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<https://doi.org/10.2216/11-116.1>

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Molecular diversity of the *Caulerpa racemosa*–*Caulerpa peltata* complex (Caulerpaceae, Bryopsidales) in New Caledonia, with new Australasian records for *C. racemosa* var. *cylindracea*

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Molecular characterization (plastid-encoded *tufA* gene) of New Caledonian members of the *Caulerpa racemosa*–*Caulerpa peltata* complex identified five lineages, each possibly harboring multiple species-level entities. On a global scale, the complex encompassed six lineages, two of which were pantropical and the remainder seemingly were restricted to the Indo-Pacific basin. *Caulerpa racemosa* var. *cylindracea*, a fast-spreading introduced taxon in the Mediterranean Sea and the Canary Islands, was previously thought to be restricted to southwestern Australia; it was newly reported here for New Caledonia and several northern Australian locations, including the Great Barrier Reef.

KEY WORDS: Alien, Biodiversity, Biogeography, *Caulerpa*, *C. peltata*, *C. racemosa*, *C. racemosa* var. *cylindracea*, *C. taxifolia*, Invasive, New Caledonia, Seaweed, *tufA*

INTRODUCTION

The genus *Caulerpa* J.V. Lamouroux is well known for its morphological plasticity (e.g. Weber-van Bosse 1898; Børgesen 1907; Eubank 1946); intermediate morphologies are known to occur under changing abiotic conditions (e.g. flow, light intensity), after field transplant experiments (Tandy 1933, 1934; Senerpont Domis *et al.* 2003) and during laboratory culture studies (Peterson 1972; Calvert 1976; Enomoto & Ohba 1987; Ohba & Enomoto 1987; Ohba *et al.* 1992; Carruthers *et al.* 1993).

The *Caulerpa racemosa*–*C. peltata* complex has a predominantly tropical distribution (see Verlaque *et al.* 2000, fig. 17), and it comprises morphologically convergent entities which develop racemes of vesicles and/or shields. Morphological variations are taxonomically classified under numerous varieties or formae, mostly within *C. racemosa* (Forsskål) J. Agardh, but some subspecific ranks are also accepted within *C. peltata* J.V. Lamouroux (Guiry & Guiry 2012). *Caulerpa peltata* is also accepted by some as a variety

of *C. racemosa* (e.g. Abbott & Huisman 2004; Kraft 2007). Overall, the morphological instability and the inconsistent use of varieties and formae have resulted in a nomenclatural tangle. We have undertaken a new investigation of the molecular diversity of the complex, which previous studies had initiated (Famà *et al.* 2000, 2002; Verlaque *et al.* 2000, 2003, 2004; Durand *et al.* 2002; Senerpont Domis *et al.* 2003; Stam *et al.* 2006; Yeh & Chen 2004).

Previous phylogenies based on the chloroplast gene *tufA* (elongation factor *Tu*) have shown that the *C. racemosa*–*peltata* complex is polyphyletic; there are multiple entities found within three main lineages of a large, rapidly diversifying and unresolved clade termed ‘modern and fast evolving’ by Famà *et al.* (2002). Additional taxa of *Caulerpa* that exhibit raceme morphologies belong to a separate clade, which is termed ‘ancient and species poor’ (Famà *et al.* 2002). However, these latter species can be readily separated from the *C. racemosa*–*peltata* complex by having an embedded pyrenoid and by having vesicles sharply delimited from the stipe by a constriction (e.g. *C. lentillifera* J. Agardh, *C. okamurae* Weber-van Bosse and *C. opposita* Coppejans & Meinesz).

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A troublesome entity of the *C. racemosa–peltata* complex is the fast-spreading alien species *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman and Boudouresque that first appeared in the Mediterranean Sea and Canary Islands in the 1990s; it represents one of the most dramatic marine invasions in terms of establishment and ecological dominance (Verlaque *et al.* 2004; Klein & Verlaque 2008). Unlike *C. taxifolia* (Vahl) C. Agardh, which was accidentally introduced from a public aquarium (Jousson *et al.* 1998), the vector of introduction of *C. racemosa* var. *cylindracea* to the Mediterranean Sea is not known. Previously, it has been hypothesized to be a Lessepsian immigrant (Alongi *et al.* 1993; Giaccone & Di Martino 1995) or a hybrid between *C. racemosa* var. *turbinata* (J. Agardh) Eubank and an unknown tropical variety (Durand *et al.* 2002). However, more recently, molecular investigations of the *C. racemosa–peltata* complex by Verlaque *et al.* (2003) identified a potential source population originally described as *C. cylindracea* Sonder in southwestern Australia (SWA; now *C. racemosa* var. *cylindracea*; Verlaque *et al.* 2003). This finding provided the basis for the Australian origin hypothesis of the invader and its endemic status in SWA prior to its introduction to the Mediterranean Sea. Since then, an invasive population was also reported in Adelaide, South Australia (SA; see also Womersley 2003; Collins *et al.* 2004), and even more recently, DNA barcoding efforts identified additional records north of SWA (C.F.D. Gurgel & G.S. Belton, unpublished).

Previous *Caulerpa* investigations have generated >70 *tufA* sequences for the *C. racemosa–peltata* complex (e.g. Famà *et al.* 2002; Senerpont Domis *et al.* 2003; Stam *et al.* 2006). Despite these studies, the geographic areas where *Caulerpa* diversity is supposedly high remain poorly sampled. For instance, the Coral Triangle, an area encompassing Southeast Asia and Melanesia (Allen 2007), represents one of the three *Caulerpa* diversity hotspots (Prud'homme van Reine *et al.* 1996); the other two hotspots are the western tropical Atlantic and the southern coast of Australia.

In the present study, we explore genetic diversity of the *C. racemosa–peltata* complex using *tufA* DNA barcodes sequenced from herbarium collections that originated predominantly from New Caledonia (NC); these were complemented with collections from Southeast Asia. We compared the diversity of these collections to that of the global *C. racemosa–peltata* complex. *Caulerpa racemosa* var. *cylindracea* is newly reported from NC and several northern Australian locations.

