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Chromosome Numbers of Some Brazilian Species of Diplopods (Diplopoda, Arthropoda)[1]

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Summary The present paper presents the chromosome numbers of five brazilian species of diplopods: *Plusioporus setiger* (2n=10 and 2n=10+1B, the distinction of the sexual pair was not possible), *Pseudonannolene ophiulus* (2n=12, XY), *Pseudonannolene halophila* (2n=16, XY), *Rhinocricus sp.* (2n=28, XY) and *Rhinocricus padbergi* (2n=20, the distinction of the sexual pair was not possible).

Key words Millipedes, Chromosomes, Cytogenetics, Diplopods.

The Myriapoda have received little attention from cytogeneticists, specially in relation to the Diplopoda Class. A bibliographic review shows that only 0.67% from the described species were studied cytogenetically. This is therefore an interesting group of research. According to Golovatch *et al.* (1995), 80.000 species were estimated for the group, being that the third largest class of Arthropoda after Insecta and Arachnida.

Most of the cytogenetic studies in this Arthropoda class, were performed by Indian researchers (Achar and Chowdaiah 1980, Achar 1983a, b, 1984a, b, 1985, 1986, 1987), restricting them therefore to zoogeographic regions different from ours. The few papers about brazilian species are from Fontanetti (1991, 1992, 1996a–c) achieving so far a total of 10 species, for an estimated number of 2000 to 3000 species in our fauna.

The scarce studies of cytogenetics in diplopods, are due greatly to technical difficulties in obtaining mitotic chromosomes, restricting the studies to meiosis and rare spermatogonial metaphases, limiting the application of more modern cytogenetic technics.

White (1979) in a short review, cites a conservatism of the group, concerning the karyotypic evolution in general, but emphasizes that the number of studied species is still very small for a definite conclusion.

The present paper deals with the chromosome number of five brazilian species of diplopods: *Plusioporus setiger* (Broleman 1901), *Pseudonannolene ophiulus* Schubart 1944, *Pseudonannolene halophila* Schubart 1949, *Rhinocricus padbergi* Verhoeff 1938 and *Rhinocricus sp.*

Material and methods

The specimens of *Plusioporus setiger* were collected in the surroundings of Rio Claro, São Paulo, specially between September and February in 1984, 1985 and 1986, the species was found widely distributed, throughout the State of São Paulo.

Pseudonanolene ophiulus was collected in some localities of São Paulo, mainly between November and February in 1984, 1989 and 1990.

Pseudonannolene halophila was collected in the Archipelago of Alcatrazes, São Paulo, in September of 1984 by F. A. G. de Mello and J. Justi.

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Rhinocricus sp. was collected at 16 km apart from Jacupiranga, São Paulo, in February of 1987 by Mesa et col.

Rhinocricus padbergi was collected in the surroundings of Rio Claro, São Paulo, almost all year long in 1989, 1990, 1991 and 1992.

Testicles of adult males were used; after dissection, the gonads were treated with hypotonic KCl 0.075 M for approximately 4 min and fixed in: Fixative I: 1.5 ml acetic acid+1.5 ml methanol+2 ml distilled water (1 min); Fixative II: 2 ml acetic acid+2 ml methanol (1 min); Fixative III: Carnoy I (20 min); Fixative IV: acetic acid (1 min). The gonads were then squashed and stained with lactoacetic orcein (1%).

Results and discussion

Plusioporus setiger showed five bivalents (2n=10) and the pair of sex chromosomes was not distinguished (Figs. 1A, B). Metaphase I showed a progressive condensation of the bivalents (Figs. 1E, F), being all chromosomes united in only one block.

In three specimens (of a total of ten), an odd, small and lightly positive heteropycnotic chromosome was observed beside the complement of ten chromosomes (Fig. 1C). It was interpreted as a small supernumeray of a kind of B chromosome, since not all specimens showed that. It was observed in diplotene (Fig. 1C) and diakinesis (Fig. 1D), but in metaphase I, the supermumerary chromosome was found situated separately from the others (Figs. 1E, F), suggesting an independent and premature movement towards the poles.

