Cryptogamie, Algol., 2002, 23 (3): 263-274

*rbc*L analysis supports the tribe Gymnothamnieae Kajimura (Ceramiaceae, Rhodophyta) [1]

Christine A. MAGGS [a] & Lynne MCIVOR [b]*

[a] School of Biology and Biochemistry, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK

[b] Present address: Martin Ryan Institute of Marine Science, National University of Ireland Galway, Galway, Ireland.

(Received 11 February 2002, accepted 12 March 2002)

Abstract — The tribe Gymnothamnieae Kajimura was proposed for the monotypic ceramiacean genus *Gymnothamnion* J. Agardh, previously placed either in the Ptiloteae Cramer or the Antithamnieae Hommersand. A bisporangial isolate of *G. elegans* (Schousboe ex C. Agardh) J. Agardh from Morocco formed only bisporangia in culture. Its smaller uninucleate cells and sporangia than those of tetrasporophytes suggested that bisporophytes may be haploid as in another member of the Ceramiaceae, *Aglaothamnion diaphanum* L'Hardy-Halos et Maggs. Phylogenetic analyses of the gene for the large subunit of rubisco (*rbc*L) from *Gymnothamnion* and representatives of eight other tribes of the Ceramiaceae confirmed that the removal of *Gymnothamnion* from the Ptiloteae and the Antithamnieae was warranted. Whereas all tribes with two or more representatives in our analyses were moderately or robustly resolved, *Gymnothamnion* did not form a strong clade with any other taxa. Analysis of *rbc*L sequences failed to resolve relationships between tribes, probably due to saturation at the high levels of sequence divergence found. In addition to reproductive features previously reported and interpreted as primitive, *G. elegans* shows a primitive vegetative feature and it is suggested that *Gymnothamnion* may be one of the most basal of the taxa presently included in the Ceramiaceae.

bisporangia / Ceramiaceae / life history / marine red algae / molecular systematics / morphology / *rbc*L

Résumé — L'analyse de la *rbc*L conforte la tribu des Gymnothamnieae Kajimura (Ceramiaceae, Rhodophyta). La tribu des Gymnothamnieae Kajimura a été proposée pour le genre monospécifique de céramiacée *Gymnothamnion* J. Agardh, placé auparavant soit dans les Ptiloteae Cramer soit dans les Antithamnieae Hommersand. Un isolat à bisporocystes de *G. elegans* (Schousboe ex C. Agardh) J. Agardh provenant du Maroc ne forme que des bisporocystes en culture. Les cellules uninucléées et les sporocystes plus petits que ceux des tétrasporophytes suggèrent que les bisporophytes pourraient être haploïdes comme chez un autre membre des Ceramiaceae, *Aglaothamnion diaphanum* L'Hardy-Halos et Maggs. Les analyses phylogénétiques du gène de la grande sous-unité de la rubisco (*rbc*L) chez *Gymnothamnion* et les représentants de huit autres tribus des Ceramiaceae

1. Dedicated to M.-T. L'Hardy-Halos, on the occasion of her retirement (see the acknowledgements section).

* Correspondence and reprints: *c.maggs@qub.ac.uk

confirment que le retrait de *Gymnothamnion* des Ptiloteae et des Antithamnieae était bien justifié. Alors que toutes les tribus avec deux ou davantage de représentants sont modérément ou solidement résolues dans nos analyses, *Gymnothamnion* ne forme de clade solide avec aucun autre taxon. L'analyse des séquences de la *rbc*L n'a pas permis de résoudre les liens de parenté entre les tribus, probablement en raison du niveau de divergence important qui existe entre les séquences et qui induit de la saturation. En plus des caractères de la reproduction précédemment décrits et interprétés comme primitifs, *G. elegans* montre un caractère végétatif primitif et il est suggéré que *Gymnothamnion* pourrait être un des taxons le plus à la base de ceux qui sont actuellement inclus dans les Ceramiaceae. (Traduit par la Rédaction)

algues rouges marines / bisporocystes / Ceramiaceae / cycle de vie / morphologie / *rbc*L / systématique moléculaire

INTRODUCTION

The type species of the monotypic ceramiacean genus *Gymnothamnion* J. Agardh (1892) was described from Algiers, Morocco, as *Callithamnion elegans* Schousboe ex C. Agardh (1828). Several species were placed in synonymy with *G. elegans* by Feldmann-Mazoyer (1941) and its reproductive development was the subject of later detailed studies by Feldmann & Feldmann (1966) and Itono (1977). Although originally assigned to the tribe Ptiloteae, *Gymnothamnion* was removed from it by Moe & Silva (1979), principally on the basis of the lack of cortication and the pattern of apical division. In *Gymnothamnion*, the plane of apical cell division was transverse in comparison to the oblique and alternating planes in the Ptiloteae. Moe & Silva (1979) noted that a primitive character in *Gymnothamnion*, the formation of the carpogonial branch directly on the basal cell of an ultimate lateral, was reminiscent of the Antithamnieae, but were unwilling to assign this enigmatic genus to a tribe. Subsequently, Athanasiadis (1987) considered that there were sufficient features linking *Gymnothamnion* to the Antithamnieae to place it in that tribe. Kajimura (1989), on the basis of his analysis of the vegetative and reproductive development of *G. elegans*, proposed the monotypic tribe Gymnothamnieae, but did not venture any suggestions regarding its relationships with the rest of the Ceramiaceae.

