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KARYOTYPES OF SOME BRAZILIAN DIPLOPODS

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ABSTRACT

The present paper shows the cytology of two neotropical diplopod species belonging to the Spirostreptidae (*Gymnostreptus acuticollis* Verhoeff and *G. olivaceus* Schubart). *G. olivaceus* showed 2n = 12, XY, while *G. acuticollis* presented 2n = 22, the sexual chromosomes being impossible to identify.

INTRODUCTION

The karyotypes of Diplopoda have been poorly studied up to now. Less than 70 of the nearly 8,000 described species have actually been analyzed. The main difficulty in working with diplopod cytology lies in the fact that most of the genera need modern taxonomic revisions and that much of the new material collected includes new species.

The first contributions on diplopod chromosomes were those by Oettinger (1909), Sokoloff (1914) and Bessière (1948), relevant from a historic point of view, but very poor in information due to technical difficulties. These early papers were followed by those by Natarajan (1959), Chowdaiah (1966a,b,c, 1967, 1969), Chowdaiah and Kanaka (1969, 1974, 1979), Sharma and Handa (1974) and more recently by those by Achar and Chowdaiah (1979, 1980) and Achar (1983a,b, 1984a,b, 1985, 1986, 1987), using modern cytogenetic techniques.

The diploid number in the species already studied is quite variable, from 2n = 8 (?) to 2n = 30, with the XY type as the most frequent sex determining mechanism in the males.

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In present paper, the chromosomes of neotropical species of diplopods are reported for the first time, through a study of two species (*Gymnostreptus olivaceus* and *G. acuticollis*), that belong to the family Spirostreptidae.

MATERIAL AND METHODS

G. acuticollis Verhoeff was collected at the Biological Station of Boracéia, Biritiba Mirim, São Paulo, in April, 1984 (three males analyzed) and *G. olivaceus* Schubart in Río Claro, São Paulo, mostly between October and March (seven specimens analyzed).

For cytological observations, testes vesicles from adult males were pretreated with 0.45% KCl and fixed in Carnoy I. The glands were squashed in a drop of 1% lacto-acetic orcein.

RESULTS

G. acuticollis (Figure 1) showed 2n = 22 chromosomes, without evidence of sex chromosomes. The beginning of prophase presented two different levels of condensation along each bivalent (Figure 1a). At premetaphase (Figure 1c) one of the bivalents showed a long uncondensed segment. At first metaphase the bivalents were highly condensed as in Figure 1d, where all the eleven bivalents are observed. At spermatogonial metaphase (Figure 1e, f) one pair shows one of the arms poorly stained along all of its length. One of the specimens studied showed four negative heteropycnotic pairs (Figure 1b) in all the diakinesis cells observed.

G. olivaceus (Figure 2) had 2n = 12, with an XY sex determining mechanism. The beginning of prophase was characterized by a "bouquet" configuration (Figure 2a). During diplotene, a positive heteropycnotic pair (possibly the sex pair) was observed. The association of this pair with an autosomal pair was also frequently noted (Figure 2b, e). The presence of a clear transversal band was observed at the end of one bivalent during diakinesis (Figure 2d) and premetaphase (Figure 2e) exactly where association takes place between the autosome pair and the sex chromosomes.

The sex chromosomes were quite similar both in meiotic as well as in mitotic nuclei. A slight difference in length was only observed between the supposed X and its partner, the former being slightly longer.

It was difficult to describe the chromosome morphology because the few "adequate" mitotic nuclei had heavily condensed chromosomes (Figure 2g). Probably a submetacentric pair (pair 1) and three metacentric pairs (pairs 3, 4, 5) exist; the remaining chromosomes do not clearly show the position of the centromere.

