

Review of the master's thesis
“Analysis of the dicyemid diversity using amplicon sequencing”
by
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The present thesis deals with diversity of Dicyemida in sampling of various cephalopod hosts. The author barcoded the hosts and extracted DNA from their renal organs, which contain dicyemids. Then she amplified rDNA, used the amplicons for PCR construction of Illumina libraries and performed bioinformatic analysis of resulting sequences including species delimitation.

The thesis is written in good English and is traditionally organized into Introduction, Main goals, Materials and methods, Results, Discussion, Conclusion, and Bibliography and contains extensive Attachments comprising supplementary figures and tables.

In the Introduction, the author provides information on dicyemids, their biology and principles of used methodologies. The text reads well but I got confused several times. Dicyemids are not properly defined and classified sooner than on p.3. They should be parasites of benthic cephalopods, other mentioned cephalopods are sepioid, teuthoid, coleoid. Unfortunately, I do not remember cephalopod system anymore so some detail on it would be nice. I learned that dicyemids are not considered parasites on p. 1. But parasitism is still invoked throughout the text to explain selection of molecular markers or their simple body plan. The author noted that dicyemids reproduce both clonally and sexually. What evidence is available for their sexual reproduction and how does it work? Are they hermaphrodites? Does axial cell correspond to a germline? When do dicyemids opt for sexual reproduction? As for amplicon sequencing, do I get it right that the used primers are universal for 16S rDNA but are “capable of amplifying 18S eukaryotic rDNA” and are “optimal for marine taxa”? What marine taxa? Is there any reason to choose 18S rDNA over ITS, which is used for barcoding in some taxa?

The aims are clearly defined. Methods are described in sufficient details. Out of interest, is it necessary for short spacers in heterogeneity primers to be random? And could you explain me again number of samples and reactions included in libraries. I got lost there. I consider tables containing PCR temperature profiles redundant. Description of clean-up of PCR reactions and DNA quantification is also repeated. Some chapters describing a bioinformatic pipeline consist of a single sentence and could be merged. The methods also refer to partial results documented in numerous figures and tables, which should be in the Results and properly commented.

The Results focus only on diversity of dicyemids. The author identified over 17 major dicyemid types, revealed their high prevalence and analysed their alpha and beta diversity. The results are nicely discussed. The author tried to assign some of the recovered types to known species based on their host spectrum, critically evaluated the methodology, and considered future research perspective.

Conclusion

Despite minor issues listed above, I enjoyed reading the thesis, which in my opinion fulfils all requirements and I recommend it for successful defence.

On May 20, 2022 in České Budějovice

Petr Nguyen