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CHROMOSOMES OF *CROSSOPRIZA LYONI* (BLACKWALL 1867), INTRAINDIVIDUAL NUMERICAL CHROMOSOME VARIATION IN *PHYSOCYCLUS GLOBOSUS* (TACZANOWSKI 1874), AND THE DISTRIBUTION PATTERN OF NORs (ARANEOMORPHAE, HAPLOGYNAE, PHOLCIDAE)

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ABSTRACT. Pholcidae (Haplogynae) encompasses 967 described species, of which only 14 have been cytogenetic analyzed. Several chromosomal features have already been described including presence of meta- and sub-metacentric chromosomes and sex determination chromosome system (SDCS) of the X, X_1X_2Y, and X_1X_2 types, which contrast with the telo- and acrocentric chromosomes and SDCS of the X_1X_2 type typical of entelegyne spiders. To obtain further cytogenetic information for the family, we examined two pholcid species, *Crossopriza lyoni* (Blackwall 1867) and *Physocyclus globosus* (Taczanowski 1874) using both conventional staining and silver staining techniques. *Crossopriza lyoni* exhibited 2n = 23 = 22 + X in males and 2n = 24 = 22 + XX in females, while *P. globosus* showed 2n = 15 = 14 + X and 4n = 30 = 28 + 2X, both in male adults, 2n = 16 = 14 + XX in female adults and embryos, and 2n = 15 = 14 + X in male embryos. Both species revealed predominately metacentric and submetacentric chromosomes and a SDCS of the X/XX type. The cytogenetic data obtained in this work and those already recorded for *C. lyoni* indicate interpopulational and intraspecific numerical chromosome variation, suggesting the presence of chromosomal races or cytotypes in this species. The intraindividual numerical chromosome variation observed in male adult specimens of *P. globosus* may be explained by the presence of cytoplasmatic bridges between germ cells. The use of the silver staining technique to reveal the nucleolar organizer region (NOR) showed that chromosome pairs 4 and 6 and the X chromosome in *C. lyoni* are telomeric NOR-bearers, and that the chromosome pair 2 in *P. globosus* possesses a proximal NOR in the long arm.

Keywords: Chiasma, chromosome rearrangements, meiosis, syncytial, tetraploidy

The family Pholcidae currently has 81 genera and 967 described species (Platnick 2007) and is included in the Haplogynae (Coddington & Levi 1991; Ramírez 2000), which is considered less morphologically derived than Entelegynae. Chromosome analyses within Pholcidae were initiated by Painter (1914) in *Spermophora senoculata* (Dugès 1836) (as *Spermophora meridionalis* Hentz 1837). Due to the inefficient cytogenetic techniques of the time, Painter noted only that the chromosome complement consisted of small metacentric chromosomes and that the sex determination chromosome system (SDCS) was of the X_1X_2 type. Since then, considering the number of named species, little cytogenetic information on Pholcidae has been added to the literature. Cytogenetic data currently exist for 14 pholcid species (Table 1), which represent less than 2% of the total known. The Indian *Crossopriza lyoni* (Blackwall 1867) has provided the greatest amount of chromosomal data that encompassed several populations. Bole-Gowda (1958) described the presence of 2n = 27 = 13II + X with metacentric autosomes and sex chromosomes; Sharma et al. (1959) recorded 2n = 24 = 11II + X_1X_2 with metacentric autosomes and acrocentric sex chromosomes; Srivastava & Shukla (1986) observed 2n = 25 = 12II + X but did not provide any information regarding chromosome morphology, and finally Parida & Sharma (1987) and Shar-

Table 1.—Cytogenetic characterized Pholcidae species with their respective diploid numbers (2n), chromosome morphology (CM) and biogeographical region of origin (BR). A = acrocentric; M = metacentric; SM = submetacentric; T = telocentric. * as *Artema atlanta*; ** as *Pholcus affinis* (Schenkel 1913); *** as *Spermophora meridionalis* Hentz 1837.

Species	2n/male	CM	BR	Authors
*Artema atlanta** Walckenaer 1837	32 = 15II + X_1X_2	——	Oriental	Parida & Sharma (1987); Sharma & Parida (1987)
Crossopriza lyoni (Blackwall 1867)	27 = 13II + X	26M + XM	Oriental	Bole-Gowda (1958)
C. lyoni	24 = 11II + X_1X_2	22M + X_1X_2A	Oriental	Sharma et al. (1959)
C. lyoni	25 = 12II + X	——	Oriental	Srivastava & Shukla (1986)
C. lyoni	23 = 11II + X	——	Oriental	Parida & Sharma (1987); Sharma & Parida (1987)
C. lyoni	23 = 11II + X	22M + XM	Neotropical	This work
Holocnemus caudatus (Dufour 1820)	23 = 11II + X	16M + 6SM + XM	Palearctic	Král et al. (2006)
Mesabolivar luteus (Keyserling 1891)	15 = 7II + X	14M + XM	Neotropical	Araujo et al. (2005b)
Micropholcus fauroti (Simon 1887)	17 = 8II + X	17M or SM	Neotropical	Araujo et al. (2005b)
*Pholcus manueli*** Gertsch 1937	25 = 12II + X	A-T + XSM	Palearctic	Xiuzhen et al. (1997)
Pholcus crypticolens Bösenberg & Strand 1906	24 = 11II + X_1X_2	22M + X_1X_2A	Palearctic	Suzuki (1954)
Pholcus phalangioides (Fuesslin 1775)	24 = 11II + X_1X_2	——	Neotropical	Rodríguez-Gil et al. (2002)
P. phalangioides	25 = 11II + X_1X_2Y	18M + 4SM + X_1M + X_2SM + YM	Palearctic	Král et al. (2006)
Pholcus sp.	26 = 12II + X_1X_2	——	Oriental	Sharma & Parida (1987)
Physocyclus californicus Chamberlin & Gertsch 1929	15 = 7II + X	14M + XM	Nearctic	Cokendolpher (1989)
Physocyclus enaulus Crosby 1926	15 = 7II + X	14M + XM	Nearctic	Cokendolpher (1989)
Physocyclus globosus (Taczanowski 1874)	15 = 7II + X	6M + 8SM + XM	Neotropical	This work
Physocyclus sp.	15 = 7II + X	14M + XM	Nearctic	Cokendolpher (1989)
*Spermophora senoculata**** (Dugès 1836)	X_1X_2	X_1X_2M	Nearctic	Painter (1914)
S. senoculata	25 = 11II + X_1X_2Y	22M + X_1X_2YM	Palearctic	Král et al. (2006)