Gymnothamnion elegans has been reported widely from around the world in warm temperate latitudes (Kajimura, 1989). Although the species has never been cultured, a typical dioecious *Polysiphonia*-type life history has been inferred from collections of gametangial, cystocarpic and tetrasporangial material in several localities including Morocco (Feldmann & Feldmann, 1967), Japan (Kajimura, 1989) and South Africa (Stegenga *et al.*, 1997). Bisporangial thalli were described from South Africa as *G. elegans* var. *bispora* Stegenga (Stegenga, 1986), considered to be a South African endemic (Stegenga *et al.*, 1997).

Following the isolation into culture of bisporangial *G. elegans* collected in Morocco, the aims of the present study were (1) to elucidate the relationship between bisporophytes and sexual/tetrasporangial thalli and (2) to determine whether molecular phylogenetic analyses supported the separate tribal status for this species, as opposed to its previous assignments to the Ptiloteae and the Antithamnieae. The molecular marker selected for phylogenetic analysis was the sequence of the plastid-encoded gene for the large subunit of rubisco, *rbc*L,

because a large database of *rbc*L sequences is available for the Ceramiales (Freshwater *et al.*, 1994; de Jong *et al.*, 1998; McIvor, 2000; Lin *et al.*, 2001; McIvor *et al.*, 2002) and in the Ceramiales this marker provides good resolution at the tribal level (Lin *et al.*, 2001) as well as at the species to genus level (Nam *et al.*, 2000).

MATERIALS AND METHODS

Field collections and cultures

Gymnothamnion elegans was collected in south-western Morocco, 11 km north-west of Agadir, near Tamrhakht (30° 26'N; 9° 36'W), on 20 December 1993. This species was abundant in small, sandy lower-shore rockpools at the edge of a moderately wave-exposed sandy bay, in a species-rich turf of ceramialean red algae such as *Antithamnion* spp., *Antithamnionella* spp. and *Dasya ocellata* (Grateloup) Harvey. Some material was fixed in 4 % seawater-formalin and the rest was returned to the laboratory in vials of boiled seawater. A culture was isolated from vegetative tips placed in sterile seawater, and grown when unialgal in half-strength modified von Stosch medium (Guiry & Cunningham, 1984) at 15°C or 20°C, in a regime of 16:8 h light:dark, at a photon irradiance of *ca* 20 µmol photons m^{-2} s^{-1}. Some replicate cultures were grown in 15 ml petri dishes in which the medium was changed weekly; others were maintained for long-term storage in 60 ml screw-cap bottles at 15°C under a photon irradiance of *ca* 5 µmol photons m^{-2} s^{-1}. For comparative analyses, various species were obtained for DNA extraction as shown in Table 1, and used fresh, dried in silica gel, or cultured until sufficient biomass was obtained (Tab. 1).

Morphology and cytology

Permanent slide mounts of cultured and field-collected material were made using a modified aniline blue staining procedure (Maggs, 1998) that is very effective for the Ceramiales, and photographed using Technical Pan film developed in Kodak HC110 liquid developer. Slide-mounted specimens have been deposited in the Ulster Museum, Belfast (BEL) and BM (London). Cell nuclei were visualized by staining formalin-fixed material in a drop of Hoechst 33258 solution (10 µg ml^{-1}) and examined and photographed under ultraviolet excitation.

DNA extraction, PCR amplification and sequencing.

DNA was extracted from *ca* 50 mg fresh weight of cultured or field-collected algae, using the DNeasy Plant Mini Kit (Quiagen UK) according to the manufacturer's instructions Primers were designed using Genbank sequence X54532 for *Antithamnionella spirographidis* (Schiffner) Wollaston (Kostrzewa *et al.*, 1990, as *Antithamnion* sp.). Ant1 (5' CAC AAC CAG GTG TTG ATC CAA TTG AAG C 3') was used as the forward external primer and Ant4 (5' CTA CGA AAG TCA GCT GTA TCT GTA GAA GTA TA 3') as the reverse external primer. PCR amplifications were carried out using either a Perkin Elmer DNA

Table 1. Collections of Ceramiaceae, listed by tribe, and outgroup taxa (Dasyaceae) from which *rbcL* sequences were obtained.

