

## MASTER'S THESIS EVALUATION

Title of the thesis: *Reconstruction of the evolution of multiple sex chromosomes in Leptidea wood white butterflies*

Author: Bc. Kristýna Pospíšilová

Supervisor: prof. RNDr. František Marec, CSc.

I read the Diploma thesis of Ms. Kristýna Pospíšilová, entitled “*Reconstruction of the evolution of multiple sex chromosomes in Leptidea wood white butterflies*”, with great interest.

Owing to the dynamic genome reshuffling and a unique species-specific system of multiple-sex chromosomes, wood-white butterflies of the genus *Leptidea* represent an interesting model for studying the various aspects of karyotype differentiation, including the role of chromosome rearrangements in species' evolution.

The thesis aims to perform a comparative sex chromosome analysis in three closely related cryptic species *L. juvernica*, *L. sinapis* and *L. reali*. In order to, reconstruct the evolution of their species-specific multiple sex chromosomes and to elucidate the role of the complex sex-chromosome system in the evolution and speciation of *Leptidea* butterflies.

To fulfil the goals of this study, Ms. Pospíšilová has firstly identified all the multiple Z chromosomes in the species of interest by FISH mapping of the BAC probes containing Z-linked *Leptidea* orthologs of *Bombix mori* genes. By comparing the assembled physical maps of the individual Z chromosomes of the triplet of species, the student has successfully identified the ancestral Z chromosome and synteny segments of autosomal origin. The acquired knowledge then served as a solid basis for revealing the chromosomal rearrangements involved in the formation of species-specific sex chromosome systems and for the reconstruction of the step-by-step evolutionary pathway of multiple sex chromosomes in *Leptidea* species.

From my point of view, the thesis exceeds the average quality expected from a master thesis, both in terms of results obtained and formal quality of presentation. The thesis is structured in a standard format and written in English. The language the student used is of quality, especially considering that Ms. Pospíšilová is not a native English speaker. The cited literature consisting of relevant scientific publications is sufficient and appropriately chosen. As a person that is not specialized in the cytogenetics of butterflies, I appreciated the clear literature survey at the beginning of the thesis and the clear definition of the objective of the study, as well as the scope of the research.

The student proves a deep understanding of both the research topic and the methods used. Ms. Pospíšilová has already demonstrated gaining the skills in her bachelor's thesis, in which she presented the first original results of an initial analysis of *Leptidea* multiple sex chromosomes using BAC-FISH.

The thesis contains a negligible amount of formal mistakes and/or typing errors and is written in a clear syntax allowing the reader to focus on its content. The figures and schemes are illustrative, accurately demonstrating the main findings. In this regard, I would like to highlight the high-quality standard of the graphics which forms an essential part of the presented results.

From my perspective, the student has accomplished all objectives of the study and I consider the thesis to be above standard. The thesis brings an original contribution to knowledge, being of interest to the broad scientific community. In this respect, the quality of the results obtained is indisputable, given the fact they constitute a relevant part of the scientific publication currently published in *Heredity*.

I have a few questions for Ms. Pospíšilová:

- I. Did the student find it difficult to obtain chromosomal preparations of satisfactory quality for BAC-FISH in the studied species? How many individuals out of the total provided suitable chromosomal material for the experiments?
- II. The three *Leptidea* species show a wide range of geographical distribution. The specimens investigated herein were collected from localities in the Czech Republic or Spain. Considering the chromosome instability in *Leptidea* species, can we confidently expect the species-specific constitution of sex chromosomes to be stable across the whole range of species distribution?
- III. After the divergence of the triplet of *Leptidea* cryptic species, the Z chromosomes tended to further differentiate in each species independently. Why is that so? Could increasing complexity of the sex chromosome system be potentially disadvantageous for the *Leptidea* species?

8.7. 2020, In Rychnov u Jablonce nad Nisou

Mgr. Jana Štundlová, Ph.D.