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Evolutionary history of the Corallinales (Corallinophycidae, Rhodophyta) inferred from nuclear, plastidial and mitochondrial genomes

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Abstract

Systematics of the red algal order Corallinales has a long and convoluted history. In the present study, molecular approaches were used to assess the phylogenetic relationships based on the analyses of two datasets: a large dataset of SSU sequences including mainly sequences from GenBank; and a combined dataset including four molecular markers (two nuclear: SSU, LSU; one plastidial: *psb*A; and one mitochondrial: COI). Phylogenetic analyses of both datasets re-affirmed the monophyly of the Corallinales as well as the two families (Corallinaceae and Hapalidiaceae) currently recognized within the order. Three of the four subfamilies of the Corallinaceae (Corallinoideae, Lithophylloideae, Metagoniolithoideae) were also resolved as a monophyletic lineage whereas members of the Mastophoroideae were resolved as four distinct lineages. We therefore propose to restrict the Mastophoroideae to the genera *Mastophora, Metamastophora,* and possibly *Lithoporella* in the aim of rendering this subfamily monophyletic. In addition, our phylogenies resolved the genus *Hydrolithon* in two unrelated lineages, one containing the gener- itype *Hydrolithon reinboldii* and the second containing *Hydrolithon onkodes,* which used to be the generitype of the now defunct genus *Porolithon*. We therefore propose to resurrect the genus *Porolithon* for the second lineage encompassing those species with primarily monomerous thalli, and trichocyte arrangements in large pustulate horizontal rows. Moreover, our phylogenetic analyses revealed the presence of cryptic diversity in several taxa, shedding light on the need for further studies to better circumscribe species frontiers within the diverse order Corallinales, especially in the genera *Mesophyllum* and *Neogoniolithon*.

Keywords: *COI,* Corallinales, Mastophoroideae, LSU rDNA, Phylogeny, Porolithon, psbA, SSU, rDNA

1. Introduction

The Corallinales, along with the Sporolithales (Corallinophycidae, Rhodophyta), is an intriguing red algal order characterized by the presence of calcite in their cell walls. This calcification capacity confers them a crucial ecological role especially in coral reef construction (Steneck 1986; Payri 1995; Amado-Filho et al., 2007) and a paleontological significance (Payri and Cabioch, 2003; Cabioch et al., 2008) due to their strong ability to become fossilized (Aguirre et al., 2010). However, coralline identification is largely hampered by phenotypic plasticity depending on environmental conditions (Steneck and Adey, 1976; Woelkerling et al., 1993a; Maneveldt and Keats, 2008) as well as the need for decalcification prior to the observation of anatomical features.

The taxonomy of the coralline algae has been extremely convoluted (e.g. Lamy and Woelkerling, 1998). The order Corallinales was formally segregated from the Cryptonemiales by Silva and Johansen (1986), who considered it with the same delimitation as the family Corallinaceae. The comprehension of the Corallinales affinities within the Florideophyceae, as well as their infra ordinal diversity, were greatly improved thanks to the advent of phyloge- nies inferred from molecular data. Molecular phylogenies based on ribosomal operons (Saunders and Bailey, 1997; Harper and Saunders, 2001a), confirmed that the Corallinales form a genetically divergent lineage among the remaining floridophycean orders. Interestingly, all taxa within the Corallinales possess primary pit plugs with two cap layers, corroborating Pueschel's (1989) hypotheses on the taxonomic importance of pit plug ultrastructures. The addition of a novel nuclear marker EF2 (elongation factor 2) (Le Gall and Saunders, 2007), as well as the mining of data available from GenBank (Verbruggen et al., 2010), greatly improved the resolution of the red algal relationships: the Coralli- nales and Rhodogorgonales were resolved and confirmed as strong allies within a lineage distinct from the remaining florideo- phycean lineages, sister to a lineage gathering together the Ahnfeltiophycidae and the Rhodymeniophycidae. The Corallinales and Rhodogorgonales were thus assigned to a new subclass, the Corallinophycidae, which members are characterized both by primary pit plugs with two cap layers and the presence of calcite (Le Gall and Saunders, 2007).

Within the Corallinales, several classifications have been proposed based solely on morphological and anatomical characters (e.g. Cabioch, 1972, 1988; Johansen, 1976; Woelkerling, 1988), which differ mainly by the weight given to vegetative and/or reproductive characters. Cabioch (1972) emphasized the importance of vegetative features (e.g. presence vs. absence of cell fusions and secondary pit connections) whereas Woelkerling (1988) considered mainly reproductive features. Bailey and

Chapman (1996, 1998) published the first molecular phylogenies of the Corallinales and confirmed the evolutionary scenario hypothesised by Cabioch (1988) that the geniculate forms had evolved independently in distinct lineages of the Corallinales. Based on their molecular data, Harvey et al. (2003) proposed the recognition of a new family within the Corallinales, the Hapalidiaceae, for taxa which tetrasporangia produce zonately arranged spores, but also which tetrasporangia develop in conceptacles beneath multiporate pore plates, and furthermore which produce tetrasporangial apical plugs. Within the Hapalidiaceae, Harvey et al. (2003) recognised three subfamilies: the Austrolithoideae, Choreonematoideae and Melobesioideae. Each of these subfamilies is defined by two morphological and anatomical characters: the presence or absence of cell fusions between cells of contiguous vegetative filaments and nature (cellular *vs.* acellular) of pore plate construction of the tet- rasporangial conceptacle (Supp. Mat. 1). The Melobesioideae are characterized by the presence of cell fusions between cells of contiguous vegetative filaments whereas the Austrolithoideae and Choreonematoideae are devoid of this feature. The Choreonematoi- deae in turn differs from the two previous subfamilies by the composition of the multiporate tetrasporangial conceptacle pore plate that is acellular at maturity, and composed only of a calcium carbonate, sieve-like matrix (Broadwater et al., 2002).

In addition, Harvey et al. (2003) conducted a thorough revision of the subfamilial circumscription among the living Corallinaceae and recognised four subfamilies, namely the Corallinoideae, Litho- phylloideae, Mastophoroideae and Metagoniolithoideae. Each of these subfamilies is defined by a combination of morphological and anatomical characters (Supp. Mat. 1).

Along with the Corallinaceae and Hapalidiaceae, Harvey et al. (2003) recognized the Sporolithaceae, proposed by Verheij (1993) for taxa characterized by cruciately divided tetrasporangia that develop individually in sori (calcified sporangial compartments) and which sori produce apical pore plugs. Le Gall et al. (2010) subsequently elevated this family to ordinal rank (the Sporolithales) because of its alliance in molecular phylogenies with the Rhodogorgonales in addition to its unique tetrasporangial development. Consequently, the Corallinales currently encompass two families namely the Corallinaceae and Hapalidiaceae, which share zonately divided tetrasporangia.

Phylogenies of the Corallinales published thus far suffer from a lack of resolution at the subfamily level, which was likely due to limited taxon sampling and the lack of signal of the molecular marker chosen to infer the phylogeny. Most of the coralline algal phylogenies published so far included only a few members (one or two) of the Mastophoroideae, whereas this subfamily currently comprises eight genera (Harvey et al., 2003). To circumvent this poor taxa sampling, Bailey et al. (2004) included in

their analyses six species belonging to three genera *(Hydrolithon, Neogoniolithon* and *Spongites)* of the Mastophoroideae and resolved the Mastopho- roideae as polyphyletic lineages. Unfortunately they did not include any representatives of the genus *Mastophora* (type genus of the subfamily) preventing them from proposing a revision of this subfamily. In addition, all the coralline algal phylogenies published until 2008 were inferred from a single marker, the SSU. Broom et al. (2008) proposed the plastidial gene *psbA* (encoding for the D1 protein of photosystem II) as a novel marker to be used in combination with SSU data to improve the phylogenetic resolution within the order. Walker et al. (2009) also showed the relevance of using a mitochondrial marker to get new insights into the genetic diversity at a lower taxonomic level; i.e. in this study the barcode marker (5' end of the COI, the cytochrome *c* oxidase subunit I) was sequenced for members of the Corallinoideae subfamily. Although promising and easy to amplify (Bittner et al., 2010), these two novel markers *(psb*A, COI) were studied for a restricted sample of morphologically identified taxa and their contribution to improve the phylogenetic resolution at the scale of the order Corallinales had yet to be tested.

The aim of the present study was thus to improve the resolution of the Corallinales infra-ordinal phylogenetic relationships. Toward this aim, two datasets were built: (1) a taxa rich SSU dataset including most sequences available in GenBank; and (2) a multi- marker dataset including two nuclear loci (SSU and LSU), one plas- tidial *(psb*A) and one mitochondrial (COI) genes. In order to meaningfully assess the delineation of the subfamily Mastophoroideae, we included up to 35 mastophoroid taxa, including representatives from the type genus *Mastophora*.

2. Material and methods

2.1 Collections and identification of taxa

Coralline algal samples were collected from a broad geographical range (Table 1) by snorkel or SCUBA diving. Specimens were dried as soon as possible after collection by placement in desiccant silica gel. Identification of the specimens was performed to the lowest possible taxonomic level possible through observation of vegetative and reproductive features on histological sections.

2.2 DNA extractions, PCR amplifications and sequencing

Coralline algal tissue was carefully removed under a dissecting microscope from part of the thallus free of epiphytes by scraping the surface with a razor blade. The excised tissue was ground using a mortar and pestle. DNA was extracted using the DNeasy Plant Kit (Qiagen Gmbh, Hilden, Germany) following the manufacturer's instructions after the lysis step, which was performed using an extraction buffer optimised for red algae (Saunders, 1993).

The SSU (18S) locus was amplified with two polymerase chain reactions (PCR) using primers G01/G08 and G04/G07, and was se- quenced using the PCR primers, as well as the internal primers G10, G06 following protocols of Saunders and Kraft (1994, 1996) and Harper and Saunders (2001a). LSU (28S) was amplified as three overlapping fragments using primers T01N/T20, T04/T08 and T05/T15, and using the PCR primers and the internal primers T10, T16N, T19N, T22, T24, T25, T30, T33, following protocols of Harper and Saunders (2001a) and Le Gall and Saunders (2010). The *psb*A was amplified and sequenced using primers psbAF1 and psbAR2 (Yoon et al., 2002) and the COI was amplified and sequenced using primers designed to amplify the barcode region in red algae: GazF1 and GazR1 (Saunders, 2005). PCR products were purified and sequenced by Genoscope (http://www.genoscope.fr).

Table 1
List of specimens used to generate DNA sequences (SSU, LSU, *psb*A and COI) for this study, their voucher numbers, collection information, and corresponding GenBank accession numbers. For these specimens, 258 sequences were newly generated and ten sequences were obtained and published in previous articles (GenBank accession numbers are underlined).

