

Chromosomal Study on Selected Small Araneomorph Spiders from Brazil, Including the First Records in Palpimanidae and Theridiosomatidae (Araneae, Araneomorphae)

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Among the 50,474 spider species, only 849 have chromosomal data available in the literature. Fifty spider families remain unknown from a cytogenetic perspective. The aim of this study was to analyze chromosomally selected araneomorph spiders from Brazil, to contribute to the cytotaxonomy and chromosome evolution of this group. The karyotypes of 12 species belonging to families Corinnidae, Linyphiidae, Oonopidae, Palpimanidae, Theridiidae, Theridiosomatidae, Trachelidae, and Zodariidae were analyzed, including the first chromosomal record for the first two families. Specimens (with the abdomen content partially exposed by perforation) were subjected to colchicine, hypotonization, and fixation. In most cases, the total content of the abdomen was used to prepare slides by spreading of cell suspension and subsequent Giemsa staining. The results were as follows: *Cinetomorpha simplex* Simon, 1892 (Oonopidae) $2n \delta = 9, X_0$; *Neotrops* sp. and *Neoxyphinus termitophilus* (Oonopidae), $2n \delta = 7, X_0$; *Otiothops birabeni* (Palpimanidae); *Agyneta* sp. (Linyphiidae), $2n \delta = 24, X_1X_2_0$; *Coleosoma floridanum*, *Thymoites* sp.1 and *Thymoites* sp.2 (Theridiidae), $2n \delta = 22, X_1X_2_0$; *Naatlo* sp. (Theridiosomatidae), $2n \delta = 30, X_1X_2_0$; *Orthobula* sp. (Trachelidae) $2n \delta = 21, X_0$; *Falconina* sp. (Corinnidae), $2n \delta = 28, X_1X_2_0$; *Epicratinus* sp. (Zodariidae) $2n \delta = 42, X_1X_2_0$. The chromosomal morphology was determined for all the samples except for Oonopidae. Most species exhibited telocentric chromosomes, with the exception of Palpimanidae and Theridiosomatidae. The main findings: 1) support an hypothesis on ancestral karyotype of Zodariidae and Oonopidae; 2) reveal a relatively high chromosome number in Palpimanidae that supports an idea on relatively high ancestral chromosome number ($2n \delta = 42$) of entelegyne spiders (Palpimanoidea is sister group of entelegyne spiders); 3) show that the karyotype found in Theridiosomatidae is exceptional within the Araneoidea.

Key words: Mitosis, Meiosis, Diploid number, Sex chromosome system

BACKGROUND

Although 50,474 spider species placed in 132 families have been described (World Spider Catalog

2022), only 849 species belonging to 80 families have chromosomal data available (Araujo et al. 2022). This scarcity of cytogenetic data makes difficult analysis of chromosomal evolution in most groups of this order,

including families of spiders with small body sizes, which are often neglected because of the difficulties in collecting, dissecting, and obtaining chromosome plates. Chromosomal studies of leaf litter spiders are also rare due to specific methodology, which is required for their collection. Many spiders from this environment are tiny, difficult to determine with the naked eye (Indicatti and Villarreal 2016).

In the present study, we analyzed species of spiders that are less than one centimeter in body length. Twelve species were evaluated, four of which belonged to families with exclusively minute species (Oonopidae and Theridiosomatidae) and eight belonged to families that also included larger species (Corinnidae, Linyphiidae, Palpimanidae, Theridiidae, Trachelidae, and Zodariidae). Most of these families are not closely related (Wheeler et al. 2017). Cytogenetic studies on most of these families are rare or do not exist. We recorded the first karyotype data for Palpimanidae and Theridiosomatidae and documented the first karyotype data for all other studied genera to fill gaps and expand the data for analysis of cytotaxonomy and karyotype evolution in araneomorph spiders.

MATERIALS AND METHODS

The specimens of *Naatlo* sp. (Theridiosomatidae) were collected over night. The remaining specimens were obtained during the day or night through litter sieving, according to Coddington et al. (1991), with some modifications. After the exclusion of leaves and larger particles with the aid of a metallic sieve with a mesh of 1 cm, the material was slowly spread in a plastic tray, and individuals were searched by tossing the sieved litter using tweezers under a strong light source, allowing the acquisition of living specimens, which are necessary for cytogenetic studies. Samples were collected exclusively in the State of Mato Grosso do Sul, Brazil, between March 2019 and September 2021. After dissection of the total abdomen content or the gonads, the specimens were identified and deposited at the Laboratory of Zoological Collections, Instituto Butantan, São Paulo, Brazil. The collection permit was provided by the Instituto Chico Mendes de Conservação da Biodiversidade, ICMBio, Ministério do Meio Ambiente, MMA (number 15382-3).

