The Taxonomy of the Green Macroalgal Genus *Caulerpa*: A Framework for the Application of Old Species Names in DNA-Based Taxonomies

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Submitted for the degree of Doctor of Philosophy in February 2016

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Abstract

Phenotypic plasticity has been a long-standing source of taxonomic difficulty in many algal groups. Nowhere is this more evident than in the siphonous green algal genera, *Caulerpa*, which although consisting of a single cell, display a complex habit with the thallus differentiating into stolons, rhizoids, and upright fronds, many also bearing branchlets. Environmentally controlled phenotypic plasticity in all morphological characters has led to much confusion, misidentification and a cumbersome classification scheme. Furthermore, recent molecular studies have added to this confusion by uncovering considerable conflict between morphological and molecular species concepts. A framework to resolve the taxonomy and match previously described names with newly obtained molecular data has remained an elusive goal to date for this group. The present thesis aimed to resolve the taxonomy of *Caulerpa* species found in Australian waters and in so doing, provide a best practice framework for integrating historical nomenclature into molecular taxonomies.

Using a combination of phylogenetic and species-delimitation approaches of partial *tuf*A and *rbc*L gene sequences, I first determined the number of genetic species within a globally sampled set of "*C. racemosa-peltata* complex" specimens. Guided by these results, comparative morphological examinations were then undertaken to gauge the extent of phenotypic plasticity within each species, as well as any morphological overlap between them. This approach was combined with detailed observations of type and herbarium specimens, and consideration of type descriptions to make informed decisions to advise on taxonomic updates. Using this framework I then tackled the taxonomies of the species rich *Caulerpa* floras of north-western and southern Australia.

Unsurprisingly, results confirmed some serious mismatches between morphological and molecular species concepts in the genus, most evident in the *C. racemosa-peltata* complex. I describe a number of new species and make a large number of taxonomic changes. A number of range extensions for some species were also uncovered, most importantly, the recent southerly extension of *C. cylindracea* into Victorian waters (previously *C. racemosa* var. *cylindracea*), one of the most notorious invasive seaweeds in the world. With an accurate DNA barcode for *C. cylindracea*, I was then able to use Genbank records and newly obtained data in order to obtain an accurate global distribution for the species. Using this dataset species distribution modelling was used to predict future spread, and identified areas at risk of invasion along the coasts of Western Europe, western Africa and the south coast of Australia.

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Originality Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Gareth Smith Belton and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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List of Publications and Associated Copyright Holders

Belton, G. S., Prud'homme van Reine, W. F., Huisman, J. M., Draisma, S. G. A. & Gurgel, C. F. D. 2014. Resolving phenotypic plasticity and species designation in the morphologically challenging *Caulerpa racemosa – peltata* complex (Caulerpacea, Chlorophyta). *Journal of Phycology*, **50**: 32-54. (John Wiley & Sons)

Belton, G. S., Huisman, J. M. & Gurgel, C. F. D. 2015. Caulerpaceae. In Huisman, J. M. ed. *Algae of Australia: Marine Benthic Algae of North-western Australia. 1. Green and Brown Algae*. (CSIRO Publishing / Australian Biological Resources Study).

Belton, G. S., Prud'homme van Reine, W. F., Huisman, J. M., Draisma, S. G. A. & Gurgel, C. F. D. Accepted, with revisions. A taxonomic reassessment of *Caulerpa* (Chlorophyta, Caulerpaceae) in southern Australia based on *tuf*A and *rbc*L sequence data. *Phycologia.* (Allen Press).

Verbruggen, H., Tyberhein, L., **Belton, G. S.**, Mineur, F., Jueterbock, A., Hoarau, G., Gurgel, C. F. D. & De Clerck, O. 2013. Improving transferability of introduced species' distribution models: new tools to forecast the spread of a highly invasive seaweed. PLoS ONE 8(6): e68337. (Public Library of Science).

Other publications produced from work in this thesis

Draisma, S. G. A., van Reine, W. F. P., Sauvage, T., **Belton, G. S.**, Gurgel, C. F. D., Lim, P.-E., Phang, S.-M. 2014. A re-assessment of the infra-generic classification of the genus *Caulerpa* (Caulerpaceae, Chlorophyta) inferred from a time-calibrated molecular phylogeny. *Journal of Phycology*, 50: 1020–1034

Sauvage, T., Payri, C., Draisma, S. G. A., Prud'homme van Reine, W. F., Verbruggen, H., **Belton, G. S.**, Gurgel, C. F. D., Gabriel, D., Sherwood, A. R. & Fredericq S. 2013. Molecular diversity of the *Caulerpa racemosa-peltata* complex (Caulerpaceae, Caulerpales) in New Caledonia, with new Australasian records for the variety *cylindracea*. *Phycologia* 52:6–13.

Acknowledgements

There are so many people to thank for getting this work completed. My supervisors, Dr. Fred Gurgel and Prof. Andrew Lowe, for giving me the opportunity and helping with all facets of this thesis. My major co-workers, Dr. John Huisman, Dr. Willem Prud-homme van Reine, Mr. Thomas Sauvage and Dr. Stefano Draisma for countless emails and hours spent dealing with the nomenclature of *Caulerpa*. Dr. Heroen Verbruggen for comments, feedback and many valuable sequences. Dr. Bob Baldock and Mrs. Carolyn Ricci and other staff at the State Herbarium of South Australia for putting up with my chaos and providing invaluable support. Thanks to Prof. Gary Saunders, Dr. Rainbo Belton and Dr. Kyatt Dixon for many specimens, sequences and good times whilst doing field work. Thank you also to the many funding bodies and institutions who provided support along the way: The University of Adelaide, State Herbarium of South Australia, State Herbarium of Western Australia, Australian Institute of Marine Sciences, Western Australian Museum, Woodside Energy Ltd, Australian Biological Resources Study, South Australian Research and Development Institute, Australian Research Council, Australian Census of Coral Reef Life, the Canadian Barcode of Life Network, Department of Water, Environment and Natural Resources South Australia, Alinytjara Wilurara Natural Resources Management Board, Wildlife Conservation Fund, I would also like to acknowledge the assistance of staff at the British Museum (BM), Leiden (L), Lund (LD), National Herbarium of Victoria (MEL), Museum National d'Histoire Naturelle (PC), and Trinity College Dublin (TCD). I would also like to acknowledge the support of Prof. Michelle Waycott and Dr. Korjent van Dijk for financial and logistical support over the past two years. Much love and thanks

should be given to my friends and community, especially those in our little valley of Basket Range who are always there for my Rainbo and I. Of course none of this could ever have happened without the encouragement of my parents, Nigel and Shelley, and brothers, Dylan and Lloyd, who have always allowed and encouraged me to do as I please. I told you I would hand this in one day! Lastly, my beautiful wife, Rainbo, you are amazing, thank you for everything and more.

Chapter 1: Introduction

Phenotypic plasticity has been a long-standing source of taxonomic difficulty in many algal groups, having led to much confusion, misidentifications, nomenclatural quagmires, incorrect biodiversity estimates, and confusing classification schemes (e.g., Bacillariophyceae: Mann 2010; Chlorophyta: Leliaert et al. 2009; Phaeophyceae: Tronholm et al. 2010; Rhodophyta: Saunders 2008). Fortunately, the advent of molecular tools has provided researchers with an independent and convenient means to delimit and identify species in taxonomically challenging groups (e.g., Verbruggen et al. 2007, Saunders 2008, Leliaert et al. 2009, Mann 2010, Tronhlm et al. 2010). These tools, however, have also presented algal taxonomists with several new problems. First, considerable conflict between morphological and molecular species concepts has been uncovered (e.g., Leliaert et al. 2009, Tronholm et al. 2012); secondly, molecular data are revealing an overwhelming diversity of species, many cryptic, at a rate far greater than formal descriptions are being made (De Clerck et al. 2013); and thirdly, as molecular-based studies continue to show increased rates of species discovery and mismatches between molecular and morphological species concepts. The greatest challenge for algal taxonomy in the future will be how to designate names, including those that have been previously described, to newly sequenced specimens. Solving this problem will speed up the process of accurately naming the many misidentified and ‘dark taxa’ (genetically distinct specimens that have not been linked to existing species or that have not been on GenBank. (De Clerck et al. 2013).

Several strategies for dealing with this challenge have been suggested. It has been proposed that newly collected and sequenced specimens (ideally topotype material similar in morphology to the type specimen) be designated as epitypes by experienced taxonomists (Tautz et al. 2003, Evans and Mann 2009, Bock et al. 2011, Saunders and McDevit 2012). However, how can one be sure that the epitype is identical to the type specimen when the type specimen is unavailable (e.g. lost, in poor condition, based on a drawing) or if the type locality is unknown, vague (e.g., "Western Australia"), significantly altered since the original collection (e.g., Suez, Egypt) or contains a number of genetic species with similar morphology? Furthermore, how does one confidently match names and type specimens to newly collected specimens in groups where high levels of phenotypic plasticity and/or cryptic species are known to occur? If we are to positively pinpoint the species in question, the only strategy leading to an unequivocal solution is to sequence the type material itself. It is for these reasons that Hughey and Gabrielson (2012), citing a number of examples, concluded that where possible, all type specimens, including their synonyms, should be sequenced. Although we agree with this in principle, as discussed by Saunders and McDevit (2012) and De Clerck et al. (2013) it is a time-consuming (and partially destructive) process hampered by the large number of synonyms and infraspecific taxa. In addition, in many instances this approach will not be possible due to type specimens being unavailable (e.g., administrative controls on destructive sampling, specimens destroyed, lost or types not yet designated) or because usable DNA cannot be extracted and amplified (e.g., original specimen was formalin-preserved). We will thus more than likely have to continue to rely on morphological information to resolve most remaining taxonomic problems. The question of how likely we are to be able to assign old names to new taxa based on morphological comparison is a question that has largely been ignored in algal systematics. However, as algal species continue to become established outside of their native ranges (e.g., *Caulerpa racemosa*, *Codium fragile*, *Undaria pinnitifida*, *Sargassum muticum*), and molecular data reveal an overwhelming diversity of species, many cryptic, at a rate far greater than formal descriptions are being made, we are at the point in algal systematics where we need to be pragmatic and revise taxonomies to the best of our abilities to move algal taxonomy forward.

The taxonomy of *Caulerpa* Lamouroux faces a number of the aforementioned issues. *Caulerpa* is a well-known and distinctive genus with over 350 associated species and infraspecific taxon names, of which about 85 species are currently accepted (Guiry and Guiry 2013). Although lacking transverse cell walls (= siphonous thallus), *Caulerpa* species display a complex habit, with the thallus differentiating into stolons, rhizoids, and upright assimilators (= fronds) that usually bear ramuli (= branchlets; Fritsch 1965). The morphology of these various structures has been historically used for species delimitation. However, environmentally controlled phenotypic plasticity in all these characters, but especially in the ramuli, has led to much confusion, a large number of synonyms, and a classification scheme involving subspecies, varieties, forms, and "ecads " (Peterson 1972, Calvert et al. 1976, Ohba and Enomoto 1987, Coppejans and Prud'homme van Reine 1992, de Senerpont Domis et al. 2003). Furthermore, there can appear to be continuous morphological gradations between many species (e.g. *C. cupressoides*/ *urvilleana*/ *serrulata*) and, as discussed by Svedelius (1906: 101), if a purely morphological standpoint was used to define species "one might just as well reduce the whole genus to one, or a few species".

Recent molecular studies of the genus have further added to this confusion with the uncovering of a considerable conflict between morphological and molecular species concepts (Fama et al. 2002, de Senerpont Domis et al. 2003, Stam et al. 2006 and Sauvage et al. 2013). However, none of these studies have dared make any taxonomical changes, with the authors largely agreeing that the matching of previously described names with newly obtained molecular data is an extremely difficult task. This has left hundreds of clearly misidentified *Caulerpa* specimens on Genbank, the taxonomy of the genus in disarray and the ability to accurately and rapidly identify *Caulerpa* species an almost impossible task for government agencies and non-specialists; a factor that is becoming increasingly important as *Caulerpa* species continue to become established outside of their native ranges (e.g., Meinesz and Hesse 1991, Jousson et al. 2000, Womersley 2003, Lapointe et al. 2005, Amat et al. 2008, Jongma et al. 2013, Perez-Estrada et al. 2013).

With this in mind the present thesis aims to resolve the taxonomy of *Caulerpa* in Australian waters, and in so doing provide a best practice framework of how to integrate historical nomenclature into molecular based taxonomies.

In Chapter 2 this framework was applied to tackle the most taxonomically troublesome taxa of the genus: those currently associated with *C. racemosa* (Forsskål) J. Agardh and *C. peltata* J. V. Lamouroux, commonly referred to as the *C. racemosa–peltata* complex, which has more than 30 described varieties and forms (most given in Guiry and Guiry 2013). First the number of genetic species were determined within a globally sampled data set through a combination of phylogenetic and species-delimitation approaches of partial elongation factor TU (*tuf*A) and RUBISCO large subunit (*rbc*L) gene sequences. Guided by these results, comparative morphological examinations were then undertaken to gauge the extent of phenotypic plasticity within each species, as well as any morphological overlap between them. A serious mismatch was found between morphological and molecular species concepts in the genus, most evident in the *C. racemosa-peltata* complex, but also clearly evident in species currently assigned to *C. brachypus* Harvey and *C. verticillata* J. Agardh. A number of new species were uncovered in the complex as well as a large number of taxonomic changes (synonyms, stat. nov., nom. nov. etc…). Based on these results, as well as detailed observations of type, type descriptions and historical herbarium specimens, taxonomic proposals of the *C. racemosa-peltata* complex taxa were made and reference sequences were provided for all examined species. A number of range extensions were also identified for some species, most importantly, *C. cylindracea* (previously *C. racemosa* var. *cylindracea*), one of the most notorious and invasive seaweeds in the world, which was shown to be an independent species with a native distribution throughout the tropical Indo-Pacific.

Using the framework developed in Chapter 2, Chapters 3 and 4 tackle the diversity of *Caulerpa* in North-western Australia and Southern Australia, respectively. A large number of specimens were newly collected and *tuf*A and *rbc*L sequences generated, including a number of species sequenced for the first time (e.g. *C. agardhii* Weber Bosse, *C. heterophylla* I.R. Price, J.M. Huisman & M.A. Borowitzka). Results indicate an underestimation of *Caulerpa* diversity in both regions, a number of important range extensions as well as a large number of required taxonomic changes. In Chapter 3, *C. delicatula* Grunow, *C. parvifolia* Harvey and *C. urvilleana* Mont. are reinstated as distinct species. In Chapter 4 *C. coppejansii* sp. nov. and *C. crispata* stat. nov. are newly described; and *C. muelleri* is reinstated. A new name, *Caulerpa lucasii* nom. nov., is proposed for *C. annulata*. *Caulerpa corynephora* and *C. fergusonii* are newly reported from the region. Furthermore, one of the most important findings of Chapter 3 was the recent extension of *C. cylindracea*, into Victorian waters around Portland. The fact that these adventive populations are found in shipping harbors, points to boating traffic as the most likely vector of introduction. From both chapters, reference *tuf*A sequences have been published and an updated nomenclature and identification key for *Caulerpa* species for both regions is provided.

Lastly, with an accurate DNA barcode for *C. cylindracea* (based on results from Chapter 2), Chapter 5 examines the use of all available Genbank records, as well as newly obtained data, to obtain an accurate global distribution (native and invasive) for the species. Using this dataset species distribution models were developed in order to predict the future spread of *C. cylindracea*. This approach identifies areas at risk from potential future invasion, including along the coasts of western Europe, western Africa and the south coast of Australia. This work demonstrates that, if available, occurrence records from the native and invasive ranges should be combined to produce models with high predictive power while reducing the sensitivity of choices made in the modelling process

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Chapter 2: Resolving phenotypic plasticity and species designation in the morphologically challenging *Caulerpa racemosa – peltata* complex

Statement of Authorship

Resolving phenotypic plasticity and species designation in the morphologically challenging *Caulerpa racemosa – peltata* complex (Caulerpacea, Chlorophyta). *Journal of Phycology*, 50: 32-54.

Belton, G. (Candidate)
Contributed to initial manuscript conceptualization, wrote the manuscript, collected specimens, generated and analyzed data, acted as lead author in drafting initial manuscript, co-ordinated addressing of reviewers comments, prepared figures and tables.

I hereby certify that the statement of contribution is accurate.

Signed…………………………….Date…………………………

Prud'homme van Reine, W. F.
Contributed to initial manuscript conceptualization and initial drafting, collected specimens, provided extensive nomenclature advice, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

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Gurgel, C. F. D.
Contributed to initial manuscript conceptualization and initial drafting, collected specimens and generated data, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

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Chapter 2

J. Phycol. **50,** 32–54 (2014)
© 2013 Phycological Society of America
DOI: 10.1111/jpy.12132

RESOLVING PHENOTYPIC PLASTICITY AND SPECIES DESIGNATION IN THE MORPHOLOGICALLY CHALLENGING *CAULERPA RACEMOSA–PELTATA* COMPLEX (CAULERPACEAE, CHLOROPHYTA)[1]

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Although recent molecular studies have indicated the presence of a number of distinct species within the *Caulerpa racemosa–peltata* complex, due to the difficulties presented by high levels of phenotypic plasticity and the large number of synonyms, infraspecific taxa, and names of uncertain affinity, taxonomic proposals are yet to be made. In this study, we aimed to resolve the taxonomy of the complex and provide an example of how historical nomenclature can best be integrated into molecular based taxonomies. We accomplished this by first determining the number of genetic species within our globally sampled data set through a combination of phylogenetic and species-delimitation approaches of partial elongation factor TU and RUBISCO large subunit gene sequences. Guided by these results, comparative morphological examinations were then undertaken to gauge the extent of phenotypic plasticity within each species, as well as any morphological overlap between them. Our results revealed the presence of 11 distinct species within the complex, five of which showed high levels of phenotypic plasticity and partial overlap with other species. On the basis of observations of a large number of specimens, including type specimens/descriptions, and geographic inferences, we were able to confidently designate names for the lineages. *Caulerpa peltata*, *C. imbricata* and *C. racemosa* vars. *laetevirens*, *occidentalis* and *turbinata* were found to represent environmentally induced forms of a single species, for which the earlier-described *C. chemnitzia*, previously regarded as a synonym of *C. racemosa* var. *turbinata*, is reinstated. *C. cylindracea*, *C. lamourouxii*, *C. macrodisca*, *C. nummularia* and *C. oligophylla* are also reinstated and two new species, *C. macra* stat. nov. and *C. megadisca* sp. nov., are proposed.

Key index words: ***Caulerpa*****;** ***chemnitzia*****; GMYC; nomenclature;** ***peltata*****; phenotypic plasticity;** ***racemosa*****;** ***rbc*****L; species delimitation; taxonomy;** ***tuf*****A**

List of Abbreviations**: BI, Bayesian inference; BP, Bootstrap percentages; C., Caulerpa; GMYC, General mixed Yule coalescence; GTR, generalized time-reversible (model); ML, Maximum likelihood; PP, posterior probability;** ***rbc*****L, RUBISCO large subunit;** ***tuf*****A, elongation factor TU**

Phenotypic plasticity has been a long-standing source of taxonomic difficulty in many algal groups,

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Editorial Responsibility: H. Verbruggen (Associate Editor)

frequently leading to misidentifications, nomenclatural quagmires, incorrect biodiversity estimates, and confusing classification schemes (e.g., Bacillariophyceae: Mann 2010, Chlorophyta: Leliaert et al. 2009, Phaeophyceae: Tronholm et al. 2010, Rhodophyta: Saunders 2008). Fortunately, the advent of molecular tools has provided researchers with an independent and convenient means to delimit and identify species in taxonomically challenging groups (e.g., Verbruggen et al. 2007, Saunders 2008, Leliaert et al. 2009, Mann 2010, Tronhlm et al. 2010). These tools, however, have also presented algal taxonomists with several new problems. First, considerable conflict between morphological and molecular species concepts has been uncovered (e.g., Leliaert et al. 2009, Tronholm et al. 2012) and, secondly, molecular data are revealing an overwhelming diversity of species, many cryptic, at a rate far greater than formal descriptions are being made (De Clerck et al. 2013). It is the naming of these species, especially the matching of previously described names with newly obtained molecular data, that is the greatest challenge currently faced by algal taxonomists, a task made more difficult by the overwhelming number of synonyms, infra-specific taxa, and names of uncertain affinity that plague many algal groups (De Clerck et al. 2013).

Several strategies for dealing with this challenge have been suggested. It has been proposed that newly collected and sequenced specimens (ideally topotype material similar in morphology to the type specimen) be designated as epitypes by experienced taxonomists (Tautz et al. 2003, Evans and Mann 2009, Bock et al. 2011, Zuccarello et al. 2011, Saunders and McDevit 2012). However, how can one be sure that the epitype is identical to the type specimen when the type specimen is unavailable (lost, in poor condition, based on a drawing etc. …) or if the type locality is unknown, vague (e.g., "Western Australia"), significantly altered since the original collection (e.g., Suez, Egypt) or contains a number of genetic species with similar morphology? Furthermore, how does one confidently match names and type specimens to newly collected specimens in groups where high levels of phenotypic plasticity and/or cryptic species are known to occur?

If we are to positively pinpoint the species in question, the only strategy leading to an unequivocal solution is to sequence the type material itself. It is for these reasons that Hughey and Gabrielson (2012), citing a number of examples, concluded that where possible, all type specimens, including their synonyms, should be sequenced. Although we agree with this in principle, as discussed by Saunders and McDevit (2012) and De Clerck et al. (2013), while it is feasible to obtain DNA information from older type specimens, it is a time-consuming (and partially destructive) process hampered by the large number of synonyms and infraspecific taxa. Furthermore, sequencing type specimens is also unlikely to be possible in many instances due to type specimens being unavailable (e.g., administrative controls on destructive sampling, specimens destroyed, lost or types not yet designated) or because usable DNA cannot be extracted and amplified (e.g., original specimen was formalin-preserved).

The taxonomy of the green macroalgal genus *Caulerpa* J.V. Lamouroux faces a number of the aforementioned issues. There are over 350 species and infraspecific taxon names associated with the genus, of which about 85 species are currently accepted (Guiry and Guiry 2013). Although lacking transverse cell walls (= siphonous thallus), *Caulerpa* species display a complex habit, with the thallus differentiating into stolons, rhizoids, and upright assimilators (= fronds) that usually bear ramuli (= branchlets; Fritsch 1965); the morphology of these various structures has been historically used for species delimitation. However, environmentally controlled phenotypic plasticity in all these characters has led to much confusion, a large number of synonyms, and a classification scheme involving subspecies, varieties, forms, and "*ecads*" (Peterson 1972, Calvert et al. 1976, Ohba and Enomoto 1987, Coppejans and Prud'homme van Reine 1992, de Senerpont Domis et al. 2003).

Within *Caulerpa*, the most taxonomically troublesome taxa are those currently associated with *C. racemosa* (Forsskål) J. Agardh and *C. peltata* J. V. Lamouroux, commonly referred to as the *C. racemosa–peltata* complex, which has more than 30 described varieties and forms (most given in Guiry and Guiry 2013; Fig. 1). One member of the complex, *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque, has recently received much scientific and public attention due to its invasion of the Mediterranean Sea, Canary Islands, and South Australia (Klein and Verlaque 2008). Although many of the *C. racemosa–peltata* complex varieties and forms were originally described as distinct species (e.g., *C. cylindracea* Sonder, *C. macrodisca* Decaisne), the subsequent discovery of apparently intermediate thalli with *C. racemosa* and *C. peltata* type morphologies resulted in these taxa being reduced to varieties or forms, mostly within *C. racemosa* (e.g., *C. racemosa* var. *macrodisca* (Decaisne) Weber-van Bosse), but with some sub-specific ranks also proposed within *C. peltata* (e.g., *C. peltata* var. *macrodisca* (Decaisne) Weber-van Bosse). However, recent molecular studies have shown the complex to consist of at least six distinct species-level entities (e.g., Sauvage et al. 2013), but as yet, no taxonomic changes have been proposed. As such, a large number of GenBank sequences (250+) remain simply labeled as either *C. racemosa* or *C. peltata*, although some sequences do include a varietal or formae epithet. This is a cause for concern as without accurately identified sequences, even with molecular sequence information available, govern-

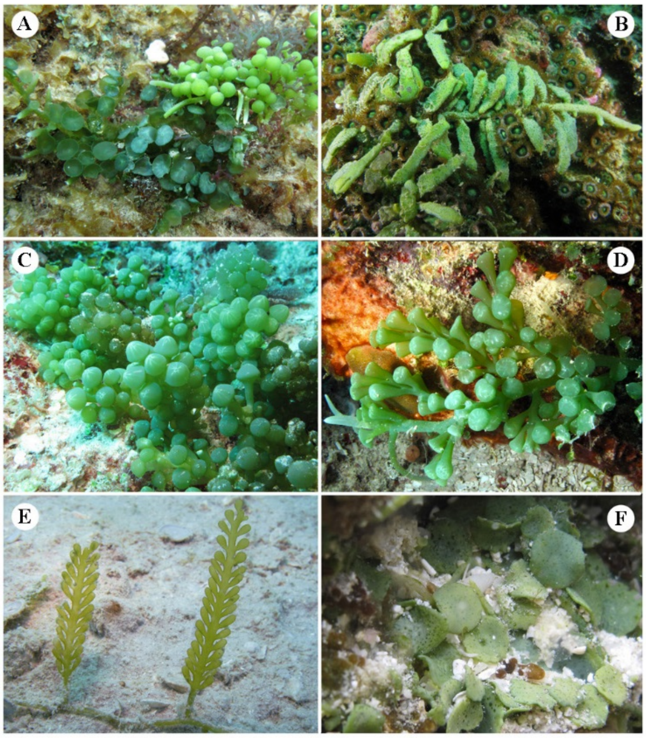


FIG. 1. Morphological variation of taxa currently assigned to the *Caulerpa racemosa-peltata* species complex. (A) *C. chemnitzia* (Esper) J.V. Lamouroux (AD-A91615) on left (previously *C. peltata* J.V. Lamouroux), growing with *C. racemosa* (Forsskål) J. Agardh (AD-A91592) on right at Ningaloo Reef, Western Australia; (B) *C. lamourouxii* (Turner) C. Agardh (PERTH 08292620) from Long Reef, north-western Australia (previously *C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse; (C) *C. chemnitzia* (AD-A92587) from Lizard Island, Great Barrier Reef (previously *C. racemosa* var. *laetevirens* (Montagne) Weber-van Bosse); (D) *C. chemnitzia* (AD-A92551) from Lizard Island, Great Barrier Reef, Australia (previously *C. racemosa* var. *turbinata* (J. Agardh) Eubank) displaying turbinate and clavate ramuli; (E) Complanate form of *C. cylindracea* Sonder (AD-A88114) from Lizard Island, Great Barrier Reef, Australia (previously *C. racemosa* f. *complanata* (J. Agardh) Weber-van Bosse); (F) *C. nummularia* Harvey ex. Agardh (AD-A91369) from Heron Island, Great Barrier Reef, Australia (previously *C. peltata*).

ment agencies and non-specialists can find it extremely difficult to correctly identify *Caulerpa* species, a factor that is becoming increasingly important as *Caulerpa* species continue to become established outside of their native ranges (e.g., Meinesz and Hesse 1991, Jousson et al. 2000, Womersley 2003, Lapointe et al. 2005, Amat et al. 2008, Jongma et al. 2013, Pérez-Estrada et al. 2013). However, designating names to sequenced *C. racemosa–peltata* complex specimens is no trivial task, as specimens can display high levels of phenotypic plasticity and in many instances crucial type specimens are lost (e.g., *C. peltata*), typification is based on illustrations only (e.g., *C. racemosa* var. *macrodisca*) or types were never designated (e.g., *C. nummularia* Harvey ex J. Agardh). Furthermore, multiple genetic species are often found at a single site (e.g., Sauvage et al. 2013).

While fully conscious of the many seemingly insurmountable problems associated with resolving the taxonomy of the complex, we feel that the current taxonomic and nomenclatural instability cannot be perpetuated. With this in mind, the present study aimed to resolve the taxonomically challenging *C. racemosa–peltata* complex and in the process provide an example of how historical nomenclature can best be integrated into molecular-based taxonomies.

MATERIALS AND METHODS

Overview. Due to the known phenotypic plasticity displayed by many members of the *C. racemosa–peltata* complex, we approached the present study in the following way. First, using a globally sampled DNA taxonomy (partial *tuf*A and *rbc*L sequences), including type locality or type region sequences, we determined the number of genetic species within our *C. racemosa–peltata* complex data set through a combination of phylogenetic and species-delimitation approaches. Then, guided by the molecular results, comparative morphological examinations were made to gauge the extent of both the phenotypic plasticity within each of the genetically distinct species and any morphological overlap between them. Using this information, plus geographic inferences and type specimen observations/descriptions, we then

designated new, current, or previously described species names to our specimens.

Taxon sampling. *Caulerpa racemosa-peltata* specimens representing a wide range of varieties and forms were collected globally, although sampling was biased toward the Indo-Pacific region. Where possible, specimens were also collected from, or close to, the type locality for each taxon. Part of the thallus was cleaned and preserved in silica gel for DNA extraction and the remainder of the specimen either press dried onto herbarium sheets, desiccated in silica gel, or preserved in 5% formalin/seawater. Specimen vouchers were housed at AD, GENT, L, PERTH, UNB, US (abbreviations follow Holmgren et al. 1990) and the personal herbarium of C. W. Schneider. Where available, type specimens, including those of varieties and forms, were observed. When types were not available, taxon concepts were based on the protologue and any subsequent descriptions and illustrations of original material. Collection information and herbarium voucher numbers of newly collected specimens used in the present study are given in Table S1 in the Supporting Information.

DNA extraction, amplification, and sequencing. Total DNA extraction was either outsourced to AGRF (Australian Genome Research Facility, Adelaide Node, SA, Australia) or processed by hand with a DNeasy Plant mini Kit (Qiagen, Hilden, Germany). A partial section (~820 bp) of the *tuf*A gene was amplified and sequenced for all specimens using the methods of Famà et al. (2002) or Saunders and Kucera (2010). The *tufA* gene was chosen as it has been shown to have sufficient species-level resolution in the Bryopsidales, including *Caulerpa* (Verbruggen et al. 2005, Saunders and Kucera 2010, Dijoux et al. 2012, Sauvage et al. 2013). On the basis of exploratory analyses of the *tuf*A data, we selected representative specimens from each lineage to sequence a partial section of the *rbc*L gene using the methods of Saunders and Kucera (2010). Purified PCR products were sent to AGRF, Macrogen (Seoul, Korea) or First BASE Laboratories Sdn Bhd (Seri Kembangan, Malaysia) for sequencing. Additional samples were extracted, amplified, and sequenced at the Centre for Environmental and Molecular Algal Research (University of New Brunswick, Fredericton, NB, Canada) following Saunders and Kucera (2010). Individual chromatograms were assembled into contigs and edited using Sequencher v.4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). Consensus sequences were compared to that of the National Centre for Biotechnology Information (NCBI) database using BLAST (http://www.ncbi.nlm.nih.gov) to check for contaminants. Newly generated sequences were submitted to GenBank and their accession numbers are given in Table S1.

Sequence alignment and phylogenetic analyses. Newly generated *tuf*A sequences from the present study were aligned with the *tuf*A alignment of Sauvage et al. (2013), which included sequences from the studies of Famà et al. (2002), de Senerpont Domis et al. (2003), Stam et al. (2006), Wynne et al. (2009), and Händeler et al. (2010). We also aligned the newly available GenBank sequences from Jongma et al. (2013), although we removed redundant sequences (i.e., 100% identical) from this data set before aligning. All available *Caulerpa rbc*L sequences were retrieved from GenBank and aligned with newly obtained *rbc*L sequences. These were from the studies of Hanyuda et al. (2000), de Senerpont Domis et al. (2003), Lam and Zechman (2006), and Maeda et al. (2012). A concatenated alignment of *tuf*A and *rbc*L sequences from de Senerpont Domis et al. (2003) and the present study was also created. GenBank sequences used in the present study are listed in Table S2 in the Supporting Information. All alignments were done using Clustal X (Larkin et al. 2007).

Alignments were analyzed using Bayesian inference (BI) and maximum likelihood (ML) with a general time-reversible substitution model incorporating a proportion of invariable sites and a gamma model of rate heterogeneity among sites (GTR+I+G) as determined by jModelTest v.1 (Posada 2008). BI was performed using BEAST v.1.7 (Drummond et al. 2006, Drummond and Rambaut 2007) with the Markov Chain Monte Carlo (MCMC) started from a random tree and run for 20 million generations, sampled every 1,000th generation, with an uncorrelated lognormal clock model. All other priors and operators were kept at their default values. A constant population size coalescent prior was used as tree prior. Tree log files were created with branch length in substitutions and MCMC runs were monitored in Tracer v.1.4.2 (Rambaut and Drummond 2007) to ensure that all final estimated sample size (ESS) values were above 200. The first 2,000 trees were removed as the burn-in before generating summary statistics and trees. Maximum-likelihood analyses were performed using RaxML (Stamatakis et al. 2008), with the reliability of each internal branch being evaluated by nonparametric bootstrapping (1,000 replicates). Final trees were edited in FigTree v.1.3.1 (Drummond and Rambaut 2007).

Species delimitation. Species were delimited using the *tuf*A data set and two different approaches. The first was to identify strongly supported clusters (i.e., bootstrap percentages (BP) ≥85, and posterior probability (PP) ≥0.95) preceded by a relatively long branch and with lower intraspecific sequence diversity (Verbruggen et al. 2005, Leliaert et al. 2009, Dijoux et al. 2012). Secondly, we used a more objective approach developed by Pons et al. (2006) and Monaghan et al. (2009) that uses a general mixed Yule coalescence (GMYC) model to define the species boundary. This method has been shown to be useful in several studies in recent years (e.g., Barraclough et al. 2009, Monaghan et al. 2009, Papadopoulou et al. 2011, Esselstyn et al. 2012, Murphy et al. 2013), including a number of macroalgal studies (e.g., Leliaert et al. 2009, Tronholm et al. 2012, Payo et al. 2013, Silberfeld et al. 2013). Using an ultrametric tree and a ML framework, the algorithm optimizes the shift in the branching patterns from interspecific branches (Yule model) to intraspecific branches (coalescent model). A threshold value is calculated such that nodes older than this threshold can be regarded as species diversification events, while younger nodes are identified as coalescence-driven clusters. The ultrametric tree was constructed using the BI methods described above, but with the tree log file being created without branch length in substitution. GMYC analysis was performed under the single-threshold model using the SPLITS package for R (R Development Core Team 2009; package available at http://r-forge.r-project.org/projects/splits/). Lineages were recognized as species if they were supported by both delimitation approaches.

Morphological observations. In order to understand the extent of phenotypic plasticity within each genetically distinct *C. racemosa–peltata* species, intra-lineage morphological variation was characterized by examining each specimen for rhizoid form, stolon width, assimilator height, and ramuli shape, size, and arrangement (Table 1). Specimens were then morphologically identified based on comparisons with type specimens, original descriptions, and numerous taxonomic references (Appendix S1 in the Supporting Information). Where available, specimen vouchers used in the studies by Famà et al. (2002), de Senerpont Domis et al. (2003), Stam et al. (2006) and Sauvage et al. (2013) were also examined. Detailed nomenclatural information of all taxa discussed in the present study is provided in Table S3 in the Supporting Information.

We based our concept of *C. racemosa* on detailed observations of the type specimen (reproduced as our Fig. 2A;

Herb. Forsskål No. 845 at C). As the type specimen of *C. peltata* is presumed lost, the illustration by Lamouroux (1809b) (reproduced as our Fig. 2B) that depicts erect assimilators bearing lateral peltate ramuli was regarded as the type form of *C. peltata*.

RESULTS

Phylogenetic analyses and species delimitation. In total, 65 *tuf*A and 20 *rbc*L sequences were newly generated (Table S1). Overall, including GenBank sequences, the final *tuf*A alignment comprised a total of 245 sequences (912 bp in length), the *rbc*L alignment 57 sequences (904 bp in length), and the concatenated data set 38 sequences (1,771 bp in length).

As the BI and ML analyses topologies were mostly congruent, only the BI topologies are shown (Figs. 3 and 4; Fig. S1 in the Supporting Information). It was difficult to compare the trees produced from the *tuf*A, *rbc*L and concatenated alignments due to data from various species not being present in all data sets. However, where *tuf*A and *rbc*L were available, the analyses produced trees with identical clades, but slightly contrasting topologies and statistical support (Figs. 3 and 4; Fig. S1). Although support in our trees was higher than in previous phylogenetic studies of *Caulerpa* (e.g., Famà et al. 2002, de Senerpont Domis et al. 2003, Sauvage et al. 2013), we still found some lack of resolution in the backbone of our single gene trees (Figs. 3 and 4). However, support was much improved in the concatenated data set analyses (Fig. S1).

We delimited 37 species with long branches and high support (PP >0.95, BS >0.85), with specimens

TABLE 1. Features of *Caulerpa* taxa that were previously assigned to either *C. racemosa* or *C. peltata*. Measurements were derived from type material and specimens collected in the present study.

	Caulerpa lamourouxii (Turner) C. Agardh	***Caulerpa oligophylla*** Montagne	***Caulerpa macra*** (Weber-van Bosse) Draisma & Prud'homme ***stat. nov.***	***Caulerpa cylindracea*** Sonder	***Caulerpa chemnitzia*** (Esper) J.V. Lamouroux
Lineage (Fig. 3)	1	2	4	5	6
Figures	1B, 5A–D	5E, F	6A, B	7A–D	1A, C, D, 8A–E
Previous accepted name(s)	*C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse	*C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse	*C. racemosa* var. *macra* Weber-van Bosse	*C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque; *C. racemosa* f. *complanata* (J. Agardh) Weber-van Bosse	*C. racemosa* var. *turbinata* (J. Agardh) Eubank; *C. imbricata* G. Murray; *C. peltata* J.V. Lamouroux; *C. racemosa* var. *laetevirens* (Montagne) Weber-van Bosse; *C. racemosa* var. *occidentalis* (J.Agardh) Børgesen
Stolon diameter, mm	1.5–3.0	1.5–2.0	(3-)4–6	0.8–1.5(2.0)	(0.8-)2–4(-6)
Assimilator					
Height, cm	2.5–8.0(-12)	2–3	3–10+	2–7(10)	1.5–7.0(-13)
Branching	Unbranched or sparingly branched	Common but irregular	Branching not observed	Branching not observed	Uncommon
Arrangement of ramuli	The number and arrangement of ramuli is very variable: from distichous and opposite to distantly spaced or completely absent.	Usually a few distantly spaced ramuli, although some specimens completely lacking ramuli	Ramuli moderately crowded, distichously or radially arranged and usually alternating.	Crowded and radially arranged to distichous and opposite	Usually very crowded and radially arranged
Ramuli					
Shape	Clavate to sub-spherical	Clavate to sub-spherical	Oviform, pyriform, claviform to slightly bulbous	Cylindrical to clavate, often flattened	Highly variable, clavate to turbinate to peltate. Often many forms on a single assimilator
Height, mm	(3-)4–8(-10)	3–5(6)	(5-)6-10(12)	(2.5-)4.0-6.0	(1.0-)3-10(-12)
Diameter, mm	2–3	2–3	2–4(6)	1.5–2.5	(1-)2–5(-8)

(continued)

TABLE 1. (continued)

	Caulerpa nummularia Harvey ex. J. Agardh	***Caulerpa megadisca*** Belton & Gurgel ***sp. nov.***	***Caulerpa macrodisca*** Decaisne	***Caulerpa racemosa*** (Forsskål) J. Agardh
Lineage (Fig. 3)	7	8	9	11
Figures	1F, 9A, B	9E	9C, D	1A, 10A–C
Previous accepted name(s)	*C. peltata* J. V. Lamouroux; *C. peltata* var. *exigua* Weber-van Bosse; *C. peltata* var. *stellata* (Harvey ex J.Agardh) Weber-van Bosse		*C. racemosa* var. *macrodisca* (Decaisne) Weber-van Bosse	*C. racemosa* (Forsskål) J. Agardh; *C. racemosa* var. *mucronata* L.N.de Senerpont Domis
Stolon diameter, mm	0.3–0.5(-0.8)	1–2	(1.0-)1.5–3.0	1.0–2.0(2.5)
Assimilator				
Height, cm	0.4–0.5	<1	1–5	1–3(6)
Branching	Common	None	Not observed	Rarely branched but becoming very branched in specimens previously assigned to var. *mucronata*
Arrangement of ramuli	Singly, or new peltate discs arising from the margin or centre of parent disc	Single peltate discs arising from the stolon	Semi-crowded, radially arranged on an upright axis	When present, irregularly arranged and uncrowded to crowded
Ramuli				
Shape	Distinctly peltate, with smooth and/or crenulated margins	Distinctly peltate and large	Distinctly peltate and large	Spherical, pyriform on a short stalk.
Height, mm	2–5	3–6	(3-)5–8(-12)	1-3(-4)
Diameter, mm	(2-)3–4(-5)	8(10)–15(20)	(4-)5-10(-18)	1.0–2.5 (3.0)

from the *C. racemosa–peltata* complex forming 12 of these (Fig. 3). A BI phylogram of the *tuf*A data set is provided in the Supplementary data to show branch lengths (Fig. S2 in the Supporting Information). The GMYC model provided a significantly better fit than a null model driven by coalescence only (confidence interval 29–46; $L_{GMYC} = 2423.955 > L_0 = 2411.179$, $P = 0.0002$) and recovered 38 lineages, with specimens from the *C. racemosa–peltata* complex forming 13 of these (Fig. 3). The two discrepancies between the methods with regard to specimens of the *C. racemosa–peltata* complex were that the long branches and high support method recovered two separate clusters within lineage 4, and lineage 6 as a single cluster (Fig. 3 and Fig. S2), whereas the GMYC method supported three separate clusters within lineage 6, and lineage 4 as a single cluster (Fig. 3 and Fig. S2). Due to these discrepancies, we did not recognize the additional clusters within lineages 4 and 6, leaving 11 recognized *C. racemosa–peltata* lineages (Fig. 3). Two of these lineages (3 and 10) were single sequences from Sauvage et al. (2013), which lacked associated *rbc*L data, and thus consideration of these lineages will not be dealt with in the present study. The *rbc*L and concatenated trees also showed high support (PP >0.95; BS >0.85) for the nine *C. racemosa–peltata* lineages (Fig. 4 and Fig. S1, respectively).

Morphological characterization of lineages. Among the genetically distinct *C. racemosa–peltata* lineages found in this study, five showed considerable levels of phenotypic plasticity and partial morphological overlap with other lineages (lineages 1, 2, 5, 6 and 11), whereas the remaining four lineages (4, 7, 8 and 9) did not.

Lineage 1 included specimens morphologically identified as *C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse (Fig. 5A) and *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse (Figs. 1B and 5, B and C) from Western Australia and the Red Sea, although some specimens could be thought to resemble *C. racemosa* var. *cylindracea* and *C. racemosa*. Some newly collected specimens were almost identical to the type specimen of *C. racemosa* var. *lamourouxii* (BM000569449, as *Fucus lamourouxii* Turner; Fig. 5D). As with previous works on *C. racemosa* var. *lamourouxii*, specimens observed in the present study varied considerably in terms of their morphologies (Table 1), with assimilators ranging from being densely covered with clavate ramuli (Fig. 5A) to being flattened and devoid of any ramuli (Figs. 1B and 5B). Very young specimens or parts of specimens often showed a very different morphology than that of mature specimens. *Caulerpa racemosa* var. *lamourouxii* specimens that bear no (or very few) ramuli (e.g., Figs. 1B and 5, B and C) are usually referred to as *C. racemosa* var.

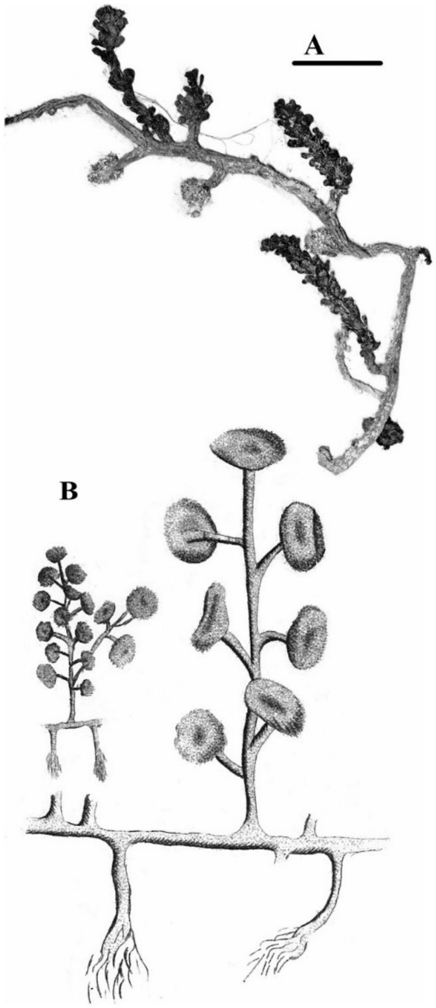


FIG. 2. (A) Photograph of the holotype of *Caulerpa racemosa* (Forsskål) J. Agardh (Herb. Forsskål No. 845 at C). Scale bar = 20 mm; (B) Illustration of *C. peltata* J.V. Lamouroux by Lamouroux (1809b). Unfortunately, as the type specimen is lost and Lamouroux's illustration does not have a scale, we were unable to provide a scale for this image.

lamourouxii f. *requienii*. However, all specimens assigned to f. *requienii* in the present study were quite different to the type specimen (see Wynne et al. 2009: 295 fig. 8, as *Herpochaeta requienii* Montagne), as well as its heterotypic synonyms *C. racemosa* var. *gracilis* (Zanardini) Weber-van Bosse (TCD0011042; Fig. S3A in the Supporting Information) and *C. clavifera* var. *nudiuscula* Zanardini (given as *nudicaulis* in herb. TCD0011044, Fig. S3B). The most notable difference was assimilator heights, with our specimens being <5 cm tall, whereas heights of the three type specimens mentioned above were up to 10–15 cm.

Lineage 2 included specimens from the Great Barrier Reef, Indonesia, Japan, New Caledonia, and the Philippines usually also attributed to *C. racemosa* var. *lamourouxii* (Fig. 5, E and F), and as with lineage 1, these specimens showed considerable morphological variation. However, these specimens were generally smaller than specimens from lineage 1 and closely resembled the type specimen of *C. oligophylla* Montagne (MA10330 at P; Fig. 5F), a species described from northeastern Australia, but currently regarded as a synonym of *C. racemosa* var. *lamourouxii* (Price 2011).

Lineage 4 included specimens from the tropical Indo-Pacific region identified as *C. racemosa* var. *macra* (Fig. 6A) based on similarity to the type specimen (Fig. 6B, L0054713 also from the Indo-Pacific region) and original description (Weber-van Bosse 1913: 107). These specimens could usually be distinguished from other *C. racemosa–peltata* complex taxa by their stolon width, large ramuli that were usually in the form of the head of a golf club, as well as elongated rhizoidal pillars (Fig. 6, A and B; Table 1). This lineage also included specimens from New Caledonia that were identified as rather similar to *C. racemosa* f. *remota* (Svedelius) Coppejans (Fig. 6C; specimens from Sauvage et al. 2013). Apart from being slightly smaller, these specimens were similar in morphology to *C. racemosa* var. *macra* specimens and also closely matched the type illustration and description by Svedelius (1906) (as *C. clavifera* f. *remota* Svedelius; reproduced in Fig. 6D) and Coppejans et al. (2009: 115, fig. 89). Unfortunately, we were unable to newly collect *C. racemosa* f. *remota* specimens from close to the type locality (Sri Lanka).

Lineage 5 included specimens collected from Australia and New Caledonia that were identified as *C. racemosa* var. *cylindracea* (Fig. 7, A and B) and *C. racemosa* f. *complanata* (J. Agardh) Weber-van Bosse (Figs. 1E and 7, C and D), although some specimens resembled *C. racemosa* var. *laetevirens* (Montagne) Weber-van Bosse. Specimens from south-western Australia (the type locality) and South Australia were identical to the type specimen of *C. racemosa* var. *cylindracea* (as *C. cylindracea*, Fig. 7B; MEL 516014), but became more varied with ramuli arrangement becoming distichous in the tropical waters of northern Australia and New Caledonia (also see Sauvage et al. 2013: 11, fig. 5), and closely resembled the type specimen of *C. racemosa* f. com-

CAULERPA RACEMOSA–PELTATA COMPLEX 39

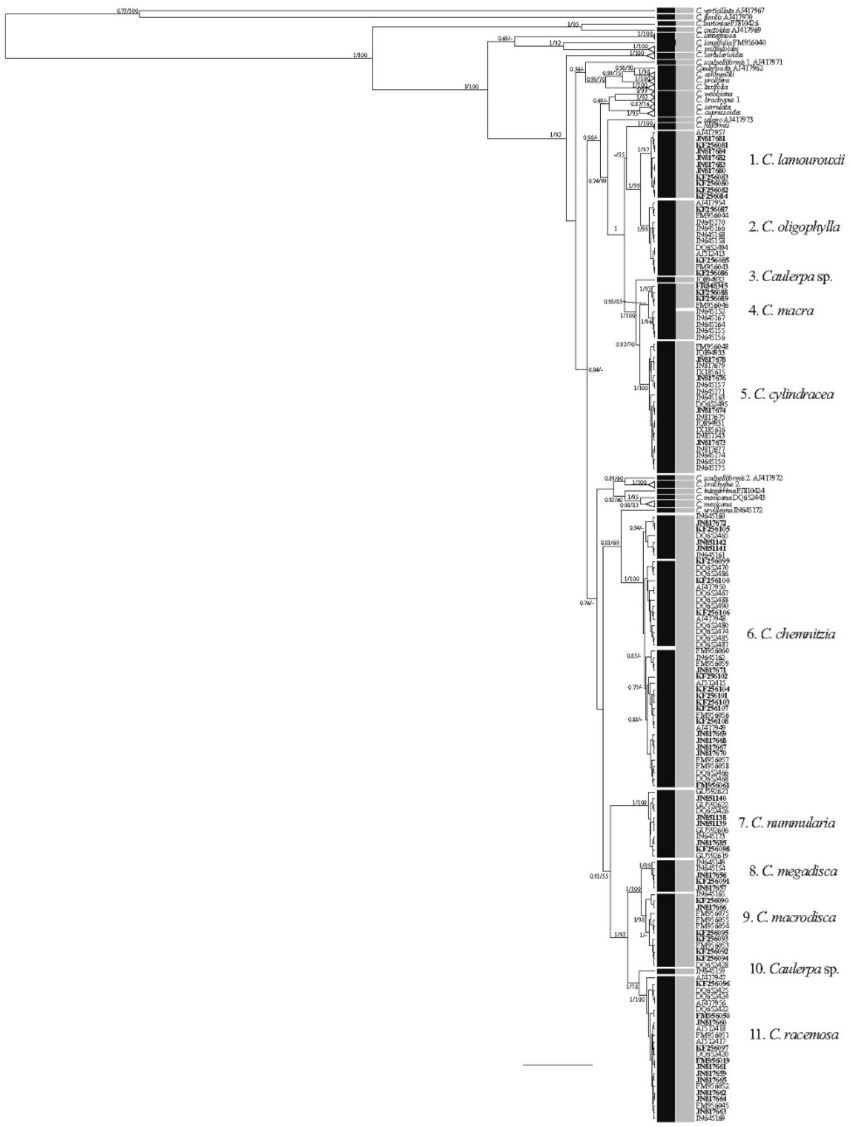


FIG. 3. Ultrametric tree of *Caulerpa* based on a Bayesian relaxed molecular clock analysis of 245 partial *tuf*A DNA sequences (912 nt, −ln = 5551.6091) with results from GMYC- and long-branch, high support species-delimitation methods. Columns to the right of the tree indicate putative species obtained under the GMYC (black) and long-branch, high support (gray) species-delimitation methods. Numbers at branch nodes correspond to BI posterior probabilities (PP) and ML bootstrap percentages (BP). PP values below 0.5 and BS values below 50% are not shown. Scale bar = 0.02 expected changes per site. Boldface = newly obtained sequences.

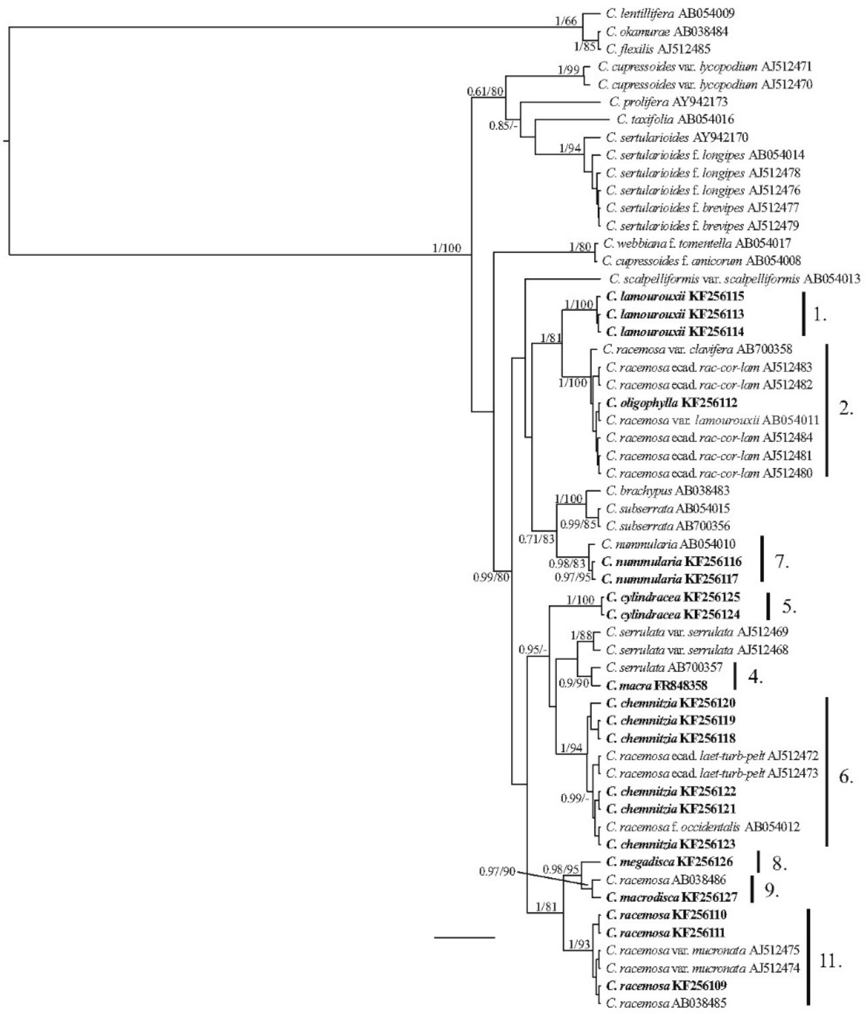


FIG. 4. Bayesian phylogenetic tree constructed from 57 partial *rbc*L DNA sequences of *Caulerpa* (alignment = 904 bp long). Numbered lineages correspond to those of Figure 3. Numbers at branch nodes correspond to BI posterior probabilities (PP) and ML bootstrap percentages (BP). PP values below 0.5 and BS values below 50% are not shown. Numbered lineages correspond to those of Figure 3. The scale is 0.008 expected changes per site. Boldface = newly obtained sequences.

planata (Fig. 7D). Although ramuli arrangement in this lineage varied from distichous to radial, ramuli were always clavate, although they were at times slightly flattened (e.g., Fig. 1E).

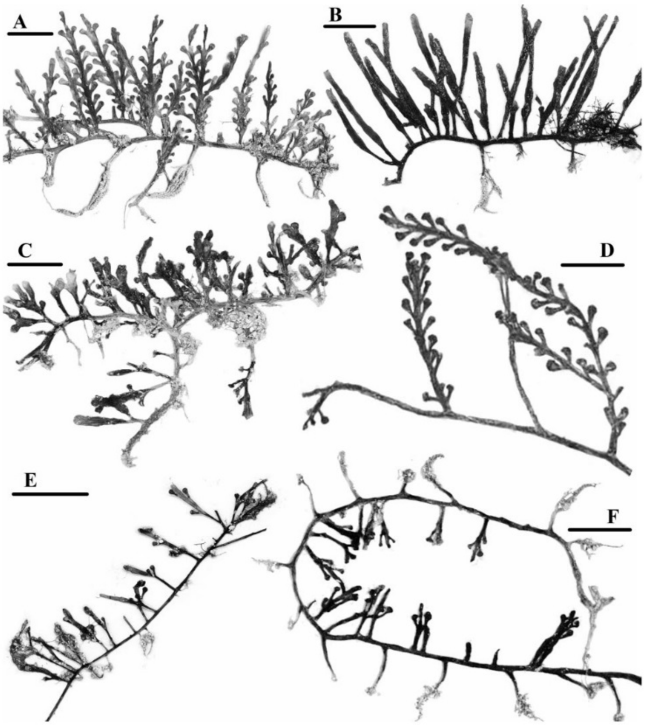


FIG. 5. Morphological variation among taxa previously assigned to the *Caulerpa racemosa* var. *lamourouxii* (Turner) Weber-van Bosse: (A) *C. lamourouxii* (Turner) C. Agardh (PERTH 08292612) from Montgomery Reef, north-western Australia; (B) *C. lamourouxii* specimen lacking ramuli (AD-A90154) from Ningaloo Reef, Western Australia; (C) *C. lamourouxii* specimen with an intermediate number of ramuli (PERTH 08428220) from Cassini Island, north-western Australia; (D) Photograph of the holotype of *C. lamourouxii* (BM000569449, as *Fucus lamourouxii* Turner); (E) Typical specimen of *C. oligophylla* Montagne (AD-A95457) from Heron Island, Great Barrier Reef, Australia; (F) Photograph of the holotype of *C. oligophylla* (MA10330 in PC) from Torres Strait, Australia. All scale bars = 20 mm.

Lineage 6 included specimens collected from the Atlantic, Indian, and Pacific Oceans and showed the largest variation in morphology (Table 1), from rather small specimens with upright assimilators bearing strictly peltate ramuli (Figs. 1A and 8A), to robust specimens with densely arranged turbinate and clavate ramuli (Figs. 1, C, D, and 8, B–D). Some specimens even bore ramuli of various forms on a single assimilator (Fig. 1D). Specimens in this lineage were identified as *C. peltata* (Figs. 1A and 8A), *C. racemosa* var. *laetevirens* (Figs. 1C and 8B), *C. racemosa* var. *occidentalis* (J. Agardh) Børgesen (Fig. 8C), *C. racemosa* var. *turbinata* (J. Agardh) Eubank (Fig. 8D), and *C. imbricata* G. Murray based on their similarity to type specimens (Figs. 2B and 8E, Figs. S4, S5 and S6A in the Supporting Information), but a myriad of intermediate specimens were also observed and some specimens were also close in morphology to *C. racemosa* var. *cylindracea* and *C. racemosa* var. *macrodisca* (Table 1). Although not from one of the Antilles Islands, a specimen from nearby Bermuda (CWS008366; Fig. 8A) was almost identical to Lamouroux's (1809b) illustration of *C. peltata* (Fig. 2B).

Lineage 7 included small, delicate, strictly peltate specimens with both crenate and entire disk margins, from which secondary peltate ramuli arose (Fig. 9A). Specimens from this lineage were only collected along the east coast of Australia (including Lord Howe Island), Micronesia, and Fiji. Although these specimens were initially identified as *C. peltata* var. *exigua* Weber-van Bosse (*sensu* Kraft 2007), *C. peltata* (*sensu* Price 2011), or *C. peltata* var. *stellata* (Harvey ex J. Agardh) Weber-van Bosse, Weber-van Bosse's (1898) illustration of the original *C. peltata* var. *exigua* concept clearly depicts secondary ramuli arising from the center of the subtending disk, not the margins. As such, these small delicate peltate plants, with secondary peltate ramuli arising from the disk margin, more accurately corresponded to *C. nummularia* (currently regarded as a heterotypic synonym of *C. peltata*, Price 2011). This identification was further confirmed by the original description of *C. nummularia* (Agardh 1873), observations

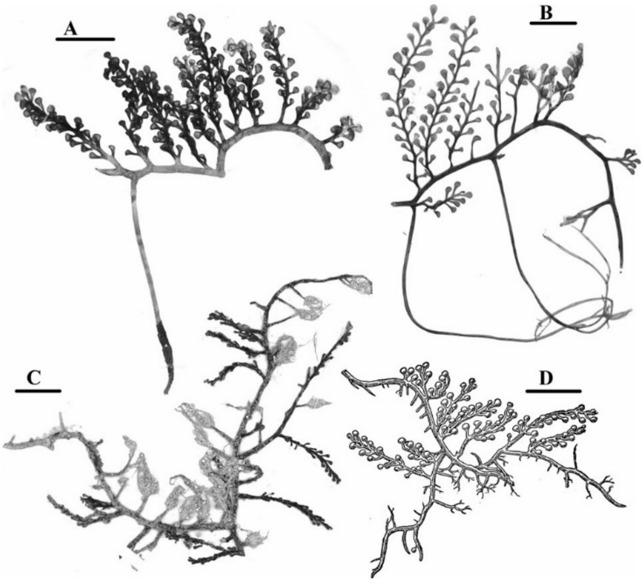


FIG. 6. Morphological variation among taxa and specimens assigned to *Caulerpa macra* (Weber-van Bosse) Draisma & Prud'homme *stat. nov.* in the present study. (A) Photograph of the holotype of *C. macra* (L 0054712 as *C. racemosa* var. *macra* Weber-van Bosse); (B) Recently collected specimen from Chuuk Island, Micronesia (L 0925909); (C) Smaller specimen from New Caledonia (= IRD5624), morphologically identified as *C. racemosa* f. *remota* (Svedelius) Coppejans); (D) Illustration of *C. racemosa* f. *remota* by Svedelius (1906) (fig. 14, as *C. clavifera* f. *remota*). All scale bars = 20 mm.

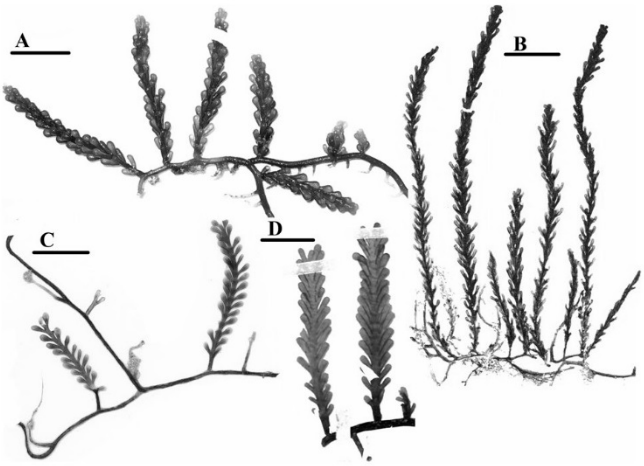


FIG. 7. Morphological variation among taxa assigned to *Caulerpa cylindracea* Sonder in the present study. (A) Specimen from south-western Australia (GWS025471); (B) Photograph of an isotype specimen (TCD001105); (C) Complanate form from Lizard Island, Great Barrier Reef, Australia (AD-A88114; previously *C. racemosa* f. *complanata* (J. Agardh) Weber-van Bosse). (D) Photograph of the holotype of *C. racemosa* f. *complanata* (originally described as *C. complanata* J. Agardh, Herb. Agardh 16718, in LD). All scale bars = 20 mm.

of syntype specimens in TCD and NSW (see Fig. 9B), and previous accounts of *Caulerpa* species from the Pacific region (i.e., Cribb and Cribb 1985, South and Skelton 2003, Abbott and Huisman 2004, Skelton and South 2007). The morphology of specimens in this lineage was unique.

Lineages 8 and 9 included specimens that were identified as *C. racemosa* var. *macrodisca* that could

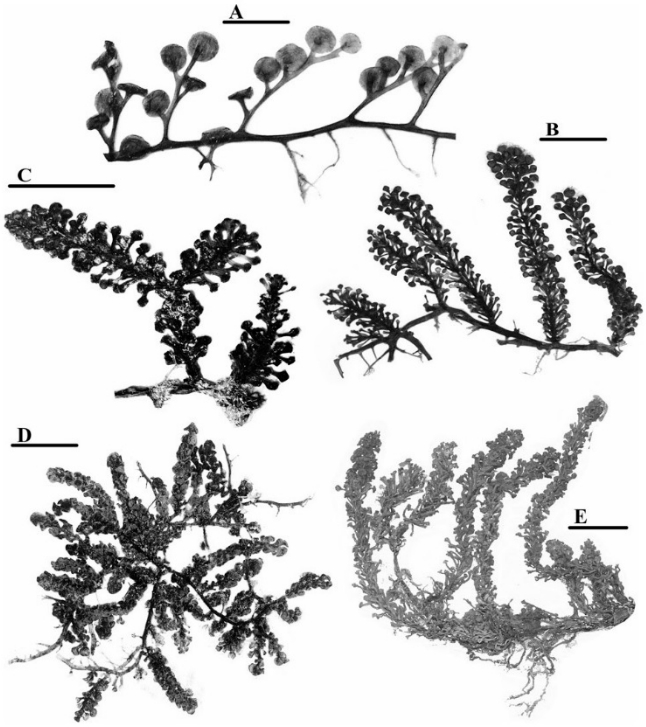


FIG. 8. Morphological variation among *Caulerpa chemnitzia* (Esper) J.V. Lamouroux taxa discussed in the present study. (A) Peltate specimen (CWS008366) from Bermuda (previously *C. peltata* J.V. Lamouroux); (B) AD-A92587 from Lizard Island, Great Barrier Reef, Australia (previously *C. racemosa* var. *laetevirens* (Montagne) Weber-van Bosse); (C) Specimen from Bermuda (= CWS008555; previously *C. racemosa* var. *occidentalis* (J. Agardh) Børgesen); (D) Pressed specimen of a recently collected individual from Sri Lanka (HEC15952); (E) Photograph of the holotype of *C. chemnitzia* (25805 in ER). Scale bars: A, E = 10 mm; B = 30 mm; C = 20 mm; D = 25 mm.

easily be morphologically distinguished from other lineages, although specimens in lineage 9 could at times resemble peltate specimens from lineage 6. Specimens in lineage 9 (Fig. 9C) closely resembled the lectotype illustration (Decaisne 1846-1864; pl. 1, fig. 1 as *C. macrodisca*; reproduced as our Fig. 9D), whereas specimens from lineage 8 had ramuli that were not arranged around an upright assimilator, but rather the assimilators consisted of a single peltate branch (Fig. 9E). No intermediates between the two forms were observed during the present study.

Lineage 11 included specimens that were collected from the Atlantic, Indian, and Pacific Ocean regions and were closest in morphology to the *C. racemosa* type morphology (compare Figs. 1A and 10A to 2A). It also included specimens that were identified as *C. racemosa* var. *macrophysa* (Sonder ex Kützing) W.R. Taylor (Fig. 10B) and *C. racemosa* var. *mucronata* L.N. de Senerpont Domis (Fig. 10C, specimen from de Senerpont Domis et al. 2003). Specimens from this lineage could usually be distinguished from other *C. racemosa–peltata* complex taxa by having ramuli that expanded abruptly from a relatively short slender stalk to a subspherical upper part, with the stalk length not being greater than the diameter of the distal regions, although some of the larger specimens could have been identified as *C. racemosa* var. *lamourouxii* (lineage 1). Unlike *C. racemosa* and *C. racemosa* var. *macrophysa*, *C. racemosa* var. *mucronata* showed considerable variation in ramulus form, with ramuli often completely absent from assimilators (Fig. 10C). Unfortunately, no specimens matching the type specimen of *C. racemosa* were located near the type locality (Red Sea) during the present study.

DISCUSSION

As molecular-based studies continue to show increased rates of species discovery and mismatches between molecular and morphological species concepts, the greatest challenge for algal taxonomy in the future will be how to designate names, includ-

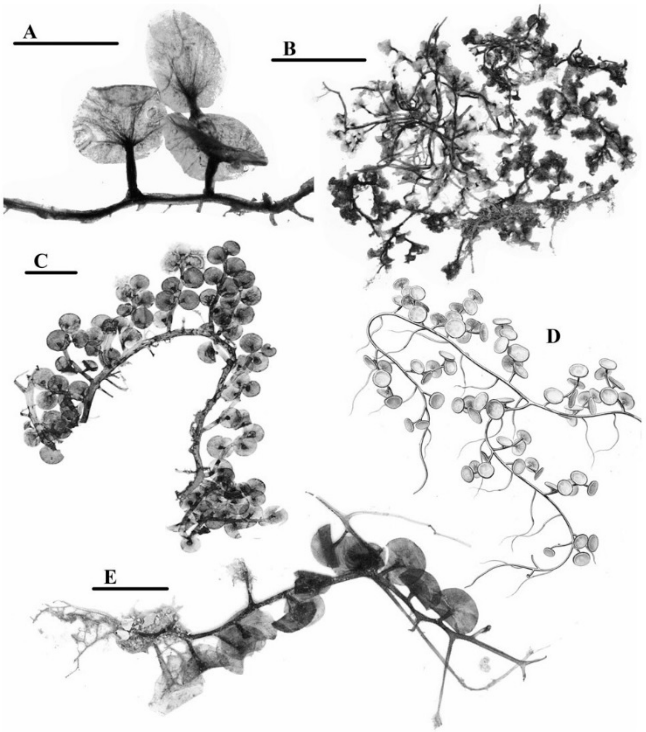


FIG. 9. Morphological variation of *Caulerpa racemosa-peltata* complex taxa discussed in the present study. (A) *C. nummularia* Harvey ex. J. Agardh (AD-A91369) from Heron Island, Great Barrier Reef, Australia, showing ramuli disk arising from margin of parent disk; (B) Syntype specimen of *C. nummularia* (TCD 00111032). (C) *C. macrodisca* Decaisne (L 0509359/FM965053) from Indonesia with characteristic upright assimilator morphology; (D) Copy of the lectotype illustration of *C. macrodisca* by Decaisne (1846 (1846-1864), pl. 1, fig. 1); (E) *C. megadisca* Belton & Gurgel *sp. nov.* (AD-A90107) from Lizard Island, Great Barrier Reef, Australia showing typical single peltate disk morphology. Scale bars: A = 0.5 mm; B = 25 mm; C, D, E = 20 mm.

ing those that have been previously described, to newly sequenced specimens (De Clerck et al. 2013). Ideally, one could aim to sequence every type specimen; however, this is a time-consuming process that may prove futile if specimens cannot be located, sequenced, or accessed. Although we acknowledge that without sequencing type specimens there may always be a level of uncertainty in the naming of lineages, including those of the present study, we are at the point in algal systematics where we need to be pragmatic and revise taxonomies to the best of our abilities. With this in mind, the primary goal in the present study was to resolve the taxonomy of the *C. racemosa–peltata* complex, one of the most taxonomically troublesome groups in the algae. The complex also has a large number of synonyms and infra-specific names that could potentially be used to name genetic lineages, and these were taken into account. We sequenced a large number of globally collected specimens to obtain a well-sampled DNA taxonomy and, for many taxa, this included sequences from type locality or regions. We then delimited species using our *tuf*A data set and two commonly used methodologies (long branch/high support and GMYC). Although the GMYC species delineation technique has its pitfalls (Lohse 2009, Papadopoulou et al. 2011, Reid and Carstens 2012, Silberfeld et al. 2013), it provides an objective tool to rapidly delimit species in large, well-sampled, single marker data sets. As our data set was large and well-sampled, the GMYC results were robust (i.e. significant difference between null model and GMYC model, and a narrow confidence interval) and results from both species-delimitation methods were largely congruent, we are confident in accepting the presence of at least 11 distinct species-level entities within the *C. racemosa–peltata* complex, although only nine of these are considered here.

We found that the morphological characterization of five of these lineages was fraught with the difficulties caused by high levels of phenotypic plasticity (Table 1), and in many instances, the morphological overlap of genetic species (e.g., lineages 1 and

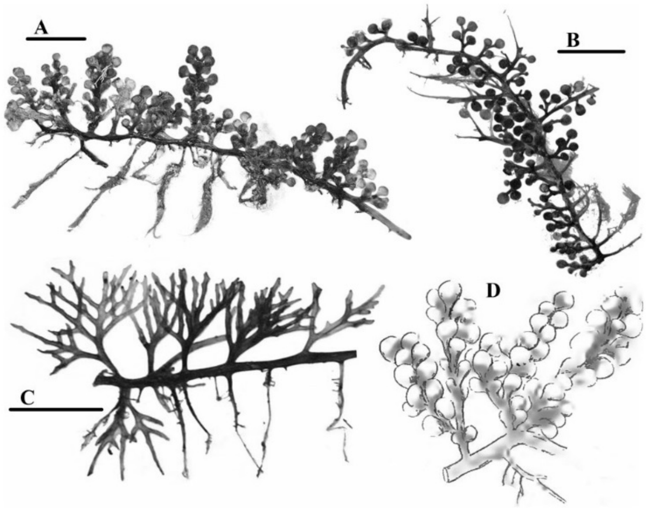
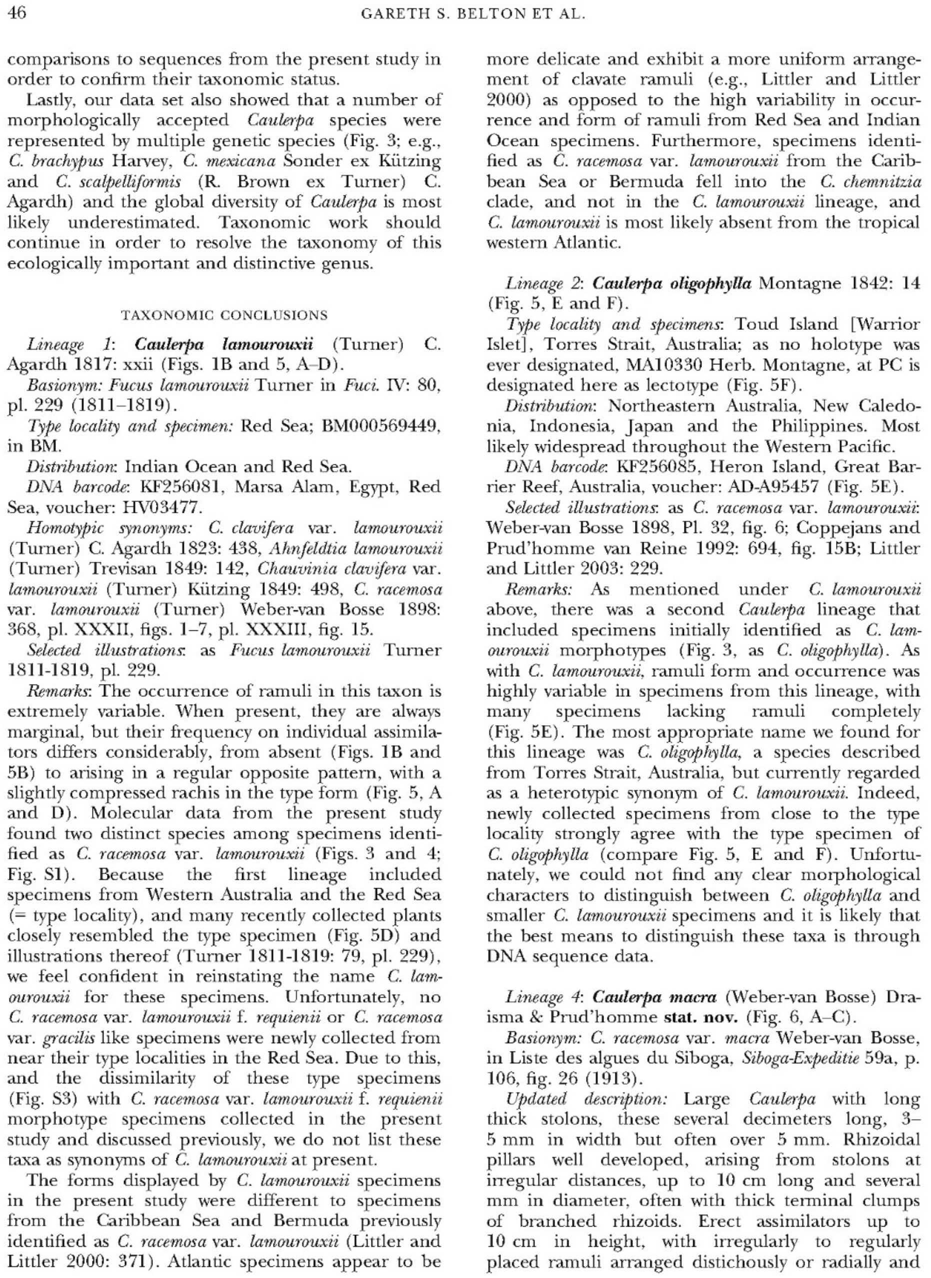


FIG. 10. Morphological variation among taxa assigned to *Caulerpa racemosa* (Forsskål) J. Agardh discussed in the present study. (A) Pressed voucher of *C. racemosa* (PERTH 08292728) from Montgomery Reef, north-western Australia; (B) *C. racemosa* var. *macrophysa* (Sonder ex Kützing) W.R. Taylor (AD-A90071) from Lizard Island, Great Barrier Reef, Australia; (C) Photograph of the holotype of *C. racemosa* var. *mucronata* L.N. de Senerpont Domis (L 0535499); (D) Illustration of *C. racemosa* var. *macrophysa* by Kützing (1857) (tab. 15 fig. II, as *Chauvinia macrophysa* Sonder ex Kützing). All scale bars = 20 mm.

2). As such, although some lineages showed distinct morphologies with minimal phenotypic plasticity (e.g., lineage 7), as an overarching conclusion for identifying our nine species, we strongly suggest the use of molecular-based identifications.

With regard to the correct nomenclatural assignment of our nine lineages, we have based our designations and nomenclatural decisions on what we regard as "best practice": molecular data obtained from specimens collected from near the type locality, that were matched with type material, original illustrations and/or descriptions. Importantly, we found that by first grouping specimens into species using molecular data, we were able to determine the true extent of morphological variation within each species. Once this was understood, we were then able to confidently match newly collected specimens with type specimens/original descriptions/illustrations (details are listed under each species below). Unfortunately, the only lineage for which we were unable to locate specimens from the type locality or type region that resembled the type specimen was *C. racemosa* (lineage 11), and we highly recommend that future studies attempt to do so. However, we are confident in assigning this lineage to *C. racemosa* for the time being as specimens closely resembled the type specimen and were in agreement with most concepts of *C. racemosa* (e.g., Coppejans and Prud'homme van Reine 1992: 698, as *C. racemosa* ecad *racemosa*; Price 2011: 185, as *C. racemosa* var. *racemosa*). In order to reduce confusion and begin the process of solving the cumbersome nature of *Caulerpa* taxonomy, we also feel it appropriate to not recognize any infra-specific ranks (i.e., varieties and forms) within the species recognized below. However, we do suggest that the use of morphological entities without formal taxonomic status (e.g., "*ecads*") may be useful for field workers when collecting highly plastic species such as *C. chemnitzia* (e.g., *C. chemnitzia* ecad *peltata*). Arrangements have also been made with GenBank to update their *tuf*A records with the newly corrected names.

The following *C. racemosa–peltata* complex taxa were not encountered during the present study: *C. laetevirens* f. *caespitosa* Svedelius; *C. racemosa* (var. *laetevirens)* f. *compressa* W. R. Taylor; *C. racemosa* (var. *uvifera*) f. *compressa* Weber-van Bosse; *C. racemosa* f. *condensata* Weber-van Bosse; *C. laetevirens* f. *depauperata* Svedelius; *C. dichotoma* Svedelius; *C. racemosa* var. *disticha* V. J. Chapman; *C. racemosa* f. *elongata* Weber-van Bosse; *C. racemosa* f. *hypocrateriaeformis* Weber-van Bosse; *C. uvifera* f. *intermedia* Weber-van Bosse; *C. racemosa* (var. *laetevirens*) f. *laxa* (Greville) Weber-van Bosse; *C. racemosa* (var. *cylindracea*) f. *laxa* (Greville) Weber-van Bosse; *C. chemnitzia* f. *major* Weber-van Bosse; *C. chemnitzia* f. *minor* Piccone; *C. racemosa* f. *mixta* Svedelius; *C. clavifera* f. *nuda* Kützing; *C. parvula* Svedelius; *C. uvifera* f. *planiuscula* Svedelius; *C. racemosa* f. *reducta* Børgesen; *C. racemosa* f. *simplicissima* Børgesen; *C. racemosa* f. *semifalcata* V. D. Chauhan & Thivy; A.D. Zinova & N.H. Dinh; *C. clavifera* f. *vulgaris* Kützing; and *C. zeyheri* Kützing. It is hoped that future studies endeavor to collect and sequence these taxa and make



comparisons to sequences from the present study in order to confirm their taxonomic status.