Species	*Collection number*	*Location of collection and/or source of culture; collector or depositor (reference if published)*	*Date*	*GenBank accession number*
Antithamnieae Hommersand				
Antithamnion cruciatum (C. Agardh) Nägeli	216	Mulroy Bay, Co. Donegal, Ireland; cultured; CAM	16.2.93	AY136277
Antithamnion nipponicum Yamada et Inagaki	365	Shimoda, Japan; cultured; CAM	4.9.93	AY136278
Callithamnieae Schmitz				
Aglaothamnion sepositum (Gunnerus) Maggs & Hommersand	498	Port Salon, Co. Donegal, Ireland; CAM	31.1.99	AF439295
Aglaothamnion tenuissimum (Bonnemaison) Feldmann-Mazoyer[a]	1026	Cudillero Harbor, Asturias, Spain; cultured; CAM	6.3.99	AF439307
Callithamnion corymbosum (J. E. Smith) Lyngbye	1003	No location; UTEX culture 1950; R.A. Lewin	–	AF439302
Callithamnion tetragonum (Withering) S.F. Gray	1001	Port Salon, Co. Donegal, Ireland; CAM	31.1.99	AF439301
Ceramieae (Dumortier) Schmitz				
Ceramium brevizonatum H. Petersen var. *brevizonatum* H. Petersen et Børgesen in Børgesen	–	Yucatan, Mexico; G. Gurgel (Lin *et al.*, 2001)	18.5.98	AF259415
Ceramium 'diaphanum' (Lightfoot) Roth	–	Wrightsville Beach, North Carolina, USA (Freshwater *et al.*, 1994)	–	U04020
Dohrnielleae Feldmann-Mazoyer				
Antithamnionella spirographidis (Schiffner) Wollaston		Villefranche-sur-Mer, Mediterranean France; culture SAG B95.79, as *Antithamnion* sp.; D.G. Müller (Kostrzewa *et al.*, 1990)	1973	X54532
Antithamnionella ternifolia (J.D. Hooker & Harvey) Lyle	224	Finavarra, Co. Clare, Ireland; cultured; CAM	26.9.92	AY136279
Griffithsieae Schmitz				
Anotrichium barbatum (C. Agardh) Nägeli	487	Oyster Bank, Pwllheli, Cardigan, Wales; cultured; CAM	20.8.98	AY136275
Gymnothamnieae Kajimura				
Gymnothamnion elegans (Schousboe) J. Agardh	493	near Agadir, Morocco; cultured; CAM	20.12.93	AF439311
Ptiloteae Cramer				
Ptilota gunneri P. Silva, Maggs et L. Irvine	456	Fanad Head, Co. Donegal, Ireland; CAM	6.7.98	AY136276
Spermothamnieae Schmitz				
Spermothamnion repens (Dillwyn) Rosenvinge	–	MA, USA (Freshwater *et al.*, 1994)	–	U04024
Sphondylothamnion multifidum (Hudson) Nägeli	471	Sarn Badrig, Cardigan Bay, Caernavon, Wales; CAM	20.8.98	AF439312
Spyridieae Schmitz				
Spyridia hypnoides (Bory) Papenfuss	–	Wrightsville Beach, North Carolina, USA (Freshwater *et al.*, 1994)	–	U04025
Dasyaceae				
Dasya ocellata (Grateloup) Harvey	337	near Agadir, Morocco; cultured; CAM (de Jong *et al.*, 1998)	20.12.93	AF083375
Heterosiphonia plumosa (Ellis) Batters	380	Skomer, Pembrokeshire, Wales; CAM (de Jong *et al.*, 1998)	4.9.96	AF083379

[a]*synonym: Aglaothamnion byssoides* (Arnott ex Harvey) L'Hardy-Halos et Rueness (see Furnari *et al.*, 1998)

Thermal Cycler 480 (Perkin Elmer Biosystems) or a PTC-100TM Programmable Thermal Cycler (MJ Research). The cycle was 5 min denaturing at 94°C, 30 cycles of 1 min at 94°C and 3 min at 60°C, followed by a final extension phase at 60°C for 10 min. Reactions contained 200 ng each primer, 20 mM dATP, dCTP, dGTP, dTTP (Ultrapure dNTP set, Amersham Pharmacia Biotech), 2.5 mM $MgCl_2$, and 5 U Taq polymerase (Biogene Ltd). PCR products (~1242 bp) were reamplified if necessary as described by Nam *et al.* (2000).

The fragments for sequencing reactions were purified using the High Pure PCR Product Purification Kit (Boehringer Mannheim) according to the manufacturer's instructions. The PCR-amplified products were directly sequenced using dideoxy chain termination methodology as described in Nam *et al.* (2000), except for the primers, which were Ant1, Ant4, and two additional primers, Ant2 (5' CGT GAG CGT ATG GAT AAA TTT GGT CGT TC 3') and Ant3 (5' TTA CTT TAC GTA AAG CAG CCC AAT CTT GTT C 3') used in various combinations to ensure that the entire 1242 bp region was sequenced.

Phylogenetic analyses

In addition to our own sequences, some *rbc*L sequences were obtained from GenBank (Tab. 1). Taxa of the Ceramiaceae were selected to represent nine tribes, including both of those that *Gymnothamnion* has previously been associated with, i.e. Antithamnieae and Ptiloteae. Sequence was obtained for only one member of the Ptiloteae, the type species *Ptilota gunneri*, but wherever possible, rather distantly related species from each tribe (as determined in more extensive and taxon-replete unpublished analyses) were used as representatives of the tribes. In all analyses, two representatives (*Dasya ocellata* and *Heterosiphonia plumosa)* from another family in the Ceramiales, the Dasyaceae, were used as outgroups.

