Cryptogamie, Algologie, 2014, 35 (1): 15-25

Detection of gametophytes in the maerl-forming species *Phymatolithon calcareum* (Melobesioideae, Corallinales) assessed by DNA barcoding

Viviana PEÑA[a,b,c], Jazmin J. HERNÁNDEZ-KANTÚN[d],*
Jacques GRALL[e], Cristina PARDO[a], Lúa LÓPEZ[a], Ignacio BÁRBARA[a],
Line LE GALL[b] & Rodolfo BARREIRO[a]

[a] *Grupo de investigación BIOCOST, University of A Coruña, Spain*

[b] *UMR 7205 ISYEB CNRS, MNHN, UPMC, EPHE, Équipe Exploration, Espèces et Évolution, Institut de Systématique, Évolution, Biodiversité. Muséum national d'Histoire naturelle (MNHN), France*

[c] *Phycology Research Group, Ghent University, Belgium*

[d] *Irish Seaweed Research Group, National University of Ireland, Ireland*

[e] *Séries Faune-Flore Observatoire Marin, UMS3113, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, France*

Résumé – Les spécimens fertiles de *Phymatolithon calcareum*, ne sont que très rarement mentionnés le long des côtes européennes de l'Atlantique. Deux spécimens ont récemment été récoltés dans un banc de maerl infralittoral de la pointe de Bretagne, sous la forme de plantes encroûtantes et épilithiques. Sur la base de leur morphologie les spécimens ont été identifiés comme appartenant à l'espèce *P. calcareum*. Cette identification a été confirmée par sur la base du critère moléculaire par l'obtention de séquences de COI-5P identiques pour ces deux spécimens et le néotype de *P. calcar*eum ainsi que des spécimens stériles en provenance de bancs de maërl européens. Les structures reproductrices étaient vides mais elles ont été interprétées comme étant des conceptacles femelles matures. En comparaison avec les deux précédentes mentions de spécimens sexués de *P. calcareum* en Atlantique européen, les conceptacles uniporés observés dans la présente étude sont plus larges et ont été collectés à une saison différente. À notre connaissance, cette étude constitue le premier lien entre l'identification de *P. calcareum* sur la base de critères morphologiques de l'appareil reproducteur et sur la base de critères moléculaires (*DNA barcodes*).

COI-5P / algues corallines encroûtantes / Côtes européennes / Reproduction / Systématique

Abstract – Fertile gametangial plants of *Phymatolithon calcareum,* which are seldom reported in the Atlantic European coasts, were collected as encrusting, epilithic plants in a subtidal maerl bed in Brittany (France). Based on their morphological features, the plants were identified as *P. calcareum*. This identification was further confirmed by DNA barcodes using as a reference COI-5P sequences obtained from the neotype together with recent collections from the Atlantic European maerl beds. The reproductive structures were empty but they were regarded as mature female conceptacles. Compared to the two previous records of gametangial plants of *P. calcareum* for the Atlantic European waters, the uniporate conceptacles observed in this study are larger, and were collected at a different time of the year. To our knowledge, this is the first time that the occurrence of gametangial plants of *P. calcareum* is corroborated with molecular tools (DNA barcodes).

COI-5P / Crustose coralline algae / European coasts / Reproduction / Systematics

---

* Corresponding author: vpena@udc.es

doi/10.7872/crya.v35.iss1.2014.15

INTRODUCTION

Maerl beds are deposits of unattached, non-geniculate coralline red algae with a variable branching density that provides a wide range of ecological niches (Birkett *et al*., 1998, BIOMAERL Team, 2003). In the North East Atlantic, they are known from Arctic regions in Iceland and Svalbard Archipelago (Adey, 1968; Gunnarsson, 1977; Teichert *et al.,* 2012) to subtropical and tropical archipelagos in the Macaronesia (Foslie, 1908; Cabioc'h, 1974; Afonso-Carrillo & Gil-Rodriguez, 1982). This latitudinal gradient entails changes in the species composition of maerl beds and the replacement of the maerl-forming species (Grall, 2003; Hall-Spencer *et al.,* 2010), being *Phymatolithon calcareum* (Pallas) Adey *et* McKibbin one of the species most commonly cited in the Atlantic European maerl beds (Hall-Spencer *et al.,* 2010).

Although thallus fragmentation is suggested as the major mechanism of propagation where extensive unattached populations occur (Bosence, 1976; Johansen, 1981), fertile unattached plants have also been found in European maerl beds. Multiporate sporangial conceptacles of *P. calcareum* have been observed by several authors on unattached specimens in maerl beds of the Atlantic European coast (Lemoine, 1910; Suneson, 1958; Cabioc'h, 1969; Adey & McKibbin, 1970; Woelkerling & Irvine, 1986; Irvine & Chamberlain, 1994; Peña & Bárbara, 2004, 2008). In comparison, uniporate gametangial conceptacles were reported only very rarely on small encrusting plants attached to gravel and dead maerl in a subtidal bed in Brittany (Mendoza & Cabioc'h, 1998). Previously, Suneson (1958) reported the occurrence of female conceptacles containing ripe carposporangia in material collected from this region, but the author did not provide further details. By contrast, fertile gametangial and sporangial plants of *P. calcareum* are common in the Mediterranean maerl beds (Bressan & Babbini, 2003). In Alaska, uniporate male conceptacles have been reported in unattached plants of *P. calcareum* (Konar *et al.,* 2006).