We dissected 218 specimens of spiders. Thirty-eight showed dividing cells and were used in this study. We analyzed 12 species, namely *Otiotrops birabeni* Mello-Leitão, 1945 (Palpimanidae), *Naatlo* sp. (Theridiosomatidae), *Agyneta* sp. (Linyphiidae), *Coleosoma floridanum* Banks, 1900, *Thymoites* sp.1 and *Thymoites* sp.2 (Theridiidae), *Falconina* sp.

(Corinnidae), *Orthobula* sp. (Trachelidae), *Epicratinus* sp. (Zodariidae), and three Oonopidae species: *Cinetomorpha simplex* Simon, 1892, *Neotrops* sp., and *Neoxyphinus termitophilus* (Bristowe, 1938). Table 1 provides the details of the analyzed individuals.

Chromosomes were prepared using the cell suspension technique and 3% Giemsa staining, according to Araujo et al. (2008), with some modifications. Due to the small size of the spiders, the whole specimens were transferred to the treatment after the perforation of the abdomen to allow the action of the reagents. The whole content of the abdomen was used to prepare slides, with the exception of *Falconina* sp. and *Epicratinus* sp. whose gonads were dissected due to their relatively large size.

The suitable plates were photographed using an AxioImager D2 (Zeiss) microscope, at 1000× magnification. If possible, the chromosomal morphology was determined using the plugin LEVAN (Sakamoto and Zacaro 2009) from ImageJ software (Rasband 1997–2021), considering the criteria proposed by Levan et al. (1964) and Green and Sessions (1991). To calculate the relative length of the X chromosome in *Orthobula* sp., chromosomes of two oogonial and two spermatogonial metaphases were measured using ImageJ software (Rasband 1997–2021).

RESULTS

Synspermiata, Dysderoidea

Oonopidae

Mitotic metaphases of *Cinetomorpha simplex* consisted of 9 (♂) or 10 (♀) chromosomes, respectively (Fig. 1A–B). In diplotene and metaphase I, spermatocytes I showed four autosomal bivalents and one univalent that corresponded to the X chromosome (Fig. 1C). Therefore, the karyotype of this species is $2n \delta = 9 (8+X0)$ and $2n \text{♀} = 10 (8+XX)$.

The spermatogonial and oogonial metaphases of *Neotrops* sp. contained 7 (♂) or 8 (♀) chromosomes, respectively (Fig. 1D–E). In *Neoxyphinus termitophilus*, oogonial metaphases contained 8 chromosomes (Fig. 1F), and diplotene and metaphase I of spermatocytes I had three autosomal bivalents and one sex chromosome univalent (Fig. 1G). Based on these data, we determined the karyotype of these two species as $2n \delta = 7 (6+X0)$ and $2n \text{♀} = 8 (6+XX)$, respectively.

We were not able to determine the chromosomal morphology of oonopids which is described as holocentric (Král et al. 2019), because: 1) the few mitotic metaphases found did not allow us to determine

