Opinion of the bachelor's thesis of Rômulo Acácio dos Santos

	☐ supervisor's opinion ☐ bachelor's thesis		☑ opponent's opinion ☐ master's thesis		
Author:		Rômulo Ao	cácio dos Santos		
Name of work:		Comparison of bioinformatics pipelines for eDNA			
		metabarco Czech rese	ding data analysis of fish porvoirs	pulations in	
Study program, spec	ialization:	Applied Inf	Formatics (Bioinformatics)		
Issue year:		2021			
Name of opponent:		Ing. Marta	Vohnoutová		
Institution:		Institution of	tion of Applied Informatics		
Contact e-mail:		mvohnouto	va@prf.jcu.cz		
Professional level of	f work:				
☑ excellent □ very	good 🗆 averag	ge 🗆 below	average unsatisfactory		
Formal mistakes: □ almost none ☑ ac	cceptable number	r 🗆 numero	ous, non-essential essentia	al	
Results: ☑ original □ original copied	nal and taken 🛚	nontrivial co	ompilation	erature 🗆	
Scope of work: ☐ large ☑ standard	l □ acceptable	□ unsatisfa	ctory		
Graphic, linguistic, ☑ excellent □ very			ow average □ unsatisfactory		
Typographical erro □ almost none □ a		er 🗆 numer	ous		
Complex level of wo ✓ excellent □ very		age □ belo	ow average □ unsatisfactory		

Verbal statements, comments and remarks of the opponent:

The aim of the thesis was to compare five pipelines (Anacapa, Barque, metaBEAT, MiFish, SEQme) for environmental DNA (eDNA) metabarcoding using data from water samples taken in three reservoirs (Klíčava, Římov, and Žlutice) both in the summer and autumn seasons. The results were analysed by comparing the number of reads assigned, number of species detected, and ecological indices (alpha and beta diversity).

Picked-up data were filtered, got rid of "genetic pollution", trimmed, and carefully prepared to avoid mistakes and false positive and false negative events. In the end, various appropriate statistical analysis ANOVA, post-hoc Tukey, and PERMANOVA were used to compare results from individual pipelines. For ecological diversities, Alpha (richness and Shannon index) and beta (Jaccard index) were used.

The compared pipelines for eDNA metabarcoding are of different logics and this thesis inspected their features and outputs thoroughly.

The student worked independently and took place in all steps of the work. He himself took the water samples from water reservoirs and processed samples in laboratories at Biology centre CAS.

The student used considerable amount of programming code (mostly Python and R), attached to this thesis. The results are described visualized in many clear graphs.

The important part of the work is also the reference database, originally derived from the reference database of the University of Hull, which the student updated. He also stored new sequences to the public databases.

It is obvious that the work comprises also a lot of study, the list of used literature is astonishing. The student describes the individual steps in logical and understandable way.

The only one objection – the student should distinguish better e.g. program name and simple text.

Conclusion:

The work is so complex and mature that could be easily accepted as a master's thesis. The result of the work has a large practical importance.

I recommend presenting the results of this thesis in some biological conference and/or publish it in some professional magazine.

Questions, discussion topics:

- 1. Reference database how did you decide what should be there and how you adjust the original reference database from the University of Hull? E.g.
- 2. Is it possible to discover in your water samples the creature who in not in your reference database (you did not simply expect it to be there in samples)?
- 3. Maylandia zebra was put intentionally to the water samples to test the pipelines? Or could you explain the role of Maylandia zebra?
- 4. How you can explain that in some cases some pipeline discovers none and another considerably high amount? E.g. Alburnus Alburnus in Římov?

Work				
☑ recommend				
☐ do not recommend				
To accept as bachelor's thesis				
With the suggested mark: ☑ excellent □ very good □ good □ unsatisfacory				
Place, date and signature of the opponent:				
In České Budějovice 5. May 2021				