# A new species of *Urostreptus* (Diplopoda, Spirostreptidae): description and chromossome number

Pedro H. B. Pierozzi & Carmem S. Fontanetti

Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Avenida 24-A, 1515, 13506-900 Rio Claro, SP, Brasil. (fontanet@rc.unesp.br)

**ABSTRACT.** This work presents the description and chromosome number of *Urostreptus atrobrunneus* sp. nov. The genus until now had not been registered yet in the São Paulo State, Brazil. The meiotic analysis showed that the species presents 2n=24, XY. The C-banding revealed large blocks of constitutive heterochromatin and two heteromorphic chromosomal pairs, one of them corresponding to the sexual pair.

**KEYWORDS.** *Urostreptus*, Diplopoda, cytogenetics, banding.

**RESUMO. Uma nova espécie de *Urostreptus* (Diplopoda, Spirostreptidae): descrição e número cromossômico**. O trabalho traz a descrição e o número cromossômico de *Urostreptus atrobrunneus* sp. nov. O gênero até o momento não havia sido registrado para o Estado de São Paulo, Brasil. A análise meiótica demonstrou que a espécie apresenta 2n=24, XY. O bandamento C revelou grandes blocos de heterocromatina constitutiva e dois pares de cromossomos heteromórficos, um deles correspondendo ao par sexual.

**PALAVRAS-CHAVE.** *Urostreptus*, Diplopoda, citogenética, bandeamento.

*Urostreptus* Silvestri, 1895 belongs to Spirostreptidae, being distributed throughout South America and characterized for presenting a median prominent carina on the epiproctum. These animals have an average size and a thin body.

The genus, described by Silvestri (1895) is composed by 12 species: *U. borellii* (Silvestri, 1895); *U. camerani* (Silvestri, 1895); *U. cultratus* (Humbert & De Saussure, 1870); *U. mineri* (Chamberlin, 1941); *U. tampiitauensis* (Schubart, 1947); *U. carvalhoi* (Schubart, 1947); *U. robustus* (Verhoeff, 1951); *U. munducurensis* (Schubart, 1957); *U. travassoi* (Schubart, 1957); *U. paxillatus* Hoffman, 1968; *U. auritus* Hoffman, 1980 e *U. fallax* Hoffman, 1980.

Six species of *Urostreptus* have been reported in Brazil, with their distribution concentrated at the central States and, according to Hoffman (1980), their absence at the eastern and southeastern parts of the country is noteworthy.

This work presents the description and chromosomal number of a new species of *Urostreptus,* widely distributed throughout the São Paulo State, southeastern Brazil.

## MATERIAL AND METHODS

The specimens were collected at different sites in the São Paulo State and in different seasons of the year. For the cytogenetical analysis, the specimens were collected at the counties of Rio Claro (22°23'59''S; 47°34'18"W) and Piracicaba (22°42'30"S; 47°38'01"W).

For chromosomal analysis, individuals were starved for one week and then injected with colchicine 0.08%. After approximately 16 hours (overnight), the specimens were anesthetized and dissected in physiological solution. For the meiotical analysis, the testes were brought to the hypotonic state by incubation in tap water for five minutes, then fixed in Carnoy I and crushed in 45% acetic acid. The material was stained in 3% Giemsa and subjected to C-banding, according to Sumner (1972), with slight modifications.

The holotype was deposited in the collection of the Zoology Museum of the University of São Paulo (MZSP), SP, Brazil and paratypes in the collection of the Biology Department, Biosciences Institute (IBRC), São Paulo State University (UNESP), Rio Claro, SP, Brazil.

## RESULTS AND DISCUSSION

### *Urostreptus atrobrunneus* sp. nov.

(Figs. 1-8)

Type material. Holotype ♂, BRAZIL, **São Paulo**: Rio Claro (Fausto Santomauro highway, km 3 North), I.1984, R. Christofoletti col. (MZSP). Paratypes: BRAZIL, São Paulo: Rio Claro, 2 ♂, 3 ♀, I.1989, E. Giannotti col. (IBRC); 2 ♂, 2 ♀, I.1996, R. Christofoletti col. (IBRC); ♂, III.1997, M. C. de Almeida col. (IBRC); (Fausto Santomauro highway, km 3 North), 2 ♀, I.1984, R. Christofoletti col.; same locality, 8 ♂, 6 ♀, II.2002, A. Mesa & C. B. Portugal col. (IBRC); (Washington Luis highway, km 177.3 North),1 immature, 4 ♂, 5 ♀, I.1989, R. Christofoletti col. (IBRC); Paulínia: 3 ♂, ♀, II.1995, L. Boccardo col. (IBRC).

Description. Small species, with a mean of 50 mm in length, 3 mm in diameter and 46 segments; color dark brown with a lighter prozona, totally covered by metazona; dark brown head, collum, telson and antennae. Collum marked laterally by one groove. Epiproct slightly projected over paraprocts.

