2018. Journal of Arachnology 46:45–49

Small pholcids (Araneae: Synspermiata) with big surprises: the lowest diploid number in spiders with monocentric chromosomes

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Abstract. Even though less than 2% of pholcid species have been karyotyped, previous studies documented a wide diversity of diploid numbers and sex chromosome systems. Here, we increase the number of native Brazilian cytogenetically investigated pholcid species from three to eight and discuss implications of chromosome evolution in this group. The species analyzed here share a X0/XX sex chromosome system and biarmed chromosomes, but vary in diploid numbers, i.e., 2n♂ = 17 in *Mesabolivar spinulosus* (Mello-Leitão, 1939) and *Mesabolivar togatus* (Keyserling, 1891), 2n♂ = 15 in *Carapoia* sp., and 2n♂ = 9 in *Micropholcus piaui* Huber, Carvalho & Benjamin, 2014 and *Micropholcus ubajara* Huber, Carvalho & Benjamin, 2014. Chromosomal data indicate that most *Mesabolivar* species share a 2n♂ = 17, X0, while *Mesabolivar luteus* shares with *Carapoia* sp. a 2n♂ = 15, X0. This lends further support to the idea that *M. luteus* is in fact misplaced and more closely related to *Carapoia* González-Sponga, 1998. The diploid number of the two *Micropholcus* species is the lowest reported so far for spiders with monocentric chromosomes. The 2n♂ = 9, differs strongly from the 2n♂ = 17 previously reported for *Micropholcus fauroti* (Simon, 1887). As the number of autosomes of *M. piaui* and *M. ubajara* is exactly half of that found in *M. fauroti*, we hypothesize that the reduction occurred by an "all or nothing" fusion event. The low diploid number observed in *M. piaui* and *M. ubajara* is the first morphological synapomorphy that would support the establishment of a new genus to allocate the New World *Micropholcus* species.

Keywords: Karyotype, meiosis, Modisiminae, Pholcinae, sex chromosome system

Knowledge regarding spider cytogenetics has grown considerably in recent years; however, less than 2% of the ca. 47,000 described species are karyotyped so far (Araujo et al. 2017; World Spider Catalog 2017). In spite of this, there is a huge variation in the diploid number, from 2n♂ = 7, in the dysderid *Dasumia carpatica* (Kulczyński, 1882) and the segestriid *Ariadna lateralis* Karsch, 1881 (Suzuki 1950, 1954; Kořínková & Král 2013), to 2n♂ = 128, in the ctenizid *Cycloscoma siamensis* Schwendinger, 2005 (Král et al. 2013). In general, lower diploid numbers are found in araneomorphs whilst higher diploid numbers prevail in non-araneomorphs (see Araujo et al. 2017).

The Pholcidae is the ninth and second most diverse family among Araneae and Synspermiata spiders, respectively, with about 1600 species and 80 genera (World Spider Catalog 2017). However, only 19 species belonging to eight genera were cytogenetically studied, with the diploid number varying from 2n♂ = 15 to 2n♂ = 32. The X0 sex chromosome system (SCS) predominates within the pholcids, occurring in 13 species, followed by the X_1X_20, X_1X_2Y and XY systems. Similar to most Synspermiata spiders, the chromosomes are, in general, biarmed (see Araujo et al. 2017).

Huber (2011), taking into account several phylogenetic hypotheses, updated a classification of pholcids in five subfamilies: Ninetinae, Arteminae, Modisiminae, Smeringopinae and Pholcinae. This classification was latter corroborated by molecular phylogenies (Dimitrov et al. 2013). Among these subfamilies, only Ninetinae has no cytogenetic data (see Araujo et al. 2017), but many gaps remain, even in the other subfamilies, which are underrepresented in this type of study. For Neotropical pholcids, there are cytogenetics data published for only three native species, all of them belonging to the genus *Mesabolivar* González-Sponga, 1998 (see Araujo et al. 2017): *M. brasiliensis* (Moenkhaus, 1898), *M. cyaneotaeniatus* (Keyserling, 1891) and *M. luteus* (Keyserling, 1891). *Mesabolivar* is a highly species-rich genus with 64 described species and dozens of hitherto undescribed species available in museums, all of them exclusively found in South America (Huber 2015; World Spider Catalog 2017; L.S. Carvalho, pers. obs.). Previous chromosomal studies in the *Mesabolivar* species revealed 2n♂ = 17, X0 and 2n♂ = 15, X0, with meta/submetacentric chromosomes (Araujo et al. 2005a; Ramalho et al. 2008).

