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CYTOGENETICS OF THREE BRAZILIAN *GONIOSOMA* SPECIES: A NEW RECORD FOR DIPLOID NUMBER IN LANIATORES (OPILIONES, GONYLEPTIDAE, GONIOSOMATINAE)

Rosângela Martins Oliveira,[1,4] **Adilson Ariza Zacaro,**[2] **Pedro Gnaspini**[3] and **Doralice Maria Cella**[1]: [1]Universidade Estadual Paulista—UNESP, Instituto de Biociências, Departamento de Biologia, Caixa Postal 199; CEP 13506-900, Rio Claro, São Paulo State, Brazil; [2]Universidade Federal de Viçosa—UFV, CCBS, Departamento de Biologia Geral; Av. P.H. Rolfs, s/n; CEP 36571-000; Viçosa, Minas Gerais State, Brazil; [3]Universidade de São Paulo—USP, Instituto de Biociências, Departamento de Zoologia, Caixa Postal 11461; CEP 05422-970, São Paulo, São Paulo State, Brazil

ABSTRACT. Currently, 60 species of harvestmen have been karyotyped and all of these are from the Nearctic and Palearctic regions. This work is the first cytogenetic report of three gonyleptid species of the suborder Laniatores: *Goniosoma* aff. *badium*, *G. proximum* and *G. spelaeum* of the Neotropical region, from the southeastern region of Brazil. Conventional Giemsa stain chromosome preparations were obtained from embryonic cells and adult male testes. Embryo mitotic plates of *G.* aff. *badium* and *G. proximum* indicated 88 chromosomes, and mitotic spermatogonial plates of *G. spelaeum* males revealed intra- and interindividual variation of chromosome number, ranging from 92–109 chromosomes. In the three analyzed species, the mitotic chromosomes were meta- or submetacentric with no obvious sex chromosomes being identified during mitosis. Prophase I spermatocytes of *G. spelaeum* also revealed intra- and interindividual bivalent number variation and furthermore indicated the presence of multivalence. The karyotypes of these three *Goniosoma* species exhibited the largest chromosome pair with a negative heteropycnosis in the distal region of the shortest arm; chromosomes of *G. spelaeum* submitted to silver impregnation evidenced this negative heteropycnotic region as nucleolus organizer region (NOR). These results, when compared with cytogenetic data of other Laniatores species from the Palearctic region, indicated that a new record for diploid chromosome number probably characterize the genus *Goniosoma* in the Neotropical region.

Keywords: Chiasma, chromosome chain, karyotype, Palpatores, meiosis

Harvestmen are cosmopolitan in distribution and are grouped into three suborders: Cyphophthalmi, with about 50 sparsely distributed species; Palpatores, with about 2000 species concentrated in the Holarctic region; and Laniatores, with about 3500 mainly Neotropical species (Pinto-da-Rocha 1999).

Cytogenetic analysis of harvestmen is well documented for the suborder Palpatores with more than 50 species of Palpatores that have been karyotyped. However, the karyotype of members of the other suborders are poorly known with only two species of Laniatores and one of Cyphophthalmi that have been characterized. Overall in the Opiliones, diploid numbers vary from 10–78 chromosomes (Sokolow 1929, 1930; Suzuki 1941, 1966, 1976, 1980; Juberthie 1956; Parthasarathy & Goodnight 1958; Tsurusaki 1982, 1985, 1986a, 1990; Tsurusaki & Cokendolpher 1990; Cokendolpher & Jones 1991). The diploid chromosome numbers found so far in the suborder Laniatores ranged from 40 for *Pseudobiantes japonicus* Hirst 1911 (Epedanidae) to 78 for *Vonones sayi* (Simon 1879) (Cosmetidae). In contrast, Tsurusaki (1985) found the suborder Palpatores had diploid chromosome numbers varying from 10–36. The highest diploid chromosome number of 52 was observed in the manubriatum (under montanum) subgroup of the *Leiobunum curvipalpe* group,

[4] Corresponding author. E-mail: characid@yahoo.com.br

due to the tetraploidy from specimens having 2n = 24 (Tsurusaki 1985). In Cyphophthalmi, *Siro rubens* Latreille 1804 was found to have 2n = 30 chromosomes (Juberthie 1956).

