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Unveiled chromosomal diversity in the Araneidae (Araneomorphae): the highest diploid number among entelegynes and the first record of the $X_1X_2X_3X_4$ Sex Chromosome System in the family

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Abstract. The Araneidae is among the most speciose spider families, but there are few karyotype studies (1.9%) and some species-rich clades are without any chromosomal study. Understanding the evolution of chromosome number and Sex Chromosome Systems is made more difficult by many uncertain evolutionary relationships within the family. In this work, the chromosomal analysis of eight araneid species (*Acacesia benigna* Glueck, 1994, *Actinosoma pentacanthum* (Walckenaer, 1841), *Alpaida bicornuta* (Taczanowski, 1878), *Dubiepeira* Levi, 1991 sp., *Gasteracantha cancriformis* (Linnaeus, 1758), *Parawixia bistriata* (Rengger, 1836), *Verrucosa meridionalis* (Keyserling, 1892) and *Verrucosa scapofracta* Lise, Kesster & Silva, 2015), contribute to discussions of some evolutionary scenarios of chromosome evolution. The gonads were submitted to colchicine treatment, hypotonization, slide preparation, and Giemsa staining. The species analyzed showed $2n♂ = 24$ ($11II + X_1X_2$), except *Dubiepeira* sp. with $2n♂ = 41$ ($19II + X_1X_2X_3$), and both *Verrucosa* species, which presented $2n♂ = 47$ ($22II + X_1X_2X_3$) in *V. meridionalis* and $2n♂ = 50$ ($23II + X_1X_2X_3X_4$) in *V. scapofracta*. The species analyzed possess all chromosomes with acro/telocentric chromosomal morphology. The $2n♂ = 24$, X_1X_2 found in most species studied here is the most frequent karyotype in the Araneidae. This study presents the first chromosomal data for the diverse clade "Micrathenines", the highest diploid number among entelegynes ($2n♂ = 50$), and the first record of an $X_1X_2X_3X_4$ in the Araneidae. The chromosome data suggest a series of fission events in the origin of *Verrucosa* karyotypes, and a close relationship between *Dubiepeira* sp. and *Araneus ventricosus* (L. Koch, 1878). Moreover, *Alpaida bicornuta* can be cytotaxonomically distinguished of other *Alpaida* species karyotyped up to now.

Keywords: Sex chromosomes, high diploid numbers, Araneae, spiders, Araneoidea.

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The Araneidae is the third most speciose spider family, with 3058 species described up to now (World Spider Catalog 2021). This great diversity makes detailed studies of the clade more difficult (Scharff et al. 2020) and, in this scenario, the use of different tools to analyze the species, such as molecular and cytogenetic data, can be helpful in unveiling the evolutionary relationships (Araujo et al. 2011; Scharff et al. 2020).

In the Araneidae, the diploid number varies from $2n♂ = 13$ in *Neoscona* Simon, 1864 sp. to $2n♂ = 49$ in *Araneus ventricosus* (L. Koch, 1878) (Parida & Sharma 1987; Sharma & Parida 1987; Youju et al. 1993). The Sex Chromosome Systems (SCS) found in the family are of the types $♂X0/♀XX$, $♂X_1X_2/♀X_1X_1X_2X_2$, $♂X_1X_2X_3/♀X_1X_1X_2X_2X_3X_3$ and $♂XY/♀XX$. Regarding the chromosomal morphology, all types have been described in the Araneidae. However, most analyzed species possess $2n♂ = 24$ (81%), $♂X_1X_2/♀X_1X_1X_2X_2$ SCS (89%) and acro/telocentric chromosomal morphology (59%) (Araujo et al. 2021). Unfortunately, only 1.9% of the araneid species were karyotyped and some clades that have genera with high species richness remain chromosomally unknown, as the "Micrathenines" clade (Scharff et al. 2020; Araujo et al. 2021).

The aim of this study was to cytogenetically analyze eight araneid species belonging to clades Eriophorines, Gasteracanthines, Micrathenines and two species with phylogenetic position not established, in order to discuss the chromosome evolution in araneid spiders and contribute with new karyotype data in conflicting and less studied taxonomic groups.

METHODS

The specimens of all analyzed species (Table 1) were collected by active night search, with exception of *Actinosoma pentacanthum* (Walckenaer, 1841) that was collected during the day on the surface of aquatic plants. The collections were performed in the states of Mato Grosso do Sul (MS) and São Paulo (SP), Brazil, from May of 2014 to April of 2018 and vouchers are deposited in the Laboratório de Coleções Zoológicas, Instituto Butantan, São Paulo, SP, Brazil (IBSP, curator: A.D. Brescovit).

The chromosome preparations of gonads and embryos were carried out following Araujo et al. (2008). All analyzed cells were photographed employing a Zeiss Axioimager D2 microscope with a monochromatic AxioCam 503 camera, using the ZEN Pro software. Chromosome morphology was determined with the software IMAGEJ (Rasband 1997–2020) and the LEVAN plugin (Sakamoto & Zacaro 2009), according to Levan et al. (1964) and Green & Sessions (1991).

RESULTS

Karyotypes with the araneid common diploid number.—The species *Acacesia benigna*, *Actinosoma pentacanthum*, *Alpaida*

Table 1.—Species, collection site and number of specimens analyzed in this work.

