Assessment of Coralline Species Diversity in the European Coasts Supported by Sequencing of Type Material: The Case Study of Lithophyllum nitorum (Corallinales, Rhodophyta)

Viviana Pena, Jazmin J Hernandez-Kantun, Walter H Adey, Line Le Gall

To cite this version:

HAL Id: hal-01787113
https://hal.sorbonne-universite.fr/hal-01787113
Submitted on 7 May 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Assessment of coralline species diversity in the European coasts supported by sequencing of type material: the case study of Lithophyllum nitorum (Corallinales, Rhodophyta).

Viviana PEÑAa,b, Jazmin J. HERNANDEZ-KANTUNE, Walter H. ADEYc, & Line LE GALLb

a BIOCOST Research Group & CICA, Universidade da Coruña, Campus de A Coruña, 15071, A Coruña, Spain.

b Equipe Exploration, Espèces et Evolution, Institut de Systématique, Evolution, 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.

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

Running title: Lithophyllum nitorum in the European coasts
Abstract

A constant effort in sequencing an extensive number of specimens originating from comprehensive sampling had return an unprecedented amount of information fostering our understanding of diversity, evolution and distribution of coralline algae; however, many sequences lack reliable assignation of a taxonomic name, specially at the species level. Recently, the sequencing of type material allowed to bridge this gap by providing a direct link between the DNA sequence and the type bearing name. For instance, in the genus *Lithophyllum*, the identity of three species, generally abundant along the European Atlantic and the Mediterranean, was demonstrated by including sequences of the type material. Nevertheless, for less conspicuous species, such as *Lithophyllum nitorum*, data are still needed to assess distribution, anatomy, phylogenetic affinities and taxonomic status. Using DNA sequences recovered from the type material of *L. nitorum*, further recent collections were resolved as conspecific and used to improve the description and refine the distribution of this species. *Lithophyllum nitorum* consisted of subtidal, thin crusts overgrowing fragments of dead maerl, pebbles and drifted fragments of fishing lines. The presence of uniporate conceptacles markedly protruding can be considered an external morphological feature useful to identify *L. nitorum* from other subtidal *Lithophyllum* species with similar habitat. *Lithophyllum nitorum* is reported for the Mediterranean Sea and confirmed in the North East Atlantic, a similar distribution as for *L. incrustans* and *L. hibernicum*.

Keywords: Biodiversity, Corallinales, DNA barcoding, distribution, *Lithophyllum*, maerl beds, Mediterranean, North East Atlantic, systematics, type collections.
INTRODUCTION

*Lithophyllum* Philippi is considered one of the most speciose non-geniculate coralline algal genera with 130 specific/infraspecific names currently accepted (Guiry & Guiry, 2017). This genus is mainly distributed in tropical and temperate waters, where it contributes to the formation of characteristic calcareous bioconstructions such as the Caribbean algal ridges (Adey, 1978), the *coralligenous* and trottoir communities in the Mediterranean Sea (Ballesteros, 2006; Pezzolesi *et al*., 2016), and singular maerl beds in the North East Atlantic (Peña *et al*., 2013; Hernández-Kantún *et al*., 2015). In this latter region, recent literature pointed out 12 species of *Lithophyllum* occurring under different growth-forms, from the intertidal to subtidal: the generitype *Lithophyllum incrustans* Philippi, together with *L. bathyporum* Athanasiadis & Ballantine, *L. byssoides* (Lamarck) Foslie, *L. crouaniorum* Foslie, *L. cystosirae* (Hauck) Heydrich, *L. decussatum* (Ellis & Solander) Philippi, *L. dentatum* (Kützing) Foslie, *L. hibericum* Foslie, *L. nitorum* W.H.Adey & P.J.Adey, *L. orbiculatum* (Foslie) Foslie, *L. papillosum* (Zanardini ex Hauck) Foslie and *L. vickersiae* Me.Lemoine (Chamberlain & Irvine, 1994; Hernandez-Kantún *et al*., 2015, reviewed in Lugilde *et al*., 2016). So far, only three taxa - *L. incrustans*, *L. hibericum* and *L. bathyporum* - have been subject of molecular studies using type material and recent collections from a wide geographic area (Hernandez-Kantun *et al*., 2015). It allowed a reassessment of the phylogenetic affinities of these species, and the consideration of earlier collections identified as *L. duckerae* (Cornwall), *L. fasciculatum* and *L. dentatum* (Ireland and Britany) as *L. hibericum* and *L. incrustans*, respectively. These results also demonstrated that *L. hibericum* and *L. incrustans* have a high morphological plasticity, for which features mainly related with epithallial cells and sporangial conceptacles were useful for their identification (Hernandez-Kantun *et al*., 2015, Vázquez & Peña, 2016). A fourth species - *L. byssoides* - has been also reassessed based on Atlantic and Mediterranean collections; however, molecular information from
the lectotype (*Nullipora byssoides* Lamarck) is still pending to be obtained for a better clarification (Pezzolesi *et al*., 2016).

