Regional Activity Centre for Specially Protected Areas (RAC/SPA)
Boulevard du Leader Yasser Arafat | B.P. 337 - 1080 Tunis Cedex -Tunisia
phone: +216 71 206 649 / +216 71 206 485 / +216 71 206 851 / +216 71 206 765
Fax: +216 71 206 490
E-mail: car-asp@rac-spa.org

Centre d'Activités Régionales pour les Aires Spécialement Protégées (CAR/ASP)
Boulevard du Leader Yasser Arafet - B.P. 337 - 1080 - Tunis Cedex - Tunisie
Téléphone: +216 71 206 649 / +216 71 206 485 / +216 71 206 851 / +216 71 206 765
Fax: +216 71 206 490
E-mail: car-asp@rac-spa.org
web: www.rac-spa.org

UNEP

MAP

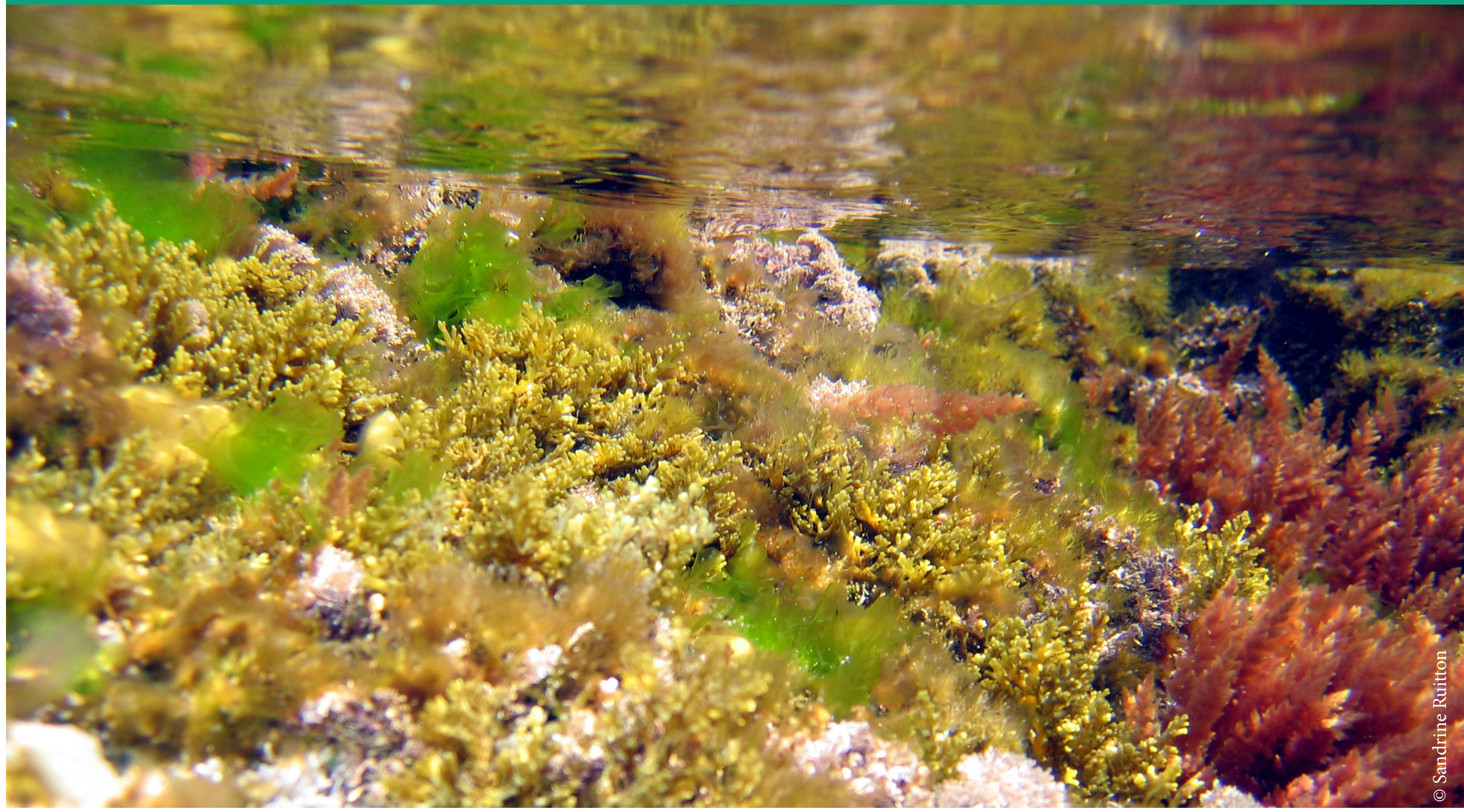
R A C / S P A

PROCEEDINGS OF THE 5th MEDITERRANEAN SYMPOSIUM ON MARINE VEGETATION

Portorož, Slovenia, 27-28 October 2014

ACTES DU 5ème SYMPOSIUM MÉDITERRANÉEN SUR LA VÉGÉTATION MARINE

Portorož, Slovenie, 27-28 octobre 2014



© Sandrine Ruitton

INSTITUTE OF THE REPUBLIC OF SLOVENIA
FOR NATURE CONSERVATION

October 2014

PROCEEDINGS OF THE 5th MEDITERRANEAN SYMPOSIUM ON MARINE VEGETATION

Portorož, Slovenia, 27-28 October 2014

ACTES DU 5ème SYMPOSIUM MÉDITERRANÉEN SUR LA VÉGÉTATION MARINE

Portorož, Slovenie, 27-28 octobre 2014

Avec le support du projet MedKeyhabitats Finance par la fondation MAVA
With the support of MedKeyhabitats project Financed by the MAVA Foundation

MKH
MEDKEYHABITATS

MAVA
FONDATION POUR LA NATURE

October 2014

The finding interpretation and the presentation of the material, expressed in this publication are entirely those of authors and should not be attributed to UNEP.

Les informations et la présentation des données, qui figurant dans cette publication sont celles des auteurs et ne peuvent être attribuées au PNUE.

Copyright :

© 2014 United Nations Environment Programme, Mediterranean Action Plan, Regional Activity Center for Specially Protected Areas (RAC/SPA)