The morphology of the chromosomes in the Opiliones has been described for some Palpatores species but for only one Laniatores species. In these species, the predominant chromosomes are meta—or submetacentric (Sokolow 1929, 1930; Suzuki 1941, 1966, 1976, 1980; Juberthie 1956; Parthasarathy & Goodnight 1958; Tsurusaki 1982, 1985, 1986a, 1990, 1993; Tsurusaki & Cokendolpher 1990; Cokendolpher & Jones 1991).

The sex chromosome system has been morphologically distinguished in only 13 species of Palpatores; most of them have an XY/XX system (Suzuki 1976, 1980; Tsurusaki 1982, 1985, 1986a, 1986b, 1989, 1990; Tsurusaki & Cokendolpher 1990), with one exception, *Mitopus morio* Fabricius 1799, which has a ZZ/ZW system (Tsurusaki & Cokendolpher 1990).

All previous karyotype descriptions of Opiliones were performed in species from Nearctic and Palearctic regions. Moreover, only a few non-systematic studies were focused on Neotropical harvestmen (Ramires & Giaretta 1994; Gnaspini 1995, 1996). This paper describes the first cytogenetic study of harvestmen belonging to suborder Laniatores (Gonyleptidae, Goniosomatinae) from the Neotropical region; the study includes embryonic metaphase analyses of *Goniosoma* aff. *badium* and *G. proximum* (Mello-Leitão 1933) and analyses of testicular mitotic and meiotic cells of *G. spelaeum* (Mello-Leitão 1933). The goal of this work was to determine the karyotypes of these three *Goniosoma* species and additionally, to describe the chromosome behavior of *G. spelaeum* during meiosis.

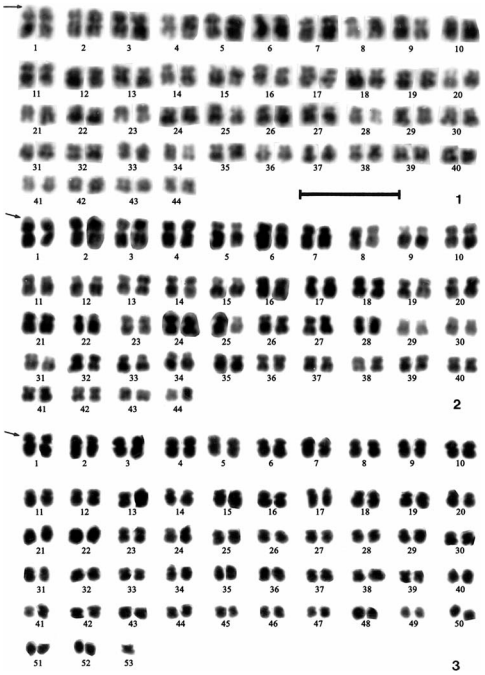
METHODS

Cytogenetic analyses were carried out on three *Goniosoma* species from southeastern Brazil: on six embryos of *G.* aff. *badium* from Barragem de Guaricana (25°43′S, 48°58′W), São José dos Pinhais, PR; on eight embryos of *G. proximum* from Gruta do Moquem, PETAR-Parque Estadual Turístico do Alto Ribeira (24°38′46″S, 48°42′2″W), Iporanga, SP; on six adult specimens (four males and two females) of *G. spelaeum* from Gruta do Tatu (24°16 05″S, 48°25′03″W), Ribeirão Grande, SP. All analyzed specimens were collected in November 1999.

To date, *G.* aff. *badium* has only been collected from the tunnels of the Guaricana Dam; *G. proximum* is widespread in the Atlantic Forest in the State of São Paulo, inhabiting small cave entrances and trees; *G. spelaeum* is a trogloxene species, living in the cave entrances in Vale do Ribeira, also in the State of São Paulo (Gnaspini 1995, 1996). These three species are nocturnal. Male and female vouchers specimens are deposited in the Museu de Zoologia da Universidade de São Paulo (MZSP), São Paulo, SP, Brazil.