ma & Parida (1987) reported the presence of 2n = 23 = 11II + X, also with no description of chromosome morphology. In *Physocyclus* Simon 1893, all cytogenetic described species (*Physocyclus californicus* Chamberlin & Gertsch 1929, *Physocyclus enaulus* Crosby 1926, and *Physocyclus* sp.) occur in the Nearctic region (Cokendolpher 1989) and show a great karyotypic uniformity in relation to diploid number (2n = 15), metacentric chromosome morphology, and X/XX sex determination chromosome system type.

Recently, cytogenetic analyses were carried out in three pholcid species. *Pholcus phalangioides* (Fuesslin 1775) revealed 2n = 24 = 11II + X_1X_2 in males with metacentric autosomes and acrocentric sex chromosomes (Rodríguez-Gil et al. 2002); *Mesabolivar luteus* (Keyserling 1891) showed 2n = 15 = 7II + X in males and 2n = 16 = 7II + XX in females with a metacentric chromosome morphology; in the male specimens of *Micropholcus fauroti* (Simon 1887), the diploid number was 2n = 17 = 8II + X, with the chromosomes being described as biarmed (Araujo et al. 2005b).

Existing karyotypic descriptions for pholcid species (Table 1) show that the diploid number varies from 2n = 15 to 2n = 32, that the predominant chromosome morphology is metacentric and that the most frequent SDCS is of the X/XX type. In addition to this type of SDCS, some species of this family presented $X_1X_2/X_1X_1X_2X_2$ and $X_1X_2Y/X_1X_1X_2X_2$ types in a decreasing succession of occurrence.

In the other 11 haplogyne families, specifically Diguetidae, Drymusidae, Dysderidae, Filistatidae, Leptonetidae, Ochyroceratidae, Plectreuridae, Scytodidae, Segestriidae, Sicariidae, and Tetrablemmidae, from which 28 species have been cytogenetically characterized (Hackman 1948; Suzuki 1954; Beçak & Beçak 1960; Diaz & Sáez 1966a, 1966b; Benavente & Wettstein 1980; Silva 1988; Tugmon et al. 1990; Silva et al. 2002; Král et al. 2006), the diploid number varies from 2n = 7 to 2n = 37, the predominant chromosome morphology is also metacentric, and the SDCS may be of the X (43%), X_1X_2 (26%), X_1X_2Y (24%) or XY (7%) types.

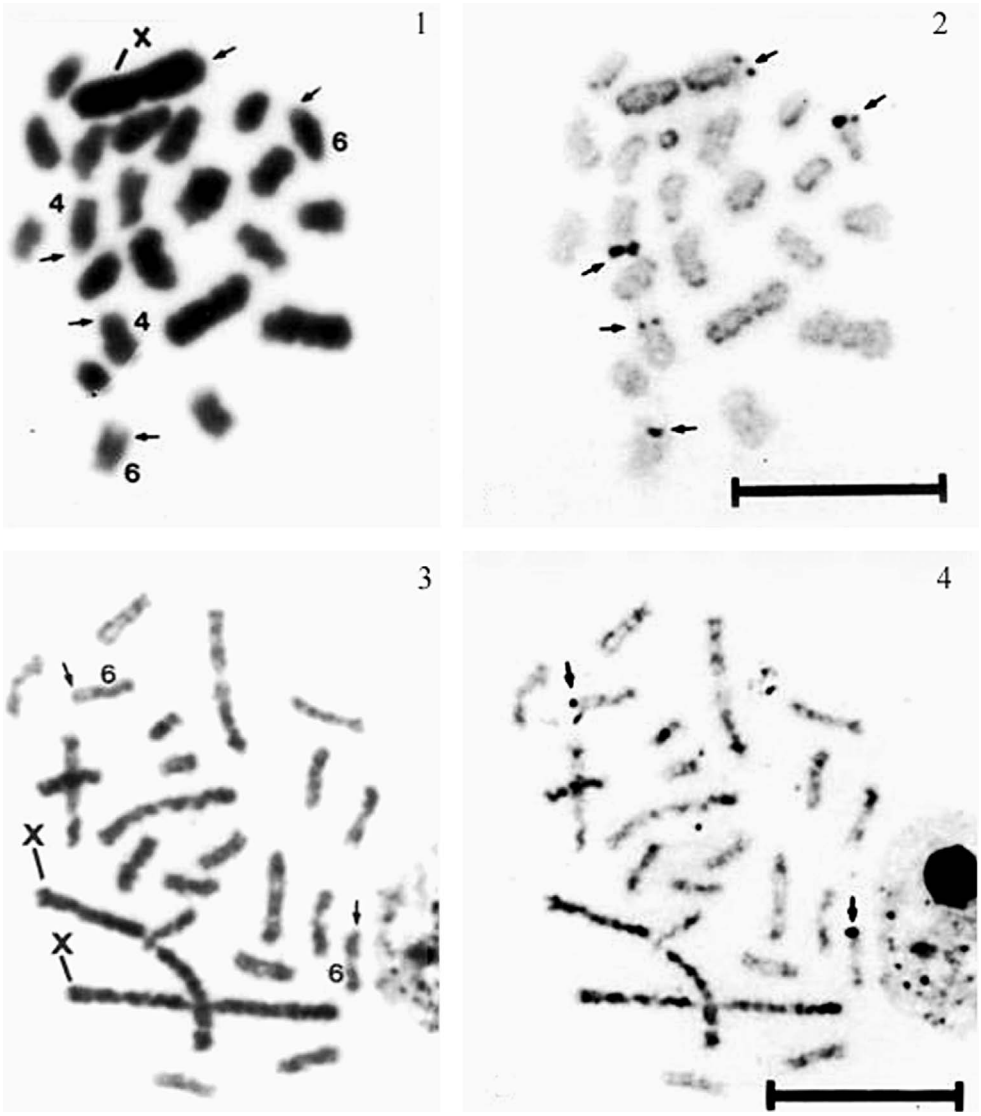
The majority of cytogenetically analyzed araneomorph species belong to Entelegynae. Approximately 500 species of entelegyne spiders, representing nearly 30 families, have a diploid number varying between 2n = 14 and 2n = 52, a predominantly acrocentric chromosome morphology, and a SDCS of the X_1X_2 type in most species. The few cytogenetically studied species of Pholcidae and other haplogyne families exhibit karyotypic peculiarities, such as predominantly metacentric chromosome morphology and X/XX sex determination chromosome system, that contrasts with those of Entelegynae. The cytogenetic analysis of other haplogyne species will probably provide additional information that can be useful in establishing some strategies of karyotype differentiation among the species of this group and also between Haplogynae and Entelegynae.

