



First assessment of the diversity of coralline species forming maerl and rhodoliths in Guadeloupe, Caribbean using an integrative systematic approach

VIVIANA PEÑA^{1,2,3}, FLORENCE ROUSSEAU², BRUNO DE REVIERS² & LINE LE GALL²

¹Grupo de investigación BIOCOST, Universidad de A Coruña, Facultad de Ciencias, Campus de A Zapateira S/N, 15071, A Coruña, Spain.

²UMR 7205 ISYEB CNRS, MNHN, UPMC, EPHE, Equipe Exploration, Espèces et Evolution, Institut de Systématique, Evolution, Biodiversité, Muséum national d'Histoire naturelle (MNHN), case postale N° 39, 57 rue Cuvier, 75231 CEDEX 05, Paris, France.

³Phycology Research Group, Ghent University, Krijgslaan 281, Building S8, 9000, Ghent, Belgium.

Corresponding author: Viviana Peña. Email address: vpena@udc.es

Abstract

The present study documents species of coralline algae that form maerl and rhodoliths in Guadeloupe, Caribbean using an integrative systematic approach of combining molecular (COI-5P, *psbA*) and morphological/anatomical data. Maerl and rhodoliths were collected by SCUBA and dredging from six localities in Guadeloupe during the *Karubenthos Expedition*, which was coordinated by the Parc National de la Guadeloupe and the Muséum National d'Histoire Naturelle. Of the twelve maerl and rhodolith specimens collected and sequenced, eight specific entities were delimited based on the analysis of molecular data: *Lithothamnion* cf. *ruptile*, five species of the genus *Lithothamnion*, one species of the genus *Spongites*, and the remaining one was either assigned to the genus *Lithoporella* or *Mastophora*. Morphological/anatomical data are presented for each of these species. Molecular analyses revealed that *Lithothamnion* cf. *ruptile* and other four species (*Lithothamnion* spp. 1–4) were resolved in a distinct lineage than the other *Lithothamnion* species examined. Nevertheless, these five species were provisionally remained in the genus *Lithothamnion* based on the presence of flared epithelial cells. Previously, only three species of *Lithothamnion* have been cited in the Caribbean. Till this study species belonging to the genus *Spongites* have not been hitherto reported for this region. The specimen identified as either *Lithoporella* or *Mastophora* shared anatomical features with both genera. Further examination of reproductive material is required to identify this specimen to a lower taxonomical rank. This study is the first to report the occurrence of maerl and rhodoliths belonging to the genus *Lithothamnion* at 110 m depth; this record being the deepest for the genus in the Caribbean Sea. Our results have revealed that species diversity of Caribbean maerl has been underestimated and further surveys coupled with an integrative taxonomic approach on this biodiversity hotspot are necessary.

Key words: Biodiversity, Caribbean, Coralline algae, DNA barcoding, Guadeloupe, maerl, rhodolith, systematics, West Indies

Introduction

In the Lesser Antilles, West Indies, the Guadeloupe Archipelago is constituted of the islands La Désirade, Les Saintes, Marie-Galante and Guadeloupe, the latter comprising two main islands Basse-Terre and Grande-Terre (Fig. 1). The Grand Cul-de-Sac Marin which extends northward is the best developed reef of the island with its outer edge bounded by a barrier reef (Spalding *et al.* 2001).

The terrestrial plant biodiversity of the region is well known. The region is considered a biodiversity hot spot with 214 plant species endemic to the Lesser Antilles, 24 species of which are endemic to Guadeloupe (IUCN 2003). However, the algal diversity of Guadeloupe has received only little attention with the majority of the main studies conducted before World War II. Mazé & Schramm (1878) were among the first to report on the algae of Guadeloupe in 1870–1877 following the initiative of Placide Duchassaing who started an herbarium of seaweeds gathered on the beach of Le Moule. Mazé & Schramm (1878) identified, with extensive exchanges with the Crouan brothers, 811 marine algae, 438 of which were red algae. In 1931, Gontran Hamel published 20 copies of an *exsiccata* entitled “Algues des Antilles Française recueillies par M.G. Hamel and Mme A. Hamel Joukov” consisting of 150 voucher specimens.

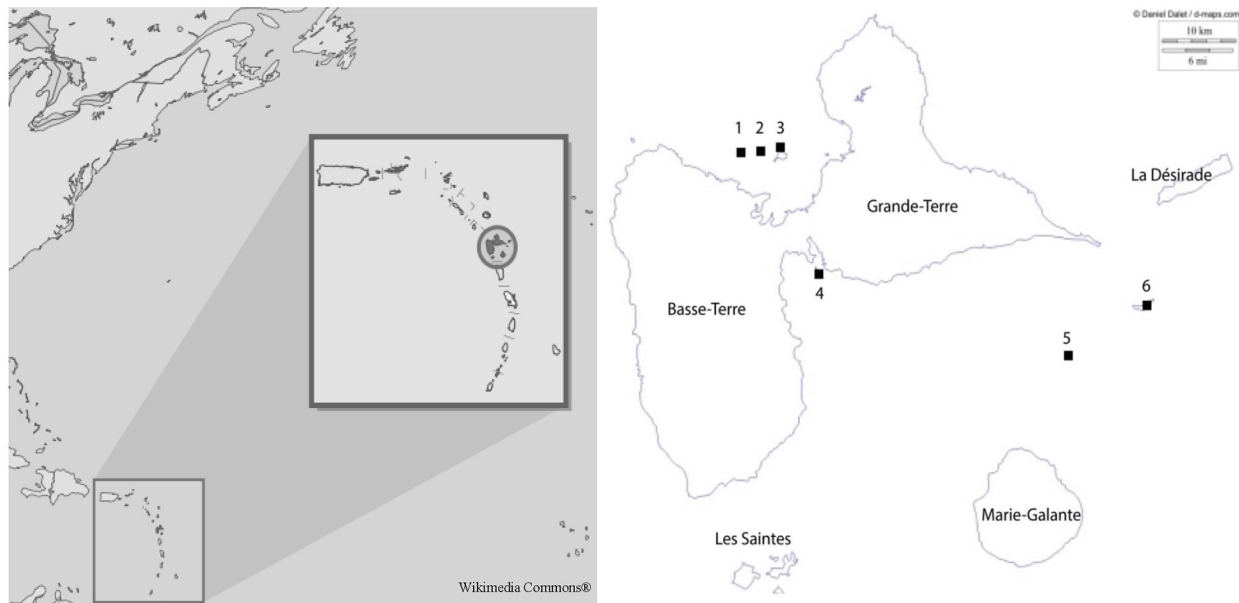


FIGURE 1. Map of the Caribbean Sea and localization of the Guadeloupe Archipelago with sampling areas: Grand Cul de-Sac-Marin (1—Passe à Caret, 2—îlet Caret, 3—îlet Fajou); Petit Cul-de-Sac Marin (4—îlet du Gosier); Saint-François (5—Banc des Vaisseaux); and îles de Petite Terre (6).

In the Caribbean, only 39 coralline taxa are currently accepted taxonomically (Guiry & Guiry 2014). These species were reported either associated with coral reefs as attached algae or as unattached free-living forms maerl or rhodoliths (Foslie 1908, Lemoine 1917, Taylor 1960, Schneider & Searles 1991, Littler & Littler 2000, Martínez-Daranas *et al.* 2000, Betancourt & Herrera-Moreno 2001, Ballantine *et al.* 2004, 2011, Athanasiadis & Ballantine 2011, Galicia-García *et al.* 2013). In Guadeloupe, Mazé & Schramm (1878) reported 32 species belonging to the Corallinales although most of them were either articulated corallines or non-articulated (crustose) epiphytes. The authors reported the presence of *Lithothamnion polymorphum* (Linnaeus) Areschoug (currently regarded as a taxonomic synonym of *Phymatolithon calcareum* (Pallas) W.H.Adey & D.L.McKibbin) on coral rubbles after a tidal wave and *Mastophora lamourouxii* Decaisne *ex* Harvey (currently regarded as a taxonomic synonym of *Metamastophora flabellata* (Sonder) Setchell) which was dredged between the reef and the beach. Within Hamel's *exsiccata* only two coralline algae were presented: the specimens 86 -*Amphiroa fragilissima* (Linnaeus) J.V.Lamouroux- and 87 -*Corallina cubensis* (Montagne *ex* Kützing) Kützing, currently regarded as a taxonomic synonym of *Jania cubensis* Montagne *ex* Kützing-.

To address the lack of knowledge on the algae and more generally on the diversity of benthic organisms, the Parc National de Guadeloupe coordinated with the Muséum National d'Histoire Naturelle, organized the *Karubenthos Expedition*. This expedition involved SCUBA and dredging surveys at several localities around Guadeloupe during May 2012. As far as we know the occurrence of maerl and rhodoliths from this archipelago was hitherto unknown.

Recently, assessments of alpha diversity of maerl, rhodoliths and crustose coralline algal species have benefited from the employment of DNA barcodes based on the mitochondrial gene coding the cytochrome c oxidase subunit 1 (COI-5P fragment) which have also been successfully applied to the identification of coralline species and the detection of cryptic taxa (Walker *et al.* 2009, Sherwood *et al.* 2010, Bittner *et al.* 2011, Pardo *et al.* 2012, Hind & Saunders 2013, Kato *et al.* 2013, Peña *et al.* 2014). The plastid-encoded *psbA* and *rbcL* genes (coding for the D1 protein of photosystem II and the large subunit of the RUBISCO, respectively) have been also previously employed for species identification and for phylogenetic reconstructions in combination with nuclear markers (Broom *et al.* 2008, Bittner *et al.* 2011, Gabrielson *et al.* 2011, Martone *et al.* 2012).

In the present study, we provided the first results obtained from this on-going project. Here we have used morphological/anatomical and molecular techniques in an integrative approach to assess the diversity of maerl and rhodolith species in Guadeloupe. The results obtained are discussed in light of the literature available for coralline red algae from the Caribbean Sea.

Material & methods

Maerl and rhodoliths were collected by SCUBA and dredging from 5 to 110 m depth during the Karubenthos expedition from five localities around Guadeloupe, Caribbean Sea (Fig. 1): Grand Cul-de-Sac Marin (1–3: Passe à Caret, îlet Caret, and îlet Fajou, respectively), Petit Cul-de-Sac Marin (4: îlet du Gosier), Saint-François (5: Banc des Vaisseaux), and îles de Petite Terre (6). Specimens were air dried and preserved in zipper bags with silica gel, and later deposited in PC (Muséum National d'Histoire Naturelle, Paris, France; acronym follows Holmgren *et al.* 1990).

Molecular studies

Twelve maerl and rhodoliths from Guadeloupe were chosen for molecular analysis. Unattached specimens with coralline algal cores were regarded as maerl, whereas unattached specimens with a non-algal core were regarded as rhodoliths (Irvine & Chamberlain 1994). The specimens were cleaned from epiphytes under a stereomicroscope and selected surfaces were ground with a 2 mm drill bit for DNA extraction. Genomic DNA was extracted using a NucleoSpin® 96 Tissue kit (Macherey-Nagel, GmbH and Co. KG, Germany). The mitochondrial COI-5P fragment was PCR amplified using the primer pairs GazF1 and GazR1 (Saunders 2005) or GazF1 and GCorR3 (reverse, 5' TGATYTTYGGACATCCTGA 3'). The *psbA* locus was amplified using *psbA*-F1 and *psbA*-R2 (Yoon *et al.* 2002). The thermal profile for PCR amplification for COI-5P followed Saunders & McDevitt (2012). Thermal profile for PCR amplification for *psbA* followed Bittner (2009). PCR reactions used the Taq PCR core kit® (Qiagen S.A.S. France) and were performed in 25 µL containing 2 µL of DNA template (diluted 1/25), 2.5 µL of 10x CoralLoad PCR buffer, 2.5 mM MgCl₂, 0.192 mM dNTPs, 0.8 µg BSA (Bovine Serum Albumin), 1% PVP (polyvinylpyrrolidone), 0.1 µM of each primer, and 0.6 U of Taq DNA Polymerase. PCR products were purified and sequenced by Genoscope (*Bibliothèque du Vivant* program, Centre National de Séquençage, France). Sequences were assembled and aligned with the assistance of CodonCode Aligner® (CodonCode Corporation, USA) and adjusted by eye using SeaView version 4 (Gouy *et al.* 2010). Sequences were submitted to the Barcode of Life Data Systems (BOLD: <http://www.boldsystems.org>; project “NGCOR”, BOLD, <http://www.boldsystems.org>; Ratnasingham & Hebert 2007) and GenBank (accession numbers detailed in Table S1). In addition, we searched in both databases for publicly available sequences from the study area (Guadeloupe) and the Caribbean Sea. For the phylogenetic analyses, the closest COI-5P sequences (> 89% similarity) and corresponding *psbA* sequences found in both databases were included, as well as available COI-5P and *psbA* sequences identified at genus or species level that pertained to different non-geniculate genera of Corallinales and Sporolithales, the latter as outgroup (Table S1). In addition, COI-5P and *psbA* sequences of temperate maerl-forming species were also included: *Lithothamnion corallioides* (P.L.Crouan & H.M.Crouan) P.L.Crouan & H.M.Crouan, *L. cf. crispatum* Hauck, *Mesophyllum sphaericum* V.Peña, Bárbara, W.H.Adey, Riosmena-Rodríguez & H.G.Choi (holotype material, Peña *et al.* 2011), *Phymatolithon calcareum* (neotype material, Woelkerling & Irvine 1986) and *Spongites cf. fruticulosa* Kützing submitted to BOLD (projects “MAERL” –Pardo *et al.* 2012- and “NGCOR”) and GenBank (Table S1).

