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The taxonomic position of *Maripelta rotata* (Rhodymeniaceae, Rhodophyta) [1]

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Abstract – *Maripelta rotata* (E.Y. Dawson) E.Y. Dawson, the type species of the genus, is a deep-water marine red alga with a distinctive morphology, presently referred to the Rhodymeniaceae. Analyses of *rbc*L sequences were used to clarify the taxonomic position of *M. rotata*. The molecular analyses placed *M. rotata* within the Rhodymeniaceae with robust support. This and previous studies suggest that, among the four peltate blade-forming genera of the Rhodymeniales, *Maripelta* and *Asteromenia* belong to the Rhodymeniaceae and *Halichrysis* and *Sciadophycus* to the Faucheaceae. These investigations show that vegetative morphological features are of little taxonomic utility within the Rhodymeniales and greater importance should be attached to reproductive characters.

Faucheaceae / *Maripelta rotata* / marine algae / molecular taxinomy / *rbc*L / Rhodymeniaceae / Rhodophyta

Résumé — Position taxonomique de *Maripelta rotata* (Rhodymeniaceae, Rhodophyta). *Maripelta rotata* (E.Y. Dawson) E.Y. Dawson, espèce type du genre, est une algue rouge marine d'eaux profondes avec une morphologie distinctive, actuellement rattachée aux Rhodymeniaceae. Les analyses des séquences *rbc*L confirment l'appartenance de *M. rotata* aux Rhodymeniaceae avec un soutien robuste. Cette étude et les études précédentes suggèrent que, parmi les quatre genres de Rhodymeniales peltés, formant des lames, *Maripelta* et *Asteromenia* appartiennent aux Rhodymeniaceae, et *Halichrysis* et *Sciadophycus* aux Faucheaceae. Ces investigations montrent que les caractères morphologiques végétatifs sont d'une moindre utilité taxinomique chez les Rhodymeniales, tandis qu'une plus grande importance doit être attachée aux caractères de la reproduction.

Algues marines / Faucheaceae / *Maripelta rotata* / *rbc*L / Rhodymeniaceae / Rhodophyta / systématique moleculaire

1. 'Warmly dedicated to Izzie Abbott on the occasion of her 85th birthday'

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INTRODUCTION

The red algal order Rhodymeniales has recently undergone a major taxonomic re-evaluation (Saunders *et al.,* 1999). Analysis of molecular data from the small-subunit ribosomal DNA (SSU rDNA) resulted in a number of taxonomic changes being proposed. The defining characters for the families within the Rhodymeniales were emended, resulting in a more restricted Rhodymeniaceae, an expanded Champiaceae, the resurrection of the Lomentariaceae and the establishment of the Faucheaceae (Saunders *et al.,* 1999).

The Rhodymeniaceae is characterized by having tetrasporangia generally formed in an intercalary position with a cruciate arrangement of tetraspores, a four-celled carpogonial branch, and elongated fusion cells (Saunders *et al.,* 1999). Twenty-five genera are currently assigned to the Rhodymeniaceae (Guiry & Nic Dhonncha, 2003) but the taxonomic position of a number of these remains little understood.

Maripelta rotata (E.Y. Dawson) E.Y. Dawson (1963) is a putative member of the family Rhodymeniaceae and is one of four genera in the Rhodymeniales known to produce peltate blades (Millar, 2001), the others being *Halichrysis* (J. Agardh) F. Schmitz (Schmitz, 1889), *Asteromenia* Huisman & A.J.K. Millar (Huisman & Millar, 1996) and *Sciadophycus* E.Y. Dawson (Dawson, 1944). Originally described as *Drouetia rotata* E.Y. Dawson (1949) from the Pacific coasts of California and Baja California, the species was proposed as the generitype of a new genus, *Maripelta,* on the basis of a number of morphological features, including its sympodial growth patterns and also its exclusively subtidal habitat (Dawson, 1963). *Drouetia coalescens* (Farlow) De Toni, also known to have peltate blades, is currently regarded as a member of the genus *Halichrysis* (Norris, 1991).

