

**Studies of Hawaiian Galaxauraceae
(Nemaliales, Rhodophyta):
Large subunit rDNA gene sequences support
conspécificity of *Galaxaura rugosa*
and *G. subverticillata*¹**

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Abstract — Species of the genus *Galaxaura* are unusual in having gametophyte and tetrasporophyte phases that, while somewhat similar in stature, display markedly different cortical morphologies. These differences have, in the past, led to many species being erected that represent no more than life-history phases of a single species. This paper discusses the identity of the tetrasporophyte phase of *G. rugosa*, the type of the genus, and concludes that it, at least, includes *G. subverticillata*, a species regarded as independent in several recent publications. Evidence for this conclusion resides in a comparison of large subunit rDNA sequence data from *G. rugosa* and “*G. subverticillata*” collected in Hawaii, *in situ* observations, plus a review of historical and recent morphological studies. In addition, evidence is presented to support the inclusion of plants with both flattened and subterete branch morphologies in *Dichotomaria marginata* (Ellis et Solander) Lamarck.

***Dichotomaria* / *Dichotomaria marginata* / *Galaxaura* / *Galaxaura rugosa* / *Galaxaura subverticillata* / Hawaii / large subunit rDNA / life history phases / Nemaliales / Rhodophyta**

Résumé — Étude des Galaxauraceae (Nemaliales, Rhodophyta) de Hawaii : les séquences du gène de la grande sous-unité des ADNr () confirment la conspécificité de *Galaxaura rugosa* et de *G. subverticillata*. Les espèces du genre *Galaxaura* ont une caractéristique inhabituelle qui est la présence de phases gamétophyte et tétrasporophyte qui, bien qu’ayant une taille à peu près semblable, présentent des morphologies corticales nettement différentes. Ces différences ont conduit, dans le passé, à élever au niveau spécifique des échantillons qui ne représentent rien de plus qu’une phase du cycle de vie d’une seule espèce. Cet article discute de l’identité de la phase tétrasporophytique de *G. rugosa*, le type du genre, et permet de conclure qu’il doit inclure, au moins, *G. subverticillata*, une espèce

1. Dedicated by the first two authors to the third, in celebration of her 85th birthday and in recognition of her contributions to phycology spanning some 60 years.

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considérée comme indépendante dans plusieurs publications récentes. Cette conclusion résulte de la comparaison des séquences du gène de la grande sous-unité des ADNr chez *G. rugosa* et « *G. subverticillata* » récoltés à Hawaii et observés *in situ*, ainsi que de la synthèse des études historiques et récentes. En outre, les résultats présentés permettent de conclure que *Dichotomaria marginata* (Ellis et Solander) Lamarck doit désormais accueillir les individus ayant des rameaux de section aplatie aussi bien que de section subarrondie. (Traduit par la Rédaction)

***Dichotomaria* / *Dichotomaria marginata* / *Galaxaura* / *Galaxaura rugosa* / *Galaxaura subverticillata* / grande sous-unité des ADNr / Hawaii / Nemaliales / phases du cycle de vie / Rhodophyta**

INTRODUCTION

Members of the genus *Galaxaura* Lamouroux (1812: 185) are widespread in tropical and warmer seas. The genus is unusual in the Rhodophyta in that the gametophyte and sporophyte phases of the life history, while relatively similar in stature, display different cortical morphologies (Huisman & Borowitzka, 1990). These differences led several early workers to recognize a large number of species in which only gametophytes or sporophytes were known, a process culminating in the study of Kjellman (1900) in which 62 species were recognized, 47 of them new.

Howe (1917, 1918) was the first to propose that some of these entities represented phases in the life histories of single species, and suggested that culture studies were desirable, although these were “not likely to be achieved in the immediate future” (Howe, 1917: 624). Based on his examination of numerous specimens, Howe went on to state that he felt the “evidence was overwhelming” (Howe, 1918: 194) that many of the “so-called species...are simply the sexual phases” of other species, despite the lack of culture studies. Howe (1918) proposed several species pairs based on his observations, the most pertinent to the present work being that of *Galaxaura subverticillata* Kjellman and *G. rugosa* (Ellis et Solander) Lamouroux. He also felt that the distinctions between some species were “vague and uncertain”, particularly those between *G. flagelliformis* Kjellman and *G. subverticillata*, and between *G. squalida* Kjellman and *G. rugosa*. In addition, Howe proposed that the tetrasporophyte of *Galaxaura cylindrica* (Ellis et Solander) Lamouroux was probably *G. lapidescens* (Ellis et Solander) Lamouroux, but this has since been shown to be incorrect. The tetrasporophyte of *G. cylindrica* is now known to be a filamentous plant (Magruder, 1984), a discovery that, in part, led to the erection of the new genus *Tricleocarpa* Huisman et Borowitzka to accommodate *G. cylindrica* (Huisman & Borowitzka, 1990). Børgeesen (1927), working with plants from the Canary Islands, combined the sexual *G. squalida* with the tetrasporic *G. flagelliformis*, adopting the name of the latter. The former was subsequently regarded as synonymous with *G. rugosa*, the latter with *G. lapidescens* (Papenfuss et al., 1982). Huisman & Borowitzka (1990) eventually treated *G. lapidescens* as a synonym of *G. rugosa*.

Howe's discoveries, and subsequently those of Børgeesen (1927) and the observations of Svedelius (1945), led to a reduction in the number of species being recognized, culminating in the studies of Papenfuss et al. (1982) and

Table 1. The complex synonymy history of *Galaxaura rugosa* and its potential tetrasporophyte phase. Species treated as synonyms are indented, including where tetrasporophyte “species” have been synonymized with *G. rugosa*.

<i>Publication</i>	<i>Reported as</i>	<i>Suggested tetrasporophyte.</i> <i>Indented names formally synonymized</i>
Howe, 1918	<i>G. rugosa</i>	<i>G. subverticillata</i>
Howe, 1918	<i>G. squalida</i>	<i>G. flagelliformis</i>
Børghesen, 1927	<i>G. squalida</i>	<i>G. flagelliformis</i>
Svedelius, 1945	<i>G. squalida</i>	Not indicated
Svedelius, 1945	<i>G. elongata</i>	<i>G. collabens</i>
Børghesen, 1949	<i>G. rugosa</i>	<i>G. mauritiana</i>
Svedelius, 1953	<i>G. rugosa</i>	<i>G. subverticillata</i> (possibly including <i>G. mauritiana</i>)
Papenfuss & Chiang, 1969	<i>G. elongata</i> <i>G. squalida</i>	<i>G. flagelliformis</i>
Papenfuss <i>et al.</i> , 1982	<i>G. rugosa</i> <i>G. elongata</i> <i>G. squalida</i>	Not indicated, <i>G. subverticillata</i> treated as independent species. <i>G. lapidescens</i> also treated as independent and including <i>G. collabens</i> and <i>G. flagelliformis</i> as synonyms.
Huisman & Borowitzka, 1990	<i>G. rugosa</i> <i>G. elongata</i> <i>G. squalida</i>	<i>G. collabens</i> (implicitly including <i>G. subverticillata</i>) <i>G. lapidescens</i>
Littler & Littler, 2000	<i>G. rugosa</i>	<i>G. lapidescens</i> <i>G. subverticillata</i> treated as independent species