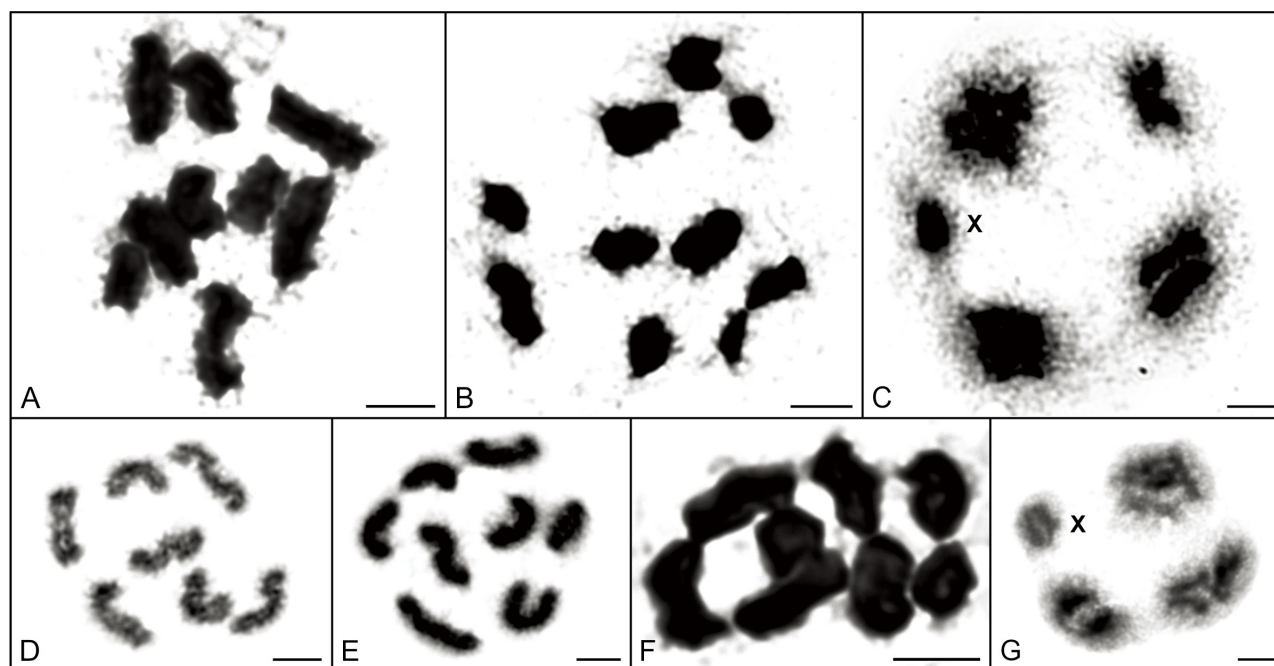


Fig. 1. Chromosomes of Onopidae (Dysderoidea). *Cinetomorpha simplex* (A–C), *Neotrops* sp. (D–E) and *Neoxyphinus termitophilus* (F–G). A. Mitotic spermatogonial metaphase, $2n \text{ ♂} = 9$. B. Mitotic oogonial metaphase, $2n \text{ ♀} = 10$. C. Spermatocyte I, metaphase I showing four autosomal bivalents and a sex chromosome univalent (4II+X). D. Mitotic spermatogonial metaphase, $2n \text{ ♂} = 7$. E–F. Mitotic oogonial metaphase, $2n \text{ ♀} = 8$. G. Spermatocyte I, diplotene consisting of three autosomal bivalents and a sex chromosome univalent (3II+X). Scale bars = 5 μm .

Table 1. Collection site, numbers of specimens (males and females), number of analyzed cells, diploid number, and sex chromosome system (SCS) in species analyzed

Family/Higher classification	Species	Collection site	Specimens	Analyzed cells	Diploid number ♂	SCS ♂
Onopidae (Dysderoidea)	<i>Cinetomorpha simplex</i>	1	2 ♂ / 1 ♀	9	9	X0
	<i>Neotrops</i> sp.	3	2 ♂ / 1 ♀	9	7	X0
	<i>Neoxyphinus termitophilus</i>	1	1 ♂ / 1 ♀	12	7	X0
Palpimanidae (Palpimanoidea)	<i>Otiothops birabeni</i>	1	1 ♀	3	36 (♀)	-
Linyphiidae (Entelegynae, Araneoidea)	<i>Agyneta</i> sp.	1	2 ♂ / 1 ♀	15	24	X ₁ X ₂ 0
Theridiidae (Entelegynae, Araneoidea)	<i>Coleosoma floridanum</i>	3	3 ♂ / 3 ♀	17	22	X ₁ X ₂ 0
	<i>Thymoites</i> sp.1	1	3 ♂ / 2 ♀	9	22	X ₁ X ₂ 0
		3				
	<i>Thymoites</i> sp.2	1	1 ♂ / 1 ♀	6	22	X ₁ X ₂ 0
Theridiosomatidae (Entelegynae, Araneoidea)	<i>Naatlo</i> sp.	2	3 ♂	22	30	X ₁ X ₂ 0
Trachelidae (Entelegynae, Clade RTA, Dionycha A)	<i>Orthobula</i> sp.	1	3 ♂ / 2 ♀	8	21	X0
		2				
		3				
Corinnidae (Entelegynae, Clade RTA, Dionycha B)	<i>Falconina</i> sp.	3	2 ♂	74	28	X ₁ X ₂ 0
Zodariidae (Entelegynae, Clade RTA, Zodarioidea)	<i>Epicratinus</i> sp.	3	2 ♂ / 1 ♀	36	42	X ₁ X ₂ 0

Collection sites: 1 - Fazenda Palmeiras II, city of Coxim, State of Mato Grosso do Sul, Brazil (18°21'45"S, 54°36'56"W) – Three field trips: 04/24/2020, 10/05//2020 and 03/09/2021. 2 - Morro do Paxixi, city of Aquidauana, State of Mato Grosso do Sul, Brazil (20°27'00"S, 55°37'18.2"W) - Two field trips: 03/19/2019 and 05/05/2020. 3 - Reserva Particular do Patrimônio Natural - RPPN/UFMS, city of Campo Grande, State of Mato Grosso do Sul, Brazil (20°30'36"S, 54°36'54"W) - Six field trips: 07/22/2020, 11/03/2020, 02/22/2021, 05/28/2021, 07/27/2021 and 09/13/2021.

the morphology, 2) metaphase II, which could help to determine chromosome morphology, was not found, and 3) our analyzes included colchicine, which reduced the number of cells showing segregation behavior during mitotic anaphase.

