Cryptogamie, Algologie, 2015, 36 (4): 429-459

Phymatolithon lusitanicum sp. nov. (Hapalidiales, Rhodophyta): the third most abundant maerl-forming species in the Atlantic Iberian Peninsula

Viviana PEÑA[a,b], Cristina PARDO[a], Lúa LÓPEZ[a], Belén CARRO[a], Jazmin HERNANDEZ-KANTUN[c], Walter H. ADEY[c], Ignacio BÁRBARA[a], Rodolfo BARREIRO[a] & Line LE GALL[b]*

[a]BIOCOST Research Group, Facultade de Ciencias, Universidade da Coruña, Campus de A Coruña, 15071, A Coruña, Spain

[b]Équipe Exploration, Espèces et Évolution, Institut de Systématique, Évolution, Biodiversité, UMR 7205 ISYEB CNRS, MNHN, UPMC, EPHE, Muséum national d'Histoire naturelle (MNHN), Sorbonne Universités, 57 rue Cuvier CP N39, F-75005, Paris, France

[c]Botany Department, National Museum of Natural History, Smithsonian Institution, MRC 166 PO Box 37012, Washington, D.C., USA

Abstract – *Phymatolithon lusitanicum* is a new maerl species described based on an integrative systematic approach including molecular (COI-5P, *psb*A) and morphological data obtained from recent collections, as well as comparison of type material from the morphologically and ecologically alike NE Atlantic species *P. lamii* and *P. laevigatum*. Molecular analyses including type material of *P. lamii* and *P. laevigatum* were congruent in delimiting *P. lusitanicum* as an independent lineage from these crustose species. The three species shared a common external morphology of multiporate asexual conceptacles, but *P. lusitanicum* has been detected only unattached as maerl while *P. lamii* and *P. laevigatum* are crustose. *Phymatolithon lusitanicum* is particularly abundant in subtidal maerl beds of the Atlantic Iberian Peninsula (Galicia and the Algarve); however it has also been detected northwards in Ireland intertidally and in Western Mediterranean Sea (Alborán Sea, Balearic Islands) down to 64 m. *Phymatolithon lusitanicum* differs from other *Phymatolithon* species reported for the European coasts mainly by the external shape of the multiporate asexual conceptacles (pore plate flush with surface or slightly sunken without a conspicuous thick raised rim) and its unattached habit as maerl/rhodolith. In addition, the lectotype of *Lithothamnion hamelii* turned out to be conspecific to *Phymatolithon calcareum*, therefore this taxon is proposed as a heterotypic synonym of *P. calcareum*. Finally, our molecular analyses detected cryptic diversity within the European collections of *Phymatolithon*, while collections identified as *P. lenormandii* from Canada or *P. repandum* from New Zealand were resolved as unrelated to the remaining *Phymatolithon*. In the light of these results, it is clear that further work is necessary to resolve species diversity within the genus *Phymatolithon* and its relationship with related genera.

Biodiversity / DNA barcoding / *Lithothamnion hamelii* / Mediterranean / NE Atlantic / *Phymatolithon laevigatum* / *Phymatolithon lamii* / systematics

* Corresponding author: vpena@udc.es.

doi/10.7872/crya/v36.iss4.2015.429

INTRODUCTION

The genus *Phymatolithon* Foslie contains non-geniculate coralline algae that include 14 currently accepted names for which seven species are reported in Europe (Guiry & Guiry, 2015) associated to coastal habitats from the intertidal to the low subtidal (Adey & Adey, 1973; Chamberlain & Irvine, 1994; Teichert *et al*., 2012). *Phymatolithon* together with *Lithothamnion* Heydrich have been regarded as the major contributors to European maerl beds in terms of species diversity as well as abundance and distribution range (Chamberlain & Irvine, 1994; Babbini & Bressan, 1997; Bressan & Babbini, 2003). In particular, *Phymatolithon calcareum* is commonly cited in the literature as the main builder of the Atlantic and Mediterranean maerl beds (Chamberlain & Irvine, 1994; Bressan & Babbini, 2003). *Phymatolithon purpureum* has also been reported as a maerl-forming species in Ireland, and more recently in the Mediterranean (Adey & Adey, 1973, as *P. polymorphum* (Linnaeus) Foslie; Blunden *et al*., 1981; Hernández-Kantún *et al*., 2014, 2015), but it is more commonly found as an Atlantic encrusting species, epilithic or epiphytic on kelp holdfasts and stipes, from the low littoral to deep subtidal down to 45 m. A similar habit is reported for *Phymatolithon laevigatum, P. lamii* and *P. lenormandii* which commonly occur in the Atlantic coasts as crustose attached plants from the mid-lower littoral to 30-35 m depth (down to 90 m for *P. lamii*, Adey & Adey, 1973; Chamberlain & Irvine, 1994). The latter two species (*P. lamii* and *P. lenormandii*) have been also cited in the Mediterranean as encrusting species, epilithic or epizoic on *Chlamys* sp. shells, down to 55 m depth (Bressan & Babbini, 2003; Kaleb *et al*., 2012). By contrast, *Phymatolithon brunneum* has been described as a rare, epilithic plant that occurs in the littoral above the *P. purpureum* zone or even higher up the shore, but only in Britain, Channel Islands and Atlantic France (Chamberlain & Irvine, 1994). Other rare species is *P. tenuissimum* (Foslie) Adey which has been cited in the Mediterranean, and formerly described in São Tomé, West coast of Africa (Foslie, 1900; Babbini & Bressan, 1997). Apart from the growth-form and habitat, the external morphology of multiporate sporangial conceptacles has been usually employed in the literature for the identification of European *Phymatolithon* taxa (Chamberlain & Irvine, 1994; Bressan & Babbini, 2003).

In the Atlantic Iberian Peninsula, other than the maerl-forming *P. calcareum* (Peña *et al.,* 2014), the only members of the genus reported so far are *P. lenormandii, P. lamii* and *P. purpureum*, but always as attached crustose plants (Ardré, 1970; Adey & Adey, 1973; Chamberlain, 1991; Chamberlain & Irvine, 1994; Bárbara *et al*., 2003). Nonetheless, new research with DNA barcodes recently detected a still undescribed species in Iberian maerl beds that, according to sequence information derived from mitochondrial and plastid markers (COI-5P and *psb*A), was tentatively assigned to *Phymatolithon* sp.3. Interestingly, this new species was a common component of maerl beds in Galicia, where it appeared mixed with the widespread *P. calcareum* and *Lithothamnion corallioides*, and it even became the dominant component of maerl beds southwards in the Algarve (Carro *et al.,* 2014; Pardo *et al.,* 2014). Additional morphological studies provided further support to the molecular-based delimitation, while the observation of multiporate asexual conceptacles with pore plates lightly protruding or flush with the surface suggested some similarities with *P. lamii* or *P. laevigatum* (Chamberlain, 1991; Chamberlain & Irvine, 1994). Nevertheless, no name could be assigned with certainty given the absence of molecular data from any other type species reported in European coasts other than the generitype *P. calcareum* (Peña *et al.,* 2014; Hernández-Kantún *et al.,*

2015). Alternatively, topotype material (specimens of the targeted species from the type locality) could be studied, but it should be done with caution given the frequent co-ocurrence of several *Phymatolithon* taxa in the same habitat. Here, we formally describe the maerl species *Phymatolithon lusitanicum* sp. nov. on the basis of novel molecular data obtained from type material of *P. lamii* and *P. laevigatum* and from recent collections of other European *Phymatolithon* species.

MATERIAL AND METHODS

Collections studied

Maerl specimens identified as *Phymatolithon* sp.3 using DNA barcoding (COI-5P/*psb*A) have been collected in recent surveys of maerl beds in the Atlantic Iberian Peninsula both in Galicia (Spain) and Algarve (Portugal) (Carro *et al.,* 2014; Pardo *et al.,* 2014). Intertidal samples identified in the field as *Phymatolithon* spp. were collected on rocky shores using a hammer and chisel in several sites along the Atlantic European coasts (France, Spain and Portugal) including the type locality of *P. purpureum* (Fort du Mingant, Brittany, France, Chamberlain & Irvine, 1994). In addition, intertidal and subtidal collections from Ireland and Western Mediterranean Sea (Alborán Sea) have been also studied (Table 1). Samples were air-dried or oven-dried (50º C), preserved in zipper bags with silica gel before molecular and morphological studies, and deposited in the herbaria of Universidade de Santiago de Compostela (SANT), Muséum National d´Histoire Naturelle (PC), Smithsonian Institution (US) and National University of Ireland (GALW) (acronyms follow Thiers, 2015). In addition, we studied the type material of *Lithophyllum lamii* Me. Lemoine (lectotype, PC0719024), *Lithothamnion laevigatum* Foslie (paratype, PC0145173), *Lithothamnion purpureum* P.L.Crouan & H.M.Crouan (lectotype – CO02256 – and isotype – CO02260 –), *Melobesia lenormandii* Areschoug (isolectotype, CN) and *Lithothamnion hamelii* (lectotype, PC0145174) deposited in the herbaria of Concarneau (CO), the Université de Caen (CN) and the Museum National d´Histoire Naturelle (PC) (Chamberlain, 1991; Chamberlain & Irvine, 1994; Woelkerling & Lamy, 1998, Table 1).

Molecular studies

A total of 38 specimens from recent intertidal and subtidal collections were cleaned under a stereomicroscope and selected surfaces were ground for DNA extraction. Genomic DNA was extracted using a NucleoSpin® 96 Tissue kit (Macherey-Nagel, GmbH and Co. KG, Germany), or the QIAamp® DNA Micro Kit (Qiagen S.A.S., France, protocol for tissues) for the type material. Samples from Ireland (E302, E113, E17, E10, Table 1) were extracted following Hernandez-Kantun *et al.* (2014), while US herbarium vouchers identified as *Phymatolihon rugulosum* and *P. lenormandii* followed Adey *et al.* (2015) The mitochondrial COI-5P fragment was PCR-amplified using primer pairs GazF1/GazR1, GazF1/GCorR3, or GWSFn/GWSRx (Saunders, 2005; Le Gall & Saunders, 2010; Saunders & McDevit, 2012; Pardo *et al.,* 2014; Peña *et al.,* 2015). The *psb*A locus was amplified using primer pairs *psb*A-F1/ *psb*A-R2 or *psb*A-F1/*psb*A600R (Yoon *et al.*, 2002).