Huisman & Borowitzka (1990), which placed many species in synonymy. This process has been driven primarily by two methods: 1) morphological examinations and broader interpretations of species limits, and 2) similar static examinations and *in situ* observations of species “pairs” leading to the recognition of life history phases. Papenfuss *et al.* (1982) were reluctant to combine possible species pairs under *G. rugosa* until they could be confirmed by “further research, involving culturing of the alga” (Papenfuss *et al.*, 1982: 423), although they did do so for *G. marginata* (Ellis *et Solander*) Lamouroux and *G. obtusata* (Ellis *et Solander*) Lamouroux based on static observations. They therefore continued to recognize species known only from one phase in the life history, including *G. lapidescens* (tetrasporophyte) and *G. rugosa* (gametophyte). *Galaxaura subverticillata* was included as a synonym of *G. lapidescens* by Papenfuss *et al.* (1982). Huisman & Borowitzka (1990) followed the lead of Børghesen (1927) and regarded *G. lapidescens* (including *G. subverticillata*) as representing the tetrasporophyte of (and therefore is synonymous with) *G. rugosa*. The complex history of *G. rugosa*, its synonyms, and proposed tetrasporophytes are given in Table 1.

Of primary relevance to the present paper are three species: *Galaxaura rugosa*, *G. lapidescens* and *G. subverticillata*. *Galaxaura rugosa* is the type of the genus and is known from many tropical locations. Based on the studies and interpretations by Howe (1918), Børghesen (1927), Svedelius (1953), Papenfuss *et al.* (1982), and Huisman & Borowitzka (1990), *G. lapidescens* and *G. subverticillata* are conspecific and represent the tetrasporophyte phase of *G. rugosa*. In *G. rugosa*,

gametophytes have a pseudoparenchymatous cortex that is glabrous or with very few epidermal filaments, whereas the putative sporophytes have an essentially filamentous cortex with a dense coating of pigmented filaments. These filaments in *G. subverticillata* are arranged in whorls, whereas in other described species (e.g. *G. lapidescens*) the filaments are evenly distributed. Both Papenfuss *et al.* (1982) and Huisman & Borowitzka (1990) felt that the degree of overlap rendered any distinctions meaningless, as many specimens displayed whorls near the apices only.

Despite these suggestions, several subsequent authors continued to recognize *G. subverticillata* as a separate species. Littler & Littler (1997, 2000), Abbott (1999: 69) and Schneider (2003: 283) all rejected the proposal made by Huisman & Borowitzka (1990) that *G. lapidescens*, *G. subverticillata*, and also *G. collabens* J. Agardh, represent the sporophyte phase of *G. rugosa*, restricting it to only those plants with *G. lapidescens* morphology and maintaining *G. subverticillata* as a separate species. As mentioned earlier, the only apparent distinction between these two entities is that the hairs in *G. subverticillata* are in whorls, whereas those of *G. lapidescens* are not. That this distinction is perhaps not of value is also demonstrated by the statement of Littler & Littler (2000: 62) that the whorls of *G. subverticillata* become "obscure with age".

Recognition of *G. subverticillata* as a separate species is made worrisome by at least four factors. Firstly, the "species" is known only by the tetrasporophyte phase, with no gametophytes recorded. If *G. subverticillata* is to be recognized as an independent species, where is the gametophyte phase? If this is the case, it presumably would be present as a cryptic entity within the specimens attributed to *G. rugosa*; an arrangement that cannot be ruled out, but seems unlikely. Secondly, if only one of *G. lapidescens* or *G. subverticillata* is to be regarded as the tetrasporophyte of *G. rugosa*, as is accepted by Littler & Littler (2000) and Schneider (2003), why *G. lapidescens*? Transverse striations are well known in *G. rugosa* gametophytes and are even depicted by Ellis & Solander in the original description of *Corallina rugosa*, the basionym (1786, pl. 22, fig. 3; also reproduced as fig. 28 in Papenfuss *et al.*, 1982). If a sporophyte is to be selected based on morphological evidence only, would not the transverse striations of *G. rugosa* lead one intuitively to the transverse whorls of *G. subverticillata*, rather than the disorganized arrangement of hairs in *G. lapidescens*? This conclusion was also reached by Howe (1918). Thirdly, in Hawaii only two relevant entities are presently recognized, *G. rugosa* for gametophytes and *G. subverticillata* for sporophytes (Abbott 1999), two species often observed growing together (Svedelius, 1953: 86 and pers. obs.).

Svedelius also reported the presence in Hawaii of *G. subfruticulosa* Chou, a species distinguished from *G. subverticillata* based on the less prominent production of surface whorls, hardly a reliable character. Abbott (1999) did not specifically mention *G. subfruticulosa* and included similar specimens under *G. subverticillata*. If we accept that only *G. rugosa* and *G. subverticillata* are of relevance here, based on the scheme of Littler & Littler (2000), neither of these species is then known by its alternate life history phase in Hawaii. Yet both are extremely common on shallow reef flats, growing side-by-side and occupying similar microhabitats. Lastly, if a separate whorled species is to be recognized, the earliest name available is *G. collabens*, a species described from Western Australia.

As proposed by Howe (1917, 1918), the obvious answer is to undertake culture studies to ascertain the morphology of the tetrasporophytes of *G. rugosa*, but as yet no-one has attempted this and the requirement to grow plants to maturity under a variety of conditions to assess the consistency of whorl production is

daunting. We have chosen a different, simpler, approach and have undertaken a DNA sequence analysis of Hawaiian plants representing *G. rugosa* gametophytes and “*G. subverticillata*”, in an attempt to answer this question: Are *G. rugosa* and *G. subverticillata*, as represented in Hawaii, the gametophyte and sporophyte phases of a single species?

The second component of the present study was prompted by the recent findings of Huisman *et al.* (2004c), based on LSU rDNA sequences, which resulted in *Galaxaura* being subdivided, with several species removed to a resurrected *Dichotomaria* Lamarck (1816: 143). *Dichotomaria marginata* (Ellis *et Solander*) Lamarck (with *Galaxaura marginata* as a homotypic synonym) was proposed as the lectotype. We have also examined some Hawaiian representatives potentially referable to *Dichotomaria*. These included two forms of *Dichotomaria marginata*, the more typical flattened type and a subterete form, the latter seemingly conforming to *Galaxaura infirma* Kjellman, a species with a type locality in Hawaii (Svedelius, 1953: fig. 18), but not mentioned in recent works. *Galaxaura infirma* was initially described from gametophytic plants, but the general habit corresponds well to the subterete tetrasporophytic plants examined herein. Svedelius (1953: 27) reported that *G. infirma* was often gathered growing together with the tetrasporophytic *G. acuminata* and *G. subverticillata*, and suggested that one of those species might represent its tetrasporophyte phase. Abbott (1999) treated both the flattened and subterete forms as representing *G. marginata*. Our objectives for this part of the study were twofold: firstly to confirm the conspecificity of Hawaiian *Dichotomaria marginata* with specimens from near the type locality, and secondly, to examine the conspecificity of the two morphotypes.