Identifying coralline species based on morphology is challenging due to the high morphological plasticity of the group (Steneck, 1986). In unattached coralline plants, morphological variation can be extreme even within a single species, and it is likely related to local hydrodynamic conditions (Bosence, 1976; Steller & Foster, 1995; Schaeffer *et al.,* 2002; Basso *et al.,* 2009; Peña & Bárbara, 2009). Fortunately, recent assessments of species diversity has benefited from the recent advent of DNA barcodes to the taxonomist's toolbox. This technique uses sequences of a fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (COI-5P) for species recognition. DNA barcodes are now well established in many red algal groups (e.g. Saunders, 2005; Le Gall & Saunders, 2010) and they have been successfully applied to the identification of coralline species and to the detection of cryptic taxa (Walker *et al*., 2009; Bittner *et al*., 2011; Hind & Saunders, 2013). In the particular case of maerl-forming species, DNA barcodes are used in an on-going Barcode of Life Database (BOLD, http://www.boldsystems.org/) project called MAERL (*maerl-NE Atlantic*) that focuses on the assessment of the diversity of maerl species along the Atlantic European coasts (Pardo *et al.,* 2012). The project promoted extensive collections of maerl and associated encrusting corallines by SCUBA diving and dredging in maerl beds scattered along the study area. As part of the project, COI-5P sequences were produced for new collections of *P. calcareum* from maerl beds of Brittany, Britain, Ireland, and Galicia; these collections included material from the neotype locality (Falmouth, UK, Woelkerling & Irvine, 1986). Moreover, our identification of

P. calcareum is supported by molecular information obtained from the neotype material deposited in the Natural History Museum (BM, London).

As part of one collection from a Breton subtidal maerl bed, we found uniporate conceptacles in two epilithic, encrusting branched plants. Both specimens were preliminary identified as *P. calcareum* based on their morphological features. However the scarcity of records with sexual uniporate conceptacles for this species in Atlantic maerl beds (Mendoza & Cabioc'h, 1998) advised that a deeper examination was required to provide further evidence in support of the species recognition. The aforementioned availability of COI-5P sequences for this species offered and excellent opportunity to obtain this corroboration.

MATERIAL AND METHODS

Samples were collected by dredging in the maerl bed of Molène archipelago (Finistère, Brittany, France) in May 2011 at 3-7 m depth (Fig 1, Table 1). Fertile encrusting attached specimens of *Phymatolithon calcareum* with uniporate conceptacles were selected for anatomical and molecular examination (CPVP-943 and CPVP-955, Table 1). They were air-dried or oven-dried (50º C), and preserved in zipper bags with silica gel. For the anatomical study, selected branches of the two specimens with uniporate conceptacles were examined under scanning electron microscope (SEM, model JEOL JSM 6400, Universidade da Coruña, Spain). The specimens were deposited in the herbarium SANT Algae

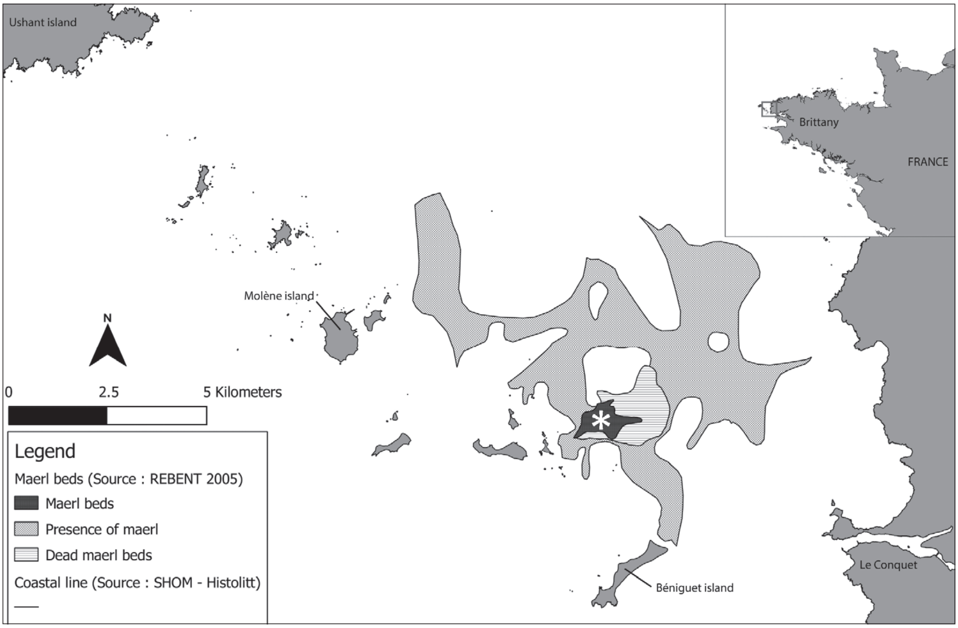


Fig 1. Location of the maerl bed in Molène Archipelago (Finistère, Brittany) and collection area (black area marked with asterisk). Resource: IFREMER.

Table 1. Sample information for the collections included in the DNA barcode analyses. GenBank accession numbers are provided for the specimens. Bold typeface: neotype of *Phymatolithon calcareum* and holotype of *Mesophyllum sphaericum*.