Sequence alignments were constructed using the Eyeball 2 sequence editor (Cabot & Beckenbach, 1989), and final adjustments were made by eye. All analyses were carried out using PAUP 4.01* beta test version (Swofford, 1998) Maximum parsimony analyses were carried out using a heuristic search, with 20 random sequence additions. Bootstrapped analyses were carried out with 1000 replications for maximum parsimony and for a neighbour-joining analysis, which used the HKY85 distance algorithm, specifying a transition-transversion ratio of 2:1 and unequal base frequencies (Hasegawa *et al.*, 1985). A maximum likelihood (ML) analysis was carried out using a heuristic search with the transition-transversion ratio set at 2:1, and unequal base frequencies, and assuming equal rates and no invariable sites. A bootstrapped analysis for ML was also carried out, although due to computational intensity, only 100 replications were performed. Pairwise sequence divergences were calculated using a Jukes Cantor distance algorithm.

RESULTS

Morphology and culture studies

Gymnothamnion elegans thalli collected near Agadir in Morocco formed a turf of erect axes 1.0-3.5 mm high arising from prostrate axes. Young erect axes bore short paired laterals, each of which formed secund adaxial branchlets, with

an overall pennate appearance, 0.3-0.5 mm wide. In older thalli some laterals resumed apical growth, and up to three orders of indeterminate branching were formed (Fig. 1). All thalli examined were bisporangial, with bisporangia (Fig. 1) formed terminally on paired branches, laterally, or terminally on short adaxial branchlets. Bisporangia were ovoid, 25-32 × 18-24 µm, and obliquely divided.

Cultures initiated from vegetative tips grew rapidly to form extensive prostrate axes that gave rise every few cells to paired erect axes and multicellular rhizoidal filaments. Prostrate axial cells were 12-15 µm in diameter, and unbranched cells were about 10 diameters long. Prostrate cells bearing branches were shorter, 3-4 diameters long, and the paired branches arose opposite each other from a position slightly anterior of the centre of the cell (Fig. 4). Specialized terminal holdfasts were not formed by the rhizoidal filaments, and instead, when prostrate axes became detached from the substratum, the rhizoidal filaments could develop into additional erect axes resulting in pairs of branched erect axes at intervals along the prostrate filament.

Erect axes (Fig. 3) freely formed bisporangia in various positions (Fig. 2), as in field-collected material. Bisporangia were 25-36 × 18-26 µm, obliqely divided, and released bispores *ca* 25 µm in diameter. Germinating bispores divided initially into two cells, one of which grew downwards as a rhizoidal cell, the other into the erect axis, and grew into bisporangial thalli similar to the parent cultures that became fertile within 3 months of germination. No other reproductive structures were observed. Clavate parasporangium-like structures 30-50 µm long were formed singly or in groups near the bases of erect axes, but did not develop further. Vegetative cells were all uninucleate (Fig. 5), as were the bispores (Fig. 6).

Molecular analyses

No insertions or deletions were present in the dataset, making the 1245 base pair alignment unambiguous. Within the Ceramiaceae, sequence divergences (Tab. 2) were lowest between congeneric species (e.g. 7.61 % for *Antithamnionella spirographidis*/*Antithamnionella ternifolia*), and highest between tribes (up to 17.23 % for *Spyridia filamentosa* – Spyridieae vs. *Callithamnion corymbosum* – Callithamnieae). Between genera of the same tribe, the sequence divergences ranged from 10.46 % (*Aglaothamnion sepositum*/*Callithamnion corymbosum* – Callithamnieae) to 11.37 % (*Spermothamnion repens*/*Sphondylothamnion multifidum* – Spermothamnieae). The sequence divergences between *Gymnothamnion elegans* and the remaining members of the Ceramiaceae ranged from 11.98 % (*Antithamnion cruciatum*) to 16.59 % (*Spyridia filamentosa*). In saturation plots for transitions and transversions (not shown), there was evidence of saturation of first and third codon-position transitions for sequence divergences above about 11 %.

Figs 1-6. Field-collected and cultured thalli of *Gymnothamnion elegans*. Fig. 1. Bisporangial thallus collected near Agadir in Morocco, with every cell of main axis bearing a pair of branched laterals, some of which have become indeterminate in growth, and bisporangia borne in various positions. Fig. 2. Obliquely divided bisporangia formed in culture terminally on main laterals, short adaxials or laterally on lateral branches. Fig. 3. Cultured erect axis with pennate form, bearing paired laterals on every axial cell, secund series of adaxial branchlets, and bisporangia. Fig. 4. Culture showing prostrate axis giving rise at intervals to erect axes paired with multicellular rhizoidal filaments. Fig. 5. Cultured vegetative cells stained with Hoechst fluorochrome to show single nuclei. Fig. 6. Bispores squashed out of bisporangium, stained with Hoechst to show single central nucleus in each spore. Scale bar represents 10 µm for Figs 5 and 6. ▶