Lastly, our data set also showed that a number of morphologically accepted *Caulerpa* species were represented by multiple genetic species (Fig. 3; e.g., *C. brachypus* Harvey, *C. mexicana* Sonder ex Kützing and *C. scalpelliformis* (R. Brown ex Turner) C. Agardh) and the global diversity of *Caulerpa* is most likely underestimated. Taxonomic work should continue in order to resolve the taxonomy of this ecologically important and distinctive genus.

TAXONOMIC CONCLUSIONS

Lineage 1: ***Caulerpa lamourouxii*** (Turner) C. Agardh 1817: xxii (Figs. 1B and 5, A–D).

Basionym: Fucus lamourouxii Turner in *Fuci.* IV: 80, pl. 229 (1811–1819).

Type locality and specimen: Red Sea; BM000569449, in BM.

Distribution: Indian Ocean and Red Sea.

DNA barcode: KF256081, Marsa Alam, Egypt, Red Sea, voucher: HV03477.

Homotypic synonyms: C. clavifera var. *lamourouxii* (Turner) C. Agardh 1823: 438, *Ahnfeldtia lamourouxii* (Turner) Trevisan 1849: 142, *Chauvinia clavifera* var. *lamourouxii* (Turner) Kützing 1849: 498, *C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse 1898: 368, pl. XXXII, figs. 1–7, pl. XXXIII, fig. 15.

Selected illustrations: as *Fucus lamourouxii* Turner 1811-1819, pl. 229.

Remarks: The occurrence of ramuli in this taxon is extremely variable. When present, they are always marginal, but their frequency on individual assimilators differs considerably, from absent (Figs. 1B and 5B) to arising in a regular opposite pattern, with a slightly compressed rachis in the type form (Fig. 5, A and D). Molecular data from the present study found two distinct species among specimens identified as *C. racemosa* var. *lamourouxii* (Figs. 3 and 4; Fig. S1). Because the first lineage included specimens from Western Australia and the Red Sea (= type locality), and many recently collected plants closely resembled the type specimen (Fig. 5D) and illustrations thereof (Turner 1811-1819: 79, pl. 229), we feel confident in reinstating the name *C. lamourouxii* for these specimens. Unfortunately, no *C. racemosa* var. *lamourouxii* f. *requienii* or *C. racemosa* var. *gracilis* like specimens were newly collected from near their type localities in the Red Sea. Due to this, and the dissimilarity of these type specimens (Fig. S3) with *C. racemosa* var. *lamourouxii* f. *requienii* morphotype specimens collected in the present study and discussed previously, we do not list these taxa as synonyms of *C. lamourouxii* at present.

The forms displayed by *C. lamourouxii* specimens in the present study were different to specimens from the Caribbean Sea and Bermuda previously identified as *C. racemosa* var. *lamourouxii* (Littler and Littler 2000: 371). Atlantic specimens appear to be more delicate and exhibit a more uniform arrangement of clavate ramuli (e.g., Littler and Littler 2000) as opposed to the high variability in occurrence and form of ramuli from Red Sea and Indian Ocean specimens. Furthermore, specimens identified as *C. racemosa* var. *lamourouxii* from the Caribbean Sea or Bermuda fell into the *C. chemnitzia* clade, and not in the *C. lamourouxii* lineage, and *C. lamourouxii* is most likely absent from the tropical western Atlantic.

Lineage 2: ***Caulerpa oligophylla*** Montagne 1842: 14 (Fig. 5, E and F).

Type locality and specimens: Toud Island [Warrior Islet], Torres Strait, Australia; as no holotype was ever designated, MA10330 Herb. Montagne, at PC is designated here as lectotype (Fig. 5F).

Distribution: Northeastern Australia, New Caledonia, Indonesia, Japan and the Philippines. Most likely widespread throughout the Western Pacific.

DNA barcode: KF256085, Heron Island, Great Barrier Reef, Australia, voucher: AD-A95457 (Fig. 5E).

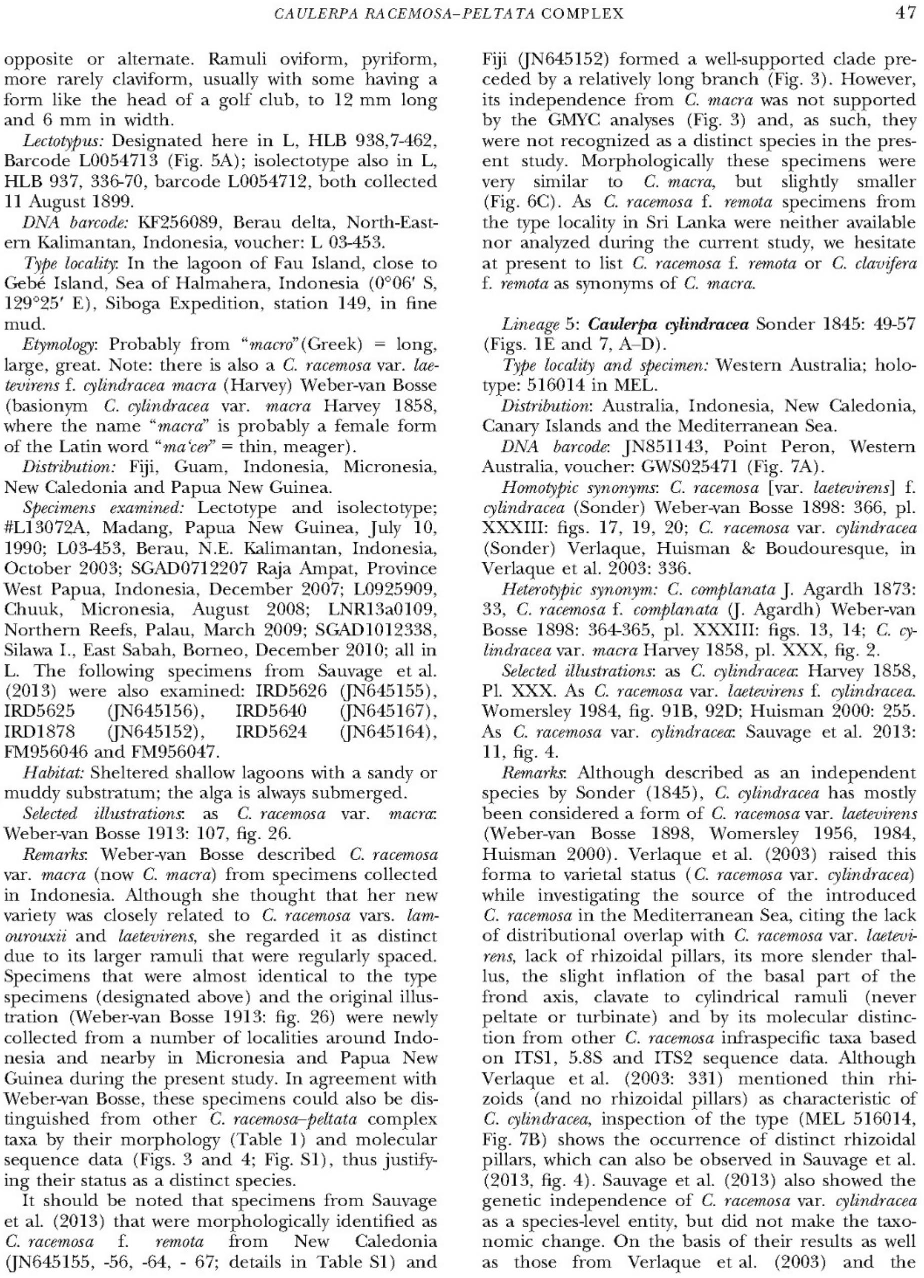
Selected illustrations: as *C. racemosa* var. *lamourouxii*: Weber-van Bosse 1898, Pl. 32, fig. 6; Coppejans and Prud'homme van Reine 1992: 694, fig. 15B; Littler and Littler 2003: 229.

Remarks: As mentioned under *C. lamourouxii* above, there was a second *Caulerpa* lineage that included specimens initially identified as *C. lamourouxii* morphotypes (Fig. 3, as *C. oligophylla*). As with *C. lamourouxii*, ramuli form and occurrence was highly variable in specimens from this lineage, with many specimens lacking ramuli completely (Fig. 5E). The most appropriate name we found for this lineage was *C. oligophylla*, a species described from Torres Strait, Australia, but currently regarded as a heterotypic synonym of *C. lamourouxii*. Indeed, newly collected specimens from close to the type locality strongly agree with the type specimen of *C. oligophylla* (compare Fig. 5, E and F). Unfortunately, we could not find any clear morphological characters to distinguish between *C. oligophylla* and smaller *C. lamourouxii* specimens and it is likely that the best means to distinguish these taxa is through DNA sequence data.

Lineage 4: ***Caulerpa macra*** (Weber-van Bosse) Draisma & Prud'homme **stat. nov.** (Fig. 6, A–C).

Basionym: C. racemosa var. *macra* Weber-van Bosse, in Liste des algues du Siboga, *Siboga-Expeditie* 59a, p. 106, fig. 26 (1913).

Updated description: Large *Caulerpa* with long thick stolons, these several decimeters long, 3–5 mm in width but often over 5 mm. Rhizoidal pillars well developed, arising from stolons at irregular distances, up to 10 cm long and several mm in diameter, often with thick terminal clumps of branched rhizoids. Erect assimilators up to 10 cm in height, with irregularly to regularly placed ramuli arranged distichously or radially and



opposite or alternate. Ramuli oviform, pyriform, more rarely claviform, usually with some having a form like the head of a golf club, to 12 mm long and 6 mm in width.

Lectotypus: Designated here in L, HLB 938,7-462, Barcode L0054713 (Fig. 5A); isolectotype also in L, HLB 937, 336-70, barcode L0054712, both collected 11 August 1899.

DNA barcode: KF256089, Berau delta, North-Eastern Kalimantan, Indonesia, voucher: L 03-453.

Type locality: In the lagoon of Fau Island, close to Gebé Island, Sea of Halmahera, Indonesia (0°06′ S, 129°25′ E), Siboga Expedition, station 149, in fine mud.

Etymology: Probably from "*macro*"(Greek) = long, large, great. Note: there is also a *C. racemosa* var. *laetevirens* f. *cylindracea macra* (Harvey) Weber-van Bosse (basionym *C. cylindracea* var. *macra* Harvey 1858, where the name "*macra*" is probably a female form of the Latin word "*ma'cer*" = thin, meager).

Distribution: Fiji, Guam, Indonesia, Micronesia, New Caledonia and Papua New Guinea.

Specimens examined: Lectotype and isolectotype; #L13072A, Madang, Papua New Guinea, July 10, 1990; L03-453, Berau, N.E. Kalimantan, Indonesia, October 2003; SGAD0712207 Raja Ampat, Province West Papua, Indonesia, December 2007; L0925909, Chuuk, Micronesia, August 2008; LNR13a0109, Northern Reefs, Palau, March 2009; SGAD1012338, Silawa I., East Sabah, Borneo, December 2010; all in L. The following specimens from Sauvage et al. (2013) were also examined: IRD5626 (JN645155), IRD5625 (JN645156), IRD5640 (JN645167), IRD1878 (JN645152), IRD5624 (JN645164), FM956046 and FM956047.

Habitat: Sheltered shallow lagoons with a sandy or muddy substratum; the alga is always submerged.

Selected illustrations: as *C. racemosa* var. *macra*: Weber-van Bosse 1913: 107, fig. 26.

Remarks: Weber-van Bosse described *C. racemosa* var. *macra* (now *C. macra*) from specimens collected in Indonesia. Although she thought that her new variety was closely related to *C. racemosa* vars. *lamourouxii* and *laetevirens*, she regarded it as distinct due to its larger ramuli that were regularly spaced. Specimens that were almost identical to the type specimens (designated above) and the original illustration (Weber-van Bosse 1913: fig. 26) were newly collected from a number of localities around Indonesia and nearby in Micronesia and Papua New Guinea during the present study. In agreement with Weber-van Bosse, these specimens could also be distinguished from other *C. racemosa–peltata* complex taxa by their morphology (Table 1) and molecular sequence data (Figs. 3 and 4; Fig. S1), thus justifying their status as a distinct species.

It should be noted that specimens from Sauvage et al. (2013) that were morphologically identified as *C. racemosa* f. *remota* from New Caledonia (JN645155, -56, -64, - 67; details in Table S1) and Fiji (JN645152) formed a well-supported clade preceded by a relatively long branch (Fig. 3). However, its independence from *C. macra* was not supported by the GMYC analyses (Fig. 3) and, as such, they were not recognized as a distinct species in the present study. Morphologically these specimens were very similar to *C. macra,* but slightly smaller (Fig. 6C). As *C. racemosa* f. *remota* specimens from the type locality in Sri Lanka were neither available nor analyzed during the current study, we hesitate at present to list *C. racemosa* f. *remota* or *C. clavifera* f. *remota* as synonyms of *C. macra.*

Lineage 5: ***Caulerpa cylindracea*** Sonder 1845: 49-57 (Figs. 1E and 7, A–D).

Type locality and specimen: Western Australia; holotype: 516014 in MEL.

Distribution: Australia, Indonesia, New Caledonia, Canary Islands and the Mediterranean Sea.

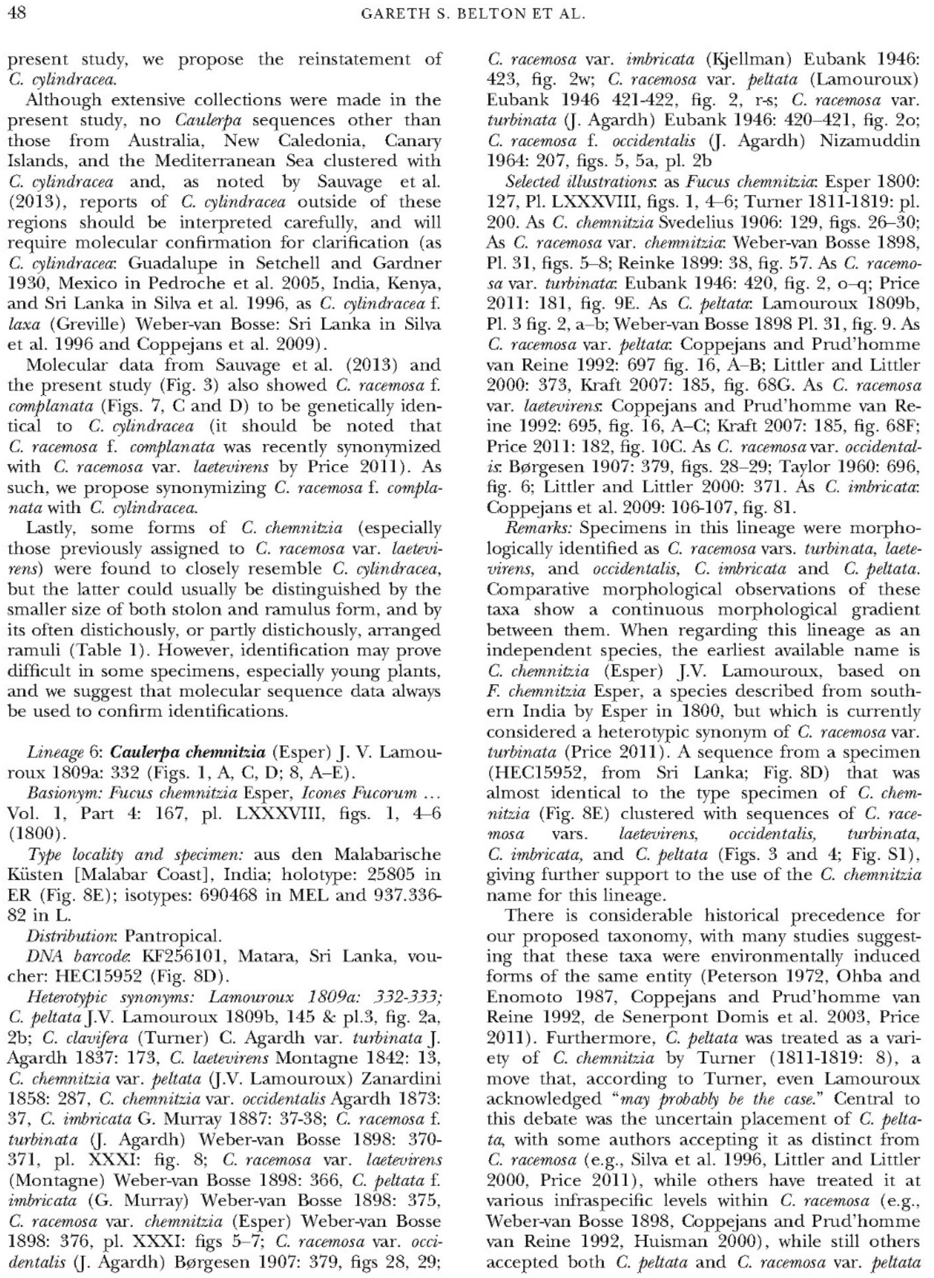
DNA barcode: JN851143, Point Peron, Western Australia, voucher: GWS025471 (Fig. 7A).

Homotypic synonyms: *C. racemosa* [var. *laetevirens*] f. *cylindracea* (Sonder) Weber-van Bosse 1898: 366, pl. XXXIII: figs. 17, 19, 20; *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque, in Verlaque et al. 2003: 336.

Heterotypic synonym: C. complanata J. Agardh 1873: 33, *C. racemosa* f. *complanata* (J. Agardh) Weber-van Bosse 1898: 364-365, pl. XXXIII: figs. 13, 14; *C. cylindracea* var. *macra* Harvey 1858, pl. XXX, fig. 2.

Selected illustrations: as *C. cylindracea*: Harvey 1858, Pl. XXX. As *C. racemosa* var. *laetevirens* f. *cylindracea.* Womersley 1984, fig. 91B, 92D; Huisman 2000: 255. As *C. racemosa* var. *cylindracea*: Sauvage et al. 2013: 11, fig. 4.

Remarks: Although described as an independent species by Sonder (1845), *C. cylindracea* has mostly been considered a form of *C. racemosa* var. *laetevirens* (Weber-van Bosse 1898, Womersley 1956, 1984, Huisman 2000). Verlaque et al. (2003) raised this forma to varietal status (*C. racemosa* var. *cylindracea*) while investigating the source of the introduced *C. racemosa* in the Mediterranean Sea, citing the lack of distributional overlap with *C. racemosa* var. *laetevirens,* lack of rhizoidal pillars, its more slender thallus, the slight inflation of the basal part of the frond axis, clavate to cylindrical ramuli (never peltate or turbinate) and by its molecular distinction from other *C. racemosa* infraspecific taxa based on ITS1, 5.8S and ITS2 sequence data. Although Verlaque et al. (2003: 331) mentioned thin rhizoids (and no rhizoidal pillars) as characteristic of *C. cylindracea,* inspection of the type (MEL 516014, Fig. 7B) shows the occurrence of distinct rhizoidal pillars, which can also be observed in Sauvage et al. (2013, fig. 4). Sauvage et al. (2013) also showed the genetic independence of *C. racemosa* var. *cylindracea* as a species-level entity, but did not make the taxonomic change. On the basis of their results as well as those from Verlaque et al. (2003) and the



present study, we propose the reinstatement of *C. cylindracea.*

Although extensive collections were made in the present study, no *Caulerpa* sequences other than those from Australia, New Caledonia, Canary Islands, and the Mediterranean Sea clustered with *C. cylindracea* and, as noted by Sauvage et al. (2013), reports of *C. cylindracea* outside of these regions should be interpreted carefully, and will require molecular confirmation for clarification (as *C. cylindracea*: Guadalupe in Setchell and Gardner 1930, Mexico in Pedroche et al. 2005, India, Kenya, and Sri Lanka in Silva et al. 1996, as *C. cylindracea* f. *laxa* (Greville) Weber-van Bosse: Sri Lanka in Silva et al. 1996 and Coppejans et al. 2009).

Molecular data from Sauvage et al. (2013) and the present study (Fig. 3) also showed *C. racemosa* f. *complanata* (Figs. 7, C and D) to be genetically identical to *C. cylindracea* (it should be noted that *C. racemosa* f. *complanata* was recently synonymized with *C. racemosa* var. *laetevirens* by Price 2011). As such, we propose synonymizing *C. racemosa* f. *complanata* with *C. cylindracea.*

Lastly, some forms of *C. chemnitzia* (especially those previously assigned to *C. racemosa* var. *laetevirens*) were found to closely resemble *C. cylindracea*, but the latter could usually be distinguished by the smaller size of both stolon and ramulus form, and by its often distichously, or partly distichously, arranged ramuli (Table 1). However, identification may prove difficult in some specimens, especially young plants, and we suggest that molecular sequence data always be used to confirm identifications.

Lineage 6: ***Caulerpa chemnitzia*** (Esper) J. V. Lamouroux 1809a: 332 (Figs. 1, A, C, D; 8, A–E).

Basionym: Fucus chemnitzia Esper, *Icones Fucorum …* Vol. 1, Part 4: 167, pl. LXXXVIII, figs. 1, 4–6 (1800).

Type locality and specimen: aus den Malabarische Küsten [Malabar Coast], India; holotype: 25805 in ER (Fig. 8E); isotypes: 690468 in MEL and 937.336-82 in L.

Distribution: Pantropical.

DNA barcode: KF256101, Matara, Sri Lanka, voucher: HEC15952 (Fig. 8D).

Heterotypic synonyms: Lamouroux 1809a: 332-333; C. peltata J.V. Lamouroux 1809b, 145 & pl.3, fig. 2a, 2b; *C. clavifera* (Turner) C. Agardh var. *turbinata* J. Agardh 1837: 173, *C. laetevirens* Montagne 1842: 13, *C. chemnitzia* var. *peltata* (J.V. Lamouroux) Zanardini 1858: 287, *C. chemnitzia* var. *occidentalis* Agardh 1873: 37, *C. imbricata* G. Murray 1887: 37-38; *C. racemosa* f. *turbinata* (J. Agardh) Weber-van Bosse 1898: 370-371, pl. XXXI: fig. 8; *C. racemosa* var. *laetevirens* (Montagne) Weber-van Bosse 1898: 366, *C. peltata* f. *imbricata* (G. Murray) Weber-van Bosse 1898: 375, *C. racemosa* var. *chemnitzia* (Esper) Weber-van Bosse 1898: 376, pl. XXXI: figs 5–7; *C. racemosa* var. *occidentalis* (J. Agardh) Børgesen 1907: 379, figs 28, 29; *C. racemosa* var. *imbricata* (Kjellman) Eubank 1946: 423, fig. 2w; *C. racemosa* var. *peltata* (Lamouroux) Eubank 1946 421-422, fig. 2, r-s; *C. racemosa* var. *turbinata* (J. Agardh) Eubank 1946: 420–421, fig. 2o; *C. racemosa* f. *occidentalis* (J. Agardh) Nizamuddin 1964: 207, figs. 5, 5a, pl. 2b

Selected illustrations: as *Fucus chemnitzia*: Esper 1800: 127, Pl. LXXXVIII, figs. 1, 4–6; Turner 1811-1819: pl. 200. As *C. chemnitzia* Svedelius 1906: 129, figs. 26–30; As *C. racemosa* var. *chemnitzia*: Weber-van Bosse 1898, Pl. 31, figs. 5–8; Reinke 1899: 38, fig. 57. As *C. racemosa* var. *turbinata*: Eubank 1946: 420, fig. 2, o–q; Price 2011: 181, fig. 9E. As *C. peltata*: Lamouroux 1809b, Pl. 3 fig. 2, a–b; Weber-van Bosse 1898 Pl. 31, fig. 9. As *C. racemosa* var. *peltata*: Coppejans and Prud'homme van Reine 1992: 697 fig. 16, A–B; Littler and Littler 2000: 373, Kraft 2007: 185, fig. 68G. As *C. racemosa* var. *laetevirens*: Coppejans and Prud'homme van Reine 1992: 695, fig. 16, A–C; Kraft 2007: 185, fig. 68F; Price 2011: 182, fig. 10C. As *C. racemosa* var. *occidentalis*: Børgesen 1907: 379, figs. 28–29; Taylor 1960: 696, fig. 6; Littler and Littler 2000: 371. As *C. imbricata*: Coppejans et al. 2009: 106-107, fig. 81.

Remarks: Specimens in this lineage were morphologically identified as *C. racemosa* vars. *turbinata, laetevirens,* and *occidentalis, C. imbricata* and *C. peltata.* Comparative morphological observations of these taxa show a continuous morphological gradient between them. When regarding this lineage as an independent species, the earliest available name is *C. chemnitzia* (Esper) J.V. Lamouroux, based on *F. chemnitzia* Esper, a species described from southern India by Esper in 1800, but which is currently considered a heterotypic synonym of *C. racemosa* var. *turbinata* (Price 2011). A sequence from a specimen (HEC15952, from Sri Lanka; Fig. 8D) that was almost identical to the type specimen of *C. chemnitzia* (Fig. 8E) clustered with sequences of *C. racemosa* vars. *laetevirens, occidentalis, turbinata, C. imbricata,* and *C. peltata* (Figs. 3 and 4; Fig. S1), giving further support to the use of the *C. chemnitzia* name for this lineage.

There is considerable historical precedence for our proposed taxonomy, with many studies suggesting that these taxa were environmentally induced forms of the same entity (Peterson 1972, Ohba and Enomoto 1987, Coppejans and Prud'homme van Reine 1992, de Senerpont Domis et al. 2003, Price 2011). Furthermore, *C. peltata* was treated as a variety of *C. chemnitzia* by Turner (1811-1819: 8), a move that, according to Turner, even Lamouroux acknowledged "*may probably be the case.*" Central to this debate was the uncertain placement of *C. peltata,* with some authors accepting it as distinct from *C. racemosa* (e.g., Silva et al. 1996, Littler and Littler 2000, Price 2011), while others have treated it at various infraspecific levels within *C. racemosa* (e.g., Weber-van Bosse 1898, Coppejans and Prud'homme van Reine 1992, Huisman 2000), while still others accepted both *C. peltata* and *C. racemosa* var. *peltata*

Eubank (Kraft 2007). This debate is further confounded by the presumed loss of the *C. peltata* type specimen, and thus our concept of the species is by necessity based on Lamouroux's (1809b) illustrations (Fig. 2B) and description (Lamouroux 1809a). Although it has been suggested that Lamouroux wrongly interpreted the stolon branches as erect axes (Price 2011: 179), our observations of material from the same region as the probable type locality (Antilles Islands) suggest that Lamouroux was indeed depicting erect assimilators bearing radially arranged peltate ramuli.

However, clarifying the concept of *C. peltata* has minimal impact on establishing an accurate taxonomy, as by far the greatest confusion stems from the occurrence of peltate ramuli in three other species within the *C. racemosa–peltata* complex (viz. *C. macrodisca*, *C. megadisca* sp. nov. and *C. nummularia*). Fortunately, these taxa can be distinguished from *C. chemnitzia* by their much larger peltate disks (*C. macrodisca* and *C. megadisca*) and branching pattern (*C. nummularia*). Furthermore, *C. chemnitzia* is pantropically distributed, whereas *C. macrodisca*, *C. megadisca*, and *C. nummularia* appear to be restricted to the central Indo-Pacific region.

It should be noted that there remains some confusion over the type locality of *C. peltata*, as although Lamouroux (1809a) gave "*Antilles*" as its (type) locality, in the paper where *C. peltata* is illustrated (1809b), he did not name any locality, only "*ded. Thuillier.*" Later, Lamouroux (1813) cited "*Oris Amer. Merid. Orient.*" and in 1823 he cited "*côtes occidentales de l'Afrique.*" Until the type specimen is located, we suggest that the eldest listed locality, the Antilles Islands, should be regarded as the probable type locality of *C. peltata*.

Interestingly, our *C. chemnitzia* sensu stricto specimen (HEC 15952) would have been identified as *C. imbricata* based on the description of Coppejans et al. (2009). However, on the basis of observations of a large number of specimens including type specimens, we concluded that *C. chemnitzia* and *C. imbricata* were the same entity. Furthermore, Coppejans et al. (2009) also suggested that *C. imbricata* specimens from Sri Lanka closely resembled *C. chemnitzia* (as *C. racemosa* var. *chemnitzia* (Esper) Weber-van Bosse. As such, we are confident of listing *C. imbricata* as a heterotypic synonym of *C. chemnitzia*.

Lastly, it should be noted that there were three *C. chemnitzia* lineages supported as distinct species in the GMYC analyses of the *tuf*A data set. However, as these lineages had poor BP and PP support and their relationships varied between the *tuf*A and *rbc*L/concatenated data sets, we do not recognize them as separate taxa, but suggest that further work be undertaken to better resolve and understand these relationships.

Lineage 7: ***Caulerpa nummularia*** Harvey ex. J. Agardh 1873: 38 (Figs. 1F and 9, A and B).

Type locality: Friendly Islands, Tonga; Nukahiva, Marquesas Islands.

Syntypes: Herb. Agardh 16809, 16811 (in part), in LD; BM; 287054, in NSW; DSC01032, in TCD. *Note*: as type material was never designated by J. Agardh, the specimen marked 77 Friendly Isl. *C. nummularia* in TCD (TCD 0011105) is designated here as the lectotype (reproduced in Fig. 9B).

Distribution: Tropical Pacific.

DNA barcode: JN817685, Heron Island, Great Barrier Reef, Australia, voucher: AD-A91369 (Figs. 1F and 9A).

Heterotypic synonyms: *C. peltata* var. *exigua* Weber-van Bosse 1898: 377, pl. XXXI, fig. 11 (Fig. S6B); *C. peltata* var. *stellata* (Harvey ex J. Agardh) Weber-van Bosse 1898: 377 (Fig. S6C).

Selected illustrations: as *C. nummularia*: Abbott and Huisman 2000: 121, fig. 44A. As *C. peltata* var. *exigua*: Weber-van Bosse 1898, Pl. 31 fig. 11; Littler and Littler 2003: 225, Kraft 2007: 173, fig. 64, D–E.

Remarks: Many authors have overlooked *C. nummularia*, treating it as a heterotypic synonym of *C. peltata* (Coppejans and Prud'homme van Reine 1992, Price 2011). However, as mentioned previously, the illustration of *C. peltata* by Lamouroux (1809b) clearly depicts a specimen with erect assimilators bearing radially arranged peltate ramuli. This morphology differs greatly from the original diagnosis of *C. nummularia* by J. Agardh (1873) who, although considering *C. nummularia* to be closely related to *C. peltata*, described the ramuli as shield like, 4–5 mm in diameter with crenulated margins and peltate branches arising from the margin of the ramulus below. The distinction between *C. nummularia* and *C. peltata*, as well as other *C. racemosa–peltata* taxa, is supported by our molecular data including sequences from close to the type locality (KF256098, Fiji), and thus *C. nummularia* warrants recognition as an independent species, a view also held by Cribb and Cribb (1985), South and Skelton (2003), Abbott and Huisman (2004), Hodgson et al. (2004), N'Yeurt and Payri (2004), and Skelton and South (2007). Many of these authors distinguished *C. nummularia* from *C. peltata* var. *exigua* (Fig. S6B), a variety originally described from Samoa and the Cook Islands, by secondary ramuli arising from the center of the disk of subtending ramuli in the latter. However, Abbott and Huisman (2004), as well as Kraft (2007), when commenting on *C. nummularia* and *C. peltata* var. *exigua* respectively, noted that secondary peltate ramuli arose either from the margin or from the center of the ramulus below, thus also removing the distinction between the *C. peltata* varieties *exigua* and *stellata*. This casts some doubt on the independence of *C. peltata* var. *exigua* and *C. peltata* var. *stellata* from *C. nummularia*, a view also shared by Price (2011). On the basis of this, the close proximity of their type localities and the variability observed in material from various regions, we synonymize both *C. pel-*

tata var. *exigua* and *C. peltata* var. *stellata* with *C. nummularia*.

Lineage 8: ***Caulerpa megadisca*** Belton & Gurgel **sp. nov.** (Fig. 9E).

Diagnosis: Thallus medium to dark green, low growing, spreading laterally to 50 cm; stolon smooth, 1–2 mm in diameter, sparsely branched; rhizoidal system well developed with rhizoidal pillars, 1.5–8 cm long, 0.8–1.5 mm wide, branching to slender apices; individual assimilators consisting of a single peltate disk attached to stolon by a terete stalk, 3–6 mm long, 0.5–1.0 mm wide, expanding abruptly into a circular, flat disk; disk fleshy to thin (8) 10–15 (20) mm wide, margin of disk entire.

Holotypus: AD-A90107a, in AD. Collected by C.F.D. Gurgel and R.R.M. Dixon, February 17, 2009 at 11 m depth from Lizard Island, Australia (Fig. 9E).

Holotype DNA barcode: JN817657 (collection details as above).

Type locality: North Direction Island, Lizard Island group, Queensland, Australia.

Etymology: This species is named after the large peltate disks born directly from the stolon.

Distribution: northeastern Australia, New Caledonia and Fiji, but most likely also in the Indonesian region (based on various samples in L).

Specimens examined: AD-A90107 b-d, North Direction Island, Lizard Island group, Queensland, Australia, at 11 m depth, collected by C.F.D. Gurgel and R.R.M. Dixon, February 17, 2009; AD-A92609, "Bommie Bay," North-side of Lizard Island, Queensland, Australia, at 12 m depth, collected by G.S. Belton and M.H.K. Marklund, September 07, 2010; DML40342, Yaukuvelailai Island, Fiji. Collected by M.M. Littler, D.M. Littler and B.L. Brooks, March 09, 1996.

Habitat: Plants were found growing over sandy substrata and coral rubble at 5–12 m depth.

Selected illustrations: as *C. peltata*: Kraft 2007: 173, fig. 64A.

Remarks: C. megadisca can be distinguished from *C. macrodisca* by its single large peltate disk assimilator and much thinner stolon (Fig. 9, C and E; Table 1). Based on these morphological observations, as well as molecular data that distinguished *C. megadisca* from *C. macrodisca* and other *Caulerpa* species (Figs. 3 and 4), its status as a distinct species is fully warranted.

Lineage 9: ***Caulerpa macrodisca*** Decaisne 1842: 336 (Fig. 9, C and D).

Type locality and specimen: Îles Anambas (Anambas Islands), Indonesia; lectotype illustration: Decaisne [1846 (1846-1864), pl. 1, fig. 1], designated by Price (2011, p. 190).

Distribution: Central Indo-Pacific.

DNA barcode: FM956053, Thousand Islands, Java Sea, Indonesia, voucher: L 0509359 (Fig. 9C).

Homotypic synonyms: C. racemosa (Forsskål) J. Agardh var. *macrodisca* (Decaisne) Weber-van Bosse 1898: 376, pl. XXXI, fig. 10. *C. peltata* J.V. Lamouroux var. *macrodisca* (Decaisne) Weber-van Bosse 1898: 376, XXVIII.

Selected illustrations: as *C. macrodisca:* Decaisne 1846-1864; Pl. 1. fig. 1; Reinke 1899: fig. 61. As *C. racemosa* ecad *peltata-macrodisca*: Coppejans and Prud'homme van Reine 1992: 697, fig. 17C.

Remarks: As *C. macrodisca* could be easily identified and distinguished from other *C. racemosa–peltata* complex taxa by molecular data and its large peltate ramuli arranged around an upright axis, its recognition as an independent species is fully justified. Most of our specimens (Fig. 9C) closely resembled the lectotype illustration (Fig. 9D); however, there was some variation, with the ramuli of some specimens (AD-A88056) having a gradual widening toward the peltate disk and others (PERTH 08292663) being slightly mushroom-like. This latter morphology is in agreement with a remark by Weber-van Bosse (1898, p. 253) who noted that after only a few minutes outside the water, the fresh ramuli become flaccid and these ramuli take different flattened forms. However, in comparison to *C. chemnitzia* specimens, the widening was always quite abrupt and the disk (including those that were slightly mushroom shaped) was always larger, 6–10 mm in diameter.

Lineage 11: ***Caulerpa racemosa*** (Forrskål) J. Agardh 1873: 35-36 (Figs. 1A and 10, A–C)

Basionym: *Fucus racemosus* Forsskål 1775: 191.

Type locality and specimen: Suez, Egypt; Herb. Forsskål No. 845 at C.

Distribution: Pantropical.

DNA barcode: JN817665, Kimberley, northwestern Australia, voucher: PERTH 08292728 (Fig. 10A).

Heterotypic synonyms: *Fucus clavifer* Turner 1807-1808: 126-127, pl. 57 *nom. illeg.* (*Fucus clavifer* Turner is a later homonym of *Fucus clavifer* Forsskål 1775, currently *Laurencia uvifera* (Forsskål) Børgesen); *Fucus uvifer* Turner 1817: 81-82, pl. 230, nom. illeg. (*Fucus uvifer* Turner is a later homonym of *Fucus uvifer* Forsskål 1775); *C. uvifera* C. Agardh 1817: xxiii; *Chauvinia clavifera* (C. Agardh) Bory de Saint-Vincent 1826–1829: 207, ; *Chauvinia clavifera* var. *uvifera* (C. Agardh) Kützing 1849, *C. racemosa* var. *clavifera* (C. Agardh) Weber-van Bosse 1898: 361-362, pl. XXXIII: figs. 1–3; *C. racemosa* var. *mucronata* L.N. de Senerpont Domis in de Senerpont Domis et al. 2003: 1035, fig. 2.

Selected illustrations: as *C. racemosa*: Littler and Littler 2003: 227, Price 2011: 176, fig. 8B. As *Fucus uvifer*: Turner 1811-1819: Pl. 230. As *C. uvifera*: Svedelius 1906: 122, fig. 15. As *C. racemosa* var. *clavifera*: Weber-van Bosse 1898, Pl. 33, figs. 1, 4–5.

Remarks: Although it has been widely accepted that *C. racemosa* exhibits considerable morphological variation, the majority of specimens from this lineage in the present study were comparatively uniform and could usually be distinguished from other *C. racemosa–peltata* taxa by their more or less pyriform ramuli that abruptly expanded above a short, stalk-like proximal region whose length was not greater than the diameter of the distal region. However, our results show *C. racemosa* var. *mucronata* (Fig. 10C) to cluster with *C. racemosa* specimens in both the *tuf*A and *rbc*L trees (Figs. 3 and 4) proving that *C. racemosa* can display quite substantial morphological variation, supporting the findings of Peterson (1972), Calvert (1976) and de Senerpont Domis et al. (2003).

We could not detect any clear morphological or genetic boundary between *C. racemosa* and specimens identified as *C. racemosa* var. *macrophysa*. It is therefore quite likely that *C. racemosa* var. *macrophysa* should be synonymized with *C. racemosa*. Unfortunately, the type specimen of *C. racemosa* var. *macrophysa* is presumed lost and no physical comparisons with the type specimen of *C. racemosa* could be made. However, the illustration of *C. racemosa* var. *macrophysa* (as *Chauvinia macrophysa* Sonder ex Kützing) by Kützing 1857: Tab. 15, fig. II; reproduced as our Fig. 10D) was detailed, clear, and accurate enough to help distinguish this morphotype and secure a correct identification of the specimens herein assigned to this taxon (e.g., Fig. 10B). But as we were unable to obtain sequences from the Pacific coast of Central America (= type locality), we suggest further work be undertaken before this synonymy is adopted.

Observations of the type material of *C. racemosa* var. *uvifera* (as *Fucus uvifer* Turner *nom. illeg.* BM000569472, Fig. S7 in the Supporting Information) and *C. racemosa* var. *clavifera* Weber-van Bosse (as *Fucus clavifer* Turner BM000569148, Fig. S8 in the Supporting Information), both originally described from the Red Sea (also the type locality for *C. racemosa*) and currently regarded as synonyms of *C. racemosa*, led us to conclude that their synonymy with *C. racemosa*, as proposed by Papenfuss and Egerod (1957), was most likely correct.

We thank G. Saunders and K. Dixon for many specimens and sequences used in this study, and for valuable comments on earlier versions of the manuscript; T. Sauvage for providing access to vouchers as well as many valuable discussions; E. Coppejans, H. Verbruggen, B. Brooks, D. Littler, M. Littler, C. Schneider, T. Popolizio, C. Lane, E. Verheij, R. Dixon, G. Kraft, L. Kraft, and G. Filloramo for help with specimen collections, providing samples and/or sequences; S. D'hondt, M. Marklund, D. McDevit and T. Moore generated some of the molecular data. The (Schure) Beijerinck Popping Fund and the TREUB maatschappij, the Royal Dutch Academy of Sciences, provided research funds to WFPVR and SGAD. Micronesia Conservation Trust to WFPVR. JMH acknowledges fieldwork support by Woodside Energy Ltd and the Western Australian Museum. The present research was funded by the Australian Research Council (LP0991083) to CFDG, the Australian Census of Coral Reef Life to GSB and CFDG, the Australian Biological Resources Study to CFDG and JMH; Canadian Barcode of Life Network from Genome Canada through the Ontario Genomics Institute, the Natural Sciences and Engineering Research Council of Canada, and other sponsors listed at www.BOLNET.ca to G. Saunders. Additional support was provided by the Canada Research Chair Program as well as infrastructure support from the Canada Foundation for Innovation and New Brunswick Innovation, Alinytjara Wilurara Natural Resources Management Board, Wildlife Conservation Fund (2009/10 #1458), the State Herbarium of SA, the WA Herbarium and the Sir Mark Mitchell Foundation. Finally, we acknowledge the assistance of staff at the British Museum (BM), Leiden (L), Lund (LD), National Herbarium of Victoria (MEL), Muséum National d'Histoire Naturelle (PC), and Trinity College Dublin (TCD).

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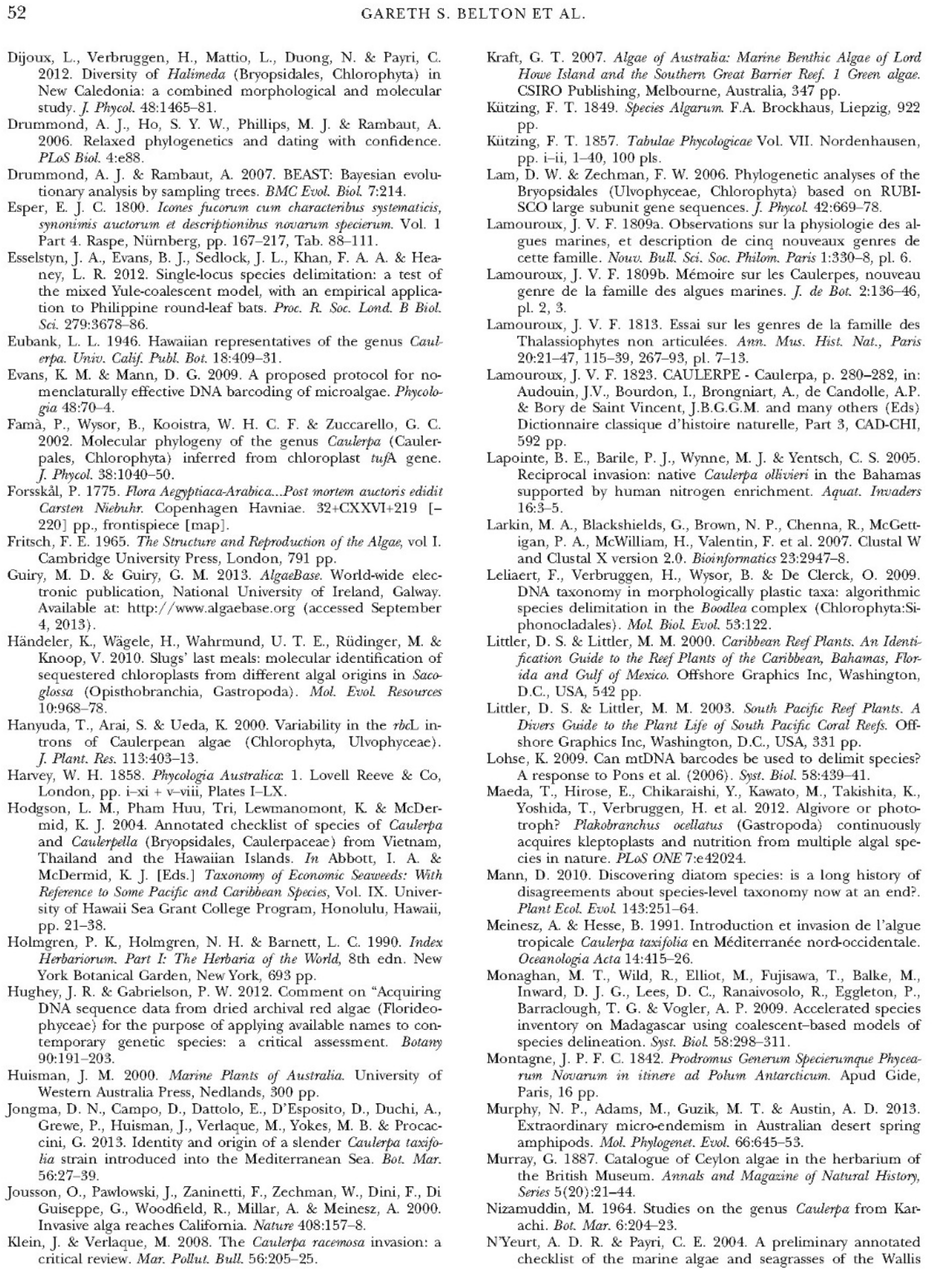

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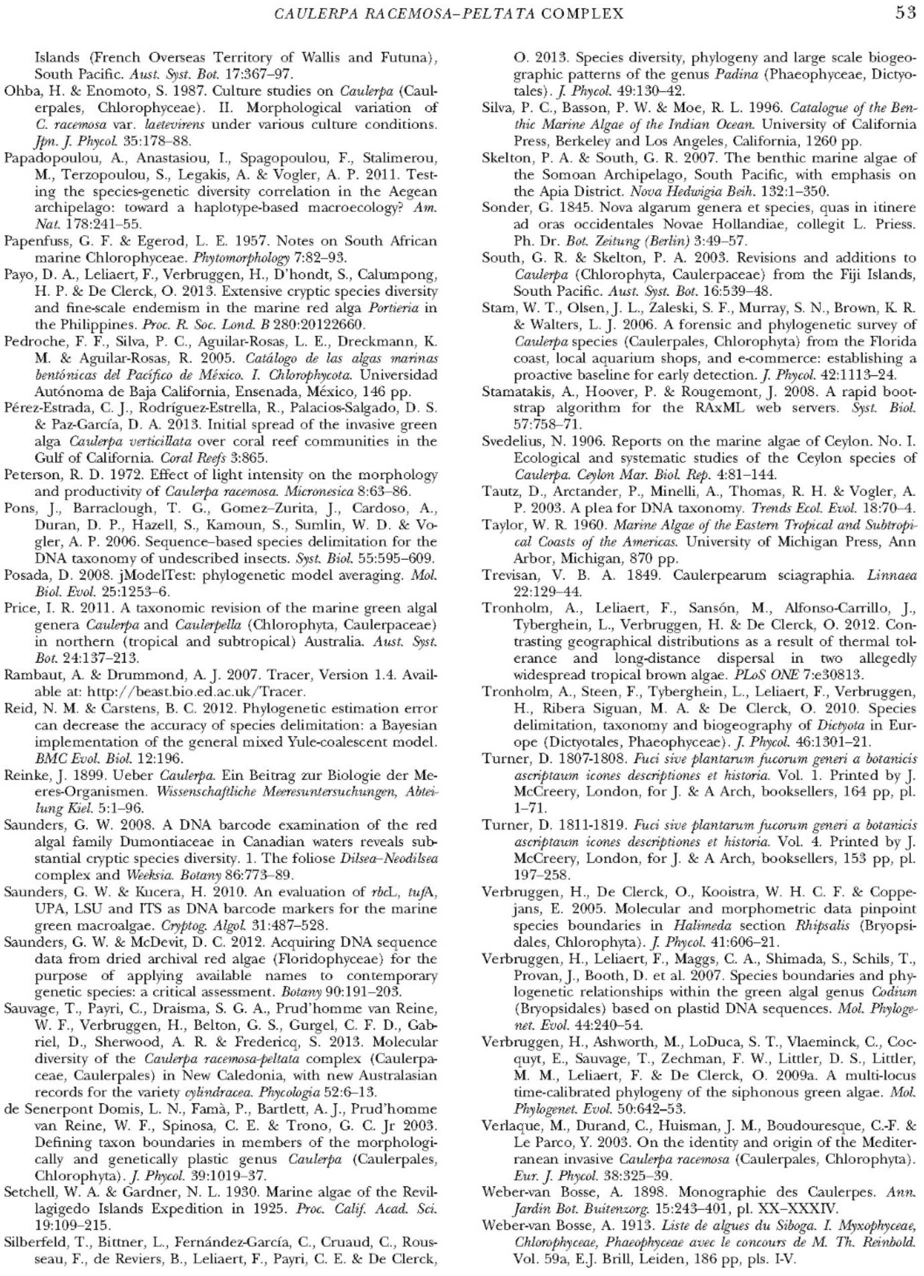

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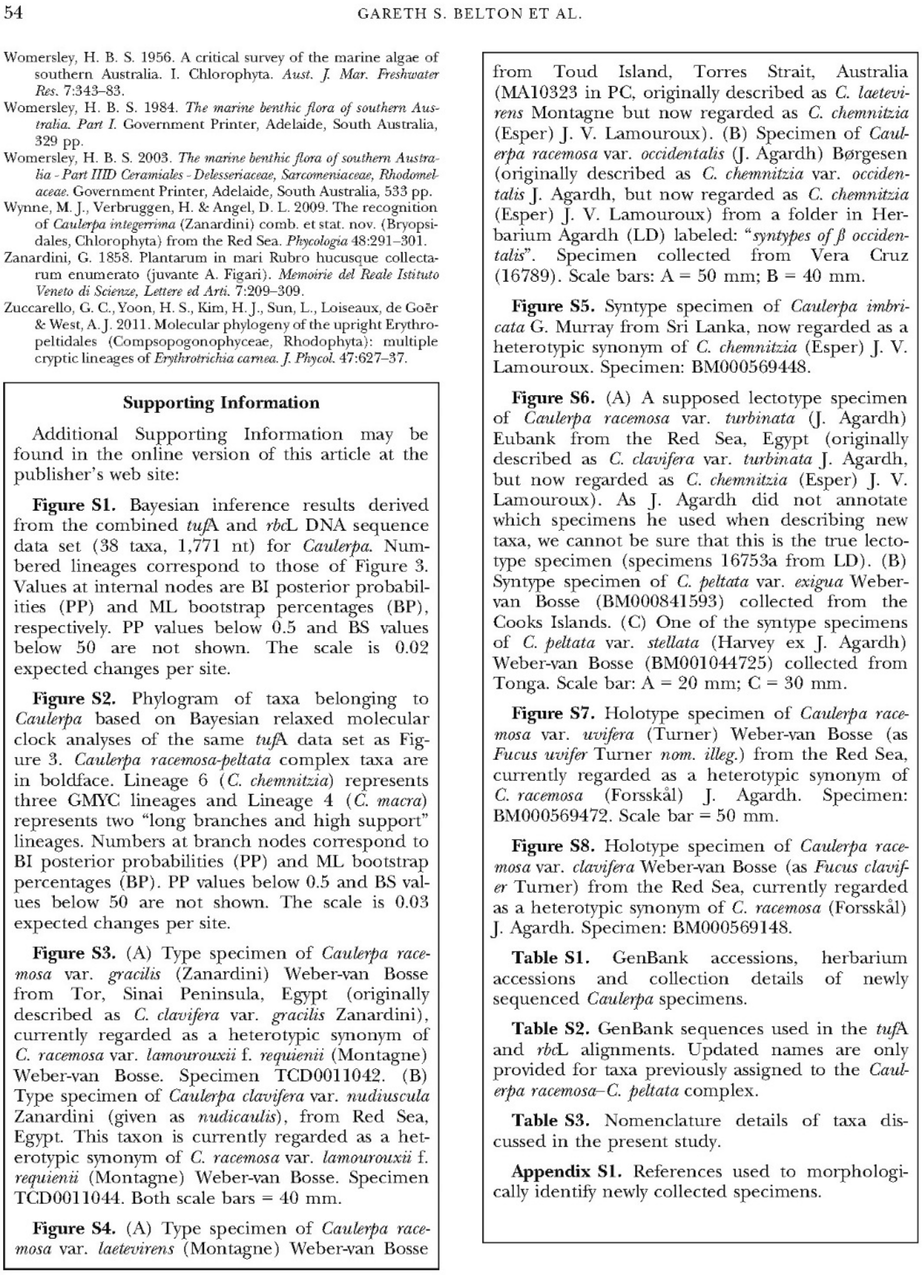

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Bayesian inference results derived from the combined *tuf*A and *rbc*L DNA sequence data set (38 taxa, 1,771 nt) for *Caulerpa*. Numbered lineages correspond to those of Figure 3. Values at internal nodes are BI posterior probabilities (PP) and ML bootstrap percentages (BP), respectively. PP values below 0.5 and BS values below 50 are not shown. The scale is 0.02 expected changes per site.

Figure S2. Phylogram of taxa belonging to *Caulerpa* based on Bayesian relaxed molecular clock analyses of the same *tuf*A data set as Figure 3. *Caulerpa racemosa-peltata* complex taxa are in boldface. Lineage 6 (*C. chemnitzia*) represents three GMYC lineages and Lineage 4 (*C. macra*) represents two "long branches and high support" lineages. Numbers at branch nodes correspond to BI posterior probabilities (PP) and ML bootstrap percentages (BP). PP values below 0.5 and BS values below 50 are not shown. The scale is 0.03 expected changes per site.

Figure S3. (A) Type specimen of *Caulerpa racemosa* var. *gracilis* (Zanardini) Weber-van Bosse from Tor, Sinai Peninsula, Egypt (originally described as *C. clavifera* var. *gracilis* Zanardini), currently regarded as a heterotypic synonym of *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse. Specimen TCD0011042. (B) Type specimen of *Caulerpa clavifera* var. *nudiuscula* Zanardini (given as *nudicaulis*), from Red Sea, Egypt. This taxon is currently regarded as a heterotypic synonym of *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse. Specimen TCD0011044. Both scale bars = 40 mm.

Figure S4. (A) Type specimen of *Caulerpa racemosa* var. *laetevirens* (Montagne) Weber-van Bosse from Toud Island, Torres Strait, Australia (MA10323 in PC, originally described as *C. laetevirens* Montagne but now regarded as *C. chemnitzia* (Esper) J. V. Lamouroux). (B) Specimen of *Caulerpa racemosa* var. *occidentalis* (J. Agardh) Børgesen (originally described as *C. chemnitzia* var. *occidentalis* J. Agardh, but now regarded as *C. chemnitzia* (Esper) J. V. Lamouroux) from a folder in Herbarium Agardh (LD) labeled: "*syntypes of β occidentalis*". Specimen collected from Vera Cruz (16789). Scale bars: A = 50 mm; B = 40 mm.

Figure S5. Syntype specimen of *Caulerpa imbricata* G. Murray from Sri Lanka, now regarded as a heterotypic synonym of *C. chemnitzia* (Esper) J. V. Lamouroux. Specimen: BM000569448.

Figure S6. (A) A supposed lectotype specimen of *Caulerpa racemosa* var. *turbinata* (J. Agardh) Eubank from the Red Sea, Egypt (originally described as *C. clavifera* var. *turbinata* J. Agardh, but now regarded as *C. chemnitzia* (Esper) J. V. Lamouroux). As J. Agardh did not annotate which specimens he used when describing new taxa, we cannot be sure that this is the true lectotype specimen (specimens 16753a from LD). (B) Syntype specimen of *C. peltata* var. *exigua* Weber-van Bosse (BM000841593) collected from the Cooks Islands. (C) One of the syntype specimens of *C. peltata* var. *stellata* (Harvey ex J. Agardh) Weber-van Bosse (BM001044725) collected from Tonga. Scale bar: A = 20 mm; C = 30 mm.

Figure S7. Holotype specimen of *Caulerpa racemosa* var. *uvifera* (Turner) Weber-van Bosse (as *Fucus uvifer* Turner *nom. illeg.*) from the Red Sea, currently regarded as a heterotypic synonym of *C. racemosa* (Forsskål) J. Agardh. Specimen: BM000569472. Scale bar = 50 mm.

Figure S8. Holotype specimen of *Caulerpa racemosa* var. *clavifera* Weber-van Bosse (as *Fucus clavifer* Turner) from the Red Sea, currently regarded as a heterotypic synonym of *C. racemosa* (Forsskål) J. Agardh. Specimen: BM000569148.

Table S1. GenBank accessions, herbarium accessions and collection details of newly sequenced *Caulerpa* specimens.

Table S2. GenBank sequences used in the *tuf*A and *rbc*L alignments. Updated names are only provided for taxa previously assigned to the *Caulerpa racemosa–C. peltata* complex.

Table S3. Nomenclature details of taxa discussed in the present study.

Appendix S1. References used to morphologically identify newly collected specimens.

Chapter 3: Caulerpaceae.

Statement of Authorship

Caulerpaceae. In Huisman, J. M. ed. *Algae of Australia: Marine Benthic Algae of North-western Australia. 1. Green and Brown Algae*. CSIRO Publishing / Australian Biological Resources Study.

Belton, G. (Candidate)
Contributed to initial manuscript conceptualization and drafting, collected specimens, generated and analyzed data, commented on and edited subsequent manuscript drafts, prepared figures and tables.

I hereby certify that the statement of contribution is accurate.

Signed… [redacted] ………………………Date…………………………

Huisman, J. M.
Contributed to initial manuscript conceptualization and drafting, collected specimens, generated data, co-ordinated addressing of reviewers comments, commented on and edited subsequent manuscript drafts.

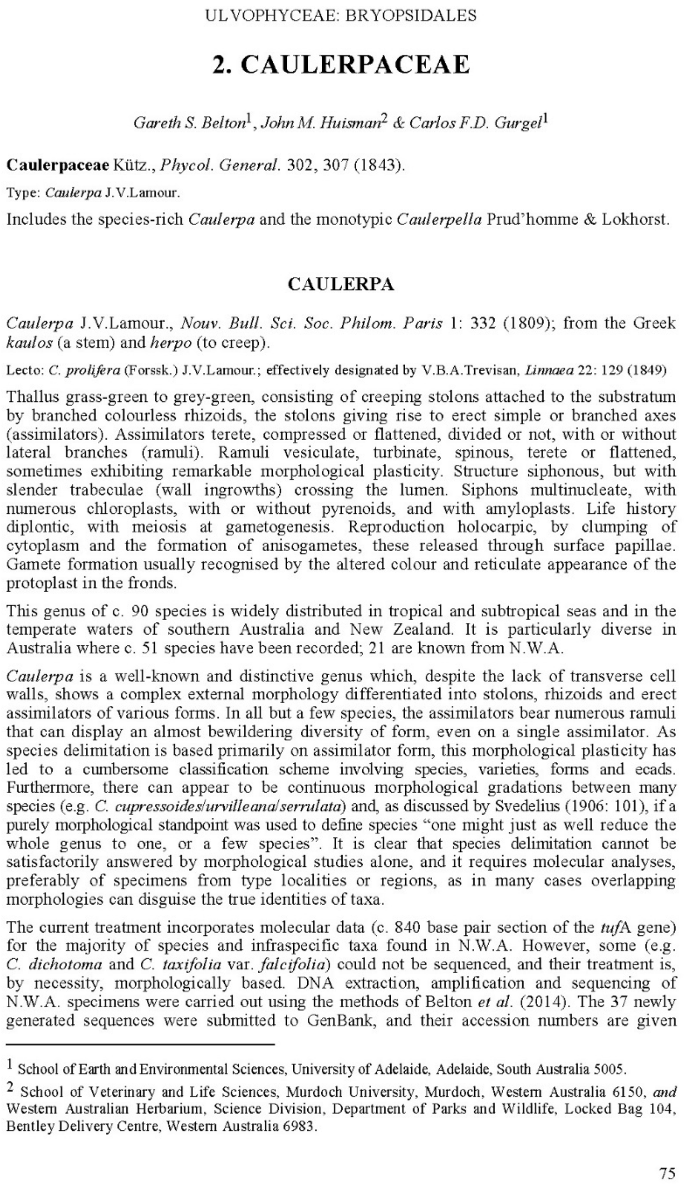
I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

Signed….. ……………Date…………………………

Gurgel, C. F. D.
Collected specimens, generated data, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

Signed.. …………………Date…………………………



ULVOPHYCEAE: BRYOPSIDALES

2. CAULERPACEAE

Gareth S. Belton[1], *John M. Huisman*[2] *& Carlos F.D. Gurgel*[1]

Caulerpaceae Kütz., *Phycol. General.* 302, 307 (1843).

Type: *Caulerpa* J.V.Lamour.

Includes the species-rich *Caulerpa* and the monotypic *Caulerpella* Prud'homme & Lokhorst.

CAULERPA

Caulerpa J.V.Lamour., *Nouv. Bull. Sci. Soc. Philom. Paris* 1: 332 (1809); from the Greek *kaulos* (a stem) and *herpo* (to creep).

Lecto: *C. prolifera* (Forssk.) J.V.Lamour.; effectively designated by V.B.A.Trevisan, *Linnaea* 22: 129 (1849)

Thallus grass-green to grey-green, consisting of creeping stolons attached to the substratum by branched colourless rhizoids, the stolons giving rise to erect simple or branched axes (assimilators). Assimilators terete, compressed or flattened, divided or not, with or without lateral branches (ramuli). Ramuli vesiculate, turbinate, spinous, terete or flattened, sometimes exhibiting remarkable morphological plasticity. Structure siphonous, but with slender trabeculae (wall ingrowths) crossing the lumen. Siphons multinucleate, with numerous chloroplasts, with or without pyrenoids, and with amyloplasts. Life history diplontic, with meiosis at gametogenesis. Reproduction holocarpic, by clumping of cytoplasm and the formation of anisogametes, these released through surface papillae. Gamete formation usually recognised by the altered colour and reticulate appearance of the protoplast in the fronds.

This genus of c. 90 species is widely distributed in tropical and subtropical seas and in the temperate waters of southern Australia and New Zealand. It is particularly diverse in Australia where c. 51 species have been recorded; 21 are known from N.W.A.

Caulerpa is a well-known and distinctive genus which, despite the lack of transverse cell walls, shows a complex external morphology differentiated into stolons, rhizoids and erect assimilators of various forms. In all but a few species, the assimilators bear numerous ramuli that can display an almost bewildering diversity of form, even on a single assimilator. As species delimitation is based primarily on assimilator form, this morphological plasticity has led to a cumbersome classification scheme involving species, varieties, forms and ecads. Furthermore, there can appear to be continuous morphological gradations between many species (e.g. *C. cupressoides*/*urvilleana*/*serrulata*) and, as discussed by Svedelius (1906: 101), if a purely morphological standpoint was used to define species "one might just as well reduce the whole genus to one, or a few species". It is clear that species delimitation cannot be satisfactorily answered by morphological studies alone, and it requires molecular analyses, preferably of specimens from type localities or regions, as in many cases overlapping morphologies can disguise the true identities of taxa.

The current treatment incorporates molecular data (c. 840 base pair section of the *tuf*A gene) for the majority of species and infraspecific taxa found in N.W.A. However, some (e.g. *C. dichotoma* and *C. taxifolia* var. *falcifolia*) could not be sequenced, and their treatment is, by necessity, morphologically based. DNA extraction, amplification and sequencing of N.W.A. specimens were carried out using the methods of Belton *et al.* (2014). The 37 newly generated sequences were submitted to GenBank, and their accession numbers are given

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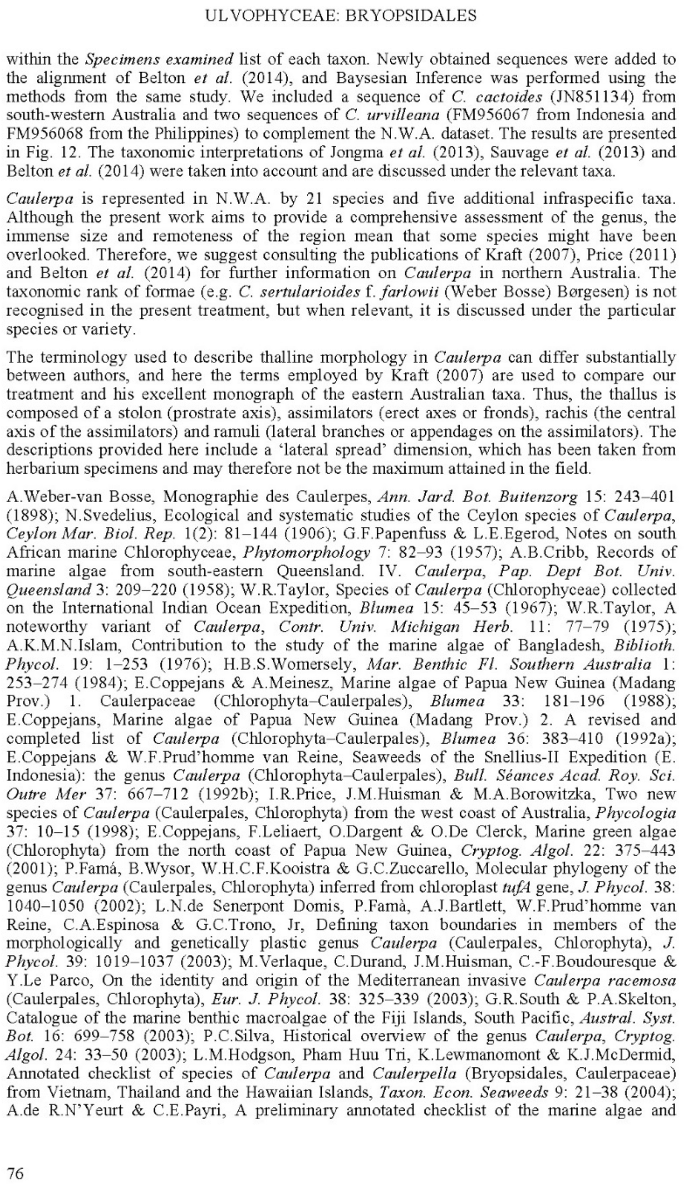
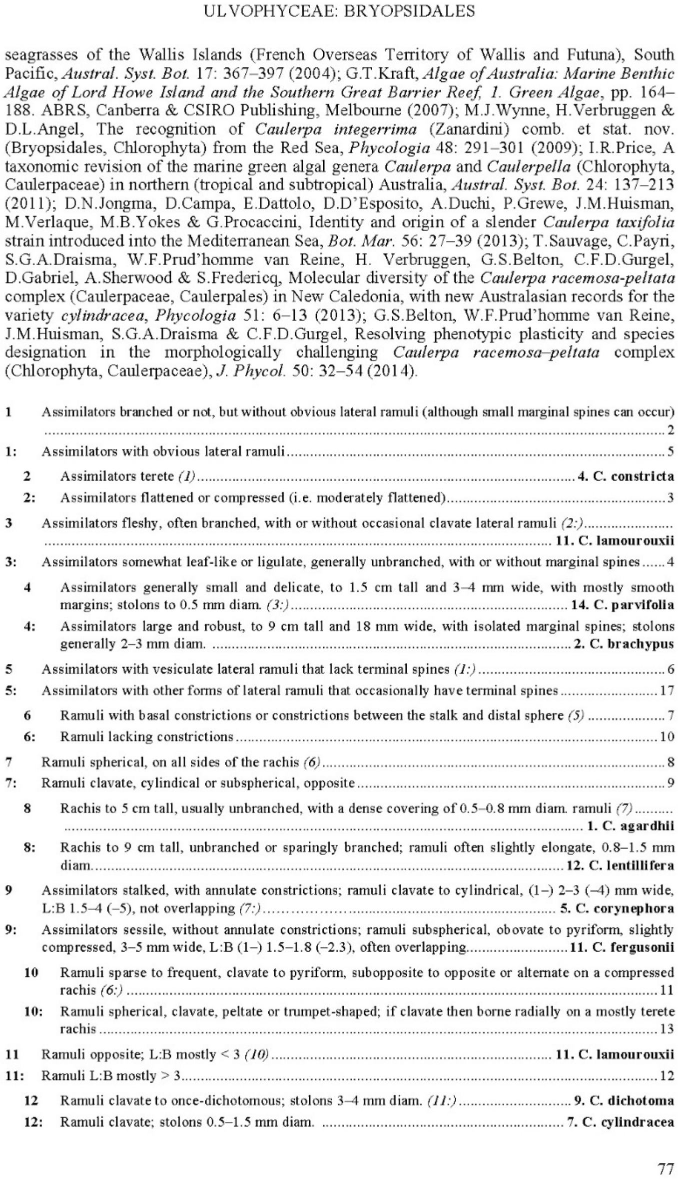


within the *Specimens examined* list of each taxon. Newly obtained sequences were added to the alignment of Belton *et al*. (2014), and Baysesian Inference was performed using the methods from the same study. We included a sequence of *C. cactoides* (JN851134) from south-western Australia and two sequences of *C. urvilleana* (FM956067 from Indonesia and FM956068 from the Philippines) to complement the N.W.A. dataset. The results are presented in Fig. 12. The taxonomic interpretations of Jongma *et al*. (2013), Sauvage *et al*. (2013) and Belton *et al*. (2014) were taken into account and are discussed under the relevant taxa.

Caulerpa is represented in N.W.A. by 21 species and five additional infraspecific taxa. Although the present work aims to provide a comprehensive assessment of the genus, the immense size and remoteness of the region mean that some species might have been overlooked. Therefore, we suggest consulting the publications of Kraft (2007), Price (2011) and Belton *et al*. (2014) for further information on *Caulerpa* in northern Australia. The taxonomic rank of formae (e.g. *C. sertularioides* f. *farlowii* (Weber Bosse) Børgesen) is not recognised in the present treatment, but when relevant, it is discussed under the particular species or variety.

The terminology used to describe thalline morphology in *Caulerpa* can differ substantially between authors, and here the terms employed by Kraft (2007) are used to compare our treatment and his excellent monograph of the eastern Australian taxa. Thus, the thallus is composed of a stolon (prostrate axis), assimilators (erect axes or fronds), rachis (the central axis of the assimilators) and ramuli (lateral branches or appendages on the assimilators). The descriptions provided here include a 'lateral spread' dimension, which has been taken from herbarium specimens and may therefore not be the maximum attained in the field.

A.Weber-van Bosse, Monographie des Caulerpes, *Ann. Jard. Bot. Buitenzorg* 15: 243–401 (1898); N.Svedelius, Ecological and systematic studies of the Ceylon species of *Caulerpa*, *Ceylon Mar. Biol. Rep.* 1(2): 81–144 (1906); G.F.Papenfuss & L.E.Egerod, Notes on south African marine Chlorophyceae, *Phytomorphology* 7: 82–93 (1957); A.B.Cribb, Records of marine algae from south-eastern Queensland. IV. *Caulerpa*, *Pap. Dept Bot. Univ. Queensland* 3: 209–220 (1958); W.R.Taylor, Species of *Caulerpa* (Chlorophyceae) collected on the International Indian Ocean Expedition, *Blumea* 15: 45–53 (1967); W.R.Taylor, A noteworthy variant of *Caulerpa*, *Contr. Univ. Michigan Herb.* 11: 77–79 (1975); A.K.M.N.Islam, Contribution to the study of the marine algae of Bangladesh, *Biblioth. Phycol.* 19: 1–253 (1976); H.B.S.Womersely, *Mar. Benthic Fl. Southern Australia* 1: 253–274 (1984); E.Coppejans & A.Meinesz, Marine algae of Papua New Guinea (Madang Prov.) 1. Caulerpaceae (Chlorophyta–Caulerpales), *Blumea* 33: 181–196 (1988); E.Coppejans, Marine algae of Papua New Guinea (Madang Prov.) 2. A revised and completed list of *Caulerpa* (Chlorophyta–Caulerpales), *Blumea* 36: 383–410 (1992a); E.Coppejans & W.F.Prud'homme van Reine, Seaweeds of the Snellius-II Expedition (E. Indonesia): the genus *Caulerpa* (Chlorophyta–Caulerpales), *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 667–712 (1992b); I.R.Price, J.M.Huisman & M.A.Borowitzka, Two new species of *Caulerpa* (Caulerpales, Chlorophyta) from the west coast of Australia, *Phycologia* 37: 10–15 (1998); E.Coppejans, F.Leliaert, O.Dargent & O.De Clerck, Marine green algae (Chlorophyta) from the north coast of Papua New Guinea, *Cryptog. Algol.* 22: 375–443 (2001); P.Famá, B.Wysor, W.H.C.F.Kooistra & G.C.Zuccarello, Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tufA* gene, *J. Phycol.* 38: 1040–1050 (2002); L.N.de Senerpont Domis, P.Famà, A.J.Bartlett, W.F.Prud'homme van Reine, C.A.Espinosa & G.C.Trono, Jr, Defining taxon boundaries in members of the morphologically and genetically plastic genus *Caulerpa* (Caulerpales, Chlorophyta), *J. Phycol.* 39: 1019–1037 (2003); M.Verlaque, C.Durand, J.M.Huisman, C.-F.Boudouresque & Y.Le Parco, On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta), *Eur. J. Phycol.* 38: 325–339 (2003); G.R.South & P.A.Skelton, Catalogue of the marine benthic macroalgae of the Fiji Islands, South Pacific, *Austral. Syst. Bot.* 16: 699–758 (2003); P.C.Silva, Historical overview of the genus *Caulerpa*, *Cryptog. Algol.* 24: 33–50 (2003); L.M.Hodgson, Pham Huu Tri, K.Lewmanomont & K.J.McDermid, Annotated checklist of species of *Caulerpa* and *Caulerpella* (Bryopsidales, Caulerpaceae) from Vietnam, Thailand and the Hawaiian Islands, *Taxon. Econ. Seaweeds* 9: 21–38 (2004); A.de R.N'Yeurt & C.E.Payri, A preliminary annotated checklist of the marine algae and



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1 Assimilators branched or not, but without obvious lateral ramuli (although small marginal spines can occur) 2

1: Assimilators with obvious lateral ramuli 5

2 Assimilators terete *(1)* .... **4. C. constricta**

2: Assimilators flattened or compressed (i.e. moderately flattened) 3

3 Assimilators fleshy, often branched, with or without occasional clavate lateral ramuli *(2:)* .... **11. C. lamourouxii**

3: Assimilators somewhat leaf-like or ligulate, generally unbranched, with or without marginal spines 4

4 Assimilators generally small and delicate, to 1.5 cm tall and 3–4 mm wide, with mostly smooth margins; stolons to 0.5 mm diam. *(3:)* .... **14. C. parvifolia**

4: Assimilators large and robust, to 9 cm tall and 18 mm wide, with isolated marginal spines; stolons generally 2–3 mm diam. .... **2. C. brachypus**

5 Assimilators with vesiculate lateral ramuli that lack terminal spines *(1:)* 6

5: Assimilators with other forms of lateral ramuli that occasionally have terminal spines 17

6 Ramuli with basal constrictions or constrictions between the stalk and distal sphere *(5)* 7

6: Ramuli lacking constrictions 10

7 Ramuli spherical, on all sides of the rachis *(6)* 8

7: Ramuli clavate, cylindical or subspherical, opposite 9

8 Rachis to 5 cm tall, usually unbranched, with a dense covering of 0.5–0.8 mm diam. ramuli *(7)* .... **1. C. agardhii**

8: Rachis to 9 cm tall, unbranched or sparingly branched; ramuli often slightly elongate, 0.8–1.5 mm diam. .... **12. C. lentillifera**

9 Assimilators stalked, with annulate constrictions; ramuli clavate to cylindrical, (1–) 2–3 (–4) mm wide, L:B 1.5–4 (–5), not overlapping *(7:)* .... **5. C. corynephora**

9: Assimilators sessile, without annulate constrictions; ramuli subspherical, obovate to pyriform, slightly compressed, 3–5 mm wide, L:B (1–) 1.5–1.8 (–2.3), often overlapping .... **11. C. fergusonii**

10 Ramuli sparse to frequent, clavate to pyriform, subopposite to opposite or alternate on a compressed rachis *(6:)* 11

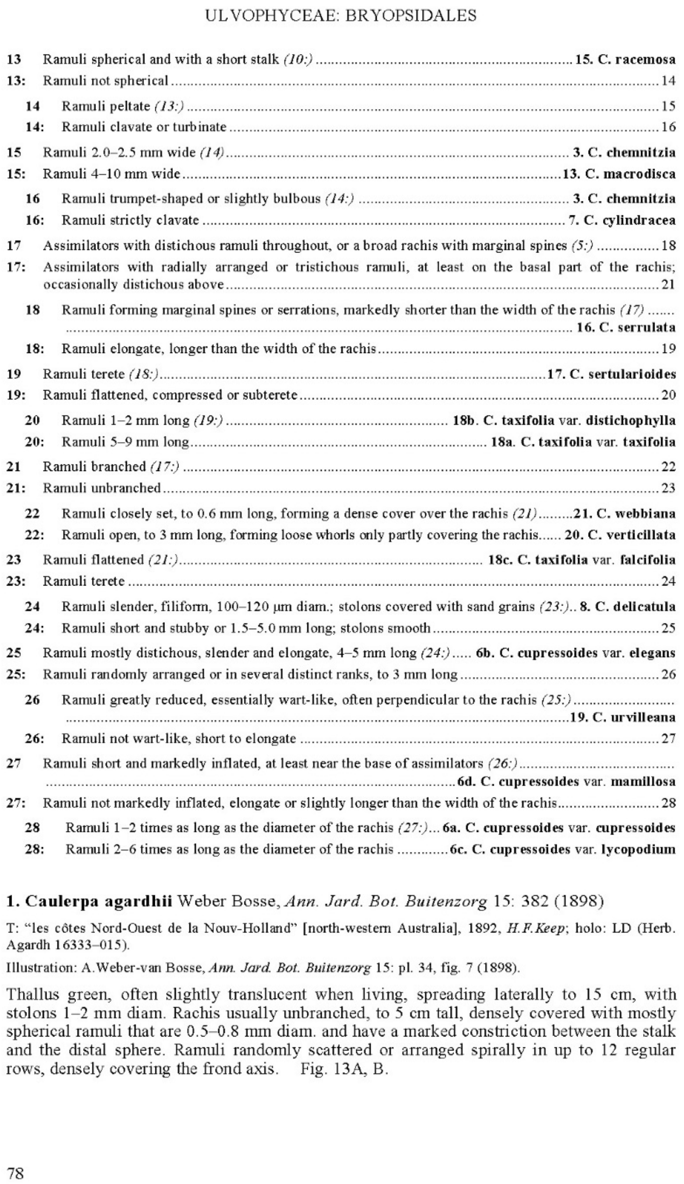
10: Ramuli spherical, clavate, peltate or trumpet-shaped; if clavate then borne radially on a mostly terete rachis 13

11 Ramuli opposite; L:B mostly < 3 *(10)* .... **11. C. lamourouxii**

11: Ramuli L:B mostly > 3 12

12 Ramuli clavate to once-dichotomous; stolons 3–4 mm diam. *(11:)* .... **9. C. dichotoma**

12: Ramuli clavate; stolons 0.5–1.5 mm diam. .... **7. C. cylindracea**



13 Ramuli spherical and with a short stalk *(10:)* .... **15. C. racemosa**
13: Ramuli not spherical 14
  14 Ramuli peltate *(13:)* 15
  14: Ramuli clavate or turbinate 16
15 Ramuli 2.0–2.5 mm wide *(14)* .... **3. C. chemnitzia**
15: Ramuli 4–10 mm wide .... **13. C. macrodisca**
  16 Ramuli trumpet-shaped or slightly bulbous *(14:)* .... **3. C. chemnitzia**
  16: Ramuli strictly clavate .... **7. C. cylindracea**
17 Assimilators with distichous ramuli throughout, or a broad rachis with marginal spines *(5:)* 18
17: Assimilators with radially arranged or tristichous ramuli, at least on the basal part of the rachis; occasionally distichous above 21
  18 Ramuli forming marginal spines or serrations, markedly shorter than the width of the rachis *(17)* .... **16. C. serrulata**
  18: Ramuli elongate, longer than the width of the rachis 19
19 Ramuli terete *(18:)* .... **17. C. sertularioides**
19: Ramuli flattened, compressed or subterete 20
  20 Ramuli 1–2 mm long *(19:)* .... **18b. C. taxifolia** var. **distichophylla**
  20: Ramuli 5–9 mm long .... **18a. C. taxifolia** var. **taxifolia**
21 Ramuli branched *(17:)* 22
21: Ramuli unbranched 23
  22 Ramuli closely set, to 0.6 mm long, forming a dense cover over the rachis *(21)* .... **21. C. webbiana**
  22: Ramuli open, to 3 mm long, forming loose whorls only partly covering the rachis .... **20. C. verticillata**
23 Ramuli flattened *(21:)* .... **18c. C. taxifolia** var. **falcifolia**
23: Ramuli terete 24
  24 Ramuli slender, filiform, 100–120 µm diam.; stolons covered with sand grains *(23:)* .... **8. C. delicatula**
  24: Ramuli short and stubby or 1.5–5.0 mm long; stolons smooth 25
25 Ramuli mostly distichous, slender and elongate, 4–5 mm long *(24:)* .... **6b. C. cupressoides** var. **elegans**
25: Ramuli randomly arranged or in several distinct ranks, to 3 mm long 26
  26 Ramuli greatly reduced, essentially wart-like, often perpendicular to the rachis *(25:)* .... **19. C. urvilleana**
  26: Ramuli not wart-like, short to elongate 27
27 Ramuli short and markedly inflated, at least near the base of assimilators *(26:)* .... **6d. C. cupressoides** var. **mamillosa**
27: Ramuli not markedly inflated, elongate or slightly longer than the width of the rachis 28
  28 Ramuli 1–2 times as long as the diameter of the rachis *(27:)* .... **6a. C. cupressoides** var. **cupressoides**
  28: Ramuli 2–6 times as long as the diameter of the rachis .... **6c. C. cupressoides** var. **lycopodium**

1. **Caulerpa agardhii** Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 382 (1898)

T: "les côtes Nord-Ouest de la Nouv-Holland" [north-western Australia], 1892, *H.F.Keep*; holo: LD (Herb. Agardh 16333–015).