	Voucher	Authorities	Location	Date	Collectors	GenBank accession numbers			
						SSU	LSU	*psb*A	COI
Sporolithon ptychoides	LLG0745	Heydrich	New Caledonia	1st November 2007	C. Payri, L. Le Gall	GQ149066	GQ149068	GQ917502	GQ917307
Sporolithon sp.	LBC0567		Vanuatu	29th August 2006	C. Payri, J.L. Menou, C. Geoffray	GQ917379	–	GQ917500	GQ917259
Sporolithon sp.	LBC0695		Fiji	14th May 2007	J.L. Menou	GQ917415	GQ917344	GQ917501	GQ917279
Renouxia sp.	LLG0730		New Caledonia	1st November 2007	C. Payri, L. Le Gall	EF033584	EF033601	GQ917503	GQ917305
Rhodogorgon sp.	LLG0743		New Caledonia	1st November 2007	C. Payri, L. Le Gall	AF006089	EF033602	GQ917504	GQ917306
Hapalidiaceae									
Lithothamnion sp.	LBC0573		Vanuatu	30th August 2006	C. Payri, J.L. Menou, C. Geoffray	GQ917395	GQ917324	GQ917450	GQ917261
Lithothamnion sp.	LBC0642		Fiji	10th May 2007	C. Payri, J.L. Menou, G. Lasne	GQ917405	GQ917334	GQ917461	GQ917270
Lithothamnion sp.	LBC0845		New Caledonia	2007	C. Payri	GQ917427	GQ917363	GQ917490	GQ917298
Mesophyllum cf. *erubescens*	LBC0541	(Foslie) M. Lemoine	Vanuatu	27th August 2006	C. Payri, J.L. Menou, C. Geoffray	GQ917390	GQ917318	GQ917444	GQ917255
Mesophyllum cf. *erubescens*	LBC0551		Vanuatu	28th August 2006	C. Payri, J.L. Menou, C. Geoffray	GQ917392	GQ917320	GQ917446	GQ917256
Mesophyllum cf. *erubescens*	LBC0676		Fiji	13th May 2007	J.L. Menou, G. Lasne	GQ917411	GQ917340	GQ917468	GQ917275
Mesophyllum lichenoïdes	LBC0031	(J. Ellis) M. Lemoine	France	15th July 2007	L. Bittner	GQ917384	GQ917312	GQ917439	GQ917249
Mesophyllum sp.	LBC0546		Vanuatu	28th August 2006	C. Payri, J.L. Menou, C. Geoffray	GQ917391	GQ917319	GQ917445	–
Phymatholithon sp.	LBC0001		France	1st July 2007	L. Bittner	GQ917381	GQ917309	GQ917436	GQ917247
Synarthrophyton patena	LLG0081	(J.D. Hooker & Harvey) R.A. Townsend	Australia	15th January 2005	L. Le Gall	U61255	EF033600	GQ917499	GQ917304
Unidentified Hapalidiaceae	LBC0005		France	1st July 2007	L. Bittner	GQ917382	GQ917310	GQ917437	–
Unidentified Hapalidiaceae	LBC0612		Indonesia	22nd November 2007	S. Draisma	GQ917401	–	GQ917456	GQ917266
Unidentified Hapalidiaceae	LBC0640		New Caledonia	20th March 2007	C. Payri, J.L. Menou	GQ917404	GQ917333	GQ917460	GQ917269
Corallinaceae									
Amphiroa fragilissima	LLG0050	(Linnaeus) J.V. Lamouroux	Belize	18th December 2004	L. Le Gall	U60744	EF033599	GQ917498	GQ917303
Amphiroa sp.	FRA0821		Guadeloupe	24.08.2007	F. Rousseau	GQ917380	GQ917308	GQ917435	GQ917246
Amphiroa sp.	LBC0708		Fiji	15th May 2007	J.L. Menou, G. Lasne	GQ917416	GQ917345	GQ917472	GQ917280
Amphiroa sp.	LBC0865		Philippines	20th September 2006	F. Lelieart	GQ917428	GQ917364	GQ917491	GQ917299
Hydrolithon onkodes	LBC0796	(Heydrich) D. Penrose & Woelkerling	New Caledonia	30th January 2008	C. Payri	GQ917371	GQ917354	GQ917480	GQ917288
	LBC0801		New Caledonia	30th January 2008	C. Payri	GQ917372	GQ917355	GQ917481	GQ917289
	LBC0820_P3		New Caledonia	27th November 2007	J.L. Menou	GQ917373	GQ917357	GQ917483	GQ917291
Hydrolithon reinboldii	LBC0822	(Weber-van Bosse & Foslie) Foslie	New Caledonia	27th November 2007	J.L. Menou	GQ917375	GQ917358	GQ917484	GQ917292
	LBC0823		New Caledonia	27th November 2007	J.L. Menou	GQ917376	GQ917359	GQ917485	GQ917293
	LBC0827		New Caledonia	27th November 2007	J.L. Menou	GQ917377	GQ917360	GQ917486	GQ917294
Hydrolithon cf. *boergesenii*	LBC0558	(Foslie) Foslie	Vanuatu	29th August	C. Payri, J.L. Menou, C. Geoffray	GQ917378	GQ917321	GQ917447	GQ917257

(*continued on next page*)

Table 1 (*continued*)

	Voucher	Authorities	Location	Date	Collectors	GenBank accession numbers			
						SSU	LSU	*psb*A	COI
				2006					
Hydrolithon sp.	LBC0635		New Caledonia	23rd March2007	C. Payri	GQ917374	GQ917330	GQ917457	GQ917267
Hydrolithon sp.	LBC0652		Fiji	12th May 2007	C. Payri, J.L. Menou, G. Lasne	GQ917407	GQ917336	GQ917463	GQ917271
Hydrolithon sp.	LBC0656		Fiji	12th May 2007	C. Payri, J.L. Menou, G. Lasne	GQ917409	GQ917338	GQ917465	GQ917273
Hydrolithon sp.	LBC0678		Fiji	13th May 2007	J.L. Menou, G. Lasne	GQ917412	GQ917341	GQ917469	GQ917276
Hydrolithon sp.	LBC0715		Fiji	15th May 2007	J.L. Menou, G. Lasne	–	GQ917348	GQ917475	GQ917283
Hydrolithon sp.	LBC0720		Fiji	16th May 2007	J.L. Menou, G. Lasne	GQ917420	GQ917349	GQ917476	GQ917284
Hydrolithon sp.	LBC0740		Fiji	21st May 2007	J.L. Menou, G. Lasne	GQ917422	GQ917352	GQ917478	GQ917286
Hydrolithon sp.	LBC0755		Fiji	23rd May 2011	C. Payri, J.L. Menou, G. Lasne	GQ917423	GQ917353	GQ917479	GQ917287
Hydrolithon sp.	LBC0882		New Caledonia (Chesterfield)	5th June 2008	C. Payri	GQ917429	GQ917366	GQ917493	GQ917301
Lithophyllum cf. *bamleri*	LBC0646	(Heydrich) Heydrich	Fiji	12th May 2007	C. Payri, J.L. Menou, G. Lasne	GQ917406	GQ917335	GQ917462	–
Lithophyllum cf. *bamleri*	LBC0713		Fiji	15th May 2007	J.L. Menou, G. Lasne	GQ917417	GQ917346	GQ917473	GQ917281
Lithophyllum cf. *pygmaeum*	LBC0639	(Heydrich) Heydrich	New Caledonia	23rd March2007	C. Payri	GQ917403	GQ917332	GQ917459	GQ917268
Lithophyllum incrustans	LBC0033	Philippi	France	15th July 2007	L. Bittner	GQ917385	GQ917313	GQ917440	GQ917250
Lithophyllum sp.	LBC0599		Vanuatu	1st September 2006	C. Payri, J.L. Menou, C. Geoffray, L. Bittner	GQ917397	GQ917326	GQ917452	GQ917263
Lithophyllum sp.	LBC0680		Fiji	13th May 2007	J.L. Menou, G. Lasne	GQ917413	GQ917342	GQ917470	GQ917277
Lithophyllum sp.	LBC0714		Fiji	15th May 2007	J.L. Menou, G. Lasne	GQ917418	GQ917347	GQ917474	GQ917282
Mastophora or Lithoporella sp.	LBC0568		Vanuatu	29th August 2006	C. Payri, J.L. Menou, C. Geoffray	GQ917394	GQ917323	GQ917449	GQ917260
Mastophora pacifica	LBC0948	(Heydrich) Foslie	Morea	17th October 2008	C. Payri	GQ917430	GQ917367	GQ917494	GQ917302
Mastophora rosea	LBC0866	(C. Agardh) Setchell	Philippines	14th September 2007	O. De Clerck	–	GQ917365	GQ917492	GQ917300
Metagoniolithon radiatum	LBC0961	(Lamarck) Ducker	Australia	17th March1999	P. Mitrovski	GQ917432	GQ917369	GQ917496	–
Metagoniolithon stelliferum	LBC0962	(Lamarck) Ducker	Australia	11th July 1999	P. Mitrovski	GQ917433	GQ917370	GQ917497	–
Neogoniolithon sp.	LBC0321		Vanuatu	17th August 2006	C. Payri, J.L. Menou, C. Geoffray, G. Lasne	GQ917387	GQ917315	GQ925909	GQ917252
Neogoniolithon sp.	LBC0433		Vanuatu	26th August 2006	C. Payri, J.L. Menou, C. Geoffray, G. Lasne	GQ917388	GQ917316	GQ917442	GQ917253
Neogoniolithon sp.	LBC0607		Philippines	13th September 2007	F. Lelieart	GQ917400	GQ917329	GQ917455	–
Neogoniolithon sp.	LBC0636		New Caledonia	21st March2007	C. Payri	GQ917402	GQ917331	GQ917458	–
Neogoniolithon sp.	LBC0662		Fiji	12th May 2007	C. Payri, J.L. Menou, G.Lasne	GQ917410	GQ917339	GQ917466	GQ917274
Neogoniolithon sp.	LBC0811		New Caledonia	30th January 2008	C. Payri	GQ917424	GQ917356	GQ917482	GQ917290
Neogoniolithon sp.	LBC0828		New Caledonia	6th February 2008	J.L. Menou	GQ917425	GU063865	GQ917487	GQ917295
Neogoniolithon sp.	LBC0840		New Caledonia	13th February 2008	S. Andrefouet	GQ917426	GQ917361	GQ917488	GQ917296
Neogoniolithon sp.	LBC0843		New Caledonia	13th February 2008	S. Andrefouet	GQ917434	GQ917362	GQ917489	GQ917297
Pneophyllum conicum	LBC0540	(E.Y. Dawson) Keats, Y.M. Chamberlain & Baba	Vanuatu	27th August 2006	C. Payri, J.L. Menou, C. Geoffray	GQ917389	GQ917317	GQ917443	GQ917254
Pneophyllum conicum	LBC0653		Fiji	12th May 2007	C. Payri, J.L. Menou, G. Lasne	GQ917408	GQ917337	GQ917464	GQ917272
Pneophyllum conicum	LBC0683		Fiji	13th May 2007	J.L. Menou, G. Lasne	GQ917414	GQ917343	GQ917471	GQ917278
Spongites hyperellus	LBC0960	(Foslie) Penrose	Australia	9th November 2003	A. Harvey	GQ917431	GQ917368	GQ917495	–
Titanoderma sp.	LBC0724		Fiji	17th May 2007	C. Payri, J.L. Menou, G. Lasne	GQ917421	GQ917350	GQ917477	GQ917285
Unidentified crustose Corallinaceae	LBC0560		Vanuatu	29th August 2006	C. Payri, J.L. Menou, C. Geoffray, L. Bittner	GQ917393	GQ917322	GQ917448	GQ917258
Unidentified crustose	LBC0584		Vanuatu	31st August 2006	C. Payri, J.L. Menou, C. Geoffray, L.	GQ917396	GQ917325	GQ917451	GQ917262

Corallinaceae				Bittner				
Unidentified crustose Corallinaceae	LBC0600	Vanuatu	1st September 2006	C. Payri, J.L. Menou, C. Geoffray, L.Bittner	GQ917398	GQ917327	GQ917453	GQ917264
Unidentified crustose Corallinaceae	LBC0601	Vanuatu	1st September 2006	C. Payri, J.L. Menou, C.Geoffray, L.Bittner	GQ917399	GQ917328	GQ917454	GQ917265
Unidentified geniculate Corallinaceae	LBC0012	France	12th July 2007	L. Bittner	GQ917383	GQ917311	GQ917438	GQ917248
Unidentified geniculate Corallinaceae	LBC0050	Spain	25th July 2007	L. Bittner	GQ917386	GQ917314	GQ917441	GQ917251

2.3 Datasets building

Sequences were edited and contigs were assembled using Sequencher TM 4.1 (Gene Codes Corporation, Michigan). Alignments were done with the assistance of MacClade version 4.06 (Maddison and Maddison, 2003) and adjusted by eye. Two datasets were built to assess infra-ordinal relationships within the Corallinales. Dataset 1, which included 191 taxa (of which 180 belong to the Corallinales), was built in order to assess relationships among the highly diverse taxa of the Corallinales by pooling the SSU sequences (61 Corallinales, Table 1) obtained in the present study with a large selection of SSU sequences available from GenBank (119 Corallinales, Tables 1 and 2). Dataset 1 encompassed representatives from each subfamily within the Corallinales (except for the Austrolithoideae) as well as "uncultured eukaryotes", which were resolved within the Corallinales. Dataset 2 included four loci (SSU, LSU, *psbA,* COI) and 70 taxa of which 65 belonged to the Corallinales. Dataset 2 was built to improve the phylogenetic resolution among representatives of each of the subfamilies within the Corallinales. Both datasets were rooted with members of the Rhodogorgonales and Sporolithales, which were resolved as sister groups to the Corallinales in recent studies (Le Gall et al., 2010). Alignments and datasets are available online in Annexes

2.4. Partitioning strategy, model choice and phylogenetic analyses

Dataset 1 included only SSU sequences and thus only one unique partition was considered. The software jModelTest (Posada, 2008), was used to select for this dataset as it was shown to be the best suited model of evolution, following the Akaike Information Criterion (AIC, Akaike, 1973), the second-order corrected AIC (AICc, Hurvich and Tsai, 1989), and the Bayesian Information Criterion (BIC, Schwarz, 1978). With dataset 1, the best model chosen by each criterion was the GTR + G8.

