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Cytogenetic Studies in Diplopoda

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Summary The Diplopoda have received little attention from cytogeneticists owing mostly to technical difficulties in obtaining mitotic chromosomes, restricting the studies to meiosis and eventual spermatogonial metaphases, which limits the use of modern cytogenetical techniques. A literature search shows that only about 0.1% of all known species have been cytogenetically studied. There are 80,000 species estimated for this group, making it the 3rd. larger class in Arthropoda, after Insecta and Arachnida. The diploid chromosomal number in diplopods varies from $2n=8$ to $2n=30$ and the sex determination mechanism commonly found is XY/XX. In meiotic prophase, the "bouquet formation" and the diffuse state in pachytene are typical events. The few works performed on Brazilian fauna add up to 16 species, out of an estimated number of 2000 to 3000 species. The present review reports all the species of diplopods that have been cytogenetically studied so far, each with its chromosome number and sex determination system.

Key words Diplopoda, Cytogenetics, Review, Chromosomes, Millipedes.

The Diplopoda are a class belonging to the Myriapoda, also known as millipedes, referring to the large number of legs they possess; in Brazil they're commonly known as *embuás*, *gôngolos*, and more frequently as *piolhos-de-cobra*, which means snake lice.

Cytogenetics of Diplopoda, as well as myriapods in general, is poorly studied and consequently poorly understood. This is greatly due to the technical difficulties in obtaining mitotic chromosomes, restricting the studies to meiosis and eventual spermatogonial metaphases, which limits the use of modern cytogenetical techniques.

White (1979) made a revision of the group and stated that it was essential to study the different kinds of cytogenetical polymorphisms that exist in the myriapods, such as the kinds of chromosomal rearrangements that have occurred in their karyotype evolution and how the chromosomes behave during meiosis in individuals from natural environments and laboratory hybrids.

A literature revision showed that only about 0.1% of the known diplopod species have been cytogenetically studied. Golovatch *et al.* (1995) estimate that there must be 80000 species in this group, making it the 3rd. major class in Arthropoda, after Insecta and Arachnida.

Achar and Chowdaiah are the leading researchers interested in cytogenetical studies of diplopods, they focused on Asian species (Achar 1983a, b, 1984a, b, 1985, 1986, 1987, Achar and Chowdaiah 1979, 1980, Chowdaiah 1966a, b, c, 1967, 1969, Chowdaiah and Kanaka 1969, 1974, 1979).

Concerning Brazilian species, Fontanetti (1987, 1991, 1996a, b, c, 1998, 2000) has catalogued the chromosomal number and sex determination system of 16 species, which is a very low number when compared to the estimated 2000 to 3000 species of diplopods in Brazil.

The diploid chromosomal number in diplopods varies from $2n=8$ to $2n=30$. The former number was reported by Bessière (1948), and it has arisen many doubts in the scientific community because of the technical difficulties that existed at the time and the fact that it hasn't been corroborat-

