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J. Wiedenmann · A. Baumstark · T. L. Pillen
A. Meinesz · W. Vogel

DNA fingerprints of *Caulerpa taxifolia* provide evidence for the introduction of an aquarium strain into the Mediterranean Sea and its close relationship to an Australian population

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Abstract The occurrence of *Caulerpa taxifolia* in the Mediterranean Sea was reported for the first time in 1984. Since then the alga has spread rapidly and is now considered to be a potential threat to sublittoral ecosystems. Two hypotheses on the origin of the Mediterranean strain of *C. taxifolia* have been discussed in the literature. One hypothesis assumed migration of the alga from the Red Sea, the other introduction via a public aquarium. The hypothesis of a descent from an aquarium strain has been supported strongly by recent studies based on DNA sequences. The DNA fingerprints of *C. taxifolia* presented here also provide evidence for the descent of the Mediterranean *C. taxifolia* from an aquarium strain. Furthermore, the present study shows that a strain of *C. taxifolia* from Manly Harbour/Moreton Bay (Australia) is closely related to the aquarium/Mediterranean strain. The feasibility of detecting similar genotypes by restriction digests of total DNA is demonstrated, which will facilitate the ongoing search for further relatives of Mediterranean *C. taxifolia*.

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J. Wiedenmann (✉)
Department of General Zoology and Endocrinology,
University of Ulm, Albert-Einstein-Allee 11,
89069 Ulm, Germany

e-mail: Joerg.Wiedenmann@biologie.uni-ulm.de
Fax: +49-731-5022581

A. Baumstark · W. Vogel
Department of Medical Genetics, University of Ulm,
Albert-Einstein-Allee 11, 89069 Ulm, Germany

T. L. Pillen
Research Station Fetovaia,
Hydra-Institute of Marine Sciences,
57034 Marina di Campo, Elba, Italy

A. Meinesz
Laboratoire Environnement Marin Littoral,
University of Nice – Sophia Antipolis,
06108 Nice Cedex 2, France

Introduction

Caulerpa taxifolia (Vahl) C. Agardh (Ulvophyceae: Caulerpales) is a common green alga of tropical seas (Meinesz et al. 1994). In 1984 a low-temperature-resistant strain of *C. taxifolia* was observed for the first time in the Mediterranean Sea, along the coast of Monaco (Meinesz and Hesse 1991; Meinesz and Boudouresque 1996; Meinesz et al. 1998). Since then the alga has spread rapidly by vegetative reproduction, and covered more than 4600 ha of sea-bed in 1997 (Meinesz 1992; Meinesz et al. 1998). With its potential to overgrow natural biotopes, it represents a major risk for Mediterranean sublittoral ecosystems (Boudouresque et al. 1995; Romero 1997).

Two hypotheses on the origin of the Mediterranean strain of *C. taxifolia* are posed in the literature: one assumes migration of *Caulerpa mexicana* from the Red Sea and metamorphosis into *C. taxifolia* (Chisholm et al. 1995); the other, an introduction of *C. taxifolia* via a public aquarium (Meinesz and Hesse 1991; Meinesz and Boudouresque 1996). The latter has been strongly supported by demonstrating that *C. taxifolia* from the Mediterranean Sea and from different aquaria have identical ITS rDNA sequences (Jousson et al. 1998) and that – also based on ITS rDNA sequences – Mediterranean *C. taxifolia* and *C. mexicana* are not conspecific (Jousson et al. 1998; Olsen et al. 1998). Knowledge of the origin of this invasive strain of *C. taxifolia* and on the population structure are fundamental for understanding the dynamics of the invasion.

In our study we used DNA fingerprinting to characterise strains of *C. taxifolia*, as this technique has an exceedingly high power for differentiating and identifying individual genotypes (Epplen et al. 1992; Housman 1995; Coffroth 1998). Moreover, as methods based on DNA sequences, like studies of ITS (internal transcribed

spacer regions), can be very time consuming (Coffroth 1998), we aimed at establishing DNA fingerprinting of *Caulerpa* as a technique to quickly distinguish among genotypes of *C. taxifolia*.