The chromosome preparations followed the method described by Webb et al. (1978), with some modifications. Dissected embryos and gonads were placed in insect saline containing 0.05% colchicine to arrest the metaphase cells. The hypotonic treatment was completed by adding a volume of tap water equal to that of insect saline containing 0.05% colchicine and was left for 15 min. The material was then fixed in methanolic Carnoy for 1 hour. Each embryo and/or gonadal tissue was macerated in a drop of 60% acetic acid by tapping the material with the flat end of metal rod on the slide. The slides were dried on a warm plate (~45° C) for 2 min. The preparations were stained with 3% Giemsa (1.5 ml of Giemsa stock solution, 45 ml of distilled water, and 1.5 ml of pH 6.8 phosphate buffer). Some testicular preparations of *G. spelaeum* were submitted to silver impregnation (Howell & Black 1980; Kodama et al. 1980) to determine the chromosomes bearing the nucleolus organizer regions (NORs).

The egg samples of *G.* aff *badium* and *G. proximum* indicated asynchronous embryo development and only a few embryos were suitable for cytogenetic analysis. The first cytogenetic results of *G. spelaeum* revealed variable diploid chromosome numbers, which was initially interpreted as a consequence of technical preparation problems. Considering that the loss and overlapping of chromosomes could be a result of, respectively, long and short duration of hypotonic treatments, decreasing times of hypotonic treatment were tried and also resulted in diploid number variation. Finally, the squash technique was also tried to avoid putative overspreading and loss of chromosomes of the mitotic and meiotic plates. Due to the presence of high diploid



Figures 1–3.—Karyotypes of three *Goniosoma* species stained with Giemsa: 1. *Goniosoma* aff. *badium* embryo (2n = 88); 2. *Goniosoma proximum* embryo (2n = 88); 3. *Goniosoma spelaeum* adult male (2n = 105). Arrows indicate the negative heteropycnotic region of pair 1. Scale bar = 10 μm.

chromosome number and the small size of the chromosomes, the squash technique did not allow any better resolution of the mitotic plates and also indicated chromosome number variation. Female gonads of *G. spelaeum* were also analyzed for chromosome preparations, but no cellular division was found.

Due to the high number and small size of the chromosomes, some restrictions were established to accomplish the karyotype characterization of these three *Goniosoma* species. This included considering only well spread mitotic plates showing low numbers of overlapped chromosomes and those having a reasonable degree of chromosome condensation to allow centromere identification.

