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Preliminary study on diversity of the *Laurencia* complex (Rhodophyta, Ceramiales) in the Macaronesian region assessed by DNA barcoding and phylogenetic inferences

Machín-Sánchez, M.[1], Le Gall, L.[2], Neto, A.I.[3], Díaz-Larrea, J.[4], Cassano, V.[5], Senties, A.[4], Fujii, M.[6] and Gil-Rodríguez, M.C.[1]

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INTRODUCTION

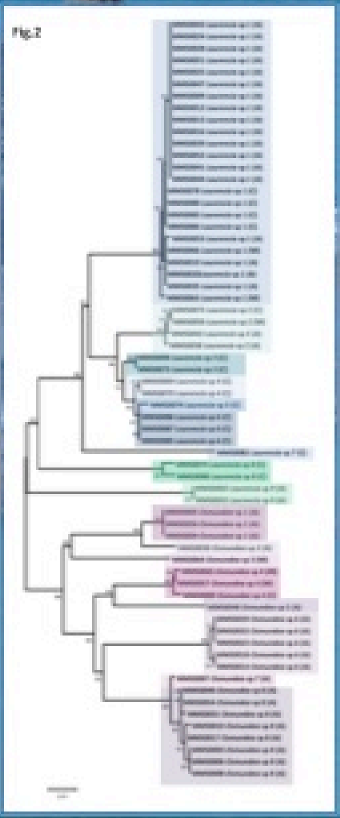
Current knowledge on the systematics of the ***Laurencia* complex** (Rhodophyta, Ceramiales) is based on molecular studies combined with traditional morphological characters. Previous phylogenetic analyses based on *rbc*L sequences have established five genera within this complex: ***Laurencia*** Lamouroux, ***Osmundea*** Stackhouse, ***Chondrophycus*** (Tokyda *et* Saito) Garbary *et* Harper, ***Palisada*** Nam and ***Yuzurua*** (Nam) Martin-Lescanne. However, generic assignment and specific identification of the members of the *Laurencia* complex is still problematic due to the high phenotypic plasticity of most species as well as the overlap between characters usually considered as taxonomically informative. In that context, **DNA barcoding** appears as a proficient alternative to morphological features for species assignment. In the present preliminary study, we assessed the diversity of the *Laurencia* complex based on partial **COI** and **LSU** markers in Macaronesian islands (**Azores**, **Madeira** and **Canary Islands**). In addition, preliminary phylogenetic analyses of the chloroplastic marker, ***rbc*L**, were carried out to infer the phylogenetic relationships and the biogeographic affinities of members of the *Laurencia* complex from Macaronesia

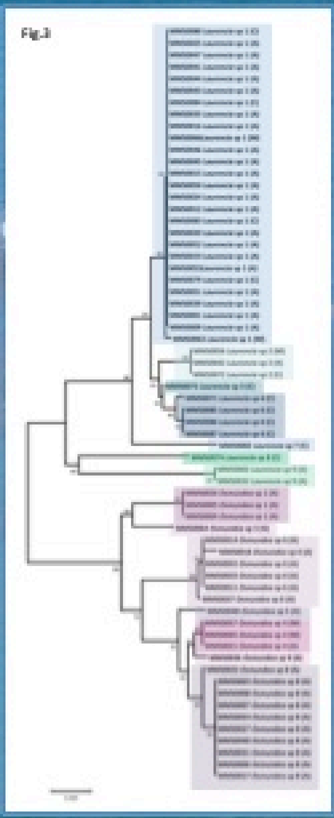
MATERIALS AND METHODS

Fig.1. Macaronesian archipelagos: Azores, Madeira, Selvagens, Canaries and Cape Verde