© 2014 Programme des Nations Unies pour l'Environnement, Plan d'Action pour la Méditerranée, Centre d'Activités Régionales pour les Aires Spécialement Protégées (CAR/ASP)

This publication may be reproduced in whole or in part, and in any form for educational or non-profit purposes, without special permission from the copyright holder, provided acknowledgement of the source is made. No use of this publication may be made, for resale or for any other commercial purpose whatsoever, without permission in writing from UNEP.

La présente publication peut être reproduite en totalité ou en partie, et sous n'importe quelle forme, dans un objectif d'éducation et à titre gracieux, sans qu'il soit nécessaire de demander une autorisation spéciale au détenteur du copyright, à condition de faire mention de la source. La présente publication ne peut être utilisée, pour la revente ou à toutes fins commerciales, sans un accord écrit préalable du PNUE.

Citation :

UNEP/MAP – RAC/SPA, 2014. Proceedings of the 5th Mediterranean Symposium on Marine Vegetation (Portorož, Slovenia, 27-28 October 2014). LANGAR H., BOUAFIF C., OUERGHI A., edits., RAC/SPA publ., Tunis: 251 p.

PNUE/PAM – CAR/ASP, 2014. Actes du 5ème Symposium Méditerranéen sur la Végétation Marine (Portorož, Slovénie, 27-28 octobre 2014). LANGAR H., BOUAFIF C., OUERGHI A., édits., CAR/ASP publ., Tunis :251 p.

Fabio RINDI, PEZZOLESI L., HERNANDEZ-KANTUN J.J., FALACE A., KALEB S., PONTI M., CERRANO C.
Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche,
Via Brecce Bianche, 60131 Ancona, Italy
E-mail: f.rindi@univpm.it

DISTRIBUTION AND GENETIC VARIATION OF TWO BIOCONSTRUCTOR CORALLINE ALGAE (*LITHOPHYLLUM BYSSOIDES* (LAMARCK) FOSLIE AND *L. STICTAEFORME* (ARESCHOUG) HAUCK) ALONG THE ITALIAN COASTS