Species	Locality	Specimens analyzed	Vouchers (IBSP)
Acacesia benigna Glueck, 1994	Campo Grande, MS (20°30′36″S/54°36′54″W)	1♂	222032
Actinosoma pentacanthum (Walckenaer, 1841)	Corumbá, MS (19°34′37″S/57°00′42″W) and Porto Murtinho, MS (21°49′26″S/57°48′48″W)	9♂/12♀ & 4 embryos	211335, 212177, 212186, 212193, 221987, 222376, 222379 - 222390, 222397, 222401 and 222403
Alpaida bicornuta (Taczanowski, 1878)	Campo Grande, MS (20°30′36″S/54°36′54″W)	2♂	222039 and 222040
Dubiepeira sp.	Campo Grande, MS (20°30′36″S/54°36′54″W)	2♂/3♀	222034 - 222038
Gasteracantha cancriformis (Linnaeus, 1758)	Campo Grande, MS (20°30′36″S/54°36′54″W)	9♀ & 6 embryos	166506, 166533, 166538, 166872, 166880, 166893, 166899, 166901, 166922
Parawixia bistriata (Rengger, 1836)	Dois Irmãos do Buriti, MS (20°31′33.83″S/ 55°25′11.67″W)	3♂/4♀	211341, 211343, 211346 - 211350
Verrucosa meridionalis (Keyserling, 1892)	Aquidauana, MS (20°27′03.93″S/55°37′17.02″W)/ Campo Grande, MS (20°30′36″S/54°36′54″W)/ Botucatu, SP (22°50′44.3″S/48°25′32.4″W) and Campo Grande, MS (20°29′19.09″S/54°39′39.06″W)	2♂/4♀	214414, 214415, 221999, 222412, 242323. 166879
Verrucosa scapofracta Lise, Kesster & Silva, 2015	Iguape, SP (24°42′13.03″S/47°32′49.98″W)	1♂	222317

MS = state of Mato Grosso do Sul. SP = state of São Paulo.

bicornuta and *Parawixia bistriata* showed in all males analyzed mitotic metaphases with 2n♂ = 24 (Figs. 1A–D). Females of *A. pentacanthum*, *P. bistriata* and *Gasteracantha cancriformis* showed 2n♀ = 26 (Figs. 1E–G). Although no adult *G. cancriformis* male was collected, two embryos presented 2n = 24 (considered males) (Fig. 1H), while four other embryos had the same chromosome number as adult females. The four embryos (3♂ and 1♀) of *A. pentacanthum* analyzed possess the same diploid number as adults.

The morphology of all chromosomes is acro/telocentric, and chromosomes show small differences in length (Fig. 1), with exception of the largest chromosome pair of *A. bicornuta*, which is almost twice the length of the second longest pair (Fig. 1C).

Spermatocytes I in diplotene of *A. benigna*, *A. pentacanthum*, *A. bicornuta* and *P. bistriata* revealed 11 autosomal bivalents and two positively heteropycnotic sex univalents (Figs. 2A–D). The SCS was confirmed as of the type X_1X_2♂/

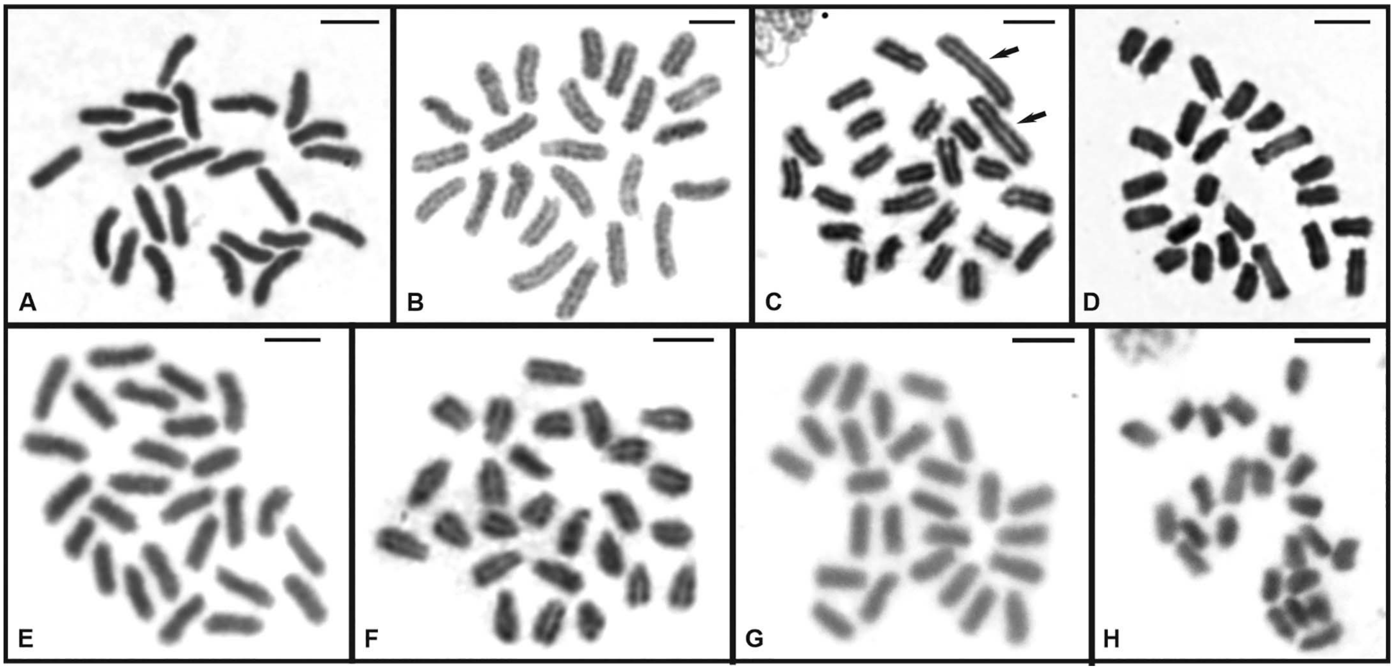

Figure 1A–H.—Araneidae mitotic cells with 2n♂ = 24 (A–D, H) and 2n♀ = 26 (E–G): A. *Acacesia benigna*. B, E. *Actinosoma pentacanthum*. C. *Alpaida bicornuta*. D, F. *Parawixia bistriata*. G, H. *Gasteracantha cancriformis*. Arrows indicate the largest chromosome pair of *A. bicornuta*. Scale bar: 5 µm.