A general mixed Yule-coalescent (GMYC) model was applied for the delimitation of species using COI-5P data (Pons *et al.* 2006, Fujisawa & Barraclough 2013). The method defines the species boundary based on an ultrametric tree obtained with a Bayesian phylogenetic analysis conducted in BEAST v1.7.4 (Drummond *et al.* 2012) under a generalized time-reversible model with gamma + invariant sites to accommodate for rate heterogeneity (GTR + G + I), an uncorrelated lognormal (UCLN) relaxed molecular clock, and using a coalescence tree prior. Two Markov Chain Monte Carlo (MCMC) analyses were run for 10 million generations, sampling every 1000th generation. The information from a sample of trees produced was summarized onto a single “target” tree (10% burnin discarded at the start of the run, 0.5 of posterior probability limit of the nodes in target tree) using Tree Annotator v 1.7.4 (<http://beast.bio.ed.ac.uk>). GMYC analyses were performed using the SPLITS package for R (<http://r-forge.r-project.org/project/splits>). Phylogenetic relationships were inferred using Maximum Likelihood (ML) and Bayesian inference (BI) using Mega 6 and MrBayes 3.2.1, respectively (Ronquist & Huelsenbeck 2003, Tamura *et al.* 2013). Models of sequences evolution were estimated using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) obtained in Modeltest 2.1.3 (Darriba *et al.* 2012). Maximum Likelihood analyses for the COI-5P and *psbA* alignments were performed under a generalized time-reversible with gamma + invariant sites heterogeneity model (GTR + G + I), and the bootstrap consisted of 1000 replicates. The Bayesian analysis was conducted for the *psbA* alignment under the same model (GTR + G + I) with four Markov Monte Carlo Chains for ten million generation, and tree sampling every thousand generations.

TABLE S1. Sample information for species included in the molecular analyses. Haplotypes observed for each species are provided. Additional COI-5P and psbA sequences available from GenBank and BOLD are detailed in alphabetical order and, in bold letters, specimens collected in the study area (Guadeloupe).

Order, family	Species	Collection details	Haplotypes	Voucher	Herbarium	Genbank Accession number (COI-5P/psbA)	Geographical coordinates (Lat, Long)
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> cf. <i>ruptile</i>	Subtidal (-32 m), Passe à Caret, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 23/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA1993	PC053581	-/KJ710353	16.3451841698, -61.6283433516
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> sp. 1	Subtidal (-5 m), Gros Mouton, îlet Caret, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 04/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA1148	PC0144033	KJ710343/ KJ710352	16.356385, -61.625596
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> sp. 1	Subtidal (-5 m), Gros Mouton, îlet Caret, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 04/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	2	FRA1152	PC0144055	KJ710342	16.356385, -61.625596
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> sp. 1	Subtidal (-6 m), îlet du Gosier, Petit Cul-de-Sac, Guadeloupe, West Indies, 03/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	3	FRA1059	PC0142665	KJ710345	16.197371, -61.490671
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> sp. 2	Subtidal (-50 m), îles de Petite Terre, Guadeloupe, West Indies, 27/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA2172	PC0144249	KJ710344/ KJ710354	16.165553, -61.131555
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> sp. 3	Subtidal (-15 m), Pente externe face à l'îlet Fajou, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 06/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA1211	PC0144042	KJ710346/ KJ710355	16.353090, -61.589891
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> sp. 4	Subtidal (-95 m), îles de Petite Terre, Guadeloupe, West Indies, 26/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA2164	PC0144248	KJ710341/-	16.165553, -61.131555
Corallinales, Hapalidiaceae	<i>Lithothamnion</i> sp. 5	Subtidal (-110 m), Banc des Vaisseaux, Saint-François, Guadeloupe, West Indies, 27/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA2177	PC0144250	KJ710337/ KJ710349	16.134224, -61.28055
Corallinales, Corallinaceae	<i>Spongites</i> sp.	Subtidal (-32 m), Passe à Caret, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 23/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA2004B	PC0144244	KJ710347/-	16.345184, -61.628343
Corallinales, Corallinaceae	<i>Spongites</i> sp.	Subtidal (-32 m), Passe à Caret, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 23/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA1997	PC0144235	KJ710338/-	16.345184, -61.628343
Corallinales, Corallinaceae	<i>Spongites</i> sp.	Subtidal (-32 m), Passe à Caret, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 23/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	2	FRA2000A	PC0144239	KJ710339/ KJ710350	16.345184, -61.628343
Corallinales, Corallinaceae	<i>Lithoporella Mastophora</i> sp.	Subtidal (-32 m), Passe à Caret, Grand Cul-de-Sac Marin, Guadeloupe, West Indies, 23/05/2012. Coll: <i>Karubenthos Expedition</i> (Le Gall <i>et al.</i>).	1	FRA1998B	PC0144236	KJ710348/ KJ710357	16.345184, -61.628343

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TABLE S1. (Continued)

Additional COI-5P and <i>psbA</i> sequences data included in the alignment ordered alphabetically		COI-5P	<i>psbA</i>
Corallinales, Corallinaceae	<i>Amphiroa</i> sp.	Guadeloupe, Bittner <i>et al.</i> (2010, 2011).	GQ917435
Corallinales, Corallinaceae	<i>Amphiroa</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010).	GQ917705
Corallinales, Corallinaceae	<i>Amphiroa</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010).	GQ917698
Corallinales, Corallinaceae	<i>Amphiroa</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010).	GQ917699
Corallinales, Corallinaceae	<i>Amphiroa fragilissima</i> (Linnaeus) J.V.Lamouroux	Belize, Bittner <i>et al.</i> (2011).	GQ917498
Corallinales, Corallinaceae	<i>A. fragilissima</i> (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010, 2011).	GQ917702
Corallinales, Corallinaceae	<i>Amphiroa</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010, 2011).	GQ917704
Corallinales, Corallinaceae	<i>Amphiroa</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010, 2011).	GQ917703
Corallinales, Hapalidiaceae	Corallinales sp.	Bermuda, Schneider <i>et al.</i> (unpublished, BOLD:AAO6764).	-
Corallinales, Hapalidiaceae	Corallinales sp.	Canada, Saunder & Dixon (unpublished, BOLD:AAM4114).	-
Corallinales, Hapalidiaceae	Corallinales sp.	Australia, Saunder & Dixon (unpublished, BOLD:AAI5865).	-
Corallinales, Corallinaceae	<i>Hydroolithon reinboldii</i> (Weber-van Bosse & Foslte).	New Caledonia, Bittner <i>et al.</i> (2010, 2011).	GQ917485
Corallinales, Corallinaceae	<i>H. reinboldii</i>	New Caledonia, Bittner <i>et al.</i> (2010, 2011).	GQ917484
Corallinales, Corallinaceae	<i>H. reinboldii</i>	Hawaii, Sherwood <i>et al.</i> (2010).	-
Corallinales, Corallinaceae	<i>Hydroolithon</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010, 2011).	GQ917700
Corallinales, Corallinaceae	<i>Hydroolithon</i> sp.	New Caledonia, Bittner <i>et al.</i> (2010, 2011).	GQ917493
Corallinales, Corallinaceae	<i>Hydroolithon</i> sp.	Hawaii, Sherwood <i>et al.</i> (2010).	-
Corallinales, Corallinaceae	<i>Lithophyllum</i> cf. <i>bamleri</i> (Heydrich) Heydrich	Fiji, Bittner <i>et al.</i> (2010, 2011).	GQ917473
Corallinales, Corallinaceae	<i>L. incrustans</i> Philippi	Atlantic France, Bittner <i>et al.</i> (2010, 2011).	GQ917440

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TABLE S1. (Continued)

Additional COL-SP and psbA sequences data included in the alignment ordered alphabetically						
Corallinales, Corallinales	<i>L. insipidum</i> Adey, Townsend & Boykins	Hawaii, Sherwood <i>et al.</i> (2010).				HQ422710 -
Corallinales, Corallinales	<i>L. koesehyannum</i> Unger	Hawaii, Sherwood <i>et al.</i> (2010).				HQ423072 -
Corallinales, Corallinales	<i>L. cf. pygmaeum</i> (Heydrich) Heydrich	New Caledonia, Bittner <i>et al.</i> (2010, 2011).				GQ917268 GQ917459
Corallinales, Corallinales	<i>L. prototypum</i> (Foslie) Foslie	Hawaii, Sherwood <i>et al.</i> (2010).				HQ423070 -
Corallinales, Hapalidiaceae	<i>Lithothamnion corallioides</i>	Atlantic France, Pardo <i>et al.</i> (2012).				KC861452 KC819264
Corallinales, Hapalidiaceae	<i>L. cf. crispatum</i> (Hauck) Hauck	Mediterranean Spain, Peña <i>et al.</i> (this study).	VPF00148	SANT-Algae 28913		- KJ1710356
Corallinales, Hapalidiaceae	<i>L. glaciale</i>	Canada, Le Gall <i>et al.</i> (unpublished).				HM918812 JQ422235
Corallinales, Hapalidiaceae	<i>L. glaciale</i>	Ireland, Hernández-Kantún <i>et al.</i> (2012).				- JQ896233
Corallinales, Hapalidiaceae	<i>L. muelleri</i>	Pacific Mexico, Hernández-Kantún <i>et al.</i> (2012).				- JQ896241
Corallinales, Corallinales	<i>Mastophora pacifica</i>	French Polynesia, Bittner <i>et al.</i> (2010, 2011).				GQ917302 GQ917494
Corallinales, Corallinales	<i>Mastophora rosea</i>	Philippines, Bittner <i>et al.</i> (2010, 2011).				GQ917300 GQ917492
Corallinales, Hapalidiaceae	<i>Mesophyllum erubescens</i> (Foslie) Mc.Lemoine	Hawaii, Sherwood <i>et al.</i> (2010).				HQ422718 -
Corallinales, Hapalidiaceae	<i>M. erubescens</i>	Japan, Kato <i>et al.</i> (2013).				AB713929 AB576043
Corallinales, Hapalidiaceae	<i>M. erubescens</i>	Canary Islands, Hernández-Kantún <i>et al.</i> (2012).				- JQ896246
Corallinales, Hapalidiaceae	<i>M. erubescens</i>	New Zealand, Broom <i>et al.</i> (2008).				- DQ167876
Corallinales, Hapalidiaceae	<i>M. cf. erubescens</i>	Fiji, Bittner <i>et al.</i> (2010, 2011).				GQ917275 GQ917468
Corallinales, Hapalidiaceae	<i>M. cf. erubescens</i>	Vanuatu, Bittner <i>et al.</i> (2010, 2011).				GQ917256 GQ917446
Corallinales, Hapalidiaceae	<i>M. cf. erubescens</i>	Vanuatu, Bittner <i>et al.</i> (2010, 2011).				GQ917255 GQ917444
Corallinales, Hapalidiaceae	<i>Mesophyllum lichenoides</i> (Ellis) Mc.Lemoine	Atlantic France, Bittner <i>et al.</i> (2010, 2011).				GQ917249 GQ917439
Corallinales, Hapalidiaceae	<i>M. prinzianum</i> Woelkerling & A.S.Harvey	New Zealand, Farr <i>et al.</i> (2009).				- FJ361577
Corallinales, Hapalidiaceae	<i>M. sphaericum</i>	Holotype material, Atlantic Spain, Pardo <i>et al.</i> (2012).				KC861526 KC819262
Corallinales, Corallinales	<i>Metamastophora</i> sp.	Hawaii, Sherwood <i>et al.</i> (2010).				HQ423073 -

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TABLE S1. (Continued)