Abstract

Coralline red algae represent a worldwide component of hard-bottom coastal communities, where they play a key role in many ecological processes. Some species are ecosystem engineers that produce biogenic platforms, reefs and other calcified structures providing a wealth of habitats for many other species, especially in temperate seas. In the Mediterranean Sea these bioconstructions are widespread and occur both in the littoral zone (trottoirs of Lithophyllum byssoides) and in the sublittoral zone (coralligenous bottoms, where coralline algae are one of the main constituents). A detailed knowledge of their distribution and composition is essential for their conservation, but molecular data assessing taxonomic identity and population structure in corallines are extremely limited. The distribution and genetic variation in two important bioconstructor corallines, Lithophyllum byssoides and L. stictaeforme (Corallinales, Corallinaceae) are investigated using sequences of the plastid psbA gene and mitochondrial cox2,3 spacer. Populations of L. byssoides occur on all parts of the Italian coastline where rocky shores exist and the molecular data show that Mediterranean populations of this species represent a sister lineage to Atlantic populations. L. stictaeforme is widespread along the Italian shores, particularly along the Ligurian and Tyrrhenian sides. In molecular phylogenies Mediterranean populations of L. stictaeforme form a well-supported clade, to which North Atlantic Lithophyllum spp. are the closest relatives. Overall the results indicate a high genetic variability in these species, with the possible existence of cryptic species.

Key-words: Bioconstructions, coralline algae, Italy, *Lithophyllum*, molecular phylogeny.

Introduction

The Mediterranean Sea is the largest (2,969,000 km^2) and deepest (average 1,460 m, maximum 5,267 m) enclosed sea on Earth (Coll *et al*., 2010). Its geological and hydrological diversity, determined by the complex geological history of this basin from the Mesozoic era (250 mya), has favoured the establishment of a large biological diversity, with approximately 17,000 species recorded (Coll *et al*., 2010).

Marine bioconstructors are algal or animal species producing rigid structures (usually calcified bodies, tubes or shells) that, accumulating over time, form new substrata. The accumulation of these structures modifies the features of the bottom and produces new surface available for the settlement of other benthic organisms. The surface of the substratum is formed by living organisms and resides above multiple layers of dead biomass, which is usually colonized by boring animals. Bioconstructors are therefore habitat-building organisms and contribute substantially to the high biodiversity of the environments where they occur. Although bioconstructor species exist in every sea, in the

Mediterranean they play a crucial role, because substrata produced by biological activity reach here a spatial and bathymetric extent unknown in other seas.

Although numerous species of seaweeds and animals are able to act as habitat builders, coralline red algae (orders Corallinales and Sporolithales) are the uncontested leaders of temperate marine bioconstruction. These organisms occur in every coastal ecosystem where stable surfaces are present and contribute significantly to the formation and consolidation of hard substrata. In the Mediterranean, encrusting corallines are the builders of two key habitats. Coralligenous concretions, which occupy large portions of the deep infralittoral and circalittoral zone, are produced by growth of encrusting corallines and other red algae (*Lithophyllum stictaeforme*, *Mesophyllum alternans*, *Neogoniolithon mamillosum*, *Peyssonnelia rosa-marina*) (Ballesteros, 2006). *Lithophyllum byssoides* a species typical of the mid littoral zone, forms hemispherical bulky thalli that in optimal conditions coalesce and grow producing rims up to 3 m thick called “trottoirs” (Verlaque 2010). These habitats are now threatened by multiple anthropic stressors (Piazzi *et al.*, 2012; Ponti *et al.*, 2014). A detailed knowledge of the taxonomic circumscription, genetic variation and population connectivity of the bioconstructor species that produce them is essential for their management, but unfortunately this information is currently lacking. In general, the body of molecular data available for coralline algae is still relatively limited and the most important studies on this topic have been published in recent years (e.g., Broom *et al.*, 2008; Bittner *et al.*, 2011). As part of a project focused on the bioconstructions of Italian shores, we are currently investigating the distribution and genetic structure of *Lithophyllum byssoides* and *Lithophyllum stictaeforme* along the coasts of Italy. We present here some results based on analysis of sequences of the plastid *psb*A gene and mitochondrial *cox*2,3 spacer. These results will be integrated by additional data to be presented in future studies, for which the work is currently in progress.