Considering the karyotypic peculiarities of Pholcidae, the present work aims to characterize the cytogenetics of two species of this family, *Crossopriza lyoni* and *Physocyclus globosus* (Taczanowski 1874). The diploid number, chromosome morphology, SDCS type, and behavior of chromosomes during meiosis were determined with conventional staining, and the distribution pattern of the active nucleolar organizer regions (NORs) in the chromosomes was established using silver staining.

METHODS

The chromosomal characterization in *C. lyoni* was performed through the analysis of 27 adult specimens (24 males and 3 females); in *P. globosus*, 10 adult specimens (7 males and 3 females) and 12 embryos were used. The individuals of both species were collected from natural populations in the city of Rio Claro (22°05′S, 47°30′W), State of São Paulo, Brazil. The adult specimens were deposited in the collection of the Butantan Institute, city of São Paulo, State of São Paulo, Brazil. All analyzed adult specimens were collected in August 2003 and *P. globosus* embryos were collected in January 2004.

The gonadal and embryonic chromosome preparations were obtained using the technique described by Webb et al. (1978), although a few were prepared from testicles not submerged in colchicine solution. Conventional staining was performed using a 3% Giemsa solution for 12 to 15 minutes. The NOR silver staining was carried out according to the



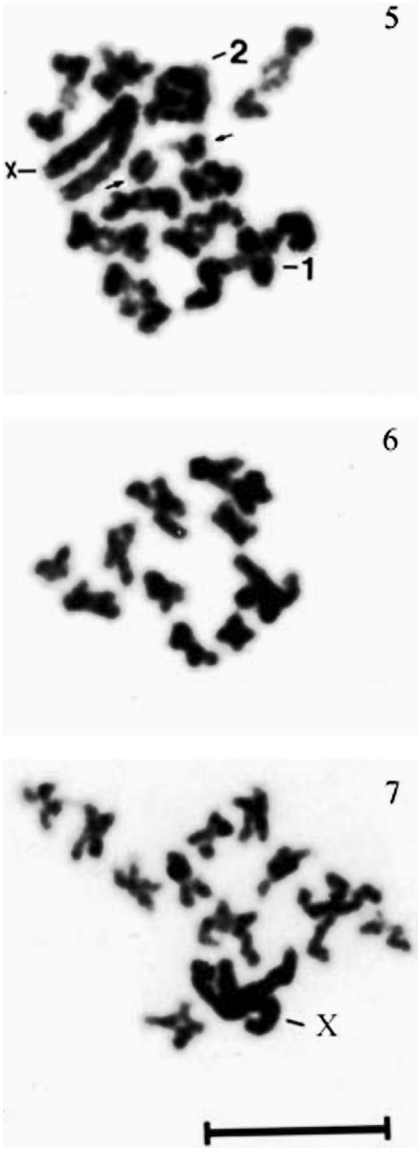
Figures 1–4.—Gonadal mitotic metaphases from adult *Crossopriza lyoni* individuals. 1, 3. Conventionally stained, male with 2n = 23 and female with 2n = 24. 2, 4. Same cells seen in 1 and 3, respectively, submitted to silver staining. Arrows indicate the NOR-bearing chromosomes. Scale bar = 10 μm.

methodology described by Howell & Black (1980).

RESULTS

Cytogenetics of *Crossopriza lyoni*.—Analysis of 356 gonadal cells of *C. lyoni* using conventional staining revealed 2n = 23 chromosomes in the spermatogonial metaphases (Fig. 1) and 2n = 24 in the oogonial metaphases (Fig. 3), a meiotic formula of 2n = 11II + X in the spermatocytes I (Fig. 5), and the occurrence of n = 11 or n = 12 = 11 + X with metacentric and submetacentric chromosomes in the metaphases II of males (Figs. 6, 7). These data showed the occurrence of the X/XX sex determining system in *C. lyoni*.

The conventionally stained mitotic metaphases of *C. lyoni* showed chromosomes with little morphological definition (Figs. 1, 3). In these cells, the chromosome elements of pair 1 and the X sex chromosome were always easily identified as being the largest elements



Figures 5–7.—Spermatocytes of adult *Crossopriza lyoni* specimens in conventional staining. 5. Meiocyte in prophase I, with 2n = 11II + X; note the cross or ring configuration of bivalents 1 and 2, evidencing the occurrence of one and two chiasmata, respectively; the arrows indicate the chromosomal elements of a bivalent with precocious separation. 6, 7. Metaphases II, with n = 11 and n = 12 = 11 + X, respectively, showing the presence of metacentric and submetacentric chromosomes. Scale bar = 10 μm.

of the complement; the other chromosomes represented a series of gradually decreasing size. Additionally, the X chromosome was positively heteropycnotic in most gonadal metaphases and spermatogonial anaphases.