Additional COI-5P and psbA sequences data included in the alignment ordered alphabetically			
Corallinales, Corallinaceae	<i>Neogoniolithon brassica-florida</i> (Harvey) Setchell & L.R.Mason	Japan, Kato <i>et al.</i> (2013).	AB713924 -
Corallinales, Corallinaceae	<i>N. brassica-florida</i>	Japan, Kato <i>et al.</i> (2013).	AB713923 -
Corallinales, Corallinaceae	<i>N. brassica-florida</i>	Japan, Kato <i>et al.</i> (2013).	AB576032
Corallinales, Corallinaceae	<i>N. brassica-florida</i>	New Zealand, Broom <i>et al.</i> (unpublished).	FJ361401
Corallinales, Corallinaceae	<i>N. brassica-florida</i>	Spain, Hernandez-Kantún <i>et al.</i> (2012).	JQ896257
Corallinales, Corallinaceae	<i>N. frutescens</i> (Foslie) Setchell & L.R.Mason	Japan, Kato <i>et al.</i> (2013).	AB713925 -
Corallinales, Corallinaceae	<i>N. megalocystum</i> (Weber-van Bosse & Foslie) Setchell & L.R.Mason	Japan, Kato <i>et al.</i> (2013).	AB713927 -
Corallinales, Corallinaceae	<i>N. trichotomum</i> (Heydrich) Setchell & L.R.Mason	Japan, Kato <i>et al.</i> (2013).	AB713928 -
Corallinales, Corallinaceae	<i>Neogoniolithon</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010).	GQ917697
Corallinales, Hapalidiaceae	<i>Phymatolithon calcareum</i>	Neotype BM, Britain, Hernández-Kantún <i>et al.</i> (2012), Peña <i>et al.</i> (2014).	KF808323 JQ896231
Corallinales, Hapalidiaceae	<i>P. lamii</i> (McLemoine) Y.M.Chamberlain	Ireland, Hernández-Kantún <i>et al.</i> (2012).	JQ896248
Corallinales, Hapalidiaceae	<i>P. lenormandii</i>	Canada, Clarkston & McDevit (unpublished).	JX571978 -
Corallinales, Hapalidiaceae	<i>P. lenormandii</i>	Ireland, Hernández-Kantún <i>et al.</i> (2012).	JQ896249
Corallinales, Hapalidiaceae	<i>P. purpureum</i> (P.L.Crouan & H.M.Crouan) Woelkerling & L.M.Irvine	Ireland, Hernández-Kantún <i>et al.</i> (2012).	JQ896232
Corallinales, Hapalidiaceae	<i>P. repandum</i>	New Zealand, Farr <i>et al.</i> (2009).	FJ361683
Corallinales, Corallinaceae	<i>Pneophyllum conicum</i> (E.Y.Dawson) Keats, Y.M.Chamberlain & Baba	Hawaii, Sherwood <i>et al.</i> (2010).	HQ422712 -
Corallinales, Corallinaceae	<i>P. conicum</i>	Hawaii, Sherwood <i>et al.</i> (2010).	HQ422716 -
Corallinales, Corallinaceae	<i>P. conicum</i>	Japan, Kato <i>et al.</i> (2011).	GQ917701
Corallinales, Corallinaceae	<i>P. conicum</i>	Vanuatu, Bittner <i>et al.</i> (2010, 2011).	GQ917254 GQ917443
Corallinales, Corallinaceae	<i>Pneophyllum</i> sp. (as Uncultured Corallinales).	Guadeloupe, Bittner <i>et al.</i> (2010).	GQ917701

.....continued on the next page

TABLE S1. (Continued)

Additional COI-5P and psbA sequences data included in the alignment ordered alphabetically						
Corallinales, Corallimaceae	<i>Porolithon gardineri</i> (Foslie) Foslie (as <i>H. gardineri</i> Verheij & Prud'homme van Reine).	Hawaii, Sherwood <i>et al.</i> (2010).		HQ423067	-	
Corallinales, Corallimaceae	<i>P. onkodes</i> (Heydrich) Foslie	New Caledonia, Bittner <i>et al.</i> (2010, 2011).		GQ917291	GQ917483	
Corallinales, Corallimaceae	<i>P. onkodes</i>	New Caledonia, Bittner <i>et al.</i> (2010, 2011).		GQ917289	GQ917481	
Corallinales, Corallimaceae	<i>Spongites</i> cf. <i>fruticulosa</i>	Rhodos Island, Greece, Mediterranean Sea, Peña <i>et al.</i> (this study).	LLG4146	KJ710340	KJ710351	PC0142664
Corallinales, Corallimaceae	<i>S. decipiens</i>	South Africa, Maneveldt <i>et al.</i> (unpublished).		-	JQ917420	
Corallinales, Corallimaceae	<i>S. discoidea</i>	South Africa, Maneveldt <i>et al.</i> (unpublished).		-	JQ917421	
Corallinales, Corallimaceae	<i>S. tunicata</i>	New Zealand, Hart <i>et al.</i> (unpublished).		-	DQ167900	
Corallinales, Corallimaceae	<i>S. yendoii</i>	New Zealand, Hart <i>et al.</i> (unpublished).		-	DQ167907	
Corallinales, Corallimaceae	<i>S. yendoii</i>	New Zealand, Hart <i>et al.</i> (unpublished).		-	DQ167878	
Corallinales, Corallimaceae	<i>Spongites</i> sp.	Hawaii, Sherwood <i>et al.</i> (2010).		HQ422714	-	
Corallinales, Corallimaceae	<i>Spongites</i> sp.	Hawaii, Sherwood <i>et al.</i> (2010).		HQ422719	-	
Sporolithales, Sporolithaceae	<i>Sporolithon durum</i> (Foslie) R.A. Townsend & Woelkerling in R.A. Townsend	New Zealand, Hart <i>et al.</i> (unpublished).		-	DQ167955	
Sporolithales, Sporolithaceae	<i>S. ptychoides</i> Heydrich	Hawaii, Sherwood <i>et al.</i> (2010).		HQ422711	-	
Sporolithales, Sporolithaceae	<i>S. ptychoides</i>	Brazil, Bahia <i>et al.</i> (2014).		-	KC870927	
Sporolithales, Sporolithaceae	<i>Sporolithon</i> sp.	Fiji, Bittner <i>et al.</i> (2010, 2011).		GQ917279	GQ917501	
Sporolithales, Sporolithaceae	<i>Sporolithon</i> sp.	Vanuatu, Bittner <i>et al.</i> (2010, 2011).		GQ917259	GQ917500	
Sporolithales, Sporolithaceae	<i>Sporolithon</i> sp.	New Zealand, Farr <i>et al.</i> (2009).		-	FJ361509	
Sporolithales, Sporolithaceae	<i>S. tenue</i> Bahia, Amado-Filho, Maneveldt <i>et al.</i> W.H. Adey	Costa Rica, Bahia <i>et al.</i> (2014).		-	KC870925	
Corallinales, Hapaliidae	<i>Synarthrophyton patena</i>	Australia, Bittner <i>et al.</i> (2011).		GQ917304	GQ917499	
Corallinales, Hapaliidae	Uncultured Corallinales	French Polynesia, Bittner <i>et al.</i> (2010, 2011).		GQ917690	-	
Corallinales, Hapaliidae	Uncultured Corallinales	French Polynesia, Bittner <i>et al.</i> (2010, 2011).		GQ917695	-	

Morphological studies

The specimens sequenced were examined under a scanning electron microscope (model JEOL JSM 6400, University of A Coruña). Selected parts of each specimen were obtained under the stereomicroscope by breaking fragments off the maerl and rhodoliths. Fragments for vertical section and surface views were mounted on carbon stubs and coated in gold before the examination.

The growth form terminology follows Woerkerling *et al.* (1993). Anatomical terminology follows Woelkerling (1988), Keats *et al.* (2009) and Peña *et al.* (2011). Cell length is the distance between primary pit connections, and cell diameter the maximum distance of the cell lumen perpendicular to the length. Conceptacle measurements follow Adey & Adey (1973) and Irvine & Chamberlain (1994).

Results

The eleven sequences obtained for the COI-5P DNA barcode region ranged from 572 to 664 bp and comprised 11 haplotypes with 193 variable sites. The COI-5P for Guadeloupe samples of *Lithothamnion cf. ruptile* (Foslie) Foslie was not obtained (Table S1). The COI-5P alignment, including publicly available sequences from GenBank and BOLD, consisted of 64 haplotype sequences. The GMYC model resolved the specimens of maerl and rhodoliths collected in the Guadeloupe as seven putative species. The fit of the likelihood of the GMYC model was significantly higher ($p = 1.67e-05$) than that of a null model of uniform coalescent branching rates. The threshold time (the estimated depth from the branch tips at which the transition from population to species level branching patterns occur) was determined at -0.006 substitutions per site. Both GMYC and ML analyses of the COI-5P alignment were congruent in delimiting the seven putative species. According to the results obtained from the ML analysis in which 13 genera belonging to the Corallinales and one genus belonging to the Sporolithales were included, the seven taxa here studied were resolved within the families Hapalidiaceae and Corallinaceae (Fig. 2). According to the analyses inferred from COI-5P, our Guadeloupian maerl and rhodoliths consisted of five species delimited within the Hapalidiaceae; three of them consisting of the specimens (PC0144033, PC0144055 and PC0142665 (“species 1”), PC0144249 (“species 2”), and PC0144042 (“species 3”) have not been resolved among any known genus. These specimens resolved as three species within the same lineage, with moderate support (88%) and are a sister lineage to the unidentified species collected in Bermuda and French Polynesia. However, the specimen PC0144250 (“species 5”) was resolved as a sister taxon to *Lithothamnion corallioides* and *L. glaciale* Kjellman, and the specimen PC0144248 (“species 4”) joined *Phymatolithon lenormandii* (Areschoug) Adey collected along the Canadian coasts (Table S1, Fig. 2). Within the Corallinaceae, specimens PC0144235, PC0144239 and PC0144244 (“species 6”) were resolved among species of the genus *Spongites* (Mastophoroideae Setchell *sensu* Harvey *et al.* 2003), while the specimen PC0144236 (“species 7”) was resolved within the lineage encompassing *Mastophora rosea* (C.Agardh) Setchell, *M. pacifica* (Heydrich) Foslie and *Metamastophora* sp. (Mastophoroideae Setchell *emend.* Kato & Baba, Kato *et al.* 2011). None of the sequences obtained from this study matched publicly available sequences; the highest percentage similarity (92 %) was between “species 6” and *Spongites cf. fruticulosa* (GenBank accession number KJ710340, Table S1, Fig. 2). This suggests that all species studied are either new to science, or possibly belong to species for which no sequence data is yet available.

The *psbA* alignment, including publicly available sequences from GenBank and BOLD, consisted of 65 haplotypes sequences. For the Guadeloupe samples, we could obtain the *psbA* sequence for *Lithothamnion cf. ruptile*, but unfortunately not for the “species 4” (PC0144248) delimited in the COI-5P analyses (Table S1). The *psbA* analyses were congruent in delimiting with strong support the species obtained from the COI-5P results (Fig. 3). None of the Guadeloupe taxa matched publicly available sequences. *Lithothamnion cf. ruptile* together with “species 1-3” and a Mediterranean specimen of *L. cf. crispatum* Hauck were resolved within a large lineage encompassing *Mesophyllum* spp., *Phymatolithon repandum* (Foslie) Wilks & Woelkerling and *Synarthrophyton patena* (Hooker & Harvey) Townsend from New Zealand and Australia, respectively (Fig. 3). The “species 5” was resolved within the *Lithothamnion* lineage that included a collection of the generitype *L. muelleri* Lenormand *ex* Rosanoff, whereas the “species 6” was resolved together with *Spongites cf. fruticulosa*, both taxa unrelated with other *Spongites* from the southern hemisphere (*S. decipiens* (Foslie) Y.M.Chamberlain, *S. discoidea* (Foslie) D.Penrose & Woelkerling, *S. tunicata* Penrose and *S. yendoii* (Foslie) Y.M.Chamberlain). The “species 7” did not ally with any of the taxa included.

As stated previously, none of the sequences obtained from this study matched publicly available or known sequences. Apart from *Lithothamnion cf. ruptile*, the rest of Guadeloupe specimens might constitute species new to science. However, the possibility that these specimens may belong to species for which no sequence data is yet available precludes us from assigning names to these specimens at this time.