Illustration: A.Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg* 15: pl. 34, fig. 7 (1898).

Thallus green, often slightly translucent when living, spreading laterally to 15 cm, with stolons 1–2 mm diam. Rachis usually unbranched, to 5 cm tall, densely covered with mostly spherical ramuli that are 0.5–0.8 mm diam. and have a marked constriction between the stalk and the distal sphere. Ramuli randomly scattered or arranged spirally in up to 12 regular rows, densely covering the frond axis. Fig. 13A, B.

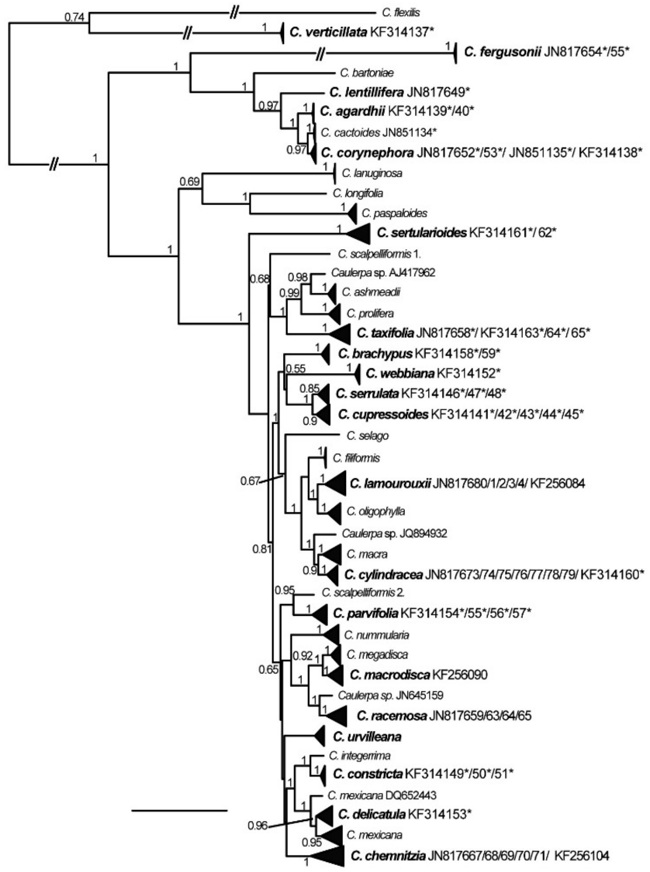


Figure 12. Phylogenetic tree constructed by Bayesian analysis of 285 partial *tuf*A sequences of *Caulerpa* taxa (840 nt). Species found in N.W.A. are in boldface, and newly obtained sequences ($n = 37$) are indicated by *. Numbers at branch nodes correspond to BI posterior probabilities (PP). PP values below 0.5 are not shown. The scale is 0.03 expected changes per site.

Endemic to northern Australia, where it is known from only a few collections. In W.A. known from the Kimberley coast and Ningaloo Reef; it appears to be associated with sandy substrata in the intertidal and shallow subtidal.

Specimens examined: in front of Jurabi sand dune, Ningaloo Reef, 3 m, 23 June 2008, *R.R.M.Dixon* (PERTH 08399646); 1 km N of Jurabi sand dune, Ningaloo Reef, drift, 17 June 2008, *C.F.D.Gurgel & R.R.M.Dixon* [AD A88266 (GenBank: KF314140*)]; Long Reef, Kimberley, intertidal, 23 Oct. 2010, *J.M.Huisman* [PERTH 08399697 (GenBank: KF314139*)].

Although Cribb (1988) recognised *Caulerpa agardhii* as a distinct species in Queensland, Price (2011) synonymised it with *C. lentillifera* due to similarities between their original descriptions and variation in the shape of ramuli in the type specimen of *C. agardhii*. Indeed, *C. agardhii* does bear a very close resemblance to *C. lentillifera*, and it can be difficult to distinguish the two. However, they are clearly separated by molecular analysis (Fig. 12) and, in N.W.A., *C. agardhii* is smaller, with more densely packed ramuli, often in regular rows, and spherical vesicles with a smaller diameter. By contrast, the vesicles of *C. lentillifera* tend to be larger and more elongate.

2. **Caulerpa brachypus** Harv., *Proc. Amer. Acad. Arts Sci.* 4: 333 (1860)

T: Tanega-shima, Kagoshima Prefecture, Japan, *C.Wright 5*; holo: TCD 0011061; iso: BM.

Illustrations: E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 674, figs 1A–C, 7A (1992); P.M.McCarthy & A.E.Orchard (eds), *Algae of Australia: Introduction* xi, pl. 9 (2007); G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 139, pl. 6A; 168, fig. 63D–F (2007); I.R.Price, *Austral. Syst. Bot.* 24: 139, fig. 1C (2011).

Thallus grass-green to olive-green, spreading laterally to 40–50 cm, with smooth terete stolons 2–3 mm diam., attached to the substratum by pillars bearing dense rhizoidal clusters. Assimilators with short basal terete stalks to 7 mm long, then flattened, simple or branched, ligulate, to 9 cm tall and 5–18 mm wide, generally of uniform width or tapering slightly, rarely sinuous or with constrictions. Ramuli absent, assimilators usually with minute widely spaced spines at the margins and an apical notch. Pl. 5A.

Widely distributed in the tropical Pacific and Indian Oceans as well as the Caribbean Sea. In W.A. it occurs south to Ningaloo Reef; epilithic in sandy areas of the lower intertidal and subtidal.

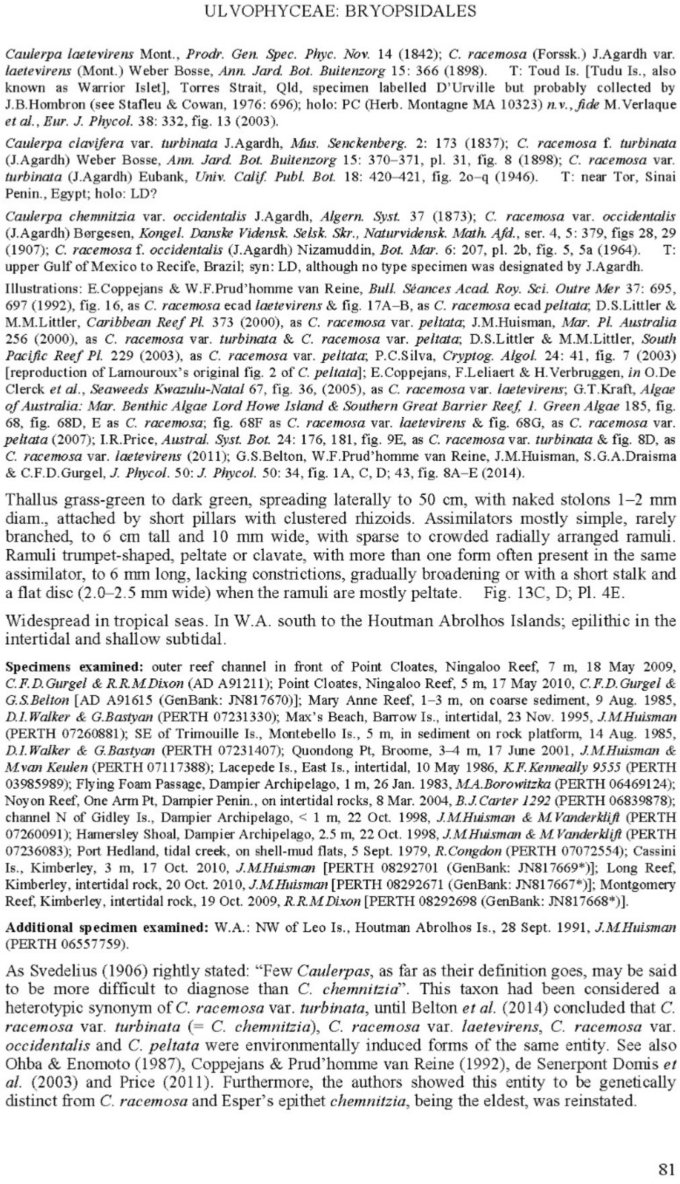
Specimens examined: Lefroy Bay, Ningaloo Reef, 1–3 m, 19 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD 91665 (GenBank: KF314158*)]; N of Winderabandi, Ningaloo Reef, 2–3 m, 16 Aug. 1995, *J.M.Huisman* (MURU NR143); *loc. id.*, 23 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90161 (GenBank: KF314159*)]; Exmouth Gulf, 10 Dec. 1987, *W.Fenical* (PERTH 07117418); Eagles Nest, Barrow Is., intertidal, 26 Nov. 1995 *J.M.Huisman* (PERTH 07262477); Hamersley Shoal, Dampier Archipelago, on coral rubble, 6 m, 27 May 1985, *J.Mercer* (PERTH 06468837); Sea Ripple Passage, between Burrup Penin. and Dolphin Is., on rock, 31 July 2000, *D.I.Walker* (PERTH 06993141); S of Nelson Rocks, Dampier Archipelago, 6 m, 8 Sept. 1999, *J.M.Huisman* (PERTH 07165714); S side of Kendrew Is., Dampier Archipelago, 3–4 m, 30 Aug. 1999, *J.M.Huisman* (PERTH 07165757); Port Hedland, intertidal on rocky shore, 23 Aug. 1985, *D.I.Walker & G.Bastyan* (PERTH 7231253).

Caulerpa brachypus is occasionally locally very common (e.g. at Barrow Island), and it can be similar to *C. parvifolia* and some forms of *C. lamourouxii*. However, it can be distinguished from *C. lamourouxii* by the usual presence of marginal spines (although these require some magnification in order to be seen) and an apical notch. The differences between *C. brachypus* and *C. parvifolia* are discussed under the latter.

3. **Caulerpa chemnitzia** (Esper) J.V.Lamour., *Nouv. Bull. Sci. Soc. Philom. Paris* 1: 332 (1809)

Fucus chemnitzia Esper, *Icones Fucorum* 4: 127–128 (err. pro 167–168), pl. 88, figs 1, 4–6 (1800); *Caulerpa racemosa* var. *chemnitzia* (Esper) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 376 (1898). T: "aus den Malabarische Küsten" [Malabar Coast], India, *Herr Prediger Chemnitz*; holo: ER; probable isotypes: BM, HBG, *fide* A.Weber-van Bosse, *op. cit.* 371, L 937.336-82, MEL 690468.

Caulerpa peltata J.V.Lamour., *Nouv. Bull. Sci. Soc. Philom. Paris* 1: 332 (1809); *C. racemosa* var. *peltata* (J.V.Lamour.) Eubank, *Univ. Calif. Publ. Bot.* 18: 421 (1946). T: Antilles, West Indies; holo: presumably CN.



Caulerpa laetevirens Mont., *Prodr. Gen. Spec. Phyc. Nov.* 14 (1842); *C. racemosa* (Forssk.) J.Agardh var. *laetevirens* (Mont.) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 366 (1898). T: Toud Is. [Tudu Is., also known as Warrior Islet], Torres Strait, Qld, specimen labelled D'Urville but probably collected by J.B.Hombron (see Stafleu & Cowan, 1976: 696); holo: PC (Herb. Montagne MA 10323) *n.v.*, *fide* M.Verlaque *et al.*, *Eur. J. Phycol.* 38: 332, fig. 13 (2003).

Caulerpa clavifera var. *turbinata* J.Agardh, *Mus. Senckenberg.* 2: 173 (1837); *C. racemosa* f. *turbinata* (J.Agardh) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 370–371, pl. 31, fig. 8 (1898); *C. racemosa* var. *turbinata* (J.Agardh) Eubank, *Univ. Calif. Publ. Bot.* 18: 420–421, fig. 2o–q (1946). T: near Tor, Sinai Penin., Egypt; holo: LD?

Caulerpa chemnitzia var. *occidentalis* J.Agardh, *Algern. Syst.* 37 (1873); *C. racemosa* var. *occidentalis* (J.Agardh) Børgesen, *Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Math. Afd.*, ser. 4, 5: 379, figs 28, 29 (1907); *C. racemosa* f. *occidentalis* (J.Agardh) Nizamuddin, *Bot. Mar.* 6: 207, pl. 2b, fig. 5, 5a (1964). T: upper Gulf of Mexico to Recife, Brazil; syn: LD, although no type specimen was designated by J.Agardh.

Illustrations: E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 695, 697 (1992), fig. 16, as *C. racemosa* ecad *laetevirens* & fig. 17A–B, as *C. racemosa* ecad *peltata*; D.S.Littler & M.M.Littler, *Caribbean Reef Pl.* 373 (2000), as *C. racemosa* var. *peltata*; J.M.Huisman, *Mar. Pl. Australia* 256 (2000), as *C. racemosa* var. *turbinata* & *C. racemosa* var. *peltata*; D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 229 (2003), as *C. racemosa* var. *peltata*; P.C.Silva, *Cryptog. Algol.* 24: 41, fig. 7 (2003) [reproduction of Lamouroux's original fig. 2 of *C. peltata*]; E.Coppejans, F.Leliaert & H.Verbruggen, *in* O.De Clerck *et al.*, *Seaweeds Kwazulu-Natal* 67, fig. 36, (2005), as *C. racemosa* var. *laetevirens*; G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 185, fig. 68, fig. 68D, E as *C. racemosa*; fig. 68F as *C. racemosa* var. *laetevirens* & fig. 68G, as *C. racemosa* var. *peltata* (2007); I.R.Price, *Austral. Syst. Bot.* 24: 176, 181, fig. 9E, as *C. racemosa* var. *turbinata* & fig. 8D, as *C. racemosa* var. *laetevirens* (2011); G.S.Belton, W.F.Prud'homme van Reine, J.M.Huisman, S.G.A.Draisma & C.F.D.Gurgel, *J. Phycol.* 50: *J. Phycol.* 50: 34, fig. 1A, C, D; 43, fig. 8A–E (2014).

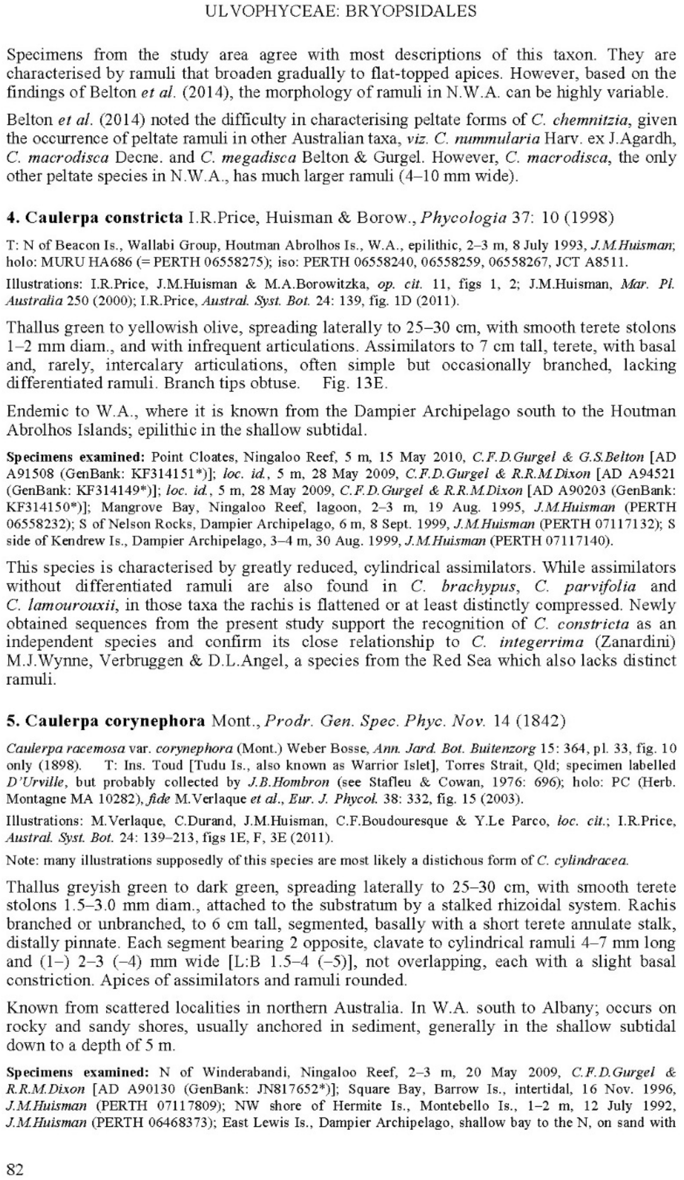
Thallus grass-green to dark green, spreading laterally to 50 cm, with naked stolons 1–2 mm diam., attached by short pillars with clustered rhizoids. Assimilators mostly simple, rarely branched, to 6 cm tall and 10 mm wide, with sparse to crowded radially arranged ramuli. Ramuli trumpet-shaped, peltate or clavate, with more than one form often present in the same assimilator, to 6 mm long, lacking constrictions, gradually broadening or with a short stalk and a flat disc (2.0–2.5 mm wide) when the ramuli are mostly peltate. Fig. 13C, D; Pl. 4E.

Widespread in tropical seas. In W.A. south to the Houtman Abrolhos Islands; epilithic in the intertidal and shallow subtidal.

Specimens examined: outer reef channel in front of Point Cloates, Ningaloo Reef, 7 m, 18 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* (AD A91211); Point Cloates, Ningaloo Reef, 5 m, 17 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91615 (GenBank: JN817670)]; Mary Anne Reef, 1–3 m, on coarse sediment, 9 Aug. 1985, *D.I.Walker & G.Bastyan* (PERTH 07231330); Max's Beach, Barrow Is., intertidal, 23 Nov. 1995, *J.M.Huisman* (PERTH 07260881); SE of Trimouille Is., Montebello Is., 5 m, in sediment on rock platform, 14 Aug. 1985, *D.I.Walker & G.Bastyan* (PERTH 07231407); Quondong Pt, Broome, 3–4 m, 17 June 2001, *J.M.Huisman & M.van Keulen* (PERTH 07117388); Lacepede Is., East Is., intertidal, 10 May 1986, *K.F.Kenneally 9555* (PERTH 03985989); Flying Foam Passage, Dampier Archipelago, 1 m, 26 Jan. 1983, *M.A.Borowitzka* (PERTH 06469124); Noyon Reef, One Arm Pt, Dampier Penin., on intertidal rocks, 8 Mar. 2004, *B.J.Carter 1292* (PERTH 06839878); channel N of Gidley Is., Dampier Archipelago, < 1 m, 22 Oct. 1998, *J.M.Huisman & M.Vanderklift* (PERTH 07260091); Hamersley Shoal, Dampier Archipelago, 2.5 m, 22 Oct. 1998, *J.M.Huisman & M.Vanderklift* (PERTH 07236083); Port Hedland, tidal creek, on shell-mud flats, 5 Sept. 1979, *R.Congdon* (PERTH 07072554); Cassini Is., Kimberley, 3 m, 17 Oct. 2010, *J.M.Huisman* [PERTH 08292701 (GenBank: JN817669*)]; Long Reef, Kimberley, intertidal rock, 20 Oct. 2010, *J.M.Huisman* [PERTH 08292671 (GenBank: JN817667*)]; Montgomery Reef, Kimberley, intertidal rock, 19 Oct. 2009, *R.R.M.Dixon* [PERTH 08292698 (GenBank: JN817668*)].

Additional specimen examined: W.A.: NW of Leo Is., Houtman Abrolhos Is., 28 Sept. 1991, *J.M.Huisman* (PERTH 06557759).

As Svedelius (1906) rightly stated: "Few *Caulerpas*, as far as their definition goes, may be said to be more difficult to diagnose than *C. chemnitzia*". This taxon had been considered a heterotypic synonym of *C. racemosa* var. *turbinata*, until Belton *et al.* (2014) concluded that *C. racemosa* var. *turbinata* (= *C. chemnitzia*), *C. racemosa* var. *laetevirens*, *C. racemosa* var. *occidentalis* and *C. peltata* were environmentally induced forms of the same entity. See also Ohba & Enomoto (1987), Coppejans & Prud'homme van Reine (1992), de Senerpont Domis *et al.* (2003) and Price (2011). Furthermore, the authors showed this entity to be genetically distinct from *C. racemosa* and Esper's epithet *chemnitzia*, being the eldest, was reinstated.



Specimens from the study area agree with most descriptions of this taxon. They are characterised by ramuli that broaden gradually to flat-topped apices. However, based on the findings of Belton *et al.* (2014), the morphology of ramuli in N.W.A. can be highly variable.

Belton *et al.* (2014) noted the difficulty in characterising peltate forms of *C. chemnitzia*, given the occurrence of peltate ramuli in other Australian taxa, *viz. C. nummularia* Harv. ex J.Agardh, *C. macrodisca* Decne. and *C. megadisca* Belton & Gurgel. However, *C. macrodisca*, the only other peltate species in N.W.A., has much larger ramuli (4–10 mm wide).

4. **Caulerpa constricta** I.R.Price, Huisman & Borow., *Phycologia* 37: 10 (1998)

T: N of Beacon Is., Wallabi Group, Houtman Abrolhos Is., W.A., epilithic, 2–3 m, 8 July 1993, *J.M.Huisman*; holo: MURU HA686 (= PERTH 06558275); iso: PERTH 06558240, 06558259, 06558267, JCT A8511.

Illustrations: I.R.Price, J.M.Huisman & M.A.Borowitzka, *op. cit.* 11, figs 1, 2; J.M.Huisman, *Mar. Pl. Australia* 250 (2000); I.R.Price, *Austral. Syst. Bot.* 24: 139, fig. 1D (2011).

Thallus green to yellowish olive, spreading laterally to 25–30 cm, with smooth terete stolons 1–2 mm diam., and with infrequent articulations. Assimilators to 7 cm tall, terete, with basal and, rarely, intercalary articulations, often simple but occasionally branched, lacking differentiated ramuli. Branch tips obtuse. Fig. 13E.

Endemic to W.A., where it is known from the Dampier Archipelago south to the Houtman Abrolhos Islands; epilithic in the shallow subtidal.

Specimens examined: Point Cloates, Ningaloo Reef, 5 m, 15 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91508 (GenBank: KF314151*)]; *loc. id.*, 5 m, 28 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A94521 (GenBank: KF314149*)]; *loc. id.*, 5 m, 28 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90203 (GenBank: KF314150*)]; Mangrove Bay, Ningaloo Reef, lagoon, 2–3 m, 19 Aug. 1995, *J.M.Huisman* (PERTH 06558232); S of Nelson Rocks, Dampier Archipelago, 6 m, 8 Sept. 1999, *J.M.Huisman* (PERTH 07117132); S side of Kendrew Is., Dampier Archipelago, 3–4 m, 30 Aug. 1999, *J.M.Huisman* (PERTH 07117140).

This species is characterised by greatly reduced, cylindrical assimilators. While assimilators without differentiated ramuli are also found in *C. brachypus*, *C. parvifolia* and *C. lamourouxii*, in those taxa the rachis is flattened or at least distinctly compressed. Newly obtained sequences from the present study support the recognition of *C. constricta* as an independent species and confirm its close relationship to *C. integerrima* (Zanardini) M.J.Wynne, Verbruggen & D.L.Angel, a species from the Red Sea which also lacks distinct ramuli.

5. **Caulerpa corynephora** Mont., *Prodr. Gen. Spec. Phyc. Nov.* 14 (1842)

Caulerpa racemosa var. *corynephora* (Mont.) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 364, pl. 33, fig. 10 only (1898). T: Ins. Toud [Tudu Is., also known as Warrior Islet], Torres Strait, Qld; specimen labelled *D'Urville*, but probably collected by *J.B.Hombron* (see Stafleu & Cowan, 1976: 696); holo: PC (Herb. Montagne MA 10282), *fide* M.Verlaque *et al.*, *Eur. J. Phycol.* 38: 332, fig. 15 (2003).

Illustrations: M.Verlaque, C.Durand, J.M.Huisman, C.F.Boudouresque & Y.Le Parco, *loc. cit.*; I.R.Price, *Austral. Syst. Bot.* 24: 139–213, figs 1E, F, 3E (2011).

Note: many illustrations supposedly of this species are most likely a distichous form of *C. cylindracea*.

Thallus greyish green to dark green, spreading laterally to 25–30 cm, with smooth terete stolons 1.5–3.0 mm diam., attached to the substratum by a stalked rhizoidal system. Rachis branched or unbranched, to 6 cm tall, segmented, basally with a short terete annulate stalk, distally pinnate. Each segment bearing 2 opposite, clavate to cylindrical ramuli 4–7 mm long and (1–) 2–3 (–4) mm wide [L:B 1.5–4 (–5)], not overlapping, each with a slight basal constriction. Apices of assimilators and ramuli rounded.

Known from scattered localities in northern Australia. In W.A. south to Albany; occurs on rocky and sandy shores, usually anchored in sediment, generally in the shallow subtidal down to a depth of 5 m.

Specimens examined: N of Winderabandi, Ningaloo Reef, 2–3 m, 20 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90130 (GenBank: JN817652*)]; Square Bay, Barrow Is., intertidal, 16 Nov. 1996, *J.M.Huisman* (PERTH 07117809); NW shore of Hermite Is., Montebello Is., 1–2 m, 12 July 1992, *J.M.Huisman* (PERTH 06468373); East Lewis Is., Dampier Archipelago, shallow bay to the N, on sand with underlying limestone at 2 m, 27 Oct. 1983, *M.A.Borowitzka* (PERTH 06468780); Cape Bossut, on intertidal rock, 30 Aug. 1985, *D.I.Walker & G.Bastyan* (PERTH 07231121); S of Port area, Broome, on rock, 25 Nov. 2003, *B.J.Carter 1256* (PERTH 6720005); S of James Price Pt, Broome, 9 Oct. 2009, *J.M.Huisman* [PERTH 08195803 (GenBank: KF314138*)]; Long Reef, Kimberley, reef flat at 3 m depth, on rock, 21 Oct. 2010, *J.M.Huisman* [PERTH 08292590 (GenBank JN817653*)].

Additional specimen examined: W.A.: Little Beach, Albany, 7 Nov. 2010, *G.W.Saunders & K.Dixon* [GWS 022470 (UNB): GenBank JN851135].

Caulerpa corynephora was commonly regarded as a variety of *C. racemosa*, based on the belief that its assimilators and ramuli were not constricted. However, morphological differences discussed by Price (2011) and molecular data from the current study confirm *C. corynephora* to be a constricted species closely related to *C. cactoides* (Turner) C.Agardh (Fig. 12). Furthermore, data from the present treatment also show that the distributions of *C. cactoides* (JN851134) and *C. corynephora* (GWS 022470) partially overlap in the south of Western Australia. *Caulerpa corynephora* differs by usually being smaller, with ramuli mostly borne on successive segments, as opposed to often being separated by a naked segment of the rachis as in *C. cactoides*.

Caulerpa cactoides has also been reported from Indonesia and Bangladesh (Silva *et al.*, 1996: 815). However, the Indonesian record was based on Coppejans & Prud'homme van Reine (1992a: 172) who listed "*Caulerpa* cf. *cactoides*". The specimens on which that record was based were later referred to *C. fergusonii* by Coppejans & Prud'homme van Reine (1992b: 690), unfortunately not listing the earlier record as a synonym. The report of *C. cactoides* from Bangladesh (Islam, 1976: pl. 12, figs 94, 95; pl. 14, fig. 103) should also be referred to *C. fergusonii*, as the specimens illustrated clearly have subspherical ramuli borne on successive segments of the rachis. Further differences between *C. corynephora* and *C. fergusonii* are discussed under the latter species.

6a. Caulerpa cupressoides (Vahl) C.Agardh, *Syn. Alg. Scand.* xxiii (1817) var. cupressoides

Fucus cupressoides Vahl, *Skr. Naturhist.-Selsk., København* 5(2): 38 (1802). T: St. Croix, Virgin Is.; neo: C (type no. A-826, specimen no. 31688), *Ryan n.v.*, *fide* R.Neilson & I.R.Price, *Taxon* 50: 830 (2001).

Illustrations: A.Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 323, pls 27, 28 (1898); D.S.Littler & M.M.Littler, *Caribbean Reef Pl.* 358–359 (2000); J.M.Huisman, *Mar. Pl. Australia* 250 (2000); I.R.Price, *Austral. Syst. Bot.* 24: 141, 142, 158; figs 2A, 3F, 4A, B (2011).

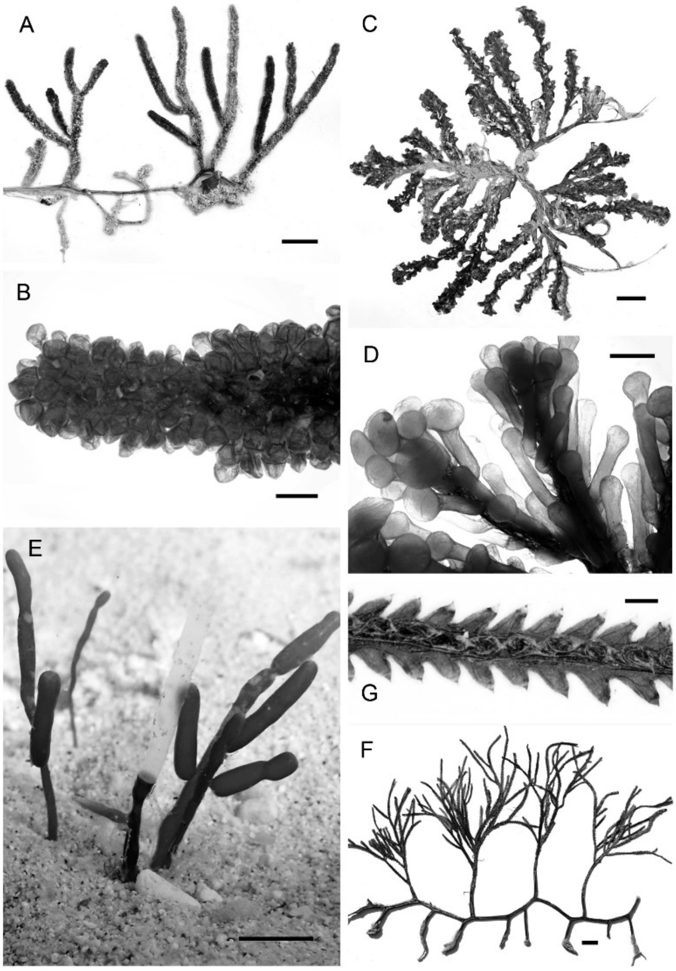
Thallus grey-green to dark green, spreading laterally to 35–40 cm, with an open spreading habit, occasionally forming extensive carpets; stolons terete, 1.5–2.5 mm diam. Rachis ±terete, branched, to 12 cm tall, 1.5–2.5 mm diam., occasionally naked near the base, above this region with short upwardly directed spinous ramuli in 2–4 ranks, the ramuli longer distally (to 2.5 mm). Fig. 13F, G.

Widely distributed in tropical seas; in W.A. south to Rottnest Island (Price, 2011: 161). Occurs in the shallow subtidal, associated with sandy or silty substrata.

Specimens examined: lagoon in front of Point Cloates, Ningaloo Reef, 6 m, 28 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90238 (GenBank: KF314141*)]; *loc. id.*, 3 m, 28 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90249 (GenBank: KF314145*)]; Point Cloates, Ningaloo Reef, on sandy bottom, 20 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91732 (GenBank KF314142*)]; Winderabandi, Ningaloo Reef, 2–3 m, 16 Aug. 1995, *J.M.Huisman* (MURU NR2); NW shore of Hermite Is., on sand at 1–2 m, 12 July 1992, *J.M.Huisman* (MURU); E side of West Lewis Is., Dampier Archipelago, in sand at 5 m, 4 Sept. 1999, *J.M.Huisman* (MURU DAR1984); Nelson Flats, Dampier Archipelago, 1 m, 27 Oct. 1983, *M.A.Borowitzka* (PERTH 06468764); near barge landing, E coast of Barrow Is., 1–2 m, Aug. 2004, *J.M.Huisman* (MURU B).

Caulerpa cupressoides is characterised by the thick smooth stolons and generally coarse erect axes bearing greatly reduced to elongate, terete ramuli with spinous tips. These can be arranged randomly on the axis, in several longitudinal rows, or distichously. Numerous infraspecific taxa have been attributed to *C. cupressoides*, and the taxonomic levels at which these are recognised have been variously interpreted. As noted by Coppejans (1992), Coppejans & Prud'homme van Reine (1992b), Kraft (2007) and Price (2011), several

ULVOPHYCEAE: BRYOPSIDALES



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seemingly distinct morphologies can be recognised and named, but intermediates are often present. Although this represents a similar case to that of the *C. racemosa-peltata* complex investigated by Belton *et al.* (2014), it appears that *C. cupressoides* from N.W.A. is monophyletic (Fig. 12) and, as such, a morphological scheme based on Price (2011) is followed here.

6b. **Caulerpa cupressoides** var. **elegans** (P.Crouan & H.Crouan) Børgesen, *Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Math. Afd.*, ser. 4, 5: 376 (1907)

Caulerpa plumaris var. *elegans* P.Crouan & H.Crouan, *in* A.Schramm & H.Mazé, *Essai Class. Alg. Guadeloupe* 39 (1865); *C. cupressoides* f. *elegans* (P.Crouan & H.Crouan) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 336 (1898). T: Guadeloupe, Caribbean Sea; holo: BM 000804772.

Illustrations: A.Weber-van Bosse, *op. cit.* pl. 27, figs 8, 9, as f. *elegans*; G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 179, fig. 66F (2007), as f. *elegans*; I.R.Price, *Austral. Syst. Bot.* 24: 141, fig. 2B; 142, fig. 3G (2011).

Thallus dark olive-green, spreading laterally to 25 cm, with smooth green to straw-coloured stolons 1–2 mm diam., attached to the substratum by pillars bearing dense rhizoids. Rachis simple or 1–4 times branched, terete but often compressed or flattened, to 8 cm tall, 1.0–1.5 mm diam., bearing mostly distichous, opposite or alternate ramuli, regularly spaced. Ramuli terete, 4–5 mm long, c. 1 mm wide, with spinous apices. Basal portions of assimilators (and occasionally elsewhere) with ramuli in several ranks. Fig. 14A, B.

Known from the Caribbean Sea, Indonesia, New Guinea, Queensland and Lord Howe Island. In northern W.A. it grows in sand in the shallow subtidal.

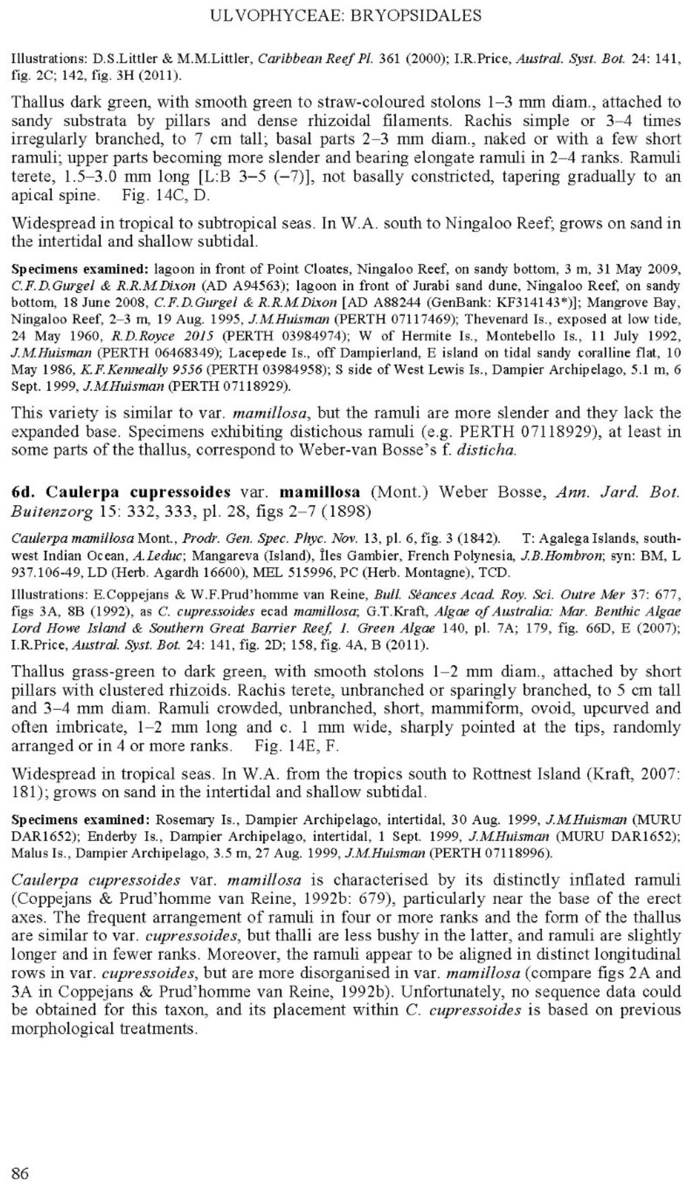
Specimens examined: Point Cloates, Ningaloo Reef, on sandy bottom, 20 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91740 (GenBank KF314144*)]; NW shore of Hermite Is., Montebello Is., 13 July 1992, *J.M.Huisman* (PERTH 06468322); E side of West Lewis Is., Dampier Archipelago, 5 m, 4 Sept. 1999, *J.M.Huisman* (PERTH 07118937); Long Reef, northern Kimberley, 22 Oct. 2010, *J.M.Huisman* (PERTH).

This differs from other varieties and forms of *C. cupressoides* in having mostly distichous ramuli 4–5 mm long. It is closest to forms of var. *lycopodium*, which differs in having shorter ramuli (less than 3 mm), but otherwise the two taxa have a similar habit and intermediates are occasionally seen (PERTH 06468322). Consequently, some authors (e.g. Weber-van Bosse, 1898; Coppejans & Prud'homme van Reine, 1992b; Kraft, 2007) have included it in the synonymy of *C. cupressoides* var. *lycopodium*. However, in almost all Australian specimens the two taxa are morphologically distinct (Price, 2011: 163). This variety can also closely resemble *C. sertularioides*, but it differs in its compressed to flattened rachis, ramuli that are mostly broader and less crowded, occasionally in several ranks, and with a few short stiff ramuli usually present on basal parts of assimilators.

6c. **Caulerpa cupressoides** var. **lycopodium** Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 335, pl. 28, fig. 11 (1898), illustrating *C. cupressoides* 'f. *typica*' Weber Bosse

Caulerpa lycopodium J.Agardh, *Öfvers. Kongl. Vetensk.-Akad. Förh.* 4: 6 (1847), *nom. illeg.*, *fide* I.R.Price, *Austral. Syst. Bot.* 24: 163 (2011). T: "e mari Brasiliae et Indiae Occidentalis"; syn: LD (Herb. Agardh 16637, 16638).

Figure 13 (opposite). *Caulerpa*. **A, B**, *C. agardhii*. **A**, Part of pressed specimen showing branched assimilators (PERTH 08399646). **B**, Detail of minute ramuli densely arranged around the rachis (AD A88266). **C, D**, *C. chemnitzia*. **C**, Pressed specimen of large plant showing upright assimilators bearing densely arranged turbinate to clavate ramuli (PERTH). **D**, Detail of assimilator bearing clavate and peltate ramuli (AD A91211). **E**, *C. constricta*. *In situ* habit showing smooth terete stolons with infrequent articulations (AD A94521). **F, G**, *C. cupressoides*. **F**, Pressed specimen from 7 m depth (AD A91732). **G**, Detail of assimilator with short, upwardly directed spinous ramuli borne in distinct rows (AD A91732). Scales: A, C, E, F = 10 mm; B, G = 1 mm; D = 3 mm.



Illustrations: D.S.Littler & M.M.Littler, *Caribbean Reef Pl.* 361 (2000); I.R.Price, *Austral. Syst. Bot.* 24: 141, fig. 2C; 142, fig. 3H (2011).

Thallus dark green, with smooth green to straw-coloured stolons 1–3 mm diam., attached to sandy substrata by pillars and dense rhizoidal filaments. Rachis simple or 3–4 times irregularly branched, to 7 cm tall; basal parts 2–3 mm diam., naked or with a few short ramuli; upper parts becoming more slender and bearing elongate ramuli in 2–4 ranks. Ramuli terete, 1.5–3.0 mm long [L:B 3–5 (–7)], not basally constricted, tapering gradually to an apical spine. Fig. 14C, D.

Widespread in tropical to subtropical seas. In W.A. south to Ningaloo Reef; grows on sand in the intertidal and shallow subtidal.

Specimens examined: lagoon in front of Point Cloates, Ningaloo Reef, on sandy bottom, 3 m, 31 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* (AD A94563); lagoon in front of Jurabi sand dune, Ningaloo Reef, on sandy bottom, 18 June 2008, *C.F.D.Gurgel & R.R.M.Dixon* [AD A88244 (GenBank: KF314143*)]; Mangrove Bay, Ningaloo Reef, 2–3 m, 19 Aug. 1995, *J.M.Huisman* (PERTH 07117469); Thevenard Is., exposed at low tide, 24 May 1960, *R.D.Royce 2015* (PERTH 03984974); W of Hermite Is., Montebello Is., 11 July 1992, *J.M.Huisman* (PERTH 06468349); Lacepede Is., off Dampierland, E island on tidal sandy coralline flat, 10 May 1986, *K.F.Kenneally 9556* (PERTH 03984958); S side of West Lewis Is., Dampier Archipelago, 5.1 m, 6 Sept. 1999, *J.M.Huisman* (PERTH 07118929).

This variety is similar to var. *mamillosa*, but the ramuli are more slender and they lack the expanded base. Specimens exhibiting distichous ramuli (e.g. PERTH 07118929), at least in some parts of the thallus, correspond to Weber-van Bosse's f. *disticha*.

6d. **Caulerpa cupressoides** var. **mamillosa** (Mont.) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 332, 333, pl. 28, figs 2–7 (1898)

Caulerpa mamillosa Mont., *Prodr. Gen. Spec. Phyc. Nov.* 13, pl. 6, fig. 3 (1842). T: Agalega Islands, south-west Indian Ocean, *A.Leduc*; Mangareva (Island), Îles Gambier, French Polynesia, *J.B.Hombron*; syn: BM, L 937.106-49, LD (Herb. Agardh 16600), MEL 515996, PC (Herb. Montagne), TCD.

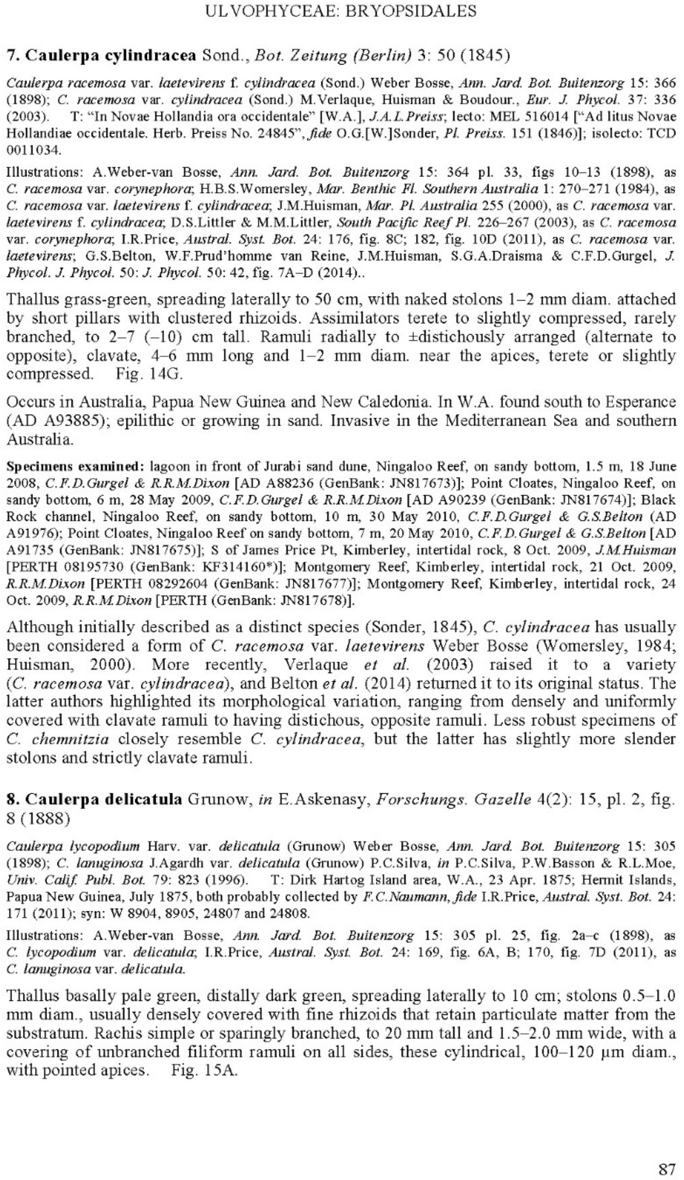
Illustrations: E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 677, figs 3A, 8B (1992), as *C. cupressoides* ecad *mamillosa*; G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 140, pl. 7A; 179, fig. 66D, E (2007); I.R.Price, *Austral. Syst. Bot.* 24: 141, fig. 2D; 158, fig. 4A, B (2011).

Thallus grass-green to dark green, with smooth stolons 1–2 mm diam., attached by short pillars with clustered rhizoids. Rachis terete, unbranched or sparingly branched, to 5 cm tall and 3–4 mm diam. Ramuli crowded, unbranched, short, mammiform, ovoid, upcurved and often imbricate, 1–2 mm long and c. 1 mm wide, sharply pointed at the tips, randomly arranged or in 4 or more ranks. Fig. 14E, F.

Widespread in tropical seas. In W.A. from the tropics south to Rottnest Island (Kraft, 2007: 181); grows on sand in the intertidal and shallow subtidal.

Specimens examined: Rosemary Is., Dampier Archipelago, intertidal, 30 Aug. 1999, *J.M.Huisman* (MURU DAR1652); Enderby Is., Dampier Archipelago, intertidal, 1 Sept. 1999, *J.M.Huisman* (MURU DAR1652); Malus Is., Dampier Archipelago, 3.5 m, 27 Aug. 1999, *J.M.Huisman* (PERTH 07118996).

Caulerpa cupressoides var. *mamillosa* is characterised by its distinctly inflated ramuli (Coppejans & Prud'homme van Reine, 1992b: 679), particularly near the base of the erect axes. The frequent arrangement of ramuli in four or more ranks and the form of the thallus are similar to var. *cupressoides*, but thalli are less bushy in the latter, and ramuli are slightly longer and in fewer ranks. Moreover, the ramuli appear to be aligned in distinct longitudinal rows in var. *cupressoides*, but are more disorganised in var. *mamillosa* (compare figs 2A and 3A in Coppejans & Prud'homme van Reine, 1992b). Unfortunately, no sequence data could be obtained for this taxon, and its placement within *C. cupressoides* is based on previous morphological treatments.



7. Caulerpa cylindracea Sond., *Bot. Zeitung (Berlin)* 3: 50 (1845)

Caulerpa racemosa var. *laetevirens* f. *cylindracea* (Sond.) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 366 (1898); *C. racemosa* var. *cylindracea* (Sond.) M.Verlaque, Huisman & Boudour., *Eur. J. Phycol.* 37: 336 (2003). T: "In Novae Hollandia ora occidentale" [W.A.], *J.A.L.Preiss*; lecto: MEL 516014 ["Ad litus Novae Hollandiae occidentale. Herb. Preiss No. 24845", *fide* O.G.[W.]Sonder, *Pl. Preiss.* 151 (1846)]; isolecto: TCD 0011034.

Illustrations: A.Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 364 pl. 33, figs 10–13 (1898), as *C. racemosa* var. *corynephora*; H.B.S.Womersley, *Mar. Benthic Fl. Southern Australia* 1: 270–271 (1984), as *C. racemosa* var. *laetevirens* f. *cylindracea*; J.M.Huisman, *Mar. Pl. Australia* 255 (2000), as *C. racemosa* var. *laetevirens* f. *cylindracea*; D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 226–267 (2003), as *C. racemosa* var. *corynephora*; I.R.Price, *Austral. Syst. Bot.* 24: 176, fig. 8C; 182, fig. 10D (2011), as *C. racemosa* var. *laetevirens*; G.S.Belton, W.F.Prud'homme van Reine, J.M.Huisman, S.G.A.Draisma & C.F.D.Gurgel, *J. Phycol. J. Phycol.* 50: *J. Phycol.* 50: 42, fig. 7A–D (2014)..

Thallus grass-green, spreading laterally to 50 cm, with naked stolons 1–2 mm diam. attached by short pillars with clustered rhizoids. Assimilators terete to slightly compressed, rarely branched, to 2–7 (–10) cm tall. Ramuli radially to ±distichously arranged (alternate to opposite), clavate, 4–6 mm long and 1–2 mm diam. near the apices, terete or slightly compressed. Fig. 14G.

Occurs in Australia, Papua New Guinea and New Caledonia. In W.A. found south to Esperance (AD A93885); epilithic or growing in sand. Invasive in the Mediterranean Sea and southern Australia.

Specimens examined: lagoon in front of Jurabi sand dune, Ningaloo Reef, on sandy bottom, 1.5 m, 18 June 2008, *C.F.D.Gurgel & R.R.M.Dixon* [AD A88236 (GenBank: JN817673)]; Point Cloates, Ningaloo Reef, on sandy bottom, 6 m, 28 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90239 (GenBank: JN817674)]; Black Rock channel, Ningaloo Reef, on sandy bottom, 10 m, 30 May 2010, *C.F.D.Gurgel & G.S.Belton* (AD A91976); Point Cloates, Ningaloo Reef on sandy bottom, 7 m, 20 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91735 (GenBank: JN817675)]; S of James Price Pt, Kimberley, intertidal rock, 8 Oct. 2009, *J.M.Huisman* [PERTH 08195730 (GenBank: KF314160*)]; Montgomery Reef, Kimberley, intertidal rock, 21 Oct. 2009, *R.R.M.Dixon* [PERTH 08292604 (GenBank: JN817677)]; Montgomery Reef, Kimberley, intertidal rock, 24 Oct. 2009, *R.R.M.Dixon* [PERTH (GenBank: JN817678)].

Although initially described as a distinct species (Sonder, 1845), *C. cylindracea* has usually been considered a form of *C. racemosa* var. *laetevirens* Weber Bosse (Womersley, 1984; Huisman, 2000). More recently, Verlaque *et al.* (2003) raised it to a variety (*C. racemosa* var. *cylindracea*), and Belton *et al.* (2014) returned it to its original status. The latter authors highlighted its morphological variation, ranging from densely and uniformly covered with clavate ramuli to having distichous, opposite ramuli. Less robust specimens of *C. chemnitzia* closely resemble *C. cylindracea*, but the latter has slightly more slender stolons and strictly clavate ramuli.

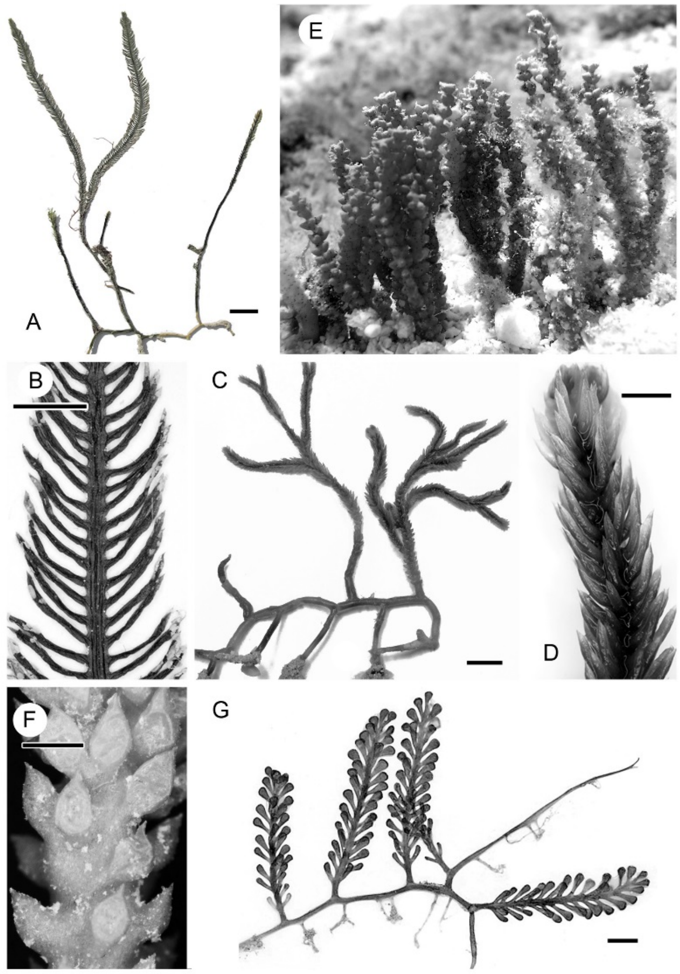
8. Caulerpa delicatula Grunow, *in* E.Askenasy, *Forschungs. Gazelle* 4(2): 15, pl. 2, fig. 8 (1888)

Caulerpa lycopodium Harv. var. *delicatula* (Grunow) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 305 (1898); *C. lanuginosa* J.Agardh var. *delicatula* (Grunow) P.C.Silva, *in* P.C.Silva, P.W.Basson & R.L.Moe, *Univ. Calif. Publ. Bot.* 79: 823 (1996). T: Dirk Hartog Island area, W.A., 23 Apr. 1875; Hermit Islands, Papua New Guinea, July 1875, both probably collected by *F.C.Naumann*, *fide* I.R.Price, *Austral. Syst. Bot.* 24: 171 (2011); syn: W 8904, 8905, 24807 and 24808.

Illustrations: A.Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 305 pl. 25, fig. 2a–c (1898), as *C. lycopodium* var. *delicatula*; I.R.Price, *Austral. Syst. Bot.* 24: 169, fig. 6A, B; 170, fig. 7D (2011), as *C. lanuginosa* var. *delicatula*.

Thallus basally pale green, distally dark green, spreading laterally to 10 cm; stolons 0.5–1.0 mm diam., usually densely covered with fine rhizoids that retain particulate matter from the substratum. Rachis simple or sparingly branched, to 20 mm tall and 1.5–2.0 mm wide, with a covering of unbranched filiform ramuli on all sides, these cylindrical, 100–120 µm diam., with pointed apices. Fig. 15A.

ULVOPHYCEAE: BRYOPSIDALES



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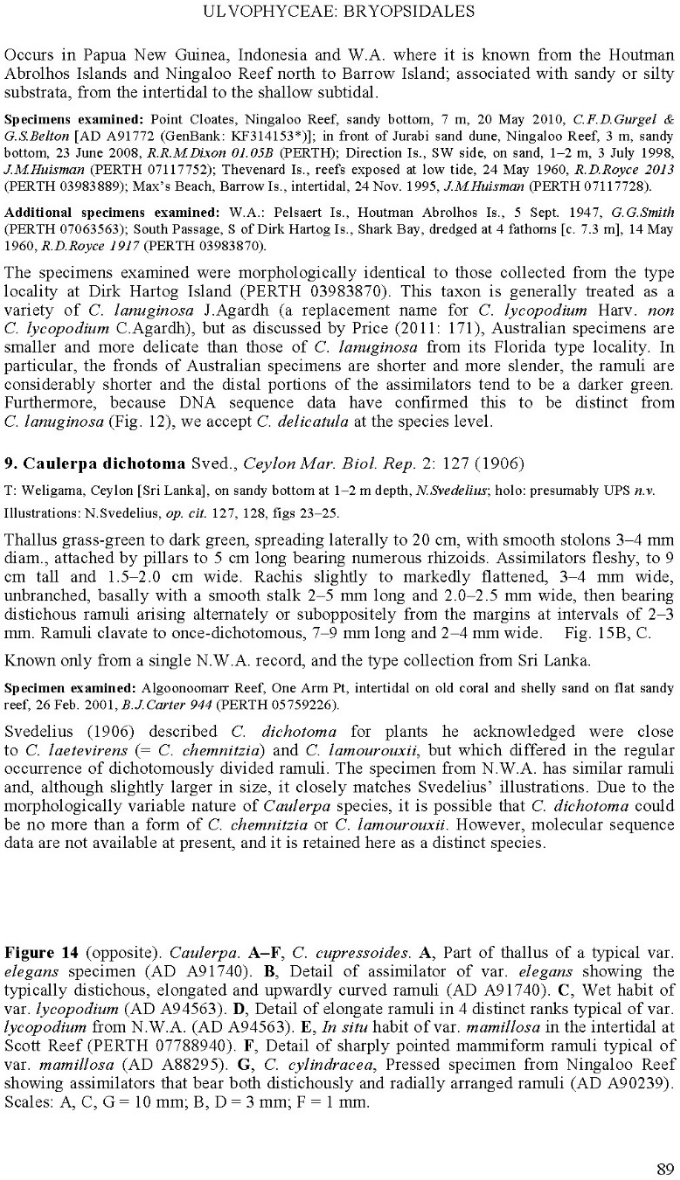


Occurs in Papua New Guinea, Indonesia and W.A. where it is known from the Houtman Abrolhos Islands and Ningaloo Reef north to Barrow Island; associated with sandy or silty substrata, from the intertidal to the shallow subtidal.

Specimens examined: Point Cloates, Ningaloo Reef, sandy bottom, 7 m, 20 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91772 (GenBank: KF314153*)]; in front of Jurabi sand dune, Ningaloo Reef, 3 m, sandy bottom, 23 June 2008, *R.R.M.Dixon 01.05B* (PERTH); Direction Is., SW side, on sand, 1–2 m, 3 July 1998, *J.M.Huisman* (PERTH 07117752); Thevenard Is., reefs exposed at low tide, 24 May 1960, *R.D.Royce 2013* (PERTH 03983889); Max's Beach, Barrow Is., intertidal, 24 Nov. 1995, *J.M.Huisman* (PERTH 07117728).

Additional specimens examined: W.A.: Pelsaert Is., Houtman Abrolhos Is., 5 Sept. 1947, *G.G.Smith* (PERTH 07063563); South Passage, S of Dirk Hartog Is., Shark Bay, dredged at 4 fathoms [c. 7.3 m], 14 May 1960, *R.D.Royce 1917* (PERTH 03983870).

The specimens examined were morphologically identical to those collected from the type locality at Dirk Hartog Island (PERTH 03983870). This taxon is generally treated as a variety of *C. lanuginosa* J.Agardh (a replacement name for *C. lycopodium* Harv. *non C. lycopodium* C.Agardh), but as discussed by Price (2011: 171), Australian specimens are smaller and more delicate than those of *C. lanuginosa* from its Florida type locality. In particular, the fronds of Australian specimens are shorter and more slender, the ramuli are considerably shorter and the distal portions of the assimilators tend to be a darker green. Furthermore, because DNA sequence data have confirmed this to be distinct from *C. lanuginosa* (Fig. 12), we accept *C. delicatula* at the species level.

9. **Caulerpa dichotoma** Sved., *Ceylon Mar. Biol. Rep.* 2: 127 (1906)

T: Weligama, Ceylon [Sri Lanka], on sandy bottom at 1–2 m depth, *N.Svedelius*; holo: presumably UPS *n.v.*

Illustrations: N.Svedelius, *op. cit.* 127, 128, figs 23–25.

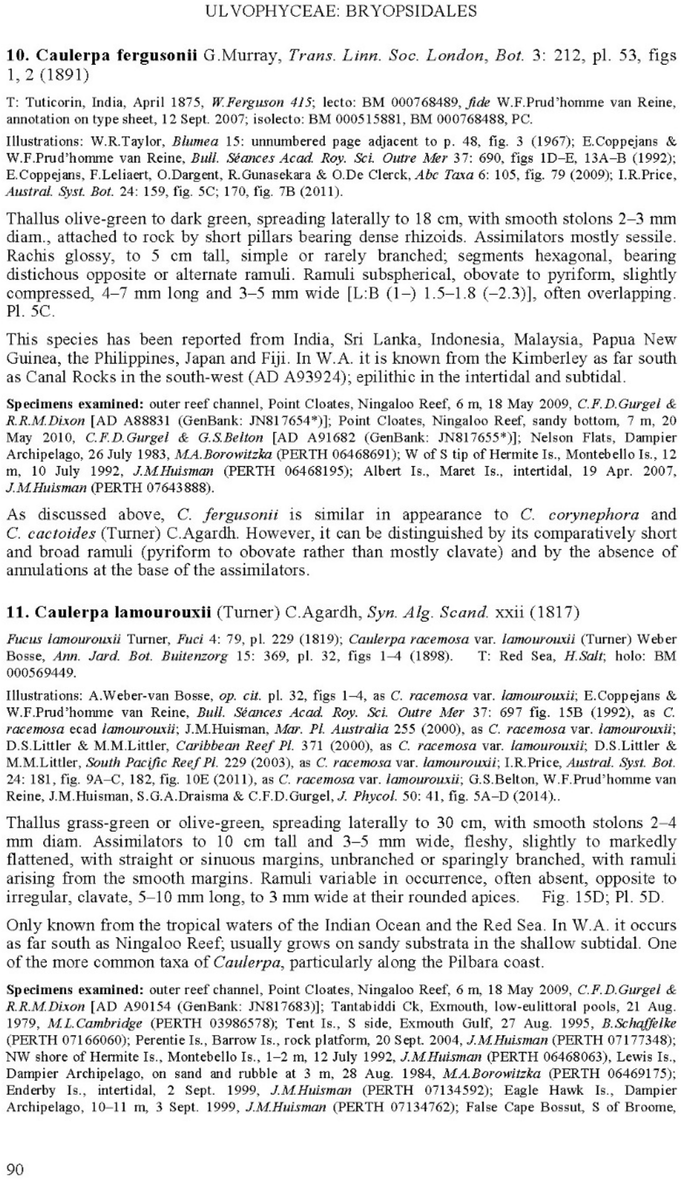
Thallus grass-green to dark green, spreading laterally to 20 cm, with smooth stolons 3–4 mm diam., attached by pillars to 5 cm long bearing numerous rhizoids. Assimilators fleshy, to 9 cm tall and 1.5–2.0 cm wide. Rachis slightly to markedly flattened, 3–4 mm wide, unbranched, basally with a smooth stalk 2–5 mm long and 2.0–2.5 mm wide, then bearing distichous ramuli arising alternately or suboppositely from the margins at intervals of 2–3 mm. Ramuli clavate to once-dichotomous, 7–9 mm long and 2–4 mm wide. Fig. 15B, C.

Known only from a single N.W.A. record, and the type collection from Sri Lanka.

Specimen examined: Algoonoomarr Reef, One Arm Pt, intertidal on old coral and shelly sand on flat sandy reef, 26 Feb. 2001, *B.J.Carter 944* (PERTH 05759226).

Svedelius (1906) described *C. dichotoma* for plants he acknowledged were close to *C. laetevirens* (= *C. chemnitzia*) and *C. lamourouxii*, but which differed in the regular occurrence of dichotomously divided ramuli. The specimen from N.W.A. has similar ramuli and, although slightly larger in size, it closely matches Svedelius' illustrations. Due to the morphologically variable nature of *Caulerpa* species, it is possible that *C. dichotoma* could be no more than a form of *C. chemnitzia* or *C. lamourouxii*. However, molecular sequence data are not available at present, and it is retained here as a distinct species.

Figure 14 (opposite). *Caulerpa*. **A–F**, *C. cupressoides*. **A**, Part of thallus of a typical var. *elegans* specimen (AD A91740). **B**, Detail of assimilator of var. *elegans* showing the typically distichous, elongated and upwardly curved ramuli (AD A91740). **C**, Wet habit of var. *lycopodium* (AD A94563). **D**, Detail of elongate ramuli in 4 distinct ranks typical of var. *lycopodium* from N.W.A. (AD A94563). **E**, *In situ* habit of var. *mamillosa* in the intertidal at Scott Reef (PERTH 07788940). **F**, Detail of sharply pointed mammiform ramuli typical of var. *mamillosa* (AD A88295). **G**, *C. cylindracea*, Pressed specimen from Ningaloo Reef showing assimilators that bear both distichously and radially arranged ramuli (AD A90239). Scales: A, C, G = 10 mm; B, D = 3 mm; F = 1 mm.



10. Caulerpa fergusonii G.Murray, *Trans. Linn. Soc. London*, *Bot.* 3: 212, pl. 53, figs 1, 2 (1891)

T: Tuticorin, India, April 1875, *W.Ferguson 415*; lecto: BM 000768489, *fide* W.F.Prud'homme van Reine, annotation on type sheet, 12 Sept. 2007; isolecto: BM 000515881, BM 000768488, PC.

Illustrations: W.R.Taylor, *Blumea* 15: unnumbered page adjacent to p. 48, fig. 3 (1967); E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 690, figs 1D–E, 13A–B (1992); E.Coppejans, F.Leliaert, O.Dargent, R.Gunasekara & O.De Clerck, *Abc Taxa* 6: 105, fig. 79 (2009); I.R.Price, *Austral. Syst. Bot.* 24: 159, fig. 5C; 170, fig. 7B (2011).

Thallus olive-green to dark green, spreading laterally to 18 cm, with smooth stolons 2–3 mm diam., attached to rock by short pillars bearing dense rhizoids. Assimilators mostly sessile. Rachis glossy, to 5 cm tall, simple or rarely branched; segments hexagonal, bearing distichous opposite or alternate ramuli. Ramuli subspherical, obovate to pyriform, slightly compressed, 4–7 mm long and 3–5 mm wide [L:B (1–) 1.5–1.8 (–2.3)], often overlapping. Pl. 5C.

This species has been reported from India, Sri Lanka, Indonesia, Malaysia, Papua New Guinea, the Philippines, Japan and Fiji. In W.A. it is known from the Kimberley as far south as Canal Rocks in the south-west (AD A93924); epilithic in the intertidal and subtidal.

Specimens examined: outer reef channel, Point Cloates, Ningaloo Reef, 6 m, 18 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A88831 (GenBank: JN817654*)]; Point Cloates, Ningaloo Reef, sandy bottom, 7 m, 20 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91682 (GenBank: JN817655*)]; Nelson Flats, Dampier Archipelago, 26 July 1983, *M.A.Borowitzka* (PERTH 06468691); W of S tip of Hermite Is., Montebello Is., 12 m, 10 July 1992, *J.M.Huisman* (PERTH 06468195); Albert Is., Maret Is., intertidal, 19 Apr. 2007, *J.M.Huisman* (PERTH 07643888).

As discussed above, *C. fergusonii* is similar in appearance to *C. corynephora* and *C. cactoides* (Turner) C.Agardh. However, it can be distinguished by its comparatively short and broad ramuli (pyriform to obovate rather than mostly clavate) and by the absence of annulations at the base of the assimilators.

11. Caulerpa lamourouxii (Turner) C.Agardh, *Syn. Alg. Scand.* xxii (1817)

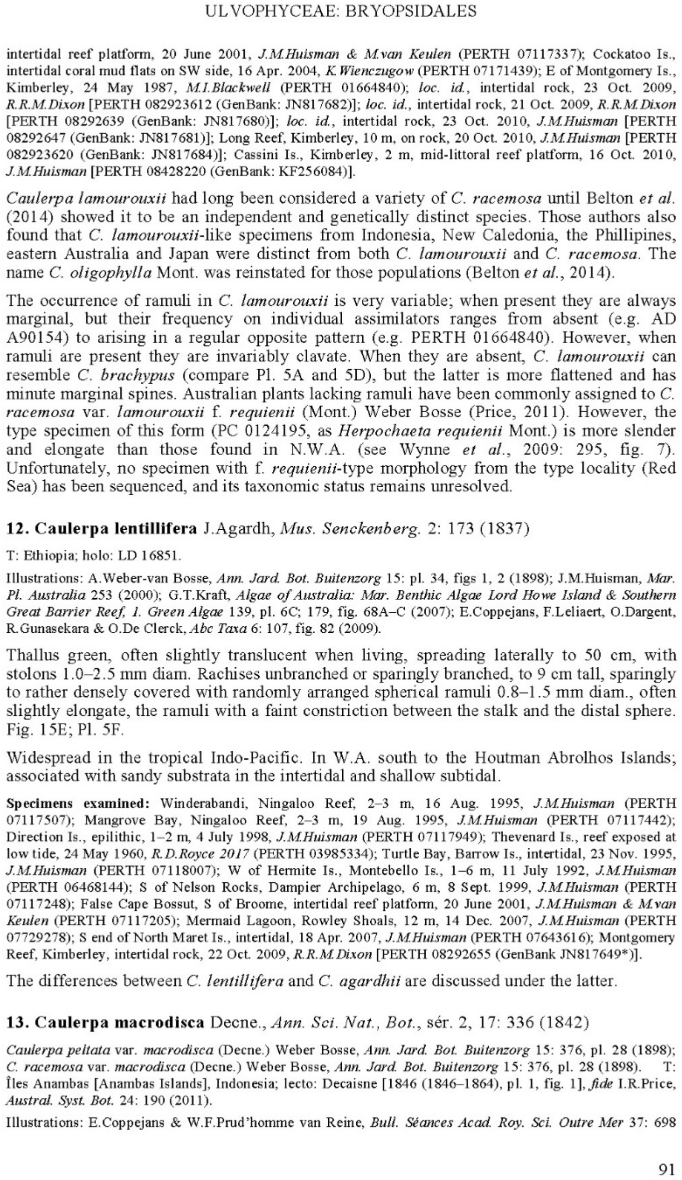
Fucus lamourouxii Turner, *Fuci* 4: 79, pl. 229 (1819); *Caulerpa racemosa* var. *lamourouxii* (Turner) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 369, pl. 32, figs 1–4 (1898). T: Red Sea, *H.Salt*; holo: BM 000569449.

Illustrations: A.Weber-van Bosse, *op. cit.* pl. 32, figs 1–4, as *C. racemosa* var. *lamourouxii*; E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 697 fig. 15B (1992), as *C. racemosa* ecad *lamourouxii*; J.M.Huisman, *Mar. Pl. Australia* 255 (2000), as *C. racemosa* var. *lamourouxii*; D.S.Littler & M.M.Littler, *Caribbean Reef Pl.* 371 (2000), as *C. racemosa* var. *lamourouxii*; D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 229 (2003), as *C. racemosa* var. *lamourouxii*; I.R.Price, *Austral. Syst. Bot.* 24: 181, fig. 9A–C, 182, fig. 10E (2011), as *C. racemosa* var. *lamourouxii*; G.S.Belton, W.F.Prud'homme van Reine, J.M.Huisman, S.G.A.Draisma & C.F.D.Gurgel, *J. Phycol.* 50: 41, fig. 5A–D (2014)..

Thallus grass-green or olive-green, spreading laterally to 30 cm, with smooth stolons 2–4 mm diam. Assimilators to 10 cm tall and 3–5 mm wide, fleshy, slightly to markedly flattened, with straight or sinuous margins, unbranched or sparingly branched, with ramuli arising from the smooth margins. Ramuli variable in occurrence, often absent, opposite to irregular, clavate, 5–10 mm long, to 3 mm wide at their rounded apices. Fig. 15D; Pl. 5D.

Only known from the tropical waters of the Indian Ocean and the Red Sea. In W.A. it occurs as far south as Ningaloo Reef; usually grows on sandy substrata in the shallow subtidal. One of the more common taxa of *Caulerpa*, particularly along the Pilbara coast.

Specimens examined: outer reef channel, Point Cloates, Ningaloo Reef, 6 m, 18 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90154 (GenBank: JN817683)]; Tantabiddi Ck, Exmouth, low-eulittoral pools, 21 Aug. 1979, *M.L.Cambridge* (PERTH 03986578); Tent Is., S side, Exmouth Gulf, 27 Aug. 1995, *B.Schaffelke* (PERTH 07166060); Perentie Is., Barrow Is., rock platform, 20 Sept. 2004, *J.M.Huisman* (PERTH 07177348); NW shore of Hermite Is., Montebello Is., 1–2 m, 12 July 1992, *J.M.Huisman* (PERTH 06468063), Lewis Is., Dampier Archipelago, on sand and rubble at 3 m, 28 Aug. 1984, *M.A.Borowitzka* (PERTH 06469175); Enderby Is., intertidal, 2 Sept. 1999, *J.M.Huisman* (PERTH 07134592); Eagle Hawk Is., Dampier Archipelago, 10–11 m, 3 Sept. 1999, *J.M.Huisman* (PERTH 07134762); False Cape Bossut, S of Broome,



intertidal reef platform, 20 June 2001, *J.M.Huisman & M.van Keulen* (PERTH 07117337); Cockatoo Is., intertidal coral mud flats on SW side, 16 Apr. 2004, *K.Wienczugow* (PERTH 07171439); E of Montgomery Is., Kimberley, 24 May 1987, *M.I.Blackwell* (PERTH 01664840); *loc. id.*, intertidal rock, 23 Oct. 2009, *R.R.M.Dixon* [PERTH 082923612 (GenBank: JN817682)]; *loc. id.*, intertidal rock, 21 Oct. 2009, *R.R.M.Dixon* [PERTH 08292639 (GenBank: JN817680)]; *loc. id.*, intertidal rock, 23 Oct. 2010, *J.M.Huisman* [PERTH 08292647 (GenBank: JN817681)]; Long Reef, Kimberley, 10 m, on rock, 20 Oct. 2010, *J.M.Huisman* [PERTH 082923620 (GenBank: JN817684)]; Cassini Is., Kimberley, 2 m, mid-littoral reef platform, 16 Oct. 2010, *J.M.Huisman* [PERTH 08428220 (GenBank: KF256084)].

Caulerpa lamourouxii had long been considered a variety of *C. racemosa* until Belton *et al.* (2014) showed it to be an independent and genetically distinct species. Those authors also found that *C. lamourouxii*-like specimens from Indonesia, New Caledonia, the Phillipines, eastern Australia and Japan were distinct from both *C. lamourouxii* and *C. racemosa*. The name *C. oligophylla* Mont. was reinstated for those populations (Belton *et al.*, 2014).

The occurrence of ramuli in *C. lamourouxii* is very variable; when present they are always marginal, but their frequency on individual assimilators ranges from absent (e.g. AD A90154) to arising in a regular opposite pattern (e.g. PERTH 01664840). However, when ramuli are present they are invariably clavate. When they are absent, *C. lamourouxii* can resemble *C. brachypus* (compare Pl. 5A and 5D), but the latter is more flattened and has minute marginal spines. Australian plants lacking ramuli have been commonly assigned to *C. racemosa* var. *lamourouxii* f. *requienii* (Mont.) Weber Bosse (Price, 2011). However, the type specimen of this form (PC 0124195, as *Herpochaeta requienii* Mont.) is more slender and elongate than those found in N.W.A. (see Wynne *et al.*, 2009: 295, fig. 7). Unfortunately, no specimen with f. *requienii*-type morphology from the type locality (Red Sea) has been sequenced, and its taxonomic status remains unresolved.

12. **Caulerpa lentillifera** J.Agardh, *Mus. Senckenberg.* 2: 173 (1837)

T: Ethiopia; holo: LD 16851.

Illustrations: A.Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg* 15: pl. 34, figs 1, 2 (1898); J.M.Huisman, *Mar. Pl. Australia* 253 (2000); G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 139, pl. 6C; 179, fig. 68A–C (2007); E.Coppejans, F.Leliaert, O.Dargent, R.Gunasekara & O.De Clerck, *Abc Taxa* 6: 107, fig. 82 (2009).

Thallus green, often slightly translucent when living, spreading laterally to 50 cm, with stolons 1.0–2.5 mm diam. Rachises unbranched or sparingly branched, to 9 cm tall, sparingly to rather densely covered with randomly arranged spherical ramuli 0.8–1.5 mm diam., often slightly elongate, the ramuli with a faint constriction between the stalk and the distal sphere. Fig. 15E; Pl. 5F.

Widespread in the tropical Indo-Pacific. In W.A. south to the Houtman Abrolhos Islands; associated with sandy substrata in the intertidal and shallow subtidal.

Specimens examined: Winderabandi, Ningaloo Reef, 2–3 m, 16 Aug. 1995, *J.M.Huisman* (PERTH 07117507); Mangrove Bay, Ningaloo Reef, 2–3 m, 19 Aug. 1995, *J.M.Huisman* (PERTH 07117442); Direction Is., epilithic, 1–2 m, 4 July 1998, *J.M.Huisman* (PERTH 07117949); Thevenard Is., reef exposed at low tide, 24 May 1960, *R.D.Royce 2017* (PERTH 03985334); Turtle Bay, Barrow Is., intertidal, 23 Nov. 1995, *J.M.Huisman* (PERTH 07118007); W of Hermite Is., Montebello Is., 1–6 m, 11 July 1992, *J.M.Huisman* (PERTH 06468144); S of Nelson Rocks, Dampier Archipelago, 6 m, 8 Sept. 1999, *J.M.Huisman* (PERTH 07117248); False Cape Bossut, S of Broome, intertidal reef platform, 20 June 2001, *J.M.Huisman & M.van Keulen* (PERTH 07117205); Mermaid Lagoon, Rowley Shoals, 12 m, 14 Dec. 2007, *J.M.Huisman* (PERTH 07729278); S end of North Maret Is., intertidal, 18 Apr. 2007, *J.M.Huisman* (PERTH 07643616); Montgomery Reef, Kimberley, intertidal rock, 22 Oct. 2009, *R.R.M.Dixon* [PERTH 08292655 (GenBank JN817649*)].

The differences between *C. lentillifera* and *C. agardhii* are discussed under the latter.

13. **Caulerpa macrodisca** Decne., *Ann. Sci. Nat., Bot.*, sér. 2, 17: 336 (1842)

Caulerpa peltata var. *macrodisca* (Decne.) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 376, pl. 28 (1898); *C. racemosa* var. *macrodisca* (Decne.) Weber Bosse, *Ann. Jard. Bot. Buitenzorg* 15: 376, pl. 28 (1898). T: Îles Anambas [Anambas Islands], Indonesia; lecto: Decaisne [1846 (1846–1864), pl. 1, fig. 1], *fide* I.R.Price, *Austral. Syst. Bot.* 24: 190 (2011).