Palpimanidae (Palpimanoidea)

Oogonial metaphases of *Otiothops birabeni* consist of 36 chromosomes. The morphology of particular chromosome pairs was not determined, but the karyotype includes metacentric, submetacentric, subtelocentric, and telocentric elements (Fig. 2). It was impossible to determine the sex chromosome system (SCS) because males were not found.

Entelegynae, Araneoidea

Linyphiidae

Analysis of the spermatogonial and oogonial metaphases of *Agyneta* sp. revealed $2n \delta = 24$ and $2n \text{♀} = 26$, respectively. Chromosomes were exclusively telocentric (Fig. 3A–B). Spermatocytes I showed 11 autosomal bivalents and two sexual univalents (X_1X_2) in early diplotene. Sex chromosomes formed a positively heteropycnotic mass (Fig. 3C). In late diplotene and metaphase I, it was possible to observe 11 autosomal bivalents (the majority with only one chiasma) and two separated sex chromosome univalents (Fig. 3D), which allowed to determine the male karyotype of the species

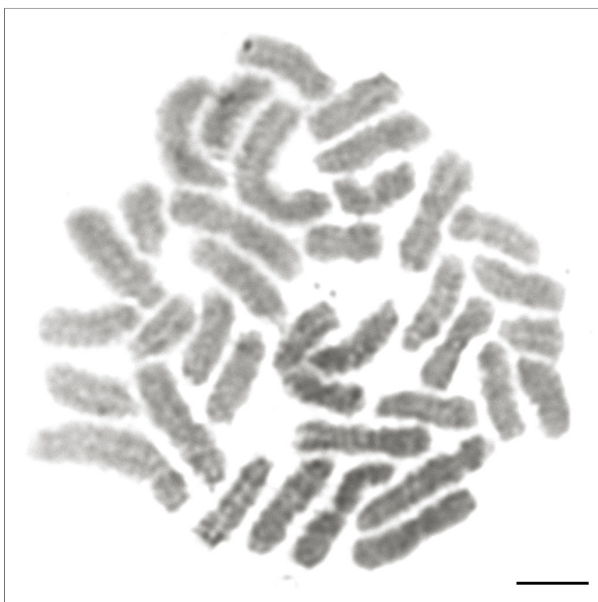


Fig. 2. Mitotic oogonial metaphase of *Otiothops birabeni* (Palpimanidae), $2n \text{♀} = 36$. Scale bar = 5 μm .

as $2n \delta = 24 (22+X_1X_20)$.

Theridiidae

Analysis of mitotic metaphases revealed $2n \delta = 22$ in *Coleosoma floridanum* and *Thymoites* sp.1 (Fig. 3E, H) and $2n \text{♀} = 24$ in *C. floridanum*, *Thymoites* sp.1, and *Thymoites* sp.2 (Fig. 3F, I, K). Plates were composed exclusively by telocentric chromosomes (Fig. 3E–F, H–I, K). In these three species, spermatocytes I showed 10 autosomal bivalents and two sex chromosome univalents (X_1X_2) in diplotene and metaphase I. The bivalents showed only one chiasma (Fig. 3G, J), except for the early diplotenes, which showed some bivalents with two chiasmata (Fig. 3L). The sex chromosome univalents were placed close to each other (Fig. 3G) or side-by-side and showed positive heteropycnosis (Fig. 3J, L), depending on the stage of diplotene/metaphase I. Based on analyzed plates, we determined the karyotype formulas of these species as $2n \delta = 22 (20+X_1X_20)$ and $2n \text{♀} = 24 (20+X_1X_1X_2X_2)$.

Theridiosomatidae

Male mitotic metaphases consisted of 30 chromosomes (Fig. 3M). Spermatocytes I in diplotene and metaphase I contained 14 autosomal bivalents and two X chromosome univalents (X_1X_2) (Fig. 3N). We found two types of metaphases II of spermatocytes II, one with $n = 14$ and another one with $n = 16 (14+X_1X_2)$ (Fig. 3O–P). In some diplotenes and metaphases II, the sex chromosomes were positively heteropycnotic. Thus, the karyotype formula of *Naatlo* sp. was $2n \delta = 30 (28+X_1X_20)$. Despite many chromosome overlaps in mitotic metaphases, which did not allow detailed analysis of chromosome morphology, the observation of mitotic plates and metaphases II indicated that all or almost all the chromosomes were biarmed (meta/submeta/subtelocentric) (Fig. 3O–P).

Entelegynae, RTA clade

Dionycha part A

Trachelidae

Analysis of mitotic metaphases of *Orthobula* sp. revealed $2n \delta = 21$ and $2n \text{♀} = 22$ chromosomes, which exhibited telocentric morphology (Fig. 4A–B). Diplotene of spermatocytes I showed 10 autosomal bivalents with one or two chiasmata and a positively heteropycnotic univalent X (Fig. 4C). Thus, the karyotype was $2n \delta = 21 (20+X0)$ and $2n \text{♀} = 22 (20+XX)$. The X chromosome was the largest element

of the complement (Fig. 4A–B), corresponding to approximately 7% of the total chromosome length of diploid karyotype in males.