Here, we provide chromosomal data for five Neotropical pholcid species: three Modisiminae—*Mesabolivar spinulosus* (Mello-Leitão, 1939), *Mesabolivar togatus* (Keyserling, 1891) and *Carapoia* sp.—and two Pholcinae—*Micropholcus piaui* Huber, Carvalho & Benjamin, 2014 and *Micropholcus ubajara* Huber, Carvalho & Benjamin, 2014. The genus *Micropholcus* Deeleman-Reinhold & Prinsen, 1987 (Pholcinae) possesses 16 species, mainly from South America and the Caribbean region (Huber et al. 2014). The only species cytogenetically characterized of this genus is its type-species, the synanthropic pantropical *Micropholcus fauroti* (Simon, 1887), that exhibits 2n♂ = 17, X0, and metacentric chromosomes in a Brazilian population (Araujo et al. 2005a). *Carapoia* González-Sponga, 1998 (Modisiminae) was never analyzed from the cytogenetic point of view.

METHODS

A total of 38 specimens from the Brazilian fauna were analyzed: 11 individuals (10♂ and 1♀) of *Mesabolivar spinulosus*, from Fazenda Bonito (05°14′S, 41°41′W), municipality of Castelo do Piauí, state of Piauí; two males of *Mesabolivar togatus*, from the municipality of Viçosa (20°45′14″S, 42°52′55″W), state of Minas Gerais; seven specimens (6♂ and 1♀) of an undescribed species of the genus *Carapoia*, from Parque Nacional de Ubajara (03°49′S, 40°59′W), municipality of Ubajara, state of Ceará; 14 topotype specimens of *Micropholcus piaui* (10♂and 4♀), from Parque Municipal da Pedra do Castelo (05°12′S, 41°41′W), municipality of Castelo do Piauí, state of Piauí; and four topotype individuals (2♂ and 2♀) of *Micropholcus ubajara*, from Gruta do Morcego Branco, Parque Nacional de Ubajara (03°49′S, 40°59′W), municipality of Ubajara, state of Ceará. After the gonads extraction, the specimens were deposited at Laboratório Especial de Coleções Zoológicas, Instituto Butantan (IBSP, curator A.D. Brescovit), São Paulo, state of São Paulo, and Coleção de História Natural, da Universidade Federal do Piauí (CHNUFPI, curator E.F.B. Lima), Floriano, state of Piauí, Brazil. The collecting permit was issued by the Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio, through the Sistema de Autorização e Informação em Biodiversidade – SISBIO (#39233-1).

The gonads were dissected out and processed for chromosomal preparations, according to Araujo et al. (2005b). The cells were photographed in an Olympus BX51 microscope, using the DP software, or in a Zeiss Axio Imager A2, using the Axio Vision software. In both cases, the total magnification was 1600x. The chromosomal morphology was identified following the proposal of Levan et al. (1964).

RESULTS

All five species analyzed here present a SCS of the X0/XX type, which was confirmed due to the difference of one chromosome between male and female mitotic metaphase cells (except in *Mesabolivar togatus*, in which females were not analyzed), presence of only one chromosomal univalent in male diplotene, and occurrence of the X chromosome in only one pole in metaphase II cells. In the five species, the X chromosome is the largest or nearly the largest element of the karyotype, and all chromosomes are biarmed.

Mitotic metaphase nuclei of *Mesabolivar spinulosus* possess 2n♂ = 17 and 2n♀ = 18 (Fig. 1A), and spermatogonia of *Mesabolivar togatus* has 2n♂ = 17 (Fig. 1B). Male diplotene nuclei of both species revealed eight autosomal bivalents with one or two interstitial or terminal chiasmata, and one sex univalent, which was easily recognized due to its length and configuration (Fig. 2A & B). Male metaphase II cells of two *Mesabolivar* species present n = 8 or n= 8 + X. The chromosome morphology of *M. spinulosus* is metacentric (pairs 1, 2, 4 and X), subtelocentric (pairs 3 and 8), and submetacentric (pairs 5, 6 and 7). *Mesabolivar togatus* presents chromosomes with metacentric (pairs 1, 3–6 and X) and submetacentric (2, 7 and 8) morphology (Fig. 1A & B).

Mitotic metaphase cells of *Carapoia* sp. reveal 2n♂ = 15 (Fig. 1C) and 2n♀ = 16. Male diplotene nuclei show seven autosomal bivalents with one or two terminal or interstitial chiasmata, and one sex univalent (Fig. 2C). Male metaphase II cells possess n = 7 or n= 7 + X (Fig. 2D). The chromosomal morphology is exclusively metacentric (Figs. 1C, 2D).