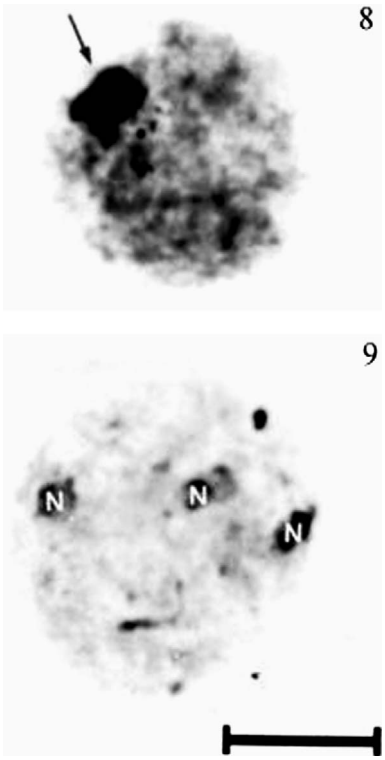
The silver-stained spermatogonial metaphases exhibited 5 telomeric NORs occupying the short arm of the chromosome elements of pair 4, the long arm of the chromosome elements of pair 6, and one of the arms of the metacentric X chromosome (Fig. 2). The oogonial metaphases showed only two telomeric NORs in the long arm of the pair 6 chromosomes (Fig. 4), reflecting an intersexual heterogeneity in the activity of these regions.

In the diplotene and diakinesis of the male *C. lyoni* specimens, the autosomal bivalents showed a regular meiotic behavior of pairing and staining, and the X chromosome appeared as an extremely large and isopycnotic univalent (Fig. 5). In these cells, most of the bivalents possessed an interstitial chiasma with a cross configuration, with the exception of bivalents 1 and 2 that showed two chiasmata, assuming a ring configuration. In some of these cells, the smallest bivalent showed a precocious separation, but exhibited a regular segregation in the subsequent meiotic phases (Fig. 5).

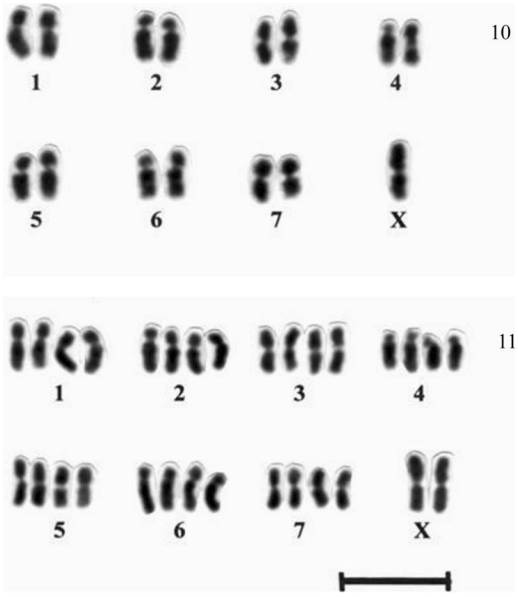
The metaphases II of male *C. lyoni*, with n = 11 or n = 12 = 11 + X, confirmed the regular reductional segregation of all the chromosomes in the preceding anaphase I and the meta- and submetacentric morphology of the chromosomes (Figs. 6, 7). In the metaphases II with n = 12, the X chromosome was identified through its large size and positive heteropycnosis.

Silver-stained spermatocytes I and II did not show NOR markings on the chromosomes. However, early prophasic nuclei I from male specimens exhibited a strongly stained nucleolus.

Conventionally stained interphasic nuclei of males and females showed one and two positive heteropycnotic chromatinic blocks, respectively, which probably corresponded to a sexual chromatin (Fig. 8). The silver staining of the interphasic nuclei of males resulted in the marking of three nucleoli (Fig. 9), corroborating the results obtained regarding the number of Ag-NOR-bearing chromosomes in the spermatogonial metaphases, i.e., pairs 4 and 6 and the X chromosome. In the inter-



Figures 8, 9.—Interphasic nuclei of male *Crossopriza lyoni*. 8. Conventionally stained, showing a conspicuous heteropycnotic-positive chromatinic block (arrow). 9. Silver-stained, evidencing three nucleoli (N). Scale bar = 10 μm.



Figures 10, 11.—Male *Physocyclus globosus* karyotypes submitted to conventional staining. 10. 2n = 15 chromosomes. 11. 4n = 30 chromosomes. Scale bar = 10 μm.