Materials and methods

Samples of *Lithophyllum byssoides* and *Lithophyllum stictaeforme* were collected by snorkelling or SCUBA diving as follows: *L. byssoides*: Gallinara Island, Italy, 13/06/2013 (34 thalli); *L.stictaeforme*: Gallinara Island, Italy, 14 June 2013 (4 thalli); Portofino, Italy, 28 June 2013 (28 thalli); Trezze of Gulf of Trieste, Italy, 5 October 2013; Voula Beach, Athens, Greece, 11 September 2011 (2 thalli). The samples were air-dried and subsequently placed in bags with silica gel for permanent storage. DNA was extracted following the modified protocol of the QIAGEN DNeasy Blood & Tissue Kit ® (QIAGEN, Crawley, U.K.) by Broom *et al.* (2008). PCR amplification of the *psb*A gene was performed following the protocol of Bittner *et al.* (2011). PCR amplification of the *cox*2,3 spacer was performed following the protocol of Hernandez-Kantun *et al.* (in press, a). PCR products were visualized and quantified in 1.5% agarose gels stained with GelRed™ (Biotium, Hayward, CA, USA) using Low DNA Mass Ladder (Invitrogen, Carlsbad, CA, USA) as reference under UV. The products of successful reactions with expected length and yield were purified and sequenced commercially (Macrogen, Amsterdam, The Netherlands).

Phylogenetic analyses were performed separately on a *psb*A dataset (844 bp) and on a *cox*2,3 dataset (326 bp) formed by sequences of Corallinaceae belonging to the subfamily Lithophylloideae. Besides new sequences generated in this study, the *psb*A dataset consisted of sequences retrieved from GenBank and sequences from Hernandez-Kantun *et al*. (in press, b); *Spongites yendoi* DQ167869 and *Hydrolithon onkodes* AB576036 were the outgroup taxa. The *psb*A alignment did not contain any gaps and its correctness was checked verifying that no stop codons occurred in the protein translation. The *cox*2,3 dataset was obtained by adding the new sequences to the dataset of Hernandez-Kantun *et al*. (in press, a), in which *Lithophorella* sp. KJ801362 was the outgroup taxon. The two datasets were aligned using Clustalw2 in Mega version 5. Neighbour Joining (NJ) distance analyses were performed also in Mega version 5 using uncorrected p-distances. Maximum Likelihood (ML) analyses were performed using RAxML 1.3 (Mac version) with the GTR (General Time Reversible) model with gamma distribution and invariant sites. Statistical support was estimated by Bootstrap (BP) analyses with 1000 resamplings.

Results

Thalli of *Lithophyllum byssoides* were collected in the mid littoral zone on the northeastern side of Gallinara Island, where this species occurred as numerous hemispherical thalli, 5-10 cm wide, and did not produce well-developed trottoirs. *Lithophyllum stictaeforme* was common in the infralittoral and circalittoral zones at the sites surveyed, ranging in depth from 20 to 50 m.

In the *psb*A phylogeny, *Lithophyllum byssoides* formed a clade with robust BP support, in which two well-supported sister lineages reflected a biogeographical separation between Atlantic and Mediterranean (Fig. 1). The Atlantic lineage was formed by two samples from Cadiz and La Coruña (Spain), whereas the Mediterranean included two samples from Gallinara and the GenBank sequence GQ917720 (obtained from a sample from Banyuls-sur-Mer, Mediterranean France). The highest uncorrected pairwise divergence was 4.38% (Gallinara 15 vs. La Coruña JQ896251).

In the *psbA* phylogeny, *Lithophyllum stictaeforme* formed a well-supported monophyletic group (Fig. 1), which belonged to a larger clade including also samples of *Lithophyllum* from Atlantic Europe and the Mediterranean. A biogeographical separation was evident within the *L. stictaeforme* group: a well-supported lineage consisted of samples from the western Mediterranean (Gallinara and Portofino); sister to it was a lineage of eastern Mediterranean samples (Gulf of Trieste and a sample from Voula Beach, Athens, Greece). The highest pairwise divergence within *L. stictaeforme* was 4.2% (Portofino/Gallinara vs. Greece). GenBank sequences of *L. stictaeforme* from other geographical regions (FJ361442, New Zealand) were not closely related to Mediterranean *L. stictaeforme* and should be referred to a different species.

The *cox*2,3 analyses yielded results in agreement with the *psbA* results (Fig. 2). Again, both *L. byssoides* and *L. stictaeforme* formed well-supported lineages. For *L. byssoides*, samples from Gallinara formed a sister lineage to a sample from Atlantic Spain (pairwise divergence: 22.49%). *L. stictaeforme* was sister to a group of *Lithophyllum* samples from Atlantic Europe.