MATERIALS AND METHODS

For the gene sequence analyses, silica gel-dried portions of plants were ground in liquid nitrogen and the DNA extracted according to the protocol of Saunders (1993). The LSU gene of the nuclear ribosomal cistron was PCR amplified in three overlapping fragments (X, Y, and Z, as described in Harper & Saunders 2001a) using the primer pairs and cycling conditions described in Harper & Saunders (2001a). The PCR products were cleaned using a QIAquick PCR Purification Kit (Valencia, California, U.S.A.) according to the manufacturer's instructions. Cleaned products were visualized on a 2 % agarose gel to ensure that sufficient product was present for the sequencing reactions. Double-stranded PCR products were sequenced in both directions using the BigDye version 3.0 sequencing chemistry (ABI; Foster City, California, U.S.A.) and an MJ Research Basestation 51 Automated DNA sequencer. Resulting sequence files were examined using the Cartographer v.1.0 software (MJ Research Inc., San Francisco, California, U.S.A.), and sequence fragments were edited and assembled using Sequencher v.3.0 (Gene Codes Corporation; Ann Arbor, Michigan, U.S.A.).

A gene sequence alignment containing the newly sequenced samples was assembled using a text editor (Table 2). The alignment was analyzed using Modeltest v.3.06 (Posada & Crandall, 1998) and was determined to be most suitable for a parameter-rich general time reversible model. In all analyses the following taxa were used as outgroups: *Halosaccion glandiforme* (Gmelin)

Table 2. GenBank accession numbers or collection information for samples included in phylogenetic analyses for this study.

<i>Taxon</i>	<i>Publication source or collection information</i>	<i>GenBank Accession number</i>
<i>Actinotrichia fragilis</i> (Forsskål) Børgesen	Huisman <i>et al.</i> (2004c)	AY570363
<i>Akalaphycus setchelliae</i> (Yamada) Huisman, Abbott <i>et</i> Sherwood #1	Huisman <i>et al.</i> (2004b)	AY523825
<i>A. setchelliae</i> #2	Huisman <i>et al.</i> (2004b)	AY523826
<i>A. setchelliae</i> #3	Huisman <i>et al.</i> (2004b)	AY523827
<i>Colaconema dasyae</i> (Collins) Stegenga <i>et al.</i>	Harper & Saunders (2001b)	AF419100
<i>C. daviesii</i> (Dillwyn) Stegenga	Harper & Saunders (2002)	AF528047
<i>C. endophyticum</i> (Batters) Harper <i>et</i> Saunders	Harper & Saunders (2001b)	AF419101
<i>C. rhizoideum</i> (Drew) Gabrielson	Harper & Saunders (2002)	AF528050
<i>Cumagloia andersonii</i> (Farlow) Setchell <i>et</i> Gardner	Harper & Saunders (2001b)	AF419137
<i>Dichotomaria australis</i> (Sonder) Huisman, Harper <i>et</i> Saunders	Harper & Saunders (2001b)	AF419138
<i>D. diesingiana</i> (Zanardini) Huisman, Harper <i>et</i> Saunders	Huisman <i>et al.</i> (2004c)	AY570364
<i>D. marginata</i> (Ellis <i>et</i> Solander) Lamarck (Hawaii - flattened)	Pupukea, O'ahu, 14 July 2003, <i>J. Huisman & D. Spafford</i> , IA 30662	AY656802
<i>D. marginata</i> (Hawaii - subterete)	Makai Pier, Waimānalo, O'ahu, 12 May 2003, <i>J. Huisman</i> , IA 30176	AY656803
<i>D. marginata</i> (Puerto Rico)	Huisman <i>et al.</i> (2004c)	AY570365
<i>D. obtusata</i> (Ellis <i>et</i> Solander) Lamarck	Huisman <i>et al.</i> (2004c)	AY570366
<i>D. tenera</i> (Kjellman) Huisman, Harper <i>et</i> Saunders	Huisman <i>et al.</i> (2004c)	AY570367
<i>Galaxaura rugosa</i> (Ellis <i>et</i> Solander) Lamouroux (Australia)	Huisman <i>et al.</i> (2004c)	AY570368
<i>G. rugosa</i> (Hawaii)	Maili, O'ahu, south of beach park in lagoon, 0.5-1m depths, 3 April 2003, <i>J. Huisman</i> , IA 30097a	AY656804
<i>G. "subverticillata"</i> Kjellman (Hawaii)	Maili, O'ahu, south of beach park in lagoon, 0.5-1m depths, 3 April 2003, <i>J. Huisman</i> , IA 30097b	AY656805
<i>Ganonema farinosum</i> (Lamouroux) Fan <i>et</i> Yung C.Wang	Huisman <i>et al.</i> (2004a)	AY424880
<i>G. papenfussii</i> (Abbott) Huisman, Abbott <i>et</i> Sherwood	Huisman <i>et al.</i> (2004a)	AY424877
<i>G. pinnatum</i> (Harvey) Huisman	Huisman <i>et al.</i> (2004a)	AY424878
<i>G. samaense</i> (Tseng) Huisman	Huisman <i>et al.</i> (2004a)	AY424879
<i>G. yoshizakii</i> Huisman, Abbott <i>et</i> Sherwood	Huisman <i>et al.</i> (2004a)	AY424876
<i>Halosaccion glandiforme</i> (Gmelin) Ruprecht	Harper & Saunders (2002)	AF528052
<i>Liagora albicans</i> Lamouroux #1	Huisman <i>et al.</i> (2004a)	AY424872
<i>L. albicans</i> #2	Huisman <i>et al.</i> (2004a)	AY523820

<i>Taxon</i>	<i>Publication source or collection information</i>	<i>GenBank Accession number</i>
<i>L. divaricata</i> Tseng #1	Huisman <i>et al.</i> (2004a)	AY424871
<i>L. divaricata</i> #2	Huisman <i>et al.</i> (2004b)	AY523821
<i>L. donaldiana</i> Abbott <i>et Huisman</i>	Huisman <i>et al.</i> (2004a)	AY424875
<i>L. julieae</i> Abbott <i>et Huisman</i>	Huisman <i>et al.</i> (2004a)	AY424873
<i>L. perennis</i> Abbott #1	Huisman <i>et al.</i> (2004a)	AY424870
<i>L. perennis</i> #2	Huisman <i>et al.</i> (2004b)	AY523822
<i>L. valida</i> Harvey	Huisman <i>et al.</i> (2004a)	AY424874
<i>Palmaria palmata</i> (L.) Kuntze	Van der Auwera <i>et al.</i> (1998)	Y11506
<i>Rhodochorton purpureum</i> (Lightfoot) Kützing	Harper & Saunders (2001b)	AF419103
<i>R. tenue</i> Kylin	Harper & Saunders (2002)	AF421126
<i>Rhodophysema elegans</i> (Crouan <i>et Crouan ex J. Agardh</i>) Dixon	Harper & Saunders (2001b)	AF419140
<i>Stenopeltis gracilis</i> (Yamada <i>et Tanaka</i>) Itono <i>et Yoshizaki</i> #1	Huisman <i>et al.</i> (2004b)	AY523823
<i>S. gracilis</i> #2	Huisman <i>et al.</i> (2004b)	AY523824
<i>Trichogloea lubrica</i> J. Agardh	Huisman <i>et al.</i> (2004b)	AY523828
<i>Tricleocarpa cylindrica</i> (Ellis <i>et Solander</i>) Huisman <i>et Borowitzka</i>	Huisman <i>et al.</i> (2004c)	AY570369
<i>Yamadaella caenomyce</i> (Decaisne) Abbott	Huisman <i>et al.</i> (2004a)	AY424881