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
***Phymatolithon lusitanicum* (holotype)**	**CPVP-676**	**KC861627**	**KC819260**	**Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.**	**SANT-Algae 29522**
***P. lusitanicum* (isotype)**	**CPVP-689**	**KC861620**	–	**Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo et al. (2014) as *Phymatolithon* sp.3.**	**SANT-Algae 29523**
***P. lusitanicum* (isotype)**	**CPVP-685**	**KC861621**	–	**Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo et al. (2014) as *Phymatolithon* sp.3.**	**SANT-Algae 29524**
P. lusitanicum	CPVP-1134	KC861646	–	Subtidal (5 m), maerl, Punta Barbafeita, Ría de Arousa, Galicia, Spain, 23/06/2011. Coll:Bárbara, I., Bunker, F. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29518
P. lusitanicum	CPVP-1064	KC861649	–	Subtidal (5 m), maerl, Punta Barbafeita, Ría de Arousa, Galicia, Spain, 23/06/2011. Coll:Bárbara, I., Bunker, F. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29520
P. lusitanicum	CPVP-1060	KC861644	–	Subtidal (5 m), maerl, Punta Barbafeita, Ría de Arousa, Galicia, Spain, 23/06/2011. Coll:Bárbara, I., Bunker, F. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29521
P. lusitanicum	CPVP-1136	KC861645	–	Subtidal (5 m), maerl, Punta Barbafeita, Ría de Arousa, Galicia, Spain, 23/06/2011. Coll:Bárbara, I., Bunker, F. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29519
P. lusitanicum	CPVP-1261	KC861650	–	Subtidal (5 m), maerl, Sálvora, Ría de Arousa, Galicia, Spain, 05/08/2011. Coll: Bárbara, I., García, V. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29546
P. lusitanicum	CPVP-1260	KC861651	–	Subtidal (5 m), maerl, Sálvora, Ría de Arousa, Galicia, Spain, 05/08/2011. Coll: Bárbara, I., García, V. & Peña, V. Published in Pardo *et al,* (2014) as *Phymatolithon* sp.3	SANT-Algae 29545

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
P. lusitanicum	CPVP-1259	KC861652	–	Subtidal (5 m), maerl, Sálvora, Ría de Arousa, Galicia, Spain, 05/08/2011. Coll: Bárbara, I., García, V. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29544
P. lusitanicum	CPVP-1258	KC861653	–	Subtidal (5 m), maerl, Sálvora, Ría de Arousa, Galicia, Spain, 05/08/2011. Coll: Bárbara, I., García, V. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29543
P. lusitanicum	CPVP-1257	KC861654	–	Subtidal (5 m), maerl, Sálvora, Ría de Arousa, Galicia, Spain, 05/08/2011. Coll: Bárbara, I., García, V. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3	SANT-Algae 29542
P. lusitanicum	CPVP-1256	KC861655	–	Subtidal (5 m), maerl, Sálvora, Ría de Arousa, Galicia, Spain, 05/08/2011. Coll: Bárbara, I., García, V. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29541
P. lusitanicum	CPVP-633	KC861660	–	Subtidal (9 m), maerl, Playa de Tulla, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29548
P. lusitanicum	CPVP-639	KC861642	KC819258	Subtidal (9 m), maerl, Playa de Tulla, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo et al. (2014) as *Phymatolithon* sp.3.	SANT-Algae 29547
P. lusitanicum	CPVP-626	KC861662	–	Subtidal (9 m), maerl, Playa de Tulla, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29550
P. lusitanicum	CPVP-627	KC861617	–	Subtidal (9 m), maerl, Playa de Tulla, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29552
P. lusitanicum	CPVP-622	KC861628	–	Subtidal (9 m), maerl, Playa de Tulla, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29549

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
P. lusitanicum	CPVP-618	KC861641	–	Subtidal (9 m), maerl, Playa de Tulla, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29551
P. lusitanicum	CPVP-611	KC861647	–	Subtidal (13 m), maerl, Isla Ons, Ría de Pontevedra Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29533
P. lusitanicum	CPVP-600	KC861648	–	Subtidal (13 m), maerl, Isla Ons, Ría de Pontevedra Galicia, Spain, 07/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29534
P. lusitanicum	CPVP-749	KC861656	–	Subtidal (5 m), maerl, Baliza Tofiño, Ría de Vigo, Galicia, Spain, 05/04/2011, coll: Barreiro, R., Bárbara, I. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29532
P. lusitanicum	CPVP-748	KC861657	–	Subtidal (5 m), maerl, Baliza Tofiño, Ría de Vigo, Galicia, Spain, 05/04/2011, coll: Barreiro, R., Bárbara, I. & Peña, V. Published in Pardo *et al.,* (2014) as *Phymatolithon* sp.3	SANT-Algae 29531
P. lusitanicum	CPVP-50	KC861659	–	Subtidal (5 m), maerl, Baliza Tofiño, Ría de Vigo, Galicia, Spain, 19/05/2009, coll: Bárbara, I. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29530
P. lusitanicum	CPVP-686	KC861622	–	Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29529
P. lusitanicum	CPVP-681	KC861623	–	Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29528
P. lusitanicum	CPVP-680	KC861624	–	Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al,* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29527

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
P. lusitanicum	CPVP-695	KC861625	–	Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29526
P. lusitanicum	CPVP-694	KC861626	–	Subtidal (5 m), maerl, Con de Pego, Ría de Vigo, Galicia, Spain, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29525
P. lusitanicum	CPVP-644	KC861635	–	Subtidal (11 m), maerl, Islas Cíes, Ria de Vigo, Galicia, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al,* (2014) as *Phymatolithon* sp.3	SANT-Algae 29538
P. lusitanicum	CPVP-645	KC861636	–	Subtidal (11 m), maerl, Islas Cíes, Ria de Vigo, Galicia, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29536
P. lusitanicum	CPVP-646	KC861637	–	Subtidal (11 m), maerl, Islas Cíes, Ria de Vigo, Galicia, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29535
P. lusitanicum	CPVP-670	KC861638	–	Subtidal (11 m), maerl, Islas Cíes, Ria de Vigo, Galicia, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29537
P. lusitanicum	CPVP-664	KC861639	–	Subtidal (11 m), maerl, Islas Cíes, Ria de Vigo, Galicia, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29540
P. lusitanicum	CPVP-669	KC861640	–	Subtidal (11 m), maerl, Islas Cíes, Ria de Vigo, Galicia, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29539
P. lusitanicum	CPVP-77	KC861643	–	Subtidal (15 m), maerl, Lagos, Algarve, Portugal, 04/09/2008. Coll: Bárbara, I. & Peña, V. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29553

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
P. lusitanicum	CPVP-453	KC861632	–	Subtidal (20 m), maerl, Armaçao de Pêra, Algarve, Portugal, 02/03/2011. Coll: Neves, P. & Rodrigues, M. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29516
P. lusitanicum	CPVP-451	KC861631	–	Subtidal (20 m), maerl, Armaçao de Pêra, Algarve, Portugal, 02/03/2011. Coll: Neves, P. & Rodrigues, M. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29515
P. lusitanicum	CPVP-452	KC861618	–	Subtidal (20 m), maerl, Armaçao de Pêra, Algarve, Portugal, 02/03/2011. Coll: Neves, P. & Rodrigues, M. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29514
P. lusitanicum	CPVP-478	KC861630	KC819253	Subtidal (20 m), maerl, Armaçao de Pêra, Algarve, Portugal, 02/03/2011. Coll: Neves, P. & Rodrigues, M. Published in Pardo et al. (2014) as Phymatolithon sp.3.	SANT-Algae 29517
P. lusitanicum	CPVP-503	KC861633	–	Subtidal (20 m), maerl, Armaçao de Pêra, Algarve, Portugal, 02/03/2011. Coll: Neves, P. & Rodrigues, M. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29511
P. lusitanicum	CPVP-480	KC861629	–	Subtidal (20 m), maerl, Armaçao de Pêra, Algarve, Portugal, 02/03/2011. Coll: Neves, P. & Rodrigues, M. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29513
P. lusitanicum	CPVP-501	KC861661	–	Subtidal (20 m), maerl, Armaçao de Pêra, Algarve, Portugal, 23/02/2011. Coll: Neves, P. & Rodrigues, M. Published in Pardo *et al.* (2014) as *Phymatolithon* sp.3.	SANT-Algae 29512
P. lusitanicum	VPF00511	KT807920	–	Subtidal (40-44 m), maerl, Alborán Sea, Mediterranean Spain, 22/09/2011. Coll: INDEMARES project.	SANT-Algae 29508
P. lusitanicum	VPF00451	KT807910	–	Subtidal (42-48 m), maerl, Alborán Sea, Mediterranean Spain, 24/09/2011. Coll: INDEMARES project.	SANT-Algae 29506
P. lusitanicum	E113	–	KT874588	Subtidal (6 m), maerl, Carraroe beach, Ireland, 09/05/2009. Coll.: Hernández, J. & Hanniffy, D. Published in Hernandez-Kantun *et al.* (2014) as *P. purpureum*.	GALW015741

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
P. lusitanicum	E17	–	KT863473	Subtidal (6 m), maerl, Carraroe beach, Ireland, 08/10/2009. Coll.: Moriarty, M.	SANT-Algae 29595
P. lusitanicum	E10	–	KT863474	Subtidal (6 m), maerl, Carraroe beach, Ireland, 08/10/2009. Coll.: Moriarty, M.	SANT-Algae 29596
P. lusitanicum	E302	–	KT863475	Intertidal, maerl, Mukinish, Ireland, 04/11/2009. Coll.: Hernández, J.	SANT-Algae 29597
Phymatolithon cf. brunneum Y.M.Chamberlain	VPF00561B	KT807916	–	Intertidal, epilithic crust, Laredo-Puerto Viejo, Cantabria, Spain, 22/02/2011. Coll: Secilla, A. & Peña, V.	SANT-Algae 29488
P. cf. brunneum	VPF00576	KT807908	–	Intertidal, epilithic crust, San Juan de Gaztelugatxe, Pais Vasco, Spain, 19/02/2011. Coll: Secilla, A. & Peña, V.	SANT-Algae 29503
P. cf. brunneum	VPF00572	KT807909	KT807938	Intertidal, epilithic crust, San Juan de Gaztelugatxe, Pais Vasco, Spain, 19/02/2011. Coll: Secilla, A. & Peña, V.	SANT-Algae 29502
Phymatolithon calcareum (Pallas) Adey & McKibbin	VPF00132	KT807904	–	Subtidal (< 10 m), crust in maerl bed, Molène, Brittany, 13/05/2011. Coll: Grall, J. & Peña, V.	SANT-Algae 29484
P. calcareum	VPF00276	KT807928	–	Subtidal (5-10 m), maerl, Osundet, Hordaland, Norway, nd. Coll: Rueness, J.	SANT-Algae 29487
P. calcareum	VPF00523	KT807921	–	Subtidal (25 m), maerl, Alborán Sea, Mediterranean Spain, 25/09/2011. Coll: INDEMARES project	SANT-Algae 29505
P. calcareum	VPF00449	KT807905	–	Subtidal (42-48 m), maerl, Alborán Sea, Mediterranean Spain, 24/09/2011. Coll: INDEMARES project	SANT-Algae 29507
***Lithothamnion hamelii* Me. Lemoine (lectotype)**	**PC0145174**	**–**	**KT807946**	**Subtidal (8 m), crust, St Servan, Pointe de Cancaval, Rance, France, 1930. Coll: Lami, R.**	**PC0145174**

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
***Phymatolithon laevigatum* (Foslie) Foslie [*Lithothamnion laevigatum* Foslie] (paratype)**	**PC0145173**	**–**	**KT807943**	**Crust, Helgoland, Germany, 29/01/1894. Coll: Kuckuck, P.**	**PC0145173**
P. cf. laevigatum	VPF00033	KT807901	KT807937	Intertidal, epilithic crust, Camelle, Galicia, Spain, 31/08/2011. Coll: Peña, V.	SANT-Algae 29496
P. cf. laevigatum	VPF00014	KT807927	–	Subtidal (9-11 m), epilithic crust, Ziérbena, Puerto Bilbao, Pais Vasco, Spain, 14/07/2011. Coll: Santolaria, A.	SANT-Algae 29479
P. cf. laevigatum	VPF00383C	KT807925	—	Intertidal, epilithic crust, Montedor, Portugal, 18/04/2011. Coll: Secilla, A. & Peña, V.	SANT-Algae 29497
P. cf. laevigatum	VPF00574	KT807918	–	Intertidal, epilithic crust, San Juan de Gaztelugatxe, Pais Vasco, Spain, 19/02/2011. Coll: Secilla, A. & Peña, V.	SANT-Algae 29504
***Phymatolithon lamii* (Me.Lemoine) Y.M.Chamberlain [*Lithophyllum lamii* Me. Lemoine] (lectotype)**	**PC0719024**	**–**	**KT807939**	**Subtidal (8 m), crust, St Servan, Pointe de Cancaval, Rance, France, 1930. Coll: Lami, R.**	**PC0719024**
P. lamii	VPF00557	KT807915	–	Intertidal, epilithic crust, Sainte-Marguerite, Landeda, Brittany, France, 19/03/2011. Coll: Peña, V.	SANT-Algae 29480
P. lamii	VPF00410	KT807923	–	Subtidal (< 10 m), epilithic crust in maerl bed, Molène, Brittany, 13/05/2011. Coll: Grall, J. & Peña, V.	SANT-Algae 29485
P.lamii	VPF00223	KT807914	KT807942	Intertidal, epilithic crust, Fort du Mingant, Brest, Brittany, France, 18/03/2011. Coll: Peña, V.	SANT-Algae 29491
P. lamii	VPF00222	KT807924	–	Intertidal, epilithic crust, Fort du Mingant, Brest, Brittany, France, 18/03/2011. Coll: Peña, V.	SANT-Algae 29493

