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Review of the Ph.D. thesis "Evolution of sex chromosomes in reptiles" by Sofia Mazzoleni, MSc

The Ph.D. thesis by Sofia Mazzoleni consists of an overview of presented papers, a nice and comprehensive review of the topic which includes the contribution of the author, a chapter summarizing and explaining methods used in presented papers, and a conclusion. The conclusion not only summarizes the results which were already published, but it also reveals the upcoming results (which made me remove some of the questions I had prepared). The thesis itself contains five papers, in three of which Sofia Mazzoleni was the first author. Out of the five papers, four were already published in respected journals, one is in the form of an unpublished manuscript. Three papers focus on chromosomes in turtles; the first paper on Chelidae, a group with differentiated sex chromosomes, the second paper analyses representatives of Geoemydidae, in which no differentiated sex chromosomes were found, and the third paper describes karyotypes of representatives of Emydidae. Further, one of the remaining papers concerns the distribution of heterochromatin regions in genomes of various reptiles, and, the final manuscript brings a new and versatile method of sexing which, unlike the methods used so far, can be used in any extant bird species.

The thesis represents an excellent work, yet there are two things I would like to comment; firstly, it is the involvement of the paper Scardino et al. (2020) into the thesis, as it does not analyze (or mention) sex chromosomes and thus, according to my opinion, does not fit into the scope of the thesis. Since the author participated in 13 papers, omitting this paper and/or replacing it with another one that would fit better should have been easy. Secondly, the supplements of the manuscript in chapter 5 are missing, and since it is an unpublished manuscript, they cannot be found elsewhere.

While I was reading the thesis, many questions crossed my mind, but often they were answered later in the text, but some remained.

1) I find the phenomenon of GATA, a microsatellite that tends to occur on sex chromosomes, fascinating. As you showed in chapter 1, GATA was present exclusively on Y chromosomes of *Elseya*, *Emydura*, and all but one species of *Chelodina*. Is there any theory why is it so? Has a

potential function of GATA in sex chromosomes been proposed? In which other species GATA occurs on sex chromosomes?

2) In chapter 2 (Mazzoleni et al., 2020), you detected a polymorphy in size of homologs of the 12th chromosome pair in *Pangschura smithii*, which was described as irrespective to sex. How often have you encountered such polymorphy not only in this species but in general? And although you disproved the existence of WZ/ZZ chromosomes in this species, could this, in fact, be the WZ pair, the sex-determining function of which has been overruled by environmental conditions?
3) In chapter 3 (Schmid et al., 2019) you visualized the 5-methylcytosine-rich regions and compared their location with C-banding, a traditional technique of heterochromatin visualization. Yet, in many cases, the conspicuous heterochromatin-rich blocks, such as centromeres in *Oplurus cuvieri*, were not detected by 5-methylcytosine antibody at all. Do you have any explanation for this? Do you think that this technique could be used for the detection of poorly degenerated sex chromosomes? Have you noticed any change of pattern of 5-methylcytosine rich regions during the chromosome condensation in prophase and metaphase? How reliable is this pattern compared to the one obtained via C-banding?
4) In chapter 4 (Scardino et al., 2020), you did not try to detect the sex chromosomes. I assume there is a reason, what is it?
5) You mentioned that you have genomic sequences of both sexes in *Chelodina novaeguineae* and *Emydura subglobosa*, which you plan to use for X-specific gene content identification. I wonder, do you also plan to compare these genomes to find other potential repeats, which could serve as sex chromosome markers (similar to the GATA microsatellite)?

To conclude, the broad range of topics as well as the table of contribution proves that the author mastered everything that a scientist needs to achieve a successful career, i.e. the wet lab work, bioinformatics, data evaluation, writing papers and grant applications. Besides this, the author participated in 13 papers, attended a number of conferences and scientific meetings, and her work has been cited over 60 times already. Her thesis represents a valuable contribution to our knowledge and I sincerely recommend it to be successfully defended.

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