

Phylogeography of the cosmopolitan red alga *Caulacanthus ustulatus* (Caulacanthaceae, Gigartinales)

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SUMMARY

Molecular analyses of mitochondrial (cox2–3 spacer) and plastidal (Rubisco spacer) genomic DNA sequence data show that specimens of *Caulacanthus ustulatus* (Turner) Kützing form two distinct lineages: a Pacific lineage containing samples from China, Korea, Australia, the Philippines and the USA, plus a cryptic introduction to Roscoff, France; and an Atlantic Ocean lineage containing samples from Spain and Portugal. Although two well-supported ocean basin lineages are evident, the position of the tropical Pacific Ocean samples, within the Pacific Ocean lineage, remains unresolved owing to apparent incongruence between the two data sets. The partition homogeneity test indicates no incongruence in data sets, although some tree topology tests indicate significant differences in tree topology. Combined data sets produce trees with reduced resolution in some branches, indicating that even in uniparentally inherited organellar DNA, the appropriateness of combining data sets must be thoroughly tested. *Caulacanthus okamurae* Yamada has been proposed as distinct from *C. ustulatus* based on axis diameter. Literature descriptions of *Caulacanthus* species showing a wide range of axis diameters have been reported. Changes in culture conditions (increased light levels, water motion) stimulated thickening of vegetative shoots, reproduction, cuticle exfoliation and hair cell formation in isolates of *C. ustulatus*. This morphological plasticity, confusion in the literature and sequencing of a putative isolate of *C. okamurae* indicates that at present only one species of *Caulacanthus* should be recognized.

Key words: *Caulacanthus okamurae*, *Caulacanthus ustulatus*, cox2–3 spacer, phylogeography, Rubisco spacer.

INTRODUCTION

Caulacanthus is a rather inconspicuous small red alga with long cylindrical stolons and short erect branches up to 3 cm tall that often forms loose irregular mats mixed with other red algae similar in form (e.g. diminutive species of *Hypnea*, *Gelidium* and *Gloiopeltis*). It can

be easily overlooked in its usual habitat, the rocky intertidal along the tropical and warm temperate coasts. *Caulacanthus* also occurs in mangroves of tropical coasts in the Philippines and Australia (West and Calumpong 1990; King and Puttock 1994).

Caulacanthus ustulatus (Turner) Kützing, the type species of the genus was described by Turner (1809) from Cadiz, Spain. Subsequently *C. ustulatus* has been observed in various localities worldwide: Azores (Schmidt 1931); Canary Islands (Børgesen 1927); Portugal (Ardré 1970); West Africa (Lawson and John 1987); Namibia (Wynne 1986); west coast of South Africa (Stegenga *et al.* 1997); KwaZulu-Natal, South Africa (Critchley *et al.* 1998); Greece (Athanasiadis 1987); India (Mairh *et al.* 1998); Malaysia (Masuda *et al.* 1999); Japan (Yoshida 1998); Mauritius (Børgesen 1950); Philippines (West and Calumpong 1990); Australia (Ngan and Price 1979; Cribb 1983; West and Calumpong 1990; Price and Scott 1992; King and Puttock 1994); New Zealand (Adams 1994); Seychelles (Wynne 1995); Tanzania (Jaasund 1976); Baja California, Mexico (Dawson 1961); Washington, USA (Norris and Wynne 1968); California, USA (M. Hommersand and S. Murray, pers comm., 2001) and Hawaii, USA (Abbott 1999). *Caulacanthus ustulatus* may also have been introduced to the northern French coast (Roscoff) in recent times (Rio and Cabioch 1988; Rueness 1997).

The genus has not yet been observed in the western Atlantic or Central and South America (Wynne 1998). There are several species described from other regions: *Caulacanthus indicus* Weber-van Bosse (1921) was described from Sulawesi, Indonesia and later was recorded in Australia (Cribb 1983). However, this species was reduced to synonymy with *C. ustulatus* by West and Calumpong (1990). *Caulacanthus spinellus* (Harvey et Hooker) (Kützing 1849) was described from New Zealand, but this has also been reduced to synonymy with *C. ustulatus* (Searles 1968; Adams 1994). Kützing (1868) described *Caulacanthus rigidus*

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Table 1. *Caulacanthus* isolates used in this study

