

Załącznik:

Streszczenie pracy doktorskiej z akceptacją promotora

Taksonomia molekularna sekcji *Batrachium* (*Ranunculus*, Ranunculaceae) w Europie

ABSTRACT

Species of *Ranunculus* sect. *Batrachium* (Ranunculaceae) are aquatic plants found in standing and flowing waters of Eurasia, North and South America, Africa and Australia. They are a valuable element of aquatic biocoenoses and play an important role in ecosystems. However, like most aquatic plants, sect. *Batrachium* are characterized by high phenotypic plasticity accomplished by frequent hybridization and polyploidization. This makes a morphological identification of particular species troublesome. Difficulties in individuals identification have a negative influence on the state of the art about the number of species and hybrids, their distribution and phylogenetic relationships among them. The aim of the study was the molecular identification of *Batrachium* species occurring in Europe. This, in turn, will accelerate the development of research on the ecology of the section members as well as the conservatory actions.

The use of the nuclear marker (sequence of ITS region) together with markers obtained from the cpDNA genome (two intergenic spaces: *rpl32-trnL* and *psbE-petL* along with the *matK* gene) allowed an identification of following species of *Batrachium* section: *R. ashibetsuensis*, *R. baudotii*, *R. circinatus*, *R. confervoides*, *R. fluitans*, *R. hederaceus*, *R. mongolicus*, *R. omiophyllus*, *R. peltatus*, *R. rionii*, *R. trichophyllus* and *R. tripartitus*.

Applied markers also allowed to analyze phylogenetic relationships between the identified species. The obtained results of the molecular data confirm that the *Batrachium* section is a monophyletic group of the *Ranunculus* genus. The results also suggest that *R. sceleratus* is the closest related to the hypothetical common ancestor of *Batrachium*. The evolutionary oldest group of this section consists following species: *R. hederaceus*, *R. omiophyllus* and *R. tripartitus*. The similarity of sequences of *R. fluitans* and *R. baudotii* suggests their close affinities, what is in agreement with some morphological traits of leaves. The analysis of sequences also reveals a

close relationship between *R. circinatus* and *R. rionii* and between species: *R. ashibetsuensis*, *R. mongolicus*, *R. confervoides* and *R. peltatus*.

The analysis showed that *R. trichophyllus* is a species of polyphyletic origin. *Ranunculus trichophyllus* as a morphological species has the widest range of distribution and wide ecological scale, it is also karyologically diverse. The genetic diversity of ITS and chloroplast sequence of the studied *R. trichophyllus* samples is not directly and clearly reflected in morphological features. It can therefore be argued that analyzed taxon are *in fact* a group of a few cryptic species.

Some species of the *Batrachium* section are able to develop morphologically diverse leaves: floating and submersed. Results obtained in this study suggests that it is a plesiomorphic feature. The development by various species of submersed leaves is an example of parallel evolution and is the result of adaptation of species to the aquatic environment.

Phenotypic plasticity determined by the influence of the environment, frequent hybridization and polyploidization mean that the morphological identification of *Ranunculus* taxa of the *Batrachium* section is not always consistent with their molecular identification. Sequences of particular species of section *Batrachium* deposited in GenBank and those obtained in the study are not always congruent. The incongruence probably comes out of incorrect morphological identification of *Batrachium* species from which the sequences were obtained.

Most taxa of section *Batrachium* form hybrids under breeding conditions as well as in natural environment. This indicates that among *Batrachium* species prezygotic isolation is not sufficiently strongly developed. The obtained results confirm that interspecific hybridization within section *Batrachium* occurs between following species: *R. aquatilis* × *R. peltatus*, *R. mongolicus* × *R. trichophyllus*, *R. fluitans* × *R. circinatus*, *R. circinatus* × *R. kauffmannii* (*R. × gluckii*) and not yet described *R. fluitans* × *R. baudotii*. Molecular markers also proved the hybrid origin of taxa: *R. aquatilis*, *R. kauffmannii*, *R. schmalhauseni*, *R. penicillatus* and *R. pseudofluitans*. These are well-defined species with distinctive morphological features, defined ecological preferences and distribution.

Not all terrestrial plant species can be identified by unified molecular barcoding, especially since some species may be evolutionary too young to have cumulative mutations in regions selected for plant barcoding. This also applies to aquatic section *Batrachium*. Hybridization and introgression processes are the forces which make the nature of phylogenetic

relationships reticular. It is indicated by a number of mismatches between signals based on nuclear and chloroplast markers. Despite the great success in molecular identification of *Batrachium* species not all doubts have been resolved by the applied analyses of individual polymorphisms. Intraspecific genetic diversity and inconsistencies with morphological identification make it difficult to draw final conclusions. New molecular methods that provide more information will allow us to return to problems that weren't able to solve until now. The use of methods based on new generation sequencing in the *Batrachium* section may make it possible to identify taxa of hybrid origin or polyploids.

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