Two species are currently attributed to *Maripelta*: *M. rotata*, the generitype, and *M. atlantica* Eiseman & R.L. Moe (Eiseman & Moe, 1981) from the Atlantic coast of Florida. Both are found in deep water, with *M. rotata* being reported from 10-80 m (Dawson, 1963) and *M. atlantica* from 60-100 m in the Atlantic and 20-60 m in the Gulf of Mexico (Eiseman & Moe, 1981). A further entity, originally named *Maripelta thivyae* E.Y. Dawson, has been referred to *Halichrysis* as *H. thivyae* (E.Y. Dawson) Eiseman & R.L. Moe (Eiseman & Moe, 1981) on the basis of its reproductive and vegetative morphology. *Maripelta rotata* and *M. atlantica* are morphologically and developmentally similar, being separated only by the arrangement of their reproductive structures. In *M. atlantica* the tetrasporangia and spermatangia are grouped in rings around the blade, whereas in *M. rotata* they are scattered in groups over the blade (Eiseman & Moe, 1981).

In the present investigation, the gene for the large subunit of the RUBISCO enzyme (*rbc*L) is used to assess the phylogenetic position of *Maripelta rotata*. *Rbc*L sequences have proved useful for taxonomic investigations of red algae, including members of the Rhodymeniales (Hommersand & Fredericq, 2003), at a number of taxonomic levels, including at both the intraspecific and supraspecific level (Harper & Saunders, 2001; McIvor *et al.,* 2001).

MATERIALS AND METHODS

Cultured material of *Maripelta rotata*, *Cordylecladia erecta, Irvinea ardreana, Botryocladia spinulifera* and *B. ebriosa* was obtained from the NUI,

Galway Marine Algal Culture Collection (Table 1). A silica-dried specimen of *B. botryoides*, collected from the Gulf of Trieste (which is in the region of the type locality, the Adriatic Sea [Silva *et al.*, 1996]), was supplied by Dr Claudio Battelli.

DNA extraction

DNA was extracted from fresh and silica dried material using a CTAB extraction method modified after Doyle & Doyle (1987) and Serrão *et al.* (1999). Silica-dried material was rehydrated in sterile seawater prior to DNA extraction. Between 0.5 - 0.05 g of blotted dry tissue was ground in liquid nitrogen in a 1.5 ml micro-centrifuge tube using a pellet-pestle. Six hundred ml of CTAB extrac-

Table 1. Specimens used for *rbc*L sequence analyses.

Species	*Location*	*Collector*	*Source*	*GenBank*	*Herbarium*[2]
Irvinea ardreana	Sines, Portugal	M.D. Guiry & J.A. West	Culture[1]	AY444177	014415
Maripelta rotata	Cordell Bank, California, USA	R. Schmieder & R. Moe	Culture[1]	AY444179	014411
Botryocladia botryoides	Gulf of Trieste, Slovenia	Claudio Battelli	Field Collection	AY444169	014413
B. ebriosa	Coffs Harbour, NSW, Australia	A.J.K. Millar, J. Huismann & M.D. Guiry	Culture[1]	AY444171	014407
B. spinulifera	St Croix, Virgin Islands	W.C.H.F. Kooistra	Culture[1]	AY444174	014403
Cordylecladia erecta	New Quay, Co. Clare, Ireland		Culture[1]	AY444178	014414
Ceratodictyon spongiosum			GenBank	U21639	
Epymenia obtusa			GenBank	AF385647	
E. capensis			GenBank	AF385646	
E. wilsonis			GenBank	AF385650	
Gastroclonium subarticulatum			GenBank	U04178	
Grateloupia filicina			GenBank	AB038603	
Halichrysis micans			GenBank	U21641	
Halymenia floresii			GenBank	AB055470	
Lomentaria hakodatensis			GenBank	U04180	
Rhodymenia pseudopalmata			GenBank	AF212195	

1. Cultures from NUI, Galway, Marine Algal Culture Collection

2. Specimen codes in GALW herbarium, NUI, Galway.

tion buffer (0.1 M Tris, 0.02 M EDTA, 1.4 M NaCl, 2% CTAB) was added along with 1% PVP and 1.5 ml ß-mercaptoethanol. Tubes were incubated at room temperature for a minimum of 1 h with constant gentle mixing. This was followed by 2 or 3 purification steps with 24:1 CIA (Chloroform: isoamyl alcohol) and precipitation with –20°C isopropanol. DNA was dissolved in 0.1 TE and incubated with RNase (Roche biochemicals) for 1 hr at 37°C. DNA was then precipitated overnight in 4 M ammonium acetate and isopropanol and the resultant DNA pellet redissolved in TE. The quality and quantity of the extracted DNA was assessed on a 1% agarose gel prior to PCR amplification.

