Review of Ph.D. thesis “Evolution of karyotype in selected groups of haplogyne and mygalomorph spiders” by Ivalú Macarena Ávila Herrera

This Ph. D. thesis is an important contribution to the knowledge of the evolution of chromosome number, sex determination systems and nucleolar organizer regions by FISH in the spider family Pholcidae, as well as allowed to revise the diploid number and sex chromosome system of mygalomorph *Atypus karschi* (Atypidae) and determine probable ancestral karyotype of the genus *Atypus*.

Pholcidae belongs to the clade of haplogyne spiders, basal to the Araneomorphae, and presents many unique features, although the number of species analyzed cytogenetically is low. The Pholcidae family is one of the most diversified within the haplogynes and has a worldwide distribution. However, before this thesis only 23 species representing nine genera has been karyotyped. In this thesis, 63 species belonging to 26 genera were analyzed, which represent an extensive cross-section through all major pholcids clades.

This huge study allows for the first time analyze the evolution of karyotype, sex chromosome systems and NORs of haplogynes at a family level. The data obtained together with the numerous bibliographies consulted allowed her to reach new hypotheses. Regarding to the ancestral pholcid karyotype, she proposed that it was close to 2n♂=33, X1X2Y, with chromosomes biarmed. The author appointed that in the karyotype evolution of pholcids were frequent the involvement of fusions, inversions, and translocations of chromosome pairs. Regarding to sex chromosome systems, the author considered, according to recent phylogenetic trees, that X1X2Y sex chromosome determination is ancestral for pholcids, as well as haplogynes. Moreover, she proposed a novel hypothesis of the origin of this system: that evolved from a cryptic sex chromosome pair via non disjunctions. She put forward that the chromosomes of X1X2Y are dynamic elements, and underwent frequent rearrangements during pholcid evolution, like translocations between X1 and X2, inversions of X, increase of Y size, loss of the Y and X fission or nondisjunction. Regarding to the nucleolar organizer regions, the author considered that that ancestral pholcids had a single biarmed pair bearing a terminal NOR. She stated that the terminal position of most NORs suggests that the increase in autosomic NORs number as well as its spread out to X chromosomes arose by ectopic recombination. Furthermore, she suggested that the sex-chromosome-linked NORs arose independently several times in pholcids and could be involved in the achiasmatic pairing

of sex chromosomes. Regarding to the ancestral haplogyne karyotype, she proposed two hypotheses. That it could be 2n=33, X1X2Y, present in the most basal clade of pholcids. Alternatively, it could be 2n=39, X1X2Y found in the haplogyne family Drymusidae. The latter is similar to that in Austrochilidae, which is an early-diverging lineage of the sister clade of haplogynes, formed by protoentelegynes and entelegynes. The author considered that cytogenetic information is not sufficient to decide which of this hypothesis is correct and a more detailed phylogenomic analysis is needed.

Atypidae is a phylogenetically basal mygalomorph family. The Infraorder Mygalomorphae is poorly cytogenetically analyzed and is not satisfactorily understood. In the family Atypidae, the four studied species belong to the genus *Atypus*. The author co-authored a paper analyzing the karyotype and genetic barcode (CO1), along with observations on habitat associations and natural history, of the only *Atypus* species present in North America. In this work it was concluded that the species called *Atypus snetsingeri* present in Pennsylvania appears to represent an introduced local population of the Asian species *Atypus karschi*.

The author valuable contribution has been to analyze the karyotype, the sex chromosomal system and the heterochromatic regions of *Atypus karschi* from Pennsylvania to compare with the cytological data of the Asian *Atypus karschi.* The revision of the karyotype allowed her to conclude that the original description of its karyotype was highly inaccurate. Her study revealed that the karyotype of the *Atypus karschi* is similar to two European species (2n=41/42, X0/XX, male/female) and predominated by metacentric chromosomes. This is probable the ancestral karyotype of the genus *Atypus.* She postulated that the X0 system, which is probably ancestral to the superfamily Atypoidea, may have arisen from the centric fusion of the X1 and X2 chromosomes in the X1X20 system, which is supposed to be ancestral in mygalomorphs. Regarding the NOR, she concluded that the location of heterochromatin and NOR revealed that NOR-associated heterochromatin is formed by inactivated rDNA. Moreover, she has been obtained the first data on heterochromatin distribution and nucleolar organizers in the family Atypidae.

The thesis is presented as a compendium of five papers published in highly-regarded journals. The introduction is very complete and covers all the cytogenetic characteristics of spiders. The materials and methods are summarized in the main text, but very well explained in the selected papers. The particular aims are clearly formulated

and fulfilled. The results, including tables and figures, are of a high standard and can be found in the published papers. The discussion at the end of the thesis is clear and detailed and integrates what has been obtained in the different published papers. Conclusions and future directions are precise, and open new perspectives for further studies. The whole thesis is written in clear language with minimal mistakes.

Questions:

1) Page 60: “Obtained data also suggest frequent integration of autosome fragments into sex chromosomes”.
Could you explain what rearrangement/s integrate autosome fragments into sex chromosomes? This integration has any genetic consequence?

2) Page 378. According to another hypothesis, the X1X2Y chromosomes evolved from CSCP chromosomes via nondisjunction.
Could you explain in more detail how sex chromosomes originated according to this hypothesis?

Final Report: I recommend this thesis for defense and I suggest to confer a scientific degree Ph.D. to Ivalú Macarena Ávila Herrera

Buenos Aires, August 20th, 2024

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