Species and collection date	Location	Culture no.	GenBank Accession no.	
			Rubisco spacer	cox2–3 spacer
<i>Caulacanthus ustulatus</i> , vi.1987	Magnetic Island, Queensland, Australia (1)	2831	AF453721	AF453732
<i>Caulacanthus ustulatus</i> , x.1995	Cooktown, Queensland, Australia (2)	3540	AF453720	AF453731
<i>Caulacanthus ustulatus</i> , v.1988	Misamis Oriental, Philippines	2920	AF453719	AF453730
<i>Caulacanthus ustulatus</i> , vii.1995	San Juan Island, WA, USA	3514	AF453722	AF453733
<i>Caulacanthus ustulatus</i> , ix.1993	Roscoff, France	4019	AF453723	AF453734
<i>Caulacanthus ustulatus</i> , vi.1994	Qingdao, China	4020	AF453724	AF453735
<i>Caulacanthus ustulatus</i> , iii.1996	Gibraltar, Spain	4021	AF453726	AF453737
<i>Caulacanthus ustulatus</i> , ii.1999	Albufeira, Portugal	4022	AF453727	AF453738
<i>Caulacanthus okamurae</i> , vi.2000	Pusan, Korea	Field	AF453725	AF453736
<i>Catenella caespitosa</i> , v.1999	Bergen, Norway	Field	AF453728	AF453741

Culture no., John A. West culture no.

from Senegal, but Searles (1968) examined this and specimens attributed to this species from Japan and concluded that they did not differ markedly from European specimens of *C. ustulatus*.

Yamada (1933) described *Caulacanthus okamurae* (a possible *nomen nudum*; Norris and Wynne 1968) from Japan separating it from *C. ustulatus* based on its narrower branches. It is also known from Korea (Lee and Kang 1986). Although West and Calumpong (1990) place it in synonymy with *C. ustulatus*, the former name continues to be occasionally applied to specimens from the northwest Pacific (Choi and Nam 2001; Lee *et al.* 2001).

The life history of *Caulacanthus* in the field is not well known because so few specimens are reproductive when collected. Female plants with carpogonial branches and postfertilization stages were observed by Bornet and Thuret (1876) and Schmitz (1883). Searles (1968), using *C. ustulatus* specimens from South Africa, provides the most complete description of spermatangial, tetrasporangial, carposporophyte development. Feldmann (1938) observed germination of tetraspores of what he thought was *C. ustulatus* from Algeria, although this was most likely *Feldmannophycus raysiae* (Feldmann et Feldmann) Augier et Boudouresque (Augier and Boudouresque 1971). Kamura (1963) also described tetraspore germination in *C. okamurae* from southern Japan. Searles (1968) examined tetrasporic and female specimens of *C. ustulatus* from New Zealand and concluded that the life history was of the *Polysiphonia* type. The complete sexual life history was investigated for *C. ustulatus* culture isolates from the Philippines and Australia by West and Calumpong (1990), and for isolates from Portugal and France by Rueness (1997). Isolates of *C. okamurae* from Korea (West and Calumpong 1990) and China (Rueness 1997) did not reproduce in culture, although recently, culture experiments have demonstrated the complete life history (*Polysiphonia* type) of an isolate of *C. okamurae* from Pusan, Korea (Choi and Nam 2001). Further isolates from Washington, USA, and

Queensland, Australia, also completed sexual life histories in culture (J. West, unpubl. obser.).

Recently, molecular analyses have been applied to clarify the taxonomic status of *Caulacanthus* species. Lee *et al.* (2001) employed DNA sequence from the plastid-encoded *rbcL* gene to assess three Korean samples and compared these with data of *C. ustulatus* samples from Namibia and France. They suggested that the specimens of *C. okamurae* from Korea and *C. ustulatus* from Brittany, France could be the same species.

Molecular studies, using the plastid encoded Rubisco spacer (intergenic spacer between ribulose-1-5-bisphosphate carboxylase/oxygenase large and small subunit genes), on samples from Japan, China, Gibraltar, Portugal and Roscoff, France, confirmed that the Roscoff sample was allied to the northwest Pacific Ocean isolates and not to the eastern Atlantic samples. The authors hypothesized the introduction of the Roscoff samples, possibly from Japan, on oyster spat (Rueness and Rueness 2000).

The present work was conducted: (1) to confirm the relationships previously determined using the plastid encoded gene with a molecular marker from the mitochondrial genome (cox2–3 spacer); (2) to investigate the relationships between further samples from the east Pacific and western tropical Pacific to isolates previously studied; (3) to test the relationships of a confirmed *C. okamurae* sample to samples of *C. ustulatus*; and (4) to investigate the plasticity of morphological characters in various culture conditions to determine their utility in species designation.

MATERIALS AND METHODS

Table 1 provides a list of the cultures and samples used. The methodology for collection, isolation and culture are as outlined in West and Zuccarello (1999).

All isolates were maintained in an air-conditioned room at temperatures ranging between 21 and 26°C with a daily photo regime of 12:12 LD using cool-white

fluorescent lighting. Light intensity was maintained at 5–10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for stock cultures.

To determine how irradiance and water motion influence morphology (shoot diameter, hair cell formation and cuticle exfoliation) and reproduction, the eight isolates of *Caulacanthus* were grown under two different culture conditions. One set was grown under the stock culture conditions, and the other set was placed in higher light (30–35 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and on a rotary shakers (75 r.p.m), both at 21–26°C with a photo regime of 12:12 LD.

