

Insights into species diversity of associated crustose coralline algae (*Corallinophycidae*, *Rhodophyta*) with Atlantic European maerl beds using DNA barcoding

Cristina Pardo*, Ignacio Bárbara¹, Rodolfo Barreiro² & Viviana Peña³

Grupo BioCost, Dpto. de Biología, Facultad de Ciencias, Univ. da Coruña, 15071 A Coruña, Spain

*Corresponding author: cristina.pardo.carabias@udc.es, <http://orcid.org/0000-0002-2534-3961>

¹ignacio.barbara@udc.es, <http://orcid.org/0000-0003-1779-0224>

²rodolfo.barreiro@udc.es, <http://orcid.org/0000-0002-6049-5215>

³vpena@udc.es, <http://orcid.org/0000-0001-7003-3850>

Abstract. DNA barcoding in combination with morpho-anatomical analysis was applied to study the diversity of crustose coralline algae associated to two maerl beds from two protected Atlantic European areas from Brittany and Galicia —France and Spain, respectively—. Given the records of gametophytes of the maerl species *Phymatolithon calcareum* under crustose growth-forms, and that associated crustose coralline algae appear to be involved in the recruitment of new maerl plants, we compared the species composition between the associated crustose coralline algae to Breton and Galician maerl beds with the maerl species identified in these beds in previous DNA barcoding surveys. Our molecular results revealed higher species diversity in associated crustose coralline algae than in maerl-forming species. Nine taxa of crustose coralline algae were found in both study areas: four in Brittany and five in Galicia. Three species from Brittany were identified as *Phymatolithon calcareum*, *Phymatolithon lamii*, and *Lithophyllum hibernicum*. The remaining six ones were assigned to the genera *Phymatolithon* and *Mesophyllum*, along with *Lithothamnion* and *Lithophyllum*. Morpho-anatomical examination of diagnostic characters corroborated our molecular identification. Our results showed that the most representative genus of crustose coralline algae in Brittany was *Phymatolithon*, while in Galicia was *Mesophyllum*. In Brittany, *Phymatolithon calcareum* was found under both growth-forms, maerl and crustose coralline algae, the latter assigned to the gametophyte stage by the presence of uniporate conceptacles. The recruitment of new maerl plants involving associated crustose coralline algae with maerl beds may occur, but only we can affirm it for *Phymatolithon calcareum* in Brittany. By contrast, the different species composition between both growth-forms in the Galician maerl beds would indicate that the fragmentation of own free-living maerl species appears to be the most common propagation mechanism.

Keywords. Brittany, COI-5P, *Corallinales*, crustose coralline algae, diversity, Galicia, *Hapalidiales*, *Lithophyllum*, *Lithothamnion*, maerl, *Mesophyllum*, morphology, *Phymatolithon*, reproduction, rhodolith, SEM.

Resumen. En este trabajo se han utilizado el sistema de códigos de barras genéticos y análisis morfo-anatómicos para estudiar la diversidad de algas coralinas costrosas asociadas a dos fondos de maerl localizados en dos áreas protegidas del Atlántico Europeo en Bretaña y Galicia —Francia and España, respectivamente—. Dadas las citas recientes de gametófitos de la especie típica de lechos de maerl, *Phymatolithon calcareum*, bajo una forma de crecimiento costrosa, y que las algas coralinas incrustantes parecen estar implicadas en el reclutamiento de nuevas plantas del maerl, en este trabajo se compara la composición entre estas algas asociadas a fondos de maerl bretones y gallegos, con las especies del maerl identificadas en estos fondos en estudios previos de códigos de barras genéticos. Los resultados moleculares del presente trabajo revelaron una diversidad más alta en las algas coralinas costrosas asociadas que en las propias especies formadoras del maerl. En las áreas estudiadas se encontraron nueve táxones de algas coralinas costrosas: cuatro en la Bretaña francesa y cinco en Galicia. Tres especies de la Bretaña fueron identificadas como *Phymatolithon calcareum*, *Phymatolithon lamii* y *Lithophyllum hibernicum*. Las seis especies restantes fueron asignadas a los géneros *Phymatolithon* y *Mesophyllum*, junto con *Lithothamnion* y *Lithophyllum*. El examen morfo-anatómico de caracteres diagnósticos concordó con la identificación molecular. Los resultados obtenidos evidenciaron que el género más representativo de algas coralinas costrosas en la Bretaña fue *Phymatolithon*, mientras que en Galicia fue *Mesophyllum*. En la Bretaña, *Phymatolithon calcareum* fue encontrado bajo ambas formas de crecimiento —maerl y morfología incrustante—, en este último caso asignada a la generación gametofítica por la presencia de conceptáculos uniporados. El reclutamiento de nuevas plantas del maerl a través de las algas coralinas costrosas asociadas a fondos de maerl puede suceder, pero solo se puede afirmar para *Phymatolithon calcareum* en la Bretaña. Por contra, en los fondos de maerl de Galicia la composición diferente de ambas formas de crecimiento podría indicar que la fragmentación de las propias especies del maerl podría ser el mecanismo de propagación más común.

Palabras clave. Algas coralinas incrustantes, Bretaña, COI-5P, *Corallinales*, diversidad, Galicia, *Hapalidiales*, *Lithophyllum*, *Lithothamnion*, maerl, *Mesophyllum*, morfología, *Phymatolithon*, reproducción, rodolito, SEM.

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INTRODUCTION

Atlantic European rhodolith beds, also known as maerl beds, are marine coastal habitats of high diversity composed of unattached non-geniculate coralline red algae —maerl/rhodolith— mixed with gravel, shells, and pebbles overgrown by crustose coralline algae (CCA; v.gr., Cabioch 1969, 1970; Bosence 1976; Peña & Bárbara 2009; Hall-Spencer & al. 2010; Adey & al. 2015). The life-history of coralline red algae involves an

alternation among three phases: a haploid gametophyte, a diploid carposporophyte —carried by the haploid female gametophyte after fertilization of carpogonium—, and a diploid tetrasporophyte (Irvine & Chamberlain 1994). In two major Atlantic European maerl species, *Phymatolithon calcareum* (Pallas) W.H.Adey & D.L.McKibbin and *Lithothamnion corallioides* (P.L.Crouan & H.M.Crouan) P.L.Crouan & H.M.Crouan, sporangial conceptacles have been occasionally reported —usually in free-living

growth forms, and rarely in encrusting plants—, while gametangial conceptacles were even more rare (i.e., sexual conceptacles), and were only found as associated CCA in maerl beds from Brittany (Cabioch 1969, 1970; Adey & McKibbin 1970; Maggs 1983; Woelkerling & Irvine 1986; Irvine & Chamberlain 1994; Mendoza & Cabioch 1998; Peña & Bárbara 2004; Peña & al. 2014). Several authors (Lemoine 1910; Cabioch 1969, 1970; Freiwald 1995; Mendoza & Cabioch 1998) have pointed out the contribution of the CCA to the development of further unattached maerl plants: after germination of the carpospores produced in CCA, juvenile tetrasporophytic plants grow also as crusts, and afterwards erect branches formed by these crusts break off, and give rise to unattached maerl plants. In Breton maerl beds, this type of recruitment was reported as the dominant (Cabioch 1969, 1970; Mendoza & Cabioch 1998). Nonetheless, others authors (Bosence 1976; Woelkerling 1988; Peña & al. 2014) have reported that fragmentation of free-living maerl thalli is the main method of propagation in the maerl beds.