With regards to the habitat, while the crustose *Lithophyllum hibernicum* (as *L. incrustans* in the literature before 2015) and *L. orbiculatum* are common in the intertidal, and *L. crouaniorum* in the subtidal (Adey & Adey, 1973; Chamberlain *et al*., 1988, 1991; Chamberlain & Irvine, 1994). *L. nitorum* is considered a rare species, occurring on rock and shells only in the sublittoral zone down to 46 m (Adey & Adey, 1973; Chamberlain & Irvine, 1994). *Lithophyllum nitorum* was described by Adey & Adey (1973), and it was mainly characterized by small, adherent crusts relatively thin, with orange-pink colour, a smooth surface without orbital ridges, highly to somewhat glossy, a single layer of epithallial cells (rarely two), short subepithallial cells, cortical cells of contiguous filaments usually laterally aligned, conceptacles markedly protruding, usually bisporangial with a pore canal tapering to the surface and lined by small papillae, and sporangial chambers not globular with a small columella (Adey & Adey, 1973; Chamberlain & Irvine, 1994). In addition, Chamberlain & Irvine (1994) pointed out the absence of secondary growth and growth over *Lithothamnion sonderi* Hauck as key characters to identify plants of *L. nitorum* in the British Isles. The type locality of *L. nitorum* is Port Erin Harbour (Isle of Man, 3-9 m depth, as epilithic in Adey & Adey, 1973), but it was also reported southwards to northern Spain (Chamberlain & Irvine, 1994); in fact, Adey & Adey (1973) recorded *L. nitorum* at most of the Bay of Biscay stations sampled, reaching 24% abundance at 37-46 m off Castro Urdiales (Cantabrian Sea) compared to 14% between 8-30 m in the British Isles, particularly abundant off the Lizard (Cornwall) while it occurs sporadically northwards (*ca.* 0.2-0.7 % mean abundance, fig. 53 in Adey & Adey, 1973). In the Mediterranean Spain, Braga & Aguirre (1995) observed plants attributed to *L. nitorum* in Almería and in Cabrera (Balearic Islands), and reported it in southern Spain as minor component of fossil coralline associations in the Neogene (Miocene and Pliocene, *ca.* 23 million years ago).

With the aim of re-assessing coralline species diversity and distributions along the
European coasts, the sequencing of type specimens coupled with extensive sampling across a wide geographic area became the most efficient practice to refine the concept of a species (Puillandre et al., 2011). In the present study, we obtained DNA sequences (psbA and COI-5P) from the holotype of *Lithophyllum nitorum*, and we inferred species delimitation among recent collections made along the European coasts to assess the distribution of this species.

**MATERIAL AND METHODS**

**Collections studied**

We examined the holotype of *Lithophyllum nitorum*, collected in Port Erin Harbour, Isle of Man, at 3-9 m depth (Adey & Adey, 1973), and preserved in the herbarium of the Smithsonian Institution (US 70-10B, 10-30, Fig. 1). In addition, we studied eleven specimens identified as *Lithophyllum* sp. collected in recent subtidal surveys in Brittany (Trevignon, Fig. 2), Galicia (Ons and Cies archipelagos, Islas Atlanticas National Park, Figs 3-7), and Mediterranean Spain (Granada, Figs 8-9) (Table 1). The Atlantic collections corresponded to thin crusts overgrowing dead maerl and pebbles associated to maerl beds between 11-15 m depth in 2011 (Figs 2-7), while the Mediterranean specimens consisted on crusts on a pebble and a drifted fragment of a fishing line at 15 m depth in 2012 (Figs 8-9). Recent collections were air-dried or oven-dried (50º C), preserved in plastic bags with silica gel before molecular and morphological studies, and deposited in the herbaria of Universidade de Santiago de Compostela (SANT) and Museum National d’Histoire Naturelle (PC) (acronyms follow Thiers, 2015).

