

***Gracilaria viridis* sp. nov. (Gracilariales, Rhodophyta): a new red algal species from the Mediterranean Sea**

ADRIANO SFRISO¹*, MARION ADELHEID WOLF²§, KATIA SCIUTO², MARINA MORABITO³, CARLO ANDREOLI² AND ISABELLA MORO²

¹*Department of Environmental Sciences, Informatics & Statistics, University of Venice, Calle Larga 2137, 30123 Venice, Italy*

²*Department of Biology, University of Padova, Via U. Bassi, 58/B 35131 Padova, Italy*

³*Department of Life Sciences “Marcello Malpighi”, University of Messina, V. Ferdinando Stagno d’Alcontres, 31 98166 Messina, Italy*

SFRISO A., WOLF M.A., SCIUTO K., MORABITO M., ANDREOLI C. AND MORO I. 2013. *Gracilaria viridis* sp. nov. (Gracilariales, Rhodophyta): a new red algal species from the Mediterranean Sea. *Phycologia* 52: 65–73. DOI: 10.2216/12-007.1

We characterized a new *Gracilaria* species from the Venice Lagoon, Italy, using molecular analyses based on the plastid large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbcL*) and the intergenic RuBisCO spacer (*rbcL-rbcS*), combined with morphology data. This new entity was recorded on the artificial substrata of the Venice Gulf from March to July, adding to 12 *Gracilaria* taxa already recorded in the Mediterranean and Adriatic seas. Thalli exhibited a green-yellowish pigmentation with pink shades, and there was dense branching in the distal portions. Tetrasporangia were scattered on thallus cortex and distributed mostly on short, stipitate branchlets. The inner pericarp was connected to the gonimoblast by tubular nutritive cells. The male gametophytic plants formed round-elliptical spermatangial *verrucosa*-type conceptacles. This species grew attached on artificial rocky substrata of the low midlittoral and upper sublittoral zone in spring and early summer. Molecular analyses based on the plastid-encoded *rbcL* gene showed a 99.66% nucleotide identity with another *Gracilaria* sp. from southern Sicily. We compared our *rbcL-rbcS* spacer sequences with those of two cryptic species, and the phylogenetic analyses confirmed that the Venice populations were a new species. We suggested that the discovery of this new species was not due to an extra-Mediterranean introduction but the consequence of its misidentification as *Gracilaria gracilis*, which has a similar gross morphology.

KEY WORDS: *Gracilaria viridis* sp. nov., Gracilariaceae, Mediterranean Sea, New species, *rbcL*, *rbcL-rbcS* spacer

INTRODUCTION

Gracilaria Greville is an important economic red algal genus because it is the major agarophyte resource in the world (Troell *et al.* 2003). It is characterized by a wide phenotypic variability and great species diversity. Thalli of this genus range from erect to prostrate and from terete to broadly flattened, with species forming branched fronds composed of cylindrical or irregularly shaped units (Guiry & Guiry 2011).

Gross morphological characters of the vegetative and reproductive structures have distinguished many genera and for years have provided the basis of Mediterranean seaweeds identification (Gargiulo *et al.* 1987, 1992; Fredericq & Hommersand 1989a, b, 1990). Unfortunately, morphological features are often limited and ambiguous, leading to misidentifications caused by convergent evolution or morphological plasticity caused by environmental influences. This makes the systematics of this group very problematic (Bird 1995).

Progress in molecular techniques solved problems in species identification and led systematic revisions for many algal groups including *Gracilaria* (Bellorin *et al.* 2002; Gurgel & Fredericq 2004). Several molecular markers have been

used: the plastid-encoded large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbcL*) (Freshwater *et al.* 1994; Fredericq *et al.* 1996; Hommersand *et al.* 1999; Gurgel & Fredericq 2004) and the *rbcL-rbcS* intergenic spacer region (Cho *et al.* 2003; Skage *et al.* 2005; Wolf *et al.* 2011), the mitochondrial-encoded *cox1* gene (Saunders 2005), and the nuclear-encoded rRNA internal transcribed spacer (Hayden & Waaland 2004).

At present Guiry & Guiry (2011) report 170 specific and intraspecific accepted taxa for *Gracilaria* and 12 species (Table 1) are present in the Mediterranean Sea (Gargiulo *et al.* 1992; Furnari *et al.* 1999, 2003; Sfriso & Curiel 2007; Cecere & Petrocelli 2009; Sfriso *et al.* 2009). However, the development of taxonomy, especially on molecular systematics, and the occurrence of introduced taxa (Sfriso *et al.* 2010) are increasing this number.

Here we report the characterization of specimens collected in the Venice Lagoon (Italy); specimens are identified using morphological features and molecular analyses of the *rbcL* gene and the more variable intergenic *rbcL-rbcS* spacer; the spacer is useful for distinguishing between cryptic species (strictly correlated species) (Maggs *et al.* 2002). The *rbcL* results show that the Venice specimens are conspecific with another *Gracilaria* species (named *Gracilaria-LI02*) from southern Sicily, Italy (Gargiulo *et al.* 2006). On the basis of our results, we propose the name *Gracilaria viridis* sp. nov.

* Corresponding authors (adriano.sfriso@unive.it, isabella.moro@unipd.it).

§ These authors contributed equally to this work.

Table 1. Morphology of the vegetative and reproductive features for the Mediterranean *Gracilaria* species.