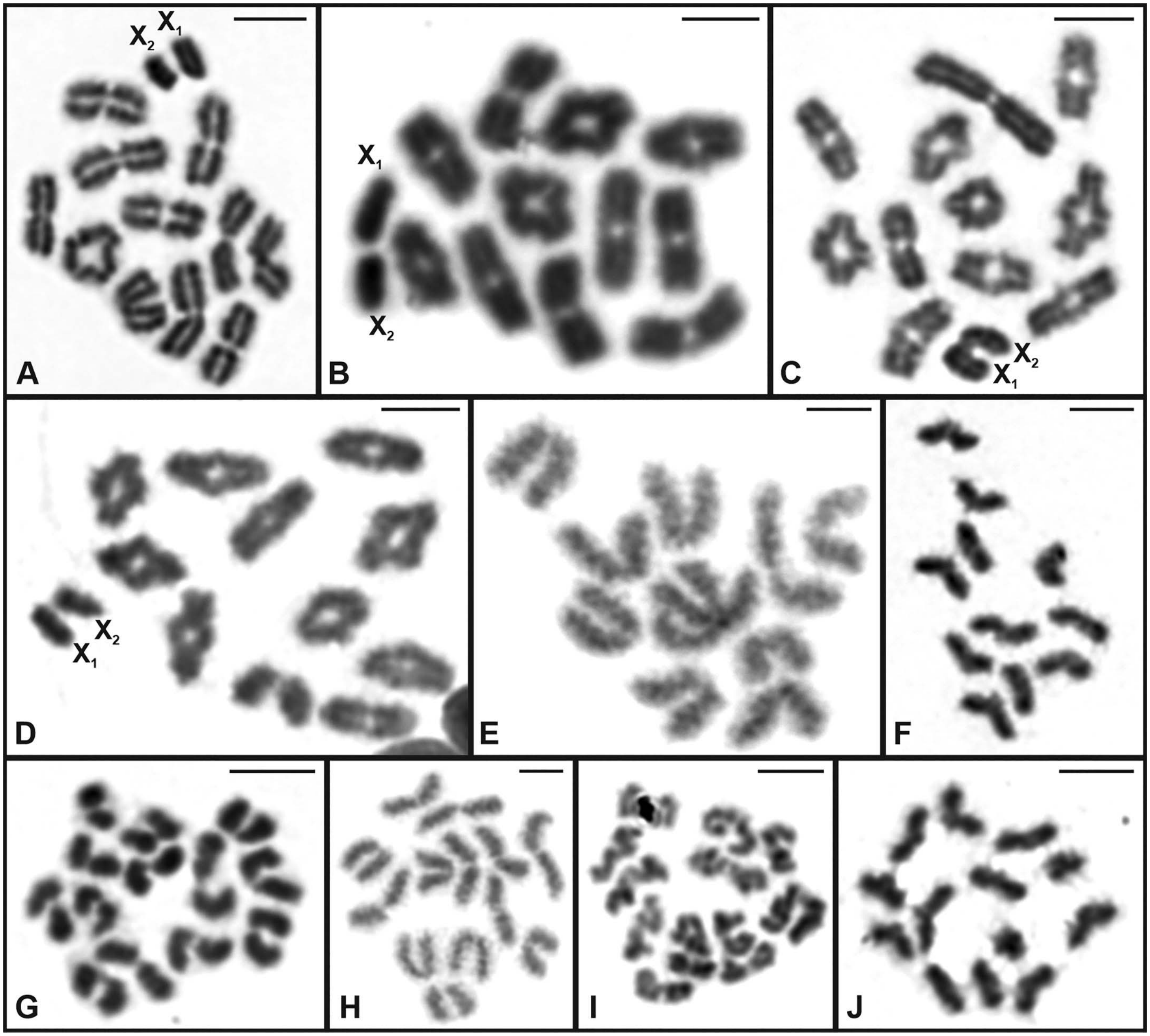

Figure 2A–J.—Meiotic cells of species with the common araneid diploid number (2n♂ = 24): A–D. Diplotenes with 11 autosomal bivalents + X_1X_2. E–J. Metaphase II with n = 11 (E, F) and n = 13 = 11 + X_1X_2 (G–J). A, G. *Acacesia benigna*. B, E, H. *Actinosoma pentacanthum*. C, I. *Alpaida bicornuta*. D, F, J. *Parawixia bistriata*. Scale bar: 5 µm.

$X_1X_1X_2X_2$♀ through spermatocytes II in metaphase with n = 11 in *A. pentacanthum* and *P. bistriata* (Fig. 2E, F) and with n = 13 = 11 + X_1X_2 in these four species (Figs. 2G–J).

Thus, in all the analyzed cells (Table 2) these five species exhibited 2n♂ = 24 = 22 + X_1X_2 and 2n♀ = 26 = 22 + $X_1X_1X_2X_2$ and chromosomes with acro/telocentric morphology.