MATERIAL AND METHODS

Herbarium specimens displaying racemose and/or peltate morphologies were selected from collections originating from NC (Table 1). These were supplemented with specimens from Southeast Asia. Following preliminary analysis confirming the presence of *Caulerpa racemosa* var. *cylindracea* in NC, taxon sampling was extended to the Mediterranean Sea (south of France and Italy) and the Canary Islands. Five sequences of the *C. racemosa* var. *cylindracea* previously generated at the University of

Adelaide were also added. Several miscellaneous samples sequenced included a *C. racemosa* sold on seafood markets in Okinawa (Japan), *C. filiformis* (Suhr) Hering, *C. longifolia* C. Agardh, *C. taxifolia* and *C. urvilleana* (Montagne). DNA was amplified and sequenced at the University of Hawaii at Mānoa, the University of Louisiana at Lafayette and at the Netherlands Centre for Biodiversity Naturalis.

DNA extraction consisted of a modified Dellaporta *et al.* (1983) protocol described by Hughey *et al.* (2001) for herbarium specimens and a DNeasy Plant MiniKit (Qiagen, Valencia, CA, USA) for silica gel-dried specimens. PCR amplification was as previously described (Famà *et al.* 2002; Stam *et al.* 2006; Saunders & Kucera 2010). The newly designed forward 157F primer (5'-GCWCCWGAA-GAAAAAGC-3'; anneals at position 157 of the *Ostreococcus tauri* Courtes & Crétiennot CR954199 complete *tufA* sequence), the *tufAF* primer (Famà *et al.* 2002) and *tufGF4* primer (Saunders & Kucera 2010) were used in combination with reverse *tufAR* primer (Famà *et al.* 2002). PCR products were purified using ExoSAP-IT® (Affymetrix, Inc., Cleveland, OH, USA) and sequenced in both directions. Individual chromatograms were assembled into contigs and edited using Sequencher™ v.4.8 (Gene Codes, Ann Arbor, MI, USA). A total of 55 sequences were newly generated (Table 1).

All previously published *Caulerpa tufA* sequences belonging to the 'modern and fast-evolving' lineage (*sensu* Famà *et al.* 2002) were retrieved from GenBank (n = 224). Redundant sequences (i.e. 100% identity) were filtered with BLAST-CLUST under high stringency parameters (-L 1 -b F -S 100; stand-alone BLAST package 2.2.18). That is, the longest sequence within a pool of identical sequences was maintained; shorter sequences were eliminated unless their epithet, subspecific rank and/or geographic origin differed. Taxonomic assignments were maintained as published except for *C. subserrata* Okamura (AJ417935; Famà *et al.* 2002), which was included within one of the *C. brachypus* Harvey clades (see Wynne *et al.* 2009), and *C. distichophylla* Sonder (AJ417940; Famà *et al.* 2002), which was included within *C. taxifolia* (Fig. S1).

In addition, five sequences isolated from chloroplasts sequestered within sacoglossan slugs (Opisthobranchia, Gastropoda) were downloaded. These corresponded to accessions HM140234, identified by BLAST as *C. serrulata* (Forsskål) J. Agardh (Wägele *et al.* 2011), and GU592606, GU592619 and GU592621-22 representing one of the lineages of the *C. racemosa–peltata* complex (Händler *et al.* 2009; Table S1). Outgroup taxa consisted of two species from a basal lineage of the Caulerpaeae, *C. verticillata* J. Agardh (AJ417967) and *C. flexilis* J.V. Lamouroux ex C. Agardh (AJ417970) and other taxa sister to the ingroup (Fig. S1; Table S2). GenBank sequences for the *C. racemosa–peltata* complex are provided in Table S1 (see Table S2 for remaining species). Overall, the final alignment comprised a total of 173 sequences (510–912 base pairs in length).

Following exploratory analysis demonstrating unequal pairwise nucleotide differences across codon position (Fig. 1), model selection was conducted in PartitionFinder (Lanfear *et al.* 2012). The three information criteria – Akaike information criterion (AIC), corrected AIC (AICc) and Bayesian information criterion (BIC) – were in

Table 1. GenBank accession numbers and collection data for newly sequenced specimens.