Taxa	Maximum axis		Shape	Medullary cells	Cystocarp	
	Length (cm)	Diameter (mm)			Position	Size (μm)
<i>G. armata</i> (C. Agardh) Greville	15–25	2–4	terete	50–250	distal branches	520–700 \times 700–800
<i>G. bursa-pastoris</i> (Gmelin) Silva	25–35	1–2(–3)	partially flattened	250–500	entire thallus	500–800 \times 700–900
<i>G. conferta</i> (Schousboe ex Montagne) Montagne	6–8	0.15	terete	180–200	distal branches	700–800 \times 800–950
<i>G. corallicola</i> Zanardini	5–10	3–7(–15)	flattened	100–180(–300)	distal branches	700–800 \times 800–900
<i>G. dendroides</i> Gargiulo <i>et al.</i>	25–30	0.2	terete with lanceolate branchlets	150–200	lanceolate branches	600–700 \times 800–900
<i>G. dura</i> (Agardh) Agardh	20–25	1–1.8	terete	75–100(–180)	entire thallus	700–800 \times 800–900
<i>G. gracilis</i> (Stackhouse) Steentoft <i>et al.</i>	15–35	0.8–1.2	terete	100–150(–200)	entire thallus	500–700
<i>G. heteroclada</i> Zhang & Xia	25–35	3–5(–8)	flattened with spatulated apices	80–120	on club-shaped branches	300–350 \times 350–400
<i>G. longa</i> Gargiulo <i>et al.</i>	100–200	1.5–3(–4)	terete	150–300(–500)	entire branches	750–850(–1200)
<i>G. multipartita</i> (Clemente) Harvey	20–60 ¹	6–10	flattened	60 \times 65–132 \times 180 ¹	entire thallus	—
<i>G. vermiculophylla</i> (Ohmi) Papenfuss	50–100–200	1.5–3(–4)	terete	200–250(–300)	entire thallus	700–900 \times 800–1300
<i>G. verrucosa</i> (Hudson) Papenfuss	15	1	terete	100–200	entire thallus	600–700 \times 700–750
<i>G. viridis</i> sp. nov.	5–35	0.5–1.3	terete	(100–)150–200	entire thallus	800–1050

¹ Neto *et al.* (2002).² Gurgel & Fredericq (2004).³ Smith (1954).⁴ Bellorin *et al.* (2004).

MATERIAL AND METHODS

Samples of *Gracilaria viridis* sp. nov. were collected on the artificial stone breakwaters perpendicular to the shore coastline of the Lido and Pellestrina islands, along the Venice sea coastline and in the jetty ‘dikes’ formed by limestone blocks placed between the sea and the Venice Lagoon (Italy) to protect the wide sea inlets of Lido, Malamocco and Chioggia (Lido: 45°23’N, 12°21’E; Malamocco: 45°20’N, 12°19’E; Pellestrina: 45°17’N, 12°18’E). Moreover, thalli of *G. viridis* were sampled in Licata (Agrigento, Sicily, Italy) (37°05’N, 13°55’E), at Marianello beach, west of the fishing harbour. Thalli were deposited in the herbarium, University of Messina (MS).

Gross morphological observations were carried out on fresh samples or on specimens fixed in 4% buffered formaldehyde–seawater solution; a stereo zoom microscope E-1654ZT45 (Euromex microscopes, Holland) and a compound light microscope-353Ph (Optika microscopes, Italy) were employed. Spermatangial conceptacles were observed on semithin sections. Parts of thalli were fixed overnight in 6% glutaraldehyde in 0.1 M cacodylate buffer (pH 6.9), and postfixed overnight in 1% OsO₄ in the same buffer. The postfixed samples were dehydrated in a graded ethanol series followed by propylene oxide, and then they were embedded in araldite resin. Semithin sections were cut with an ultramicrotome (Reichert Ultracut S), stained with 1% toluidine blue and examined with a DMR Leica microscope (Wetzlar, Germany) equipped with a digital image acquisition system.

Genomic DNA was extracted from dried samples using the Genomic DNA purification kit (Fermentas International Inc., Burlington, Ontario, Canada) following the manufacturer’s instructions. For amplification and sequencing reactions of the plastid *rbcL* gene, two primer pairs (F7-R753, F577-R1381) were used (see Freshwater & Rueness 1994). For the *rbcL-rbcS* spacer amplification and sequencing, the forward primer F1320 (Freshwater *et al.* 2006) was used in combination with the reverse primer RrbcSstart (Freshwater & Rueness 1994). PCR amplifications were performed as follows: an initial denaturation step of 2 min at 94°C; 30 cycles at 94°C for 40 s, at 50°C for 40 s, and at 72°C for 50 s; and a final extension step at 72°C for 5 min. The amplification products were cleaned with ExoSAP-IT™ kit (GE Healthcare, Uppsala, Sweden, Europe) following the manufacturer’s protocol. DNA sequencing was performed at the BMR Genomics Sequencing Service (Padova University, Italy) on automated ABI DNA sequencers (Applied Biosystem, Foster City, CA, USA). Final consensus sequences were assembled using the SeqMan II program from the Lasergene software package (DNASTar®, Madison, WI, USA). Identity of new sequences was checked with the BLAST program (Altschul *et al.* 1990) available at the USA National Center for Biotechnology Information (NCBI) web server (<http://www.ncbi.nlm.nih.gov>), and the sequence alignments were obtained by the ClustalW computer program (Thompson *et al.* 1994).

For phylogenetic analyses of the *rbcL* gene, a dataset of 73 sequences was constructed using our new sequences plus sequences obtained from DDBJ/GenBank/EBI Data Bank.

Table 1. Extended

Tubular nutritive cells to			Male conceptacles		Tetrasporangia	
Inner pericarp	Outer pericarp	Fusion cell type	Type	Size (µm)	Position	Size (µm)
+	+	restricted	<i>textorii</i>	25–38 × 25–38	distal branches	15–20 × 25–30
+	+	expanded	<i>textorii</i>	20–25 × 20–25(–38)	entire thallus	15–25 × 20–35
+	+	restricted	<i>verrucosa</i>	15–20 × 20–25	distal branches	18–20 × 25–35
+	+	restricted	<i>chorda</i>	8–10 × 15–20	entire thallus	18–20 × 22–26
+	+	expanded	<i>verrucosa</i>	30–50 × 65–75	lanceolate branches	35–40 × 50–60
–	+	expanded	<i>verrucosa</i>	25–30 × 40–50	distal region	25–30 × 35–40
–	+	expanded	<i>verrucosa</i>	25–30 × 30–40	entire thallus	25–30 × 30–40
+	+	restricted	<i>chorda</i>	30–40 × 40–50	spatulated branches	18–20 × 30–40
–	+	expanded	<i>verrucosa</i>	30–45 × 50–70	entire thallus	15–20 × 18–40(–50)
+ ²	+ ²	expanded ³	<i>textorii</i> ²	—	scattered, younger parts of the blade ¹	23–29 × 34–60 ¹
– ⁴	+(rare)	restricted ⁴	<i>verrucosa</i>	25–40(–50) × 40–100(–125)	entire thallus	20–30 × 20–40(–50)
–	+	expanded	<i>verrucosa</i>	20–25 × 30–40	entire thallus	25–30 × 30–40
+	+	restricted	<i>verrucosa</i>	15–35(–50) × 50–100(–125)	entire thallus	12–15 × 20–35