Dataset 2 included ribosomal loci (SSU, LSU) and encoding markers (psbA, COI). An appropriate partitioning scheme was chosen by applying a partitioned model selection pipeline, implemented in the software 'Partitioned Model Tester' (PMT, version 1.0.1). The PMT software (developed by Heroen Verbruggen, downloadable on his webpage: http://www.phycoweb.net/) is a Perl program that evaluates different partitioning strategies and models of sequence evolution for a given alignment. Akaike and Bayes- ian information criteria (AIC, AICc, BIC) were calculated with PMT for five partitioning strategies and for 36 models of sequence evolution (details in Supp. Mat. 2). Finally, the preferred combination partitioning strategy was that in which dataset 2 was partitioned by marker and by codon position within protein coding genes (8 partitions: 1 with SSU, 1 with LSU, and 3 partitions for each positions of *psb*A and COI). With dataset 2, the best model chosen by the AIC was the GTR + G8, and the best model chosen by the AICc and BIC was the GTR + G4 + I.

Subsequent to the partitioning strategy and the model choice steps, phylogenetic analyses of Maximum likelihood (ML) were performed using the RAxML software version 7.2.0 (Stamatakis, 2006) on the Cipres portal 2 (CIPRES cluster). Analyses were performed for each dataset at least four times, with different starting trees, using the partition strategy and the model of sequence evolution detailed in the previous paragraph. With dataset 2, for each partition, the GTR + G4 + I was selected.

For dataset 1 and dataset 2, bootstrap supports (BS) (Felsenstein, 1985) analyses consisting of 2000 replicates, were calculated with the RAxML rapid bootstrap algorithm (Stamatakis et al., 2008) on the same portal. With dataset 2, prior to inferring phylogeny with combined markers, analyses were performed for each included loci and no strongly conflicting nodes were found by visually comparing topologies (except for psbA and COI tree with the specimens LBC0796, LBC0801 and LBC0820, see Supp. Mat. 3). With reference to these latter three specimens, psbA and COI trees strongly disagree, whereas LSU and SSU trees show the same phylogenetic relationships hypotheses than the plastidial tree with low BS support. These dissimilar phylogenetic patterns could be due to incomplete lineage sorting, or processes of hybridization/recombination. Considering this conflict, the COI sequence from LBC0796 was removed from the concatenated dataset (dataset 2) before performing the analyses.

Table 2
List of GenBank accession numbers of the SSU sequences included in dataset 1. When more than one sequence was allocated to the same species name, information about the sampling locality (when indicated in the original publication) or the voucher number were retained in the labelling of the specimen, to help the reader identify the taxa in Fig. 1. AUS = Australia, NZ = New Zealand, SAF = South Africa. NB: Classification and more specifically genus and species names have been reported in the table herein as they are indicated on GenBank.

Order, family, subfamily, species, voucher number [details on sampling locality]	GenBank accession no. (SSU sequences)
Sporolithales	
Heydrichia homalopasta [AUS]	AF411629
Heydrichia homalopasta [NZ Chatham I]	EF628210
Heydrichia woelkerlingii	U61253
Sporolithon durum [AUS]	U61254
Sporolithon durum [NZ Cable Bay South I]	EF628211
Sporolithale sp. [Rhodolith d'Urville I]	EF628212
Corallinales	
Hapalidiaceae	
Choreonematoideae	
Choreonema thuretii	AY221254
Melobesioideae	
Clathromorphum compactum	U60742
Clathromorphum parcum	U61252
'Leptophytum' acervatum	U62119
'Leptophytum' ferox	U62120
Lithothamnion glaciale	U60738
Lithothamnion sp. BISH 689378	DQ629010
Lithothamnion tophiforme	U60739
Mastophoropsis canaliculata	U62118
Melobesioideae sp. BISH 683176	DQ628972
Mesophyllum engelhartii [SAF]	U61256
Mesophyllum erubescens [Brazil]	U61257
Mesophyllum erubescens [NZ Chatham I]	EF628222
Mesophyllum erubescens [NZ Golden Bay 1]	EF628220
Mesophyllum erubescens [NZ Golden Bay 2]	EF628221
Mesophyllum erubescens [NZ Wellington]	EF628223
Mesophyllum erubescens [NZ Whararíki Beach]	EF628219
Mesophyllum printzianum [NZ Chatham I]	EF628224
Mesophyllum sp. [NZ Chatham I]	EF628218
Phymatolithon laevigatum	U60740
Phymatolithon lenormandii	U60741
Phymatolithon repandum [NZ Kaikoura]	EF628216
Phymatolithon repandum [NZ Chatham I]	EF628215
Synarthrophyton schielianum	EF628217
Corallinaceae	
Corallinoideae	
Arthrocardia carinata CH968	EU095601
Arthrocardia filicula	U61258
Arthrocardia flabellata	EU095603
Arthrocardia sp. ASD200 [NZ Northland]	EF628230
Bossiella californica ssp. *schmittii*	U60945
Bossiella orbigniana ssp. *dichotoma*	U60746
Bossiella orbigniana ssp. *orbigniana*	EU095604
Calliarthron cheilosporioides	U60943
Calliarthron tuberculosum	U60944
Cheilosporum cultratum	EU095605
Cheilosporum sagittatum [AUS]	U60745
Cheilosporum sagittatum ASD165 [NZ Gisborne]	EF628226
Corallina elongata	U60946
Corallina elongata CH989	EU095607
Corallina elongata IRV50	FM180099
Corallina officinalis	L26184
Corallina officinalis ASE091 [NZ Wellington]	EF628232
Corallina officinalis CH507	EU095606
Corallina sp. 343a	FM180101
Haliptilon roseum [AUS]	U60947
Haliptilon roseum CH750	EU095614
Haliptilon roseum [NZ Stewart I 1 ASE0277]	EF628229
Haliptilon roseum OK244	EU095609
Haliptilon sp. CH935	EU095616
Haliptilon squamatum CH985	EU095617
Jania adhaerens	EU095620
Jania crassa	U62113
Jania rubens	U61259
Jania sp. KC145	EU095627
Jania sp. OK239	EU095625
Jania ungulata	EU095627
Jania verrucosa CH735	EU095628
Marginisporum declinata	EU095632

Table 2 (*continued*)

Order, family, subfamily, species, voucher number [details on sampling locality]	GenBank acc
Serraticardia macmillanii	U62114
Lithophylloideae	
Amphiroa sp. [AUS]	U62115
Amphiroa sp. [SAF]	U62116
Amphiroa hancockii	AY234233
Amphiroa tribulus	AY234234
Lithophyllum incrustans	AF093410
Lithophyllum kotschyanum	U62117
Lithophyllum koschianum BISH 683166	DQ628975
Lithophyllum koschianum BISH 683245	DQ628974
Lithophyllum cf. *koschianum* BISH 699887	DQ628976
Lithophyllum sp. [NZ Northland]	EF628242
Lithophyllum sp. [NZ Wharakiki Beach]	EF628240
Lithophyllum stictaeforme	EF628241
Lithothrix aspergillum	U61249
Titanoderma pustulatum	AF093409
Mastophoroideae	
Hydrolithon gardineri BISH 683169	DQ628993
Hydrolithon gardineri BISH 683171	DQ628992
Hydrolithon gardineri BISH 689388	DQ628991
Hydrolithon improcerum NZC0667	EF628239
Hydrolithon onkodes	AY234237
Hydrolithon cf. *onkodes* BISH 683248	DQ628996
Hydrolithon cf. *onkodes* BISH 689384	DQ628997
Hydrolithon pachydermum	AY234235
Hydrolithon reinboldii BISH 689383	DQ628999
Hydrolithon reinboldii BISH 699815	DQ628998
Hydrolithon reinboldii BISH 699817	DQ629003
Hydrolithon reinboldii BISH 699824	DQ629002
Hydrolithon cf. *reinboldii* BISH 689378	DQ629001
Hydrolithon cf. *reinboldii* BISH 689382	DQ629000
Hydrolithon samoense	AY234236
Hydrolithon sp. BISH 683179	DQ628990
HyMastophoroideae sp. BISH 699814	DQ629006
HyMetamastophora flabellata clone 1	AY234239
HyMetamastophora flabellata clone 2	AY234240
HyNeogoniolithon brassica-florida	AY233346
HyNeogoniolithon spectabile	AY234238
HyPneophyllum cf. *conicum* BISH 666750	DQ628995
HyPneophyllum cf. *conicum* BISH 683242	DQ628994
HyPneophyllum cf. *conicum* BISH 699889	DQ628989
HyPneophyllum conicum BISH 683243	DQ628985
HyPneophyllum conicum BISH 683253	DQ628987
HyPneophyllum conicum BISH 683255	DQ628983
HySpongites yendoi [AUS]	U60948
HySpongites yendoi NZC0090	EF628237
HySpongites yendoi NZC0482	EF628236
HySpongites yendoi NZC0507	EF628233
HySpongites yendoi NZC0627	EF628234
HySpongites yendoi NZC0779	EF628235
HySpongites yendoi NZC0781	EF628238
HyMetagoniolithoideae	
HyMetagoniolithon chara	U60743
HyMetagoniolithon radiatum	U61250
HyMetagoniolithon stelliferum	U61251
Unidentified Corallinales	
Corallinales sp. CB-2003	AY247408
Uncultured eukaryot clone 15	FJ153777
Uncultured eukaryot clone 16	FJ153760
Uncultured eukaryot clone 17a	FJ153778
Uncultured eukaryot clone 18a	FJ153779
Uncultured eukaryot clone 37	FJ153768
Uncultured eukaryot clone 51a	FJ153771
Uncultured eukaryot clone 52	FJ153772

2.5 Detection of long branches attraction (LBA)

SlowFaster software (Kostka et al., 2008) was used to detect potential long branches attraction artifacts. SlowFaster was designed to: (i) assess the substitution rate of all the aligned positions assuming that some monophyletic groups are known *a priori;* (ii) identify slow and fast evolving sites; and (iii) create new alignments with different proportions of slow/fast evolving sites. Using an initial alignment and a tree topology (including nodes with constraint monophyly), SlowFaster counts the maximum number of changes in a position of the alignment. Once the largest number of changes per position is defined, SlowFaster partitions the dataset in new alignments. For instance, if the maximum number of changes per position in an alignment is four, SlowFaster will from the original dataset build four new alignments, labelled S0, S1, S2 and S3. S0 alignment is the shortest one and contains no homopla- sic signal (no changes per position) within the admitted monophy- letic groups. S1 alignment is longer than S0 and includes all positions with at most one change in the admitted monophyletic groups, and so on for S2 and S3. Both datasets (one marker in data- set 1, four markers in dataset 2) were analysed with SlowFaster, and we assumed the monophyly of the Corallinales as the single constraint to build sub-datasets. Phylogenetic analyses of ML and BS support (of 2000 replicates) calculations were then performed on each of these sub-datasets with the same partitioning strategy and the same model of evolution than previously selected (see Section 2.4). Comparisons of the phylogenies and of the BS obtained with these sub-datasets were then made to see if the results obtained with the initial alignments were influenced by fast evolving sites and potential LBA artifacts. Moreover, in order to test whether the loss of informative positions in the sub-datasets influenced the statistical support of the resulting tree topology, for each of the sub-datasets (for instance S0-S3), alignments of same length, but comprising a random selection of positions (e.g. a random mix of fast and slow evolving sites), were prepared. Ten Jackknife datasets were then built for each sub-dataset using the Jackknife option of the SlowFaster and the same analyses (phylogenetic analyses of ML and BS calculations, with the same partitioning strategy and model of evolution than selected previously) were performed on each of these random shortened alignments.

