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Taxonomic considerations of a foliose *Grateloupia* species from the Straits of Messina

R.J. Wilkes[1], M. Morabito[2] & G.M. Gargiulo[2]*
[1]*Irish Seaweed Centre, Martin Ryan Institute, National University of Ireland, Galway, Ireland;* [2]*Department of Botanical Sciences, University of Messina, Salita Sperone, 31, S. Agata, 98166 Messina, Italy*

*Author for correspondence: e-mail: ggargiulo@unime.it

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Abstract

Grateloupia turuturu Yamada is the currently accepted name for the invasive red alga that is present on coasts of the North Atlantic. Previously considered as *G. doryphora* (Montagne) M.A. Howe, populations of this invasive species were examined and their taxonomic position revised using molecular and morphological techniques. It was also thought that similar invasive populations in the Mediterranean should be identified as *G. turuturu*. This investigation used *rbc*L based molecular analyses to clarify the taxonomic position of *Grateloupia "doryphora"* from the Straits of Messina. Our results indicate that this population is neither *G. doryphora* nor *G. turuturu*. It was placed separately in all analyses and grouped consistently with other *Grateloupia* species from the Pacific. On the basis of molecular data from this and previous investigations, it is evident that the status of the foliose Atlantic and Mediterranean entities is still unclear and a re-evaluation of the old names connected to them should be undertaken.

Introduction

Grateloupia turuturu Yamada is the currently accepted name for the invasive red alga, previously considered as *Grateloupia doryphora* (Montagne) M.A. Howe, present on coasts of the UK, France and north eastern America (Gavio & Fredericq, 2002). Until recently, both Atlantic and Mediterranean foliose *Grateloupia* was referred to as *G. doryphora* based on the papers of Ardré and Gayral (1961) and Dawson et al. (1964). Ardré and Gayral (1961) placed in synonymy some foliose Atlantic and Pacific entities under the name *G. lanceola* (J. Agardh) J. Agardh *emend.* Ardré et Gayral, originally described as *Halymenia lanceola* J. Agardh (1841: 19) for the Atlantic coasts of Morocco and South Spain. *Grateloupia doryphora* was not included in this due to an absence of data (Ardré & Gayral, 1961). Subsequently, Dawson et al. (1964) associated *G. doryphora* to the *G. lanceola*-complex, keeping the former name as the first validly published. Since then reports for different localities, both Mediterranean and Atlantic, such as the records from Morocco and Senegal (Gayral, 1958; Ardré & Gayral, 1961) or those from southern Spanish coasts (Pérez-Cirera et al., 1989) of *G. lanceola*, have been considered *G. doryphora.*

Recent investigations have compared some invasive foliose *Grateloupia* populations with specimens from the type localities of *G. doryphora* (Peru) and of *G. turuturu* (Japan), and, based on *rbc*L gene sequences and anatomical observations, these were shown to be conspecific to *G. turuturu* (Gavio & Fredericq, 2002). This result was previously hypothesised by Verlaque (2001) on the basis of phytogeographical considerations. *Grateloupia "doryphora"* specimens from the Mediterranean Sea were also supposed to be *G. turuturu* (Verlaque, 2001; Gavio & Fredericq, 2002), but molecular investigations had not been carried out to test this hypothesis.

Grateloupia "doryphora" was first reported from the Straits of Messina, Mediterranean Sea (De Masi & Gargiulo, 1982) but specimens of a foliose *Grateloupia*

species, identified as *G. cuneifolia* J. Agardh *ex* Kützing in Giaccone's herbarium (CAT), date back to 1969 (Giaccone, 1969). Subsequently, a similar foliose species was recorded in Thau lagoon (Riouall et al., 1985) and in Venice lagoon (Gargiulo et al., 1992; Tolomio, 1993). In this paper comparative *rbc*L molecular analyses have been undertaken in order to clarify the taxonomic position of *Grateloupia "doryphora"* from the Straits of Messina.