Karyotypes with high diploid numbers.—*Dubiepeira* sp. showed males with 2n♂ = 41 and females with 2n♀ = 44, with all chromosomes acro/telocentric gradually decreasing in size (Figs. 3A, B). Spermatocytes I in diplotene of *Dubiepeira* sp. exhibit 19 autosomal bivalents and three sex univalents (Fig. 3C). Only spermatocytes II in metaphase with n = 19 were found (Fig. 3D). Altogether, these characteristics confirm the SCS of the type $X_1X_2X_3$♂/$X_1X_1X_2X_2X_3X_3$♀

Verrucosa meridionalis and *V. scapofracta* showed 2n♂ = 47 / 2n♀ = 50 and 2n♂ = 50, respectively, with all chromosomes of both species with acro/telocentric chromosome morphology and gradually decreasing in size (Figs. 3E–G). Spermatocytes I in diplotene of *V. meridionalis* possess 22 autosomal bivalents and three positively heteropycnotic sex univalents (Fig. 3H), while the spermatocytes II in metaphase showed n = 22 and n = 25 = 22 + $X_1X_2X_3$ (Fig. 3I). Spermatocytes I in diplotene from the single male analyzed of *V. scapofracta* exhibit 23 autosomal bivalents and four positively heteropycnotic sex

Table 2.—Number of analyzed cells, diploid number and sex chromosome system (SCS) of species analyzed in this work.

Species	Analyzed cells	Diploid number (2n♂)	SCS (♂)
Acacesia benigna Glueck, 1994	50	24	X_1X_2
Actinosoma pentacanthum (Walckenaer, 1841)	236	24	X_1X_2
Alpaida bicornuta Taczanowski, 1878)	87	24	X_1X_2
Gasteracantha cancriformis (Linnaeus, 1758)	35	24	X_1X_2
Parawixia bistriata (Rengger, 1836) Acro/telocentric	74	24	X_1X_2
Dubiepeira sp.	104	41	$X_1X_2X_3$
Verrucosa meridionalis (Keyserling, 1892)	64	47	$X_1X_2X_3$
Verrucosa scapofracta Lise, Kesster & Silva, 2015	10	50	$X_1X_2X_3X_4$

univalent (Fig. 3J), and only spermatocytes II in metaphase with $n = 27 = 23 + X_1X_2X_3X_4$ were found (Fig. 3K).

Thus, based on all cells analyzed (Table 2) *Dubiepeira* sp. possess 2n♂ $= 41 = 38 + X_1X_2X_3$ and 2n♀ $= 44 = 38 + X_1X_1X_2X_2X_3X_3$, *V. meridionalis* has 2n♂ $= 47 = 44 + X_1X_2X_3$ and 2n♀ $= 50 = 44 + X_1X_1X_2X_2X_3X_3$ and *V. scapofracta* 2n♂ $= 50 = 46 + X_1X_2X_3X_4$ and we suggest that females have the corresponding diploid number 2n♀ $= 54 = 46 + X_1X_1X_2X_2X_3X_3X_4X_4$. All species with acro/telocentric chromosomes.