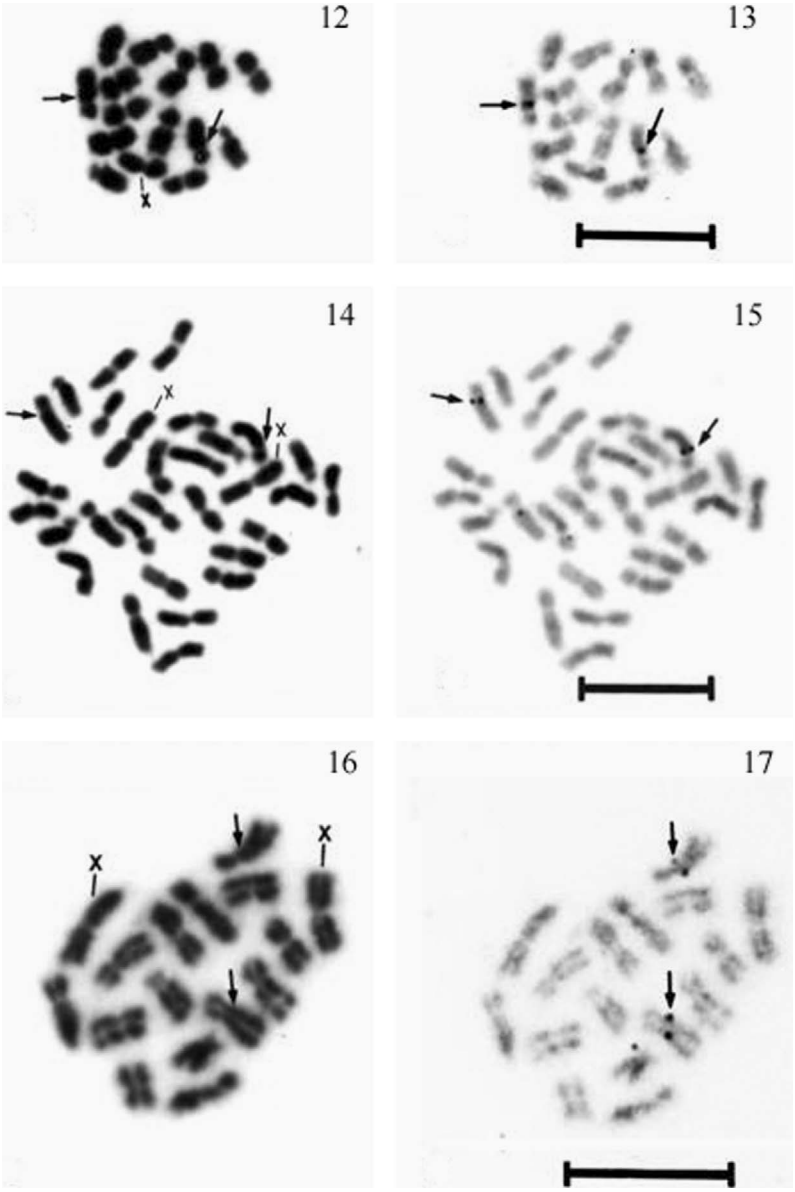
phasic nuclei of female specimens, only one strongly stained nucleolus was observed, confirming the number of active NORs verified in the oogonial metaphases.

Cytogenetics of *Physocyclus globosus*.—The testicular cells of the 7 analyzed adult specimens of *P. globosus* showed intraindividual variation in the number of chromosomes, i.e., of the 208 metaphases obtained from these individuals, 125 exhibited 15 = 14 + X chromosomes (Figs. 10, 12), and 83 showed 30 = 28 + 2X chromosomes (Figs. 11, 14). Of approximately 30 spermatogonial metaphases obtained from each individual, about 60% of cells showed 15 chromosomes and about 40% possessed 30 chromosomes. The analysis of 136 oogonial and embryonic metaphases showed the occurrence of 16 = 14 + XX chromosomes (Fig. 16) in 9 females (3 adults and 6 embryos) and 15 = 14 + X chromosomes in 6 male embryos.

The spermatocytes of the adult specimens of *P. globosus* also exhibited intraindividual variation in the number of chromosomes. Spermatocytes I showed the meiotic formula 7II + X (Fig. 18) or 14II + 2X (Fig. 19) in prophase I and metaphase I; spermatocytes II exhibited 7 chromosomes (Fig. 20) or 8 = 7 + X chromosomes (Fig. 21) in the metaphases II, indicating that these came from the spermatocytes I with 7II + X. Or they possessed 15 = 14 + X chromosomes (Fig. 22), indicating that they originated from spermatocytes I with 14II + 2X.

Considering the chromosome numbers obtained in the testicular, ovarian and embryonic cells of *P. globosus*, the diploid number and the chromosomal sex determination system were established: 2n = 15 = 14 + X = 7II + X in males and 2n = 16 = 14 + XX = 7II + XX in females. The testicular cells with 30 = 28 + 2X = 14II + 2X were interpreted as tetraploids, indicating an intraindividual numerical chromosome variation in the male specimens of adult *P. globosus*.

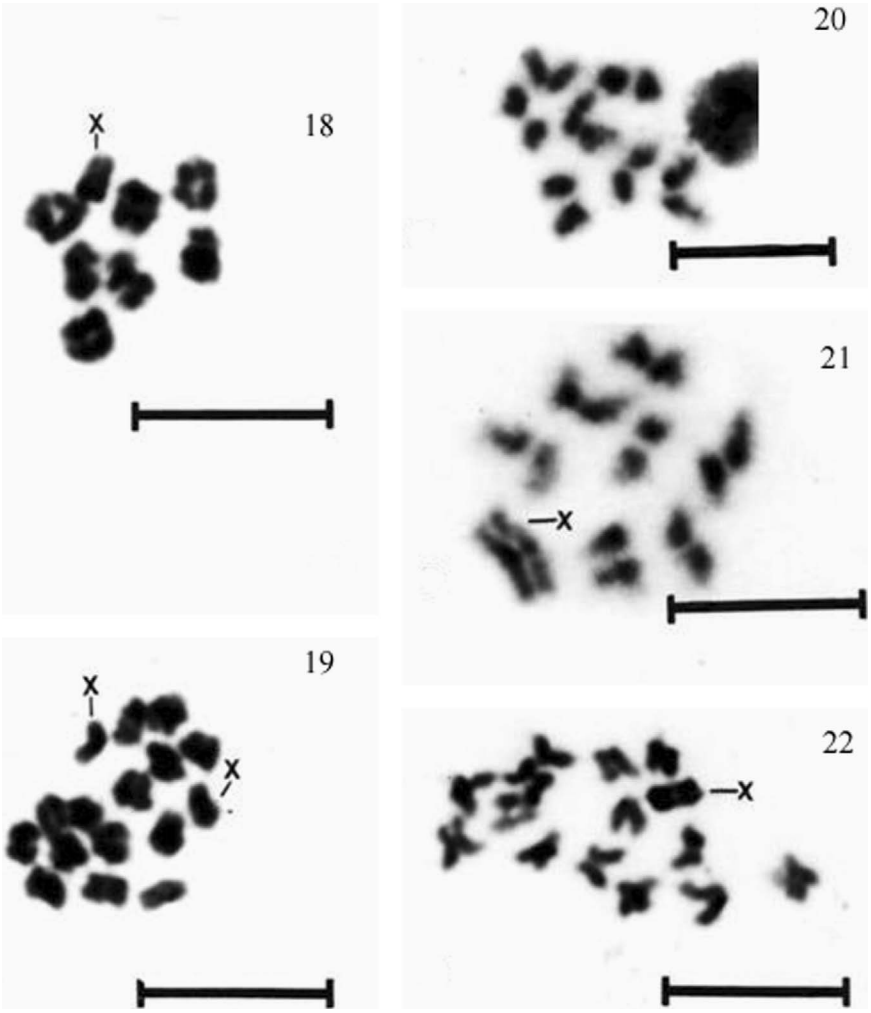
The gonadal and embryonic somatic metaphases revealed that pairs 1, 2, 4 and 6 of the *P. globosus* karyotype are submetacentric, and