2.6. Ancestral state reconstructions

Based on previous publications and on the examination of the histological sections of our specimens, a matrix of morphological and anatomical characters was built. The states of five features traditionally involved in the identification of coralline algal orders, families and subfamilies, were encoded (matrix is provided in Supp. Mat. 4). These included: (1) the absence or presence of genic- ula (genicula refer to the uncalcified joints that alternate with calcified segments of the thallus; the presence of genicula separates the articulated (geniculate) coralline algae from the crustose or non-geniculate corallines); (2) cell fusions common or not (cells of contiguous vegetative filaments may be joined secondarily by cell fusions that correspond to the

break down of a part of the cellular wall and the melding of the cell content); (3) secondary pit- connections common or not (cells of contiguous filaments may be linked secondarily by pit-connection that correspond to an adjoining opening in the cell walls); (4) the absence or presence of uniporate or multiporate tetrasporangial conceptacles (Tetrasp- orangia are produced either in conceptacles where the roof may have a single pore (uniporate) or a number of pores (multiporate) through which spores are released, or are produced in sori that possess only a single pore); and (5) the absence or presence of tet- rasporangial pore plugs (within conceptacles/sori, individual tet- rasporangia may form an apical pore plug that occupies a space in the roof directly above the sporangium.
A consensus tree of the Corallinales (a cladogram) was drawn considering the major, well-resolved lineages (BS>85) recovered with the phylogenetic analyses of dataset 1 and 2 (Figs. 1 and 2). All characters were then encoded as discrete, unordered states, and their evolution was traced on the previously described Coral- linales tree using parsimony reconstruction implemented in Mes- quite version 2.6 (Maddison and Maddison, 2006).

3. Results

This study provided 258 new sequences deposited in GenBank (accession numbers are listed in Table 1): 63 sequences of SSU, 63 sequences of LSU, 62 sequences of COI and 70 sequences of *psb*A. A noticeable low percentage of missing data in the concatenated dataset can be pointed out. In dataset 2, only 4% of the sequences were missing. Phylograms resulting from the ML analyses are presented in Fig. 1 for dataset 1 and in Fig. 2 for data- set 2. Lineages were named with letters (A to V) to facilitate the reading of the following sections. In Fig. 1, lineages B, N, U are not recovered. In Fig. 2, lineages D, I, J, K R only include one taxon, and the lineage H is not represented in dataset 2. The average number of statistically well-resolved nodes and details of statistical support of the lineages A to U is reported for each topology (Figs. 1 and 2) and for each analyse in Supp. Mat. 5A and 5B.

3.1. Phylogenetic signal of the two datasets

3.1.1 Basic metrics

Dataset 1 (191 taxa and 1549 base pairs (bp)) included 1068 constant characters (CC) and 341 parsimony-informative characters (PI). Dataset 2 (70 taxa and 5503 bp) included 3837 CC and 1390 PI. The contribution of each loci of dataset 2 was as follows: 1549 bp of SSU (CC = 1186, PI = 273), 2502 bp of LSU (CC = 1816, PI = 547), 645 bp of COI (CC = 336, PI = 285) and 807 bp of *psbA* (CC = 499, PI = 285). The ratio PI vs. sequence length calculated for the dataset 2, clearly showed that the SSU was the least variable marker with the ratio of 0.17 followed by LSU (ratio = 0.22) and then *psbA* (ratio = 0.35); the marker containing the most PI was COI (ratio = 0.44).

3.1.2 Phylogenetic resolution

BS was compared for several datasets (Supp. Mat. 5A). Dataset 1 had the advantage of covering a large diversity of coralline species, but rose only 30.7% of well resolved nodes (i.e. BS p 80, Supp. Mat. 5A) in the whole phylogenetic tree. In contrast, the tree resulting from the ML analysis of the gene-rich, but "taxa-poor" dataset (dataset 2) had nearly 73% of its nodes well resolved (Supp. Mat. 5A). Analyses of single loci included in dataset 2 clearly showed that LSU trees were more resolved than trees obtained with the other single marker. Deep phylogenetic relationships (lineages B, E, G, U; Supp. Mat. 5B) were better resolved by nuclear markers (SSU and LSU) than organelle genes. Recent nodes (corresponding to generic or species level) benefited both from the organellar *(psbA* and COI) and the nuclear genetic information (Supp. Mat. 5B).

3.1.3 SlowFaster analyses

Assuming the monophyly of the Corallinales, the maximum number of observed changes in a position of the alignments was four for each dataset (dataset 1, and the four loci of the dataset 2). Thus, four new alignments were created. These sub-datasets were labelled S0 up to S3, and contained gradually from S0 to S3 more saturated positions. S0 was the shortest alignment and contained only slow evolving sites. S3 was the longest alignment and contained the highest number of fast evolving sites (compared to S0, S1 and S2); S1 and S2 were intermediate. Comparisons of BS evolution showed a similar trend with all datasets. S0 alignments contained no information (except the monophyly of the Coralli- nales).

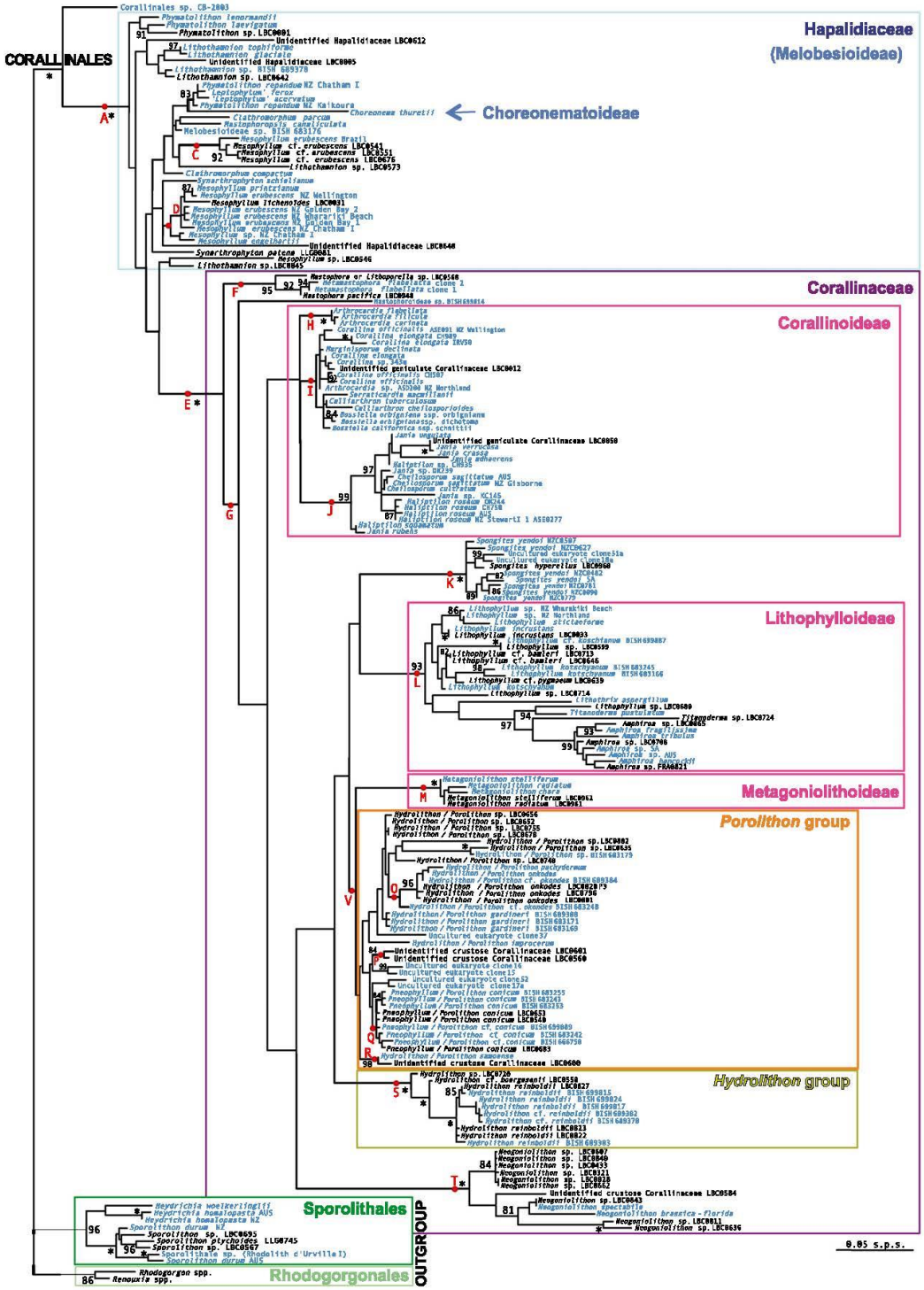

Fig. 1. Phylogram inferred from ML analyses with dataset 1 (SSU sequences, 1549 bp, 192 taxa). s.p.s. means number of substitutions per site. Sequences coloured in blue have been downloaded from GenBank. Values above or below nodes indicate BS (for 1000 replicates): * indicates BS of 100. BS < 80 are not indicated. Information on voucher number or / and on sampling area have been added in the specimens names in order to help to distinguish them when they shared the same species name (See list of specimens in Tables 1 and 2).