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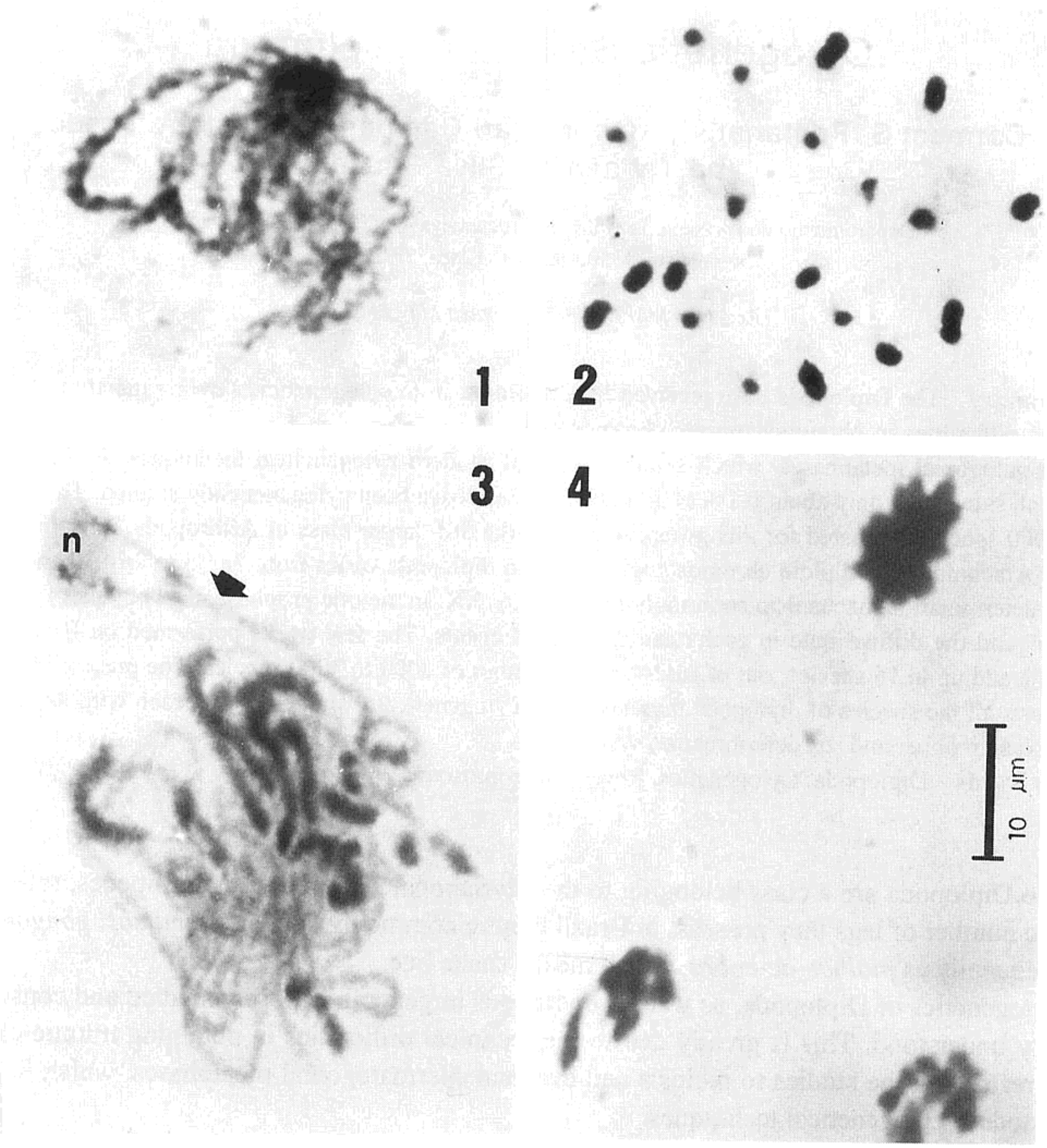
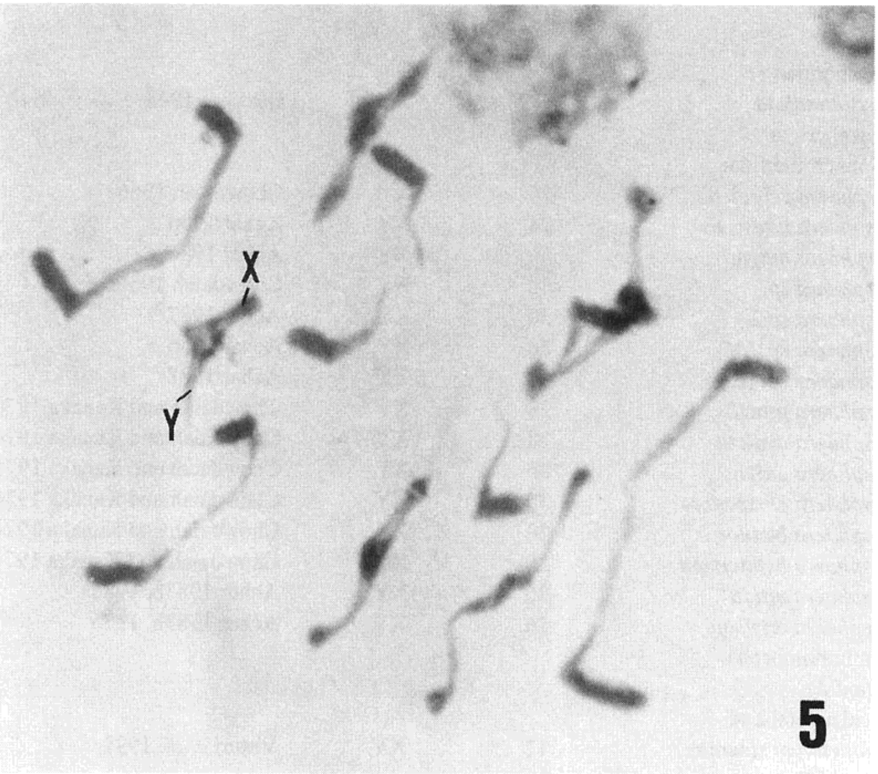