DNA extractions and amplification, and sequencing of the Rubisco spacer are as outlined in Zuccarello *et al.* (1999b). Amplification and sequencing of the *cox2–3* spacer followed Zuccarello *et al.* (1999a). Automated sequencing was performed on an ABI Prism 377 DNA Sequencer (Perkin-Elmer) after cycle sequencing of the purified polymerase chain reaction product with the ABI PRISM Big Dye Terminator Cycle Sequencing Reaction Kit. Samples were cleaned up by the ethanol precipitation method following the manufacturer's recommendations.

Sequences were assembled using the computer software supplied with the sequencer. Sequences were aligned with Clustal X (Thompson *et al.* 1997) and refined by eye. Phylogenetic relationships were inferred with PAUP* version 4.0b (Swofford 2001). In all analyses, gaps were considered as missing data. The outgroup used was *Catenella caespitosa* (Withering) L. M. Irvine; other outgroups were used, including *Catenella nipae* Zanardini (isolate 3972, *cox2–3* spacer, GenBank Accession no. AF453739) and various *Hypnea* sp. (isolate 4085, *cox2–3* spacer, GenBank Accession no. AF453740; isolate 2281, Rubisco spacer, GenBank Accession no. AF453729), which lead to no significant changes in the monophyly or topology of the ingroup (data not shown). Maximum parsimony (MP) trees were constructed with PAUP*, using the heuristic search option, 500 random sequence addition and tree bisection–reconnection branch swapping. Distance trees were constructed using Kimura 2-parameter estimate of distance and neighbor-joining (NJ) reconstruction. Maximum likelihood (ML) was also used to construct the most likely tree from the data set (2–5 random additions). The ML parameters were estimated using the ML ratio test. The program MODELTEST version 3.0 (Posada and Crandall 1998) was used to find the model of sequence evolution that best fitted each data set by a hierarchical likelihood ratio test ($\alpha = 0.05$). When the best sequence evolution model was determined, ML tree searches were performed with PAUP* using the estimated parameters (gamma distribution, proportion of invariable sites, transition/transversion ratio).

Support for individual internal branches were determined by bootstrap analysis (Felsenstein 1985) as implemented in PAUP*, and a decay index (Donoghue

et al. 1992). For bootstrap analysis, 1000 bootstrap data sets were generated from resampled data (5 random sequence additions), for both the MP and NJ analysis. Decay indices, on a strict consensus of the most parsimonious trees, were calculated with AUTODECAY version 4.0.2 (Eriksson 1998). All sequences are deposited at GenBank. Only MP reconstruction topologies are shown.

Data sets (Rubisco spacer and *cox2–3* spacer) were combined after testing for character incongruence using the incongruence length difference test (Farris *et al.* 1994), also known as the partition homogeneity test, as implemented in PAUP*. This test compares the incongruence length differences, the difference in the number of steps in separate and combined analyses of the original partitions (different genomic regions), to a series of randomized partitions generated from the data (Farris *et al.* 1994). If the datasets have the same evolutionary history, the sums of the lengths of the gene trees from the original data and the resampled data should be similar; if not, then the sums of the tree lengths should be longer than the actual data. Significance was assessed by comparing the summed tree lengths from the observed data to those of 1000 resampled data sets. Maximum parsimony, NJ and ML reconstructions were performed on this combined data set.

Apparent differences in phylogenetic tree topologies with the different genetic regions were tested using the Kishino–Hasegawa test for statistical significance of log-likelihood differences (Kishino and Hasegawa 1989) between trees with alternate topologies. The test was performed using PHYLIP version 3.5c (Felsenstein 1993). Another test performed on the alternate tree topologies was the topology-dependent permutation tail probability test (T-PTP) (Faith 1991; Faith and Ballard 1994). In this test, observed differences in length (number of steps) between two trees (most parsimonious tree for that data set vs most parsimonious tree given the topological constraints, in this instance, the topology produced by the alternate data set) are evaluated relative to a difference in lengths inferred from randomized data sets. If the differences in length between the two tree topologies using the true data is outside 95% ($P < 0.05$) of the tree-length-difference distribution based on randomized data (1000 replicates, 10 random sequence additions), it can be concluded that the observed length differences between the two topologies is significant.

