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Identification of partial MHC class II B exon 2 sequences in 3 European Ranidae species

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The major histocompatibility complex (MHC) genes are the most polymorphic genes of the vertebrate genome and play a key role in the adaptive immune system. Due to their high variability they can be used as molecular markers for assessment of population genetic structure and populations' phylogeography. The use of MHC genes for species phylogeny is still disputed. In this study we report the partial MHC class II B exon 2 sequences of three Ranidae species: Rana arvalis, Pelophylax kurtmuelleri and Pelophylax lessonae, which according to our knowledge have not been described so far. For our study we used 3 individuals of Rana arvalis, 2 individuals of Pelophylax lessonae and 2 individuals of Pelophylax kurtmuelleri. Species were identified by morphological traits and in case of the two Pelophylax species we ensured identification with the amplification of a partial 16 S gene sequence from the 4 individuals. The 16S sequences of these frog species can be found in the NCBI database, thus the BLAST of our sequences proved that the morphological identification was correct. In case of all 3 species the degenerate MHC primer products were cloned and 10 colonies were analyzed for each individual. We do not claim that with this method we were able to find all the alleles and genes, but our initial aim was only the identification of the target sequences. For the Rana arvalis the target sequence length excluding primers was 186 bps and we found 8 different sequences. For the Pelophylax kurtmuelleri the target sequence length excluding primers was 196 bps and we found only one allele. For the Pelophylax lessonae the target sequence length excluding primers was 196 bps and we found 4 different sequences. Codons involved in antigen binding were identified as well.

A phylogenetic analysis of our sequences together with sequences of other frog species form the NCBI database indicates that there are overlapping alleles among species and in this case the MHC class II B exon 2 sequences are not a precise tool for species delimitation. Our findings open the possibility for further population analyses of these frog species, based on the MHC class II B gene sequences.