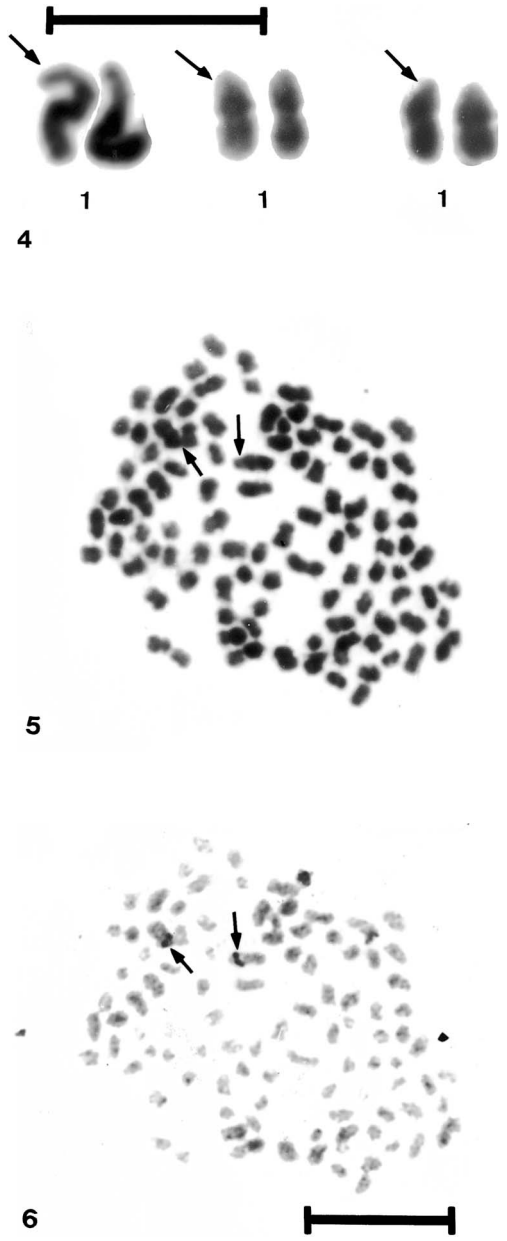
RESULTS

The majority of embryo metaphases of *G.* aff. *badium* and *G. proximum* revealed a karyotype of 2n = 88 chromosomes (Figs. 1, 2); in a few embryo metaphase cells, the diploid chromosome number varied in the same individual or different specimens, which was interpreted as accidental gaines or losses of chromosomes among cells during slide preparation. From a total of 76 spermatogonial metaphases preparations of *G. spelaeum*, only 26 were suitable for cytogenetic analysis. The selected *G. spelaeum* spermatogonial metaphases showed intra- and interindividual variation in relation to the diploid chromosome number that ranged from 92–109 chromosomes (Table 1, Fig. 3). Considering the high chromosome number variation, the diploid number for the *G. spelaeum* was not determined. The chromosomes of these three *Goniosoma* species exhibited mainly meta- or submetacentric morphology (Figs. 1–3). Chromosomes that were differentiated by size and/or morphological heterogeneity, which could indicate sex chromosomes, were not noted in the karyotypes of the *Goniosoma* species.

A notable karyotypic feature shared by these *Goniosoma* species was the presence of a negative heteropycnosis in the distal region of the short arm of the largest chromosome pair of the karyotype (Figs. 1–4). Other differentially stained chromosome regions were also noted in these karyotypes. However, these regions did not constitute common characteristics to the three analyzed *Goniosoma* species. The identification of all these differentially stained regions depended on the degree of chromosome condensation.

Table 1.—Number of chromosomes observed in diploid cells (2n) and first meiotic metaphases (n) of four males of *Goniosoma spelaeum*.

Male number	2n					n				
	# of preps	Range	mean	mode	median	# of preps	range	mean	mode	median
1	6	92–103	97.5	96	97.5	6	39–46	42	40	40.5
2	6	93–105	96.8	97	96	7	40–47	43.6	42	42
3	5	97–100	98.6	98	98	1	43–43	43	43	43
4	9	99–101	103.1	101	101	8	44–53	47	45	45.5



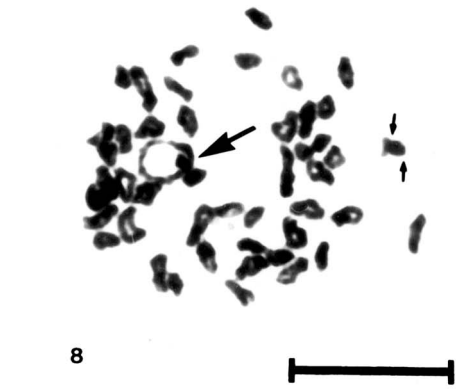
Figures 4–6.—Mitotic chromosomes of *Goniosoma spelaeum*: 4. Pairs 1 submitted to Giemsa staining showing different degrees of condensation and clearly visible negative heteropycnotic regions (arrows); 5. Spermatogonial metaphase (2n = 100) stained with Giemsa; 6. The same spermatogonial metaphase showed in Fig. 5 submitted to silver nitrate impregnation, exhibiting NORs (arrows) in the pair 1 chromosomes. Scale bar = 10 μm.

Silver impregnation of Giemsa stained mitotic metaphases of *G. spelaeum* evidenced active NOR in the distal region of the short arm of the pair 1 chromosomes (see arrow in Figs. 5, 6). The NOR labeling was coincident with the negative heteropycnotic region of pair 1.