Materials and methods

Sample collection and storage

Specimens of *Caulerpa taxifolia* originating from different geographical regions were analysed in this study (Table 1). Distances of 50–300 m were kept between each sampling location in which more than one sample was taken from the same region (Elba, Italy and St. Cyprien, France). All samples of *Caulerpa* spp. were cultivated in aquaria for further analysis, except the sample of *C. taxifolia* from Starigrad (Hvar, Croatia), which was collected and preserved in ethanol (100%).

DNA fingerprinting

The specimens were kept in the dark for 48 h to reduce their starch content prior to DNA isolation. Approximately 8 g of photoassimilatory fronds of each sample were used for analysis. Total DNA was isolated using CTAB (*N*-cetyl-*N*, *N*, *N*, trimethyl ammonium bromide) instead of SDS (sodium dodecyl sulphate) as described by Dellaporta et al. (1983). DNA was dissolved in 1× TE buffer (Tris/EDTA) and purified by phenol/chloroform extraction and isopropanol precipitation according to Sambrook et al. (1989).

RNase digests were performed by adding RNase A to selected samples. After a 1 h incubation at 37 °C, phenol/chloroform extraction and isopropanol precipitation was repeated.

About 10 µg DNA per sample was digested by the restriction enzyme *Taq*I for 6 h at 65 °C as described by the manufacturer (Pharmacia). DNA fragments were separated on agarose gels [1.2%, 50 V, 14 h, 1× TBE buffer (Tris/Borate/EDTA)]. Gels were stained with ethidium bromide and documented with a Fluor-S Multi-Imager (BIO-RAD). Subsequently, DNA was transferred to a Hybond-N^+-membrane according to Southern (1975) and probed with $(CAC)_5$ (Epplen et al. 1992) or $(GAA)_6$ (Epplen et al. 1991), labelled with ^{32}P γ-ATP. Membranes were rinsed in 6 × SSC (sodium chloride/trisodium citrate) for 0.5–1 h at room temperature. Autoradiography was performed at –80 °C for up to 48 h. The buffers are described in Ausubel et al. (1995).

Results

Detection of different genotypes of *Caulerpa taxifolia* by DNA fingerprinting

To ensure that DNA fingerprints are suitable to distinguish different genotypes of *C. taxifolia*, samples of the tropical strains from Ryukyu and Martinique were compared to the aquarium strains from Stuttgart, Ulm and Enoshima. *Caulerpa prolifera* was included in the study in order to examine the variability of genotypes detectable among different species. The $(CAC)_5$-hybridisation patterns obtained from *C. prolifera* and the tropical strains could be clearly distinguished from each other and from the aquarium strains. In contrast, all strains from the aquaria showed identical fingerprints (Fig. 1).

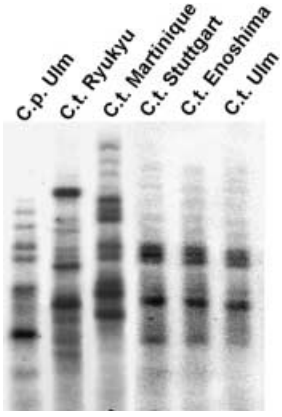


Fig. 1 *Caulerpa* spp. DNA fingerprints after *Taq*I digestion of total DNA and hybridisation with $(CAC)_5$ of *C. prolifera* (C.p.) and *C. taxifolia* (C.t.) from Ryukyu, Martinique and the aquaria of Stuttgart, Enoshima and Ulm. The control samples (lanes 1–3) can clearly be distinguished from the strains from the aquaria. In contrast, the aquarium samples show identical fingerprints