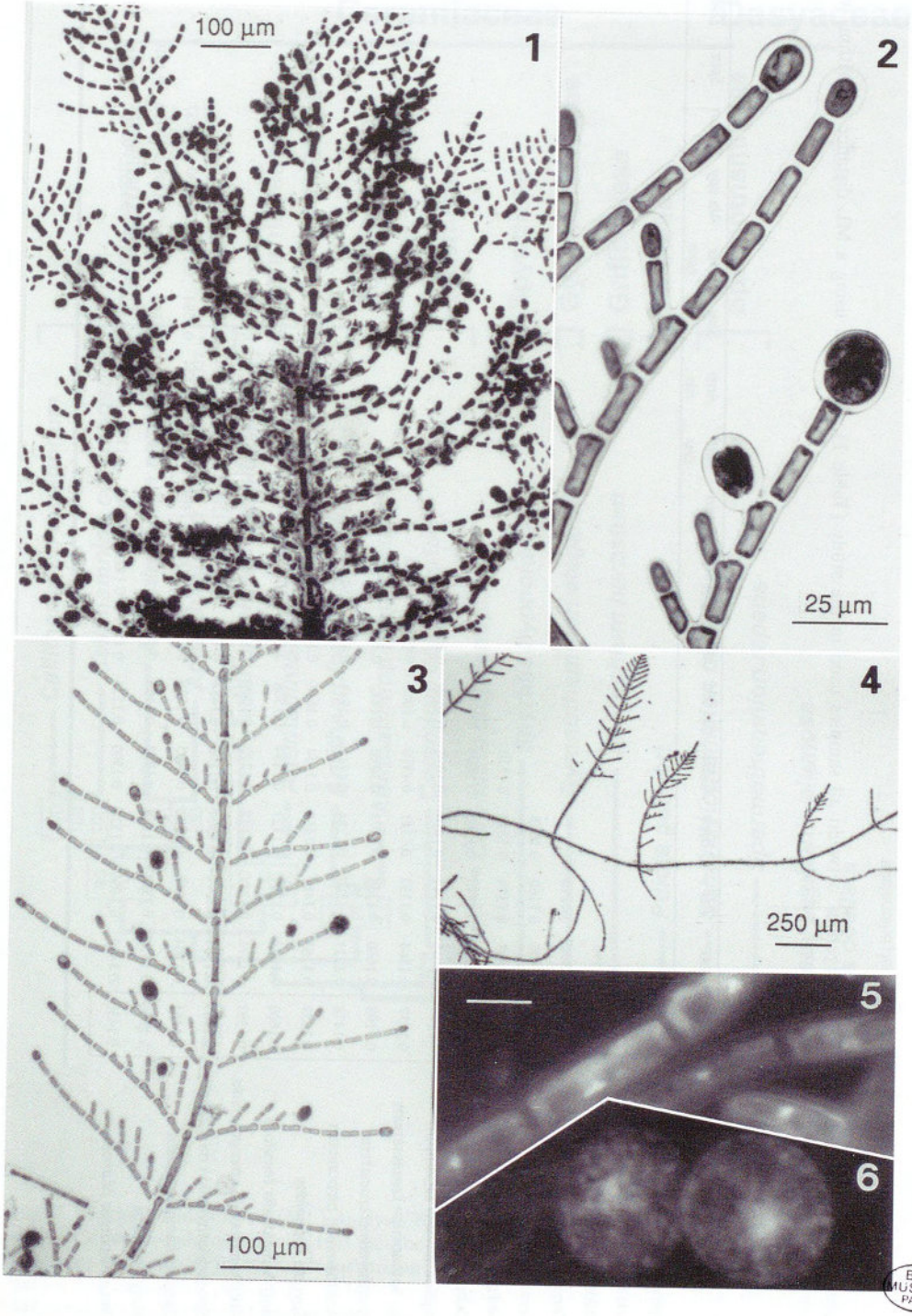

Table 2. Pairwise *rbcL* sequence divergences between all samples used in the study (Table 1), calculated using a ML distance algorithm, with divergences from *Gymnothamnion elegans* indicated in the box.

	Gymn	*Ano*	*Ptil*	*C brev*	*C dia*	*Cal tet*	*Cal cor*	*Ag sep*	*A ten*	*Anti cru*	*Anti nip*	*Spy*	*Ala tern*	*Ala spy*	*Sphon*	*Sper*	*Dasya*
Gymnothamnion elegans																	
Anotrichium barbatum	0.1636																
Ptilota gunneri	0.1326	0.1596															
Ceramium brevizonatum	0.1464	0.1534	0.1212														
Ceramium diaphanum	0.1472	0.1490	0.1160	0.1058													
Callithamnion tetragonum	0.1623	0.1603	0.1393	0.1333	0.1310												
Callithamnion corymbosum	0.1579	0.1618	0.1340	0.1360	0.1468	0.0775											
Aglaothamnion sepositum	0.1585	0.1641	0.1341	0.1404	0.1433	0.1046	0.1059										
Aglaothamnion tenuissimum	0.1457	0.1564	0.1333	0.1341	0.1310	0.1068	0.1074	0.0922									
Antithamnion cruciatum	0.1198	0.1660	0.1164	0.1173	0.1240	0.1482	0.1474	0.1343	0.1290								
Antithamnion nipponicum	0.1478	0.1521	0.1287	0.1284	0.1111	0.1348	0.1486	0.1460	0.1407	0.0872							
Spyridia hypnoides	0.1659	0.1763	0.1487	0.1419	0.1454	0.1646	0.1723	0.1611	0.1539	0.1506	0.1542						
Antithamnionella ternifolia	0.1600	0.1649	0.1397	0.1327	0.1434	0.1508	0.1539	0.1544	0.1370	0.1200	0.1289	0.1580					
Antithamnionella spirographidis	0.1393	0.1576	0.1331	0.1149	0.1227	0.1269	0.1319	0.1356	0.1219	0.1121	0.1246	0.1404	0.0761				
Sphondylothamnion multifidum	0.1335	0.1508	0.1070	0.1304	0.1196	0.1464	0.1372	0.1322	0.1289	0.1318	0.1275	0.1386	0.1384	0.1279			
Spermothamnion repens	0.1461	0.1704	0.1298	0.1382	0.1405	0.1535	0.1574	0.1485	0.1406	0.1439	0.1484	0.1594	0.1594	0.1390	0.1137		
Dasya ocellata	0.1346	0.1656	0.1327	0.1364	0.1377	0.1465	0.1404	0.1490	0.1412	0.1240	0.1344	0.1644	0.1598	0.1399	0.1190	0.1510	
Heterosiphonia plumosa	0.1430	0.1492	0.1466	0.1526	0.1500	0.1526	0.1531	0.1428	0.1461	0.1385	0.1506	0.1586	0.1614	0.1351	0.1320	0.1378	0.1217

