PhD thesis referee report

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Title: Contrasting patterns of karyotype and sex chromosome evolution in Lepidoptera

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PhD thesis by Jindra Šíchová is a study on various aspects of chromosome (genome) evolution in Lepidoptera. Text is well composed, it is easy to understand and the facts are clearly explained. Most of the work is presented as separate articles that have already been published in the international (highly impacted) journals, in other words they have already been revised and corrected. Therefore, it is difficult and almost inadequate to re-review these results. The main body of the thesis is based on 4 papers in excellent journals (PNAS, PLoS One, BMC Evolutionary Biology and Biological Journal of the Linnean Society). My aim as a referee is rather to open a discussion on relevant topics mentioned in PhD thesis than to evaluate the quality of published results.

Comments and questions:

Author claims (page 2) that the common ancestor of Lepidoptera had a sex determination Z0/ZW (W appeared later in evolution). If we go deeper into the evolutionary history – how Z0/ZW system has established?

How much is the knowledge on Lepidopteran sex chromosomes based on just cytogenetics? What is known about genetics of sex chromosomes in these species (sex determination genes, degeneration of sex chromosomes *sensus stricto*)?

Generally, XX/XY and ZZ/ZW systems are considered as analogous systems. How do these systems differ (pace of degeneration....)?

In FISH experiments, fluorochrome-labelled probes or biotin and dig based probes are used. What makes a difference in terms of quality (e.g. background problems) and sensitivity? What is the size limit of low copy probes (e.g. genic region derived) for signal detection? As for BAC-FISH, I have negative experience with this approach in plant models (widespread repetitive sequences). Pictures presented by the author (mainly PNAS paper) are beautiful. Does your BAC-FISH work routinely? How many BACs (percentage) are removed from the study because of multiple or spread signals? How much is the final (published) picture dependent on *in silico* image processing?

It is claimed that exceptional variability in chromosome numbers and localization of cytogenetic markers contrasts with intraspecific stability of sex chromosome constitution (page 81). On the other hand, it is known that e.g. Y chromosome size varies significantly among human races. Do you see such phenomenon in butterflies? Moreover, recent study on human and ape species reveals that human and gorilla Y chromosomes are almost identical while chimpanzee possess (gene content) very divergent Y chromosome. Can you comment this observation that little bit contradicts your claim. Based on your knowledge of genome structure and evolution in butterflies what is your preferred theory about evolution of sex chromosomes – 1. Inevitable doomsday because of degeneration (popularized by Jenifer Graves) or 2. Existence of molecular mechanisms suppressing degeneration (e.g. supported on David Page observation in human).

My recent interest is to compare sex chromosome evolution between the plants and animals. It seems there are specific evolutionary pathways forming sex chromosomes in these two kingdoms because of differences in development (e.g. short haploid phase in animals versus long haploid phase in plants). Do you have some idea why and how could plant and animal context make sex chromosome evolution different?

There are many economically important species studied in the thesis. Based on the papers (and discussion in these papers) published by Jindra Šíchová it seems that knowledge about genome structure in these species is rather limited. I am quite surprised since there are many ongoing sequencing projects on various less interesting and important species. Can you introduce the context of genomic research (number of international groups, extent of genomic studies....) running in this area?

The most striking result I found in the thesis is Z-autosome fusion bringing two genes (gene for insecticide resistance and gene for detoxification of plant secondary metabolites) under sex-linked inheritance in *Cydia pomonella*. The consequences of the fusion for the evolution of *Tortricidae* genome is suggested but should be confirmed. Have you any idea how to design and manage such project? Do you plan to continue in this direction?

As I wrote at the beginning, it is difficult to evaluate published results. Anyway, I am curious how did reviewers comment submitted manuscripts. I am interested just in major (if any) issues.

As a summary of my comments I have to declare that Jindra Šíchová has carried out excellent research. She has studied interesting biological topics and revealed her ability to answer and discuss many opened questions. Results of her research have been published in peer reviewed international journals. On the basis of all her excellent achievements, Jindra Šíchová should be awarded by PhD degree.

Brno, June 10th 2016

RNDr. Roman Hobza, Ph.D.

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External review

The major part of the thesis consists of four papers, all are already published in reputable journals (*PNAS, PlosONE, BMC Evolutionary Biology* and *Biological Journal of the Linnean Society*). In three of them, Jindra Šíchová is the first author and her contribution to all of them is clearly stated in the thesis and it is substantial. This nice collection of papers demonstrates that Jindra Šíchová is able to design experiments, that she managed a lot of molecular-cytogenetic techniques, that she is able to perform experiments and to interpret the results and also to put together very well-written papers acceptable in solid scientific journals. Her writing skills demonstrate also the interesting introductory part of the thesis, which represents a brief, adequate and correct introduction to lepidopteran cytogenetics, evolution of sex chromosomes and speciation. Equally useful is the concluding part synthesizing all results with a short description of future plans.