Discussion

Lithophyllum byssoides is a well-defined species, characterized by its superficial anastomosed lamellae, slightly protruding conceptacles and mid littoral habitat. Its morphology is homogeneous throughout its geographical range and does not reflect the clear genetic differentiation between Atlantic and Mediterranean specimens unravelled by our analyses. The unexpectedly high divergence in the *psbA* sequences suggests that the recognition of the Mediterranean and Atlantic lineages as different species may be an appropriate solution, but this requires corroboration based on additional samples from other regions. If this solution is adopted, the correct identity of *L. byssoides* would become a major taxonomic problem because the type locality is dubious (supposedly the English Channel, where this species, however, does not occur). *Lithophyllum stictaeforme* is

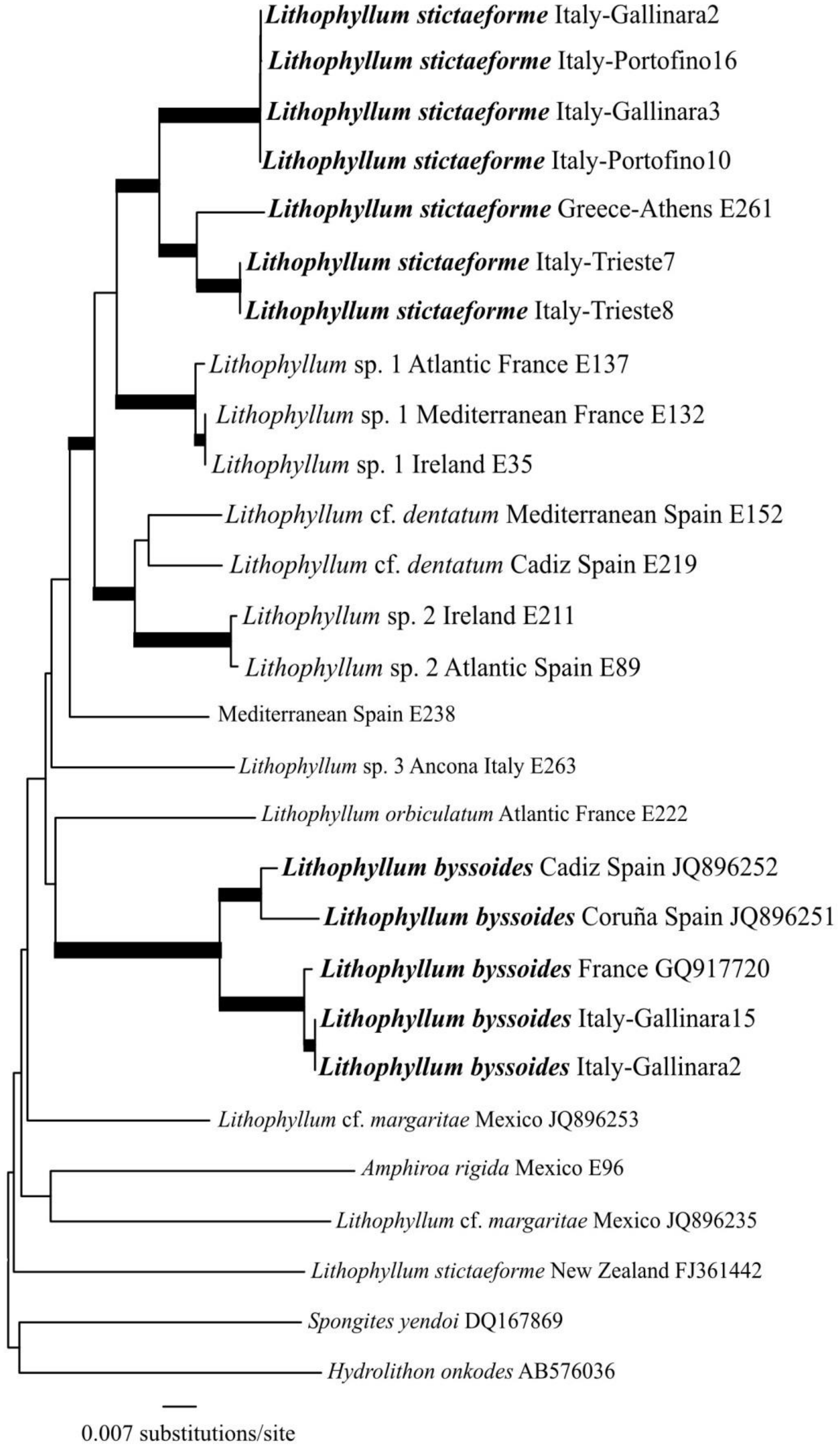


Fig. 1: Phylogeny of the *psb*A gene in the subclass Lithophylloideae inferred by NJ analysis. Sequences of *Lithophyllum byssoides* and *L. stictaeforme* are indicated in bold. Thick lines indicate branches receiving high support in bootstrap analyses (>80% BP for both ML and NJ).

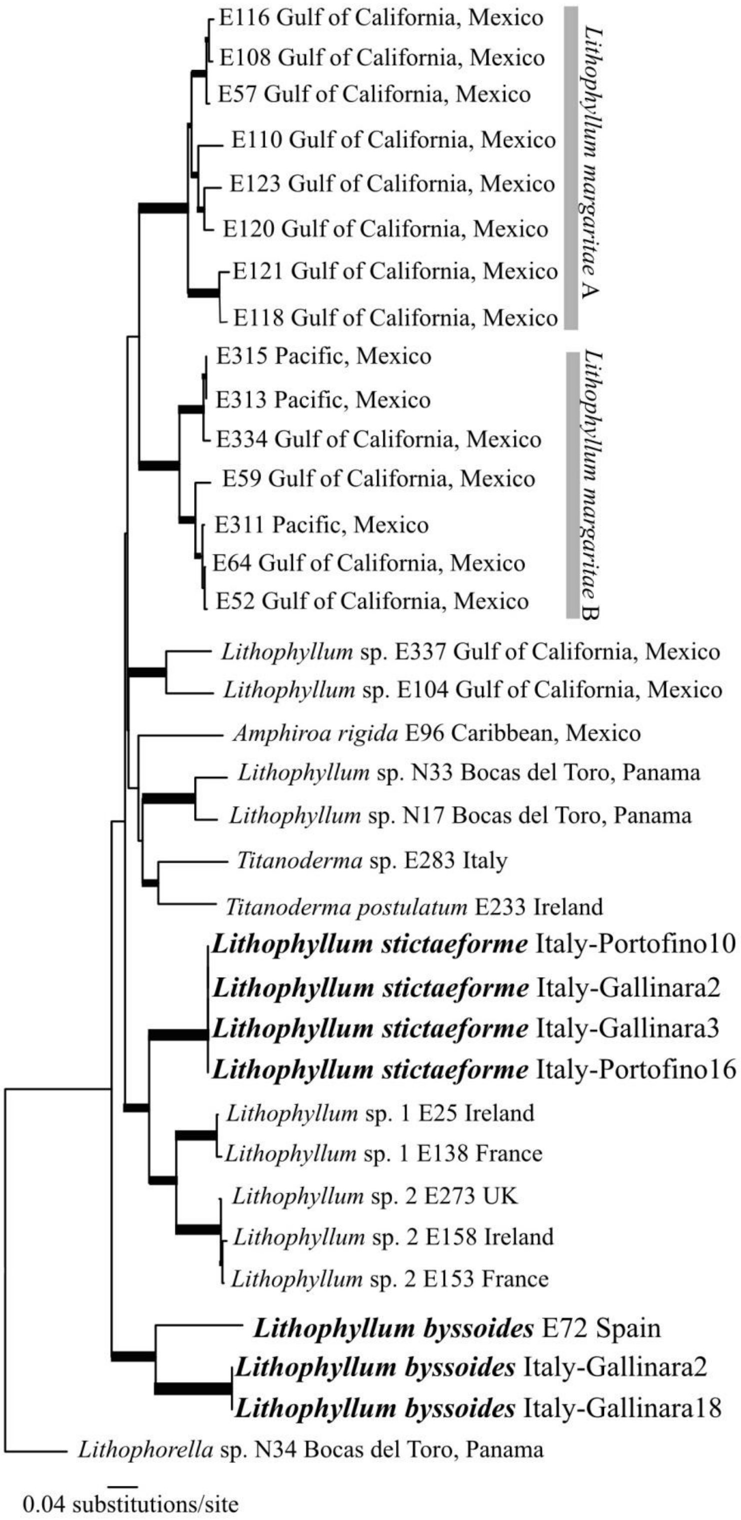


Fig. 2: Phylogeny of the cox2,3 in the subclass Lithophylloideae inferred by NJ analysis. Sequences of *Lithophyllum byssoides* and *L. stictaeforme* are indicated in bold. Thick lines indicate branches receiving high support in bootstrap analyses (>80% BP for both ML and NJ).