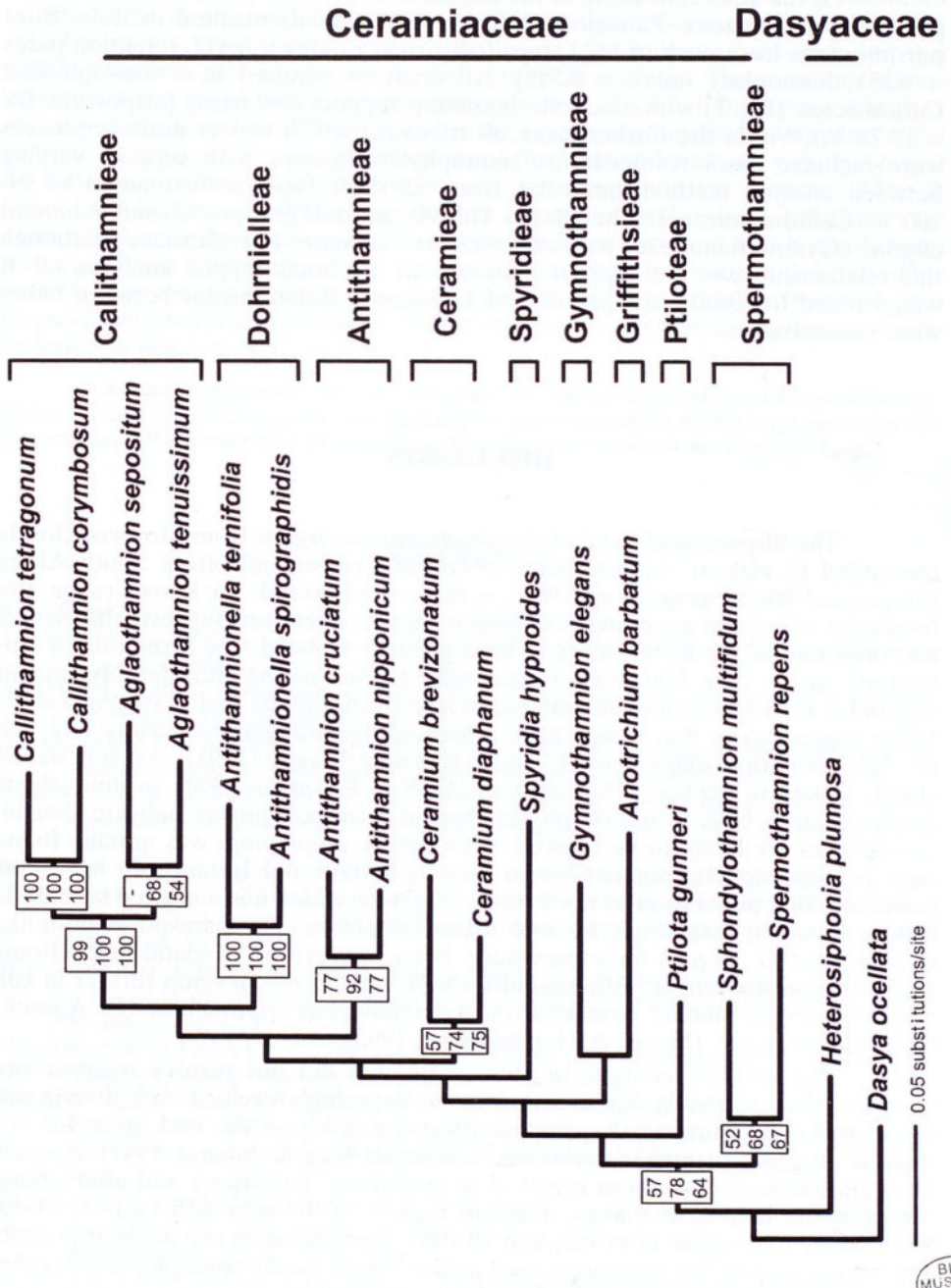

Fig. 7. Maximum likelihood (ML) analysis of *rbcL* alignment for representatives of nine tribes of the Ceramiaceae, rooted with two members of the Dasyaceae, using a Jukes-Cantor model of sequence change and transition/transversion ratio of 2.0. Values in boxes at nodes are bootstrap values for maximum parsimony (1000 resamplings), neighbour-joining (1000 resamplings) and ML analyses (100 resamplings) respectively.