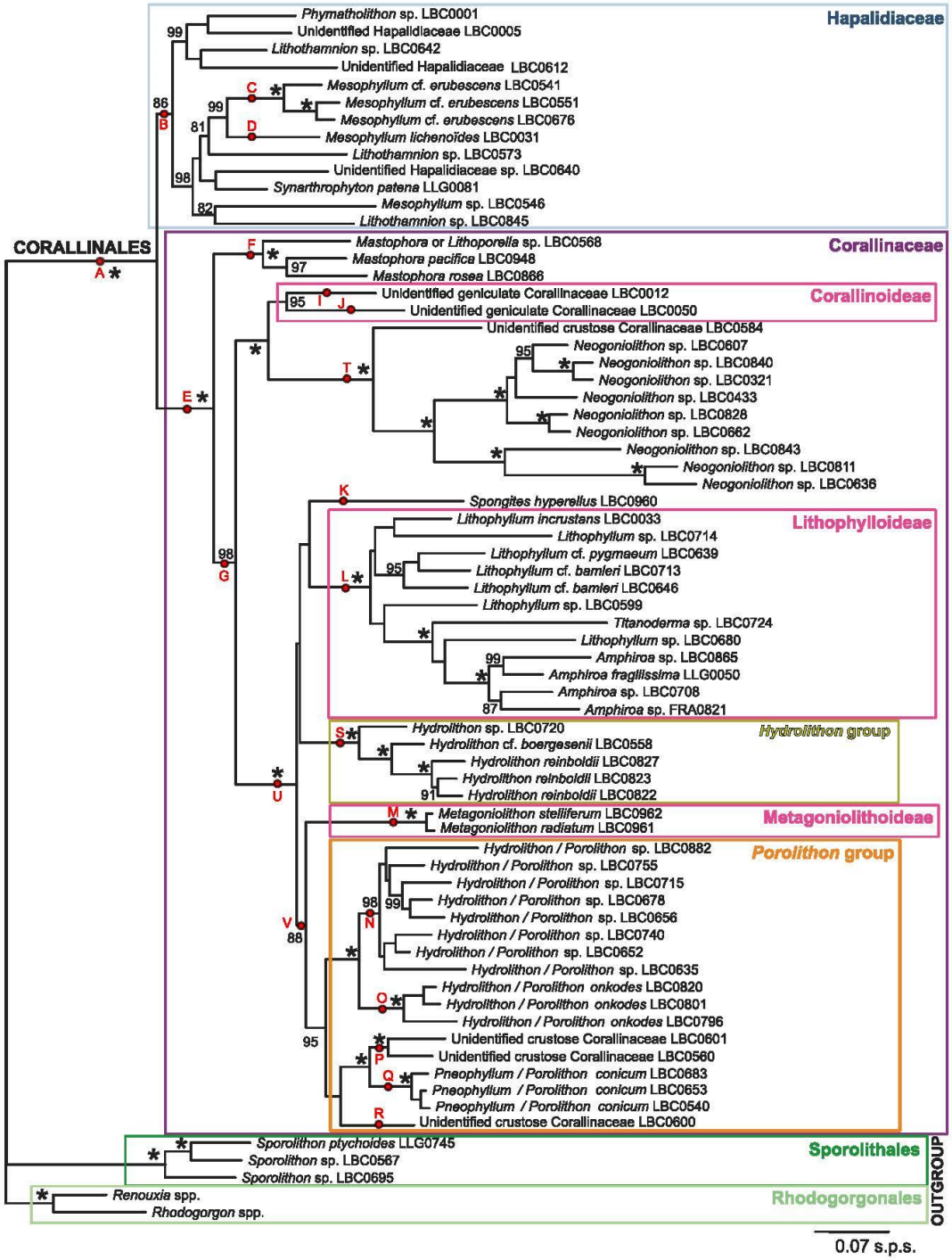

Fig. 2. Phylogram inferred from ML analyses with dataset 2 (4 genes sequences, 5503 bp, 70 taxa). s.p.s. means number of substitutions per site. Values above or below nodes indicate BS (for 1000 replicates): * indicates BS of 100. BS < 80 are not indicated. Information on voucher number or / and on sampling area have been added in the specimens names in order to help to distinguish them when they shared the same species name (See list of specimens in Table 2.).

S1 hardly resolved a few nodes (except for the LSU) and BS increased suddenly with the alignment of S2 (Supp. Mat. 5B). The highest BS were obtained with either the initial alignments (for the majority of the pointed out nodes), or with the S3 alignments (Supp. Mat. 5B). The only group that behaved slightly differently was the

lineage (N + O + P + Q + R) in dataset 2, which had a higher BS with the dataset LSU - S3 or S2. This lineage was nevertheless also strongly supported in the concatenated analyses of dataset 2 (BS = 88).

Jackknife datasets of the same length as the two informative datasets (S2, S3), but shortened by random deletion of positions, were also analysed for dataset 1 and for each partition of dataset 2. Ten of these randomly shortened datasets were analysed (20 alignments per locus, in total: 100 analyses). The average of the BS obtained with the Jackknifed datasets was always lower than the BS found with S2 and S3 sets (details of the analyses not provided here).

Finally, the SlowFaster analyses suggested that in our datasets BS increased with the length of the alignment analysed. BS was thus not due to phylogenetic noise.

3.2. Phylogenetic inferences resolved relationships

3.2.1. Among the Corallinales

Phylogenies inferred from dataset 1 and 2 recovered with full support the monophyly of the Corallinaceae (Figs. 1 and 2). In contrast, the Hapalidiaceae (node B) was resolved as a monophyletic lineage only when the multi-marker dataset was analysed (BS = 86, Fig. 2). Single locus analyses (Supp. Mat. 5) seldom resolved the Hapalidiaceae as monophyletic whereas the Corallina- ceae (node E) form a strongly supported monophyletic lineage in phylogenies inferred from nuclear markers (Supp. Mat. 5B).

3.2.2. Within the Hapalidiaceae

Our analyses included representatives of the Melobesioideae and Choreonematoideae (represented by a single monospecific genus), two of the three subfamilies currently recognised in the Hapalidiaceae. The only member of the Choreonematoideae, *Cho- reonema thuretii* (Bornet) F. Schmitz, was resolved as a long branch with low support for its position within the Hapalidiaceae (Fig. 1). Dataset 1 included twelve different sequences of specimens identified as *Mesophyllum erubescens* from various locations (nine from GenBank and three generated in the present study), which were resolved within two distant and unrelated lineages (node C and D). Specimens from the Melanesian region (Vanuatu, Fiji) allied with one specimen from the type locality (Brazil) of the species. All specimens from New Zealand were resolved along with other congeneric species within the lineage D. The specimens from Wellington (New Zealand) joined *Mesophyllum printzianum* and together they were resolved as the sister lineage of *Mesophyllum lichenoides*

3.2.3. Within the Corallinaceae

Lithophylloideae and Metagoniolithoideae (lineages L and M, respectively) were recovered as monophyletic lineages with strong support (Supp. Mat. 5B, Figs. 1 and 2). Corallinoideae (lineages I+J + H) were also resolved as monophyletic with both

datasets (Figs. 1 and 2). However, only the multi-markers dataset strongly supported the monophyly of the Corallinoideae (BS(dataset 1) = 69, BS(dataset 2) = 95). Within the lineage I, three specimens identified as *Corallina officinalis* and three specimens identified as *Corallina elongata* displayed distinct SSU sequences and phylogenetic analyses split these two species into several distinct lineages (Fig. 1).

In our multi-marker analyses members of the subfamily Masto- phoroideae were resolved into four distinct strongly supported lineages (nodes F, K, T, N + O + P + Q+ R) (Fig. 2). Analyses of both datasets resolved the lineage F as the earliest divergence within the Corallinaceae and encompassed species of *Mastophora, Meta- mastophora* and possibly *Lithoporella* (Figs. 1 and 2). Species of *Neo- goniolithon* included in both datasets clustered together with the unidentified specimen LBC0584 within the lineage T despite their high genetic divergence. Species of the genus *Spongites* were resolved as the sister lineage (node K) to the Lithophylloideae in both analyses albeit without statistical support. Analyses of both data- sets recovered species of *Pneophyllum* as a monophyletic lineage (node Q), which allied with full support in combined loci analyses with unidentified specimens (nodes P and R) forming altogether the sister taxa of *Hydrolithon onkodes* (node O), and an unidentified species of *Hydrolithon* (node N). The lineages N, O, P, Q and R clus- tered with the Metagoniolithoideae (lineage M) with high support (lineage labelled V, BS = 88, dataset 2). The remaining representatives of the genus *Hydrolithon (Hydrolithon reinboldii, Hydrolithon* cf. *boergesenii* and *Hydrolithon* sp. (LBC0720)), allied together and formed the lineage S, which phylogenetic position was unclear within the lineage U.

Several specimens included in dataset 1 were annotated on GenBank as 'uncultured eukaryotes' (Medina-Pons et al., 2009). On Fig. 1, some of them were resolved among members of *Spong- ites* and others as relatives to *Pneophyllum* and *Hydrolithon* species characterized by a dimerous thallus structure.

3.3. Ancestral states reconstruction

Ancestral state reconstructions have been performed for five morpho-anatomical characters (Fig. 3). Combinations of these character states are traditionally used to identify families and subfamilies in the Corallinales (details in Supp. Mat. 1). Parsimony reconstructions of the evolution of these characters highlight a high degree of homoplasy of these features. The first feature (i.e. absence or presence of uniporate or multiporate tetrasporangial conceptacles) is the only one useful as a diagnostic character. Each character state associated with this feature corresponds to a family. The Hapalidiaceae possess multiporate tetrasporangial con- ceptacles, whereas the Corallinaceae possess uniporate tetraspo- rangial conceptacles. The second feature shows the presence of tetrasporangial pore plugs in both Sporolithales and Hapalidiaceae. It is, however, not possible to infer if pore plugs in these two lin- eages were derived from a common ancestor. Cell fusions are common (feature 3) in

the Corallinales except in Lithophylloideae (Lineage L), and have also been described for taxa from the out- group Rhodogorgonales. Further developmental studies are thus required to evaluate whether this character state is autapomorphic to the Lithophylloideae. The predominance or frequent presence of secondary pit-connections (feature 4) and the presence of genicula (feature 5) occur several times in the corallinalean tree. In the majority of the Corallinales secondary pit-connections are absent or rare; the subfamily Lithophylloideae and some species from the Mastophoroid genus *Metamastophora* are exceptions. Similarly, genicula appear at least four times in the corallinean tree (twice in lineage L). All the features and their character states appear to have evolved independently from each other.

4. Discussion

4.1. Improvement of phylogenetic resolution within the Corallinales

Simulation studies have established that the accuracy of phylo- genetic trees determined from molecular data can be improved by adding more taxa and more markers (Rokas and Carroll, 2005).

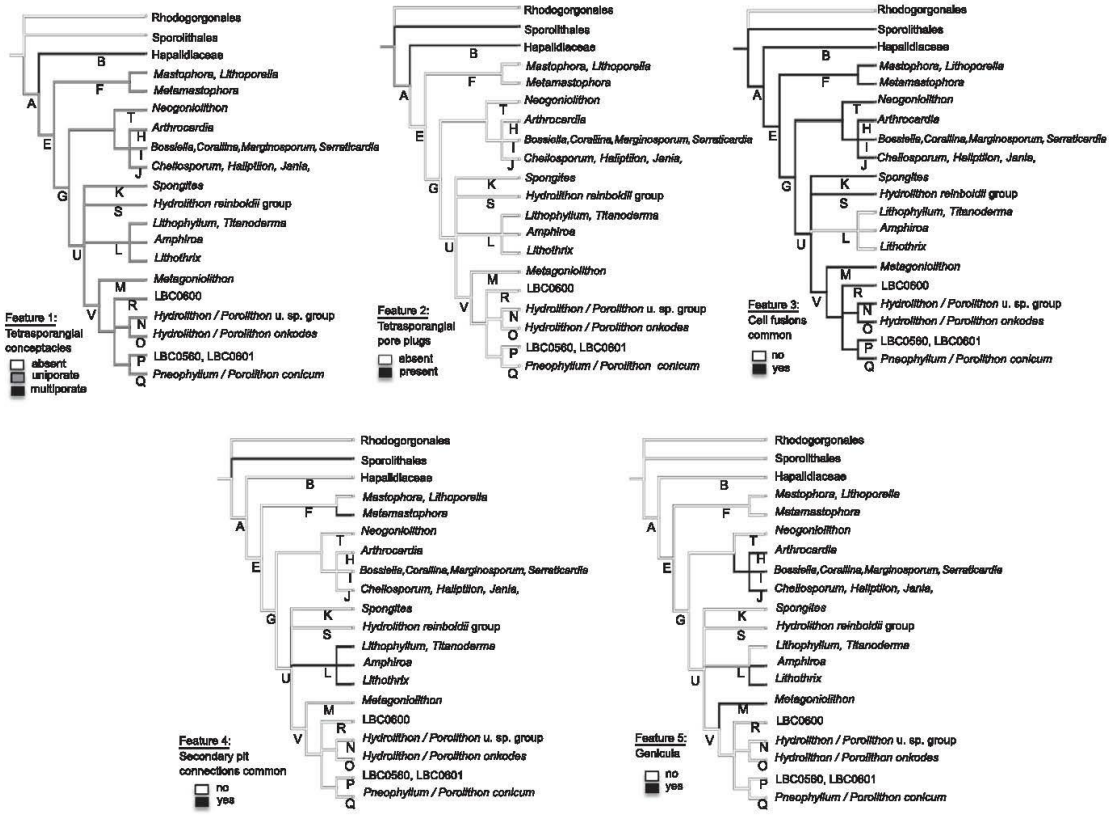

Fig. 3. Mapping of morpho-anatomical characters classically employed to describe the Corallinales families and subfamilies (*Supp.* Mat. 1). A combination topology for main lineages from Figs. 1 and 2 has been made, and character states (see *Supp.* Mat. 4) were mapped onto this Corallinales phylogeny. Ancestral states were inferred under a parsimony criterion with Mesquite version 2.6 (Maddison and Maddison, 2006). Character states encoding is provided as legend next to each tree u. sp. means unidentified species.