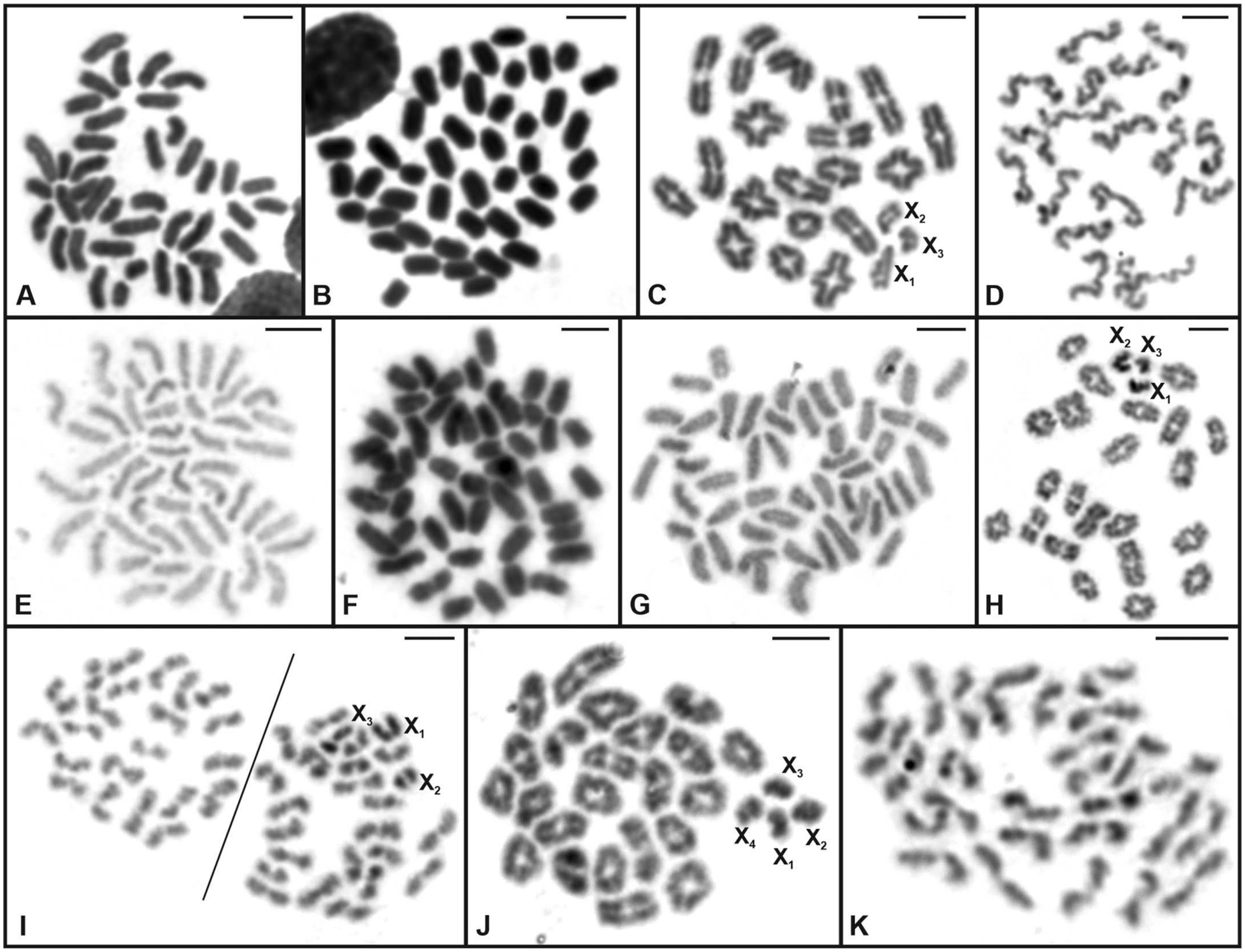

Figure 3A–K.—Cell divisions in *Dubiepeira* sp. (A–D), *Verrucosa meridionalis* (E–F, H–I) and *Verrucosa scapofracta* (G, J–K). A. Spermatogonial metaphase with 2n♂ = 41. B. Oogonial metaphase with 2n♀ = 44. C. Diplotene with 19 autosomal bivalents + $X_1X_2X_3$. D. Metaphase II with n = 19. E. Spermatogonial metaphase with 2n♂ = 47. F. Oogonial metaphase with 2n♀ = 50. G. Spermatogonial metaphase with 2n♂ = 50. H. Diplotene with 22 autosomal bivalents + $X_1X_2X_3$. I. Metaphase II with n = 22 (left) and $n = 25 = 22 + X_1X_2X_3$ (right). J. Diplotene with 23 autosomal bivalents + $X_1X_2X_3X_4$. K. Metaphase II with $n = 27 = 23 + X_1X_2X_3X_4$. Scale bar: 5 µm.

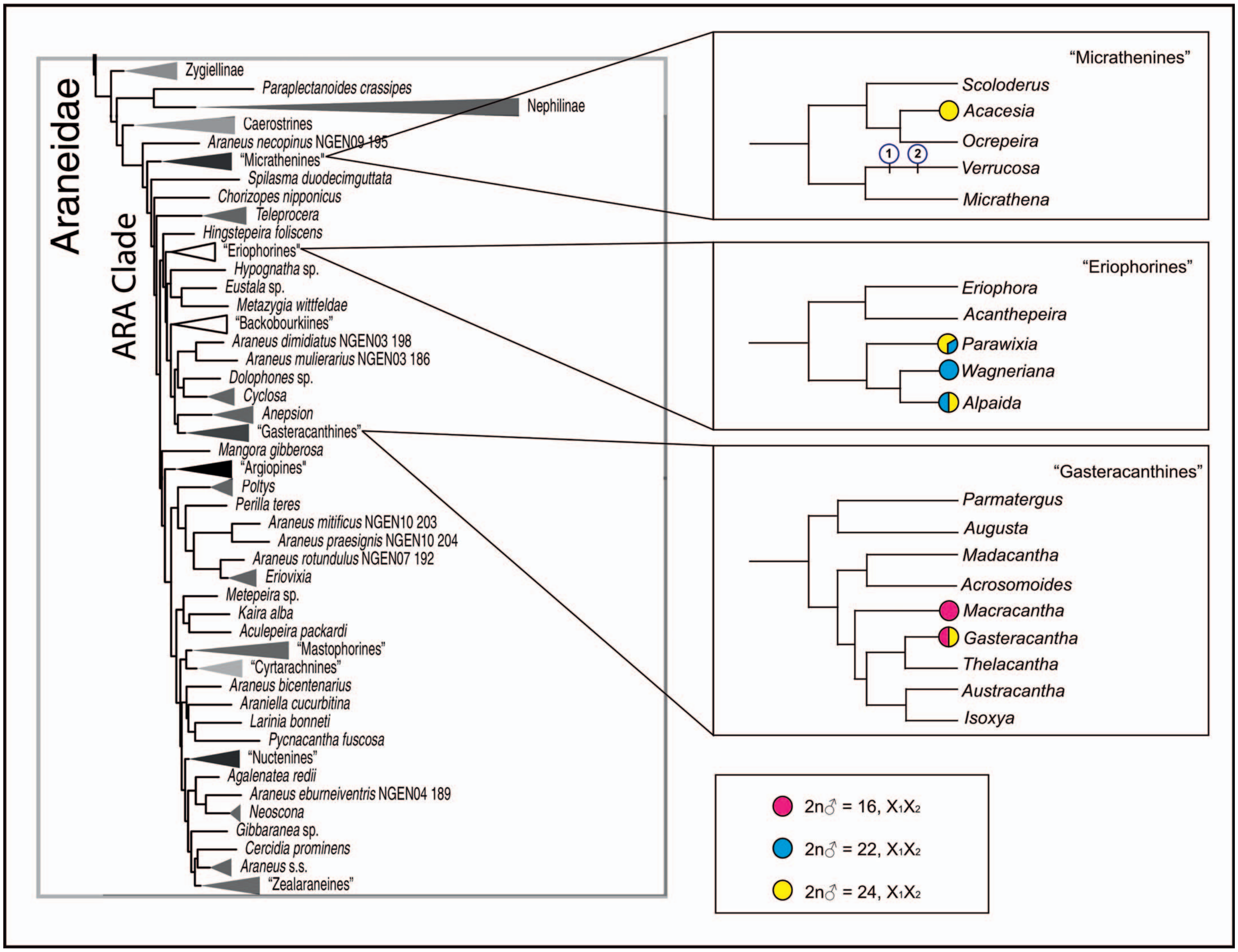

Figure 4.—Araneidae summary tree extracted from Scharff et al. (2020) showing the cytogenetic data available for "Micrathenines," "Eriophorines," and "Gasteracanthines" in detail.