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
P. lamii	VPF00225	KT807907	–	Intertidal, epilithic crust, Fort du Mingant, Brest, Brittany, France, 18/03/2011. Coll: Peña, V.	SANT-Algae 29489
P. lamii	VPF00236	KT807926	–	Intertidal, epilithic crust, Fort du Mingant, Brest, Brittany, France, 18/03/2011. Coll: Peña, V.	SANT-Algae 29495
P. lamii	VPF00088	KT807902	–	Subtidal (7 m), epilithic crust, Astondo, Bizkaia, Pais Vasco, Spain, 18/07/2011. Coll: Santolaria, A.	SANT-Algae 29501
P. lamii	VPF00561A	KT807903	–	Intertidal, epilithic crust, Laredo-Puerto Viejo, Cantabria, Spain, 22/02/2011. Coll: Secilla, A. & Peña, V.	SANT-Algae 29488
P. lamii	VPF00074	KT807917	–	Intertidal pool, epilithic crust, Cadavedo, Asturias, Spain, 03/08/2011. Coll: Peña, V.	SANT-Algae 29499
P. lamii	VPF00075	KT807906	–	Intertidal pool, epilithic crust, Cadavedo, Asturias, Spain, 03/08/2011. Coll: Peña, V.	SANT-Algae 29498
P. lamii	VPF00369B	–	KT807947	Subtidal (2 m), epilithic crust, Peinzás, Galicia, Spain, 09/02/2011. Coll: Pardo, C., Bárbara, I, Maneiro, I., Peña, V.	SANT-Algae 29118
P. lamii	VPF00320	KT807919	–	Intertidal, epilithic crust, Cala Encendida, Cadiz, Spain, 18/02/2011. Coll: Díaz, P. & Bárbara, I.	SANT-Algae 29478
P. lamii	VPF00325	KT807912	KT807941	Intertidal, epilithic crust, Cala Encendida, Cadiz, Spain, 18/02/2011. Coll: Díaz, P. & Bárbara, I.	SANT-Algae 29477
Phymatolithon cf. lenormandii (Areschoug) Adey	VPF00081	KT807911	KT807940	Intertidal pool, epilithic crust, Cadavedo, Asturias, Spain, 03/08/2011. Coll: Peña, V.	SANT-Algae 29500
P. lenormandii	US 170885	KT863470	KP142718	Intertidal, epilithic crust, Nichol Is, off Ship Harbour Nova Scotia, Canada, 3/10/1964. Coll: Adey, W.H.	US 170885
P. lenormandii	US 170941	KT863471	KP142719	Subtidal (0-3 m), epilithic crust, Samlenfjord, Hardangerfjord, Norway, 31/07/1967. Coll: Adey, W.H.	US 170941

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank accession number (COI-5P)	GenBank accession number (*psb*A)	Collection details	Herbarium
Phymatolithon rugulosum W.H.Adey	US 170942	KT863472	KP142717	Subtidal (3-8 m), epilithic crust, Schoodic pt., ME, Gulf of Maine, US, 26/06/1962. Coll: Adey, W.H.	US170942
Phymatolithon sp.	VPF00425A	–	KT807945	Subtidal (11 m), epilithic crust in maerl bed, Islas Cíes, Ria de Vigo, Galicia, 05/04/2011. Coll: Bárbara, I., Barreiro, R., Peña, V.	SANT-Algae 29481
Phymatolithon sp.	VPF00432	KT807922	–	Subtidal (2 m), epilithic crust, Peinzás, Galicia, Spain, 25/02/2012. Coll: Pardo, C., Bárbara, I, Maneiro, I., Peña, V.	SANT-Algae 29476
Phymatolithon sp.	VPF00615A	KT807913	–	Subtidal, maerl, Douarnenez, Brittany, France, 22/08/2012. Coll: Le Gall, L.	PC0145175

Additional sequences analysed

Species	Voucher	GenBank Accession numbers (COI-5P/*psb*A)	Collection details
***Phymatolithon calcareum* (Pallas) Adey &McKibbin (neotype)**	**000712373BM**	**KF808323/JQ89623**	**St Mawes Bank, Falmouth, England(Woelkerling & Irvine 1986; Peña *et al.*, 2014, Hernández-Kantún *et al.*, 2015)**
P. calcareum	CPVP-781	KC861616	Ireland, Pardo *et al.*, (2014)
P. calcareum	CPVP-157	KC861615	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-151	KC861614	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-135	KC861613	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-956	KC861612	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-130	KC861612	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-275	KC861611	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-899	KC861610	Brittany, France, Pardo *et al.*, (2014)

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank Accession numbers (COI-5P/*psb*A)	Collection details
P. calcareum	CPVP-897	KC861609	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-906	KC861608	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-903	KC861607	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-909	KC861606	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-912	KC861605/KC819243	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-47	KC861604	Cornwall, England, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-696	KC861603	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-779	KC861602	Ireland, Pardo *et al.*, (2014)
P. calcareum	CPVP-654	KC861601	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-648	KC861600	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-665	KC861599	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-167	KC861598	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-766	KC861597	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-565	KC861596	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-566	KC861595	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-310	KC861594	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-554	KC861593	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-560	KC861592	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-955	KC861591	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-558	KC861590	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-916	KC861589	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-629	KC861587	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-628	KC861586	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-48	KC861585	England, UK, Pardo *et al.*, (2014)

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank Accession numbers (COI-5P/*psb*A)	Collection details
P. calcareum	CPVP-207	KC861584	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-174	KC861583	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1078	KC861582	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1067	KC861581	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1065	KC861580	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1129	KC861579	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1121	KC861578	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1120	KC861577	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1119	KC861576	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1118	KC861575	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1110	KC861574	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1109	KC861573	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1108	KC861572	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1104	KC861571	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1103	KC861570	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-655	KC861569	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-170	KC861568	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-164	KC861567	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1104	KC861571	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-163	KC861566	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-162	KC861565	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-46	KC861564	England, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-783	KC861563	Ireland, Pardo *et al.*, (2014)
P. calcareum	CPVP-780	KC861562	Ireland, Pardo *et al.*, (2014)

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank Accession numbers (COI-5P/*psb*A)	Collection details
P. calcareum	CPVP-778	KC861561	Ireland, Pardo *et al.*, (2014)
P. calcareum	CPVP-1195	KC861557	England, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1187	KC861556	England, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-961	KC861555	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-959	KC861554	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-1234	KC861559	La Rochelle, France, Pardo et al. (2014)
P. calcareum	CPVP-1236	KC861560	La Rochelle, France, Pardo et al. (2014)
P. calcareum	CPVP-858	KC861553	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-615	KC861552	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-607	KC861551	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-603	KC861550	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-1450	KC861549	Norway, Pardo *et al.*, (2014)
P. calcareum	CPVP-44	KC861548	England, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-43	KC861547	England, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-753	KC861546	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	CPVP-929	KC861545	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-921	KC861544	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-920	KC861543	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-1254	KC861542	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1253	KC861541	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1252	KC861540	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1251	KC861539	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1247	KC861538	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1246	KC861537	Northern Ireland, UK, Pardo *et al.*, (2014)

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

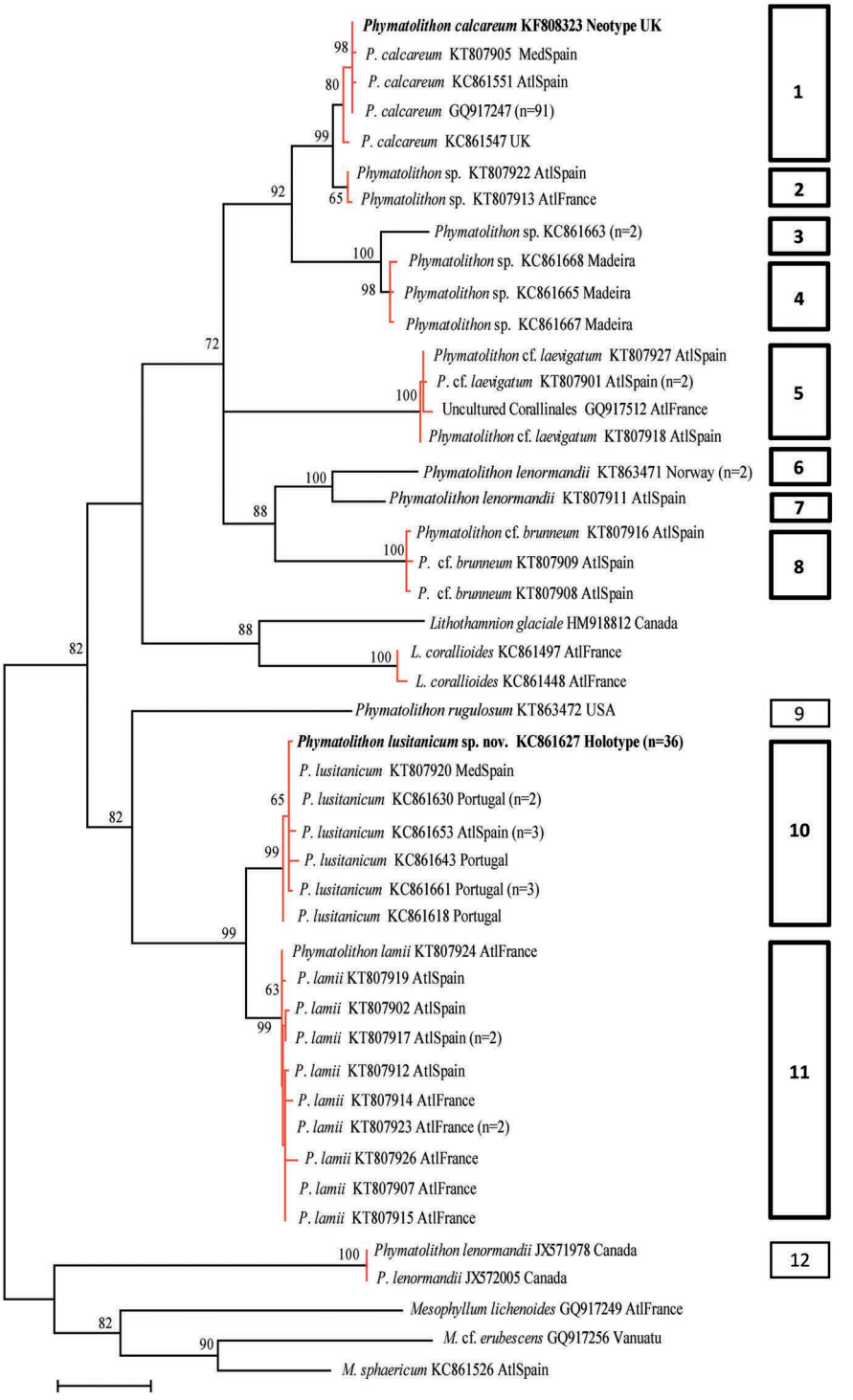
Species	Voucher	GenBank Accession numbers (COI-5P/*psb*A)	Collection details
P. calcareum	CPVP-1245	KC861536	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1244	KC861535	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1243	KC861534	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-1242	KC861533	Northern Ireland, UK, Pardo *et al.*, (2014)
P. calcareum	CPVP-900	KC861532	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-901	KC861531	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-910	KC861530	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-943	KC861529	Brittany, France, Pardo *et al.*, (2014)
P. calcareum	CPVP-555	KC861528	Galicia, Spain, Pardo *et al.*, (2014)
P. calcareum	LBC0001	GQ917247/GQ917436	France, Bittner *et al.*, (2010)
P. calcareum	CPVP-1188	KC861558	Milford Haven, UK, Pardo *et al.*, (2014)
Phymatolithon lamii	GALW15780	–/JQ896248	Ireland, Hernández-Kantún *et al.*, (2015)
Phymatolithon lenormandii	GWS005398	JX572005	Canada, Saunders & McDevit, unpublished
P. lenormandii	GWS005396	JX571978	Canada, Saunders & McDevit, unpublished
P. lenormandii	GALW15781	–/JQ896249	Ireland, Hernández-Kantún *et al.*, (2015)
Phymatolithon purpureum (P.L. Crouan & H.M. Crouan) Woelkerling & L.M.Irvine	GALW15782	–/JQ896232	Kingstown Bay, Ireland, Hernández-Kantún *et al.*, (2015)
Phymatolithon repandum (Foslie) Wilks & Woelkerling	ND114	–/FJ361450	New Zealand, Broom et al., unpublished
P. repandum	ND175	–/FJ361477	New Zealand, Broom et al., unpublished
P. repandum	ND659	–/FJ361683	New Zealand, Broom et al., unpublished
Phymatolithon sp.	GALW15783	–/JQ896247	Ireland, Hernández-Kantún *et al.*, (2015)
Phymatolithon sp.	CPVP-441	KC861668/KC819250	Madeira, Pardo *et al.*, (2014)
Phymatolithon sp.	CPVP-440	KC861667	Madeira, Pardo *et al.*, (2014)