Ruprecht, *Palmaria palmata* (Linnaeus) Kuntze, *Rhodochorton purpureum* (Lightfoot) Rosenvinge, *R. tenue* Kylin and *Rhodophysema elegans* (P. Crouan *et H. Crouan ex J. Agardh*) Dixon (Table 2). Distance analyses (using PAUP, version 4; Swofford 2001) were used to construct trees under minimum evolution, using the parameters determined by Modeltest. A Bayesian estimate of the phylogeny was obtained using the program MrBayes v.2.01 (Huelsenbeck & Ronquist, 2001). The analysis used four Markov chains, the temperature was 0.2 and 10⁶ generations were run with sampling every 100 generations. Log-likelihood values became stabilized around 32,000 generations and we used the final 6000 trees to calculate posterior probabilities. Parsimony trees were constructed using the heuristic search option with random sequence addition (100 replicates), steepest descent, and tree bisection-reconnection (TBR) branch swapping. Gaps were coded as “missing data”. Both parsimony and distance analyses were subject to bootstrap resampling (2000 replicates) to estimate robustness of nodes on the trees (Felsenstein, 1985).

Specimens

Galaxaura rugosa and *G. “subverticillata”*, Maili, O’ahu, south of beach park in lagoon, 0.5-1m depths, 3 April 2003, *J. Huisman*, IA 30097a & b (Figs 1 & 2).

Dichotomaria marginata (flattened form), Pupukea, O’ahu, from 9 m depth, 14 July 2003, *J. Huisman* & *D. Spafford*, IA 30662. (Figs 7 & 8).

Dichotomaria marginata (subterete form), Makai Pier, Waimānalo, O’ahu, 12 May 2003, *J. Huisman*, IA 30176 (Figs 9, 10).

RESULTS

Morphology

Galaxaura rugosa (Ellis et Solander) Lamouroux and “*G. subverticillata* Kjellman”

The habit and structure of the specimens used are depicted in Figs 1-6. Plants are similar in stature (Figs 1, 2), but differ in their cortical morphologies. The surface of *G. rugosa* (Fig. 3) is mostly glabrous (short hairs are sometimes present in lower portions of the plants) and the cortex in section shows a pseudoparenchymatous construction (Fig. 4). Plants *in situ* are generally a pale to bright orange colour and stand out from their surroundings. In contrast, the surface of *G. subverticillata* has prominent whorls of emergent filaments (Fig. 5) and in section the cortex is essentially filamentous in structure (Fig. 6). Plants *in situ* are a deep red colour and often accumulate detritus; as such they are generally less conspicuous.

Dichotomaria marginata (Ellis et Solander) Lamarck

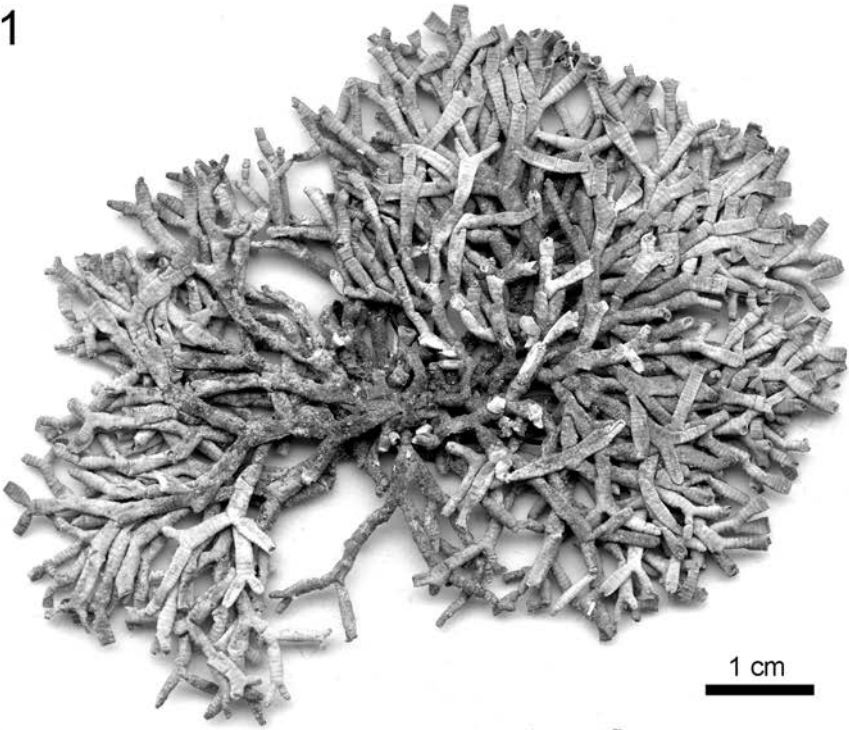
The specimens used in this study were similar in stature, but differed in either having broad and flattened branches with thickened margins (Figs 7, 8), conforming to typical *D. marginata*, or narrower, subterete branches (Fig. 9), conforming to *Galaxaura infirma*. In section the cortex of the specimens used was typical of tetrasporophytes of *Dichotomaria marginata*, having a surface layer of paired ellipsoid cells, each with a small terminal spine (Fig. 10).