Analyses were performed according to maximum likelihood (ML) method with the PHYML 2.4.4 program (Guindon & Gascuel 2003) applying the GTR+I+Γ evolutionary model (Lanave *et al.* 1984). Nonparametric bootstrap resampling (Felsenstein 1985) was performed to test the robustness of the tree topology (1000 replicates). The models that best fitted our data were found using the program jMODEL-TEST under the Akaike Information Criterion (Posada & Crandall 1998; Posada & Buckley 2004). Bayesian Inference analyses were carried out using MrBayes version 3.1 (Ronquist & Huelsenbeck 2003). The substitution model was the GTR+I+Γ and the Bayesian analyses were performed with four search chains for 3,000,000 generations, sampling trees every 100 generations. The first 7500 trees were discarded as burn-in. Parameter stability was estimated by plotting log-likelihood values against generation time, and a consensus tree with posterior probabilities was then produced. The nexus files for the Bayesian analyses were generated with the Mesquite 2.71 software package (Maddison & Maddison 2009).

The *rbcL* gene and the *rbcL-rbcS* spacer sequences of the Venice isolate were deposited in DDBJ/GenBankTM/EBI Data Bank with the accession numbers HE614144 and HE614145, respectively, while the *rbcL* sequence of *Gracilaria conferta* Montagne from Morocco was assigned the accession number HE964956.

RESULTS

Gracilaria viridis Sfriso, Wolf, Sciuto, Morabito, Andreoli & Moro sp. nov.

Figs 1–11

DIAGNOSIS: *Thalli tereti 0.5–1.3 mm diametro, 5–35 cm alti, saxicolae, irregularis, fere horizontaliter effusi, haptero parvo discoideo affixi. Color viridis-flavidus, aliquando purpureus. Rami alterni, dense fruticosi, ad apices angustati et saepe incurvati. In transversa sectio frondes multistrato constitutas: ab exterior parte cellulae corticalis per 1–2 stratos; mediae cellulae medullariae subcorticalesque, 10–20 µm diametro, per 2–3 stratos; interioris cellulae medullariae magnae, 100–200 µm diametro, parietibus tenuibus. Cystocarpia matura ellipsoidea, ~ 900 µm diametro, basi constricta, per totem fronda sparsa. Gonimoblasti per cellulas nutrices tubulares ad cellulas basales pericarpium affixi. Tetrasporangia, ~ 30 µm longa ~ 13 µm lata, filamentis corticalibus circumcinctis, in brevis ramulis, per totam frondem sparsa. Spermatangia in conceptaculis, ~ 35 µm diametro ~ 75 µm altis, in cellula corticalis et subcorticalibus mersi.*

HOLOTYPE DESIGNATED HERE: A000008, a voucher specimen deposited at the Herbarium Patavinum (PAD); a tetrasporophytic plant with short stipitate unbranched to tribranched branchlets (see Fig. 1).

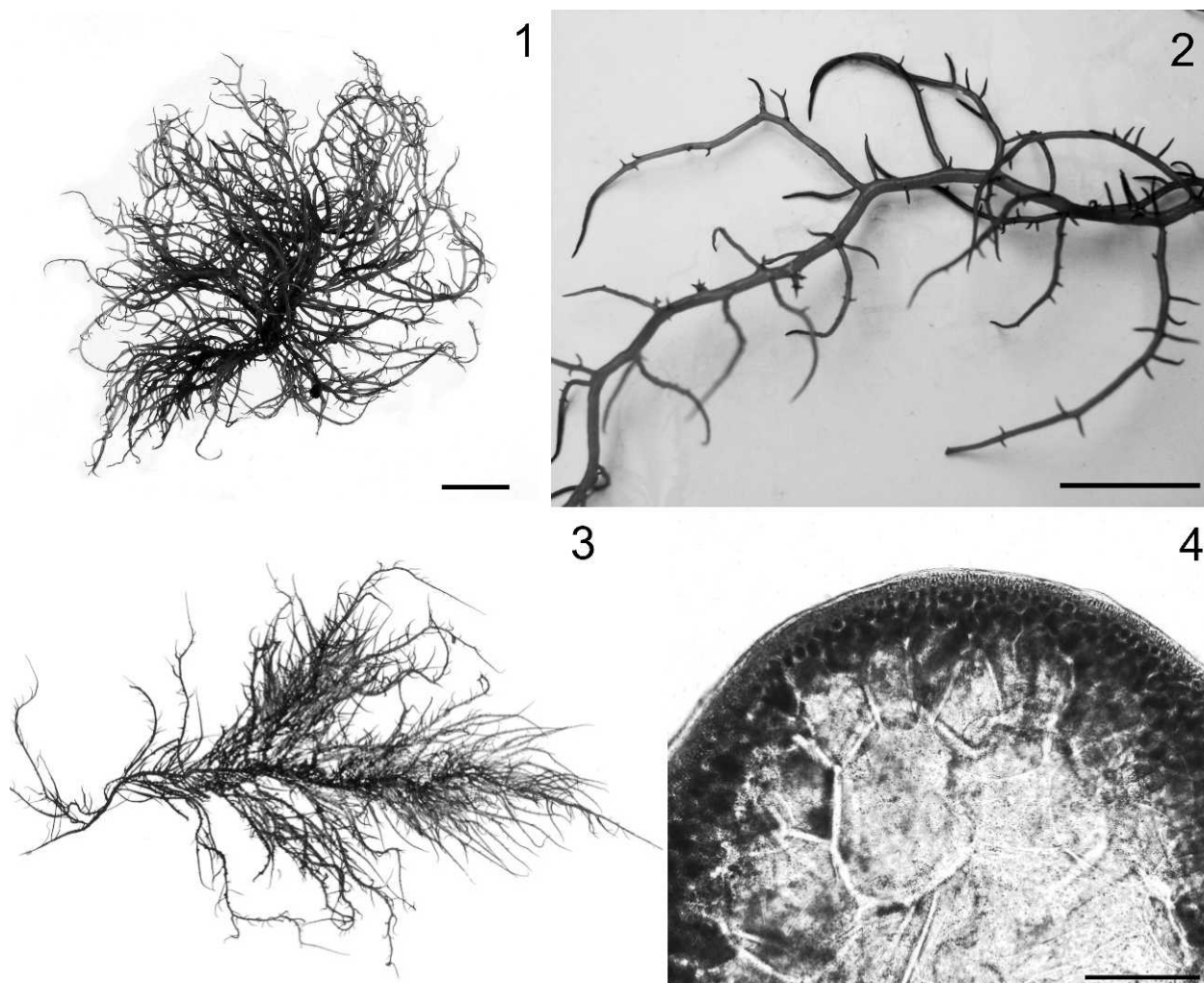
TYPE LOCALITY: Jetty, marine coast, Lido, Venice, Italy (45°22'140"N, 12°20'362"E).