widespread in the Mediterranean Sea, but its taxonomic status is not settled (there is no general agreement whether *Lithophyllum cabiochae* should be considered a synonym or a separate species from it). Even for this species our analyses indicate a possible biogeographic separation, in this case between western and eastern Mediterranean populations. Overall, our results show a high genetic variation in *L. byssoides* and *L. stictaeforme*, and suggest that geographic distributions reflect molecular phylogenetic patterns better than morphological data. Recent studies for other coralline algae are delineating a similar scenario (Hernandez-Kantun *et al*., in press a, in press b).

Again, the addition of samples from other geographical regions will be essential to obtain an accurate taxonomic circumscription and a detailed assessment of the phylogeography of these two important bioconstructor species. We are currently processing collections obtained from other sites of the Italian coasts, which will allow to draw stronger conclusions in forthcoming studies.

Acknowledgements

The study was supported by a grant of M.I.U.R. (Italian Ministry for Education, Universities and Research) through a P.R.I.N. 2011 Project (Coastal bioconstructions: structure, function and management).

References

BALLESTEROS E. (2006) - Mediterranean coralligenous assemblages: a synthesis of present knowledge. *Ocean. Mar. Biol. Ann. Rev.* 44: 123-195.

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

BROOM J.E., HART D.R., FARR T.J., NELSON W.A., NEIL K.F., HARVEY AS, WOELKERLING WJ. (2008) - Utility of *psb*A and nSSU for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. *Mol. Phylogen. Evol.* 46: 958–973.

COLL M., PIRODDI C., STEENBEK J., KASCHNER K., LASRAM F.B.R. AGUZZI J., BALLESTEROS E., BIANCHI C.N., CORBERA J., DAILIANIS T., DANOVARO R., ESTRADA M., FROGLIA C., GALIL B.S., GASOL J.M., GERTWAGEN R., GIL J., GUILHAUMON F., KESNER-REYES K., KITSOS M.S., KOUKOURAS A., LAMPADARIOU N., LAXAMANA E., LÓPEZ-FÉ DE LA CUADRA C.M., LOTZE H.K., MARTIN D., MOUILLOT D., ORO D., RAICEVICH S., RIUS-BARILE J., SAIZ-SALINAS J.I., SAN VICENTE C., SOMOT S., TEMPLADO J., TURON X., VAFIDIS D., VILLANUEVA R., VOULTSIADOU E. (2010) - The biodiversity of the Mediterraneran Sea: estimates, patterns and threats. *PLoS One* 5: e11842.

HERNANDEZ-KANTUN J.J., RIOSMENA-RODRIGUEZ R., ADEY W., RINDI F. (in press, a) - Analysis of the *cox*2-3 spacer region for population diversity and taxonomic implications in rhodolith-forming species (Rhodophyta: Corallinales). *Phytotaxa*.

HERNANDEZ-KANTUN J.J., RIOSMENA-RODRIGUEZ R., HALL-SPENCER J.M., PEÑA V., MAGGS C.A., RINDI F. (in press, b) - Phylogenetic analysis of rhodolith formation in the Corallinales (Rhodophyta). *Eur. J. Phycol*.

PIAZZI L., GENNARO P., BALATA D. (2012) - Threats to macroalgal coralligenous assemblages in the Mediterranean Sea. *Mar. Poll. Bull.* 64: 2623-2629.

PONTI M., PERLINI R.A., VENTRA V., GRECH D., ABBIATI M., CERRANO C. (2014) - Ecological shifts in Mediterranean coralligenous assemblages related to gorgonian forest loss. *PLoS One* 9: e102782.

VERLAQUE M. (2010) - Field methods to analyse the condition of Mediterranean *Lithophyllum byssoides* (Lamarck) Foslie rims. *Sci. Rep. Port-Cros Natl. Park* 24: 185-196.