In recent years, several molecular studies have been focused on the diversity and systematics of maerl-forming species in Atlantic Europe (Carro & al. 2014; Hernández-Kantún & al. 2014, 2015a, 2015b; Pardo & al. 2014; Peña & al. 2015b). However, the diversity and specific composition of their associated CCA are little-known. In Brittany, two DNA barcoding works found crustose plants of *Phymatolithon calcareum* and *Phymatolithon lamii* (Me.Lemoine) Y.M.Chamb., which showed that species composition of associated CCA could share some similarities with unattached maerl plants (Peña & al. 2014, 2015b). Based on previous results obtained for maerl-forming species of two study areas from Brittany and Galicia (Pardo & al. 2014), we identified their associated CCA using a combination of DNA barcoding—COI-5P—and morpho-anatomical features. Apart from the species diversity of associated CCA, we compared the composition of both growth-forms (maerl and CCA) in order to know if the associated CCA could be involved in the recruitment of new unattached maerl plants.

MATERIAL AND METHODS

Sampling collection

A total of 16 CCA specimens, all of them epilithic over pebbles, were collected in two Atlantic European maerl beds in 2011 (table 1, fig. 1): Molène Archipelago—Brittany, France— and Ons Archipelago—Galicia, Spain—. Both archipelagos are located in two protected marine areas: Parc Naturel Marin d'Iroise—PNMI—, and Parque Nacional Marítimo Terrestre das Illas Atlánticas de Galicia—PNMTIAG—, respectively. Collections were carried out by dredging at < 10 m in Brittany, and SCUBA diving at 13 m in Galicia, as complementary to maerl-

forming species surveys (Carro & al. 2014; Pardo & al. 2014; Peña & al. 2014). After collection, material was air-dried, and vouchered in silica. Specimens were observed and photographed under stereomicroscope, and were deposited in the herbarium SANT (Thiers 2016; table 1).

Molecular studies

For the DNA extraction, a subsample was obtained by grounding of a small portion of each plant selected, choosing free epiphytes areas under stereomicroscope. DNA was extracted and purified using the DNeasy® Blood & Tissue Kit—Qiagen, Valencia, CA— following manufacture's recommendations. A fragment of 664 bp of the 5' end of the mitochondrial gene cytochrome oxidase I—COI-5P— was amplified using primers GazF1 and GazR1 (Saunders 2005). Amplifications PCR were performed in a Biometra TProfessional Basic thermocycler following Saunders & McDevit (2012). Amplification success was evaluated by running the reactions in agarose gels. After excess of primers and nucleotides were removed with shrimp alkaline phosphatase and exonuclease I enzymes. PCR products were sequenced using the Sanger method at MacroGen facilities—<http://www.macrogen.com>—. The sequences obtained were checked, edited and aligned with the program Geneious v. 5.6.6—Biomatters, New Zealand—, and lodged in BOLD and GenBank (table 1). Haplotypes obtained were searched for matches in BOLD and GenBank databases to their molecular taxonomic identification (table 2). For the molecular analyses, publicly available COI-5P sequences for both maerl-forming and CCA taxa, particularly from Atlantic Europe, were included (Carro & al. 2014; Pardo & al. 2014; Peña & al. 2014, 2015a, 2015b; Hernández-Kantún & al. 2015a), as well as a sequence of *Corallina officinalis* L. as outgroup (Pardo & al. 2015; table 3). In total, 30 sequences were used to generate a Maximum Likelihood—ML— tree. A bootstrap with 1,000 replicates was applied in Mega v. 6.0 with defaults settings (Tamura & al. 2013), using the General Time-Reversible model, Gamma distributed with Invariant sites—GTR+G+I—, identified previously in ModelTest v. 2.1.3 (Darriba & al. 2012) as the best-fitting substitution model using an Akaike Information Criterion—AIC— and Bayesian Information Criterion—BIC—.

Morphological studies

CCA selected for molecular studies were examined under stereomicroscope, and representative fragments were examined by Scanning Electron Microscope—SEM, model JEOL JSM 6400, Univ. da Coruña—. Vegetative and reproductive features considered diagnostic and anatomical terminology followed Irvine & Chamberlain (1994).

Table 1. Information and collection data of the epilithic CCA collected in Brittany and Galicia.

Haplotype ML-tree	Species identification	Voucher	BOLD Process ID GenBank accession no. SANT-Algae	Collection details
CCA_hap-1	<i>Phymatolithon calcareum</i>	VPF00132/CPVP-945	CCOR002–17 MF133371 SANT-Algae 29484	Molène Archipelago Depth < 10 m 13–V–2011 48° 23.150' N, 04° 51.233' W
CCA_hap-2	<i>Phymatolithon</i> sp. 5	CPVP-758	CCOR001–17 MF133370 SANT-Algae 00273	Ons Archipelago Depth 13 m 07–IV–2011 42° 23.678' N, 08° 54.915' W
CCA_hap-3	<i>Phymatolithon</i> sp. 6	VPF00407B	CCOR003–17 MF133369 SANT-Algae 29168	Molène Archipelago Depth < 10 m 13–V–2011 48° 23.150' N, 04° 51.233' W
		VPF00407C	CCOR004–17 MF133366 SANT-Algae 29168	
		VPF00411B	CCOR005–17 MF133368 SANT-Algae 29169	
		VPF00406A	CCOR006–17 MF133367 SANT-Algae 00272	
CCA_hap-4	<i>Phymatolithon lamii</i>	VPF00410	CCOR007–17 MF133372 SANT-Algae 29485	Molène Archipelago Depth < 10 m 13–V–2011 48° 23.150' N, 04° 51.233' W
CCA_hap-5	<i>Lithothamnion</i> sp. 2	VPF00483	CCOR008–17 MF133360 SANT-Algae 00274	Ons Archipelago Depth 13 m 7–IV–2011
CCA_hap-6		CPVP-763	CCOR009–17 MF133361 SANT-Algae 00275	42° 23.678' N, 08° 54.915' W
CCA_hap-7	<i>Mesophyllum</i> sp. 3	VPF00479	CCOR010–17 MF133363 SANT-Algae 00276	Ons Archipelago Depth 13 m 7–IV–2011
CCA_hap-8		CPVP-767	CCOR011–17 MF133362 SANT-Algae 00277	42° 23.678' N, 08° 54.915' W
CCA_hap-9	<i>Mesophyllum</i> sp. 4	VPF00480	CCOR012–17 MF133365 SANT-Algae 00278	Ons Archipelago Depth 13 m 7–IV–2011
CCA_hap-10		CPVP-769	CCOR013–17 MF133364 SANT-Algae 00279	42° 23.678' N, 08° 54.915' W
CCA_hap-11	<i>Lithophyllum</i> sp. 3	CPVP-762	CCOR014–17 MF133359 SANT-Algae 00280	Ons Archipelago Depth 13 m 7–IV–2011 42° 23.678' N, 08° 54.915' W
CCA_hap-12	<i>Lithophyllum hibernicum</i>	VPF00411A	CCOR015–17 KR733526 SANT-Algae 29169	Molène Archipelago Depth < 10 m Date: 13–V–2011
CCA_hap-13		VPF00407A	CCOR016–17 KR733456 SANT-Algae 29168	48° 23.150' N, 04° 51.233' W