Figs. 1–4. 1) Bouquet formation in *Gymnostreptus olivaceus* (Spirostreptida), 2) Diffuse state in *Pseudonannolene tocaiensis* (Spirostreptida), 3) Zygotene in *P. tocaiensis*, where observed two different states of chromosome condensation with pairing of homologues occurring in the less condensed portion (arrow), 4) Clumping in metaphase I in *Gymnostreptus acuticollis* (Spirostreptida).

ed since. The sex determination mechanism commonly found is XY in males and XX in females.

Achar (1987), based on species from India, states that the main rearrangements that occurred during the evolution of diplopod karyotype are the Robertsonian rearrangements and the pericentric inversions. He proposed that a family or group has the type (modal) chromosome number and the species that showed the most similar number would be nearer to the ancestral condition than those that deviate from that number. These deviations from the type number would be caused by centric fusions, centric fissions and polyploidy. Species with a high chromosome number have more acrocentric chromosomes in relation to the metacentric ones, and *vice versa*. However, definitive conclusions about karyotypic evolution in this group are far away of being reached, once only aproximately 0.1% of the species have been studied.

In more studied groups like Arachnida and Insecta, affirmations like these are made in some taxa, in which the studies are more concentrated. In Arachnida, studies in many Aranae families have shown that karyotypic evolution seem to be done through the reduction in the chromosome number; however the karyotype diversification in spiders seem to appear independently in different families with similar evolution strategies, because in various families seem to predominate the same type of rearrangements (White 1977, Oliveira 1997). In Insecta, the different orders present their own karyotype pattern, with basic number and sex determination system. Thus, a major number of studies in the Diplopoda class is necessary to understand the evolution of the mechanisms involved in the karyotype of these groups. Considering the low vagility rate of these animals to be hope that



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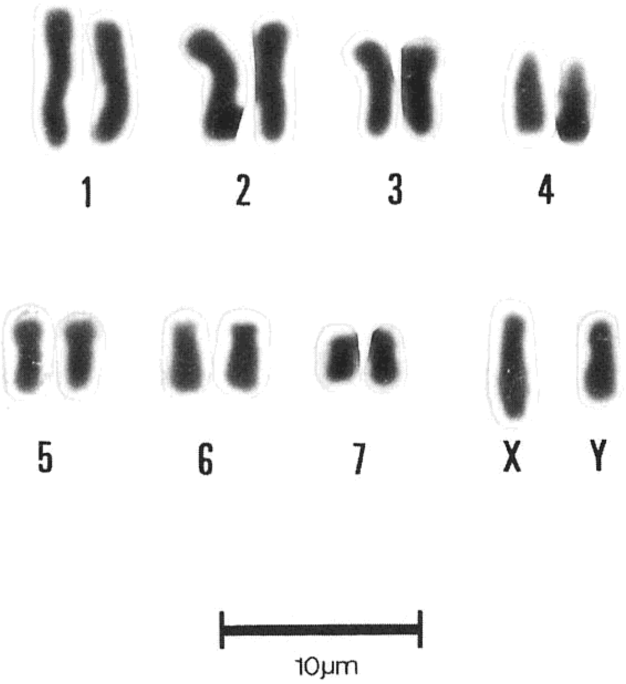