Species	*Collection details*	*Voucher*	*Genbank accession number*
Phymatolithon calcareum	St. Mawes Bank, Falmouth Harbour, Cornwall, UK, 11/12/1983. Coll: W.F. Farnham. Neotype material.	BM000712373	KF808323
P. calcareum	St. Mawes Bank, Falmouth Harbour, Cornwall, UK, –18 m, 02/06/2010. Coll: J.M. Hall-Spencer.	CPVP-47	KC861604
P. calcareum	Milford Haven, Wales, UK, –4 m, 14/07/2011. Coll: F. Bunker	CPVP-1188	KC861558
P. calcareum	Zara Shoal, Strangford Lough, Northern Ireland, –10 m, 25/07/2011. Coll: L. Kregting, D. Pritchard	CPVP-1243	KC861534
P. calcareum	Gleesk Pier, Co. Kerry, Ireland, –10 m, June 2008. Coll: M. Moriarty.	CPVP-778	KC861561
P. calcareum	Carraroe, Co. Galway, Ireland, –5 m, 21/10/2010. Coll: N. Nolan, J. Hernandez	CPVP-781	KC861616
P. calcareum	Guerheon, Baie de Morlaix, Brittany, –11 m, 10/05/2011. Coll: T. Wilfried, Y. Fontana	CPVP-906	KC861608
P. calcareum	Molène Archipelago, Finistère, Brittany, –10 m, 10/03/2011. Coll: V. Peña	CPVP-858	KC861553
P. calcareum	Molène Archipelago, Finistère, Brittany, –10 m, 10/05/2011. Coll: J. Grall, V. Peña. Uniporate conceptacles.	CPVP-955	KC861591
P. calcareum	Molène Archipelago, Finistère, Brittany, –10 m, 10/05/2011. Coll: J. Grall, V. Peña. Uniporate conceptacles.	CPVP-943	KC861529
P. calcareum	Le Dragon, Trevignon, Brittany, –15 m, 13/05/2011. Coll: T. Wilfried, Y. Fontana.	CPVP-916	KC861589
P. calcareum	Ensenada de Bornalle, Ría de Muros-Noia, Galicia, –11 m, 31/03/2011. Coll: I. Bárbara, C. Pardo, R. Barreiro, V. Peña.	CPVP-560	KC861592
P. calcareum	Nido do Corvo, Ría de Arousa, Galicia, –6 m, 23/11/2010. Coll: I. Bárbara, C. Pardo, R. Barreiro, V. Peña.	CPVP-164	KC861567
P. calcareum	Punta Barbafeita, Ría de Arousa, Galicia, –5 m, 23/06/2011. Coll: I. Bárbara, F. Bunker, V. Peña.	CPVP-1067	KC861581
P. calcareum	Isla Benencia, Ría de Arousa, Galicia, –3 m, 23/06/2011. Coll: I. Bárbara, F. Bunker, V. Peña.	CPVP-1108	KC861572
P. calcareum	Isla de Ons, Ría de Pontevedra, Galicia, –13 m, 07/04/2011. Coll: I. Bárbara, R. Barreiro, V. Peña.	CPVP-607	KC861551
P. calcareum	Playa de Tulla, Ría de Pontevedra, Galicia, –9 m, 07/04/2011. Coll: I. Bárbara, R. Barreiro, V. Peña.	CPVP-628	KC861586
P. calcareum	Islas Cíes, Ría de Vigo, Galicia, –11 m, 03/04/2011. Coll: I. Bárbara, R. Barreiro, V. Peña.	CPVP-655	KC861569
P. calcareum	Baliza Tofiño, Ría de Vigo, Galicia, –11 m, 03/04/2011. Coll: I. Bárbara, R. Barreiro, V. Peña.	CPVP-753	KC861546
Uncultured Corallinales	Atlantic France, Bittner *et al.* (2010).	LBC0001	GQ917247
Lithothamnion corallioides	Baie de Douarnenez, Finistère, Brittany, –10 m, 08/03/2011. Coll: J. Grall.	CPVP-802	KC861447
Lithothamnion glaciale Kjellman	English Harbour Eastern Cove, Newfoundland and Labrador. *Unpublished.*	GWS007542	HM918812
Mesophyllum sphaericum	Isla Benencia, Ría de Arousa, Galicia, –3 m, 14/10/2008. Coll: I. Bárbara, V. Peña. Holotype material.	CPVP-776. SANT-Algae 21804	KC861526
Mesophyllum erubescens (Foslie) Me. Lemoine	Hawaii, Pacific Ocean, Sherwood *et al.* (2010).	ARS02835	HQ422717

(Universidade de Santiago de Compostela, Spain). For the molecular study, the specimens were cleaned under stereomicroscope and clean surfaces were ground with a drill bit of 2 mm in diameter. Genomic DNA was extracted using Qiagen DNeasy Blood and Tissue Kit® (Qiagen Iberia, S.L., Spain) following manufacturer's recommendations. A fragment of the 5' end of the COI gene (COI-5P) was PCR-amplified with the primers GazF1 and GazR1 (Saunders, 2005). PCR amplification followed Saunders & McDevit (2012). PCR products were purified with exonuclease I and shrimp alkaline phosphatase –ExoSAP- (Fermentas, Thermo Fisher Scientific, Spain) and sequenced at Macrogen® (Macrogen Europe, the Netherlands). Forward and reverse sequences were assembled and aligned with the assistance of CodonCode Aligner® (CodonCode Corporation, USA) and Geneious 6.1.2. (Biomatters Ltd, New Zealand). Sequences were submitted to the on-going BOLD project MAERL (http://www.boldsystems.org/) and GenBank (Table 1). They were compared with 17 COI-5P sequences of *P. calcareum* obtained from the neotype (BM Box 1626, Woelkerling & Irvine 1986) as well as from recent collections from the neotype locality (Falmouth, Cornwall, UK) and from other Atlantic maerl beds of Britain, Ireland, Galicia and Brittany (Table 1). Collections from Brittany included the locality where the gametophytes were previously recorded (Guerheon, Brittany, Mendoza & Cabioc'h 1998, Table 1). COI-5P sequences were also obtained for two other maerl species *Lithothamnion corallioides* (P.L.Crouan *et* H.M.Crouan) P.L.Crouan *et* H.M.Crouan and *Mesophyllum sphaericum* Peña, Bárbara, Adey, Riosmena-Rodríguez *et* Choi, the latter were produced from the holotype material (Peña *et al.,* 2011, Table 1). DNA barcodes of *P. calcareum* were compared to public records in Genbank and BOLD. Available COI-5P sequences from Genbank and BOLD for the subfamily Melobesioideae were included in the analyses as outgroup (Table 1). Distance analyses for the total of 24 COI-5P sequences were performed using neighbor-joining algorithm (Jukes-Cantor method) with default settings in Mega v. 5.02 (Tamura *et al.,* 2011).