RESULTS

Molecular results

COI-5P sequences were obtained for the 16 CCA specimens collected (table 1). They were grouped in 13

haplotypes —CCA_hap-1 to CCA_hap-13—: five from Molène Archipelago and eight from Ons Archipelago (table 1). These 13 haplotypes were delimited in nine CCA species belonging to four genera (fig. 2): *Phymatolithon* sp. 5 —CCA_hap-2—, *Phymatolithon* sp. 6 —CCA_hap-3—, *Phymatolithon calcareum* —CCA_hap-1—,

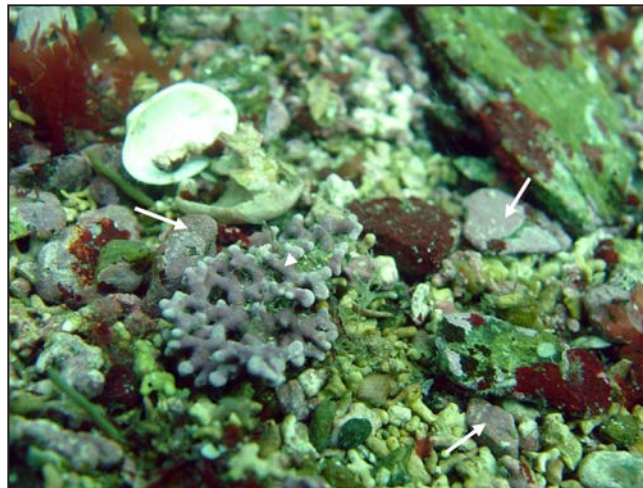


Fig. 1. Close up of the maerl bed studied in Ons Archipelago. [Arrow: epilithic CCA; arrowhead: a maerl-forming species.]

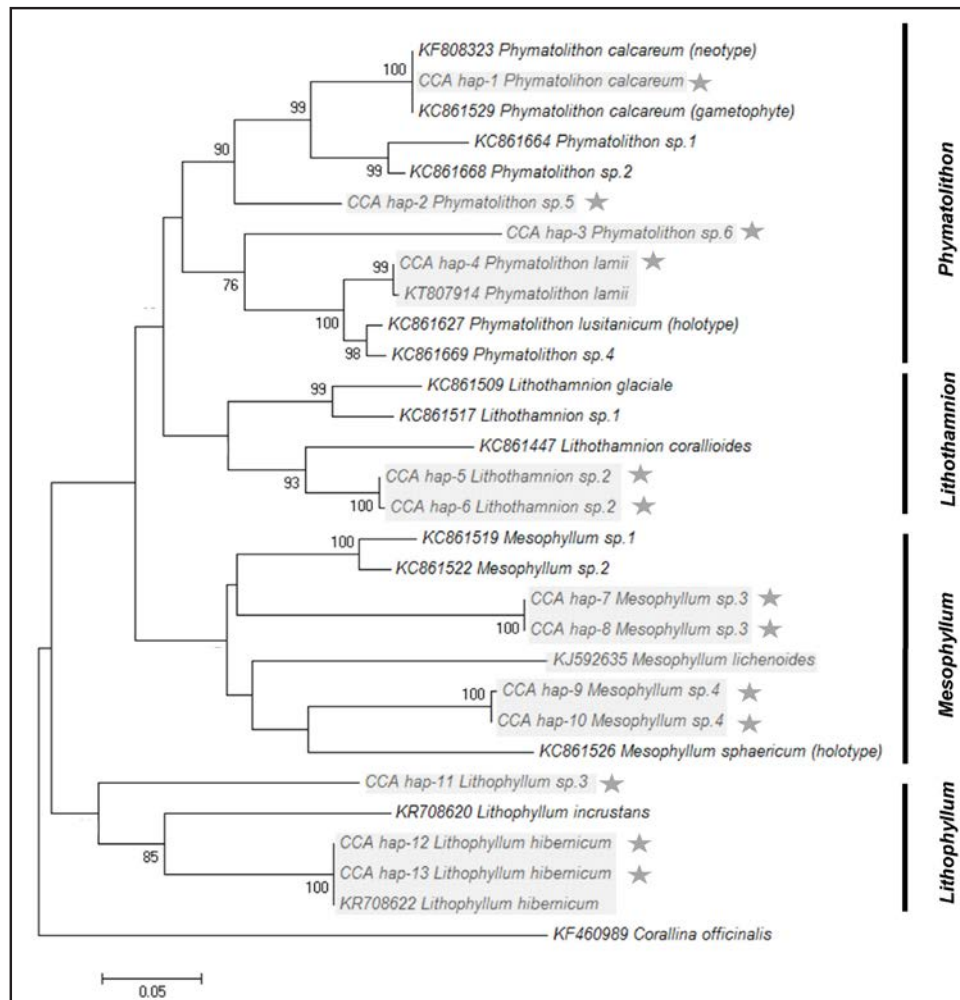


Fig. 2. Maximum-Likelihood —ML— tree for the haplotypes —i.e., DNA barcodes, in grey colour with a star— of the CCA associated to maerl beds from Ons Archipelago and Molène Archipelago showing relationships with other sequences of genus *Lithophyllum*, *Phymatolithon*, *Lithothamnion*, and *Mesophyllum*. [Encrusting growth-forms are indicated in grey colour; bootstrap support values > 75% —1,000 replicates— are shown; outgroup: *Corallina officinalis* L.; scale bar refers to base substitutions per site.]