The *rbc*L gene was amplified using a either a single primer pair (F8 & R1381) or a set of three pairs (F8 & R646; F481 & R1150; F765 & R1381, Wang *et al.* 2000). The reaction mix consisted of 2.5 µl of 10x PCR buffer, 2.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM primers and 0.6 units of Taq (Sigma UK Ltd or Biogene UK Ltd) in each 25 µl reaction. Reaction profile included an initial denaturation of 93°C for 1 min followed by 35 cycles of 94° C for 30 s, 55°C for 30 s and 72°C for 45 s with a final extension at 72°C for 5 min. PCR reactions were run on a Hybaid OMN-E or Hybaid PCR-express themocycler. PCR products were agarose-gel purified using the Roche High-Pure PCR product purification kit according to the manufacturer's instructions. Purified products were commercially sequenced (MWG, Biotech, UK, Ltd. or Lark Technologies UK, Ltd).

Phylogenetic analysis

Sequences were aligned by eye using Genedoc 2.6.002 (Nicholas & Nicholas, 1997). Published sequence data for other members of the Rhodymeniales were included in the analyses using two members of the Cryptonemiales, *Grateloupia filicina* (J.V. Lamouroux) C. Agardh and *Halymenia floresii* (Clemente) C. Agardh, as outgroups. The ingroup taxa are detailed in Table 1. Sequence data were analysed with PAUP* 4.0b10 (Swofford 2002) for maximum parsimony (MP), maximum likelihood (ML) and for neighbour joining (NJ), using a Kimura 2-parameter distance matrix as input. MP analysis was performed using a heuristic search with 50 random sequence additions. Modeltest (Posada & Crandall 1998) was used to determine the parameters for ML analysis, and specified a General Time Reversible model with a gamma distribution (GTR+G). The rate matrix was specified as [A-C] = 0.9190, [A-G] = 7.0651, [A-T] = 3.9553, [C-G] = 1.6323, [C-T] = 21.4635 and [G-T] = 1.0000, with the base frequencies at A = 0.3043, C = 0.1436, G = 0.2155, and T = 0.3366 and a gamma distribution of 0.2292.

The robustness of the phylogenetic trees produced was tested by boostrapping (Felsenstein, 1985) with 1000 replicates for MP, NJ and 100 replicates for ML analyses.

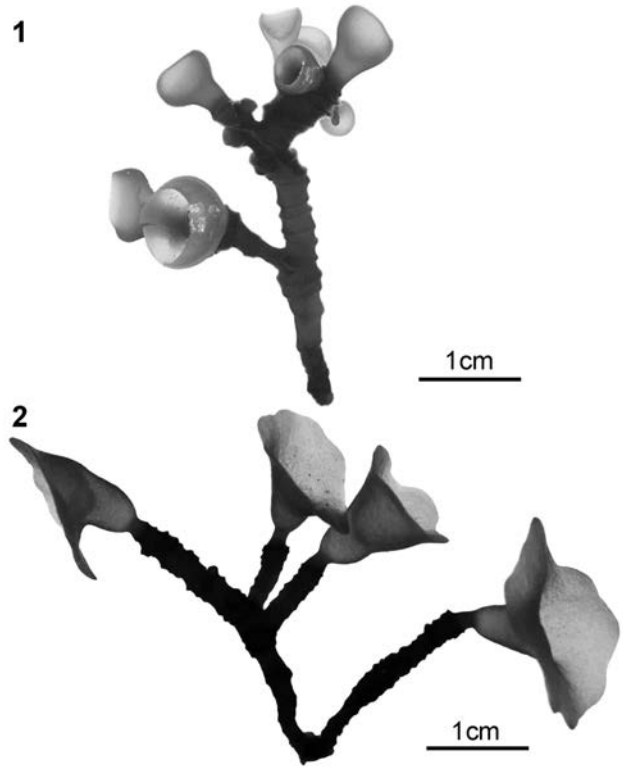
RESULTS

The morphologically distinctive thalli of *Maripelta rotata* consist of a sympodially produced stipe formed from the scars of the deciduous peltate blades. The stipe elongates sequentially with the production of each new stipe-increment and blade after the loss of the previous blade. These blades are approximately

circular and are attached centrally to the stipe. In culture, these blades are cup-shaped when young (Fig. 1) and as they develop they flatten out to form an obconical blade (Fig. 2).

Between 1056 and 1305 base pairs of *rbc*L sequences were obtained. These were combined with published data to give a 1305-bp sequence alignment for analyses. The alignment contained 320 parsimony informative sites and 848 invariable characters. No insertions or deletions were found, making the alignment unambiguous.