Of the 1245 characters in the alignment, 746 were constant and 354 were parsimony-informative. Parsimony analyses (not shown) resulted in three most parsimonious trees, each of 1522 steps (consistency index = 0.442, retention index = 0.351, homoplasy index = 0.558). All analyses resulted in a monophyletic Ceramiaceae (Fig. 7) with moderate bootstrap support (bootstrap proportion, BP = 57-78 %) Within the Ceramiaceae, all tribes for which two or more sequences were included were resolved into monophyletic groups with support varying between analysis method and tribe, from 52-68 % (Spermothamnieae) to 99-100 % (Callithamnieae, Dorhnielleae). The ML analysis grouped *Gymnothamnion elegans* (Gymnothamnieae) with *Anotrichium barbatum* (Griffithsieae), although this relationship was not supported by any of the bootstrapped analyses, all of which failed to resolve the position of *G. elegans*. Relationships between tribes were unresolved.

DISCUSSION

The bisporangial thalli of *Gymnothamnion elegans* from Morocco closely resembled *G. elegans* var. *bisporum*, previously known only from South Africa (Stegenga, 1986; Stegenga *et al.*, 1997), so this record extends the known range. The formation of several generations of bisporophytes in culture suggests that sexual and bisporangial life histories are at least partially isolated. The formation of uninucleate rather than binucleate bisporangia, as well as the considerably smaller vegetative cells and sporangia than found in tetrasporangial thalli (Stegenga *et al.*, 1997), suggest to us that it may have a haploid bisporangial life history like that of *Aglaothamnion diaphanum* L'Hardy-Halos *et* Maggs (1991). As cell size is closely linked to nuclear DNA content (Goff & Coleman, 1990), in uninucleate species smaller cells in bisporophytes than in tetrasporophytes indicate that bisporophytes are likely to be haploid. Although *A. diaphanum* was initially found only as bisporophytic populations in France, Britain and Ireland, we have now found a sexual population in the Lizard, Cornwall, which has a normal sexual life history involving gametophytes and tetrasporophytes. The parasporangium-like structures of *G. elegans* have previously been interpreted as gland cells (Itono, 1977) or monosporangia (Athanasiadis, 1987), but did not develop further in culture, resembling similar structures in *Aglaothamnion tripinnatum* (C. Agardh) Feldmann-Mazoyer (Maggs & Hommersand, 1993).

Phylogenetic analysis of *rbc*L sequences did not resolve relationships between tribes, probably due to saturation at these high levels of *rbc*L divergence. Functional constraints on the enzyme affect the utility of the *rbc*L gene for systematic purposes at higher taxonomic levels (Kellogg & Juliano, 1997). Because some positions, such as those involved in interdimer, intradimer and intersubunit reactions, are unable to change, different regions of the gene will saturate at different rates. The apparent monophyly of the Ceramiaceae in our analysis is probably an artefact of the outgroups used as this family is not monophyletic in other analyses (Freshwater *et al.*, 1994; Saunders *et al.*, 1996).

All tribes with two or more representatives were resolved into clades, with at least moderate bootstrap support. *G. elegans* did not clade either with the Antithamnieae or the only included representative of the Ptiloteae, the type species *Ptilota gunneri*. Our analysis thus supports the removal of *Gymnothamnion*

from the Ptiloteae (Moe & Silva, 1979) and from the Antithamnieae by Kajimura (1989). In the lowest log-likelihood tree in ML analyses, *G. elegans* formed a clade with the only representative of the Griffithsieae, *Anotrichium barbatum*, but this relationship was not supported by bootstrapped analyses. The sequence divergence between these two taxa was extremely high (16.3 %), indicating that they are only distantly related. Tribal status of the Gymnothamnieae thus appears to be justified at present. No possible affinities of the Gymnothamnieae were proposed by Kajimura (1989). In addition to its primitive reproductive features (Moe & Silva, 1979; Kajimura, 1989), *G. elegans* has an unusual and possibly primitive vegetative feature in its unspecialized rhizoidal filaments, initials of which can develop into erect axes. Determining the relationships of the Gymnothamnieae to other ceramiacean tribes will require the use of a more conserved marker than *rbc*L, but its morphological features suggest that it may be quite basal among taxa curently included in this family.

Acknowledgements. We are delighted to acknowledge Marie-Thérèse L'Hardy-Halos for her practical support and enthusiastic encouragement of our exploration of the Ceramiaceae. We would also like to thank Max Hommersand for his constant help.

REFERENCES

AGARDH C.A., 1828 — *Species algarum...* 2 (1). Greifswald.

AGARDH J.G., 1892 — *Analecta algologica. Observationes de speciebus algarum minus cognitis earumque dispositione*. Lund.

ARDRÉ F., 1970 — Contribution à l'étude des algues marines du Portugal. 1. La flore. *Portugaliae Acta Biologica B* 10: 1-423.

ATHANASIADIS A., 1987 — *A survey of the seaweeds of the Aegean Sea with taxonomic studies on species of the tribe Antithamnieae (Rhodophyta)*. Göteborg.

CABOT E.L. & BECKENBACH A.T., 1989 — Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Computer Applications in the Biosciences*, 5: 233-234.