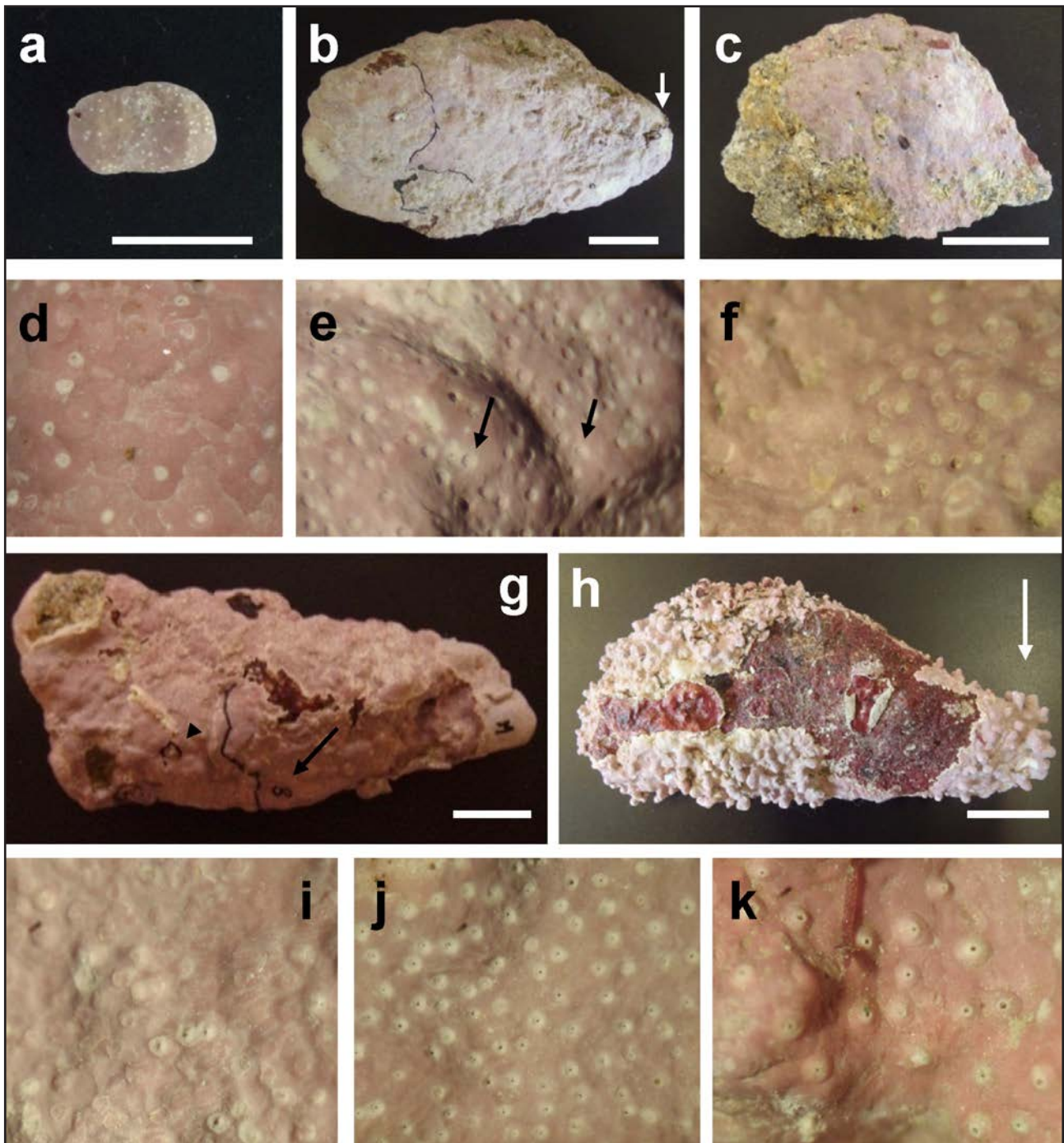


Fig. 3. Epilithic crusts associated with maerl beds in Galicia and in Brittany: **a**, *Phymatolithon* sp. 5 from Ons Archipelago (CPVP-758); **b**, *Phymatolithon* sp. 6 from Molène Archipelago (VPF00406A); **c**, *Phymatolithon lamii* from Molène Archipelago (VPF00410); **d**, *Phymatolithon* sp. 5 with asexual multiporate conceptacles (CPVP-758); **e**, *Phymatolithon* sp. 6 with sexual uniporate conceptacles (VPF00406A); **f**, *Phymatolithon lamii* with asexual multiporate conceptacles (VPF00410); **g**, *Phymatolithon* sp. 6 from Molène Archipelago (VPF00407B arrow, and VPF00407C arrowhead); **h**, *Phymatolithon calcareum* from Molène Archipelago (VPF00132); **i**, *Phymatolithon* sp. 6 with sexual uniporate conceptacles (VPF00407B); **j**, *Phymatolithon* sp. 6 with sexual uniporate conceptacles (VPF00407C); **k**, *Phymatolithon calcareum* with sexual uniporate conceptacles (VPF00132). [Scale bars: a, 1 cm; b, c, h, 2.5 cm; g, 1.5 cm.]

Table 2. Matches with our sequences in public databases (BOLD and GenBank). Only hits with an identity beyond a minimum threshold are reported in this table (98%).

Haplotype ML-tree	BOLD				GenBank			
	Number	Identity	Taxonomy	Locality	Accession no.	Identity	Taxonomy	Locality
CCA_hap-1	BOLD:ACD0016	100%	<i>Phymatolithon calcareum</i>	Falmouth (British Isles)	KC861551	99%	<i>Phymatolithon calcareum</i>	Galicia (NW Spain)
CCA_hap-3	BOLD Process ID: ABMMC9521-10 BOLD Sample ID: GWS006296	100%	<i>Phymatolithon</i> sp.3AT crust	New Brunswick (Atlantic Canada)	No match	–	–	–
CCA_hap-4	BOLD Process ID: NGCOR259-15 BOLD Process ID: VPF00075	100%	<i>Phymatolithon lamii</i>	Brittany (NW France)	No match	–	–	–
CCA_hap-12	BOLD Process ID: NGCOR158-14	99.85%	<i>Lithophyllum hibernicum</i>	Brittany (NW France)	KR733526	100%	<i>Lithophyllum hibernicum</i>	Brittany (NW France)
CCA_hap-13	BOLD Process ID: VPF00230	100%	<i>Lithophyllum hibernicum</i>	Brittany (NW France)	KR733438	100%	<i>Lithophyllum hibernicum</i>	Brittany (NW France)

Table 3. COI-5P sequences downloaded from GenBank and BOLD used in the ML tree (fig. 2).