**DNA sequencing and phylogenetic analyses**

Eleven specimens from recent 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), and DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA) following manufacture’s recommendations. DNA extractions and amplifications of the holotype of *Lithophyllum nitorum* housed in US were performed separately from recent collections and with negative controls run throughout at Smithsonian Laboratories of Analytical Biology, Washington DC, US; extraction of total genomic DNA of the holotype followed the modified protocol by Broom *et al.* (2008) of the Qiagen DNeasy Blood and Tissue Kit® (Qiagen, Crawley, UK). The mitochondrial COI-5P fragment was PCR-amplified using primer pairs GazF1/GazR1 (Saunders, 2005). The chloroplast *psbA* gene was amplified using primer pairs *psbA*-F1/*psbA*-R2 or *psbA*-F1/*psbA*600R (Yoon *et al.*, 2002), this latter used for the holotype. PCR reactions, thermal profiles, cleaning for sequencing and sequencing of the resulting products of the holotype followed Adey *et al.* (2015). Thermal profiles for PCR amplification of COI-5P and *psbA* fragments of recent collections followed Saunders & McDevit (2012) and Bittner (2009), respectively; PCR reactions followed Peña *et al.* (2015a). Excess primers and nucleotides from PCR products (labeled CPVP, Table 1) were removed with shrimp alkaline phosphatase and exonuclease I enzymes, and sequenced using the Sanger method at Macrogen facilities (http://www.macrogen.com). PCR products labelled VPF and LLG (Table 1) were purified and sequenced by Genoscope (Bibliothèque du Vivant program, Centre National de Séquençage, France). Sequences were assembled and aligned with the assistance of CodonCode Aligner® (CodonCode Corporation, USA) and adjusted by eye using SeaView version 4 (Gouy *et al.*, 2010). Sequences were submitted to the Barcode of Life Data Systems (project ‘NGCOR’, BOLD, http://www.boldsystems.org; Ratnasingham & Hebert, 2007) and GenBank (accession numbers listed in Table 1). Our analyses also comprised publicly available GenBank sequences of Atlantic and Mediterranean *Lithophyllum* spp., including those conspecific with type collections of the generitype *L. incrustans*, *L. bathyporum* and *L. hibernicum* (Hernández-Kantun *et al.*, 2015) as well as the most relevant matches found with *L. nitorum* (> 90% similarity). These databases also...
encompassed sequences assigned to the genus *Lithophyllum*, and collected as associated crusts in Galician and Brittany maerl beds (Pardo *et al.*, 2017). According to recent phylogenetic studies (Van der Merwe *et al.*, 2015; Rösler *et al.*, 2016), publicly available sequences for *Spongites* spp. corresponding to the “Southern Hemisphere group” were selected as outgroups for the *psbA* alignment; while sequences of *Porolithon onkodes* were selected as outgroups for the COI-5P alignment (Table 1).

The alignments comprised 25 COI-5P and 16 *psbA* sequences. 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 *psbA* 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.

**Morphological studies of DNA-sequenced specimens**

According to the previous phylogenetic analyses, representative specimens resolved conspecific to *Lithophyllum nitorum* were selected for anatomical examination with a scanning electron microscope (SEM, model JEOL JSM 6400, Universidade da Coruña, Spain). The examined specimens were deposited in the herbarium of Universidade de Santiago de Compostela (SANT). 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 holotype of *Lithophyllum nitorum* was successfully sequenced only for the plastid *psb*A gene (fragment of 450 bp), but it did not result in any match in GenBank database obtaining the highest similarity (94%) with *Lithophyllum impressum* from NE Pacific and Uncultured Corallinales -LBC0599- from Vanuatu (Table 1, Fig. 10; searched on 9 November 2017). Our phylogenetic analyses of the *psb*A alignment (22 sequences ranging from 450 to 822 bp, with 222 variable sites) resolved this species, albeit with low support values, as the sister taxa of *L. byssoides*. Moreover, this phylogeny recovered a recent collection obtained from a Galician maerl bed (voucher SANT-Algae 31426, Ons archipelago) with high similarity with the holotype of *L. nitorum* (0.07 % divergence, 3 bp difference, Fig. 10). Based on this recent collection, the phylogenetic tree obtained from the ML analysis of the COI-5P alignment (25 sequences resulting 17 haplotypes, ranging from 445 to 664 bp in length, consisting of 216 variable sites) resolved another ten additional specimens (grouped into four haplotypes) from Atlantic and Mediterranean waters as conspecific with it (Fig. 11). Thus, our results confirm the occurrence of *L. nitorum* along the Atlantic European coasts of Brittany and the Northwest of the Iberian Peninsula (Galicia), and they also expand its distribution range southwards to the Mediterranean Spain (Granada).