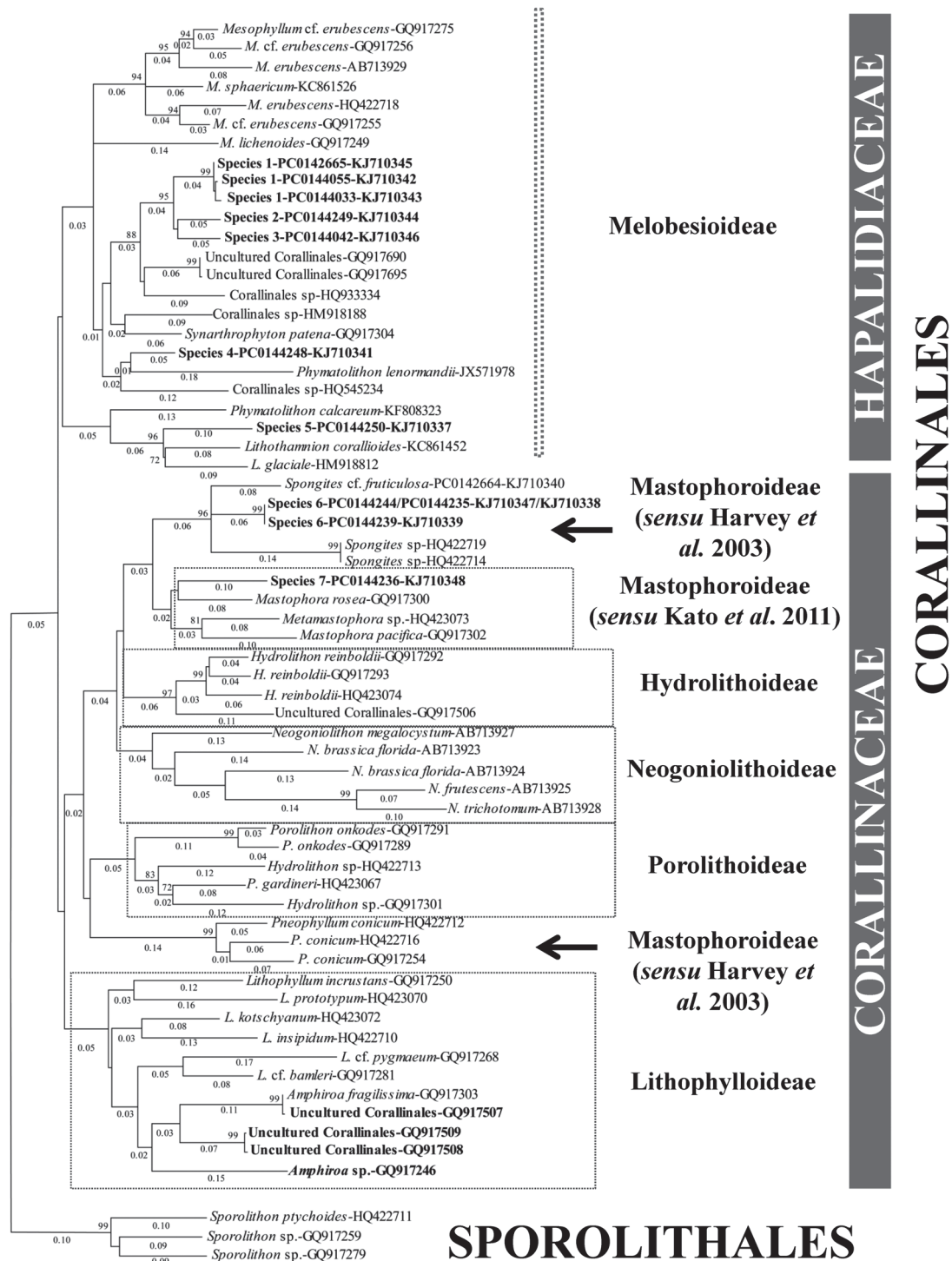


FIGURE 2. ML tree inferred from the DNA barcode sequences (COI-5P) of haplotypes observed from each species delimited according to the GMYC model. Text in bold letters refers to specimens collected in the study area during the present study and earlier studies. The subfamilies represented are indicated; within Corallinaceae, the subfamilies Neogoniolithoideae, Porolithoideae, Hydroolithoideae and Mastophoroideae *sensu* Kato *et al.* (2011), and Mastophoroideae *sensu* Harvey *et al.* (2003). Bootstrap values > 70 % are shown for each node. Members of the order Sporolithales were used as outgroup. Scale bar: 0.05 substitutions per site.

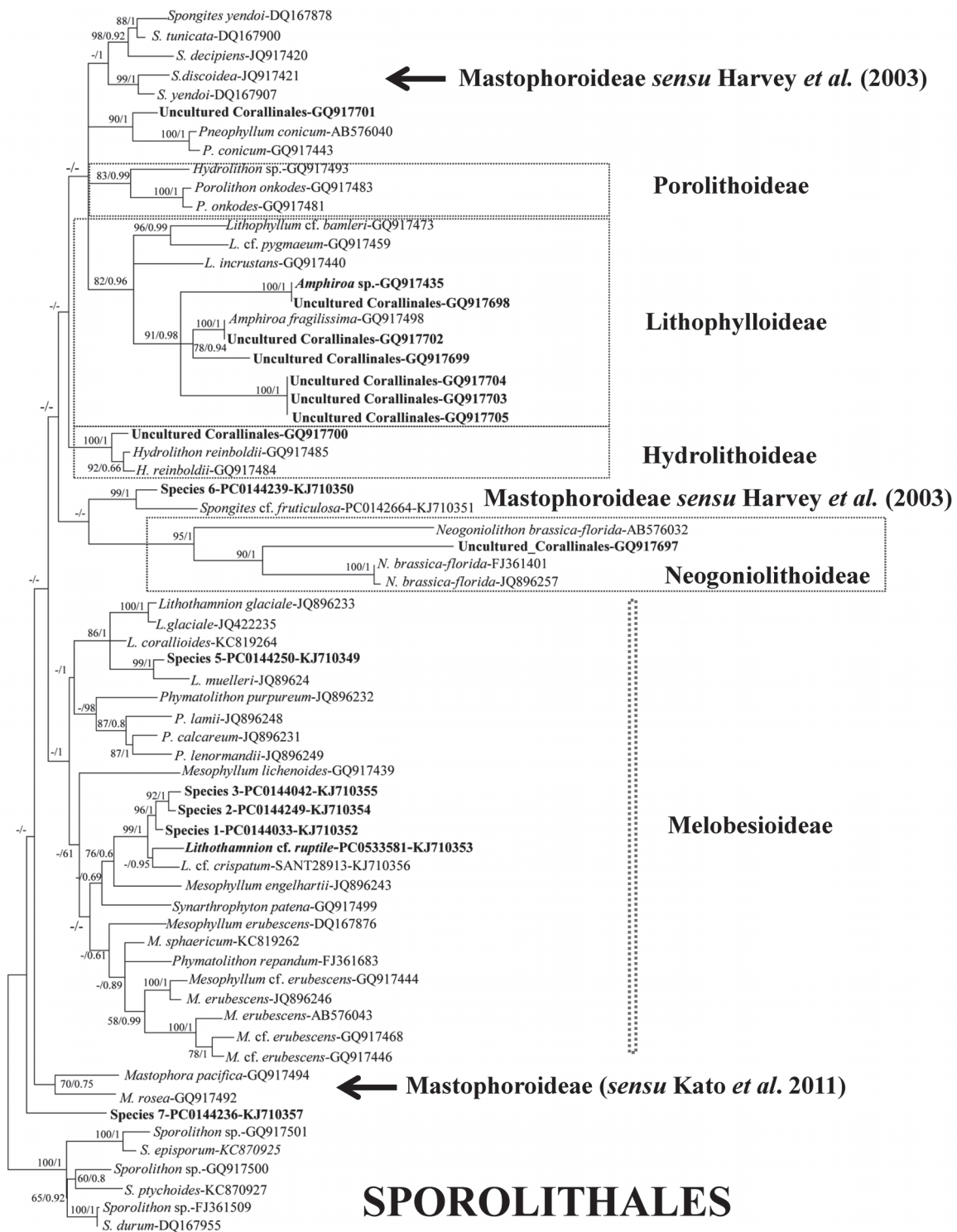


FIGURE 3. Phylogenetic tree inferred from ML and BI analyses of the *psbA* sequences of the Guadeloupe and publicly available sequences. Text in bold letters refers to specimens collected in the study area during the present study and earlier studies. The subfamilies represented are indicated; within Corallinaceae, the subfamilies Neogoniolithoideae, Porolithoideae, Hydrolithoideae and Mastophoroideae sensu Kato *et al.* (2011), and Mastophoroideae sensu Harvey *et al.* (2003). Bootstrap ML values > 50% and posterior probabilities > 0.50 from Bayesian inference are shown for each node. Members of the order Sporolithales were used as outgroup. Scale bar: 0.05 substitutions per site.

Lithothamnion cf. rutilum (Foslie) Foslie
(Fig. 4A–F)

Specimens examined:—West Indies, Guadeloupe: Passe à Caret, Grand Cul-de-Sac Marin, at 32 m depth (23.v.2012, *Karubenthos Expedition*, PC0144242, PC0533581, PC0533582, PC0533583, PC0533584); Pente externe face à l'îlet Fajou, Grand Cul-de-Sac Marin, at 29 m depth (24.v.2012, *Karubenthos Expedition*, PC0142246).

Appearance and vegetative structure—Thalli unattached as maerl and rhodoliths, fruticose, spherical to ellipsoidal, up to 3 cm in diameter, densely branched with broadly fluted branch apices up to 5 mm in length (Fig. 4A–B). Thallus construction monomerous, radially organized in branches (Fig. 4C). Cell fusions predominate (Fig. 4D); secondary pit connections were not observed. Medullary cells are 25–30 μm in length by 10–15 μm in diameter. Cortical cells are 5–10 μm in length by 5–7 μm in diameter. Subepithallial initials cells were 5–6 μm in length by 9–10 μm in diameter. Subepithallial cells are as long as or shorter than the cells subtending them. Epithallial cells occur in a single layer and have flared outermost walls, 1 μm in length by 5–9 μm in diameter (Fig. 4D). In surface view, the epithallial cells are polygonal, thick walled, 10–12 μm in diameter (Fig. 4E).

Reproduction:—No gametangial conceptacles were observed. Tetra/bisporangial conceptacles multiporate were only found buried within the thallus and refilled with new tissue, chambers 70–90 μm in height by 211–275 μm in diameter (Fig. 4F).

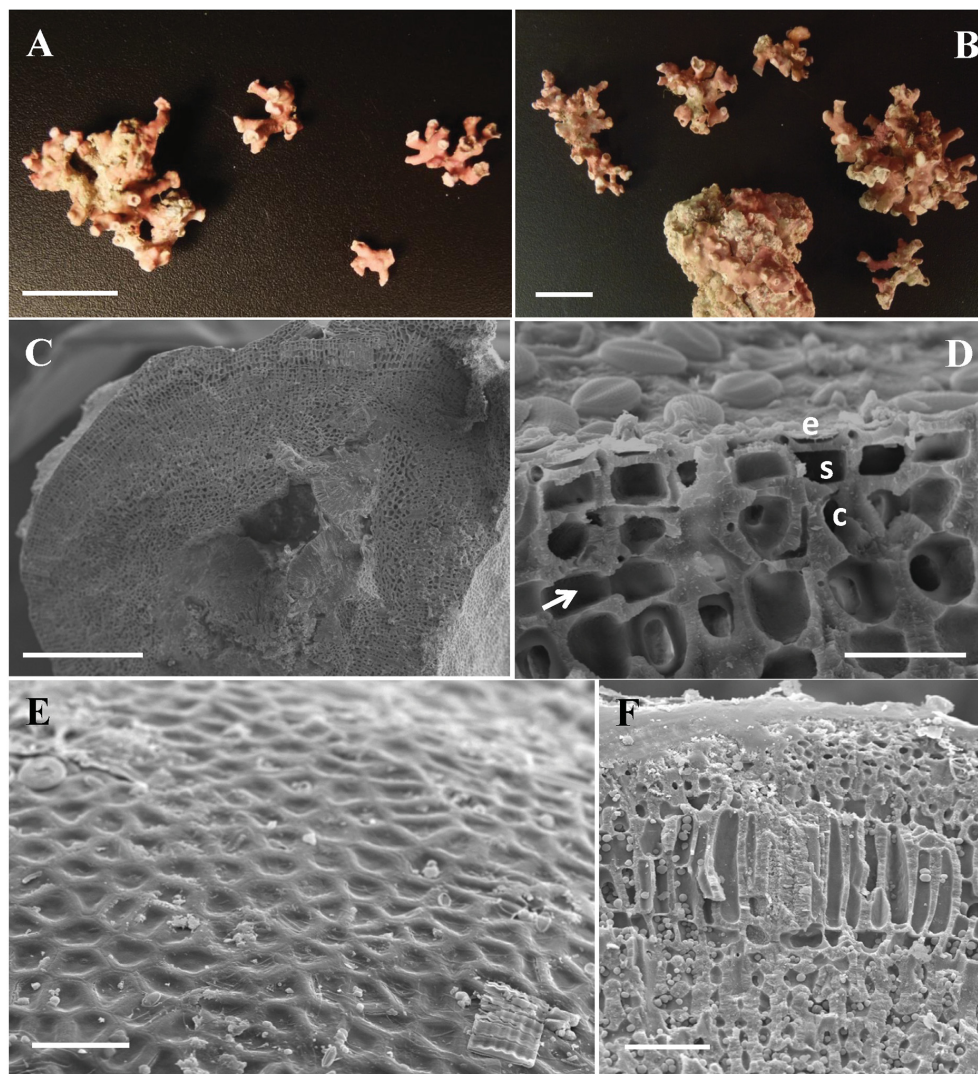


FIGURE 4. *Lithothamnion cf. rutilum* (PC0144242, PC0144246). **A–B** maerl densely branched and with broadly fluted branch apices; **C** vertical section of the branch radially organized; **D** vertical section of the branch showing a single layer of flared epithallial cells (e) derived from short subepithallial initials (s), and cortical cells (c) joined by cell fusions (arrow); **E** surface view of polygonal epithallial cells; **F** multiporate sporangial conceptacle buried within the thallus and refilled with new tissue. A, C–F: PC0144242, B: PC0144246. Scale bars: A–B: 1 cm, C= 300 μm , D=20 μm , E= 20 μm , F=50 μm .