Species	GenBank accession no.	BOLD Process ID
<i>Lithophyllum incrustans</i>	KR708620	–
<i>Lithophyllum hibernicum</i>	KR708622	–
<i>Phymatolithon calcareum</i> neotype, maerl plant	KF808323	MAERL237-13
<i>Phymatolithon calcareum</i> gametophyte, encrusting plant	KC861529	MAERL235-13
<i>Phymatolithon</i> sp. 1 maerl plant	KC861664	MAERL069-11
<i>Phymatolithon</i> sp. 2 maerl plant	KC861668	MAERL067-11
<i>Phymatolithon lamii</i> encrusting plant	KT807914	–
<i>Phymatolithon lusitanicum</i> holotype, maerl plant	KC861627	MAERL086-11
<i>Phymatolithon</i> sp. 4 maerl plant	KC861669	MAERL087-11
<i>Lithothamnion glaciale</i> maerl plant	KC861509	MAERL224-13
<i>Lithothamnion</i> sp. 1 maerl plant	KC861517	MAERL011-11
<i>Lithothamnion corallioides</i> maerl plant	KC861447	MAERL017-11
<i>Mesophyllum</i> sp. 1 maerl plant	KC861519	MAERL019-11
<i>Mesophyllum</i> sp. 2 maerl plant	KC861522	MAERL217-13
<i>Mesophyllum lichenoides</i> encrusting plant	KJ592635	–
<i>Mesophyllum sphaericum</i> holotype, maerl plant	KC861526	MAERL015-11
<i>Corallina officinalis</i>	KF460989	COR006-12

Phymatolithon lamii —CCA_hap-4—, *Lithothamnion* sp. 2 —CCA_hap-5 and 6, both differing in 1 bp—, *Mesophyllum* sp. 3 —CCA_hap-7 and 8, both differing in 1 bp—, *Mesophyllum* sp. 4 —CCA_hap-9 and 10, both differing in 1 bp—, *Lithophyllum hibernicum* —CCA_hap-12 and 13—, and *Lithophyllum* sp. 3 —CCA_hap-11— (figs. 3, 4).

Four CCA species were detected in the Breton maerl bed, and were successfully identified by matching with BOLD and GenBank databases (table 2): *Phymatolithon calcareum*, *Phymatolithon lamii*, and *Lithophyllum hibernicum*. Likewise, the species named as *Phymatolithon* sp. 6 —CCA_hap-3; table 1) scored with *Phymatolithon* sp. 3AT crust from NW Atlantic —Canada—. By contrast, the Galician CCA haplotypes did not match with any available sequence, but according to the ML-tree performed they were resolved into five different taxa (fig. 2): *Phymatolithon* sp. 5, *Lithothamnion* sp. 2, *Mesophyllum* sp. 3, *Mesophyllum* sp. 4, and *Lithophyllum* sp. 3.

Morpho-anatomical examination

Phymatolithon sp. 5 (fig. 3a) showed domed epithallial cells, and subepithallial cells as short as cells subtending them (fig. 5a); cell fusions between contiguous filaments were common (fig. 5b); multiporate asexual conceptacles showed white pore plates (fig. 3d), surrounded by rim and with roof slightly depressed (fig. 5c). *Phymatolithon* sp. 6 (figs. 3b, e, g, i, j, 4q) and *Phymatolithon calcareum* (fig. 3h, k) showed sexual uniporate conceptacles, white coloured in surface view, flushed with thallus surface to sunken in *Phymatolithon* sp. 6, while raised in *Phymatolithon calcareum*. *Phymatolithon lamii* (fig. 3c, f) showed asexual multiporate conceptacles with white pore plates flushed with thallus surface or slightly sunken.

Lithothamnion sp. 2 (fig. 4a, b) showed epithallial cells mainly flared and somewhat flattened, subepithallial cells as long or longer than cells subtending them (fig. 5d, e). Cell fusions between cells of contiguous filaments were common (fig. 5f); asexual multiporate conceptacles with sunken pore plate and white in colour (fig. 4d, e).

Mesophyllum sp. 3 (fig. 4c, g) showed epithallial cells somewhat flared, with subepithallial cells longer than cells subtending them (fig. 6d); cell fusions between contiguous filaments (fig. 6e); multiporate asexual conceptacles with sunken pore plates (figs. 4f, k, 6a); conceptacle chambers were elliptical, 90 µm height by 137 µm in diameter, and conceptacle roof of 11 µm thickness (fig. 6b, c). *Mesophyllum* sp. 4 (fig. 4h, j) showed epithallial cells somewhat flared, subepithallial cells as long as or longer than cells subtending them, and cell fusions common between contiguous filaments (fig. 6f); uniporate sexual conceptacles were prominent on thallus surface (fig. 4l, n); multiporate asexual conceptacles showed sunken pore

plates (fig. 4l, n), and also raised pore plates at the same time in the plant CPVP-769 (fig. 4n); conceptacle chambers were elliptical, 77 µm height by 189 µm in diameter, and conceptacle roof of 40 µm thickness (fig. 6g, h).

Lithophyllum hibernicum (fig. 4o-q), and *Lithophyllum* sp. 3 (fig. 4i, m), showed uniporate conceptacles slightly flushed with thallus surface.

DISCUSSION

In our study, nine CCA species were recorded associated with two Atlantic European maerl beds: Molène —Brittany— and Ons —Galicia— Archipelagos. Despite that these CCA collections consisted on epilithic crustose corallines growing partial or entirely over pebbles, we are aware that could be interpreted as “rhodolith” given that this term is extensively applied to unattached nodules with non-algal core (Irvine & Chamberlain 1994; v.gr. see fig. 6 in Basso & al. 2009). While the four CCA species associated with the Breton maerl bed matched with available sequences from northern Atlantic coast —*Phymatolithon calcareum*, *Phymatolithon lamii*, *Lithophyllum hibernicum*, and *Phymatolithon* sp. 3ATcrust—, the five CCA taxa from the Galician maerl bed did not obtain any record. However, according to the molecular and morphological data obtained, CCA collected in Galicia were identified up to genus level as *Phymatolithon* sp. 5, *Lithothamnion* sp. 2, *Mesophyllum* sp. 3, *Mesophyllum* sp. 4, and *Lithophyllum* sp. 3. The absence of any match with public databases containing more than 4,000 COI-5P sequences of coralline red algae pointed out the cryptic diversity still uncovered in coralline algae.