Illustrations: E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 698

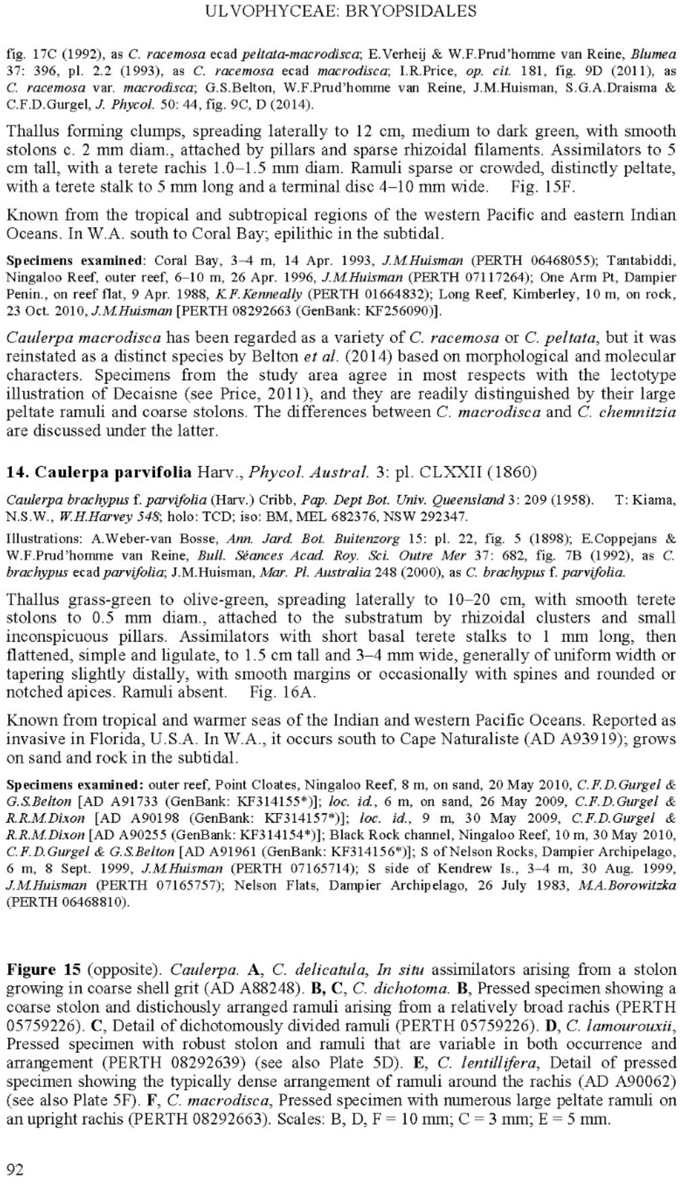


fig. 17C (1992), as *C. racemosa* ecad *peltata-macrodisca*; E.Verheij & W.F.Prud'homme van Reine, *Blumea* 37: 396, pl. 2.2 (1993), as *C. racemosa* ecad *macrodisca*; I.R.Price, *op. cit.* 181, fig. 9D (2011), as *C. racemosa* var. *macrodisca*; G.S.Belton, W.F.Prud'homme van Reine, J.M.Huisman, S.G.A.Draisma & C.F.D.Gurgel, *J. Phycol.* 50: 44, fig. 9C, D (2014).

Thallus forming clumps, spreading laterally to 12 cm, medium to dark green, with smooth stolons c. 2 mm diam., attached by pillars and sparse rhizoidal filaments. Assimilators to 5 cm tall, with a terete rachis 1.0–1.5 mm diam. Ramuli sparse or crowded, distinctly peltate, with a terete stalk to 5 mm long and a terminal disc 4–10 mm wide. Fig. 15F.

Known from the tropical and subtropical regions of the western Pacific and eastern Indian Oceans. In W.A. south to Coral Bay; epilithic in the subtidal.

Specimens examined: Coral Bay, 3–4 m, 14 Apr. 1993, *J.M.Huisman* (PERTH 06468055); Tantabiddi, Ningaloo Reef, outer reef, 6–10 m, 26 Apr. 1996, *J.M.Huisman* (PERTH 07117264); One Arm Pt, Dampier Penin., on reef flat, 9 Apr. 1988, *K.F.Kenneally* (PERTH 01664832); Long Reef, Kimberley, 10 m, on rock, 23 Oct. 2010, *J.M.Huisman* [PERTH 08292663 (GenBank: KF256090)].

Caulerpa macrodisca has been regarded as a variety of *C. racemosa* or *C. peltata*, but it was reinstated as a distinct species by Belton *et al.* (2014) based on morphological and molecular characters. Specimens from the study area agree in most respects with the lectotype illustration of Decaisne (see Price, 2011), and they are readily distinguished by their large peltate ramuli and coarse stolons. The differences between *C. macrodisca* and *C. chemnitzia* are discussed under the latter.

14. Caulerpa parvifolia Harv., *Phycol. Austral.* 3: pl. CLXXII (1860)

Caulerpa brachypus f. *parvifolia* (Harv.) Cribb, *Pap. Dept Bot. Univ. Queensland* 3: 209 (1958). T: Kiama, N.S.W., *W.H.Harvey 548*; holo: TCD; iso: BM, MEL 682376, NSW 292347.

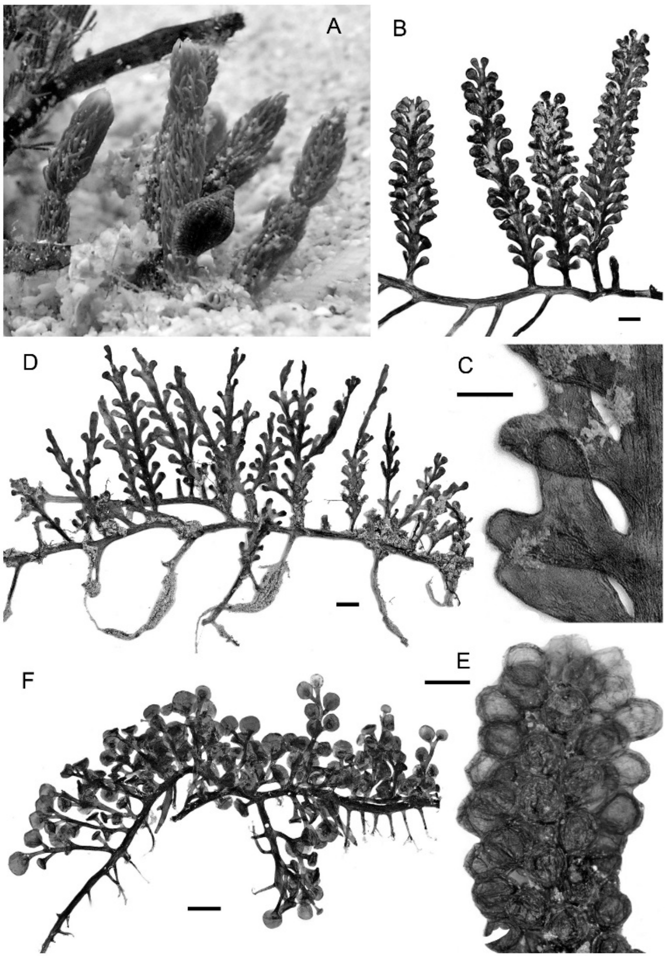
Illustrations: A.Weber-van Bosse, *Ann. Jard. Bot. Buitenzorg* 15: pl. 22, fig. 5 (1898); E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 682, fig. 7B (1992), as *C. brachypus* ecad *parvifolia*; J.M.Huisman, *Mar. Pl. Australia* 248 (2000), as *C. brachypus* f. *parvifolia*.

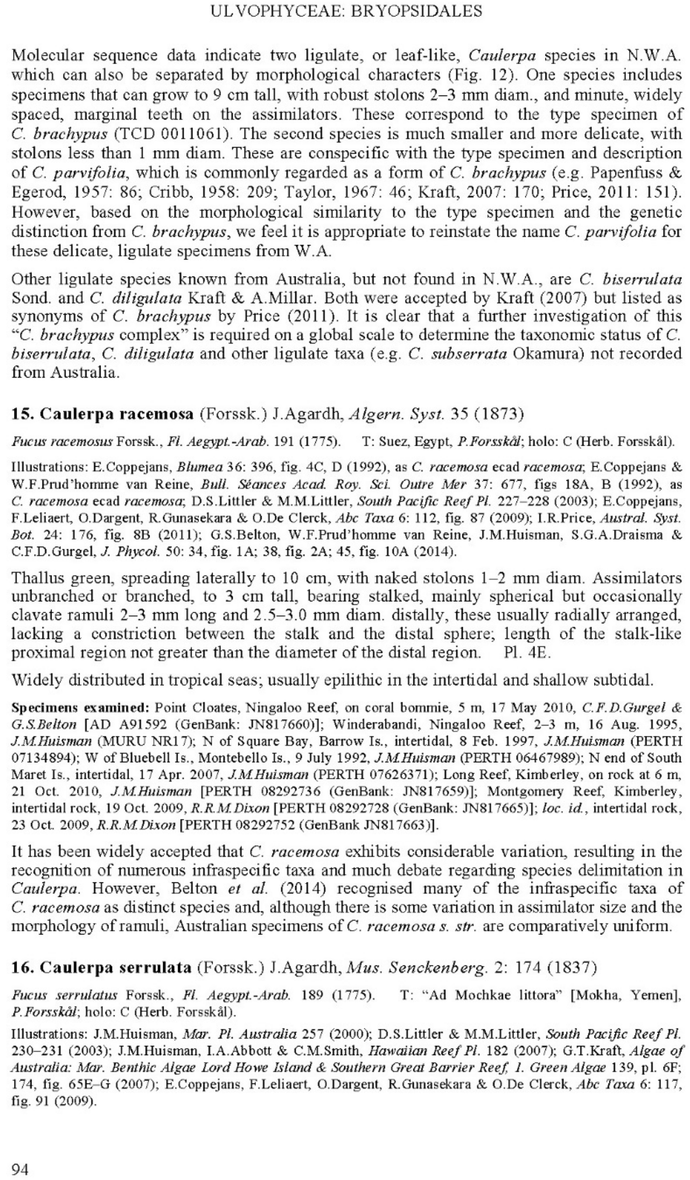
Thallus grass-green to olive-green, spreading laterally to 10–20 cm, with smooth terete stolons to 0.5 mm diam., attached to the substratum by rhizoidal clusters and small inconspicuous pillars. Assimilators with short basal terete stalks to 1 mm long, then flattened, simple and ligulate, to 1.5 cm tall and 3–4 mm wide, generally of uniform width or tapering slightly distally, with smooth margins or occasionally with spines and rounded or notched apices. Ramuli absent. Fig. 16A.

Known from tropical and warmer seas of the Indian and western Pacific Oceans. Reported as invasive in Florida, U.S.A. In W.A., it occurs south to Cape Naturaliste (AD A93919); grows on sand and rock in the subtidal.

Specimens examined: outer reef, Point Cloates, Ningaloo Reef, 8 m, on sand, 20 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91733 (GenBank: KF314155*)]; *loc. id.*, 6 m, on sand, 26 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90198 (GenBank: KF314157*)]; *loc. id.*, 9 m, 30 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90255 (GenBank: KF314154*)]; Black Rock channel, Ningaloo Reef, 10 m, 30 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91961 (GenBank: KF314156*)]; S of Nelson Rocks, Dampier Archipelago, 6 m, 8 Sept. 1999, *J.M.Huisman* (PERTH 07165714); S side of Kendrew Is., 3–4 m, 30 Aug. 1999, *J.M.Huisman* (PERTH 07165757); Nelson Flats, Dampier Archipelago, 26 July 1983, *M.A.Borowitzka* (PERTH 06468810).

Figure 15 (opposite). *Caulerpa*. **A**, *C. delicatula*, *In situ* assimilators arising from a stolon growing in coarse shell grit (AD A88248). **B, C**, *C. dichotoma*. **B**, Pressed specimen showing a coarse stolon and distichously arranged ramuli arising from a relatively broad rachis (PERTH 05759226). **C**, Detail of dichotomously divided ramuli (PERTH 05759226). **D**, *C. lamourouxii*, Pressed specimen with robust stolon and ramuli that are variable in both occurrence and arrangement (PERTH 08292639) (see also Plate 5D). **E**, *C. lentillifera*, Detail of pressed specimen showing the typically dense arrangement of ramuli around the rachis (AD A90062) (see also Plate 5F). **F**, *C. macrodisca*, Pressed specimen with numerous large peltate ramuli on an upright rachis (PERTH 08292663). Scales: B, D, F = 10 mm; C = 3 mm; E = 5 mm.


A
B
C
D
E
F



Molecular sequence data indicate two ligulate, or leaf-like, *Caulerpa* species in N.W.A. which can also be separated by morphological characters (Fig. 12). One species includes specimens that can grow to 9 cm tall, with robust stolons 2–3 mm diam., and minute, widely spaced, marginal teeth on the assimilators. These correspond to the type specimen of *C. brachypus* (TCD 0011061). The second species is much smaller and more delicate, with stolons less than 1 mm diam. These are conspecific with the type specimen and description of *C. parvifolia*, which is commonly regarded as a form of *C. brachypus* (e.g. Papenfuss & Egerod, 1957: 86; Cribb, 1958: 209; Taylor, 1967: 46; Kraft, 2007: 170; Price, 2011: 151). However, based on the morphological similarity to the type specimen and the genetic distinction from *C. brachypus*, we feel it is appropriate to reinstate the name *C. parvifolia* for these delicate, ligulate specimens from W.A.

Other ligulate species known from Australia, but not found in N.W.A., are *C. biserrulata* Sond. and *C. diligulata* Kraft & A.Millar. Both were accepted by Kraft (2007) but listed as synonyms of *C. brachypus* by Price (2011). It is clear that a further investigation of this "*C. brachypus* complex" is required on a global scale to determine the taxonomic status of *C. biserrulata*, *C. diligulata* and other ligulate taxa (e.g. *C. subserrata* Okamura) not recorded from Australia.

15. Caulerpa racemosa (Forssk.) J.Agardh, *Algern. Syst.* 35 (1873)

Fucus racemosus Forssk., *Fl. Aegypt.-Arab.* 191 (1775). T: Suez, Egypt, *P.Forsskål*; holo: C (Herb. Forsskål).

Illustrations: E.Coppejans, *Blumea* 36: 396, fig. 4C, D (1992), as *C. racemosa* ecad *racemosa*; E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 677, figs 18A, B (1992), as *C. racemosa* ecad *racemosa*; D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 227–228 (2003); E.Coppejans, F.Leliaert, O.Dargent, R.Gunasekara & O.De Clerck, *Abc Taxa* 6: 112, fig. 87 (2009); I.R.Price, *Austral. Syst. Bot.* 24: 176, fig. 8B (2011); G.S.Belton, W.F.Prud'homme van Reine, J.M.Huisman, S.G.A.Draisma & C.F.D.Gurgel, *J. Phycol.* 50: 34, fig. 1A; 38, fig. 2A; 45, fig. 10A (2014).

Thallus green, spreading laterally to 10 cm, with naked stolons 1–2 mm diam. Assimilators unbranched or branched, to 3 cm tall, bearing stalked, mainly spherical but occasionally clavate ramuli 2–3 mm long and 2.5–3.0 mm diam. distally, these usually radially arranged, lacking a constriction between the stalk and the distal sphere; length of the stalk-like proximal region not greater than the diameter of the distal region. Pl. 4E.

Widely distributed in tropical seas; usually epilithic in the intertidal and shallow subtidal.

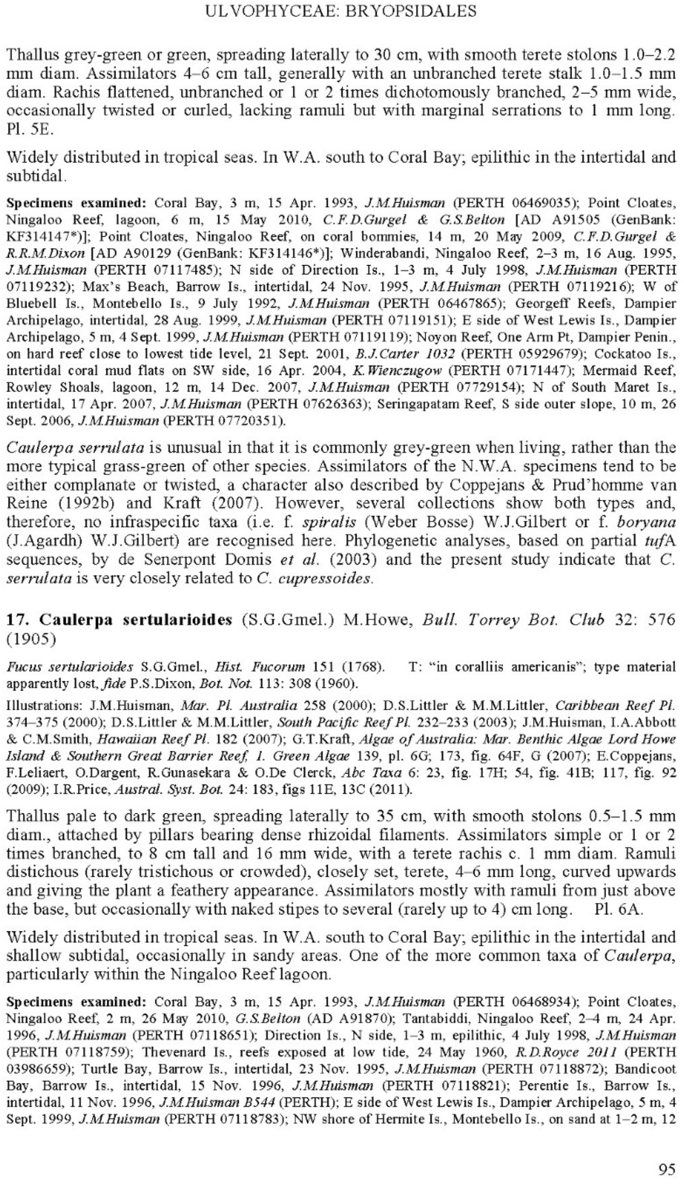
Specimens examined: Point Cloates, Ningaloo Reef, on coral bommie, 5 m, 17 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91592 (GenBank: JN817660)]; Winderabandi, Ningaloo Reef, 2–3 m, 16 Aug. 1995, *J.M.Huisman* (MURU NR17); N of Square Bay, Barrow Is., intertidal, 8 Feb. 1997, *J.M.Huisman* (PERTH 07134894); W of Bluebell Is., Montebello Is., 9 July 1992, *J.M.Huisman* (PERTH 06467989); N end of South Maret Is., intertidal, 17 Apr. 2007, *J.M.Huisman* (PERTH 07626371); Long Reef, Kimberley, on rock at 6 m, 21 Oct. 2010, *J.M.Huisman* [PERTH 08292736 (GenBank: JN817659)]; Montgomery Reef, Kimberley, intertidal rock, 19 Oct. 2009, *R.R.M.Dixon* [PERTH 08292728 (GenBank: JN817665)]; *loc. id.*, intertidal rock, 23 Oct. 2009, *R.R.M.Dixon* [PERTH 08292752 (GenBank JN817663)].

It has been widely accepted that *C. racemosa* exhibits considerable variation, resulting in the recognition of numerous infraspecific taxa and much debate regarding species delimitation in *Caulerpa*. However, Belton *et al.* (2014) recognised many of the infraspecific taxa of *C. racemosa* as distinct species and, although there is some variation in assimilator size and the morphology of ramuli, Australian specimens of *C. racemosa s. str.* are comparatively uniform.

16. Caulerpa serrulata (Forssk.) J.Agardh, *Mus. Senckenberg.* 2: 174 (1837)

Fucus serrulatus Forssk., *Fl. Aegypt.-Arab.* 189 (1775). T: "Ad Mochkae littora" [Mokha, Yemen], *P.Forsskål*; holo: C (Herb. Forsskål).

Illustrations: J.M.Huisman, *Mar. Pl. Australia* 257 (2000); D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 230–231 (2003); J.M.Huisman, I.A.Abbott & C.M.Smith, *Hawaiian Reef Pl.* 182 (2007); G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 139, pl. 6F; 174, fig. 65E–G (2007); E.Coppejans, F.Leliaert, O.Dargent, R.Gunasekara & O.De Clerck, *Abc Taxa* 6: 117, fig. 91 (2009).



ULVOPHYCEAE: BRYOPSIDALES

Thallus grey-green or green, spreading laterally to 30 cm, with smooth terete stolons 1.0–2.2 mm diam. Assimilators 4–6 cm tall, generally with an unbranched terete stalk 1.0–1.5 mm diam. Rachis flattened, unbranched or 1 or 2 times dichotomously branched, 2–5 mm wide, occasionally twisted or curled, lacking ramuli but with marginal serrations to 1 mm long. Pl. 5E.

Widely distributed in tropical seas. In W.A. south to Coral Bay; epilithic in the intertidal and subtidal.

Specimens examined: Coral Bay, 3 m, 15 Apr. 1993, *J.M.Huisman* (PERTH 06469035); Point Cloates, Ningaloo Reef, lagoon, 6 m, 15 May 2010, *C.F.D.Gurgel & G.S.Belton* [AD A91505 (GenBank: KF314147*)]; Point Cloates, Ningaloo Reef, on coral bommies, 14 m, 20 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90129 (GenBank: KF314146*)]; Winderabandi, Ningaloo Reef, 2–3 m, 16 Aug. 1995, *J.M.Huisman* (PERTH 07117485); N side of Direction Is., 1–3 m, 4 July 1998, *J.M.Huisman* (PERTH 07119232); Max's Beach, Barrow Is., intertidal, 24 Nov. 1995, *J.M.Huisman* (PERTH 07119216); W of Bluebell Is., Montebello Is., 9 July 1992, *J.M.Huisman* (PERTH 06467865); Georgeff Reefs, Dampier Archipelago, intertidal, 28 Aug. 1999, *J.M.Huisman* (PERTH 07119151); E side of West Lewis Is., Dampier Archipelago, 5 m, 4 Sept. 1999, *J.M.Huisman* (PERTH 07119119); Noyon Reef, One Arm Pt, Dampier Penin., on hard reef close to lowest tide level, 21 Sept. 2001, *B.J.Carter 1032* (PERTH 05929679); Cockatoo Is., intertidal coral mud flats on SW side, 16 Apr. 2004, *K.Wienczugow* (PERTH 07171447); Mermaid Reef, Rowley Shoals, lagoon, 12 m, 14 Dec. 2007, *J.M.Huisman* (PERTH 07729154); N of South Maret Is., intertidal, 17 Apr. 2007, *J.M.Huisman* (PERTH 07626363); Seringapatam Reef, S side outer slope, 10 m, 26 Sept. 2006, *J.M.Huisman* (PERTH 07720351).

Caulerpa serrulata is unusual in that it is commonly grey-green when living, rather than the more typical grass-green of other species. Assimilators of the N.W.A. specimens tend to be either complanate or twisted, a character also described by Coppejans & Prud'homme van Reine (1992b) and Kraft (2007). However, several collections show both types and, therefore, no infraspecific taxa (i.e. f. *spiralis* (Weber Bosse) W.J.Gilbert or f. *boryana* (J.Agardh) W.J.Gilbert) are recognised here. Phylogenetic analyses, based on partial *tuf*A sequences, by de Senerpont Domis *et al.* (2003) and the present study indicate that *C. serrulata* is very closely related to *C. cupressoides*.

17. Caulerpa sertularioides (S.G.Gmel.) M.Howe, *Bull. Torrey Bot. Club* 32: 576 (1905)

Fucus sertularioides S.G.Gmel., *Hist. Fucorum* 151 (1768). T: "in coralliis americanis"; type material apparently lost, *fide* P.S.Dixon, *Bot. Not.* 113: 308 (1960).

Illustrations: J.M.Huisman, *Mar. Pl. Australia* 258 (2000); D.S.Littler & M.M.Littler, *Caribbean Reef Pl.* 374–375 (2000); D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 232–233 (2003); J.M.Huisman, I.A.Abbott & C.M.Smith, *Hawaiian Reef Pl.* 182 (2007); G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 139, pl. 6G; 173, fig. 64F, G (2007); E.Coppejans, F.Leliaert, O.Dargent, R.Gunasekara & O.De Clerck, *Abc Taxa* 6: 23, fig. 17H; 54, fig. 41B; 117, fig. 92 (2009); I.R.Price, *Austral. Syst. Bot.* 24: 183, figs 11E, 13C (2011).

Thallus pale to dark green, spreading laterally to 35 cm, with smooth stolons 0.5–1.5 mm diam., attached by pillars bearing dense rhizoidal filaments. Assimilators simple or 1 or 2 times branched, to 8 cm tall and 16 mm wide, with a terete rachis c. 1 mm diam. Ramuli distichous (rarely tristichous or crowded), closely set, terete, 4–6 mm long, curved upwards and giving the plant a feathery appearance. Assimilators mostly with ramuli from just above the base, but occasionally with naked stipes to several (rarely up to 4) cm long. Pl. 6A.

Widely distributed in tropical seas. In W.A. south to Coral Bay; epilithic in the intertidal and shallow subtidal, occasionally in sandy areas. One of the more common taxa of *Caulerpa*, particularly within the Ningaloo Reef lagoon.

Specimens examined: Coral Bay, 3 m, 15 Apr. 1993, *J.M.Huisman* (PERTH 06468934); Point Cloates, Ningaloo Reef, 2 m, 26 May 2010, *G.S.Belton* (AD A91870); Tantabiddi, Ningaloo Reef, 2–4 m, 24 Apr. 1996, *J.M.Huisman* (PERTH 07118651); Direction Is., N side, 1–3 m, epilithic, 4 July 1998, *J.M.Huisman* (PERTH 07118759); Thevenard Is., reefs exposed at low tide, 24 May 1960, *R.D.Royce 2011* (PERTH 03986659); Turtle Bay, Barrow Is., intertidal, 23 Nov. 1995, *J.M.Huisman* (PERTH 07118872); Bandicoot Bay, Barrow Is., intertidal, 15 Nov. 1996, *J.M.Huisman* (PERTH 07118821); Perentie Is., Barrow Is., intertidal, 11 Nov. 1996, *J.M.Huisman B544* (PERTH); E side of West Lewis Is., Dampier Archipelago, 5 m, 4 Sept. 1999, *J.M.Huisman* (PERTH 07118783); NW shore of Hermite Is., Montebello Is., on sand at 1–2 m, 12

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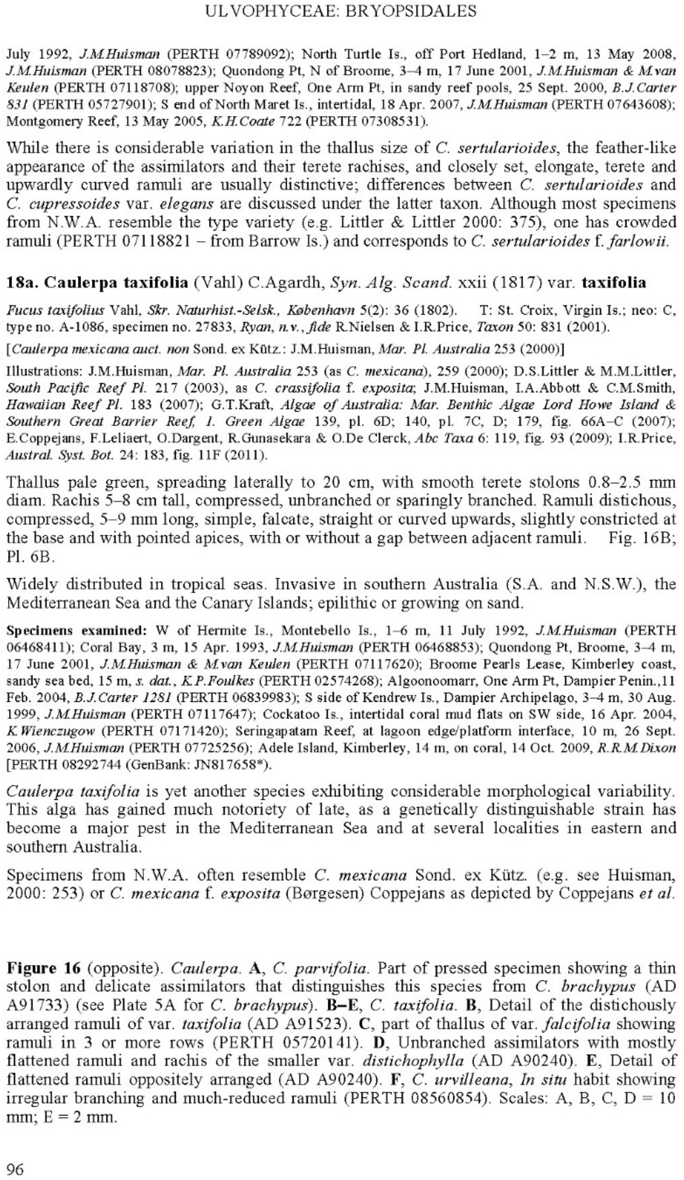


July 1992, *J.M.Huisman* (PERTH 07789092); North Turtle Is., off Port Hedland, 1–2 m, 13 May 2008, *J.M.Huisman* (PERTH 08078823); Quondong Pt, N of Broome, 3–4 m, 17 June 2001, *J.M.Huisman & M.van Keulen* (PERTH 07118708); upper Noyon Reef, One Arm Pt, in sandy reef pools, 25 Sept. 2000, *B.J.Carter 831* (PERTH 05727901); S end of North Maret Is., intertidal, 18 Apr. 2007, *J.M.Huisman* (PERTH 07643608); Montgomery Reef, 13 May 2005, *K.H.Coate* 722 (PERTH 07308531).

While there is considerable variation in the thallus size of *C. sertularioides*, the feather-like appearance of the assimilators and their terete rachises, and closely set, elongate, terete and upwardly curved ramuli are usually distinctive; differences between *C. sertularioides* and *C. cupressoides* var. *elegans* are discussed under the latter taxon. Although most specimens from N.W.A. resemble the type variety (e.g. Littler & Littler 2000: 375), one has crowded ramuli (PERTH 07118821 – from Barrow Is.) and corresponds to *C. sertularioides* f. *farlowii*.

18a. Caulerpa taxifolia (Vahl) C.Agardh, *Syn. Alg. Scand.* xxii (1817) var. taxifolia

Fucus taxifolius Vahl, *Skr. Naturhist.-Selsk., København* 5(2): 36 (1802). T: St. Croix, Virgin Is.; neo: C, type no. A-1086, specimen no. 27833, *Ryan, n.v., fide* R.Nielsen & I.R.Price, *Taxon* 50: 831 (2001).

[*Caulerpa mexicana auct. non* Sond. ex Kütz.: J.M.Huisman, *Mar. Pl. Australia* 253 (2000)]

Illustrations: J.M.Huisman, *Mar. Pl. Australia* 253 (as *C. mexicana*), 259 (2000); D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 217 (2003), as *C. crassifolia* f. *exposita*; J.M.Huisman, I.A.Abbott & C.M.Smith, *Hawaiian Reef Pl.* 183 (2007); G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 139, pl. 6D; 140, pl. 7C, D; 179, fig. 66A–C (2007); E.Coppejans, F.Leliaert, O.Dargent, R.Gunasekara & O.De Clerck, *Abc Taxa* 6: 119, fig. 93 (2009); I.R.Price, *Austral. Syst. Bot.* 24: 183, fig. 11F (2011).

Thallus pale green, spreading laterally to 20 cm, with smooth terete stolons 0.8–2.5 mm diam. Rachis 5–8 cm tall, compressed, unbranched or sparingly branched. Ramuli distichous, compressed, 5–9 mm long, simple, falcate, straight or curved upwards, slightly constricted at the base and with pointed apices, with or without a gap between adjacent ramuli. Fig. 16B; Pl. 6B.

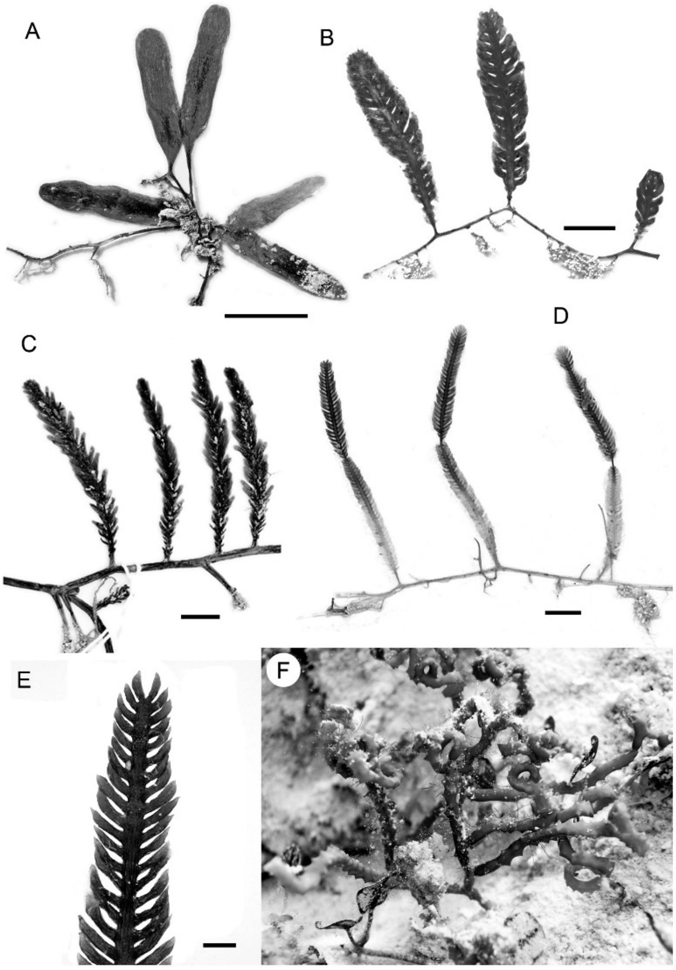
Widely distributed in tropical seas. Invasive in southern Australia (S.A. and N.S.W.), the Mediterranean Sea and the Canary Islands; epilithic or growing on sand.

Specimens examined: W of Hermite Is., Montebello Is., 1–6 m, 11 July 1992, *J.M.Huisman* (PERTH 06468411); Coral Bay, 3 m, 15 Apr. 1993, *J.M.Huisman* (PERTH 06468853); Quondong Pt, Broome, 3–4 m, 17 June 2001, *J.M.Huisman & M.van Keulen* (PERTH 07117620); Broome Pearls Lease, Kimberley coast, sandy sea bed, 15 m, *s. dat.*, *K.P.Foulkes* (PERTH 02574268); Algoonoomarr, One Arm Pt, Dampier Penin.,11 Feb. 2004, *B.J.Carter 1281* (PERTH 06839983); S side of Kendrew Is., Dampier Archipelago, 3–4 m, 30 Aug. 1999, *J.M.Huisman* (PERTH 07117647); Cockatoo Is., intertidal coral mud flats on SW side, 16 Apr. 2004, *K.Wienczugow* (PERTH 07171420); Seringapatam Reef, at lagoon edge/platform interface, 10 m, 26 Sept. 2006, *J.M.Huisman* (PERTH 07725256); Adele Island, Kimberley, 14 m, on coral, 14 Oct. 2009, *R.R.M.Dixon* [PERTH 08292744 (GenBank: JN817658*).

Caulerpa taxifolia is yet another species exhibiting considerable morphological variability. This alga has gained much notoriety of late, as a genetically distinguishable strain has become a major pest in the Mediterranean Sea and at several localities in eastern and southern Australia.

Specimens from N.W.A. often resemble *C. mexicana* Sond. ex Kütz. (e.g. see Huisman, 2000: 253) or *C. mexicana* f. *exposita* (Børgesen) Coppejans as depicted by Coppejans *et al.*

Figure 16 (opposite). *Caulerpa*. **A**, *C. parvifolia*. Part of pressed specimen showing a thin stolon and delicate assimilators that distinguishes this species from *C. brachypus* (AD A91733) (see Plate 5A for *C. brachypus*). **B–E**, *C. taxifolia*. **B**, Detail of the distichously arranged ramuli of var. *taxifolia* (AD A91523). **C**, part of thallus of var. *falcifolia* showing ramuli in 3 or more rows (PERTH 05720141). **D**, Unbranched assimilators with mostly flattened ramuli and rachis of the smaller var. *distichophylla* (AD A90240). **E**, Detail of flattened ramuli oppositely arranged (AD A90240). **F**, *C. urvilleana*, *In situ* habit showing irregular branching and much-reduced ramuli (PERTH 08560854). Scales: A, B, C, D = 10 mm; E = 2 mm.


A
B
C
D
E
F

(2009) and Littler & Littler (2003: 217, as *C. crassifolia* f. *exposita* Børgesen), but all were referable to *C. taxifolia* based on DNA sequence analyses (Fig. 12). Furthermore, extensive DNA barcoding of *Caulerpa* from eastern Australia has failed to detect *C. mexicana* (C.F.D.Gurgel, *pers. comm.*), and it is likely that the species is absent from the Australian marine flora.

18b. Caulerpa taxifolia var. **distichophylla** (Sond.) Verlaque, Huisman & Procaccini, *in* Jongma *et al.*, *Bot. Mar.* 56: 36 (2013)

Caulerpa distichophylla Sond., *Bot. Zeitung (Berlin)* 3: 50 (1845). T: W.A. [probably near Fremantle], *J.A.L.Preiss*; holo: MEL 503814.

Illustrations: H.B.S.Womersley, *Mar. Benthic Fl. Southern Australia* 1: 257–258, fig. 86C (1984); J.M.Huisman, *Mar. Pl. Australia* 255 (2000), both as *C. distichophylla*; D.N.Jongma, D.Campa, E.Dattolo, D.D'Esposito, A.Duchi, P.Grewe, J.M.Huisman, M.Verlaque, M.B.Yokes & G.Procaccini, *op. cit.* 32, figs 2–8.

Thallus medium to dark green, spreading laterally to 15 cm, with smooth slender stolons 0.5–1.0 mm diam. Assimilators to 8 cm tall, simple to several times branched. Rachis basally terete, becoming distally compressed, 0.5–1.0 mm wide, bearing opposite distichously arranged closely adjacent ramuli. Ramuli flattened, 1–2 (–3) mm long, slightly upwardly curved and broadest (0.2–0.5 mm) just above the slightly constricted base, tapering to a broad and pointed apex. Fig. 16D, E.

In W.A., this species is known from Ningaloo Reef south to Albany (AD A51659); invasive in Sicily and Turkey.

Specimen examined: Point Cloates, Ningaloo Reef, sandy bottom, 6 m, 28 May 2009, *C.F.D.Gurgel & R.R.M.Dixon* [AD A90240 (GenBank: KF314165*)].

Caulerpa taxifolia var. *distichophylla* was previously thought to be an independent species (*C. distichophylla*) confined to temperate waters in Western Australia. However, while investigating the identity and origin of slender *C. taxifolia* populations in Sicily and Turkey, Jongma *et al.* (2012) found *C. distichophylla* and *C. taxifolia* to be genetically indistinguishable. Data from the present N.W.A. study agrees with their findings and supports the reduction of *C. distichophylla* to a slender and more delicate variety of *C. taxifolia*.

18c. Caulerpa taxifolia var. **falcifolia** (Harv. & J.W.Bailey) W.R.Taylor, *Contr. Univ. Michigan Herb.* 11: 77 (1975)

Caulerpa falcifolia Harv. & J.W.Bailey, *Proc. Boston Soc. Nat. Hist.* 3: 373 (1851). T: Friendly Islands [Tonga], July–Oct. 1855; holo: TCD [W.H.Harvey, *Friendly Islands Algae* No. 70]; iso: NSW 292355.

Caulerpa taxifolia f. *tristichophylla* Sved., *Ceylon Mar. Biol. Rep.* 2: 112 (1906). T: Paumben, Rameswaran Is., South India, 3 Apr. 1903, *N.Svedelius*; holo: presumably UPS *n.v.*, *fide* N.Svedelius, *op. cit.* 113, fig. 5.

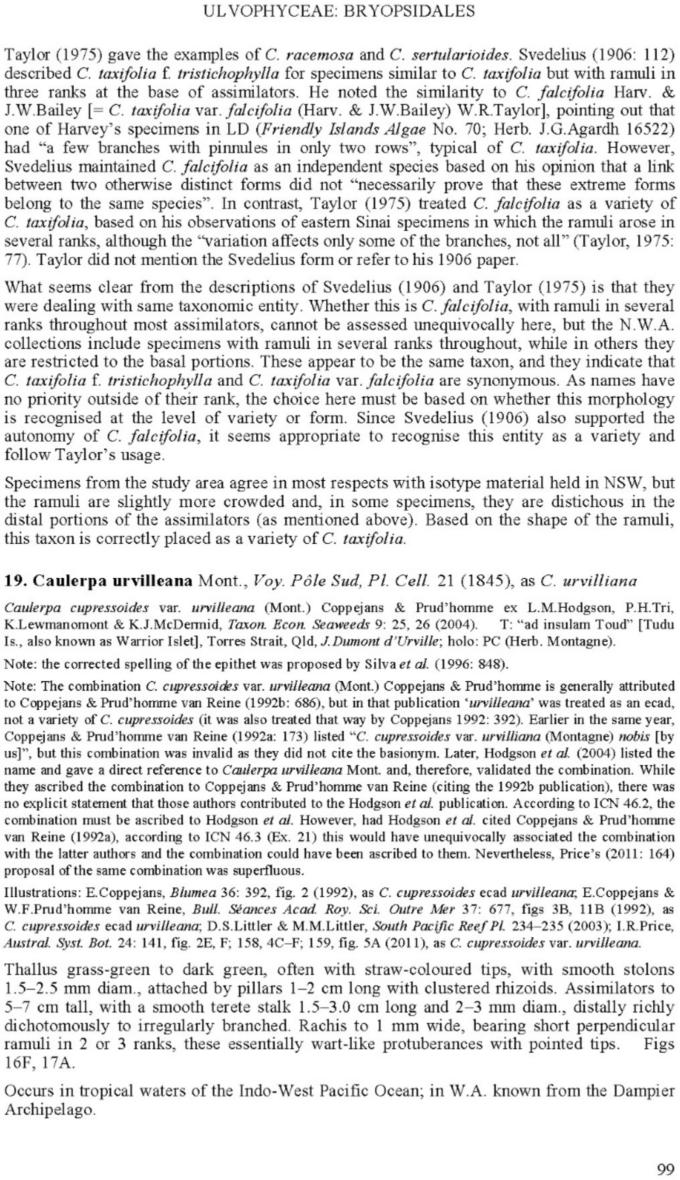
Illustrations: J.W.Bailey & W.H.Harvey, *U.S. Explor. Exped., Atlas to vol. 17, Algae* pl. 8, figs 4, 5 (1862); I.R.Price, *Austral. Syst. Bot.* 24: 198, fig. 12A (2011).

Thallus medium to dark green, spreading laterally to 25 cm, with smooth stolons 1–2 mm diam., attached by short or long pillars and dense rhizoidal filaments. Assimilators to 7 cm tall, simple or occasionally branched, bearing ramuli in 3 or more rows, or basally in several rows but distally bilateral. Ramuli flattened, 3–6 mm long, to 1 mm wide, constricted at the base, mostly linear and slightly curved upwardly, with broadly rounded apices each with a terminal spine. Fig. 16C.

Known from Egypt, India, Sri Lanka, Indonesia, Tonga, Qld and N.W.A.; inhabits intertidal sand flats.

Specimens examined: Adele Is., on fringing reef at low tide, 8 July 1990, *K.F.Kenneally 11027* (PERTH 01210084); Algoonoomarr Reef, One Arm Pt, on sandy reef flat, 13 Dec. 2000, *B.J.Carter 901* (PERTH 05720141).

As noted by Svedelius (1906: 113) and Taylor (1975: 77), several typically bilateral species of *Caulerpa* include variants in which the ramuli are arranged on all sides of the rachis, for which



Taylor (1975) gave the examples of *C. racemosa* and *C. sertularioides*. Svedelius (1906: 112) described *C. taxifolia* f. *tristichophylla* for specimens similar to *C. taxifolia* but with ramuli in three ranks at the base of assimilators. He noted the similarity to *C. falcifolia* Harv. & J.W.Bailey [= *C. taxifolia* var. *falcifolia* (Harv. & J.W.Bailey) W.R.Taylor], pointing out that one of Harvey's specimens in LD (*Friendly Islands Algae* No. 70; Herb. J.G.Agardh 16522) had "a few branches with pinnules in only two rows", typical of *C. taxifolia*. However, Svedelius maintained *C. falcifolia* as an independent species based on his opinion that a link between two otherwise distinct forms did not "necessarily prove that these extreme forms belong to the same species". In contrast, Taylor (1975) treated *C. falcifolia* as a variety of *C. taxifolia*, based on his observations of eastern Sinai specimens in which the ramuli arose in several ranks, although the "variation affects only some of the branches, not all" (Taylor, 1975: 77). Taylor did not mention the Svedelius form or refer to his 1906 paper.

What seems clear from the descriptions of Svedelius (1906) and Taylor (1975) is that they were dealing with same taxonomic entity. Whether this is *C. falcifolia*, with ramuli in several ranks throughout most assimilators, cannot be assessed unequivocally here, but the N.W.A. collections include specimens with ramuli in several ranks throughout, while in others they are restricted to the basal portions. These appear to be the same taxon, and they indicate that *C. taxifolia* f. *tristichophylla* and *C. taxifolia* var. *falcifolia* are synonymous. As names have no priority outside of their rank, the choice here must be based on whether this morphology is recognised at the level of variety or form. Since Svedelius (1906) also supported the autonomy of *C. falcifolia*, it seems appropriate to recognise this entity as a variety and follow Taylor's usage.

Specimens from the study area agree in most respects with isotype material held in NSW, but the ramuli are slightly more crowded and, in some specimens, they are distichous in the distal portions of the assimilators (as mentioned above). Based on the shape of the ramuli, this taxon is correctly placed as a variety of *C. taxifolia*.

19. **Caulerpa urvilleana** Mont., *Voy. Pôle Sud, Pl. Cell.* 21 (1845), as *C. urvilliana*

Caulerpa cupressoides var. *urvilleana* (Mont.) Coppejans & Prud'homme ex L.M.Hodgson, P.H.Tri, K.Lewmanomont & K.J.McDermid, *Taxon. Econ. Seaweeds* 9: 25, 26 (2004). T: "ad insulam Toud" [Tudu Is., also known as Warrior Islet], Torres Strait, Qld, *J.Dumont d'Urville*; holo: PC (Herb. Montagne).

Note: the corrected spelling of the epithet was proposed by Silva *et al.* (1996: 848).

Note: The combination *C. cupressoides* var. *urvilleana* (Mont.) Coppejans & Prud'homme is generally attributed to Coppejans & Prud'homme van Reine (1992b: 686), but in that publication '*urvilleana*' was treated as an ecad, not a variety of *C. cupressoides* (it was also treated that way by Coppejans 1992: 392). Earlier in the same year, Coppejans & Prud'homme van Reine (1992a: 173) listed "*C. cupressoides* var. *urvilliana* (Montagne) *nobis* [by us]", but this combination was invalid as they did not cite the basionym. Later, Hodgson *et al.* (2004) listed the name and gave a direct reference to *Caulerpa urvilleana* Mont. and, therefore, validated the combination. While they ascribed the combination to Coppejans & Prud'homme van Reine (citing the 1992b publication), there was no explicit statement that those authors contributed to the Hodgson *et al.* publication. According to ICN 46.2, the combination must be ascribed to Hodgson *et al.* However, had Hodgson *et al.* cited Coppejans & Prud'homme van Reine (1992a), according to ICN 46.3 (Ex. 21) this would have unequivocally associated the combination with the latter authors and the combination could have been ascribed to them. Nevertheless, Price's (2011: 164) proposal of the same combination was superfluous.

Illustrations: E.Coppejans, *Blumea* 36: 392, fig. 2 (1992), as *C. cupressoides* ecad *urvilleana*; E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 677, figs 3B, 11B (1992), as *C. cupressoides* ecad *urvilleana*; D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 234–235 (2003); I.R.Price, *Austral. Syst. Bot.* 24: 141, fig. 2E, F; 158, 4C–F; 159, fig. 5A (2011), as *C. cupressoides* var. *urvilleana*.

Thallus grass-green to dark green, often with straw-coloured tips, with smooth stolons 1.5–2.5 mm diam., attached by pillars 1–2 cm long with clustered rhizoids. Assimilators to 5–7 cm tall, with a smooth terete stalk 1.5–3.0 cm long and 2–3 mm diam., distally richly dichotomously to irregularly branched. Rachis to 1 mm wide, bearing short perpendicular ramuli in 2 or 3 ranks, these essentially wart-like protuberances with pointed tips. Figs 16F, 17A.

Occurs in tropical waters of the Indo-West Pacific Ocean; in W.A. known from the Dampier Archipelago.

Specimen examined: East Lewis Is., Dampier Archipelago, shallow bay to the N, on sand with underlying limestone, 2 m, 27 Oct. 1983, *M.A.Borowitzka* (PERTH 06468748).

The ramuli of *C. urvilleana* are greatly reduced and often perpendicular to the rachis. Although Coppejans (1992: 391) and Coppejans & Prud'homme van Reine (1992b: 686) treated this taxon as an 'ecad' of *C. cupressoides*, Coppejans *et al.* (2001) recognised it as an independent species without giving an explanation for the change of status. More recently, the phylogenetic trees of Sauvage *et al.* (2013) demonstrated the independence of *C. urvilleana*. Unfortunately, we have been unable to obtain molecular data for N.W.A. specimens but, based on the data of Sauvage *et al.* (2013), and the two *C. urvilleana* sequences (FM956067 from Indonesia and FM956068 from the Phillipines) added to our dataset (Fig. 12), we see no reason to doubt the status of *C. urvilleana* as a distinct species.

20. Caulerpa verticillata J.Agardh, *Öfvers Förh. Kongl. Svenska Vetensk.-Akad.* 4: 6 (1847)

T: "Ad insulas Indiae occidentalis" [West Indies], *fide* J.Agardh (1873: 7); syn: LD (Herb. Agardh).

Illustrations: E.Coppejans & W.F.Prud'homme van Reine, *Bull. Séances Acad. Roy. Sci. Outre Mer* 37: 705, fig. 21B (1992); D.S.Littler & M.M.Littler, *Caribbean Reef Pl.* 376–377 (2000); D.S.Littler & M.M.Littler, *South Pacific Reef Pl.* 234–235 (2003); E.Coppejans, F.Leliaert, O.Dargent, R.Gunasekara & O.De Clerck, *Abc Taxa* 6: 29, fig. 22D; 48, fig. 36H; 119, fig. 94 (2009); I.R.Price, *Austral. Syst. Bot.* 24: 198, fig. 12C, D (2011).

Thallus green, spreading laterally to 9 cm, forming dense low turfs or mats, with smooth terete stolons 250–300 µm diam., attached to the substratum by rhizoids 20–30 µm diam. Rachis 1–5 cm tall, 140–180 µm diam., bearing loosely whorled terete ramuli 2.5–3.0 mm long and 5–6 times dichotomously branched. Ramuli 75–90 µm diam. near the base, tapering gradually to 25–30 µm near the obtuse or pointed and often closely forked apices. Fig. 17B; Pl. 6C.

Widely distributed in warmer waters of the Indian and Pacific Oceans and the Caribbean Sea; epilithic on sand-covered rock in the shallow subtidal.

Specimens examined: NW shore of Hermite Is., Montebello Is., 12 July 1992, *J.M.Huisman* (PERTH 06468446); S side of Kendrew Is., Dampier Archipelago, 3–4 m, 30 Aug. 1999, *J.M.Huisman* (PERTH 07117159); Huay Is., Dampier Archipelago, 2 m, 23 Oct. 1998, *J.M.Huisman* (PERTH 07121296); Quondong Pt, Broome, 2 m, 16 June 2001, *J.M.Huisman & M.van Keulen* (PERTH 07117167); Seringapatam Reef, at lagoon edge/platform interface, intertidal, 26 Sept. 2006, *J.M.Huisman* (PERTH 07725205); Cassini Is., N Kimberley, 20 m, 18 Oct. 2010, *C.Bryce* [PERTH 08399654 (GenBank: KF314137*)].

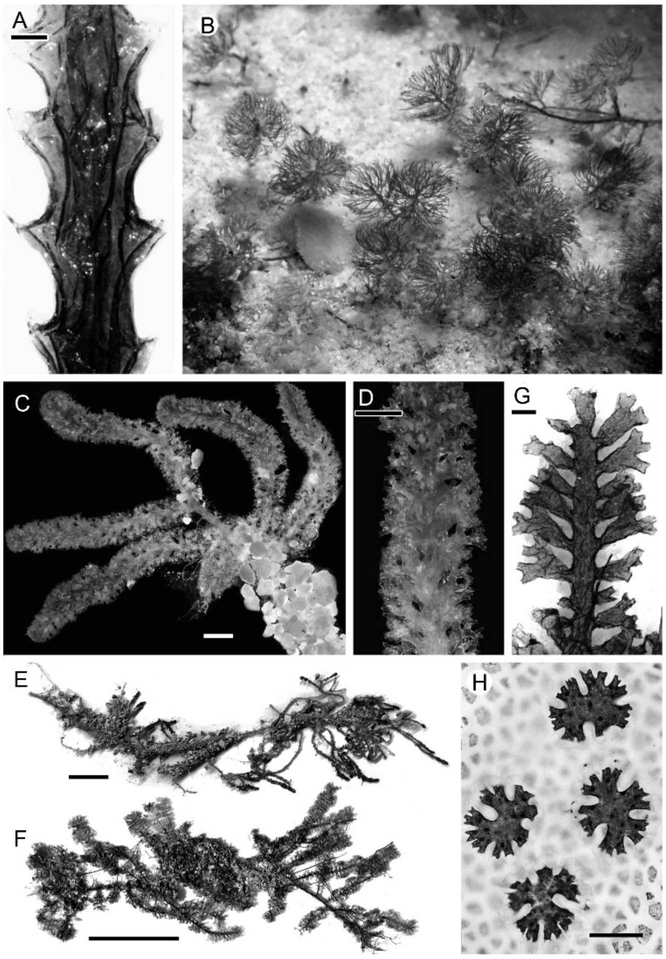
21. Caulerpa webbiana Mont., *Ann. Sci. Nat., Bot.*, sér. 2, 8: 354 (1837)

T: Arrecife, Isla Lanzarote [Canary Islands], *P.B.Webb & S.Berthelot*; holo: PC (Herb. Montagne); possible iso: BM, MEL 690464, PC (Herb. Montagne), TCD (Herb. Montagne).

Illustrations: J.M.Huisman, *Mar. Pl. Australia* 259 (2000); J.M.Huisman, I.A.Abbott & C.M.Smith, *Hawaiian Reef Pl.* 183 (2007); G.T.Kraft, *Algae of Australia: Mar. Benthic Algae Lord Howe Island & Southern Great Barrier Reef, 1. Green Algae* 139, pl. 6H; 180, fig. 67A–C, E (2007); I.R.Price, *Austral. Syst. Bot.* 24: 198, fig. 12E, G; 199, fig. 13F (2011).

Thallus bright green, spreading laterally to 12 cm, very low-growing and forming dense clumps, with slender stolons c. 0.5 mm diam. and a dense covering of sand-binding rhizoids. Rachis terete, to 2.5 cm tall, 200–250 µm diam., covered by whorls of (2–) 4 (–5) times dichotomously branched ramuli 450–600 µm long and closely set; apices of ramuli sharply pointed and occluded. Fig. 17C–G.

Figure 17 (opposite). *Caulerpa* and *Blastophysa*. **A**, *C. urvilleana*, Detail of wart-like ramuli arising perpendicular from the rachis (PERTH 08560854). **B**, *C. verticillata*, At 10 m depth at Seringapatam Reef (see also Plate 6C). **C–G**, *C. webbiana*. **C**, Wet habit showing the typical radial arrangement of multifid ramuli (PERTH 07788827). **D**, Detail of branched ramuli (PERTH 07788827). **E**, Pressed specimen showing habit of specimen with radially arranged ramuli (PERTH 07118627). **F**, Habit of plant with distichously arranged ramuli (AD A91788). **G**, Detail of branched ramuli of distichously arranged specimen (AD A91788). **H**, *Blastophysa rhizopus* thalli *in situ*. Scales: A, C = 1 mm; D, G = 0.2 mm; E, F = 10 mm; H = 50 µm.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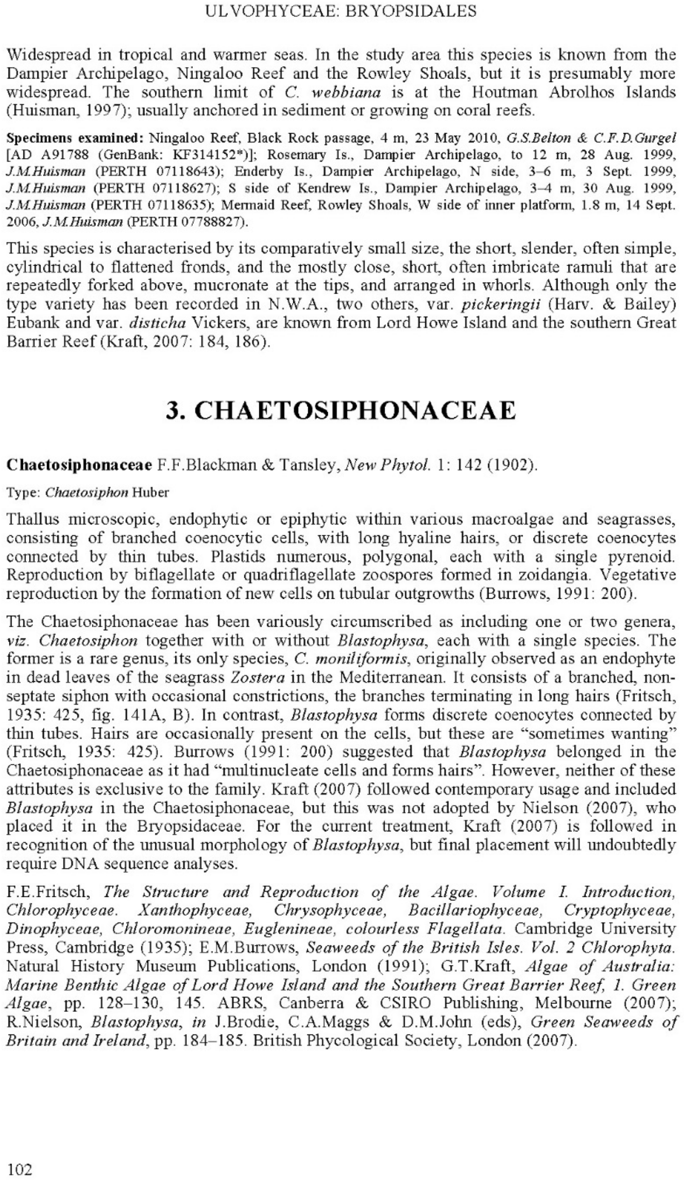

Widespread in tropical and warmer seas. In the study area this species is known from the Dampier Archipelago, Ningaloo Reef and the Rowley Shoals, but it is presumably more widespread. The southern limit of *C. webbiana* is at the Houtman Abrolhos Islands (Huisman, 1997); usually anchored in sediment or growing on coral reefs.

Specimens examined: Ningaloo Reef, Black Rock passage, 4 m, 23 May 2010, *G.S.Belton & C.F.D.Gurgel* [AD A91788 (GenBank: KF314152*)]; Rosemary Is., Dampier Archipelago, to 12 m, 28 Aug. 1999, *J.M.Huisman* (PERTH 07118643); Enderby Is., Dampier Archipelago, N side, 3–6 m, 3 Sept. 1999, *J.M.Huisman* (PERTH 07118627); S side of Kendrew Is., Dampier Archipelago, 3–4 m, 30 Aug. 1999, *J.M.Huisman* (PERTH 07118635); Mermaid Reef, Rowley Shoals, W side of inner platform, 1.8 m, 14 Sept. 2006, *J.M.Huisman* (PERTH 07788827).

This species is characterised by its comparatively small size, the short, slender, often simple, cylindrical to flattened fronds, and the mostly close, short, often imbricate ramuli that are repeatedly forked above, mucronate at the tips, and arranged in whorls. Although only the type variety has been recorded in N.W.A., two others, var. *pickeringii* (Harv. & Bailey) Eubank and var. *disticha* Vickers, are known from Lord Howe Island and the southern Great Barrier Reef (Kraft, 2007: 184, 186).

3. CHAETOSIPHONACEAE

Chaetosiphonaceae F.F.Blackman & Tansley, *New Phytol.* 1: 142 (1902).

Type: *Chaetosiphon* Huber

Thallus microscopic, endophytic or epiphytic within various macroalgae and seagrasses, consisting of branched coenocytic cells, with long hyaline hairs, or discrete coenocytes connected by thin tubes. Plastids numerous, polygonal, each with a single pyrenoid. Reproduction by biflagellate or quadriflagellate zoospores formed in zoidangia. Vegetative reproduction by the formation of new cells on tubular outgrowths (Burrows, 1991: 200).

The Chaetosiphonaceae has been variously circumscribed as including one or two genera, *viz. Chaetosiphon* together with or without *Blastophysa*, each with a single species. The former is a rare genus, its only species, *C. moniliformis*, originally observed as an endophyte in dead leaves of the seagrass *Zostera* in the Mediterranean. It consists of a branched, non-septate siphon with occasional constrictions, the branches terminating in long hairs (Fritsch, 1935: 425, fig. 141A, B). In contrast, *Blastophysa* forms discrete coenocytes connected by thin tubes. Hairs are occasionally present on the cells, but these are "sometimes wanting" (Fritsch, 1935: 425). Burrows (1991: 200) suggested that *Blastophysa* belonged in the Chaetosiphonaceae as it had "multinucleate cells and forms hairs". However, neither of these attributes is exclusive to the family. Kraft (2007) followed contemporary usage and included *Blastophysa* in the Chaetosiphonaceae, but this was not adopted by Nielson (2007), who placed it in the Bryopsidaceae. For the current treatment, Kraft (2007) is followed in recognition of the unusual morphology of *Blastophysa*, but final placement will undoubtedly require DNA sequence analyses.

F.E.Fritsch, *The Structure and Reproduction of the Algae. Volume I. Introduction, Chlorophyceae. Xanthophyceae, Chrysophyceae, Bacillariophyceae, Cryptophyceae, Dinophyceae, Chloromonineae, Euglenineae, colourless Flagellata*. Cambridge University Press, Cambridge (1935); E.M.Burrows, *Seaweeds of the British Isles. Vol. 2 Chlorophyta*. Natural History Museum Publications, London (1991); G.T.Kraft, *Algae of Australia: Marine Benthic Algae of Lord Howe Island and the Southern Great Barrier Reef, 1. Green Algae*, pp. 128–130, 145. ABRS, Canberra & CSIRO Publishing, Melbourne (2007); R.Nielson, *Blastophysa*, *in* J.Brodie, C.A.Maggs & D.M.John (eds), *Green Seaweeds of Britain and Ireland*, pp. 184–185. British Phycological Society, London (2007).

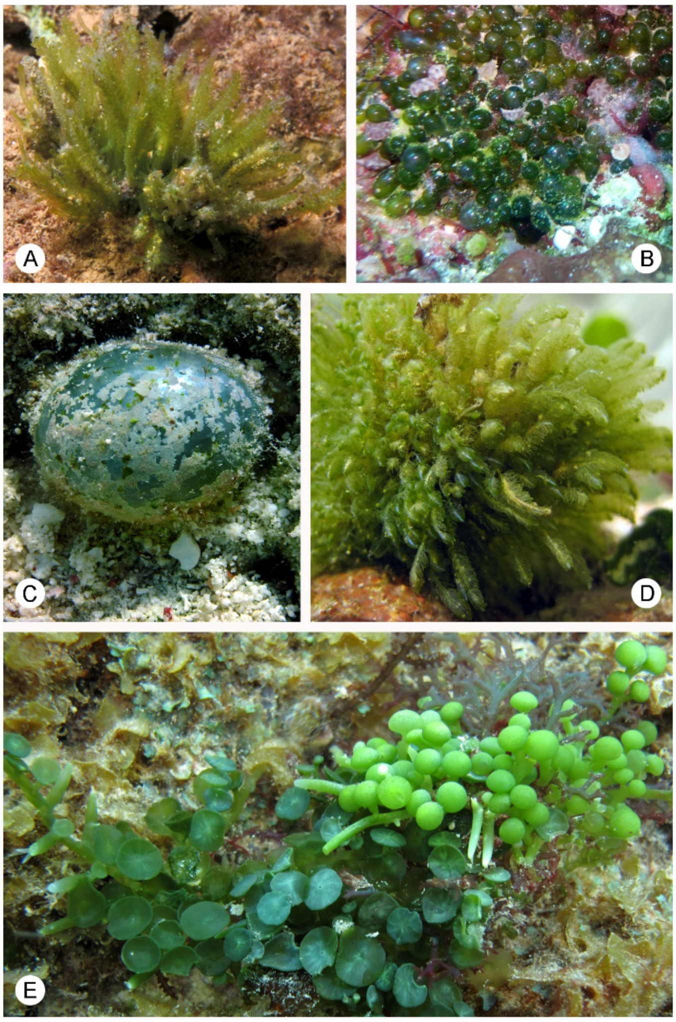

Plate 4. Cladophorales (*Siphonocladus* and *Valonia*) and Bryopsidales (*Bryopsis* and *Caulerpa*).

Plate 5. Bryopsidales: *Caulerpa*.

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Plate 6. Bryopsidales: *Avrainvillea*, *Caulerpa* and *Codium*.

Chapter 4: A taxonomic reassessment of Caulerpa (Chlorophyta, Caulerpaceae) in southern Australia based on tufA and rbcL sequence data.

Statement of Authorship

A taxonomic reassessment of *Caulerpa* (Chlorophyta, Caulerpaceae) in southern Australia based on *tuf*A and *rbc*L sequence data. *Phycologia.*

Belton, G. (Candidate)
Contributed to initial manuscript conceptualization, wrote the manuscript, collected specimens, generated and analyzed data, acted as lead author in drafting initial manuscript, co-ordinated addressing of reviewers comments, prepared figures and tables.

I hereby certify that the statement of contribution is accurate.

Signed… ……………………Date…………………………

Prud'homme van Reine, W. F.
Contributed to initial manuscript conceptualization and initial drafting, collected specimens, provided extensive nomenclature advice, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

Signed……………… …………………Date 6/8/2016

Huisman, J. M.
Contributed to initial manuscript conceptualization and initial drafting, collected specimens, provided extensive nomenclature advice, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

Signed…… ……………Date…………………………

Draisma, S. G. A.
Collected specimens and generated data, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

Signed……………. …………………Date…………………………

Gurgel, C. F. D.
Contributed to initial manuscript conceptualization and initial drafting, collected specimens, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Gareth Belton PhD thesis.

Signed…… ……………………Date…………………………

Chapter 4

A taxonomic reassessment of *Caulerpa* (Chlorophyta, Caulerpaceae) in southern Australia based on *tuf*A and *rbc*L sequence data

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Running title: Southern Australia *Caulerpa*

Key words: Australia, Bryopsidales, *Caulerpa*, *Caulerpa coppejansii, Caulerpa crispata*, *Caulerpa lucasii, Caulerpa muelleri, Caulerpa parvifolia,* green algae, molecular phylogeny, *rbc*L, species delimitation, *tuf*A.

ABSTRACT

Although recent molecular based taxonomic investigations of the green algal genus *Caulerpa* have shown a mismatch between morphological and molecular species concepts, no studies have specifically focused on the species rich *Caulerpa* flora of southern Australia. The present study investigated the molecular variation of *Caulerpa* using *tuf*A and *rbc*L DNA sequences of specimens from 24 of the 29 taxa known from the region. Based on both molecular and morphological data, *Caulerpa coppejansii* sp. nov. and *Caulerpa crispata* stat. nov. are described; and *Caulerpa muelleri* and *Caulerpa parvifolia* are reinstated. A new name, *Caulerpa lucasii* nom. nov., is proposed for *Caulerpa annulata*. *Caulerpa corynephora* and *Caulerpa fergusonii* are newly reported from the region and a newly established population of *Caulerpa cylindracea* from Portland, Victoria is also reported. Reference *tuf*A sequences, an updated nomenclature and identification key for southern Australian *Caulerpa* species are provided.

INTRODUCTION

The green algal genus *Caulerpa* J.V. Lamouroux is one of the most widespread, diverse and conspicuous green macroalgal genera in the world. Nowhere is this diversity more evident than in the temperate and floristically rich waters of southern Australia, where *Caulerpa* species can grow to over 50 cm in height and cover large areas of reef as monospecific stands (Edgar 2008). The taxonomy of

Caulerpa in southern Australia has a history dating back more than 200 years, since the first species description based on specimens collected by R. Brown and described by Turner in 1809–1811 (as *Fucus cactoides* R. Brown *ex* Turner; *F. hypnoides* R. Brown *ex* Turner; *F. scalpelliformis* R. Brown *ex* Turner; *F. sedoides* R. Brown *ex* Turner; *F. simpliciusculus* R. Brown *ex* Turner). Other notable works on the genus include several new species described by Harvey (1858–1863) and J. Agardh (1873), through to the more recent account by Womersley (1984). Womersley (1984) provided the most comprehensive and complete work on the genus in southern Australia to date, listing 19 species, two varieties and one forma. Since then, populations of *C. taxifolia* (H. West) C. Agardh have become established in South Australia (Womersley 2003) and New South Wales (Schaffelke *et al.* 2002). Furthermore, five taxa have been listed (Huisman & Walker 1990; Huisman 2000) or described (Carruthers *et al.* 1993; Price *et al.* 1998) from the region, whilst others have had their taxonomic status changed (Millar & Kraft 1994; Silva *et al.* 1996; Price 2011; Jongma *et al.* 2012; Belton *et al.* accepted). As it currently stands, there are 24 species (17 of these endemic), three varieties and two forms known from the region (Table 1). However, although *C. sedoides* C. Agardh has priority over *C. geminata* Harvey, being described 38 years prior (Silva *et al.* 1996), many authors have incorrectly used *C. geminata* for *C. sedoides* f. *geminata* (Harvey) Weber-van Bosse specimens (e.g. Huisman 2000; Littler & Littler 2003; Edgar 2008), most likely due to the incorrect use of *C. geminata* by Womersley (1984).

The morphological structure of *Caulerpa* (and the monospecific genus *Caulerpella* Prud'homme & Lokhorst) is unique in that, despite being unicellular and lacking transverse cell walls, specimens can grow to a remarkably large size and be differentiated into creeping stolons, highly branched rhizoids, and erect upright assimilators, which usually bear branchlets, termed ramuli. Species have historically been characterized by differences in assimilator branching patterns, shape of ramuli, and stolon morphology, as no genus-wide cellular or anatomical features (e.g. reproductive structures) have been found to aid in delimiting species, although the presence/ absence of pyrenoids have been used to distinguish between some species (Calvert *et al.* 1976; Coppejans and Prud'homme van Reine 1992; Price 2011). Unfortunately, *Caulerpa* species can display remarkably high levels of plasticity in all nearly morphology characters (Peterson 1972; Calvert *et al.* 1976; Ohba & Enomoto 1987; de Senerpont Domis *et al.* 2003; Belton *et al.* accepted) and this has lead to much confusion, the description of a large number taxa (> 350, Guiry & Guiry 2013) and a classification scheme involving varieties, forms and 'ecads'.

As a result, in recent years molecular tools have been readily embraced by researchers working on the genus (Famà *et al.* 2002; de Senerpont Domis *et al.* 2003; Verlaque *et al.* 2003; Stam *et al.* 2006; Wynne *et al.* 2009; Jongma *et al.* 2013; Sauvage *et al.* 2013; Belton *et al.* 2014). However, rather than alleviating taxonomic issues, these studies have found the genus to be plagued by an unstable taxonomy and a nomenclature difficult to untangle. Even under recently generated molecular evidence, researchers have hesitated to make taxonomic changes. One major factor deterring taxonomic changes is the absence of molecular data from type material or type localities (= topotype material), particularly from species with apparently widespread geographic distribution and high levels of phenotypic plasticity. Moreover, although genetic information can be obtained from older type specimens (e.g. Hughey & Gabrielson 2012), it is unlikely to be possible in the majority of cases due to type specimens being unavailable (e.g. administration controls, destroyed, lost, based on an illustration or have not yet designated) or because high quality DNA cannot be extracted (e.g. original specimen was formalin-preserved). Furthermore, many *Caulerpa* type localities are unknown, vague (e.g. “Southern coast of Australia”) or contain a number of genetic species with similar morphologies (Belton *et al.* 2014).

It is for this reason that many authors (De Clerck *et al.* 2012; Saunders & McDevit 2012) have called for more pragmatic approaches based on well sampled DNA phylogenies (e.g. Belton *et al.* 2014) in order to resolve the taxonomy of the genus and thus speed up the process of accurately naming the many misidentified and ‘dark taxa’ (genetically distinct specimens that have not been linked to existing species or that have not been formally described as new species; Page 2013) on GenBank. As a number of *Caulerpa* species have recently become established outside of their native ranges (e.g. *C. cylindracea* Sonder (Klein & Verlaque 2008), *C. taxifolia* (Meinesz & Hesse 1991), *C. webbiana* Montagne (Amat *et al.* 2008), *C. brachypus* f. *parvifolia* (Harvey) A. B. Cribb (Lapointe *et al.* 2006), *C. ollivieri* Dostál (Lapointe *et al.* 2005), *C. taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procacini (Jongma *et. al.* 2013) and *C. verticillata* J. Agardh (Pérez-Estrada *et al.* 2013)), having accurately identified GenBank sequences is especially important for rapid identification of newly established populations.

As no molecular based studies have focused on the *Caulerpa* rich flora of southern Australia, the taxonomic status of the many infra-specific taxa and synonyms from this region remains unknown. As such, the goal of the present study was to investigate the molecular and morphological variation of *Caulerpa* from southern Australian in order to: (1) assess *Caulerpa* species diversity in the region; (2) provide an updated taxonomic (i.e. nomenclatural and classification) revision for the region to complement the recent revisions of the genus in tropical Australia by

Kraft (2007), Price (2011), Belton *et al.* (2014) and Belton *et al.* (2015), and (3) provide reference DNA sequences for species from the region to assist with rapid and accurate molecular assisted identifications in future studies.

MATERIALS AND METHODS

Specimens were newly collected for the present study along the coastline of southern Australia (*sensu* Womersley 1984) between Perth, Western Australia and the Victorian/ New South Wales border and Tasmania (Table 2). We aimed to collect species from across their ranges, and where possible, also from their type localities. Although not considered as being a part of the southern Australian flora (*sensu* Womersley 1984), *C. filiformis* (Suhr) Hering is known from the Sydney region of New South Wales (Millar & Kraft 1994), and as this region experiences annual sea surface temperatures similar to those of southwestern Australia (www.bom.gov.au), we felt it appropriate to include it in the present study. *Caulerpa filiformis* from South Africa was also collected for comparison to Australian specimens of the same species. *Caulerpa hodgkinsoniae* J. Agardh specimens from northern New South Wales (= type locality) were also newly collected. *Caulerpa sedoides*, a species described from southern Australia but often reported from tropical locations (Coppejans *et al.* 1998; Littler & Littler 2003 as *C. geminata*), was also collected from tropical Australia (Heron, Lord Howe and Norfolk Islands) and Fiji for comparison to southern Australian (= type locality) specimens. In order to further clarify the identity of the tropical *C. sedoides* entity, we also collected specimens with vesiculate ramuli and constricted pedicels from tropical Australia, including: *C. corynephora* Montagne, *C. fergusonii* Grunow *ex* G. Murray, *C. opposita* Coppejans & Meinesz and *C. lentillifera* J.Agardh.

With all newly collected specimens, a clean part of the thallus was preserved in silica gel for molecular analysis with the remainder of the specimen either preserved in 5% formalin/seawater, desiccated in silica gel or press dried fresh and free of chemicals onto herbarium sheets. Specimen vouchers were housed at AD, GENT, L, PERTH, UNB or US (abbreviations follow Holmgren *et al.* 1990). Details of all the specimens collected and used in the present study are listed in Table 3.

Molecular phylogenetic analyses and species delimitation were based on *tuf*A and *rbc*L DNA sequences. DNA extraction, PCR amplification and sequencing were performed using the methods described in Belton *et al.* (accepted) with representative specimens from each lineage also being sequenced for the *rbc*L gene. Newly generated sequences were compared to that of the National Centre for Biotechnology Information (NCBI) database using BLAST (http://www.ncbi.nlm.nih.gov) to check for contaminants and were then submitted to GenBank (Table 3). Newly generated *tuf*A and *rbc*L sequences were added to the associated alignments from Belton *et al.* (accepted) using Clustal X (Larkin *et al.* 2007), which included sequences from the works of Hanyuda *et al.* (2000), Famà *et al.* (2002), de Senerpont Domis *et al.* (2003), Stam *et al.* (2006), Verbruggen *et al.* (2009), Wynne *et al.* (2009), Maeda *et al*. (2012) Jongma *et al*. (2013), Sauvage *et al.* (2013), Belton *et al*. (2014) and Belton *et al*. (). A concatenated alignment was not created, as in many instances we were unable to obtain sequences of both markers for all lineages.

Bayesian Inference was performed using BEAST v.1.7 (Drummond *et al.* 2006; Drummond & Rambaut 2007) with the three codon positions completely unlinked, an uncorrelated lognormal clock model and branch lengths in substitution. The *tuf*A and *rbc*L alignments were both analyzed using the GTR+I+G model of sequence evolution as determined by the Akaike Information Criterion (AIC) in jModelTest v.1

(Guindon & Gascuel 2003; Posada 2008). The Markov Chain Monte Carlo (MCMC) was started from a UPGMA tree and run for 20 million generations, sampled every 1,000th generation. A constant population size coalescent prior was used as tree prior and all other priors and operators were kept at their default values. MCMC runs were monitored in Tracer v.1.4.2 (Rambaut & Drummond 2007) and the ESS values were all above 200 for each run. The first 1,000 trees were removed as the burn-in before generating summary statistics and final consensus trees. Final trees were created and edited in FigTree v.1.3.1 (Drummond & Rambaut 2007).

Using the *tuf*A dataset, species were delimited using the Generalized Mixed Yule Coalescent (GMYC) model to define the species boundary (Pons *et al.* 2006; Monaghan *et al.* 2009). The GMYC method has been shown to be useful in a number of macroalgal studies (e.g. Leliaert *et al.* 2009; Tronholm *et al.* 2012; Payo *et al.* 2013; Silberfeld *et al.* 2013; Belton *et al.* accepted). The ultrametric tree required for the GMYC analysis was constructed using the Bayesian Inference methods described above but with the tree log file being created without branch length in substitution. GMYC analyses were performed under the single-threshold model using the SPLITS package for R (R Development Core Team 2009; package available at http://r-forge.r-project.org/projects/splits/). GMYC approach used in this study is also the same described in Belton *et al.* (accepted). GMYC lineages were recognized as species only if they were also strongly supported (Posterior Probability (PP) = 0.95) and preceded by a relatively long branch and with low intraspecific sequence diversity (Leliaert *et al.* 2009; Dijoux *et al.* 2013; Belton *et al.* accepted).

Specimens were then morphologically identified based on observations of type specimens, descriptions and the following references: Turner (1809–1819), Harvey (1855a; 1855b; 1858–1863), Agardh (1873), Weber-van Bosse (1898; 1910), Reinke

(1900), Lucas (1936), Chapman (1956), Womersley (1984), Adams (1994), Coppejans *et al.* (1998) and Price (2011). Morphological variation within and between each molecularly defined species was then characterized by examining each specimen for rhizoid form, stolon width, assimilator height, and ramuli shape, size and arrangement as well as the presence/absence of pyrenoids (visible under light microscope after Lugol's iodine stain).

RESULTS

Over 200 specimens were newly collected from southern Australia during the present study, representing 21 morphological species, two varieties and two forma (Table 2). The following southern Australian species were unable to be located during the present study: *C. articulata* Harvey*, C. ellistoniae* Womersley, *C. cupressoides* (H. West) C. Agardh, *C. lagara* Carruthers, Walker & Huisman and *C. simpliciuscula* var. *laxa* Womersley. Until these taxa are freshly collected and sequenced, we are unable to provide detailed comments on their taxonomic and phylogenetic status. However, specimens corresponding to *C. fergusonii*, not previously reported from southern Australia, were newly collected in southwestern Australia during the present study.