The recent collections resolved conspecific with the holotype of *Lithophyllum nitorum* (Fig. 1) and consisted of small crusts overgrowing fragments of dead maerl (ca. <3 cm), pebbles, cobbles, and a drifted fragment of fishing line (Figs 2-9). In contrast with the colour features provided in the literature for *L. nitorum*, the colour of our specimens was dark to pale pink, and the surface was somewhat glossy only in some specimens (i.e. Fig. 3). Uniporate conceptacles were conspicuously protruding (Figs 12,13) and surrounded by a white rim (i.e. Figs 5,12). The SEM examination of several specimens showed very thin crusts, <500 µm thick, very adherent to the substrate with a monomorous construction and non-coaxial medulla (Fig. 14). Epithallial cells were mostly flattened, 1-
2 µm tall x 7-8 µm diameter, disposed in 1-2 layers. Subepithallial cells were 5-8 µm tall x 3-10 µm diameter, as short as or shorter than cells subtending them (Figs 16, 17). Cortical cells of contiguous filaments were laterally aligned, 6-14 µm tall x 5-8 µm diameter, containing inclusions usually interpreted as floridean starch (Figs 15-17). Secondary pit-connections were observed; cell fusions were absent (Fig. 15). Uniporate conceptacles were protruding (Fig. 18); old conceptacles were buried, not infilled, with elliptical chambers of 54-100 µm in height x 180-290 µm in diameter (Fig. 22).

According to the shape of the chamber, some buried and empty conceptacles in one plant collected in Ons archipelago (Galicia, voucher SANT-Algae 31426) corresponded to spermatangial conceptacles with chambers 21-22 µm in height x 84-87 µm in diameter (Fig. 19). While another recent collection from the same Galician locality (voucher SANT-Algae 31421) showed an uniporate tetrasporangial conceptacle protruding (Fig. 20), with pore canal of 50 µm in length, tapering towards the surface (from 60 µm to 20 µm diameter) (Fig. 21); the chamber was 150 µm in height x 250 µm in diameter, with a small calcified columella and tetrasporangia elongate, 117 µm in height x 24 µm in diameter, zonately divided (Fig. 20).

