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SEARCHING FOR SCAR MARKERS NEEDED FOR DIVERSIFICATION OF THREE GROUPS WITHIN *RANUNCULUS TRICHOPHYLLUS* (RANUNCULACEAE, SUBGEN. *BATRACHIUM*)

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Batrachium is a group of aquatic plants classified within the genus *Ranunculus* L. (Ranunculaeceae). It is a widely distributed and one of the most difficult group of aquatic plants.

During the analysis of the ITS sequences of *Ranunculus trichophyllus* Chaix ex Vill. specimens from different parts of Europe significant differences were detected, and as a result intraspecific diversification of this taxon into three groups was made. Specimens forming different groups have different ecological preferences, partly also differ in range. Most likely, these groups correspond to a separate species but the full picture still needs morphological analysis. However one of the adaptations to the aquatic environment is their plastic vegetation response to changing environmental conditions, manifested in their morphological variability. This phenotypic variability is taxonomically insignificant but impedes the identification of individual species. That is why molecular analysis can be useful. Although amplification and direct sequencing of ITS (Internal Transcribed Spacers) is a reliable method to determine species but this method is relatively time-consuming and expensive. As an alternative to sequencing, RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter-Simple Sequence Repeats) are often chosen as cheap and simple methods. It is because they do not require an information sequence and are effective in obtaining the characteristic DNA profile. The main drawback of these methods is low repeatability. Conversion RAPD markers to the SCAR increase the specificity and stability, allowing for convenient and fast way to identify groups.

For the analysis there were selected 8 species of the genus *Ranunculus* and 3 groups of *R. trichophyllus*. Other taxa were treated as a reference point that allows the exclusion of products varying within *R. trichophyllus*, but being alike with other species. In each species, a different number of samples were taken. The differences in numbers of samples were determined by the availability of previously defined species based on the analysis of ITS sequences.

Samples were combined and treated as a single sample during the primers test. From 26 tested RAPD primers 11 were selected for further analysis. It was these primers which gave profiles showing the specific products for each group. Next, primers were tested whether specific products were present while using DNA from individual samples within a group. From analyzed profiles five products were chosen to be cloned into plasmids and few of them sequenced. On the basis of obtained sequences there were designed at least one pair of primers for each sequence. From four pairs of starters designed for *trichophyllus* type I one gave products for individuals from this group and didn't for individuals from other two. For *trichophyllus* type II two pairs of starters were tried and one of them worked as expected. Also one pair of starter gave products for individuals from for *trichophyllus* type I group without products for DNA from other groups. These

markers however work well for deliberate population but usually didn't mark hybrids. This is the worse flaw however shouldn't impact the significance of their role.

These markers will give the possibility to examine a much larger number of samples than it would be possible by direct sequencing. This can lead to obtain knowledge of distribution of particular groups within *R. trichophyllus*, as well as provide new information about their habitat preferences and morphological variation and shed a new light on taxonomical aspects of this group.

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