From the newly collected specimens, 95 *tuf*A and 43 *rbc*L sequences were generated (Table 3). The *tuf*A alignment consisted of 335 sequences and was 820 bp in length. The *rbc*L alignment consisted of 83 sequences and was 677 bp in length. After several attempts were unable to obtain *rbc*L sequences for *C. alternans* Womersley, *C. corynephora*, *C. hodgkinsoniae, C. lentillifera* and the tropical *C. sedoides* specimens. Support in both the *tuf*A and *rbc*L trees was greater towards the terminal nodes and in the deepest nodes, but was relatively poor in the mid-nodes of the trees (Figs 1 & 2). The *rbc*L and *tuf*A datasets produced trees with slightly different topologies and statistical support, but the lineages including southern Australian specimens were identical (Figs 1 & 2). The most significant incongruence between *rbc*L and *tuf*A tree topologies referred to the positions of the *C. hedleyi* Weber-van Bosse, *C. cupressoides* and *C. serrulata* (Forsskål) J. Agardh lineages. In the *tuf*A phylogram, *C. hedleyi* formed a sister lineage to the section that included *C. alternans*, *C. heterophylla* I.R. Price, J.M. Huisman & M.A. Borowitzka, *C. obscura* Sonder, *C. flexilis* J.V. Lamouroux, *C. flexilis* var. *muelleri* (Sonder) Womersley, *C. trifaria* Harvey and *C. brownii* (C. Agardh) Endlicher (PP = 1, Fig. 1), whereas in the *rbc*L tree it formed a poorly supported sister lineage to *Caulerpella ambigua* (PP < 0.5, Fig. 2). The position of *C. flexilis* var. *muelleri* (as *C. muelleri* Sonder in Figs. 1 & 2) also varied from being sister to *C. flexilis* in the *rbc*L tree (PP = 1) to falling outside the *C. trifaria*/ *C. brownii*/ *C. flexilis* lineage in the *tuf*A tree (unsupported). *Caulerpa cupressoides* and *C. serrulata* were closely related in the *tuf*A tree (PP = 0.92) but were distantly related in the *rbc*L tree (Figs 1 & 2). In both the *tuf*A and *rbc*L trees, *Caulerpella ambigua* clustered within *Caulerpa* and not as a separate genus (Figs 1 & 2).

Southern Australian species with stolon appendages were highly divergent from other *Caulerpa* species (Figs. 1 & 2). However, *C. alternans*, a southern Australian endemic with a smooth stolon also clustered with southern Australian species with stolon appendages (Fig. 1). Southern Australian species were also found to make up the majority of species in the lineage of species with vesiculate constricted ramuli (Figs. 1 & 2). *Caulerpa cliftonii* Harvey, which has a smooth stolon, does not belong to either of these sections but formed its own highly divergent monospecific lineage that was sister to the southern Australian species with stolon appendages lineage

(Figs. 1 & 2).

The GMYC model provided a significantly better fit than a null model driven by coalescence only (confidence interval 40 – 81; $L_{GMYC} = 3371.605 > L_0 = 3361.659$, $p = 0.00017$). The GMYC analysis delimited 61 species in the *tuf*A dataset, with specimens from southern Australia found in 27 of those (Fig. 1). However, only 58 of these lineages had high PP support (> 0.95), although all 27 southern Australia lineages were well supported (Fig. 1). The extra lineages found by the GMYC analysis corresponded to an extra GMYC lineage in each of the *C. chemnitzia* (Esper) J.V. Lamouroux, *C. macra* (Weber-van Bosse) Draisma & Prud'homme and *C. mexicana* Sonder ex Kützing (Fig. 1).

With regards to the lineages containing southern Australian specimens, the majority of these corresponded to the currently accepted morpho-species that did not show high levels of intra-lineage morphological plasticity. However, a number of morpho-species (*C. brachypus* Harvey, *C. flexilis* and *C. longifolia* C. Agardh,) were represented by multiple, genetically distinct species (Figs. 1 & 2). Furthermore, the southern Australian specimens of *C. hodgkinsoniae C. scalpelliformis* (R. Brown *ex* Turner) C. Agardh and *C. sedoides* were genetically distinct from specimens of the same species collected outside of the region (Fig. 1).

In the *tuf*A tree we found two distinct lineages that could be identified as containing *C. hodgkinsoniae* specimens (Fig. 1). The first included specimens from Victoria and South Australia (Fig. 3) and the second, specimens from northern New South Wales (Fig. 4). As the New South Wales specimens were collected just south of the type locality (Ballina, New South Wales) and were similar in appearance to the type specimen (Fig. 5), we designated specimens from that lineage as the true *C. hodgkinsoniae* (Fig. 1). The other we described as *C. lucasii* Prud'homme, Draisma

& Belton *nom. nov.* based on *C. annulata* Lucas *nom. illeg.* (Fig. 6). *Caulerpa hodgkinsoniae* and *C. lucasii* specimens differed in their ramuli morphology, stolon diameter (Figs. 3 & 4) and *C. lucasii* was also found to have pyrenoids associated with its chloroplasts (visible under light microscope), whereas these were absent in *C. hodgkinsoniae*.

Specimens identified as *C. sedoides* in southern Australian showed considerable variation in ramuli arrangement, from distichously to densely arranged on all sides of the rachis. Furthermore, we found no genetic distinction between specimens from southern Australia that were morphologically identified as *C. sedoides, C. sedoides* f. *geminata* and *C. sedoides* f. *tasmanica* Weber-van Bosse. Supposed *C. sedoides* specimens collected at Heron Island and Fiji (Figs 7–9) were genetically distinct from southern Australian *C. sedoides* specimens (Fig. 1), including those collected from close to the type locality (Kent I., Bass Strait). The tropical specimens were also genetically distinct from specimens that closely resembled the type specimen of *C. sedoides* f. *geminata* collected from the type locality (= Rottnest I., Western Australia). Furthermore, these specimens were also genetically distinct from other *Caulerpa* species with constricted vesiculate ramuli known from Australia (*C. corynephora, C. fergusonii, C. lentillifera* and *C. opposita,* Fig. 1). Interestingly, sequence data from specimens morphologically identified as *C. sedoides* from Norfolk Island clustered with the *C. sedoides* specimens from southern Australia, and not with the Heron Island and Fijian samples (Fig. 1, as *C. coppejansii*). Molecular data has also recently confirmed the presence of true *C. sedoides* in New Caledonia (T. Sauvage, *pers. comm.*) As such, although not from the southern Australian region, we believe that due to confusion over having two tropical taxonomic *C. sedoides* entitites, we should take this opportunity to describe a new species, *C. coppejansii* Belton & Prud'homme *sp. nov.* based on the Heron Island and Fijian specimens (see below).

Caulerpa flexilis var. *muelleri* and *Caulerpa longifolia* f. *crispata* (Harvey) Womersley were found to be genetically and morphologically distinct from their nominal infraspecific counterparts, i.e. *C. flexilis* and *C. longifolia*, respectively (Figs 1, 2, 11–20). We therefore propose the reinstatement of *C. muelleri* and describe *C. crispata* (Harvey) Belton & Gurgel *stat. nov.*, based on *C. longifolia* f. *crispata*, below. *Caulerpa heterophylla*, which closely resembles both *C. flexilis* and *C. muelleri*, was found to be sister to *C. muelleri*, although the relationship was poorly supported (PP = 0.58; Fig. 1). The *C. flexilis* specimen from Famà *et al.* (2002; AJ417970) formed a separate lineage (supported by GMYC and PP) to our *C. flexilis* and *C. muelleri* lineages (Fig. 1).

Our results support Belton et al. (2015) who found *C. brachypus f. parvifolia* to be distinct from *C. brachypus,* and thus reinstated *C. parvifolia*. However, in the present study, specimens morphologically identified as *C. parvifolia* from south-western Australia and New South Wales (including Lord Howe and Norfolk Islands), formed two distinct lineages, supported by both markers and species delimitation methods (Figs. 1 & 2). Morphologically, specimens from these two lineages could not be distinguished from one another (Figs. 23 & 24).

Caulerpa scalpelliformis specimens from southern Australia (the type locality; no more specific details are known) were found to be unrelated to a *C. scalpelliformis* sequence (AJ417972) from Lebanon (Fig. 1). Furthermore, molecular results showed that, in many instances, specimens morphologically identified as *C. scalpelliformis* were actually *C. remotifolia* Sonder and that the distribution of *C. remotifolia* is more widespread (Table 2) than was previously thought (Womersley 1984).

Molecular data not only confirmed the initial morphological identification of *C. fergusonii* (Fig. 25, JN851136) specimens from southwestern Australia, which is the first report of this species from southern Australia, but also found the distribution of *C. corynephora* to extend into south-western Australia (Fig. 26, GWS024470) confirming Harvey's (1855) listing of the species from south-western Australia, although he later recorded the species for southern Australia as a synonym of *C. cactoides* (Harvey 1858). Both *C. fergusonii* and *C. corynephora* closely resemble *C. cactoides* (Turner) C. Agardh (Fig. 27) with the *C. corynephora* specimen (Fig. 26, GWS024470) initially being identified as *C. cactoides*. However, on closer inspection, it differed from *C. cactoides* by its smaller habit and ramuli mostly borne on successive rachis segments, as opposed to *C. cactoides* whose ramuli were usually separated by a naked segment of the rachis (compare Figs 25 and 27). However, some *C. cactoides* specimens have ramuli borne on each successive rachis segments and thus DNA sequence data is the most reliable and accurate means to tell these species apart. *Caulerpa fergusonii* could be distinguished from *C. cactoides* and *C. corynephora* by its relatively short and broad ramuli, the absence of annulations at the base of the assimilators (Fig. 26) and lack of pyrenoids visible under the light microscope.

As with Jongma *et al.* (2013), we found a single nucleotide difference between *C. taxifolia* and *C. taxifolia* var. *distichophylla* specimens, which also formed separate clusters within the *C. taxifolia* lineage in the *tuf*A alignment (not supported by GMYC, high PP values or *rbc*L data; Figs 1 & 2). The *C. filiformis* sequence from South Africa was found to be genetically identical (no bp differences) to the *C. filiformis* from Sydney, New South Wales (Fig. 1).

Lastly, whilst collecting for the present study, we located a newly established population of *C. cylindracea* in Portland, Victoria, which was herein confirmed by molecular data (Fig. 1).

Caulerpa coppejansii* Belton & Prud'homme *sp. nov.

Figs 7–9

DESCRIPTION: Stolon naked, 1.5–2.5 mm in diameter, pale light green to yellow in color; rhizoids, when present, 2–4 cm long, often composed of thick bushes of branched rhizoids at their ends; assimilators upright, 2–4 cm high, reaching 7 cm, bearing distichously arranged, opposite, ramuli; ramuli sub–sphaerical, 2.5–3.5 mm in diameter, clearly stalked. Pyrenoids present.

HOLOTYPE: in BRI (ex. AD-A88473a), collected on 03 September 2008, at 30 m (Fig. 7–9).

ISOTYPES: AD-A88473b and AD-A88473c collected on 03 September 2008, at 30 m; AD-A88423, collected on 01 September 2008, at 30 m.

TYPE LOCALITY: Wistari Channel, Heron Island, Queensland, Australia.

DNA BARCODE (*TUFA*): KF649871, from type specimen (in BRI (ex. AD-A88473a)

ETYMOLOGY: after the epithet honors Eric Coppejans who first suggested that there was a distinct tropical "*C. sedoides*" species (see Coppejans *et al.* 1998).

DISTRIBUTION: Currently known from the Great Barrier Reef, Fiji and Papua New Guinea, but most likely also found in the South Pacific.

SPECIMENS EXAMINED: Holotype and isotypes; DML40305, North Astrolabe Reef, Fiji; AD-A95458A and AD-A95458B, Heron Island, Australia (further details provide for each specimen in Table 3). We also examined the specimens from Coppejans et al. (1998): HEC10187, Motuporo Island, Papua New Guinea; HEC10280 & HEC10371, Loloata Island, Papua New Guinea.

COMMENTS: *Caulerpa coppejansii* specimens from the present study were genetically distinct from *C. sedoides* specimens from southern Australia as well as other known species from the region with oppositely arranged constricted vesiculate ramuli (i.e. *C. annulata* (now *C. lucasii*), *C. cactoides, C. corynephora, C. fergusonii, C. hodgkinsoniae, C. lentillifera* and *C. opposita*; Fig. 1) and thus deserves recognition as a distinct species.

Caulerpa coppejansii is most likely the same as the *C. sedoides* specimens collected in Papua New Guinea (listed above) by Coppejans *et al.* (1998; listed as *C. sedoides* var. *geminata* in that study). Although Coppejans *et al.* (1998) initially thought these specimens were a distinct species (they suggested the name *C. micropposita* sp. nov. provis.), after observations of the type specimen of *C. sedoides* f. *geminata* (Coppejans *et al.* 1998, figs 15 & 16), they concluded that they "had collected specimens of this taxon in its most characteristic growth form". However, morphological observations of the Papua New Guinea specimens, as well as the newly collected specimens from Heron Island and Fiji, showed that they could be distinguished from southern Australian *C. sedoides*, by their larger ramuli that were spherical to sub-spherical and arranged in two opposite rows (Figs. 7–9). However, *C. sedoides* specimens from Norfolk Island were almost identical in morphology to *C. coppejansii* and smaller specimens of *C. opposita* could easily be mistaken for *C. coppejansii.* Furthermore, pyrenoids were observed in *C. coppejansii, C. sedoides* and *C. opposita* and thus, the presence/absence of pyrenoids is not a useful character to distinguish between species. As such, we strongly suggest the use of DNA sequence data to distinguish between these three species in future studies in tropical waters.

Reports of *C. sedoides* from India, Indonesia (Java and Semau), Mauritius, (Silva *et al*. 1996); Kenya (Coppejans *et al.* 1998); and Sri Lanka (Svedelius 1906; Silva *et al.* 1996) require verification as no sequence data were available from these localities and it is unknown how those specimens are related to *C. coppejansii* or *C. sedoides*. However, the fact that Norfolk Island and New Caledonian *C. sedoides* specimens clustered with *C. sedoides* specimens from southern Australia, and not with *C. coppejansii,* reports of *C. sedoides* from tropical waters could be legitimate.

As we found no genetic distinction between specimens identified as *C. sedoides, C. sedoides* f. *geminata* and *C. sedoides* f. *tasmanica* in southern Australia, we see no reason for these forms to continue to be recognized.

Caulerpa crispata **(Harvey) Belton & Gurgel** ***stat. nov.***

Figs 11–13, 15

BASIONYM: *Caulerpa harveyi* var. *crispata* Harvey *Phycologia Australica* 2: pl. XCV (1859).

SYNONYMS: *Caulerpa curvifolia* J. Agardh *nomen nudum* in J.B. Wilson (1882: 188), *Caulerpa longifolia* f. *crispata* (Harvey) Womersley (1950: 147).

EXTENDED DESCRIPTION: Stolon relatively slender, 1–2 mm in diameter, naked, pale light green to yellow in color; assimilators medium green in color up to 30 cm high, simple or with a few, often clustered, branches produced several cm above the base; rachis terete, naked near the base, bearing irregularly placed, slender, 0.5–1 (–3) cm long and 200–300 µm in diameter, upwardly incurved, terete, ramuli with spiny tips.

LECTOTYPE: TCD0011013, in TCD (see Fig. 13), designated here.

TYPE LOCALITY: Port Phillip Heads, Victoria, Australia

DNA BARCODE (*TUFA*): KF649917 from specimen GWS016662, collected from Port Phillip Heads, Victoria, Australia.

ETYMOLOGY: adapted from the Latin *crispare*, meaning to curl; referring to the strongly incurved and frequently curled nature of this species (Fig. 15).

DISTRIBUTION: southern Australian endemic, from Jurien Bay, Western Australia to Wilsons Promontory, Victoria and around Tasmania.

HABITAT: epilithic in rock pools and sheltered bays.

SPECIMENS EXAMINED: Type specimen; AD-A89139, Jurien Bay, Western Australia; AD-A50856, Whitfords Beach, Perth, Western Australia; GWS025588, Rottnest Island, Western Australia; GWS025171, Canal Rocks, Western Australia; AD-A87764, Coombra, Great Australian Bight, South Australia; AD-A79031, Kangaroo Island, South Australia; AD-A92236, Kangaroo Island, South Australia; GWS016662, Pt Lonsdale, Victoria; TCD0011013, Port Phillip Bay, Victoria; TCD0011015, Westernport Bay, Victoria; TCD0011014, Philip Island, Victoria; GWS016226, Boat Beach Harbor, Tasmania; AD-A42101, Bruny Island, Tasmania. Further details provide for each specimen in Table 3.

COMMENTS: Figure 1 shows that specimens previously assigned to *C. longifolia* f. *crispata* (now *C. crispata*) are actually an independent sister species to *C. longifolia* and as such we propose this new combination. *Caulerpa crispata* can be morphologically distinguished from *C. longifolia* by its more slender stolon (1-2 mm versus 2–4 mm diameter) and assimilators with randomly arranged, upwardly incurved ramuli (Fig. 15) compared to the much larger assimilators of *C. longifolia* (up to 65 cm high), of which the ramuli are only slightly curved upwards and found in 4–6 distinct rows (Fig. 14). Morphological characteristics of *C. crispata* specimens collected during the present study matched those of the type specimen (Fig. 13), with the exception of some cases, especially those from Western Australia, which had assimilators producing a cluster of branches several cm (2–12) above the base and smaller, more closely arranged, ramuli (Fig. 12). Although Harvey (1858-1863) and Womersley (1984) noted that intergrades between *C. longifolia* and *C. crispata* (then as *C. longifolia* f. *crispata*) do occur, these were not observed during the present study.

Caulerpa lucasii **Prud'homme, Draisma & Belton** ***nom. nov.***

Figs 3 & 6.

REPLACED NAME: *Caulerpa annulata* A. Lucas *Proceedings of the Linnean Society of New South Wales* 56: 410 pl. 27 fig. 1 (1936), non *Caulerpa annulata* (Ettinghousen) W.P. Schimper (Schimper 1869: 159).

TYPE LOCALITY: Port Arthur, Tasmania

HOLOTYPE: NSW288774 (Fig. 6).

ISOTYPES: NSW288775 and NSW288776.

DNA BARCODE (TUFA): KF649876 from specimen AD-A88572, Sir Joseph Banks Islands Group, South Australia (Fig. 3).

DISTRIBUTION: Southern Australia

SPECIMENS EXAMINED: Holotype and isotypes; AD-A88572, Sir Joseph Banks Group, South Australia; AD-A93737, Portland, Victoria. Further details provided for each specimen in Table 3.

COMMENTS: Genetic data showed the separation of *C. hodgkinsoniae* and specimens referable to *C. annulata*, a species that was synonymised with *C. hodgkinsoniae* by Millar & Kraft (1994). However, *C. annulata* Lucas cannot be used as it is a later homonym of the fossil alga *Caulerpa annulata* [Ettinghousen] W. P. Schimper

(Schimper 1869:159 in Schimper 1869–1874; basionym *Caulerpites annulatus* Ettinghousen and now known as *Keckia annulata* (Ettinghousen) W.P. Schimper), thus making *C. annulata* Lucas illegitimate. As such we have proposed the name *C. lucasii* after the author who first described this species. Morphologically, we found *C. lucasii* and *C. hodgkinsoniae* to differ in ramuli L/B and stolon diameter (compare Figs 3–6, see Identification Key). *Caulerpa lucasii* was also found to have pyrenoids associated with its chloroplasts (visible under light microscope), whereas *C. hodgkinsoniae* did not.

Lastly, we suggest that future studies endeavor to collect *C. articulata* from New Zealand (type locality), a species that lacks pyrenoids (W.H. Prud'homme van Reine pers. obs. of type specimen) and is very similar in morphology to *C. hodgkinsoniae*, and which has also been recorded from Western Australia (Huisman 2000).

DISCUSSION

The present study represents the first molecular-based revision of southern Australia *Caulerpa* and, as with previous molecular investigations of *Caulerpa*, shows the genus to be more diverse than previously appreciated (de Senerpont Domis *et al.* 2003; Stam *et al.* 2006; Sauvage *et al.* 2013; Belton *et al.* accepted). Although the genus has been reported as displaying high levels of phenotypic plasticity, the majority of species found in southern Australia did not show much variation in their morphologies and could be distinguished morphologically (with the exception of some specimens of *C. coppejansii*/ *C. opposita*/ *C. sedoides*; *C. corynephora*/ *C. cactoides* and *C. remotifolia/ C. scalpelliformis*, as discussed above). Furthermore, as the majority of species are endemic to the region, we did not encounter difficulties in obtaining type locality sequences and sequences from across the known extent of the species range. Based on this, as well as a well-sampled molecular phylogeny, we are confident in describing three new species (*C. coppejansii*, *C. crispata*, *C. lucasii*) and reinstating *C. parvifolia* and *C. muelleri* (see more details on the latter two species below). An updated taxonomy and nomenclature (including reference *tuf*A sequences) is provided in Table 2. Twenty-nine species, approximately one third of all known *Caulerpa* species in the world are now known from the region, further highlighting southern Australia as a significant macroalgal biodiversity hotspot (Phillips 2001).

Species endemic to southern Australian made up the majority of the so called ‘species poor ancient lineages’ (*sensu* Famà 2002) of specimens with stolon appendages and specimens that have vesiculate ramuli with a constricted pedicel (Figs. 1 & 2). The fact that species in both these highly divergent lineages were mainly from southern Australia does appear to support Calvert *et al.* (1976) who, based on characters of plastid ultrastructure and the assumption that the large pyrenoid-containing chloroplast of members of the Section *Sedoideae* (sensu Weber-van Bosse 1898: specimens which have vesiculate ramuli with a constricted pedicel) was the most primitive form, hypothesized that the warm-temperate waters of southern Australia were the geographic origin of the genus. However, the time calibrated phylogeny of the siphonous green algae by Verbruggen *et al.* (2009) shows that the *C. flexilis*/ *Caulerpella ambigua* lineage diverged from other *Caulerpa* species approximately 185 million years ago when the entire present-day southern Australia coastline was still connected to the Antarctic plate. Further work is clearly needed to better understand the origin of the genus and the reason for its radiation in southern Australia.

Our results agree with the phylogenetic trees of Verbruggen *et al.* (2009), which showed that *Caulerpella ambigua* is a sister lineage to the *Araucarioideae*/ *C. cliftonii* lineage and does not belong to a separate genus (Figs 1 & 2). However, as the two *Caulerpella ambigua* sequences available on GenBank represent different species, we suggest further work be undertaken before their reinstatement within *Caulerpa*.

Caulerpa brachypus f. *parvifolia* specimens were found in two lineages corresponding to specimens from south-western Australia and New South Wales (including Lord Howe and Norfolk Islands). These lineages were also shown to be distinguished from *C. brachypus sensu stricto* by genetic data and by their smaller and more delicate habit (Figs 1, 22–23). With this in mind, we propose the reinstatement of *C. parvifolia* for specimens belonging to the New South Wales lineage (type locality) but do not describe the additional *C. parvifolia* lineage from Western Australia as the relationship between our lineage, and other delicate ligulate *Caulerpa* species (*C. biserrulata* Sonder, *C. diligulata* Kraft & A.J.K. Millar, *C. spathulata* Womersley & Bailey and *C. subserrata* Okamura as well as *C. brachypus* f. *brasiliana* A.B. Joly & Semir, *C. brachypus* f. *nordestina* Womersley & Semir and *C. mauritiana* Børgesen), remains unknown. However, as the description of *C. parvifolia* predates all these other delicate ligulate species, it would take priority if any of these species were shown to be genetically identical to *C. parvifolia*. Interestingly the aquarium trade specimens identified as *C. brachypus* in Stam *et al.* (2006) clustered with the *C. parvifolia* specimens from N.S.W. Furthermore, *C. brachypus* f. *parvifolia* has also been reported as invasive in Florida by Lapointe *et al.* (2006), but specimens from these populations are yet to be sequenced and further work should be undertaken to establish the rue identity of the Florida populations.

We also propose the reinstatement of *C. muelleri* as a distinct species as it was distinguished from *C. flexilis* by genetic data (Figs 1 & 2) and by its strictly distichous second-order laterals and densely arranged ramuli that are always more robust (300 µm in diameter) and upwardly curved so as to overlap each other, compared to ramuli of *C. flexilis* (200 µm in diameter) which do not overlap (Figs. 16–20). The morphological distinction between these taxa has been previously documented by Womersley (1984) who noted that "very few plants are intermediate between the above varieties". In regards to the *C. flexilis* specimens used by Famà *et al.* (2002, AJ417970), which formed a separate lineage (supported by GMYC) to our *C. flexilis* and *C. muelleri* specimens, this specimen is presumed lost (W. Prud'homme van Reine, *pers. infor.*) and we cannot provide any comment on these sequences or specimens. However, the *C. flexilis* specimen used by Famà *et al.* (2002) is from the same sample later used by De Senerpont Domis *et al.* (2003, AJ512426), and therefore we suggest that future molecular studies on *Caulerpa* omit these sequences until vouchers are located. *Caulerpa heterophylla*, a species that closely resembles *C. flexilis* and *C. muelleri* but which is distinguished by its unforked ramuli and stolons covered with broad conical protuberances, was herein confirmed as a valid species by *tuf*A sequence data (Fig. 1).

Womersley (1984) believed that *C. remotifolia* (Figs. 28–30) and *C. scalpelliformis* (Fig. 31) were distinct in habit and in habitat, with the density of the ramuli being very variable in the former (Figs. 28–30). Indeed, the type specimens of these species are very distinct and could not be confused. However, data from the present study showed *C. remotifolia* to have a much larger geographic distribution than previously considered (Table 2), including exposed coastlines, and at times, to be indistinguishable from *C. scalpelliformis* (compare Figs. 28 & 31). Consequently, DNA sequence data seems to be the most accurate means of distinguishing between these two species. Unfortunately, C. *ellistoniae*, a species that closely resembles both *C. remotifolia* and *C. scalpelliformis*, was not found during the present study and its taxonomic status remains based on morphology alone.

With regards to the two *C. scalpelliformis* lineages (Fig. 1), as the type locality for this species is the southern coast of Australia, it is likely that *C. denticulata* Decaisne (originally described from the Red Sea and currently regarded as *C. scalpelliformis* var. *denticulata* (Decaisne) Weber-van Bosse), should be reinstated and used to recognize specimens from outside southern Australia. However, we suggest that further collections should be obtained from the Red Sea in order to confirm the reinstatement of *C. denticulata* and to determine if they can be distinguished apart morphologically.

In regards to *C. fergusonii*, our observations of older herbarium specimens (e.g. AD-A00127 and AD-A50563) of *C. cactoides* from Western Australia found a number of specimens lacking assimilators with an annulated base and corresponding to *C. fergusonii* (also alluded to by Womersley 1984: 269). It is therefore likely that this species has previously being simply overlooked by previous researchers due to its morphological similarity with *C. cactoides* (e.g. Womersely 1984; Huisman & Walker 1990). However, as both *C. corynephora* and *C. cactoides* have annulations at the base of their assimilators (Fig. 26 & 27), without sequencing herbarium specimens we are unable to confidently determine if *C. corynephora* has previously been overlooked in south-western Australia or has recently undergone a southward shift in its distribution (i.e. Wernberg *et al.* 2011).

The discovery of a new population of *C. cylindracea* in Portland, Victoria represents the most southerly record for this species and is a serious cause for concern as it is located at more than 600 km from Adelaide, South Australia, the only other populations of this species in south-eastern Australia (Womersley 2003). The fact that these adventive populations are found in shipping harbors, points to boating traffic as the most likely vector of introduction. As the potential distribution of this species encompasses the entire southern coastline of the Australian mainland, including northern Tasmania (Verbruggen *et al.* 2013), it is likely that the species will continue to spread to other localities unless stricter quarantine rules are put in place.

Although originally described in 1841 from specimens collected in Cape Town, South Africa, *C. filiformis* is found around the Sydney and Wollongong regions of New South Wales where it was first recorded in 1922 (NSW723391) and has since also been reported from Peru (Acleto 1973). However, although there have been extensive collections by various phycologists in the temperate waters of Australia, no other *C. filiformis* populations have been found in the region besides those in New South Wales. Due to this very disjunct distribution, the lack of sequence divergence between South Africa and Australian populations (Fig. 1), and the fact that the temperate waters of Australia and South Africa share no other *Caulerpa* species in common, we suggest that further population level studies take place in order to better understand the true status of the populations in Australia and Peru.

Lastly, although we were unable to collect and sequence some species known from southern Australia (e.g. *C. ellistoniae*), with the exception of *C. articulata*, our observations of herbarium material and type specimens of these non-sequenced species found that they could be easily distinguishable from other southern Australian *Caulerpa* species on morphological grounds alone. As such, we feel confident in providing an updated identification key to the *Caulerpa* of southern Australia below. However, although discussed in detail in the present study, we do not include *C. brachypus* and *C. coppejansii* as these species are not found in southern Australian waters.

Revised Identification key for southern Australian Caulerpa *(adapted from Womersley 1984).*

1 Assimilators without obvious lateral ramuli (although small marginal spines can be present) 2

1. Assimilator with obvious ramuli 3

2 Assimilators small (< 5cm), leaf like, mostly with smooth margins .... *C. parvifolia*

2. Stolons robust, assimilators bifurcate with annulations at base....... *C. filiformis*

3 Ramuli compressed or terete (simple or branched), not vesiculate.................. 4

3. Ramuli vesiculate, unbranched 19

4 Assimilator bearing distichous, simple, terete or compressed ramuli.............. 5

4. Assimilator and/or secondary laterals bearing ramuli usually on all sides or in more than two rows; ramuli terete, simple or branched 10

5 Ramuli terete, linear, (1–) 1.5–2.5 (–3) mm long, and less than 0.5 mm in diameter, alternately arranged .... *C. alternans*

5. Ramuli compressed 6

6 Ramuli scattered, often absent, otherwise separated by at least their basal width, (2–) 4–6 (–10) mm long, 0.5–1 (–1.5) mm broad .... *C. remotifolia*

6. Ramuli regular and adjacent, separated by less than or about their basal width 7

7 Rachis 0.5–1.5 mm broad, ramuli opposite, 2–8 mm long .... *C. taxifolia*

7\. Rachis 2–8(– 10) mm broad, ramuli alternate, compressed, 3–10 mm long 8

8 Assimilator obviously branched, older ramuli separated by about the width of a ramulus, ramuli basally constricted ....*C. ellistoniae*

8\. Assimilator rarely branched, ramuli usually broadest at the base.................... 9

9 Ramuli scalpelliform, usually curved on both sides, with small alternating ramuli present at apex of assimilator.................................... *C. scalpelliformis*

9\. Ramuli convex on lower side, straighter on upper side, small alternating ramuli absent at apex of assimilator .... *C. remotifolia*

10 Erect axes without second-order laterals but bearing simple or 1–4 times branched ramuli in rows or on randomly arranged on all sides.....................11

10\. Erect axes bearing numerous distichously or radially arranged second–order laterals, each of which bears numerous simple or furcate ramuli.................15

11 Ramuli unbranched..12

11\. Ramuli once or more branched 14

12 Ramuli in distinct longitudinal rows, relatively straight 13

12\. Assimilator often branched as a cluster, ramuli not in distinct longitudinal rows, incurved, 1–3 cm long ....*C. crispata*

13 Ramuli in 3 rows (2 when juvenile), 3–5 (–9) mm long.................. *C. trifaria*

13\. Ramuli usually in 5(4–6) rows, 0.5–1.5 cm long .... *C. longifolia*

14 Ramuli once, or more usually twice, furcate near their base, rigid, straight to slightly curved, 1.5–4 mm long; stolon bearing simple ramuli 0.5–2.5 mm long... *C. brownii*

14\. Ramuli 1–5 times laterally branched in their lower half, 1–3 cm long; stolon naked .... *C. cliftonii*

15 Second-order laterals irregularly radially arranged around erect axes...........16

15. Second-order laterals distichously arranged on erect axes 17

16 Second-order laterals irregularly arranged around erect axes, 1–4 cm long, bearing usually simple ramuli 2–10 mm long, subdistichously to irregularly arranged; stolon covered with spinous ramuli .... *C. obscura*

16. Ramuli once furcate usually near their base, 1–3 mm long, with a single or twinned spinous apex .... *C. flexilis*

17 Ramuli simple, stolons covered with broad conical protuberances ....*C. heterophylla*

17. Ramuli branched, stolon densely covered branched appendages...................18

18 Ramuli minute (0.2–0.5 mm long), much-branched and spinous, covering the thallus ....*C. hedleyi*

18. Ramuli once furcate usually near their base, 1–3 mm long, with a single or twinned spinous apex.. *C. muelleri*

19 Ramuli distichously arranged on axes..20

19. Ramuli usually on all sides of axes,...27

20 Rachis without constrictions 21

20. Rachis with constrictions...22

21 Ramuli ovoid to elongate-ovoid, 3–7 mm long, usually L/B<2...... *C. sedoides*

21. Ramuli terete, generally of similar diameter throughout, 5.0–13.5 mm in length, <1 mm in width .... *C. lagara*

22 Rachis without annulated stalk, ramuli subspherical, obovate to pyriform, slightly compressed, <1cm long, arising from every segment and often overlapping...*C. fergusonii*

22 Base of assimilator bare and annulated..23

23 Plant robust, stolon coarse, >2 mm in diameter, ramuli clavate, with obvious pyrenoids 24

23. Plant delicate, stolon <2 mm in diameter, ramuli cylindrical to 10 mm long and 1–2 mm in diameter, without obvious pyrenoids 26

24. Ramuli with rounded tip and base 6–9 mm long, 1.5–2.5 mm broad, uncommon .... *C. lucasii*

24 Ramuli 3–10 mm broad 25

25 Stolon 3–5 mm diameter, ramuli 1–3 cm long, 3–10 mm broad often arising from every second segment, common .... *C. cactoides*

25 Stolon 2–3 mm in diameter, ramuli 0.5–1 cm long, 3–5 mm broad, arising from every segment, rare, tropical but known from south-western Australia*C. corynephora*

26 Assimilators usually over 10 cm in height, narrow, constricted at attachment, ramuli approx. 10 mm long, <2 mm in diameter .... *C. articulata*

26. Assimilators up to 5 cm in height, ramuli with obtuse tip, >8 mm long, approx. 1 mm in diameter .... *C. hodgkinsoniae*

27 Ramuli without constrictions, elongate clavate........................ *C. cylindracea*

27. Ramuli constricted, sub-spherical to ovoid, elongate-ovoid or pyriform, usually less than 4 mm long...28

28 Ramuli with a slight to prominent swollen base protruding from the axes, then constricted with a sub–spherical or ovoid terminal part.......................29

28. Ramuli with the constriction adjacent to the axis, ovoid to clavate or ovoid–pyriform..30

29 Ramuli with a prominent papillate base, subspherical above the constriction and 150–550 (–600) µm in diameter .... *C. papillosa*

29. Ramuli with a slight, convex papilla from the axis, ovoid above the constriction and 1–2 mm long, 0.5–1.5 mm in diameter, fewer than 10 around the rachis..*C. vesiculifera*

30 Ramuli usually loosely and irregularly arranged, 2–6 around the rachis (subdistichous to radial), ovoid, L/B 1.5–2, (1.5–) 2–4 (–7) mm long, 1–3 mm broad.. *C. sedoides*

30. Ramuli densely arranged on the axes, touching, (6–) 8–14 around the rachis, 0.7–1.5 mm long and 300–700(450) µm in diameter....................................31

31 More than 10 ramuli around the rachis broad........................ *C. simpliciuscula*

31. Less than 10 ramuli around the rachis.....................*C. simpliciuscula* var. *laxa*

ACKNOWLEDGMENTS

This work is part of the PhD dissertation by G. S. Belton submitted to the School of Earth & Environmental Sciences, the University of Adelaide. We would like to thank O. De Clerck, H. Verbruggen, B. Brooks, D. and M. Littler, A. Kurihara, R. Dixon, G. Kraft and L. Kraft for help with specimen collections; M. Marklund, K. Dixon, D. McDevit, T. Moore, T. Sauvage and G. Saunders for collections and generating molecular data; E. Verheij, I.R. Price and W.T. Stam for discussions; and W.T. Stam, A. Millar and J. Parnell for making available herbarium vouchers. Hilda Prud'homme van Reine-de Jager made many photographs of *Caulerpa* specimens in herbarium collections. This research was funded by the Australian Research Council (LP0991083), a Alinytjara Wilurara Natural Resources Management Board grant, and the Australian Census of Coral Reef Life (CReefs) to CFDG. We thank G. Saunders and the Canadian Barcode of Life Network, Genome Canada, the Ontario Genomics Institute, the Natural Sciences and Engineering Research Council of Canada, and other sponsors listed at www.BOLNET.ca for providing unpublished *tuf*A DNA sequences. Additional support was provided by the Canada Research Chair Program, the Canada Foundation for Innovation, New Brunswick Innovation, the SA Wildlife Conservation Fund (2009/10 #1458) and the Sir Mark Mitchell Foundation to GSB; the Netherlands Organization for Scientific Research (WOTRO–NWO grant R 85–363) Naturalis Biodiversity Center, and the Research Centre for Oceanography of the Indonesian Institute of Sciences (PPO–LIPI) to SGAD and WFPR. JMH acknowledges the support of the Australian Biological Resources Study. Finally we acknowledge the assistance of staff at AD, BM, L, LD, MEL, NSW, PC and TCD. Finally we acknowledge the assistance of staff at AD, BM, L, LD, MEL, NSW, PC and TCD.

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Table 1. Accepted *Caulerpa* taxonomy from southern Australia prior to the present study

Species	**Most recent reference**
C. alternans Womersley 1956: 364	Womersley 1984
C. articulata Harvey in J.D. Hooker 1855b: 261	Huisman 2000
C. brachypus Harvey 1860: 333	Price 2011
C. brownii (C. Agardh) Endlicher 1843: 16	Womersley 1984
C. cactoides (Brown *ex* Turner) C. Agardh 1817: xxiii	Womersley 1984
C. cliftonii Harvey 1863: LVII	Womersley 1984
C. cupressoides (H.West) C. Agardh 1817: xxiii	Huisman & Walker 1990
C. cylindracea Sonder 1845: 50	Belton et al. accepted
C. ellistoniae Womersley 1955: 387, fig. 2	Womersley 1984
C. flexilis J.V. Lamouroux 1813: 283, pl. 7, fig.	Womersley 1984
var. *muelleri* (Sonder) Womersley 1956: 367	Womersley 1984
C. hedleyi Weber-van Bosse 1910: 1, pl. 1, figs. 1-4	Womersley 1984
C. heterophylla I.Price, Huisman & M.A. Borowitzka 1998: 12-14, Figs 3-7	Price *et al.* 1998
C. hodgkinsoniae J. Agardh 1887: 129	Millar & Kraft 1994
C. lagara Carruthers, Walker & Huisman 1993: 595, fig. 16	Carruthers *et al.* 1993
C. longifolia C. Agardh 1823: 437	Womersley 1984
f. *crispata* (Harvey) Womersley (1950: 147)	Womersley 1984
C. obscura Sonder 1845: 50	Womersley 1984

C. papillosa J. Agardh 1873: 42	Womersley 1984
C. remotifolia Sonder 1853: 660	Womersley 1984
C. scalpelliformis (R. Brown *ex* Turner) C. Agardh 1817: XXII	Womersley 1984
C. sedoides (R. Brown *ex* Turner) C. Agardh 1817: XXIII	Silva *et al.* 1996
f. geminata (Harvey) Weber-van Bosse 1898: 387-388	Coppejans *et al.* 1998
C. simpliciuscula (R. Brown *ex* Turner) C. Agardh 1823: 439	Womersley 1984
var. *laxa* Womersley 1956: 370	Womersley 1984
C. taxifolia (H.West) C. Agardh 1817: xxii	Womersley 2003
var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini	Jongma *et al*. 2012
C. trifaria Harvey 1863 lvii, pl. CCLXI	Womersley 1984
C. vesiculifera (Harvey) Harvey 1863: LVI	Womersley 1984

Table 2. Updated list and nomenclature of *Caulerpa* species known from southern Australia with reference *tuf*A accession numbers. Species not collected in the present study are shaded in grey. *C.* = *Caulerpa*.

Species	Type locality	Homotypic synonyms	Heterotypic synonyms	Distribution	Reference *tuf*A sequence
C. alternans Womersley 1956: 364	Port Phillip heads, Victoria		*C. alternifolia* J. Agardh	Port Phillip heads, Victoria to the South Australian Gulfs	KF649862
C. articulata Harvey in J.D. Hooker 1855b: 261	East coast of New Zealand			South-western Australia and New Zealand	–
C. brownii (C. Agardh) Endlicher 1843: 16	Kent Islands, Bass Strait	*C. selago* var. *brownii* C. Agardh; *Chauvinia selago* var. *brownii* (C. Agardh) Trevisan	*C. furcifolia* J.D. Hooker & Harvey; *Chauvinia furcifolia* (J.D. Hooker & Harvey) Trevisan	Perth, Western Australia to Walkerville, Victoria and around Tasmania	KF649856

C. cactoides (Brown *ex* Turner) C. Agardh 1817: xxiii	Southern coast of Australia	*Fucus cactoides* Brown *ex* Turner; *Ahnfeldtia cactoides* (Brown *ex* Turner) Trevisan; *Chauvinia cactoides* (Brown *ex* Turner) Kutzing	*C. cactoides* var. *gracilis* G.Murray; *Tricladia australis* Decaisne	Houtman Albrolhos Is., Western Australia around southern Australia and Tasmania to Ballina, New South Wales	JN817650
C. cliftonii Harvey 1863: LVII	Western Australia			Houtman Albrolhos Is., Western Australia around southern Australia to Port Phillip heads, Victoria	KF649854
C. corynephora Montagne 1842: 14	Torres Strait, Queensland		*Caulerpa racemosa* var. *corynephora* (Mont.) Weber-van Bosse	Northern Australia south to Albany in Western Australia	JN817653

C. crispata (Harvey) Belton & Huisman *stat. nov.*	Port Phillip heads, Victoria	*C. harveyi* var. *crispata* Harvey; *C. curvifolia* J. Agardh *ex* Bracebridge Wilson (*nomen nudum*); *C. longifolia* f. *crispata* (Harvey) Womersley		From Jurien Bay, Western Australia to Wilsons Promontory, Victoria and around Tasmania	KF649917
C. cupressoides (H.West) C. Agardh 1817: xxiii	St Croix, Virgin Islands	*Fucus cupressoides* Vahl; *Chauvinia cupressoides* (M.Vahl) Trevisan	*Chauvinia indica* Sonder ex Kützing 1857 *C. triangularis* Mazé & Schramm	Widespread in tropical waters. In southern Australia, only known from a single specimen on Rottnest Island, Western Australia	AJ417929*
C. cylindracea Sonder 1845: 50	Western Australia	*Ahnfeldtia cylindracea* (Sonder) Trevisan;		New Caledonia around northern Australia to Cape	JN851143 (from

		Chauvinia cylindracea (Sonder) Kutzing; *C. racemosa* [var. *laetevirens*] f. *cylindracea* (Sonder) Weber-van Bosse; *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque	le Grand, Western Australia. Invasive in the Mediterranean Sea, Canary Islands, South Australia and Victoria.	Sauvage *et al.* 2013)
C. ellistoniae Womersley 1955: 387, fig. 2	Elliston, South Australia		Rottnest I. and Cape Hamelin, Western Australia; Pearson I., Elliston, and Kangaroo I., South Australia	–

C. fergusonii Grunow *ex* G.Murray 1891: 212, pl. 53, figs 1,2	Sri Lanka			In Western Australia from the Kimberley south to Canal Rocks. Also reported from India, Sri Lanka, Japan, Indonesia, Malaysia, the Philippines, Papua New Guinea and Fiji.	JN851136
C. filiformis (Suhr) Hering 1841: 91	Algoa Bay, South Africa	*Amphibolis filiformis* Suhr; *Himandactylius filiformis* (Suhr) Trevisan	*C. ligulata* Harvey *ex* J. Agardh; *C. flagelliformis* var. *ligulata* (Harvey *ex* J, Agardh)Weber-van Bosse	Around Sydney, New South Wales, the southern coast of South Africa and Peru	FM956035
C. flexilis J.V. Lamouroux 1813: 283, pl. 7, fig. 3	Esperance, Western Australia	*Chauvinia flexilis* (J.V. Lamouroux ex C. Agardh) Trevisan	*Fucus hypnoides* R. Brown *ex* Turner; *C. hypnoides* (R. Brown *ex* Turner) C.	Geraldton, Western Australia to Collaroy, New South Wales and around	KF649866

			Agardh; *Chauvinia hypnoides* (R. Brown *ex* Turner) Trevisan	Tasmania. New Zealand (North Island).	
C. hedleyi Weber-van Bosse 1910: 1, pl. 1, figs. 1-4	Kangaroo Island, South Australia			Rottnest I., Western Australia to Kangaroo I., South Australia	KF649869
C. heterophylla I.Price, Huisman & M.A. Borowitzka 1998: 12-14, Figs 3-7	Rottnest Island, Western Australia			Rottnest I. and Albany, Western Australia	Victor harbor
C. hodgkinsoniae J. Agardh 1887: 129 (as *C. hodkinsoniae*)	Richmond River, Ballina, New South Wales			New South Wales	KF649883

C. lagara Carruthers, Walker & Huisman 1993: 595, fig. 16	Perth, Western Australia			Swan River, Perth, Western Australia	–
C. longifolia C. Agardh 1823: 437	Southern Australia		*Corradoria longifolia* (J. Agardh) Trevisan; *C. harveyi* F.Mueller *ex* Harvey	Eucla, Western Australia to Wilsons Promontory, Victoria and around Tasmania,	KF649914
C. lucasii Prud'homme, Draisma & Belton	Tasmania	*C. annulata* A. Lucas *nom. illeg.*		Sir Joseph Banks Is., S.A. to Walkerville, Victoria and around Tasmania	KF649877
C. muelleri Sonder 1853: 661	Rivoli Bay, South Australia	*C. hypnoides* var. *muelleri* (Sonder) Weber-van Bosse; *C. flexilis* var.		Rottnest Island, Western Australia to Waratah Bay, Victoria, and around Tasmania.	JN817643

		muelleri (Sonder) Womersley			
C. obscura Sonder 1845: 50	Western Australia	*Chauvinia obscura* (Sonder) Trevisan	*C. sonderi* F. Mueller; *C. obscura* f. *sonderi* (Mueller) Weber-van Bosse	Yanchep Beach, Western Australia to Walkerville, Victoria and around Tasmania.	JN817645
C. papillosa J. Agardh 1873: 42	Queenscliff, Victoria			Recherche Archipelago, Western Australia to Walkerville, Victoria and northern Tasmania	KF649879
C. parvifolia Harvey 1860b, pl. 172	Kiama, New South Wales	*C. brachypus* f. *parvifolia* (Harvey) A.B. Cribb 1958: 209		New South Wales including the Norfolk I. and Lord Howe I. Specimens from Ningaloo Reef south to	KF649905

			Cape Naturaliste, Western Australia are most likely a different species.	
C. remotifolia Sonder 1853: 660	Lefevre Pen., South Australia		Jurien Bay, Western Australia to Westernport Bay, Victoria and northern Tasmania.	KF649891
C. scalpelliformis (R. Brown *ex* Turner) C. Agardh 1817: XXII	Southern coast of Australia	*Fucus scalpelliformis* R. Brown *ex* Turner; *Corradoria scalpelliformis* (R. Brown *ex* Turner) Trevisan	Perth, Western Australia to Jervis Bay, New South Wales and around Tasmania	KF649897

C. sedoides (R. Brown *ex* Turner) C. Agardh 1817: XXIII	Kent Islands, Bass Strait	*Fucus sedoides* R. Brown *ex* Turner; *Chauvinia sedoides* (R. Brown *ex* Turner) Kützing		Port Denison, Western Australia to Coffs Harbour, New South Wales (incl. Norfolk I.) and around Tasmania	KF649888
C. simpliciuscula (R. Brown *ex* Turner) C. Agardh 1823: 439	Kent Island, Bass Strait	*Ahnfeldtia simpliciuscula* (R.Brown) Trevisan; *Fucus simpliciuscula* R. Brown *ex* Turner; *Chauvinia simpliciuscula* (R. Brown *ex* Turner) Kützing		Port Denison, Western Australia to Wilson Promontory, Victoria and around Tasmania	KF649875

var. *laxa* Womersley 1956: 370	Georgetown, Tasmania			In calm sheltered waters. North coast of Kangaroo I., to Phillip I., Victoria and around Tasmania	–
C. taxifolia (H.West) C. Agardh 1817: xxii	St. Croix, Virgin Islands	*Fucus taxifolius* H.West		Globally distributed in tropical waters. Invasive in Adelaide, South Australia, estuaries of southern New South Wales and the Mediterranean Sea.	AJ417938*
var. *distichophylla* (Sonder) Verlaque, Huisman &	Western Australia	*C. distichophylla* Sonder		Ningaloo Reef south to Cape Naturaliste, Western Australia and the Mediterranean Sea.	KF649925

Procaccini, in Jongma *et al*. (2012)					
C. trifaria Harvey 1863 lvii, pl. CCLXI	Port Phillip Heads, Victoria			Cottesloe, Western Australia to Western Port, Victoria and around Tasmania	KF649860
C. vesiculifera (Harvey) Harvey 1863: LVI	Westernport, Victoria	*C. simpliciuscula* var. *vesiculifera* Harvey	*C. ethelae* Weber-van Bosse	Shark Bay, Western Australia to Phillip I., Victoria and the north coast of Tasmania	KF649878

* Proposed reference sequences not from the present study but from specimens collected closer to the type localities by Famà et al. (2002)

Table 3. Collection data and GenBank accessions for newly sequenced specimens of *Caulerpa* from the present study. All specimens were collected in Australia unless specified (Vic.=Victoria; SA=South Australia; WA=Western Australia, NSW=New South Wales; Qld=Queensland). AD-A specimens are housed in AD; DML specimens are housed in US; GWS specimens are housed in UNB; HV specimens are housed in GENT; L specimens are housed in L; and PERTH specimens are housed in PERTH (abbreviations follow Holmgren *et al.* 1990).

					GenBank accession no.	
Species	**Voucher**	**Location**	**Collection date**	**Collector**	***tuf*A**	***rbc*L**
C. alternans	AD-A88966	drift, Lacepede Bay, SA	06 May 2009	R.N. Baldock	KF649862	–
C. brachypus	TS0518	Tomioka, Amakusa, Kumamoto Prefecture, Japan	17 May 2010	A. Kurihara	KF649910	–
C. brachypus	TS0523	Izumo, Kushimoto, Wakayama Prefecture, Japan	10 June 2010	A. Kurihara	KF649909	–
C. brownii	AD-A74759	Mexican Hat, Great Australian Bight, SA	25 Sept. 2008	F. Gurgel & R.N. Baldock	KF649857	–

C. brownii	AD-A89132	Hamelin Bay, WA	09 Dec. 2009	M. Thomsen & T. Wernberg	KF649858	–
C. brownii	AD-A92244	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649856	KF649933
C. brownii	L 81(5)	Wellington, N.Z.	31 Oct. 1998	W. Prud’homme van Reine	FM956037	-
C. cactoides	GWS016222	Boat Harbour Beach, Tas.	27 Jan. 2010	G.W. Saunders, K. Dixon & L. Kraft	JN851133	–
C. cactoides	AD-A74763	Coombra, Great Australian Bight, SA	25 Sept. 2008	F. Gurgel & R.N. Baldock	JN817650	–
C. cactoides	GWS024974	Cosy Corner, WA	10 Nov. 2010	G.W. Saunders & K. Dixon	JN851134	KF649951
C. cliftonii	AD-A93731	Pt. Lonsdale, Vic.	09 Nov. 2011	G.S. Belton & R. Dixon	KF649855	KF649931

C. cliftonii	AD-A79076	Kangaroo Island, SA	07 Feb. 2008	F. Gurgel & K. Dixon	KF649854	KF649930
C. coppejansii	BRI (tbd)	Heron I., Great Barrier Reef, Qld.	01 Sep. 2008	F. Gurgel, R. Dixon & J.M. Huisman	KF649871	–
C. coppejansii	DML40154	North Astrolab Reef, Fiji	17 Feb. 1996	D. Littler, M. Littler & B. Brooks	KF649872	–
C. coppejansii	AD-A95458A	Heron I., Great Barrier Reef, Qld.	Sept. 2012	G.S. Belton	KF649880	–
C. coppejansii	AD-A95458B	Heron I., Great Barrier Reef, Qld.	Sept. 2012	G.S. Belton	KF649881	–
C. corynephora	GWS024470	Little Beach, WA	07 Nov. 2010	G.W. Saunders & K. Dixon	JN851135	–
C. corynephora	PERTH08292590	Long Reef, Kimberley, WA	21 Oct. 2010	J.M. Huisman	JN817653	–
C. corynephora	AD-A90130	Ningaloo Reef, W.A.	20 May 2009	F. Gurgel & R. Dixon	JN817652	–

C. crispata	GWS025171	Canal Rocks, WA	11 Nov. 2010	G.W. Saunders & K. Dixon	KF649916	–
C. crispata	GWS016662	The Springs, Point Lonsdale, Vic.	02 Feb. 2010	G.W. Saunders, L. Kraft & K. Dixon	KF649917	–
C. crispata	GWS016226	Boat Harbour Beach, Tas.	27 Jan. 2010	G.W. Saunders, K. Dixon & L. Kraft	KF649918	–
C. crispata	AD-A89139	Hamelin Bay, WA	09 Dec. 2009	M. Thomsen & T. Wernberg	KF649912	KF649955
C. crispata	AD-A92236	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649913	KF649956
C. cylindracea	GWS025468	Pt. Peron, WA	09 Nov. 2010	G.W. Saunders & K. Dixon	KF649921	–
C. cylindracea	GWS025467	Pt. Peron, WA	09 Nov. 2010	G.W. Saunders & K. Dixon	KF649922	KF649968

C. cylindracea	AD-A93727	O' Sullivans Beach, SA	07 Apr. 2011	G. Belton, W.M. Grant & S. Taylor	JQ894933 (from Sauvage et al. (2013)	KF649967
C. cylindracea	AD-A98125	Portland Harbor, Vic	17 Nov. 2011	C. White	KF649903	–
C. fergusonii	GWS025196	Canal Rocks, WA	11 Nov. 2010	G. Saunders & K. Dixon	JN851136	KF649942
C. fergusonii	GWS025259	Geographe Bay, WA	12 Nov. 2010	G. Saunders & K. Dixon	JN851137	–
C. fergusonii	AD-A93587	Rottnest Island, WA	17 Nov. 2010	G. Belton	KF649870	–
C. filiformis	AD-A95186	Botany Bay, NSW	18 Feb. 2012	F. Gurgel, W. Grant & S. Taylor	KF649902	–
C. filiformis	HV2140	Sydney, NSW	22 Nov. 2009	H. Verbruggen	KF649901	–

C. filiformis	L2005-31	Rock Bay, South Africa	17 Aug. 2005	W. Prud'homme van Reine	FM956035 (from Sauvage et al. 2013)	FR848349
C. flexilis	GWS015249	Burying Ground Point, Tas.	21 Jan. 2010	G.W. Saunders & K. Dixon	JN851146	KF649938
C. flexilis	AD-A79649	Kangaroo I., SA	10 Feb. 2008	F. Gurgel & K. Dixon	KF649867	–
C. flexilis	GWS024748	Cape Leeuwin, WA.	09 Nov. 2010	G.W. Saunders & K. Dixon	KF649866	–
C. flexilis	AD-A92238	Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649865	KF649939
C. hedleyi	GWS025664	Rottnest I., WA	18 Nov. 2010	G.W. Saunders & K. Dixon	KF649868	KF649928

C. hedleyi	AD-A89143	Marmion, WA	Nov. 2009	M. Thomsen & T. Wernberg	KF649869	KF649929
C. heterophylla		Victor Harbour, SA	Dec 2012		TBD	
C. hodgkinsoniae	GWS032688	Coffs Harbour, NSW	10 Dec. 2012	G. Filloramo & L. Kraft	KF649883	–
C. hodgkinsoniae	GWS032711	Coffs Harbour, NSW	11 Dec. 2012	G. Saunders & K. Dixon	KF649884	–
C. lentillifera	PERTH08292655	Montgomery Reef, Kimberley, WA	22 Oct. 2009	R. Dixon	JN817649	–
C. longifolia	AD-A92232	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649914	KF649953
C. longifolia	AD-A93781	Port Macdonnell, Vic.	13 Nov. 2011	G. Belton & R. Dixon	KF649915	KF649954
C. lucasii	AD-A88572	Sir Joseph Banks Group, SA	05 May 2009	K. Dixon	KF649876	KF649948

C. lucasii	AD-A93737	Portland, Vic.	14 Nov. 2011	G. Belton & R. Dixon	KF649877	–
C. muelleri	GWS016521	Stanley Breakwater, Tas.	29 Jan. 2010	G. Saunders, L. Kraft & K. Dixon	KF649863	KF649936
C. muelleri	AD-A92239	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	JN817643	KF649937
C. muelleri	AD-A74721	Kings Head, SA	09 Nov. 2007	F. Gurgel & C. Ricci	JN817644	KF649935
C. muelleri	GWS025334	Canal Rocks, WA	11 Nov. 2010	G. Saunders & K. Dixon	KF649864	KF649934
C. obscura	GWS016619	Point Lonsdale, Vic	02 Feb. 2010	G. Saunders, L. Kraft & K. Dixon	JN817644	–
C. obscura	AD-A90909	Pt Peron, WA	04 June 2010	G. Belton & F. Gurgel	JN817646	KF649941

C. obscura	GWS024445	Little Beach, WA	07 Nov. 2010	G. Saunders & K. Dixon	JN851145	–
C. obscura	AD-A92237	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	JN817645	KF649940
C. opposita	AD-A92544	Lizard Island, Great Barrier Reef, Qld.	Sept. 2010	G. Belton & M. Marklund	JN817647	KF649949
C. opposita	L03-038	Berau Archipelago, Indonesia	Oct. 2003	W. Prud'homme van Reine & L.N. de Senerpont Domis	FM956026	–
C. papillosa	AD-A92242	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649879	KF649952
"*C. parvifolia*"	GWS025307	Canal Rocks, WA	11 Nov. 2010	G. Saunders & K. Dixon	KF649923	–

"*C. parvifolia*"	AD-A93919	Geographe Bay, WA	12 Nov. 2010	G. Belton & R. Dixon	KF649924	KF649963
C. parvifolia	GWS023177	Lord Howe Island, NSW	22 Nov. 2010	G. Saunders & K. Dixon	KF649927	–
C. parvifolia	GWS032313	Norfolk Island, NSW	01 Dec. 2012	G. Saunders & K. Dixon	KF649926	–
C. parvifolia	GWS032309	Norfolk Island, NSW	01 Dec. 2012	G. Saunders & K. Dixon	KF649908	–
C. parvifolia	GWS032319	Norfolk Island, NSW	01 Dec. 2012	G. Saunders & K. Dixon	KF649907	–
C. parvifolia	GWS032622	Coffs Harbour, NSW	09 Dec. 2012	G. Saunders & K. Dixon	KF649905	KF649964
C. parvifolia	GWS032623	Coffs Harbour, NSW	09 Dec. 2012	G. Saunders & K. Dixon	KF649906	–

C. parvifolia	GWS032634	Coffs Harbour, NSW	09 Dec. 2012	G. Saunders & K. Dixon	KF649904	–
C. remotifolia	GWS025195	Canal Rocks, WA	11 Nov. 2010	G. Saunders & K. Dixon	KF649920	KF649961
C. remotifolia	GWS025258	Eagle Bay Bommie, WA	12 Nov. 2010	G. Saunders & K. Dixon	KF649919	–
C. remotifolia	AD-A89138	Jurien Bay, WA	12 Dec. 2009	M. Thomsen & T. Wernberg	KF649892	KF649959
C. remotifolia	AD-A92285	American River, Kangaroo I., SA	27 Feb. 2011	G. Belton & T. Spokes	KF649891	KF649960
C. remotifolia	AD-A92287	American River, Kangaroo I., SA	27 Feb. 2011	G. Belton & T. Spokes	KF649894	–
C. remotifolia	AD-A93751	Port Philip Bay, Vic.	10 Nov. 2011	G. Belton & R. Dixon	KF649893	KF649962

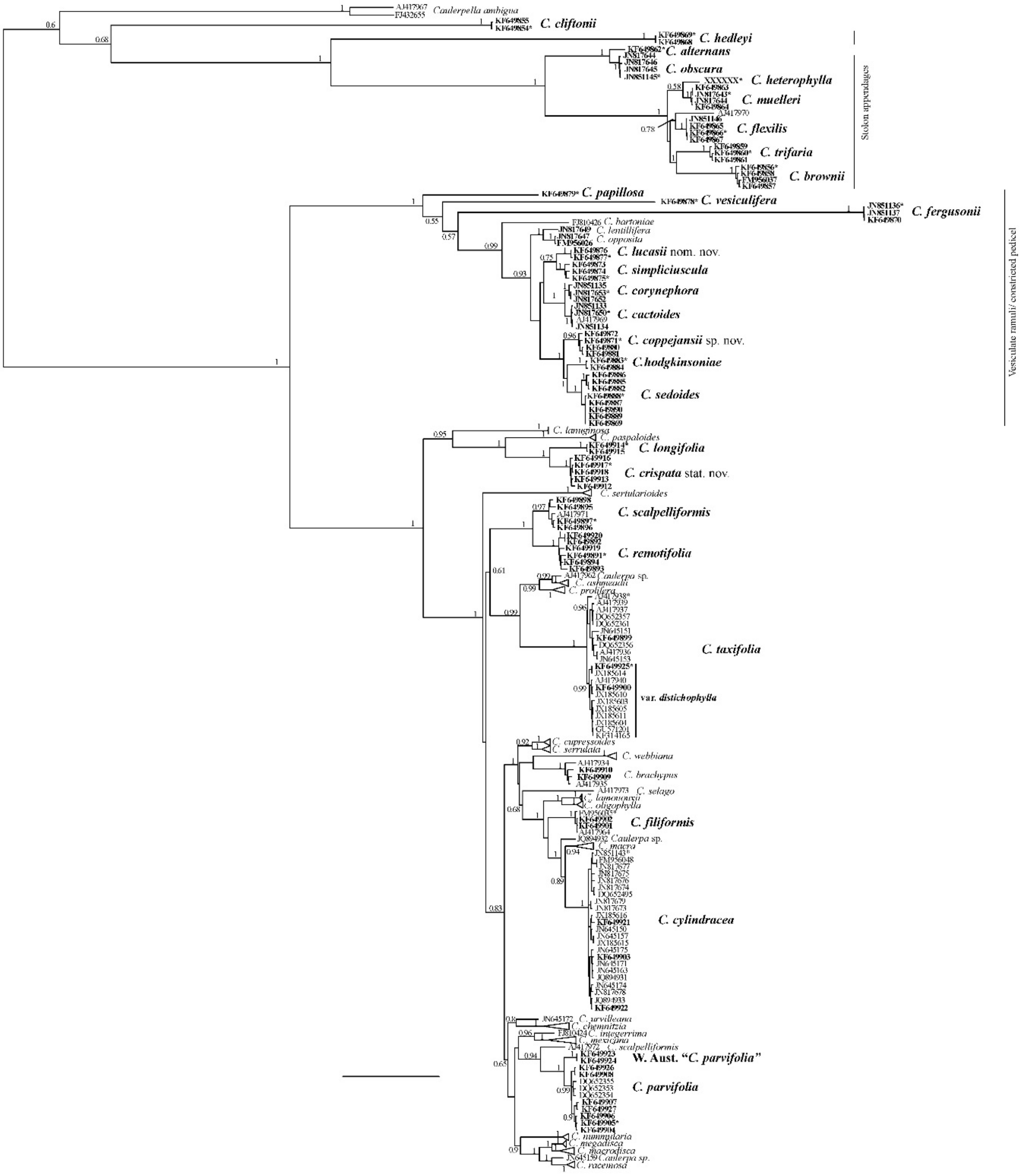
C. remotifolia	FR848355	Port Philip Bay, Vic	06 Nov. 2009	W. Prud’homme van Reine	–	FR848355
C. scalpelliformis	GWS015575	Nine Pin Point, Tas.	22 Jan. 2010	G.T. Kraft & L. Kraft	KF649898	KF649957
C. scalpelliformis	AD-A92213	Pennington Bay, Kangaroo I., SA	24 Feb. 2011	G. Belton & F. Gurgel	KF649895	–
C. scalpelliformis	AD-A92252	Vivonne Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton, R. Dixon & T. Spokes	KF649897	KF649958
C. scalpelliformis	HV2246	Flinders, Vic.	27 Nov. 2009	H. Verbruggen	KF649896	–
C. sedoides f. *tasmanica*	GWS015273	Burying Ground Point, Tas.	21 Jan. 2010	G. Saunders & K. Dixon	KF649889	KF649945
C. sedoides	GWS016227	Boat Harbour Beach, Tas.	27 Jan. 2010	G. Saunders, K. Dixon & L. Kraft	KF649888	–

C. sedoides	GWS016545	Stanley Breakwater, Tas.	29 Jan. 2010	G. Saunders, L. Kraft & K. Dixon	KF649887	–
C. sedoides	GWS025332	Canal Rocks, WA	11 Nov. 2010	G. Saunders & K. Dixon	KF649890	KF649946
C. sedoides	GWS032239	Norfolk Island, NSW	29 Nov. 2012	G. Saunders & K. Dixon	KF649885	–
C. sedoides	GWS032273	Norfolk Island, NSW	30 Nov. 2012	G. Saunders & K. Dixon	KF649882	–
C. sedoides	GWS032314	Norfolk Island, NSW	01 Dec. 2012	G. Saunders & K. Dixon	KF649886	–
C. sedoides f. geminata	AD-A87725	Pt. Peron, WA	27 Apr. 2009	M. Thomsen & T. Wernberg	KF649969	KF649947
C. simpliciuscula	AD-A92243	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649875	KF649944

C. simpliciuscula	AD-A89133	Jurien Bay, WA	11 Dec. 2009	M. Thomsen & T. Wernberg	KF649873	KF649943
C. simpliciuscula	GWS015874	Tinderbox, Tas.	23 Jan. 2010	G.W. Saunders & K. Dixon	KF649874	–
C. taxifolia	AD-A94929	Port River, Adelaide, SA	08 Dec. 2011	W. Grant & S. Taylor	KF649899	KF649966
C. taxifolia var. *distichophylla*	AD-A89134	Jurien Bay, WA	11 Dec. 2009	M. Thomsen & T. Wernberg	KF649900	KF649965
C. taxifolia var. *distichophylla*	GWS025589	Rottnest I., WA	17 Nov. 2010	G. Saunders & K. Dixon	KF649925	–
C. trifaria	AD-A92257	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649860	KF649932
C. trifaria	AD-A88364	Pt Peron, WA	27 Mar. 2009	M. Thomsen & T. Wernberg	KF649861	–

C. trifaria	AD-A79664	Kangaroo Island, SA	10 Feb. 2008	F. Gurgel & K. Dixon	KF649859	–
C. vesiculifera	AD-A92234	Hanson Bay, Kangaroo I., SA	25 Feb. 2011	G. Belton & F. Gurgel	KF649878	KF649950

Fig. 1. Phylogenetic tree of *Caulerpa* based on a Bayesian Inference analysis of 335 *tuf*A sequences (820 nt) showing GMYC lineages supported by long branch/high posterior probabilities. Southern Australian species are in larger bold font and numbers at nodes correspond to posterior probabilities resulting from the Bayesian analysis. Boldface = newly obtained sequences. * indicates proposed reference sequence. Scale bar = 0.003 expected changes per site.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KF649854*
C. cliftonii
KF649869*
KF649868
C. hedleyi
KF649862*
C. alternans
JN817644
JN817646
JN817645
JN851145*
C. obscura
XXXXXX*
C. heterophylla
KF649863
JN817643*
JN817644
KF649864
C. muelleri
AJ417970
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C. flexilis
KF649859
KF649860*
KF649861
C. trifaria
KF649856*
KF649858
FM956037
KF649857
C. brownii
Stolon appendages
KF649879*
C. papillosa
KF649878*
C. vesiculifera
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C. fergusonii
FJ810426
C. bartoniae
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C. lentillifera
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C. opposita
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C. simpliciuscula
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C. corynephora
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C. cactoides
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KF649880
KF649881
C. coppejansii sp. nov.
KF649883*
KF649884
C.hodgkinsoniae
KF649886
KF649885
KF649882
KF649888*
KF649887
KF649890
KF649889
KF649869
C. sedoides
Vesiculate ramuli/ constricted pedicel
C. lanuginosa
C. paspaloides
KF649914*
KF649915
C. longifolia
KF649916
KF649917*
KF649918
KF649913
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C. crispata stat. nov.
C. sertularioides
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C. scalpelliformis
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C. prolifera
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C. serrulata
C. webbiana
AJ417934
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C. brachypus
AJ417973
C. selago
C. lamourouxii
C. oligophylla
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C. filiformis
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C. macra
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C. cylindracea
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C. urvilleana
C. chemnitzia
FJ810424
C. integerrima
C. mexicana
AJ417972
C. scalpelliformis
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W. Aust. "C. parvifolia"
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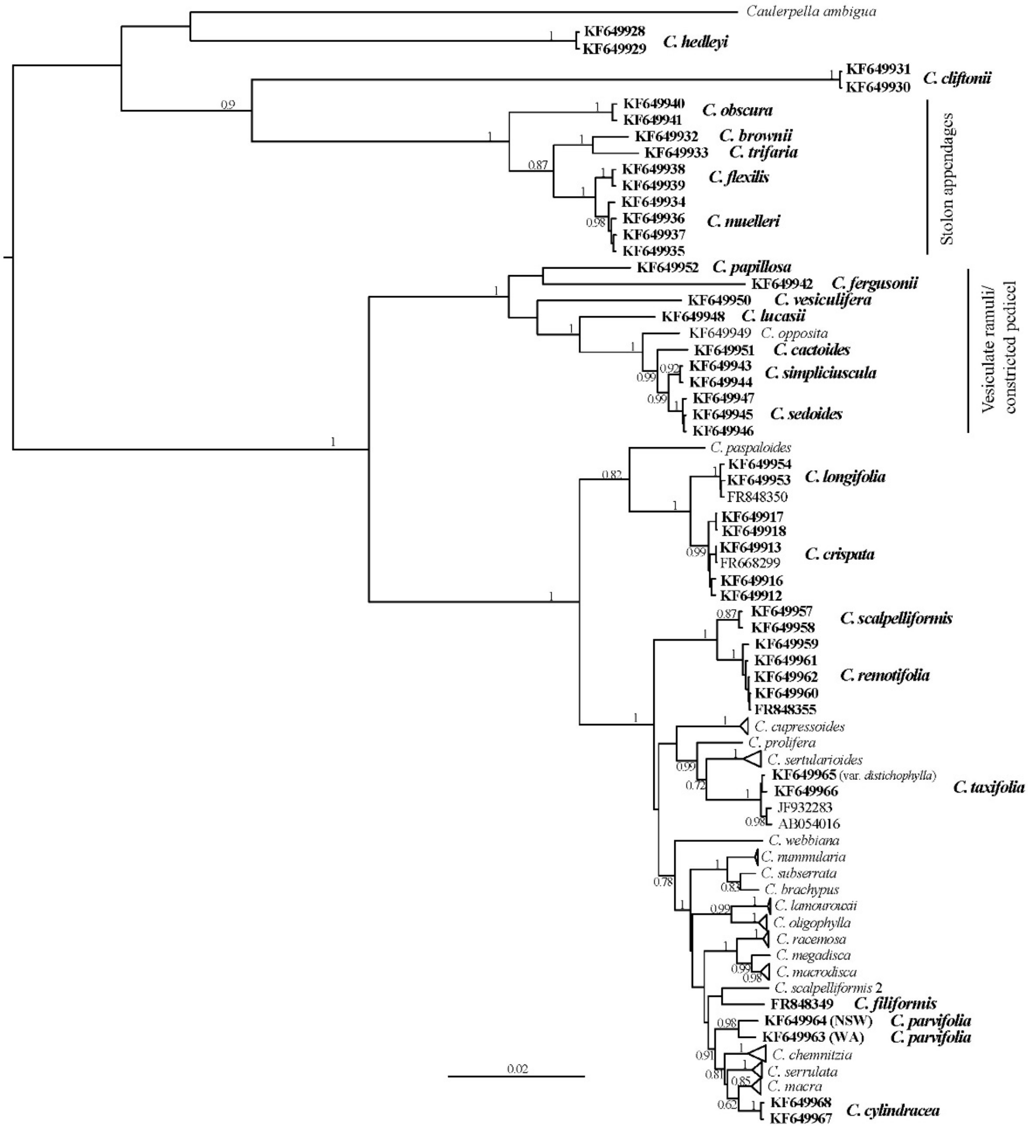
KF649905*
KF649904
C. parvifolia
C. nummularia
C. megadisca
C. macrodisca
JN645159
Caulerpa sp.
C. racemosa

Fig. 2. Phylogenetic tree of Caulerpa based on a Bayesian Inference analysis of 83 rbcL sequences (677 nt). Numbers above branches correspond to posterior probabilities resulting from Bayesian Inference analysis. Boldface = newly obtained sequences. Scale bar = 0.02 expected changes per site.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KF649940
KF649941
C. obscura
1
1
KF649932
C. brownii
KF649933
C. trifaria
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1
KF649938
KF649939
C. flexilis
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KF649937
KF649935
C. muelleri
Stolon appendages
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C. papillosa
KF649942
C. fergusonii
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KF649950
C. vesiculifera
KF649948
C. lucasii
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KF649949
C. opposita
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C. cactoides
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C. simpliciuscula
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1
KF649947
KF649945
KF649946
C. sedoides
Vesiculate ramuli/ constricted pedicel
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C. paspaloides
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1
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KF649953
FR848350
C. longifolia
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KF649917
KF649918
0.99
KF649913
FR668299
KF649916
KF649912
C. crispata
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0.87
KF649957
KF649958
C. scalpelliformis
1
1
KF649959
KF649961
KF649962
KF649960
FR848355
C. remotifolia
1
1
C. cupressoides
C. prolifera
1
C. sertularioides
0.99
0.72
KF649965 (var. distichophylla)
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KF649966
JF932283
0.98
AB054016
C. taxifolia
C. webbiana
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C. nummularia
C. subserrata
0.78
0.83
C. brachypus
1
0.99
1
C. lamourouxii
1
C. oligophylla
1
C. racemosa
1
C. megadisca
0.99
C. macrodisca
0.98
C. scalpelliformis 2
FR848349
C. filiformis
0.98
KF649964 (NSW)
C. parvifolia
KF649963 (WA)
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C. chemnitzia
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1
0.81
C. serrulata
0.85
C. macra
0.62
1
KF649968
KF649967
C. cylindracea

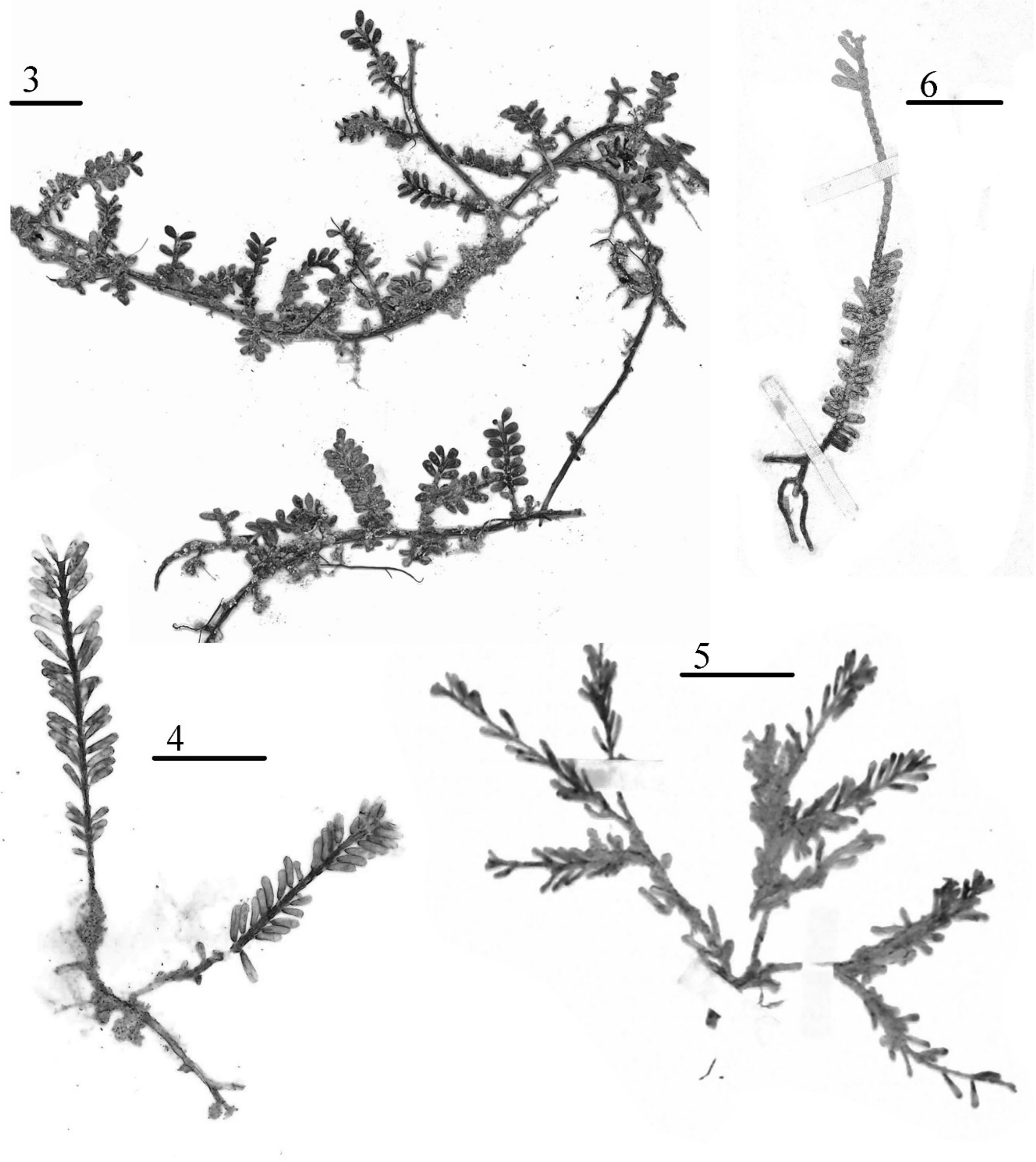
Figs. 3–6. *Caulerpa lucasii* nom. nov. and *C. hodgkinsoniae*

Fig. 3. Characteristic specimen of *C. lucasii* (previously *C. annulata*; AD-A88572). Scale bar = 20 mm

Fig. 4. Characteristic specimen of *C. hodgkinsoniae* (GWS032711). Scale bar = 15 mm

Fig. 5. Image of holotype specimen of *C. hodgkinsoniae* (LD Herb. Agardh 16587). Scale bar = 20 mm

Fig. 6. Image of lectotype specimen of *C. lucasii* (NSW288774, as *C. annulata*). Scale bar = 20 mm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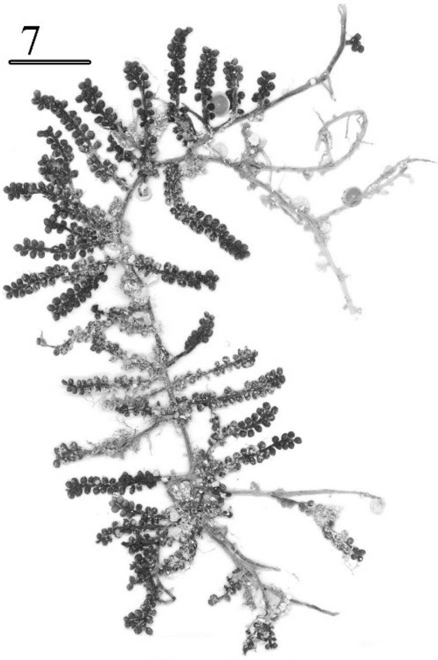
Figs. 7–10. *Caulerpa coppejansii* sp. nov. and *Caulerpa sedoides*

Fig 7. Image of the holotype specimen of *C. coppejansii* (in BRI). Scale bar = 3 cm.

Fig 8. In-situ image of *C. coppejansii* (AD-A88473b) taken on 03 Sept. 2008 at 28 m in the Wistari Channel, Heron Island, Australia.

Fig 9. Detail of the sub-spherical and opposite ramuli of *C*. *coppejansii* (in BRI). Scale bar = 0.5 mm.