Mitotic metaphase cells of *Micropholcus piaui* and *Micropholcus ubajara* (Pholcinae) present 2n♂ = 9 (Fig. 1D & E) and 2n♀ = 10. Male diplotene/metaphase I nuclei showed four autosomal bivalents, with one terminal or interstitial chiasma in *M. piaui*, and one or two terminal or interstitial chiasmata in *M. ubajara*. The sex chromosome is univalent, identified due to its positive heteropycnosis (Fig. 2E & F). In both species, male metaphase II nuclei reveal n = 4 or n= 4 + X (Fig. 2G & H). In these species, the chromosomal morphology is exclusively metacentric, except in the submetacentric pair 2 of *M. ubajara* (Fig. 1D & E).

DISCUSSION

The karyotype characteristics (X0 SCS, a large biarmed X chromosome, and biarmed autosomes) observed in the five species studied here are the most common among the Pholcidae (see Araujo et al. 2017). Within *Mesabolivar* (Modisiminae), the 2n♂ = 17, X0 was previously found in *M. brasiliensis* and *M. cyaneotaeniatus* (Ramalho et al. 2008). However, the Pholcidae molecular phylogenetic analyses carried out by Dimitrov et al. (2013) did not recover the monophyly of *Mesabolivar* representatives analyzed.

The phylogenetic position of *M. spinulosus* and *M. togatus* within *Mesabolivar* is unknown as these species were not included in the only available phylogenetic analysis of the Pholcidae (Dimitrov et al. 2013). However, these species, along with *M. brasiliensis, M. cyaneotaeniatus,* (all with 2n♂ = 17), and others, forms a group of *Mesabolivar* species with a "distinctively curved procursus", called "southern group" (Huber 2000). Moreover, these four species of *Mesabolivar* with 2n♂ = 17 present a mixture of meta/submeta/subtelocentrics, or at least meta/submetacentrics, while *M. luteus* presents exclusively metacentric chromosomes, reinforcing that this last species is not closely related to the species with 2n♂ = 17.

Carapoia is the second genus of Modisiminae chromosomally studied, and the results shown herein suggest a close phylogenetic relationship between *M. luteus* and *Carapoia* species based on the chromosome number and morphology. In fact, the generic allocation of *M. luteus* has a long history of uncertainty (see Huber 2000, 2005; Astrin et al. 2007), and the new chromosomal data corroborates the most recent molecular data (Dimitrov et al. 2013) and the female morphology (Huber 2000), pointing towards its position in the genus *Carapoia*.

Interestingly, within pholcids, the karyotype 2n♂ = 15, X0, such as herein observed in *Carapoia* sp., was previously found only in the modisimine *M. luteus* (Araujo et al. 2005a), and in representatives of the arteminae genus *Physocyclus* Simon, 1893 (Cokendolpher & Brown 1985; Cokendolpher 1989; Oliveira et al. 2007; Golding & Paliulis 2011). According to the phylogenetic hypothesis of Dimitrov et al. (2013), Arteminae and Modisiminae are sister clades, but the considerably lack of cytogenetic data prevents any further comparison.

The phylogenetic analysis of Huber et al. (2014) recovered the monophyly of *Micropholcus* species, but with some uncertain relationship between the South American clade,

