Review of the Ph.D. thesis "Evolution of sex determination and karyotypes in lacertid lizards and related lineages (Lacertoidea)" by Grzegorz Tomasz Suwała

The Ph.D. thesis by Grzegorz Tomasz Suwała builds on the studies conducted by Michail Rovatsos and Lukáš Kratochvíl, who are highly successful in reconstructing the evolution of sex chromosomes and karyotypes across reptiles. In the case of this dissertation, the model group represents lacertid lizards, which initially appear to have highly uniform karyotypes and consistent sex chromosomes. However, by extending the scope to the entire superfamily Lacertoidea, a comprehensive perspective on the evolutionary changes that led to the diversification of karyotypes within this major evolutionary lineage of squamate reptiles was achieved. This was facilitated by a broad methodological approach, which enabled the confirmation of the homology of the sex chromosomes across a wide phylogenetic spectrum of lacertids and their outgroups through the comparison of gene copy numbers between sexes in certain genes (Chapter 3). Furthermore, the significance of repetitive motifs in the size differentiation of W chromosomes was identified (Chapter 2). The amount of information about the karyotypes within selected families and relatively good understanding of phylogenetic relationships also enabled a comparison of the rate of chromosome number evolution among four Lacertoidea families, confirming the uniformity and lower evolutionary rate in the family Lacertidae (Chapter 1). Additionally, the availability of genomic data allowed precise identification of synteny among selected reptilian lineages, leading to the identification of specific rearrangements and mechanisms in the karyotype evolution of Lacertoidea (Chapter 1). From this perspective, the presented Ph.D. thesis represents a comprehensive study that provides highly valuable insights into the evolution of karyotypes and sex chromosomes in lizards.

The main body of work includes a concise and clear introduction that reviews fundamental cytogenetic characteristics and key hypotheses concerning karyotype evolution within the studied group. This section is also complemented by an overview of the diversity and distribution of all families. This approach enables readers fast orientation of the topic, even those who are not familiar with lacertid lizards. I expected more detailed information on specific karyotypes and a more extensive discussion of the results in this part. However, these information are thoroughly addressed in the individual chapters that follow.

The results of the dissertation are presented in three chapters. They are introduced by clearly defined aims and a specification of the student's contributions to the included articles. In the cases of Chapters 1 and 2, these contributions are substantial, as evidenced by Grzegorz Tomasz Suwała's role as the first author in both cases. The three separate chapters consist of one

manuscript and two published articles. Owing to the important results and student's significant contributions to the preparation of the presented texts, it is evident that he adopted a wide range of methods and approaches to scientific research. Therefore, based on the presented dissertation, the author should be awarded a Ph.D. degree.

I have a few minor comments and additional questions regarding some Chapters.

For the manuscript (Chapter 1), it is evident that this is a draft prepared for submission (probably still waiting for the final control). This can be demonstrated by some errors such as the omission of the important cited article Zheng & Wiens 2015 from the list of references, as well as discrepancies in the number of analyzed species in Table S1. Specifically, I found 193 species listed, whereas the methods section in the text states that 196 species were included in the time-calibrated tree. Is there any specific reason for this discrepancy? Regarding the manuscript, I would like to recommend placing the legends next to the figures (this might prevent misplacement: Fig. 2 is presented as the first one). In Fig. 1, it would be more comprehensible if both narrow sections of the phylogenetic tree were displayed side by side on one page, facilitating easier comparison of all families. Additionally, the resolution of Fig. 2 is insufficient, and the lack of contrast in colors makes it difficult to track some minor shifts between chromosomes. For this figure, I also have a question regarding how chromosome numbers are determined in the schematic representation. For example, in the results section, it is mentioned: "Tegu's sixth and seventh chromosomes aligned to a single (macro-)chromosome…". However, in *Salvator merianae*, the sixth and seventh chromosomes are microchromosomes. I must admit that I found the text describing synteny somewhat confusing. The figure clarifies much of the information, but some details are not clearly visible. From Fig. 2, it appears that chromosomes across different groups vary in size — is this correct? What factors contribute to this size differences, and does it correlate, for example, with genome size?

In the results section discussing evolutionary rates in chromosome number changes, it is mentioned that "The family Lacertidae revealed to exhibit an approximately five-times slower rate of chromosome number evolution ($\sigma 2L = 88$) than the other lacertoidean lineages ($\sigma 2nL = 442$) (Fig. 1)." I am not sure if such a specific difference is really visible in Figure 1. Is it possible to calculate the rate relative to time scale, as can be obtained using ChromEvol, which can also be utilized for reconstructing karyotypic evolution?

In the methodology, it is stated that 196 species were used out of a total of 249 karyotyped lacertoidean species. Only species included in the phylogenetic analysis could be analyzed. However, is there any distinct variability among the 53 species that were excluded that might somewhat change the results? Within the family Lacertidae, the genera *Iberolacerta* and *Parvilacerta* show clearly different karyotypes compared to the otherwise highly uniform karyotypes of other genera. Is there any explanation for the accumulation of chromosomal changes in these two groups (e.g., a specific evolutionary event affecting these genera)?

For Chapter 2, I only have questions regarding the methods. How were the motifs for the 22 microsatellites chosen? The article only generally states that they are related to the sex chromosomes. Could you please provide further details on the specific relationship of these motifs to the sex chromosomes for example in other animal groups? Was there truly no visible signal for any of these motifs on the autosomes? This is not documented in the article with an overall image of the entire mitosis, and I may have overlooked this information in the text. Could you also clarify whether sequential staining was applied to the microsatellites (except for GATA and telomeric sequences, which are mentioned)? Alternatively, what method of washing probes did you use? And how did you identify the W chromosome without C-staining?

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