**DISCUSSION**

Based on a phylogenetic analysis of *psb*A sequences including the sequence obtained for the holotype of *Lithophyllum nitorum*, we were able to identify conspecific specimens in our recent collections from Atlantic and Mediterranean coasts (Fig. 11). However, none of the publicly available sequences from recent studies in Atlantic and Mediterranean *Lithophyllum* taxa (i.e. Hernández-Kantún *et al.*, 2015; Pezzolesi *et al.*, 2016; Pardo *et al.*, 2017) matched sequences from *L. nitorum*. According to the literature (Adey & Adey, 1973; Chamberlain & Irvine, 1994), *Lithophyllum nitorum* is considered a rare, subtidal species distributed from the British Isles to northern Spain, and recorded on rocks and shells. Our present study based on DNA sequence data confirmed the presence of
Lithophyllum nitorum in Brittany, the Northwest of the Iberian Peninsula (Galicia) and Mediterranean Spain. Furthermore, this study revealed the presence of this species on other substrates such as dead maerl, small pebbles or even drifted fragments of fishing lines. It is noteworthy to remark that all specimens collected are of small size and do not develop into large, conspicuous crusts; therefore, L. nitorum is likely to be easily overlooked. In agreement with the literature reports (Adey & Adey, 1973; Chamberlain & Irvine, 1994) and our morphological observations, the presence of uniporate conceptacles markedly protruding can be considered an external morphological feature useful to identify L. nitorum from other Lithophyllum species that also occur in the subtidal, such as L. crouaniorum, L. bathyporum, L. hibernicum or L. incrustans (Chamberlain & Irvine, 1994; Hernández-Kantún et al., 2015). On the other hand, L. nitorum is described in the literature as orange-pink crusts, highly to somewhat glossy (Adey & Adey, 1973; Chamberlain & Irvine, 1994) while in our collections the colour varied from dark to pale pink and only some specimens were somewhat glossy. Most of our specimens of L. nitorum have empty uniporate conceptacles, but one Galician plant is considered a male gametophyte based on the shape of spermatangial conceptacles. Chamberlain & Irvine (1994) pointed out that gametangial plants are unknown in the British Isles; Adey & Adey (1973, Table XII) recorded spermatangial conceptacles, however collection details are not indicated. According to the measurements provided in Adey & Adey (1973), the Galician specimen had smaller chambers (21-22 µm in height x 84-87 µm in diameter vs. 22 µm in height x 90-118 µm in diameter). In our study, only one Galician specimen examined showed tetrasporangial conceptacles that are in agreement with the literature (chamber 150 µm in height x 250 µm in diameter vs 100-135 x 255-385 µm µm and 94-156 µm x 245-385 µm in Adey & Adey, 1973 and Chamberlain & Irvine, 1994, respectively). For the British Isles, Chamberlain & Irvine (1994) reported only bisporangial conceptacles, while Adey & Adey (1973) indicated that tetrasporangia were rare in the distribution range hitherto known for L. nitorum along the Atlantic European coasts. Likewise, we observed buried conceptacles not infilled which has not been
reported earlier (Adey & Adey, 1973; Chamberlain & Irvine, 1994). The distribution range hitherto reported for *L. nitorum* is restricted from the British Isles to northern Spain (Adey & Adey, 1973; Chamberlain & Irvine, 1994); our results confirm records for Brittany and Galicia (Northwest of Spain), and they also expand the distribution of the species southwards to the Spanish coast of the Mediterranean Sea (Granada). Our Mediterranean record based on molecular evidences are congruent with previous observations of extant plants and Neogene fossil records in southern Spain and Balearic Islands (Braga & Aguirre, 1995). All recent collections assigned to *L. nitorum* were subtidal, made between 11-15 m, in agreement with the wide depth range provided in the literature (3-30 m in the British Isles, down to 46 m in northern Spain, Adey & Adey, 1973; Chamberlain & Irvine, 1994). The Atlantic collections from Brittany and Galicia were found on dead maerl or small pebbles associated with maerl beds. A recent DNA barcoding study carried out on maerl beds from these two Atlantic regions (Pardo *et al.*, 2017) revealed higher species diversity in associated crustose coralline algae than in maerl-forming species (9 vs 3, as total taxa obtained for each growth-form); two out of the nine crustose taxa corresponded to *Lithophyllum hibernicum* (Brittany) and an unidentified *Lithophyllum* sp.3 (Galicia) overgrowing pebbles, both with uniporate conceptacles not protruding (Fig. 3 in Pardo *et al.*, 2017). The record of *L. nitorum* as an additional encrusting species associated with maerl beds in Brittany and Galicia increases the associated diversity reported for this coastal habitat in the North East Atlantic (Peña *et al.*, 2014a).

The present work confirms the effectiveness of sequencing type specimens of coralline algae to link the type bearing name to the many sequences obtained from recent specimens (i.e. Peña *et al.* 2014b, 2015b; Adey *et al.*, 2015; Hernandez-Kantun *et al.*, 2015) in the aim to establish a DNA Library of Life (Le Gall *et al.*, 2017). The sequence of the holotype of *Lithophyllum nitorum* allowed us to identify recent collections that expanded its distribution range southwards to the Mediterranean. *Lithophyllum nitorum* is
considered a rare species in the British Isles, while our results indicate that it occurs on
different substrates in the subtidal, even associated to maerl beds in Brittany and Galicia.
According to the literature (Adey & Adey 1973), *L. nitorum* occurs sporadically to the
north while increased southwards in Cornwall and the Cantabrian Sea. Together with our
confirmed records in the Northwest of Spain (Galicia) and in the Mediterranean, it might
represent a temperate *Lithophyllum* species with warm-water affinities; a similar Atlantic-
Mediterranean distribution is also reported for *L. incrustans* and *L. hibernicum*
(Hernández-Kantún *et al.*, 2015). Despite the considerable number of publicly available
sequences of *Lithophyllum* taxa (more than 275 COI-5P and *psbA* GenBank sequences),
none of them matched with *L. nitorum*; the presence of this uncommon, subtidal species
with somewhat restricted distribution further supports *Lithophyllum* as a genetically
diverse genus in the European coasts (i.e. Pezzolesi *et al.*, 2016). In this regard, if the
diversification of the subfamily Lithophylloideae was estimated during the early and
middle Miocene (~ 23-12 million years ago, Rösler *et al*., 2017), the Miocene fossil
records of *L. nitorum* in Mediterranean Spain (Braga & Aguirre, 1995) would indicate an
early divergence of this species. Finally, our results based on type material confirmed
*Lithophyllum nitorum* as a distinct species from other European *Lithophyllum* taxa
recently re-assessed (Hernández-Kantun *et al.*, 2015). Numerous additional *Lithophyllum*
species from the North East Atlantic and worldwide need to have their type specimens
sequenced to correctly apply names.

