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Note

A nuclear small-subunit ribosomal DNA perspective on the taxonomic affinities of *Maripelta* (Rhodymeniales, Rhodophyta)

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Abstract – Small subunit ribosomal DNA sequence has been determined for *Maripelta rotata* confirming its taxonomic position in the Rhodymeniaceae *sensu stricto*. Among the included genera, *Maripelta* was associated specifically with *Chrysymenia sensu stricto* and *Gloiosaccion* with which it shares no particular aspects of gross morphology or vegetative anatomy. This observation is discussed in light of recent modifications to rhodymenialean taxonomy that have deemphasized an earlier reliance on vegetative features at the family level.

***Maripelta* / Rhodymeniaceae / Rhodymeniales / small subunit ribosomal DNA / systematics / Rhodophyta**

Résumé – L'ADN codant la petite sous-unité de l'ARNr éclaire la position systématique de *Maripelta* (Rhodymeniales, Rhodophyta). La séquence de la petite sous-unité de l'ADN ribosomique a été déterminée pour *Maripelta rotata* confirmant sa position taxinomique au sein des Rhodymeniaceae *sensu stricto*. Parmi les genres inclus dans les analyses de ce travail, *Maripelta* a été spécifiquement associé à *Chrysymenia sensu stricto* et *Gloiosaccion* avec lesquels il ne partage aucun aspect morphologique ou anatomique particulier. Cette observation est discutée à la lumière des récents changements dans la taxinomie des Rhodymeniales qui ont permis d'établir qu'il ne fallait plus mettre l'accent sur les caractères végétatifs pour établir leur systématique au niveau de la famille.

***Maripelta* / Rhodymeniaceae / Rhodymeniales / petite sous-unité de l'ADN ribosomique / systématique / Rhodophyta**

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Maripelta is one of four genera of Rhodymeniales that produce peltate or hemipeltate blades, and the type species *M. rotata* (E.Y. Dawson) E.Y. Dawson is particularly distinct due to its sympodial growth pattern (see Wilkes *et al.*, 2005). One additional species, *M. atlantica* Eiseman *et* R.L. Moe, is currently included in the genus; it differs from the type species largely in the disposition of its reproductive structures on the vegetative fronds, which are aggregated in rings around the blade rather than scattered in localized sori as in *M. rotata* (Eiseman & Moe, 1981).

Saunders *et al.* (1999) recently used small-subunit ribosomal DNA sequences to restructure classification within the Rhodymeniales. In that study four families were recognized. The Rhodymeniaceae was considered to include species of various vegetative constructions, but generally having four-celled carpogonial branches and intercalary tetrasporangia with cruciately arranged spores. *Maripelta* was provisionally included within this family despite its unusual morphological attributes, as its reproductive features were considered consistent with placement here. Material for inclusion in the molecular trees was not available at that time.

A recent study by Wilkes *et al.* (2005) provided the first molecular insights into the taxonomic affinities of the red algal genus *Maripelta*. Using data from the plastid *rbc*L gene, these authors established that *Maripelta* was correctly positioned in the Rhodymeniaceae, and that it did not associate with a species of another hemipeltate genus, *Halichrysis micans* (Hauptfleisch) P. Huvé *et* H. Huvé. Unfortunately, the taxon sampling was low in that study (only six genera of the family were included), which precluded a more detailed understanding of the phylogenetic affinities of this species in the Rhodymeniaceae. We have determined the SSU (small subunit ribosomal DNA) sequence for *Maripelta rotata* and have added it to a more genus-rich (ca. 15; listed below) alignment covering most of the currently recognized genera of the Rhodymeniaceae *sensu stricto* (*cf.* Saunders *et al.*, 1999, fig. 1).

The SSU was PCR amplified from *Maripelta rotata* DNA provided from an earlier study (Wilkes *et al.*, 2005) following published protocols (Saunders *et al.*, 2006). The new sequence (DQ343159) was added to an alignment including SSU data for: *Botryocladia ebriosa* A. Millar (AF085255); *Botryocladia leptopoda* (J. Agardh) Kylin (DQ343160); *Botryocladia sonderi* P.C. Silva (AF085256); *Cephalocystis furcellata* (J. Agardh) A. Millar, G.W. Saunders, I.M. Strachan *et* Kraft (U23949); *Cephalocystis leucobotrys* A. Millar, G.W. Saunders, I.M. Strachan *et* Kraft (U23950); *Chrysymenia ornata* (J. Agardh) Kylin (AF085257); *Chrysymenia wrightii* (Harvey) Yamada (AF117129); *Coelarthrum opuntia* (Endlicher) Børgesen (AF085258); *Cordylecladia erecta* (Greville) J. Agardh (U23952); *Erythrocolon podagricum* (Harvey) J. Agardh (U23953); *Fryeella gardneri* (Setchell) Kylin (AF085273); *Gloiosaccion brownii* Harvey (AF085259); *Halichrysis concrescens* (J. Agardh) De Toni (DQ343654); *Halichrysis micans* (Hauptfleisch) P. Huvé *et* H. Huvé (DQ343655); *Hymenocladiopsis prolifera* (Reinsch) M.J. Wynne (AF085274); *Irvinea ardreana* (Brodie *et* Guiry) Guiry (AF085254); *Rhodymenia leptophylla* J. Agardh (U09621); *Rhodymenia obtusa* (Greville) Womersley (U33128); *Rhodymenia stenoglossa* J. Agardh (AF085262); South Africa "*Drouetia*" (DQ343653; *cf.* Saunders *et al.*, 2006); *Sparlingia pertusa* (Postels *et* Ruprecht) G.W. Saunders, I.M. Strachan *et* Kraft (AF085261); and an unidentified entity from Western Australia (WA-unknown; AF085253; see Saunders *et al.*, 2006). Based on previous work by Saunders *et al.* (2006), *Fryeella gardneri* and *Hymenocladiopsis prolifera* were used to root the Rhodymeniaceae *sensu stricto*.