RESULTS

Phylogenetic analysis

The plastid-encoded Rubisco spacer produced an unambiguously aligned data set of 316 characters with 36 potentially parsimony informative characters. All

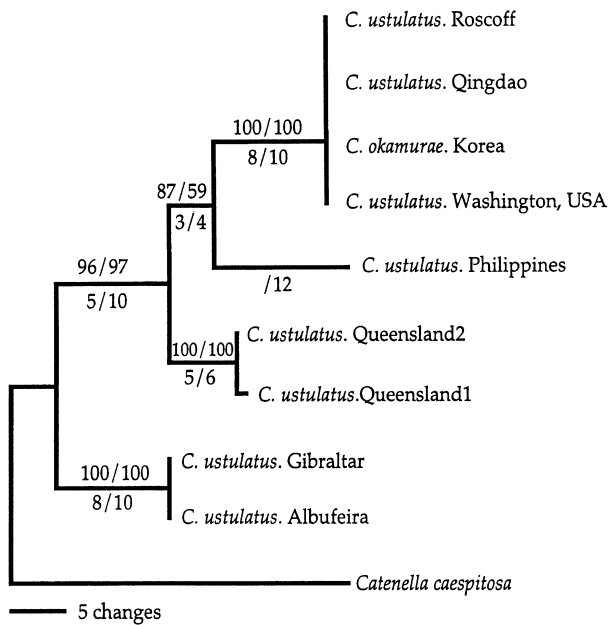


Fig. 1. Topology of one of the most parsimonious trees based on Rubisco spacer DNA sequence. Numbers: above branch left of forward slash, parsimony-based bootstrap values in percentages (1000 bootstrap replicates, 5 random sequence additions); above branch right of forward slash, distance-based bootstrap values (Kimura 2-parameter distances, 1000 bootstrap replicates); below branch left of forward slash, decay values; below branch right of forward slash, number of base pair changes along branch.

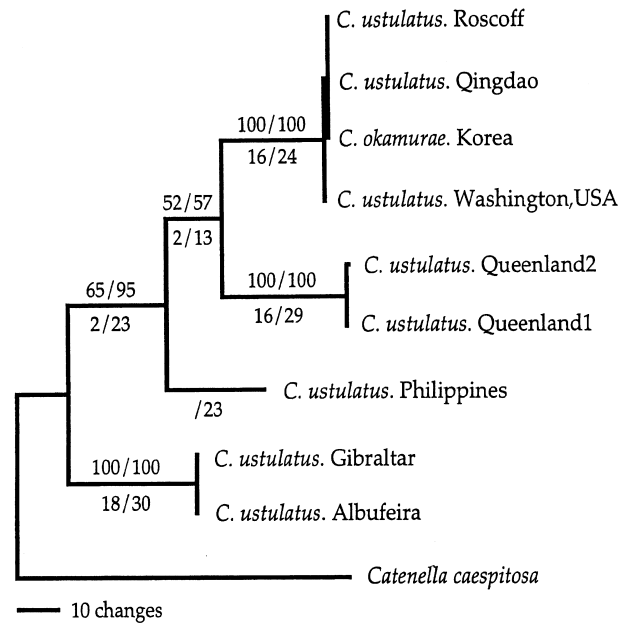


Fig. 2. Topology of single most parsimonious trees based on *cox2-3* spacer DNA sequence. Numbers: above branch left of forward slash, parsimony-based bootstrap values in percentages (1000 bootstrap replicates, 5 random sequence additions); above branch right of forward slash, distance-based bootstrap values (Kimura 2-parameter distances, 1000 bootstrap replicates); below branch left of forward slash, decay values; below branch right of forward slash, number of base pair changes along branch.

phylogenetic reconstruction methods (MP, NJ and ML) produced similar tree topologies. Maximum parsimony produced 13 equally most parsimonious trees of 87 steps (CI = 0.9195; RI = 0.9067). One of the most parsimonious trees is shown in Fig. 1. This data is congruent with results presented in Rueness and Rueness (2000). Two clades revealed in that study are also present in our analysis. The first clade contains samples from the eastern Atlantic (Gibraltar, Albufeira) and is well supported (100% bootstrap support for parsimony analysis and distance analysis, and a decay index of 8) and forms a sister group to all the other isolates. The second clade from the Rueness and Rueness (2000) study contained isolates from the northwest Pacific (China, Japan) and the isolate from Roscoff, France. Our data also strongly supports this group (100% bootstrap support for parsimony analysis and distance analysis, and a decay index of 8). This group contains the additional samples of *C. ustulatus* from the eastern Pacific (Washington, USA) and *C. okamurae* from Korea. Samples from the tropical western Pacific are in an intermediate position between these two previously analyzed clades and form a well-supported group of Pacific samples (96% bootstrap support for parsimony analysis and 97% for distance analysis, and a decay index of 5) separate from the Gibraltar and Albufeira samples. The Australian samples

form a well-supported lineage (100% bootstrap support for parsimony analysis and distance analysis, and a decay index of 5) as a sister group to the other Pacific samples. The Philippine sample forms a moderately supported (87% bootstrap support for parsimony analysis and 59% for distance analysis, and a decay index of 3) sister group to the northwest Pacific and Roscoff sample.