DE JONG Y.S.D.M., van der WURFF, A.W.G., STAM, W.T. & OLSEN J.L., 1998 — Studies on Dasyaceae 3. Towards a phylogeny of the Dasyaceae (Ceramiales, Rhodophyta), based on comparative *rbc*L gene sequences and morphology. *European Journal of Phycology* 33: 187-202.

FELDMANN J. & FELDMANN G., 1966 — Sur le *Gymnothamnion elegans* (Schousboe) J. Ag. et la situation des organes femelles chez les Céramiacées. *Revue générale de Botanique* 73: 5-17.

FELDMANN-MAZOYER G., 1941 — *Recherches sur les Céramiacées de la Méditerranée*. Minerva; Koenigstein: reprinted 1977 by Otto Koeltz.

FRESHWATER D.W., FREDERICQ S., BUTLER B.S., HOMMERSAND M.H. & CHASE M.W. 1994 — A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbc*L. *Proceedings of the National Academy of Sciences of the USA* 91: 7281-7285.

FURNARI G., L'HARDY-HALOS M.T., RUENESS, J. & SERIO D., 1998 — On the conspecificity of *Aglaothamnion tenuissimum* and *A. byssoides* (Ceramiaceae, Rhodophyta). *Taxon* 47: 843-849.

GOFF L.J. & COLEMAN A.W., 1990 — DNA: microspectrofluorometric studies. *In*: Cole K. M. & Sheath R. G. (eds), *Biology of the Red Algae*. Cambridge: Cambridge University Press, pp. 43-72.

GUIRY M.D. & CUNNINGHAM E.C., 1984 — Photoperiodic and temperature responses in the reproduction of north-eastern Atlantic *Gigartina acicularis* (Rhodophyta: Gigartinales). *Phycologia* 23: 357-367.

HASEGAWA M., KISHINO H. & YANO T., 1985 — Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 21: 205-285.

HOMMERSAND M.H., 1963 — The morphology and classification of some Ceramiaceae and Rhodomelaceae. *University of California Publications in Botany* 35: 165-366.

ITONO H., 1977 — *Studies on the ceramiaceous algae (Rhodophyta) from southern parts of Japan.* Vaduz: J. Cramer.

KAJIMURA M., 1989 — Gymnothamnieae trib. nov. (Ceramiaceae, Rhodophyta). *Botanica Marina* 32: 121-130.

KELLOG E. A. & JULIANO N. D., 1997 — The structure and function of RubisCO and their implications for systematic studies. *American Journal of Botany* 84: 413-428.

KISHINO H. & HASEGAWA M.J., 1989 — Evaluation of the maximum-likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* 29: 170-179.

KOSTRZEWA M., VALENTIN K., MAID U., RADETZKY R. & ZETSCHE K., 1990 — Structure of the rubisco operon from the multicellular red alga *Antithamnion* spec. *Current Genetics* 18: 465-469.

L'HARDY-HALOS M.-T. & MAGGS C.A., 1991 — A novel life history in *Aglaothamnion diaphanum* sp. nov. from France and the British Isles. *Phycologia* 30: 467-479.

LIN S.-M., FREDERICQ S. & HOMMERSAND M.H., 2001 — Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbc*L sequences, including the Phycodryoideae, subfam. nov. *Journal of Phycology* 37: 881-899.

MAGGS C.A., 1998 — Life history variation in *Dasya ocellata* (Dasyaceae, Rhodophyta). *Phycologia* 37: 100-105.

MAGGS C.A. & HOMMERSAND M.H., 1993 — *Seaweeds of the British Isles Volume 1 Rhodophyta, Part 3A Ceramiales*. London: Natural History Museum/HMSO.

McIVOR L.M., 2000 — *Molecular systematics of the red algal order Ceramiales.* PhD thesis, Queen's University Belfast, N. Ireland.

McIVOR L.M., MAGGS C.A. & STANHOPE M.J., 2002 – *Rbc*L sequences indicate a single evolutionary origin of multinucleate cells in the red algal tribe Callithamnieae. *Molecular Phylogenetics and Evolution* 23: 433-446.

MOE R.L. & SILVA P.C., 1979 — Morphological and taxonomic studies on Antarctic Ceramiaceae (Rhodophyceae). I. *Antarcticothamnion polysporum* gen. et sp. nov. *British Phycological Journal* 14: 385-405.

NAM K. W., MAGGS C. A., MCIVOR L. & STANHOPE M.J., 2000 — Taxonomy and phylogeny of *Osmundea* (Rhodomelaceae, Rhodophyta) in Atlantic Europe. *Journal of Phycology* 36: 759-772.

SAUNDERS G.W., STRACHAN I.M., WEST J.A. & KRAFT G.T., 1996 — Nuclear small-subunit ribosomal RNA gene sequences from representative Ceramiaceae (Ceramiales, Rhodophyta) *European Journal of Phycology* 31: 23-29.

STEGENGA H., 1986 — The Ceramiaceae (excl. *Ceramium*) (Rhodophyta) of the South West Cape Province, South Africa. *Bibliotheca Phycologica* 74: 1-149.

STEGENGA H., BOLTON J.J. & ANDERSON R.J., 1997 — *Seaweeds of the South African West Coast.* Bolus Herbarium publication n° 18, Cape Town: University of Cape Town.

SWOFFORD D.L. 1998 — *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.* Sinauer Associates, Sunderland, Massachusetts.