The origin of B chromosomes, seems to vary in different organisms and it is probably not due

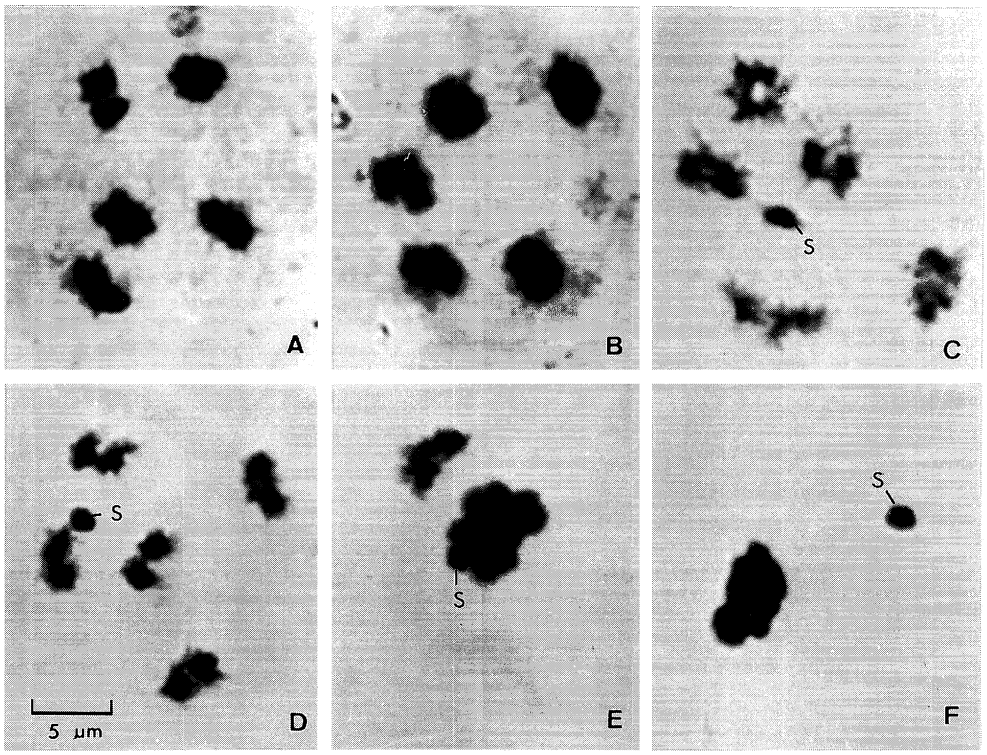

Fig. 1. Meiosis in *Plusioporus setiger*. A, B. Diakinesis in specimens that do not present the supernumerary chromosome; C. Diplotene; D. Diakinesis; E, F. Metaphase I. Figs. C, D and E show the presence of the supernumerary chromosome (S).

to only one kind of evolutionary mechanism. In some cases strong evidence has been found that they originate from centric segments of a chromosomes. In some insects, evidence has been found that they could have originated from X-chromosomes (Guerra 1988). However, any evolutionary advantage caused by B chromosomes to the species is so far unknown.

The supermumerary chromosome observed here in *P. setiger* is the first time found in diplopods. The application of more sofisticated technics and observation of a great deal of individuals will be necessary to enable speculations about the origin of the supernumerary chromosome in *P. setiger.*

The number 2n=10 has been observed so far, only in this species of diplopods. A smaller number (2n=8) was observed by Bessière (1948) in *Polydesmus complanatus* is dubious, since the technics used at that time, histological cuts, presented frequent errors of interpretation (White 1979).

In *Pseudonannolene ophiulus* six bivalents (2n=12) were observed in the meiocytes (Figs. 2A–C). In the diplotene (Fig. 2A), part of the less condensed chromatin was observed returning from the diffuse stage (arrows), a characteristic of prophase I in this group, as proposed by Fontanetti (1990). The components of the heteromorphic bivalent observed in metaphase I (Figs. 2B, C), implies X (larger) and Y (smaller). This pair is the largest of all the complement.

The number 2n=12, found in *P. ophiulus* has already been reported for two other species, in brazilian fauna, *Gymnostreptus olivaceus* (Fontanetti 1991) and *Sandalodesmus gasparae* (Fontanetti 1996a), as well as for other species of different regions: *Polydesmus gracilis* (Achar 1984b), *Thyroglutus sp.*, *Xenobulus acuticonus* (Natarajan 1959), *Harpurostreptus sp.* (Chowdaiah 1966), *H. hamifer*, *H. robustior*, *Jonespeltis splendidus* (Chowdaiah and Kanaka 1979), *Carlogonus*