DISCUSSION

Araneidae karyotypes under the light of phylogenetic and taxonomic studies.—The karyotype configuration 2n♂ = 24 = 22 + X_1X_2 with acro/telocentric chromosomes found in the majority of species analyzed here corresponds to the most frequent complement in Araneidae (Table 2) (Araujo et al. 2021). Even though only 1.9% of all species of this family has been cytogenetically studied, the basic karyotype features seem to be relatively homogeneous in this group, with occasional rearrangements in some internal clades (Araujo et al. 2021; World Spider Catalog 2021).

"*Micrathenines*": following Scharff et al. (2020), this clade clusters the genera *Acacesia* Simon, 1895*, Scoloderus* Simon, 1887*, Ocrepeira* Marx, 1883*, Verrucosa* McCook, 1888 and *Micrathena* Sundevall, 1833, all of them occurring in the New World (World Spider Catalog 2021). Within this clade are two groups, one clustering *Acacesia*, *Ocrepeira* and *Scoloderus* and another contatining *Micrathena* and *Verrucosa* (see fig. 3 in Scharff et al. 2020).

Here, we show the first chromosomal data for Micrathenines with members of both Micrathenine clades. *Acacesia benigna* presents the standard karyotype of the family, while *Verrucosa meridionalis* and *V. scapofracta* have a much larger diploid number and unusual SCS (Table 2) (Fig. 4). Thus, the events which generated the chromosomal patterns in *Verrucosa* probably are restricted to the group comprising *Micrathena* and *Verrucosa* or occurred only in *Verrucosa* species. Therefore, karyological studies in *Micrathena* are needed in a next step of the research. Independently, due to the differences of diploid number and SCS between *V. meridionalis* and *V. scapofracta* we could affirm that some rearrangements have already occurred after *Verrucosa* divergence (Table 2).

"*Eriophorines*": A clade that includes species of the genera *Acanthepeira* Marx, 1883, *Alpaida* O. Pickard-Cambridge, 1889, *Eriophora* Simon, 1864, *Parawixia* F. O. Pickard-Cambridge, 1904 and *Wagneriana* F. O. Pickard-Cambridge, 1904 (Scharff et al. 2020). Only six species of this clade were cytogeneticaly analyzed up to now, belonging to the genera

Alpaida, *Parawixia* and *Wagneriana* (Fig. 4). *Alpaida leucogramma* (White, 1841) and *Parawixia velutina* (Taczanowski, 1878) showed the common 2n♂ $= 24 = 22 + X_1X_2$ (Araujo et al. 2011), the same chromosome configuration of both *A. bicornuta* and *P. bistriata* (present study), whilst *Alpaida truncata* (Keyserling, 1865), *Alpaida veniliae* (Keyserling, 1865), *Parawixia kochi* (Taczanowski, 1873) and *Wagneriana* sp. exhibit a reduction of one autosomal pair, with 2n♂ $= 22 = 20 + X_1X_2$ (Araujo et al. 2011).

The only species of *Alpaida* with mitotic metaphases analyzed were *A. leucogramma* (Araujo et al. 2011) and *A. bicornuta* (present study). Despite both species presenting 2n♂ $= 24 = 22 + X_1X_2$, the size of the largest autosomal pair of *A. bicornuta* corresponds to almost twice the size of the second pair (Fig. 1C), a chromosomal characteristic that was not observed in *A. leucogramma*. Thus, *A. bicornuta* possesses a complement different from the rest of the *Alpaida* species that have been cytogenetically studied to date.

Parawixia bistriata and *P. velutina* possess the same complement (present study; Araujo et al. 2011), suggesting a closer relationship chromosomal between them than to *P. kochi*. Unfortunately, there is no phylogeny of the genus so far that corroborates any hypothesis related to the species of *Parawixia*. However, the few *Parawixia* species included in phylogenetic analysis of the Araneidae by Scharff et al. (2020) showed as polyphyletic (part belonging to "Eriophorines" and part to "Backobourkiines"). Molecular data have been used to find species-specific characters, but are relatively scarce in Araneae (Agnarsson et al. 2013; Garrison et al. 2016; Scharff et al. 2020), and morphological characters have limitations for determining species in some groups (Scharff et al. 2020). Better approaches to these cases are those that include characteristics of different natures, such as behavior data (Coddington 1986, 1990; Dimitrov et al. 2017) and cytogenetic data (Araujo et al. 2008, 2011; Řezáč et al. 2018), for example. The karyotype of some *Parawixia* species presented variability and can be helpful in further studies of this polyphyletic genus, as suggested by Araujo et al. (2011). Cytogenetic studies in all "Eriophorines" genera are needed to establish a better understanding of the chromosome evolution in the clade.

"*Gasteracanthines*": despite this clade possessing nine genera, the vast majority of species richness belongs to *Gasteracantha* Sundevall, 1833 (Scharff et al. 2020; World Spider Catalog 2021). Cytogenetically, only *Macracantha hasselti* (C. L. Koch, 1837) and *Gasteracantha kuhli* C. L. Koch, 1837 were analyzed and found to exhibit 2n♂ $= 16 = 14 + X_1X_2$ (Datta & Chatterjee 1983, 1988), diverging from diploid number 2n♂ $= 24 = 22 + X_1X_2$ of *G. cancriformis* studied here (Fig. 4). In the Araneidae, several instances of intrageneric reduction in diploid number have been reported. In *Larinioides* Caporiacco, 1934 these range from 2n♂ $= 14$ in *Larinioides patagiatus* (Clerck, 1757) to 2n♂ $= 23$ and 24 in *L. cornutus* (Clerck, 1757) (Berry 1906; Painter 1914; Hackman 1948; Qingtao et al. 1998, 1999). In *Neoscona* Simon, 1864 diploid numbers of 2n♂ $= 13$, 14, 21, 23 and 24 have been reported (Suzuki 1951a, b; Mittal 1960, 1966a; Datta & Chatterjee 1983, 1988; Parida & Sharma 1987; Sharma & Parida 1987; Amalin 1988; Amalin et al. 1993; Doan & Paliulis 2009; Prakash & Prakash 2014). Finally, *Alpaida* and *Parawixia* have just the reduction of one autosomal pair (Araujo et al. 2011).