Figures 12–17.—Gonadal mitotic metaphases of *Physocyclus globosus*. 12, 14, 16. Conventionally stained with 2n = 15 (male), 4n = 30 (male tetraploid cell), and 2n = 16 (female), respectively. 13, 15, 17. Same cells seen in 12, 14 and 16, respectively, stained with silver nitrate, showing the NORs in the chromosomes of pair 2 (arrows). Scale bar = 10 μm.

that pairs 3, 5 and 7 and the X chromosome are metacentric (Fig. 10). The autosomal pairs could be arranged in a series of gradually decreasing size and the X chromosome was a size intermediate between chromosome pairs 1 and 2.

Only mitotic metaphases of males and females were subjected to silver staining, which revealed a NOR in the proximal region of the long arm of the second pair of chromosomes (Figs. 13, 15, 17). The metaphases with 2n = 15 and those with 2n = 16 exhibited a maximum number of two NOR-bearing chromosomes, while the metaphases with 30 chromosomes presented a maximum number of four NOR-bearing chromosomes; in these



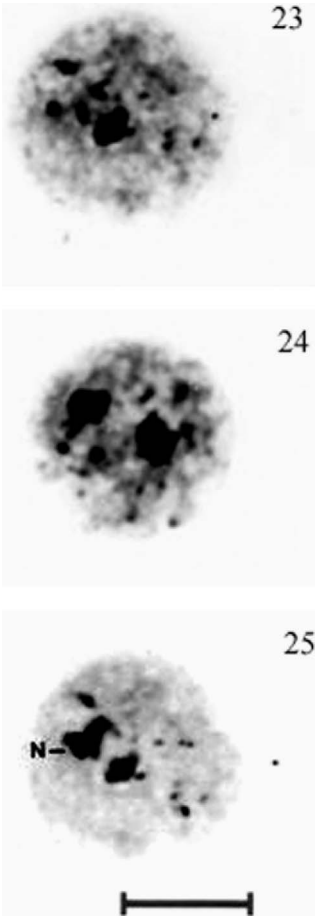
Figures 18–22.—Conventionally stained meiotic cells of male *Physocyclus globosus*. 18, 19. Diplotenes exhibiting 2n = 7II + X and 4n = 14II + 2X, respectively. 20, 21, 22. Metaphases II with 7 chromosomes, 8 = 7 + X chromosomes and 15 = 14 + X chromosomes, respectively. Scale bar = 10 μm.

metaphases, the NORs were always marked in the chromosomes of pair 2.

In the *P. globosus* testicular chromosome preparations, meiocytes with 15 or 30 chromosomes were found in all the phases of meiosis I. In the subphases prophase I and metaphases I, autosomal bivalents and the X univalent always occurred, even in the cells with 30 = 14II + 2X (Figs. 18, 19). In the diplotene cells, the occurrence of an interstitial chiasma was observed in three bivalents, in the cells with 15 = 7II + X, and in six bivalents, in the cells with 30 = 14II + 2X. Metaphases II showed chromosome numbers that confirmed the reductional segregation of the chromosomes during the preceding anaphase I, including the asynaptic X chromosomes present in the cells with 30 = 14II + 2X.

The silver-stained testicular meiocytes provided no information on NORs or nucleolar material, with the exception of pachytene cells that exhibited a single strongly stained block of nucleolar material.

Conventionally stained testicular and ovarian interphasic nuclei revealed the presence of one or two large heteropycnotic-positive blocks, respectively, which are probably related to the sex chromatin (Figs. 23, 24). The occurrence of two chromatinic blocks in a few



Figures 23–25.—Testicular interphasic nuclei of *Physocyclus globosus*. 23, 24. Conventionally stained, emphasizing one and two conspicuous heteropycnotic-positive chromatinic blocks, respectively. 25. Same nucleus seen in 23 submitted to silver staining, evidencing one nucleolus (N). Scale bar = 10 μm.

testicular interphasic nuclei (Fig. 24) suggested the presence of cells with two X chromosomes in males. Silver-stained interphasic nuclei exhibited a single marked nucleolus (Fig. 25).

DISCUSSION

The cytogenetical data obtained from *C. lyoni* and *P. globosus* in relation to the metacentric and submetacentric morphology of all the chromosomes of the complement and the presence of an X/XX sex determination system are similar to those described for related species belonging to the Nearctic, Neotropical, Oriental and Palearctic regions (Bole-Gowda 1958; Srivastava & Shukla 1986; Parida & Sharma 1987; Sharma & Parida 1987; Cokendolpher 1989; Araujo et al. 2005b; Král et al. 2006). However, the studied species showed some particularities regarding chromosome number when compared with related species described in the literature.

