A network of liverwort-specific miRNAs and their targets in the sexual reproduction of *Marchantia polymorpha*

Liverworts (Marchantiophyta) belong to the group of the oldest living land plants. Due to their evolutionary history, they are considered as a very good object for conducting evolutionary analyzes of developmental biology ("evo-devo"). The material for our research is the common liverwort (*Marchantia polymorpha*) belonging to the Marchantiopsida class. It is a dioecious plant - female thalli produce archegoniophores containing archegonia (Fig. 1A) and separately - male thalli produce antheridiophores containing antheridia (Fig. 1B). These complex generative organs are crucial for sexual reproduction of *Marchantia*. In the higher plants, flower development, flowering time and fertilization process are regulated by molecules called - microRNAs (miRNAs), which are short, non-coding RNAs. These molecules regulate gene expression level by mRNA cleavage or blocking the translation of mRNA. In this project, we will examine whether the formation of generative organs, their development and plant fertility are regulated by miRNAs. In our opinion, particularly interesting are these miRNAs that occur only in the liverworts. Thus, the key tasks to be performed in our project will be **(1) to characterize liverwort-specific miRNAs that exhibit differential expression profile in *Marchantia* generative organs: characterization of their genes, primary transcripts (pri-miRNAs), and detailed expression pattern, (2) to describe the functions of selected miRNAs and their target mRNAs and creating a model showing the miRNA network and their target mRNAs in controlling the sexual reproduction process of the oldest terrestrial plants.** Our preliminary results show that seven liverwort-specific miRNAs exhibit a differential expression pattern in *Marchantia* generative organs when compared to vegetative thalli. For these miRNAs, we also identified their target mRNAs. We intend to clarify the function of the genes encoding the target mRNAs for the miRNAs studied. We will prepare a number of mutant lines overexpressing selected miRNAs and their mRNA target genes and CRISPR/ Cas9 mutants by "switching off" the genes which encode selected microRNAs and their mRNA target genes. Characterization of mutant phenotypes (including estimation of their sexual reproduction capacity) will allow us to determine the functions of these miRNAs and their targets. Characterization of the mutant phenotypes (including the assessment of their ability to reproduce sexually) will enable us to determine the actual functions performed by these miRNAs and by the genes under their control. One such target for a specific miRNA (miR8185) is a dual-specificity phosphatase - DUSP12. By carrying out protein co-immunoprecipitation and protein identification experiments by mass spectrometry, we will designate potential DUSP12-interacting protein partners and examine their role in the generative reproduction of *Marchantia*. Using transcriptomic studies we will compare the transcriptomes of the mutants with the absence of miR8185, DUSP12 overexpression, and wild-type plants. We will search for genes that change their expression profile in the same way in both mutants, and we will compare the obtained results to the results of the co-IP experiment, thus characterizing the mutual networks of connections between the identified proteins. We will propose a model of the network of relationships between the known miRNAs - target mRNAs in the control of the development of generative organs and fertilization in liverworts. The results obtained by us will significantly fill the gap in the knowledge about the role of miRNAs in the sexual reproduction of the first terrestrial plants, and will also have a significant impact on the expansion of our understanding about the regulation of the expression of genes responsible for the development of generative reproductive organs in plants.

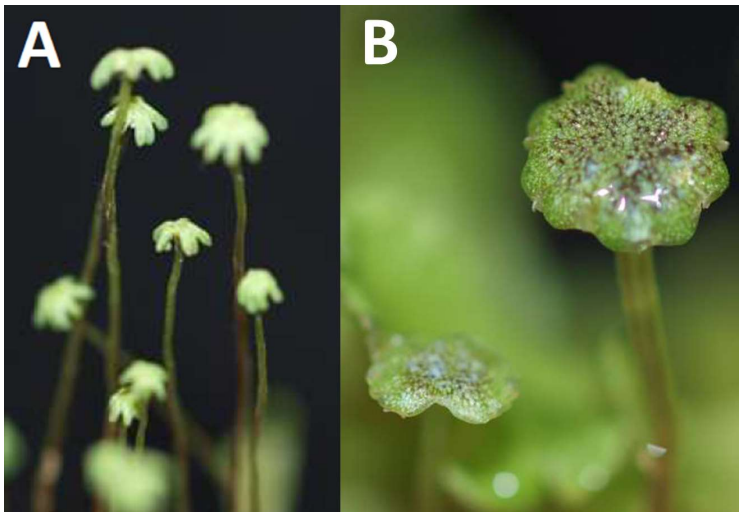

Fig.1. Archegoniophores (A) and antheridiophores (B) of *M. polymorpha*