

Review of the master thesis

“Molecular mechanisms of predation of planktonic protist *Hemistasia phaeocysticola*”
by Bc. Tomáš Knobloch

The present thesis deals with analyses of differential expression between cultures of marine diplomonid *Hemistasia phaeocystida* feeding on various food sources (none, rich medium, and bacteria). The thesis is written in English, consists of 42 pages and is organized into Introduction, Aim of the thesis, Materials and methods, Results, Discussion, Summary, and References. The text is supplemented with 19 Figures and 11 Tables.

In the Introduction, the author provides information on ocean ecosystems and food webs, heteromorphic protists, diplomonids, and model species used in the study, *H. phaeocysticola*. The text reads well although there are some surprising moments. Such as introduction of a bacterial loop supported by experiments with fluorescently labelled prey analogues (here I am not sure whether the article referred to is Pachiadaki et al. 2016 or Rocke et al. 2015), which is followed by information that the bacterial loop is outdated. This could be elaborated and evidenced by more references. There is also inconsistency between information that “a significant number of planktonic protists cannot be easily categorized into heterotroph and autotroph categories” and concluding sentence of Chapter 1.3 that “phototrophy and mixotrophy are generally excluded as probable feeding strategies of diplomonids due to large abundance of diplomonids in deep ocean and phylogenetic relations”. Reference to Fig. 1 is missing in the text. I would also appreciate some overview of similar studies and methodologies used to identify food behaviour in protists.

Aims were to record growth curves under various conditions, test for intake of bacteria, and perform differential expression analyses. This section contains also working hypotheses that *H. phaeocysticola* is a generalist and does not discriminate between different food sources. These hypotheses should be explicitly addressed in the text. I am confused by the claim made in the Discussion that „*H. phaeocysticola* might be a generalist with a wide range of nutrient sources“. Could you explain it.

Materials and methods are described in sufficient details. I miss primer concentrations in Tables 1 and 4. Table 3 shows composition of standard LB medium and could be omitted. Final concentration of SDS is missing in Table 7. As for the bioinformatics pipeline, I would like to ask what are advantages of rnaSPAdes and Salmon compared to generally used Trinity and RSEM, respectively?

The Results evidence growth curves, localization of bacteria inside of *H. phaeocysticola* vacuoles, and differential expression analyses. *H. phaeocysticola* grew better in presence of bacteria but eventually died “possibly from the lack of available bacteria”. Fluorescence *in situ* hybridization and DAPI staining shows presence of bacteria in vacuoles. The text states that bacteria in vacuoles are partially digested. Is there any evidence supporting this claim? I would say that the growth curves can be explained by inability of *H. phaeocysticola* to digest *Paracoccus* bacteria. Is it testable? The author used N50 statistic to characterize his transcriptome assembly. Is it meaningful? Are there any other ways to evaluate transcriptome assemblies? As for the differential expression analysis, all samples were compared against each other? However, are not you interested also in

genes which are upregulated in feeding compared to starving *H. phaeocysticola* and common to both food sources? What were null hypotheses for Fisher's exact tests?

The Discussion reads well but as mentioned above, the hypotheses set in the Aim are not clearly addressed as well as digestion of bacteria. I am also not sure what is a relevance of *Paracoccus carotenoids* (p. 34).

There are some typos and awkward formulations, but overall the thesis is written in good English. I find the format of citations in the text where reference follows full stop at the end of sentence weird.

Conclusion

Despite minor issues listed above, I liked the thesis. The author acquired hands on experience with bioinformatics analyses and met the aims. The present thesis in my opinion fulfils all requirements and I recommend it for successful defence.

On May 24, 2021, in České Budějovice

Petr Nguyen