Figs. 5–6. 5) Metaphase I in *Pseudonannolene tocaiensis*, 6) Karyotype of *Pseudonannolene strinatii*.

Table 1. Checklist of cytogenetically studied millipedes

Taxa/species	2*n*	Sexual system	Author
Subclass Penicillata			
Order Polyxenida			
Family Polyxenidae			
Polyxenus sp.	16	XY	Sokoloff 1914
Subclass Chilognatha			
Infraclass Pentazonia			
Order Glomerida			
Family Glomeridae			
Glomeris annulata	20?	XY	Bessière 1948
Order Sphaerotheriida			
Family Sphaerotheriidae			
Arthrosphaera zebraica	26	XY	Chowdaiah 1966c
Arthrosphaera lutescens	26	XY	Achar 1986
Arthrosphaera magna	30	XY	Achar 1986
Arthrosphaera sp.1	30	XY	Chowdaiah 1966c
Arthrosphaera sp.2	30	XY	Achar 1987
Arthrosphaera sp. (*M*)	30	XY	Achar 1986
Arthrosphaera sp. (*C*)	30	XY	Achar 1987
Arthrosphaera gracilis	28	XY	Chowdaiah and Kanaka 1974
Arthrosphaera disticta	28	XY	Chowdaiah and Kanaka 1974, Achar 1986
Arthrosphaera dalyi	30	XY	Chowdaiah and Kanaka 1974, Achar 1986
Arthrosphaera craspedota	30	XY	Chowdaiah and Kanaka 1974
Arthrosphaera bicolor	30	XY	Chowdaiah and Kanaka 1974
Arthrosphaera hendersoni	30	XY	Chowdaiah and Kanaka 1974
Arthrosphaera nitida	30	XY	Achar 1983b, 1986
Arthrosphaera davisoni	26	XY	Achar 1983b, 1986
Infraclass Helminthomorpha			
Order Callipodida			
Family Schizopetalidae			
Acanthopetalum sicanum	12	XY	Vitturi *et al.* 1997
Order Polydesmida			
Family Chelodesmidae			
Sandalodesmus gasparae	12	?	Fontanetti 1996c
Family Polydesmidae			
Polydesmus complanatus	8?	?	Bessière 1948
Polydesmus gracilis	12	XY	Achar 1984b
Family Xystodesmidae			
Parafontaria circula	12	?	Tanabe 1992
Parafontaria sp.	12	?	Tanabe 1992
Levizonus montanus	12	?	Tanabe 1992
Riukiaria semicircularis	16	?	Tanabe 1992
Family Paradoxomatidae			
Strongylosoma sp.	24	XY	Chowdaiah 1966a
Chordomorpha mammifera	14	XY	Chowdaiah 1966b
Anoplodesmus (*Jonespeltis*) *splendidus*	12	XY	Chowdaiah and Kanaka 1979
Order Chordeumatida			
Family Chordeumatidae			
Melogona (*Microchordeuma*) sp.	24	XY	Chowdaiah and Kanaka 1979
Order Julida			
Family Julidae			
Pachyulus varius	24/25?	X0?	Oettinger 1999
Schyzophyllum albolineatum	24?	?	Bessière 1948
Schyzophyllum sabulosum	24?	?	Bessière 1948
Enologus oxypygum	22	XY	Vitturi *et al.* 1997