Table 1 *Caulerpa* spp. Origin of plant material used in the present study

Species	Origin	Collector (date of collection)
Caulerpa taxifolia	Aquarium Tornseifer Co., Ulm (Germany)	Wiedenmann (1996)
	Aquarium Enoshima (Japan)	Komatsu (1997) via Aquarium Meinesz
	Aquarium Wilhelma, Stuttgart (Germany)	Koch, Wiedenmann (1998)
	Cala d'Or, Mallorca (Spain)	Meinesz (1992) via Aquarium Meinesz
	St. Cyprien Harbour 1–3 (France)	Wiedenmann (1997)
	Monaco (France)	Cottalorda (1998) via Aquarium Meinesz
	Marina di Campo, Island Elba 1–3 (Italy)	Pillen (1998)
	Strait of Messina, Sicily (Italy)	Spohr (1998)
	Malinska Harbour, Krk (Croatia)	Meinesz (1995) via Aquarium Meinesz
	Starigrad, Hvar (Croatia)	Zuljevic (1998)
	Manly Harbour, Moreton Bay (Australia)	Pillen (1998)
	Martinique (France)	Blachier (1998) via Aquarium Meinesz
	Ryukyu (Japan)	Komatsu (1997) via Aquarium Meinesz
Caulerpa prolifera	Aquarium University Ulm (Germany)	Wiedenmann (1998)

Comparison of the strains from aquaria and the Mediterranean

The Mediterranean strains of *C. taxifolia* from Monaco, Krk and Sicily were compared to the aquarium strains from Stuttgart and Enoshima and the control strains (*C. taxifolia* from Martinique, *C. prolifera*). The control samples could be clearly distinguished, while the aquarium and Mediterranean strains of *C. taxifolia* revealed identical DNA fingerprints after *Taq*I digestion and $(CAC)_5$-hybridisation (Fig. 2). The same DNA fingerprints were obtained from the Mediterranean strains from Mallorca, Elba and St. Cyprien. Furthermore, samples from aquaria and the Mediterranean Sea revealed identical restriction patterns on Southern blots hybridised with $(GAA)_6$ (Fig. 3).

Comparison of the aquarium/Mediterranean strain and the Australian strain

Only slight differences between Australian and Mediterranean *C. taxifolia* in the position of a single band could be detected when Southern blots were probed with $(CAC)_5$ (Fig. 2). Furthermore, on $(GAA)_6$-hybridised Southern blots, no distinction could be made between the samples of *C. taxifolia* from Manly Harbour and from aquaria/Mediterranean Sea, whereas the sample from Martinique and *C. prolifera* were obviously different (Fig. 3).

*Taq*I-digested nucleic acids showed distinct bands in the range of 1–6.5 kb in ethidium-bromide-stained agarose gels. Some of these fragments exhibit a higher fluorescence intensity compared to the others. These fragments have apparent lengths of about 3.5 kb (*C. prolifera*, *C. taxifolia* from Martinique and Stuttgart), and of 2 and 6.5 kb (*C. taxifolia* from Martinique) (Fig. 4). Two further fragments of this category, with lengths of ca. 3 and 4 kb, are visible in the sample of *C. taxifolia* from Ryukyu (Fig. 5). They could be shown to represent a group of RNA species as they disappear

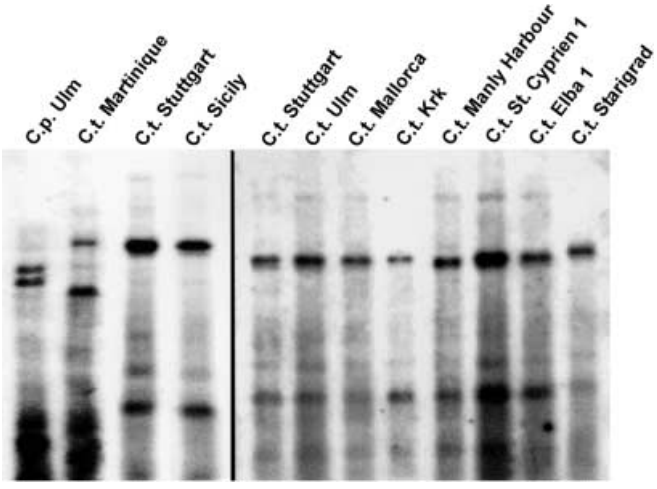


Fig. 3 *Caulerpa* spp. Fingerprints of total DNA after *Taq*I digestion and hybridisation with $(GAA)_6$. In contrast to the control samples (*C. prolifera*, *C. taxifolia* from Martinique), which can clearly be distinguished, the samples of *C. taxifolia* from aquaria (Stuttgart, Ulm), Mediterranean Sea and Manly Harbour (Australia) reveal identical restriction patterns. The Mediterranean samples are represented by Sicily, Mallorca, Krk, St. Cyprien 1, Elba and Starigrad

