

The breeding system plays an important role in the evolution of plants and mechanisms of sex determination and reproduction models have long attracted the attention of scientists. However, despite intensive research many of the problems have not yet been explained. Plants in the evolution process acquired different reproduction models: vegetative (asexual) and generative (sexual). Among sexually reproducing plants, dioecious and monoecious species are identified. In monoecious species, different distribution of male and female generative organs in one individual can be distinguished. Also diverse mechanisms responsible for sex determination are present, and hence they are barely known. In plants sex determination can take place at the genetic level via the presence of sex chromosomes, or individual genes, at the physiological level by phytohormone regulation, at the epigenetic level through regulation of gene expression by DNA methylation and, last but not least, by the ratio of sex chromosomes to autosomes. However, research aimed at understanding the factors of sex determination are focused primarily on higher plants of great economic importance, e.g. spinach, asparagus, and papaya. In liverworts - plants, which according to recent studies, are the first line of development of terrestrial plants, the mechanisms of sex determination are still very poorly understood.

The aim of the project is to recognize the mechanisms of sex determination in the Calypogeiaceae family of leafy liverwort as well as to understand the evolutionary consequences that entail reproduction model in the process of speciation and whether it is the driving force of evolution in this family. Another goal of the project is to analyse phylogenetic relationships and taxonomic status of genera and subgenera within the family. The Calypogeiaceae family comprises about 120 species grouped in 4 genera: *Eocalypogeia*, *Metacalypogeia*, *Mnioloma* and *Calypogeia* with a different geographical range, from a very limited (neotropical or paleotropical) to a wide Holarctic one. The family includes both dioecious and monoecious species. Moreover, some species have the ability to produce propagules (gemmae) for vegetative propagation, while in other species such capacity has not been developed. The above features of the liverwort family selected for studies give wide possibilities to analyze the mechanisms that determine sex in this group of plants.

The basic research planned in the project concerns looking for mechanisms of sex determination in hitherto unexplored species of liverworts belonging to the family Calypogeiaceae. The mechanisms responsible for the development of female or male gametophyte will be looked for at the genome level, i.e. sex-specific sequences among the sequences obtained by transcriptome sequencing and analyzing the differences between sterile and fertile (female and male) gametophytes as well as at the cytogenetic level in order to localize on chromosomes the detected specific to sex and autosome chromosomes sequences. In the first stage of the research, analyses will be conducted on the basis of genetically determined living material obtained from *in vitro* cultures for species of the *Calypogeia* genus - dioecious: *C. suecica*, *C. arguta*, which are often fertile and have ability to produce asexual propagules and monoecious: *C. azurea*, *C. sphagnicola* that have arisen as a result of allopolyploidisation. In the next step, in order to check whether the detected mechanisms are universal for the whole family, other species representing remaining genera and subgenera will be included in analyses. In addition, phylogenetic analysis on the basis of complete organellar genomes generated by NGS method will be carried out.

Our interest in the liverwort family stems from the fact that the mechanisms responsible for sex determination in this group of plants so far have not been studied. Until now, only a few papers were devoted to liverworts, which, almost exclusively, focused on the oldest evolutionary species - thallose liverworts. The object of the proposed research is one of the evolutionarily youngest leafy liverworts families, thus the obtained results will help fill an important gap in our knowledge about the different models of sex determination in plants of different evolutionary age. The selected research object includes both haploid and polyploid, dio- and monoecious species, growing on different substrates, which will provide comprehensive possibilities of results interpretation. Liverworts constitute one of three (along with mosses and hornworts) divisions of plants that traditionally are called bryophytes and comprise about 7000 species in the world. In liverworts, the dominant phase of the life cycle is the haploid gametophyte and in, contrasts to vascular plants, dioecious species prevail here accounting for about 65%. Liverworts are organisms that played a key role in land plants evolution. Fossil records together with evidence from phylogenetic studies based on chloroplast, mitochondrial and nuclear genomes support the hypothesis that liverworts were the first plants that colonized land and were present on land approximately 475 million years ago. Heteromorphic sex chromosomes were described for the first time in land plants in *Sphaerocarpos donnellii* - species belonging to thallose liverworts. Until now, heteromorphic sex chromosomes in liverworts were detected in 4 species representing the oldest evolutionary lines: *Sphaerocarpos*, *Marchantia*, *Pellia*, whose age is estimated at over 370 mln years. The Calypogeiaceae family is one of the youngest families of leafy liverworts, its evolutionary age is estimated at about 50 mln years. An interesting question arises, whether evolutionary age was sufficient for the accumulation of changes and emergence of heteromorphic sex chromosomes in the dioecious species of this family.