The mitochondrial encoded *cox2-3* spacer produced an aligned data set of 352 characters with 97 potentially parsimony informative characters. All phylogenetic reconstruction methods (MP, NJ and ML) produced similar tree topologies. Maximum parsimony produced one most parsimonious tree of 234 steps (CI = 0.8889; RI = 0.8639). The most parsimonious tree is shown in Fig. 2. The topology is very similar to the topology produced using the Rubisco spacer DNA sequence data. The Atlantic samples form a well-supported clade that is a sister group to all the other samples. The north Pacific and Roscoff samples form a well-supported clade. The Australian samples form a well-supported lineage as a sister group to the northern Pacific samples. The only incongruence between this tree topology and the Rubisco spacer data is the position of the Philippine isolate. Using the *cox2-3* data set, the Philippine isolate forms a sister group to all the Pacific and Roscoff isolates. This sister group

Table 2. Summary of tree topology tests

Trees	No. of trees†	Steps‡	Kishino–Hasegawa test§			T-PTP¶
			Diff. log-L	SD	Significantly worse	
Rubisco spacer data set						
Most parsimonious (Fig. 1)	13	87	–	–	Best	
Cox2–3 spacer topology (Fig. 2)	3	90	0.067635	3.3154	No	$P = 0.004$
Cox2–3 spacer data set						
Most parsimonious (Fig. 2)	1	234	–	–	Best	
Rubisco spacer topology (Fig. 1)	1	236	0.05093	5.5402	No	$P = 0.002$

†Number of equally most parsimonious trees with and without topology constraints; ‡number of steps for those trees. §Maximum-likelihood statistical test (Kishino and Hasegawa 1989) for constrained vs unconstrained most parsimonious tree; Diff. log-L, difference in log-likelihood between unconstrained and constrained tree; significantly worse, a tree is considered significantly worse at $P < 0.05$ if the difference in log-likelihood from the best tree is greater than 1.96 standard deviations. ¶Parsimony analysis with T-PTP testing based on 1000 replicate data sets (10 randomized sequence addition), null hypothesis: length differences between most parsimonious tree and constraint tree is due to change alone.

relationship is not well supported (52% bootstrap support for parsimony analysis and 57% for distance analysis, and a decay index of 2).

The apparent incongruent position of the Philippine isolate between the two molecular markers was tested using a partition homogeneity test. The P value was above the incongruence threshold of 0.05 ($P = 0.4880$), indicating that data sets could be combined. The ML approach (Kishino–Hasegawa test) was also used to test for significant differences between the tree topologies of the two data sets. The topology of one data set was used as a constraint tree using the other data set. This analysis shows that the apparent incongruence between data sets was not statistically significant (Table 2). An alternate test used for estimating the incongruence of the trees based on the different data sets was the T-PTP test. Using the Rubisco spacer data, the most parsimonious unconstrained tree was of 87 steps and the most parsimonious tree using the tree topology constraint of the cox2–3 spacer data set was 90 steps (Table 2). The T-PTP was significant at the $P = 0.004$ level and the null hypothesis that the length difference between the two trees is owing to chance alone can be rejected (difference between the most parsimonious tree with and without constraints was +3; length difference using randomized data was +2 to +24). Using the cox2–3 spacer data, the most parsimonious unconstrained tree was of 234 steps and the most parsimonious tree using the topology constraint of the Rubisco spacer data set was 236 steps (Table 2). The T-PTP was significant at the $P = 0.002$ level and the null hypothesis that the length difference between the two trees is owing to chance alone can also be rejected (difference between the most parsimonious tree with and without constraints was +2; length difference using randomized data was +5 to +36).

Although the results on whether the two data sets were incongruent are contradictory (the partition homogeneity test indicates that the data sets are congruent

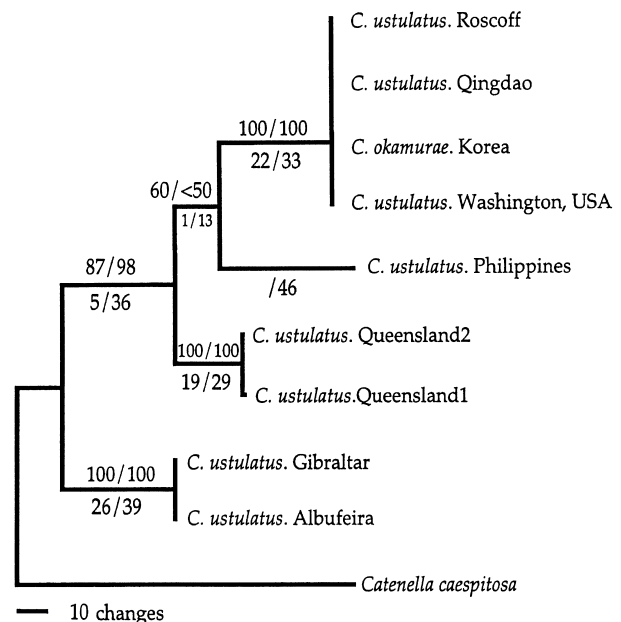


Fig. 3. Topology of one of the most parsimonious trees based on combined Rubisco spacer/cox2–3 spacer DNA sequence. Numbers: above branch left of forward slash, parsimony-based bootstrap values in percentages (1000 bootstrap replicates, 5 random sequence additions); above branch right of forward slash, distance-based bootstrap values (Kimura 2-parameter distances, 1000 bootstrap replicates); below branch left of forward slash, decay values; below branch right of forward slash, number of base pair changes along branch.