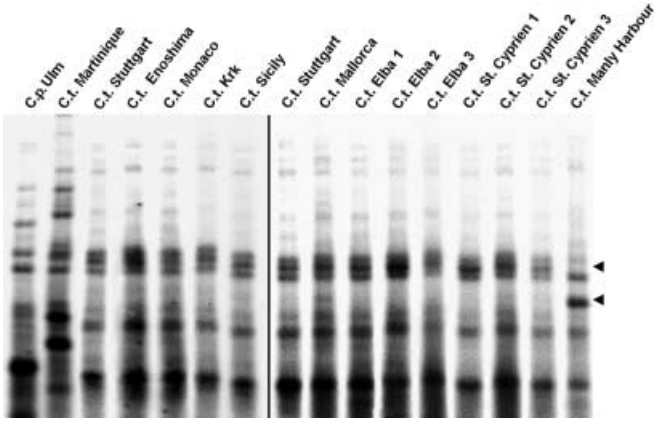


Fig. 2 *Caulerpa* spp. Comparison of *C. taxifolia* strains from aquaria, the Mediterranean Sea and from Manly Harbour (Australia) on $(CAC)_5$-hybridised Southern blots of total DNA after *Taq*I digestion. In contrast to the differing control samples (*C. prolifera*, *C. taxifolia* from Martinique), the aquarium strains from Stuttgart and Enoshima reveal identical restriction patterns as samples from the Mediterranean Sea (Monaco, Krk, Sicily, Mallorca, Elba 1–3, St. Cyprien 1–3). Only slight differences in the position of a single band (indicated by *arrowheads*) was detected between the sample from Manly Harbour (lane 16) and the specimens from aquaria and the Mediterranean Sea

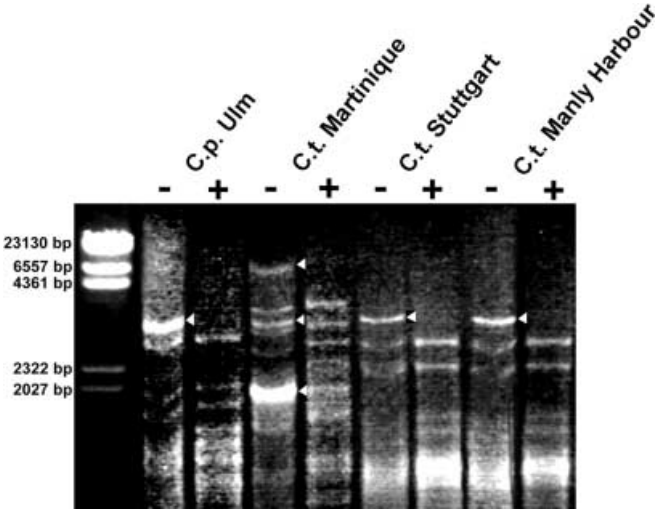


Fig. 4 *Caulerpa* spp. Total nucleic acids of *C. prolifera* (lanes 1, 2) and *C. taxifolia* from Martinique (lanes 3, 4), Stuttgart (lanes 6, 7) and Manly Harbour (lanes 7, 8) after digestion with *Taq*I and separation in ethidium-bromide-stained agarose gels. Some of the fragments in samples without additional RNase digestion (lanes marked with a *minus sign*) are characterised by a higher intensity of ethidium bromide fluorescence (*arrowheads*). These bands were revealed to belong to a group of RNA species, with apparent lengths between 2000 and 6500 bp, as they disappear when the samples are additionally digested with RNase A (lanes marked with a *plus sign*)

