

Reviewer of the dissertation thesis – Azucena Claudia Reyes Lerma

PART A: General comments on the thesis

This PhD Thesis belongs to Azucena Claudia Reyes Lerma and focuses on the chromosomal evolution of spiders. She used haplogyne spiders (with monocentric and holocentric chromosomes), and two other families (Charinidae and Phrynidae) from the closely related order Amblypygi. She used a multipronged set of timely-updated techniques, including conventional and molecular cytogenetics techniques, coupled with morphological and phylogenetical analysis. Azucena had Dr. Alexandr Sember as supervisor. His research group represents one of the most prestigious ones in animal cytogenetics, mainly focused on fishes, with large expertise in conventional and molecular cytogenetics and the quality of this work well illustrates this.

The introduction and the literature overview are very well written in a complete and well-referenced text. The author bring general information about cytogenetic and genomic studies in Arachnida, then specific information about the karyotype features, holocentric and sex chromosomes are highlighted, focusing on the karyotype evolution in this group. It's very brilliant and complete data that provide a timely-updated review about the state-of-art of all relevant issues for this work. The aims are correctly described where cytogenetic, morphological, and genomic approaches were used to study the mechanisms underlying their karyotype evolution.

The Material and Methods section is quite concise and does not bring the protocols and references used in the experiments. However, such information is provided in each chapter, which is fine to avoid the repetition of information. However, in papers the MM section normally is quite concise, and, on the thesis, it would be a great opportunity to have such long and more complete protocol details. But I presume that such a short format is the standard in your Institution. Besides, images from the animals could also be included to illustrate the target group.

The results and discussion sections of this Doctoral thesis were divided into 3 chapters, each corresponding to a scientific paper published were in just one of them the PhD candidate figures in the first authorship.

The first chapter is a paper entitled: "Insights into the karyotype and genome evolution of haplogyne spiders indicate a polyploid origin of lineage with holokinetic chromosomes" published in the journal: Scientific Reports (IF =4.37). Conventional cytogenetic data highlighted the presence of holocentric chromosomes and a very unusual Xy-derived sex chromosome system, with more than 10 sex chromosomes that formed a meiotic chain. They also correlate the data with their genome sizes, making interesting correlations. One must recognize the high difficulty in dealing with spider's chromosomes and the authors performed great work regarding their quality. They also recorded the species with the new highest chromosome count among spiders *Caponia natalensis* (Caponiidae), $2n = 152$. It's a very nice piece of work despite only

conventional cytogenetic techniques being applied, which impairs some deeper conclusions about the results. I will make specific questions on this issue in the next section.

The second chapter is a paper entitled: "Insights into the karyotype evolution of Charinidae, the early-diverging clade of whip spiders (Arachnida: Amblypygi) published in the journal: *Animals* (IF =2.752). In this study, she applied both conventional and molecular cytogenetic methods to check the ancestral traits and evolutionary pathways of amblypygid karyotype evolution. The results are illustrated in high-quality figures with very clear results. The authors concluded that these sets of analyzed species harbor highly variable $2n$ and karyotypes shaped by chromosomal fusions and inversions.

The third chapter is a paper entitled: "Cryptic diversity in the whip spider genus *Paraphrynus* (Amblypygi: Phrynidae): integration morphology, karyotype, and DNA" published in the journal: *Arthropod Systematics and Phylogeny* (IF =2.50). In this study, she coupled cytogenetic, genomic, and morphological data to provide insights into species delimitation, diversity, and phylogenetic inferences in *Paraphrynus* species. The pictures, especially those from morphological analyses are of great quality. The authors propose some hypotheses of karyotype evolution but I am afraid the quality of preparations and limitations of the techniques are obstacles for such an attempt and still need further confirmation. I will make specific questions on this issue in the next section.

The final part brings a discussion with the main findings of this Ph.D. thesis together with some conclusions and main perspectives of studies. Undoubtedly, the results advanced our understanding of the karyotype and genome evolution in arachnids and open new windows for further studies with deeper analysis.

PART B: Questions to the ph.D candidate

1) Evolution of the XY-derived multiple and complex sex chromosome system in Caponiidae.

In the first chapter, the authors bring a nice hypothesis related to the origin of this unique and unusual multiple sex chromosome system. However, all of them were strictly supported by conventional cytogenetic techniques and small sampling size.

Questions:

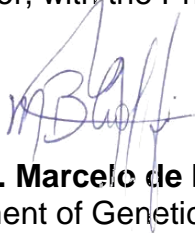
- 1) Are you fully convinced about the occurrence of such multiple systems only based on meiotic analysis under conventional techniques checked in a small number of males?
- 2) Assuming the real occurrence of such multiple systems, several hypotheses of the main steps involved were presented. Why i) WCP experiments (i.e: Isolation of this chromosomal multivalent by microdissection and further construction of probes) or ii) Simple CGH experiments searching for any male-specific regions were performed since these techniques are largely available and used by your research group?
- 3) Despite the phylogeny present in Figure 5. I missed information on the sex chromosomes systems coupled with such phylogeny that could explain the differentiation of such putative systems. Why haven't the authors correlated these points to explain such a rare scenario? Besides, can you link some ecological behavior or genomic reason (i.e, identification of Evolutionary Breakpoint Regions) on these animals that explain such a scenario?

2) Karyotype Evolution of Charinidae

Considering the complex scenario present among Charinidae species, the second chapter brings insights over the karyotype evolution present in this group but based their results only on some old-fashioned chromosomal markers that do not bring too much to the history. In addition to this fact, the quality of chromosomal preparations usually obtained in spiders also represents a problem even for proper identification of the main chromosomal types (m-sm-a-st).

- 1) As nowadays NGS techniques can be largely used for the isolation of a larger set of repetitive sequences (TEs, SatDNAs, microsatellites, among others). Why haven't the authors just based their results on this small set of markers and did not consider the analysis of their relationships based on a larger set of genomic markers?

Final Report: I recommend this thesis for defense and subsequent graduation of the author, with the Ph.D. title.

A handwritten signature in blue ink, appearing to read 'M. Bello Cioffi', written over a faint, larger version of the same signature.

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