First pair of male legs. Strait coxa and divergent prefemural processes, orally projected.

Gonopods (Figs. 1,2). Coxite with about half-length of telocoxite. Telocoxite enlarged at the distal portion and guided towards the inside. Paragonocel long, prominent, covered with bristles, distal margin oblique. Telopodite free, long, narrow, directed caudad, tapering final portion only. Prefemural processes C-shaped.

Discussion. *Urostreptus atrobrunneus* sp. nov. is similar to *U. borelli*, which was described from Paraguay and Argentina (SILVESTRI, 1895; HOFFMAN, 1974, 1980) by the gonopods morphology, but *Urostreptus atrobrunneus* sp. nov. is smaller and probably the smallest species of the genus. It is agile, commonly found in buildings, underneath the masonry of house structures causing infestations, or in migratory groups. With relation to other species, the configuration of the gonopods is different, mainly the morphology of telocoxite.

Cytogenetical analysis. The species presents 2n=24 and a sexual determination system of the type XY in the males. The bivalent chromosomes appear close together and in some nuclei we observed the typical aspect of chromosomal chains formed by associations of chromatin (arrows in Figs. 3, 4). This fact is probably due to the high amount of heterochromatin found in this species.

The meiotic analysis revealed two heteromorphic pairs, one of them presents a slight size difference between the homologues and was identified as the sexual pair (Figs. 5, 7). The heteromorphism was highly evident with regards to their C-banding patterns (Figs. 6, 8). One of the chromosomes of the sexual pair was marked by a large C+ block (the X chromosome) whereas the other had a much smaller block (Y chromosome) (Figs. 6, 8). The other bivalent pair presents a chromosome marked by a large C+ block while the other chromosome had no apparent marks (arrows in Figs. 6, 8).

The *Urostrepus* species here analyzed was the first of the genus that has been studied from the cytogenetical point of view, which hampers a comparison of the results obtained. Members of the Spirostreptidae have been poorly studied cytogenetically, comprising a total of six species (CHOWDAIAH & KANAKA, 1969; ACHAR & CHOWDAIAH, 1979, 1980; ACHAR 1983; FONTANETTI, 1991, 1998) and none of them presents the diploid number found in *Urostrepus atrobrunneus*, sp. nov. Nevertheless, this number has already been observed in other millipedes belonging to different families (FONTANETTI *et al.*, 2002).

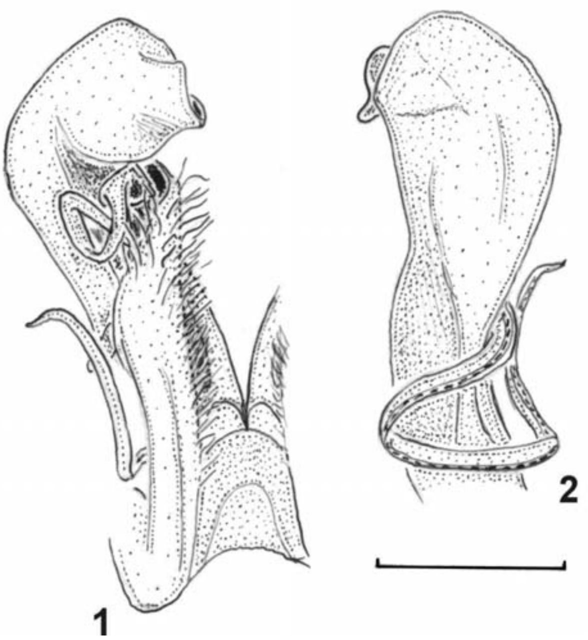
The XY sex determination system observed in the males is the most frequent in millipedes. While studying *Spirostrepus asthenes*, ACHAR (1983) commented that the sex determination mechanism in the Diplopoda is in a primitive stage, since in most species the sexual pairs are poorly differentiated from the autosomes and, even between the X and Y chromosomes, the differences are very slight. Such a discrete sexual differentiation was clearly apparent in the species here presented; the heteromorphism of the sexual pair was thin when compared to the other chromosomes, and even between X and Y, the difference is subtle.

The large amount of constitutive heterochromatin found in *Urostrepus atrobrunneus* sp. nov. was also observed in other species of diplopods both in Brazilian fauna and in representatives of other regions. *Acanthopetalum sicanum* (Berlese, 1883) (Callipodida) presents about 60 and 56% of heterochromatin, respectively in males and females and *Enologus oxypygum* (Julida) about 67%, with this value being equal in males and females (VITTURI *et al.*, 1997, 2001). The Brazilian species *Pseudonannolene tocaiensis* Fontanetti, 1996, *P. silvestris* Schubart, 1944 and *P. strinatii* Mauriès, 1974 also present a large amount of constitutive heterochromatin, representing in the later about 65% of the genome (CAMPOS & FONTANETTI, 2004; SOUZA *et al.*, 2005).