GenBank	Specimen	Lineage ¹ /species	Geographical origin ²	Day/month/year
FM956056	SGAD0509640	A	Thousand Islands, Java Sea, Indonesia	23 Sep. 2005
FM956057	03-227	A	Berau delta, East Kalimantan, Indonesia	Oct.2003
FM956058	—	A	Koh Kham, Songkhla, Thailand	12 Nov. 2006
FM956059	SGAD0712145	A	Raja Ampat, West Papua, Indonesia	23 Nov. 2007
FM956060	FL1176	A	Philippines	19 Sep. 2007
JN645160	IRD5627	A	Ile des Pins, NC	28 Nov. 2005
JN645162	IRD5638	A	Goro, Grande Terre, NC	08 Feb. 2005
JN645173	HV2132	B	Minnie Waters, NSW, Australia	21 Nov. 2009
FM956053	SGAD0509359	C	Thousand Islands, Java Sea, Indonesia	16 Sep. 2005
FM956054	03-341	C	Berau Delta, East Kalimantan, Indonesia	Oct. 2003
FM956055	SGAD0712635	C	Raja Ampat, West Papua, Indonesia	10 Dec. 2007
FM956075	HEC161156	C	Klong Yang, Krabi province, Thailand	12 Apr. 2007
JN645149	IRD5639	C	Ile aux Canards, Grande Terre, NC	10 Feb. 2005
JN645154	IRD5636	C	Ile des Pins, NC	27 Nov. 2003
JN645165	IRD5635	C	Ilot Bayes, Grande Terre, NC	23 Oct. 2001
FM956045	SGAD0712247	D	Raja Ampat, West Papua, Indonesia	28/11/2007
FM956051	SGAD0509638	D	Thousand Islands, Java Sea, Indonesia	23 Sep. 2005
FM956052	03-342	D	Berau delta, East Kalimantan, Indonesia	Oct. 2003
JN645159	IRD5634	D	Ile des Pins, NC	28 Nov. 2005
JN645169	IRD5641	D	Ouano, Grande Terre, NC	24 Feb. 2008
FM956043	SGAD0509242	E	Thousand Islands, Java Sea, Indonesia	08 Sep. 2005
FM956044	03-340	E	Berau delta, East Kalimantan, Indonesia	Oct. 2003
JN645158	IRD5623	E	Ile des Pins, NC	18 Jun. 2007
JN645166	IRD5622	E	Ile des Pins, NC	05 Nov. 2004
JN645168	IRD5633	E	Ile des Pins, NC	28 Nov. 2005
JN645170	IRD5642	E	Ilôt Larégnère, Grande Terre, NC	29 Feb. 2008
FM956046	SGAD0712251	F	Raja Ampat, West Papua, Indonesia	28 Nov. 2007
FM956048	SGAD0706109	F	Ischia Ponte, Gulf of Naples, Italy	15 Jun. 2007
JN645150	TS0133	F	Porquerolles, France	03 Aug. 2007
JN645152	IRD1878	F	Fiji	18 May 2007
JN645155	IRD5626	F	Ile des Pins, NC	18 Jun. 2007
JN645156	IRD5625	F	Ile des Pins, NC	18 Jun. 2007
JN645157	IRD5631	F	Ile des Pins, NC	18 Jun. 2007
JN645163	IRD5629	F	Baie Sainte Marie, Grande Terre, NC	14 Sep. 2002
JN645164	IRD5624	F	Ile des Pins, NC	18 Jun. 2007
JN645167	IRD5640	F	Balabio, Grande Terre, NC	05 Oct. 2004
JN645171	IRD5630	F	Ile des Pins, NC	27 Nov. 2005
JN645174	HV2537	F	Hopetoun Beach, SWA, Australia	13 Dec. 2009
JN645175	HV2634	F	Leander Reef, Port Denison, SWA, Australia	19 Dec. 2009
JN817675	AD-A91736	F	Ningaloo Reef, WA, Australia	20 May 2010
JN817677	PERTH08292604	F	Montgomery Reef, WA, Australia	21 Oct. 2009
JN817679	AD-A92474	F	Lizard Island, GBR, Qld, Australia	31 Aug. 2010
JN851143	GWS025471	F	Point Peron, WA, Australia	13 Nov. 2011
JQ894932	TS1197	F	Fish market, Okinawa, Japan	Jan. 2012
JQ894933	AD-A93727	F	O'Sullivan's Beach, SA, Australia	09 May 2011
JQ894931	CAN-06-30	F	Gran Canaria, Canary Islands, Spain	Jun. 2006
FM956035	L2005-31	<i>C. filiformis</i>	Rocky Bay, South Africa	17 Aug. 2005
FM956040	L15	<i>C. longifolia</i>	Tasmania, Australia	25 Jan. 2007
JN645153	IRD5637	<i>C. taxifolia</i>	Goeland Island, NC	16 Nov. 2007
JN645151	TS0134	<i>C. taxifolia</i>	Porquerolles, France	03 Aug. 2007
JN645172	TS0372	<i>C. urvilleana</i>	Rose Atoll, Samoa	05 Mar. 2006

¹See Fig. 2 for lineage identity of the *C. racemosa*-*C. peltata* complex.²GBR = Great Barrier Reef; NC = New Caledonia; NSW = New South Wales; Qld = Queensland; SA = South Australia; SWA = Southwestern Australia; WA = Western Australia.

agreement on a three-codon position partition scheme, (1)(2)(3), with respective models determined as (GTR+G)(F81+H)(GTR+G) (Table 2).

Bayesian phylogenetic inference was carried out with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003) with default priors and the model of evolution specified as above. Markov chain Monte Carlo searches consisted of two independent runs of four increasingly heated chains run for 6,000,000 generations, sampled every 100 generations. Convergence was visualized in Tracer v1.5, and the first 5000 trees were discarded as burn-in. A majority-rule

consensus tree of the remaining trees was obtained with MrBayes 'sumt' command and displayed in Fig. 2, from which outgroup and intermediate taxa to the 'modern and fast-evolving' lineage were pruned for clarity. Pairwise genetic distances for this lineage were calculated from the branch lengths of the Bayesian tree with the Package APE in R (Paradis *et al.* 2004). The resulting distance matrix was used to build a histogram and to extract the maximum genetic distance of representative clusters of sequences reported on the phylogenetic tree.

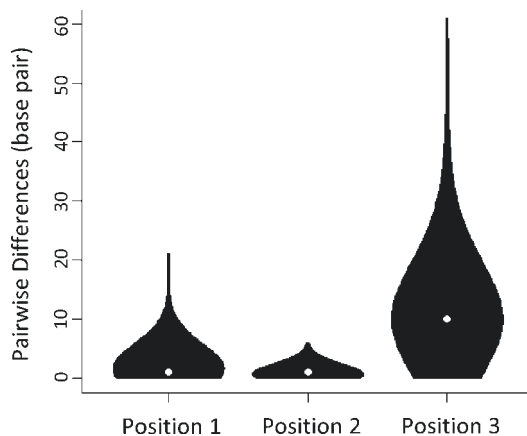


Fig. 1. Violin plot depicting the smoothed distribution of pairwise nucleotide differences (uncorrected) for the first, second, and third codon positions from the 912-base-pair *tufA* alignment.

RESULTS

The analysis performed in MrBayes rapidly reached convergence, resulting in a majority-rule consensus tree that displayed topological patterns and support values (Fig. 2) similar to those found in previous *Caulerpa* studies with *tufA* (Famà *et al.* 2002; Stam *et al.* 2006; Wynne *et al.* 2009). Some resolution was found near the tips of the tree, but *tufA* failed to resolve the backbone of the ‘modern and fast-evolving’ lineage, to the exception of *C. sertularioides* (S.G. Gmelin) M.A. Howe, which branched off first with strong support. A clade harboring *C. scalpelliformis* (R. Brown ex Turner) C. Agardh, *C. taxifolia*, *C. ashmeadii* Harvey, an unknown taxon (*Caulerpa* sp.) and *C. prolifera* (Forsskål) J.V. Lamouroux (the type species) branched next, but with low support. The remainder of the taxa, including the polyphyletic *C. racemosa–peltata* complex, were found within a large polytomy (Fig. 2). Other polyphyletic complexes, such as *C. scalpelliformis* and *C. brachypus*, were also present in the phylogeny. *Caulerpa serrulata* was paraphyletic with the nested species *C. cupressoides* (West) C. Agardh, as previously demonstrated (Famà *et al.* 2002; Stam *et al.* 2006).