Lithothamnion sp. 1 (“species 1”)
(Fig. 5A–F)

Specimens examined:—West Indies, Guadeloupe: Gros Mouton, îlet Caret, Grand Cul-de-Sac Marin, at 5 m depth (04.v.2012, *Karubenthos Expedition*, PC0144033, PC0144055); îlet du Gosier, Petit Cul-de-Sac Marin, at 6 m depth (03.v.2012, *Karubenthos Expedition*, PC0142665).

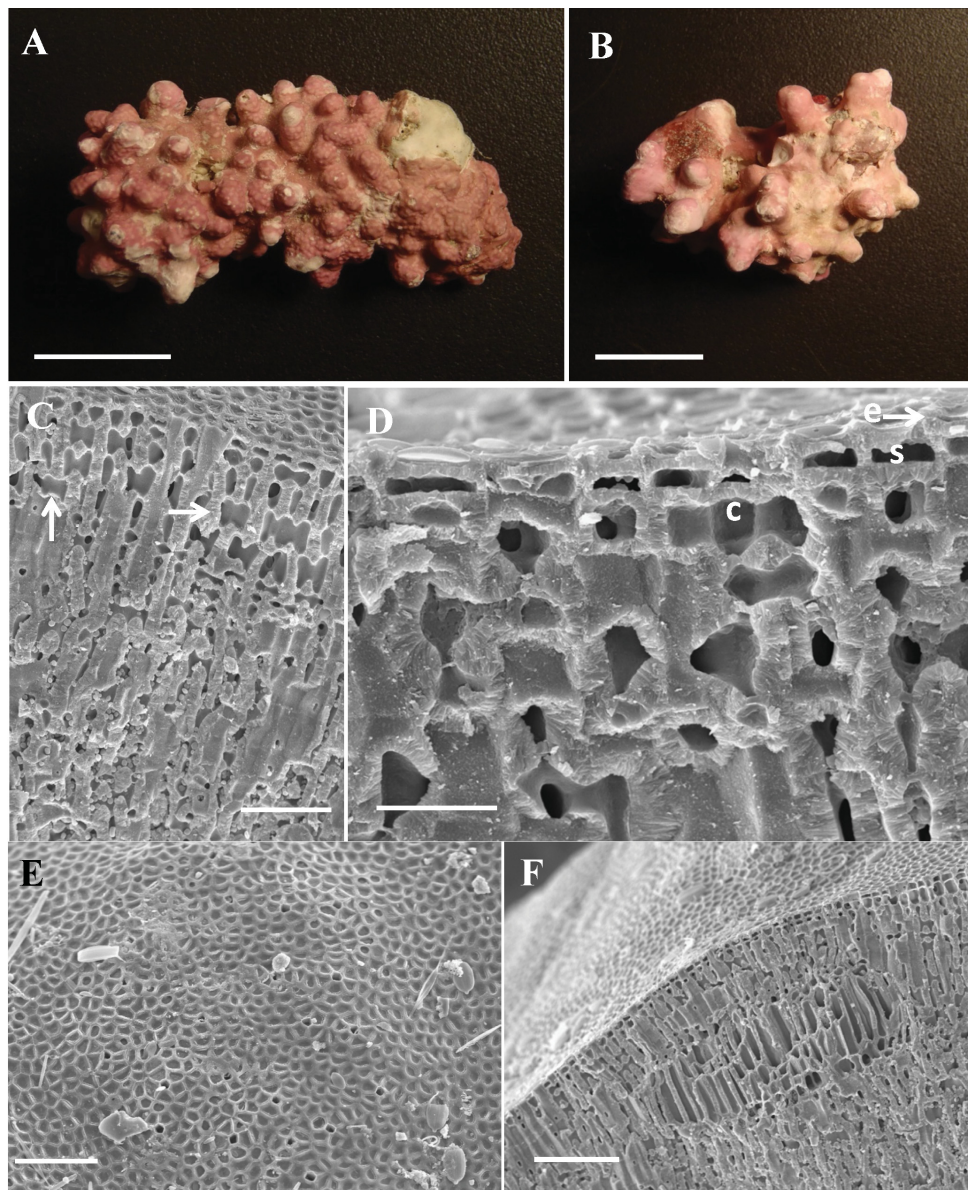


FIGURE 5. Morphological, vegetative and reproductive anatomy of *Lithothamnion* sp. 1 (PC0144033, PC0144055). **A–B** warty rhodolith and maerl with short protuberances; **C** vertical section through a protuberance showing abundant cell fusions (arrows); **D** magnified view of the outer thallus showing a single layer of flared epithallial cells (e) derived from short subepithallial initials (s), and cortical cells (c) joined by cell fusions; **E** surface view a multiporate tetra/bisporangial conceptacle showing pores surrounded by 4–6 rosette cells that are similar in appearance to surrounding roof cells; **F** vertical section of the outer thallus showing a multiporate tetra/bisporangial conceptacle buried and infilled within the thallus. A, C, E, F: PC0144033; B,D: PC0144055. Scale bars: A–B= 1 cm, C= 50 µm, D= 20 µm, E= 50 µm, F= 100 µm.

Appearance and vegetative structure:—Thalli unattached as maerl and rhodoliths, warty, spherical to ellipsoidal, 2–4 cm in diameter with unbranched protuberances *ca* 2 mm in diameter and 2–3 mm in length (Figs 5A–B). Thallus construction monomerous, and radially organized in branches. Cell fusions predominate (Fig. 5C); secondary pit connections were not observed. Medullary cells are 12–19 µm in length by 4–7 µm in diameter (Fig. 5C). Cortical cells 6–12 µm in length by 4–6 µm in diameter (Fig. 5D). Subepithallial initials are 2–3 µm in length by 7–13 µm in diameter. Subepithallial initials are as long as or shorter than the cells subtending them (Fig. 5D). Epithallial cells

occur in a single layer and have flared outermost walls. Epithallial cells are squat, 1–2 μm in length by 10–11 μm in diameter (Fig. 5D). In surface view, the epithallial cells are polygonal, 6–12 μm in diameter (Fig. 5E).

Reproduction:—No gametangial conceptacles were observed. Tetra/bisporangial conceptacles multiporate slightly raised with a flattened pore plates lacking a peripheral rim (Fig. 5E). Conceptacle pores surrounded by 4–6 (mostly 5) rosette cells similar in appearance to surrounding roof cells. In vertical section, tetra/bisporangial conceptacle chambers were only observed buried within the thallus and refilled with new tissue, chambers 270–280 μm in diameter by 114–136 μm in height (Fig. 5F).

***Lithothamnion* sp. 2** (“species 2”)

(Figs 6A–D)

Specimen examined:—West Indies, Guadeloupe: îles de Petite Terre, at 50 m depth (27.v.2012, *Karubenthos Expedition*, PC0144249).

Appearance and vegetative structure:—Thallus unattached as maerl, warty, ellipsoidal, up to 2 cm in diameter with short protuberances 2–3 mm in diameter and 3–4 mm in length (Fig. 6A). Thallus construction monomerous, radially organized in branches (Fig. 6B). Cell fusions predominate (Fig. 6C–D); secondary pit connections were not observed. Medullary cells are 13–20 μm in length by 10–13 μm by diameter. Cortical cells are 10–15 μm in length by 5–7 μm by diameter (Fig. 6D). Subepithallial initials are 6–10 μm in length by 6–10 μm in diameter. Subepithallial initials are as long as or shorter than the cells subtending them (Fig. 6D). Epithallial cells occur in a single layer and have flared outermost walls. Epithallial cells are squat, 1–2 μm in length by 6–9 μm in diameter (Fig. 6D). In surface view, the epithallial cells are polygonal.

Reproduction:—Reproductive structures were not observed.

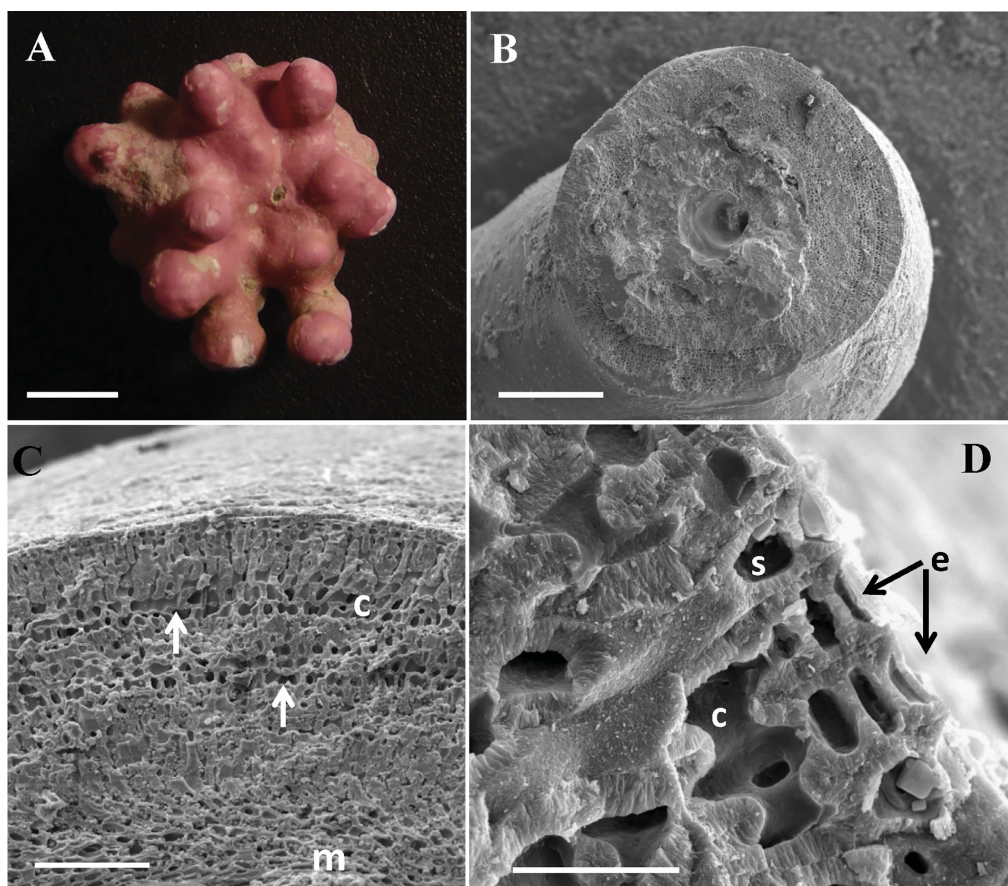


FIGURE 6. Morphological and vegetative anatomy of *Lithothamnion* sp. 2 (PC0144249). **A** warty maerl specimen with short protuberances; **B** vertical section of a protuberance; **C** vertical section of a protuberance showing the monomerous thallus construction, the medulla (m), cortex (c), and abundant cell fusions (arrows); **D** magnified view of the outer thallus showing a single layer of flared epithallial cells (e) derived from short subepithallial initials (s) and cortical cells (c) joined by cell fusions. Scale bars: A= 0.5 cm, B= 500 μm , C =100, D= 20 μm .

Lithothamnion sp. 3 (“species 3”)
(Fig. 7A–D)

Specimen examined:—West Indies, Guadeloupe: Pente externe face à l’îlet Fajou, Grand Cul-de-Sac, at 15 m depth (06.v.2012, *Karubenthos Expedition*, PC0144042).

Appearance and vegetative structure:—Thallus unattached as maerl, warty, spherical up to 3 cm in diameter, with short spherical protuberances 2–3 mm in diameter and 2–3 mm in length (Fig. 7A). Thallus construction monomerous, and radially organized in branches (Fig. 7B). Cell fusions predominate (Figs. 7C–D); secondary pit-connections were not observed. Medullary cells are 22–26 μm in length by 7–9 μm in diameter. Cortical cells are 9–16 μm in length by 6–11 μm in diameter. Subepithallial initials are 3–9 μm in length by 8–12 μm in diameter. Subepithallial initials are as long as or shorter than the cells subtending them (Figs 7C–D). Epithallial cells occur in a single layer and have flared outermost walls. Epithallial cells are squat, 1–2 μm in length by 5–7 μm in diameter (Figs 7C–D). In surface view, the epithallial cells are polygonal, 9–13 μm in diameter.