Phylogenetic relationships inferred from our combined loci analyses are largely congruent with those inferred from SSU published by Bailey et al. (2004). Moreover, in the present study these relationships are statistically more strongly supported, suggesting that the incorporation of many taxa and addition of new molecular markers greatly improved the resolution of phylogenetic relationships within the Corallinophycidae. LSU sequences in particular contributed to improve the resolution of phylogenetic relationships observed when analyses where performed using the multi- marker dataset (Supp. Mat. 4, Fig. 2). This was likely due to its length (here 2502 bp) as well as its phylogenetic signal. In a recent study Broom et al. (2008) stated that *psb*A has considerable potential as a marker for the Corallinales because it is easily amplified and considerably more variable than SSU. COI is another gene that has recently been used to assess subfamilial relationships within the Corallinales (Walker et al., 2009) and this marker, selected as the DNA-barcode for the Rhodophyta, is currently widely se- quenced by the barcode community to populate the Barcode Of Life Database (Ratnasingham and Hebert, 2007). Nevertheless, our analysis of the proportion of nodes with high bootstrap for each marker show that LSU is significantly more informative than the other markers. This is followed by *psb*A and then COI and SSU. This result confirmed empirically that LSU is an efficient marker to assess phylogenetic relationships within the Corallinales at several taxonomic levels. Within the Rhodophyta several studies (e.g. Harper and Saunders, 2001b, 2002; Saunders and Lehmkuhl, 2005; Le Gall and Saunders, 2007; Le Gall et al., 2008) have highlighted that LSU provide good resolution at both deep and terminal nodes. We therefore recommend that LSU, rather than SSU sequences, be used to pursue further phylogenetic inferences within the Corallinales. However, considering that *psb*A sequences (1) are easy to amplify, (2) only require two sequencing reactions (one forward, one reverse), (3) can be aligned unambiguously and (4) provide significant phylogenetic signal in recent and deep branching (Broom et al., 2008), focusing on the use of new plastid- ial sequences other than LSU sequences, might also be an attractive strategy to access coralline algal relationships in future analyses. The studies of sub-datasets (built with the SlowFaster software, Kostka et al., 2008), where fast-evolving sites were removed, showed that our alignments were not affected by phylogenetic noise. It seems therefore likely that our trees are not suffering from long branches attraction.

4.2. Suprageneric relationships among the Corallinales

Our phylogenies confirm the monophyly of the coralline algal families Corallinaceae and Hapalidiaceae, as well as most of their subfamilies as delineated by Harvey et al. (2003).

4.2.1 Hapalidiaceae

When analyses were performed with the multi-marker dataset, the Hapalidiaceae (node B) were well supported (BS = 86) in comparison to the few previous studies that also recovered this lineage as monophyletic (Bailey and Chapman, 1998 [as the

Melobesioi- deae: BS (with a Maximum of Parsimony analyse, MP) = 61], Harvey et al., 2003 [BS(ML)<50 and BS(MP) = 64]). Broom et al. (2008) only found the monophyly of Hapalidiaceae with their worldwide dataset based on SSU sequences (BS(Neighbour-Joining analyse) = 99, BS(ML) = 91, Posterior probabilities for Bayesian analyses = 1.00). In Fig. 1, the phylogetenic tree shows an outgroup situated on a long ingroup branch and an ingroup constituted from a highly unequal root-to-tip path lengths with a comb-like structure (branch lengths are slightly shorter near the base and are then increasingly longer moving through the Hapalidiaceae towards the Corallinaceae). This distinct structure suggests that the paraphyly of the Hapalidiaceae from the SSU dataset may not be a true biological pattern: it could have resulted from a methodological bias (Shavit et al., 2007). Nevertheless, SlowFaster analyses (Kostka et al., 2008) show that alignments (from datasets 1 and 2) did not appear to be affected by phylogenetic noise. The monophyly of the Hapalidiaceae is in fact mainly due to the phylogenetic signal of the LSU marker. The Hapalidiaceae as delineated by Harvey et al. (2003) based on morphological and anatomical characters (zonately arranged tetra/bisporangia born in multiporate concep- tacles that bear apical pore plugs) is therefore supported to form a natural lineage within the Corallinales. However, our multi-marker analyses only included members of the Melobesioideae; representatives from the other two subfamilies (Austrolithoideae and Choreonematoideae) should be included in future multi-marker studies to strengthen these results (as to date only one SSU sequence from *C. thuretii* is available). The latter two subfamilies are poorly known and respectively include three and one mono- specific genera that are mostly endophytic or parasitic on genicu- late species from the Corallinaceae subfamily, Corallinoideae (Townsend and Huisman, 2004).

2.2.2. Corallinaceae: a revision from the subfamilies boundaries

An updated taxonomic scheme (Fig. 4) of the Corallinaceae is presented based on the phylogenetic relationships inferred from our datasets.

Emendation of the Mastophoroideae. Within the fully supported lineage corresponding to the Corallinaceae (node E), three of the four subfamilies namely the Corallinoideae (nodes H + I + J), Lithophyl- loideae (node L) and Metagoniolithoideae (node M) were resolved as monophyletic. However, the fourth subfamily, the Mastophoroi- deae was resolved as several independent lineages. This result is consistent with the phylogenies inferred by Bailey et al. (2004) who first highlighted the polyphyly of this subfamily. Unfortunately, their dataset did not include any representatives of the type genus *Mastophora* preventing them from proposing a revision to this subfamily. Our analyses, which included several species of *Mastophora,* including the type species *M. rosea* (Figs. 1 and2)(Setchell, 1943), re-solved this genus as a sister group to the genera *Lithoporella* and *Metamastophora* within a lineage sister to the remaining Corallina- ceae. Based on the phylogenetic position of *Mastophora,* we propose to restrict the subfamily Mastophoroideae to

only the genera *Litho- porella, Mastophora* and *Metamastophora* (Lineage F, Figs. 1 and 2). As emended here, Mastophoroideae includes taxa of the Corallinaceae with a ventral or central layer of predominantly palisade cells throughout the thallus. This character has already been used by Woelkerling (1988) to distinguish *Mastophora* from other genera within the subfamily Mastophoroideae *sensu lato*.
Affinities within the lineage G Lineages H, I and J correspond to the Corallinoideae *sensu* (J.E. Areschoug) Foslie and are restricted to geniculate genera. In the combined analyses, they are resolved as the sister group to lineage T, which encompasses taxa from the genus *Neogoniolithon*. These data corroborate Bailey et al.'s (2004) results and support Cabioch's (1972,1988) assessment that *Neogoniolithon* is more closely related to the Corallinoideae than to other non-geniculate groups.

Neogoniolithon fosliei (Heydrich) Setchell & L.R. Mason, the type species of the genus *Neogoniolithon* is regarded as an heterotypic synonym of *Neogoniolithon brassica-florida* (Harvey) Setchell et Mason (Woelkerling et al., 1993b). Numerous taxa including *Neogon- iolithon frutescens* and *Neogoniolithon laccadivicum* have been transferred to *N. brassica-florida* (Guiry and Guiry, 2011). However, Kato et al. (2009) refined the delineation of *N. brassica-florida* using molecular data (SSU) and concluded that the circumscription of the species based on Verheij (1994) is not appropriate. The crustose and fruticose specimens analysed in their study and referred to *N. fosliei* and *N. frutescens* respectively formed several distinct clades, a result which is usually considered to reflect different species. In our dataset, several distinct clades correspond to *Neogoni- olithon* crusts with large conceptacles assigned to the complex *N. fosliei/brassica-florida*. Thorough morphological studies are thus required to better delineate this complex and supplementary phylo- genetic analyses have to be performed to unravel the true taxonomic affinities of all the species currently recognised within the genus *Neogoniolithon*.

Neogoniolithon and Corallinoideae specimens share common reproductive features namely: (1) the position of the spermatangia on the floor, walls and roof of the male conceptacles; (2) the distribution of gonimoblast filaments across the dorsal surface of the fusion cell; and (3) the similar peripheral development of the tetrasporangial conceptacle roofs in both lineages. This later character, however, is also observed in the Mastophoroideae *sensu lato* genera *Spongites, Lesueria, Mastophora* and *Metamastophora,* and so it is not diagnostic for the lineage (H + I + J + T). Nevertheless the first two characters differ from all other mastophoroids and can thus be used to distinguish members of this lineage (H + I + J + T) from others in the lineage G. Bailey et al. (2004) had suggested transferring the genus *Neogoniolithon* from the Mastophoroideae to the Corallinoideae. In light of the current findings, a global revision of the taxonomy and a re-defining of the ranks of the classification within the Corallinaceae have to be undertaken.

The lineage U, which comprises the Lithophylloideae, Metago- niolithoideae and the remaining genera of the Mastophoroideae *sensu lato (Spongites, Hydrolithon, Pneophyllum)* is strongly supported in our multi-markers dataset. This grouping was previously shown by Bailey (1999) and Bailey et al. (2004), but was not well supported. Similarly, in the current study, inter-group relationships within the lineage U remain poorly resolved.

The affinity of the genus *Spongites* (lineage K) needs to be confirmed by studying the generitype *Spongites fructiculosus* (Kutzing), a species unfortunately not included in our datasets. The lineage (L) corresponds to the Lithophylloideae *sensu* Cabioch (1972). It includes the type genus and species *Lithophyllum incrustans,* and encompasses both geniculate *(Amphiroa* and *Lithothrix* only in dataset 1) and non-geniculate *(Lithophyllum/Titanoderma)* genera. These results are consistent with Bailey's (1999) work. The Litho- phylloideae are characterized by the predominance of secondary pit-connections between cells of contiguous filament with cell fusions being absent or comparatively rare. Surprisingly, our results failed to resolve the controversial taxonomic status of the genus *Titanoderma*. The limited molecular evidence available favours placing the type species of *Lithophyllum* and *Titanoderma* in separate genera (Bailey, 1999; present study). The morphological criteria proposed to separate the two genera (basal layer of palisade cells and bistratose margins *vs.* basal layer of non-palisade cells and non-bistratose margins for *Titanoderma* vs. *Lithophyllum* respectively), however, do not stand up to rigorous testing because all these characters can occur together in the same thallus to varying degrees (Campbell and Woelkerling, 1990; Woelkerling and Campbell, 1992). Thus it is impossible to draw meaningful, reliable generic boundaries on the morphological grounds currently proposed as the material studied here had the *Titanoderma-type* diagnostic characters (namely a basal layer of palisade cells and bistratose margins), but did not join the generitype *Titanoderma pustulatum*. More morphological, anatomical and molecular analyses are thus needed to better circumscribe these two taxa *(Litho- phyllum/Titanoderma)*.