Testicular preparations of *G. spelaeum* indicated cells in pachytene and diplotene stages. Pachytene cells of *G. spelaeum* showed at least 46 filamentous structures, regular in width, which were probably formed by separated or end-to-end associated bivalents (Fig. 7); nevertheless, the exact number of associated chromosomes was impossible to determine.

Diplotene spermatocytes of *G. spelaeum* also revealed variable bivalent numbers and additionally showed the occurrence of a typical chromosome chain. The diplotene cells exhibited a maximum of 53 bivalents plus a chain (Figs. 8, 9). From 100 studied diplotene cells, only 24 permitted a count of the bivalent number and clearly showed the chromosome chain. Table 1 shows the bivalent number variation in the analyzed diplotene cells. Early diplotene cells exhibited ring multivalence (Fig. 8) and late ones showed a linear multivalence (Fig. 9). Probably the latest multivalent configuration appeared due to the precocious chiasma terminalization (Fig. 9).

Although sufficient number of diplotene cells have been analyzed, the number of chromosomes in the multivalent was not determined because the chromosomes were highly condensed, and lacked morphological resolution. Additionally, the precocious chiasma terminalization events, involving the chromosomes in the multivalent and perhaps the bivalents, allied with the high degree of chromosome condensation, also compromised the establishment of the exact bivalent number. As a consequence, some diplotene chromosome elements could not be identified as univalent or bivalent. Moreover, the type sexual determination system was not established, considering that none of the meiotic chromosome elements showed any particular feature that could permit sex chromosome recognition. Giemsa stained interphasic nuclei of *G. spelaeum* indicated several positive heteropycnotic dots, which were variable in number and probably representative of heterochromatin. Therefore, neither large nor frequent het-



Figures 7–9.—*Goniosoma spelaeum* prophase I cells stained with Giemsa: 7. Pachytene cell with approximately 45 filamentous structures. The arrows indicate linear chromosome association; 8. Diplotene cell with approximately 45 bivalents plus a ring multivalent (large arrow). The small arrows indicate a bivalent with interstitial and terminal chiasmata; 9. Late diplotene cell with about 47 bivalents plus a linear multivalent (arrowhead). The medium size arrow indicates a bivalent with one interstitial chiasma. Scale bar = 10 μm.

eropycnotic blocks were noted in interphasic nuclei that could be interpreted as sex chromatin.