RESULTS

The DNA barcodes produced for these gametophytes were 100% identical to COI-5P sequences obtained from neotype material for *Phymatolithon calcareum* (possessing multiporate sporangial conceptacles, Woelkerling & Irvine, 1986) and from recent collections of this taxon along Atlantic European maerl beds (Fig. 2). In the NJ tree, *P. calcareum* clearly separated from other Melobesioideae (*Lithothamnion* and *Mesophyllum*). Genbank and BOLD searches revealed that only one accession matched our DNA barcodes for *P. calcareum*; the matching sequence was produced by a collection from the Atlantic coast of France labeled as *Uncultured Corallinales* (accession number GQ917247, Bittner *et al.,* 2010, Table 1).

The gametophytes of *Phymatolithon calcareum* were encrusting thalli with short branches, up to 5 cm of diameter, growing on pebbles associated to the maerl bed (Figs 3-4). The uniporate conceptacles examined here were raised, appearing as white circles scattered on the thallus surface (Figs 5-6). The anatomical examination by SEM revealed branches with abundant uniporate

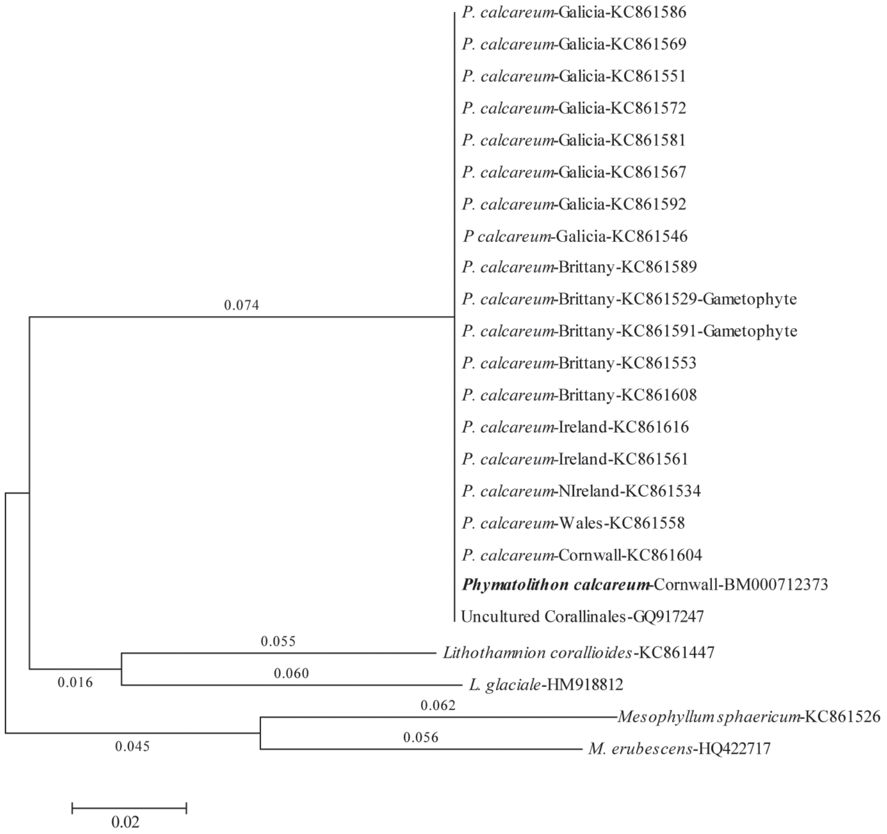
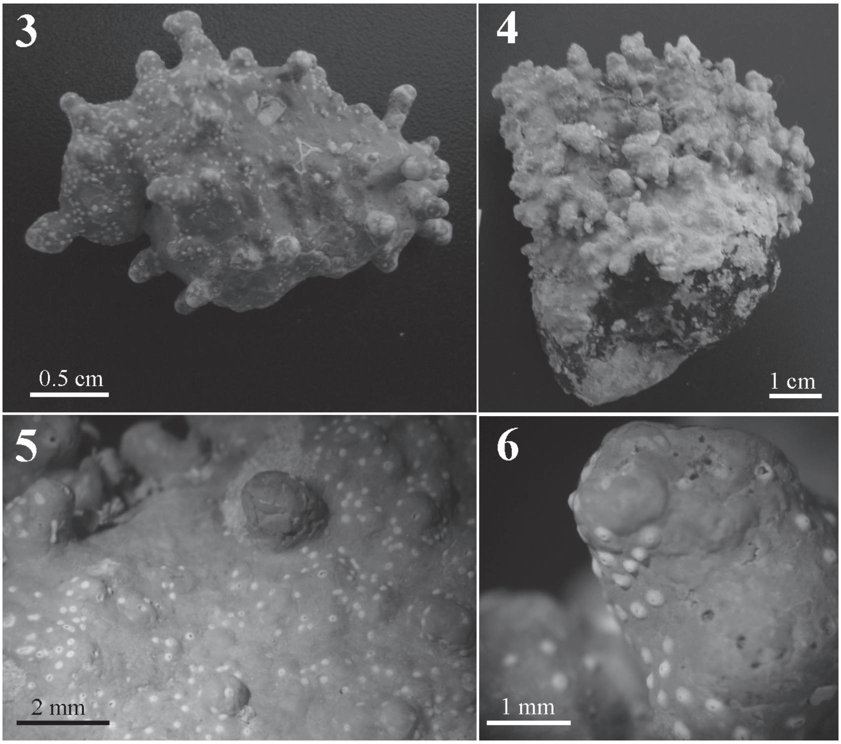