Table 1. Sample information for species included in molecular analyses. Type material in bold: holotype and isotype specimens of *Phymatolithon lusitanicum sp. nov.*, lectotype specimens of *P. lamii* and *Lithothamnion hamelii*, paratype of *P. laevigatum*, and neotype of *P. calcareum*. Additional COI-5P and *psb*A sequences donwloaded from GenBank are detailed *(continued)*

Species	Voucher	GenBank Accession numbers (COI-5P/*psb*A)	Collection details
Phymatolithon sp..	CPVP-443	KC861665/KC819251	Madeira, Pardo *et al.*, (2014)
Phymatolithon sp.	CPVP-510	KC861664/KC819255	Algarve, Portugal, Pardo *et al.*, (2014)
Phymatolithon sp.	CPVP-868	KC861663	Brittany, France, Pardo *et al.*, (2014)
Uncultured Corallinales	LBC0028	GQ917512/GQ917711	France, Bittner *et al.*, (2010)
Uncultured Corallinales	LBC0008	-/GQ917707	France, Bittner *et al.*, (2010)
Uncultured Corallinales	LBC0013	-/GQ917708	France, Bittner *et al.*, (2010)
Unidentified Hapalidiaceae	LBC0005	-/GQ917437	France, Bittner *et al.*, (2010)
Lithothamnion corallioides (P.L.Crouan & H.M.Crouan) P.L.Crouan & H.M.Crouan	CPVP-817	KC861448/KC819265	Brittany, France, Pardo *et al.*, (2014)
L. corallioides	CPVP-1238	KC861497	La Rochelle, France, Pardo *et al.*, (2014)
Lithothamnion glaciale Kjellman	GWS007542	HM918812/JQ422235	Newfoundland and Labrador, Canada, Le Gall *et al.*, (unpublished)
Mesophyllum cf. *erubescens* (Foslie) Me.Lemoine	LBC0551	GQ917256/GQ917446	Vanuatu, Bittner *et al.*, (2010)
M. lichenoides (J.Ellis) Me.Lemoine	LBC0031	GQ917249/GQ917439	Atlantic France, Bittner *et al.*, (2010)
M. *sphaericum* V.Peña, Bárbara, W.H.Adey, Riosmena-Rodriguez & H.G.Choi (holotype)	CPVP-776	KC861526/KC819262	Galicia, Spain, Pardo *et al.*, (2014)

Thermal profiles for PCR amplification of COI-5P and *psb*A fragments followed Saunders & McDevit (2012) and Bittner (2009), respectively. PCR reactions followed Peña *et al.* (2015). DNA extractions and amplifications of type material were performed separate from recent collections, and with negative controls run throughout. PCR products were purified and sequenced by Genoscope (Bibliothèque du Vivant program, Centre National de Séquençage, France); except for those obtained from specimens from Ireland or US specimens that followed Hernandez-Kantun *et al.* (2014) and Adey *et al.* (2015), respectively. 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 (project 'NGCOR', BOLD, http://www.boldsystems.org; Ratnasingham & Hebert, 2007) and GenBank (accession numbers listed in Table 1). Our analyses also included COI-5P and *psb*A sequences of *Phymatolithon* sp.3 generated in previous studies of Atlantic Iberian maerl beds (Carro *et al.*, 2014; Pardo *et al*., 2014), and publicly available sequences assigned to the genus *Phymatolithon* in BOLD and GenBank databases, of the latter included other maerl-forming species detected in the OSPAR region (BOLD project "MAERL", Pardo *et al.,* 2014). Finally, the alignments comprised 177 COI-5P and 42 *psb*A sequences, including the generitype *P. calcareum* (Peña *et al.,* 2014; Hernández-Kantun *et al.,* 2015) and other European taxa in the subfamily Melobesioideae (*Lithothamnion* and *Mesophyllum*) that were used as outgroups (Table 1). A general mixed Yule–coalescent (GMYC) model was applied to delimit *Phymatolithon* species (Pons *et al*., 2006; Fujisawa & Barraclough, 2013). The ultrametric tree derived from Bayesian phylogenetic analyses of the COI-5P alignment run in BEAST v1.7.4 (Drummond *et al.*, 2012) under a generalized time-reversible model with gamma+invariant sites to accommodate rate heterogeneity (GTR+G+I), an uncorrelated log normal (UCLN) relaxed molecular clock, and using a coalescence tree prior. In BEAST, two Markov Chain Monte Carlo (MCMC) analyses were run for 10 million generations, sampling every 1000th generation. The information from a simple of trees was summarized onto a single 'target' tree (10% burn-in 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 sequence evolution were estimated using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) obtained in jModeltest 2.1.3 (Darriba *et al*., 2012). Maximum likelihood and Bayesian analyses for the COI-5P and *psb*A alignments were performed under a generalized time-reversible with gamma+invariant sites heterogeneity model (GTR+G+I). The Bayesian analyses were performed under the same model with four Markov Chain Monte Carlo for 10 million generations, and tree sampling every 1000 generations.

Fig. 1. ML tree inferred from DNA barcode (COI-5P) for each species delimited according to the GMYC model (red colour). In bold, holotype of *Phymatolithon lusitanicum sp. nov.* and neotype of *P. calcareum*. The twelve supported lineages of *Phymatolithon* are indicated; in bold, the European ones. Bootstrap values > 60 % are shown for each node. Scale bar: 0.05 substitutions per site ▶


Phymatolithon calcareum KF808323 Neotype UK
98
P. calcareum KT807905 MedSpain
80
P. calcareum KC861551 AtlSpain
P. calcareum GQ917247 (n=91)
99
P. calcareum KC861547 UK
Phymatolithon sp. KT807922 AtlSpain
92
65
Phymatolithon sp. KT807913 AtlFrance
Phymatolithon sp. KC861663 (n=2)
100
Phymatolithon sp. KC861668 Madeira
98
Phymatolithon sp. KC861665 Madeira
Phymatolithon sp. KC861667 Madeira
72
Phymatolithon cf. laevigatum KT807927 AtlSpain
P. cf. laevigatum KT807901 AtlSpain (n=2)
100
Uncultured Corallinales GQ917512 AtlFrance
Phymatolithon cf. laevigatum KT807918 AtlSpain
100
Phymatolithon lenormandii KT863471 Norway (n=2)
Phymatolithon lenormandii KT807911 AtlSpain
88
Phymatolithon cf. brunneum KT807916 AtlSpain
100
P. cf. brunneum KT807909 AtlSpain
P. cf. brunneum KT807908 AtlSpain
88
Lithothamnion glaciale HM918812 Canada
82
100
L. corallioides KC861497 AtlFrance
L. corallioides KC861448 AtlFrance
Phymatolithon rugulosum KT863472 USA
Phymatolithon lusitanicum sp. nov. KC861627 Holotype (n=36)
P. lusitanicum KT807920 MedSpain
65
P. lusitanicum KC861630 Portugal (n=2)
82
P. lusitanicum KC861653 AtlSpain (n=3)
99
P. lusitanicum KC861643 Portugal
P. lusitanicum KC861661 Portugal (n=3)
P. lusitanicum KC861618 Portugal
99
Phymatolithon lamii KT807924 AtlFrance
63
P. lamii KT807919 AtlSpain
99
P. lamii KT807902 AtlSpain
P. lamii KT807917 AtlSpain (n=2)
P. lamii KT807912 AtlSpain
P. lamii KT807914 AtlFrance
P. lamii KT807923 AtlFrance (n=2)
P. lamii KT807926 AtlFrance
P. lamii KT807907 AtlFrance
P. lamii KT807915 AtlFrance
100
Phymatolithon lenormandii JX571978 Canada
P. lenormandii JX572005 Canada
Mesophyllum lichenoides GQ917249 AtlFrance
82
M. cf. erubescens GQ917256 Vanuatu
90
M. sphaericum KC861526 AtlSpain
1
2
3
4
5
6
7
8
9
10
11
12