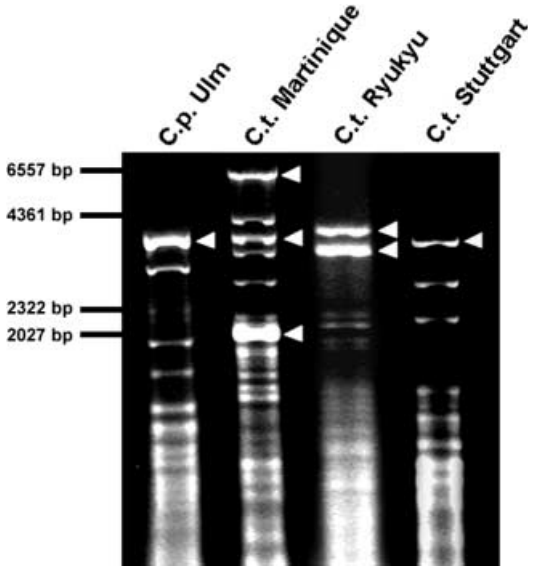


Fig. 5 *Caulerpa* spp. *Taq*I-digested total DNA after separation in ethidium-bromide-stained agarose gels. Samples of *C. prolifera* and *C. taxifolia* from Martinique, Ryukyu and Stuttgart exhibit different restriction patterns. The RNA bands present in these samples are marked by *arrowheads* (compare Fig. 4)

after digestion with RNase A, as demonstrated for *C. prolifera* and *C. taxifolia* from Martinique, Stuttgart and Manly Harbour (Fig. 4). Visualised on agarose gels, ribosomal RNAs contained in these samples were highly degraded, indicating RNase activity in the storage buffer (data not shown). The unknown RNA species must be highly stable, as they were not degraded by this RNase activity.

The other bands visible in ethidium-bromide-stained agarose gels after *Taq*I digestion most likely belong to plastidal or mitochondrial DNA, as complex nuclear DNA produces a homogeneous smear when digested with a frequent-cutting enzyme such as *Taq*I.

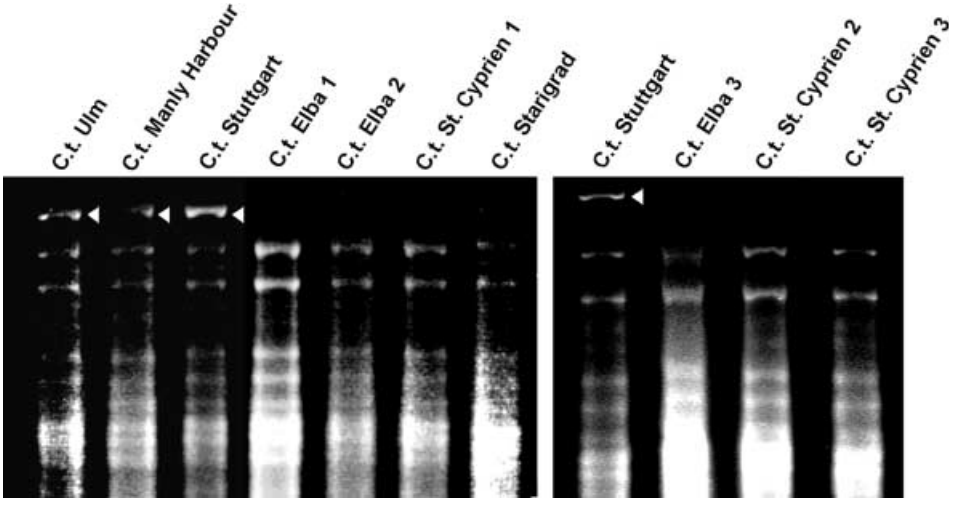
The restriction patterns show clear differences between *C. prolifera*, the tropical strains of *C. taxifolia* and the aquarium strain of *C. taxifolia* from Stuttgart (Fig. 5). In contrast, it was not possible to distinguish between the strains of *C. taxifolia* from Manly Harbour (Australia) and from the public aquaria or the Mediterranean Sea (Fig. 6).

Discussion

The application of oligonucleotide probes such as $(CAC)_5$ and $(GAA)_6$ in DNA fingerprinting has been shown to be useful for identifying individual genotypes in many species of plants, fungi and animals (review in Epplen et al. 1991). In contrast, no data are available for marine green algae. We compared the hybridisation patterns of *Taq*I-digested total DNA of *Caulerpa taxifolia* from Martinique (France), Ryukyu (Japan), several strains from the Mediterranean Sea, from different public aquaria and of the related taxon *Caulerpa prolifera*. Clear differences among these patterns demonstrate that oligonucleotide fingerprinting is suitable to distinguish different genotypes of *Caulerpa* spp.