MP analysis produced three equally parsimonious trees. These trees were the same as the single trees produced from the NJ and ML analyses differing only in the position of a subgroup containing *Halychrysis* and *Gastroclonium*. The single maximum likelihood tree with bootstrap values from each analysis overlaid on the branches, is shown in Figure 3. Robust monophyletic Rhodymeniales clades (BP 96;100;100 (MP;NJ;ML)%) were produced in all analysis. The species currently regarded as members of the Rhodymeniaceae formed a robust subclade within the Rhodymeniales (BP 92;100;88%). *Lomentaria* and *Ceratodictyon*, the two members of the Lomentariaceae included in this investigation, grouped together with moderate support of 78;93;78%. While a *Halichrysis* and *Gastroclonium* group was resolved in the MP analysis, this relationship had very



Figs 1-2. *Maripelta rotata* from NUI, Galway Marine Algal Culture. **1**. Thallus with small cup-shaped blades and the striped axis resulting from the incremental loss of older blades. **2**. Mature thalli showing fully developed obconical peltate blades.

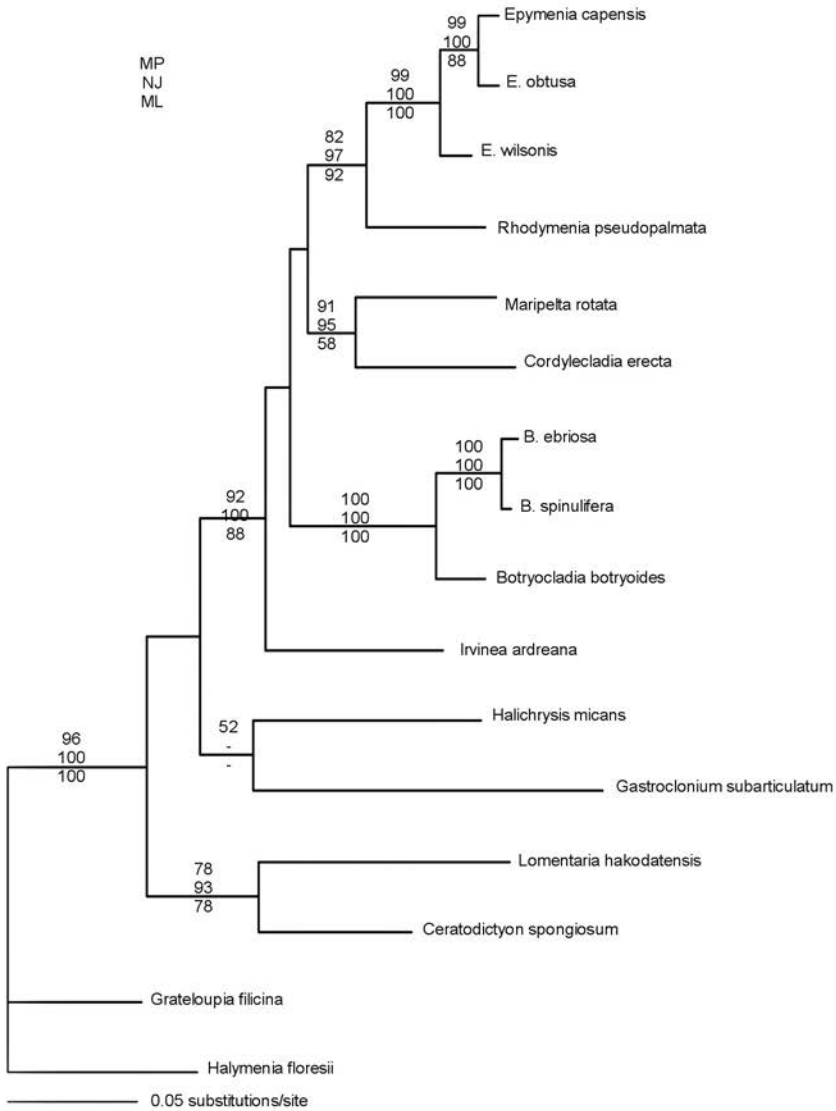


Fig. 3. Maximum Likelihood tree for *rbc*L sequences, with bootstrap values from MP (1000), NJ (1000) and ML (100) analyses overlaid on the branches.

low support (BP 52%) and no support from NJ or ML analysis. *Maripelta* was consistently placed with *Cordylecladia* (BP 91;95;58%) within the Rhodymeniaceae clade although the support from the ML analysis was low. *Epymenia* and *Rhodymenia* formed a moderately-supported group (BP 82;97;92%). The *Botryocladia* isolates lie separately within the family in robust group (100% support) with *Irvinea* being placed basally in each analysis.