Table 1. (Continued)

Taxa/species	2*n*	Sexual system	Author
Order Spirobolida			
Family Rhinocricidae			
Rhinocricus padbergi	20	?	Fontanetti 1998
Rhinocricus cachoeirensis	24	XY	Fontanetti 1987
Rhinocricus sp.	28	XY	Fontanetti 1998
Family Pachybolidae			
Aulacobolus thrustoni	26	XY	Chowdaiah and Kanaka 1969
Aulacobolus levissimus	26	XY	Chowdaiah and Kanaka 1979
Aulacobolus variolosus	26	XY	Chowdaiah and Kanaka 1979
Aulacobolus excellens	12	XY	Achar 1985
Xenobolus acuticonus	12	XY	Natarajan 1959
Xenobolus carnifex	26	XY	Achar 1987
Cingalobolus bugnioni	16	XY	Achar 1987
Cingalobolus sp.	26	XY	Achar 1987
Trigoniulus sp.	24	XY	Achar 1984a
Order Spirostreptida			
Family Pseudonannolenidae			
Pseudonannolene strinatii	16	XY	Fontanetti 1996b Campos and Fontanetti 2000
Pseudonannolene tricolor	14	XY	Fontanetti 1996a
Pseudonannolene tocaiensis	20	XY	Fontanetti 1996b
Pseudonannolene silvestris	16	XY	Fontanetti 1996a Prado and Fontanetti 2000
Pseudonannolene ophiulus	12	XY	Fontanetti 1998
Pseudonannolene mesai	16	XY	Fontanetti 2000
Pseudonannolene halophila	16	XY	Fontanetti 1998
Family Harpagophoridae			
Phyllogonostreptus nigrolabiatus	24/25?	X0/XX0?	Natarajan 1959
Phyllogonostreptus nigrolabiatus	24	X0	Sharma and Handa 1974
Phyllogonostreptus nigrolabiatus	24	XY	Achar 1987
Phyllogonostreptus sp.	24	XY	Achar 1987
Phyllogonostreptus negotiosus	26	XY	Achar 1987
Gonostreptus malayus	15	X0	Sharma and Handa 1974
Gonoplectus (*Thyroglutus*) sp.	12	XY	Natarajan 1959
Gonoplectus (*Gongylorrhus*) *sulcatulus*	24	XY	Chowdaiah and Kanaka 1979
Ktenostreptus sp.	20	XY	Chowdaiah 1966b
Ktenostreptus calcaratus	28	XY	Achar 1983b
Ktenostreptus costulatus	20	XY	Chowdaiah and Kanaka 1979
Leptostreptus sp.	22	XY	Chowdaiah and Kanaka 1979
Harpurostreptus sp.	12	XY	Chowdaiah 1966b
Harpurostreptus hamifer	12	XY	Chowdaiah and Kanaka 1979
Harpurostreptus robustior	12	XY	Chowdaiah and Kanaka 1979
Thyropygus nigrolabiatus	24	XY	Parida and Mohanty 1972 *apud* Achar 1987 Chowdaiah and Kanaka 1979
Thyropygus descriptus	26	XY	Chowdaiah and Kanaka 1979
Thyropygus induratus	28	XY	Chowdaiah and Kanaka 1979
Thyropygus sp.1	24	XY	Chowdaiah 1966b
Thyropygus sp.2	26	XY	Achar 1984a
Thyropygus alienus	28	XY	Chowdaiah and Kanaka 1979, Achar 1984a
Carlogonus palmatus	12	XY	Achar and Chowdaiah 1979
Carlogonus acifer	12	XY	Achar and Chowdaiah 1980
Fageostreptus hyathi	22	XY	Achar 1987
Family Spirostreptidae			
Spirostreptus asthenes	16	XY	Chowdaiah and kanaka 1969, Achar 1983a
Spirostreptus sp.	26	XY	Achar and Chowdaiah 1979
Gymnostreptus acuticollis	22	XY	Fontanetti 1991
Gymnostreptus olivaceus	12	XY	Fontanetti 1991
Alloporus principes	18	XY	Fontanetti 1987
Plusioporus setiger	10	?	Fontanetti 1998

estasipatric speciation cases is found if a greater number of species were studied.