Dionycha part B

Corinnidae

Spermatogonial metaphases of *Falconina* sp. showed 28 telocentric chromosomes (Fig. 4D). Spermatocytes I showed 13 autosomal bivalents and

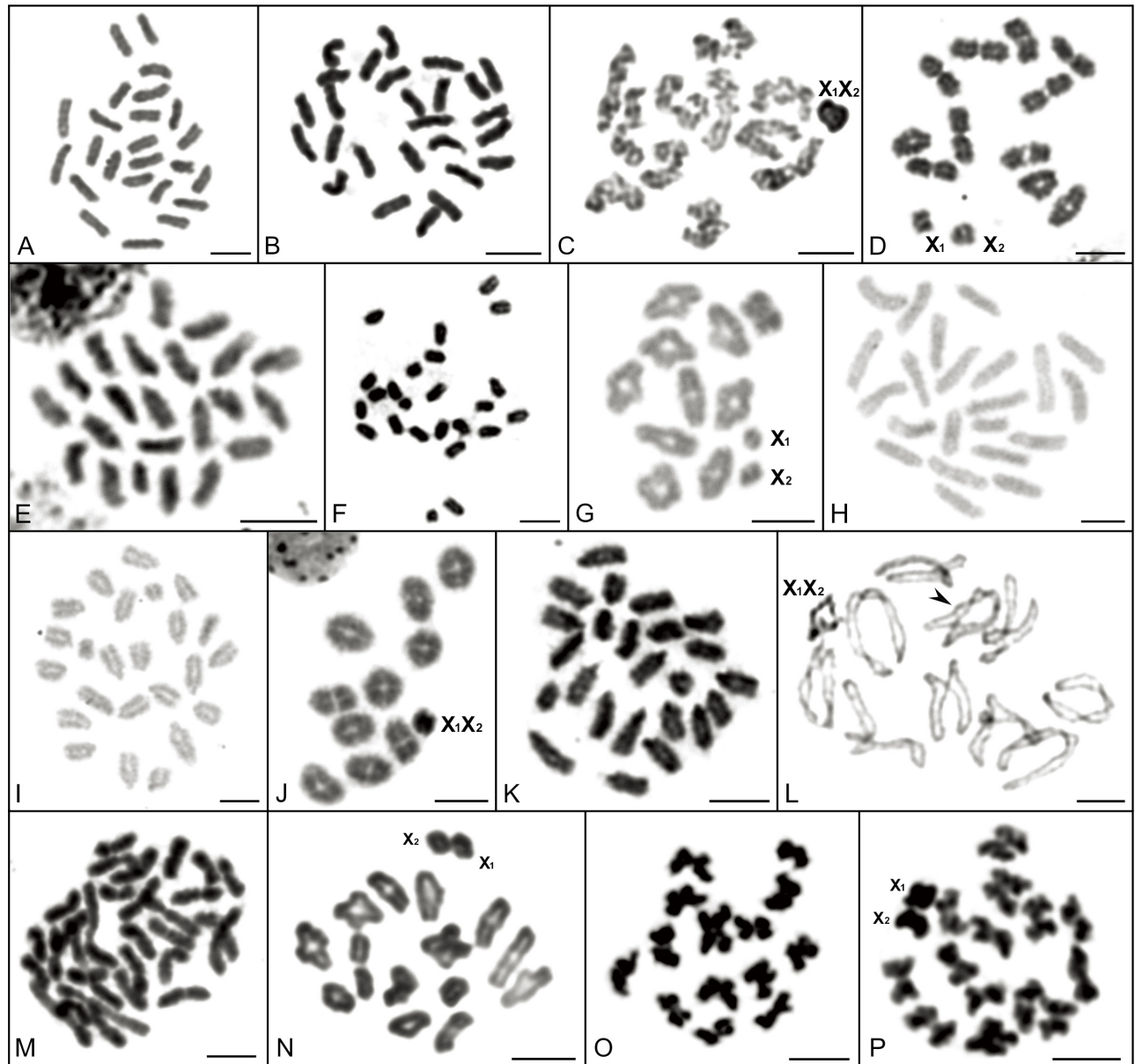


Fig. 3. Chromosomes of Linyphiidae (A–D), Theridiidae (E–L) and Theridiosomatidae (Araneoidea). *Agyneta* sp. (A–D), *Coleosoma floridanum* (E–G), *Thymoites* sp.1 (H–J), *Thymoites* sp.2 (K–L) and *Naatlo* sp. (M–P): A: Mitotic spermatogonial metaphase composed of 24 telocentric chromosomes. B: Mitotic oogonial metaphase with 26 telocentric chromosomes. C: Spermatocyte I (early diplotene) with 11 autosomal bivalents and two positively heteropycnotic sex chromosome univalents arranged in parallel (11II+X₁X₂). D: Spermatocyte I (metaphase I) with 11 autosomal bivalents and two sex chromosome univalents (11II+X₁X₂). E, H: Mitotic spermatogonial metaphase, 2n ♂ = 22 telocentric chromosomes. F, I, K: Mitotic oogonial metaphases, 2n ♀ = 24 telocentric chromosomes. G, J, L: Spermatocyte I, metaphase I (G, J) and early diplotene (L) consisting of 10 autosomal bivalents and two sex chromosome univalents (10II+X₁X₂). Sex chromosomes are positively heteropycnotic at J and L. While they are associated at G, they pair in parallel in J. Mode of pairing is unclear at L. M: Mitotic spermatogonial metaphase, 2n ♂ = 30. N: Spermatocyte I, metaphase I. Note 14 autosomal bivalents and two sex chromosome univalents (14II+X₁X₂). O: Metaphase II, n = 14. P: Metaphase II, n = 16 (14+X₁X₂). Sex chromosomes are positively heteropycnotic. Arrowhead = bivalent with two chiasmata. Scale bars = 5 μm.