Samples of members of the *Laurencia* complex were collected in **2011** in Azores, Madeira and Canary Islands (Fig.1.) Sub samples were dried and preserved in silica gel for **molecular analyses**. Total DNA was extracted, using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The **COI-5'**region was PCR amplified using the forward primers **GazF1** (Saunders, 2005) and **GWSFn** (Le Gall and Saunders, 2010) variously combined with the reverse primers **GazR1** (Saunders, 2005) **GWSRx** (Saunders, 2009). The ***rbc*L** gene was amplified with the primer pairs **F-*rbc*Lstart _R-753** (Freshwater and Rueness, 1994) for the 5' end, ***rbc*LFC_1011R** (Nam *et al*., 2000) or **F-577_R1381** (Freshwater and Rueness, 1994) for the middle fragment, and **F-993_R-*rbc*S start** (Freshwater and Rueness, 1994) for the 3' end. The 5'end of the **LSU** was amplified with **T01N** (Harper and Saunders, 2001) and **T20** (Le Gall and Saunders, 2010). Sequencing reactions were performed by Genoscope (www.genoscope.fr, Evry, France). Forward and reverse sequence reads were assembled with the software Codoncode (Dedham, MA) and a multiple sequence alignment was constructed in MEGA 4.02. A **Neighbor-joining tree** was inferred from COI-5' and LSU markers to assess the **species delineation**. *rbc*L loci was used for inferred **phylogenetic relationships** with MrBayes v.3.0 beta 4 (Huelsenbeck and Ronquist, 2001)

PRELIMINARY RESULTS





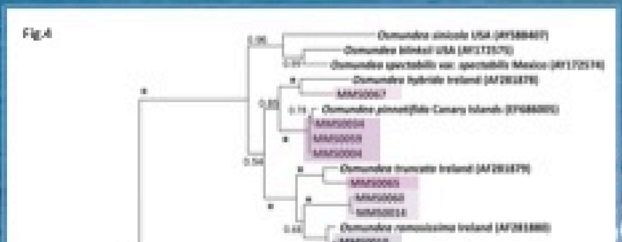
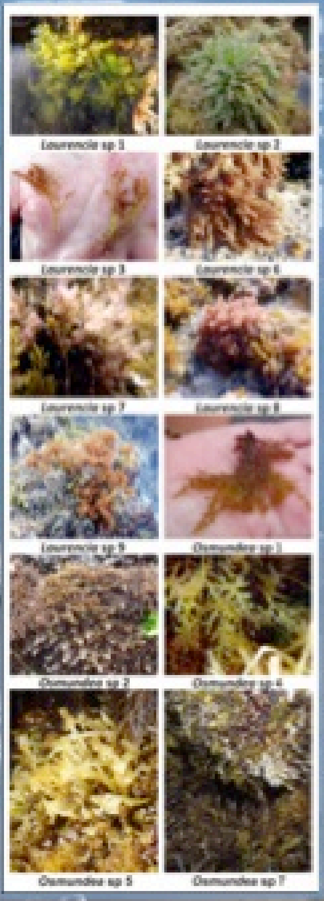


Fig.2. Neighbor-joining tree inferred from COI-5'. Bootstrap values are indicated at the nodes. **Fig.3.** Neighbor-joining tree inferred from LSUx. Bootstrap values are indicated at the nodes. **Fig.4.** Bayesian analysis of *rbc*L DNA sequences. Bayesian posterior probability values are indicated at the nodes,* posterior probability=1. **(A)** Azores ; **(C)** Canaries; **(M)** Madeira



CONCLUSIONS

1. The information obtained from the analysis of the COI and LSU sequences are consistent. Both markers are shown as powerful tools for the delineation of species

2. The phylogenetic reconstruction achieved from *rbc*L sequence analysis of specimens from Macaronesia and sequences from GenBank shows the presence of seven known species including the genera *Laurencia* and *Osmundea* and eight potential new taxa included in the *Laurencia* complex

3. Our results reveal that is necessary to conduct further analysis of the sequences in order to obtain robust phylogenetic reconstructions which reflect relations between different genus, since these are not sufficiently consistent

4. Morphological and chemical studies of the samples in combination with the study of molecular characters will help us in the delimitation of species

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CERTIFICADO DE PARTICIPACIÓN

El Comité Organizador del III Simposio Internacional en Ciencias del Mar y del XVI Seminario Ibérico de Química Marina, certifica que el trabajo titulado:

“PRELIMINARY STUDY ON DIVERSITY OF THE LAURENCIA COMPLEX RHODOPHYTA, CERAMIALES IN THE MACARONESIAN REGION ASSESSED BY DNA BARCODING AND PHYLOGENETIC INFERENCES”

del cual son autores **Machín-Sánchez, M.; Le Gall, L.; Neto, A.I.; Díaz-Larrea, J.; Cassano, V.; Sentíes, A.; Fujii, M. y M.C. Gil-Rodríguez,** ha sido presentado como póster.

Cádiz, a 27 de Enero de 2012

Por la Comisión Organizadora

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