I do not have a lot of critical comments, as all parts of the thesis are excellent; however, not to be suspected that I did not fulfil my role and to contribute to discussion, here are some of my reflections and questions. Most of them came from comparison of butterfly cytogenetics not so familiar to me with vertebrates.

Besides interesting findings in tortricid moths, the **paper I.** was extremely important even for our work on amniotes, as we used the qPCR mapping of a gene to sex chromosomes introduced here in a series of papers on reptile sex chromosomes.

The **paper II.** added new cytogenetic data on tortricids. I would like to ask what is the favourite explanation of the author for a stability of karyotypes in butterflies in spite of the holokinetic nature of their chromosomes and for large dynamics in the position of NOR-site (which we observe also in vertebrates, see e.g. our paper on oplurid iguanas: Altmanová et al. 2016, *Biol. J. Linn Soc.*).

Here, I would also like to stress that I am not totally sure whether we should describe the Y or W chromosomes or their parts as non-differentiated versus differentiated based only on their euchromatic/heterochromatic nature. What is your definition and understanding of the stage of differentiation of sex chromosomes? To me, heterochromatic sex chromosome are clearly differentiated, but highly degenerated unpaired sex chromosomes can still be euchromatic (see again e.g. our oplurid paper mentioned above).

Papers III-IV:

The system of multiple sex chromosomes discovered in all species, especially in *Leptidea amurensis* is fascinating and it was very inspiring for me how such a system can be studied by careful examination of female meiotic chromosomes. Also, I like a lot that a progeny of a single female was studied in some cases, which highly contributed to the insight into processes responsible for the variability in karyotypes in the genus. I highly appreciated the schematic depicting of the multiple sex chromosomes in *L. amurensis* in the paper IV. It is a very clever scheme showing the very complicated pairing of the multiple sex chromosomes during female meiosis, successfully illustrated in 2D. I was missing it in the paper III., where I was a little bit confused by so many sex chromosomes

and I was extremely happy to find it for other species in the concluding part of the thesis. I hope that this great figure will be published in one of the next papers on *Leptidea* cytogenetics.

I have also a question concerning the explanation how and why such a complicated system of sex chromosomes might evolved in a group with female heterogamety. The other similar systems approaching the complexity of sex chromosomes in the genus *Leptidea* are hence known in the groups with male heterogamety, e.g. in monotremes, spiders and hemipteran insects. At least in vertebrates, it seems that male heterogamety allows much more frequent emergence/fixation of multiple neo-sex chromosomes than female heterogamety (Pokorná et al. 2015 *Chromosome Research* 2015; Kitano et al. 2015 *Plos Biology*). The two most popular hypotheses explaining this striking difference are 1) driving centromeres and their effect of sex ratio, and 2) higher evolutionary rates in males. Could lineages with holokinetic chromosomes help to test these hypotheses?

In summary, I have no doubt that the Ph.D. thesis is of very high quality and that Jindra Šíchová should be awarded by the Ph.D. title. I congratulate her to such a nice and stimulating work.

In Lomy u Kunžaku, June 14, 2016

Doc. Mgr. Lukáš Kratochvíl, Ph.D.

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PhD thesis review

Title: Contrasting patterns of karyotype and sex chromosome evolution in Lepidoptera
Author: Jindra Šíchová

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The PhD thesis by Jindra Šíchová titled 'Contrasting patterns of karyotype and sex chromosome evolution in Lepidoptera' was a pleasure to read. It is written in a clear accessible manner even when complicated subjects are addressed.

The PhD candidate used cytogenetic techniques on Tortricid moths and Pyrid butterflies to study chromosome evolution in a context of speciation, insecticide resistance, sterile insect technique, the dynamics of (neo) sex-chromosomes, and complex sex-chromosome compositions. The main findings are the convincing taxonomic reconstruction which pinpoints the origin of the neo sex chromosomes in Tortricid moths and the fate of the W and Z representatives. The exploration of the extraordinary complex multiple sex chromosome configurations in Leptidea butterflies is impressive both as an achievement as well as a biological phenomenon which shows the importance to look beyond the Silkworm Lepidoptera model to understand the extreme variety and complexity in Lepidoptera.

One fascinating aspect is how new chromosome arrangements manage to propagate and eventually fixate in the context of speciation. Novel chromosomal arrangements would have a disadvantage in the first generations if there is are negative hybrid effects which eventually drive speciation. It would take a few generations at least before homozygotes of the new configuration occur. I intend to ask the candidate to share her thoughts on the evolutionary dynamics of major structural chromosomal events and the selective forces in generations immediately after the rise of such chromosomal restructuring.

The thesis demonstrates that the author is capable of producing scientific output which meets the highest standards, and it provides valuable knowledge and further thoughts on the subject to the scientific community. Therefore I recommend the thesis to be defended.

yours sincerely,

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