The nine taxa delimited corresponded to the same four maerl-forming genera in Atlantic Europe —*Lithothamnion*, *Phymatolithon*, *Mesophyllum*, and *Lithophyllum* (Irvine & Chamberlain 1994; Pardo & al. 2014; Hernández-Kantún & al. 2015a)—. Nevertheless, the number of associated CCA species recorded was higher than the number of maerl species cited in both studied areas (Carro & al. 2014; Pardo & al. 2014; Peña & al. 2014, 2015b): four CCA vs. two maerl species in Molène Archipelago —*Phymatolithon calcareum* and *Lithothamnion corallioides*—; and five CCA vs. three maerl species in Ons Archipelago —*Phymatolithon calcareum*, *Phymatolithon lusitanicum*, and *Lithothamnion corallioides*—. The similarity in the species composition between the both growth-forms is approximately 25% in the Breton maerl bed, but totally dissimilar —0% shared species— in the Galician bed.

In Molène Archipelago the dominant genus in the CCA studied was *Phymatolithon*, while in Galicia was *Mesophyllum*, this latter having a southern distribution along the Atlantic European coasts (Guiry & Guiry 2016). This gradual replacement of both species with latitude has been also observed in the composition of major maerl-

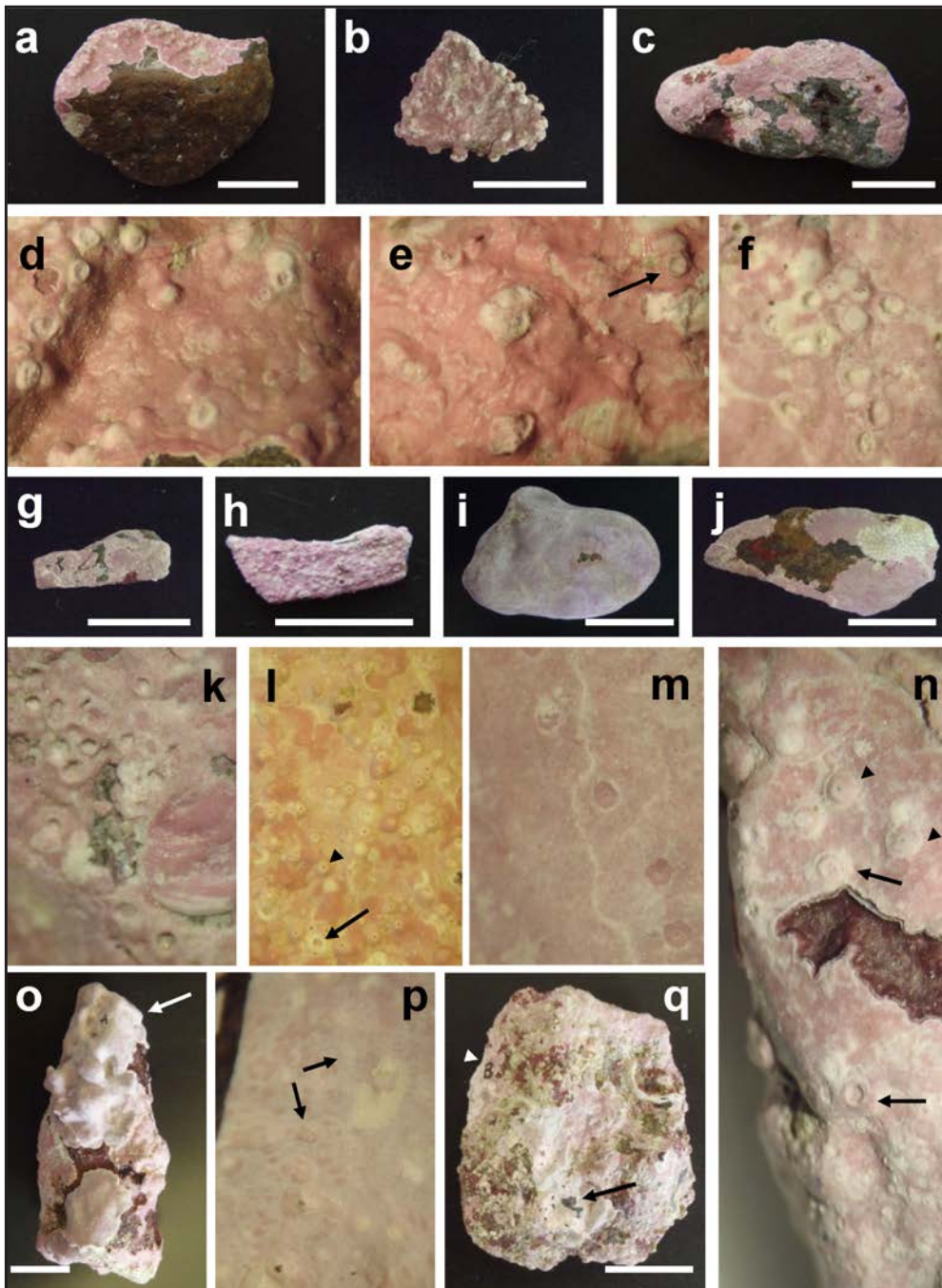


Fig. 4. Epilithic crusts associated with maerl beds in Galicia and in Brittany: **a**, *Lithothamnion* sp. 2 from Ons Archipelago (VPF00483); **b**, *Lithothamnion* sp. 2 from Ons Archipelago (CPVP-763); **c**, *Mesophyllum* sp. 3 from Ons Archipelago (VPF00479); **d**, *Lithothamnion* sp. 2 with asexual multiporate conceptacles (VPF00483); **e**, *Lithothamnion* sp. 2 with asexual multiporate conceptacles (CPVP-763); **f**, *Mesophyllum* sp. 3 with asexual multiporate conceptacles (VPF00479); **g**, *Mesophyllum* sp. 3 from Ons Archipelago (CPVP-767); **h**, *Mesophyllum* sp. 4 from Ons Archipelago (VPF00480); **i**, *Lithophyllum* sp. 3 from Ons Archipelago (CPVP-762); **j**, *Mesophyllum* sp. 4 from Ons Archipelago (CPVP-769); **k**, *Mesophyllum* sp. 3 with asexual multiporate conceptacles (CPVP-767); **l**, *Mesophyllum* sp. 4 with asexual multiporate (arrow) and sexual uniporate (arrowhead) conceptacles (VPF00480); **m**, *Lithophyllum* sp. 3 with uniporate conceptacles (CPVP-762); **n**, *Mesophyllum* sp. 4 with asexual multiporate (arrow) and sexual uniporate (arrowhead) conceptacles (CPVP-769); **o**, *Lithophyllum hibernicum* from Molène Archipelago (VPF00407A) habit; **p**, *Lithophyllum hibernicum* with uniporate conceptacles (VPF00407A); **q**, sample from Molène Archipelago, arrow shows *Lithophyllum hibernicum* (VPF00411A), arrowhead shows *Phymatolithon* sp. 6 (VPF00411B). [Scale bars: a, b, c, g, h, j, 1cm; i, o, q, 2 cm.]

forming species in the OSPAR maritime area, including Brittany and Galicia (Carro & al. 2014; Pardo & al. 2014).