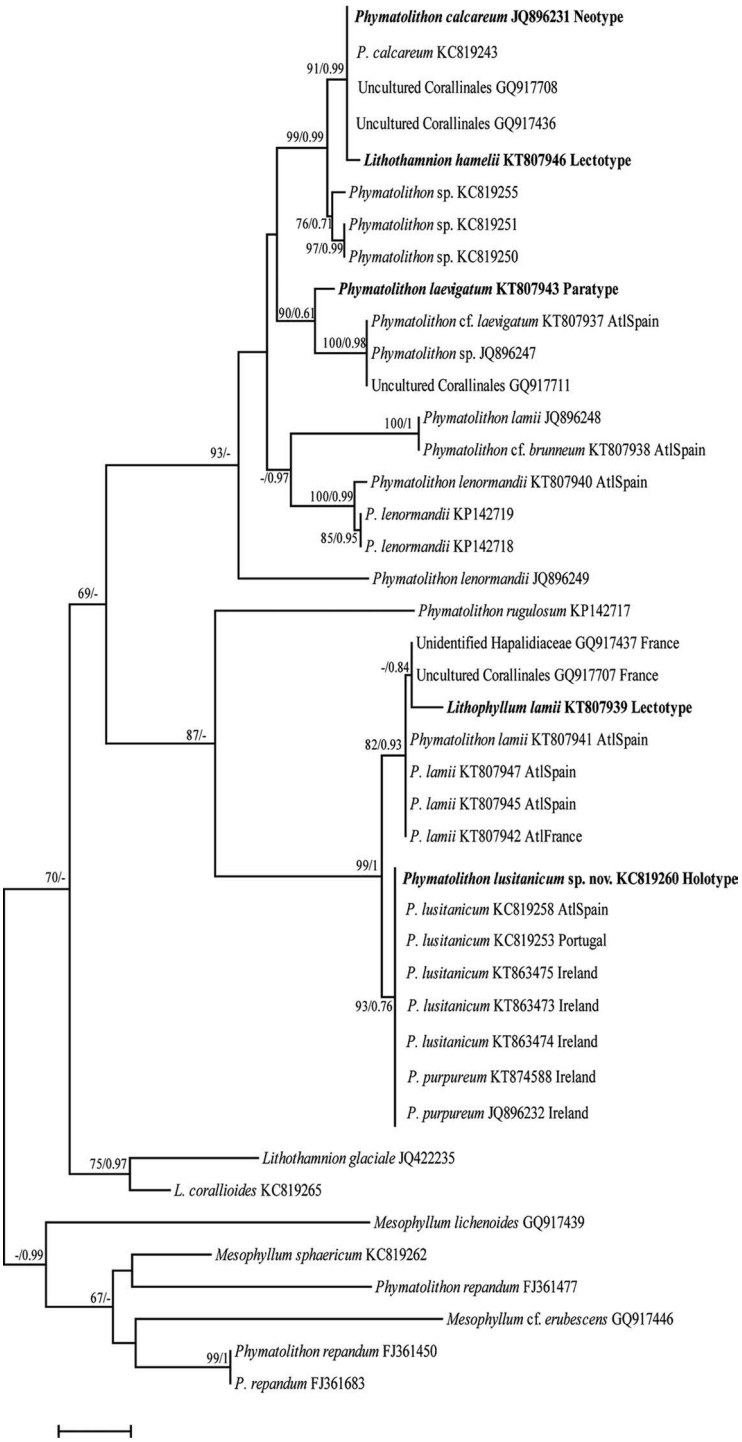
Morphological studies

Representative specimens of *Phymatolithon lusitanicum* and other *Phymatolithon* species from the Atlantic Iberian Peninsula were selected for anatomical examination with a scanning electron microscope (SEM, model JEOL JSM 6400, Universidade da Coruña, Spain). SEM analyses for specimens from Ireland followed Hernandez-Kantun *et al.* (2014). Particular attention was directed towards key features historically used to delimit *Phymatolithon* species regarding habit and growth form, and characters related to the multiporate sporangial conceptacles such as external shape and the presence or absence of a conspicuous rim (Chamberlain, 1991; Chamberlain & Irvine, 1994; Kaleb *et al.*, 2012). The anatomical terms medulla (equivalent to the terms hypothallium and core) and cortex (equivalent perithallium and peripheral region) follows Chamberlain & Irvine (1994). Cell length is the distance between primary pit-connections, and cell diameter is the measurement taken perpendicularly to this across the middle of the cell lumen. Conceptacle measurements were taken according to Adey & Adey (1973) and Chamberlain & Irvine (1994).

RESULTS

The COI-5P alignment of 177 sequences, including publicly available sequences from GenBank and BOLD, ranged from 538 to 664 base pairs (bp), and consisted of 46 haplotype sequences with 262 variable sites. The phylogenetic tree obtained from the ML analysis of the COI-5P alignment resolved *Phymatolithon* taxa studied into 12 supported lineages, 10 of them corresponding to European collections (Fig. 1, Table 1). Infralineage variation (uncorrected p-distance) ranged from 0% to 1.8%. The GMYC model of the COI-5P alignment supported the 12 *Phymatolithon* lineages, and it estimated a total of 17 putative Hapalidiales species with a confidence interval ranging from 16 to 19 taxa. The fit of the likelihood of the GMYC model was significantly higher ($P<0.001$) than that of a null model of uniform coalescent branching rates. The threshold time (estimated depth from the branch tips at which the transition from population to species level branching patterns occur) was determined at –0.00686 substitutions per site. Both GYMC and ML analyses of COI-5P data were congruent in delimiting the maerl collections from Atlantic Iberia and western Mediterranean Sea as an independent lineage separated from crustose specimens identified as *P. lamii*. Based on our collections, the diversity of *Phymatolithon* taxa in the European coasts is high (10 putative species). It is noteworthy that the two Canadian collections identified as *P. lenormandii* were remotely related to the remaining *Phymatolithon* included in the analyses (Fig. 1). The *psb*A alignment comprised 42 sequences ranging from 543 to 851 bp, with 204 variable sites. It included sequences from the type material of *Phymatolithon*

Fig. 2. Phylogenetic tree inferred from ML and BI analyses of *psb*A sequences of European *Phymatolithon* and publicly available sequences for this genus. In bold, holotype of *Phymatolithon lusitanicum sp. nov.*, and type material of *P. calcareum, P. lamii, P. laevigatum* and *Lithothamnion hamelii*. Bootstrap ML values > 60% and posterior probabilities > 0.60 from Bayesian inference are shown for each node. Members of subfamily Melobesioideae were used as outgroup. Scale bar: 0.02 substitutions per site.


Phymatolithon calcareum JQ896231 Neotype
P. calcareum KC819243
91/0.99
Uncultured Corallinales GQ917708
Uncultured Corallinales GQ917436
99/0.99
Lithothamnion hamelii KT807946 Lectotype
Phymatolithon sp. KC819255
76/0.77
Phymatolithon sp. KC819251
97/0.99
Phymatolithon sp. KC819250
Phymatolithon laevigatum KT807943 Paratype
90/0.61
Phymatolithon cf. *laevigatum* KT807937 AtlSpain
100/0.98
Phymatolithon sp. JQ896247
Uncultured Corallinales GQ917711
100/1
Phymatolithon lamii JQ896248
Phymatolithon cf. *brunneum* KT807938 AtlSpain
93/-
-/0.97
Phymatolithon lenormandii KT807940 AtlSpain
100/0.99
P. lenormandii KP142719
85/0.95
P. lenormandii KP142718
Phymatolithon lenormandii JQ896249
69/-
Phymatolithon rugulosum KP142717
Unidentified Hapalidiaceae GQ917437 France
-/0.84
Uncultured Corallinales GQ917707 France
Lithophyllum lamii KT807939 Lectotype
87/-
82/0.93
Phymatolithon lamii KT807941 AtlSpain
P. lamii KT807947 AtlSpain
P. lamii KT807945 AtlSpain
P. lamii KT807942 AtlFrance
99/1
70/-
Phymatolithon lusitanicum sp. nov. KC819260 Holotype
P. lusitanicum KC819258 AtlSpain
P. lusitanicum KC819253 Portugal
P. lusitanicum KT863475 Ireland
93/0.76
P. lusitanicum KT863473 Ireland
P. lusitanicum KT863474 Ireland
P. purpureum KT874588 Ireland
P. purpureum JQ896232 Ireland
75/0.97
Lithothamnion glaciale JQ422235
L. corallioides KC819265
Mesophyllum lichenoides GQ917439
-/0.99
Mesophyllum sphaericum KC819262
Phymatolithon repandum FJ361477
67/-
Mesophyllum cf. *erubescens* GQ917446
99/1
Phymatolithon repandum FJ361450
P. repandum FJ361683

calcareum, P. lamii, P. laevigatum and *Lithothamnion hamelii* while our attempts to amplify the *psb*A region for the type material of *P. lenormandii* and *P. purpureum* were unsuccessful. According to the molecular information obtained from type material of *Phymatolithon lamii*, our crustose collections were correctly identified (match = 98.9-99.1 %). Among the specimens identified as *P. lamii* were those collected at the type locality of *P. purpureum* (Fort du Mingant, France, VPF00223 represented in Fig. 2, together with VPF00222, VPF00225 and VPF00236, represented in Fig. 1, Table 1), indicating that the specimens belong to *P. lamii* and not to *P. purpureum*. Both ML and BI resolved the maerl-forming *P. lusitanicum* as a sister taxon to *P. lamii* with full support (99%/1 for ML and BI, respectively, Fig. 2). The clade of *P. lusitanicum* comprised collections from the Iberian Peninsula as well as maerl specimens from Ireland, the latter included one specimen previously assigned to *P. purpureum* (E28 as JQ896232, E10, E17, E113 and E302, Fig. 2, Table 1). Additionally, the paratype of *P. laevigatum* was closely related to collections from Ireland and from the Atlantic coasts of France and Spain, while the lectotype of *Lithothamnion hamelii* was conspecific with the neotype (Falmouth, England) and recent collections of *Phymatolithon calcareum* from Atlantic Europe (Fig. 2, Table 1). On the other hand, specimens identified as *P. lenormandii* from Norway, Ireland and Atlantic Spain represented three different clades within the genus *Phymatolithon*. By contrast, New Zealand collections of *P. repandum* were resolved as unrelated with other *Phymatolithon* species included in our analyses.

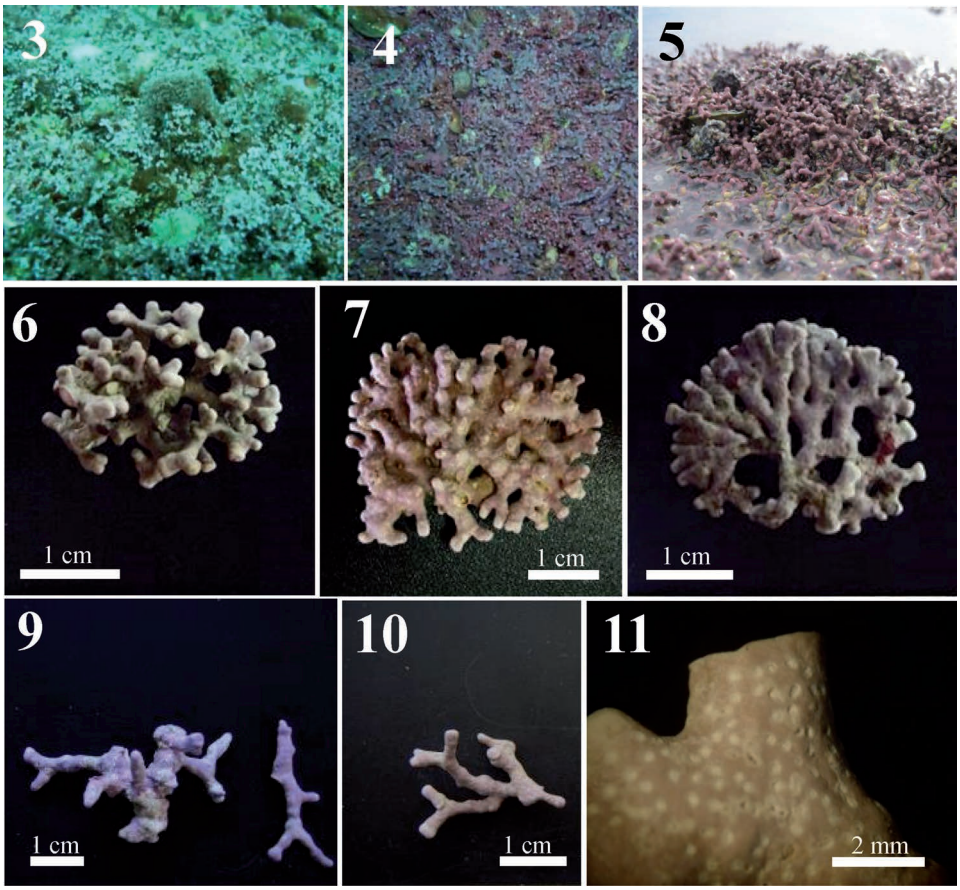
Given the molecular evidence shown above, we proposed to describe the new species of maerl as *Phymatolithon lusitanicum*.