The corrected genetic distance matrix computed from the branch lengths of the Bayesian tree allowed examination of intraspecific distances for species clusters that were well delimited morphologically, and they were compared to those of the *C. racemosa–peltata* complex. These monophyletic species ranged between 0.072 for *C. filiformis* (Suhr) Hering to 0.426 for *C. mexicana* Sonder ex Kützing. *Caulerpa*

species that were sequenced over a broad geographical scale (Atlantic and Indo-Pacific regions) revealed large intraspecific distances, such as *C. mexicana* and *C. taxifolia* (0.426 and 0.289, respectively; Fig. 2). In accordance, taxa with more restricted geographic ranges (and less sampling), showed lower intraspecific values such as the Atlantic species *C. ashmeadii* and *C. prolifera* (0.220 and 0.185, respectively). The paraphyletic species *C. serrulata* and nested species *C. cupressoides* showed an overall maximum distance of 0.321 in the range of some monophyletic species. Examination of the distribution of corrected genetic distances with a histogram demonstrated a sharp drop in frequencies between distances of 0.25 and 0.65 (Fig. 3), which may be considered as the minimum and maximum cutoff values to assess species boundaries for *Caulerpa* taxa. Based on these values, branch support and topological relationships for members of the *C. racemosa–peltata* complex, six lineages named A through F were delineated with distances of between 0.128 and 0.514. Lineage A displayed paraphyletic haplotypes and also the greatest divergence (0.514). Within some of these lineages (A, C, D, E, F), discrete subclades with distances of between 0.058 and 0.341 were highlighted. These may correspond to species-level entities.

Among the six lineages delineated, B was found in eastern Australia. Lineage A and D were found both in the Atlantic and in the Indo-Pacific. Lineages C, E and F were sequenced only from the Indo-Pacific. Overall, NC members of the complex were found in five of the six lineages, namely, A, C, D, E and F.

Caulerpa racemosa var. *cylindracea* clustered in lineage F as one of three strongly supported clades. The four sequences obtained from Ile des Pins and Baie St. Marie, NC, clustered tightly with those from Australia (i.e. from the type locality in SWA; Leander Reef, Hopetoun Beach, Point Peron) and around the continent in northwestern Australia (NWA; Ningaloo Reef, Montgomery Reef) and eastern Australia (EA; Lizard Island, Great Barrier Reef) (Figs 2, S2). The invasive populations from O’Sullivan’s Beach, South Australia (SA) as well as populations from France, Italy and Gran Canaria were also found in this cluster. A last sequence of the *C. racemosa* var. *cylindracea* corresponded to a specimen collected from an aquarium shop in California by Stam *et al.* (2006). Within the newly generated sequences of *C. racemosa* var. *cylindracea*, two *tufA* haplotypes differed by a single base pair at position 54 of the alignment (third codon position). The first haplotype corresponded to specimens from EA and NC and the second to those from the Mediterranean, Canary, NWA, SA and SWA.

Table 2. Model and partition scheme selection for the protein gene *tufA* (912 base pairs) based on AIC, AICc, and BIC. Schemes tested are given between parentheses, and numerals 1, 2 and 3 correspond to positions of the codons. The best-fitting partitioning scheme and models are in boldface.

Scheme	lnL	Parameters	AIC	AICc	BIC	Best models
(1,2,3)	-5041.641	353	10,789.282	11,237.174	12,489.203	(GTR+I+G)
(1,3)(2)	-4960.147	357	10,634.294	11,095.688	12,353.478	(GTR+G)(F81+I)
(1)(2,3)	-4899.840	361	10,521.680	10,996.888	12,260.127	(GTR+G)(TVM+G)
(1,2)(3)	-4775.261	360	10,270.521	10,742.245	12,004.152	(TVMef+I+G)(GTR+I+G)
(1)(2)(3)	-4724.498	368	10,184.997	10,685.151	11,957.152	(GTR+G)(F81+I)(GTR+G)