A recent work that used morphology and DNA sequence data in a review of Gasteracanthines transferred *G. hasselti* to the genus *Macracanta* (Macharoenboon et al. 2021) corroborating the study of Tan et al. (2019), who found a paraphyly in *Gasteracantha*, specifically related to *G. hasselti*. However, both analyses used limited taxon sampling for a robust phylogeny of Gasteracanthines, and Macharoenboon et al. (2021) comment that the taxonomy in the group is complicated due to the difficulty of finding male specimens for morphological and molecular studies. The karyotype data suggest a closer proximity between *M. hasselti* and *G. kuhli* than between *G. cancriformis* and *G. kuhli*, which is concordant with the geographic distribution of these three species (Datta & Chatterjee 1983, 1988; World Spider Catalog 2021). Thus, further cytogenetic, morphological and phylogeographic work with several species are needed to help clarifying the relationships in this group.

Actinosoma pentacanthum and Dubiepeira sp.: the two largest phylogenies of the family do not include *Actinosoma* Holmberg, 1883 and *Dubiepeira* Levi, 1991 (Scharff & Coddington 1997; Scharff et al. 2020). However, the phylogenetic inference of *Wagneriana* by Cabra-García & Hormiga (2020) suggests a proximity of *Actinosoma* with *Alpaida*, *Rubrepeira* Levi, 1992 and some species of *Wagneriana*. From the cytogenetic point of view, *A. pentacanthum* presents the same diploid number (2n♂ $= 24$) found in two of the four *Alpaida* species chromosomally analyzed up to now (*A. leucogramma* and *A. benigna*), whilst the other two *Alpaida* species and *Wagneriana* sp. presents 2n♂ $= 22$ (present study; Araujo et al. 2011), though further studies in these related genera are needed to determine more accurately the relationship among them.

In spite of the fact that *Dubiepeira* was not sampled in any phylogeny, Levi (1991) notice that females of *Dubiepeira* and *Araneus* Clerck, 1757 are very similar. Interestingly, *A. ventricosus* presented one of the highest diploid numbers in the Araneidae (2n♂ $= 32$, 46 and 49) (Suzuki 1951a, b; Zhang & Tong 1990; Youju et al. 1993), a characteristic also observed in *Dubiepeira* sp. (2n♂ $= 41$) in the present study and the same SCS (♂$X_1X_2X_3$/♀$X_1X_1X_2X_2X_3X_3$) in one analyzed population (Youju et al. 1993). Moreover, *Araneus* is well known in the literature as a polyphyletic genus in which many unresolved species are inserted (Scharff & Coddington 1997; Scharff et al. 2020), thus, the karyotype found in *Dubiepeira* sp. suggests a proximity with *A. ventricosus* and chromosomal characters may be an alternative to help clarify relationships within the polyphyletic genus *Araneus*.

Chromosomal evolution of the araneid karyotypes with high diploid numbers.—Based on the high diploid numbers found in Mesothelae and several clades of Mygalomorphae, the chromosomal evolution in Araneae has been suggested to occur mainly through rearragements that decrease the diploid number, such as Robertsonian fusions (Suzuki 1954; Král et al. 2006; Araujo et al. 2021. Nevertheless, even in the clades that possess mostly species with relatively low diploid numbers—such as the Araneidae—chromosomal fissions may occur, originating karyotypes with high diploid numbers like those found in *Dubiepeira* sp., *V. meridionalis* and *V.*