The *C. lyoni* specimens analyzed in this work showed a diploid number (2n = 23 = 22 + X) similar to the one found by Parida & Sharma (1987) and Sharma & Parida (1987) in specimens from the same species from two different Indian populations. Nevertheless, this diploid number differs from those described by Bole-Gowda (1958) - 2n = 27 = 26 + X, Sharma et al. (1959) - 2n = 24 = 22 + X_1X_2, and Srivastava & Shukla (1986) - 2n = 25 = 24 + X, for individuals belonging to other, more geographically distant Indian populations.

Chromosome rearrangements of the centric fission, followed or not by pericentric inversion, and/or centric fusion types are suggested in order to explain the interpopulational and intraspecific numerical variation found in *C. lyoni*. The presence of predominantly metacentric or submetacentric autosomes and of a SDCS of the X_1X_2 type, with acrocentric X chromosomes, or of the X type, with a metacentric X chromosome, corroborate such mechanisms of karyotypic differentiation.

Chromosomal variations of the diploid number have already been described for some species of entelegyne spiders, such as *Agelena limbata* Thorell 1897 (Agelenidae), *Delena cancerides* Walckenaer 1837 (Sparassidae) and *Evarcha hoyi* (Peckham & Peckham1883) [as *Pellenes hoyi* (Peckham & Peckham 1909)] (Salticidae). In each one of these species, karyotypes belonging to different populations were characterized as chromosomal races that appeared to have originated mainly by centric or tandem fusion, involving only autosomes or autosomes and sex chromosomes (Maddison 1982; Rowell 1985, 1990, 1991; Tsurusaki et al. 1993; Hancock & Rowell 1995). Likewise, the different karyotypes present in *C. lyoni* could represent chromosomal races or cytotypes.

There are three works that focus on the phylogeny of pholcid spiders (Huber 2000; Bruvo-Madaric et al. 2005; Astrin et al. 2006) and could be used to hypothesize the origin

of the *C. lyoni* cytotypes. However, the study of Astrin et al. (2006) did not include *Spermophora senoculata*, which Bruvo-Madaric et al. (2005) considered basal to all pholcines and part of Holocneminae. Due to its type of SDCS, this species was also considered to be the most basal of all pholcid species already analyzed from the cytogenetic point of view (Král et al. 2006). The phylogeny proposed by Huber (2000) was based on morphological characters and that of Bruvo-Madaric et al. (2005) was made using both morphological and molecular data.

Considering the phylogeny described by Bruvo-Mararic et al. (2005), *S. senoculata*, with 2n = 25 = 11II + X_1X_2Y (Král et al. 2006), is basal in relation to *C. lyoni*. The X_1X_2Y system of basal pholcid species could give rise to an X_1X_2 system, such as that registered for one *C. lyoni* Oriental population with 2n = 24 = 11II + X_1X_2 (Sharma et al. 1959), by gradual heterochromatinization and erosion of the Y sex chromosome. Taking into account this process of SDCS differentiation, the 2n = 24 = 11II + X_1X_2 could represent the basic karyotype of *C. lyoni*. The other diploid numbers obtained for this species could be derived from this basic number. The process of Y sex chromosome heterochromatinization and erosion have been detected in many groups of arthropods and considered a usual event involved in SDCS evolution (White 1973; Smith & Virkki 1978; Steinemanm & Steinemanm 1998). On the other hand, the possibility of conversion of an X_1X_2Y system into an X system, as postulated by Král et al. (2006) for some Haplogynae species, can not be excluded, especially if we consider the 2n = 23 = 11II + X of *Holocnemus caudatus* (Král et al. 2006), which represents a genus closely related to *Crossopriza* (Huber 2000; Bruvo-Madaric et al. 2005). If so, the *C. lyoni* populations with 2n = 23 = 11II + X could be ancestral.

The proposal that 2n = 24 originated the chromosomal races in *C. lyoni* suggests karyotypic evolution by raising or lowering the diploid number of chromosomes and an origin of the X_1X_2 sex determination chromosome system from the X_1X_2Y system. The proposal that 2n = 23 is the ancestral condition requires an increase in the chromosome number and origin of an X system from an X_1X_2Ysystem. These propositions differ from those elaborated by other researchers, such as Suzuki (1951, 1954), Postiglioni & Brum-Zorrila (1981), Maddison (1982), Rowell (1985, 1990) and Datta & Chatterjee (1988), in order to explain the karyotypic differentiation of most spider species.

Considering that the highest chromosome numbers occur in some less morphologically derived spider species (Mesothelae and Mygalomorphae) and that the acrocentric chromosome morphology and X_1X_2 sex determination chromosome system are the most frequent among Araneae, these researchers have suggested that the above-mentioned karyotypic characteristics would be ancestral to Araneae. Lower chromosome numbers and other types of chromosome morphology and sex determination systems, particularly of the X and $X_1X_2X_3Y$ types, would be derived mainly from centric fusions followed or not by pericentric inversions, or by tandem fusions. Alternatively, some of these researchers postulated that the existence of an X sex determination chromosome system as an ancestral condition can not be ruled out and X chromosome centric fission from species with a metacentric X chromosome could give rise to an X_1X_2 system.

Nevertheless, cladistic analyses have indicated that Mesothelae, Mygalomorphae and Araneomorphae (Haplogynae and Entelegynae) have undergone independent processes of morphological differentiation. Independent processes also seem to have promoted the diversification between haplogyne and entelegyne spiders (Platnick et al. 1991; Griswold et al. 1999; Ramírez 2000). These data raise the possibility of an independent karyotypic differentiation in the Mesothelae, Mygalomorphae, Haplogynae and Entelegynae spiders, i.e., the karyotypic differentiation of extant spiders may occur by a raise or lowering of the basic chromosome number.