DNA analysis

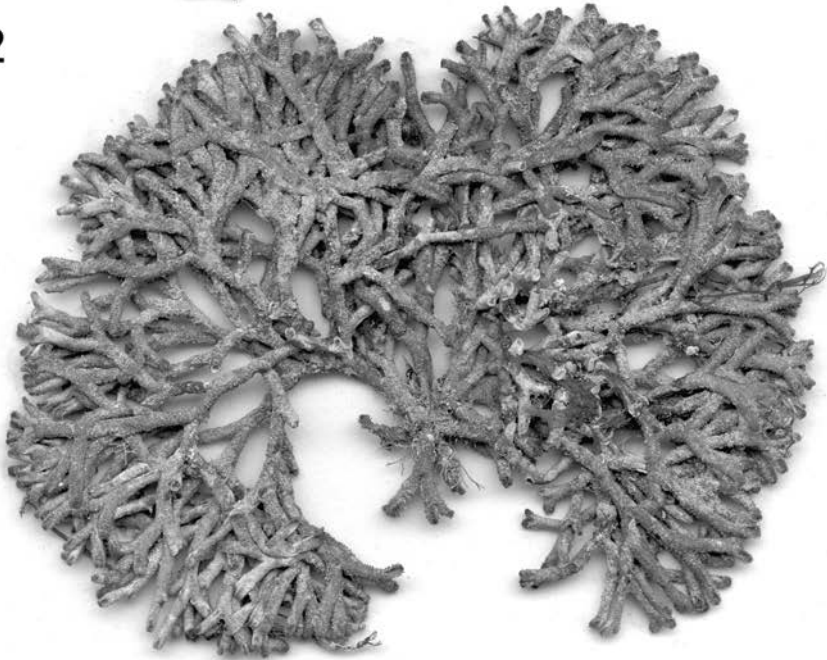
Parsimony analysis of the LSU alignment containing members of the Nemaliales, Colaconematales and a selection of outgroup taxa yielded 310 most-parsimonious trees with a length of 1111 steps. The final alignment was 2577 bases long and contained 2037 constant characters and 340 phylogenetically informative characters. Trees produced by parsimony analysis, minimum evolution distance analysis and Bayesian analysis were all similar topologically, and so only the distance tree is shown, with the support values for all three forms of analysis superimposed at the respective nodes (Fig. 11). The outgroup taxa are clearly separated from the ingroup, and the Colaconematales forms a well-supported monophyletic clade that is sister to the Nemaliales. Large-subunit rRNA gene sequences obtained for *Galaxaura rugosa* and *G. subverticillata* from the Hawaiian islands were identical, strongly supporting the unity of these two taxa. These two taxa form a clade that is sister to *Galaxaura rugosa* from Australia (differing by only four nucleotide positions, thus supporting all three specimens belonging to the same taxon). The flattened and subterete specimens of *Dichotomaria marginata* also formed a well supported clade that is sister to *Dichotomaria marginata* from Puerto Rico.

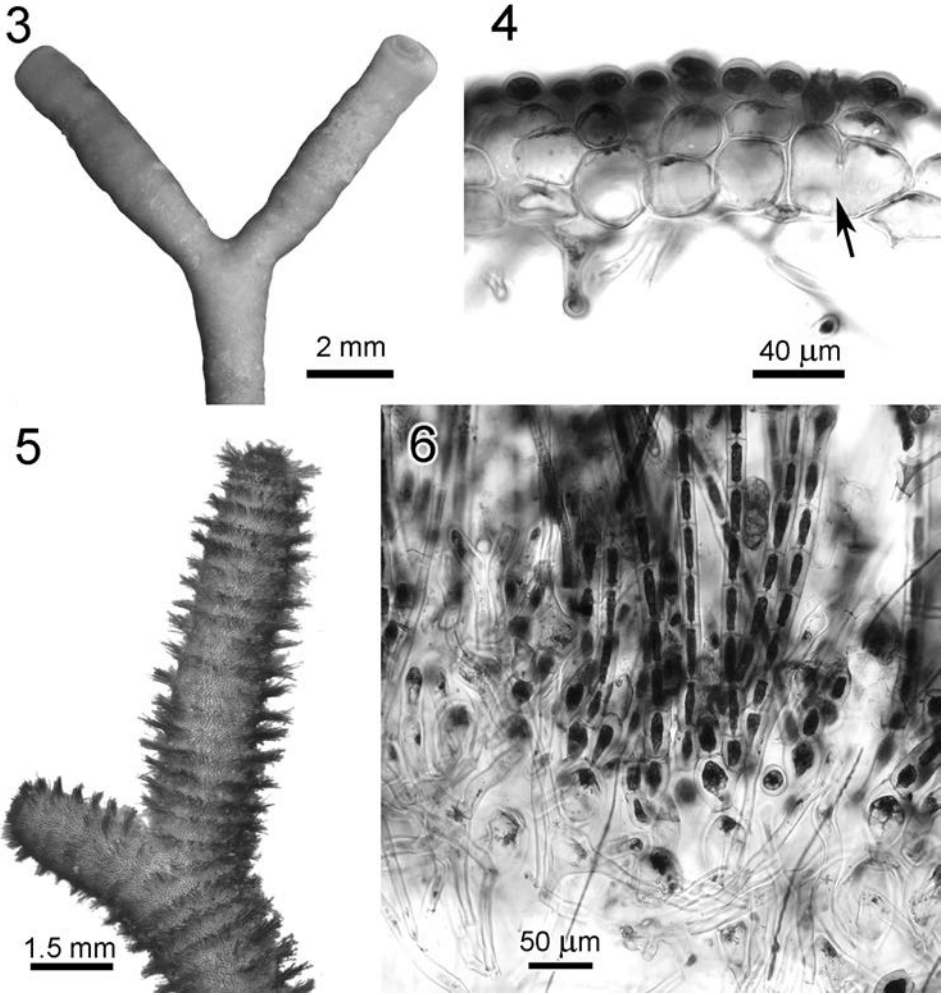
Figs 1-2. *Galaxaura rugosa* (Ellis et Solander) Lamouroux and “*G. subverticillata*”. Fig. 1. Morphology of pressed voucher specimen of *G. rugosa* from Maili, O’ahu (IA 30097a). Fig. 2. Morphology of pressed voucher specimen of “*G. subverticillata*” from Maili, O’ahu (IA 30097b).

1



2

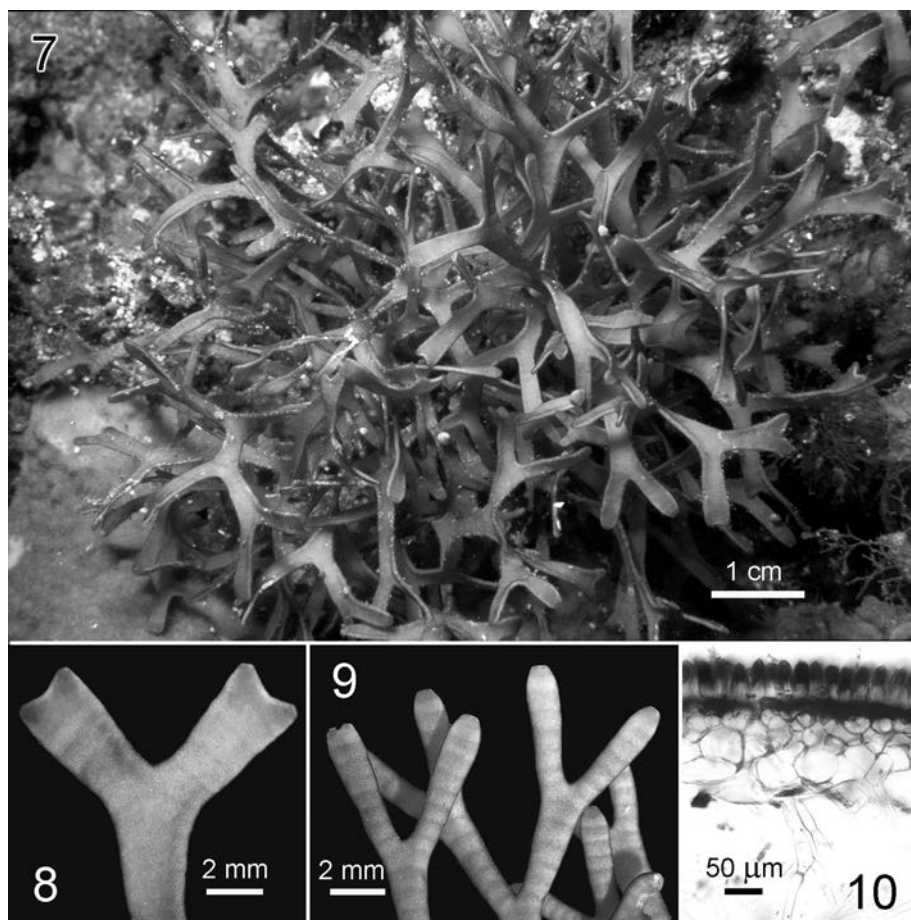




Figs 3-6. Fig. 3. Detail of branch apex of *G. rugosa* showing glabrous surface (IA 30097a). Fig. 4. Detail of cortical structure in *G. rugosa* gametophyte phase (IA 30097a). Fig. 5. Detail of branch apex of "*G. subverticillata*" showing whorls of surface filaments (IA 30097b). Fig. 6. Details of cortical structure in "*G. subverticillata*" sporophyte (IA 30097b).