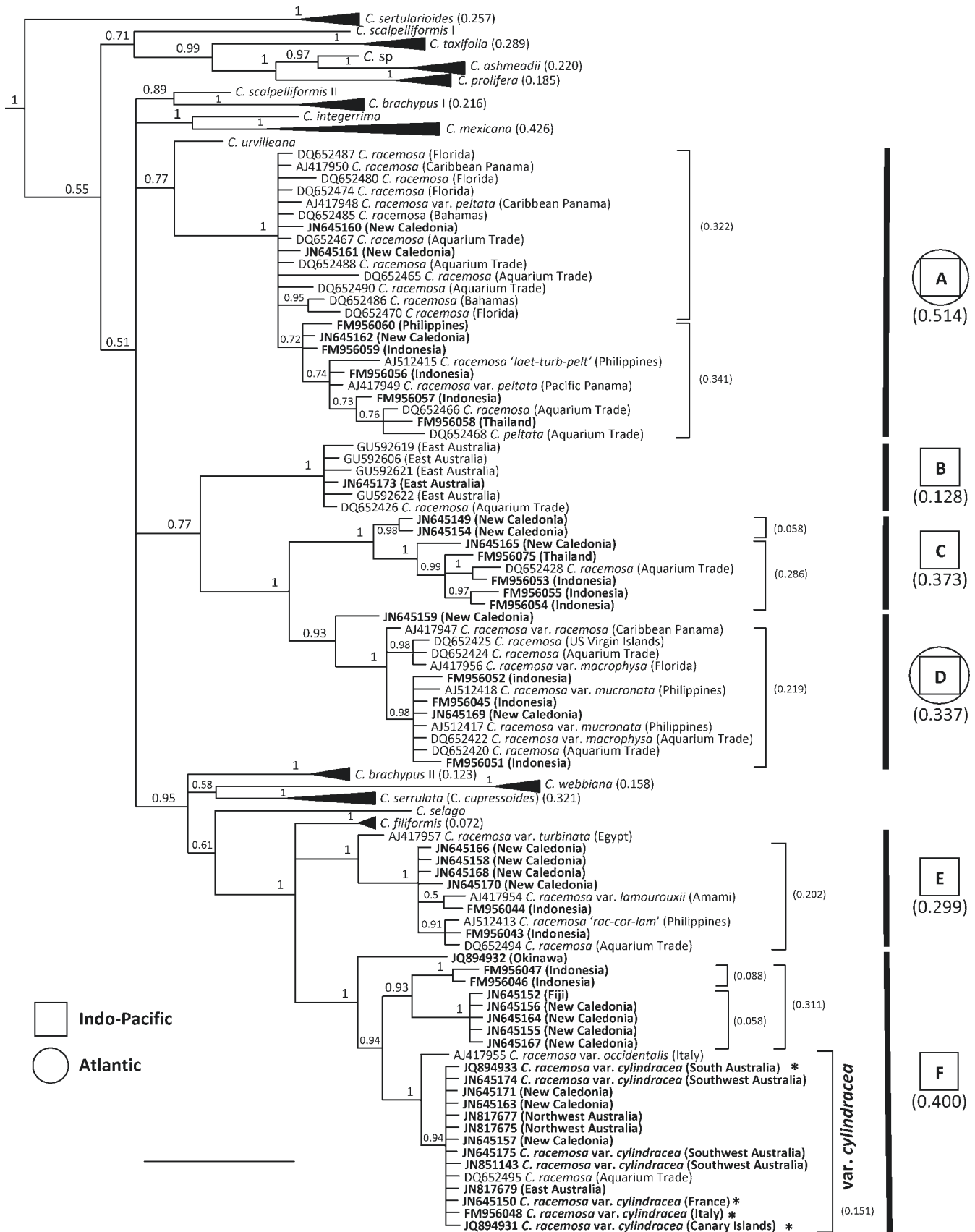


Fig. 2. Phylogenetic tree displaying the genetic diversity of the *Caulerpa racemosa*–*Caulerpa peltata* complex. Boldface = new sequences; numbers above branches = posterior probabilities; numbers in parentheses = corrected maximum genetic distances; circle = Atlantic; square = Indo-Pacific; * = alien specimen; scale bar = 0.35 substitutions per site.

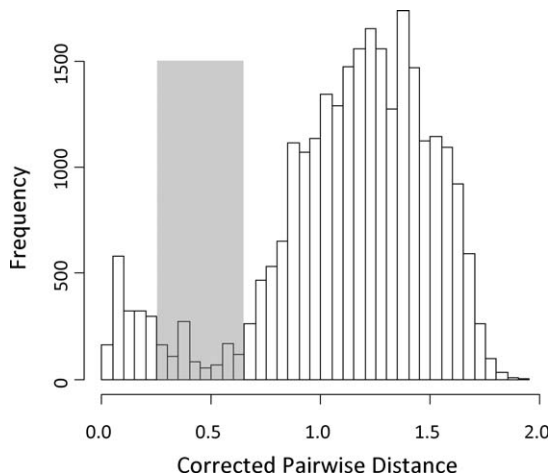


Fig. 3. Frequency distribution of corrected pairwise genetic distances for the ‘modern and fast-evolving’ *Caulerpa* lineage (*sensu* Famà *et al.* 2002). Distances were computed from branch lengths of the phylogenetic tree resulting from the Bayesian inference. The estimated range of genetic distances representative of species boundaries (minimum and maximum cutoff) is shaded in gray (0.25–0.65).

DISCUSSION

The lack of resolution in the backbone of *Caulerpa* phylogenies is a recurrent problem in molecular studies of the genus that have employed *tufA* or other markers (e.g. Famà *et al.* 2000; Senerpont Domis *et al.* 2003; Famà *et al.* 2003; Stam *et al.* 2006; Wynne *et al.* 2009); thus, little information is currently available on the relatedness and order of diversification of the different lineages of the *C. racemosa–peltata* complex with other species found in the polytomy. Nonetheless, *tufA* provides sufficient resolution to highlight six lineages (A–F) that may each harbor multiple species-level entities. These, drawn as eight (possibly 10) discrete subclades, displayed comparable genetic distances to morphologically well-delimited monophyletic species (0.058–0.341 vs 0.072–0.426, respectively), but their accurate delineation represents a hypothesis to be further addressed with faster-evolving markers (yet to be developed) and/or further geographical sampling with *tufA*. In addition, within some of the six lineages, several taxa lie on long branches (e.g. AJ417957, JN645159 and JQ894932) and may represent distinct entities as well; their separation from closely related clusters of sequences also needs to be addressed with further sequencing. Finally, the existence of paraphyletic taxa in the phylogeny is to be taken into consideration when trying to estimate species boundaries within the *C. racemosa–peltata* complex. For instance, the paraphyletic taxon *C. serrulata* and nested species *C. cupressoides* (West) C. Agardh are not resolved into monophyletic clades with *tufA* (overall distance 0.321) in spite of their clear morphological distinction. Lineage A displays paraphyletic haplotypes (overall distance 0.514) and likewise may harbor several entities.

Overall, NC members of the complex are found in five of the six documented lineages, namely, A, C, D, E and F. Lineage B, sequenced from EA, has not been found in the NC collections but could be present locally considering the

floristic affinities between EA and NC (Millar & Payri 2006). The taxonomic assignment of the NC specimens and their potential correspondence to varieties recognized locally (Payri 2007) is currently under way based on morphological observations and DNA-assisted identification.

