

GENETIC DIVERSITY OF SUBMERGED MACROPHYTES DETECTED BY INTER-SIMPLE SEQUENCE REPEAT (ISSR) AND CHLOROPLAST MICROSATELLITE (cpSSR) MARKERS

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Among plant taxa, aquatic macrophytes are considered as being among the most widely dispersed, however, not much is known about their bio-geographic patterning in ecologically different aquatic systems. Submerged macrophyte like *Callitriche*, *Ceratophyllum*, *Potamogeton* and *Zannichellia* from two different aquatic habitats, namely freshwater and brackish water were considered in this study to test the hypothesis whether within species genotype differentiation correlates with the habitat differentiation. In this study, Inter-simple sequence repeats (ISSRs) and chloroplast microsatellites (cpSSRs) were used as genetic markers because of their high level of intra-specific variability.

By using ISSR and cpSSR markers on target submerged macrophyte species, the objectives of this study could be expressed as followed: (1) to examine the usefulness of ISSR and cpSSR markers in detecting variation within macrophyte species, (2) to determine the genetic variation of each macrophyte within and between habitat groups namely freshwater and brackish water; and (3) to determine genotype distribution within the macrophytes in relation to environmental factors.

A total number of 70 leaf samples of *Callitriche*, *Ceratophyllum*, *Potamogeton pectinatus* and *Zannichellia* was collected at 13 freshwater and 12 brackish water sites in Belgium in 2004. In addition, we also used dried fruits of *Zannichellia* provided by APNA laboratory-VUB. These fruits were widely collected in the period 1983-1985 from two different habitats in Europe including 8 freshwater populations and 14 brackish water populations. For the localities in Belgium (2004), water samples also were collected for measuring water quality variables. STATISTICA 6.0 was used to produce Box and Whisker plots and Mann-Whitney Test for water quality data coming from two independent location groups, namely freshwater and brackish water. Also, this program was used for creating the matrix plots and the Spearman rank tests which express the trend of relationship between an environmental variable and genetic variation of the macrophytes. In addition, genetic variation between fresh and brackish water population groups was measured by using POPGENE 1.32 program. The NTSYS-pc version 2.1 program was used to construct UPGMA dendrograms and PCA plots. Lastly, by using CANOCO 4.5, canonical correspondence analysis (CCA) was applied to determine relative contribution of environmental factors in structuring detected genotypes among *Callitriche* populations in Belgium by ISSR and cpSSR analysis.

A total of 6 ISSR primers was used to detect genetic variation of *Callitriche*, *Ceratophyllum*, *P. pectinatus* and *Zannichellia* which were collected in Belgium. The amplified products were reproducible and clearly visualised. Generally, brackish water macrophyte populations were diverse. ISSR data showed a clear association of each macrophyte group (*Ceratophyllum*, *P. pectinatus*, *Zannichellia*) with their different habitats, specially, freshwater versus brackish water. These two habitats also contrast significantly in conductivity, oxygen, chloride, silicate, orthophosphate and total phosphate. In addition, a clorotic trend in genetic variation among brackish water populations of *P. pectinatus* was found.

Chloroplast microsatellites showed a potential in detecting different haplotypes of *Callitriche* species and of *Zannichellia* species. In the case of *Callitriche*, there was no clear and distinguishable ecodemes (freshwater and brackish water populations) observed when using ISSRs and cpSSRs. However, the relationships between genetic communities and water quality variables are detected by using CCA of ISSR and cpSSR data. The results strongly support a correlation of genotype differentiation and environmental factors. Additionally, a significant geographical trend in genetic variation among individuals of a *Callitriche* taxon was detected. This indicates "Isolation by Distance" from coast to inland.

For *Zannichellia*, a comparison between freshwater and brackish water populations in Europe was investigated. Brackish water populations gave higher values of percentage of polymorphic loci, number of alleles per locus, gene diversity and Shannon's information index and number of chloroplast haplotypes. In addition, the geographical distribution of unique haplotypes also show the cpSSR potential in phylogeography.