



Supervisor' review of dissertation thesis by Pragya Tripathi

When Pragya came to our lab from her master studies in France, she knew surprisingly little about the trade of doing research, including the basics of cell and molecular biology, giving me a disconcerting impression about the quality of some French universities. Pragya was trying to make up for it by enthusiasm and easygoing communication, which certainly helped but of course there are also limits to that. When she arrived, our former PhD student Sameer Dixit was leaving for the US, taking with him the complex methodology of the iCLIP protocol that he established in our lab for *Trypanosma brucei*. Hence, I asked her to learn that method from Sameer and try to establish it in *Diplonema* (*Paradiplonema*) *papillatum*, the model of our lab and from that moment on also for Pragya. The aim was to use this powerful method to identify RNA targets of a peculiar RNA ligase identified in the mitochondrion of this *Diplonema* by a Canadian group. Unfortunately, this was too challenging for several reasons, also for the guys in Montreal, so Pragya at least overexpressed the protein and generated polyclonal antibodies against the ligase. This will hopefully be useful in its future studies but was not included in the submitted thesis.

We sat down and pondered what to do next. The decision was to stick to *Diplonema* because by that time two members of the lab, Drahuska and Binny, managed to introduce gene tagging and homologous recombination into this flagellate. This also corresponded well with our funding at that time and the intention of a substantial part of our lab to focus intensely on this long ignored yet ecologically and evolutionary extremely interesting group of marine protists.

The switch was much delayed by Pragya's pregnancy and successful giving birth to her son, followed by a relatively short maternity leave. All this was not helped by the covid pandemics, which stranded Pragya and her family in India, as they were simply not allowed to return to Europe from their holiday for several months. Hence, getting on the track of projects that will be presented today by Pragya took very long time, certainly more than is a standard in our lab. But as we all know well, c'est la vie sometimes and one has coping with it.

And I can say that Pragya successfully did cope, despite all the odds. Indeed, initially I and others in the lab had serious doubts about the prospects of her PhD, given the motherhood in a foreign country, having scientific husband with demanding duties working elsewhere in the institute, by which I mean the Zikova lab, and keeping in mind the abandonment of the initial main project. But as you will hopefully see, Pragya managed to push several projects, some of which are still halfway, mostly for the reasons described above. In all of them,



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we aimed to target protein complexes that are either omnipresent in eukaryotes, and yet are extremely derived in euglenozoans, where they were intensely studied usually only in the model parasitic protist, *Trypanosoma brucei*.

Alternatively, as in the case of the paraflagellar rod proteins, they represent a hallmark feature of an important eukaryotic supergroup, whether you name it Excavata, Discoba or otherwise.

Although the methodology of tagging a gene, getting it into the genome of *Diplonema* and pulling down its interacting partners was just established in our lab, it turned out not to be trivial at all. In fact, Pragya's projects represent, along with Drahuska's Sec13, the very first applications of this methodology that we hope will turn diplomonids into organism much more amenable for molecular biologists. Each project thus required specific adjustments for generating a suitable construct and obtaining overexpressing clones, as well as specific conditions of electroporation and mass spectrometry, requiring some decent level of stubbornness.

Despite her husband getting a job at the University of Varanasi in India, Pragya managed to arrange her private life so that she did her share of experiments and the lab. Especially the previous year and the present one is turning out into being pretty productive, as Pragya contributed also to other projects not included in her PhD, such as the expression of modified tRNAs in trypanosomatids.

In parallel, Pragya made a substantial progress in her abilities to critically think about experiments, designing cloning strategies, preparing controls, and so on, as well as presenting her work. The impression from her PhD might be that her contributions were mostly methodological, and I would not deny that. It is actually methodological advances that in cell and molecular biology drive many fields or may even have transformative power. Due to Pragya's tagging projects we now have tools necessary to genetically modify *Diplonema* (and that it is not just another protocol on some other protist may be reflected by Drahuska et al. paper on genetic tools for marine protists, with key position kept by *Diplonema*, published well in Nature Methods, picked as a highlight by the editor of Science etc.), and also several highly promising protein complexes worth an in-depth characterization. While the thesis does not belong to the top ones produced in our lab, it is in my opinion a decent amount of work.

Therefore, I would like to state that the presented dissertation thesis fulfils, in my opinion, all postulations and I recommend it to be accepted as a partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Faculty of Science of the University of South Bohemia.



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Thanks, Pragya for a good work and all the best in your next life.

June 19, 2023 in České Budějovice