Unfortunately, we do not have enough evidence to determine the process of karyotypic differentiation among the *C. lyoni* cytotypes. Cytogenetical analysis of other *Crossopriza* species will certainly provide additional information that will allow a more secure establishment of the characteristics of the basic karyotype of the species of this genus, as well as the mechanisms involved in the origin of the chromosomal races or the cytotypes of *C. lyoni*.

Considering that *C. lyoni* is a species with a wide geographic distribution, the karyotype diversity recorded for this species is not surprising and the occurrence of other cytotypes can not be excluded. Therefore, it is not possible to disregard the hypothesis that *C. lyoni* represents a species complex. Cytogenetic analyses may be useful in understanding the taxonomy of this species, that is, if distinct cytotypes are sympatric and there are not hybrid karyotypes. In a few animal groups whose species are very morphologically similar, cytogenetic studies coupled with morphological analyses have promoted the discovery of new species (Silva & Yonenaga-Yassuda 1998; Bertollo et al. 2000).

In the *P. globosus* sample analyzed, the number of chromosomes found in females, 2n = 16 = 14 + XX, and male embryos, 2n = 15 = 14 + X, is coincident with those described by Cokendolpher (1989) for other *Physocyclus* species, namely *P. californicus*, *P. enaulus* and *Physocyclus* sp. However, an intraindividual variation in the number of chromosomes, i.e., 2n = 15 = 14 + X and 4n = 30 = 28 + 2X, was observed in the testicular cells of the adult specimens of *P. globosus*.

The presence of tetraploid cells in the male germ line of *P. globosus* is probably related to the occurrence of cytoplasmatic bridges between cells of the same cyst. These bridges form a syncytium and promote synchronization in cell division and cell differentiation (Alberti & Weinmann 1985; Alberts et al. 2002; Michalik et al. 2003). Due to some peculiarities of these cytoplasmatic bridges, cell couples from a single cyst remained connected during chromosome preparation, leading to the formation of cells that were apparently tetraploid, and of interphasic nuclei with two sexual chromatinic blocks. In fact, the chromosomes of the resulting tetraploid cells exhibited the same degree of condensation and meiotic behavior. In the pholcid *Mesabolivar luteus*, some diplotene cells appeared in pairs, and the authors also suggested that the organization of the testicular cells was responsible for this apparent tetraploidy (Araujo et al. 2005b).

Cokendolpher & Brown (1985) also verified the presence of a few polyploid cells in *Physocyclus* sp., which was attributed to the cell treatment with a colchicine solution. In *P. globosus*, the numerical chromosome variation was certainly not due to the cell treatment with the colchicine solution, because this variation was also observed in preparations where the cells were not subjected to this solution.

The meiotic testicular cells of *C. lyoni* and *P. globosus* showed that the autosomal bivalents and the univalent X chromosome exhibited a regular behavior similar to those described by Suzuki (1954), Bole-Gowda (1958), Cokendolpher (1989), Tugmon et al. (1990), Gorlov et al. (1995), Gorlova et al. (1997), Shyh-Hwang (1999) and Rodríguez-Gil et al. (2002) for most Araneae in terms of condensation, synapsis, chiasma number and chromosome segregation.

The occurrence of the nucleolus or NORs associated with specific chromosomes has been described in some spiders based on ultrastructural analysis of bisected testicular cells using transmission electron microscopy (Benavente & Wettstein 1980; Wise 1983) or on analysis of silver impregnated gonadal and embryonic metaphases using light microscopy (Araujo et al. 2005a; Král et al. 2006). In these analyses, the nucleolar material was associated with the X chromosome in some haplogyne spiders, such as *Dysdera crocata* Koch 1838 (Dysderidae), with 2n = 11 = 10 + X (Benavente & Wettstein 1980), *Ochyrocera* sp. Simon 1891 (Ochyroceratidae), with 2n = 13 = 12 + X (Král et al. 2006), *Scytodes thoracica* (Latreille 1802) (Scytodidae), with 2n = 19 = 18 + X (Král et al. 2006), and *Monoblemma muchmorei* Shear 1978 (Tetrablemmidae), with 2n = 23 = 22 + X (Král et al. 2006), and with autosomes of some entelegyne species, such as two autosomal bivalents of *Allocosa georgicola* (Walckenaer 1837) (as *Lycosa georgicola*) (Lycosidae, Entelegynae), with 2n = 28 = 26 + X_1X_2 (Wise 1983), and three autosomal pairs of *Nephilengys cruentata* (Fabricius 1775) (Nephilidae), with 2n = 22 + $X_1X_1X_2X_2$ (Araujo et al. 2005a).

In *C. lyoni*, five NORs were found occupying the telomeric regions of the pair 4, pair 6 and X chromosomes, while in *P. globosus*, two NORs were found in the interstitial region of the pair 2 chromosomes. The presence of a NOR in the X chromosome of *C. lyoni*, *D. crocata*, *Ochyrocera* sp., *Scytodes thoracica*, and *Monoblemma muchmorei*, all bearing an X/XX sex determination system type, sug-

gests that the X chromosome can represent one of the elements that constitutes the basic NOR pattern in ''Higher Haplogynes''(*sensu* Coddington & Levi 1991). The absence of a NOR marking in the X chromosome of *P. globosus* is possibly due to chromosome rearrangements or differential activation of this region. Considering the low number of species whose chromosomes have been subjected to silver staining, it is not yet possible to determine a quantitative pattern of active NORs in spiders.

The interpopulational and intraindividual numerical variations respectively found in *C. lyoni* and *P. globosus* have different origins and meanings in the two species. In *C. lyoni*, the interpopulational variation shows that structural chromosome rearrangements are acting upon the karyotypic evolution of this species. On the other hand, the apparent tetraploidy of the spermatogonial metaphases in *P. globosus* is a result of the tissue organization of the spermatogonia. Thus, variation in *P. globosus* seems to have no relationship to chromosomal evolution of this species, but reflects a mechanisms which guarantees that great quantities of spermatozoids are simultaneously differentiated at the moment of reproduction.

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