Reproduction:—Reproductive structures were not observed.

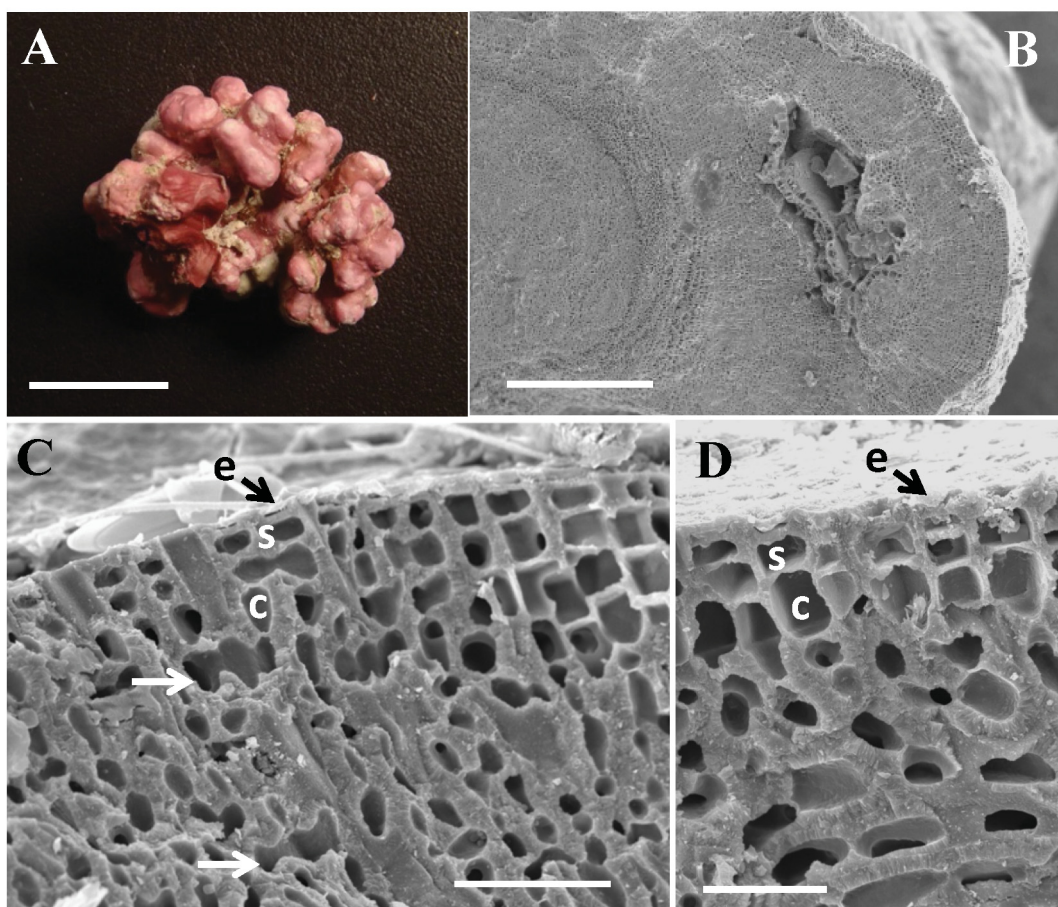


FIGURE 7. Morphological and vegetative anatomy of *Lithothamnion* sp. 3 (PC0144042). **A** warty maerl specimen with short protuberances; **B** vertical section of a protuberance showing the radial organization; **C–D** magnified view of the outer thallus showing a single layer of flared epithallial cells (e) derived from short subepithallial initials (s), and cortical cells (c) joined by cell fusions (arrows) Scale bars: A= 1 cm, B= 300 μm , C= 50 μm , D= 20 μm .

Lithothamnion sp. 4 (“species 4”)
(Fig 8A–D)

Specimen examined:—West Indies, Guadeloupe: îles de Petite Terre, at 95 m depth (26.v.2012, *Karubenthos Expedition*, PC0144248).

Appearance and vegetative structure:—Thallus unattached as rhodolith, warty to lumpy, ellipsoidal, up to 3 cm in diameter with very short protuberances < 1 mm in diameter and length (Fig. 8A). Thallus construction monomerous, and radially organized in branches (Fig. 8B). Cell fusions predominate (Figs 8C–D); secondary pit connections were not observed. Medullary cells are 14–18 μm in length by 4–6 μm in diameter. Cortical cells are 6–9 μm in length by 4–6 μm in diameter (Figs 8C–D). Subepithallial initials are 3–4 μm in length by 4–6 μm in diameter. Subepithallial initials are shorter than the cells subtending them (Fig. 8D). Epithallial cells occur in a single layer and have somewhat flared outermost walls, although most of them were found collapsed. They are squat, 2 μm in length by 3–5 μm in diameter (Fig. 8D). In surface view, the epithallial cells are very thick walled and polygonal, 5–9 μm in diameter.

Reproduction:—Reproductive structures were not observed.

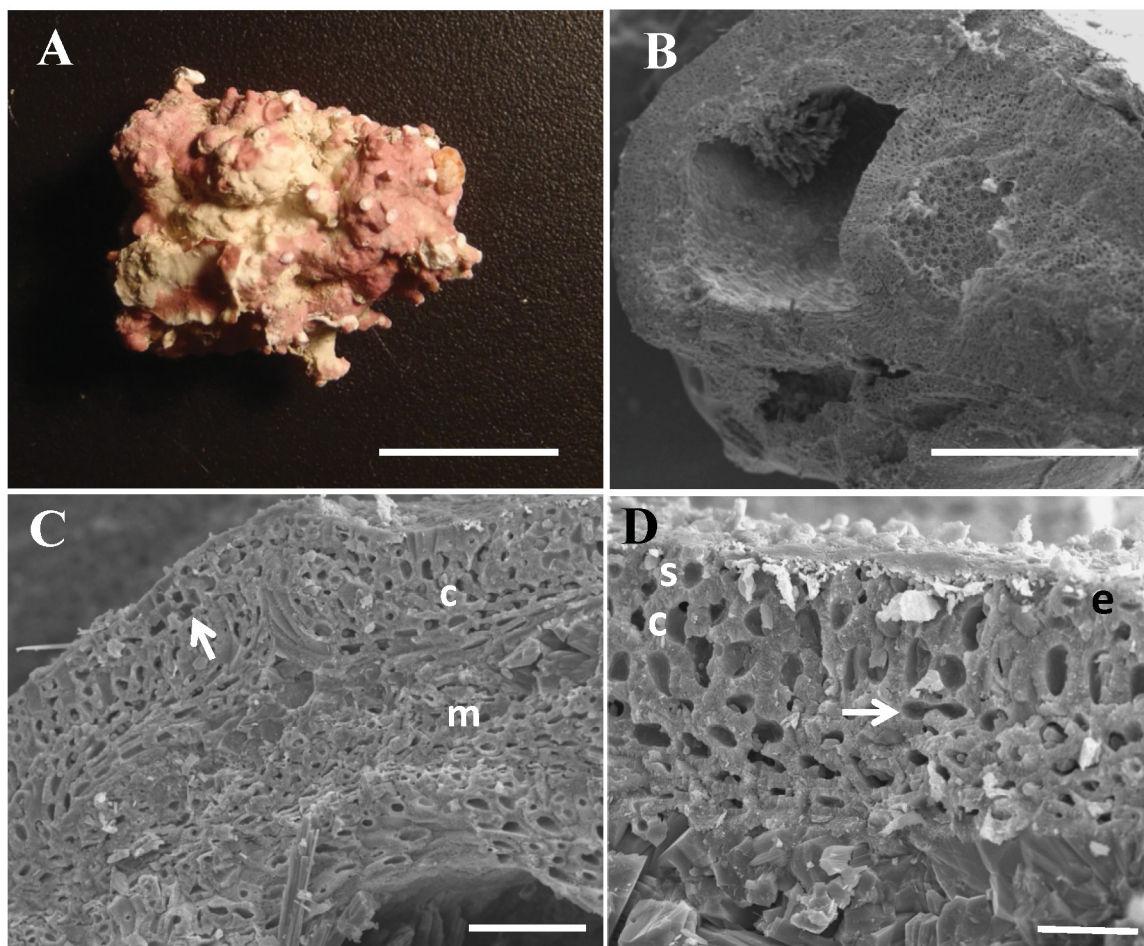


FIGURE 8. Morphological and vegetative anatomy of *Lithothamnion* sp. 4 (PC0144248). **A** warty to lumpy rhodolith specimen with short protuberances; **B** vertical section of the branch radially organized; **C** vertical section through the thallus showing the monomerous construction, the medulla (m), the cortex (c) and abundant cell fusions (arrow); **D** magnified view of the outer thallus showing a single layer of collapsed epithallial cells with flared outermost walls (e) derived from subepithallial initials (s), and cortical cells (c) joined by cell fusions (arrow). Scale bars: A= 1 cm, B= 400 μm , C = 50 μm , D= 20 μm .

***Lithothamnion* sp. 5** (“species 5”)
(Figs 9A–D)

Specimen examined:—West Indies, Guadeloupe: Banc des Vaisseaux, Saint-François, at 110 m depth (27.v.2012, *Karubenthos Expedition*, PC0144250).

Appearance and vegetative structure:—Thallus unattached as rhodoliths, warty to lumpy, discoidal, up to 2 cm in diameter with very short protuberances < 1 mm in diameter and length (Fig. 9A). Thallus construction monomerous, and radially organized in branches (Fig. 9B). Cell fusions predominate (Figs 9C–D); secondary pit

connections were not observed. Medullary cells 5–9 μm are in length by 4–8 μm in diameter. Cortical cells are 5–20 μm in length by 9–10 μm in diameter (Fig. 9C). Subepithallial initials are 4–7 μm in length by 4–9 μm in diameter. Subepithallial initials are as long as or shorter than the cells subtending them (Fig. 9D). Epithallial cells occur in a single layer and have somewhat flared outermost walls. They are squat, 2 μm in length by 5 μm in diameter (Fig. 9D). In surface view, the epithallial cells are polygonal, thin to thick walled, 7–11 μm in diameter.

Reproduction:—Reproductive structures were not observed.

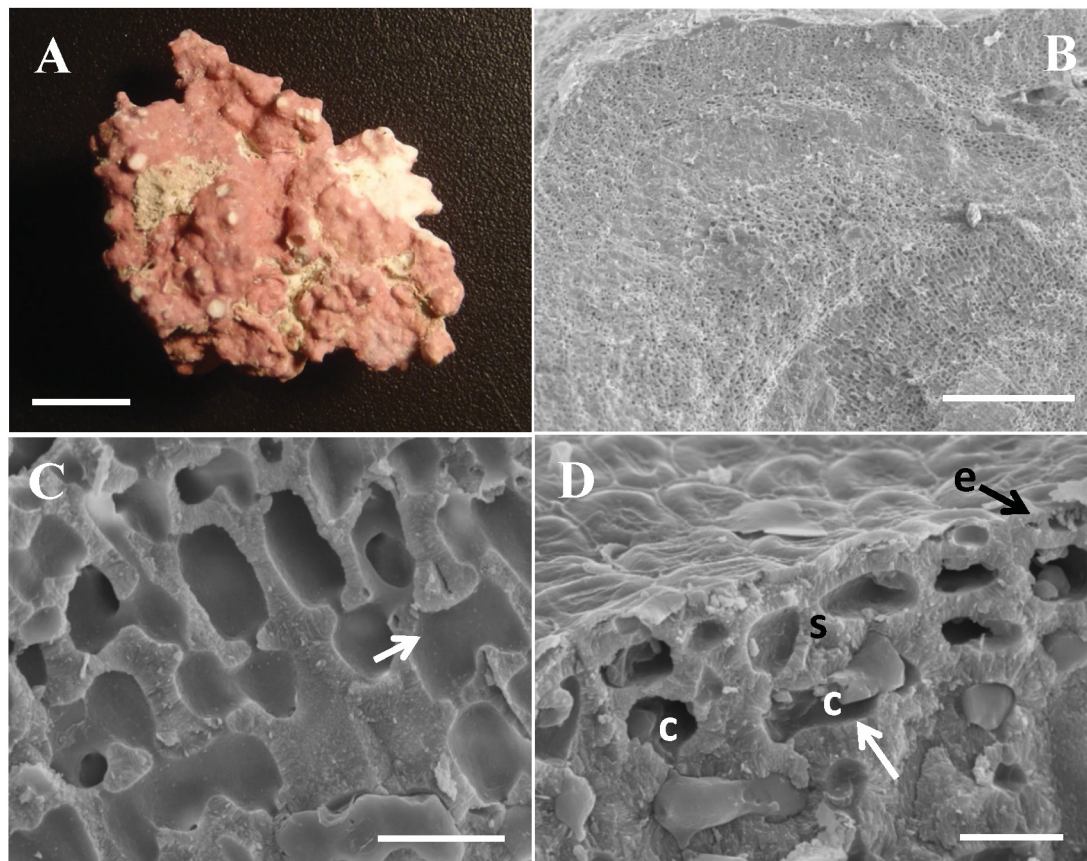


FIGURE 9. Morphological and vegetative anatomy of *Lithothamnion* sp. 5 (PC0144250). **A** warty to lumpy rhodolith specimen with short protuberances; **B** vertical section of a protuberance showing the radial organization; **C** vertical section through the thallus showing abundant cell fusions (arrow); **D** magnified view of the outer thallus showing a single layer of flared epithallial cells (e) derived from subepithallial initials (s), and cortical cells (c) joined by cell fusions (arrow). Scale bars: A=0.5 cm, B=200 μm , C=20 μm , D=10 μm .