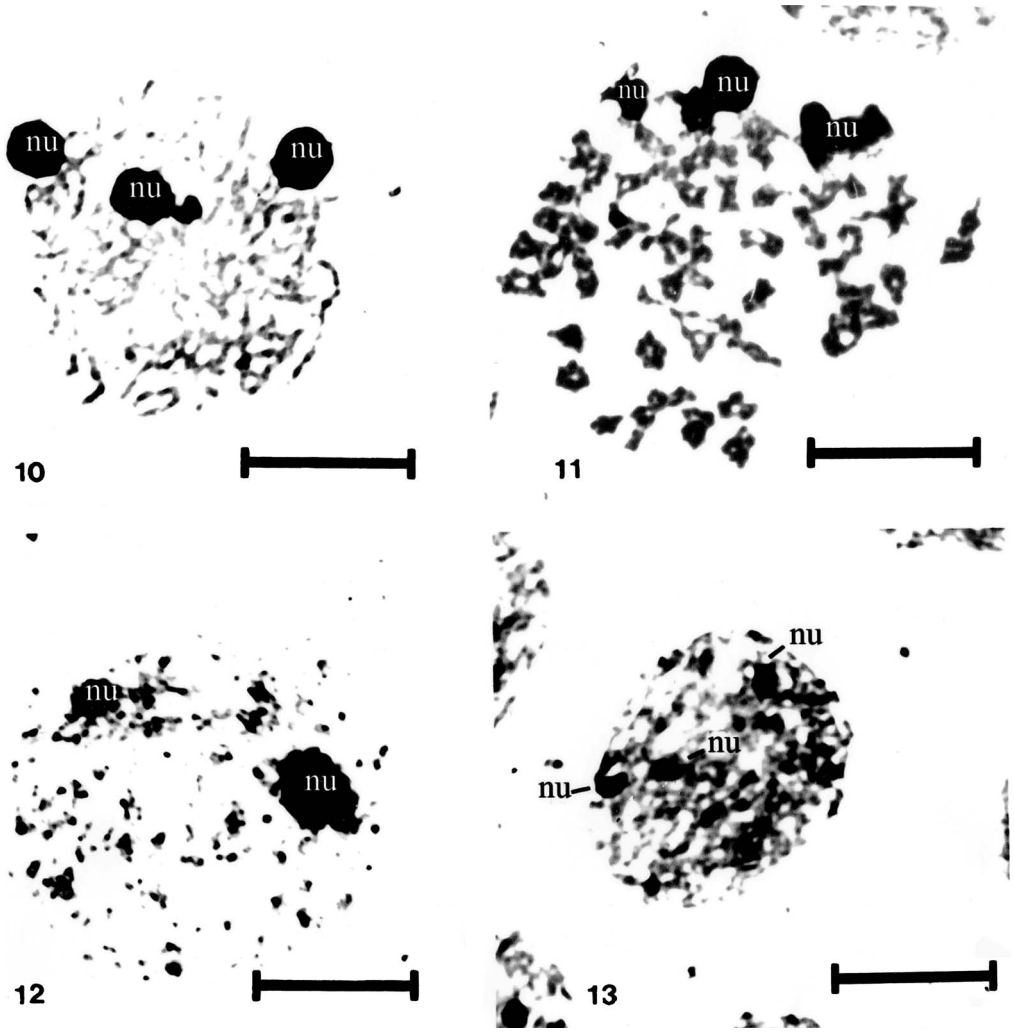
Early prophase I meiocytes and interphasic nuclei of *G. spelaeum* submitted to the silver impregnation revealed the presence of one to three blocks of nucleolar material deeply impregnated (Figs. 10–13), suggesting that there are at least one and a maximum of three active NORs. Late diplotene cells did not provide detectable labeling of nucleolar material or bivalent carrier of the NORs.

DISCUSSION

The karyological results of these three gonyleptid species are similar to *Vonones sayi* (Simon 1879) (Laniatores, Cosmetidae) in relation to the high number and morphology of the chromosomes and no obvious sex chromosomes. *V. sayi* has 2n = 78, with most of the chromosomes being metacentric or submetacentric (Cokendolpher & Jones 1991). However, the high chromosome numbers observed in these species that belong to the suborder Laniatores, contrast with those described for most of the species of Palpatores, whose diploid chromosome numbers vary from 10–36 (Tsurusaki 1986a; Tsurusaki & Cokendolpher 1990).

In comparison to the total number of species of Opiliones, only a small percentage have been karyotyped, and those are members of Laniatores. In considering the cytogenetic data described for Cyphophthalmi, Palpatores, and Laniatores species, the *Goniosoma* species karyotype differentiation seems to have occurred by an increase of the chromosome number. Probably chromosomal centric fissions followed by pericentric inversions were responsible for the chromosome number increase and meta- or submetacentric morphology maintenance of the *Goniosoma* species.

Using phylogenetic analyses based on mor-



Figures 10–13.—Prophase I and interphasic nuclei of *Goniosoma spelaeum* submitted to silver nitrate impregnation: 10,11. Early prophase I and diplotene nuclei, respectively, both exhibiting three blocks of nucleolar material (nu); 12,13. Interphasic nuclei, showing respectively, two and three blocks of nucleolar material (nu). Scale bar = 10 μm.

phological and molecular data from several species belonging to Cyphophthalmi, Palpatores, and Laniatores, Giribet et al. (1999) found that Gonyleptoidea (Laniatores) includes species with highly derived characteristics. This differs from other species of the Laniatores superfamily. These data corroborate the possibility of the *Goniosoma* species having a highly derived karyotype.

The diploid number variation noted in the *G. spelaeum* testes cells could be a consequence of differential chromosome non-disjunction, involving heterozygous chromosomes for translocation or supernumerary chromosomes, or random gaines or losses of chromosomes among cells during the procedures of chromosomal preparations.