ETYMOLOGY: *viridis* (Latin) = green, and refers to the green-yellowish thallus colour.

HABITAT: Artificial rocky substrata of the low midlittoral and upper sublittoral zones.

DISTRIBUTION: Gulf of Venice, Adriatic Sea; Licata, Agrigento, southern Sicily.

The thallus was green-yellowish in colour but sometimes it was a partially reddish colour (Figs 1,2). Thalli were



Figs 1–4. *Gracilaria viridis*.

Fig. 1. Habit of the thallus collected in the Venice Lagoon, Italy (image of the holotype, A000008). Scale bar = 2 cm.

Fig. 2. Branches and branchlets of thallus (A000008). Scale bar = 0.5 cm.

Fig. 3. Habit of the thallus collected in Sicily, Italy (*Gracilaria*-LI02). Scale bar = 2 cm.

Fig. 4. Cross-section of an axis showing cortical, sub-cortical and medullary cells (A000008). Scale bar = 200 μ m.

attached by a small discoid holdfast bearing erect fronds; thalli were 5–35 cm high and 0.5–1.3 mm thick, they were smooth throughout, and they were irregularly branched at the basal and median regions, with dense entangled branching in the apical parts, especially on the oldest specimens. Branches and branchlets formed without order, but they were mostly perpendicular and frequently sigmoid or recurvate (Fig. 3). Branching insertions were feebly restricted at the base.

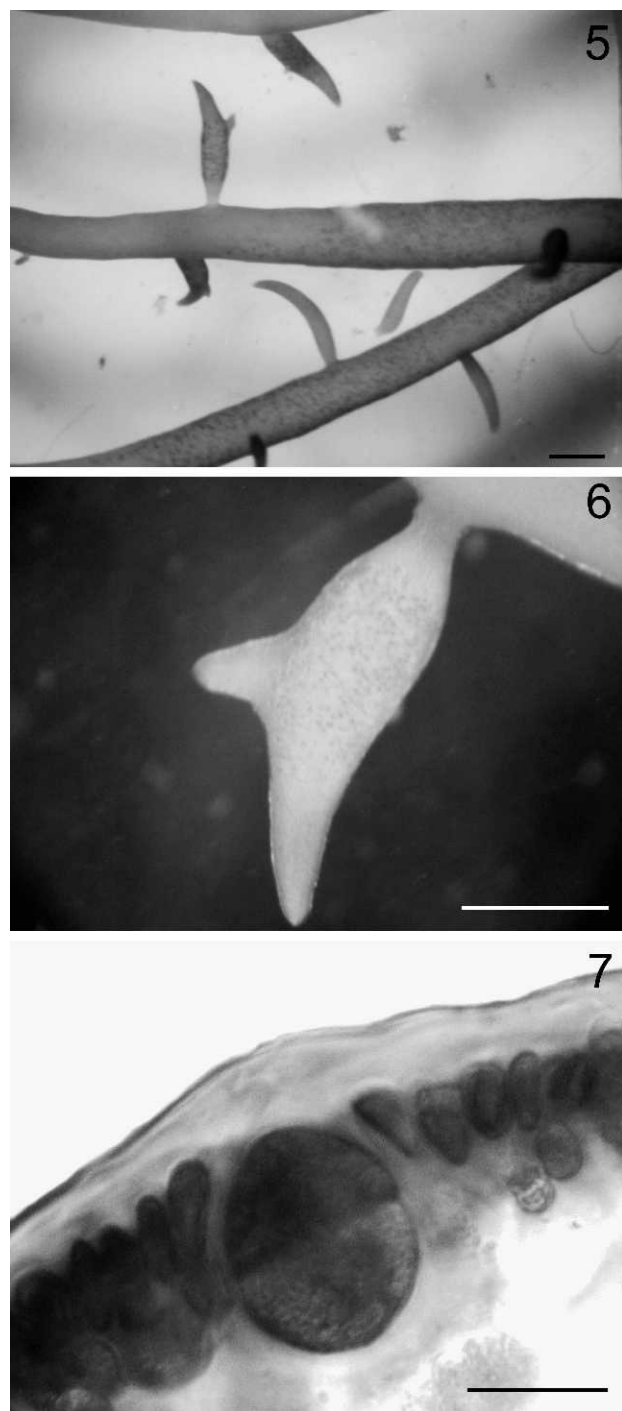
The same thallus morphology was observed for the Sicily strain, which was collected in 2002 (Fig. 3).

Cortical cells had a rounded-polyhedric shape, *c.* 7–10 μ m in surface view. In cross-section, they were 3–6 μ m in width and 7–10 μ m long. There were one or two layers of subcortical cells, 10–20 μ m in diameter, and these were surrounded by the larger colourless thin-walled medullary cells (*c.* 150–200 to 300 μ m in diameter; Fig. 4). Subspherical tetrasporangia, *c.* 12–15 \times 20–35 μ m, were produced throughout the main branches (Fig. 5) and on short stipitate

unbranched to tribranched branchlets that were frequently enlarged in the median region (branches 0.3–0.45 mm diameter 1–2(–3) mm in length) (Fig. 6). Tetrasporangia were \sim 30 μ m long and 13 μ m broad, and they were immersed in the cortex (Fig. 7).