Fig 10. Western Australian specimen of *C. sedoides* (GWS025322) similar in morphology to the type specimen of *C. geminata.* Scale bar = 20 mm.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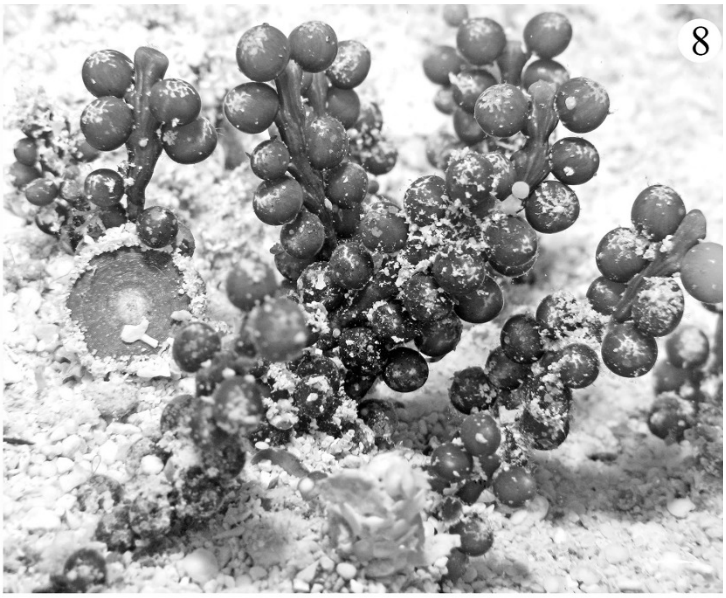
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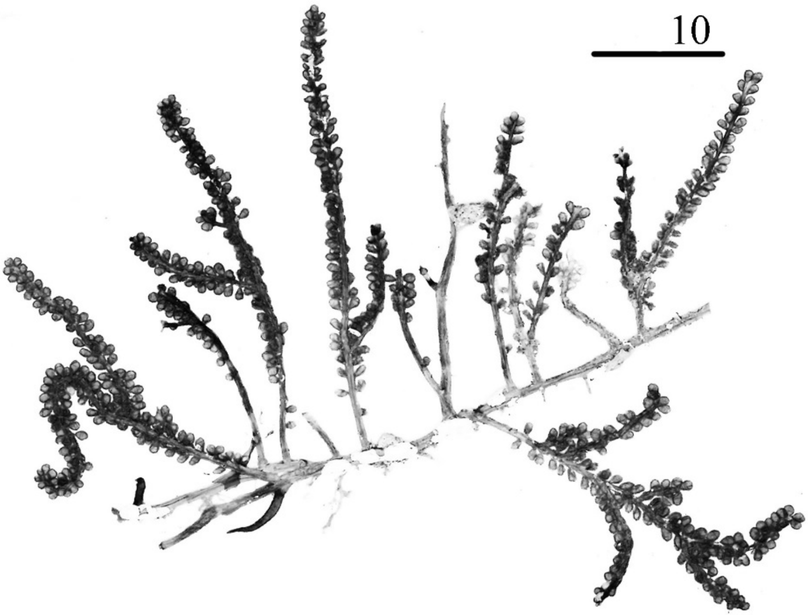
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Figs 11–15. *Caulerpa crispata* stat. nov. and *Caulerpa longifolia*.

Fig. 11. Habit of a characteristic specimen of *C. crispata* (previously *C. longifolia* f. *crispata*; AD-A92236). Scale bar = 20 mm.

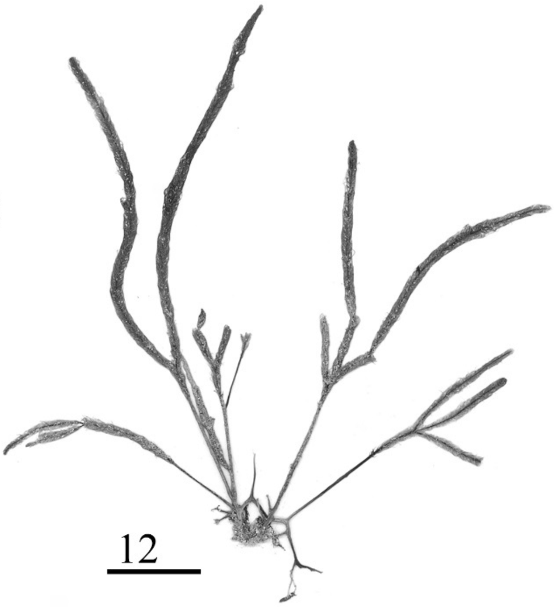
Fig. 12. Western Australian specimen of *C. crispata* with distinctive clustered branching (AD-A93870). Scale bar = 20 mm.

Fig. 13. Image of holotype specimen of *C. crispata* (TCD0011013, as *C. harveyi* var. *crispata*). Scale bar = 5 mm.

Fig. 14. Details of ramuli of *C. longifolia* (AD-A92232) in distinct rows. Scale bar = 0.5 mm.

Fig. 15. Details of randomly arranged and incurved ramuli of *C. crispata* (AD-A92236). Scale bar = 0.3 mm.

11



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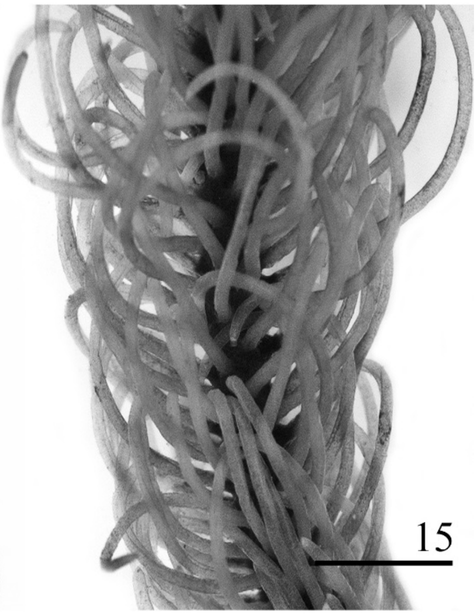

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Figs. 16–20. *Caulerpa muelleri* and *Caulerpa flexilis*.

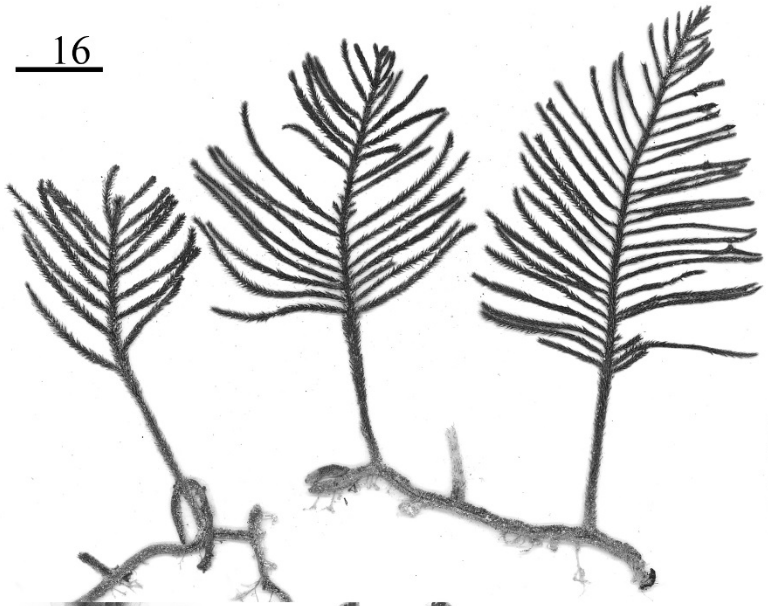
Fig. 16. General habit of *C. muelleri* (previously *C. flexilis* var. *muelleri*) showing distichously arranged ramuli (AD-A92239). Scale bar = 20 mm.

Fig. 17. Type specimen of *C. muelleri* (MEL503815). Scale bar = 30 mm.

Fig. 18. Densely arranged and upwardly curved ramuli of *C. muelleri* (AD-A92239). Scale bar = 1 mm.

Fig. 19. Habit of *C. flexilis* (AD-A92238). Scale bar = 30 mm.

Fig. 20. Arrangement and detail of ramuli of *C. flexilis* (AD-A92238). Scale bar = 1 mm.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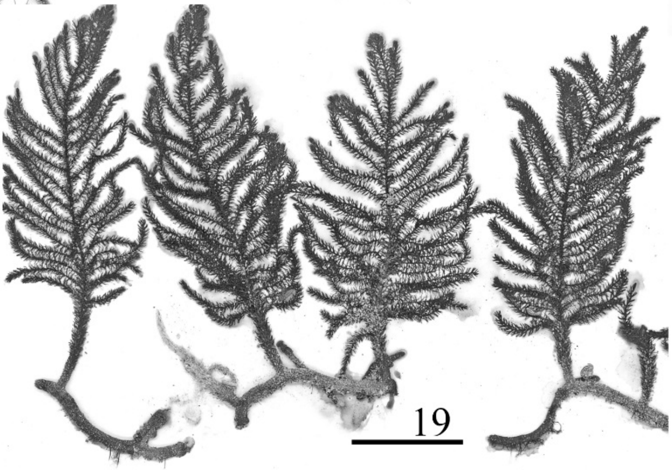
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Fig. 21. Image of the holotype specimen of *C. brachypus* (TCD0011061). Scale bar = 10 mm

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Fig. 23. Characteristic specimen of *C. parvifolia* from New South Wales (GWS032622). Scale bar = 20 mm

Fig. 24. Characteristic specimen of *C. parvifolia* from Western Australia (AD-A93919). Scale bar = 20 mm

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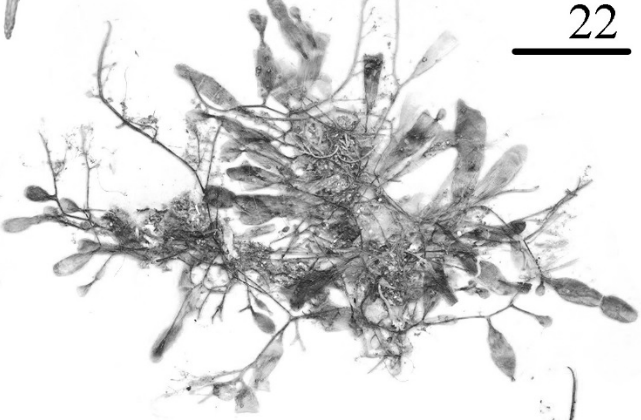

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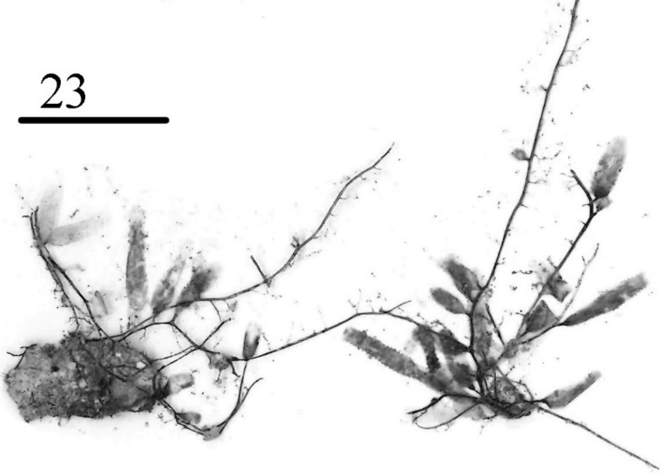

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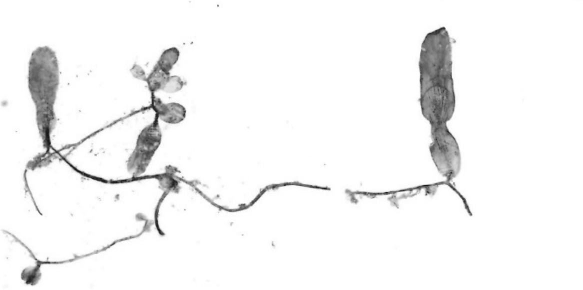

Figs. 25–27. *Caulerpa corynephora*, *Caulerpa fergusonii* & *Caulerpa cactoides*

Fig. 25. Characteristic specimen of *C. fergusonii* (AD-A93587) from Western Australia with relatively short and broad ramuli and lacking annulations at the base of the assimilator. Scale bar = 20 mm

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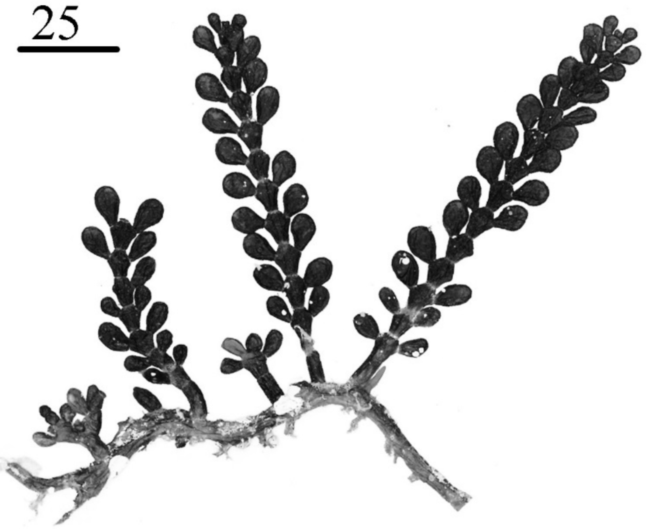
Fig. 27. Specimen of *C. cactoides* (AD-A74763) with typical habit. Scale bar = 20 mm



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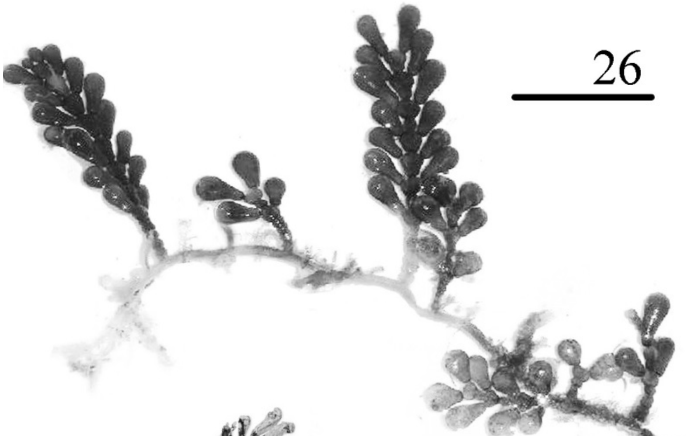

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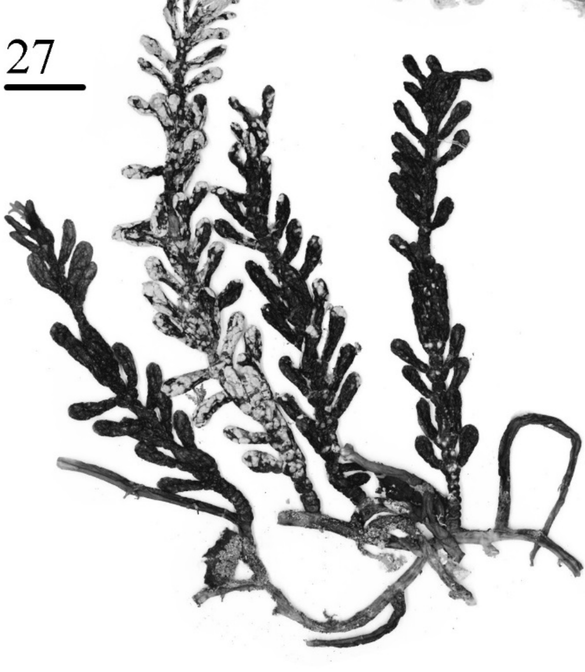

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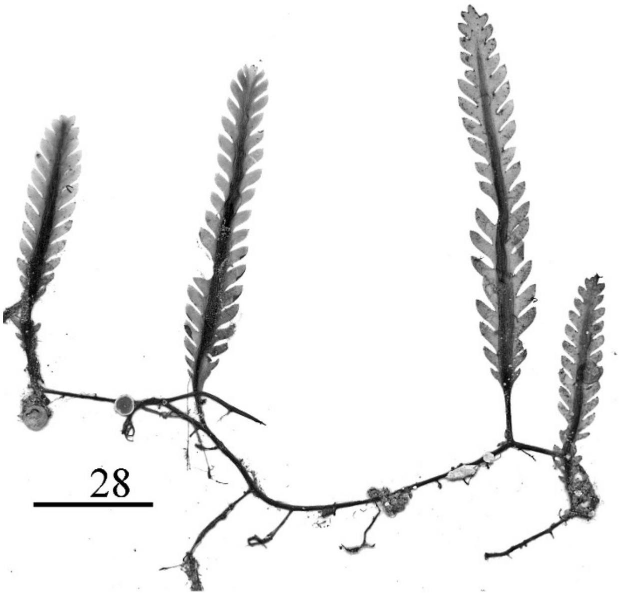
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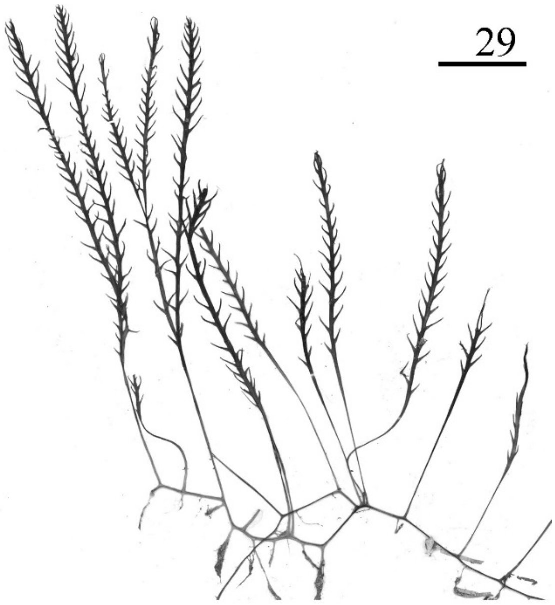
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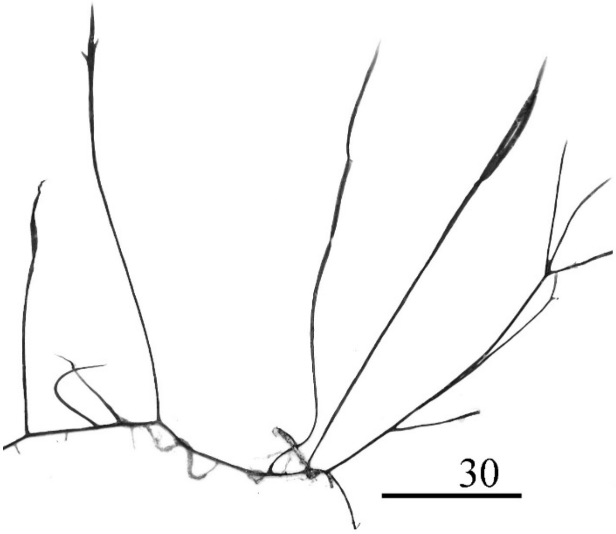
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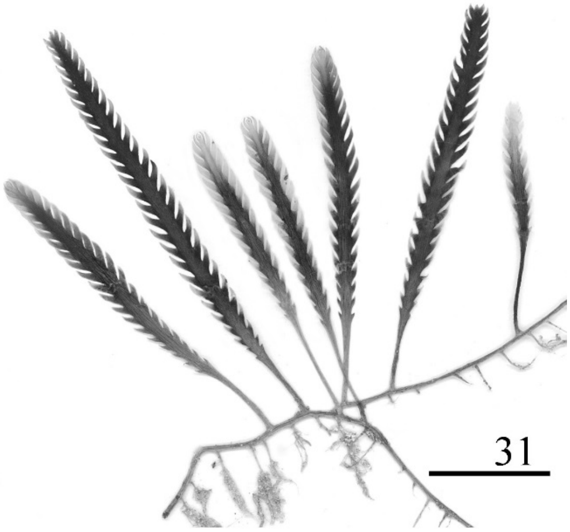
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Chapter 5: Improving transferability of introduced species' distribution models: new tools to forecast the spread of a highly invasive seaweed.

Statement of Authorship

Improving Transferability of Introduced Species' Distribution Models: New Tools to Forecast the Spread of a Highly Invasive Seaweed. Plos One

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Contributed to initial manuscript conceptualization, wrote the manuscript, collected specimens, designed the experiments, generated and analyzed data, acted as lead author in drafting initial manuscript, co-ordinated addressing of reviewers comments, prepared figures and tables.

I hereby certify that the statement of contribution is accurate.

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Improving Transferability of Introduced Species' Distribution Models: New Tools to Forecast the Spread of a Highly Invasive Seaweed

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Abstract

The utility of species distribution models for applications in invasion and global change biology is critically dependent on their transferability between regions or points in time, respectively. We introduce two methods that aim to improve the transferability of presence-only models: density-based occurrence thinning and performance-based predictor selection. We evaluate the effect of these methods along with the impact of the choice of model complexity and geographic background on the transferability of a species distribution model between geographic regions. Our multifactorial experiment focuses on the notorious invasive seaweed *Caulerpa cylindracea* (previously *Caulerpa racemosa* var. *cylindracea*) and uses Maxent, a commonly used presence-only modeling technique. We show that model transferability is markedly improved by appropriate predictor selection, with occurrence thinning, model complexity and background choice having relatively minor effects. The data shows that, if available, occurrence records from the native and invaded regions should be combined as this leads to models with high predictive power while reducing the sensitivity to choices made in the modeling process. The inferred distribution model of *Caulerpa cylindracea* shows the potential for this species to further spread along the coasts of Western Europe, western Africa and the south coast of Australia.

Citation: Verbruggen H, Tyberghein L, Belton GS, Mineur F, Jueterbock A, et al. (2013) Improving Transferability of Introduced Species' Distribution Models: New Tools to Forecast the Spread of a Highly Invasive Seaweed. PLoS ONE 8(6): e68337. doi:10.1371/journal.pone.0068337

Editor: Mark R. Muldoon, Manchester University, United Kingdom

Received January 14, 2013; **Accepted** May 28, 2013; **Published** June 28, 2013

Funding: Funding was provided by the Australian Research Council (FT110100585 to HV, LP0882109 and LP0991083 to CFDG), Research Foundation–Flanders, the AXA Research Fund (to FM), the Adelaide & Mt Lofty Ranges Natural Resource Management Board, the Australian Census of Coral Reef Life and the Australia Biological Research Study (ABRS 209-62 to CFDG). LT is a doctoral fellow of the Agency for Innovation by Science and Technology (IWT). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: Author Heroen Verbruggen is a PLoS One Editorial Board member. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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Introduction

Species distribution models (SDMs) help us understand and map species' distributions, play a key role in forecasting range expansion of introduced species and can help us predict the effects of climate change on species distributions [1-4]. An SDM characterizes the species' response to relevant environmental variables, using either physiological information from experimental work (mechanistic models) or by relating the presence and/or absence of the species to environmental information (correlative models) [5]. This response is subsequently projected into geographic space using gridded environmental layers, resulting in a map showing the potential distribution of the species. Because experimental physiological work has not been carried out for a great majority of species, correlative approaches dominate species distribution modeling. Furthermore, it is quite troublesome to assess the absence of species from an area while species occurrence data are abundant in museum databases and the literature. As a consequence, most SDMs rely on presence-only techniques [1].

A crucial assumption in using SDMs to forecast the spread of introduced species or distribution changes in response to environmental change is that the model is transferable to the new conditions [6]. In the case of introduced species, models trained primarily on distribution data from the species' native range need to be transferred to the region where it has been introduced. This often implies projecting the species response to climatic conditions that are not present in the native (training) range, which is an innately difficult task. For such situations, it is valuable to visualize those areas where extrapolation beyond observed conditions was required and consider those distribution predictions as uncertain [7,8]. In addition, the ability of presence-only methods to capture a species' ecological response is affected by the choice of background points [7], predictor variables [9], model complexity [10,11] and the geographic spread of occurrence records in relation to environmental gradients [12,13]. Besides these problems, it is also possible that biotic interactions limit the utility of models based on abiotic predictors [14] and, of course, there is always the possibility that the fundamental niche of the introduced population has changed due to natural selection [15,16].

This study focuses on the choices made during the modeling process that affect the transferability and overall predictive performance of the resulting model. We introduce two new methods that have the potential to increase the transferability of correlative SDMs: density-based occurrence thinning and performance-based predictor selection. As a case study, we apply these to the highly invasive seaweed species, *Caulerpa cylindracea*, in order to assist in assessing the risk of further spreading as well as predicting areas with suitable environmental conditions worldwide.

Methods

Experimental Design

The overarching goal of the present study is to examine and improve the overall performance and the transferability between regions of maximum entropy (Maxent) presence-only models of introduced species. The experimental design centers on the impact of four important choices that have to be made during the modeling process: (1) the amount of geographic autocorrelation in occurrence records, (2) the choice of predictor variables, (3) the complexity of the model, and (4) the selection of background points.

Because most environmental variables show spatial autocorrelation, geographically biased sampling of occurrence records (e.g. heterogeneous accessibility and local expertise) naturally results in environmental biases in the data used to train the SDM, leading to model misspecification [12,17] and issues related to its evaluation [18]. We introduce a method that thins occurrence records in densely sampled regions to obtain a more even geographic distribution (details given below). To examine the effect of this method, models with and without occurrence thinning are compared.

The choice of predictor variables is arguably one of the most studied elements affecting the transferability of SDMs, with several papers showing differences in transferability depending on which predictor set is used [9,19,20]. This has also led to the recognition of predictor variables as more conserved or relaxed, depending on whether they match between native and invaded species occurrences or not [9,21]. We introduce a method that surveys the performance of all possible predictor sets (explained below) and evaluate the transferability between regions of models built with two different sets of predictors.

The complexity of an SDM is also known to impact on its predictive performance, with overfitting often leading to poor transferability [10,22,23]. By default, Maxent determines the types of features it allows automatically, based on the number of samples available for model training [24], but this standard behavior has been reported to result in overfitted models [11]. We compare models with automatically determined model complexity to models forced to be simple.

Finally, the selection of background points is known to affect the outcome of presence-only SDMs [7,25,26]. To examine this, we compare SDMs built with global background points to models built with a regional background.

Using *C. cylindracea* as a case study, model transferability was assessed by training models on samples from either the native or the invaded range and measuring the overlap of the two models, as well as by calculating how well they predict presences in the other range. We also compare the overall predictive performance of SDMs trained with occurrences from either range to that of models combining occurrences from both ranges.

Study Species and Environmental Data

This study focuses on the introduced and highly invasive seaweed species *Caulerpa cylindracea* Sonder [27]. Specimens of the *Caulerpa* genus are well known for their rampant morphological plasticity that, due to the inconsistent use of varieties and forms amongst taxonomists, has resulted in a confusing nomenclature. Most of this confusion has existed around the *C. racemosa/peltata* complex that has more than 30 described varieties and forms [28]. Until recently this included *C. cylindracea*, which, although originally described as an independent species, had long been considered a form of *C. racemosa* var. *laetevirens* until it was raised to varietal status [29] and it is now due to be reinstated as an independent species [28].

Since the early 1990s *C. cylindracea* has rapidly and aggressively spread in the Mediterranean Sea and Canary Islands, representing one of the most dramatic marine invasions in terms of establishment and ecological dominance [30,31]. The species has been reported from all kinds of substrata and depths, as part of a variety of benthic assemblages, and thrives in disturbed habitats of the heavily urbanized Mediterranean coastlines [30,32]. Invasive populations of *C. cylindracea* establish dense and compact monospecific stands, which easily overgrow and outcompete and/or negatively impact other seaweed [33,34], seagrass [35] and invertebrate species [36,37] leading to biotic homogenization [38] and an overall decrease of species diversity in affected areas [30]. To date only partial recovery of the assemblages could be observed after eradication of *C. cylindracea* in Italy and France [33,39].

Unlike *C. taxifolia*, which was accidentally introduced from a public aquarium [40], the vector of introduction of *C. cylindracea* to the Mediterranean Sea is unknown. It was initially hypothesized to be a Lessepssian immigrant [41,42], or a hybrid between *C. racemosa* var. *turbinata* and an unknown tropical variety [43], until molecular investigations identified a potential source population in southwestern Australia [29]. However, recent findings indicate that the native range of *C. cylindracea* is much larger than previously thought (extending from Western Australia around northern Australia into the Great Barrier Reef and New Caledonia), and that the source of the invasive *C. cylindracea* populations in the Mediterranean Sea is not known with certainty [44].

Whatever the vector and source population, *C. cylindracea* is spreading rapidly with reports of its presence in 12 Mediterranean countries including all the large islands [29,45], and has more recently been reported from two locations on the southern coast of Australia (Adelaide, SA and Portland, VIC, e.g. references 46,47 and unpublished data GSB). As *C. cylindracea* is only found near shipping ports and had not been reported from this area prior to 2003 [48], it is most likely that this species is a recent introduction. The rapid spread of this species through the European invaded range makes it a suitable case study for the question at hand.

A total of 191 distribution records were assembled from the native range in and around Australia (65 records), the invaded range in Europe (111) and the recently invaded areas in southern Australia (15). The data sources for these records are: Australia Virtual Herbarium (http://chah.gov.au/avh/), new collections from Victoria by GSB deposited in the AD herbarium, the data gathered by FM for the ERC FP5 ALIENS project, and the literature [31,41,44,48-60]. The absence of the species in various DNA bar coding surveys of *Caulerpa* from some other parts of the Indo-Pacific (Philippines, Japan, Tanzania, Red Sea) suggests that the native range may be limited to Australia and some closeby locations (unpublished data: Stefano Draisma, Thomas Sauvage, Heroen Verbruggen).

We used the Bio-ORACLE dataset [61] as a source of marine environmental grids (90° N–90°S, real values). To make the distribution records compatible with the grids, occurrence coordinates situated on land according to the Bio-ORACLE grids were moved to the closest cell in the ocean. When multiple records were situated in the same Bio-ORACLE grid cell, a single record was retained and as a result, the dataset reduced to 95 distribution records.

Occurrence Thinning

Geographical biases in the occurrence records were dampened by thinning the distribution points with OccurrenceThinner 1.03 [62]. We developed this program to filter occurrence records using a probability-based procedure. The probability that any specific occurrence record is removed is proportional to the density of occurrence records in the area as defined by a kernel density grid. The two-dimensional binned kernel density grid used in this procedure was computed from the occurrence records with the bkde2D function in the R package KernSmooth v.2.23-7 [63,64], with a bandwidth of 3.0. The thinning procedure with thresholds t_1=0.5 and t_2=1.0 was repeated 10 times, resulting in 10 occurrence-thinned datasets. These datasets had on average 25 records from the native range, 46 from the European invaded range, and three from the southern Australian invasive populations.

To evaluate whether occurrence thinning influences model transferability and performance, we compared Maxent models based on a thinned subset of samples with models using all occurrence records (but limited to one per cell as mentioned above).

Predictor Sets

The predictor variables were chosen in two steps. The first step consisted of *a priori* selection of a set of 8 predictors. This selection was based on knowledge of the physiological determinants of seaweed distributions [65], and takes the structure of the Bio-ORACLE dataset into account by not using multiple closely correlated predictors. The eight resulting predictors were mean sea surface temperature (SSTmean), the range in sea surface temperature (SSTrange) as a measure of seasonality, mean photosynthetically active radiation (PARmean), salinity, pH, mean diffuse attenuation (DAmean) as a measure of water transparency, dissolved oxygen (dissox) and the phosphate concentration. Nitrate concentration was not included because it is correlated with the phosphate concentration [61].

In the second step, the predictive ability of those eight variables was explored using Maxent Model Surveyor (MMS) version 1.03 [66]. We developed this software to evaluate the performance of all possible subsets of variables (2^8 - 1 = 255 for our eight predictors), using the test AUC (Area Under the receiver operating characteristic Curve) to measure model performance [67]. The program was run multiple times: (1) on samples from native range with global background, (2) on samples from invaded range with global background, (3) on samples from both ranges with global background, (4) on samples from native range with background restricted to native range, and (5) on samples from invaded range with background restricted to invaded range. The program used 50% of the samples for training and 50% for testing. It worked from the thinned set of occurrences and restricted the model complexity to linear and quadratic features. Each of the five runs listed above was repeated ten times (i.e., on each of the ten replicate sets of thinned occurrences). The training and test data were randomly drawn from the occurrence records and do not represent a subdivision into the native vs. invaded ranges. As a consequence, the model performance used to evaluate predictor combinations does not represent transferability between regions. From the MMS results, a consensus was derived as to which variables are most important across the different runs. We retained only those variables that were present in more than 60% of the top-scoring models for at least two out of three regions (native, Europe, combined, i.e. conditions 1, 2 and 3 described above). The 60% threshold criterion is essentially arbitrary – we chose it because it halved the number of predictor variabes from eight to four (specified in results). Retaining variables important in at least two regions was done because it would prefer variables of global, rather than regional, relevance.

In order to evaluate whether this predictor selection approach can improve the transferability of models across regions, Maxent models were run with all eight variables listed above as well as the subset of four variables generated with the predictor selection procedure.

Model Complexity

Model complexity was varied to verify its impact on the predictive power and transferability. The first condition used the default behavior of Maxent (auto-features), which determines which features are used based on the number of samples [24]. The second condition forced the use of smooth response curves by allowing only linear and quadratic features to be fitted.

Background Selection

Data for background points was extracted from the Bio-ORACLE grids [61]. Three sets of 10,000 random background points were created: (1) from the entire globe, (2) from the native range defined as a box around Australia with latitude between 5° S and 45° S and longitude between 100° E and 175° E, and (3) from the invaded range defined as western Europe extending to Africa and the Mediterranean Sea, between latitude 20° N and 60° N and longitude 35° W and 40° E. These boxes roughly correspond to the maps of the native and invaded ranges presented in the results. In each of these three cases, the background selection corrected for unequal areas at different latitudes (i.e. they correspond to random pixel draws from equal area grids).

To compare the effect of background selection on transferability, regional models with corresponding regional backgrounds were compared to regional models with global background. Models trained with combined samples from the native and invaded ranges always used the global background.

Niche Model Inference

Niche models were inferred with Maxent 3.3.3f [24,68,69]. The analyses were automated via a Perl script and carried out on a multicore linux server. All analyses were run with 10,000 random background points as specified above. The training, test and background points, were provided as SWD files and the resulting models were projected onto the Bio-ORACLE grid [61]. Maxent's jackknife function was activated and samples were not added to the background to avoid complicating model comparisons. The models resulting from the ten replicate occurrence-thinned training sets were averaged for visualization but other interpretations were based on the individual models.

Downstream Analyses

Models were compared to identify which choices lead to better-performing models. In order to evaluate the transferability of models, we compared models built on the native and invaded ranges in a pairwise fashion, using the Schoener's D niche similarity measure [70] and reciprocal test AUC (i.e. native training samples with test samples in invaded range and vice versa). The overall predictive power of models was compared with the test AUC, taking care to only compare models built with identical geographic background datasets.

Results

Exploration of new methods

We implemented two methods that tackle issues related to the overall quality and transferability of niche models. The first of these, occurrence thinning, clearly reduced the geographic sampling bias present in the occurrence points, as indicated by the kernel density plots before and after occurrence thinning (Figure 1). In this figure, the red blob with dense sampling along the French Riviera and nearby localities disappears entirely after the thinning procedure (Figure 1A-B). Geographic sampling bias was less of a problem in the native range (Figure 1C-D).

The results of the second method, which surveyed all combinations of predictor combinations, is summarized in Figure 2. As could be anticipated from previous studies, the representation frequency of variables among the top-scoring models is sensitive to whether the analysis was done on the native range, the invaded range, or both combined. Using local or global background points resulted in qualitatively similar results (Figure S1). The consensus made across the three boxes in Figure 2, including only variables that are likely to be of global significance (present in at least 60% of the top-scoring models for at least 2 out of 3 regions), consisted of 4 predictors: DAmean, phosphate, salinity and SSTmean.

The effect of these two methods on model performance was evaluated by including them as factors in our experimental design. So all Maxent analyses were run with all samples and thinned samples. Similarly, models were run with all eight variables included and with only the four consensus variables selected from the survey.

Transferability as a function of modeling choices

Our multifactorial experiment showed that reducing the number of predictors, based on our surveying method, yielded much better models with higher test AUCs (Figure 3A) and Schoener's D (Figure 3B) than models with the full set of eight predictors. This is clearly visible in both figures: the leftmost two columns of both panels of the figure have warmer colors than the rightmost two columns. A Wilcoxon signed-rank test (WSRT) indicated that the difference in test AUC and Schoener's D between matching models is significant ($p = 0.0078$ in both cases, $N = 8$).

With test AUC as the measure of transferability (Figure 3A), the two upper rows had warmer colors than the lower two rows, suggesting better performance of models that use global background samples compared to models in which background samples are restricted to the region in which the model is trained. This pattern was not present in the Schoener's D values (Figure 3B), where models with global background and auto-features had remarkably low values of D, and the WSRT outcomes conflicted strongly ($p = 0.0078$ for AUC, $p = 0.9453$ for Schoener's D, $N = 8$). The higher AUC with global backgrounds may thus be a consequence of the sensitivity of AUC to background choice rather than an actual increase in predictive power with global backgrounds.

Model complexity and occurrence thinning did not have a large effect on transferability between regions. However, the

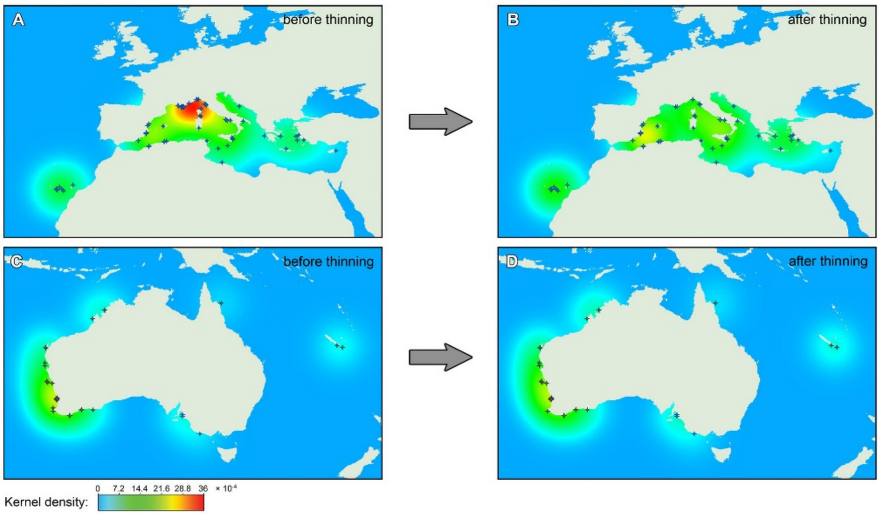


Figure 1. Effect of occurrence thinning on geographical sample bias. The colors on the map represent the regional sampling density, warmer colors indicating higher sample densities. Occurrence thinning substantially reduces the geographic sampling bias, as illustrated by the disappearance of the red blob along the French Riviera and closeby localities (panel A → B). There is less geographic sampling bias in the native range, so occurrence thinning does not have a big influence on the kernel density maps of that region (panel C → D). Note that the slightly elevated density close to the Spanish-French border in the Bay of Biscay (panels A and B) is caused by samples in the Mediterranean of which the kernel extends across land; there are no occurrences of *C. cylindracea* known from that area.

doi: 10.1371/journal.pone.0068337.g001

second row in Figure 3B shows substantially lower Schoener's D for a set of models with auto-features compared to the same set of models with enforced simple models (the row above). This difference was not present for the regional background case (3rd vs. 4th row).

Overall predictive performance of SDMs

Models built with occurrences from throughout the native and invaded ranges have considerably higher predictive power than models trained on one range and projected onto the other (WSRT, $p = 0.0156$ and 0.0078 for AUC_{global} vs. $AUC_{native \to invaded}$ and AUC_{global} vs. $AUC_{invaded \to native}$ respectively, $N = 8$, for pairs with global background only). These models' test AUC values, calculated on 50% random test occurrences from throughout the range, are all close to 1 (Table 1), indicating strong overall predictive power. The predictive performance of models based on pooled occurrences from native and invaded regions barely differ between conditions, indicating that models built with occurrences from both ranges are less sensitive to choices made during the modeling process (Table 1).

An SDM for *Caulerpa Cylindracea*. The various SDMs with high predictive power were visually similar, and we present environmental suitability maps of one of the top-scoring models in Figure 4. The global map, which uses a threshold to indicate predicted suitable areas, clearly highlights large parts of the coasts of Australia (native region) and the Mediterranean Sea (invaded region) as having suitable macroecological conditions. In addition, the model predicts suitable environmental conditions along the East Coast of the USA, parts of the Caribbean region, the tropical to warm-temperate coast of Brazil, parts of the coasts of Madagascar and Southeast Africa, as well as Taiwan and the main Japanese islands.

Within the native region (Australia, Figure 4B), the model predicts suitable macroecological conditions along almost the entire coast of southern Australia, including northern Tasmania, the west and east Australian coasts except for a region in SE Queensland, and parts of the north coast, where some regions had intermediate predicted suitability. These predictions are a considerable extension of the presently known range of the species (Figure 1C), and high environmental suitability is predicted in the various embayments of southern Australia where the species has recently established and become a

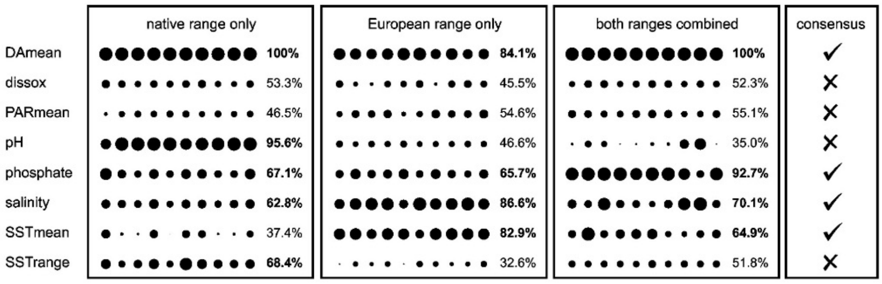
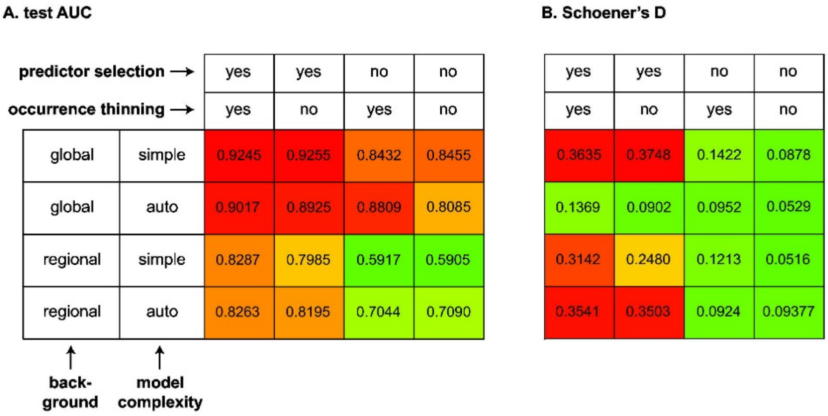


Figure 2. Results of the surveying procedure to identify the predictors present in top-scoring models. Each box contains the results of the survey for occurrence records from the native range, the invaded European range, or both ranges combined. Each column within a box represents a single survey carried out on one set of thinned coordinates. The circle diameter represents how often the variable in question occurred in the top 10 highest-scoring models (test AUC) for that set of thinned occurrences. The representation of each predictor in the top 10 is also summarized across columns (percentage indicates how many of the top 10 models had the predictor), and the consensus predictor set across ranges is indicated in the box on the right.

doi: 10.1371/journal.pone.0068337.g002



A. test AUC

	predictor selection →	yes	yes	no	no
	occurrence thinning →	yes	no	yes	no
global	simple	0.9245	0.9255	0.8432	0.8455
global	auto	0.9017	0.8925	0.8809	0.8085
regional	simple	0.8287	0.7985	0.5917	0.5905
regional	auto	0.8263	0.8195	0.7044	0.7090
↑ back-ground	↑ model complexity				

B. Schoener's D

yes	yes	no	no
yes	no	yes	no
0.3635	0.3748	0.1422	0.0878
0.1369	0.0902	0.0952	0.0529
0.3142	0.2480	0.1213	0.0516
0.3541	0.3503	0.0924	0.09377

Figure 3. Impact of modeling choices on the transferability of SDMs. The transferability of models is approximated by test AUC (panel A) and the global niche overlap (Schoener's D, panel B). Columns and rows represent the combinations of the four factors that were varied in our experimental design and are identical in both panels. The values are also plotted as colors along a color gradient to permit rapid visual assessment of the important factors, with warmer colors indicating higher values. Each AUC value in panel A represent the average of the $\text{AUC}_{\text{native-invaded}}$ and $\text{AUC}_{\text{invaded-native}}$ for the corresponding condition.

doi: 10.1371/journal.pone.0068337.g003

conspicuous member of the benthic community. The multivariate environmental similarity surface (MESS) map is positive in almost the entire range (Figure 4D, blue colors), which indicates that the conditions present in the region were observed in the training data and gives extra credibility to the model prediction. Given that the MESS map is mostly positive the "most dissimilar" (MoD) variable map is nearly blank (Figure 4F).

In the invaded region (Figure 4C), the model also predicted beyond the known occurrences of the species (Figure 1A),

Table 1. Predictive performance of models built with occurrences from native and invaded ranges as a function of choices made in the modeling process.

occurrence thinning	predictor selection	model complexity	performance (test AUC)
yes	no	simple	0.975
yes	no	auto	0.990
yes	yes	simple	0.982
yes	yes	auto	0.988
no	no	simple	0.972
no	no	auto	0.991
no	yes	simple	0.974
no	yes	auto	0.992

The overall predictive performance, as measured by the test AUC, is very high and the factors have only a minor influence on the outcome. All models compared in this table use the same set of 10,000 background points (global, equal area).

including Portugal, the NW of Spain and the NW of Africa. In the East, suitable macroecological conditions were inferred for the northern Red Sea, although the MESS map indicates that there is extrapolation beyond observed environmental conditions (Figure 4E), with the MoD map highlighting the (high) salinity occurring in the northern Red Sea as the most dissimilar variable.

The entire Maxent run including input data and all outputs is available for examination on FigShare (http://dx.doi.org/10.6084/m9.figshare.681723). Besides showing the main results presented here in more detail, this resource also allows examining limiting factors and exploring the components of the prediction for particular sites with Maxent's explain tool.

Discussion

Our results have implications for the invasion biology of *Caulerpa cylindracea* as well as the more general question of how best to model the distribution of species introduced outside their native range. We will first highlight the effects of the different distribution modeling practices on model transferability and performance, as well as some limitations of the procedures described here. Then we will discuss the meaning of our SDMs for the spread of *C. cylindracea* in Europe and Australia.

Building more reliable SDMs of introduced species

Niche conservatism is a central assumption when extrapolating correlative SDMs of introduced species to an area outside the bounds of training occurrences. The poor predictive power of SDMs trained in the native range and projected onto the invaded range that has been observed in many studies led to the conclusions that ecological niches can shift in association with introductions outside of the native range (e.g. [19,21,71-73], but see 6). In interpreting such niche shifts, it is important to realize that correlative models estimate a species' realized niche and that, as a consequence, observed niche shifts do not necessarily reflect physiological changes (i.e., modifications of the fundamental niche). In other words, the perceived niche shift can result from two different realizations of the same fundamental niche in different areas, and it has been argued that this scenario is more parsimonious than that in which the fundamental niche changes [9,74]. However, changes in the fundamental niche of introduced species are certainly possible [4,15].

Regardless of whether niche shifts observed in correlative SDMs are a consequence of changes in the realized or fundamental niche, it would certainly be useful to have a set of procedures that improve the predictive power of SDMs outside the training range in order to inform conservation planning and decision making. The methods used here were applied hoping they would improve the transferability of the SDMs of introduced species built using the popular presence-only method Maxent. We found that reducing the number of predictor variables drastically improved the transferability of our SDMs. Limiting the model complexity, reducing geographic sampling bias by occurrence thinning and choosing a global background had comparably small effects.

The effect of the choice of predictors has long been known to have a drastic effect on the transferability of SDMs of introduced species (e.g., [4,9,19,20,21]). The method used here, which surveys all combinations of variables for the native as well as the invaded region, attempts to identify variables that are likely to be of global rather than regional significance. Models based on the set of variables identified by this approach were more transferable than models with a more comprehensive set of variables, irrespective of whether reciprocal test AUC or Schoener's D were used to measure transferability. Although the use of procedures to select predictors and model complexity in an automated manner is common practice in many types of modeling including niche modeling [75-77], to our knowledge such approaches have not been used commonly in combination with Maxent. However, we do acknowledge that such predictor selection methods are no substitute for physiological knowledge of the organism [78], and here they were used to further refine a set of predictors that was already reduced from the full Bio-ORACLE dataset based on what we know are important factors determining algal growth.

Previous studies have also shown that reducing the complexity of models to fit smoother responses yields the best correspondence to physiological knowledge and as such, the models achieve better overall performance and have higher transferability [7,10,11,79]. For these reasons, the use of simple environmental response surfaces to avoid overfitting has been recommended for SDMs of invasive species [4,7,11]. Generally, the complexity of maximum entropy models is adjusted by using L_1 regularization [68], which varies along a continuous scale and has been used in other studies aimed at improving the performance of Maxent SDMs [10]. We chose to use a simple dichotomy between Maxent's auto-features versus the use of only linear and quadratic features to keep the experimental setup simple. Our results did not show a meaningful difference between the transferability of models built under both conditions and thus we did not observe the improvement of predictions with simpler models that other

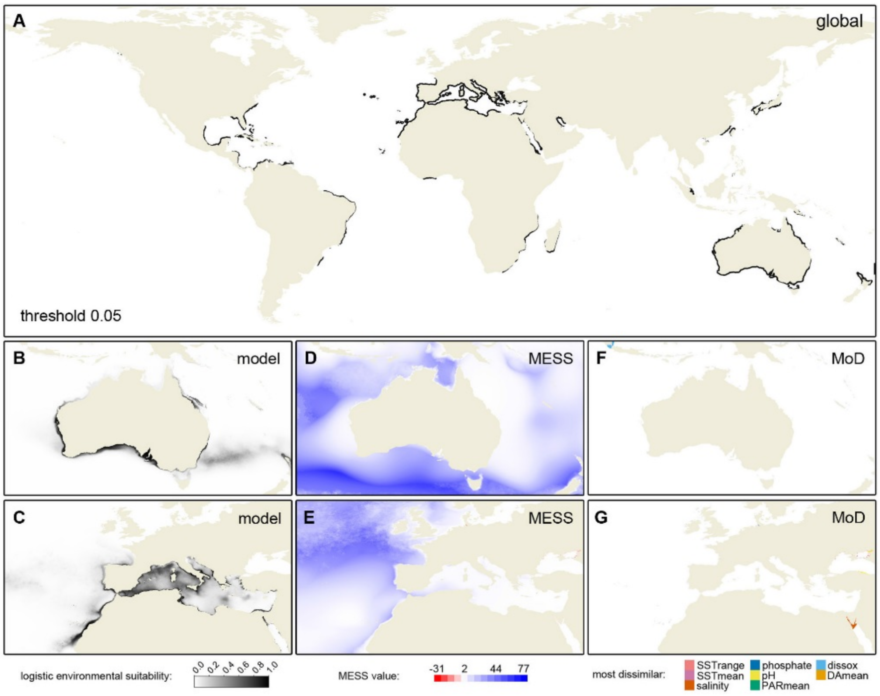


Figure 4. Species distribution model for *Caulerpa cylindracea*. Panel A shows global areas predicted to have suitable macroecological conditions for the species. This map uses a threshold for Maxent's logistic suitability corresponding to the 10% training presences (threshold = 0.053) and predictions are plotted only for coastal areas (less than 7 pixels from shore), with predictions in the open ocean masked. Panels B and C show the continuous logistic model output for the native and invaded ranges, respectively. The corresponding multivariate environmental similarity surface (MESS) maps are shown in panels D and E, and the most dissimilar (MoD) variables in those areas that require extrapolation are shown in panels F and G.
doi: 10.1371/journal.pone.0068337.g004

studies have [7,10]. This can probably be attributed to the fact that model complexity does not differ much between the two conditions in our experimental setup: the auto-features condition only differed in having hinge features in addition to the linear and quadratic features used in the "simple" condition. Nonetheless, we follow previous authors in their conclusion that correlative models with smooth responses will generally outperform those with complex responses. This is especially true if the number of occurrence points used to build models is large, because this increases the potential for overfitting. Since the identification of suitable predictors and an appropriate level of model complexity are related to one another, it may be advisable to integrate these two into a single procedure as commonly done in classical model selection procedures [75].

The use of thinned occurrences generally resulted in SDMs with better transferability, but the effect was not significant in a Wilcoxon signed-rank test and small compared to that obtained from predictor selection. Nonetheless, we anticipate that this approach may be useful in situations where the geographical bias is stronger than in our dataset and/or in situations with stronger spatial autocorrelation in the environmental grids. Other approaches that have been proposed to deal with geographic bias in occurrence records are to introduce the same sort of bias in the background points by specifying a target-group background, using bias grids in Maxent, or through application of trend surface analysis [7,25,26]. Various statistical approaches to address spatial autocorrelation have also been used [13]. In our case study, the background selection had a rather limited effect on the transferability of SDMs and in this context, it is worth noting that there were differences between the transferability results depending on whether they were measured as test AUC or as Schoener's D. The difference was most pronounced for models with global backgrounds and auto-features (compare second row in Figure 3A with second row in Figure 3B). It is well known that AUC is sensitive to background choice, with larger backgrounds inflating AUC values while not yielding more informative models [26,80,81]. Our observation of higher AUC values for global backgrounds compared to regional backgrounds, which was not paralleled in Schoener's D, is completely in line with this. As such, for comparisons of the transferability of models built with different backgrounds, we suggest the use of Schoener's D rather than test AUC. Regarding the transferability of models as a function of the background selection, a previous study concluded that using background in reachable areas provides a "less risky prediction space" [7]. Our experiments did not confirm this conclusion but suggested that transferability (as measured by Schoener's D) is indifferent to the choice of background.

From the results discussed above it is clear that the usefulness (i.e., the predictive power) of reciprocal niche models is quite variable and strongly depends on the choices made. While they barely outperform random models under some conditions (some test AUC < 0.6 in Figure 3A), making the right choices outlined above improves the predictive power of models trained in one range and projected onto the other (0.90 < test AUC < 0.93 for the best models, Figure 3A). Nevertheless, if distribution data are available from both the native and invaded ranges, it is advisable to build models from a combined set of occurrences. For our data, models based on combined occurrences outperformed reciprocal models (test AUC > 0.99 for best models). In this case, it is appropriate to use test AUC to compare performance, as all these models are built and evaluated using identical background points. Similar conclusions regarding the better predictive power of models using combined native-invaded datasets were reached in studies of other species (e.g., [4,82]). Our results also suggest that the combined data have the advantage of being more insensitive to the modeling choices that need to be made, but this generalization should be verified with other case studies.

Potential limitations

Besides discussing the performance of the various methods applied, it is also useful to point out their assumptions and potential caveats.

Firstly, our case study had the advantage of having relatively large sets of occurrence records for the native as well as the invaded range. In many cases, however, one will want to build reliable predictive models for species that were recently introduced and for which only a few occurrences have been recorded in the invaded range. How could a suitable set of predictors be identified in this case? Our approach relied on having sufficient data to identify those variables with predictive power in both geographic regions separately and combined. As an alternative, one could first identify the predictors achieving predictive power in the native range and subsequently compare the frequency distribution of those variables between samples from the native and invaded ranges with the aim of avoiding variables for which the invaded samples are outside of the range of values of native samples. It may also be beneficial to upweight the scarce samples from the invaded range in the model-building step. It is worth noting that we used an essentially arbitrary threshold to retain predictor variables, i.e. they had to be present in 60% or more of the top-scoring models for at least two out of three regions (Figure 2). This approach was chosen because variables important in multiple regions are more likely to be of global importance, and secondly because the 60% threshold resulted in a halving of the number of predictors. However, this raises the question of how these criteria influence the results and whether more objective criteria could be used. The evaluation of all these ideas as well as other possible approaches is an attractive avenue for further research.

Our general approach towards increasing the transferability of SDM does not make explicit assumptions about whether or not a niche shift between ranges is present, or if it is, whether it is situated at the level of the fundamental or the realized niche. The ideal scenario is that there are no niche shifts between the populations and transferability is not an issue. However, if a niche shift is present, our predictor reduction approach will eliminate those predictors that have poor predictive power in one or both ranges, regardless of whether any changes in predictive power between regions are due to differences in the realized or fundamental niche. While we expect that eliminating predictors that have regional rather than general relevance will be sound in a majority of cases, there are scenarios imaginable where this will not work. For example, if the correlation structure of predictor variables differs between regions, an indirect variable (i.e. one that does not affect the distribution but is correlated with another one that does affect it) may be identified as important in both regions but have very different response curves in both areas and thus lead to poor transferability. Similarly, variables that are directly relevant to the distribution may differ systematically between regions, decreasing the transferability of the SDMs built from them [74].

Even though it can be expected that the distance-based thinning will improve most models, this may not always be the case. In fact, this procedure may discard useful data when regions of dense sampling coincide with steep ecological gradients over short geographic distances. Also, if sampling reflects population densities, geographic autocorrelation of records can add a potentially desirable quantitative aspect to the model. This will, of course depend on the specific goal and the dataset being studied.

Finally, our evaluation of methods is based on a single case study, and there are no guarantees that our results will extrapolate to other introduced species. A logical next step is to apply these methods to a range of suitable case studies. The time since the introduction and dispersal potential of the species should be prime criteria in selecting species to further test these methods. Species that were introduced a long time ago and have had the chance to disperse widely in the invaded range are more likely to have spread through their entire potential niche and thus make good case studies.

An additional approach towards testing the degree to which these methods can be generalized, as well as to explore the various other questions raised in the discussion, is to carry out simulation experiments. Simulation is a powerful tool for testing the logical consistency of ideas as well as the efficiency and reliability of methods. They have not been widely used to evaluate presence-only SDM methods, although there appears to be a trend towards their increased use in recent years [17,74,79,83-87]. Besides identifying the circumstances in which niche modeling algorithms perform well and those in which they are more likely to fail, simulation is a powerful tool to assess the effectiveness of procedures such as those described here. Such insights would obviously be beneficial to the whole SDM field.

Invasion and spread of *Caulerpa Cylindracea*

The distribution model presented for *C. cylindracea* predicted potential expansions in the invaded range along East Atlantic coastlines of Europe and Africa as well as a substantial potential expansion along the southern coast of Australia (Figure 4A). Admittedly, the logistic values in Maxent lack a clear-cut interpretation [88] and determining thresholds for presence-only SDMs is not an exact science [89,90]. Based on several thresholds tested (e.g. 10-percentile training presence, equal training sensitivity and specificity), the inferred range boundaries are quite far beyond the known occurrences of the species (Figure 1 vs. Figure 4A). This suggests that our current knowledge may underestimate the potential range of this species in these areas. In the Mediterranean and East Atlantic region, the species has only been present for only about 20 years and, despite the species' relatively rapid colonization rate [91], it is likely that it has not reached its distributional limits yet. In Australia, the native area of the species, it was known best from the Western Australian coast [48]. However, the recent observations of invasive populations of this species along the southern coast, where it did not previously occur (reference [46] and pers. obs.), prompted us to generate SDMs for this species in order to investigate whether the species could potentially colonize more of the coast. Our models do indeed suggest that the macroecological conditions are highly favorable and that *C. cylindracea* could colonize the entire southern coastline of Australia. Besides these potential expansions in regions where the species is present already, several other coastlines are predicted to be suitable environment where the species could establish if it were to be introduced (Figure 4A).

Needless to say our models only incorporate macroecological predictor variables. Besides this, the microhabitat, as well as possible biotic interactions, also need to be favorable for the species to establish itself in the areas that are predicted to be suitable. In its native range, *C. cylindracea* is usually found on rocky substrata close to the low-tide mark but in more tropical locations (NW Australia and the Great Barrier Reef) it is typically found growing on sand in lagoons and around reefs. In the Mediterranean Sea, it has been found between 1 and 60 meters depth, on all types of hard and soft substrata and in different communities, with the only exception being unstable sandy substrata [29]. A number of studies have studied the microhabitat preferences of the species in some detail in the Mediterranean, showing that it thrives on rocky substrata among other macroalgae as well as in dead seagrass beds [92-94], and that it tolerates near-bottom orbital velocities below 15 cm s^{-1} [93]. In summary, the species occurs in a wide range of common microhabitats, so it is likely that it could establish in the great majority of areas predicted by our SDM if there are no biotic interactions inhibiting its settlement and expansion.

The correlative model from this study can also be used to inform experimental studies on the physiological tolerances of *C. cylindracea*. Even though we have not shown or discussed detailed response curves in the main paper, these are available as supplementary materials on FigShare (http://dx.doi.org/10.6084/m9.figshare.681723). Most correspond to our expectations based on physiological knowledge of other algae, including other *Caulerpa* species [95], but some do not. For example, the correlative model indicates that the species is mainly found in phosphate-poor waters with the response curve rapidly dropping at concentrations over 0.4 μmol L^{-1}. Studies on other species indicate that macroalgae have an increasing response curve for macronutrients and that low rather than high concentrations may be limiting seaweed species in nature [96-99]. This suggests that our correlative model may be misled in this case. It is also interesting to note that models built from occurrences in the native range predicted a much broader range of suitable temperatures than models from occurrences in the invaded range. More specifically, the model from invasive occurrences has a response curve that peaks at ca. 20°C, dropping off quickly at higher temperatures. The curve from a model with native occurrences also peaks at ca. 20°C, but drops much more gently at higher temperatures. Whether this should simply be interpreted as an indication that warmer areas are yet to be colonized in the invaded range (i.e. that the model is biased towards colder temperature due to the current distribution), or that the introduced strain has a reduced range of temperature tolerance compared to the native population, remains to be investigated. To further characterize the most relevant features determining the species' range, it would be informative to evaluate the gradients of predictors occurring across the inferred range boundaries, and put those to the test in physiological experiments.

Conclusions

In order for Maxent presence-only SDMs to be useful in predicting and managing introduced and invasive species, a number of problems related to their accuracy and transferability have to be overcome. The methods introduced, explored and evaluated here aim to improve the situation. Reducing the set of predictors to those anticipated to be of global significance resulted in a strong improvement of SDM transferability, with occurrence thinning, model complexity and background choice having relatively minor effects. If available, occurrences from the native and invaded regions should be combined, as this yields the best-performing models and apparently reduces their sensitivity to choices made in the modeling process. We also presented an SDM of *Caulerpa cylindracea* that achieves very high predictive power, illustrating the applicability of these methods in the marine realm for which comparably little niche modeling has been done [100]. The procedures introduced here are available for further evaluation with other case and simulation studies, which should provide further insights into the degree to which our results can be generalized. We hope and anticipate that they will form a useful strategy to improve predictive SDMs and in turn, help to better inform environmental decision makers.

Supporting Information

Figure S1. Model surveying results indicating qualitatively similar results when analyses are carried out with global or regional backgrounds.
(PDF)

Acknowledgements

We thank Thomas Sauvage and Claude Payri for sharing their work on New Caledonian *Caulerpa* and providing New Caledonian occurrence records of *C. cylindracea*. We thank Stefano Draisma and Willem Prud'Homme van Reine for sharing information about their DNA bar codes survey of *Caulerpa* collections and John Huisman for providing many of the occurrence records of *C. cylindracea* from NW Australia. Niels Raes and Dirk Maes provided valuable feedback on a previous version of this manuscript.

Author Contributions

Conceived and designed the experiments: HV LT. Performed the experiments: LT GB FM HV. Analyzed the data: HV LT. Contributed reagents/materials/analysis tools: AJ CFDG GH ODC. Wrote the manuscript: HV GB.

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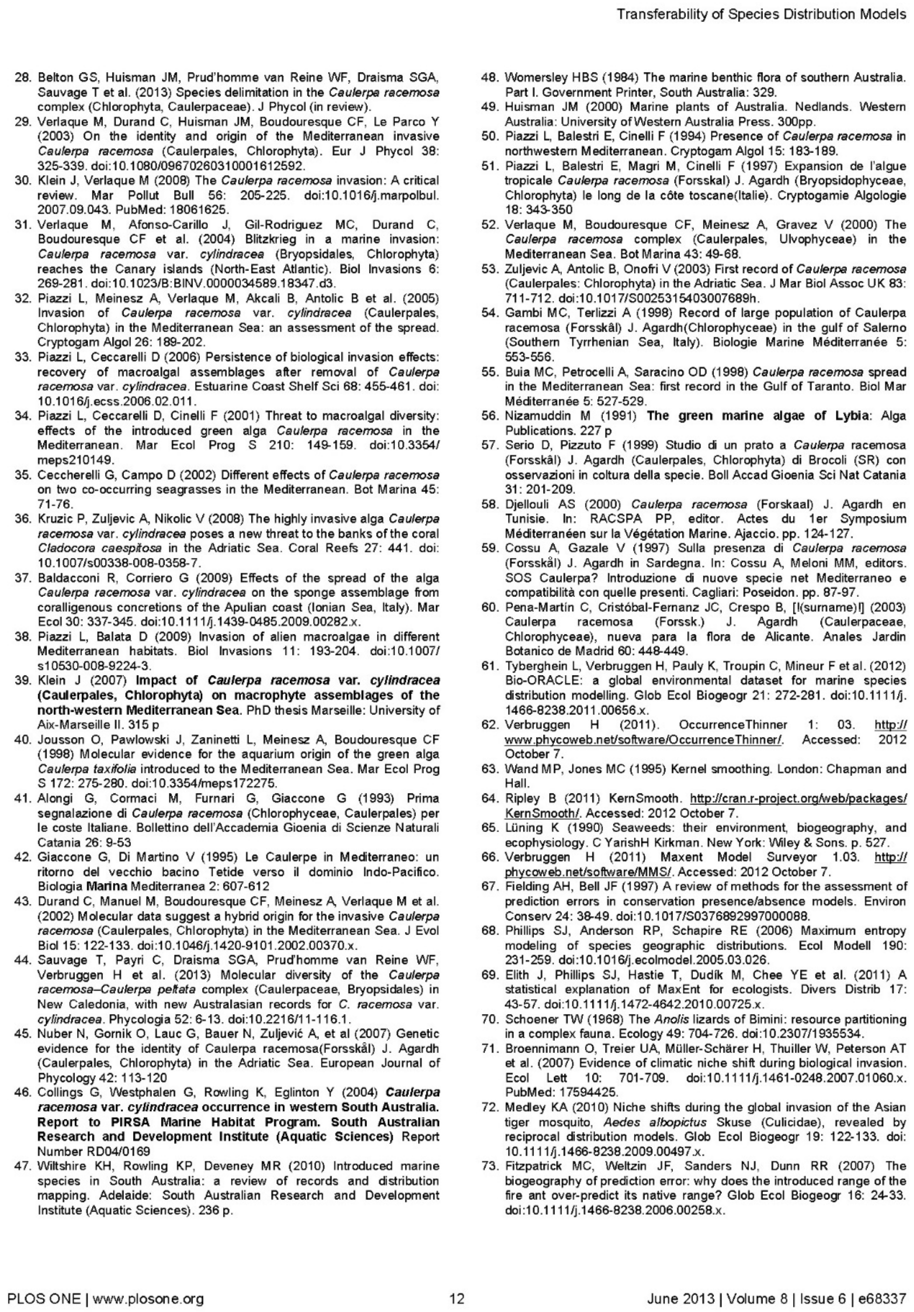

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Chapter 6: Conclusion

This thesis has been written as a selection of manuscripts, either published, or in preparation for submission for publication. Each chapter has its own discussion where results are considered in light of the chapter aims and associated literature. Here, in this final chapter, I present a more cohesive synthesis of the overall contribution of this body of work to our understanding of the taxonomy of *Caulerpa* and in so doing, provide what we consider, best practice framework for integrating historical nomenclature into molecular taxonomies in the face of many challenges (e.g. type specimen unavailable, usable DNA cannot be extracted and amplified from type specimens etc…).

Chapter 2 developed this best practice framework by tackling the most taxonomically troublesome taxa of *Caulerpa*: the 30+ described varieties and forms currently associated with *C. racemosa* and *C. peltata*. Unsurprisingly, results confirmed some serious mismatches between morphological and molecular species concepts with our molecular results revealing the presence of 11 distinct species within the complex, five of which showed high levels of phenotypic plasticity (*C. chemnitzia*, *C. cylindracea*, *C. lamourouxii*, *C. oligophylla* and *C. racemosa*) and partial overlap with other species. On the basis of an in-depth morphological examination of each species clade, as well as observations of a large number of herbarium specimens, including type specimens/descriptions, and geographic inferences, we were able to confidently designate names for the lineages and describe a number of new species and make a large number of taxonomic changes. For examples, *C. peltata*, *C. imbricata* and *C. racemosa* vars. *laetevirens*, *occidentalis* and *turbinata* were found to represent environmentally induced forms of a single species, for which the earlier-described *C. chemnitzia* , previously regarded as a synonym of *C. racemosa* var. *turbinata* , was reinstated. Furthermore, we were able to provide the proper species name for one of the most notorious invasive seaweeds of the world (= *C. cylindracea*) as well as show it to have a native distribution throughout the tropical Indo-Pacific.

Using the framework developed in Chapter 2, Chapters 3 and 4 examined the diversity of *Caulerpa* in North-western Australia and Southern Australia, respectively. A large number of specimens were newly collected and a number of species sequenced for the first time (e.g. *C. agardhii*). Results indicated an underestimation of *Caulerpa* diversity in both regions and a number of taxonomic changes were made, including new species descriptions (e.g. *C. coppejansii*). One of the most important findings was a newly reported population of the highly invasive *C. cylindracea* in the waters around Portland, Victoria in Chapter 4.

In Chapter 5, with an accurate DNA barcode for *C. cylindracea* (based on results from Chapters 2 and 4), we were able to use all available Genbank records, as well as newly obtained data, to obtain an accurate global distribution (native and invasive) for the species. We then developed species distribution models to predict the future spread of the species. This approach identifies areas at risk from potential future invasion, including along the coasts of western Europe, western Africa and the south coast of Australia. This work also allowed us to demonstrate that occurrence records from the native and invasive ranges should be combined to produce models with higher predictive power.

In general, although we resolved a large number of taxonomic issues, what was clear from our data is that the taxonomy and true diversity of *Caulerpa* is still poorly understood, with a number of other species complexes still in need of investigation, especially *C. brachypus* and *C. verticillata*. This lack of an accurate understanding of the *Caulerpa* genus is surprising given that it is a well-known and distinctive genus with a number of infamous invasive species and some instances, a high level of ecological importance. In a broader sense, our data clearly suggests that one should not assume that because two individuals look alike, they are going to belong to the same species because a substantial proportion of species can be expected to be cryptic or not all species will have unique morphologies. This has consequences for how field-work is carried out. Rather than sampling one or a few individuals of each morphological type, we should move to a sampling design where many individuals of similar morphology are investigated in detail and with the assistance of molecular data. This will allow for more accurate biodiversity estimates and thus improved conservation management and decision-making. Although we base this conclusion on our studies on *Caulerpa*, it is likely true for a large proportion of other algal groups as one can only imagine how poorly understood are other more cryptic or uncommon algal groups.

Finally, I feel it is important to highlight the limitations of our study in order to generate further debate in order to find solutions or reduce uncertainties. Most obviously, we were unable to sequence any type specimens and obtain newly collected material from several type localities. Also, as pointed out by Verbruggen (2014), we did not formally quantify morphological variation or use statistical tools to match the morphology of types with that of sequenced samples. We do agree that in doing so, we would have reduced uncertainty somewhat, but it would certainly have not eliminated it. We therefore accept the possibility that some of the applied names will need to be revised in the future or that multi-marker work may alter some of the species boundaries. But, as stated by Verbruggen (2014) "*the Pareto principle states, 80% of the effects come from 20% of the causes, or translated to this situation, a majority of correct conclusions can be reached with limited information*". It is therefore our opinion that, through careful consideration and pragmatic decision-making, the methodologies developed in these chapters provide a tool for taxonomists to revise taxonomies to the best of their abilities, with limited information and in the face of uncertainty. Any move to increase the speed of taxonomic work is important as molecular data continues to reveal an overwhelming diversity of algal species at a rate far greater than formal descriptions are being made and extinction rates now as high as 27,000 of the known species per annum (Wilson 1992).

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Appendix 1: Molecular diversity of the *Caulerpa racemosa–Caulerpa peltata* complex (Caulerpaceae, Bryopsidales) in New Caledonia, with new Australasian records for *C. racemosa var. cylindracea.*

Appendix: 1

Appendix: 1

Phycologia (2013) Volume 52 (1), 6–13 Published 4 January 2013

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 *tuf*A 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, *tuf*A

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 *tuf*A (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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Appendix: 1

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 *tuf*A 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 *tuf*A 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′-GCWCCWGAAGAAAAAGC-3′; anneals at position 157 of the *Ostreococcus tauri* Courtes & Crétiennot CR954199 complete *tuf*A sequence), the *tuf*AF primer (Famà *et al.* 2002) and *tuf*GF4 primer (Saunders & Kucera 2010) were used in combination with reverse *tuf*AR 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 tuf*A 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 BLASTCLUST 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 (Händeler *et al.* 2009; Table S1). Outgroup taxa consisted of two species from a basal lineage of the Caulerpaceae, *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

Appendix: 1

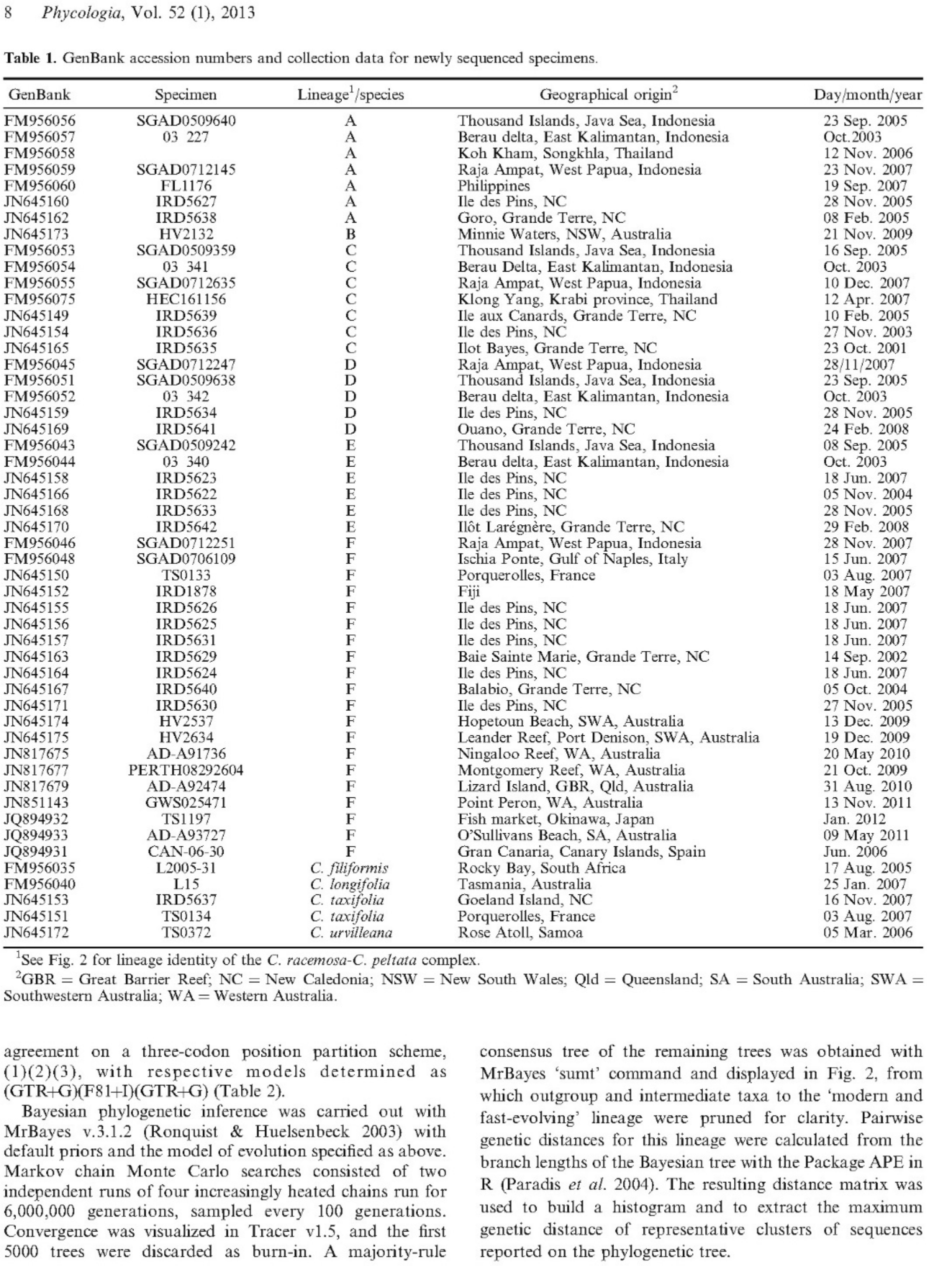

Table 1. GenBank accession numbers and collection data for newly sequenced specimens.

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'Sullivans Beach, SA, Australia	09 May 2011
JQ894931	CAN-06-30	F	Gran Canaria, Canary Islands, Spain	Jun. 2006
FM956035	L2005-31	*C. filiformis*	Rocky Bay, South Africa	17 Aug. 2005
FM956040	L15	*C. longifolia*	Tasmania, Australia	25 Jan. 2007
JN645153	IRD5637	*C. taxifolia*	Goeland Island, NC	16 Nov. 2007
JN645151	TS0134	*C. taxifolia*	Porquerolles, France	03 Aug. 2007
JN645172	TS0372	*C. urvilleana*	Rose Atoll, Samoa	05 Mar. 2006

[1] See Fig. 2 for lineage identity of the *C. racemosa*-*C. peltata* complex.

[2] GBR = 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+I)(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.

Appendix: 1

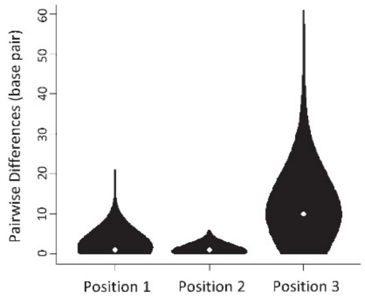

Fig. 1. Violin plot depicting the smoothed distribution of pairwise nucleotide differences (uncorrected) for the first, second, and third codon positions from the 912-base-pair *tuf*A alignment.

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 *tuf*A (Famà *et al.* 2002; Stam *et al.* 2006; Wynne *et al.* 2009). Some resolution was found near the tips of the tree, but *tuf*A 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'Sullivans 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 *tuf*A 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 *tuf*A (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)**

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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.

Appendix: 1

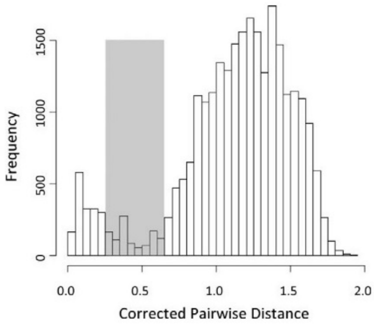

Fig. 3. Frequency distribution of corrected pairwise genetic distances for the 'modern and fast-evolving' *Caulerpa* lineage (*sensu* Famà *et al*. 2002). Distances were computed from branch lengths of the phylogenetic tree resulting from the Bayesian inference. The estimated range of genetic distances 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 *tuf*A 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, *tuf*A 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 *tuf*A. 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 *tuf*A (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'Sullivans Beach). Unlike the newly identified records from NWA, EA and NC, which show no signs of overabundance, SA populations found at O'Sullivans 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

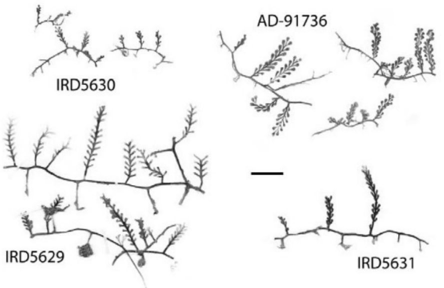

Fig. 4. Herbarium specimens of *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman and Boudouresque sequenced from New Caledonia (IRD5629 from Baie Sainte Marie, Grande Terre; IRD5630 and IRD5631 from Ile des Pins) and Australia (AD-91736 from Ningaloo Reef, Western Australia). Note radial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

Appendix: 1

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 Huisman 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.