***Phymatolithon lusitanicum* V. Peña sp. nov.** **Figs 3-21**

Diagnosis: Plant unattached, monomerous, scarcely to densely branched (Figs 3-11). Epithallial cells disposed in 1 (2) layers, cells domed, 1.5-2.5 µm long by 4 µm wide in vertical section (Fig. 14), 5-6 µm in diameter in surface view, polygonal with thick walls (Fig. 15). Subepithallial initials as short as or shorter than cells subtending them, 2-3 µm long by 4-8 µm wide. Cortex composed of cells 10-14 µm long by 5-7 µm wide. Cell fusions present (Fig. 14). Secondary pit-connections absent. Trichocytes absent. Sporangial conceptacles multiporate with a white color, without a conspicuous, thick raised rim, sometimes covered by a calcified cap (Figs 11, 16, 19), pore plate flushed with surface or slightly sunken, up to 30 pores, 130-170 µm in diameter (Figs 17-18). Chambers elliptical, (35) 40-75 µm high by 70-166 (175) µm wide with roof composed of 2-3 (4) cells, 12-25 µm thick (Figs 19-21). Buried conceptacles within the tissue not observed. Sexual uniporate conceptacles unknown.

Morphology: Non-geniculate, unattached, sparsely to densely branched (up to fifth-order branching), size ranged 0.20-7.88 cm^3, shape mainly discoidal and ellipsoidal (Figs 3-11). Colour pink – greyish to pale pink – to purple, texture smooth and matt when dried.

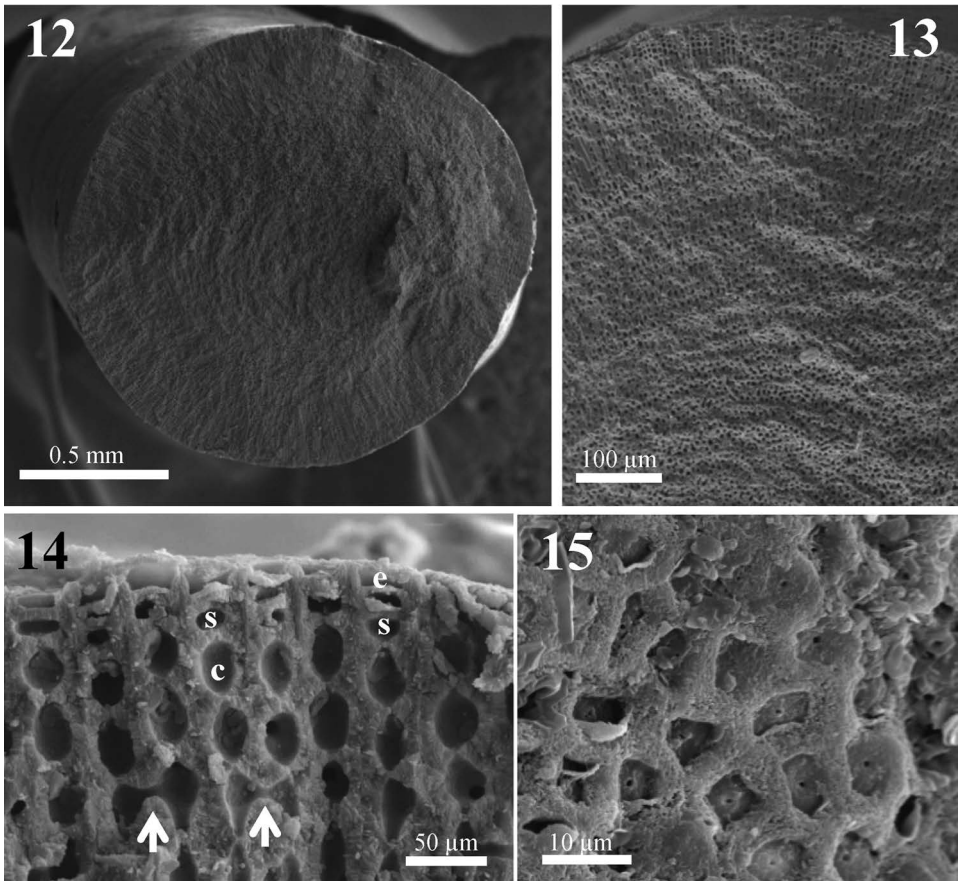
Anatomy: Pseudoparenchymatous, monomerous, and radially organized (Figs 12-13). Medullary cells 4-7 µm in diameter, cortical cells 10-14 µm × 5-7 µm. The subepithallial initials are as short as or shorter than cells subtending them, 2-3 µm long by 4-8 µm in diameter (Fig. 14). Each cortical filament produces one epithallial cell domed in transverse section; they are 1.5-2.5 µm long by 4 µm wide, and are disposed in 1 (2) layers (Fig. 14); in surface view (Fig. 15), epithallial cells are 5-6 µm in diameter, polygonal with thick walls of 2-3 µm (*Phymatolithon*-type SEM, as suggested by Chamberlain & Irvine 1994). Fusions cells present between



Figs 3-11. *Phymatolithon lusitanicum* sp. nov. **3.** *Phymatolithon lusitanicum* mixed with *P. calcareum* and *Lithothamnion corallioides* at the type locality (Con de Pego, Ría de Vigo, Galicia). **4.** *Phymatolithon lusitanicum* forming maerl at 20 m depth (Armaçao de Pêra, Algarve, south Portugal). **5.** Intertidal maerl of *P. lusitanicum* in Muckinish, Ireland. **6.** Holotype of *P. lusitanicum* showing an ellipsoidal shape (voucher SANT-Algae 29522). **7-8**. Discoidal specimens of *P. lusitanicum* collected in Galicia and the Algarve (vouchers SANT-Algae 29547 and SANT-Algae 29517, respectively). **9-10.** Collections of *P. lusitanicum* in Alborán Sea, Mediterranean (vouchers SANT-Algae 29506 and SANT-Algae 29508, respectively). **11.** Maerl branch showing multiporate asexual conceptacles without conspicuous raised rim and white pore plate (collection from Armaçao de Pêra, Algarve, south Portugal, SANT-Algae 29509).

cells of neighbouring filaments, secondary pit connections are absent (Fig. 14). Trichocytes are absent.

Reproductive structures: Sexual uniporate conceptacles not observed. Sporangial conceptacles multiporate, white coloured, without a conspicuous, thick raised rim, sometimes covered by a calcified cap (Figs 11, 16, 19), pore plate flush with surface or slightly sunken, 130-170 µm in diameter, up to 30 pores (Figs 17-18). Chambers were found empty, elliptical, (35) 40-75 µm high by 70-166 (175) µm wide, roof of mature conceptacles composed of 2-3 (4) cells, 12-25 µm thick

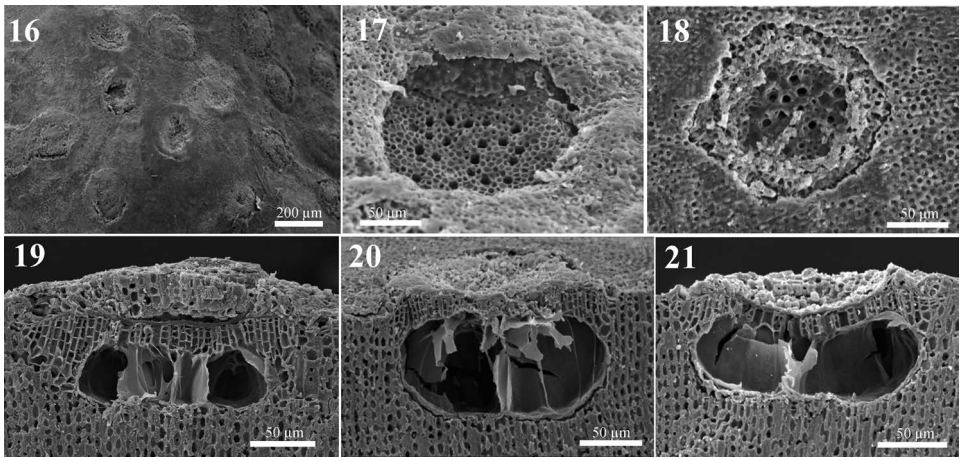


Figs 12-15. *Phymatolithon lusitanicum* sp. nov. **12-13.** Vertical section of the branch showing a monomerous structure (voucher SANT-Algae 29534). **14.** Vertical section showing domed epithallial cells (e), subepithallial cells as short as or shorter than cells subtending them (s), and cortical cells (c) joined by cell fusions (arrows) (voucher SANT-Algae 29534). **15.** Surface view of epithallial cells polygonal with thick walls (*Phymatolithon*-type) (voucher SANT-Algae 29510)

(Figs 19-21). Tetrasporangia or bisporangia not observed. Buried multiporate senescent conceptacles not observed within the thallus. Sporangial conceptacles occasionally recorded, from the Algarve collections in May, June and September, and from Ireland in autumn (September- November).

Holotype: voucher CPVP-676 (COI-5P: KC861627; *psb*A: KC819260), Con de Pego, Ría de Vigo, Galicia, Spain; 42º 15.498 N, 8º 45.087 W, 5 m depth, collectors V. Peña, I. Bárbara & R. Barreiro, 05-iv-2011, SANT-Algae 29522 (Fig. 6, Table 1). Isotypes: CPVP-689 (COI-5P: KC861620; SANT-Algae 29523) and CPVP-685 (COI-5P: KC861621; SANT-Algae 29524).

Complementary collections: voucher CPVP-639 (COI-5P: KC861642, *psb*A: KC819258), Playa de Tulla, Ría de Pontevedra, Galicia, Spain, 9 m depth, collectors V. Peña, I. Bárbara & R. Barreiro, 07-iv-2011, SANT-Algae 29547



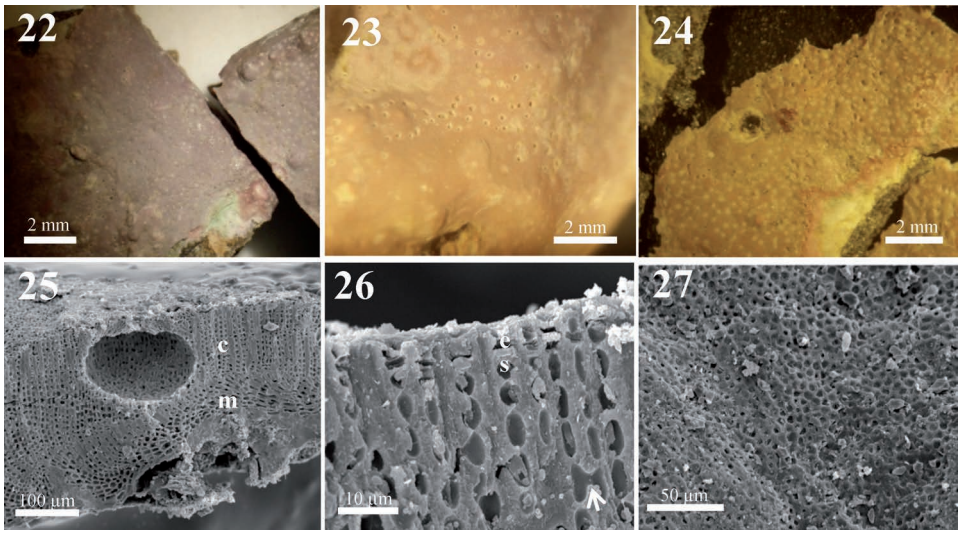
Figs 16-21. *Phymatolithon lusitanicum* sp. nov. **16.** Surface view of multiporate asexual conceptacles with calcified cap, without a conspicuous, thick raised rim (voucher E302, SANT-Algae 29597). **17-18.** Surface view of multiporate asexual conceptacles without a conspicuous raised rim and pore plate flush with the surface or slightly sunken (voucher SANT-Algae 29509). **19.** Vertical section of a multiporate sporangial conceptacle covered by a calcified cap (voucher E302; SANT-Algae 29597). **20-21.** Vertical section showing multiporate sporangial conceptacles with an elliptical chamber empty and clearly laminated, roof composed of 2-3 (-4) cells thick (voucher E302; SANT-Algae 29597).