Fig 2. Neighbor-joining tree for DNA barcodes (COI-5P) of *Phymatolithon calcareum* (detailed in Table 1). The outgroup contained other members of the subfamily Melobesioideae from our on-going project and from Genbank: *Lithothamnion (L. corallioides, L. glaciale*), and *Mesophyllum (M. sphaericum, M. erubescens).* Evolutionary distances of the branches were computed using the Jukes-Cantor method. Scale bar refers to base subtitutions per site.

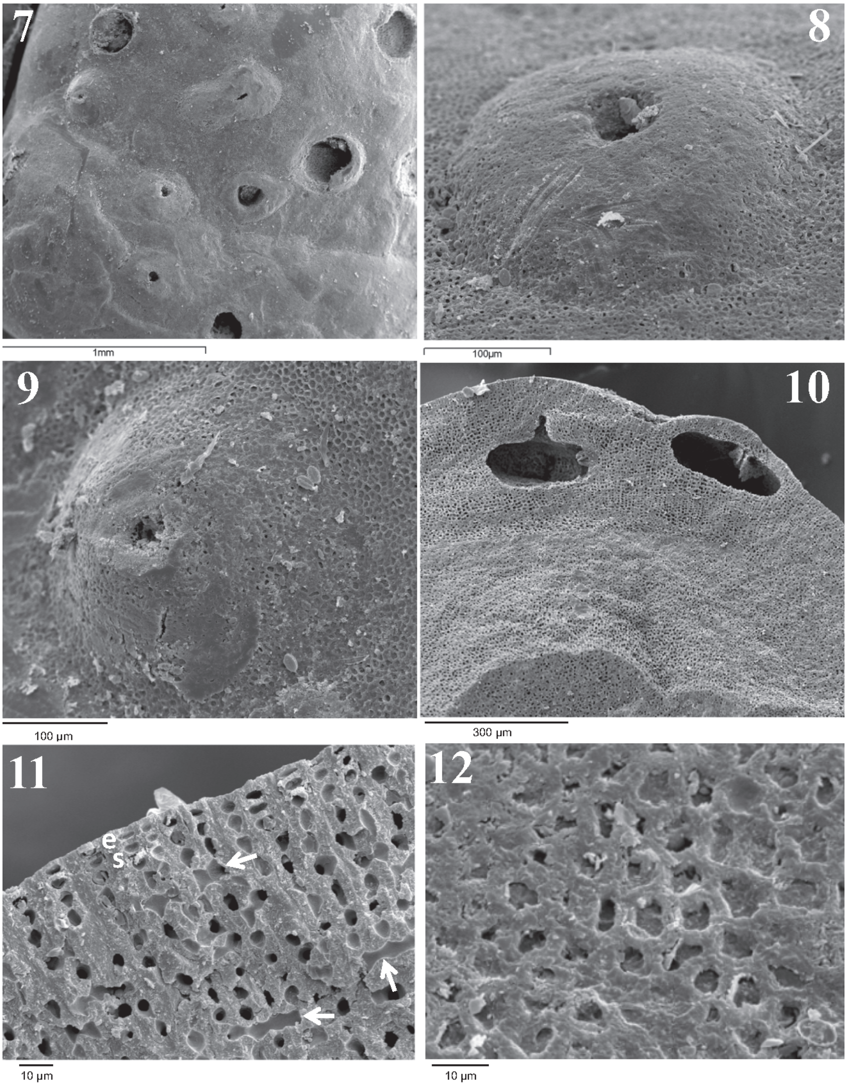
conceptacles raised, conical to hemispherical, 250-300 µm external diameter in surface view, with a pore of 15-17 µm of diameter (Figs 7-9). In transverse section (Fig. 10), the uniporate conceptacles were empty and were located within the cortex. The conceptacle chambers were elliptical-ovoid, 226-260 µm diameter by 85-140 µm height. The pore canal was 130 µm long, tapering from 83 µm at the base to 23 µm wide at the surface, with lining filaments oriented to the canal. Although we only found empty chambers, they were interpreted as mature female conceptacles given their shape and large size. The epithallial cells were domed, the subepithallial cells were short and cells fusions were abundant between adjacent cortical cells (Fig. 11). The epithallial cells surface was *Phymatolithon*-type with thickened calcareous ridges surrounding central concavities (Fig. 12).