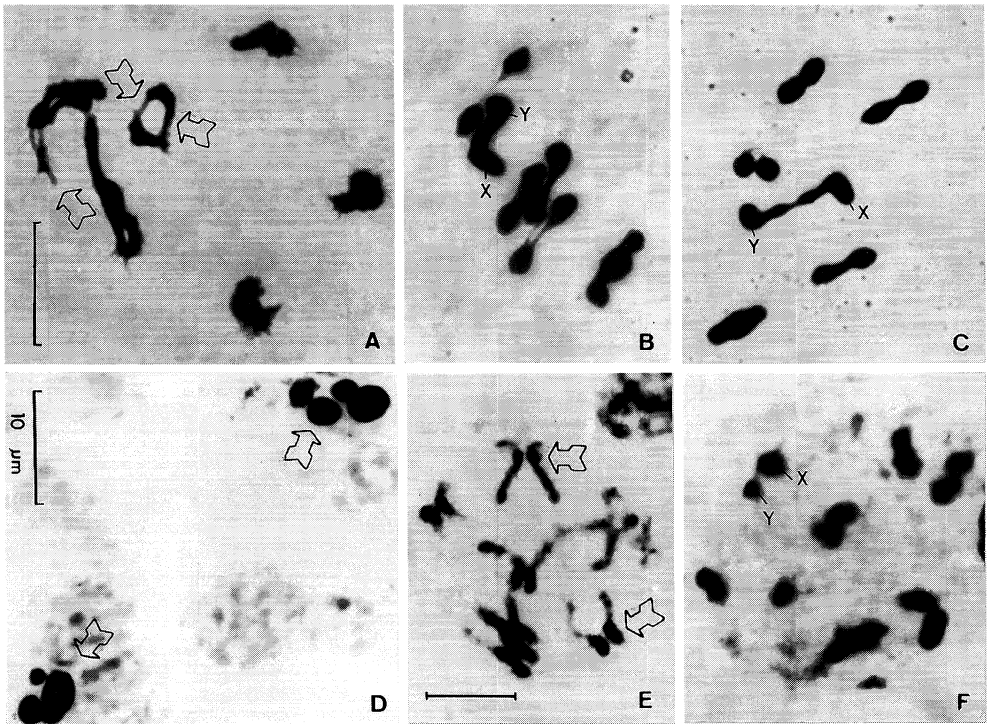

Fig. 2. Meiosis in *P. ophiulus* (A–C) and in *P. halophila* (D–F). A. Diplotene in *P. ophiulus* (arrows indicate part of the less condensed chromatin, returning from the diffuse stage); B, C. Metaphase I in *P. ophiulus*; D. Clumping (arrows) in *P. halophila*; E. Metaphase II in *P. halophila* (arrows indicating positive heteropycnotic blocks); F. Diakinesis in *P. halophila.*

palmatus (Achar and Chowdaiah 1979), *C. acifer* (Achar and Chowdaiah 1980) and *Aulacobolus excellens* (Achar 1985).

In *Pseudonannolene halophila* eight bivalents (2n=16) was found in the meiocytes (Fig. 2F). The difference in size between components of a bivalent implies X and Y. In metaphase I, the chromosomes come together (clumping), turning into compact blocks (arrows, Fig. 2D) making it difficult to distinguish the eight bivalents. The chromosome at metaphase II showed considerably large positive heteropycnotic blocks (arrows, Fig. 2E).

The same chromosome number as found in *P. halophila* (2n=16), has been reported in four other species of *Pseudonannolene*, by Fontanetti (1992, 1996b, c) and in species of other regions by different authors: *Polyxenus* sp. (Sokoloff 1914), *Cingalobolus bugnioni* (Achar 1987) and *Spirostreptus asthenes* (Chowdaiah and Kanaka 1969, Achar 1983a).

Rhinocricus sp. showed fourteen bivalents (2n=28), and its mechanism of sexual determination was XY type; the XY pair was distinguished from others by the difference in size between the paired chromosomes (Figs. 3A, B), X chromosome is much larger than Y chromosome. This chromosome number was also reported for the species *Ktenostreptus calcaratus* (Achar 1983b), *Thyropygus alienus* (Chowdaiah and Kanaka 1979, Achar 1984a), *Arthrosphaera gracillis* (Chowdaiah