and the Kishino–Hasegawa test indicates that the two tree topologies are not significantly different; the T-PTP test indicates that the differences are significant between the two tree topologies) we combined the data. This combined data set contained 665 characters, 133 of which are informative, producing tree topologies similar to the individual data sets with increased bootstrap support at some nodes (Fig. 3). Maximum parsimony produced three equally most parsimonious

Table 3. Changes in shoot diameter, reproduction, cuticle exfoliation and hair cell formation in different light intensities and water motion

Culture no.		Irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	Water motion (r.p.m)	Vegetative shoot diam. (μm)†	Reproductive stichidia diam. (μm)‡	Reproduction	Cuticle exfoliation	Hairs
2831	Week 0	5–8	0	130–140 (135)	—	No	No	No
	Week 4	30–35	75	150–160 (155) 15%	170–200 (190) 22%	Tetrasporangia	Yes	No
2920	Week 0	5–8	0	110–150 (130)	—	No	No	No
	Week 4	30–35	75	110–170 (140) 110–170 (145) 10%	190–220 (205) 46% 190–200 (195) 34%	Tetrasporangia Spermatangia	No No	Yes Yes
3514	Week 0	5–8	0	110–120 (115)	—	No	No	No
	Week 4	30–35	75	110–200 (165) 43%	160–200 (175) 6%	Tetrasporangia	No	No
3540	Week 0	5–8	0	130–140 (135)	—	No	No	No
	Week 4	30–35	75	140–155 (150) 155–185 (170) 18%	140–170 (155) 3% 160–190 (180) 6%	Spermatangia Carpogonia	Yes No	No No
4019	Week 0	5–8	0	140–150 (145)	—	No	No	No
	Week 4	30–35	75	200–240 (225) 55%	250–280 (265) 18%	Tetrasporangia	No	Yes
4020	Week 0	5–8	0	110–120 (115)	—	No	No	No
	Week 4	30–35	75	160–200 (185) 52%	180–210 (195) 5%	Tetrasporangia	Yes	Yes
4021	Week 0	5–8	0	200–220 (210)	—	No	No	No
	Week 4	30–35	75	240–310 (270) 29%	240–310 (285) 6%	Carpogonia	Yes	No
4022	Week 0	5–8	0	180–200 (185)	—	No	No	No
	Week 4	30–35	75	180–250 (205) 11%	220–250 (235) 15%	Tetrasporangia	Yes	No

†Values represent range (mean) % increase. ‡Values represent range (mean) % difference with vegetative shoot.

trees of 323 steps (CI = 0.8916; RI = 0.8684). The topology of one of the most parsimonious trees is presented in Fig. 3; this topology is more similar to the topology derived from the Rubisco spacer data (Fig. 1), although the bootstrap and decay values for the relationships of the Queensland and Philippine samples to each other and to the north Pacific isolates is lowered.

Morphological plasticity

Initially, all plants grown in low irradiance (5–8 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) without water motion showed slow growth, with narrow axes, no reproduction, no hair formation and no outer cell wall (cuticle) exfoliation. However, when placed in higher light and shaker cultures for four weeks, differences were seen (Table 3). Vegetative shoot diameters showed increases that ranged from 10 to 55%. The reproductive stichidia in all isolates were somewhat greater in diameter than vegetative shoots.

Unicellular hairs developed from cortical cells in high light and water motion in one Queensland isolate (2831), the Roscoff isolate (4019) and the Qingdao isolate (4020), but not in other isolates. Cuticle exfoliation was evident in the tetrasporophytes from Queensland (1) (2831), China (4020) and Portugal

(4022), the male of Queensland (2) (3540) and the female from Spain (4021). Under the enhanced conditions, all isolates became reproductive. Isolates 2831, 2920, 3514, 4019, 4020 and 4022 formed tetrasporangia, tetraspores were released and germinated. In isolate 3540, only males and females were maintained, and by the fourth week these had abundant spermatangia and carpogonia. Isolate 4021 was a female that also developed numerous carpogonia.

