

Project title: „Male-specific microRNAs in *Marchantia polymorpha*: key regulators of spermatogenesis and sexual reproduction efficiency”

The aim of the project is to determine the role of microRNAs (miRNAs) specific to the male reproductive organs of the liverwort *Marchantia polymorpha* in regulating spermatogenesis and sexual reproduction. MiRNAs are short (typically 18–21 nucleotides in length), non-coding RNAs (i.e., they do not encode proteins) that perform regulatory functions in eukaryotic organisms. Their role involves controlling the levels of target mRNAs (messenger RNAs). MiRNAs regulate their target mRNAs in two ways: either by participating in the cleavage of the mRNA molecule, leading to its degradation, or by blocking the process of translation, i.e., the synthesis of proteins. The importance of these molecules is highlighted by the fact that Viktor Ambros and Gary Ruvkun were awarded the Nobel Prize in Physiology or Medicine this year for their discovery of miRNAs and their crucial role in regulating gene expression.

Why did we choose *Marchantia* for our studies? *Marchantia* belongs to the liverwort phylum and is one of the oldest terrestrial plants, having inhabited Earth for over 400 million years. Due to its ancient origins, *Marchantia* serves as a "living laboratory" for studying the evolution of plant metabolic and developmental processes at the molecular level. Additionally, it has a small genome and is easy to genetically modify. Furthermore, *Marchantia* is a dioecious plant, meaning it produces separate male and female plants. Male plants produce motile, elongated sperm cells equipped with two flagella (Fig. 1A). Spermatogenesis, the process of sperm maturation, occurs in specialized organs called antheridia (Fig. 1B), which are embedded in antheridiophores (Fig. 1C).

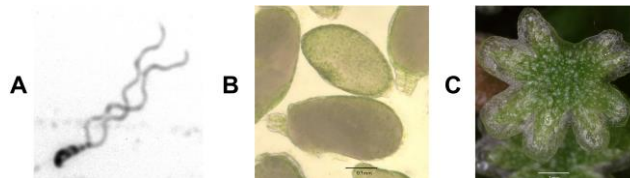


Fig. 1. A mature sperm cell with two flagella (A); antheridia isolated from an antheridiophore (B); a mature antheridiophore (C).

The microtranscriptome (the complete set of miRNAs) of *Marchantia* consists of seven evolutionarily conserved miRNA families (those found in all land plants) and over two hundred miRNA families specific to liverworts. Until now, only two miRNA families, MpmiR11889 and MpmiR11887, have been identified as being exclusively expressed in male reproductive organs of *Marchantia*. The research presented in my project focuses on three main scientific objectives: (1) Analysis of specific miRNA-mRNA regulatory modules, such as MpmiR11887-MpATX1; (2) Characterization of novel, previously unidentified male-specific miRNAs (including those encoded on the male sex chromosome) and their target mRNAs.

Notably, we have confirmed, for the first time in plants, the presence of miRNA-encoding genes on the male sex chromosome. Preliminary studies indicate that the target mRNA for MpmiR11887 is likely MpATX1, which encodes a histone methyltransferase enzyme involved in chromatin organization. *Marchantia* mutant plants lacking MpmiR11887 have larger antheridiophores compared to wild-type plants.

In this project, we aim to thoroughly examine the morphology of sperm cells in these mutants, as well as in plants with overexpression and/or loss of MpATX1 expression. Additionally, we will investigate whether altered levels of MpATX1 affect sexual reproduction efficiency.

Transcriptome sequencing (RNA-seq) and quantitative proteomic analysis will help identify other genes and proteins with altered expression levels in the studied *Marchantia* mutants. Similar analyses will be conducted for the newly discovered male-specific miRNAs and their target mRNAs.

The final step in my project (3) will involve integrating the obtained results to uncover a broader miRNA-mRNA interaction network involved in the control of spermatogenesis and reproductive success in *Marchantia*. Our previous studies have shown that mRNAs regulated by male-specific miRNAs (MpmiR11889 and MpmiR11887) are evolutionarily conserved and present in both higher plants and vertebrates, including humans. These findings suggest that our research could contribute to the identification of novel genetic factors with previously unknown roles in the development of male gametes, both in higher plants and vertebrates.

The project will expand our understanding of spermatogenesis and plant sexual reproduction regulated by miRNAs, and the results will serve as a starting point for future studies on the evolution of molecular mechanisms shaping reproductive strategies in land plants.