Figs 3-6. Gametangial plants of *Phymatolithon calcareum*. **3-4.** Encrusting branched plants growing on pebbles (CPVP-955, and CPVP-943, respectively). **5.** Uniporate female conceptacles scattered along the thallus surface. **6.** Uniporate female conceptacles raised, on branches.

DISCUSSION

Our study illustrates the usefulness and accuracy of DNA barcodes for assessing the diversity of maerl-forming species. In agreement with the literature, our results indicate that *Phymatolithon calcareum* is a common component of maerl beds in Brittany, Ireland, Britain and Galicia (Lemoine, 1910; Cabioc'h 1969; Bosence, 1976, Adey & McKibbin, 1970; Irvine & Chamberlain, 1994; Peña & Bárbara, 2004, 2008). Our molecular results, which include the neotype of *P. calcareum* (Woelkerling & Irvine, 1986), confirm that the encrusting, epilithic plants found in an Atlantic European bed were gametophytes of *P. calcareum*. The anatomical examination of the gametophytes showed raised conceptacles with empty chambers that were interpreted as mature female conceptacles, although previous authors described the uniporate female conceptacles as not prominent, with only the canal pore slightly protruding and smaller dimensions (120-150 µm of diameter by 60-110 µm in height with a canal pore 50 µm long, Mendoza & Cabioc'h, 1998; Bressan & Babbini, 2003). However, the vegetative features observed in our specimens matched the diagnostic characters described for *P. calcareum* in the literature (Cabioc'h, 1966; Adey & McKibbin, 1970; Chamberlain 1990; Irvine & Chamberlain, 1994).



Figs 7-12. Uniporate female conceptacles of *Phymatolithon calcareum* and vegetative diagnostic features (SEM images). **7.** Surface of a branch with abundant uniporate conceptacles. **8-9.** Surface view of uniporate female conceptacles raised, hemispherical to conical. **10.** Vertical section of the thallus with empty uniporate female conceptacles showing chambers elliptical to ovoid with a canal pore. **11.** Vertical section of the thallus showing domed epithallial cells (e), short subepithallial initials (s), and cell fusions between contiguous cortical cells (arrows). **12.** Surface view of the thallus showing epithallial cells *Phymatolithon*-like with thickened calcareous ridges surrounding central concavities.

A previous study carried out also in the same Atlantic region (Brittany) reported uniporate conceptacles only in crustose plants attached to gravel or dead maerl (Mendoza & Cabioc'h, 1998). Interestingly, however, gametangial plants have been reported to grow as maerl in the Mediterranean and Alaska (Bressan

& Babbini, 2003; Konar *et al.,* 2006). A previous study in European waters detected fertile sexual plants of *P. calcareum* in September-October (Mendoza & Cabioc'h, 1998) while our specimens were collected in May. On the other hand, Konar *et al.* (2006) found male gametangial plants in summer at much higher latitude (Alaska). In this regard, the fact that our conceptacles were empty might indicate that they had released their content some time before collection. However, the conceptacles were abundant along the surface of the plant rather than buried within the thallus.

Based on our collections of more than 1000 unattached and encrusting plants from a range of Atlantic European maerl beds sampled at different times of the year, the occurrence of uniporate sexual conceptacles in *P. calcareum* seems a rather infrequent phenomenon. In Britain, gametangial plants of *P. calcareum* remain unreported and it is the only species of this genus with unrecorded sexual structures in this region (Irvine & Chamberlain, 1994). In comparison, multiporate sporangial conceptacles were commonly observed on unattached plants throughout the year and they even occur in the neotype material (Lemoine, 1910; Suneson, 1958; Cabioc'h, 1969; Adey & McKibbin, 1970; Woelkerling & Irvine, 1986; Irvine & Chamberlain, 1994; Peña & Bárbara, 2004, 2008). The extremely low frequency of uniporate sexual conceptacles could be indicative that the gametophytes spend most of their life history as vegetative plants. Alternatively, their scarcity could be just a consequence of a low ratio of gametophytes in Atlantic European beds. Cabioc'h (1969) suggested that the rarity of sexual and asexual reproduction in Breton maerl beds possibly indicated that reproduction follows a cyclical pattern of at least 6 years with alternate fertile periods between the main maerl-forming species *P. calcareum* and *Lithothamnion corallioides*. On the other hand, the low occurrence of fertile plants in Brittany and Norway was interpreted as evidence that recruitment in maerl populations depends primarily on the breakage of branches of attached plants (Cabioc'h, 1969; Freiwald, 1995). However, given the absence, or at least scarcity, of encrusting thalli of *P. calcareum* reported so far, it seems unlikely that they might be the providers of unattached plants in most of the Atlantic European beds. According to Irvine & Chamberlain (1994), encrusting *P. calcareum* has not ever been recorded in Britain (Irvine & Chamberlain, 1994). Likewise, other than the collections from Breton maerl beds reported here, we never detected encrusting *P. calcareum* in any other Atlantic European bed. In this regard, our results seem consistent with the proposal that thallus fragmentation of the maerl itself must be the main mechanism of propagation in Atlantic beds as previously suggested for unattached populations in the literature (Bosence, 1976; Johansen, 1981; Steller & Foster, 1995). However, solving the precise recruitment strategy followed by Atlantic maerl populations surely warrants more detailed investigations.

