verlaque & Latala 1996).

A bibliographical analysis of the introduced flora of the Mediterranean lagoons (M. Verlaque, unpublished observations) shows that (1) most of the introductions of marine macroalgae occurred in the lagoons harbouring shellfish aquaculture facilities (oysters and or mussels) and (2) more than 80% of the introduced taxa were native to Japan and (or) Korea, the world’s two major oyster exporters. However, in the case of *G. luxurians* and *G. turuturu*, it is possible that the introduction occurred via oyster-spat importations from populations of Japanese oysters already established in the Atlantic because both species seem to have been introduced in the NE Atlantic before the 1970s (see Farnham & Irvine 1968, 1973, as *G. doryphora* and *G. filicina* var. *luxurians*). On the basis of this study, it is not possible to determine the exact source population(s) of the introduced species. It is particularly difficult considering that there were probably multiple introductions of each species into Europe and that these species have a very wide distribution in the warm and temperate seas of Japan and Korea (Okamura 1935; Kawabata 1962; Lee & Kang 1986; Noda 1987; Chihara 1990; H.B. Lee & I.K. Lee 1993; Tokuda et al. 1994; Yoshida 1998; Kawaguchi et al. 2001; Faye et al. 2004).

Probability of spread and risks of invasion

The five introduced *Grateloupia* species in the Thau lagoon constitute a case study of multiple introductions. Numerous studies of plants have documented the occurrence of intra- and interspecific hybridizations after species introductions (Ellstrand & Schierenbeck 2000; Lee 2002). The risk of hybridization increases as the number of introduced allopatric populations of the same taxon or different taxa increases. Although in this study none of the specimens we examined appears to be a hybrid (i.e. gene trees based on nuclear, chloroplast, and mitochondrial sequences do not exhibit well-supported incongruities in the relative positions of taxa), the presence of multiple, closely related, introduced species makes the Thau Lagoon a ‘melting pot’ from which new genetic combinations could arise. Although hybridization in Rhodophyta is extremely rare (Rueness 1978; Brodie et al. 1993, 1997), this should be of concern because there are many examples of plants in which hybridization preceded the emergence of successful invasive populations (Ellstrand & Schierenbeck 2000).

In the NW and NE Atlantic, *G. turuturu* (as *G. doryphora*) is an invasive species (Harlin & Villalard-Bohn sack 2001; Simon et al. 2001; Villalard-Bohnsack & Harlin 2001; Bábara & Crema des 2004). This species withstands a large range of salinities, temperatures, and other environmental conditions (Simon et al. 1999, 2001; Harlin & Villalard-Bohn sack 2001). In the Thau Lagoon, four Asiatic *Grateloupia* species (e.g. *G. asiatica, G. lanceolata, G. luxurians* and *G. turuturu*) have been successfully established for the past 20 to 25 yr and have developed reproductive populations without becoming invasive. Nevertheless because the Thau Lagoon is an important site for the exportation of living bivalve molluscs (*C. gigas, Ostrea edulis, Mytilus galloprovincialis, Tapes* spp.), these species are likely to spread, if they have not already, to other regions and countries in the Mediterranean and throughout Europe.

NOTE ADDED IN PROOF

According to Art. 11.2 of the ICBN (Greuter et al. 2000), priority only applies in the same rank. *G. luxurians* was described as a variety of *G. filicina* and hence cannot take priority over *G. subpectinata*. Consequently, the correct name for this species is *G. subpectinata* Holmes.

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