Dark red mature ostiolate cystocarps, *c.* 800–1050 μ m in diameter but constricted at the base, were borne throughout the main axis and branches (Figs 8,9). Their dark-red pigmentation stands out on the green-yellowish plants. The inner pericarp was connected to the gonimoblast by tubular nutritive cells (Fig. 10). The gonimoblast tissue consisted of cellular rows producing ovoid carposporangia of 15–18 \times 20–30 μ m.

The male gametophytic plants formed round to elliptical spermatangial *Gracilaria verrucosa*-type conceptacles that were scattered on the cortical layer (Fig. 11). Sometimes they were confluent, showing a cristated shape in surface view. Conceptacle cavities were \sim 20–30 μ m diameter and 25–35 μ m deep.



Figs 5–7. *Gracilaria viridis*.

Fig. 5. Tetrasporophytic branches with short stipitate uni-tri-branched branchlets (A000008). Scale bar = 1 mm.

Fig. 6. Tetrasporophytic branches with short stipitate bi-branched branchlets (A000008). Scale bar = 0.5 mm.

Fig. 7. Tetrasporangium immersed in the cortex (A000008). Scale bar = 20 μ m.

Thalli were common from March to July in the artificial stone breakwaters along the Lido and Pellestrina islands; the alga also occurred on the first part of the limestone block jetties (dikes) that were placed between the seaside and the lagoon side for protection of the wide

lagoon inlets of Lido, Malamocco and Chioggia. They colonized the lower intertidal and the upper sublittoral zones where they formed a fringe of isolated thalli that were strongly entangled with many epiphytic taxa, especially *Ceramium*, *Polysiphonia* and *Centroceras*. Thalli were always haptophytic and were not able to grow in a free-floating form.

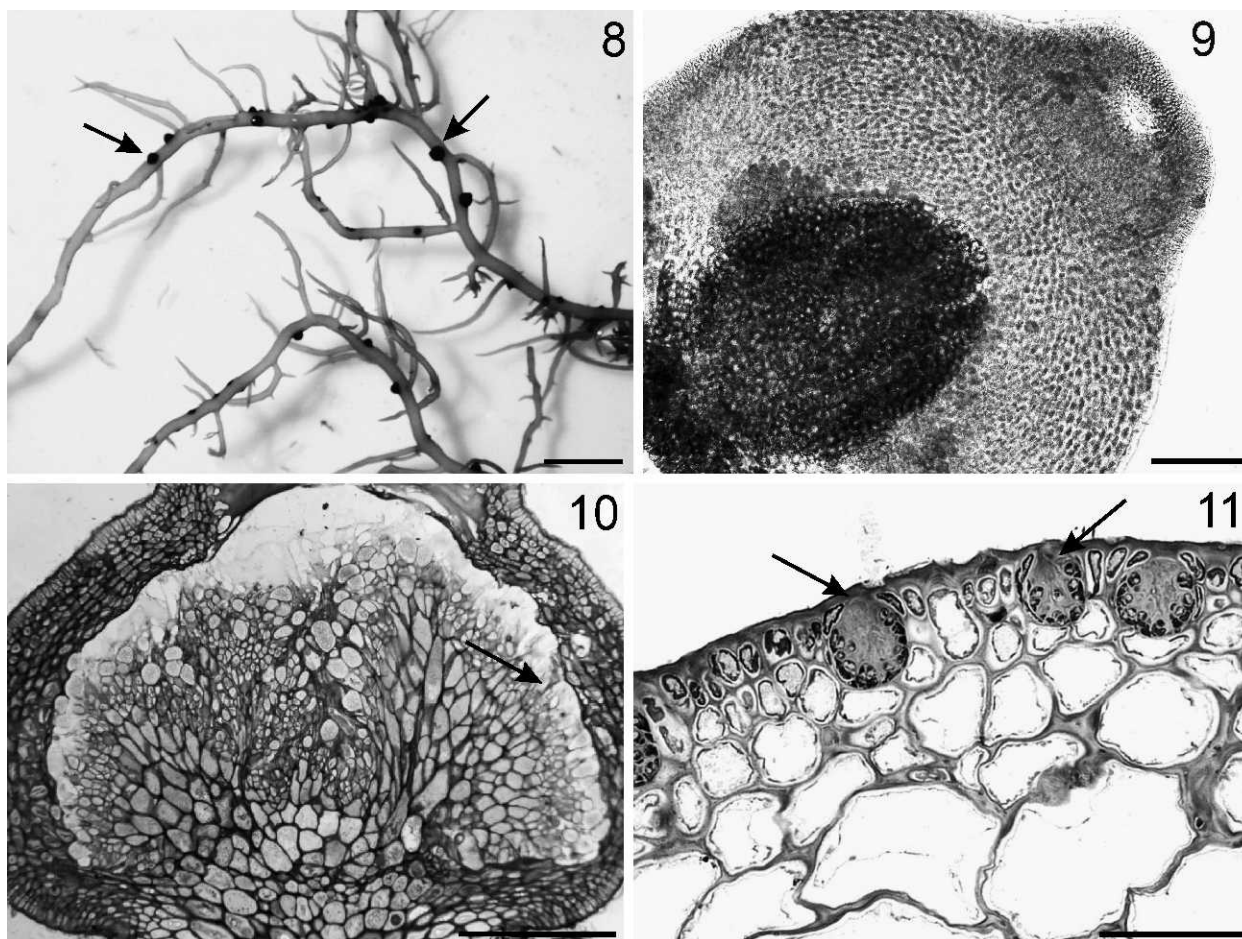
The *rbcL* and *rbcL-rbcS* spacer sequences from the five isolates were all identical. The partial *rbcL* sequence showed 99.66% identity with *Gracilaria* sp. (GenBank AY651040). The *rbcL-rbcS* spacer was most similar to *Gracilaria conferta* (Schousboe ex Montagne) Montagne and *Gracilaria gracilis* (Stackhouse) Stentoft, Irvine & Farnham. The *rbcL-rbcS* spacer region sequences were 220 bp long; they consisted of the *rbcL-rbcS* spacer (116 bp), a fragment of the *rbcL* gene (85 bp) and a fragment of the *rbcS* gene (19 bp). The spacer region showed a nucleotide divergence of 2.27% with *G. conferta* and of 5.45% with *G. gracilis*.