DISCUSSION

Our molecular data reveals two distinct groups of *C. ustulatus* from two ocean basins. Atlantic samples form a distinct lineage that is a sister group to all the other samples. The 5' end of *rbcL* gene of a further South Atlantic sample from Namibia (GenBank accession no. AF099687) can be aligned to our Rubisco spacer data and suggests that this sample also groups with the other Atlantic samples (85 bp aligned, 2 synapomorphies, data not shown). The other grouping contains samples from the Pacific and the introduced sample in France. The North Pacific samples form a very distinct group with little genetic variation. This is expected in the newly introduced sample, but also suggests an introduction across the Pacific to the USA. It is known

that both genic regions studied have a relatively slow mutation rate (Zuccarello and West 2002), so lack of genetic variation between east and west Pacific samples does not necessarily indicate a recent introduction. Without further sampling, and more variable genic regions, conclusions about recent dispersal based solely on molecular data are premature.

Comparing phylogenetic relationships between the two genetic regions (Fig. 1 vs Fig. 2) shows incongruence in the position of the Philippine isolate with respect to the other Pacific samples. Although an incongruence length difference test revealed no significant incongruence between these data sets and this data could be combined (P values are non-significant above the incongruence threshold of 0.05, $P = 0.488$), almost half the trees are longer (more steps, extra homoplasy) than the original partition. The Kishino–Hasegawa test, using the ML approach, also found no significant difference between the alternate topologies, whereas the T-PTP test, using a parsimony approach, showed that the length difference between the two alternate topologies was significantly different.

Topological incongruence between trees produced from different genes may be the result of: (1) differences in phylogenetic histories (Doyle 1992); (2) differences in the power of phylogenetic resolution between the genes; or (3) differences in the rates or modes of evolution of the genetic regions (Bull *et al.* 1993). Both plastid and mitochondrial genomes are inherited maternally in red algae in the order Ceramiales (Zuccarello *et al.* 1999a, 1999b) and presumably in other red algae, although this has not been confirmed for the family Caulacanthaceae. Thus, we have no evidence to indicate different histories for these two genomes. Differences in phylogenetic resolution and in rates of evolution for the two genetic elements is likely, as it is known that these two genetic regions evolve at different rates (Zuccarello *et al.* 1999a; Zuccarello and West 2002), with the mitochondrial spacer evolving more quickly. The tree topology of the combined data set also shows signs that the combined data was misapplied. Certain branches that show relatively high bootstrap support with either one or the other data set have reduced bootstrap (and decay value) support when the data are combined (cf. Figs 1,2 and Fig. 3). These results show the danger of assuming congruence between data sets and combining data without careful analysis (Bull *et al.* 1993). Data sets should be treated as phylogenetically incongruent until statistical and phylogenetic tests show otherwise. A resolution to the phylogenetic inconsistencies and a more reliable estimate of the relationship of the tropical Pacific isolates to the north Pacific isolates, will have to await more sampling, especially from the tropical Pacific region, and/or the use of a more phylogenetically informative region.

Caulacanthus is generally characterized as small uniaxial plants, less than 3 cm tall, with terete, erect,

laterally branched shoots arising from stoloniferous branches that produce well-defined multicellular rhizoidal discs arising from cortical cells at uniform intervals. The apical cell divides by oblique septa, each axial cell cutting off two periaxial cells that divide repeatedly forming the compact cortex.

In general, the vegetative shoots of field-collected plants and cultured isolates noted by other authors (Table 4) were similar to those seen in isolates in the present study. However, there are sometimes errors in scale bars used in published figures, for example Searles (1968) shows a cystocarpic plant of *C. ustulatus* (fig. 19) with a shoot diameter of 40 μm , and Lee *et al.* (2001) show plants (figs 2,3) with an alleged shoot diameter of 30 μm , whereas in figs 4 and 5, the dimensions of 245–247 μm are more accurate, as is the diameter of 180–310 μm stated in their table 2. Clearly, this leads to serious errors in comparing specimens from different regions.

Tetraspore and carpospore germination described by Rueness (1997) and Choi and Nam (2001) is similar to that which we have observed in various strains from other regions (J. West, unpubl. obser.). When firmly attached to the substrate, tetraspores developed into multicellular discs. Thus, it appears quite uniform among the isolates in culture. *Catenella*, a related genus in the Caulacanthaceae, also show a similar germination pattern with the formation of rhizoids usually found on sporelings not firmly attached to the substrate (Zablackis *et al.* 1993).

When cultures were grown in higher light with water motion, outer cell wall layers (cuticle) developed and exfoliated in several of the isolates, and this may have a protective function. Cuticle formation and exfoliation is common in the red algae such as *Chondrus crispus* Stackhouse (Craigie *et al.* 1992), *Chondracanthus johnstonii* (Hollenberg) Guiry (West and Guiry 1982) and *Stictosiphonia intricata* (Bory) Silva (West *et al.* 1996). The cuticle may function in various ways to deter epi-/endophytes from attaching (Bouarab *et al.* 2001).

