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Examination Report for the PhD thesis "*Chromera velia* heme pathway localization", submitted by Mgr. Jitka Richtová

Dear thesis committee,

the thesis submitted by Jitka Richtová describes findings on the biology and on biochemical pathways of *Chromera velia*, a photosynthetic relative of the apicomplexa. One focus of the thesis is on the heme pathway, which is delivering building blocks for tetrapyrrole biosynthesis, required for generation of chlorophylls, hemes, phycobilins, and other compounds. *C. velia* had been discovered only a couple of years ago and was positioned within the alveolata, comprising groups like the apicomplexa and the dinoflagellates. Although apparently being a sister group to the secondarily heterotrophic/parasitic apicomplexa, *C. velia* and related chromerids, like the also investigated *Vitrella brassicaformis*, are photosynthetic organisms. This leads to interesting evolutionary questions regarding the biology, genetics, biochemistry, and cell biology of these organisms in comparison to the well-studied apicomplexa.

The submitted thesis is a cumulative PhD thesis (thesis by compilation), and comprises three main parts: a general introduction/discussion of the topic of the thesis, followed by five already published manuscripts with Jitka Richtová as first author/co-author, as well as a CV with a publication list. The introduction first describes the general aspects of *C. velia* with regard to its discovery, morphology, evolutionary origin and life cycle. Next, the biochemistry of the heme pathway in different organisms is presented as well as the individual enzymes involved in the pathway. Furthermore, a detailed description of intracellular protein targeting within different organisms and into different organelles presents detailed information on targeting sequences and mechanisms. This general part of the thesis is very well-written and gives a comprehensive view on these topics. The citations in this chapter include also the latest publications in the field. It demonstrates that Jitka Richtová is aware of the current literature and the state-of-the-

art lab methods. It was a pleasure to read this chapter, also because of the often hand-drawn figures that complement well the descriptions in the text.

Five of the six manuscripts mentioned in the list at the end of the dissertation have been included in this thesis. The first manuscript "Using Diatom and Apicomplexan Models to Study the Heme Pathway of *Chromera velia*" has been published in 2021 with Jitka Richtová as the first author in the "International Journal of Molecular Sciences". It tries to unravel the heme pathway in *C. velia* by comparing it to the better studied model systems, diatoms and apicomplexa. By using prediction software as well as experimental approaches, the intracellular location of heme pathway enzymes are studied. While in organisms with primary plastids the heme pathway is located within the plastids, the results here demonstrate that in *C. velia* the enzymes may have different evolutionary origins and may be located in different cellular compartments. The second methodological manuscript "Isolation of plastids and mitochondria from *Chromera velia*" published in "Planta" (2019) with Jitka Richtová as a co-author describes a new protocol for the isolation of organelles of *C. velia*. An important step here is the fractionation of the ruptured cells by differential centrifugation and by applying density gradients. Remarkably the authors had been able to isolate a plastid fraction that is free of mitochondria which was proven via Westrn blots using organelle-specific antisera. Similarly, a fraction of mitochondria being free of cellular contaminations had been obtained. These methods may be very valuable for future research when pure organelles are required. The third manuscript "Budding of the Alveolate Alga *Vitrella brassicaformis* Resembles Sexual and Asexual Processes in Apicomplexan parasites" in "Protist" (2017) with Jitka Richtová (Kručinská) as co-author now deals with a different topic, which is the formation of zoospores. Via electron microscopy, gene analyses, and fluorescence microscopy, it is shown that *V. brassicaformis* shows two types of zoosporangia with different structural features. Furthermore, the fusion of zoospores has been observed, allowing to improve the model of the life cycle. Manuscript number IV "Separation and identification of lipids in the photosynthetic cousins of Apicomplexa *Chromera velia* and *Vitrella brassicaformis*" from 2017 with Jitka Richtová (Kručinská) as co-author shows a biochemical approach. It appeared in the "Journal of Separation Science", and describes the analysis of the lipid composition of the two species by sophisticated approaches like chromatography and mass spec. The lipid profile shows a large number of compounds with a rather high proportion of neutral triacylglyeroles, which may be storage compounds. One though has to take into account that the cells had been cultivated under optimal light and nutrient conditions, which partially may explain a large amount of storage lipids. The last manuscript "Life cycles of chromerids resemble those of colpodellids and apicomplexan parasites" from 2016 with Jitka Richtová (Kručinská) as co-author is a review article that appeared in "Perspectives in Phycology". It gives a comprehensive overview on the current knowledge in this field.

Overall, this thesis covers a large area and different aspects of the biology of chromerids and demonstrates that Jitka Richtová has been using successfully different technical methods and approaches. Being involved in a total of six manuscripts in good to very good journals during the PhD thesis is impressive. There is only one first author paper included, however, according to the contribution lists, Jitka Richtová had been actively involved in the experiments and in writing of the other manuscripts. The period of ten years for developing this thesis is quite long, but can be explained by a longer maternity leave. Furthermore, the manuscripts presented here date back only to the last five years. It is always difficult to compare PhD theses in different systems, if this thesis would be submitted at the University of Konstanz, I would estimate that it should be among the upper 30%.

On the basis of the elements presented in this report, I clearly recommend accepting this thesis to be defended.

Questions for the defense:

- The phylogenetic tree in Fig. 2 (page 6) shows that the branching of the apicomonada within the alveolata is shortly after the dinoflagellates separated from the apicomplexa. I would like to know on which basis this tree has been generated, how stable this branching is, and whether other aspects (cellular, biochemical, etc.) could argue that the chromerids are closer to the apicomplexa.

- Based on your protein targeting results, what is your model for plastid protein targeting in *C. velia*?

- Could you observe any light-dependent oxygen evolution in the isolated *C. velia* plastids? Considering that some of the envelope membranes of the plastids remain intact, one would expect that photosynthesis might still be functional.

- Reading the Protist (2017) manuscript, I became a bit confused regarding the life cycle of *V. brassicaformis*. It was shown show that zoospores can fuse, which is something I would expect from gametes. Could you please explain to me how this works in *V. brassicaformis*?

- The growth conditions of the chromerids for the lipid analysis are surely optimized with respect to light and nutrients, which partially may explain the high content in storage lipids. Is there also data available (GC-Ms or fluorescence staining) whether this is also the case under sub-optimal conditions?