According to the literature, *Phymatolithon calcareum* is considered as a major maerl-forming species in Atlantic Europe (Irvine & Chamberlain 1994; Carro & al. 2014; Pardo & al. 2014). Our record of CCA with uniporate sexual conceptacles in the Breton maerl bed agrees with previous reports of gametophytes—including the carposporophyte—under crustose forms, all of them from this region (Mendoza & Cabioch 1998; Peña & al. 2014). This observation would corroborate that, at least in Brittany, the gametangial stage of *Phymatolithon calcareum* occurs as associated crusts—i.e. attached—, while unattached maerl plants of this species are sporophytes (Cabioch 1969, 1970; Mendoza & Cabioch 1998). However, in adjacent areas such as the British Isles or Atlantic Iberian Peninsula where maerl beds have been intensively studied, fertile gametophytes—crusts or unattached—have been never recorded yet (Irvine & Chamberlain 1994; Peña & al. 2014, 2015b). Given that all Atlantic European records of gametangial stages of *Phymatolithon calcareum* are still restricted to Brittany (Mendoza & Cabioch 1998; Peña & al. 2014), we presume that this Atlantic region plays an important role in the life-history of this major maerl-forming species.

Phymatolithon lamii is a crustose species occurring from the intertidal to subtidal rocky shores of the Atlantic

coasts from Arctic Norway to South Atlantic Iberian Peninsula (Chamberlain 1991; Irvine & Chamberlain 1994; Peña & al. 2015b). Our collection in the Breton maerl bed as associated CCA corroborated recent records of this species from the same maerl bed—Molène archipelago (Peña & al. 2015b).

A recent molecular study (Hernández-Kantún & al. 2015a) confirmed that *Lithophyllum hibernicum* is a common intertidal crust that has been repeatedly misidentified in the Atlantic European literature as *Lithophyllum incrustans*, which also occurs as both encrusting and maerl forms—i.e., Pardo & al. (2014), as *Lithophyllum fasciculatum* (Lam.) Foslie and *Lithophyllum dentatum* (Kütz.) Foslie—. In our study, *Lithophyllum hibernicum* was recorded subtidally in the Breton maerl bed as associated CCA.

A non-common morpho-anatomical observation was detected in the Galician specimen identified as *Mesophyllum* sp. 4 (CPVP-769): both types of conceptacles—uniporate sexual and multiporate asexual—occurred in the same crust surface. A similar observation was found in the Galician maerl species *Mesophyllum sphaericum* V.Peña & al. in Galicia (see fig. 2a in Peña & al. 2011).

Regarding the development of new maerl plants in the Atlantic European maerl beds, two main mechanisms have been suggested in the literature: i) vegetative fragmentation

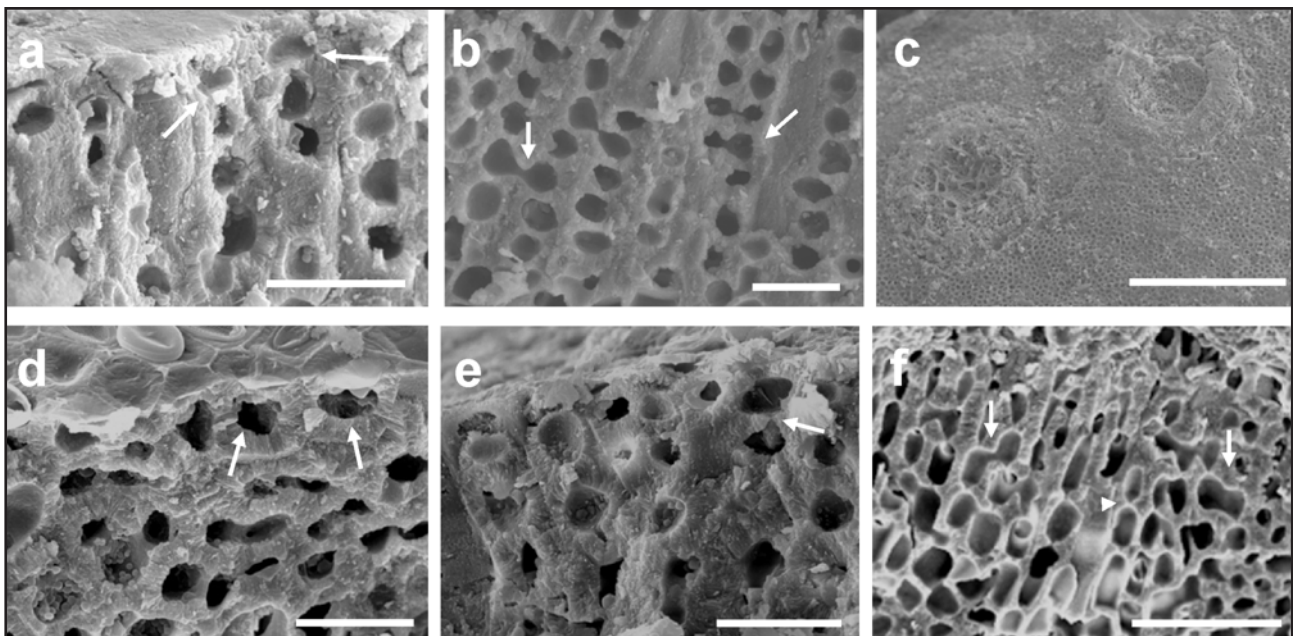


Fig. 5. Anatomical features of *Phymatolithon* sp. 5 (CPVP-758) and *Lithothamnion* sp. 2 (CPVP-763) under SEM: **a**, *Phymatolithon* sp. 5, vertical section of more or less domed epithallial cells (arrows); **b**, *Phymatolithon* sp. 5, vertical section of fusion cells (arrows); **c**, *Phymatolithon* sp. 5, surface view of multiporate conceptacles; **d**, *Lithothamnion* sp. 2, vertical section showing flared epithallial cells (arrows); **e**, *Lithothamnion* sp. 2, vertical section showing primary pit connections (arrow); **f**, Vertical section showing fusion cells (arrows) and primary pit connections (arrowhead). [Scale bars: a, b, d, e, 15 μ m; c, 200 μ m; f, 50 μ m.]