Acknowledgements. Financial support: Spain's Ministerio de Economía y Competitividad (CTM2010-18787), CGL2009-09495/BOS (partially founded by ERDF) and Xunta de Galicia (10MMA103003PR). ASSEMBLE (grant agreement nº. 227799) supported sampling in Brittany. VP acknowledges support by the postdoctoral programs Axudas de apoio á etapa inicial de formación posdoutoral do Plan I2C (Xunta de Galicia) and Programa Nacional de Movilidad de Recursos Humanos (Spain´s Ministerio de Economía y Competitividad), CP acknowledges support by Spain's Ministerio de Educación (Programa de Formación del Profesorado Universitario, 2010), LL acknowledges support by Universidade da Coruña (Contratos Predoutorais, 2012). JHK is supported by CONACyT-211950 (National Council for Science and Technology, Mexico) and SEP (Secretariat for Public Education, Mexico) with a PhD scholarship. We thank Jo Wilbraham at BM for assistance with obtaining specimens on loan, and Jason

M. Hall-Spencer, Francis Bunker, Louise Kregting and Daniel Pritchard for field collaboration and/or providing samples. We also thank REBENT survey. We are also grateful to both reviewers for their comments on the manuscript.

REFERENCES

ADEY W.H., 1968 — The distribution of crustose corallines on the Icelandic coast. *Scientia Islandica* 1: 16-25.

ADEY W.H. & MCKIBBIN D.L., 1970 — Studies on the maerl species *Phymatolithon calcareum* (Pallas) nov. comb. and *Lithothamnium coralloides* Crouan in the Ría de Vigo. *Botanica marina* 13(2): 100-106.

AFONSO-CARRILLO J. & GIL-RODRÍGUEZ M. C., 1982 — Sobre la presencia de un fondo de "Maerl" en las Islas Canarias. *Collectanea botanica* 13(2): 703-708.

BASSO D., NALIN R. & CAMPBELL N., 2009 — Shallow-water Sporolithon rhodoliths from North Island (New Zealand). *Palaios* 24: 92-103.

BARBERÁ C., BORDEHORE C., BORG J.A., GLÉMAREC M., GRALL J., HALL-SPENCER J.M., DE LA HUZ C., LANFRANCO E., LASTRA M., MOORE P.G., MORA J., PITA M.E., RAMOS-ESPLÁ A.A., RIZZO M., SÁNCHEZ-MATA A., SEVA A., SCHEMBRI P.J. & VALLE C., 2003 — Conservation and management of northeast Atlantic and Mediterranean maerl beds. *Aquatic conservation: marine and freshwater ecosystems* 13: S65-S76.

BIRKETT D.A., MAGGS C.A & DRING M.J., 1998 — Maerl. An overview of dynamics and sensitivity characteristics for conservation management of marine SACs. Scottish association for marine science (UK Marine SACs Project). 117 p.

BITTNER L., HALARY S., PAYRI C., CRUAUD C., REVIERS B. DE, LOPEZ P. & BAPTESTE E., 2010 — Some considerations for analyzing biodiversity using integrative metagenomics and gene networks. *Biology direct* 5: 47

BITTNER L., PAYRI C., MANEVELDT G., COULOUX A., CRUAUD C., REVIERS B. DE & LE GALL L., 2011 — Evolutionary history of the Corallinales (Corallinophycidae, Rhodophyta) inferred from nuclear, plastidial and mitochondrial genomes. *Molecular phylogenetics and evolution* 61: 697-713.

BOSENCE, D.W., 1976 — Ecological studies on two unattached coralline algae from western Ireland. *Palaeontology* 19(2): 365-395.

BRESSAN, G. & BABBINI L., 2003 — Corallinales del mar Mediterraneo: guida alla determinazione. *Societá Italina di biologia marina* 10(2): 1-237.

CABIOC'H J., 1966 — Contribution à l'étude morphologique, anatomique et systematique de deux Mélobésiées: *Lithothamnium calcareum* (Pallas) Areschoug et *Lithothamniun corallioides* Crouan. *Botanica marina* 9(1-2): 33-53.

CABIOC'H J., 1969 — Les fonds de maerl de la Baie de Morlaix et leur peuplement végétal. *Cahiers de biologie marine* 10: 139-161.

CABIOC'H J., 1974 — Un fond de maerl de l'Archipel de Madère et son peuplement végétal. *Bulletin de la société phycologique de la France* 19: 74-82.

CHAMBERLAIN Y.M., 1990 — The genus *Leptophytum* (Rhodophyta, Corallinaceae) in the British Isles with descriptions of *Leptophytum bornetii*, *L. elatum* sp. nov. and *L. laeve*. *British phycological journal* 25(2): 179-199.

FOSLIE M., 1908 — Die lithothamnien der deutschen Südpolar-Expedition 1901-1903. *Deutsche Südpolar-Expedition* 1901-1903 8: 205-219.

FREIWALD A., 1995 — Sedimentological and biological aspects in the formation of branched rhodoliths in northern Norway. *Beiträge zur Paläontologie Österreichs* 20: 7-19.

GRALL J., 2003 — *Fiche de synthése sur les biocénoses: le bancs de maerl*. IFREMER, 20 p.

GUNNARSSON K., 1977 — Dörungar á kóralsetlögum í Arnarfirdi. *Hafrannsóknir* 10: 1-10.

HALL-SPENCER J.M., KELLY J. & MAGGS C.A., 2010 — *Background document for maerl beds*. OSPAR Commission Biodiversity Series, 491/2010, 36 p.

HIND K.R. & SAUNDERS G.W., 2013 — A molecular phylogenetic study of the tribe Corallineae (Corallinales, Rhodophyta) with an assessment of genus-level taxonomic features and descriptions of novel genera. *Journal of phycology* 49: 103-114.