As noted by Searles (1968), the two main characters used to delimit species were the diameter of the main axes and dimorphism of vegetative and reproductive shoots. It is clear that the first character is not acceptable in defining a species, because variations in growth environments lead to changes in axis diameter. The second character, dimorphism, is not satisfactory because we have noted that with all reproductive strains in culture, the reproductive stichidia showed a significant increase in diameter from that of the vegetative branches. This increase is certainly not unusual in red algae during reproductive growth.

The basic difficulty in circumscribing species within the genus *Caulacanthus* is that too few reliable morphological features have been employed. This appears to be because so few reproductive specimens have

Table 4. Features used to separate taxa within the genus *Caulacanthus* from the literature

Species	Reference	Locality	Main axes diam (μm)	Tetrasporangial stichidia diam. (μm)	Spermatangial stichidia diam. (μm)	Cortical cells diam (μm)	Tetrasporangia (μm)
<i>C. indicus</i>	Weber-van Bosse (1921)	Sulawesi, Indonesia	40–60	—	—	—	—
<i>C. indicus</i>	Cribb (1983)	Queensland, Australia	80–200	—	—	6–15	—
<i>C. okamurae</i>	Lee <i>et al.</i> (2001)	Korea	180–310	—	—	—	—
<i>C. okamurae</i>	Yamada (1933)	Japan	—	—	—	—	—
<i>C. ustulatus</i>	Abbott (1999)	Hawaii, USA	< 100	—	—	—	—
<i>C. ustulatus</i>	Adams (1994)	New Zealand	—	—	—	—	—
<i>C. ustulatus</i>	Børgeesen (1950)	Mauritius	150–300	—	—	—	—
<i>C. ustulatus</i>	Lawson and John (1987)	West Africa	about 500	—	—	—	—
<i>C. ustulatus</i>	Masuda <i>et al.</i> (1999)	Malaysia	160–240	—	—	—	36–44 × 24–30
<i>C. ustulatus</i>	Norris and Wynne (1968)	Washington, USA	< 250	—	—	—	—
<i>C. ustulatus</i>	Rueness (1997)	Roscoff, France	—	422 (from fig. 4)	150 (from fig. 7)	—	—
<i>C. ustulatus</i>	Rueness and Rueness (2000)	Gibraltar	430 (from fig. 3)	—	—	11.55 (7.4–16.6)	104 × 54–68 (from fig. 5)
<i>C. ustulatus</i>	Rueness and Rueness (2000)	Qingdao, China	200–250	—	—	11.04 (6.4–14.7)	—
<i>C. ustulatus</i>	Rueness and Rueness (2000)	Roscoff, France	200–250	—	—	9.65 (4.6–13.8)	—
<i>C. ustulatus</i>	Searles (1968)	Qingdao, China	200–250	—	—	—	40 × 20 (from fig. 21D)
<i>C. ustulatus</i>	Searles (1968)	Gibraltar	350–400	—	—	—	70 × 25
<i>C. ustulatus</i>	Searles (1968)	South Africa	1000–1500.	—	—	—	—
<i>C. ustulatus</i>	Stegenga <i>et al.</i> (1997)	South Africa	40 (from fig. 19)?	—	—	—	—
<i>C. ustulatus</i>	Stegenga <i>et al.</i> (1997)	Philippines	< 1000	—	—	—	—
<i>C. ustulatus</i>	West and Calumpong (1990)	Australia	140–155	—	—	—	—
<i>C. ustulatus</i>	Wynne (1995)	Yoshida (1998)	125–133	—	—	—	—
<i>C. ustulatus</i>	Wynne (1995)	Yoshida (1998)	110–120	—	—	—	40–46 × 20–24
<i>C. ustulatus</i>	Yoshida (1998)	Yoshida (1998)	175–350	—	—	4–7	—

been available in field collections. The most useful information on reproduction has been obtained through laboratory culture investigations in which many of the isolates have completed a *Polysiphonia* type life history. However, these characters were generally not available in specimens from the field.

Although the current available data suggests there are probably two species worldwide (an Atlantic and Pacific entity), as yet we have no reliable morphological characters to support this separation. Choi and Nam (2001) suggest cystocarpic spines as a possible characteristic to differentiate *C. ustulatus* from *C. okamurae*, but they based their data on only one isolate from Korea. Besides its lack of utility in the field, owing to infrequent reproduction, more samples will have to be investigated, especially from the tropical Pacific, before this character can be utilized.

Our data, plus previous data, strongly suggest that the Atlantic isolates are different from the Pacific isolates (including the introduced French sample). Because of the plasticity of morphological characters and lack of consistency in character descriptions, we believe that at present morphological characters do not exist to distinguish the Atlantic lineage from the Pacific lineage. We suggest that *C. ustulatus* be used for all samples until further studies can be conducted.

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