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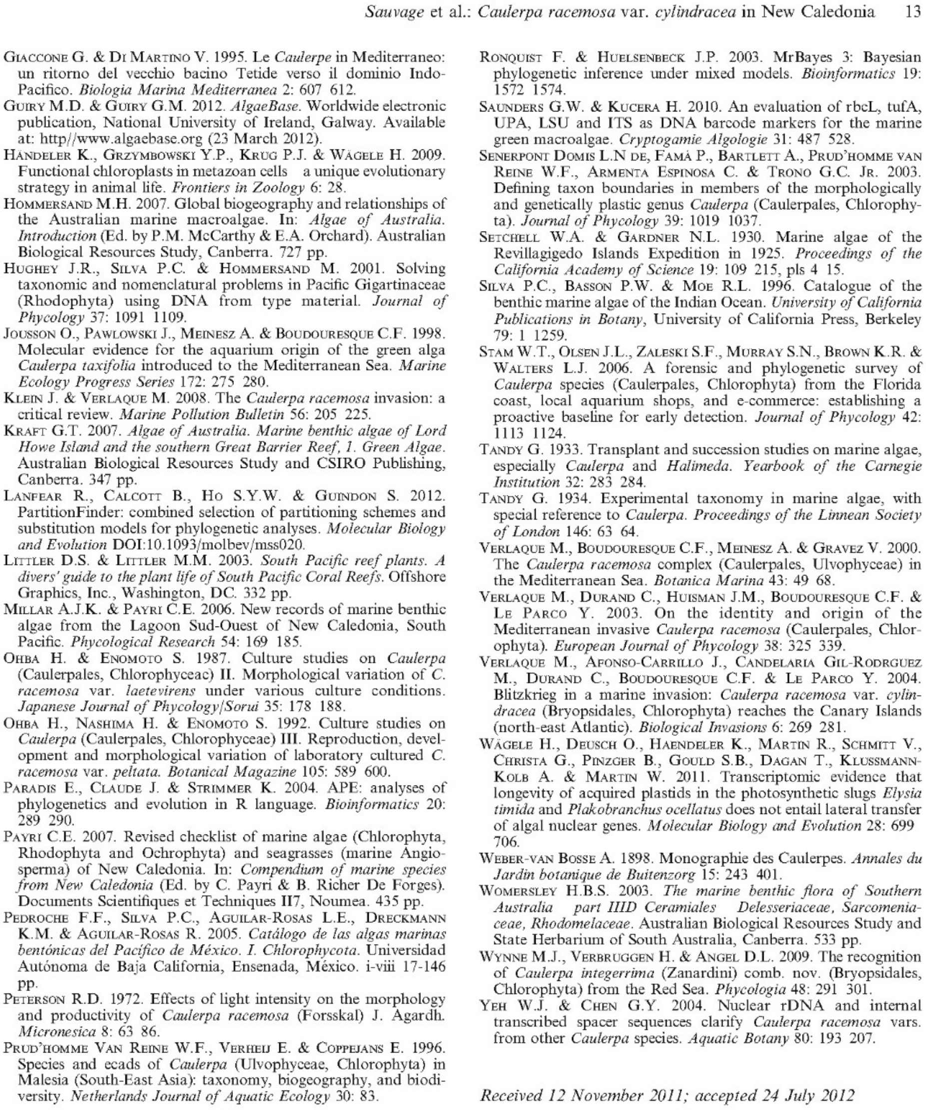
Appendix: 1



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Received 12 November 2011; accepted 24 July 2012

Appendix: 1

Appendix: 2

Appendix 2: A re-assessment of the infra-generic classification of the genus C*aulerpa* (Caulerpaceae, Chlorophyta) inferred from a time-calibrated molecular phylogeny.

Appendix: 2

Appendix: 2

J. Phycol. **50,** 1020–1034 (2014)
© 2014 Phycological Society of America
DOI: 10.1111/jpy.12231

A RE-ASSESSMENT OF THE INFRA-GENERIC CLASSIFICATION OF THE GENUS *CAULERPA* (CAULERPACEAE, CHLOROPHYTA) INFERRED FROM A TIME-CALIBRATED MOLECULAR PHYLOGENY[1]

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The siphonous green algal family Caulerpaceae includes the monotypic genus *Caulerpella* and the species-rich genus *Caulerpa*. A molecular phylogeny was inferred from chloroplast *tuf*A and *rbc*L DNA sequences analyzed together with a five marker dataset of non-caulerpacean siphonous green algae. Six Caulerpaceae lineages were revealed, but relationships between them remained largely unresolved. A *Caulerpella* clade representing multiple cryptic species was nested within the genus *Caulerpa*. Therefore, that genus is subsumed and *Caulerpa ambigua* Okamura is reinstated. *Caulerpa* subgenus status is proposed for the six lineages substantiated by morphological characters, viz., three monotypic subgenera *Cliftonii*, *Hedleyi*, and *Caulerpella*, subgenus *Araucarioideae* exhibiting stolons covered with scale-like appendages, subgenus *Charoideae* characterized by a verticillate branching mode, and subgenus *Caulerpa* for a clade regarded as the *Caulerpa* core clade. The latter subgenus is subdivided in two sections, i.e., *Sedoideae* for species with pyrenoids and a species-rich section *Caulerpa*. A single section with the same name is proposed for each of the other five subgenera. In addition, species status is proposed for *Caulerpa filicoides* var. *andamanensis* (W.R. Taylor). All *Caulerpa* species without sequence data were examined (or data were taken from species descriptions) and classified in the new classification scheme. A temporal framework of *Caulerpa* diversification is provided by calibrating the phylogeny in geological time. The chronogram suggests that *Caulerpa* diversified into subgenera and sections after the Triassic-Jurassic mass extinction and that infra-section species radiation happened after the Cretaceous-Tertiary mass extinction.

Key index words: ***Caulerpa andamanensis*** **stat. nov.;** ***Caulerpa denticulata*****;** ***Caulerpella*****; chronogram; group IIA intron; molecular phylogeny; pyrenoid;** ***rbc*****L; relaxed molecular clock;** ***tuf*****A**

***Abbreviations*:** **AIC, Akaike information criterion; AICc, corrected AIC; *atp*B, beta subunit of the ATP synthase gene; BI, Bayesian Inference; BIC, Bayesian information criterion; BP, Bootstrap Percentage;**

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Editorial Responsibility: H. Verbruggen (Associate Editor)

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Ma, Mega-annum; ML, Maximum Likelihood; nt, nucleotide(s); PP, posterior probability; *rbc*L, large subunit of the D-ribulose 1,5-bisphosphate carboxylase-oxygenase gene; *tuf*A, elongation factor Tu gene

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The Caulerpaceae Kützing (Bryopsidales, Chlorophyta) is a siphonous green algal family characterized by the presence of ubiquitous trabeculae (i.e., cell wall ingrowths) traversing the cell lumen to provide structural support. The thallus is differentiated into creeping stolons, downward growing rhizophores (with which it can anchor in soft substrate), and upright fronds termed assimilators that bear branchlets termed ramuli of various shapes (Weber-van Bosse 1898, De Senerpont Domis et al. 2003). This cosmopolitan tropical to temperate marine family currently includes two genera, i.e., the species-rich genus *Caulerpa* J.V. Lamouroux and the monotypic genus *Caulerpella* Prudhomme & Lokhorst. The latter genus was created to separate *Caulerpa ambigua* Okamura from the former on the basis of differences in reproductive structures (Prud'homme van Reine and Lokhorst 1992). In *Caulerpa*, the entire content of the vegetative plant divides up into reproductive cells to be released as gametes, resulting in the death of the thallus (i.e., holocarpy). *Caulerpella ambigua* (Okamura) Prudhomme & Lokhorst presumably survives gamete release by forming compound zoidangia on lateral branches cut-off from sterile parts of the thallus by a transverse cell wall (i.e., non-holocarpy). Vegetative, asexual reproduction by detached fragments is considered most common in *Caulerpa* (Prud'homme van Reine et al. 1996, Varela-Álvarez et al. 2012), but is unknown in *Caulerpella*.

Species of the genus *Caulerpa* exhibit a wide array of assimilator morphology and are renowned for their phentotypic plasticity (Peterson 1972, Calvert 1976, Ohba et al. 1992). This plasticity has resulted in an unstable classification of numerous varieties and forms. There are 360 species and infra-specific names in the online database *AlgaeBase* of which 87 species and 117 varieties and forms have been flagged as currently taxonomically accepted (Guiry and Guiry 2013). However, several recent molecular studies by Sauvage et al. (2013) and Belton et al. (2014) have shown the genus to have a taxonomy in need of revision. Species status is proposed for some varieties of taxa in the studies by Belton et al. (2014) and G.S. Belton et al. (unpublished data) although species cannot always be distinguished from each other based on morphology alone, and the authors suggested that it is likely that the best means to distinguish many *Caulerpa* species is through DNA sequence data.

Agardh (1873) subdivided the genus *Caulerpa* into thirteen tribes based on morphological similarities. However, these names were illegitimate because a tribe is a supra-generic rank. Agardh's names were validated by De Toni (1889) who used the rank of section. Weber-van Bosse (1898) recognized twelve of these sections, but considered the Opuntioideae J. Agardh *ex* De Toni as one of four series in the section Sedoideae J. Agardh *ex* De Toni. However, in a molecular phylogenetic study of interspecific relationships in the genus based on the chloroplast-encoded *tuf*A gene, Famà et al. (2002) found that most of these sections are polyphyletic. Their sampled *Caulerpa* species were divided into four clades of which two were monotypic; (i) Australasian endemic *Caulerpa flexilis* J.V. Lamouroux, (ii) *Caulerpa verticillata* J. Agardh, (iii) a clade comprised of species that have a pyrenoid associated with large chloroplasts and vesiculate ramuli with constricted pedicels (i.e., *C. cactoides* [R. Brown *ex* Turner] C. Agardh, *Caulerpa microphysa* [Weber-van Bosse] Feldmann, and *Caulerpa sedoides* C. Agardh [as *C. geminata* Harvey]), and (iv) a clade containing Caribbean *Caulerpa lanuginosa* J. Agardh and *C. paspaloides* (Bory de Saint-Vincent) Greville, and the remaining fifteen sampled *Caulerpa* species which grouped together in an internally largely unresolved crown clade. The crown clade taxa with vesiculate ramuli do not have constricted pedicels and do not contain pyrenoids. The analysis of Stam et al. (2006) revealed the same four *Caulerpa* clades as in Famà et al. (2002), and both studies used *Caulerpella ambigua* as outgroup in their *tuf*A analysis. However, in more recently published multi-locus molecular phylogenies of the Bryopsidales and Dasycladales (Verbruggen et al. 2009a,b), *Caulerpella ambigua* showed conflicting positions with respect to four sampled *Caulerpa* species. The simple diminutive siphon *Pseudochlorodesmis abbreviata* (Gilbert) Abbott & Huisman from Hawaii was revealed by Verbruggen et al. (2009b) as sister to the entire Caulerpaceae, thus representing the closest documented extant lineage to the family. The temperate waters of Southern Australia have been hypothesized to be the geographic origin of the genus (Calvert et al. 1976), but relaxed molecular clock models calibrated with the fossil record (Verbruggen et al. 2009a) indicate that the Caulerpaceae lineage split from the other Halimedineae lineages in the Carboniferous or Permian when southern Australia was still attached to Antarctica (Hommersand 2007). *Pseudochlorodesmis* was, however, not included by Verbruggen et al. (2009a) and would have shortened the branch leading to *Caulerpa*.

This study aims to investigate the deeper phylogenetic relationships within the Caulerpaceae using a wider sampled outgroup and a longer alignment than in Famà et al. (2002) and Stam et al. (2006), as well as a wider sampled ingroup than in Verbruggen et al. (2009a,b), using chloroplast-encoded *tuf*A and *rbc*L gene sequences. In addition it aims to provide for the first time a temporal framework of caulerpacean diversification by calibrating the phylogeny in geological time. The inferred phylogenetic chronogram (i.e., timetree) is subsequently

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used to revise the subdivision of the family by giving equal rank to clades equivalent in time. The earlier hypothesized geographic origin of *Caulerpa* is discussed on the basis of the timetree, which may illuminate causal geological events and processes in the history of life (Avise 2009).

MATERIALS AND METHODS

Taxon sampling and sequencing. For this study, diverse caulerpacean collections were gathered mainly from two of the main Caulerpaceae biodiversity centers (Australia and Southeast Asia) and included a number of representatives previously unsequenced (e.g., *C. agardhii* Weber-van Bosse, *C. elongata* Weber-van Bosse, *Caulerpa filicoides* Yamada). Some species are new records for Indonesia, Malaysia, or Palau (indicated in Table S1 in the Supporting Information). The traditional twelve sections and four series are each represented by at least two species, except for the Zosteroideae J. Agardh *ex* De Toni. The Zosteroideae originally contained *Caulerpa filiformis* (Suhr) Hering and *C. flagelliformis* C. Agardh. Newly collected specimens were identified based on references from literature as well as examination of type specimens. Specimen vouchers used in the studies by Stam et al. (2006) and Famà et al. (2002) were also re-examined, although not all specimens of the latter study were available (indicated in Table S1 in the Supporting Information). In addition, new collections of the Caulerpaceae sister-clade *Pseudochlorodesmis* were made.

Genomic DNA was extracted from silica dried or herbarium dried algal tissue using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions or was outsourced to AGRF (Australian Genome Research Facility, Adelaide Node, SA, Australia). Double-stranded *tuf*A amplifications were performed in 25 μL following Stam et al. (2006) using the tufAF (5′-TGAAACAGAA MAWCGTCATTATGC-3′; Famà et al. 2002) and tufAR1 (5′-CCATAGGAATTGGACTATCA-3′; Stam et al. 2006) forward and reverse primers. A few samples (indicated in Table S1) were amplified with the newly designed reverse primer tufA652R (5′-GAGTATGGGGTGTAATAGAT-3′) resulting in 164 nucleotides (nt) shorter fragments. Amplification products were purified using the Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI, USA), the NucleoSpin Extract II kit (Machery-Nagel, Düren, Germany), or the LaboPass Gel and PCR Clean-up Kit (Cosmo Genetech, Seoul, Korea) following the manufacturer's instructions. Purified PCR products were sent to Macrogen (Seoul, Korea) or First BASE Laboratories Sdn Bhd (Seri Kembangan, Malaysia) for sequencing using the amplification primers. A few samples were extracted, amplified, and sequenced at the Centre for Environmental and Molecular Algal Research (University of New Brunswick, Fredericton, NB, Canada) following Saunders and Kucera (2010). Partial *rbc*L sequences were determined for a subset of the samples. *Rbc*L was amplified as two overlapping fragments of, respectively, 633 and 651 nt using the primer combinations CR-F/CR-mR and CR-mF/CR-R which were designed for this study, the latter fragment on the downstream side of the former. Primer CR-F anneals 26 nt after the intron reported in two *Caulerpa* species in Hanyuda et al. (2000). Primer sequences are CR-F 5′-CTGGWGRSA WAATCARTATATTGC-3′, CR-mF 5′-GGACATTATTTAAAT GCWACTGC-3′, CR-mR 5′-CAATAACAGCATGCATWGCAC G-3′, and CR-R 5′-AGGACTCCATYKAGCAGCATCACG-3′. Both fragments were amplified in 25 μL reaction volumes using the *i*-Taq *plus*DNA Polymerase kit (iNtRON Biotechnology, Seongnam-Si, Korea) and applying the general reaction mixture recommended by the manufacturer. An initial denaturation step of 94°C for 2 min was followed by 10 cycles of 20 s at 94°C 1 min at 45°C, and 2 min at 72°C, and then 25 cycles of 20 s at 94°C, 30 s at 48°C, and 2 min at 72°C. The amplification was ended with a final step of 72°C for 8 min. *Rbc*L PCR products (in case of low yield multiple reactions were pooled) were prepared for sequencing in the same way as the *tuf*A amplifications. The sequence of the two *rbc*L fragments combined was 1,039 nt in length excluding the CR-F and CR-R primers sites and encompasses the nt positions 297–1,335 in a typical green algal *rbc*L gene of 1,428 nt (e.g., GenBank AB260909). For some specimens only the CR-mF/CR-R amplifications were successful (indicated in Table S1). The chromatograms were assembled and edited as described in Draisma et al. (2010a,b).

Dataset assembly and model selection. In addition to 150 newly generated sequences, *tuf*A and *rbc*L sequences representing *Caulerpella* and *Caulerpa* species were downloaded from the GenBank/EMBL database. Only a selection of the sequences representing the *Caulerpa* crown clade was used for analysis to represent high diversity but low sequence redundancy. Some Genbank sequences representing non-crown taxa were also excluded from analyses. The *rbc*L sequences of *Caulerpa brownii* (C. Agardh) Endlicher (GenBank EU380530) and *C. verticillata* (EF583684) were excluded because they were short and largely outside the alignment of this study. The *C. filiformis* *rbc*L sequence AY004763 was excluded because it is a chimera of *C. filiformis* (nt 1–605) and a member of the angiosperm order Poales (nt 606–1,356). The *Caulerpa flexilis* J.V. Lamouroux *rbc*L sequence AJ512485 was left out because it is identical to that of *Caulerpa okamurae* Weber-van Bosse AB038484. Moreover, these four species were already represented by other specimens. All *Caulerpa, Caulerpella,* and *Pseudochlorodesmis* taxa used in this study are listed in Table S1. *Tuf*A and *rbc*L sequences were aligned separately by eye in the BioEdit Sequence Alignment Editor v.7.2.1 (Hall 1999). Identical or nearly identical sequences were

TABLE 1. Selection of partitioning strategy using the AIC, AICc, and BIC.

lnL	# parameters	# partitions	AIC	AICc	BIC	Partition scheme
−57273.62	227	1	115,001.24	115,020.56	116,505.84	(12345)
−56295.111	454	2	113,498.22	113,578.73	116,507.43	(123) (45)
−54303.861	454	2	109,515.72	109,596.23	112,524.92	(3) (1245)
−53891.12	681	3	109,144.24	109,333.62	113,658.04	(12) (3) (45)
−53635.417	908	4	109,086.83	109,439.71	115,105.24	(1) (2) (3) (45)
−53485.99	1,135	5	109,241.98	109,821.34	116,764.98	(1) (2) (3) (4) (5)

The log-likelihood, number of parameters and the three criterion scores are listed for six partitioning strategies. Lower criterion score values indicate a better fit of the model to the data. Light gray indicates the best scoring for each criterion, darker gray the second best scoring. The best model for all partitions was GTR+G+I. In the partition scheme column 1 = 1st codon position of protein-coding gene, 2 = 2nd codon postition, 3 = 3rd codon position, 4 = 16S cp rDNA, and 5 = 18S nrDNA.

pruned from the dataset (indicated in Table S1). The two aligned markers were then concatenated and incorporated in the five markers (plastid-encoded *tuf*A, *rbc*L, *atp*B, and 16S rDNA and nuclear 18S rDNA) dataset of Verbruggen et al. (2009a; table 1) comprising five Ulvophyceae (outgroup), seventeen Dasycladales, and 34 Bryopsidales. Five *Pseudochlorodesmis* taxa, three *Caulerpella* taxa, and 46 *Caulerpa* taxa were selected to be analyzed together with the five markers dataset of 56 non-caulerpaceans. Five *Caulerpa* taxa were represented by *tuf*A and *rbc*L sequences from different individuals, namely *C. lentillifera* J. Agardh, *C. paspaloides*, *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, *C. scalpelliformis* var. *denticulata* (Decaisne) Weber-van Bosse, and *C. taxifolia*. Eight taxa were represented only by *tuf*A, namely *C. cactoides*, *C. fastigiata*, *C. lanuginosa*, *C. manorensis* Nizamuddin, *Caulerpella ambigua*-3, and three *Pseudochlorodesmis* spp. Table S1 indicates which *tuf*A and *rbc*Lsequences were used in the analysis with the five markers dataset of non-caulerpaceans.

Model testing was performed in PartitionFinder (Lanfear et al. 2012) to determine the best models and partitioning strategy according to the selection criteria Akaike information criterion (AIC), corrected AIC (AICc) and Bayesian information criterion (BIC). The PartitionFinder analysis pointed to a three partitions scheme: (i) 1st + 2nd codon positions of protein-coding genes, (ii) 3rd codon positions, and (iii) rDNA. A General Time Reversible model (GTR, Yang 1994) along with among-sites rate heterogeneity (G) and an estimated proportion of invariable sites (I) was selected as best model for all three partitions. This partitioning strategy scored very closely to the four partitions scheme adopted in Verbruggen et al. (2009a) in which 1st and 2nd codon positions represented separate partitions rather than a single one. The three partitions scheme was favored here considering the greater support among the three criteria AIC, AICc, and BIC (Table 1).

Phylogenetic analyses. Maximum Likelihood (ML) estimation was performed in RAxML v. 7.2.8 (Stamatakis 2006) with the Ulvophyceae as outgroup and with model and partitioning scheme determined as above. Branch support was assessed with non-parametric bootstrapping of 1,000 replicates (Felsenstein 1985). ML bootstrap percentages (BP) were considered as strong (80%–100%), moderate (70%–79%), weak (50%–69%) or no (<50%) support.

Bayesian inference (BI) was performed with the BEAST package v. 1.4 (BEAST, BEAUti and LogCombiner; Drummond et al. 2006, Drummond and Rambaut 2007), which was also used to produce a time-calibrated phylogeny (chronogram, timetree). Three Markov Chain Monte Carlo chains of 40,000,000 generations (with logging every 4,000 generations) were run independently from a randomly generated starting tree under an uncorrelated lognormal relaxed clock and Yule speciation process. To produce a chronogram, the age (in Ma) of six well supported nodes were input as priors. Ages were set to the mean (μ) and standard deviation (σ) obtained from a normal distribution matching the 95% confidence intervals (CI) reported in Verbruggen et al. (2009a). The six calibrated nodes (indicated in Fig. 1) were (A) the node where the Dasycladales diverge from the Bryopsidales ($\mu = 571$, $\sigma = 30$, 95% CI = 521.7–620.3), (B) the node where Dasycladales diversify ($\mu = 458$, $\sigma = 25$, 95%CI = 416.9–499.1), (C) the node where *Ostreobium* sp. splits from the other Bryopsidales ($\mu = 479$, $\sigma = 20$, 95% CI = 446.1–511.9), (D) the node where the Bryopsidineae diversify ($\mu = 351$, $\sigma = 32$, 95% CI = 298.4–403.6), (E) the node where the Halimedineae diversify ($\mu = 391$, $\sigma = 20$, 95% CI = 358.1–423.9), and (F) the divergence point of the core Halimedineae ($\mu = 303$, $\sigma = 25$, 95% CI = 261.9–344.1). The traces of trees –lnL values from the three independent runs were visualized in Tracer v. 1.5.0 (Rambaut and Drummond 2009) revealing rapid chain convergence, and high run quality (high Effective Sampling Size values). The default 10% burnin period was thus appropriate, and the logs of runs were then combined in LogCombiner, resulting in the exclusion of the first 4,000,000 generations representing the first 1,000 trees from each run. A maximum clade credibility chronogram with mean node heights was calculated from the set of post-burnin trees with TreeAnnotator v.1.6.1 (Rambaut and Drummond 2010). BI posterior probability (PP) values 0.95–1.00 were considered as strong support, values 0.90–0.94 as weak support, and values <0.90 as no support.

Morphological examination. All currently accepted *Caulerpa* species (Table S2 in the Supporting Information) were examined for the presence of pyrenoids associated with the chloroplasts (visible under light microscope after Lugol's iodine stain), assimilators with or without constricted rachis, presence of constricted ramuli pedicels, rhizoids on stolons, and scale-like appendages on stolons. When a species was not available for examination these data were taken, if possible, from the literature description of the species.

RESULTS

Sequence alignment and model selection. EMBL accession numbers of newly generated sequences are given in Table S1. We generated 89 new *tuf*A sequences representing two *Pseudochlorodesmis* spp., three *Caulerpella* spp., and 33 *Caulerpa* spp. (nine representing the crown clade). Alignment was unambiguous for *tuf*A, but gaps to restore alignment were needed in the *tuf*A of *Caulerpa scalpelliformis* (R. Brown *ex* Turner) C. Agardh (three positions), *Caulerpa papillosa* J. Agardh (six positions) and *Caulerpella ambigua* (nine positions). The final *tuf*A alignment was 882 nt in length. We generated 61 new *rbc*L sequences representing one *Pseudochlorodesmis* sp., two *Caulerpella* spp., and 32 *Caulerpa* spp. (ten representing the crown clade). Alignment of the *rbc*L sequences was also straightforward (final alignment was 1,384 nt) after removal of introns found in two specimens. The CR-F/CR-mR PCR fragment of *Caulerpa fergusonii* PERTH 6.10.9.27 contained a 638 nt intron between nt positions 612–613 (based on 1,428 nt complete *rbc*L), which was submitted to EMBL/GenBank separately (accession number FR848361). The secondary structure of the 638 nt intron of *C. fergusonii* G. Murray (specimen PERTH 6.10.9.27) was predicted using the program mfold 3.4 (Zuker et al. 1999) on The mfold Web Server (http://mfold.rna.albany.edu/) of the University at Albany, USA. The predicted secondary structure (Fig. S1 in the Supporting Information) had a group IIA intron structure with six recognizable domains (Bonen and Vogel 2001, Dai et al. 2003). *Caulerpa brownii* specimen L 09.10.057 also contained an intron at the same position, but its sequence was not completely determined because of its great length estimated at ~3,300–3,400 nt by electrophoresis on a 2% agarose gel. Respectively, 693 nt of the 5′-end (FR848362) and 628 nt of the 3′-end (FR848363) were determined. The first 553 nt of the 638 nt *C. fergusonii* intron were alignable with

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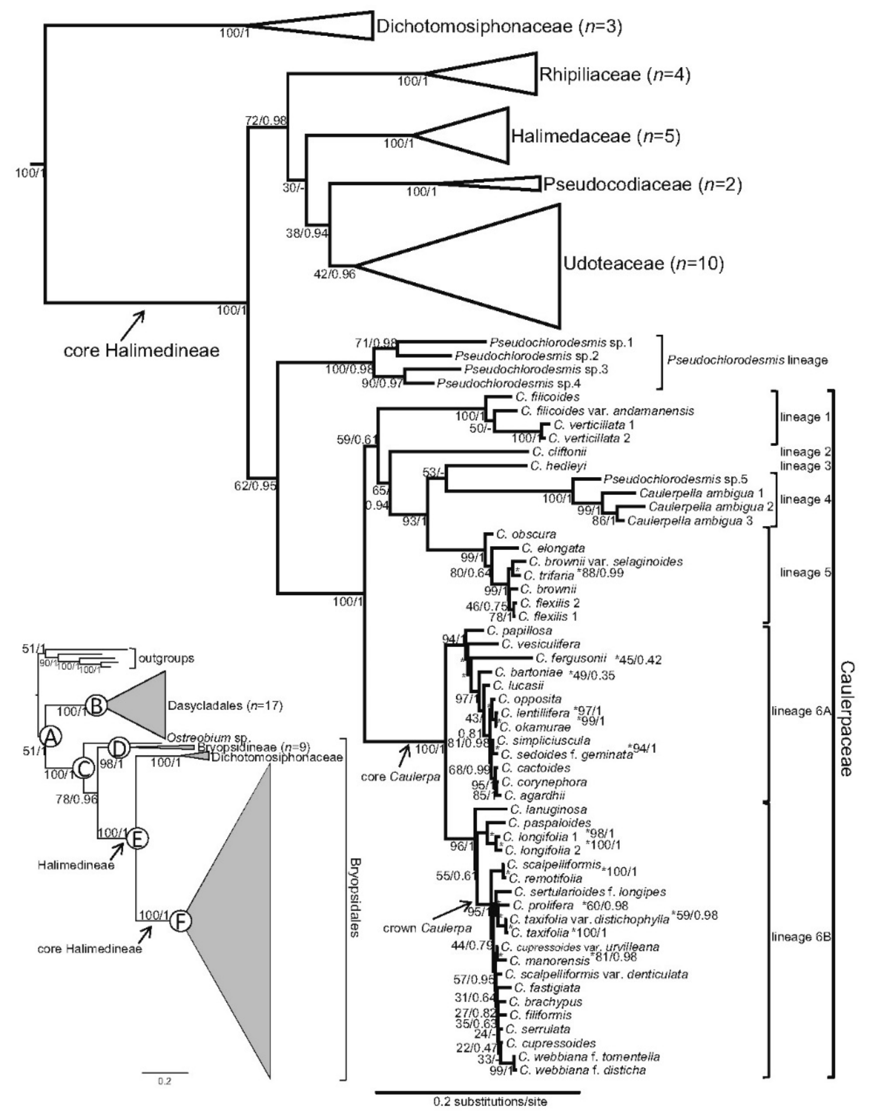

FIG. 1. Five markers Maximum Likelihood (ML) tree of 110 taxa. Only the Caulerpaceae and its sister-clade are shown in detail. The other Halimedineae families are summarized and all other taxa are pruned from the tree. A summary of the complete tree is shown in the lower left corner and the original tree in Figure S3. Branch support values (ML bootstrap percentage/Bayesian Inference [BI] posterior probability) are given near nodes (or right from taxon labels in case of insufficient space near the node, indicated with *). A dash (-) indicates that the branch does not occur in the BI tree. Seven Caulerpaceae lineages (1-6A and B) discussed in the main text are indicated right from the tree. Calibration points (encircled letters A–F) for the chronogram in Figure 2 are indicated in the summarized tree. *C.* = *Caulerpa.*

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the 5′-end of the *C. brownii* intron (24 substitutions and three indels) and the last 85 nt (554–638) with the 3′-end (1 substitution). It is also a group IIA intron (Fig. S2 in the Supporting Information).

Phylogeny of the Caulerpaceae. Figure 1 shows the five markers ML tree of the Dasycladales and Bryopsidales with five Ulvophyceae outgroup taxa (110 taxa in total). The outgroup, *Ostreobium* sp., Dasycladales, and Bryopsidineae are pruned from the tree and the Halimedineae families are summarized except for the *Pseudochlorodesmis* clade (*incertae sedis*) and the Caulerpaceae clade, which are shown in detail. The complete tree is summarized in the lower left corner of Figure 1 and shown in full in the Figure S3 in the Supporting Information. ML boostrap percentages (BP) are plotted on the topology of the trees (Fig. 1 and Fig. S3) as well as the BI PP from the BI analysis (not shown) of the same dataset. The ML and BI trees were in general agreement, revealing the same main clades and only differed in a few unsupported topology differences within the main clades. Pair-wise phylogenetic distances, i.e., branch lengths between taxa, were derived from the ML tree and plotted in the Table S3 in the Supporting Information and their frequency distribution is shown in Figure S4 in the Supporting Information. A pilot analyzing the *tuf*A and *rbc*L alignments separately revealed the same Caulerpaceae main clades in the *tuf*A tree and *rbc*L tree (not shown).

Four *Pseudochlorodesmis* specimens consistently formed a sister-clade to the Caulerpaceae. *Pseudochlorodesmis* sp. 5, however, was nested inside the Caulerpaceae and sister to a *Caulerpella ambigua* clade with maximum support. Six main clades can be discerned within the Caulerpaceae and these are indicated as lineages 1-6 in Figure 1. Maximum supported lineage 6 splits into two strongly supported lineages 6A and 6B. Lineage 6B includes a strongly supported *Caulerpa* crown clade. Taxa in Table S1, but not included in Figure 1 and Figure S1, could each be assigned to one of the Caulerpaceae lineages based on the pilot analysis and this is indicated in Table S1. *Tuf*A sequences were not able to differentiate *C. lentillifera* from *C. microphysa* and *C. matsueana* Yamada from *C. opposita* Coppejans & Meinesz (no *rbc*L data of *C. microphysa* and *C. matsueana*). *C. filicoides* var. *filicoides* and *C. filicoides* var. *andamanensis* W.R. Taylor differed by 35 of 744 nt in *tuf*A (4.7%) and 21 of 604 nt in *rbc*L (3.5%). *C. verticillata* 1 and *C. verticillata* 2 differed by a minimum of 12 of 786 nt in *tuf*A (1.5%) and 9 of 604 nt in *rbc*L (1.5%). *Caulerpa scalpelliformis* is clearly not monophyletic. Typical *C. scalpelliformis* and *C. scalpelliformis* var. *denticulata* differ by 23 and a 3 nt indel of 820 nt in *tuf*A (2.9%) and 15 of 663 nt in *rbc*L (2.3%). *Caulerpa brownii* is seemingly not monophyletic. Australian *C. brownii* and New-Zealandish *C. brownii* var. *selaginoides* J. Agardh differ by 17 of 632 nt in *tuf*A (2.7%) and 10 of 604 nt in *rbc*L (1.7%). Sequence divergence within the *Caulerpella ambigua* clade (lineage 4 excluding *Pseudochlorodesmis* sp. 5) is 6.7% in *tuf*A and 4.7% in *rbc*L.

A chronogram of the Caulerpaceae phylogeny with estimated node ages is shown in Figure 2. According to this timetree the Caulerpaceae probably diverged from their sister-clade *Pseudochlorodesmis* during the Paleozoic. The main lineages within the Caulerpaceae were formed in the first half of the Mesozoic and most diversification within these lineages took place during the Cenozoic.

Morphological observations. The morphology of 99 *Caulerpa* species was examined and the observations are reported in Table S2 ordered by phylogenetic lineage.

DISCUSSION

The Caulerpaceae phylogeny. The analysis of the *tuf*A gene and the *rbc*L gene both support the existence of six main lineages in the Caulerpaceae. De Senerpont Domis et al. (2003) mentioned briefly the incongruence between *tuf*A and *rbc*L in *Caulerpa*, but this incongruence was probably caused by the *rbc*L sequence that represented *C. flexilis* (lineage 5) that actually belonged to *C. okamurae* (lineage 6A). The combined analysis of *tuf*A and *rbc*L (in a five marker alignment, Fig. 1 and Fig. S1) resulted in higher support values than when the genes were analyzed separately. Lineages 2 and 3 are both monotypic and revealed here for the first time. Lineages 1, 4, 5, 6A, and 6B were also revealed by Famà et al. (2002) and Stam et al. (2006), but their phylogenies included only a single representative for each of the lineages 1, 4, and 5. Within lineage 6B, *C. lanuginosa*, *C. paspaloides*, and *C. longifolia* do not belong to the strongly supported species-rich *Caulerpa* crown clade. *C. longifolia* was not included in the studies by Famà et al. (2002) and Stam et al. (2006). Relationships between the six lineages are largely unresolved. Lineage 6 is sister to a weakly supported (ML BP 59) or unsupported (BI PP 0.61) clade comprising the other five lineages. The support for the clade comprising lineages 2–5 is weak (ML BP 65, BI PP 0.94). Only the clade with lineages 3–5 gains strong support (ML BP 93, BI PP 1.00). Lineage 3 is sister to lineage 4 in the ML tree (BP 53, Fig. 1) and to lineage 5 in the BI tree (PP 0.53, Fig. 2). The latter hypothesis is most likely on morphological grounds. *Caulerpa hedleyi* (lineage 3) and the members of lineage 5 have stolons covered in scaly appendages. It is clear that more DNA markers need to be added to the *Caulerpa* alignment to resolve phylogenetic relationships between the deeper lineages of the Caulerpaceae as well as relationships within some of these lineages, notably the *Caulerpa* crown clade for which a more variable marker is needed.

The origin of the genus Caulerpa *in place and time.* Calvert et al. (1976; fig. 20) illustrated a hypothetical

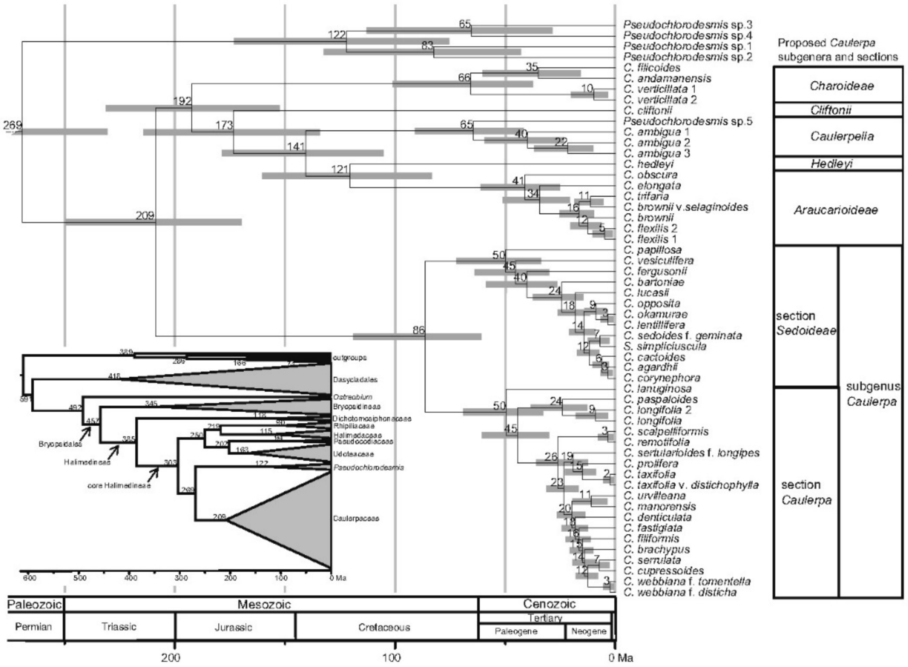

FIG. 2. Chronogram of the Caulerpaceae (all other taxa except for its sister-clade were pruned). Node ages were inferred using Bayesian inference assuming a relaxed molecular clock and a set of node age constraints derived from a chronogram in Verbruggen et al. (2009a) that was calibrated with data from the fossil record. Values at nodes indicate average node ages (in Ma) and gray bars represent 95% confidence intervals. The calibration points used for this analysis are A–F in Figure 1 and explained in the Materials & Methods section. The complete chronogram based on 110 taxa is summarized in the lower left corner including node ages, but without confidence intervals. Major geological eras are indicated along the timescale bar at the bottom. The summarized chronogram has its own timescale. A newly proposed subgeneric classification of the Caulerpaceae is shown right from the chronogram.

scheme for the evolutionary development of the chloroplast in *Caulerpa* and a corresponding phylogenetic tree of the generic sections generated from it. They presumed that the large pyrenoid-containing chloroplast in all but one of their sampled *Sedoideae* was "the most primitive" and speculated that southern Australia, being the apparent center of distribution of pyrenoid-containing species, may also be the geographic origin of *Caulerpa*. However, the pyrenoid-containing chloroplast is not the ancestral type according to the results of this study and appears relatively recent in geological time (nested inside lineage 6A, Fig. 2). The oldest fossil attributed to *Caulerpa* was recovered from the Palo Duro Basin in Texas and dated 280 Ma old (Gustavson and Delevoryas 1992). It resembles the extant species *Caulerpa racemosa* var. *clavifera* (Turner) Weber-van Bosse, but the placement of vesiculate ramuli in the fossil is more regular than in extant *Caulerpa* species with vesiculate ramuli all around the rachis. The phylogenetic chronogram in Figure 2 must be interpreted with great caution as it is calibrated with node ages taken from a timetree that was calibrated with fossils of non-caulerpaceans (fig. 4 in Verbruggen et al. 2009a). The Caulerpaceae tree was essentially grafted into the Dasycladales-Bryopsidales tree. Although the 280 Ma old Palo Duro Basin fossil falls within the 95% confidence interval of the split of the Caulerpaceae from *Pseudochlorodesmis* (indicated by the gray bar around the node at 269 Ma in Fig. 2), the morphology displayed by the fossil seems temporally incongruent since extant *Caulerpa* species with vesiculate ramuli are only found within lineages 6A and 6B, which diversified much later. The validity of this fossil as belonging to the Caulerpaceae is thus questionable, but the possibility that it is indeed a *Caulerpa* cannot be excluded. No other extant macroalgal taxon resembles the morphology of the fossil. Yi et al. (2014) interpreted the

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non-calcified thallophytic fossil alga *Menieria minuta* Wang, Jin *et* Zhan from the Lower Silurian (Middle Aeronian, 440 Ma) of eastern Canada (tropical at that time) as *Caulerpa*-like on the basis of branch morphology and attachment structure, but it does not resemble any extant *Caulerpa* species. According to the timetree (Fig. 2), the Caulerpaceae split from the sister-clade *Pseudochlorodesmis* sometime in the late Carboniferous, the Permian, or early Triassic (the 95% confidence interval bar around the node at 269 Ma spans this time-frame). In the Triassic, the supercontinent Pangaea had not yet started to break up, southern Australia was still connected to the Antarctic plate, and the Atlantic Ocean had not yet formed, suggesting a Tethyan origin of *Caulerpa* along the eastern shores of Pangea. The Palo Alto Basin was on the West coast of Pangea. The tropical East Pacific is poor in *Caulerpa* species with only six confirmed species and no endemics (C. Fernández-García et al., unpublished data). During the Triassic, the Tethys Sea was at that time divided by the Cimmerian superterrane into a Paleotethys (North) and a Neotethys (South; Dèzes 1999). Both the Paleotethys and Neotethys were tropical and the extant species of the sister-clade of the Caulerpaceae are only known from the tropics. The other Halimedineae also have a predominantly tropical distribution. The Caulerpaceae lineages 1 and 4 are exclusively tropical, whereas the monotypic lineages 2 and 3 only occur in temperate Australia. Lineage 5 consists of temperate Australasian species with the exception of *C. elongata* which occurs in the tropical Indo-Pacific. Lineages 6A and 6B both contain tropical, temperate, and tropical-temperate species.

A diversification of the genus *Caulerpa* into at least six lineages during the late Triassic to early Cretaceous is congruent with the rediversification of life after the Permian-Triassic (251.4 Ma ago) and Triassic-Jurassic (199.6 Ma ago) mass extinction events in which the majority of marine life on Earth perished (Benton 2003, Tanner et al. 2004). *Caulerpa* may have diverged into more than six lineages during this period, but the extant six *Caulerpa* lineages are the surviving *Caulerpa* lineages of the Cretaceous-Tertiary/Paleogene (K-T) extinction event (65.5 Ma ago). Species radiation within the six lineages took place after the K-T extinction, resulting in the present day *Caulerpa* diversity. Species richness is highest in lineage 6B with more than fifty currently accepted species (Table S2) which is more than 60% of the total number of extant *Caulerpa* species. This is the first study that gives us a sense of the age of the *Caulerpa* lineages. Although, the genus appears to be ancient, most species radiations appear to be of relative recent date. A similar scenario was found by Verbruggen et al. (2009c) in the genus *Halimeda* J.V. Lamouroux (Halimedaceae, Bryopsidales) where five main lineages (given the rank of *Halimeda* sections) evolved during the Cretaceous and diverged within the last 65 Ma. *Halimeda* is probably of tropical origin and in one of the five sections colonized temperate waters multiple times during global cooling in the Paleogene-Neogene. However, it rather seems that in *Caulerpa* lineages 5 and 6 a colonization from temperate to tropical waters happened. The other four lineages are either exclusively tropical (species poor lineages 1 and 4) or exclusively temperate (monotypic lineages 2 and 3). All species in lineage 5 are endemic to temperate Australasia, except for *C. elongata*, which has a tropical Indo-West-Pacific distribution. The eight tropical taxa within lineage 6A are monophyletic and nested within the clade (node age of 14 Ma in Fig. 2) indicating that they evolved from temperate species. Within the *Caulerpa* crown clade the two temperate species *C. scalpelliformis* and *C. remotifolia* are together sister to the rest of the crown clade which contains temperate and tropical taxa. About half of the species of the crown clade occurs in the Indo-Malay archipelago, the biodiversity hotspot of *Caulerpa* diversity, albeit with a low level of endemism (Prud'homme van Reine et al. 1996). Their evolution in the early neogene coincides with the time that this region became a hotspot of marine biodiversity (Renema et al. 2008). Perhaps tropical species moved to lower latitudes during global cooling, whereas extant temperate species are the descendents of species that did not move, but adapted to cooler temperatures.

On the validity of some Caulerpa *species.* Although challenging, the validity of *Caulerpa* species was not the aim of this study, the present authors take the view that a taxonomic revision should be proposed if it is supported by the collected data. Saunders and Kucera (2010) proposed to adopt *tuf*A as the universal DNA barcode marker for marine green macro-algae (with the exception of the Cladophoraceae) because it showed the largest difference between maximum intra- and minimum interspecific divergence of six tested markers. The 3′-end *rbc*L also showed a large barcode gap, but had moderate amplification success, caused, at least in part, by the presence of introns in some taxa, hence reducing its utility as barcode. However, taxon sampling by Saunders and Kucera (2010) focused on the genus *Ulva* L. and only five bryopsidaleans were included in the study (one *Bryopsis* and four *Codium*). Sauvage et al. (2013) used *tuf*A as a barcode to differentiate between species of the *C. racemosa*–*C. peltata* complex, but did not demonstrate a *tuf*A barcode gap for *Caulerpa*. However, if two true biological species are considered to be a single morphological species, then the observed maximum intraspecific variation will be greater than the observed interspecific variation (unless they are sister-species). Within the *Caulerpa* crown clade phylogenetic resolution is limited and the monophyly of some morphologically well-defined species is not resolved. For example, *C. cupressoides* is nested within *C. serrulata* (Forsskål) J. Agardh in Sauvage

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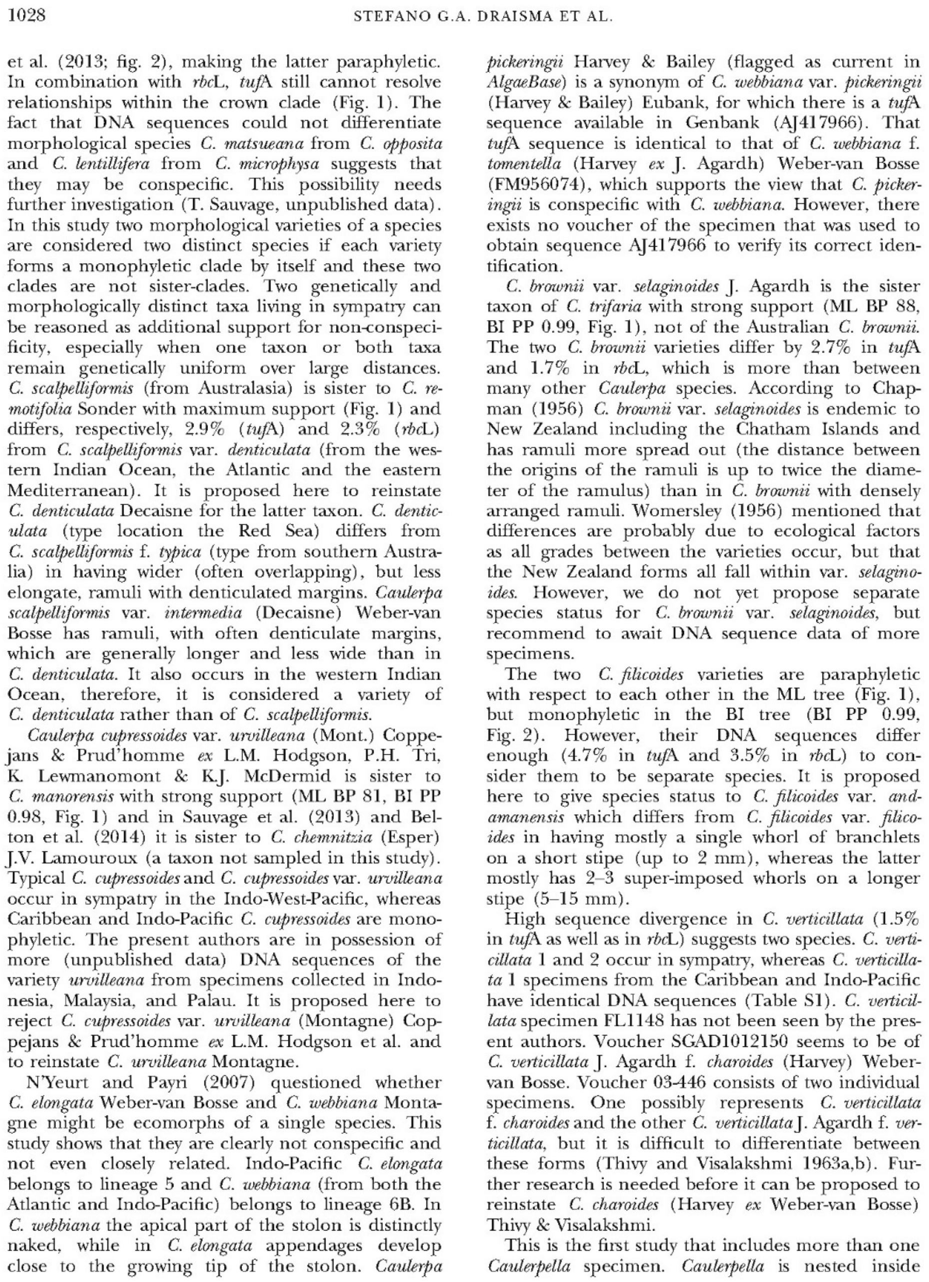

et al. (2013; fig. 2), making the latter paraphyletic. In combination with *rbc*L, *tuf*A still cannot resolve relationships within the crown clade (Fig. 1). The fact that DNA sequences could not differentiate morphological species *C. matsueana* from *C. opposita* and *C. lentillifera* from *C. microphysa* suggests that they may be conspecific. This possibility needs further investigation (T. Sauvage, unpublished data). In this study two morphological varieties of a species are considered two distinct species if each variety forms a monophyletic clade by itself and these two clades are not sister-clades. Two genetically and morphologically distinct taxa living in sympatry can be reasoned as additional support for non-conspecificity, especially when one taxon or both taxa remain genetically uniform over large distances. *C. scalpelliformis* (from Australasia) is sister to *C. remotifolia* Sonder with maximum support (Fig. 1) and differs, respectively, 2.9% (*tuf*A) and 2.3% (*rbc*L) from *C. scalpelliformis* var. *denticulata* (from the western Indian Ocean, the Atlantic and the eastern Mediterranean). It is proposed here to reinstate *C. denticulata* Decaisne for the latter taxon. *C. denticulata* (type location the Red Sea) differs from *C. scalpelliformis* f. *typica* (type from southern Australia) in having wider (often overlapping), but less elongate, ramuli with denticulated margins. *Caulerpa scalpelliformis* var. *intermedia* (Decaisne) Weber-van Bosse has ramuli, with often denticulate margins, which are generally longer and less wide than in *C. denticulata*. It also occurs in the western Indian Ocean, therefore, it is considered a variety of *C. denticulata* rather than of *C. scalpelliformis*.

Caulerpa cupressoides var. *urvilleana* (Mont.) Coppejans & Prud'homme *ex* L.M. Hodgson, P.H. Tri, K. Lewmanomont & K.J. McDermid is sister to *C. manorensis* with strong support (ML BP 81, BI PP 0.98, Fig. 1) and in Sauvage et al. (2013) and Belton et al. (2014) it is sister to *C. chemnitzia* (Esper) J.V. Lamouroux (a taxon not sampled in this study). Typical *C. cupressoides* and *C. cupressoides* var. *urvilleana* occur in sympatry in the Indo-West-Pacific, whereas Caribbean and Indo-Pacific *C. cupressoides* are monophyletic. The present authors are in possession of more (unpublished data) DNA sequences of the variety *urvilleana* from specimens collected in Indonesia, Malaysia, and Palau. It is proposed here to reject *C. cupressoides* var. *urvilleana* (Montagne) Coppejans & Prud'homme *ex* L.M. Hodgson et al. and to reinstate *C. urvilleana* Montagne.

N'Yeurt and Payri (2007) questioned whether *C. elongata* Weber-van Bosse and *C. webbiana* Montagne might be ecomorphs of a single species. This study shows that they are clearly not conspecific and not even closely related. Indo-Pacific *C. elongata* belongs to lineage 5 and *C. webbiana* (from both the Atlantic and Indo-Pacific) belongs to lineage 6B. In *C. webbiana* the apical part of the stolon is distinctly naked, while in *C. elongata* appendages develop close to the growing tip of the stolon. *Caulerpa pickeringii* Harvey & Bailey (flagged as current in *AlgaeBase*) is a synonym of *C. webbiana* var. *pickeringii* (Harvey & Bailey) Eubank, for which there is a *tuf*A sequence available in Genbank (AJ417966). That *tuf*A sequence is identical to that of *C. webbiana* f. *tomentella* (Harvey *ex* J. Agardh) Weber-van Bosse (FM956074), which supports the view that *C. pickeringii* is conspecific with *C. webbiana*. However, there exists no voucher of the specimen that was used to obtain sequence AJ417966 to verify its correct identification.

C. brownii var. *selaginoides* J. Agardh is the sister taxon of *C. trifaria* with strong support (ML BP 88, BI PP 0.99, Fig. 1), not of the Australian *C. brownii*. The two *C. brownii* varieties differ by 2.7% in *tuf*A and 1.7% in *rbc*L, which is more than between many other *Caulerpa* species. According to Chapman (1956) *C. brownii* var. *selaginoides* is endemic to New Zealand including the Chatham Islands and has ramuli more spread out (the distance between the origins of the ramuli is up to twice the diameter of the ramulus) than in *C. brownii* with densely arranged ramuli. Womersley (1956) mentioned that differences are probably due to ecological factors as all grades between the varieties occur, but that the New Zealand forms all fall within var. *selaginoides*. However, we do not yet propose separate species status for *C. brownii* var. *selaginoides*, but recommend to await DNA sequence data of more specimens.

The two *C. filicoides* varieties are paraphyletic with respect to each other in the ML tree (Fig. 1), but monophyletic in the BI tree (BI PP 0.99, Fig. 2). However, their DNA sequences differ enough (4.7% in *tuf*A and 3.5% in *rbc*L) to consider them to be separate species. It is proposed here to give species status to *C. filicoides* var. *andamanensis* which differs from *C. filicoides* var. *filicoides* in having mostly a single whorl of branchlets on a short stipe (up to 2 mm), whereas the latter mostly has 2–3 super-imposed whorls on a longer stipe (5–15 mm).

High sequence divergence in *C. verticillata* (1.5% in *tuf*A as well as in *rbc*L) suggests two species. *C. verticillata* 1 and 2 occur in sympatry, whereas *C. verticillata* 1 specimens from the Caribbean and Indo-Pacific have identical DNA sequences (Table S1). *C. verticillata* specimen FL1148 has not been seen by the present authors. Voucher SGAD1012150 seems to be of *C. verticillata* J. Agardh f. *charoides* (Harvey) Weber-van Bosse. Voucher 03-446 consists of two individual specimens. One possibly represents *C. verticillata* f. *charoides* and the other *C. verticillata* J. Agardh f. *verticillata*, but it is difficult to differentiate between these forms (Thivy and Visalakshmi 1963a,b). Further research is needed before it can be proposed to reinstate *C. charoides* (Harvey *ex* Weber-van Bosse) Thivy & Visalakshmi.

This is the first study that includes more than one *Caulerpella* specimen. *Caulerpella* is nested inside

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Caulerpa with strong support (Fig. 1). This supports the opinion of Silva et al. (1996) who retained *C. ambigua* in the genus *Caulerpa* based on the shared internal trabeculate structure and thought that non-holocarpic reproduction should have infrageneric taxonomic value. Therefore, it is proposed to reinstate the binomial *C. ambigua* Okamura. High *tuf*A (6.7%) and *rbc*L (4.7%) sequence divergence between *C. ambigua* specimens suggests multiple species. *C. ambigua* 1 and 2 occur in sympatry (Hawaii), whereas *C. ambigua* 1 from Hawaii and Texas have identical DNA sequences (Table S1). The species status of one or more of the synonymized taxa *Caulerpa vickersiae* Børgesen and *Caulerpa biloba* Kempermann & Stegenga might be restored in the future, but the present data are insufficient. Remarkably, one of the *Pseudochlorodesmis* specimens was also nested within *Caulerpa* ("*Caulerpella*" lineage 4), whereas the other four *Pseudochlorodesmis* specimens formed a strongly supported sister-clade to *Caulerpa* with multiple cryptic species. It is outside the scope of this study to clarify the taxonomy of *Pseudochlorodesmis* any further. In Figure 1, Figure S3 and Table S1 old taxon names are applied, and in Figure 2 and Tables S2 and S3 the newly proposed names are applied.

Inferring a new infrageneric classification of Caulerpa. The traditional *Caulerpa* sections were based on overall thallus morphology, especially of the erect fronds. It has become clear since Famà et al. (2002) that these sections are polyphyletic and do not reflect phylogeny. Vesiculate, terete, and flattened ramuli all evolved multiple times. Only section *Charoideae* J. Agardh *ex* De-Toni remains monophyletic in this study (lineage 1). Nine sections are represented in lineage 6B and five of them also outside lineage 6B (Table S1). Subgenera have also been described in *Caulerpa*. Decaisne (1842) described the *Caulerpa* subgenera *Chauvinia* (Bory) Decaisne (type *Chauvinia paspaloides* Bory = *Caulerpa paspaloides* [Bory] Greville) and *Chemnitzia* Decaisne (type *C. chemnitzia* [Esper] J.V. Lamouroux). The subgenus *Caulerpa* was automatically formed when Decaisne separated these subgenera. The lectotype (*C. prolifera*) was later selected by Eubank Egerod (1952). The subgenus *Eucaulerpa* Endlicher (1843) is a synonym of the subgenus *Caulerpa*, which has priority. The type species of these subgenera all belong to lineage 6B. Although relationships between the six main lineages (Fig. 1) were not unambiguously resolved, the six lineages are clearly distinct clades at the end of relatively long branches. The maximum pair-wise phylogenetic distance within the six lineages is 0.156 (lineage 6) and the minimum pair-wise distance between the six lineages is 0.163 (between lineage 3 and 5; Table S3). The minor gap between these values cannot be discerned in the histogram of Figure S4 where distances are divided in cohorts of 0.005. The low minimum pair-wise distance between lineages can be ascribed to *C. hedleyi* (lineage 3) and would be 0.236 if this species is ignored. The high maximum pair-wise distance within lineages can be attributed to the long branch leading to *C. fergusonii* (lineage 6) and would be 0.125 if this species is ignored. If lineages 6A and 6B are considered separate main lineages, the maximum pair-wise distance within lineages would be 0.099, but minimum pair-wise distance between lineages 0.091 and thus no gap.

In the previous section, the family of the Caulerpaceae has been reduced to a single genus *Caulerpa* when the genus *Caulerpella* was abolished. It is proposed here to ascribe subgenus rank to each of the lineages 1, 2, 3, 4, 5, and 6. The autonym *Caulerpa* is available for lineage 6, because it includes the type. No subgenus names are available for the other lineages, because the types of the other available subgenus names are also included in lineage 6. It is proposed to give subgenus status to the sections *Charoideae* and *Araucarioideae* J. Agardh *ex* De Toni and to apply them to, respectively, lineage 1 and 5. It is proposed to give *Caulerpa* subgenus rank to the genus *Caulerpella* (lineage 4). New *Caulerpa* subgenus names are proposed for monotypic lineage 2 (*Cliftonii*) and lineage 3 (*Hedleyi*). Furthermore, it is proposed to treat the two lineages 6A and 6B of the *Caulerpa* core clade (i.e., subgenus *Caulerpa*) as sections. The other five proposed *Caulerpa* subgenera each contain only a single section bearing the same name as the subgenus. Characteristics of the newly proposed infrageneric taxa are discussed in the next paragraphs and the names are indicated in Figure 2. In Table S2, all the currently accepted *Caulerpa* species names as listed in *AlgaeBase* (searched September 18, 2013) are listed and ordered according to the newly proposed classification.

The *Caulerpa* subgenus *Charoideae* comb. et stat. nov. is proposed for lineage 1 with a single section *Charoideae* for which *C. verticillata* is the lectotype. The unsampled species *Caulerpa kempfii* A.B. Joly & S. Pereira, *Caulerpa murrayi* Weber-van Bosse, and *Caulerpa pusilla* (Kützing) J. Agardh are also assigned to this subgenus. The former two species are only known from northeast Brazil. The last mentioned species has also been found in Brazil as well as in several Caribbean locations. *C. filicoides* and *C. andamanensis* stat. nov. are known only from the tropical Indo-Pacific. Specimens identified as *C. verticillata* are known from both the Indo-Pacific and the Atlantic Ocean. The species in the subgenus and section *Charoideae* are characterized by repeatedly branching ramuli, which are arranged in whorls (i.e., a verticillate branching mode) and stolons, which can be glabrous, densely or sparsely covered by rhizoids or tuberculate.

The *Caulerpa* subgenus *Cliftonii* subgen. nov. is proposed for lineage 2 with a single section *Cliftonii* sect. nov. for which Australian endemic *Caulerpa cliftonii* is the type and currently the only included species. The *Caulerpa* subgenus *Hedleyi* subgen. nov.

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is proposed for lineage 3 with a single section *Hedleyi* sect. nov. for which Australian endemic *C. hedleyi* is the type and currently the only included species.

The *Caulerpa* subgenus *Caulerpella* comb. et stat. nov. is proposed for lineage 4 with a single section *Caulerpella* comb. et stat. nov. for which *C. ambigua* is the type and currently the only included species with a cosmopolitan tropical distribution. However, the high DNA sequence divergence between the *C. ambigua* specimens included in this study indicates that the taxon actually comprises multiple (cryptic) species. *Pseudochlorodesmis* sp. 5 should also be included in the subgenus *Caulerpella*. The occurrence of compound zoidangia distinguishes the subgenus *Caulerpella* from the other subgenera, but neither holocarpy nor zoidangia have been reported for *Pseudochlorodesmis* spp. (Abbott and Huisman 2003, 2004). However, compound zoidangia also occur in the halimedinean genera *Halimeda* J.V. Lamouroux and *Chlorodesmis* Harvey & Bailey and thus appear not to be phylogenetically informative in the Bryopsidales (Vroom et al. 1998).

The *Caulerpa* subgenus *Araucarioideae* comb. et stat. nov. is proposed for lineage 5 with a single amended section *Araucarioideae* for which *C. flexilis* is the type. All members of the subgenus and section *Araucarioideae* have conspicuous simple branched or unbranched appendages growing from the surface of the stolon, giving them a scaly or spiny appearance. However, the stolons of *C. webbiana* (lineage 6B) are also covered with outgrowths, but these are identical to the ramuli on the upright assimilators, whereas the stolon appendages in lineage 5 differ from those on the assimilators. *Caulerpa seuratii* Weber-van Bosse is an unsampled species with stolons densely covered by rhizoids, resembling *C. elongata* and *C. webbiana* and is expected to belong to the *Caulerpa* crown clade (lineage 6B). The stolons of *C. lanuginosa* and *C. antoensis* Yamada (both belonging to lineage 6B) are also covered by rhizoids (not by squamulate outgrowths) and so are the stolons of the *Charoideae* species (lineage 1). *C. hedleyi* (lineage 3) has squamulate stolons, but does not belong to lineage 5. However, lineage 3 and 5 might be sister lineages, which would mean that the stolon-covering scale-like appendages could be a synapomorphy. All other *Caulerpa* species have naked (glabrous) stolons, except *Caulerpa heterophylla* I.R. Price, J.M. Huisman & M.A. Borowitzka from West-Australia, which has stolons covered by conical protuberances and is therefore classified here in the *Caulerpa* subgenus and section *Araucarioideae*. *Caulerpa alternans* Womersley has glabrous stolons and is not sampled in this study but is included in the *Araucarioideae* based on unpublished DNA sequence data (G. Belton).

The *Caulerpa* subgenus *Caulerpa* (autonym) is proposed for lineage 6 for which *C. prolifera* is the type. Two *Caulerpa* subgenus *Caulerpa* sections are proposed: an amended section *Sedoideae* J. Agardh *ex* De Toni (lectotype: *C. sedoides*) for lineage 6A and a section *Caulerpa* (autonym) for lineage 6B. Section *Caulerpa* includes the strongly supported *Caulerpa* crown clade (ML BP 95, BI PP 1.00, Fig. 1), as well as *C. longifolia*, *C. paspaloides*, and *C. lanuginosa*. The amended section *Sedoideae* includes *Caulerpa* species that have glabrous stolons. All species in lineage 6A have an Indo-Pacific distribution, except *C. microphysa*, which also occurs in the Atlantic. Several species exhibit assimilators bearing vesiculate (including elongate-ovoid to clavate) ramuli with a constricted pedicel. There are also species without vesiculate ramuli which mostly exhibit a rachis with regularly interspaced constrictions (i.e., annulate). Many species have pyrenoids associated with relatively large chloroplasts, 7–11 μm in length (Calvert 1974, Calvert et al. 1976, Famà et al. 2002, Wynne et al. 2009, present study). In *Caulerpa* species without pyrenoids, chloroplasts are 3–5 μm. No *Caulerpa* species with pyrenoids are known outside lineage 6A. No pyrenoids have been reported for four Australasian species in lineage 6A, i.e., *C. fergusonii*, *C. hodgkinsoniae* J. Agardh, *C. papillosa* J. Agardh, and *C. vesiculifera* (Harvey) Harvey. All have vesiculate ramuli with constricted pedicels, but the rachis is without constrictions in the latter two species. The present authors neither observed pyrenoids when inspecting herbarium vouchers of these species stained with iodine (to make starch around the pyrenoids visible) under the light microscope. However, many chloroplasts in *C. papillosa* showed a 1.5 μm light-colored area. This might be the "presumptive pyrenoid region or pyrenoid-like region" that Borowitzka (1976) reported for the chloroplast of *C. papillosa*. Calvert et al. (1976) did not observe pyrenoids, nor a pyrenoid-like region, in *C. papillosa*, but measured 5–7 μm long chloroplasts which is longer than the 3–5 μm measured in other species without pyrenoids. However, the present authors measured 3–5 μm in voucher material of *C. papillosa* under the light microscope. Hori (1974) stated that pyrenoids usually are recognized by the formation of starch plates and that they are rarely without limiting membranes, but that this is not the case in *C. fergusonii* from Japan. In Japanese *C. fergusonii*, the centrally located matrix of the pyrenoids in the chloroplasts is only set with many small starch grains and is thus less elaborate than the pyrenoid in *C. okamurae*, the other species of the pyrenoid clade studied by Hori by use of an electron microscope. The presence of pyrenoids (observable under the light microscope) might be a synapomorphy within *Caulerpa*. All pyrenoid-containing species form a strongly supported monophyletic clade within lineage 6A (ML BP 81, BI PP 0.98, Fig. 1), except for *Caulerpa bartoniae* G. Murray which is outside this clade, albeit without support (ML BP 43, BI PP 0.81). *C. bartoniae* lacks an annulate rachis and vesiculate ramuli. *C. filiformis* (lineage 6B) has an annulate rachis, but no pyrenoids and neither ramuli with constricted pedicels. Three species without

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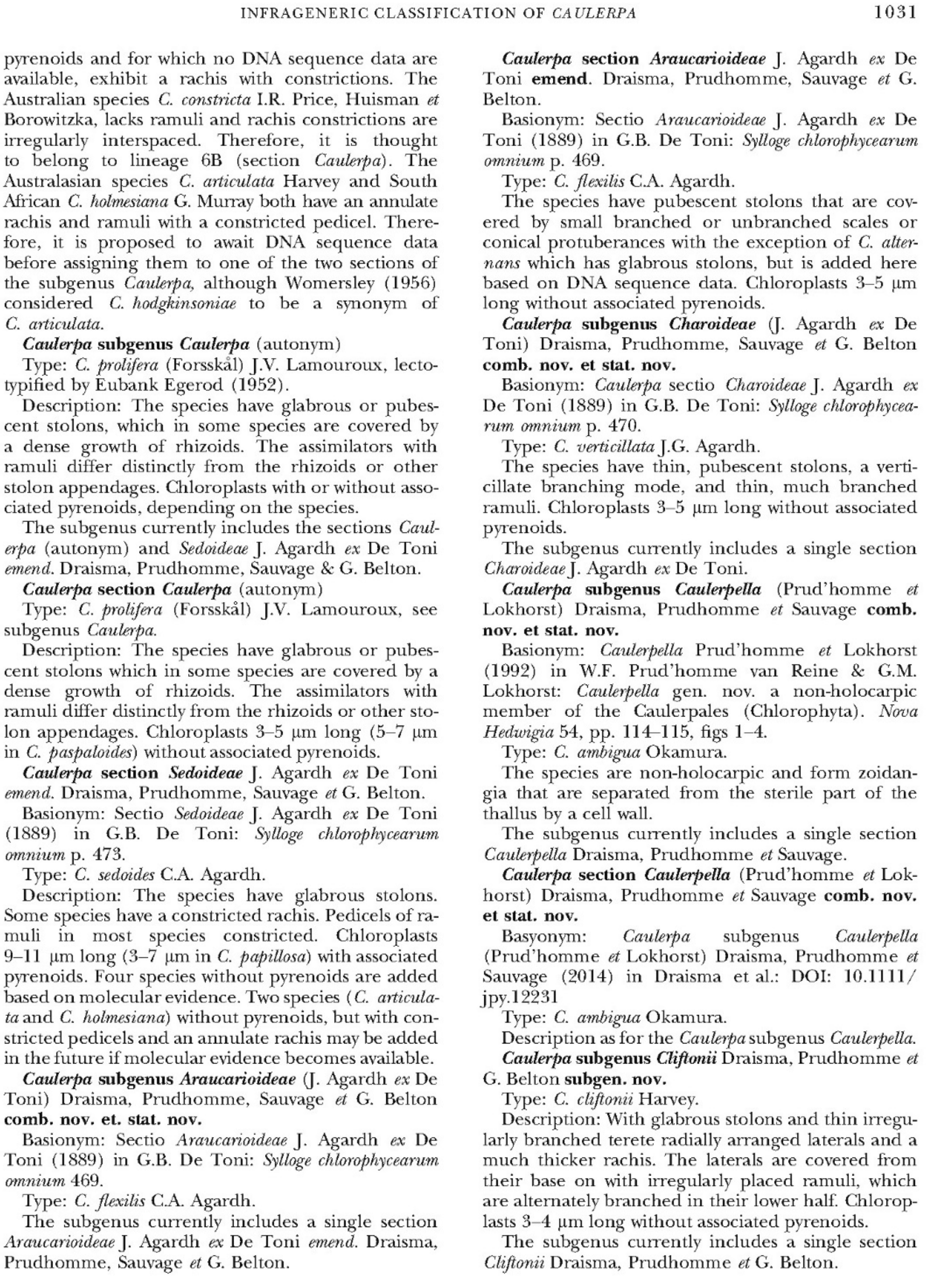

pyrenoids and for which no DNA sequence data are available, exhibit a rachis with constrictions. The Australian species *C. constricta* I.R. Price, Huisman *et* Borowitzka, lacks ramuli and rachis constrictions are irregularly interspaced. Therefore, it is thought to belong to lineage 6B (section *Caulerpa*). The Australasian species *C. articulata* Harvey and South African *C. holmesiana* G. Murray both have an annulate rachis and ramuli with a constricted pedicel. Therefore, it is proposed to await DNA sequence data before assigning them to one of the two sections of the subgenus *Caulerpa*, although Womersley (1956) considered *C. hodgkinsoniae* to be a synonym of *C. articulata*.

Caulerpa* subgenus *Caulerpa (autonym)

Type: *C. prolifera* (Forsskål) J.V. Lamouroux, lectotypified by Eubank Egerod (1952).

Description: The species have glabrous or pubescent stolons, which in some species are covered by a dense growth of rhizoids. The assimilators with ramuli differ distinctly from the rhizoids or other stolon appendages. Chloroplasts with or without associated pyrenoids, depending on the species.

The subgenus currently includes the sections *Caulerpa* (autonym) and *Sedoideae* J. Agardh *ex* De Toni *emend.* Draisma, Prudhomme, Sauvage & G. Belton.

Caulerpa* section *Caulerpa (autonym)

Type: *C. prolifera* (Forsskål) J.V. Lamouroux, see subgenus *Caulerpa.*

Description: The species have glabrous or pubescent stolons which in some species are covered by a dense growth of rhizoids. The assimilators with ramuli differ distinctly from the rhizoids or other stolon appendages. Chloroplasts 3–5 μm long (5–7 μm in *C. paspaloides*) without associated pyrenoids.

Caulerpa* section *Sedoideae J. Agardh *ex* De Toni *emend.* Draisma, Prudhomme, Sauvage *et* G. Belton.

Basionym: Sectio *Sedoideae* J. Agardh *ex* De Toni (1889) in G.B. De Toni: *Sylloge chlorophycearum omnium* p. 473.

Type: *C. sedoides* C.A. Agardh.

Description: The species have glabrous stolons. Some species have a constricted rachis. Pedicels of ramuli in most species constricted. Chloroplasts 9–11 μm long (3–7 μm in *C. papillosa*) with associated pyrenoids. Four species without pyrenoids are added based on molecular evidence. Two species (*C. articulata* and *C. holmesiana*) without pyrenoids, but with constricted pedicels and an annulate rachis may be added in the future if molecular evidence becomes available.

Caulerpa* subgenus *Araucarioideae (J. Agardh *ex* De Toni) Draisma, Prudhomme, Sauvage *et* G. Belton **comb. nov. et. stat. nov.**

Basionym: Sectio *Araucarioideae* J. Agardh *ex* De Toni (1889) in G.B. De Toni: *Sylloge chlorophycearum omnium* 469.

Type: *C. flexilis* C.A. Agardh.

The subgenus currently includes a single section *Araucarioideae* J. Agardh *ex* De Toni *emend.* Draisma, Prudhomme, Sauvage *et* G. Belton.

Caulerpa* section *Araucarioideae J. Agardh *ex* De Toni **emend.** Draisma, Prudhomme, Sauvage *et* G. Belton.

Basionym: Sectio *Araucarioideae* J. Agardh *ex* De Toni (1889) in G.B. De Toni: *Sylloge chlorophycearum omnium* p. 469.

Type: *C. flexilis* C.A. Agardh.

The species have pubescent stolons that are covered by small branched or unbranched scales or conical protuberances with the exception of *C. alternans* which has glabrous stolons, but is added here based on DNA sequence data. Chloroplasts 3–5 μm long without associated pyrenoids.

Caulerpa* subgenus *Charoideae (J. Agardh *ex* De Toni) Draisma, Prudhomme, Sauvage *et* G. Belton **comb. nov. et stat. nov.**

Basionym: *Caulerpa* sectio *Charoideae* J. Agardh *ex* De Toni (1889) in G.B. De Toni: *Sylloge chlorophycearum omnium* p. 470.

Type: *C. verticillata* J.G. Agardh.

The species have thin, pubescent stolons, a verticillate branching mode, and thin, much branched ramuli. Chloroplasts 3–5 μm long without associated pyrenoids.

The subgenus currently includes a single section *Charoideae* J. Agardh *ex* De Toni.

Caulerpa* subgenus *Caulerpella (Prud'homme *et* Lokhorst) Draisma, Prudhomme *et* Sauvage **comb. nov. et stat. nov.**

Basionym: *Caulerpella* Prud'homme *et* Lokhorst (1992) in W.F. Prud'homme van Reine & G.M. Lokhorst: *Caulerpella* gen. nov. a non-holocarpic member of the Caulerpales (Chlorophyta). *Nova Hedwigia* 54, pp. 114–115, figs 1–4.

Type: *C. ambigua* Okamura.

The species are non-holocarpic and form zoidangia that are separated from the sterile part of the thallus by a cell wall.

The subgenus currently includes a single section *Caulerpella* Draisma, Prudhomme *et* Sauvage.

Caulerpa* section *Caulerpella (Prud'homme *et* Lokhorst) Draisma, Prudhomme *et* Sauvage **comb. nov. et stat. nov.**

Basyonym: *Caulerpa* subgenus *Caulerpella* (Prud'homme *et* Lokhorst) Draisma, Prudhomme *et* Sauvage (2014) in Draisma et al.: DOI: 10.1111/jpy.12231

Type: *C. ambigua* Okamura.

Description as for the *Caulerpa* subgenus *Caulerpella.*

Caulerpa* subgenus *Cliftonii Draisma, Prudhomme *et* G. Belton **subgen. nov.**

Type: *C. cliftonii* Harvey.

Description: With glabrous stolons and thin irregularly branched terete radially arranged laterals and a much thicker rachis. The laterals are covered from their base on with irregularly placed ramuli, which are alternately branched in their lower half. Chloroplasts 3–4 μm long without associated pyrenoids.

The subgenus currently includes a single section *Cliftonii* Draisma, Prudhomme *et* G. Belton.

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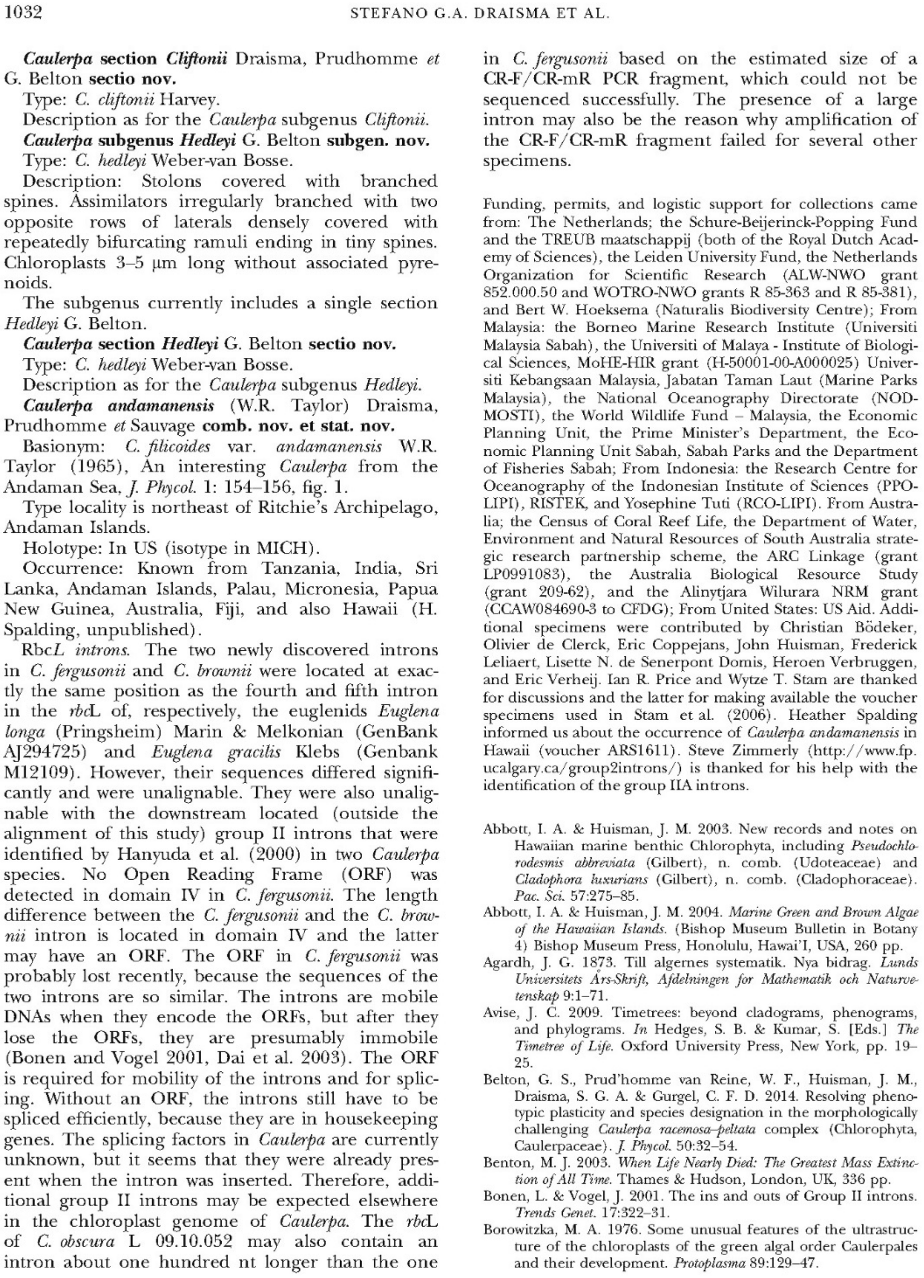

Caulerpa* section *Cliftonii Draisma, Prudhomme *et* G. Belton **sectio nov.**

Type: *C. cliftonii* Harvey.

Description as for the *Caulerpa* subgenus *Cliftonii.*

Caulerpa* subgenus *Hedleyi G. Belton **subgen. nov.**

Type: *C. hedleyi* Weber-van Bosse.

Description: Stolons covered with branched spines. Assimilators irregularly branched with two opposite rows of laterals densely covered with repeatedly bifurcating ramuli ending in tiny spines. Chloroplasts 3–5 µm long without associated pyrenoids.

The subgenus currently includes a single section *Hedleyi* G. Belton.

Caulerpa* section *Hedleyi G. Belton **sectio nov.**

Type: *C. hedleyi* Weber-van Bosse.

Description as for the *Caulerpa* subgenus *Hedleyi.*

Caulerpa andamanensis (W.R. Taylor) Draisma, Prudhomme *et* Sauvage **comb. nov. et stat. nov.**

Basionym: *C. filicoides* var. *andamanensis* W.R. Taylor (1965), An interesting *Caulerpa* from the Andaman Sea, *J. Phycol.* 1: 154–156, fig. 1.

