



Project no. 043251

EDEN

ECOLOGICAL DIVERSITY AND EVOLUTIONARY NETWORKS

Instrument: Specific Targeted Project (STREP)

Thematic Priority: Integrating and strengthening the European Research Area
NEST Pathfinder initiative Tackling Complexity in Science

D1.2 (D9). Database of hypervariable neutral microsatellite loci for all the macrophyte (seagrass and algae) species and populations considered. Development of TAG loci for *Z. noltii* and *Fucus* spp

Due date of deliverable: Month 24

Actual submission date: Month 24

Start date of project: 1 January 2007

Duration: 36 months

Organisation name of lead contractor for this deliverable: CCMAR

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

NOTE: This is a short description of deliverable D1.2 . The deliverable itself is in
<http://ifisc.uib-csic.es/EDEN/fichaOutput.php?idOutput=12>

DESCRIPTION OF MACROPHYTE DATA FILES AVAILABLE AT THE EDEN WEBSITE, CONFORMING DELIVERABLE D1.2 (D9).

Because of journal embargo policies, public access is being given to the datafiles only immediately after the corresponding scientific paper becomes published.

MICROSATELLITE LOCI

Cymodocea nodosa microsatellite data

Data file names:

[Data Biogeografia Cymodocea nodosa ramets MLGs.xls](#)

[Geographical coordinates for C nodosa.txt](#)

These files contain the microsatellite genotypes for every ramet sampled in Alberto et al (2008). There are columns for Population (1st), Ramet labels (2nd), x and y spatial coordinates inside the population (3rd and 4th) when available, genet label (5th). From the 6th column onwards there is allelic information, two columns per locus. There are a total of eight loci. The second file has the latitude and longitude of sites (populations) sampled.

References:

Alberto F, Massa S, Manent P, Diaz-Almela E, Arnaud-Haond S, Duarte CM, Serrão EA (2008) Genetic differentiation and secondary contact zone in the seagrass *Cymodocea nodosa* across the Mediterranean-Atlantic transition region. *Journal of Biogeography* 35: 1279-1294

Posidonia microsatellite data

Data file name: [AllDataOK.txt](#)

This file contains data for microsatellite repetitions, at seven nuclear loci, obtained from approximately 40 shoots of *Posidonia oceanica* sampled in each of 37 Mediterranean coastal localities. This data set has been analysed and described in Arnaud-Haond et al. (2007).

Data were analyzed by network tools in [[Rozenfeld et al. \(2007\)](#)], [[Rozenfeld et al. \(2008\)](#)], [[Hernandez-Garcia et al. \(2006\)](#)], [[Hernandez-Garcia et al. \(2007\)](#)].

The format of the data file (illustrated by the first file entries) is:

```
population name  population number
abbreviation name
geographical location (Mallorca, Malta, ...)
geographical coordinates
number of ramets
```

and then each line contains the 7 pairs (each pair is a locus with two chromosomes) of repetition numbers for each ramet sampled followed by the separator %%%

and then a new population begins

References:

Arnaud-Haond S, Migliaccio M, Diaz-Almela E, Teixeira S, van de Vliet MS, Alberto F, Procaccini G, Duarte CM, Serrão EA (2007) Vicariance patterns in the Mediterranean Sea: East-West cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. *Journal of Biogeography*, 34: 963-976

Zostera marina microsatellite data

Data file name: [FullgenotypesZosteramarinaBrittany.122009.xls](#)

The seagrass *Zostera marina* has an extensive distribution in the Northern hemisphere and this dataset of 7 meadows collected with two quadrates per replicate was gathered to focused on i) its diversity in Brittany, that has been shown as a hotspot for genetic diversity, ii) also its high phenotypic plasticity as we also aimed at assessing the status of the morph 'angustifolia' that appears as a connecting entity in the core part of the network, supporting its status as an ecotype rather than a different species, and iii) its fine grained genetic structure that has shown a for of genetic patchiness similar to that observed for many marine invertebrates, suggesting a patchwork and heterogeneous composition of meadows likely due to the integration of successive events of recruitment through 'windows of opportunity', and questioning the usefulness of the genetic concept of population for this kind of clonal species.

TAG LOCI

Fucus vesiculosus SNP genotyping.