two sex chromosome univalents (X_1X_2) in pachytene, diplotene, and metaphase I (Fig. 4E–F), with one chiasma per bivalent, except for two or three bivalents with two chiasmata in late prophase I (Fig. 4F). We found two types of metaphases II, namely with $n = 13$ and $n = 15$ chromosomes ($13+X_1X_2$) (Fig. 4G–H). The sex chromosomes were positively heteropyknotic in pachytenes, diplotenes, and metaphases II. Based on these data, karyotype was determined as $2n \delta = 28$ ($26+X_1X_2$ 0).

Zodarioidea

Zodariidae

The oogonial metaphases of *Epicratinus* sp. were composed of 44 chromosomes (Fig. 4I). All chromosomes were telocentric, with the exception of the second largest pair, which was submetocentric and

corresponded to the X_1 chromosomes. These elements show the same morphology at male prophase I (Fig. 4I–J). Diplotene of spermatocytes I consisted of 20 autosomal bivalents and two sex chromosome univalents (X_1X_2). Bivalents exhibited one chiasma, except for two bivalents with two chiasmata. Subtelocentric X_1 is larger than X_2 (Fig. 4J). We found two types of metaphases II in spermatocytes II, one with $n = 20$ and another one with $n = 22$ chromosomes ($20+X_1X_2$) (Fig. 4K–L). Based on these data, karyotype was determined as $2n \delta = 42$ ($40+X_1X_2$ 0) and $2n \text{♀} = 44$ ($40+X_1X_1X_2X_2$).

DISCUSSION

Oonopidae (Synspermiata, Dysderoidea)

Members of *Neoxyphinus* Birabén, 1953, *Neotrops* Grismado & Ramírez, 2013, *Xestaspis* Simon, 1884