**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”), Spain’s
Ministerio de Economía y Competitividad (CTM2010-18787, partially founded by FEDER) and Xunta de Galicia (10MMA103003PR); ASSEMBLE (grant agreement nº. 227799) supported sampling in Brittany. Acquisition of molecular data was carried out at the CNRS-UMS 2700 in Service de Systématique Moléculaire (MNHN, Paris). VPF acknowledges support by Universidade da Coruña (Contrato programa-Campus Industrial de Ferrol). JJHK acknowledges support from the Smithsonian Institution through a postdoctoral fellowship. We sincerely thank Ignacio Bárrbara, Rodolfo Barreiro and Cristina Pardo for fieldwork and/or lab collaboration. We acknowledge to Parque Nacional Marítimo Terrestre das Illas Atlánticas de Galicia for permission to carry out our surveys. We are also grateful to both reviewers for their helpful comments on the manuscript.

REFERENCES


GUIRY M.D. & GUIRY G.M., 2017 – *AlgaeBase*. World-wide electronic publication,
National University of Ireland, Galway. http://www.algaebase.org; searched on 08 June 2017.


PEÑA V., HERNANDEZ-KANTUN J.J., GRALL J., PARDO C., LÓPEZ L.,
BÁRBARA I., LE GALL L. & BARREIRO R., 2014b– Detection of gametophytes in
the maerl-forming species *Phymatolithon calceum* (Melobesioideae, Corallinales)

PEÑA V., DE CLERCK O., AFONSO-CARRILLO J., BALLESTEROS E., BÁRBARA
I., BARREIRO R. & LE GALL L., 2015a – 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.

PEÑA V., PARDO C., LÓPEZ L., CARRO B., HERNANDEZ-KANTUN J.J., ADEY
lusitanicum* sp. nov. (Hapalidiales, Rhodophyta): the third most abundant maerl-

PEZZOLESI L., FALACE A., KALEB S., HERNÁNDEZ-KANTÚN J.J., CERRANO C.
& RINDI F., 2016 – Genetic and morphological variation in an ecosystem engineer,

PUILLANDRE N., MACPHERSON E., LAMBOURDIÈRE J., CRUAUD C.,
COULOUX A., BOISSELIER-DUBAYLE M-C. & SAMADI S., 2011. - Barcoding
type specimens helps to identify synonyms and an unnamed new species in Eumunida

RATNASINGHAM S. & HEBERT P.D.N., 2007 – BOLD: The Barcode of Life Data

RICHARDS J.L., GABRIELSON P.W. & FREDERICQ S., 2014 – New insights into the
genus *Lithophyllum* (Lithophyloideae, Corallinaeae, Corallinales) from deepwater
rhodolith beds offshore the NW Gulf of Mexico. *Phytotaxa* 190: 162-175.


Table 1. Sample information for species included in molecular analyses. In bold, holotype specimen of *Lithophyllum nitorum*. Additional COI-5P and *psb* A sequences downloaded from GenBank are detailed.