The *rbcL* dataset (1191 bp) included one sequence from a Venice specimen, a *G. conferta* sequence, obtained from an herbarium specimen kindly provided by Prof. C. Destombe (Morocco), 70 *Gracilaria* sequences from public databases and the outgroup sequence of *Hypnea spinella* (C. Agardh) Kützing (AF385635). The tree showed the Venice specimen in a well-supported clade with *Gracilaria* sp. from Sicily (100% of bootstrap support, 100 BT, and 1.00 of posterior probability, 1.00PP) (Fig. 12). *Gracilaria conferta* from Morocco was placed as sister taxon (100BT/1.00PP), while *G. pacifica* from Indian Island (USA) and two specimens of *G. gracilis* from France (AY049399) and Italy (EF434946) occupied a basal position.

DISCUSSION

The molecular and morphological features strongly suggest that our strain represents a new species, which we named *Gracilaria viridis* sp. nov. The *rbcL* phylogenetic analyses (Fig. 12) show that our strain is not conspecific with the cryptic species *G. gracilis* or with another Mediterranean terete species, *G. conferta*. *Gracilaria viridis* forms a separate clade with *Gracilaria* sp. LI02 from Sicily, and the clade is supported by 100BT/1.00PP values. Moreover, the small nucleotide divergence between the Sicilian and the Venetian specimens (0.34%) strongly suggests that they could be considered the same species.

The comparison of *rbcL-rbcS* spacer sequences shows that the closest species, *G. gracilis* and *G. conferta*, are distinctly different (divergence = 5.45% and 2.27%, respectively). That is, although the divergences are small, they fall with interspecific range reported for this genus (1.05–17.07%) and these differences are comparable with other *Gracilaria* species [1.94% for *G. gracilis* and *G. dura* (C. Agardh) J. Agardh; 2.72% for *G. conferta* and *G. dura* and 3.92% for *G. conferta* and *G. gracilis*] (Iyer *et al.* 2005). Moreover our results agree with those of other authors, i.e. no sequence variation was found between individuals assigned to the same morphospecies (Guillemin *et al.* 2008; Hommersand & Freshwater 2009).



Figs 8–11. *Gracilaria viridis*.

Fig. 8. Dark red ostiolate cystocarps (arrows) on green-yellowish thalli (*Gracilaria* VE01). Scale bar = 2 mm.

Fig. 9. Longitudinal section of cystocarp (*Gracilaria* VE01). Scale bar = 200 μ m.

Fig. 10. Longitudinal section of cystocarp with tubular nutritive cells (arrow) (*Gracilaria* LI02). Scale bar = 250 μ m.

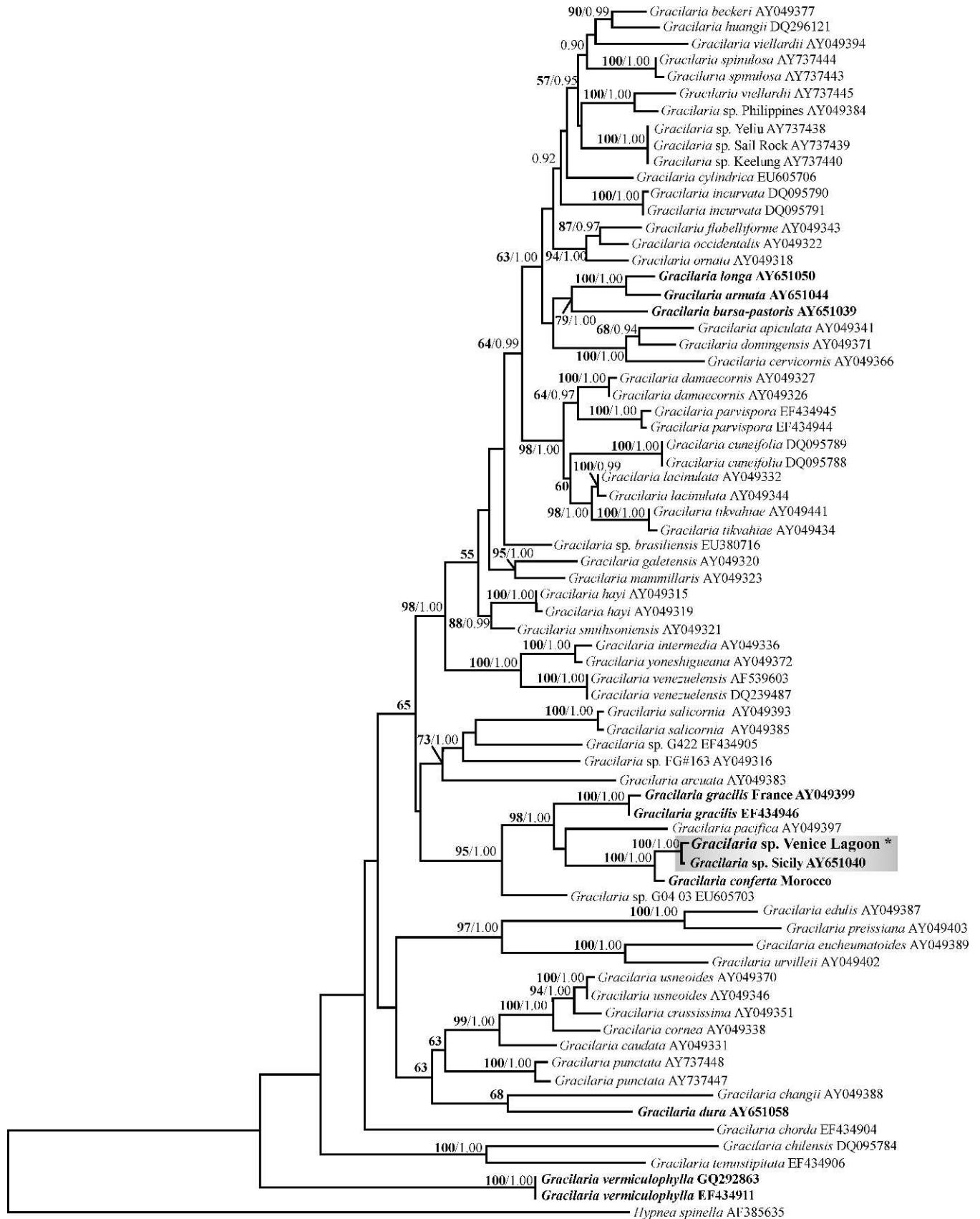
Fig. 11. Cross-section of a male gametophytic plant showing round-elliptical spermatangial *verrucosa*-type conceptacles (arrow) scattered on the cortical layer (*Gracilaria* VE02). Scale bar = 50 μ m.