DISCUSSION

The results of our study show that the sequences of *G. rugosa* and *G. subverticillata* as represented in Hawaii are identical. While this result does not unequivocally prove that the two represent the same species, it is a strong indication of such. In all independent species subjected to the same analyses, some differences in DNA sequences were always present. Thus we feel that our results provide compelling evidence for the recognition of *G. subverticillata* as representing



Figs 7-10. *Dichotomaria marginata* (Ellis et Solander) Lamarck. Fig. 7. Habit of plant *in situ* at Pupukea, O'ahu (IA 30662). Fig. 8. Detail of branch apex of the flattened form (IA 30662). Fig. 9. Detail of branch apex of the subterete form (IA 30167). Fig. 10. Cortical structure, showing apiculate outer cells (IA 30176).

the sporophyte of *G. rugosa*. This result agrees with the interpretation given by several earlier workers.

As we were unable to sequence any plants conforming to *G. lapidescens*, we cannot confirm the conspecificity of that species with *G. rugosa*/*G. subverticillata*. In our view it is unlikely, however, that *G. lapidescens* represents an independent species, for all of the same reasons given above that cast doubt on the autonomy of *G. subverticillata*. Virtually all authors have commented on the variability of the feature that separates these two "species" (the arrangement of surface filaments in whorls) and the various synonymies proposed (Table 1) lead to the conclusion that *G. lapidescens* and *G. subverticillata* represent the same taxon. If these two are not synonymous and are regarded as separate entities, then equally there must

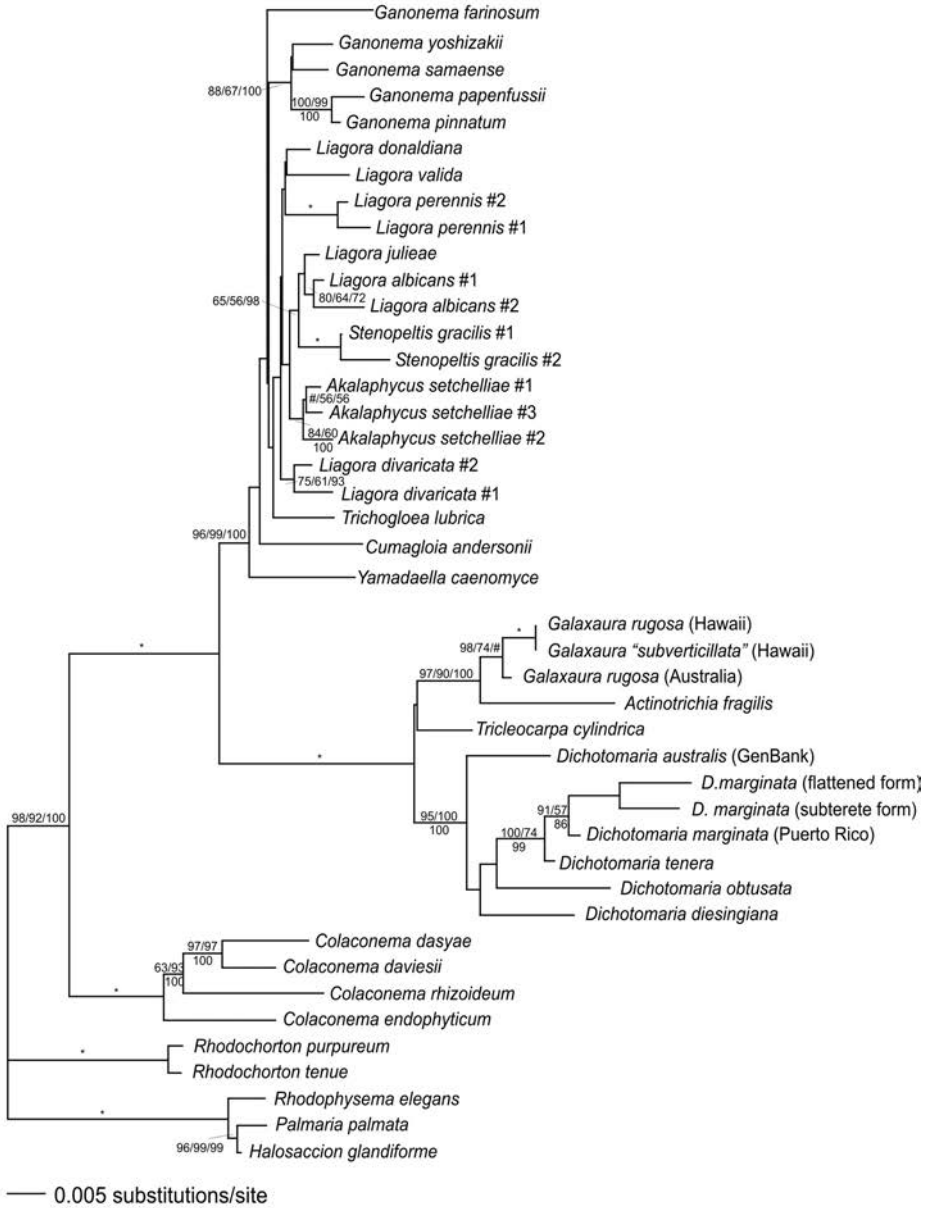


Fig. 11. Minimum evolution distance analysis illustrating the close phylogenetic relationship between *G. rugosa* and "*G. subverticillata*". Support values for all three forms of analysis are indicated at relevant nodes as follows: first value = minimum evolution bootstrap, second value = parsimony bootstrap, third value = Bayesian posterior probability. Values < 50% are either not shown or indicated by (#). Nodes with 100% support from all three analyses are labeled (*).

be two corresponding gametophytes and *G. rugosa* would be a complex including at least one cryptic species. While we cannot discount this, it is not consistent with the available data and our present understanding of the species. Further sequence data from a range of plant morphologies and from specimens collected at or near type localities are desirable, however, to confirm our interpretation. And yes, as proposed by Howe and many others, culture studies would be most illuminating.