Type locality is northeast of Ritchie's Archipelago, Andaman Islands.

Holotype: In US (isotype in MICH).

Occurrence: Known from Tanzania, India, Sri Lanka, Andaman Islands, Palau, Micronesia, Papua New Guinea, Australia, Fiji, and also Hawaii (H. Spalding, unpublished).

Rbc*L introns.* The two newly discovered introns in *C. fergusonii* and *C. brownii* were located at exactly the same position as the fourth and fifth intron in the *rbc*L of, respectively, the euglenids *Euglena longa* (Pringsheim) Marin & Melkonian (GenBank AJ294725) and *Euglena gracilis* Klebs (Genbank M12109). However, their sequences differed significantly and were unalignable. They were also unalignable with the downstream located (outside the alignment of this study) group II introns that were identified by Hanyuda et al. (2000) in two *Caulerpa* species. No Open Reading Frame (ORF) was detected in domain IV in *C. fergusonii.* The length difference between the *C. fergusonii* and the *C. brownii* intron is located in domain IV and the latter may have an ORF. The ORF in *C. fergusonii* was probably lost recently, because the sequences of the two introns are so similar. The introns are mobile DNAs when they encode the ORFs, but after they lose the ORFs, they are presumably immobile (Bonen and Vogel 2001, Dai et al. 2003). The ORF is required for mobility of the introns and for splicing. Without an ORF, the introns still have to be spliced efficiently, because they are in housekeeping genes. The splicing factors in *Caulerpa* are currently unknown, but it seems that they were already present when the intron was inserted. Therefore, additional group II introns may be expected elsewhere in the chloroplast genome of *Caulerpa.* The *rbc*L of *C. obscura* L 09.10.052 may also contain an intron about one hundred nt longer than the one in *C. fergusonii* based on the estimated size of a CR-F/CR-mR PCR fragment, which could not be sequenced successfully. The presence of a large intron may also be the reason why amplification of the CR-F/CR-mR fragment failed for several other specimens.

Funding, permits, and logistic support for collections came from: The Netherlands; the Schure-Beijerinck-Popping Fund and the TREUB maatschappij (both of the Royal Dutch Academy of Sciences), the Leiden University Fund, the Netherlands Organization for Scientific Research (ALW-NWO grant 852.000.50 and WOTRO-NWO grants R 85-363 and R 85-381), and Bert W. Hoeksema (Naturalis Biodiversity Centre); From Malaysia: the Borneo Marine Research Institute (Universiti Malaysia Sabah), the Universiti of Malaya - Institute of Biological Sciences, MoHE-HIR grant (H-50001-00-A000025) Universiti Kebangsaan Malaysia, Jabatan Taman Laut (Marine Parks Malaysia), the National Oceanography Directorate (NOD-MOSTI), the World Wildlife Fund – Malaysia, the Economic Planning Unit, the Prime Minister's Department, the Economic Planning Unit Sabah, Sabah Parks and the Department of Fisheries Sabah; From Indonesia: the Research Centre for Oceanography of the Indonesian Institute of Sciences (PPO-LIPI), RISTEK, and Yosephine Tuti (RCO-LIPI). From Australia; the Census of Coral Reef Life, the Department of Water, Environment and Natural Resources of South Australia strategic research partnership scheme, the ARC Linkage (grant LP0991083), the Australia Biological Resource Study (grant 209-62), and the Alinytjara Wilurara NRM grant (CCAW084690-3 to CFDG); From United States: US Aid. Additional specimens were contributed by Christian Bödeker, Olivier de Clerck, Eric Coppejans, John Huisman, Frederick Leliaert, Lisette N. de Senerpont Domis, Heroen Verbruggen, and Eric Verheij. Ian R. Price and Wytze T. Stam are thanked for discussions and the latter for making available the voucher specimens used in Stam et al. (2006). Heather Spalding informed us about the occurrence of *Caulerpa andamanensis* in Hawaii (voucher ARS1611). Steve Zimmerly (http://www.fp.ucalgary.ca/group2introns/) is thanked for his help with the identification of the group IIA introns.

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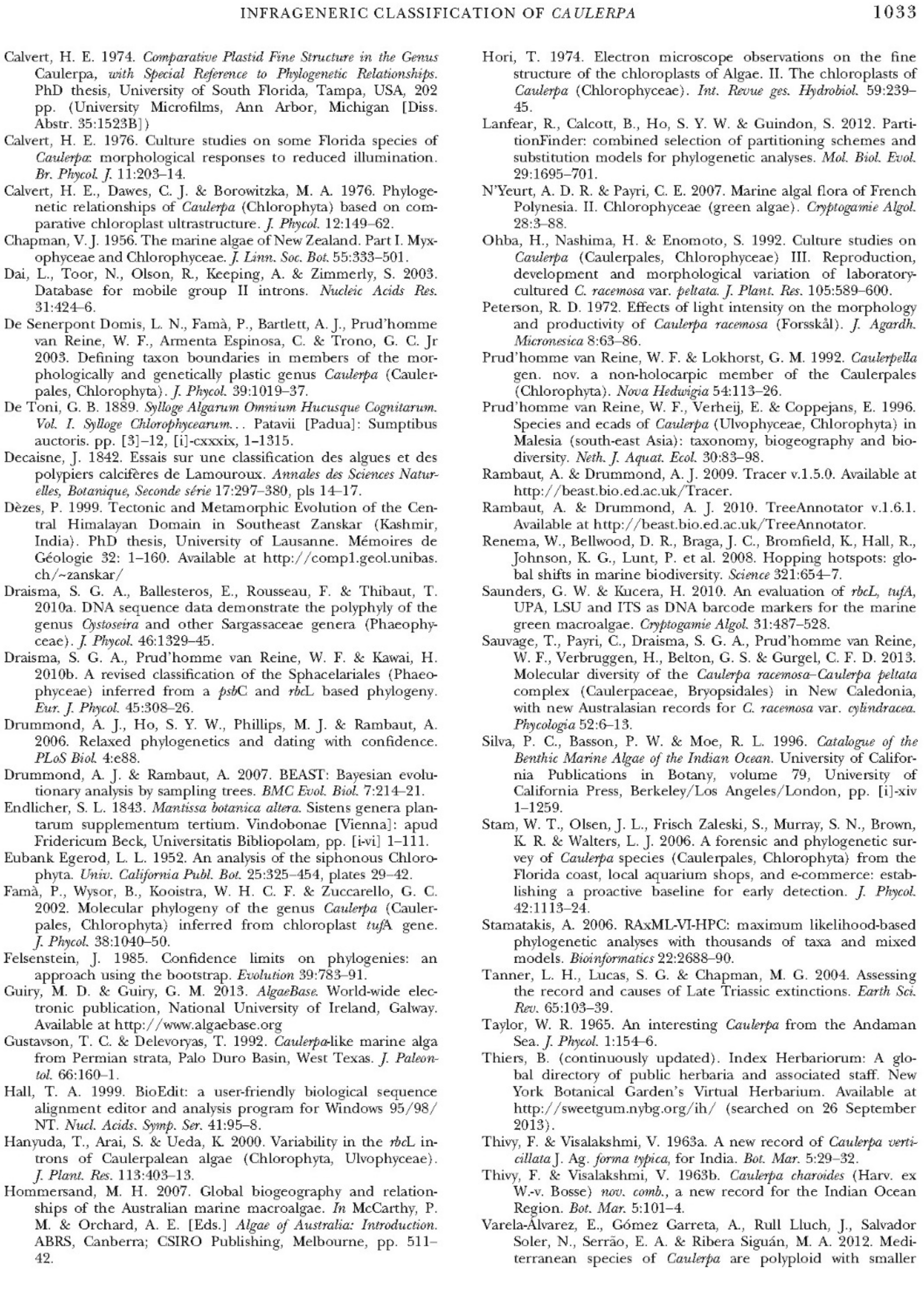
Appendix: 2



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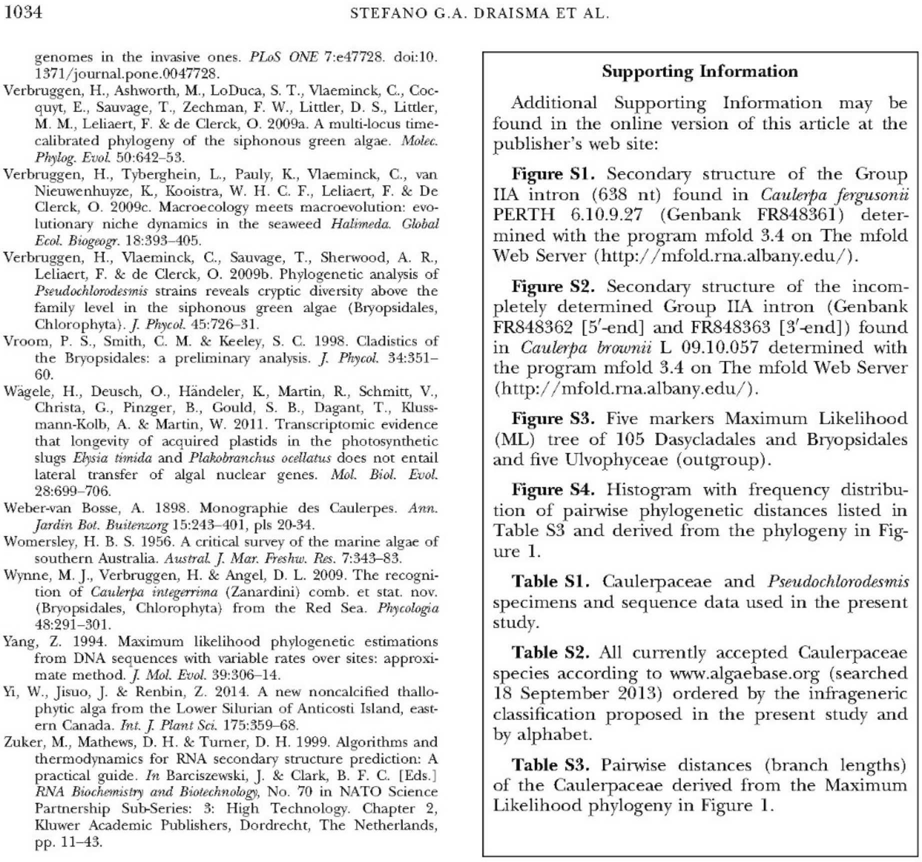
Appendix: 2



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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Secondary structure of the Group IIA intron (638 nt) found in *Caulerpa fergusonii* PERTH 6.10.9.27 (Genbank FR848361) determined with the program mfold 3.4 on The mfold Web Server (http://mfold.rna.albany.edu/).

Figure S2. Secondary structure of the incompletely determined Group IIA intron (Genbank FR848362 [5′-end] and FR848363 [3′-end]) found in *Caulerpa brownii* L 09.10.057 determined with the program mfold 3.4 on The mfold Web Server (http://mfold.rna.albany.edu/).

Figure S3. Five markers Maximum Likelihood (ML) tree of 105 Dasycladales and Bryopsidales and five Ulvophyceae (outgroup).

Figure S4. Histogram with frequency distribution of pairwise phylogenetic distances listed in Table S3 and derived from the phylogeny in Figure 1.

Table S1. Caulerpaceae and *Pseudochlorodesmis* specimens and sequence data used in the present study.

Table S2. All currently accepted Caulerpaceae species according to www.algaebase.org (searched 18 September 2013) ordered by the infrageneric classification proposed in the present study and by alphabet.

Table S3. Pairwise distances (branch lengths) of the Caulerpaceae derived from the Maximum Likelihood phylogeny in Figure 1.

Appendix: 3

1

Appendix 3: Supplementary material for Chapter 2.

Appendix: 3

Fig. S1. Bayesian Inference results derived from the combined *tuf*A and *rbc*L DNA sequence dataset (38 taxa, 1,771 nt) for *Caulerpa*. Numbered lineages correspond to those of Fig. 3. Values at internal nodes are BI posterior probabilities (PP) and ML bootstrap percentages (BP), respectively. PP values below 0.5 and BS values below 50 are not shown. The scale is 0.02 expected changes per site.

Appendix: 3

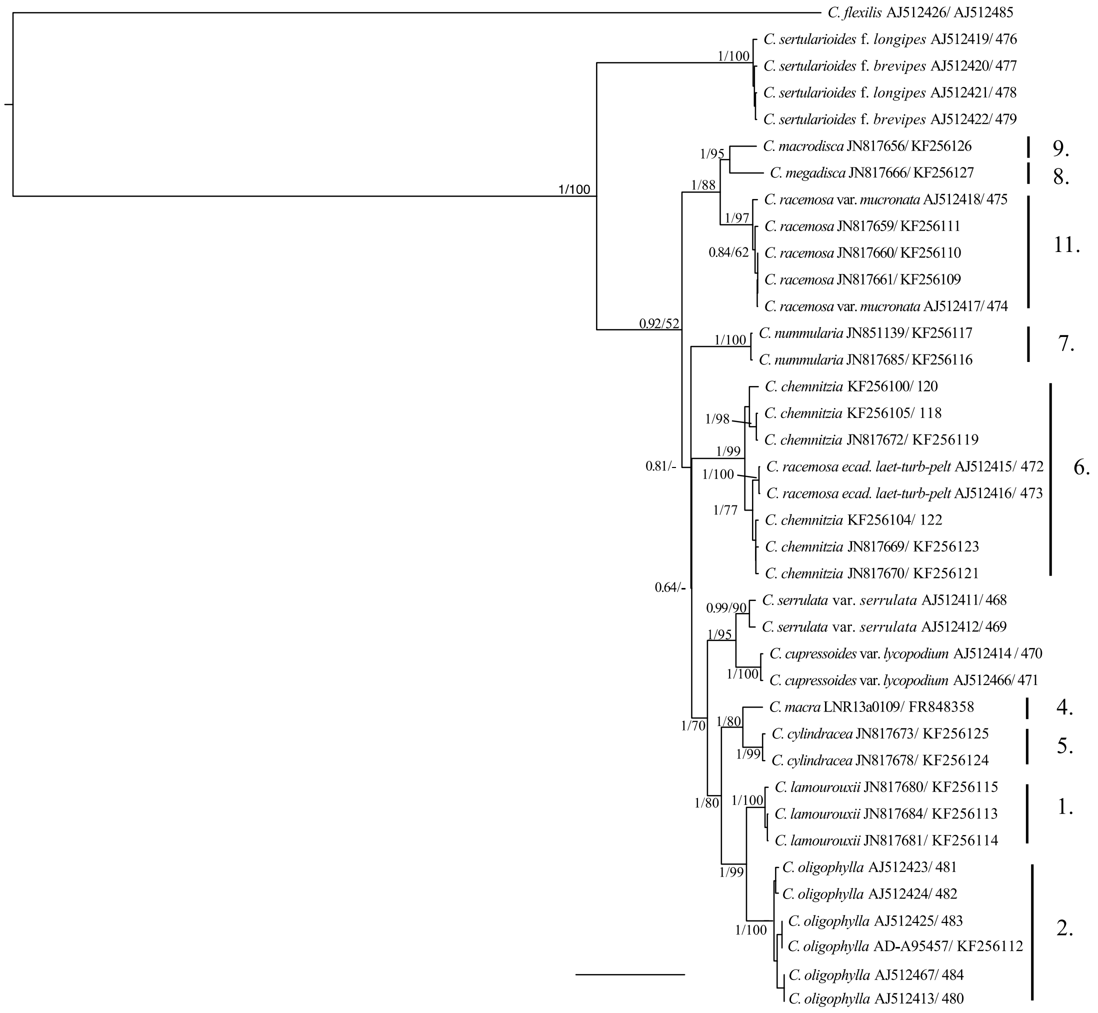
C. flexilis AJ512426/ AJ512485
1/100
C. sertularioides f. longipes AJ512419/ 476
C. sertularioides f. brevipes AJ512420/ 477
C. sertularioides f. longipes AJ512421/ 478
C. sertularioides f. brevipes AJ512422/ 479
1/95
C. macrodisca JN817656/ KF256126
9.
C. megadisca JN817666/ KF256127
8.
1/100
1/88
1/97
C. racemosa var. mucronata AJ512418/ 475
C. racemosa JN817659/ KF256111
0.84/62
C. racemosa JN817660/ KF256110
11.
C. racemosa JN817661/ KF256109
C. racemosa var. mucronata AJ512417/ 474
0.92/52
1/100
C. nummularia JN851139/ KF256117
7.
C. nummularia JN817685/ KF256116
C. chemnitzia KF256100/ 120
1/98
C. chemnitzia KF256105/ 118
C. chemnitzia JN817672/ KF256119
1/99
0.81/-
1/100
C. racemosa ecad. laet-turb-pelt AJ512415/ 472
6.
C. racemosa ecad. laet-turb-pelt AJ512416/ 473
1/77
C. chemnitzia KF256104/ 122
C. chemnitzia JN817669/ KF256123
C. chemnitzia JN817670/ KF256121
0.64/-
0.99/90
C. serrulata var. serrulata AJ512411/ 468
C. serrulata var. serrulata AJ512412/ 469
1/95
C. cupressoides var. lycopodium AJ512414 / 470
1/100
C. cupressoides var. lycopodium AJ512466/ 471
C. macra LNR13a0109/ FR848358
4.
1/70
1/80
C. cylindracea JN817673/ KF256125
5.
1/99
C. cylindracea JN817678/ KF256124
C. lamourouxii JN817680/ KF256115
1/80
1/100
C. lamourouxii JN817684/ KF256113
1.
C. lamourouxii JN817681/ KF256114
C. oligophylla AJ512423/ 481
1/99
C. oligophylla AJ512424/ 482
C. oligophylla AJ512425/ 483
1/100
2.
C. oligophylla AD-A95457/ KF256112
C. oligophylla AJ512467/ 484
C. oligophylla AJ512413/ 480

Fig. S2. Phylogram of taxa belonging to *Caulerpa* based on Bayesian relaxed molecular clock analyses of the same *tuf*A dataset as Fig. 3. *Caulerpa racemosa-peltata* complex taxa are in boldface. Lineage 6 (*C. chemnitzia*) represents three GMYC lineages and Lineage 4 (*C. macra*) represents two “long branches and high support” lineages. Numbers at branch nodes correspond to BI posterior probabilities (PP) and ML bootstrap percentages (BP). PP values below 0.5 and BS values below 50 are not shown. The scale is 0.03 expected changes per site.

Appendix: 3

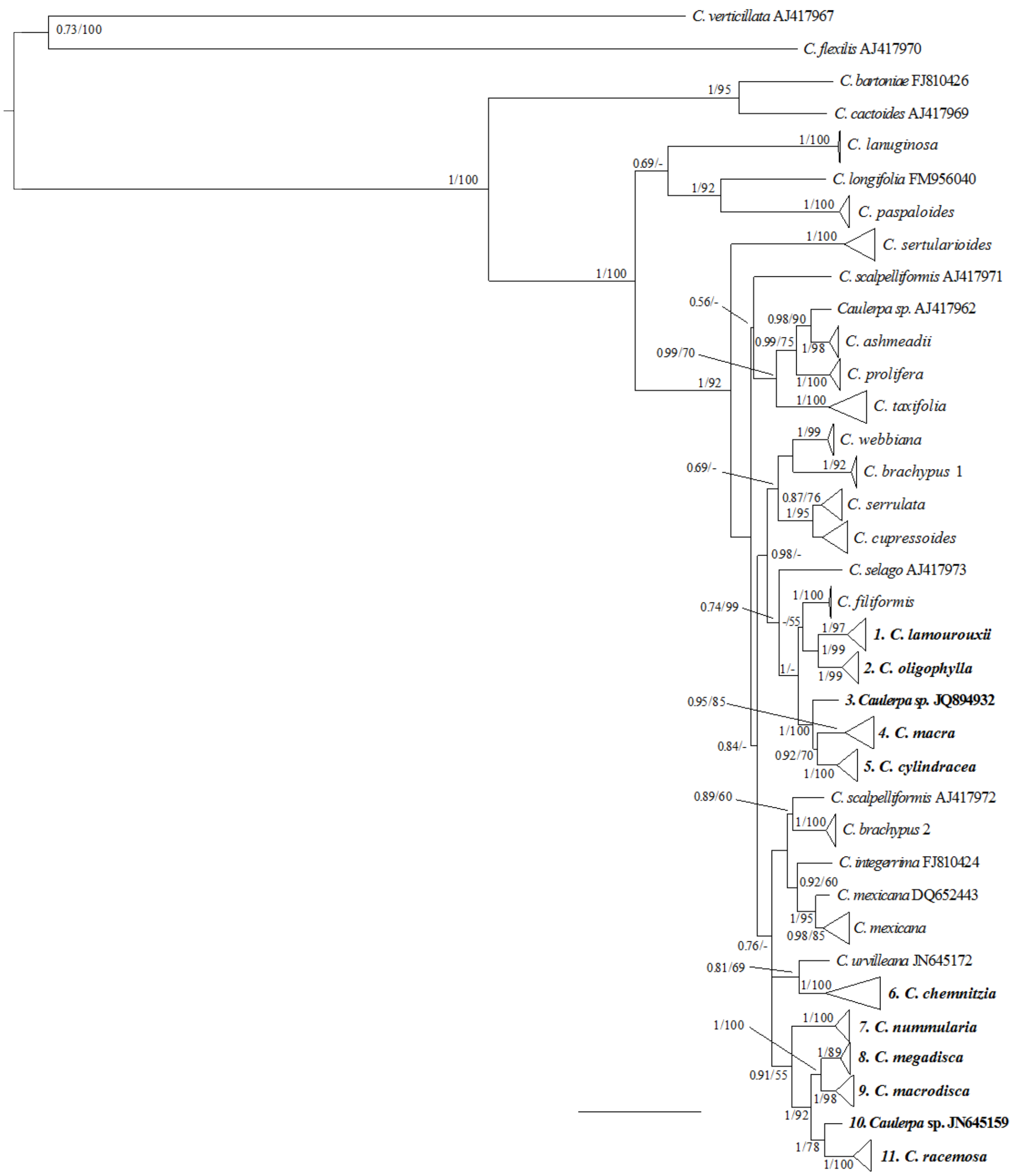
C. verticillata AJ417967
C. flexilis AJ417970
0.73/100
1/100
1/95
C. bartoniae FJ810426
C. cactoides AJ417969
1/100
0.69/-
1/100
C. lanuginosa
1/92
C. longifolia FM956040
1/100
C. paspaloides
1/100
C. sertularioides
1/92
C. scalpelliformis AJ417971
0.56/-
0.98/90
Caulerpa sp. AJ417962
0.99/75
1/98
C. ashmeadii
0.99/70
1/100
C. prolifera
1/100
C. taxifolia
1/99
C. webbiana
0.69/-
1/92
C. brachypus 1
0.87/76
C. serrulata
1/95
C. cupressoides
0.98/-
C. selago AJ417973
1/100
C. filiformis
0.74/99
-/55
1/97
1. C. lamourouxii
1/99
1/-
1/99
2. C. oligophylla
0.95/85
3. Caulerpa sp. JQ894932
1/100
4. C. macra
0.84/-
0.92/70
5. C. cylindracea
1/100
0.89/60
C. scalpelliformis AJ417972
1/100
C. brachypus 2
C. integerrima FJ810424
0.92/60
C. mexicana DQ652443
1/95
0.98/85
C. mexicana
0.76/-
0.81/69
C. urvilleana JN645172
1/100
6. C. chemnitzia
1/100
1/100
7. C. nummularia
1/89
8. C. megadisca
0.91/55
1/98
9. C. macrodisca
1/92
10. Caulerpa sp. JN645159
1/78
1/100
11. C. racemosa

Fig. S3. (A) Type specimen of *Caulerpa racemosa* var. *gracilis* (Zanardini) Weber-van Bosse from Tor, Sinai Peninsula, Egypt (originally described as *C. clavifera* var. *gracilis* Zanardini), currently regarded as a heterotypic synonym of *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse. Specimen TCD0011042. (B) Type specimen of *Caulerpa clavifera* var. *nudiuscula* Zanardini (given as *nudicaulis*), from Red Sea, Egypt. This taxon is currently regarded as a heterotypic synonym of *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse. Specimen TCD0011044. Both scale bars = 40 mm.

Appendix: 3


TRIN. COLL. DUBLIN.
No.
TCD0011042
Type! of Caulerpa clavifera (Turner) ~~Bory~~ Lamouroux var. gracilis Zanardini
Determinavit: W.F. Prud'homme van Reine
= Caulerpa racemosa var. Lamourouxii f. requienii (Mont.) W.v.B
Date: 17 / 09 / 2007
A
e var. rubra
Caulerpa clavifera var. gracilis Zan
Trinity College Herbarium
Dublin Ireland
TCD0011044
Type! of Caulerpa clavifera var. nudiuscula Zanardini = C. racemosa var Lamourouxii f. requienii (Mont.) Weber v. Bosse
Determinavit: W.F. Prud'homme van Reine
Date: 17 / 09 / 2007
B
var. rubra
Caulerpa clavifera var. nudiuscula Zan.

Fig. S4. (A) Type specimen of *C. racemosa* var. *laetevirens* (Montagne) Weber-van Bosse from Toud Island, Torres Strait, Australia (originally described as *C. laetevirens* Montagne but now regarded as *C. chemnitzia* (Esper) J. V. Lamouroux). (B) Specimen of *Caulerpa racemosa* var. *occidentalis* (J.Agardh) Børgesen (originally described as *C. chemnitzia* var. *occidentalis* J.Agardh but now regarded as *C. chemnitzia* (Esper) J. V. Lamouroux) from a folder in Herbarium Agardh (LD) labeled: *“syntypes of ß occidentalis”*. Specimen collected from Vera Cruz (16789). Scale bars: A = 50 mm; B = 40 mm.

Appendix: 3


A
MA 10323
BOTANISKA MUSEET
91/65-3881
LUND
01/023 0395
B
16789

Fig. S5. Syntype specimen of *C. imbricata* G.Murray from Sri Lanka, now regarded as a heterotypic synonym of *C. chemnitzia* (Esper) J. V. Lamouroux. Specimen: BM000569448.

Appendix: 3

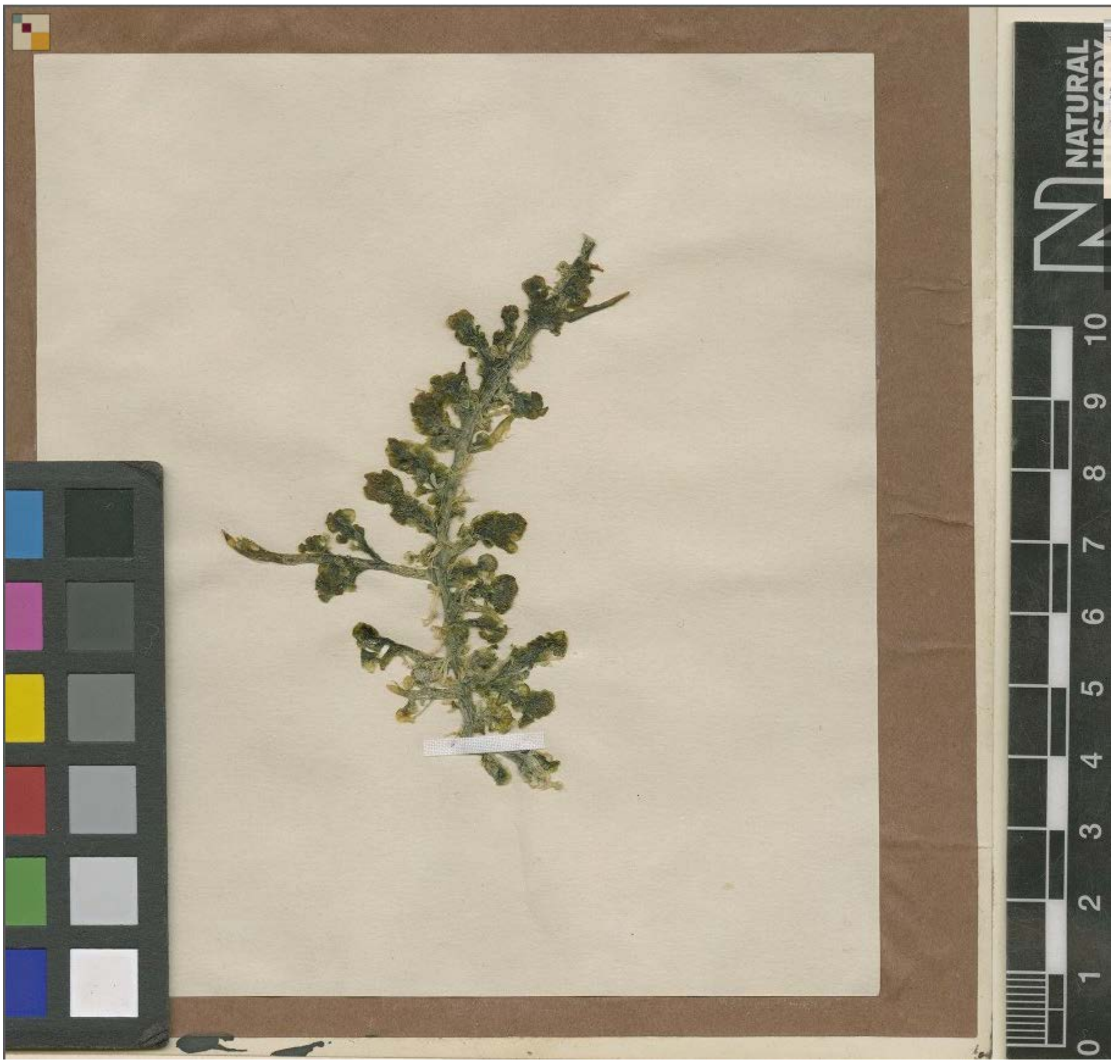

Fig. S6. (A) The supposed syntype specimen of *Caulerpa racemosa* var. *turbinata* (J.Agardh) Eubank from the Red Sea, Egypt (originally described as *C. clavifera* var. *turbinata* J.Agardh but now regarded as *C. chemnitzia* (Esper) J. V. Lamouroux). As J. Agardh did not annotate which specimens he used when described new taxa, we cannot be sure that this is the true type specimen. Specimen 16753a from LD. (B) Syntype specimen of *C. peltata* var. *exigua* Weber-van Bosse (BM000841593) collected from the Cooks Islands. (C) One of the syntype specimens of *C. peltata* var. *stellata* (Harvey ex J.Agardh) Weber-van Bosse (BM001044725) collected from Tonga. Scale bar: A = 20 mm; C = 30 mm.

Appendix: 3

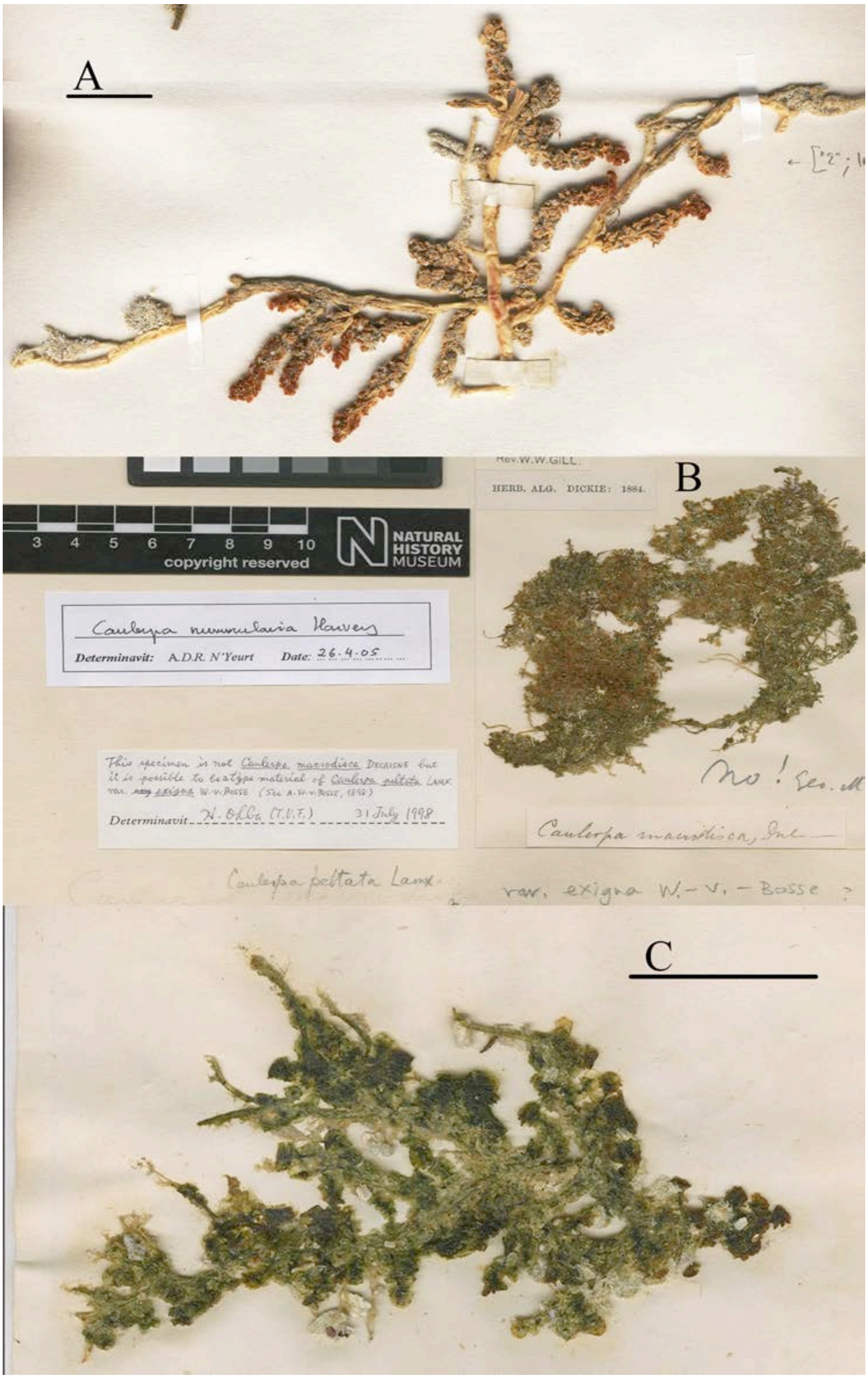
A
B
C
Rev.W.W.GILL.
HERB. ALG. DICKIE: 1884.
3 4 5 6 7 8 9 10
copyright reserved
NATURAL HISTORY MUSEUM
Caulerpa nummularia Harvey
Determinavit: A.D.R. N'Yeurt Date: 26.4.05
This specimen is not Caulerpa macrodisca Decaisne but it is possible to be a type material of Caulerpa peltata Lamx. var. exigua W.v.Bosse (See A.W.v.Bosse, 1898)
Determinavit H. Ohba (T.U.F.) 31 July 1998
no! Geo. M
Caulerpa macrodisca, Dne
Caulerpa peltata Lamx.
var. exigua W.-v.-Bosse ?

Fig. S7. Type specimen of *C. racemosa* var. *uvifera* (Turner) Weber-van Bosse (as *Fucus uvifer* Turner *nom. illeg.*) from the Red Sea, currently regarded as a heterotypic synonym of *C. racemosa* (Forrskål) J. Agardh. Specimen: BM000569472. Scale bar = 50 mm.

Appendix: 3


HERB. HORT. BOT. REG. KEW.
THE NATURAL HISTORY MUSEUM, LONDON
DEPARTMENT OF BOTANY
000569472 (BM)
HERBARIUM HOOKERIANUM 1867
Caulerpa racemosa (Forsskål) J. Agardh
holotype of Fucus uvifer Turner
det. L.N. de Senerpont Domis
Nationaal Herbarium Nederland,
Universiteit Leiden branch
12-01 .2004
Apparently the specimen figured in Turner 1819, pl. 230 and thus the type of Fucus uvifer Turner, now generally referred to Caulerpa racemosa var. uvifera (Turner)
DET
C. racemosa var. uvifera

Fig. S8. Type specimen of *C. racemosa* var. *clavifera* Weber-van Bosse (as *Fucus clavifer* Turner) from the Red Sea, currently regarded as a heterotypic synonym of *C. racemosa* (Forrskål) J. Agardh. Specimen: BM000569148.

Appendix: 3

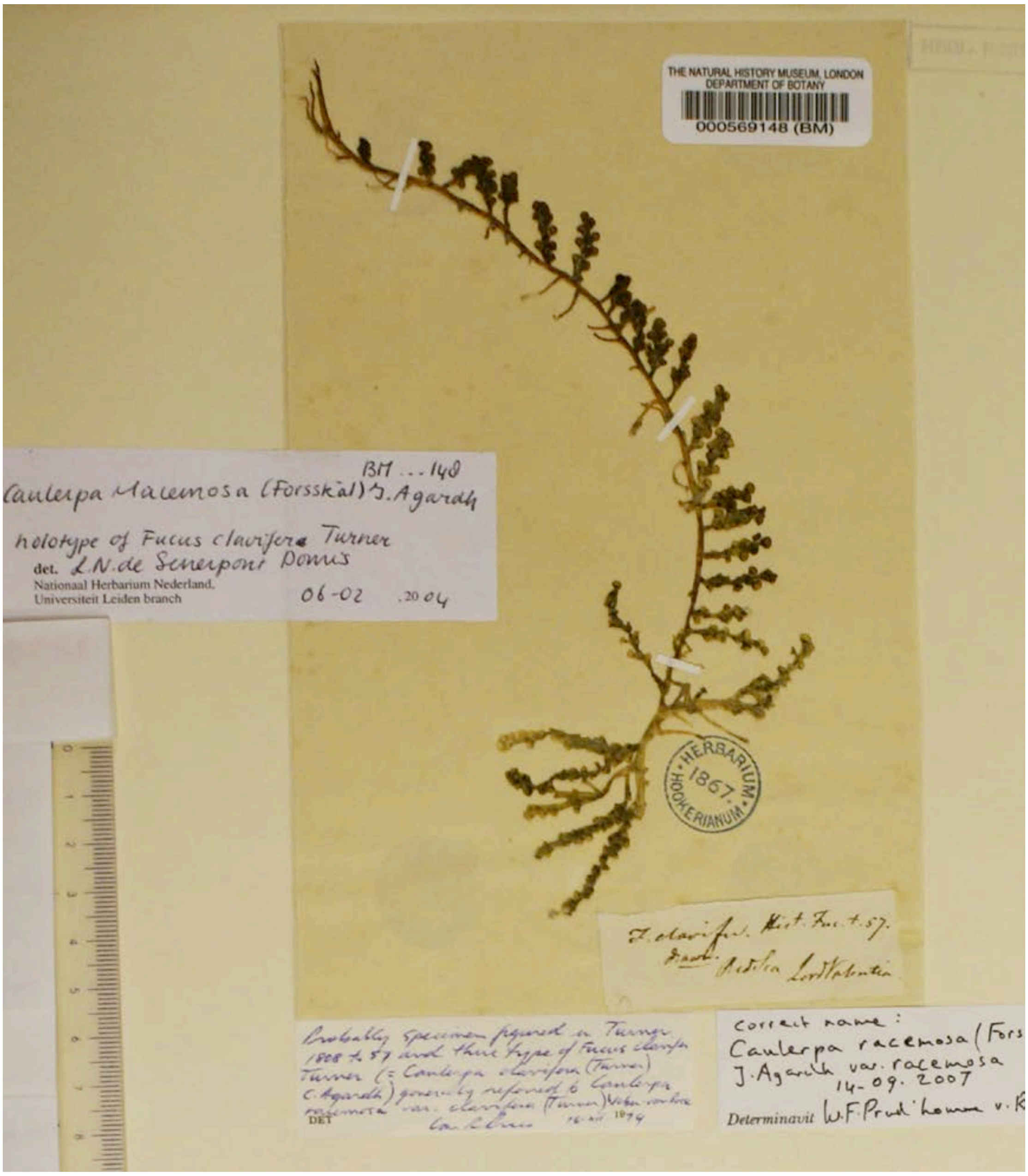
THE NATURAL HISTORY MUSEUM, LONDON
DEPARTMENT OF BOTANY
000569148 (BM)
BM ...148
Caulerpa racemosa (Forsskål) J. Agardh
holotype of Fucus clavifera Turner
det. L.N. de Senerpont Domis
Nationaal Herbarium Nederland,
Universiteit Leiden branch
06-02 .2004
HERBARIUM 1867 HOOKERIANUM
F. clavifer. Hist. Fuc. t. 57.
Probably specimen figured in Turner 1808 t. 57 and thus type of Fucus clavifera Turner (= Caulerpa clavifera (Turner) C. Agardh) generally referred to Caulerpa racemosa var. clavifera (Turner) Weber-van Bosse
DET
16-xii-1974
correct name:
Caulerpa racemosa (Fors
J. Agardh var. racemosa
14-09. 2007
Determinavit W.F. Prud'homme v. R

Table S1: GenBank accessions, herbarium accessions and collection details of newly sequenced *Caulerpa* specimens.

Taxon	Voucher	Collection site	Collection date	*tuf*A	*rbc*L	Morphological identification
C. chemnitzia (Esper) J.V. Lamouroux (Lineage 6)						
	PERTH08292671	Montgomery Reef, Australia	Oct. 2010	JN817667	-	*C. racemosa* var. *laetevirens*
	PERTH08292701	Cassini I., Australia	Oct. 2010	JN817669	KF256123	*C. racemosa* var. *turbinata*
	PERTH08292698	Montgomery Reef, Australia	Oct. 2009	JN817668	-	*C. racemosa* var. *laetevirens*
	AD-A92551	Lizard I., Australia	Sept. 2010	KF256105	KF256118	*C. racemosa* var. *laetevirens*
	GWS023897	Lord Howe I., Australia	Nov. 2010	JN851142	-	*C. racemosa* var. *laetevirens*
	GWS023816	Lord Howe I., Australia	Nov. 2010	JN851141	-	*C. peltata*

AD-A92587	Lizard I., Australia	Sept. 2010	JN817672	KF256119	*C. racemosa* var. *laetevirens*
AD-A89144	Houtman Abrolhos Is., Australia	Oct. 2009	KF256104	KF256122	*C. racemosa* var. *laetevirens*
AD-A91615	Ningaloo, Australia	May 2010	JN817670	KF256121	*C. peltata*
AD-A90831	Heron I., Australia	Nov. 2009	JN817671	-	*C. peltata*
CWS008555	St George’s, Bermuda	Aug. 2010	KF256099	-	*C. racemosa* var. *occidentalis*
CWS008366	Blue cut channel, Bermuda	Aug. 2010	KF256100	KF256120	*C. peltata*
DML70471	Exumas, Bahamas	July 2009	KF256106	-	*C. racemosa* var. *occidentalis*
L0712298	Raja Ampat, West Papua, Indonesia	Dec. 2007	FM906061	-	*C. peltata*
HEC16045	Dickwella, Sri Lanka	Jan. 2007	KF256102	-	*C. peltata*

	HEC15952	Matara, Sri Lanka	Jan. 2006	KF256101	-	*C. racemosa* var. *turbinata*
	SGAD0509018	Thousand Islands, Jakarta, Indonesia	Sept. 2006	KF256108	-	*C. racemosa* var. *laetevirens*
	DML68817	Islas Ladrones, Panama	Sept. 2007	KF256103	-	*C. peltata*
	SGAD0509305	Thousand Islands, Jakarta, Indonesia	Sept. 2006	KF256107	-	*C. racemosa* var. *laetevirens*
C. cylindracea Sonder (Lineage 5)						
	AD-A88236	Ningaloo Reef, Australia	June 2008	JN817673	KF256125	*C. racemosa* var. *cylindracea*
	PERTH(RD)24.10.9.1.2	Montgomery Reef, Kimberley, Australia	Oct. 2009	JN817678	KF256124	*C. racemosa* var. *cylindracea*
	AD-A88114	Lizard I., Australia	April 2008	JN817676	-	*C. racemosa* f. *complanata*

AD-A90239	Ningaloo Reef. Australia	June 2009	JN817674	-	*C. racemosa* var. *cylindracea*
C. lamourouxii (Turner) C. Agardh (Lineage 1)					
PERTH08292612	Montgomery Reef, Australia	Oct. 2009	JN817682	-	*C. racemosa* var. *lamourouxii*
PERTH08292620	Long Reef, Australia	Oct. 2010	JN817684	KF256113	*C. racemosa* var. *lamourouxii* f. *requienii*
PERTH08292639	Montgomery Reef, Australia	Oct. 2009	JN817680	KF256115	*C. racemosa* var. *lamourouxii*
PERTH08292647	Long Reef, Kimberley, Australia	Oct. 2010	JN817681	KF256114	*C. racemosa* var. *lamourouxii*
AD-A90154	Ningaloo Reef, Australia	June 2009	JN817683	-	*C. racemosa* var. *lamourouxii* f. *requienii*

HV03462	Marsa Alam, Red Sea, Egypt	Dec. 2011	KF256083	-	*C. racemosa* var. *lamourouxii*
HV03464	Marsa Alam, Red Sea, Egypt	Dec. 2011	KF256082	-	*C. racemosa* var. *lamourouxii*
HV03481	Marsa Alam, Red Sea, Egypt	Dec. 2011	KF256080	-	*C. racemosa* var. *lamourouxii*
HV03477	Marsa Alam, Red Sea, Egypt	Dec. 2011	KF256081	-	*C. racemosa* var. *lamourouxii*
PERTH08428220	Cassini I., Kimberley, Australia	Oct. 2010	KF256084	-	*C. racemosa* var. *lamourouxii* f. *requienii*
C. macra (Weber-van Bosse) Draisma & Prud'homme *stat. nov.* (Lineage 4)					
L03-453	Berau delta, North-Eastern Kalimantan, Indonesia	Oct. 2003	KF256089	-	*C. racemosa* var. *macra*

LNR13a0109	Northern Reefs, Palau	Mar. 2009	KF256088	-	*C. racemosa* var. *macra*
L 0925909	Chuuk Island, Micronesia	Aug. 2008	FR848345	-	*C. racemosa* var. *macra*
SGAD1012338	Silawa I., East Sabah, Borneo	Dec. 2010	-	FR848358	*C. racemosa* var. *macra*
C. macrodisca Decaisne (Lineage 9)					
SGAD0509510	Thousand Islands, Jakarta, Indonesia	Sept. 2006	KF256094	-	*C. racemosa* var. *macrodisca*
SGAD0509390	Thousand Islands, Jakarta, Indonesia -	Sept 2006	KF256093	-	*C. racemosa* var. *macrodisca*
SGAD0509415	Thousand Islands, Jakarta, Indonesia	Sept 2006	KF256092	-	*C. racemosa* var. *macrodisca*

SGAD0712405	Raja Ampat, West Papua, Indonesia	Dec. 2007	KF256095	-	*C. racemosa* var. *macrodisca*
PERTH08292663	Long Reef, Kimberley, Australia	Oct. 2010	KF256090		*C. racemosa* var. *macrodisca*
AD-A88056	Lizard I., Australia	April 2008	JN817666	KF256127	*C. racemosa* var. *macrodisca*
C. megadisca Belton & Gurgel *sp. nov.* (Lineage 8)					
AD-A92609	Lizard I., Australia	Sept. 2010	JN817656	KF256126	*C. racemosa* var. *macrodisca*
DML40342	Yaukuvelailai Island, Fiji	Mar. 1996	KF256091	-	*C. racemosa* var. *macrodisca*
AD-A90107 (holotype)	Lizard I., Australia	Feb. 2009	JN817657	-	*C. racemosa* var. *macrodisca*
C. nummularia Harvey ex J. Agardh (Lineage 7)					
GWS023932	Lord Howe I., Australia	Nov. 2010	JN851139	KF256117	*C. peltata*

	GWS023933	Lord Howe I., Australia	Nov. 2010	JN851138	-	*C. peltata*
	AD-A91369	Heron I., Australia	Nov. 2009	JN817685	KF256116	*C. peltata*
	DML40015	Dravuni, Fiji	Feb. 1996	KF256098	-	*C. peltata*
	GWS023180	Lord Howe I., Australia	Nov. 2010	JN851140	-	*C. peltata*
C. oligophylla Montagne (Lineage 2)						
	SGAD0712229	Raja Ampat, W. Papua, Indonesia	Dec. 2007	KF256087	-	*C. racemosa* var. *lamourouxii*
	AD-A95457	Heron I., Australia	Sept. 2012	KF256085	KF256112	*C. racemosa* var. *lamourouxii*
	SGAD0509292	Thousand Islands, Jakarta, Indonesia	Sept. 2006	KF256086	-	*C. racemosa* var. *lamourouxii*
C. racemosa (Forsskål) J. Agardh (Lineage 11)						
	SGAD0509057	Thousand Islands, Jakarta, Indonesia	Sept 2006	FM956050	-	*C. racemosa*

DML60836	Pelican Cays, Belize	May 2001	KF256096	-	*C. racemosa*
L0789171	Raja Ampat, West Papua, Indonesia	Dec. 2007	KF256097	-	*C. racemosa*
SGAD0509636	Thousand Islands, Jakarta, Indonesia	Sept. 2006	FM956019	-	*C. racemosa* var. *macrophysa*
PERTH08292752	Montgomery Reef, Kimberley, WA	Oct. 2009	JN817663	-	*C. racemosa*
PERTH08292728	Montgomery Reef, Kimberley, Australia	Oct. 2009	JN817665	-	*C. racemosa*
AD-A92441	Lizard I., Australia	Sept. 2010	JN817661	KF256109	*C. racemosa*
PERTH08292736	Long Reef, Kimberley, Australia	Oct. 2010	JN817659	KF256111	*C. racemosa* var. *macrophysa*
AD-A88524	Heron I., Australia	Sept. 2008	JN817662	-	*C. racemosa*

AD-A91592	Ningaloo, Australia	May 2010	JN817660	KF256110	*C. racemosa* var. *macrophysa*
AD-A90071	Lizard I., Australia	Feb. 2009	JN817664	-	*C. racemosa* var. *macrophysa*

Appendix: 3

Table S2. GenBank sequences used in the *tuf*A and *rbc*L alignments. Updated names are only provided for taxa previously assigned to the *C. racemosa–C. peltata* complex.

GenBank Accession	Marker	Original published or GenBank name	Updated name	Publication details
AB038483	*rbc*L	*C. brachypus*	-	Hanyuda et al. (2000)
AB038484	*rbc*L	*C. okamurae*	-	Hanyuda et al. (2000)
AB038485	*rbc*L	*C. racemosa* f. *macrophysa*	*C. racemosa*	Hanyuda et al. (2000)
AB038486	*rbc*L	*C. racemosa*	*C. macrodisca*	Hanyuda et al. (2000)
AB054008	*rbc*L	*C. cupressoides*	-	Hanyuda et al. Unpublished data
AB054009	*rbc*L	*C. lentillifera*	-	Hanyuda et al. Unpublished data
AB054010	*rbc*L	*C. nummularia*	*C. nummularia*	Hanyuda et al. Unpublished data
AB054011	*rbc*L	*C. racemosa* var. *lamourouxii*	*C. oligophylla*	Hanyuda et al. Unpublished data
AB054012	*rbc*L	*C. racemosa* f. *occidentalis*	*C. chemnitzia*	Hanyuda et al. Unpublished data
AB054013	*rbc*L	*C. scalpelliformis*	-	Hanyuda et al. Unpublished data
AB054014	*rbc*L	*C. sertularioides*	-	Hanyuda et al. Unpublished data
AB054015	*rbc*L	*C. subserrata*	-	Hanyuda et al. Unpublished data

AB054016	*rbc*L	*C. taxifolia*	-	Hanyuda et al. Unpublished data
AB054017	*rbc*L	*C. webbiana*	-	Hanyuda et al. Unpublished data
AB700356	*rbc*L	*C. subserrata*	-	Maeda et al. (2012)
AB700357	*rbc*L	*C. serrulata*	-	Maeda et al. (2012)
AB700358	*rbc*L	*C. racemosa* var. *clavifera*	*C. oligophylla*	Maeda et al. (2012)
AJ417928	*tuf*A	*C. cupressoides*	-	Famà et al. (2002)
AJ417929	*tuf*A	*C. cupressoides*	-	Famà et al. (2002)
AJ417930	*tuf*A	*C. cupressoides*	-	Famà et al. (2002)
AJ417931	*tuf*A	*C. serrulata*	-	Famà et al. (2002)
AJ417932	*tuf*A	*C. serrulata*	-	Famà et al. (2002)
AJ417933	*tuf*A	*C. serrulata*	-	Famà et al. (2002)
AJ417934	*tuf*A	*C. brachypus* 1.	-	Famà et al. (2002)
AJ417935	*tuf*A	*C. subserrata* (*C. brachypus* 1)	-	Famà et al. (2002)
AJ417936	*tuf*A	*C. taxifolia*	-	Famà et al. (2002)
AJ417937	*tuf*A	*C. taxifolia*	-	Famà et al. (2002)

AJ417938	*tuf*A	*C. taxifolia*	-	Famà et al. (2002)
AJ417939	*tuf*A	*C. taxifolia*	-	Famà et al. (2002)
AJ417940	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Famà et al. (2002)
AJ417941	*tuf*A	*C. ashmeadii*	-	Famà et al. (2002)
AJ417942	*tuf*A	*C. prolifera*	-	Famà et al. (2002)
AJ417943	*tuf*A	*C. prolifera*	-	Famà et al. (2002)
AJ417944	*tuf*A	*C. sertularioides*	-	Famà et al. (2002)
AJ417945	*tuf*A	*C. sertularioides*	-	Famà et al. (2002)
AJ417946	*tuf*A	*C. sertularioides*	-	Famà et al. (2002)
AJ417947	*tuf*A	*C. racemosa* var. *macrophysa*	*C. racemosa*	Famà et al. (2002)
AJ417948	*tuf*A	*C. racemosa* var. *peltata*	*C. chemnitzia*	Famà et al. (2002)
AJ417949	*tuf*A	*C. racemosa* var. *peltata*	*C. chemnitzia*	Famà et al. (2002)
AJ417950	*tuf*A	*C. racemosa*	*C. chemnitzia*	Famà et al. (2002)
AJ417951	*tuf*A	*C. mexicana*	-	Famà et al. (2002)
AJ417952	*tuf*A	*C. mexicana*	-	Famà et al. (2002)

AJ417953	*tuf*A	*C. mexicana*	-	Famà et al. (2002)
AJ417954	*tuf*A	*C. racemosa* var. *lamourouxii*	*C. oligophylla*	Famà et al. (2002)
AJ417955	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Famà et al. (2002)
AJ417956	*tuf*A	*C. racemosa* var. *macrophysa*	*C. racemosa*	Famà et al. (2002)
AJ417957	*tuf*A	*C. racemosa* var. *turbinata*	*C. lamourouxii*	Famà et al. (2002)
AJ417958	*tuf*A	*C. webbiana*	-	Famà et al. (2002)
AJ417959	*tuf*A	*C. lanuginosa*	-	Famà et al. (2002)
AJ417960	*tuf*A	*C. geminata*	-	Famà et al. (2002)
AJ417961	*tuf*A	*C. microphysa*	-	Famà et al. (2002)
AJ417962	*tuf*A	*Caulerpa* sp.	-	Famà et al. (2002)
AJ417963	*tuf*A	*Caulerpella ambigua*	-	Famà et al. (2002)
AJ417964	*tuf*A	*C. filiformis*	-	Famà et al. (2002)
AJ417965	*tuf*A	*C. paspaloides*	-	Famà et al. (2002)
AJ417966	*tuf*A	*C. webbiana*	-	Famà et al. (2002)
AJ417967	*tuf*A	*C. verticillata*	-	Famà et al. (2002)

AJ417968	*tuf*A	*C. geminata*	-	Famà et al. (2002)
AJ417969	*tuf*A	*C. cactoides*	-	Famà et al. (2002)
AJ417970	*tuf*A	*C. flexilis*	-	Famà et al. (2002)
AJ417971	*tuf*A	*C. scalpelliformis* 1	-	Famà et al. (2002)
AJ417972	*tuf*A	*C. scalpelliformis* 2	-	Famà et al. (2002)
AJ417973	*tuf*A	*C. selago*	-	Famà et al. (2002)
AJ512411	*tuf*A	*C. serrulata* var. *serrulata*	-	de Senerpont Domis et al. (2003)
AJ512412	*tuf*A	*C. serrulata* var. *serrulata*	-	de Senerpont Domis et al. (2003)
AJ512413	*tuf*A	*C. racemosa* ecad. *rac-cor-lam*	*C. oligophylla*	de Senerpont Domis et al. (2003)
AJ512415	*tuf*A	*C. racemosa* ecad. *laet-turb-pelt*	*C. chemnitzia*	de Senerpont Domis et al. (2003)
AJ512417	*tuf*A	*C. racemosa* var. *mucronata*	*C. racemosa*	de Senerpont Domis et al. (2003)
AJ512418	*tuf*A	*C. racemosa* var. *mucronata*	*C. racemosa*	de Senerpont Domis et al. (2003)
AJ512419	*tuf*A	*C. sertularioides* f. *longipes*	-	de Senerpont Domis et al. (2003)
AJ512420	*tuf*A	*C. sertularioides* f. *brevipes*	-	de Senerpont Domis et al. (2003)
AJ512421	*tuf*A	*C. sertularioides* f. *longipes*	-	de Senerpont Domis et al. (2003)

AJ512422	*tuf*A	*C. sertularioides* f. *brevipes*	-	de Senerpont Domis et al. (2003)
AJ512426	*tuf*A	*C. flexilis*	-	de Senerpont Domis et al. (2003)
AJ512466	*tuf*A	*C. cupressoides* var. *lycopodium*	-	de Senerpont Domis et al. (2003)
AJ512468	*rbc*L	*C. serrulata* var. *serrulata*	-	de Senerpont Domis et al. (2003)
AJ512469	*rbc*L	*C. serrulata* var. *serrulata*	-	de Senerpont Domis et al. (2003)
AJ512470	*rbc*L	*C. cupressoides* var. *lycopodium*	-	de Senerpont Domis et al. (2003)
AJ512471	*rbc*L	*C. cupressoides* var. *lycopodium*	-	de Senerpont Domis et al. (2003)
AJ512472	*rbc*L	*C. racemosa* ecad. *laet-turb-pelt*	*C. chemnitzia*	de Senerpont Domis et al. (2003)
AJ512473	*rbc*L	*C. racemosa* ecad. *laet-turb-pelt*	*C. chemnitzia*	de Senerpont Domis et al. (2003)
AJ512474	*rbc*L	*C. racemosa var. mucronata*	*C. racemosa*	de Senerpont Domis et al. (2003)
AJ512475	*rbc*L	*C. racemosa var. mucronata*	*C. racemosa*	de Senerpont Domis et al. (2003)
AJ512476	*rbc*L	*C. sertularioides* f. *longipes*	-	de Senerpont Domis et al. (2003)
AJ512477	*rbc*L	*C. sertularioides* f. *brevipes*	-	de Senerpont Domis et al. (2003)
AJ512478	*rbc*L	*C. sertularioides* f. *longipes*	-	de Senerpont Domis et al. (2003)
AJ512479	*rbc*L	*C. sertularioides* f. *brevipes*	-	de Senerpont Domis et al. (2003)

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AJ512480	*rbc*L	*C. racemosa* ecad. *rac-cor-lam*	*C. oligophylla*	de Senerpont Domis et al. (2003)
AJ512481	*rbc*L	*C. racemosa* ecad. *rac-cor-lam*	*C. oligophylla*	de Senerpont Domis et al. (2003)
AJ512482	*rbc*L	*C. racemosa* ecad. *rac-cor-lam*	*C. oligophylla*	de Senerpont Domis et al. (2003)
AJ512483	*rbc*L	*C. racemosa* ecad. *rac-cor-lam*	*C. oligophylla*	de Senerpont Domis et al. (2003)
AJ512484	*rbc*L	*C. racemosa* ecad. *rac-cor-lam*	*C. oligophylla*	de Senerpont Domis et al. (2003)
AJ512485	*rbc*L	*C. flexilis*	-	de Senerpont Domis et al. (2003)
AY942170	*rbc*L	*C. sertularioides*	-	Lam and Zechman (2006)
AY942171	*rbc*L	*C. paspaloides*	-	Lam and Zechman (2006)
AY942173	*rbc*L	*C. prolifera*	-	Lam and Zechman (2006)
DQ652329	*tuf*A	*C. cupressoides*	-	Stam et al. (2006)
DQ652330	*tuf*A	*C. cupressoides*	-	Stam et al. (2006)
DQ652345	*tuf*A	*C. cupressoides*	-	Stam et al. (2006)
DQ652346	*tuf*A	*C. serrulata*	-	Stam et al. (2006)
DQ652350	*tuf*A	*C. serrulata*	-	Stam et al. (2006)
DQ652353	*tuf*A	*C. brachypus* 2	-	Stam et al. (2006)

DQ652354	*tuf*A	*C. brachypus* 2	-	Stam et al. (2006)
DQ652355	*tuf*A	*C. brachypus* 2	-	Stam et al. (2006)
DQ652356	*tuf*A	*C. taxifolia*	-	Stam et al. (2006)
DQ652361	*tuf*A	*C. taxifolia*	-	Stam et al. (2006)
DQ652362	*tuf*A	*C. ashmeadii*	-	Stam et al. (2006)
DQ652365	*tuf*A	*C. ashmeadii*	-	Stam et al. (2006)
DQ652368	*tuf*A	*C. ashmeadii*	-	Stam et al. (2006)
DQ652372	*tuf*A	*C. prolifera*	-	Stam et al. (2006)
DQ652385	*tuf*A	*C. prolifera*	-	Stam et al. (2006)
DQ652390	*tuf*A	*C. prolifera*	-	Stam et al. (2006)
DQ652393	*tuf*A	*C. sertularioides*	-	Stam et al. (2006)
DQ652395	*tuf*A	*C. sertularioides*	-	Stam et al. (2006)
DQ652400	*tuf*A	*C. sertularioides*	-	Stam et al. (2006)
DQ652405	*tuf*A	*C. sertularioides*	-	Stam et al. (2006)
DQ652408	*tuf*A	*C. sertularioides*	-	Stam et al. (2006)

DQ652409	*tuf*A	*C. sertularioides*	-	Stam et al. (2006)
DQ652414	*tuf*A	*C. sertularioides*	-	Stam et al. (2006)
DQ652420	*tuf*A	*C. racemosa*	*C. racemosa*	Stam et al. (2006)
DQ652422	*tuf*A	*C. racemosa*	*C. racemosa*	Stam et al. (2006)
DQ652424	*tuf*A	*C. racemosa*	*C. racemosa*	Stam et al. (2006)
DQ652425	*tuf*A	*C. racemosa*	*C. racemosa*	Stam et al. (2006)
DQ652426	*tuf*A	*C. racemosa*	*C. nummularia*	Stam et al. (2006)
DQ652428	*tuf*A	*C. racemosa*	*C. macrodisca*	Stam et al. (2006)
DQ652465	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652466	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652467	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652468	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652470	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652474	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652480	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)

DQ652485	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652486	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652487	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652488	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652490	*tuf*A	*C. racemosa*	*C. chemnitzia*	Stam et al. (2006)
DQ652494	*tuf*A	*C. racemosa*	*C. oligophylla*	Stam et al. (2006)
DQ652495	*tuf*A	*C. racemosa*	*C. cylindracea*	Stam et al. (2006)
DQ652496	*tuf*A	*C. lanuginosa*	-	Stam et al. (2006)
DQ652499	*tuf*A	*C. paspaloides*	-	Stam et al. (2006)
DQ652520	*tuf*A	*C. microphysa*	-	Stam et al. (2006)
DQ652521	*tuf*A	*C. microphysa*	-	Stam et al. (2006)
DQ652525	*tuf*A	*C. verticllata*	-	Stam et al. (2006)
DQ652530	*tuf*A	*C. verticllata*	-	Stam et al. (2006)
DQ652532	*tuf*A	*C. flexilis*	-	Stam et al. (2006)
FJ432654	*tuf*A	*C. sertularioides*	-	Verbruggen et al. (2009)

FJ810424	*tuf*A	*C. integerrima*	-	Wynne et al. (2009)
FJ810425	*tuf*A	*C. integerrima*	-	Wynne et al. (2009)
FJ810426	*tuf*A	*C. bartoniae*	-	Wynne et al. (2009)
FM956026	*tuf*A	*C. filiformis*	-	Sauvage et al. (2013)
FM956043	*tuf*A	*C. racemosa-peltata*	*C. oligophylla*	Sauvage et al. (2013)
FM956044	*tuf*A	*C. racemosa-peltata*	*C. oligophylla*	Sauvage et al. (2013)
FM956045	*tuf*A	*C. racemosa-peltata*	*C. racemosa*	Sauvage et al. (2013)
FM956046	*tuf*A	*C. racemosa* var. *cylindracea*	*C. macra*	Sauvage et al. (2013)
FM956047	*tuf*A	*C. racemosa* var. *cylindracea*	*C. macra*	Sauvage et al. (2013)
FM956048	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
FM956051	*tuf*A	*C. racemosa-peltata*	*C. racemosa*	Sauvage et al. (2013)
FM956052	*tuf*A	*C. racemosa-peltata*	*C. racemosa*	Sauvage et al. (2013)
FM956053	*tuf*A	*C. racemosa-peltata*	*C. macrodisca*	Sauvage et al. (2013)
FM956054	*tuf*A	*C. racemosa-peltata*	*C. macrodisca*	Sauvage et al. (2013)
FM956055	*tuf*A	*C. racemosa-peltata*	*C. macrodisca*	Sauvage et al. (2013)

FM956056	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
FM956057	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
FM956058	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
FM956059	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
FM956060	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
FM956075	*tuf*A	*C. racemosa-peltata*	*C. macrodisca*	Sauvage et al. (2013)
GU571201	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Jongma et al. (2013)
GU592621	*tuf*A	Ulvophyceae	*C. nummularia*	Händeler et al. (2010)
GU592622	*tuf*A	Ulvophyceae	*C. nummularia*	Händeler et al. (2010)
GU592606	*tuf*A	Ulvophyceae	*C. nummularia*	Händeler et al. (2010)
GU592619	*tuf*A	Ulvophyceae	*C. nummularia*	Händeler et al. (2010)
JN645149	*tuf*A	*C. racemosa-peltata*	*C. megadisca*	Sauvage et al. (2013)
JN645150	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN645151	*tuf*A	*C. taxifolia*	-	Sauvage et al. (2013)
JN645152	*tuf*A	*C. racemosa* var. *cylindracea*	*C. macra*	Sauvage et al. (2013)

JN645153	*tuf*A	*C. taxifolia*	-	Sauvage et al. (2013)
JN645154	*tuf*A	*C. racemosa-peltata*	*C. megadisca*	Sauvage et al. (2013)
JN645155	*tuf*A	*C. racemosa* var. *cylindracea*	*C. macra*	Sauvage et al. (2013)
JN645156	*tuf*A	*C. racemosa* var. *cylindracea*	*C. macra*	Sauvage et al. (2013)
JN645157	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN645158	*tuf*A	*C. racemosa-peltata*	*C. oligophylla*	Sauvage et al. (2013)
JN645159	*tuf*A	*C. racemosa-peltata*	*Caulerpa* sp.	Sauvage et al. (2013)
JN645160	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
JN645161	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
JN645162	*tuf*A	*C. racemosa-peltata*	*C. chemnitzia*	Sauvage et al. (2013)
JN645163	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN645164	*tuf*A	*C. racemosa-peltata*	*C. macra*	Sauvage et al. (2013)
JN645165	*tuf*A	*C. racemosa* var. *cylindracea*	*C. macrodisca*	Sauvage et al. (2013)
JN645166	*tuf*A	*C. racemosa-peltata*	*C. oligophylla*	Sauvage et al. (2013)
JN645167	*tuf*A	*C. racemosa* var. *cylindracea*	*C. macra*	Sauvage et al. (2013)

JN645168	*tuf*A	*C. racemosa-peltata*	*C. oligophylla*	Sauvage et al. (2013)
JN645169	*tuf*A	*C. racemosa-peltata*	*C. racemosa*	Sauvage et al. (2013)
JN645170	*tuf*A	*C. racemosa-peltata*	*C. oligophylla*	Sauvage et al. (2013)
JN645171	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN645172	*tuf*A	*C. urvilleana*	-	Sauvage et al. (2013)
JN645173	*tuf*A	*C. racemosa-peltata*	*C. nummularia*	Sauvage et al. (2013)
JN645174	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN645175	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN817675	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN817677	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN817679	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JN851143	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JQ894931	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)
JQ894932	*tuf*A	*C. racemosa-peltata*	*Caulerpa* sp.	Sauvage et al. (2013)
JQ894933	*tuf*A	*C. racemosa* var. *cylindracea*	*C. cylindracea*	Sauvage et al. (2013)

JX185603	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Jongma et al. (2013)
JX185604	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Jongma et al. (2013)
JX185605	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Jongma et al. (2013)
JX185610	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Jongma et al. (2013)
JX185611	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Jongma et al. (2013)
JX185614	*tuf*A	*C. taxifolia* var. *distichophylla*	-	Jongma et al. (2013)
JX185615	*tuf*A	*C. racemosa var. cylindracea*	*C. cylindracea*	Jongma et al. (2013)
JX185616	*tuf*A	*C. racemosa var. cylindracea*	*C. cylindracea*	Jongma et al. (2013)

Appendix: 3

Table S3. Nomenclature details of taxa discussed in the present study.

Lineage	**New taxonomy**	**Previous taxonomy**	**Basionym**	**Original publication**	**Type locality**
1	*Caulerpa lamourouxii* (Turner) C. Agardh	*C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse, 1898: 368, pl. XXXII, figs 1-7, pl. XXXIII, fig. 15	*Fucus lamourouxii* Turner, 1811-1819: 79, pl. 229	Turner, D. 1811-1819. *Fuci* ….. Vol. 4. Printed by J. McCreery, London. p. 79, pl. 229	Red Sea
2	*Caulerpa oligophylla* Montagne	Syn. of *C. racemosa* var. *lamourouxii*		Montagne, J. P. F. C. 1842. *Prodromus Generum Specierumque Phycearum Novarum in itinere ad Polum Antarcticum* …. Paris. p. 14	Toud Island [Warrior Islet], Torres Strait, Australia

4	*Caulerpa macra* (Weber-van Bosse) Draisma & Prud'homme *stat. nov.*	*C. racemosa* var. *macra* Weber-van Bosse		Weber-van Bosse, A. 1913. *Liste de algues du Siboga. I. Myxophyceae, Chlorophyceae, Phaeophyceae avec le concours de M. Th. Reinbold*. Vol. 59a, p. 107, fig. 26	Fau Island, close to Gebé Island, Sea of Halmahera, Indonesia
5	*Caulerpa cylindracea* Sonder	*C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque, 2003: 336.		Sonder, G. 1845. Nova algarum genera et species, quas in itinere ad oras occidentales Novae Hollandiae, collegit L. Priess, Ph. Dr. *Bot. Zeitung (Berlin)* 3: 49–57	Western Australia
		C. racemosa f. *complanata* (J. Agardh) Weber-van Bosse, 1898:	*C. complanata* J.Agardh	Agardh, J. G. 1873. Till algernes systematik. Nya Bidrag. *Lunds Univ. Årsskr.* 9: 33	Port Denison (Bowen), Queensland, Australia

		364-365, pl. XXXIII: figs. 13, 14			
6	*Caulerpa chemnitzia* (Esper) J.V.Lamouroux	Syn. of *C. racemosa* var. *turbinata*	*Fucus chemnitzia* Esper	Esper, E. J. C. 1800. *Icones fucorum* …..*Vol. 1* Part 4., Raspe, Nürnberg, Tab. 88-111, pp. 167-217	Malabar Coast, India
		C. peltata J.V.Lamouroux, 1809a: 332-333, 1809b, 145 & pl.3, fig. 2a, 2b		Lamouroux, J.V. 1809a. Observations sur la physiologie des algues marines, …. *Nouv. Bull. Sci. Soc. Philom. Paris* 1:332–333, pl. 6	Antilles Islands
		C. racemosa var. *turbinata* (J. Agardh) Eubank,	*C. clavifera* (Turner) C. Agardh var.	Agardh, J. G. 1837. Novae species algarum, quas in itinere ad oras maris rubri	Near Tor, Sinai Peninsula, Egypt

1946: 420–421, fig. 2o	*turbinata* J. Agardh	collegit Eduardus Rüppell; …. *Mus. Senckenberg.* 2:173	
C. racemosa var. *laetevirens* (Montagne) Weber-van Bosse, 1898: 366	*C. laetevirens* Montagne	Montagne, J. P. F. C. 1842. *Prodromus Generum Specierumque Phycearum Novarum in itinere ad Polum Antarcticum* …. Paris. p. 13	Toud Island, Torres Strait, Australia
C. racemosa var. *occidentalis* (J.Agardh) Børgesen 1907: 379, figs 28, 29	*C. chemnitzia* var. *occidentalis* J.Agardh	Agardh, J. G. 1873. Till algernes systematik. Nya Bidrag. *Lunds Univ. Årsskr.* 9: 37	Upper Gulf of Mexico to Recife, Brazil
C. imbricata G.Murray 1887: 37–38		Murray, G. 1887. Catalogue of Ceylon algae in the herbarium of the British	Galle, Sri Lanka

				Museum. *Annals and Magazine of Natural History, Series 5* 20: 21-44.	
7	*Caulerpa nummularia* Harvey ex. J. Agardh	Syn. of. *C. peltata*		Agardh, J. G. 1873. Till algernes systematik. Nya Bidrag. *Lunds Univ. Årsskr*. 9: 38	Friendly Islands, Tonga; Nukahiva, Marquesas Islands
		C. peltata var. *exigua* Weber-van Bosse, 1898: 377, pl. XXXI, fig. 11		Weber-van Bosse, A. 1898. Monographie des Caulerpes. *Ann. Jardin Bot. Buitenzorg*. 15: 377, pl. XXXI, fig. 11	Samoa and Mangia
		C. peltata var. *stellata* (Harvey ex J.Agardh) Weber-van Bosse 1898: 377	*Caulerpa stellata* Harvey ex J.Agardh 1873: 38-39	Agardh, J. G. 1873. Till algernes systematik. Nya Bidrag. *Lunds Univ. Årsskr*. 9: 38-39	Tonga

8	*Caulerpa megadisca* Belton & Gurgel, *sp. nov.*	*C. racemosa* (Forsskål) J. Agardh var. *macrodisca* (Decaisne) Weber-van Bosse, 1898: 376, pl. XXXI, fig. 10	Present paper	Lizard Island, Queensland, Australia
9	*Caulerpa macrodisca* Decaisne	*C. racemosa* (Forsskål) J. Agardh var. *macrodisca* (Decaisne) Weber-van Bosse, 1898:	Decaisne, J. 1842. Essais sur une classification des algues et des polypiers calcifères de Lamouroux. *Ann. Sci. Nat.*, *Bot.*, sér. 2. 17: 336	Îles Anambas [Anambas Islands], Indonesia

		376, pl. XXXI, fig. 10			
11	*Caulerpa racemosa* (Forsskål) J.Agardh	Current	*Fucus racemosus* Forsskål	Forsskål, P. 1775. *Flora Aegyptiaco-Arabica sive descriptiones plantarum, …..* Post mortem auctoris edidit Carsten Niebuhr. p. 191	Suez, Egypt
		C. racemosa var. *mucronata* L.N.de Senerpont Domis		Senerpont Domis, L.N. de, Famà, P., Bartlett, A.J., Prud'homme van Reine, W.F., Espinosa, C.A. & Trono, Jr G.C. 2003. Defining taxon boundaries in members of the morphologically and genetically plastic genus *Caulerpa* (Caulerpales, Chlorophyta). *Journal of Phycology* 39: 1035, fig. 2.	Bolinao, Philippines

	Caulerpa racemosa var. *uvifera* (Turner) Weber-van Bosse	*Fucus uvifer* Turner *nom. illeg.*	Turner, D. 1811-1819. *Fuci* ….. Vol. 4. Printed by J. McCreery, London. p. 81, pl. 230	Red Sea, Egypt
	Caulerpa racemosa var. *clavifera* Weber-van Bosse	*Fucus clavifer* Turner	Turner 1807-1808: *Fuci* ….. Vol. 1. Printed by J. McCreery, London. p. 126-127, pl. 57	Red Sea, Egypt

Additional taxa discussed but not assigned to a species in the present study

	Current taxonomy	**Basionym**	**Original publication**	**Type locality**
Caulerpa racemosa var. *macrophysa* (Sonder ex	Current	*Chauvinia macrophysa* Sonder ex Kützing	Kützing, F.T. 1857. *Tabulae phycologicae*; oder, Abbildungen der Tange. pp. 6, pl. 15: fig. II	Central America, Eastern Pacific

Kützing) W.R.Taylor				
Caulerpa racemosa var. *gracilis* (Zanardini) Weber-van Bosse	Syn. of *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse	*C. clavifera* var. *gracilis* Zanardini	Zanardini, G. 1851. Algae novae vel minus cognitae in mari Rubro a Portiero collectae. *Flora* 34: 37	Red Sea, Egypt
Caulerpa clavifera var. *nudiuscula* Zanardini	Syn. of *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse	*C. clavifera* var. *nudicaulis* Zanardini	Zanardini, G. 1851. Algae novae vel minus cognitae in mari Rubro a Portiero collectae. *Flora* 34: 37	Red Sea, Egypt

Caulerpa racemosa var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse	Current	*Herpochaeta requienii* Montagne	Montagne, C. 1856. *Sylloge generum specierumque cryptogamarum* quas in variis operibus descriptas iconobusque illustratas, nunc ad diagnosim reductas, nonnullasque novas interjectas, ordine systematijco disposuit. Parisiis [Paris] & Londini [London]: sumptibus J.-B. Baillière...; H. Baillière....p. 454	Red Sea, Egypt
Caulerpa racemosa f. *remota* (Svedelius) Coppejans	Current	*C. clavifera* f. *remota* Svedelius	Svedelius, N. 1906. Reports on the marine algae of Ceylon. No. 1. Ecological and systematic studies of the Ceylon species of *Caulerpa*. *Reports of the Ceylon Marine Biological Laboratory* 2(4): 120, fig. 14	Sri Lanka

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Appendix: 4

Appendix 4: Supplementary Material for Chapter 5.

Appendix: 4

Appendix: 4

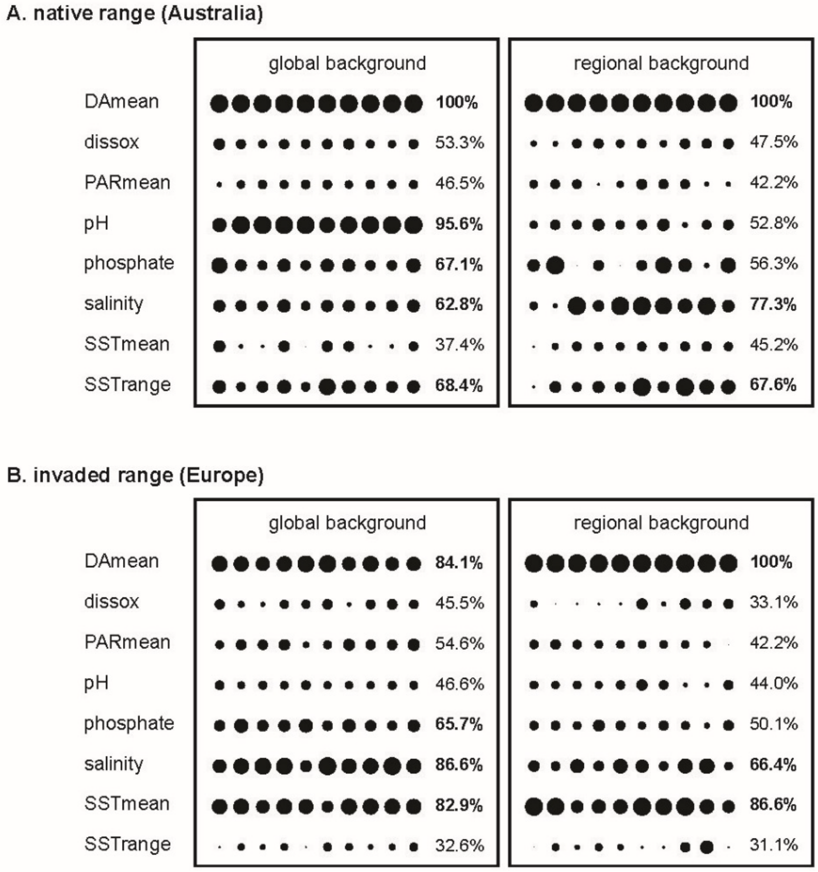

Fig. S1: Model surveying results indicating qualitatively similar results when analyses are carried out with global or regoinal backgrounds.