Thalli of *Gracilaria viridis* are distinguishable from other Mediterranean terete species of *Gracilaria* (Table 1). For example, *G. longa* Gargiulo, De Masi & Tripodi and *G. vermiculophylla* (Ohmi) Papenfuss differ for the much bigger size of the thallus and *G. armata* (C. Agardh) Greville for thicker axes. The phylogenetically related species *G. conferta* presents both cystocarps and tetrasporangia only on distal branches and not on the entire thallus as occurs on *G. gracilis* and *G. viridis*. Therefore when only vegetative thalli are examined, *G. viridis* could be misidentified as *G. conferta* or *G. gracilis* (e.g. Sfriso & Curiel 2007; Sfriso *et al.* 2009). Superficially, *G. gracilis* thalli resemble those of our new species except for the dark red to purple colour of *G. gracilis*. However, with careful examination, *G. gracilis* has

bigger cortical cells (10- μ m width, 14 μ m long), subcortical cells (40–80 in diameter) and tetrasporangia (30 \times 40 μ m) (Iyer *et al.* 2004); furthermore, *G. gracilis* has smaller cystocarps (500–700 μ m) and less deep male conceptacles (30–40 μ m).

In 1992 Gargiulo and collaborators identified an exsiccata from Chioggia (Levi, no date, MS-33076-04) as *G. conferta*; the thalli were characterized by some morphological features similar to those of *G. viridis*, e.g. terete filaments with a main axis diameter < 1.5 μ m and dense bushy or spiny branching in the distal portions. However, our new species is larger, reaching an height of 30–35 cm, instead of the 8 cm reported by Gargiulo *et al.* (1992). Also, our species never has a wine-red colour – it is always green-yellowish, with at most a few

Fig. 12. ML tree inferred from *rbcL* gene sequences, calculated using the GTR+I+ Γ model of evolution. Numbers near nodes indicate bootstrap values (>50%) for ML analysis (in bold black font), and posterior probabilities (in normal font). The new species sequence determined in this work is evidenced in bold, in higher size and marked with an asterisk. The Mediterranean species sequences are indicated in bold. Scale bar = 0.02 nucleotide substitutions per site.



reddish parts. Unfortunately, there is only a morphological description for the exsiccata, and no molecular data are available for a comparison.

ACKNOWLEDGEMENTS

The authors thank Prof. G.M. Gargiulo and Dr G. Genovese for their helpful observations of the material from Licata. We also thank Prof. C. Destombe for providing a specimen of *G. conferta* from Morocco. This work was supported by grants provided by Progetto di Ateneo of the University of Padova.