Materials and methods

Samples of *Grateloupia* sp. were collected from both sides of the Straits of Messina, on rocks and concrete blocks near the water line. Samples used in the present study, with voucher numbers and collection information, are: Gra002 (Villa S. Giovanni, Reggio Calabria, Italy, 16/02/2000); Gra004 (Villa S. Giovanni, Reggio Calabria, Italy, 16/02/2000); Gra015 (Villa S. Giovanni, Reggio Calabria, Italy, 17/03/2004); Gra016 (Villa S. Giovanni, Reggio Calabria, Italy, 17/03/2004); Gra017 (Villa S. Giovanni, Reggio Calabria, Italy, 17/03/2004); Gra018 (Torre Faro, Messina, Italy, 22/03/2004).

DNA was isolated from freshly collected thalli (within 48 h) and from silica gel and herbarium preserved samples, with a modified CTAB protocol (Doyle & Doyle, 1987). Voucher specimens were preserved in 4% formalin in seawater, dried in silica gel, pressed as herbarium sheets and deposited in the Phycological Herbarium of the Department of Botanical Sciences of the University of Messina (MS). Anatomical observations were made on hand sections of the thalli. Micrographs were taken by a Diaplan Leica microscope equipped with a camera. In order to prevent errors in sorting of samples, each DNA isolation was performed from a single individual, a fragment of which was kept as voucher formalin preserved and/or pressed for further inspections.

The *rbc*L gene was PCR amplified using primers listed in Wang et al. (2000). Sequencing was performed by an external company (MWG Biotech AG, Ebersberg, Germany). Six separate thalli from the samples of the Straits of Messina were sequenced but as they differed by only 1bp only one was used for the analyses (Gra017). Sequences were aligned manually using GeneDoc 2.6.002 (Nicholas & Nicholas, 1997). No insertions or deletions were found, making the alignment unambiguous. Previously published sequences were obtained from Genbank and added to the dataset to give a 1253-bp sequence alignment for analyses (Table 1). Trees were rooted with *Sebdenia monardiana* (Montagne) Berthold, which is the generitype of the family Sebdeniaceae, a sister group to the Halymeniaceae (Saunders & Kraft, 1996).

The data were analysed for maximum parsimony (MP), neighbour joining (NJ), using a Kimura 2-parameter distance matrix as input, and for maximum likelihood (ML) using PAUP* 4b10 (Swofford, 2002). MP analysis was performed with 50 random sequence additions. Modeltest version 3.06 (Posada & Crandall, 1998) was used to determine the parameters for the ML analyses, and specified a Transition model with a proportion of invariable sites and a gamma distribution (TIM+I+G). The rate matrix was specified as [A−C] = 1.0, [A-G] = 4.4776, [A−T] = 0.6298, [C−G] = 0.6298, [C−T] = 11.0935 and [G−T] = 1.0, with the base frequencies at A = 0.3164, C = 0.1435, G = 0.2059, and $T = 0.3342$. The proportion of invariable sites was set at 0.4904 with a gamma distribution of 0.6904. The robustness of each analysis was tested by bootstrapping the dataset 1000 times for MP and NJ, and 100 times for ML analysis (Felsenstein, 1985).

Results

Thalli of *Grateloupia* sp. from the Straits of Messina are solitary or gregarious, purplish-red to yellowish-red. Fronds are lanceolate or irregularly lobate, with one or more blades connected to a short stipe, arising from a discoid holdfast (Figure 1). They are up to 70 cm long and 15 cm large, and 500–2000 μm thick. The consistency of the frond ranges from leathery to somewhat gelatinous.Transverse sections show a compact outer cortex of 4–6 cells periclinally oriented, with the most external ones elongated (6–8 × 1.5–2.5 μm), and the others roundish (5–6 μm in diameter), an inner cortex of 2–3 irregularly stellate cells (8–10 × 16–18 μm), anticlinally oriented (Figure 2). The medulla is loose, with filaments mainly anticlinally oriented (1.5–2.5 × 25–30 μm). The cortex pattern is very constant both at different levels of the blade and in variously aged thalli.

The auxiliary ampullae are branched up to secondary order (Figure 3).

The alignment of *rbc*L gene sequences contained 263 parsimony informative sites with 869 invariable positions. The single maximum-likelihood tree with bootstrap values from each analysis overlaid on the branches is shown in Figure 4. NJ and MP trees had topologies similar to ML and are not shown. All the *Grateloupia* samples were resolved in a single large