We provide further evidence for the descent of the Mediterranean strain of *C. taxifolia* from an aquarium strain with this method, by comparing samples from 11 locations in the Mediterranean Sea with three representatives from public aquaria. Total DNA obtained from these specimens was digested with *Taq*I. Hybridisation patterns revealed no differences by probing DNA with $(CAC)_5$ or with $(GAA)_6$. The uniformity of hybridisation patterns indicates that representative specimens from the Mediterranean and aquaria belong to the same clone. In this light, the hypothetical descent of

Fig. 6 *Caulerpa taxifolia*. *Taq*I-digested total nucleic acids in ethidium-bromide-stained agarose gels. The restriction patterns of total DNA are identical for the aquarium strains (Ulm, Stuttgart), the Australian sample from Manly Harbour and Mediterranean strains (Elba 1–3, St. Cyprien 1–3, Starigrad). The lanes representing the samples from Ulm, Manly Harbour and Stuttgart show an additional band (*arrowheads*). This topmost band represents RNA, compare with Fig. 4. It is not visible after complete RNase digestion (Elba 1–3, St. Cyprien 1–3)



Mediterranean *C. taxifolia* from an aquarium strain (Meinesz and Hesse 1991; Meinesz and Boudouresque 1996) is quite likely.

Among the examined strains of *Caulerpa* spp., a group of highly stable RNA species, with apparent lengths between 2000 and 6500 bp, could be identified in ethidium-bromide-stained agarose gels. These RNAs possibly belong to one of the different types of ''non-infectious'' RNAs described for plant cells (Boccardo et al. 1986). Examples of such molecules, which are synthesised in an RNA-dependant manner, are genomes of cryptic viruses and double-stranded RNAs enclosed in a nucleocapsid structure. The algal genus *Bryopsis* has already been demonstrated to contain double-stranded RNAs present in nucleoprotein particles that are associated with both chloroplasts and mitochondria (Ishihara et al. 1992).

After *Taq*I digestion and separation of DNA fragments, distinct bands could be observed in ethidium-bromide-stained agarose gels. These fragments most likely belong to the plastidal or mitochondrial genomes, as the complex nuclear genome produces a homogeneous smear after digestion with a frequent-cutting enzyme such as *Taq*I. The restriction patterns obtained from *C. prolifera* and *C. taxifolia* from Martinique, Ryukyu and Stuttgart reveal clear differences. These differences show the possibility to detect both inter- and intraspecific variations by digesting total DNA with *Taq*I. In contrast, *C. taxifolia* from Manly Harbour (Australia) and the representatives of the aquarium/Mediterranean populations show identical restriction patterns in ethidium-bromide-stained agarose gels, indicating that they carry very similar chloroplast and mitochondria strains. Specimens of *C. taxifolia* from Australia, the Mediterranean Sea and public aquaria also cannot be distinguished on $(GAA)_6$-hybridised Southern blots. Slight differences in genotypes associated with the position of a single band can be found by probing Southern blots with $(CAC)_5$. As the control samples *C. prolifera* and *C. taxifolia* from Ryukyu (Japan) and Martinique (France) are always clearly different, these similarities indicate a close relationship of the Australian and the aquarium/Mediterranean strain. However, this result should be confirmed by an analysis involving more tropical strains. The observed differences may reflect the divergent evolution of two isolated parts of one ancestor strain or relations via sexual reproduction. In any case, the Australian population of Manly Harbour/Moreton Bay is well suited for comparative studies of the role of *C. taxifolia* in a non-Mediterranean ecosystem because of the close relationship to the aquarium/Mediterranean strain.

Overall, our results show that similarities between different strains of *C. taxifolia* can be detected in *Taq*I-digested DNA on the level of ethidium-bromide-stained agarose gels. In the ongoing search for further relatives of the aquarium/Mediterranean strain of *C. taxifolia*, this inexpensive technique enables a fast pre-screening of large sample sizes of *C. taxifolia* in order to select possible relatives for an evaluation with more sensitive methods such as hybridisation experiments or sequence-based analysis.