Expressed Sequence Tags (EST) were developed (Pearson et al. 2009) and these EST were used to define SNP, Single Nucleotide Polymorphisms, in populations of the algae *Fucus* spp. Several data files are given here, listing the following:

Data file names:

[F vesiculosus SNP alleles high shore from WB](#)

[F vesiculosus SNP alleles low shore from WB](#)

[F vesiculosus SNP Genotyping high shore from WB](#)

[F vesiculosus SNP Genotyping low shore from WB](#)

[Metadata SW UK Fucus sampling locations.pdf](#)

These first two data files correspond to the allelic frequencies and the following two data files to genotype frequencies per population of the macroalga *Fucus vesiculosus* in different habitats (high and low shore). The first column lists the SNP loci developed in this project and the following columns list the allele frequencies of the various possible nucleotide states at each locus or the genotypes. The 5th file contains the metadata describing the sampling locations. In summary, the 5 files contain:

- F vesiculosus SNP alleles high shore from Widemouth Bay, UK
- F vesiculosus SNP alleles low shore from Widemouth Bay, UK
- F vesiculosus SNP Genotyping high shore from Widemouth Bay, UK
- F vesiculosus SNP Genotyping low shore from Widemouth Bay, UK
- Metadata SW UK Fucus sampling locations.pdf

References:

Pearson GA, Hoarau G, Lago-Leston A, Coyer JA, Kube M, Henckel K, Serrão EA, Corre E, Stam WT, Olsen JL (2009). An Expressed Sequence Tag (EST) Analysis of the Intertidal Brown Seaweeds *Fucus serratus* (L.) and *F. vesiculosus* (L.) (Heterokontophyta, Phaeophyceae) in Response to Abiotic Stressors. Marine Biotechnology DOI 10.1007/s10126-009-9208-z

Zostera noltii EST data

Data file name: [EDEN report 11022010.xls](#)

The dwarf seagrass *Zostera noltii* dominates the intertidal habitats of the Ria Formosa, either in low-water intertidal pools or in waterless patches where temperatures can reach the lethal limits for the species (Massa et al. 2009). The predicted increase in sea surface temperatures of 0.2°C/decade (SST) (IPCC) is raising concerns as to the ability of this species to survive the expected global warming, especially in this kind of habitats, where shallow water temperature is more affected by air temperature than SST.

Here we present the result of five EST libraries, corresponding to four moments of the response of *Zostera noltii* to a sub-lethal temperature stress (30 min, 2h, 4h and 24h) and its corresponding negative control. We aim to identify non-neutral genes that may be implicated in the seagrass' response to heat shock by comparison of the heat shock libraries to the control one, as well as determine if expressed genes change over time.

A total of 8594 clones from all libraries were sequenced, with a total of 7799 high quality sequences. After screening for cloning relics and contamination, a total of 113 sequences were removed, remaining a total of 7686 successful sequences. Average percentage of successfully blasted sequences was 82%, successfully mapped sequences 85% and successfully annotated sequences 80%. Detailed information for each library can be found in table 1.

Fisher's Exact Test shows a significant difference ($p < 0.05$) between the 30 min, 2 hours and 4 hours libraries and the control, but not between the 24 hours library and the blank. Detailed results can be found in worksheets 2-4.

Each library was blasted against a pooled database of all stress libraries to find homologues. Identification of differentially expressed genes was performed using the IDEG6 web tool, by comparing the number of reads of each TC in all 5 libraries (worksheet 5). These were then blasted against Dr. Zompo EST databases for *Zostera marina* and *Posidonia oceanica* for matching hits. A bar graph with the number of counts for each one of these genes in each library can be found in worksheet 6; hits to Dr. Zompo's databases are also indicated.

Worksheets 7 and 8 contain pie charts for the "photosynthesis" and "response to stress" related genes, respectively, and their evolution through time, showing the number of counts in each library.

A total of 4734 TCs were screened for EST-SSRs. We found a total of 86 microsatellites, of which 43 are dinucleotides, 36 trinucleotides, 4 tetranucleotides and 3 hexanucleotides (worksheet 9).

References:

Massa SI, Arnaud-Haond S, Pearson GA, Serrao EA (2009) Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal) *Hydrobiologia*. 619: 195-201