Spongites sp. (“species 6”)

(Figs 10A–H)

Specimens examined:—West Indies, Guadeloupe: Passe à Caret, Grand Cul-de-Sac Marin, at 32 m depth (23.v.2012, *Karubenthos Expedition*, PC0144235, PC0144239 and PC0144244).

Appearance and vegetative structure:—Thalli unattached as maerl and rhodoliths, warty to lumpy, ellipsoidal, up to 4 cm in diameter with densely branched protuberances up to 1–2 m in diameter and 2–3 mm in length (Figs 10A–C). Thallus construction monomerous, and radially organized in branches (Fig. 10D). Cell fusions predominate (Figs 10D–E); secondary pit connections were not observed. Medullary cells are 15–26 μm in length by 10–15 μm in diameter. Cortical cells are 13–18 μm in length by 9–12 μm in diameter. Subepithallial initials are 9–14 μm in length by 8–12 μm in diameter (Figs. 10E–F). Epithallial cells occur in a single layer and have rounded corners. Epithallial cells are squat, 2–3 μm in length by 7–9 μm in diameter (Fig. 10F). Solitary, bottle-shaped trichocytes measuring 38 μm in length by 20 μm in diameter (8 μm at the apex) were observed (Figs 10G–H). Small groups of trichocytes were also observed (Fig. 10H).

Reproduction:—Reproductive structures were not observed.

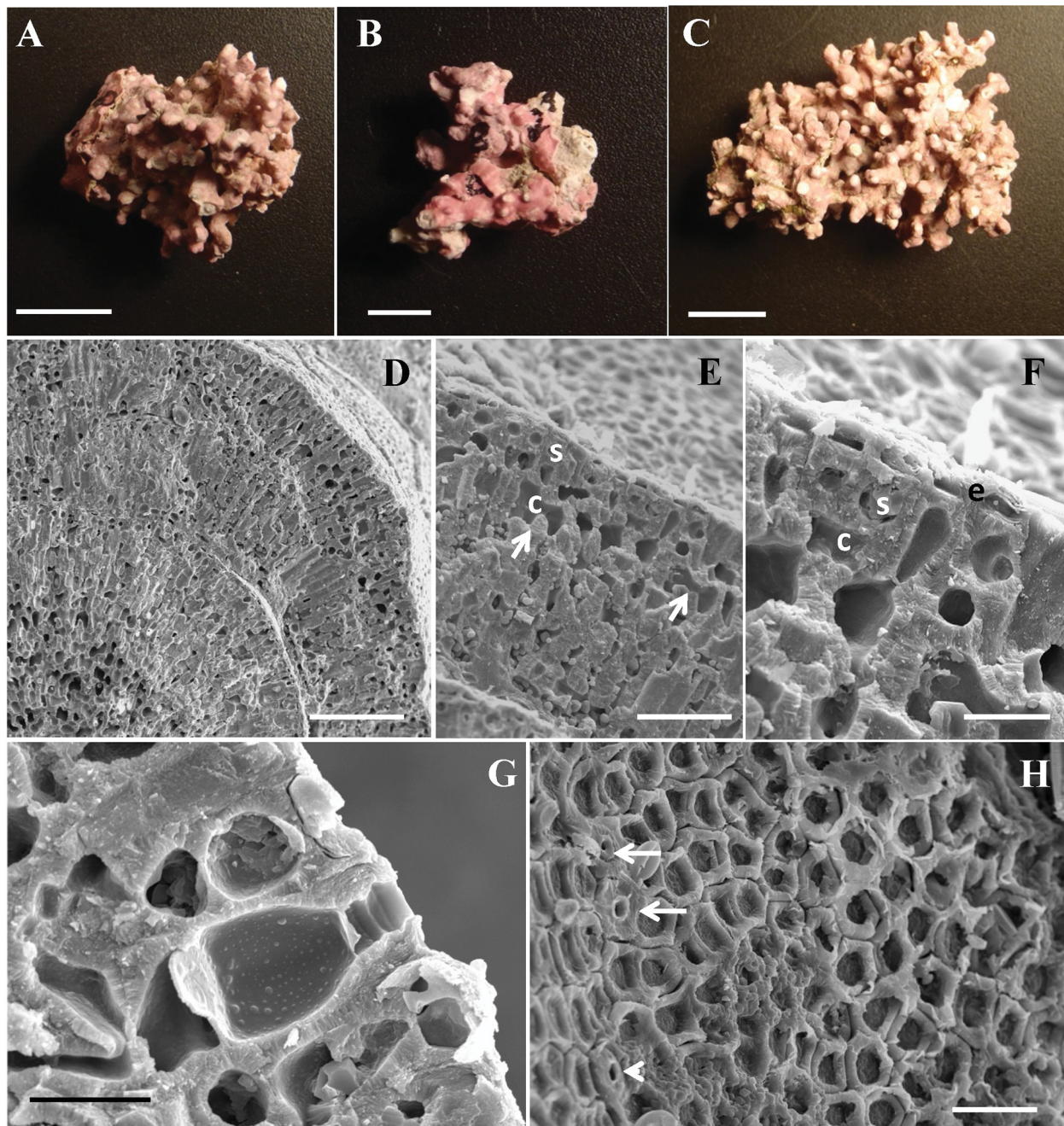


FIGURE 10. Morphological and vegetative anatomy of *Spongites* sp. (PC0144235, PC0144239, PC0144244). **A** warty rhodolith specimen with densely branched protuberances; **B** lumpy rhodolith specimen with densely branched protuberances; **C** warty maerl specimen with densely branched protuberances; **D** vertical section of a protuberance showing the radial organization; **E–F** magnified view of the outer thallus showing a single layer of flattened epithallial cells (e) derived from subepithallial initials (s), and cortical cells (c) joined by cell fusions (arrows); **G** magnified view of the outer thallus showing a solitary bottle-shaped trichocyte; **H** surface view of the thallus showing thick-walled epithallial cells and trichocytes occurring singly (arrowhead) and in small groups (arrows). A, D, E, F, H: PC0144239; B: PC0144244, C, G: PC0144235. Scale bars: A, C=1 cm, B=0.5 cm, D= 150 μ m, E=50 μ m, F–H= 20 μ m.

***Lithoporella/Mastophora* sp. (“species 7”)**

(Figs 11A–G)

Specimen examined:—West Indies, Guadeloupe: Passe à Caret, Grand Cul-de-Sac Marin, at 32 m depth (23.v.2012, *Karubenthos Expedition*, PC0144236).

Appearance and vegetative structure:—Thalli unattached as rhodolith, lumpy, discoidal, up to 3 cm in diameter (Fig. 11A). Rhodolith consist of successive layers of thalli overgrowing the core (Figs 11B–C). Thallus

construction is dimerous. The vegetative thallus is bistratose comprising a single basal layer of non-palisade cells and a single layer of epithallial cells (Fig 11D). Haustoria were not observed. Cells of the basal layer are 30 μm in length by 12–15 μm in diameter. Cell fusions are common between cells of the basal layer (Fig. 11D). Epithallial cells are 2 μm in length by 8–11 μm in diameter (Fig. 11D). Solitary trichocytes occur in clusters; their pores are 7 μm in diameter (Fig. 11E).

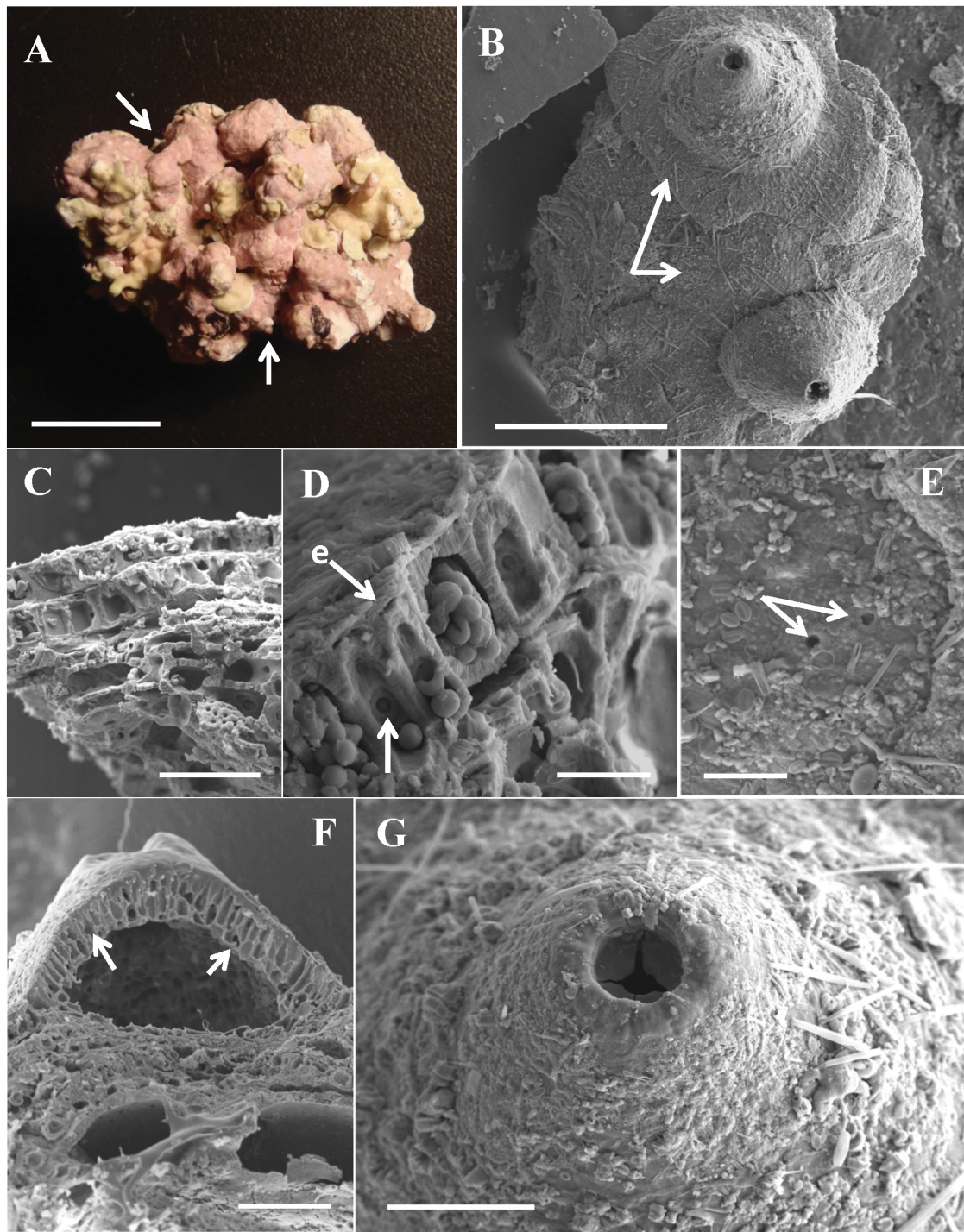


FIGURE 11. Morphological, vegetative and reproductive anatomy of the *Lithoporella/Mastophora* sp. (PC0144236). **A** mostly lumpy rhodolith specimen bearing uniporate conceptacles (arrows); **B** surface view of the thallus showing successive layers of thalli (arrows) and conical uniporate conceptacles; **C** vertical section showing successive layers of thalli overgrowing the rhodolith core; **D** magnified view of the thallus showing a bistratose, dimerous thallus composed of alternating non-palisade basal cells with fusion cells (arrow) and a single layer of epithallial cells (e); **E** surface view of the thallus showing solitary trichocytes (arrows); **F** vertical section through a conical uniporate conceptacle with a roof composed of several layers of cells joined by cell fusions (arrows); **G** magnified view of a uniporate conceptacle. Scale bars: A= 1 cm, B=500 μm , C, G=100 μm , D= 25 μm , E=50 μm , F=150 μm .

Reproduction:—Reproductive thalli are several cell layers thick. Only empty uniporate conceptacles observed. They are raised and conical, measuring 300 µm in height by 500 µm in diameter (Figs 11F–G). Conceptacle chambers 325 µm in diameter by 200 µm in height (Fig 11F). Conceptacle roofs are 100 µm thick and composed of 2–3 layers of cells (Fig. 11F). In surface view, conceptacle pores are 60 µm in diameter (Figs 11B, G).