In a report concerning meiotic prophase I in diplopods, Fontanetti (1990) mentions as typical events the "bouquet formation" (Fig. 1), observed at the initial stages, and a diffuse state (Fig. 2), observed at the pachytene. At this latter phase, the nucleus shows parts of the chromatin condensed (constitutive heterochromatin) and part uncondensed (euchromatin). Other authors have also reported similar events in species from other regions (Sharma and Handa 1974, Chowdaiah 1966b, Achar and Chowdaiah 1979, Achar 1984a, b, 1985, 1986). All the authors agree that the number of heteropicnotic blocks of the diffuse stage corresponds to the total number of chromosomes of the species, never exceeding it, which suggests that only one end of each chromosome becomes heteropicnotic in order to indicate the beginning of the condensation and spiralization of the chromosomes from its telomeric regions.

According Fontanetti (1990) the prophase I present 2 different states of chromosome condensation (Fig. 3), at zygotene, the pairing of homologues occurring in the less condensed portion (Arrow in Fig. 3). In addition, Fontanetti (1990) stated that prophase I is rather long in diplopods, the process is accelerated from the diplotene, because it is difficult to find nuclei after this phase. Another typical event that occurs during the meiosis in this group is the chromosome clumping observed at metaphase I (Fig. 4).

Achar (1983a) suggest that the sex determination mechanism in Diplopoda is in a primitive state, since in most of the species the sexual chromosomes are poorly differentiated from the autosomes. This observation agrees with studies made on a variety of placental mammals, suggesting that the X and Y chromosomes evolved from a couple of autosomes without morphological differentiation. In species from India, the XY chromosomes are recognized as the largest chromosomes, even though there is a small difference in size (Achar 1987). Nevertheless, in many known species, from the Brazilian fauna (Figs. 5, 6) as well as from other regions, it is not possible to identify the sexual chromosomes as the larger pair of chromosomes (Fontanetti 1991, 1996a, b, 1998, 2000, Tanabe 1992).

According to White (1979), the sexual chromosomes of diplopods are more conserved than the ones from Chilopoda, which is the group that has been better cytogenetically studied among the Myriapoda. The Diplopoda could also be the most conserved group with regards to general karyotype evolution.

The use of differential cytogenetical techniques on diplopod's chromosomes is rare, mainly because of the technical difficulties in obtaining mitotic chromosomes.

Achar and Chowdaiah (1980) used the C banding technique on mitotic chromosomes of *Carlogonus acifer*; they observed that the constitutive heterochromatin was confined to the centromeric regions. The size and shape of the C bands, including the coloring intensity, are almost identical in all chromosomes, a significant characteristic was the absence of intermediary and telomeric bands.

The G banding technique was employed by Achar (1983a) on *Spirostreptus asthenes*. This species showed a good characterization of the banding pattern on its chromosomes, which was demonstrated in detail by means of a diagram.

More recently, Vitturi *et al.* (1997) used C banding, silver ion and fluorochromes AT-GC specifics in 2 species from Spain (from different orders). They detected a high amount of heterochromatin, adding up to almost 60% of the genome. In one of the species, they also detected a large amount of rDNA, which was analyzed by FISH (Colomba and Vitturi 1998).

In Brazilian species, it was recently possible to use a technique that provided a reasonable number of mitotic nuclei from midgut cells of individuals subjected to a prolonged period of starvation. By using this technique, it was possible to begin a more comprehensive study employing modern cytogenetical techniques in different species of the genus *Pseudonannolene* (Prado and Fontanetti 2000, Campos and Fontanetti 2000).