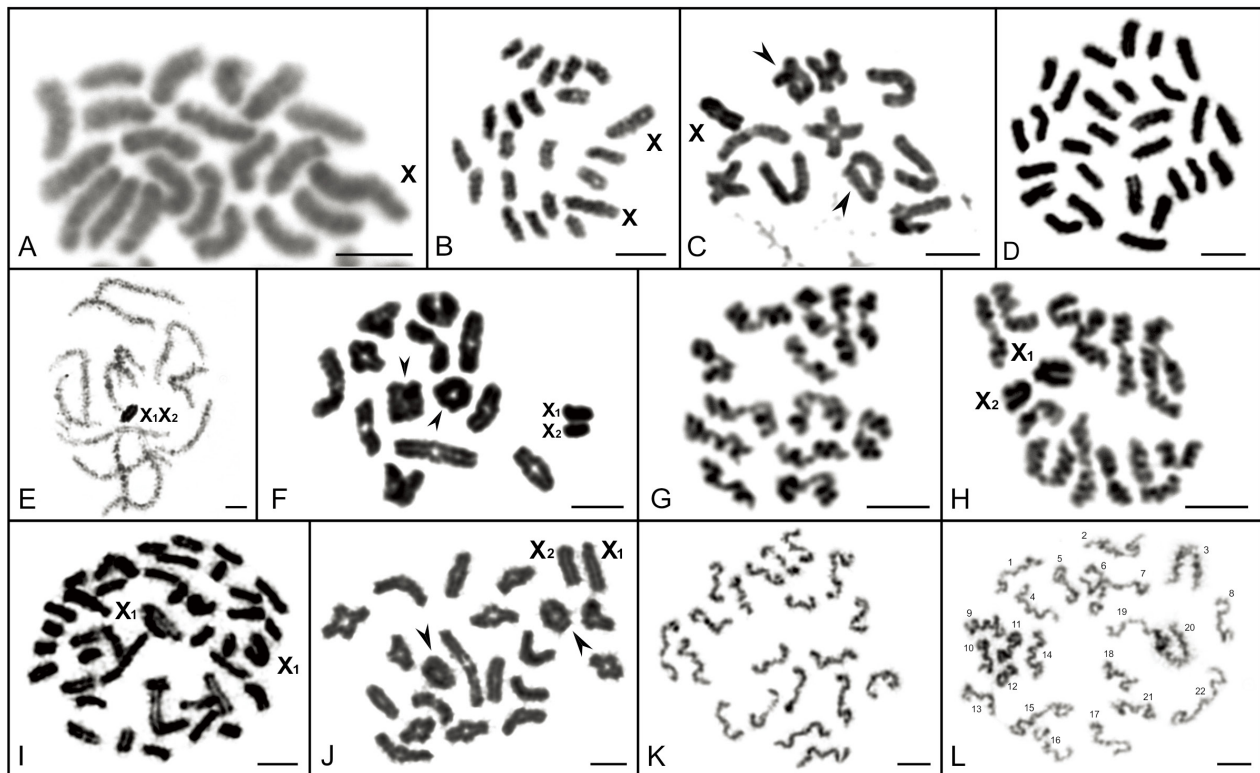


Fig. 4. Chromosomes of Corinnidae, Trachelidae, and Zodariidae (RTA Clade). *Falconina* sp. (Corinnidae) (A–E), *Orthobula* sp. (Trachelidae) (F–H) and *Epicratinus* sp. (Zodariidae) (I–L): A: Mitotic spermatogonial metaphase, $2n \delta = 28$ telocentric chromosomes. B: Pachytene, $13II+X_1X_2$. Sex chromosomes pair in parallel; they are positively heteropyknotic. C: Spermatocyte I, diplotene. Note 13 autosomal bivalents and two sex chromosome univalents ($13II+X_1X_2$). Sex chromosomes pair in parallel on the periphery of the plate. D: Metaphase II, $n = 13$ telocentric chromosomes. E: Metaphase II, $n = 15$ ($13+X_1X_2$), telocentric chromosomes. Sex chromosomes are associated and positively heteropyknotic. F: Mitotic spermatogonial metaphase, $2n \delta = 21$. G: Mitotic oogonial metaphase, $2n \text{♀} = 22$ telocentric chromosomes. H: Spermatocyte I, diplotene consisting of 10 autosomal bivalents and a sex chromosome univalent ($10II+X$). Sex chromosome is positively heteropyknotic. I: Mitotic oogonial metaphase, $2n \text{♀} = 44$ telocentric chromosomes, except for the submetocentric X_1 . J: Spermatocyte I, diplotene composed of 20 autosomal bivalents and two sex chromosome univalents ($20II+X_1X_2$). Sex chromosomes are associated in parallel. K: Metaphase II, $n = 20$ telocentric chromosomes. L: Metaphase II, $n = 22$ ($20+X_1X_2$). Arrowhead = bivalent with two chiasmata. Scale bars = 5 μm .

(present study), as well as *Oonops* Templeton, 1835 and *Ischnothyreus* Simon, 1893 (Král et al. 2019), have $2n \hat{=} 7, X0$. These genera, with the exception of *Xestaspis*, had already been included in the phylogeny of De Busschere et al. (2014). Formula $2n \hat{=} 7, X0$ is considered ancestral for Dysderoidea and Oonopidae by Král et al. (2019). Our study supports this hypothesis for oonopids. This pattern was found in two of the three genera analyzed in the present work. A derived karyotype was found in *Cinetomorpha simplex* ($2n \hat{=} 9, X0$). This genus was not included in the phylogeny of De Busschere et al. (2014). The karyotype of this spider contains an additional autosomal pair in comparison to previously analyzed oonopids.

Palpimanidae

To date, there are no chromosomal studies on the Palpimanoidea clade (Araujo et al. 2022). According to Wood et al. (2012 2013) and Dimitrov et al. (2017), Palpimanoidea is a sister group of Entelegynae. The relatively high chromosome number found in *O. birabeni* (Palpimanidae) ($2n \hat{=} 36$) is consistent with the relatively high diploid number ($2n \hat{=} 42$) of the ancestral Entelegynae karyotype proposed by Král et al. (2006).

Linyphiidae, Theridiidae and Theridiosomatidae (Entelegynae, Araneoidea)

Agyneta sp. ($2n \hat{=} 24, X_1X_20$, telocentric) is the first Neotropical member of Linyphiidae to be karyotyped. However, it shows the same pattern as most karyotyped species of the family (10 of 15 species karyotyped) (Araujo et al. 2022), including the sister genus *Helophora* Menge, 1866. Species with different karyotype compositions (Araujo et al. 2022) have been found in genera which are not closely related to *Agyneta* (Arnedo et al. 2009; Wang et al. 2015).

