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Genotyping of *Callitriche* L. is useful to estimate relationships of clones with environmental variables through multivariate analysis

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Species of the aquatic macrophyte *Callitriche* L. (Callitrichaceae) exhibit considerable diversity in chromosome numbers, pollination systems and ecology. Most species are amphibious and the vegetative plasticity of the leaf shape as well as the absence of mature fruits, render taxonomic identification difficult. In running water, clonal patches of most *Callitriche* species possess floating rosettes of spatulate leaves that develop when stems reach the surface. However, submerged leaves and even plants may show only linear leaves. Although *Callitriche* taxa are widespread in rivers of various water quality and often represent the only tolerant macrophyte in more polluted waters, this vegetative plasticity limits the practical use of *Callitriche* taxa as bio-indicators. The objective of this study was to investigate the genotypes of *Callitriche* populations in headwaters of rivers in Flanders (Belgium) and to estimate the clonality among rivers as well as the relationship of these clones and their genotypes with environmental variables.

The genetic variation and clonality in *Callitriche* L. populations from 16 headwaters in Belgium was determined by random amplified polymorphic DNA (RAPD). The presence and absence of amplified products after RAPD were scored for multivariate analysis with NTSYSpc 2.1 (Principal coordinate analysis – PCORDA- using the simple matching coefficient; Neighbour Joining tree using Manhattan distance coefficient). Water quality variables were temp, pH, cond, %O₂, DO, BOD, alkalinity, T-hardn, Ca-hardn, Mg-hardn, Si, Cl, OPO₄, TP, NO₂, NO₃, NH₄. Substrate quality variables were T-Kjeldahl N, TP and particle sizes in 6 classes. Habitat descriptors were indices of diverse organisms (macrophytes, diatoms, invertebrates, fish). Correlations (Pearsons product-moment) between the first three axes of the PCORDA and environmental variables (chemical variables of water and substrates; habitat descriptions) were done with STATISTICA and multivariate analysis of genotypes and environmental variables with CANOCO (CCA with forward selection using Monte Carlo permutation test, after checking the gradient length in DCA).

The correlation of multilocus genotypes with the environmental variables revealed a highly significant relationship of the first axis (PC1) with the saprobity of the water as was estimated from benthic diatom assemblages. A higher number of significant relationships of water and soil variables with PC2 is due to the limited number of samples from harder waters. The CCA showed that the variability among different clones, as characterized by their genotypes, can be explained significantly through ecological indicator values such as the saprobity index (as estimated by diatom assemblages), the index of biotic integrity (based on fishes), total Kjeldahl nitrogen of the sediment, alkalinity, sediment particle size and macrophyte scores

This study showed that multilocus genotypes or gene pools might have a non random distribution in the rivers and in the particular environments. However, the observation that there is no clear relationship along a first axis (PC1) between a gene pool and a particular water or soil quality

parameter is compensated by the highly significant relationship with more general indicators of ecological quality such as the diatom saprobity index and fish index. Indeed, these indices integrate water and habitat quality over longer periods and are an advantage over the punctual water quality estimations. Therefore, the latter might not be sensitive enough to elucidate relationships of gene pools with the environment. Nevertheless, genotypes from harder waters showed clear differences along the second axis (PC2) suggesting that the water typology can be an important factor for understanding the distribution of genotypes and taxa.