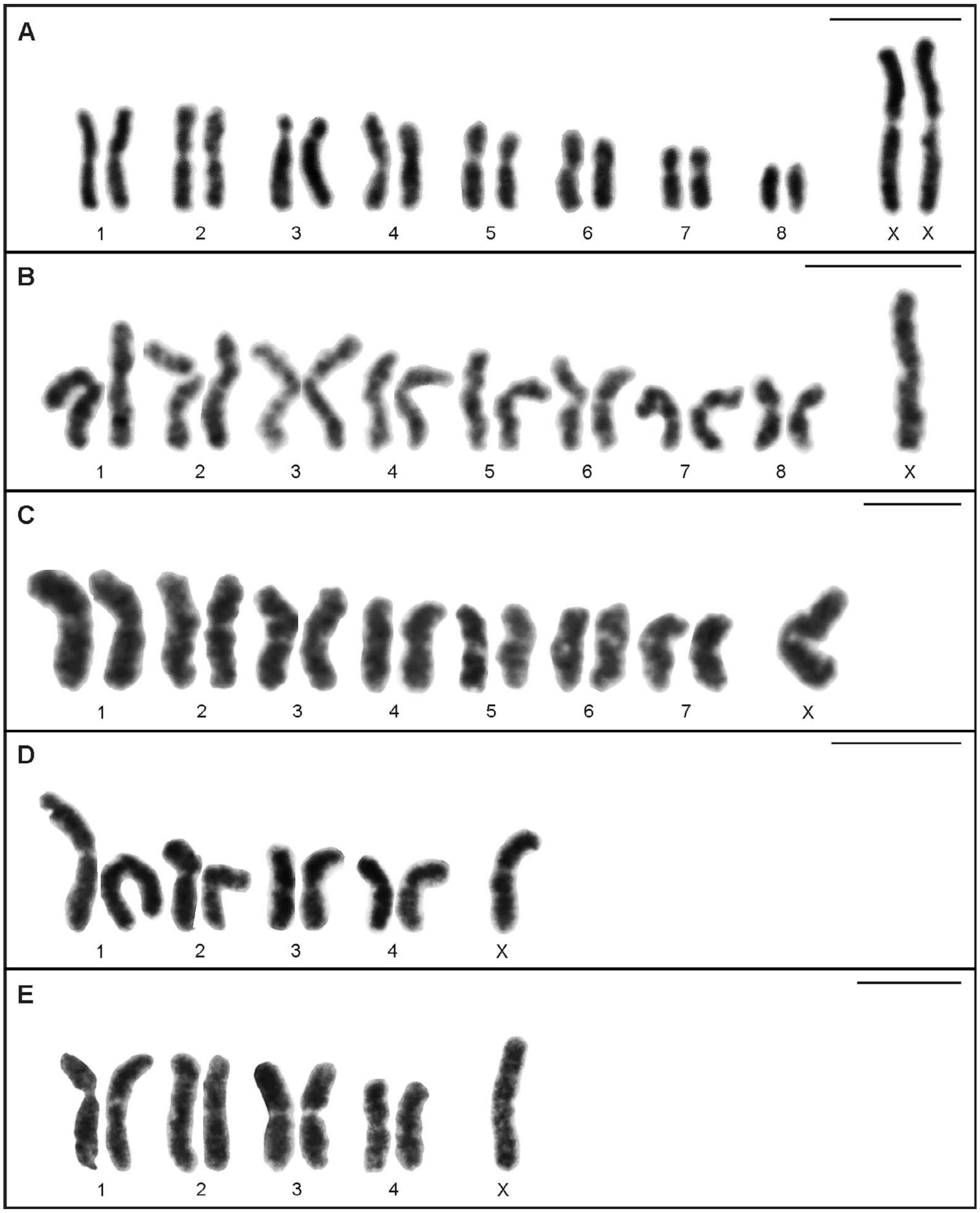

Figure 1.—Karyotypes of five pholcid species. A. *Mesabolivar spinulosus*, 2n♀ = 18, XX. B. *Mesabolivar togatus*, 2n♂ = 17, X0. C. *Carapoia* sp., 2n♂ = 15, X0. D–E. *Micropholcus piaui* and *Micropholcus ubajara*, respectively, 2n♂ = 9, X0. Scale bars = 10 μm.

the Caribbean clade and the *Micropholcus* type-species, the pantropical *M. fauroti*. The chromosomal data obtained in *M. piaui* and *M. ubajara* (2n♂ = 9, X0), both Brazilian species, strongly differ from that of *M. fauroti* (2n♂ = 17, X0) (Araujo et al. 2005a). Huber et al. (2014) placed the New World *Micropholcus* species in this genus mostly because they were not able to find any morphological synapomorphies that would have supported the establishment of a new genus. The lower diploid number observed in *M. piaui* and *M. ubajara* is the first morphological putative synapomorphy that would support a new genus including the New World *Micropholcus* species. Analyses of Caribbean *Micropholcus*, unknown from the cytogenetic point of view, reveals to be important to verify whether a low diploid number is also present in this clade of *Micropholcus*.