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References

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1995) Short protocols in molecular biology. Wiley, New York

Boccardo G, Lisa V, Vuisoni E, Milne RG (1986) Cryptic plant viruses. Adv Virus Res 32: 171–213

Boudouresque C-F, Meinesz A, Ribera MA, Ballesteros E (1995) Spread of the green alga *Caulerpa taxifolia* (Caulerpales, Chlorophyta) in the Mediterranean: possible consequences of a major ecological event. Sci Mar 59[Suppl 1]: 21–29

Chisholm JRM, Jaubert JM, Giaccone G (1995) *Caulerpa taxifolia* in the Northwest Mediterranean: introduced species or migrant from the Red Sea? C R Acad Sci Ser III Sci Vie 318: 1219–1226

Coffroth MA (1998) Molecular approaches to the study of clonal organisms: deciphering the alphabet soup. In: Lessios HA, MacIntyre (eds) Proceedings 8th international coral reef symposium. Smithsonian Tropical Research Institute, Balboa, Panama

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation, Ver II. Plant Mol Biol 1(4): 19

Epplen JT, Ammer H, Epplen C, Kammerbauer C, Mitreiter R, Roewer L, Schwaiger W, Steimle V, Zischler H, Albert E (1991) Oligonucleotide fingerprinting using simple repeat motifs: a convenient, ubiquitously applicable method to detect hypervariability for multiple purposes. In: Burke T, Dolf G, Jeffreys J, Wolff R (eds) DNA fingerprinting: approaches and applications. Birkhäuser, Basel, pp 50–69

Epplen JT, Melmer G, Schmidt L (1992) On the potential of simple repetitive DNA for fingerprinting in clinical, forensic, and evolutionary dynamic studies. Clin Investig Med 70: 1043–1051

Housman DE (1995) DNA on trial – The molecular basis of DNA fingerprinting. New Engl J Med 332: 534–535

Ishihara J, Pak JY, Fukuhara T, Nitta T (1992) Association of particles that contain double-stranded RNAs with algal chloroplasts and mitochondria. Planta 187: 475–482

Jousson O, Pawlowski J, Zaninetti L, Meinesz A, Boudouresque C-F (1998) Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. Mar Ecol Prog Ser 172: 275–280

Meinesz A (1992) Mode de dissémination de l'algue *Caulerpa taxifolia* introduite en Méditerranée. Rapp P-V Reun Comm Int Explor Sci Mer Mediterr 33B: 44

Meinesz A, Boudouresque CF (1996) On the origin of *Caulerpa taxifolia* in the Mediterranean Sea. C R Acad Sci Ser III Sci Vie 319: 603–613

Meinesz A, Hesse B (1991) Introduction et invasion de l'algue tropicale *Caulerpa taxifolia* en Méditerranée nord-occidentale. Oceanol Acta 14: 415–426

Meinesz A, Pietkiewicz D, Komatsu T, Caye G, Blachier J, Lemee R, Renoux-Meunier A (1994) Notes taxinomiques préliminaires sur *Caulerpa taxifolia* et *Caulerpa mexicana*. In: Boudouresque

C-F, Meinesz A, Gravez V (eds) 1st international workshop on *Caulerpa taxifolia*. GIS Posidonie, Marseille, pp 105–114

Meinesz A, Cottalorda J-M, Chiaverini D, Cassar N, Vaugelas J (1998) Suivi de l'invasion de l'algue tropicale *Caulerpa taxifolia* en Méditerranée: situation au 31 décembre 1997. Laboratoire Environnement Marin Littoral, University of Nice – Sophia Antipolis, Nice

Olsen JL, Valero M, Meusnier I, Boele-Bos S, Stam WT (1998) Mediterranean *Caulerpa taxifolia* and *C. mexicana* (Chlorophyta) are not conspecific. J Phycol 34: 850–856

Romero J (1997) Consequences de l'expansion de *Caulerpa taxifolia* en Méditerranée. In: Dynamique d'espèces marines invasives: application à l′ expansion de *Caulerpa taxifolia* en Méditerranée. Académie des Sciences, Paris, pp 241–254

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. Cold Spring Harbour Laboratory Press, New York

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98: 503–517