The presence of *C. racemosa* var. *cylindracea* in NC was unexpected, and it is also reported for the first time from northern Australia (Fig. S2). The four sequences obtained from Ile des Pins and Baie St. Marie, NC, clustered tightly with those from Australia, that is, from the type locality in SWA (Leander Reef, Hopetoun Beach, Point Peron), NWA (Ningaloo Reef, Montgomery Reef) and EA (Lizard Island, Great Barrier Reef; (Figs 2, 4). *Caulerpa racemosa* var. *cylindracea* is alien and invasive in the Mediterranean Sea, Canary Islands (Verlaque *et al.* 2003, 2004) and more recently in SA (O’Sullivan’s Beach). Unlike the newly identified records from NWA, EA and NC, which show no signs of overabundance, SA populations found at O’Sullivan’s Beach and the Port Adelaide area form dense blanketing over the substratum (C.F.D. Gurgel & G.S. Belton, personal observations). Both the presence of SA populations, at close proximity of major harbors, and their isolation from populations of SWA, as indicated by intensive fieldwork in the region, support a recent introduction to the Adelaide area (Collins *et al.* 2004).

An intriguing sequence was published from Italy by Famà *et al.* (2002) as *Caulerpa racemosa* var. *occidentalis* (J. Ag.) Børgesen a name previously given to the Mediterranean invasive before Verlaque *et al.* (2003) narrowed its identity to *C. racemosa* var. *cylindracea*. This sequence was sister to the *C. racemosa* var. *cylindracea* cluster (Fig. 2) and differs from the newly sequenced Mediterranean and Canary Islands specimens by two base pairs located at sites 151 and 839 of the alignment, both of which correspond to slow-evolving positions of the codons (first and second position, respectively; Fig. 1). Whether a closely related taxon is found in the Mediterranean or PCR/sequence assembly error occurred in

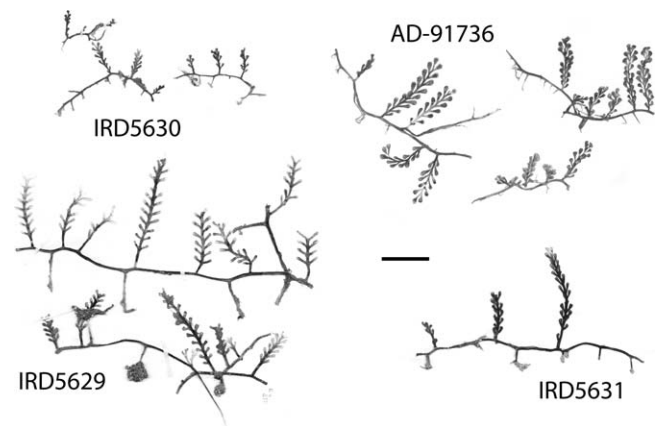


Fig. 4. Herbarium specimens of *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman and Boudouresque sequenced from New Caledonia (IRD5629 from Baie Sainte Marie, Grande Terre; IRD5630 and IRD5631 from Ile des Pins) and Australia (AD-91736 from Ningaloo Reef, Western Australia). Note radial or distichous arrangement of branchlets. Scale bar = 4.5 cm. Specimens are maintained at IRD, Nouméa and AD, Adelaide.

Famà *et al.* (2002) study needs to be clarified. The remainder of the sequences of *Caulerpa racemosa* var. *cylindracea* differed by a single base pair from one another at site 54 of the alignment on a fast-evolving position of the corresponding codon (third position), a minimal variation which nonetheless appears consistent with regional differences in the collections, that is, western Pacific haplotypes (EA and NC) vs eastern Indian Ocean (NWA and SWA) and the Mediterranean. This pattern needs to be confirmed with additional sampling and/or further explored with faster-evolving markers.

The NC and Australian specimens exhibit a radial and/or distichous arrangement with upright clavate branchlets with rounded apices (Fig. 4), which fits the description of *C. racemosa* var. *cylindracea* from SWA and alien specimens from the Mediterranean Sea (Verlaque *et al.* 2003). The distichous and alternating arrangement of ramuli closely resembles illustrations of *C. racemosa* f. *complanata* (J. Agardh) Weber-van Bosse and *C. racemosa* var. *corynephora* Weber-van Bosse (1898, p. 364, pl. XXXIII, figs 10–14) from northern Australia and SWA, which taxonomic status in relation to *C. racemosa* var. *cylindracea* needs to be clarified. The reports of *C. racemosa* f. *complanata* and *C. racemosa* var. *corynephora* from India, Indonesia and Papua New Guinea (e.g. Silva *et al.* 1996; Littler & Littler 2003, p. 227) suggest that the distribution of *C. racemosa* var. *cylindracea* could extend northward of Australia and NC into the Coral Triangle and Southeast Asia. In the literature, several records of *C. racemosa* var. *laetevirens* f. *cylindracea* can also be found from the eastern tropical Pacific at Isla Guadalupe (Setchell & Gardner 1930), the Pacific coast of Mexico (Pedroche *et al.* 2005), and the Indian Ocean from India, Kenya and Sri Lanka (Silva *et al.* 1996). These records should be interpreted with caution until sequencing of specimens from these regions confirms their identity.

In summary, our analysis reveals a large diversity underlying the *Caulerpa racemosa*–*peltata* complex in NC with five identified lineages locally, which most likely harbor multiple species-level entities. A large amount of diversity within the complex seems to be restricted to the Indo-Pacific, although further sampling is required in the Atlantic. The discovery of *C. racemosa* var. *cylindracea* in NC and new records from NA, including Lizard Island on the Great Barrier Reef, support a much larger native range than SWA, which might possibly extend into the Coral Triangle and beyond. These new Australasian records of *C. racemosa* var. *cylindracea* obviously have implications for the interpretation of its biogeography and raise new questions about the origin of the Mediterranean alien strain and its potential vectors of introduction. Overall, sequencing of *Caulerpa* from Australasia and Southeast Asia needs to be continued for further insights into distributional patterns at this regional scale as well as globally (Hommersand 2007).