The number of autosomes in *M. piaui* and *M. ubajara* (eight) is exactly half of that found in *M. fauroti* (sixteen). Thus, one plausible hypothesis for the origin of the karyotype observed in *M. piaui* and *M. ubajara* is the occurrence of an “all or nothing” fusion event, in which all autosomes from the 2n♂ = 17 = 16 + X0 have fused with each other. This type of mechanism has been proposed to explain the origin of the karyotype of some spider species (Suzuki 1954; Rowell 1990; Stávale et al. 2011). As in all these *Micropholcus* the chromosomes are biarmed, it is possible that tandem fusions, instead of centric fusion, were implied in the process. This rearrangement was preceded or followed by pericentric

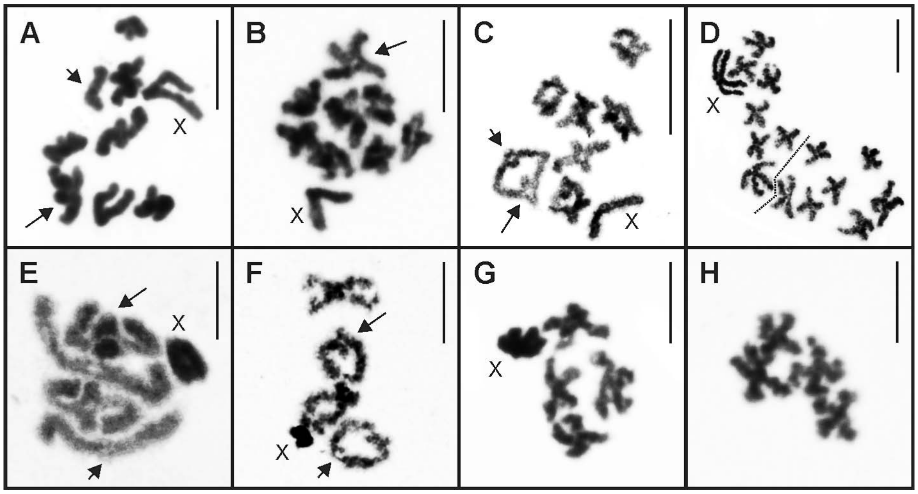

Figure 2.—Male meiotic cells of five pholcid species. A–B. Diplotene nuclei of *Mesabolivar spinulosus* and *Mesabolivar togatus*, respectively, with 8 autosomal bivalents and the X univalent. C. Diplotene of *Carapoia* sp., with 7 autosomal bivalents and one X univalent. D. Metaphase II cells of *Carapoia* sp., n = 7 + X (left pole) and n = 7 (right pole). E–F. Metaphase I and diplotene cells of *Micropholcus piaui* and *Micropholcus ubajara*, respectively, with 4 autosomal bivalents and the X univalent. G–H. Metaphase II nuclei of *Micropholcus piaui*, with n = 4 + X (G) and n = 4 (H). Long arrows = interstitial chiasma, short arrows = terminal chiasma. Scale bars = 10 μm.

inversions, in a way that keep both chromosome arms of each chromosome of similar sizes. The proposition that tandem fusions, instead of centric fusions, occurred in spiders that experienced a reduction in chromosome number and maintenance of chromosomal morphology was already postulated by Král et al. (2006), but referring to acrocentric karyotypes. In the case of *M. piaui* and *M. ubajara*, it is possible that the putative dicentric chromosomes, products of the fusion, undergo an inactivation of one centromere, a process already well documented in several organisms (Stimpson et al. 2012).

The 2n♂ = 9, observed in *M. piaui* and *M. ubajara* is the lowest diploid number recorded for spiders with monocentric chromosomes. Low diploid numbers are found in *Ariadna lateralis* (Segestriidae) and *Dasumia carpatica* (2n♂ = 7), *Dysdera crocata* C.L Koch, 1838 (2n♂ = 8) (Dysderidae), and *Ariadna bosenbergi* Keyserling, 1877 (2n♀ = 8) (Suzuki 1950, 1954; Diaz et al. 2010; Kořínková & Král 2013), however, these species possess holocentric chromosomes.

In conclusion, the present cytogenetical data support a separate evolutionary position of *Mesabolivar luteus* in relation to the remaining *Mesabolivar* representatives and show that, within the Pholcidae, the 2n♂ = 15, X0 is present only in the sister subfamilies Modisiminae and Arteminae. Moreover, we could show the lowest diploid number in spiders with monocentric chromosomes, 2n = 9. Thus, our findings raise questions on the evolution of the low diploid number in the genus *Micropholcus*, requiring further investigation in Caribbean species or hitherto undescribed Old World species.

ACKNOWLEDGMENTS

This work is part of LSC's Ph.D. thesis and part of the Programa de Pesquisas em Biodiversidade do Semiárido (CNPq 558317/2009-0 and 457471/2012-3). This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (2011/21643-1 and 2012/10679-8). We are grateful to Bernhard A. Huber and anonymous referees for their critical reading of the manuscript.

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Manuscript received 5 May 2017, revised 6 October 2017.