Discussion

This study provides the first assessment of the taxonomic diversity of Caribbean maerl and rhodolith-forming species using molecular tools such as DNA barcoding. Our molecular analyses supported by two molecular markers (COI-5P and *psbA*) have revealed the occurrence of eight different species forming maerl and rhodoliths in Guadeloupe. Most importantly, none of the sequences generated in this study matched any publicly available sequences. Apart from *Lithothamnion* cf. *ruptile*, this observation suggests that all species studied are either new to science, or possibly belong to species for which no sequence data is yet available. In either case, our result show that we are far from having a comprehensive view of the maerl and rhodolith-forming species of non-geniculate coralline diversities.

Lithothamnion cf. *ruptile* was identified based on the morphological examination of several unattached forms forming maerl. However, the molecular results obtained pointed out that this taxon is unrelated with other *Lithothamnion* spp. included in our study (*L. corallioides*, *L. glaciale* and *L. muelleri*). The same result was obtained for the Mediterranean specimen *Lithothamnion* cf. *crispatum*, and both species were resolved as sister taxa in our analyses. In the literature, Lemoine (1917) already pointed out that *L. ruptile* is morphologically similar to *L. crispatum*, although the latter has tetra/bisporangial conceptacle roofs pitted with depressions, each one hosting a pore and the surrounding rosette cells (Bressan & Babbini 2003, Basso *et al.* 2011). On the other hand, another resembling species, *L. occidentale* (Foslie) Foslie, is also reported for the Caribbean Sea. Based on the Caribbean literature, this species also develops branches with tips projections and form unattached forms (Lemoine 1917, Taylor 1960, Littler & Littler 2000, Ballantine *et al.* 2004, 2011). Further studies on the type material *L. ruptile*, *L. occidentale* and *L. crispatum* involving DNA sequencing are required to assess the boundaries of these taxonomical entities in a modern context, and to elucidate the phylogenetic relationship among these closely resembling species.

Based primarily on phylogenetic analyses and secondarily on morphological/anatomical examination, five of the species studied were assigned to the genus *Lithothamnion*. Of the five species, only *Lithothamnion* sp. 5 was resolved in a lineage encompassing other species (*L. muelleri*, *L. corallioides*, *L. glaciale*, the two latter species common maerl-forming species in temperate and subarctic regions—Irvine & Chamberlain 1994, Mendoza & Cabioch 1998, Adey *et al.* 2005). The remaining four species were resolved unrelated to the clade bearing *Lithothamnion* sp. 5, appearing related with other Melobesioideae such as *Mesophyllum* spp, *Synarthrophyton patena*, *Phymatolithon repandum* and *P. lenormandii*. However, they were assigned to this genus based on the presence of flared epithallial cells and a monomeric thallus construction. Within the family Hapalidiaceae, flared epithallial cells are considered diagnostic of the genera *Lithothamnion* and *Exilicrusta*. The two genera are separated from each other by the presence of either a monomeric (*Lithothamnion*) or a dimerous (*Exilicrusta*) thallus construction (Woelkerling 1983, Chamberlain 1992). On the other hand, the genus *Lithothamnion* is characterized within the Hapalidiaceae by subepithallial initials that are as long as or longer than cells subtending them (Johansen 1981, Woelkerling 1983, Irvine & Chamberlain 1994, Mendoza & Cabioch 1998, Adey *et al.* 2005). However, the species recorded in this study bear subepithallial initials that are usually shorter than cells subtending them. The presence of short subepithallial initials in species belonging to the genus *Lithothamnion* was also reported in *L. carpoklonion* A. Athanasiadis & D.L. Ballantine (Athanasiadis & Ballantine 2011) and in *L. ruptile* (Littler & Littler 2000). These observations suggest either that: i) we need to re-examine our concept of the use of flared epithallial cells as a diagnostic character for generic delimitation within the Hapalidiaceae; or ii) an active cell division processes in subepithallial initials of Caribbean species is a common occurrence. Apart from *L. ruptile* and *L. occidentale*, only one other Caribbean species of *Lithothamnion* is currently recognized, namely *L. carpoklonion*. This latter species is as an epiphytic encrusting species associated with mangrove prop roots and bears characteristic conceptacle protuberances (Athanasiadis & Ballantine 2011). Based on these other and our findings, it is clear that the diversity within the genus *Lithothamnion* from the Caribbean has been highly underestimated.

As noted previously, due to the limitations of publicly available sequence data, at this stage we are unable to conclude whether the species documented here are new to science, or simply belong to species for which no sequence data is yet available. Further molecular studies involving taxa described from neighbouring areas and the study of type material or topotype material are therefore necessary to fully identify our Caribbean species of *Lithothamnion*. In addition, further analyses combining different phylogenetic markers for our specimens are required to clarify the taxonomic status of these Caribbean species. In Guadeloupe, *Lithothamnion* species were found forming maerl and rhodoliths from shallow to as deep as 110 m. In the Caribbean, unattached forms of *Lithothamnion* were usually reported up to 80 m depth (Lemoine 1917, Taylor 1960, Schneider & Searles 1991, Littler & Littler 2000). A deeper record (up to 290 m) was reported on a seamount in the Bahamas although the coralline cover of the nodules was less than 15% (Littler *et al.* 1991).

The species 6 was assigned to the genus *Spongites* based on the molecular results and the anatomical examination of vegetative features, although we are aware that *Spongites* share with *Neogoniolithon* vegetative features, differing in the spermatangial development and in the carposporophyte features (Penrose 1991, Penrose & Woelkerling 1992, Kato *et al.* 2011). In our molecular analyses, the group composed of the species 6 and *S. cf. fruticulosa* were not resolved within *Neogoniolithoideae*. According to the COI-5P analyses, both taxa were resolved together with *Spongites* sp. from Hawaii; for the *psbA* analyses, there were not available sequences for the Hawaiian specimens, and the clade composed of species 6 and *S. cf. fruticulosa* appeared unrelated with taxa identified as *S. decipiens*, *S. discoidea*, *S. tunicata* and *S. yendoii* collected in South Africa and New Zealand. The original material of the type species of *Spongites* (*S. fruticulosa*) was collected in an unspecified locality in the Mediterranean Sea (Woelkerling 1985). The Mediterranean specimen included in the present study and identified as *S. cf. fruticulosa* consisted on a sporophyte maerl specimen collected in Greece. The vegetative features reproductive features of this specimen matched previous descriptions of the Mediterranean *S. fruticulosa* (Bressan & Babbini 2003, Basso & Rodondi 2006).

Till now there were no previous records of any representatives of this genus reported for the Caribbean Sea (Lemoine 1917, Taylor 1960, Schneider & Searles 1991, Guiry & Guiry 2014). Some species of *Spongites* have, however, been reported from other localities in the tropical Atlantic. *Spongites absimile* (Foslie & M.Howe) Afonso-Carrillo and *S. yendoii* have been cited for the tropical western Atlantic (Wynne 2011). *Spongites decipiens*, a crustose species originally described from California (Chamberlain 1993), was reported along the Pacific coast of Costa Rica (Fernández-García *et al.* 2011). However, according to our molecular analyses that included data from *S. decipiens* and *S. yendoii* from South Africa and New Zealand respectively, the species 6 was resolved unrelated with both taxa. The type species of the genus, *S. fruticulosa*, has been also cited in the Pacific coast of Mexico and Central America under the synonym *Lithothamnion fruticulosum* (Kützinger) Foslie (Dawson 1960, Fernández-García *et al.* 2011). This species has been also widely recorded in the Atlantic and Indian coasts (Guiry & Guiry 2014). In a comparison of the type material and Mediterranean collections of *S. fruticulosa* with Australian specimens described in the literature, Basso & Rodondi (2006) noted that the Mediterranean populations had smaller sexual and asexual conceptacles. Further investigations involving molecular and anatomical data from the type material of *S. fruticulosa* are required to examine the phylogenetic relationship among collections reported in different regions. Regarding our species 6 assigned to *Spongites*, new collections with sexual reproductive structures are necessary to confirm its taxonomical position. In addition, further molecular and anatomical data from the *Spongites* species reported in neighbouring areas as well as from their type material are required to properly resolve the identity and distribution of this Caribbean taxon.

Finally, our molecular phylogeny resolved the species 7 together with other species of Mastophoroideae *sensu* Harvey *et al.* (2003). This rhodolith comprised of successive layers of largely bistratose, dimerous thalli that could be assigned to either the genus *Lithoporella* or *Mastophora*. There are no previous records of species from the genus *Mastophora* in the Caribbean Sea, except for *Mastophora atlantica* Foslie and *M. lamourouxii* Decaisne ex Harvey that are currently regarded as taxonomic synonyms of *Lithoporella atlantica* (Foslie) Foslie and *Metamastophora flabellata*, respectively (Mazé & Schramm 1878, Woelkerling 1988, Keats *et al.* 2009, Guiry & Guiry 2014). *Metamastophora flabellata*, however, is the only mastophoroid characterized by a distinctive arborescent (tree-like) and flabelliform (fan-shaped) habit, with compressed to flattened upright axes that arise from a discoid to applanate holdfast (Woelkerling 1980). Although our specimen has non-palisade basal cells that are smaller than those reported for *L. atlantica* by Lemoine (1974, 32–60 µm in height by 18–40 µm in diameter), it does match those reported for the *L. atlantica* in the Caribbean earlier by the same author (Lemoine 1917, 18–32

(–60) μm in height by 10–25 (40) μm in diameter). *Lithoporella atlantica* has been described from our study area (Virgin Islands) by Woelkerling & Lamy (1998) and later reported in other Caribbean Islands and Central America (Guiry & Guiry 2014). Unfortunately, there are no diagnostic characters to tell apart the genera *Lithoporella* and *Mastophora* based on vegetative features. Lemoine (1974) suggested that these genera could be distinguished by differences in the degree of thallus calcification, but Turner & Woelkerling (1982a) observed that the degree of calcification as well as the thallus form, varied within specimens, and was likely influenced by the nature of the substrate. However, both genera do show differences in tetra/bisporangial conceptacle anatomy and are distinguished from each other primarily by the mode of the tetra/bisporangial conceptacle roof development and the shape of the cells at the base of the pore canal; and secondarily by the presence or absence of a central columella in the tetra/bisporangial conceptacle (Turner & Woelkerling 1982b, Woelkerling 1988). In *Lithoporella* the pore canals of tetra/bisporangial conceptacles are lined by a ring of conspicuously enlarged cells that arise from filaments interspersed among and surrounding the sporangial initials; these cells do not protrude into the pore canal and are oriented more-or-less perpendicularly to the roof surface. In addition, tetra/bisporangial conceptacles lack a central columella. In *Mastophora* the pore canals of tetra/bisporangial conceptacles are lined by cells that arise from peripheral roof filaments; these cells protrude into the pore canal as papillae and are oriented more or less parallel or at a sharp angle to the conceptacle roof surface. In addition, tetras/bisporangial conceptacles possess a central columella. In our material the uniporate conceptacles were found to be empty without a central columella, and they could be interpreted to be tetra/bisporangial conceptacles of *L. atlantica* according to the conical shape and diameter (500–800 μm) previously reported for Caribbean material by Lemoine (1917). However, due to the limitations of SEM prepared material (e.g. weakly calcified material such as columella often does not show up) further studies detailing the anatomy of the uniporate conceptacles will be necessary to confirm the identity of this specimen. In addition, a detailed examination of the type material of *L. atlantica* in a modern context may be required to clarify its generic status.

As far as we know, this is the first record of maerl and rhodolith-forming species in Guadeloupe. Specimens were recorded from a wide bathymetric range and they can be considered as one of the deepest reports for the Caribbean Sea (110 m depth). The occurrence of deep-water rhodoliths was also reported from the Bahamas (San Salvador Seamount) at 290 m depth, although the highest rhodolith cover was recorded at 67–91 m (Littler *et al.* 1991). However, these rhodoliths were morphologically different from most of the Guadeloupe collections as they were described as algal nodules containing a mixture of encrusting corallines and non-coralline species and patches of small corals, sponges and other boring organisms (Littler *et al.* 1991), whereas the specimens collected in the *Karubenthos Expedition* were almost solely formed by coralline algae.

Conclusion

Maerl and rhodolith-forming species have been encountered in various locations in the Guadeloupe Archipelago extending from 5 to at least 110 m depth. Molecular and morphological/anatomical results revealed eight species of maerl and rhodolith-forming species: *Lithothamnion* cf. *ruptile*, five (species 1 to 5) were assigned to the genus *Lithothamnion*, one to the genus *Spongites* and one to either the genus *Lithoporella* or *Mastophora*. Our results have revealed that species diversity of Caribbean maerl and rhodolith-forming species of coralline algae have been underestimated and further ongoing molecular studies on marine algae from various biodiversity hotspots are still necessary.

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