(Fig. 7); voucher CPVP-478 (COI-5P: KC861630; *psb*A: KC819253), Armaçao de Pêra, Algarve, Portugal, 20 m depth; collectors P. Neves & M. Rodrigues, 02-iii-2011, SANT-Algae 29517 (Fig. 8); voucher VPF00451 (COI-5P:KT807910), Alborán Sea, Mediterranean Sea, 48 m depth, collector INDEMARES project, 24-ix-2011, SANT-Algae 29506 (Fig. 9); voucher VPF00511 (COI-5P:KT807920), Alborán Sea, Mediterranean Sea, 40–44 m depth, collector INDEMARES project, 22-ix-2011, SANT-Algae 29508 (Fig. 10); voucher SANT-Algae 29509, Armaçao de Pêra, Algarve, Portugal, 20 m depth; collectors V. Peña & I. Bárbara, 02-ix-2008, (Fig. 11); voucher SANT-Algae 29510, Armaçao de Pêra, Algarve, Portugal, 20 m depth; collectors V. Peña & I. Bárbara.

Etymology: The specific epithet refers to the range of distribution in which the species is more abundant, the Lusitania biogeographic province.

Habitat: *Phymatolithon lusitanicum* mainly occurs subtidally as maerl (rhodoliths) in relatively shallow beds in Galicia (4-13 m, Fig. 3), and in Ireland (6 m), but deeper in southern Portugal (15-20 m, Fig. 4), in the Mediterranean Alborán Sea (40-48 m), and in the-Balearic Islands (54-64 m, Hernandez-Kantun *et al.*, 2014, as *P. purpureum*). The species is uncommonly reported from an intertidal bed in Muckinish, Ireland (E302, Table 1, Fig. 5, Hernandez-Kantun *et al.*, 2014, as *P. purpureum*). In Galicia, *P. lusitanicum* is usually found mixed with *P. calcareum* and *Lithothamnion corallioides*, and with *Mesophyllum sphaericum* in the only locality where the latter has been reported in the Atlantic Iberian Peninsula.

Distribution: Ireland (Kingstown Bay, Carraroe beach and Muckinish), Atlantic Iberian Peninsula (Galicia and the Algarve), and western Mediterranean Sea (Alborán Sea and the Balearic Islands).



Figs 22-27. *Phymatolithon lamii.* **22-24.** Crustose, epilithic specimens showing conceptacles without conspicous raised rim, pore plates white-coloured, initially sunken forming deep holes or flush with surface at maturity (vouchers SANT-Algae 29501, SANT-Algae 29477 and SANT-Algae 29491, respectively). **25.** Vertical section showing medullary (m) and cortical (c) cells, and uniporate sexual conceptacle empty (voucher SANT-Algae 29501). **26.** Vertical section showing domed epithallial cells (e), subepithallial cells as short as or shorter than cells subtending them (s), and cortical cells joined by cell fusions (arrow) (voucher SANT-Algae 29498). **27.** Surface view of epithallial cells polygonal with thick walls (*Phymatolithon*-type) (voucher SANT-Algae 29498).

Comments*: Phymatolithon lusitanicum* differs from other *Phymatolithon* species reported from European coasts mainly by the external shape of the multiporate asexual conceptacles (pore plate flush with surface or slightly sunken and without a conspicuous, thick raised rim), and its unattached habit as maerl/rhodolith. In addition, recent collections of *P. lamii* matched the habit and ecology described in the literature (Chamberlain 1991; Chamberlain & Irvine, 1994, Kaleb *et al.,* 2012) because they were crustose, epilithic specimens with uniporate sexual and multiporate asexual conceptacles appearing initially as deep holes, or more or less flush with surface and showing white pore plates at maturity (Figs 22-27). They were collected from the intertidal to the shallow subtidal (down to 10 m), in rocky shores but also associated with maerl beds in Brittany (Table 1).

DISCUSSION AND CONCLUSIONS

Phymatolithon lusitanicum is described as the third major maerl-forming species in the Atlantic Iberian Peninsula although its distribution range extends to subtidal and also intertidal maerl beds from Ireland to Western Mediterranean Sea (Alborán Sea and the Balearic Islands). In Galician maerl beds, *P. lusitanicum* has been usually detected mixed with *P. calcareum* and *Lithothamnion corallioides* as

well as *Mesophyllum sphaericum*, although in southern Portugal it is reported as the dominant maerl species (Carro *et al.,* 2014; Pardo *et al.,* 2014, as "*Phymatolithon* sp.3"). Based on this evidence, it is likely that some of the previous records of *P. calcareum* in the Atlantic Iberian Peninsula (Peña & Bárbara, 2008; Hall-Spencer *et al.,* 2010), and particularly in southern Portugal (Peña *et al.,* 2009), might be actually referred to *P. lusitanicum*. Likewise, the detection of *P. lusitanicum* in maerl collections from Ireland that had previously been assigned to *P. purpureum* (E28, E113, Hernandez *et al.,* 2014, 2015) suggests that other misidentifications may exist in the literature (Adey & Adey 1973; Blunden *et al.,* 1981), and highlights that more detailed studies are required to get a better delimitation of these two species and to clarify the occurrence of *P. purpureum* as a maerl-forming species. According to Chamberlain & Irvine (1994), *P. purpureum* is characterized by showing multiporate sporangial conceptacles with thick, tyre-like, raised rims up to 100 μm wide. Similarly, the Mediterranean maerl collections from the Balearic Islands originally assigned to *P. purpureum* (E242, E245, E269-E270 in Hernández-Kantún *et al.,* 2014) must be considered *P. lusitanicum* according to their conspecificity with Irish collections (E113), challenging the occurrence of *P. purpureum* in the Mediterranean. According to the evidence presented here and in previous studies that used molecular tools to investigate extensive collections of maerl along Atlantic European coasts (Carro *et al.,* 2014; Pardo *et al.,* 2014, Hernández-Kantún *et al*., 2015), *P. lusitanicum* reaches western Ireland in the north but has gone undetected in Britain and France. In this regard, the disjunct distribution of *P. lusitanicum* contrasts with the pattern shown by the other two maerl species that typically occur mixed with it and that overwhelmingly dominate maerl beds in France and southern Britain (*P. calcareum* and *Lithothamnion corallioides*). Hence, further research with appropriate molecular tools on maerl beds from both regions (Britain, France) are required to clarify the discontinuity observed in the range of *P. lusitanicum*.

The collections studied here together with results derived from Hernández-Kantún *et al.* (2014, 2015) show that *Phymatolithon lusitanicum* occurs in shallow maerl beds in Galicia and in Ireland (even intertidally), while it was found at greater depths (from 15-64 m) in Algarve and Western Mediterranean Sea (Alborán Sea, Balearic Islands). The migration to greater depths from the Atlantic to the Mediterranean is shared with other Atlantic maerl-forming taxa recorded in the Mediterranean (*P. calcareum, Lithothamnion corallioides, Mesophyllum sphaericum*) as well as with other non-geniculate species (*M. expansum* (Philippi) Cabioch & M.L.Mendoza) (Ballesteros, 1988; Bressan & Babbini 2003; Peña *et al.,* 2015). In agreement with the predominance of sterile specimens typical of the main maerl-forming species reported in temperate Atlantic European beds (*P. calcareum, L. corallioides*), *P. lusitanicum* rarely showed reproductive structures in our collections. Nonetheless, we still were able to detect multiporate sporangial conceptacles in some collections of *P. lusitanicum* from southern Portugal (May, June and September) and from Ireland (September - November). In the literature, previous records of sexual uniporate conceptacles in crustose forms of *P. calcareum* (Mendoza & Cabioch, 1998) were recently confirmed with DNA barcodes (Peña *et al.*, 2014), which also suggested that vegetative multiplication by fragmentation could be the main mechanism of propagation in maerl beds.

Our molecular results that included the lectotype of *P. lamii* and the paratype of *P. laevigatum* allowed us to delimit *P. lusitanicum* by an integrative systematic approach. The three species shared a common external morphology of multiporate asexual conceptacles. However, *P. lamii* and *P. laevigatum* have never been reported as maerl-forming taxa (Adey & Adey, 1973; Chamberlain, 1991; Chamberlain &

Irvine, 1994). The sequence from the paratype of *P. laevigatum* was resolved as closely related to recent collections from Ireland and Atlantic coasts of France and Spain. This species was described from Helgoland (Germany), and has been reported as epilithic crusts that have multiporate sporangial conceptacles with a thickened rim, somewhat raised, and pore plate flush with surface, sunken or slightly raised, up to *ca.* 5 cells thick (Chamberlain & Irvine 1994). The present study also confirms literature records of *P. lamii* in intertidal and subtidal rocky shores along the Atlantic Iberian Peninsula (Ardré, 1970; Chamberlain & Irvine, 1994), being Algarve and Cádiz the southernmost localities. To date, *P. lamii* has been found only epilithic as a crustose growth-form, even in locations where it was found in maerl beds. Moreover, this is the first time that *P. lamii* is reported for subtidal maerl in Brittany (Adey & Adey, 1973; Chamberlain, 1991; Chamberlain & Irvine, 1994). Finally, the comparison of our sequences from the type material of *P. lamii* with one specimen from NW Atlantic identified as *P. rugulosum* (voucher US170942) does not support the synonymization of these species proposed in Chamberlain (1991). *Phymatolithon rugulosum* was described by Adey (1964) from the Gulf of Maine, the same locality of our collection, but DNA sequences from its type material will be needed to draw more definitive conclusions.

Our phylogenetic analyses resolved the new maerl species *P. lusitanicum* and the crustose *P. lamii* as a sister taxon with full support, indicating that both species share a very recent common ancestor. Furthermore, the presence of crustose *P. lamii* in subtidal maerl beds of Brittany suggests that the speciation event is correlated to two clearly delimited growth forms able to live under similar ecological conditions. Moreover, *P. lusitanicum* and *P. lamii* were resolved in a separate clade distantly related to other European *Phymatolithon* species that included the generitype *P. calcareum*.

An additional result derived from our molecular analyses is that *P. calcareum* and *Lithothamnion hamelii* must be regarded as conspecific. Importantly, this conclusion is based on sequence information obtained from neotype and lectotype material, respectively. Marie Lemoine (1931) described *L. hamelii* but the lectotype material was designated more recently by Woelkerling & Lamy (1998). According to the priority principle of the International Code of Nomenclature (article 11, ICN 2012), we propose *L. hamelii* as a heterotypic synonym of *P. calcareum.* Contrary to the common habit of *P. calcareum* as maerl, the lectotype of *L. hamelii* was an epilithic, crustose specimen collected by dredge in La Rance, Saint Servan (France) by Robert Lami in 1930. Apart from *L. hamelii*, Marie Lemoine (1931) reported other Melobesioideae species in Saint Servan such as *P. calcareum*, also subtidally in the same locality.

Finally, our COI-5P analyses revealed that *Phymatolithon* contains a high diversity of species in Atlantic European coasts with up to 10 supported lineages, while only seven are reported in the literature (Guiry & Guiry, 2015). In addition, the analyses of COI-5P and *psb*A resolved several lineages for which specimens were not identified up to the species level, while other lineages encompassed specimens assigned to different species name. Further efforts in sequencing type material from other European species that failed in the present study (*Melobesia lenormandii* and *Lithothamnion purpureum*) will be required to provide an appropriate taxonomic baseline to clarify their identity. Alternatively, recent topotype material could be selected, but it should be studied with caution given the frequent co-occurrence of several *Phymatolithon* species in the same habitat. This observation has been pointed out in the literature (Chamberlain & Irvine, 1994), and has been confirmed in the present study where specimens collected in the intertidal of the type

locality of *P. purpureum* belonged to *P. lamii*. The presence of different clades with the name *P. lenormandii* and the absence of a DNA sequence from the type material cannot guaranty that the actual species is represented in our phylogeny. In addition, collections identified as *P. lenormandii* from Canada and *P. repandum* from New Zealand were resolved unrelated with other *Phymatolithon* taxa that, importantly, comprised sequences derived from type material for the generitype (*P. calcareum*). Hence, the inclusion of these taxa in our data set strongly suggests that the current concept and definition of the genus *Phymatolithon* and its relationship with related genera (e.g. *Lithothamnion, Mesophyllum*) requires further evaluation.