The karyotype found in the three species of Theridiidae analyzed (*C. floridanum*, *Thymoites* sp.1, *Thymoites* sp.2, subfamily Theridiinae) ($2n \hat{=} 22, X_1X_20$, telocentric chromosomes) is the most common for the family (23 of 32 karyotyped species) (Araujo et al. 2022), and it is also found in *Theridion* Walckenaer, 1805, a notoriously problematic and non-monophyletic genus that includes species phylogenetically close to those studied here (Agnarsson 2004; Liu et al. 2016).

The Theridiosomatidae family (Entelegynae, Araneoidea) was also chromosomally analyzed for the first time. Its position within the superfamily Araneoidea is controversial (Griswold et al. 1998; Dimitrov et al. 2017; Wheeler et al. 2017; Fernandez et al. 2018). Chromosomal data has been published only for the two

families proposed as sister groups, namely Synotaxidae (1 species, $2n \hat{=} 24, XY$) and Araneidae, whose male diploid numbers range from 13 to 50, with $2n \hat{=} 24$ being more frequent (52 species of 66 karyotyped). The diploid number found in *Naatlo* sp. ($2n \hat{=} 30, X_1X_20$) was not found in the last two families, as well as in any Araneoidea so far (Araujo et al. 2022).

Another peculiarity of *Naatlo* sp. is the exclusively or almost exclusively biarmed karyotype. Among Araneoidea species that have been karyotyped, only seven species of Araneidae show the same pattern. In some cases, this change in morphology was accompanied by a considerable reduction in the diploid number ($2n \hat{=} 14$) (Hackman 1948; Suzuki 1951; Amalin et al. 1993), whereas in others, the diploid number $2n \hat{=} 24$ has been retained (Carandang and Barrion 1994) or slightly increased to $2n \hat{=} 26$ (Prakash and Prakash 2014).

Trachelidae, Corinnidae, and Zodariidae (Entelegynae, RTA clade)

Concerning Trachelidae (Clade RTA, Dionycha part A), *Orthobula* sp. ($2n \hat{=} 21, X0$) differs from *Afroseto plana* Lyle & Haddad, 2010 and *Trachelas japonicus* Bösenberg & Strand, 1906 ($2n \hat{=} 22, X_1X_20$) (Suzuki 1952; Šťáhlavský et al. 2020) by sex chromosome system. The number of autosomal pairs in these trachelids is reduced in comparison to *Trachelas* sp. ($2n \hat{=} 24, X_1X_20$) (Datta and Chatterjee 1983). Telocentric X of *Orthobula* possibly involves tandem fusion of X_1 and X_2 . The same type of rearrangement is possibly involved in the autosome number reduction from the $2n \hat{=} 24, X_1X_20$, found in *Trachelas* sp. (for review, see Araujo et al. 2012).

In Corinnidae (Clade RTA, Dionycha part B), the diploid number of the Neotropical genus *Falconina* sp. ($2n \hat{=} 28$) differs from members of *Castianeira* Keyserling, 1879 ($2n \hat{=} 26$) (Bole-Gowda 1958; Mittal 1966), which is a globally distributed genus (World Spider Catalog 2022).

Concerning Zodariidae, the karyotype $2n \hat{=} 42, X_1X_20$ has been found in two taxa, namely Neotropical *Epicratinus* sp. (Storeninae) and *Pax islamita* (Simon, 1873) (Zodariinae) (Král et al. 2011; Kumbıçak et al. 2014) from the Near and Middle East (Jocqué 1991; Henrard 2019). This karyotype is considered by Král et al. (2006) as ancestral for Entelegynae. Presence of this karyotype in two zodariid subfamilies occurring in distinct geographical areas suggests that this pattern is also an ancestral karyotype for the family. Karyotype evolution of some zodariid clades was accompanied by the reduction of diploid numbers as found in the genera *Mallinella* Strand, 1906 ($2n \hat{=} 22$) (Datta and

Chatterjee 1983 1989) and *Zodariion* Walckenaer, 1826 ($2n \hat{=} 21, 24, 25, 26$ and 29) (Pekár and Král 2001; Pekár et al. 2005a b). *Epicratinus* sp. differentiated from an ancestral pattern found in *Pax islamita* by the subtelo-centric morphology of the X_1 chromosome, possibly originated by pericentric inversion.

CONCLUSIONS

The main findings of the study are as follows: 1) the confirmation of an ancestral karyotype for Zodariidae and Oonopidae; 2) the relatively high chromosome number in Palpimanidae which supports an hypothesis on relatively high diploid number in ancestral Entelegynae; 3) the peculiar karyotype in theridiosomatid representative.

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