Reviewer Report: Liam Dolan (Gregor Mendel Institute, Vienna, Austria)
Candidate: Bharti Aggarwal
Thesis Title: Functional characterization of liverwort-specific miRNAs in sexual organ development in *Marchantia polymorpha*

1. **Achievements / Contributions of the Candidate**
This is an outstanding thesis worth of the award of a PhD.

The thesis set out to define the functions of a number of miRNAs in liverworts. miRNAs control the expression of genes by targetting the respective mRNAs for destruction. miRNAs are key regualtors of genes that conttl development, plant physiology and biochemistry. In the Introduction of the thesis (Chapter 1) , the candidate reviewed the literature on miRNAs in plants. They highlighted that some miRNAs are ancient and conserved between liverworts and vascualr plants. The candidate also demonstrated that some miRNAs were speficic to liverworts and some are restrictred to sub groups of liverworts. These lieverwort-specific miRNAs were the focus of her research because only two of these had prebiously bee characterised (MpoMir113 and Mp FRH1). The comprehensive introduction was also a good summary of the state of the art and eloquently described the role of micro RNAs in loverwort development, while also highlighting the liverwort life cycle, which is considerably different from the live cycle of angisoperms, from which most of our knowledge aobut plant miRNAs comes.

The second chapter outlines in detail the Material and Methods used in the course of the research and these adequately describe experimentally procedures. The third chapter details the discoveries the candidate made during the course of her research.

2. **Highlight novelty, significance**
The candidate carried out a great deal of research on a numebr of different micro RNAs. Valuable discoveries have been made for all of these molecules

The candidate has made important discoveries bout the role of MIR 11796 in plant development. Not only have they described gene structure and sequence, but they also have considerable evidenec for the function of this miRNA in the devleopment of the plant. For me this was a highlight of the thesis. By examining a wild type transcriptome and RNA detection by Northern blotting, the candidate showed that the miRNA is present in developing reproductive structures (gametangiophores). Promoter:GUS lines were generated and restricted patterns of GUS expression were observed, suggesting that the gene is expressed in a subset of cells. IN aparticualr a clear strang of expression was seen along the gametangiophore corresonding to the position of rhizoid cells suggesting a funciton in this cell type. To test this hypothesis the candidate generated loss of fcuntion mutants in the micro RNA and characterised the phenothpes of the mutants. Mutants developed fewere rhizoids than wild type, suggesting that the micro RNA was required for rhizoid cell development. It could target a gene that represses rhiozid cell development, which is derepressed in the absence of the miRNA. Identificaiton of the target of MiR 11796 in the future will test this hypothesis. The candidate made sensible efforts at identifying the mRNA target of MiR11796, but at the time of writing had not managed to identify and versify the identity of the target (although a number of possible targets were investigated). This part of the research could be written up as an elegant publication on its own because the findings are novel. Obviosuly the identify of the target would increase the impact of such a publication. This was an elegant and informative piece of research.

The candidate demonstrate dht the MIR11887 is functional in Marchantia. Loss of funciton mutants develop defective antheridiophores, suggesting that it modualted the function of an mRNA expressed in the make preproductive organ. This is a very interesting observation.

The candidate also demonstrated the expression patterns of a number of other miRNAs. These include a characterissation of MIR 11737a and MIR 11737b which may target similar mRNAs for slicing. However, MIR 11737a and MIR 11737b are differentiatlly expressed and this may reflect tissue specialisation, wehre these micro RNAs are expressed in different tissues. They may therefore operate in different developmental or physiological contexts.

MIR11865 and MIR11865* are encoded in the same transcript and are expressed in diferent, complementary tissues. This leads to the hypothesis that the same transcript controls the expression of two different mRNAs in different tissues. This is an important discovery and probably the first of its kind in liverworts.

The candidate also identified putative targets of MIR11887 and future characterisdation of thes emutants will be informative.

The candidate has published a very good paper in *RNA Biology* in 2024 and has contributed to a review on miRNA in palnts focussing on what we have learned from Marchantia in 2023. I think there is a further very interesting paper to be puboished based on the information of MiR11796, and perhaps more.

3. **Critical Evaluation:**

The introduction was very well written and focussed on the main points in the published literature. It would be impossible to write a comprehensive review of the function of micro RNAs in plants. Many students find it difficult to focus their Introduction when there is such an abundance of knowledge. However, this candidate found their focus. This is important because it indicates that the candidate understands the field and is therefore in a position to identify the important, relevant parts that need to be included in a focussed introduction.

Clarifications
I have no major recommendations for changes (because the thesis is excellent).

Queries
I have few queries but I would like the candidates opinion on a few topics. I will ask these uring the oral examination. For example, I found the text in section 5.1.2.1 a little confusing and I would like to get clarity on this. I would like to hear the candidates view on the priority experiments to be carried out for MIR 11796. Why are so many micro RNAs differentially expressed in reproductive structures?

The candidate has produced an outstanding thesis that includes many important discoveries. I look forward to discussing the exciting data with the candidate.

4. Conclusion / Recommendation:
The research presented in the written thesis meets the standards of a PhD dissertation. This is based on my experience as an internal examiner at the University of Oxford, University of

East Anglia, and the University of Vienna, as well as my experience as an external examiner at numerous universities around the world.

I enthusiastically recommend that the candidate proceed to the next stage of PhD defense procedure.

Liam Dolan

Vienna

27/7/25