The Table 1 reports all the species of diplopods that have been cytogenetically studied so far, each with its chromosome number and sex determination system. The names of the taxa are cited according to Hoffman (1979) and Enghoff (1984); the names in parenthesis refer to the original names cited by the authors who performed the cytogenetic study.

References

Achar, K. P. 1983a. The use of G-banding techinique in the chromosome studies of a millipede species—*Spirostreptus asthenes*. Curr. Sci. **52**: 540–543.

— 1983b. Karyological studies in nine species of Indian Diplopoda (Myriapoda). Nucleus **26**:191–197.

— 1984a. Analysis of male meiosis in five species of Indian Diplopoda (Myriapoda). Caryologia **37**: 373–386.

— 1984b. Chromosome studies in Indian Diplopoda (Myriapoda). I. A note on the occurrence of polyploidy in *Polydesmus gracilis*. Curr. Sci. **53**: 764–767.

— 1985. Chromosome studies in Indian Diplopoda (Myriapoda). II. A note on the occurrence of translocation in *Alacobolus excellens*. Curr. Sci. **54**: 1057–1060.

— 1986. Analysis of male meiosis in seven species of Indian (Diplopoda: Myriapoda). Caryologia **39**: 89–101.

— 1987. Chromosomal evolution in Diplopoda (Myriapoda: Arthropoda). Caryologia **40**: 145–155.

— and Chowdaiah, B. N. 1979. The Use of Air Drying in the Study of Diplopod Chromosomes. In: Camatini, M. (ed.). Myriapod Biology. Academic Press, London. pp. 21–23.

— and — 1980. The use of C-banding technique in the chromosome studies of millipede species *Carlogonus acifer*. Caryologia **33**: 185–191.

Bessière, C. 1948. La spermatogènese de quelques Myriapods diplopods. Arch. Zool. Expt. **85**: 149–236.

Campos, K. A. and Fontanetti, C. S. 2000. Caracterização cromossômica do diplópodo cavernícola *Pseudonanolene strinatti* coletado na gruta Ressurgência do Córrego das Areias de Águas quentes. Genet. Mol. Biol. **23** suppl.: 3.

Chowdaiah, B. N. 1966a. Cytological investigations on some Indian Diplopoda (Myriapoda). Nature **210**: 847.

— 1966b. Cytological studies of some Indian Diplopoda (Myriapoda). Cytologia **31**: 294–301.

— 1966c. Chromosome studies in two species of pill millipedes. Caryologia **19**: 135–141.

— 1967. A note on the abnormal sex chromosome behavior in the male of *Thyropygous species*. Curr. Sci. **36**: 105–106.

— 1969. A note on the ocurrence of chromatid bridges in the male of *Arthrosphaera* sp. A pill-millipede. Curr. Sci. **38**: 1969–1970.

— and Kanaka, R. 1969. Cytological studies of Indian Diplopoda. II. (Myriapoda). Bull. Mus. Nat. D'His. Natur. **41** Suppl.: 43–47.

— and — 1974. Cytological studies in six species of pill-millipedes (Diplopoda-Myriapoda). Caryologia **27**: 55–64.

— and — 1979. Chromosome Cytology of Seven Species of Indian Diplopoda (Myriapoda). In: Camatini, M. (ed.). Myriapod Biology. Academic Press, London. pp. 9–20.

Colomba, M. S. and Vitturi, R. 1998. High rDNA amount in Enologus oxypygum (Diplopoda) analyzed by FISH. Cytogenet. Cell Genet. **81**: 107.

Enghoff, H. 1984. Phylogeny of millipedes—cladistic analysis. Zeits. fur Zool. Syst. und Evolutionsforschung **22**: 8–26.