Our sequence analyses also show that *G. rugosa* from Hawaii is sister to *G. rugosa* from Australia. Some minor differences were present, but these are consistent with minor differences found in other purportedly conspecific samples and probably do not warrant the recognition of additional taxa. Until further sampling is undertaken, and sequences of *G. rugosa* from near the type locality are obtained, we defer any speculation. A similar result was found with *Dichotomaria marginata*, with the two Hawaiian samples forming a clade sister to *D. marginata* from Puerto Rico [regarded by Huisman *et al.* (2004c) as representative of the type]. Again, the importance of these differences cannot be ascertained without further sampling. It is clear, however, that the flattened and subterete forms of *D. marginata* form a strongly supported clade and most likely represent a single species. This observation agrees with the interpretation of *D. marginata* by Børgesen (1915: 108) and Huisman *et al.* (2004c), that not all plants are distinctly flattened, some are terete.

Three additional species of *Galaxaura* are recorded for the Hawaiian Islands by Abbott (1999). Of those, *G. obtusata* was reinstated in *Dichotomaria* by Huisman *et al.* (2004c), as *D. obtusata* (Ellis *et Solander*) Lamarck. *Galaxaura filamentosa* Chou is unknown reproductively and its status is yet to be clarified. Huisman & Borowitzka (1990) regarded Australian plants morphologically comparable to *G. filamentosa* as representing a basal, undifferentiated portion of *G. rugosa*, but they maintained the former until further studies could be undertaken. *Galaxaura fasciculata* Kjellman was referred to the synonymous *G. cohaerens* Kjellman by Huisman & Borowitzka (1990), to avoid possible confusion regarding the type specimen, and that species was subsequently synonymized with *Galaxaura divaricata* (Linnaeus) Huisman *et Townsend* (1993: 100). *Galaxaura divaricata* displays an unusual cortical morphology for a species of *Galaxaura* and it was suggested by Huisman & Borowitzka (1990) that further studies might show the entity to be a member of *Actinotrichia*. A DNA sequence study incorporating *G. filamentosa* and *G. divaricata* is clearly highly desirable to clarify their relationships with other members of the Galaxauraceae.

In addition, several species of *Galaxaura* recorded for Hawaii have subsequently been included in the genus *Tricleocarpa* Huisman *et Borowitzka* (1990: 164). Two species are presently recognized in the genus: *T. cylindrica* (Ellis *et Solander*) Huisman *et Borowitzka* and *T. fragilis* (Linnaeus) Huisman *et Townsend* [including *Galaxaura oblongata* (Ellis *et Solander*) Lamouroux]. Unfortunately, the placement of *G. cylindrica* in the synonymy of *G. oblongata* [proposed by various authors including Papenfuss *et al.* (1982), but later rejected by Huisman & Borowitzka (1990)], resulted in many records of the former being included in the latter, and therefore subsequently being attributed to *T. fragilis* when they are probably more appropriately placed in *T. cylindrica*. For example, Magruder's study of the life history of "*G. oblongata*" (Magruder, 1984) describes a cystocarp with gonimoblasts lining the pericarp, which is clearly referable to *T. cylindrica* as defined by Huisman & Borowitzka (1990). It would appear (Huisman, unpublished observations) that *T. cylindrica* is the more common of the species in Hawaii, yet very few of the older records have been attributed to this species. Further study is needed to establish the identities of the Hawaiian *Tricleocarpa*.

Table 3. Species of *Galaxaura* recorded for the Hawaiian Islands by Butters (1911), Svedelius (1953), Magruder (1984), and Abbott (1999), and their current placement.

<i>Species</i>	<i>Current Name</i>	<i>References</i>
<i>G. acuminata</i> Kjellman ex Butters.	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	Abbott (1999: 67)
<i>G. annulata</i> Lamouroux	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Abbott (1999: 69)
<i>G. arborea</i> Kjellman	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	Huisman & Borowitzka (1990: 157); Huisman <i>et al.</i> (2004c)
<i>G. contigua</i> Kjellman	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	Abbott (1999: 67); Huisman <i>et al.</i> (2004c)
<i>G. cuculligera</i> Kjellman	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Papenfuss <i>et al.</i> (1982: 422); Abbott (1999: 69)
<i>G. fastigiata</i> Decaisne	<i>Tricleocarpa cylindrica</i> (Ellis et Solander) Huisman et Borowitzka	Huisman & Borowitzka (1990: 164)
<i>G. fasciculata</i> Kjellman	<i>G. divaricata</i> (Linnaeus) Huisman et Townsend	Huisman & Townsend (1993)
<i>G. filamentosa</i> Chou	<i>G. filamentosa</i> Chou	Abbott (1999: 67)
<i>G. fragilis</i> Lamarck	<i>Tricleocarpa fragilis</i> (Linnaeus) Huisman et Townsend	Abbott (1999: 74)
<i>G. glabriuscula</i> Kjellman	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Papenfuss <i>et al.</i> (1982: 422); Abbott (1999: 69)
<i>G. hawaiiiana</i> Butters	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Abbott (1999: 68)
<i>G. hystrix</i> Kjellman	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	Suggested by Papenfuss <i>et al.</i> (1982: 414); Abbott (1999: 68)
<i>G. infirma</i> Kjellman	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	herein
<i>G. intricata</i> Kjellman	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Abbott (1999: 68)
<i>G. marginata</i> (Ellis et Solander) Lamouroux	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	Huisman <i>et al.</i> (2004c)
<i>G. mauiana</i> Butters	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	Abbott (1999: 67); Huisman <i>et al.</i> (2004c)
<i>G. oblongata</i> (Ellis et Solander) Lamouroux p.p.	<i>Tricleocarpa cylindrica</i> (Linnaeus) Huisman et Borowitzka	Huisman & Borowitzka (1990); Abbott (1999: 74)
<i>G. oblongata</i> (Ellis et Solander) Lamouroux p.p.	<i>Tricleocarpa fragilis</i> (Linnaeus) Huisman et Townsend	Huisman & Borowitzka (1990); Huisman & Townsend (1993); Abbott (1999: 74)
<i>G. obtusata</i> (Ellis et Solander) Lamouroux.	<i>Dichotomaria obtusata</i> (Ellis et Solander) Lamarck	Huisman <i>et al.</i> (2004c)
<i>G. pacifica</i> Tanaka	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Papenfuss <i>et al.</i> (1982: 422); Abbott (1999: 69)
<i>G. schimperi</i> Decaisne	<i>Tricleocarpa fragilis</i> (Linnaeus) Huisman et Townsend	Abbott (1999: 74)
<i>G. stupocaula</i> Kjellman	<i>Dichotomaria marginata</i> (Ellis et Solander) Lamarck	Papenfuss <i>et al.</i> (1982: 411)
<i>G. subfruticulosa</i> Chou	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Huisman & Borowitzka (1990); herein.
<i>G. subverticillata</i> Kjellman	<i>G. rugosa</i> (Ellis et Solander) Lamouroux	Huisman & Borowitzka (1990); herein.

The fates of the various species of *Galaxaura* recorded for the Hawaiian Islands by Butters (1911), Svedelius (1953), Magruder (1984), and Abbott (1999) are summarized in Table 3.