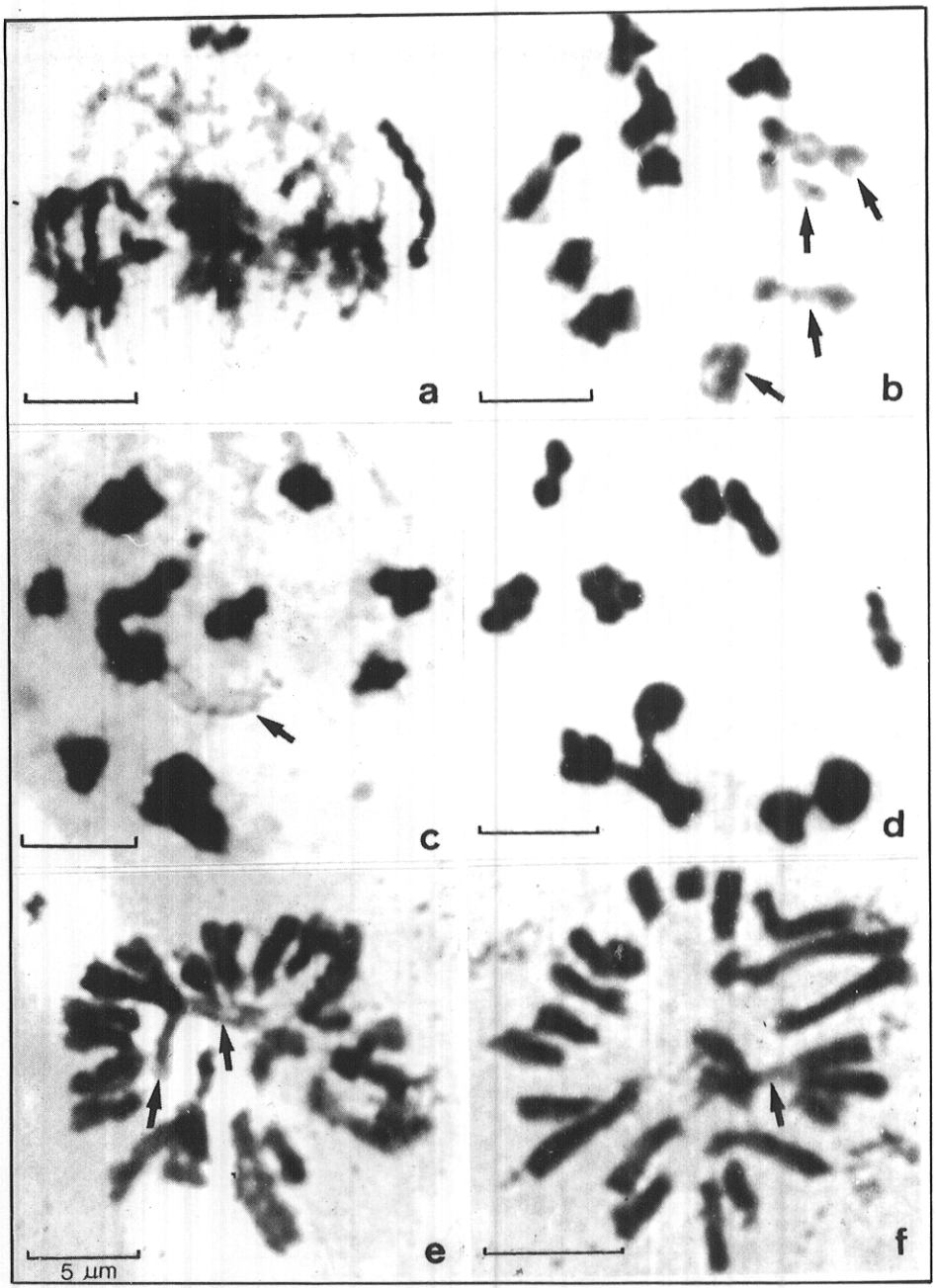

Figure 1 - Chromosomes of *Gymnostreptus acuticollis*. a. initial prophase (observe the two different levels of chromosomes condensation). b. diakinesis observed in one specimen, where four negative heteropycnotic pairs are noted (arrows). c. premetaphase (observe the descondensed region in one bivalent - arrow). d. metaphase I. e, f. spermatogonial metaphases (observe one pair that presents a negative heteropycnotic arm - arrow).