The variation in intra-individual chromosome number is not common, but there are descriptions about its occurrence in some species that possess heterozygous specimens for structural chromosome rearrangements (Ohno et al. 1965; Beçak et al. 1966; Hartley & Horne 1984; Thode et al. 1985; Giles et al. 1985). This intra-individual variation seems to be a consequence of somatic chromosome

non-disjunction that could favor the reconstitution of homozygous chromosomes. The chromosome non-disjunction presumably functions as an ''accumulation mechanism'' and is particularly evident in some polymorphic species for supernumerary chromosomes (White 1973; Gorlov & Tsurusaki 2000a, b; Tsurusaki & Shimada 2004).

The presence of supernumerary chromosomes, which are extra chromosomes, in Opiliones is well documented in *Psathyropus tenuipes* Koch 1878 (formerly *Metagagrella tenuipes* Suzuki 1949) (Tsurusaki 1993; Gorlov & Tsurusaki 2000a, b; Tsurusaki & Shimada 2004). This species showed extensive variation in the number of chromosomes, ranging from 0–19, among cells of a single individual and among individuals of a single population, as well as among populations. They behave as univalents during meiosis, though some of them seem to form a chain with end-to-end associations in diakinesis (Gorlov & Tsurusaki 2000b).

The *G. spelaeum* multivalence was identified in all prophase I cells analyzed, but this feature does not constitute an accurate parameter to determine if these chromosomes are or are not supernumeraries. The description of multivalent-like associations between supernumerary chromosomes during meiosis are rare in the literature (Jones & Rees 1982; Gorlov & Tsurusaki 2000b).

The *G. spelaeum* meiotic multivalent configurations suggest the occurrence of heterozygous chromosomes for serial translocations. Although pachytene filaments with special configurations, such as loops or open crosses, are indicative of heterozygosity for structural chromosome rearrangements, these were not seen in the analyzed cells, probably due to the occurrence of heterosynapsis. The *G. spelaeum* multivalent configuration is similar to that found in *Delena cancerides* Walckenaer 1837 (Araneae), *Neotermes fulvescens* (Silvestri) 1901 (Isoptera) and *Keyacris scurra* (Rehn) (Orthoptera), which arose from heterozygous and serial interchanges, such as centric fusions and reciprocal translocations (Rowell 1985, 1991a, 1991b; Hancock & Rowell 1995; Martins & Mesa 1995; White 1973).

In Opiliones there is only one description about NORs in *Psathyropus tenuipes* (=*Metagagrella tenuipes*) (Gorlov & Tsurusaki 2000b). In this species, the NOR was associated with a single A-chromosome. Although this species has an XY-XX sex chromosome system (Tsurusaki 1993), whether this single NOR is on one of the sex chromosomes in unclear. In *G. spelaeum*, the NORs were associated with the largest pair, which could possibly be sex chromosomes. In Palpatores harvestmen species, whose sex determination system has already been established (Tsurusaki 1985, 1989, 1990, 1993; Tsurusaki & Cokendolpher 1990) the X and Z sex chromosomes displayed similar size to the largest chromosomes of the diploid complement.

Considering the range of nucleolar material observed at interphase and prophase I, it is likely that more than two NOR-bearing chromosomes might be present, since activation or inactivation mechanisms in these regions could be playing a role on the gene transcription.

This work characterized three species of Brazilian harvestmen *G.* aff. *badium* (2n = 88), *G. proximum* (2n = 88) and *G. spelaeum* (2n = 92–109). These species showed a high chromosome diploid number and several meta- or submetacentric chromosomes. The results suggests that chromosomal evolution in the genus *Goniosoma* occurred by an increase of chromosomal number through centric fissions, which were followed by pericentric inversions. *G. spelaeum* evidenced both intra- and interindividual variable diploid numbers which could be explained by the presence of translocated chromosomes in heterozygous. The presence of multivalents during meiosis in this species corroborates the hypothesis of occurrence of heterozygous chromosomes for serial translocations.

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