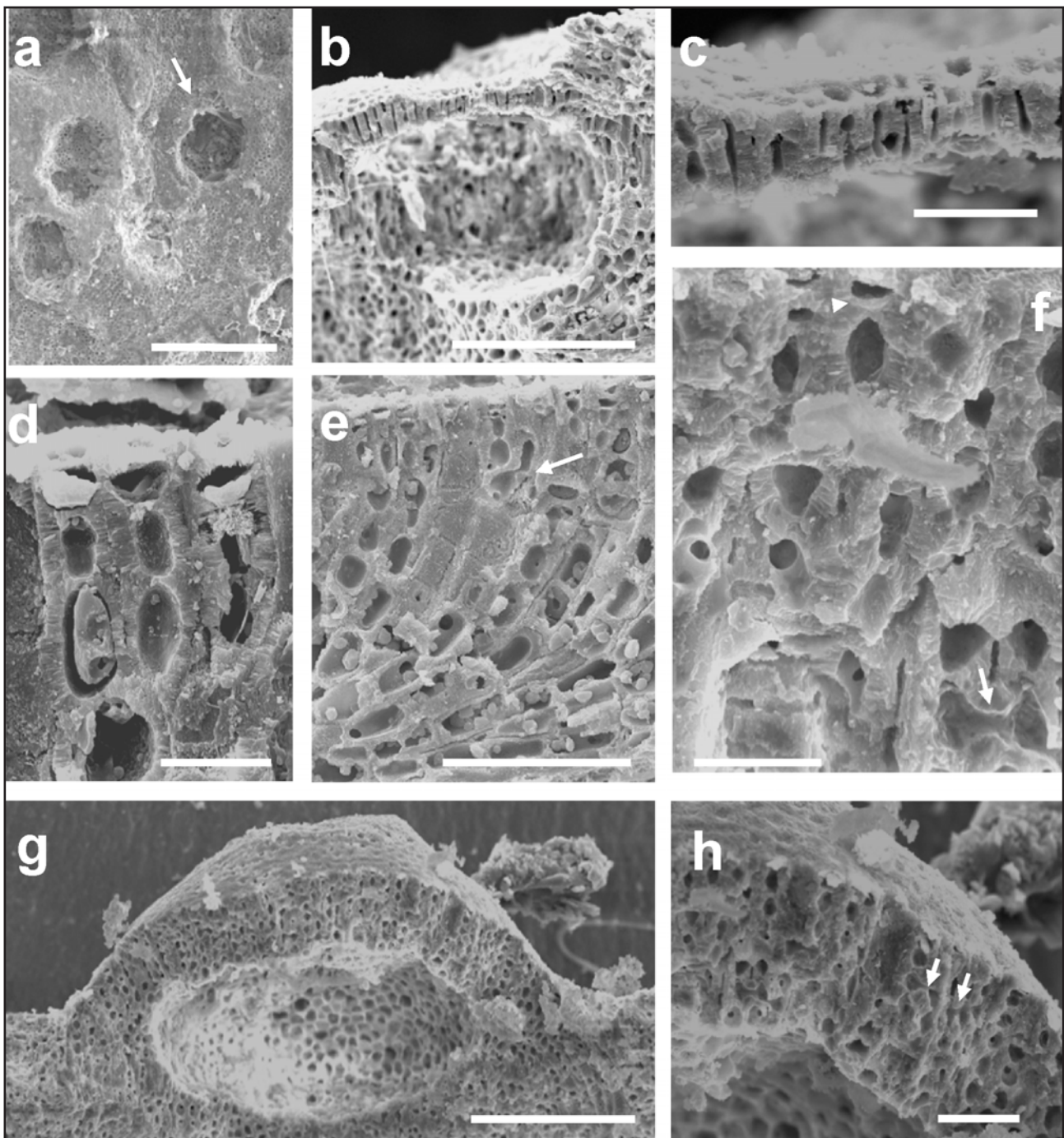


Fig. 6. Anatomical features of *Mesophyllum* sp. 3 (CPVP-767) and *Mesophyllum* sp. 4 (CPVP-769) under SEM: **a**, *Mesophyllum* sp. 3, surface view of asexual multiporate conceptacles; **b**, *Mesophyllum* sp. 3, vertical section of a asexual multiporate conceptacle; **c**, *Mesophyllum* sp. 3, vertical section showing the roof of an asexual multiporate conceptacle; **d**, *Mesophyllum* sp. 3, vertical section of somewhat flared epithallial cells; **e**, *Mesophyllum* sp. 3, vertical section showing cells fusions (arrow); **f**, *Mesophyllum* sp. 4, vertical section showing flattened epithallial cells (arrowhead) and fusion cells (arrow); **g**, *Mesophyllum* sp. 4, vertical section of an asexual multiporate conceptacle; **h**, *Mesophyllum* sp. 4, vertical section showing roof of an asexual multiporate conceptacle with fusion cells (arrows). [Scale bars: a, 250 μ m; b, e, g, 100 μ m; c, h, 20 μ m; d, f, 10 μ m.]

of own free-living maerl plants (Bosence 1976; Woelkerling 1988; Peña & al. 2014); ii) involvement of associated CCA in the recruitment of new maerl plants —i.e., detached branches of crusts (Lemoine 1910; Cabioch 1969, 1970; Freiwald 1995; Mendoza & Cabioch 1998)—. Our study pointed out that the latter hypothesis could be applied, but only for *Phymatolithon calcareum* in the Breton maerl beds, where gametangial —plus carposporangial— and sporangial stages occur under different growth-forms —CCA and maerl, respectively (Cabioch 1969, 1970; Mendoza & Cabioch 1998; Peña & al. 2014)—. However, in the Galician maerl beds the species composition of associated CCA in comparison with the main maerl-forming taxa —*Phymatolithon calcareum*, *Phymatolithon lusitanicum*, and *Lithothamnion corallioides*— appeared to be different. This together with the absence of maerl species records bearing gametangial —and carposporangial— structures in this region —attached or unattached—, and the occasional records of sporangial conceptacles observations (Peña & Bárbara 2004), the fragmentation of own free-living maerl plants should be considered as the main propagation method in the Galician maerl beds.

CONCLUSION

The present study carried out in two protected Atlantic European maerl beds pointed out that maerl beds harbours a high species diversity of associated CCA, which may not share the same species composition that maerl-forming algae. Also a high cryptic diversity was found in the associated CCA, as occurs in the maerl-forming species (Carro & al. 2014; Pardo & al. 2014) and in geniculate coralline algae (Walker & al. 2009; Pardo & al. 2015). Further extensive studies in other European maerl beds are necessary to understand this unexpected diversity of associated CCA. Nonetheless, according to our results, the crustose growth-forms should be taken into account in management actions of Atlantic European maerl beds.

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