

**Supervisor's opinion to bachelor thesis Comparison of bioinformatics pipelines for eDNA
metabarcoding data analysis of fish populations in Czech reservoirs written by
Rômulo Acácio dos Santos**

The aim of the bachelor thesis was the application of five bioinformatic pipelines for one environmental DNA (eDNA) metabarcoding dataset. The aim was based on the practical need for comparison of different pipelines outputs to verify the trustfulness of eDNA metabarcoding for ecological studies. Therefore, the thesis is not a student exercise, but very important contribution to science and practical management.

I highly appreciate the complexity of the thesis. Romulo has proved his orientation in all aspects of bioinformatic workflow. Although the main aim was the comparison of outputs of five different pipelines, Romulo dedicated a huge effort to improve the reference database. He updated the reference database developed at the University of Hull by sequences from a public database and added new *de novo* sequences (these he submitted to the public database). Romulo managed to run all selected pipelines, which cover the main trends in the current bioinformatic processing (e.g., free MiFish x private SEQme; identical parts of the processing in Barque & MetaBEAT x highly different algorithms in Anacapa). The outputs were tested by appropriate statistical tests and the outcomes were schematically visualized. In the discussion, Romulo critically evaluated the results in the context of the main community characteristics, ecological requirements of detected species, published literature, and recent contributions at international conferences. The cited literature covers 120 sources composed mainly of scientific literature and software references. Moreover, Romulo attended the collection of water for eDNA in the field and the following processing in dedicated laboratories at Biology centre CAS.

As the supervisor, I have to highlight Romulo's diligence and purposefulness. Romulo is following the modern trends not only by studying literature, but also attendance at international conferences. I can imagine, the thesis would be finished earlier, but I am aware of high-quality work needs time.

To sum my opinion up, the submitted thesis exceeds in many aspects the minimum requirements of Bachelor thesis at the Faculty of Science, University of South Bohemia in České Budějovice. Therefore, I recommend the thesis for acceptance with excellent evaluation.

In České Budějovice April-18 2021

RNDr. Petr Blabolil, Ph.D.