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Referee's evaluation of PhD thesis of Mgr. **Marie Altmanová** entitled „**Evolution of sex chromosomes and karyotypes in iguanas (Squamata: Pleurodonta)**“

Evaluation of thesis:

The **thesis** is a relatively short (17 pages) but dense and informative text without typing errors where a larger part represents a brief introduction to the six main papers that is valuable in itself. The introduction has a clear line leading readers through the story. The text contains two nice figures, I only think that more figures in such an attractive topic would improve the text significantly.

The thesis clearly demonstrates that the applicant has not only successfully applied several classical **methods** of cytogenetics (FISH) and molecular biology (qPCR) but also studied a large number of relevant papers on sex determination and sex chromosomes. The number of studied reptile species is admirable. She has achieved many important **results** that have made a significant contribution to the understanding of sex determination in reptiles.

The candidate is the first author in two out of **five papers** that clearly testify to her significant contribution to realized experiments. These papers have been published in respected journals with good impact factors like *Evolution*, *Biology Letters*, *Chromosome Research* and *PLoS One*. It is beyond any doubt that the applicant is able to produce results of sustained high quality and will be able to successfully progress in her scientific carrier.

Questions of referee:

Since all the papers presented have undergone strict peer review, my role is limited here. I would rather raise a few questions as a good basis for the discussion that will form the substance of the respectable defense:

1. (paper II) Can you provide more information about the **primers for qPCR** – how many primers were used, what was their specificity in various species? Are only five genes enough for making conclusions about sex chromosomes homology?
2. (paper III) You conclude that your study showed that **old and differentiated sex chromosomes** are present not only in mammals and birds but also in some reptiles. Is this

statement based only on qPCR detection of genes localized on the X and Y chromosome or is it also supported by cytogenetic experiments?

3. (paper IV) You mentioned that **multiple sex chromosomes** are more often present in **XX/XY** (mammals) than in **ZZ/ZW** (birds) systems and you explain this phenomenon by a female meiotic drive and by more frequent chromosomal rearrangement in mammals. You also showed that sex chromosomes of iguanas fuse more often than in other taxa. Are such fusions and other rearrangements somehow in relation to genome size, frequency of recombination or number of chromosomes?

4. (paper V) You describe the **miniature Y chromosome** with a few functional genes in iguanas and claim that this example supports the hypothesis proposing that the degeneration of the Y chromosome is slowed down during evolution, which prevents the final extinction of the Y chromosome. How common are such examples and how strongly does it support this hypothesis?

Conclusion:

The PhD thesis of Mgr. Marie Altmanová clearly shows that she has produced a large amount of valuable results that were published in respected journals. She is a mature scientist who is able to creatively perform successful experiments and interpret obtained data thanks to her theoretical background. Her thesis fulfills the standard requirements of a PhD thesis in Ecology.

Brno, August 30, 2017

Eduard Kejnovský