Our understanding of the diversity of non-geniculate coralline algae in Europe has been much improved in the last five years by with new DNA and morpho-anatomical data; as a result, two new species are now added as maerl-forming: *Phymatolithon lusitanicum* and *Mesophyllum sphaericum*. Previous reports that describe *P. calcareum* and *Lithothamnion corallioides* as the major builders of maerl beds in Europe need to be updated in both European and national legislation in order to ensure appropriate protection of these sensitive biogenic habitats.

Acknowledgments. Financial support came from the following institutions and projects: Action Transversale du Muséum National d'Histoire Naturelle ("*Taxonomie moléculaire: DNA Barcode et gestion durable des collections*" and "*Biodiversité actuelle et fossile. Crises, stress, restaurations et panchronisme: le message systématique*" and"*Emergences*"), British Phycological Society (Small Grant Scheme-Project Award 2011-2012), Spain´s Ministerio de Economía y Competitividad (CTM2010-18787, partially founded by ERDF) and Xunta de Galicia (10MMA103003PR). Acquisition of molecular data was carried out at the CNRS-UMS 2700 in Service de Systématique Moléculaire, MNHN, Paris. This project was supported by the network 'Bibliothèque du Vivant' funded by CNRS, Muséum National d'Histoire Naturelle, INRA and CEA (Centre National de Séquençage). VPF acknowledges support by the postdoctoral programmes Axudas de apoio á etapa inicial de formación posdoutoral do Plan I2C (Xunta de Galicia). CP acknowledges support by Programa FPU-2010 (Spain´s Ministerio de Educación). JJHK acknowledges support by the Smithsonian Institution through a postdoctoral fellowship and Gabriel Johnson through technical assistance. We sincerely thank Jacques Grall, Antonio Secilla, Alberto Santolaria, Jan Rueness, Francis Bunker, Verónica García, Pilar Díaz, Isabel Maneiro, Pedro Neves, Meadhbh Moriarty, Declan Hanniffy and Miguel Rodrigues for field collaboration and/or for providing samples. We also thank project INDEMARES for providing samples from Alborán Sea. We are also grateful to both reviewers for their helpful comments on the manuscript.

REFERENCES

ADEY W.H., 1964 — The genus *Phymatolithon* in the Gulf of Maine. *Hydrobiologia* 24(2): 377-420.

ADEY W.H. & ADEY P.J., 1973 — Studies on the biosystematics and ecology of epilithic crustose Corallinaceae of the British Isles. *British phycological journal* 8: 343-407.

ADEY W.H., HERNANDEZ-KANTUN J.J., JOHNSON G. & GABRIELSON P.W., 2015 — DNA sequencing, anatomy, and calcification patterns support a monophyletic, subarctic, carbonate reef-forming *Clathromorphum* (Hapalidiaceae, Corallinales, Rhodophyta). *Journal of phycology* 51: 189-203.

ARDRÉ F., 1970 — Contribution à l'étude des algues marines du Portugal. I. La Flore. *Portugaliae acta biologica, Série B, Sistemática, ecologia, biogeografia e paleontologia* 10: 137-555.

BABBINI L. & BRESSAN G., 1997 — *Recensement de Corallinacées de la Mer Méditerranée et considérations phytogéographiques*. In: *Bibliotheca phycologica*. Berlin, J. Cramer, 421 p.

BALLESTEROS E., 1988 — Composición y estructura de los fondos de maërl de Tossa de Mar (Gerona, España). *Collectanea botanica* 17: 161-82.

BÁRBARA I., CALVO S., CREMADES J., DÍAZ P., DOSIL J., PEÑA V., LÓPEZ VARELA C., NOVO T., VEIGA A.J, & LÓPEZ RODRÍGUEZ M.C., 2003 — Fragmenta Chorologica Occidentalia, Algae, 8641-8747. *Anales del jardín botánico de Madrid* 60: 409-416.

BITTNER L., 2009 — *Phylogénie des Corallinales (Rhodophyta) et analyse de leur diversité génétique dans le Pacifique Sud.* PhD Thesis, Muséum National d´Histoire Naturelle, Paris.

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.

BLUNDEN G., FARNHAM W.F., JEPHSON N., BARWELL C.J., FENN R.H. & PLUNKETT B.A., 1981 — The composition of maërl beds of economic interest in Northern Brittany, Cornwall and Ireland. *Xth International Seaweed Symposium* 10: 651-6.

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

CARRO B., LÓPEZ L., PEÑA V., BÁRBARA I. & BARREIRO R., 2014 — DNA barcoding allows the accurate assessment of European maerl diversity: a Proof-of-Concept study. *Phytotaxa* 190: 176-89.

CHAMBERLAIN Y.M., 1991 — Observations on *Phymatolithon lamii* (Lemoine) Y. Chamberlain comb. nov. (Rhodophyta, Corallinales) in the British Isles with an assessment of its relationship to *P. rugulosum*, *Lithophyllum lamii* and *L. melobesioides*. *British Phycological Journal* 26: 219-33.

CHAMBERLAIN Y.M. & IRVINE L.M., 1994 — Melobesioideae Bizzozero. In: Irvine L.M. & Chamberlain Y.M. (Eds), *Seaweeds of the British Isles*. Volume 1. Rhodophyta Part 2B Corallinales, Hildenbrandiales, London: HMSO, pp. 159-234

DARRIBA D., TABOADA G.L., DOALLO R. & POSADA D., 2012 — jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772.

DRUMMOND A.J., SUCHARD M.A., XIE D. & RAMBAUT A., 2012 — Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular biology and evolution* 29: 1969-73.

FOSLIE M., 1900 — New or critical calcareous algae. *Det kongelige Norske videnskabernes selskab, Skrifter* 1899: 1-34.

FUJISAWA T. & BARRACLOUGH T.G., 2013 — Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent (GMYC) approach: a revised method and evaluation on simulated datasets. *Systematic biology* 62: 707-24.

GOUY M., GUINDON S. & GASCUEL O., 2010 — SeaView Version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular biology and evolution* 27: 221-4.

GUIRY M.D. & GUIRY G.M., 2015 — *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 08 June 2015.

HALL-SPENCER J.M., KELLY J. & MAGGS C.A., 2010 — *Background document for maërl beds.* (O. C. B. S. 491/2010, ed.) OSPAR Commission. 36 p.

HERNANDEZ-KANTUN J.J., RIOSMENA-RODRIGUEZ R., ADEY W.H. & RINDI F., 2014 — Analysis of the *cox*2-3 spacer region for polulation diversity and taxonomic implications in rhodolith- forming species (Rhodophyta: Corallinales). *Phytotaxa* 190: 331-54.

HERNANDEZ-KANTUN J.J, RIOSMENA-RODRÍGUEZ R., HALL-SPENCER J.M., PEÑA V., MAGGS C.A. & RINDI F., 2015 — Phylogenetic analysis of rhodolith formation in the Corallinales (Rhodophyta). *European journal of phycology* 50: 46-61.

ICN-International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). 2012. *Regnum Vegetabile 154*. Koeltz Scientific Books. ISBN 978-3-87429-425-6.

KALEB S., FALACE A. & WOELKERLING W.J., 2012 — *Phymatolithon lamii* (Hapalidiaceae, Corallinales, Rhodophyta): a first report for the Mediterranean Sea. *Botanica marina* 55:377-85.

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: 374-89.

LEMOINE M., 1931 — Les algues mélobésiées de la région de Saint-Servan. *Bulletin du laboratoire maritime du Muséum d'histoire naturelle de Saint-Servan* 7:1-20.

MENDOZA M.L. & CABIOCH 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: 1433-45.

PARDO C., LOPEZ L, PEÑA V., HERNANDEZ-KANTUN J.J., LE GALL L., BARBARA I. & BARREIRO R., 2014 — A multilocus species delimitation reveals a striking number of maërl species in the OSPAR area. *PLoS ONE* 9: e104073.

PEÑA V. & BÁRBARA I., 2008 — Maërl community in the north-western Iberian peninsula: a review of floristic studies and long-term changes. *Aquatic conservation: Marine and freshwater ecosystems* 18: 339-66.

PEÑA V., BÁRBARA I., BERECIBAR E. & SANTOS R., 2009 — Present distribution of maërl beds in the Atlantic Iberian Peninsula. *Museologia scientifica e Naturalistica, volumen speciale 6th Regional Symposium of the International Fossil Algae Association*: 46.

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 maërl-forming species from the northeast Atlantic. *Journal of phycology* 47: 911-27.

PEÑA V., HERNANDEZ-KANTUN J.J., GRALL J., PARDO C., LÓPEZ L., BÁRBARA I., LE GALL L. & BARREIRO R., 2014 — Detection of gametophytes in the maerl-forming species *Phymatolithon calcareum* (Melobesioideae, Corallinales) assessed by DNA barcoding. *Cryptogamie, Algologie* 35: 15-25.

PEÑA V., DE CLERCK O., AFONSO-CARRILLO J., BALLESTEROS E., BÁRBARA I., BARREIRO R. & LE GALL L., 2015 — An integrative systematic approach to species diversity and distribution in the genus *Mesophyllum* (Corallinales, Rhodophyta) in Atlantic and Mediterranean Europe. *European journal of phycology* 50: 20-36.

PONS J., BARRACLOUGH T.G., GOMEZ-ZURITA J., CARDOSO A., DURAN D.P., HAZELL S., KAMOUN S., SUMLIN W.D. & VOGLER A.P., 2006 — Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic biology* 55: 595-609.

RATNASINGHAM S. & HEBERT P.D.N., 2007 — BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular ecology notes* 7: 355-364.

RONQUIST F. & HUELSENBECK J.P., 2003 — MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

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: 1879-88.

SAUNDERS G.W. & MCDEVIT D.C., 2012 — Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. In: Kress W.J. & Erickson D.L. (eds), *DNA Barcodes: Methods and Protocols,* New York, Human Press, pp. 207-222.

TAMURA K., STECHER G., PETERSON D., FILIPSKI A. & KUMAR S., 2013 — MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular biology and evolution* 30: 2725-9.

TEICHERT S., WOELKERLING W., RÜGGEBERG A., WISSHAK M., PIEPENBURG D., MEYERHÖFER M., FORM A., BÜDENBENDER J. & FREIWALD A., 2012 — Rhodolith beds (Corallinales, Rhodophyta) and their physical and biological environment at 80º 31´N in Nordkappbukta (Nordaustlandet, Svalbard Archipelago, Norway). *Phycologia* 51: 371-90.

THIERS B., 2015 — Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/. Revised June 2015.

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

WOELKERLING W.J., 1988 — *The Coralline Red Algae: an analysis of the genera and subfamilies of nongeniculate Corallinaceae*. London, Oxford, Oxford University Press, 268 p.

WOELKERLING W.J. & LAMY D., 1998 — *Non-geniculate coralline red algae and the Paris Muséum: systematics and scientific history.* Paris, Publications Scientifiques du Muséum, 767 p.

YOON S.H., HACKETT J.D. & BHATTACHARYA D., 2002 — A single origin of the peridinin — and fucoxanthin — containing plastids in dinoflagellates through tertiary endosymbiosis. *Proceedings of the national academy of sciences USA* 99: 11724-9.