Fig. 4. Corallinaceae updated classification. Left: Corallinales classification from Harvey et al. (2003) modified by Le Gall et al. (2010). Right: Proposed Corallinaceae classification based on this multi-markers study (SSU, LSU, *psb*A, COI). Lineages written in purple and underlined correspond to specimens that required further study, and in particular an exhaustive morphological and nomenclatural work.

Our analyses resolved the genus *Hydrolithon* (Foslie) Foslie in two unrelated lineages ((N + O) and S). Interestingly, the anatomical structure of the thallus (monomerous *vs.* dimerous) is a character, which distinguishes each of the two lineages. This result confirms the phylogenetic significance of this feature, which was emphasized by Maneveldt (2005) to distinguish two morphological groups within the genus. Our phylogenies, however, clearly support the presence of two unrelated entities and we propose to restrict the genus *Hydrolithon* for the lineage (S), which includes *H. reinboldii* (Weber-van Bosse & Foslie) Foslie, the type species of the genus. As emended here the genus *Hydrolithon* is restricted to those species with a primarily dimerous thallus construction (thalli rarely become secondarily monomerous, and when they do it is probably in response to wound healing) and possessing trichocytes singly, in pairs and/or in small horizontal rows in which trichocytes are quite often separated from one another by normal vegetative filaments. The second lineage (O) encompasses a number of other *Hydrolithon* species as well as *H. onkodes* (Heyd- rich) D. Penrose & Woelkerling, which was the type species of the defunct genus *Porolithon* Foslie before it was subsumed in the genus *Hydrolithon* by Penrose and Woelkerling (1992). According to our phylogenetic results (Fig. 2) and observations of the anatomical features by

Maneveldt (2005), we propose to resurrect the genus *Porolithon* for those species displaying a primarily mono- merous thallus construction and possessing trichocytes in large horizontal, pustulate (as "pustulous" byAdey, 1970) fields without any normal vegetative filaments between the individual tricho- cytes. Accordingly, we also propose to re-assign *Hydrolithon craspe- dium,* (Foslie) P.C. Silva *Hydrolithon gardineri* (Foslie) Verheij & Prud'homme van Reine, *Hydrolithon improcerum* (Foslie & M.A. Howe) Foslie, *Hydrolithon munitum* (Foslie & M.A. Howe) Penrose, *Hydrolithon pachydermum* (Foslie) J.C. Bailey, J.E. Gabel, & D.W. Freshwater, *Hydrolithon samoense* (Foslie) Keats & Y.M. Chamberlain, *Hydrolithon superficiale* Keats & Y.M. Chamberlain and *Hydroli- thon rupestris* (Foslie) Penrose to the genus *Porolithon* (Maneveldt, 2005).

The status of *Pneophyllum conicum* (E.Y. Dawson) Keats, Y.M. Chamberlain & Baba and its relationships with the genera *Hydroli- thon* and *Porolithon* also needs to be reconsidered. *Hydrolithon conicum* E.Y. Dawson was transferred to *Pneophyllum* by Keats et al. (1997) because the species has the tetrasporangial concepta- cle roof development said to be diagnostic of the genus *Pneophyl- lum*. However, *Pneophyllum conicum* (lineage Q) and presently several unidentified crustose specimens (LBC0601, LBC0560, lineage P; LBC0600, lineage R) ally with the genus *Porolithon* (lineages N + O). Incidentally, these specimens also have a monomerous thallus organisation. We propose to also attribute these latter taxa to the genus *Porolithon* and suggest transferring *Pn. conicum* to *Porolithon conicum* comb. nov. In future studies, it would be worthwhile including other *Pneophyllum* species (and particularly the type species *Pneophyllum fragile* Kutzing), which all possess a dimerous thallus construction, to ascertain the phylogenetic position of this genus. It is also worth mentioning that Cabioch (1972) highlighted the similarity of the thallus development between the genus *Metagoniolithon* Weber-van Bosse and branched (protuberant) species of *Porolithon*.

Finally, our molecular data shows that the large lineage U, which is well supported, comprises five distinct evolutionarily lineages. Significant taxonomic changes at subfamily and lower ranks are clearly in need. This has to be addressed in future studies with exhaustive nomenclatural investigation.

Cryptic diversity in the Corallinales

The Corallinales are reported to be the third most diverse order within the Rhodophyta with 564 (Brodie and Zuccarello, 2007) to 601 (Guiry and Guiry, 2011) morpho-species currently recognized. Several taxa are supposedly cosmopolitan. However, their diversity has not been evaluated in light of molecular data.

Our phylogenies show clearly that re-appraisals of the genera *Neogoniolithon* as well as *Mesophyllum* (particularly *M. erubescens)* are necessary. The type species of

Mesophyllum is *M. lichenoides* (Woelkerling and Irvine, 1986, 2007). While this species is included in our analyses, our species-rich dataset (Fig. 1) shows that specimens of *M. erubescens* from New Zealand are more closely related to *M. lichenoides* (lineage D) from France (Channel Sea) than to specimens of *M. erubescens* from the type-locality (Brazil), or from the South-Pacific Ocean (Vanuatu, Fiji) (lineage C). Broom et al. (2008) already highlighted the cryptic diversity of *M. erubes- cens* and our results confirm that this morpho-species has been overlooked. These findings thus warrant a thorough study of the species from various geographical locations combining morpho- anatomic observations and molecular phylogenies (inferred from a more variable marker than the SSU) to better delineate species frontiers within this complex.

4.4. Considerations concerning diagnostic characters

Mapping of the character states that are traditionally used to identify families and subfamilies in the Corallinales shows that, except for the absence or presence of uniporate or multiporate tet- rasporangial conceptacles, none are diagnostic and useful to define lineages at an infra-ordinal rank. Since sexual reproductive structures are rarely observable (Woelkerling, 1988), efforts should focus on finding additional vegetative structures, for example, trichocyte arrangements and presence of megacells are character states that have to be re-investigated. We advocate also that detailed studies of developmental features (as thallus ontogeny) can certainly shed new light into the evolutionary story of the numerous lineages within the Corallinales, as predicted by Cabioch (1972, 1988) a few decades ago.

5. Conclusion and prospective studies

This study used four molecular markers and included numerous representative taxa from all but one **(Austrolithoideae)** subfamily within the Corallinales, rendering it, to the best of our knowledge, the most comprehensive study of its kind to date. Our study shows that multi-marker analyses improves the resolution of the Corallinales phylogeny and that LSU and *psbA* sequences provide a better phylogenetic resolution than SSU, the most commonly used marker for Corallinales phylogeny. Amplification and sequencing of supplementary plastidial markers, or of nuclear encoding markers (such as EF2) would likely bring additional signal to clarify the phylogenetic relationships within the lineage U of the Corallinaceae, which includes representatives of the genera *Amphiroa, Hydrolithon, Litho- phyllum, Metagoniolithon, Pneophyllum, Spongites* and *Titanoderma.*

In order to render the taxonomy of the Corallinales closer to a natural system of classification, new taxonomic delineations within the Corallinaceae (as the emendation of the Mastophoroideae only to the genera *Lithoporella, Mastophora* and *Metamastophora)* and the resurrection of the genus *Porolithon* are proposed. Despite our well-resolved and taxon-rich dataset, phylogenetic affinities of many

coralline algal taxa still need to be addressed. The genera *Lithothamnion* and *Lithophyllum,* which encompass 80 and 112 species respectively (Guiry and Guiry, 2011), should be studied in further detail to better delineate taxon boundaries. Efforts should also be made toward including more 'rare' species such as the mono-specific taxa *Lesueuria minderiana* Woelkerling & Ducker (described as a Mastophoroideae, Woelkerling and Ducker, 1987) and *Boreoli- thon van-heurckii* (Heydrich) A.S. Harvey & Woelkerling, as well as various parasitic forms (as listed in Townsend and Huisman, 2004).

Finally, Corallinales show an extensive and robust fossils records because of the calcification of their cell walls (Aguirre et al., 2010). However some specimens, because of the poor preservation and/or absence of diagnostic morpho-anatomical characters, cannot be pinpointed easily to current living clades. Next challenges will certainly be to produce and then include sequences from fossils for comparison against extant lineages (Hughey et al., 2008). The present study provides a reliable phylogeny which, coupled with few strong reliable calibration points inferred from the fossil record, could be used to improve molecular clock analyses within the Corallinales. To date, splitting events were inferred without representatives of the Mastophoroideae due to the suspected paraphyly of this subfamily (Aguirre et al., 2010). The molecular data set that we have provided in the present article will most likely contribute to understanding evolutionary scenarios on the diversification (speciation/extinction), colonisation, and recurrent morpho-anatomical convergence events within the coralline algae, as well as the calibration of the red algal tree of life.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.07.019.

References

Adey, W.H., 1970. A revision of the Foslie crustose coralline herbarium. Det K. Nor. Vidensk. Selsk. Skr. 1, 1-46.

Aguirre, J., Perfectti, F., Braga, J.C., 2010. Integrating phylogeny, molecular clocks, and the fossil record in the evolution of coralline algae (Corallinales and Sporolithales, Rhodophyta). Paleobiology 36, 519-533.

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov, B.N., Csaki, F., (Eds), Second International Symposium on Information Theory. Akademiai Kiado, Budapest, Hungary, pp. 267-281.

Amado-Filho, G.M., Maneveldt, G., Manso, R.C.C., Marins-Rosa, B.V., Pacheco, M.R., Guimaraes, S.M.P.B., 2007. Structure of rhodolith beds from 4 to 55 meters deep along the southern coast of Espfrito Santo State, Brazil. Cienc. Mar. 33, 399-410.

Bailey, J.C., 1999. Phylogenetic positions of *Lithophyllum incrustans* and *Titanoderma pustulatum* (Corallinaceae, Rhodophyta) based on 18S rRNA gene sequence analyses, with a revised classification of the Lithophylloideae. Phycologia 38, 208-216.

Bailey, J.C., Chapman, R.L., 1996. Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) inferred from 18S rRNA gene sequence analysis. In: Chaudhary, B.R., Agrawal, S.B. (Eds.), Cytology, Genetics and Molecular Biology of Algae. SPB Academic Publishing, Amsterdam, pp. 363-376.

Bailey, J.C., Chapman, R.L., 1998. A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. J. Phycol. 34, 692-705.

Bailey, J.C., Gabel, J.E., Freshwater, D.W., 2004. Nuclear 18S rRNA gene sequence analyses indicate that the Mastophoroideae (Corallinaceae, Rhodophyta) is a polyphyletic taxon. Phycologia 43, 3-12.

Bittner, L., Halary, S., Payri, C., Cruaud, C., de Reviers, B., Lopez, P., Bapteste, E., 2010. Some considerations for analyzing biodiversity using integrative metagenomics and gene networks. Biol. Direct 5, 47.

Broadwater, S.T., Harvey, A.S., Lapointe, E.A., Woelkerling, W.J., 2002. Conceptacle structure of the parasitic coralline red alga *Choreonema thuretii* (Corallinales) and its taxonomic implications. J. Phycol. 38,1157-1168.

Brodie, J., Zuccarello, G.C., 2007. Systematics of the species rich algae: red algal classification, phylogeny and speciation. In: Hodkinson, T.R. (Ed.), Reconstructing the Tree of Life: Taxonomy and Systematics of Species Rich Taxa. CRC/Taylor and Francis, Boca Raton, Florida, pp. 317-330.

Broom, J.E.S., Hart, D.R., Farr, T.J., Nelson, W.A., Neill, K.F., Harvey, A.S., Woelkerling, W.J., 2008. Utility of *psb*A and nSSU for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. Mol. Phylogenet. Evol. 46, 958-973.