ACKNOWLEDGEMENTS

We thank Sofie D'hondt for technical assistance and Martha Diaz-Ruiz and Peter Vroom for providing *Caulerpa urvilleana* from Rose Atoll (U.S. Fish and Wildlife Service permit # USFWS-SUP 12521-06011) and Rainbo Dixon and

John Huismans for providing samples from the Kimberley, Australia. We also thank the 'Institut de Recherche pour le Développement (IRD) de Nouméa' for supporting field collections in NC onboard the Alis Vessel. Support was provided by the U.S. National Science Foundation grants DEB-0743024 and NSF-DEB 0919508; the Schure-Beijerinck-Popping Fund and TREUB maatschappij (Royal Dutch Academy of Sciences); the Netherlands Organization for Scientific Research grants ALW-NWO 852.000.50, WOTRO-NWO R 85-363 and WOTRO-NWO R 85-381; the Netherlands Centre for Biodiversity *Naturalis*; the Research Centre for Oceanography of the Indonesian Institute of Sciences (PPO-LIPI); the NOAA Hawaii Coral Reef Initiative; the Australian Research Council grants ARC Linkage LP0991083 and FT110100585; the Australian Biological Resource Study grant ABRS 209–62; and the Australian Census of Coral Reef Life.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at <http://dx.doi.org/10.2216/11-116.1.s1>.

REFERENCES

- ABBOTT I.A. & HUISMAN J.M. 2004. *Marine green and brown algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu, Hawai'i. 259 pp.
- ALLEN G. R. 2007. Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems* DOI:10.1002/aqc.880.
- ALONGI G., CORMACI M., FURNARI G. & GIACCONE, G. 1993. Prima segnalazione di *Caulerpa racemosa* (Chlorophyceae, Caulerpales) per le coste italiane. *Bollettino dell'Accademia Gioenia di Scienze Naturali Catania* 26: 9–53.
- BØRGESEN F. 1907. Ecological and systematic account of the *Caulerpa* of the Danish West Indies. *Det Kongelige Danske Videnskabernes Selskabs Skrifter* 4: 337–392.
- CALVERT H.E. 1976. Culture studies on some Florida species of *Caulerpa*: morphological responses to reduced illumination. *British Phycological Journal* 11: 203–214.
- CARRUTHERS T.J.B., WALKER D.I. & HUISMAN J.M. 1993. Culture studies on two morphological types of *Caulerpa* (Chlorophyta) from Perth, Western Australia, with a description of a new species. *Botanica Marina* 36: 589–596.
- COLLINS G., WESTPHALEN G., ROWLING K., & EGLINTON Y. 2004. *Caulerpa racemosa* var. *cylindracea* occurrence in western South Australia. Report to PIRSA Marine Habitat Program. South Australian Research and Development Institute (Aquatic Sciences) Report Number RD04/0169.
- DELLAPORTA S.L., WOOD J., & HICKS J.B. 1983. A plant DNA mini preparation: version II. *Plant Molecular Biology Reporter* 1: 19–21.
- DURAND C., MANUEL M., BOUDOURESQUE C.F., MEINESZ A., VERLAQUE M. & LE PARCO Y. 2002. Molecular data suggest a hybrid origin for the invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta) in the Mediterranean Sea. *Journal of Evolutionary Biology* 15: 122–133.
- ENOMOTO S. & OHBA H. 1987. Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) I. Reproduction and development of *C. racemosa* var. *laetevirens*. *Japanese Journal of Phycology/Sorui* 35: 167–177.
- EUBANK L.L. 1946. Hawaiian representatives of the genus *Caulerpa*. *University of California Publications in Botany* 18: 409–432.
- FAMÀ P., OLSEN J.L., STAM W.T. & PROCACCINI G. 2000. High levels of intra- and inter-individual polymorphism in the rDNA ITS1 of