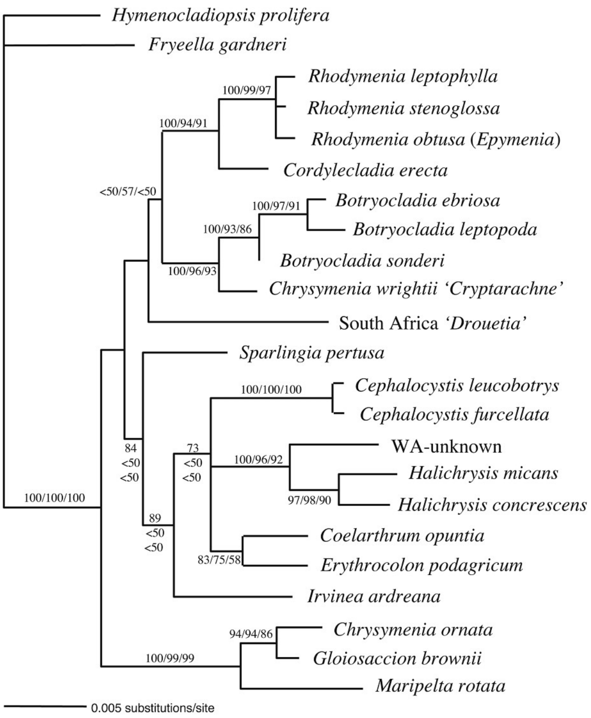

Fig. 1. Bayesian consensus tree with the posterior probabilities, distance and parsimony bootstrap results, respectively, appended to the appropriate branches. Branches lacking values were not supported in any of the bootstrap analyses.

The final alignment had 23 taxa and 1782 nucleotide positions of which 53 were excluded as ambiguous or owing to homology of the original PCR primers. Neighbor-joining (model parameters estimated in Modeltest v 3.06; Posada & Crandall, 1998) and maximum parsimony analyses were implemented in PAUP (v.4.0b10; Swofford, 2002). For parsimony (characters unweighted and unordered, gaps treated as missing data) 100 random sequence additions were implemented and the TBR swapping option was used. Distance and parsimony (100 random additions) analyses were subjected to 2000 rounds of bootstrap resampling to estimate robustness of the inferred topology. Bayesian analyses were conducted with the assistance of Mr. Bayes v3.1.1 (Huelsenbeck & Ronquist, 2001) utilizing the GTR + I + G model and two million generations (analyses completed in triplicate). Trees were sampled every 100 generations with log-likelihood scores stabilized at ca. 30,000 generations - the first 5000 of a possible 20,000 trees were discarded as burning. The remaining trees were imported into PAUP to generate a majority rule consensus tree to estimate posterior probabilities.

Using the protocols outlined here, we successfully amplified the SSU from *Maripelta rotata.* In bayesian inference, neighbor-joining and parsimony analyses *Maripelta* was allied solidly as sister to a lineage including the genera *Chrysymenia sensu stricto* (*cf.* Saunders *et al.*, 1999, pp. 37-38) and *Gloiosaccion* (Fig. 1).

Gloiosaccion brownii Harvey, the generitype of the genus, consists of simple to rarely branched ovoid to clavate mucilage-filled sacs with secretory cells on the inner surfaces of the cells lining the medulla (Womersley, 1996). *Chrysymenia ornata* (J. Agardh) Kylin has irregularly cleft, flattened blades, which are nonetheless hollow and mucilage-filled with the inner layer of cells bearing secretory cells (Millar, 1990). Aspects of their vegetative anatomy thus support a strong alliance between these two closely related genera in the SSU trees, whereas the more distantly related *Maripelta* is morphologically and anatomically distinct. Our results further support the earlier conclusions of Saunders *et al.* (1999) that vegetative attributes are only useful within, and between, closely related genera in the Rhodymeniales. Most significantly, *Maripelta* failed to ally with other peltate samples included in our analyses, *viz*. *Halichrysis concrescens* (J. Agardh) De Toni, *H. micans*, and unknown species of peltate rhodymeniaceous algae from Western Australia and South Africa (WA-unknown and South Africa "*Drouetia*" in Fig. 1., respectively). Of the remaining rhodymenialean peltate genera, *Maripelta* is distinct from *Drouetia* based on reproductive anatomy, from *Asteromenia*, which falls outside the Rhodymeniaceae *sensu stricto* in SSU analyses, and from *Sciadophycus*, whose features are more consistent with inclusion in the Faucheaceae (Saunders *et al.*, 1999, 2006).

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