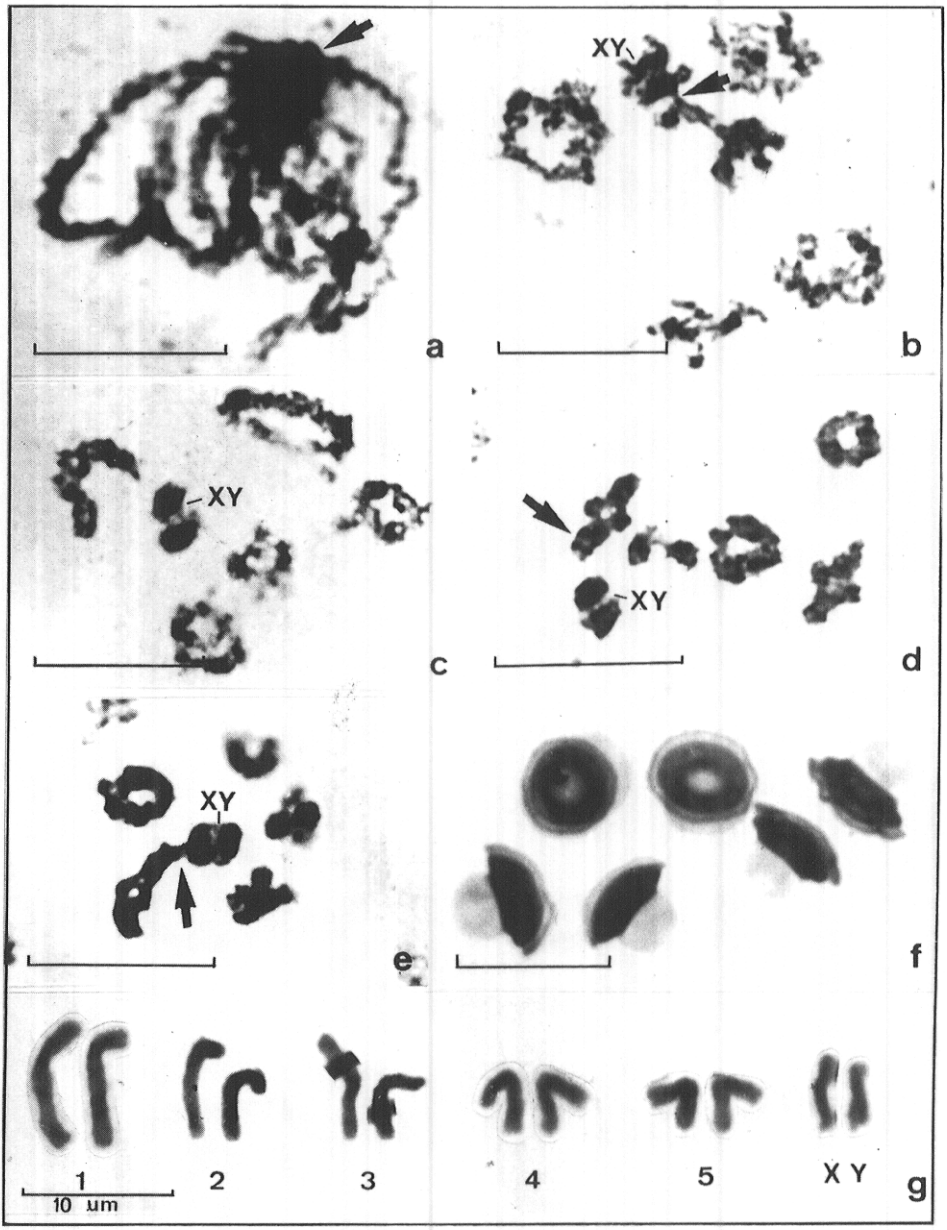

Figure 2 - Chromosomes of *Gymnostreptus olivaceus*. a. "bouquet" configuration (arrow indicating polarized material). b. diplotene (observe the association between the sex and autossomal pairs). c. diplotene. d. diakinesis (observe a clear transversal band in one bivalent - arrow). e. premetaphase (observe the association (arrow) of the pair (that shows a clear transversal band) with the XY). f. spermatozoids. g. karyotype from a spermatogonial metaphase.

The spermatozoids of both species were immobile and "hat" shaped, as is usual in the majority of Spirostreptida (Figure 2f).

C-banding was attempted, but the results were not clear.

DISCUSSION

The XY sex determining mechanism observed in *G. olivaceus* is the most frequently found in the group. The sex pair is difficult to distinguish from the remaining pairs due to the slight difference in length between X and Y.

Achar (1983a), who studied *S. asthenes*, comments on this fact: that the sex mechanism in this species remains in a primitive stage of development, the sex chromosomes being poorly differentiated from the remaining autosomic pairs. This observation agrees with studies done on placental mammals where X and Y have evolved from an autosomal pair without morphological differentiation. In the diplopod species studied until now, it was not possible to demonstrate the heteromorphic nature of this pair based on meiosis alone. In addition, according to Achar (1983a), no female chromosome has yet reported due to technical difficulties.

The "bouquet" arrangement has frequently been found in the Diplopoda species studied. According to Schulz-Schaeffer (1980) this characteristic observed during leptotene and pachytene appears because of a polarization of the chromosomes, where one of the ends attachs to the nuclear membrane close to the position where the centrosome remains in the cytoplasm. This arrangement apparently helps ensure homologous pairing during zygotene.

Differential staining of one of the chromosome arms of pair 1 of *G. acuticollis*, suggests a difference in the chromatin composition. The negative heteropycnosis observed in four chromosomes in one individual of this species during diakinesis, suggests a different degree of condensation in relation to the remaining pairs, but limited only to that stage of meiosis.

The association of the sex pair to one of the autosomic pairs in *G. olivaceus*, is probably due either to the presence of constitutive heterochromatin in both pairs or to the presence of NOR in those chromosomes.

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RESUMO

São apresentadas as citologias de duas espécies de diplópodos neotropicais, pertencentes a família Spirostreptidae, *Gymnostreptus acuticollis* Verhoeff e *G. olivaceus* Schubart. *G. acuticollis* apresenta 2n = 22, não sendo possível a distinção do mecanismo de determinação sexual, enquanto *G. olivaceus* apresenta 2n = 12, XY.

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