Cabioch, J., 1972. Etude sur les Corallinacees. II. La morphogenese: consequences systematiques et phylogenetiques. Cah. Biol. Mar. 13, 137-288.

Cabioch, G., Montaggioni, L., Franck, N., Seard, C., Salle, E., Payri, C.E., Pelletier, B., Paterne, M., 2008. Successive reef depositional events along the Marquesas foreslopes (French Polynesia) since 26 ka. Mar. Geol. 250, 157-179.

Cabioch, J., 1988. Morphogenesis and generic concepts in coralline algae—a reappraisal. Botanica 49, 493-509, Botanica 49,1-98.

Campbell, S.J., Woelkerling, W.J., 1990. Are *Titanoderma* and *Lithophyllum* (Corallinaceae, Rhodophyta) distinct genera? Phycologia 29, 114-125.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783-791.

Guiry, M.D., Guiry, G.M., 2011. AlgaeBase. World-Wide Electronic Publication, National University of Ireland, Galway. <http://www.algaebase.org>.

Harper, J.T., Saunders, G.W., 2001a. Molecular systematics of the Florideophyceae (Rhodophyta) using nuclear large and small subunit rDNA sequence data. J. Phycol. 37, 1073-1082.

Harper, J.T., Saunders, G.W., 2001b. The application of sequences of the ribosomal cistron to the systematics and classification of the florideophyte red algae (Florideophyceae, Rhodophyta). Cah. Biol. Mar. 42, 25-38.

Harper, J.T., Saunders, G.W., 2002. A re-classification of the Acrochaetiales based on molecular and morphological data, and establishment of the Colaonematales ord. nov. (Florideophyceae, Rhodophyta). Eur. J. Phycol. 37, 463-476.

Harvey, A.S., Broadwater, S.T., Woelkerling, W.J., Mitrovski, P.J., 2003. *Choreonema* (Corallinales, Rhodophyta): 18S rDNA phylogeny and resurrection of

the Hapalidiaceae for the subfamilies Choreonematoideae, Australithoideae, and Melobesioideae. J. Phycol. 39, 988-998.

Hughey, J.R., Braga, J.C., Aguirre, J., Woelkerling, W.J., Webster, J.M., 2008. Analysis of ancient DNA from fossil corallines (Corallinales, Rhodophyta). J. Phycol. 44, 374-383.

Hurvich, C.M., Tsai, C.L., 1989. Regression and time series model selection in small samples. Biometrika 76, 297-307.

Johansen, H.W., 1976. Current status of generic concepts in coralline algae (Rhodophyta). Phycologia 15, 221-244.

Kato, A., Baba, M., Suda S., 2009. A Systematic Study of Crustose Coralline Alga *Neogoniolithon brassica-florida* in the Ryukyu islands, Japan. In: Poster at the IPC9 (2nd-8th August, Tokyo, Japan).

Keats, D.W., Chamberlain, Y.M., Baba, M., 1997. *Pneophyllum conicum* (Dawson) comb. nov. (Rhodophyta, Corallinaceae), a widespread Indo-Pacific non- genienlate coralline alga that overgrows and kills coral. Bot. Mar. 40, 263-279.

Kostka, M., Uzlikova, M., Cepicka, I., Flegr, J., 2008. SlowFaster, a user-friendly program for slow-fast analysis and its application on phylogeny of Blastocystis. BMC Bioinform. 9, 341.

Lamy, D., Woelkerling, W.J., 1998. The Paris Museum and non-geniculate coralline systematics. In: Woelkerling, W.J., Lamy, D. (Eds.), Non-geniculate Coralline Red Algae and the Paris Museum: Systematics and Scientific History. Publications Scientifiques du Museum/A.D.A.C, Paris, pp. 15-242.

Le Gall, L., Dalen, J.D., Saunders, G.W., 2008. Phylogenetic analyses of the red algal order Rhodymeniales supports recognition of the Hymenocladiaceae fam. nov., Fryeellaceae fam. nov., and *Neogastroclonium* gen. nov. J. Phycol. 44, 15561571.
Le Gall, L., Payri, C.E., Bittner, L., Saunders, G.W., 2010. Multigene phylogenetic analyses support recognition of the Sporolithales ord. nov. Mol. Phylogenet. Evol. 54, 302-305.

Le Gall, L., Saunders, G.W., 2007. A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: establishing the Corallinophycidae subclassis nov. Mol. Phylogenet. Evol. 43, 1118-1130.

Le Gall, L., Saunders, G.W., 2010. DNA barcoding is a powerful tool to uncover algal diversity: a case study of the Phyllophoraceae (Gigartinales, Rhodophyta) in the Canadian flora. J. Phycol. 46, 374-389.

Maddison, W., Maddison, D., 2003. MacClade, v. 4.06, Sinauer Associates.
Maddison, W., Maddison, D., 2006. Mesquite: a modular system for evolutionary analysis. Version 2.6. <http://mesquiteproject.org>.

Maneveldt, G.W., 2005. A Global Revision of the Nongeniculate Coralline Algal Genera *Porolithon* Foslie (Defunct) and *Hydrolithon* Foslie (Corallinales, Rhodophyta). PhD manuscript, University of the Western Cape, Bellville, South Africa. pp 520 (pls 187).

Maneveldt, G.W., Keats, D.W., 2008. Effects of herbivore grazing on the physiognomy of the coralline alga *Spongites yendoi* and on associated competitive interactions. Afr. J. Mar. Sci. 30, 581-593.

Medina-Pons, F.J., Terrados, J., Lopez-Lopez, A., Yarza, P., Rossello-Mora, R., 2009. Evaluation of the 18S rRNA clone library approach to study the diversity of the macroeukaryotic leaf-epiphytic community of the seagrass *Posidonia oceanica* (L.). Delile. Mar. Biol. 156, 1963-1976.

Payri, C.E., 1995. Production carbonatee de quelques algues calcifiees sur un recif corallien de Polynesie fran^aise". Bull. Soc. Geol. Fr. 166 (1), 77-84.

Payri, C.E., Cabioch, G., 2003. The systematics and significance of coralline red algae in the rhodolith sequence of the Amedee 4 drill core (South-West New- Caledonia). Palaeogeogr. Palaeoclimatol. Palaeoecol. 204, 187-208.

Penrose, D., Woelkerling, W.J., 1992. A reappraisal of *Hydrolithon* and its relationship to *Spongites* (Corallinaceae, Rhodophyta). Phycologia 31, 81-88.
Posada, D., 2008. JModelTest: phylogenetic modelaveraging. Mol. Biol. Evol. 25, 1253-1256.

Pueschel, C.M., 1989. An expanded survey of the ultrastructure of red algal pit plugs. J. Phycol. 25, 625-636.

Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: the barcode of life data system. Mol. Ecol. Note 7, 355-364.

Rokas, A., Carroll, S.B., 2005. More genes or more taxa? The relative contribution of gene number and taxon number to phylogenetic accuracy. Mol. Biol. Evol. 90, 1337-1344.

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. J. Phycol. 29, 251-254.

Saunders, G.W., 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philos. Trans. R. Soc. London, B 360, 1879-1888.

Saunders, G.W., Bailey, J.C., 1997. Phylogenesis of pit-plug-associated features in the Rhodophyta: inferences from molecular systematic data. Can. J. Bot. 75,14361447.

Saunders, G.W., Kraft, G.T., 1994. Small-subunit rRNA gene sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 1. Evidence for the Plocamiales ord. nov. Can. J. Bot. 72, 12501263.

Saunders, G.W., Kraft, G.T., 1996. Small-subunit rRNA gene sequences from reprensentatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 2. Recognition of the Halymeniales ord. nov.. Can. J. Bot. 74,694707.

Saunders, G.W., Lehmkuhl, K.V., 2005. Molecular divergence and morphological diversity among four cryptic species of *Plocamium* (Plocamiales, Florideophyceae) in northern Europe. Eur. J. Phycol. 40, 293-312.

Schwarz, G., 1978. Estimating the dimension of a model. Ann. Stat. 6, 461-464.
Setchell, W.A., 1943. *Mastophora* and the Mastophoreae: genus and subfamily of Corallinaceae. Proc. Natl. Acad. Sci. U. S. A. 29, 127-135.

Silva, P.C., Johansen, H.W., 1986. A reappraisal of the order Corallinales (Rhodophyceae). Brit. Phycol. J. 21, 245-254.

Shavit, L., Penny, D., Hendy, M.D., Holland, B.R., 2007. The problem of rooting rapid radiations. Mol. Biol. Evol. 24, 2400-2411.

Stamakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 26882690.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML Web-servers. Sys. Biol. 75, 758-771.

Steneck, R.S., 1986. The ecology of coralline algal crusts: convergent patterns and adaptive strategies. Am. Rev. Ecol. Syst. 17, 273-303.

Steneck, R.S., Adey, W.H., 1976. The role of environment control of morphology in *Lithophyllum congestum,* a Caribbean algal ridge builder. Bot. Mar. 19, 197215.

Townsend, R.A., Huisman, J.M., 2004. *Epulo multipedes* gen. et. sp. nov. (Corallinaceae, Rhodophyta), a coralline parasite from Australia. Phycologia 43, 288-295.

Verbruggen, H., Maggs, C.A., Saunders, G.W., Le Gall, L., Yoon, H.S., De Clerck, O., 2010. Data mining approach identifies research priorities and data requirements for resolving the red algal tree of life. BMC Evol. Biol. 10,16.

Verheij, E., 1993. The genus *Sporolithon* (Sporolithaceae fam. nov., Corallinales, Rhodophyta) from the Spermonde Archipelago, Indonesia. Phycologia 32,184196.

Verheij, E., 1994. Nongeniculate Corallinaceae (Corallinales, Rhodophyta) from the Spermonde Archipelago, SW Sulawesi, Indonesia. Blumea 39, 95-137.

Walker, R.H., Brodie, J., Russell, S., Irvine, L.M., Orfanidis, S., 2009. Biodiversity of Coralline algae in the north-eastern Atlantic including *Corallina caespitosa* sp. nov. (Corallinoideae, Rhodophyta). J. Phycol. 45, 287-297.

Woelkerling, W.J., 1988. The coralline red algae : an analysis of the genera and subfamilies of nongeniculate Corallinaceae. Brithish Museum (Natural History), London and Oxford University Press, Oxford, 268 pp.

Woelkerling, W.J., Campbell, S.J., 1992. An account of the southern Australian species of *Lithophyllum* (Corallinaceae, Rhodophyta). Bull. Brit. Mus. (Nat. Hist.) Bot. 22, 1-107.

Woelkerling, W.J., Ducker, S.C., 1987. *Lesueuria minderiana* gen. et sp. nov. (Corallinaceae, Rhodophyta) from southern and western Australia. Phycologia 26, 192-204.

Woelkerling, W.J., Irvine, L.M., 1986. The neotypification and status of *Mesophyllum* (Corallinaceae, Rhodophyta). Phycologia 25, 379-396.

Woelkerling, W.J., Irvine, L.M., 2007. The genus *Mesophyllum* (Hapalidiaceae, Corallinales, Rhodophyta): typification update. Phycologia 46, 230-231.

Woelkerling, W.J., Irvine, L.M., Harvey, A.S., 1993a. Growth-forms in non-geniculate coralline red algae (Corallinales, Rhodophyta). Aust. Syst. Bot. 6, 277-293.

Woelkerling, W.J., Penrose, D., Chamberlain, Y.M., 1993b. A reassessment of type collections of non-geniculate Corallinaceae (Corallinales, Rhodophyta) described by C. Montagne and L. Dufour, and of *Melobesia brassica-florida* Harvey. Phycologia 32, 323-331.

Yoon, H.S., Hackett, J.D., Bhattacharya, D., 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. Proc. Natl. Acad. Sci. U. S. A. 99,11724-11729.