- Caulerpa racemosa* (Chlorophyta). *European Journal of Phycology* 35: 349–356.
- FAMÁ P., WYSOR B., KOOISTRA W.H.C.F. & ZUCCARELLO G. 2002. Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from the chloroplast *tufA* gene. *Journal of Phycology* 38: 1040–1050.
- GIACCONE G. & DI MARTINO V. 1995. Le *Caulerpe* in Mediterraneo: un ritorno del vecchio bacino Tetide verso il dominio Indo-Pacifico. *Biologia Marina Mediterranea* 2: 607–612.
- GUIRY M.D. & GUIRY G.M. 2012. *AlgaeBase*. Worldwide electronic publication, National University of Ireland, Galway. Available at: <http://www.algaebase.org> (23 March 2012).
- HÄNDELER K., GRZYMBOWSKI Y.P., KRUG P.J. & WÄGELE H. 2009. Functional chloroplasts in metazoan cells – a unique evolutionary strategy in animal life. *Frontiers in Zoology* 6: 28.
- HOMMERSAND M.H. 2007. Global biogeography and relationships of the Australian marine macroalgae. In: *Algae of Australia. Introduction* (Ed. by P.M. McCarthy & E.A. Orchard). Australian Biological Resources Study, Canberra. 727 pp.
- HUGHEY J.R., SILVA P.C. & HOMMERSAND M. 2001. Solving taxonomic and nomenclatural problems in Pacific Gigartinales (Rhodophyta) using DNA from type material. *Journal of Phycology* 37: 1091–1109.
- JOUSSON O., PAWLOWSKI J., MEINESZ A. & BOUDOURESQUE C.F. 1998. Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. *Marine Ecology Progress Series* 172: 275–280.
- KLEIN J. & VERLAQUE M. 2008. The *Caulerpa racemosa* invasion: a critical review. *Marine Pollution Bulletin* 56: 205–225.
- KRAFT G.T. 2007. *Algae of Australia. Marine benthic algae of Lord Howe Island and the southern Great Barrier Reef, 1. Green Algae*. Australian Biological Resources Study and CSIRO Publishing, Canberra. 347 pp.
- LANFEAR R., CALCOTT B., HO S.Y.W. & GUINDON S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* DOI:10.1093/molbev/mss020.
- LITTLER D.S. & LITTLER M.M. 2003. *South Pacific reef plants. A divers' guide to the plant life of South Pacific Coral Reefs*. Offshore Graphics, Inc., Washington, DC. 332 pp.
- MILLAR A.J.K. & PAYRI C.E. 2006. New records of marine benthic algae from the Lagoon Sud-Ouest of New Caledonia, South Pacific. *Phycological Research* 54: 169–185.
- OHBA H. & ENOMOTO S. 1987. Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) II. Morphological variation of *C. racemosa* var. *laetevirens* under various culture conditions. *Japanese Journal of Phycology/Sorui* 35: 178–188.
- OHBA H., NASHIMA H. & ENOMOTO S. 1992. Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) III. Reproduction, development and morphological variation of laboratory cultured *C. racemosa* var. *peltata*. *Botanical Magazine* 105: 589–600.
- PARADIS E., CLAUDE J. & STRIMMER K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290.
- PAYRI C.E. 2007. Revised checklist of marine algae (Chlorophyta, Rhodophyta and Ochrophyta) and seagrasses (marine Angiosperma) of New Caledonia. In: *Compendium of marine species from New Caledonia* (Ed. by C. Payri & B. Richer De Forges). Documents Scientifiques et Techniques II7, Noumea. 435 pp.
- PEDROCHE F.F., SILVA P.C., AGUILAR-ROSAS L.E., DRECKMANN K.M. & AGUILAR-ROSAS R. 2005. *Catálogo de las algas marinas bentónicas del Pacífico de México. I. Chlorophycota*. Universidad Autónoma de Baja California, Ensenada, México. i-viii 17-146 pp.
- PETERSON R.D. 1972. Effects of light intensity on the morphology and productivity of *Caulerpa racemosa* (Forsskal) J. Agardh. *Micronesica* 8: 63–86.
- PRUD'HOMME VAN REINE W.F., VERHEIJ E. & COPPEJANS E. 1996. Species and ecads of *Caulerpa* (Ulvophyceae, Chlorophyta) in Malesia (South-East Asia): taxonomy, biogeography, and biodiversity. *Netherlands Journal of Aquatic Ecology* 30: 83.
- RONQUIST F. & HUELSENBECK J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- SAUNDERS G.W. & KUCERA H. 2010. An evaluation of *rbcL*, *tufA*, *UPA*, *LSU* and *ITS* as DNA barcode markers for the marine green macroalgae. *Cryptogamie Algologie* 31: 487–528.
- SENERPONT DOMIS L.N DE, FAMÁ P., BARTLETT A., PRUD'HOMME VAN REINE W.F., ARMENTA ESPINOSA C. & TRONO G.C. JR. 2003. Defining taxon boundaries in members of the morphologically and genetically plastic genus *Caulerpa* (Caulerpales, Chlorophyta). *Journal of Phycology* 39: 1019–1037.
- SETCHELL W.A. & GARDNER N.L. 1930. Marine algae of the Revillagigedo Islands Expedition in 1925. *Proceedings of the California Academy of Science* 19: 109–215, pls 4–15.
- SILVA P.C., BASSON P.W. & MOE R.L. 1996. Catalogue of the benthic marine algae of the Indian Ocean. *University of California Publications in Botany*, University of California Press, Berkeley 79: 1–1259.
- STAM W.T., OLSEN J.L., ZALESKI S.F., MURRAY S.N., BROWN K.R. & WALTERS L.J. 2006. A forensic and phylogenetic survey of *Caulerpa* species (Caulerpales, Chlorophyta) from the Florida coast, local aquarium shops, and e-commerce: establishing a proactive baseline for early detection. *Journal of Phycology* 42: 1113–1124.
- TANDY G. 1933. Transplant and succession studies on marine algae, especially *Caulerpa* and *Halimeda*. *Yearbook of the Carnegie Institution* 32: 283–284.
- TANDY G. 1934. Experimental taxonomy in marine algae, with special reference to *Caulerpa*. *Proceedings of the Linnean Society of London* 146: 63–64.
- VERLAQUE M., BOUDOURESQUE C.F., MEINESZ A. & GRAVEZ V. 2000. The *Caulerpa racemosa* complex (Caulerpales, Ulvophyceae) in the Mediterranean Sea. *Botanica Marina* 43: 49–68.
- VERLAQUE M., DURAND C., HUISMAN J.M., BOUDOURESQUE C.F. & LE PARCO Y. 2003. On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta). *European Journal of Phycology* 38: 325–339.
- VERLAQUE M., AFONSO-CARRILLO J., CANDELARIA GIL-RODRIGUEZ M., DURAND C., BOUDOURESQUE C.F. & LE PARCO Y. 2004. Blitzkrieg in a marine invasion: *Caulerpa racemosa* var. *cylindracea* (Bryopsidales, Chlorophyta) reaches the Canary Islands (north-east Atlantic). *Biological Invasions* 6: 269–281.
- WÄGELE H., DEUSCH O., HAENDELER K., MARTIN R., SCHMITT V., CHRISTA G., PINZGER B., GOULD S.B., DAGAN T., KLUSSMANN-KOLB A. & MARTIN W. 2011. Transcriptomic evidence that longevity of acquired plastids in the photosynthetic slugs *Elysia timida* and *Plakobranthus ocellatus* does not entail lateral transfer of algal nuclear genes. *Molecular Biology and Evolution* 28: 699–706.
- WEBER-VAN BOSSE A. 1898. Monographie des Caulerpes. *Annales du Jardin botanique de Buitenzorg* 15: 243–401.
- WOMERSLEY H.B.S. 2003. *The marine benthic flora of Southern Australia – part IIID Ceramiales – Delesseriaceae, Sarcoceniaceae, Rhodomelaceae*. Australian Biological Resources Study and State Herbarium of South Australia, Canberra. 533 pp.
- WYNNE M.J., VERBRUGGEN H. & ANGEL D.L. 2009. The recognition of *Caulerpa integerrima* (Zanardini) comb. nov. (Bryopsidales, Chlorophyta) from the Red Sea. *Phycologia* 48: 291–301.
- YEH W.J. & CHEN G.Y. 2004. Nuclear rDNA and internal transcribed spacer sequences clarify *Caulerpa racemosa* vars. from other *Caulerpa* species. *Aquatic Botany* 80: 193–207.