REFERENCES

- ALTSCHUL S.F., GISH W., MILLER W., MYERS E.W. & LIPMAN D.J. 1990. Basic Local Alignment Search Tool. *Journal of Molecular Biology* 215: 403–410.
- BELLORIN A.M., OLIVEIRA M.C. & OLIVEIRA E.C. 2002. Phylogeny and systematics of the marine algal family Gracilariaceae (Gracilariales, Rhodophyta) based on small subunit rDNA and ITS sequences of Atlantic and Pacific species. *Journal of Phycology* 38: 551–563.
- BELLORIN A.M., OLIVEIRA M.C. & OLIVEIRA E.C. 2004. *Gracilaria vermiculophylla*: A western Pacific species of Gracilariaceae (Rhodophyta) first recorded from the eastern Pacific. *Phycological Research* 52: 69–79.
- BIRD C.J. 1995. A review of recent taxonomic concepts and developments in the Gracilariaceae (Rhodophyta). *Journal of Applied Phycology* 7: 255–267.
- CECERE E. & PETROCELLI A. 2009. The Mar Piccolo of Taranto. In: *Flora and vegetation of the Italian transitional water systems* (Ed. by Cecere E., Petrocelli A., Izzo G. & Sfriso A.), pp. 195–228. CORILA, Spinea, Italy.
- CHO T.O., FREDERICQ S. & BOO S.M. 2003. *Ceramium inkyuui* sp. nov. (Ceramiaceae, Rhodophyta) from Korea; a new species based on morphological and molecular evidence. *Journal of Phycology* 39: 237–247.
- FELSENSTEIN J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* 39: 783–791.
- FREDERICQ S. & HOMMERSAND M.H. 1989a. Proposal of the Gracilariales ord. nov. (Rhodophyta) based on analysis of the reproductive development of *Gracilaria verrucosa*. *Journal of Phycology* 25: 213–227.
- FREDERICQ S. & HOMMERSAND M.H. 1989b. Comparative morphology and taxonomic status of *Gracilariopsis* (Gracilariales, Rhodophyta). *Journal of Phycology* 25: 228–241.
- FREDERICQ S. & HOMMERSAND M.H. 1990. Diagnoses and key to the genera of the Gracilariaceae (Gracilariales, Rhodophyta). *Hydrobiologia* 204/5: 172–178.
- FREDERICQ S., HOMMERSAND M. & FRESHWATER D.W. 1996. The molecular systematics of some agar- and carrageenan-containing marine red algae based on *rbcL* sequence analysis. *Hydrobiologia* 326/327: 125–135.
- FRESHWATER D.W. & RUENESS J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia* 33: 187–194.
- FRESHWATER D.W., FREDERICQ S., BUTLER B.S., HOMMERSAND M.H. & CHASE M.W. 1994. A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbcL*. *Proceedings of the National Academy of Science USA* 91: 7281–7285.
- FRESHWATER D.W., MONTGOMERY F., GREENE K.J., HAMNER M.R., WILLIAMS M. & WHITFIELD E.P. 2006. Distribution and identification of an invasive *Gracilaria* species that is hampering commercial fishing operations in southeastern North Carolina, USA. *Biological Invasions* 8: 631–637.
- FURNARI G., CORMACI M. & SERIO D. 1999. Catalogue of the benthic marine macroalgae of the Italian coast of the Adriatic Sea. *Boccone* 12: 1–214.
- FURNARI G., GIACCONE G., CORMACI M., ALONGI G. & SERIO D. 2003. Biodiversità marina delle coste italiane: catalogo del macrofitobenthos. *Biologia Marina Mediterranea* 10: 1–482.
- GARGIULO G.M., DE MASI F. & TRIPODI G. 1987. Structure and reproduction of *Gracilaria longa* sp. nov. (Rhodophyta, Gigartinales) from the Mediterranean Sea. *Giornale Botanico Italiano* 121: 247–257.
- GARGIULO G.M., DE MASI F. & TRIPODI G. 1992. Morphology, reproduction and taxonomy of the Mediterranean species of *Gracilaria* (Gracilariales, Rhodophyta). *Phycologia* 31 (1): 53–80.
- GARGIULO G.M., MORABITO M., GENOVESE G. & DE MASI F. 2006. Molecular systematics and phylogenetics of gracilariacean species from the Mediterranean Sea. *Journal of Applied Phycology* 18: 497–504.
- GUILLEMIN M.-L., AIT AKKI S., GIVERNAUD T., MOURADI A., VALERO M. & DESTOMBE C. 2008. Molecular characterisation and development of rapid molecular methods to identify species of Gracilariaceae from the Atlantic coast of Morocco. *Aquatic Botany* 89(3): 324–330.
- GUINDON S. & GASCUEL O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.
- GUIRY M.D. & GUIRY G.M. 2011. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>.
- GURTEL C.F.D. & FREDERICQ S. 2004. Systematics of the Gracilariaceae (Gracilariales, Rhodophyta): a critical assessment based on *rbcL* sequence analyses. *Journal of Phycology* 40: 138–159.
- HAYDEN H.S. & WAALAND J.R. 2004. A molecular systematic study of *Ulva* (Ulveae, Ulvales) from the northeast Pacific. *Phycologia* 43: 364–382.
- HOMMERSAND M.H. & FRESHWATER D.W. 2009. *Gracilaria hummii* sp. nov. (Gracilariales, Rhodophyta), a new name for the agarophyte “*Gracilaria confervoides*” harvested in North Carolina during World War II. *Journal of Phycology* 45(2): 503–516.
- HOMMERSAND M.H., FREDERICQ S., FRESHWATER D.W. & HUGHEY J. 1999. Recent developments in the systematics of the Gigartinales (Gigartinales, Rhodophyta) based on *rbcL* sequence analysis and morphological evidence. *Phycological Research* 47: 139–151.
- IYER R., DE CLERCK O., BOLTON J.J. & COYNE V.E. 2004. Morphological and taxonomic studies of *Gracilaria* and *Gracilariopsis* species (Gracilariales, Rhodophyta) from South Africa. *South African Journal of Botany* 70: 521–539.
- IYER R., TRONCHIN E.M., BOLTON J.J. & COYNE V.E. 2005. Molecular systematics of the Gracilariaceae (Gracilariales, Rhodophyta) with emphasis on Southern Africa. *Journal of Phycology* 41: 672–84.
- LANAVE C., PREPARATA G., SACCONI C. & SERIO G. 1984. A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* 20: 86–93.
- MADDISON W.P. & MADDISON D.R. 2009. Mesquite: a modular system for evolutionary analysis. Version 2.71. <http://mesquiteproject.org>.
- MAGGS C.A., MCIVOR L.M., EVANS C.E., RUENESS J. & STANHOPE M.J. 2002. Molecular analyses elucidate the taxonomy of fully corticated, nonspiny species of *Ceramium* (Ceramiaceae, Rhodophyta) in the British Isles. *Phycologia* 41: 409–420.
- NETO A.I., TERRA M.R. & HAROUN R.J. 2002. New foliose and gelatinous red macroalgae (Rhodophycota) from the Azores: morphological and geographical observations. *Aquatic Botany* 72: 1–11.
- POSADA D. & BUCKLEY T.R. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.
- POSADA D. & CRANDALL K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

- RONQUIST F. & HUELSENBECK J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- SAUNDERS G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transaction of the Royal Society B* 360: 1879–1888.
- SFRISO A. & CUIEL D. 2007. Check-list of marine seaweeds recorded in the last 20 years in Venice lagoon and a comparison with the previous records. *Botanica Marina* 50: 22–58.
- SFRISO A., CUIEL D. & RISMONDO A. 2009. The Venice Lagoon. In: *Flora and vegetation of the Italian transitional water systems* (Ed. by Cecere E., Petrocelli A., Izzo G. & Sfriso A.), pp. 17–80. CORILA, Spinea, Italy.
- SFRISO A., MAISTRO S., ANDREOLI C. & MORO I. 2010. First record of *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) in the Po Delta lagoons, Mediterranean Sea (Italy). *Journal of Phycology* 46: 1024–1027.
- SKAGE M., GABRIELSEN T.M. & RUENESS J. 2005. A molecular approach to investigate the phylogenetic basis of three widely used species groups in the red algal genus *Ceramium* (Ceramiales, Rhodophyta). *Phycologia* 44: 353–360.
- SMITH G.E. 1954. Cytological observations on *Gracilaria multipartita*. (In) *Inaugural meeting of the British Phycological Society — Edinburgh, July, 1952*. *British Phycological Bulletin*, 1(2): 2–7.
- THOMPSON J.D., HIGGINS D.G. & GIBSON T.J. 1994. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.
- TROELL M., HALLING C., NEORI A., CHOPIN T., BUSCHMANN A.H., KAUTSKY N. & YARISH C. 2003. Integrated mariculture: asking the right questions. *Aquaculture* 226: 69–90.
- WOLF M.A., SCIUTO K., MAGGS C.A., BARROS-BARRETO M.B.B., ANDREOLI C. & MORO I. 2011. *Ceramium* Roth (Ceramiales, Rhodophyta) from Venice lagoon (Adriatic Sea, Italy): comparative studies of Mediterranean and Atlantic taxa. *Taxon* 60: 1584–1595.

Received 19 January 2012; accepted 13 September 2012
Associate Editor: Mariana Cabral Oliveria