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REFERENCES

- ABBOTT I.A., 1999 — *Marine Red Algae of the Hawaiian Islands*. Honolulu, Bishop Museum Press, xv + 477 pp.
- BØRGESEN F., 1927 — Marine algae from the Canary Islands especially from Teneriffe and Gran Canaria. III. Rhodophyceae. Part I. Bangiales and Nemalionales. *Kongelige Danske Videnskabernes Selskab, Biologiske Meddelelser* 6(6): 1-97.
- BØRGESEN F., 1915 — The marine algae of the Danish West Indies. Part 3. Rhodophyceae (1). *Dansk Botanisk Arkiv* 3: 1-80.
- BØRGESEN F., 1949 — Some marine algae from Mauritius. Additions to the parts previously published. *Kongelige Danske Videnskabernes Selskab, Biologiske Meddelelser* 21(5): 1-48.
- BUTTERS F.K., 1911 — Notes on the species of *Liagora* and *Galaxaura* of the central Pacific. *Minnesota Botanical Studies* 4: 161-184, pl. 24.
- ELLIS J. & SOLANDER D., 1786 — *The natural history of many curious and uncommon zoophytes, collected from various parts of the globe by the late John Ellis...Systematically arranged and described by the late Daniel Solander*. London: B. White & Son, xii + 208 pp., 63 plates.
- FELSENSTEIN J., 1985 — Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- HARPER J.T. & SAUNDERS G.W., 2001a — The application of sequences of the ribosomal cistron to the systematics and classification of the florideophyte red algae (Florideophyceae, Rhodophyta). *Cahiers de Biologie Marin*. 42: 25-38.
- HARPER J.T. & SAUNDERS G.W., 2001b — Molecular systematics of the Florideophyceae (Rhodophyta) using nuclear large and small subunit rDNA sequence data. *Journal of Phycology* 37: 1073-1082.
- HARPER J.T. & SAUNDERS G.W., 2002 — A re-classification of the Acrochaetiales based on molecular and morphological data, and establishment of the Colaconematales ord. nov. (Florideophyceae, Rhodophyta). *European Journal of Phycology* 37: 463-476.
- HOWE M.A., 1917 — A note on the structural dimorphism of sexual and tetrasporic plants of *Galaxaura obtusata*. *Bulletin of the Torrey Botanical Club* 43: 621-624.
- HOWE M.A., 1918 — Further notes on the structural dimorphism of sexual and tetrasporic plants in the genus *Galaxaura*. *Brooklyn Botanic Garden Memoirs* 1: 191-197, 4 figs, Plates III, IV.
- HUELSENBECK J.P. & RONQUIST F.R., 2001 — MRBAYES: Bayesian inference of phylogeny. *Biometrics* 17: 754-755.
- HUISMAN J.M. & BOROWITZKA M.A., 1990 — A revision of the Australian species of *Galaxaura* (Rhodophyta, Nemaliales), with a description of *Tricleocarpa* gen. nov. *Phycologia* 29: 150-172.
- HUISMAN J.M. & TOWNSEND R.A., 1993 — An examination of Linnaean and pre-Linnaean taxa referable to *Galaxaura* and *Tricleocarpa* (Galaxauraceae, Rhodophyta). *Botanical Journal of the Linnean Society* 113: 95-101.

- HUISMAN J.M., ABBOTT I.A. & SHERWOOD A.R. 2004a — The Liagoraceae (Nemaliales, Rhodophyta) of the Hawaiian Islands III: The genus *Ganonema*, with a description of *Ganonema yoshizakii* sp. nov. *Phycologia* 43: 296-310.
- HUISMAN J.M., ABBOTT I.A. & SHERWOOD A.R. 2004b — Large subunit rDNA gene sequences and reproductive morphology reveal *Stenopeltis* as a member of the Liagoraceae, with a description of *Akalaphycus* gen. nov. *European Journal of Phycology* 39: 257-272.
- HUISMAN J.M., HARPER J.T. & SAUNDERS G.W., 2004c — Phylogenetic study of the Nemaliales (Rhodophyta) based on large-subunit ribosomal DNA sequences supports segregation of the Scinaiaaceae fam. nov. and resurrection of *Dichotomaria* Lamarck. *Phycological Research* 52: 224-234.
- KJELLMAN F.R., 1900 — Om Floridé-släget *Galaxaura*, dess organografi och systematik. *Kongl. Svenska Vetenskaps-Akademiens Handlingar [ser. 4]* 33(1): 1-109, 20 plates.
- LAMARCK J.B. DE, 1816 — *Histoire naturelle des animaux sans vertèbres...* Vol. 2. Paris, 568 pp.
- LAMOUREUX J.V.F., 1812 — Sur la classification des Polypiers coralligènes non entièrement pierreux. *Nouveau Bulletin des Sciences, par la Société Philomathique de Paris* 3: 181-188.
- LITTLER D.S. & LITTLER M.M., 1997 — An illustrated flora of the Pelican Cays, Belize. *Bulletin of the Biological Society of Washington* 9: 1-149, 190 figs.
- LITTLER D.S. & LITTLER M.M., 2000 — *Caribbean reef plants. An identification guide to the reef plants of the Caribbean, Bahamas, Florida and Gulf of Mexico*. Washington: Offshore Graphics, 542 pp.
- MAGRUDER W.H., 1984 — Reproduction and life history of the red alga *Galaxaura oblongata* (Nemaliales, Galaxauraceae). *Journal of Phycology* 20: 402-409.
- PAPENFUSS G.F. & CHIANG, Y.-M., 1969 — Remarks on the taxonomy of *Galaxaura* (Nemaliales, Chaetangiaceae). In: *Proceedings of the Sixth International Seaweed Symposium, Santiago de Compostela...1968*. Subsecretaría de la Marina Mercante, Madrid, pp. 303-314.
- PAPENFUSS G.F., MSHIGENI K.E. & CHIANG Y.-M., 1982 — Revision of the red algal genus *Galaxaura* with special reference to the species occurring in the western Indian Ocean. *Botanica Marina* 25: 401-444.
- POSADA D. & CRANDALL K.A., 1998 — Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- SAUNDERS G.W., 1993 — Gel purification of red algal genomic DNA: an inexpensive and rapid method for the isolation of polymerase chain reaction-friendly DNA. *Journal of Phycology* 29: 251-254.
- SCHNEIDER C.W., 2003 — An annotated checklist and bibliography of the marine macroalgae of the Bermuda Islands. *Nova Hedwigia* 76: 275-361.
- SVEDELIUS N., 1945 — Critical notes on some species of *Galaxaura* from Ceylon. *Arkiv för Botanik* 32A(6): 1-74, 22 figs, IX plates.
- SVEDELIUS N., 1953 — Critical studies of some species of *Galaxaura* from Hawaii. *Nova Acta Regiae Societatis Scientiarum Upsaliensis Ser. IV* 15(9): 1-92.
- SWOFFORD D.L., 2001 — PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- VAN DER AUWERA G., HOFMANN C.J., DE RIJK P. & DE WACHTER R., 1998 — The origin of red algae and cryptomonad nucleomorphs: A comparative phylogeny based on small and large subunit rRNA sequences of *Palmaria palmata*, *Gracilaria verrucosa*, and the *Guillardia theta* nucleomorph. *Molecular Phylogenetics and Evolution* 10: 333-342.