IRVINE L.M. & CHAMBERLAIN Y.M., 1994 — *Seaweeds of the British Isles. Volume 1. Rhodophyta, Part 2B Corallinales, Hildenbrandiales*. London, The Natural History Museum, 276 p.

KONAR B., RIOSMENA-RODRÍGUEZ R. & IKEN K., 2006 — Rhodolith bed: a newly discovered habitat in the North Pacific Ocean. *Botanica marina* 49: 355-359.

JOHANSEN H.W., 1981 — *Coralline algae, A First Synthesis*. Boca Raton, Florida, CRC Press, 239 p.

LE GALL L. & SAUNDERS G.W., 2010 — DNA barcoding is a powerful tool to uncover algal diversity: a case study of the Phyllophoraceae (Gigartinales, Rhodophyta) in the Canadian flora. *Journal of phycology* 46: 374-389.

LEMOINE M. P., 1910 — Répartition et mode de vie du Maerl (*Lithothamnium calcareum*) aux environs de Concarneau (Finistère). *Annales de Institut Océanographique. Fondation Albert Ier, Prince de Monaco* 1(3): 27.

MENDOZA M.L. & CABIOC'H J., 1998 — Étude comparée de la reproduction de *Phymatolithon calcareum* (Pallas) Adey & McKibbin et *Lithothamnion corallioides* (P. & H. Crouan) P. & H. Crouan (Corallinales, Rhodophyta), et reconsidérations sur le définition des genres. *Canadian journal of botany* 76: 1433-1445.

PARDO C., LÓPEZ L., PEÑA V., HERNÁNDEZ-KANTÚN J., LE GALL L., BÁRBARA I. & BARREIRO R., 2012 — A DNA-barcoding study fosters our understanding of the distribution and magnitude of maerl biodiversity in the OSPAR area. *Book of Abstracts of the IV International Rhodolith Workshop*: 58.

PEÑA V. & BÁRBARA I., 2004 — Diferenciación morfológica y anatómica entre *Lithothamnion corallioides* y *Phymatolithon calcareum* (Corallinales, Rhodophyta) en dos bancos de maerl de la Ría de Arousa (N. O. Península Ibérica). *Anales de biología* 26: 21-27.

PEÑA V. & BÁRBARA I., 2008 — Biological importance of an Atlantic European maerl bed off Benencia Island (northwest Iberian Peninsula). *Botanica marina* 51: 493-505.

PEÑA V. & BÁRBARA I., 2009 — Distribution of the Galician maërl beds and their shape classes (Atlantic Iberian Peninsula): proposal of areas in future conservation actions of the Galician maërl. *Cahiers de biologie marine* 50: 353-368.

PEÑA V., ADEY W.H., RIOSMENA-RODRÍGUEZ R., JUNG M-Y., CHOI H-G., AFONSO-CARRILLO J. & BÁRBARA I., 2011 — *Mesophyllum sphaericum* sp. nov (Corallinales, Rhodophyta): a new maerl-forming species from the northeast Atlantic. *Journal of phycology* 47: 911-927.

SAUNDERS G.W., 2005 — Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical transactions of the Royal Society B* 360: 1879-1888.

SAUNDERS G.W. & MCDEVIT D.C., 2012 — Methods for DNA Barcoding Photosynthetic Protists Emphasizing the Macroalgae and Diatoms. *Methods in molecular biology* 858: 207-222.

SCHAEFFER T.N., SMITH J & FOSTER M.S., 2002 — Genetic differences between two growth-forms of *Lithophyllum margaritae* (Rhodophyta) in Baja California Sur, Mexico. *Journal of phycology* 38: 1090-1098.

SHERWOOD A.R., KURIHARA A., CONKLIN K.Y., SAUVAGE,T. & PRESTRESTING G.G., 2010 — The Hawaiian Rhodophyta Biodiversity Survey (2006-2010): a summary of principal findings. *BMC plant biology* 10: 258.

STELLER D.L. & FOSTER M.S., 1995 — Environmental factors influencing distribution and morphology of rhodoliths in Bahía Concepción, B.C.S., México. *Journal of experimental marine biology and ecology* 194: 201-212.

STENECK R.S., 1986 — The ecology of coralline algal crusts: Convergent patterns and adaptative strategies. *Annual review of ecology and systematics* 17: 273-303.

SUNESON S., 1958 — *Lithothamnion calcareum* vid svenska västkusten. *Botaniska notiser* 111: 197-199.

TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M. & KUMAR S., 2011 — MEGA 5: Molecular evolutionary genetics analyses using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution* 28(10): 2731-2739.

TEICHERT S., RÜGGEBERG A., WISSHAK M., PIEPENBURG D., MEYERHÖFER M., FORM A., BÜDENBENDER J., FREIWALD A. & WOELKERLING W.J, 2012 — Rhodolith beds (Corallinales, Rhodophyta) and their physical and biological environment at 80º 31´N in Nordkappbukta (Nordaustlandet, Svalbard Archipelago, Norway). *Phycologia* 51(4): 371-390.

WALKER R.H., BRODIE J, RUSSEL S. & IRVINE L.M., 2009 — Biodiversity of coralline algae in the northeastern Atlantic including *Corallina caespitosa* sp. nov. (Coralllinoideae, Rhodophyta). *Journal of phycology* 45: 287-297.

WOELKERLING W.J. & IRVINE L.M., 1986 — The typification and status of *Phymatolithon* (Corallinaceae, Rhodophyta*). British phycological journal* 21(1): 55-80.