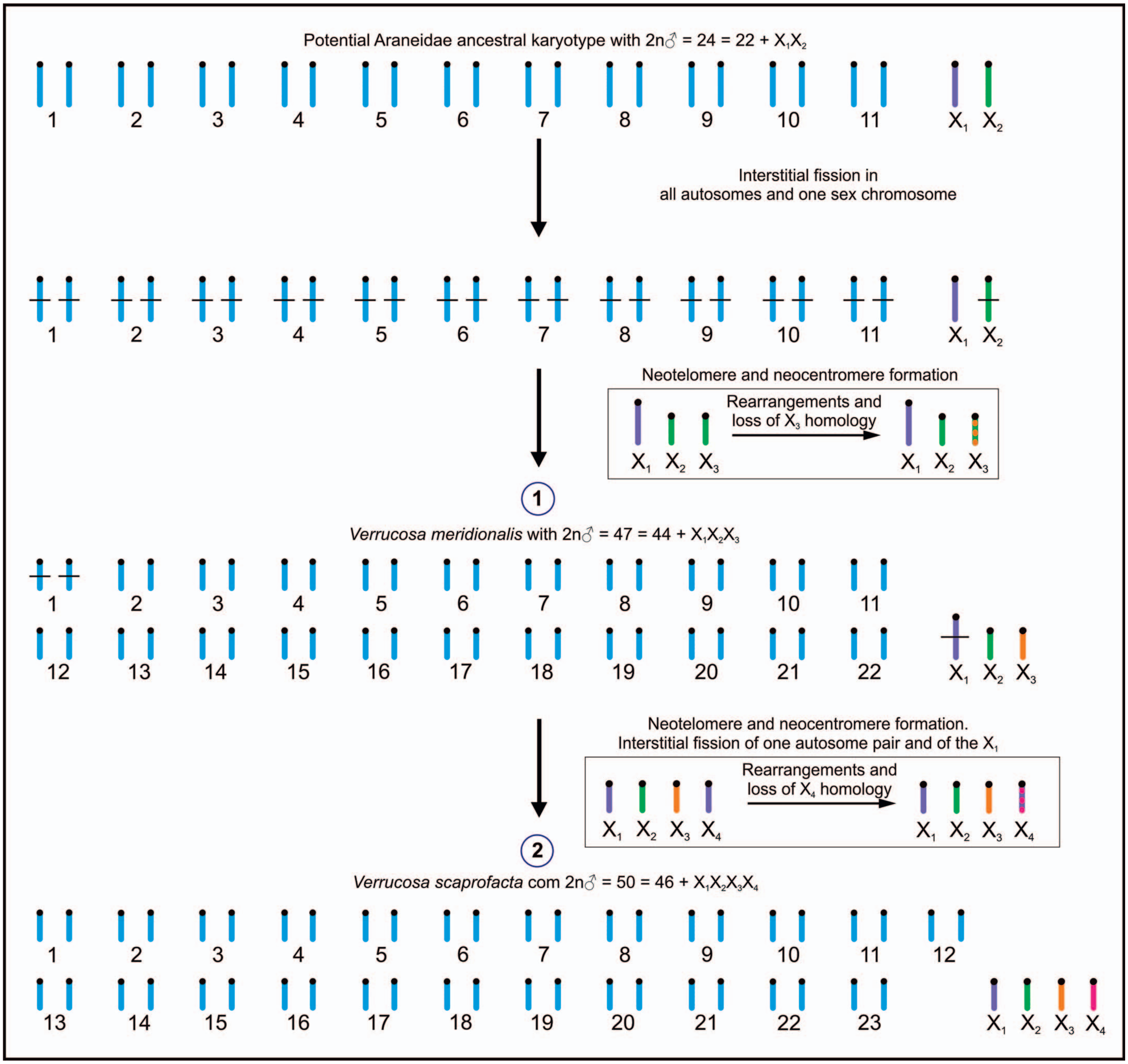

Figure 5.—Scheme showing hypothetical origins of the karyotypes 2n♂ = 47 = 44 + $X_1X_2X_3$ and 2n♂ = 50 = 46 + $X_1X_2X_3X_4$, found in *Verrucosa meridionalis* and *Verrucosa scapofracta*, respectively. The rearrangement events represented by the numbers "1" and "2" would have occurred within the *Verrucosa* genus, as represented by the same numbers on "Micrathenines" (detail in Fig. 4).

scapofracta analyzed here. Interestingly, the 2n♂ = 50 of the latter species is the largest known diploid number within all Entelegynae spiders. A former study on *Agelenopsis naevia* (Walckenaer, 1841) (Agelenidae) mentioned around 50 chromosomes, but emphasizes that chromosome counting is not accurate, which is understandable considering that the study relies on histological sections. The same author had already described around 40 chromosomes previously to the same species (Wallace 1905, 1909).

The SCS of the type ♂$X_1X_2X_3$/♀$X_1X_1X_2X_2X_3X_3$ is commonly found in spider families such as Sparassidae (Hackman 1948; Suzuki & Okada 1950; Bole-Gowda 1952; Suzuki 1952; Mittal 1961, 1966b; Datta & Chatterjee 1983; Rowell 1985, 1988, 1990, 1991; Parida & Sharma 1986, 1987; Srivastava & Shukla 1986; Sharma & Parida 1987; Hancock & Rowell 1995), Agelenidae, in the genus *Tegenaria* Latreille, 1804 and Tetragnathidae, in the genus *Leucauge* White, 1841 (Sokólska 1925; Sharma et al. 1959; Datta & Chatterjee 1983, 1988; Xiuzhen et al. 1996; Král 2007; Kořínková & Král 2013). However, in the Araneidae it is very rare, restricted to *Araneus ventricosus* (Youju et al. 1993), *Dubiepeira* sp. and *V. meridionalis* (present study).

The SCS of the type ♂$X_1X_2X_3X_4$/♀$X_1X_1X_2X_2X_3X_3X_4X_4$ found in *V. scapofracta*, has been observed in nine species of spiders so far (Datta & Chatterjee 1983, 1988; Král et al. 2011, 2013; Kořínková & Král 2013; Souza et al. 2021). Souza et al. (2021) suggest its independent appearance sometimes in the Araneae because of the phylogenetic distance between the clades that have this SCS.

Based on the chromosome characteristics above described, we hypothesize a scenario to the origins of the *Verrucosa* karyotypes, that we will start from a karyotype with 2n♂ = 24, X_1X_2, because it is also present in other Araneoidea and probably the ancestor of the family (Araujo et al. 2021), which suffered events of chromosomal fission in all autosomal pairs and the X_2 chromosome, originating a karyotype with 2n♂ = 47, $X_1X_2X_3$, as observed in *V. meridionalis*. In a next step, one autosomal pair and the X_1 would be fissioned, originating the karyotype with 2n♂ = 50, $X_1X_2X_3X_4$, presented in *V. scapofracta* (Fig. 5).

Rearrangement events involving all chromosomes of the complement are called "all or nothing" and have already been described in the spider families Oxyopidae, Pholcidae, Sparassidae and Theridiidae (Rowell 1990; Stávale et al. 2010, 2011; Lomazi et al. 2018). However, more extensive research within the speciose genera *Verrucosa* and *Micrathena* can reveals karyotypes with intermediate diploid numbers, indicating a more gradual occurrence of the rearrangments.

Alternatively, the origin of the ♂$X_1X_2X_3$/♀$X_1X_1X_2X_2X_3X_3$ and ♂$X_1X_2X_3X_4$/♀$X_1X_1X_2X_2X_3X_3X_4X_4$ SCS could have happened through nondisjunctions followed by loss of homology of the neo X chromosomes, as suggested by some authors in other spider species (Postiglioni & Brum-Zorrilla 1981; Datta & Chatterjee 1988; Král et al. 2011; Araujo et al. 2012).

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