Fontanetti, C. S. 1987. Estudo citotaxonômico em espécies brasileiras de diplópodos. M. Sc. thesis, Instituto de Biociências, UNESP.

— 1990. Meiotic prophase in Diplopoda. Revta. Brasil. Genet. **13**: 697–703.

— 1991. Karyotype of some brazilian diplopods. Revta. Bras. Genet. **14**: 645–651.

— 1996a. Description of a new species and karyotype of cavernicolous millipedes *Pseudonannolene strinatii* Mauriès, 1974 (Diplopoda, Pseudonannolenida, Pseudonannolenidae). Revta. Bras. Zool. **13**: 419–426.

— 1996b. The use of cytogenetics to certify a Diplopoda species (Pseudonannolenida, Pseudonannolenidae). Revta. Bras. Biol. **56**: 775–781.

— 1996c. Karyotype of a termitophilic species of Diplopoda (Polydesmida, Chelodesmidae). Brazilian J. Genetics **19**: 593–595.

— 1998. Chromosome numbers of some brazilian species of diplopods (Diplopoda, Arthropoda). Cytologia **63**: 149–154.

— 2000. Description and chromosome number of a species of *Pseudonannolene* Silvestri (Arthropoda, Diplopoda, Pseudonannolenidae). Revta. Bras. Zool. **17**: 187–191.

Golovatch, S. I., Hoffman, R. L., Adis, J. and Morais, J. W. 1995. Identification plate for the millipede orders populating the neotropical region south of central Mexico (Myriapoda, Diplopoda). Studies on Neotropical Fauna and Environment **30**: 159–164.

Hoffman, R. L. 1979. Classification of the Diplopoda. Museum D'Histoire Naturelle, Geneve.

Natarajan, R. 1959. Cytological studies in Diplopoda (Myriapoda). J. Zool. Soc. India **11**: 91–101.

Oettinger, R. 1999. Zur kenntnis Spermatogenese bei den Myriapoden. Archiv. F. Zellforschung **3**: 563–626.

Oliveira, E. D. 1997. Evolução Cariotípica nos Araneae (Arachnida). Texto de Qualificação, Instituto de Biociências, UNESP.

Parida, B. B. and Mohanty, S. 1972. Studies on the chromosomes of a millipede, *Thyropygus nigrolabiatus* (Newport) (Harpagophoridae: Diplopoda: Arthropoda). Prakruti, Utkal Univ. J. Sci. **9**: 97–104. *apud* Achar, K. P. 1987. Chromosomal evolution in Diplopoda (Myriapoda: Arthropoda). Caryologia **40**: 145–155.

Prado, R. A. and Fontanetti, C. S. 2000. Citogenética de *Pseudonannolene silvestris* (Diplopoda, Pseudonannolenidae): Bandamento C, AgNOR e FISH com sonda de rDNA. Genet. Mol. Biol. **23** suppl.: 4.

Sharma, G. P. and Handa, S. M. 1974. Comparative cariological studies on *Gonoplectus malayus* and *Phyllogonostreptus nigrolabiatus* (Diplopoda: Myriapoda). Cytologia **39**: 673–680.

Sokoloff, J. 1914. Uber die Spermatogenese bei *Polyxenus* sp. Zoo. Anz. **44**: 558–566.

Tanabe, T. 1992. Karyotypes of four Xystodesmid millipeds from Japan. Acta Arachnologica **41**: 87–90.

Vitturi, R., Colomba, M. S., Caputo, V., Sparacio, I. and Barbieri, R. 1997. High heterochromatin content in somatic chromosomes of two unrelated species of Diplopoda (Myriapoda). Chrom. Research **5**: 407–412.

White, M. J. D. 1977. Animal Cytology and Evolution. Cambridge University Press, London. 961 p.

— 1979. The Present Status of Myriapod Cytogenetics. In: Camatini, M. (ed.). Myriapoda Biology. Academic Press, London. pp. 3–8.