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher</th>
<th>GenBank accession number (COI-5P)</th>
<th>GenBank accession number (psbA)</th>
<th>Collection details</th>
<th>Herbarium</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lithophyllum nitorum</em></td>
<td>US 70-10B</td>
<td>MG648773</td>
<td></td>
<td>Port Erin Harbour, Isle of Man, UK (Adey &amp; Adey, 1973)</td>
<td>US 70-10B</td>
</tr>
<tr>
<td><em>L. nitorum</em></td>
<td>CPVP913</td>
<td>MG648771</td>
<td></td>
<td>Subtidal (15 m), maerl bed, Le Dragon, Trevignon, France, 13/05/2011. Coll. W. Thomas, Y. Fontana.</td>
<td>SANT-Algae 31427</td>
</tr>
<tr>
<td><em>L. nitorum</em></td>
<td>CPVP760</td>
<td>MG648763</td>
<td></td>
<td>Subtidal (13 m), maerl bed, Ons, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td>SANT-Algae 31420</td>
</tr>
<tr>
<td>L. nitorum</td>
<td>CPVP765</td>
<td>MG648764</td>
<td>Subtidal (13 m), maerl bed, Ons, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>----------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. nitorum</td>
<td>CPVP772</td>
<td>MG648765</td>
<td>Subtidal (13 m), maerl bed, Ons, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. nitorum</td>
<td>CPVP774</td>
<td>MG648767</td>
<td>Subtidal (13 m), maerl bed, Ons, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. nitorum</td>
<td>VPF00474</td>
<td>MG648769</td>
<td>Subtidal (13 m), maerl bed, Ons, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. nitorum</td>
<td>VPF00475</td>
<td>MG648762</td>
<td>Subtidal (13 m), maerl bed, Ons, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Accession 1</td>
<td>Accession 2</td>
<td>Additional Information</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. nitorum</em></td>
<td>VPF00482</td>
<td>MG648766</td>
<td>Subtidal (13 m), maerl bed, Ons, Ría de Pontevedra, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VPF00388</td>
<td>MG648770</td>
<td>Subtidal (11 m), maerl bed, Cíes, Ría de Vigo, Galicia, Spain, 07/04/2011. Coll. I. Bárbara, R. Barreiro, V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LLG4596I</td>
<td>MG648768</td>
<td>Subtidal (15 m), La Herradura, Granada, Spain, 19/09/2012. Coll. Le Gall, L., V. Peña.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LLG4608A</td>
<td>MG648772</td>
<td>Subtidal (15 m), La Herradura, Granada, Spain, 19/09/2012. Coll. Le Gall, L., V. Peña.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Additional sequences analysed**

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession 1</th>
<th>Accession 2</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lithophyllum bathyporum</em></td>
<td>-</td>
<td>KR736255</td>
<td>Brittany, Atlantic France</td>
</tr>
<tr>
<td><em>L. bathyporum</em></td>
<td>KR733435</td>
<td>-</td>
<td>Galicia, Atlantic Spain</td>
</tr>
<tr>
<td><em>Lithophyllum byssoides</em></td>
<td>-</td>
<td>JQ896252</td>
<td>Cádiz, Atlantic Spain</td>
</tr>
<tr>
<td><em>L. byssoides</em></td>
<td>-</td>
<td>JQ896251</td>
<td>Galicia, Atlantic Spain</td>
</tr>
<tr>
<td>Species</td>
<td>Accession 1</td>
<td>Accession 2</td>
<td>Location</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><em>Lithophyllum dentatum</em> (Kützing) Foslie</td>
<td>KR708623</td>
<td>KR708611</td>
<td>Sitges, Mediterranean Spain</td>
</tr>
<tr>
<td><em>Lithophyllum hibernicum</em></td>
<td>KR708622</td>
<td>KR708594</td>
<td>Ireland</td>
</tr>
<tr>
<td><em>L. hibernicum</em></td>
<td>-</td>
<td>KR708614</td>
<td>Cornwall, UK</td>
</tr>
<tr>
<td><em>L. hibernicum</em></td>
<td>KR733526</td>
<td>-</td>
<td>Brittany, Atlantic France</td>
</tr>
<tr>
<td><em>L. hibernicum</em></td>
<td>KR733530</td>
<td>KR736256</td>
<td>Galicia, Atlantic Spain</td>
</tr>
<tr>
<td><em>L. hibernicum</em></td>
<td>KR733464</td>
<td></td>
<td>Port de Sète, Mediterranean France</td>
</tr>
<tr>
<td><em>L. hibernicum</em></td>
<td>KR733463</td>
<td>-</td>
<td>Granada, Mediterranean Spain</td>
</tr>
<tr>
<td><em>Lithophyllum impressum</em></td>
<td>-</td>
<td>JQ917416</td>
<td>NE Pacific</td>
</tr>
<tr>
<td><em>Lithophyllum incrustans</em></td>
<td>KR708619</td>
<td>JQ896237</td>
<td>Ireland</td>
</tr>
<tr>
<td><em>L. incrustans</em></td>
<td>KR708620</td>
<td>JQ896238</td>
<td>Brittany, Atlantic France</td>
</tr>
<tr>
<td><em>L. incrustans</em></td>
<td>KR733470</td>
<td>-</td>
<td>Galicia, Atlantic Spain</td>
</tr>
<tr>
<td><em>L. incrustans</em></td>
<td>KR733480</td>
<td></td>
<td>Granada, Mediterranean Spain</td>
</tr>
<tr>
<td><em>L. incrustans</em></td>
<td>-</td>
<td>KR708602</td>
<td>Granada, Mediterranean Spain</td>
</tr>
<tr>
<td><em>Lithophyllum orbiculatum</em></td>
<td>KR708625</td>
<td>KR708609</td>
<td>Brittany, Atlantic France</td>
</tr>
<tr>
<td><em>Lithophyllum pustulatum</em> (J.V.Lamouroux) Foslie</td>
<td>-</td>
<td>KM407551</td>
<td>Mediterranean Spain</td>
</tr>
<tr>
<td>Species</td>
<td>Accession</td>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>-----------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Lithophyllum stictaeforme</em> (J.E. Areschoug)</td>
<td>KM407553</td>
<td>Mediterranean Spain</td>
<td></td>
</tr>
<tr>
<td>Hauck</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. stictaeforme</em></td>
<td>KM407554</td>
<td>Mediterranean Spain</td>
<td></td>
</tr>
<tr>
<td><em>Lithophyllum</em> sp.</td>
<td>KJ418414</td>
<td>Louisiana, USA</td>
<td></td>
</tr>
<tr>
<td><em>Lithophyllum</em> sp.</td>
<td>KJ418415</td>
<td>Louisiana, USA</td>
<td></td>
</tr>
<tr>
<td><em>Lithophyllum</em> sp3</td>
<td>MF133359</td>
<td>Galicia, Atlantic Spain</td>
<td></td>
</tr>
<tr>
<td><em>Porolithon onkodes</em> (Heydrich) Foslie</td>
<td>GQ917289</td>
<td>New Caledonia</td>
<td></td>
</tr>
<tr>
<td><em>Spongites agulhensis</em> Maneveldt, E. van der Merwe &amp; P.W. Gabrielsons</td>
<td>KT184812</td>
<td>South Africa</td>
<td></td>
</tr>
<tr>
<td><em>Spongites hyperellus</em> (Foslie) D.L. Penrose</td>
<td>GQ917495</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Uncultured Corallinales</td>
<td>GQ917452</td>
<td>Vanuatu</td>
<td></td>
</tr>
</tbody>
</table>
**Figure legends**