As several of the sequences used had varying amount of missing data a second analysis was undertaken using a truncated dataset. The resultant trees (not shown) had the same topologies but lower bootstrap support for the arrangements. Similar results using partial *rbc*L sequences have been reported by previous authors (Freshwater *et al.*, 1995).

DISCUSSION

The arrangement of the taxa within the Rhodymeniales as inferred here from the *rbc*L data concurs with that of previous investigations using SSU rDNA (Saunders *et al.,* 1999) and *rbc*L (Hommersand & Fredericq, 2003) sequences. The arrangement of the *Botryocladia* and *Irvinea* is in close agreement with the results obtained from SSU rDNA data and provides further support for the separation of these two genera. This is the first molecular investigation to include the genus *Maripelta* and the results place it firmly within the Rhodymeniaceae. Based on its reproductive morphology, *viz*, cruciately arranged, intercalary tetrasporangia and three- or four-celled carpogonial branch, this species is currently included in the recently emended Rhodymeniaceae (Saunders *et al.,* 1999). The present *rbc*L analyses support this taxonomic placement of *Maripelta*.

The differences in vegetative and reproductive morphology of the peltate blade-forming genera in the Rhodymeniales were summarised by Huisman & Millar (1996). *Maripelta* has a monostromatic medulla and develops tetrasporangia from an intercalary cell (Eiseman & Moe, 1981; Norris, 1991); *Halichrysis,* on the other hand, has a polystromatic medulla and terminal, cruciately arranged tetrasporangia, characters that it shares with another peltate blade-forming genus, *Sciadophycus* (Norris, 1991; Millar, 2001). Although *Halichrysis* had been previously considered to belong to the Rhodymeniaceae (Eiseman & Moe, 1981; Norris, 1991), Saunders *et al.* (1999) concluded that it belonged in a new family, the Faucheaceae. These results indicate that *Halichrysis* is not a member of the Rhodymeniaceae and, whereas it cannot be directly inferred from the present study, it would seem likely that it is correctly regarded as a member of the Faucheaceae based on the morphological features previously described (Eiseman & Moe, 1981; Norris, 1991; Saunders *et al*., 1999).

The other two peltate blade-bearing genera in the Rhodymeniales are currently considered members of the Rhodymeniaceae. *Asteromenia* differs considerably from *Halichrysis* and *Sciadophycus* in the development of tetrasporangia. It produces intercalary tetrasporangia with a cruciate arrangement of the tetraspores (Huisman & Millar, 1996). These features place it within the recently emended Rhodymeniaceae and previous SSU rDNA molecular data also confirmed this placement (Saunders *et al.,* 1999).

The taxonomic difficulties when using vegetative morphology within the Rhodymeniales have long been remarked upon (Irvine & Guiry, 1980; Guiry & Irvine, 1981). These problems were also highlighted by Saunders *et al.* (1999) who concluded that reproductive features should be given greater importance. The grouping of *Maripelta rotata* and *Cordylecladia erecta* by our molecular analyses gives further weight to this suggestion. While both species have widely varying

vegetative morphologies they share the same tetrasporangial and gametangial development patterns (Eiseman & Moe, 1981; Brodie & Guiry, 1988).

The distribution of the peltate blades as highlighted here suggests that gross morphology is of little taxonomic utility within the Rhodymeniales These seemingly characteristic blades appear to have arisen independently more than once in the evolution of species and genera within the Rhodymeniales. Further morphological and molecular analyses with a greater number of species will allow a better understanding of how this feature has developed within the Rhodymeniales.

In conclusion, despite the relatively few taxa included in the molecular analyses, the *rbc*L data presented here confirm that *Maripelta rotata*, the generitype, is correctly placed within the family Rhodymeniaceae. The results from this dataset compare well with previous morphological and molecular investigations (Huisman & Millar, 1996; Saunders *et al.,* 1999) and while the addition of further sequences would provide greater resolution of the relationships between the species, the overall phylogeny is likely to remain basically the same, confirming that both *Maripelta* and *Asteromenia* are correctly regarded as members of the Rhodymeniaceae.

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