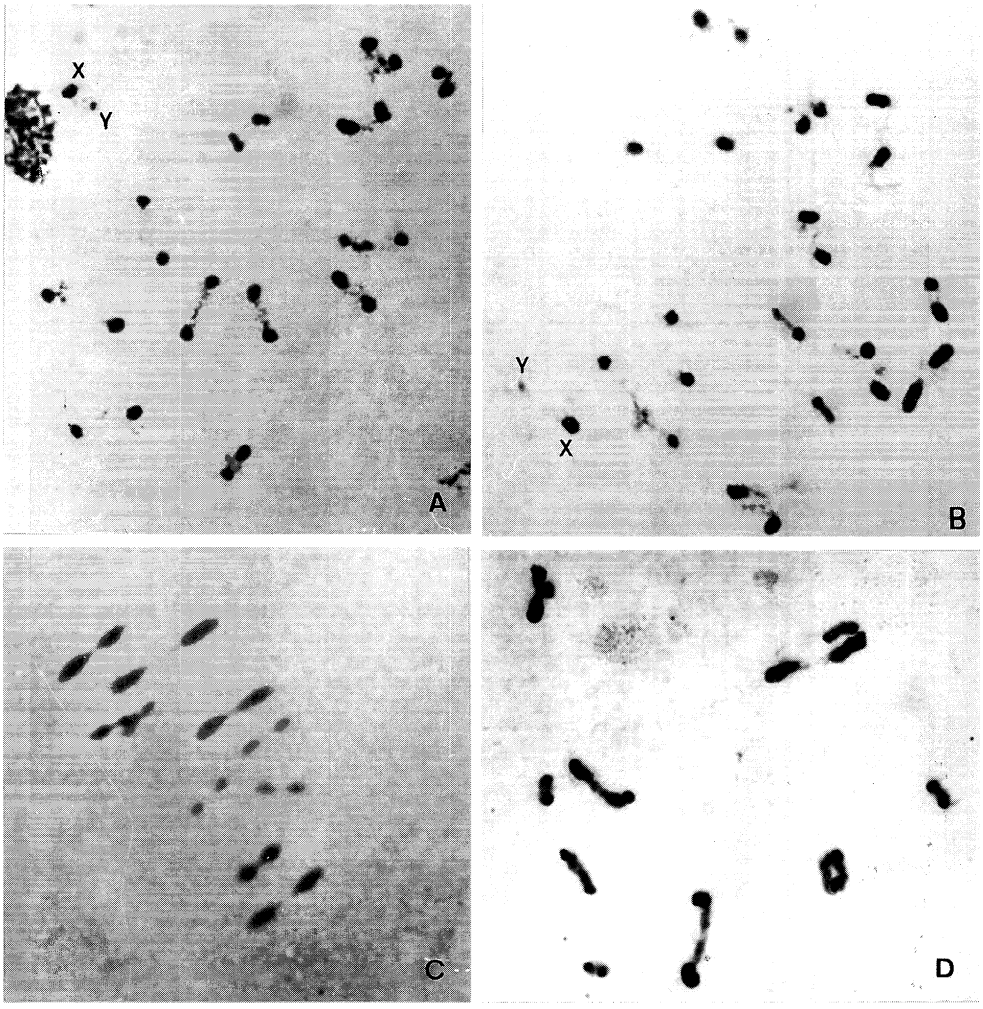

Fig. 3. Meiosis in *Rhinocricus sp.* (A, B) and in *Rhinocricus padbergi* (C, D); A, B. Diplotenes; C. Metaphase I; D. Diakinesis.

and Kanaka 1974), *A. disticta* (Chowdaiah and Kanaka 1974, Achar 1986) all from India.

Rhinocricus padbergi showed clear configuration of ten bivalents (2n=20) in the meiocytes (Figs. 3C, D) and the distinction of the sex chromosome pair was not possible. This number was found in the species *Glomeris annulata* (Bessière 1948), *Ktenostreptus* sp. (Chowdaiah 1966b), *K. costulatus* (Chowdaiah and Kanaka 1979), and the brazilian species, *Pseudonannolene tocaiensis* (Fontanetti 1996c).

The diploid numbers in diplopods range from 2n=10 to 2n=30, excepting the number 2n=8 reported by Bessière in 1948.

In many species of diplopods, chromosomes at metaphase of the primary spermatocytes were found clumping (Chowdaiah and Kanaka 1969, 1974) as observed here in *Pseudonannolene halophila* and *Plusioporus setiger*. The clumping and the high condensation of chromosomes often make it difficult to count the chromosome number and to study the morphology of the chromosomes; furthermore, there is no tissue favourable to study mitotic chromosomes apart from rare cases of spermatogonial metaphases.

It is suggested that the mechanism of sexual determination in diplopods is in a primitive stage, since in most species the sexual chromosomes are little differentiated from the autosomes (Achar 1983a). In the diplopod species of India, the largest pair of the complement is recognized as XY by the little difference in size between the constituent chromosomes of this pair in some species (Achar 1987); in many analized species of millipedes, both in our fauna as in other regions, it is not possible to distinguish the pair of sex chromosomes from the autosomes, not to suggest the largest pair of the complement to be the XY (Fontanetti 1991, 1992, 1996b, c, Tanabe 1992).

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