Fig. 10. Phylogenetic tree inferred from ML and BI analyses of psbA sequences of *Lithophyllum* recorded from European coasts, and highest similarity matches found in GenBank (> 94%). In bold, holotype of *Lithophyllum nitorum* and other *Lithophyllum* species corroborated by type collections. Stars indicate species names linked to type specimens by DNA sequences; quotation marks for species names based on morpho-anatomy or from toptype material (i.e. *L. impressum*). Bootstrap ML values > 60% and posterior probabilities > 0.60 from Bayesian inference are shown for each node. *Spongites agulhensis* and *S. hyperellus* from the Southern Hemisphere were used as outgroup.

Fig. 11. ML tree inferred from DNA barcode (COI-5P). In red colour, recent collections resolved conspecific with the holotype of *Lithophyllum nitorum*, and their geographical location in the European coasts. Stars indicate species names linked to type specimens by DNA sequences; quotation marks for species names based on morpho-anatomy. Bootstrap values > 60 % are shown for each node. *Porolithon onkodes* (subfamily Metagoniolithoideae, Corallinales) was used as outgroup.

15. Vertical section showing cortical cells of contiguous filaments laterally aligned, and joined by secondary pit conncetions –arrows-; lumen cells containing inclusions usually interpreted as floridean starch (voucher SANT-Algae 31421). 16-17. Vertical section showing 1-2 layers of mostly flattened epithallial cells (e), and subepithallial cells (s) as short as or shorter than cells subtending them (voucher SANT-Algae 31428). 18. Surface view showing uniporate conceptacles markedly protruding (voucher SANT-Algae 31422).

Lithophyllum hibanicum ★

“Lithophyllum dentatum”

Lithophyllum bathyaporum ★

Lithophyllum incrustans ★

Lithophyllum nitorum

Lithophyllum sp.3

“Lithophyllum orbiculatum”

“Porolithon onkodes”