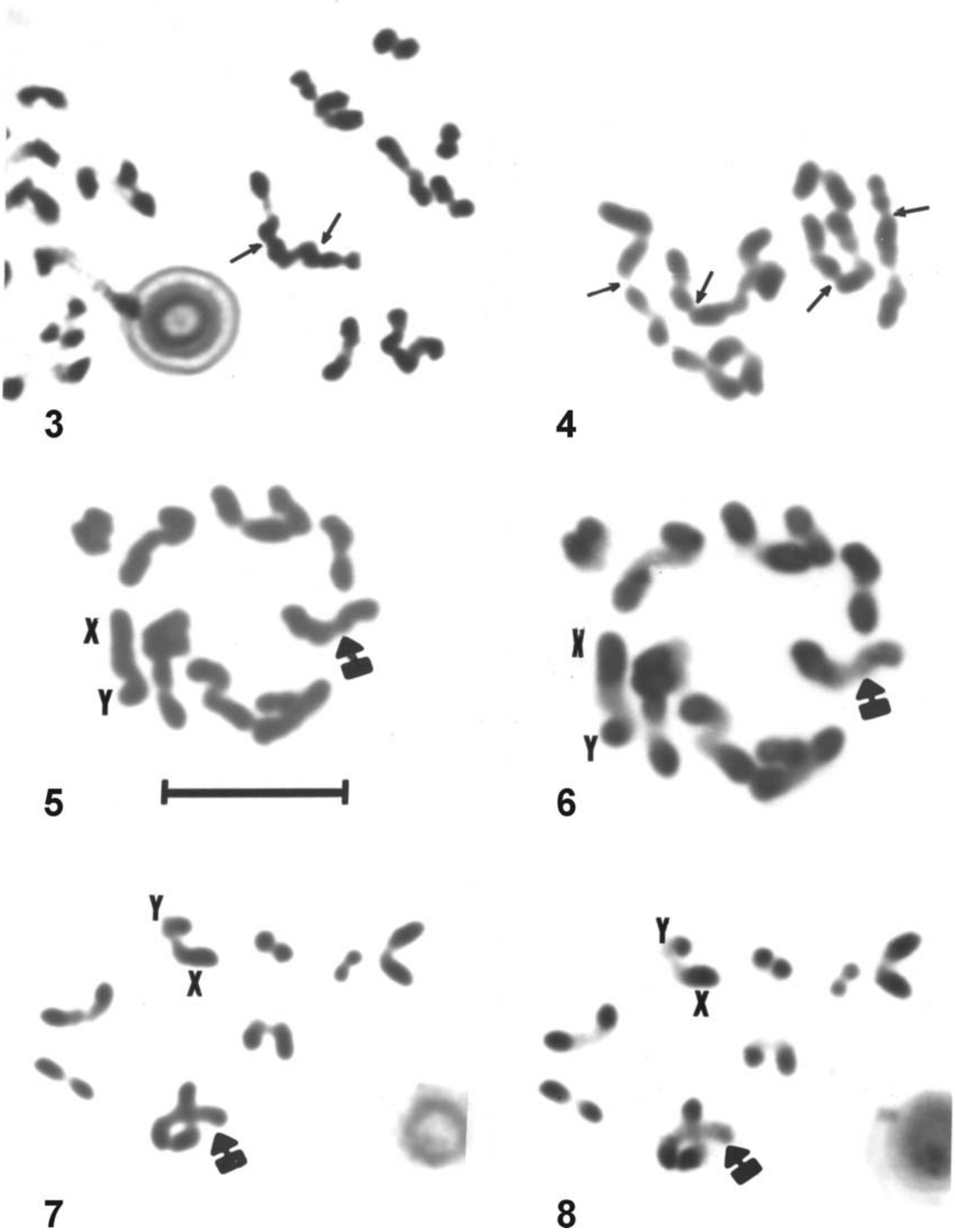
The differential staining between the homologues, with regards to the constitutive heterochromatin, has been reported by several authors. KING (1991) also observed the same phenomenon on amphibians and, according to this author, these pairs are heteromorphic due to an addition of heterochromatin to one of the elements of the pair.

In a given species, homologous chromosomes might differ not only in the number and location of the C-bands (qualitative difference) but also on the amount of heterochromatin in any given C-band (quantitative difference). The detection of both types of differences in the types of C-bands (qualitative and quantitative) depends on different factors, such as the extent of the variation and/or degree of chromosomal condensation. Since a measure of the amount of heterochromatin in a specific band is difficult to acquire and given that chromosomal contractions might lead to the apparent fusion of neighboring C-bands, only large differences can be detected by this method, resulting in inexact estimations of the C-band models (SANTOS & GIRÁLDEZ, 1982).

Although still rare in millipedes, the C-banding method has been applied in several animal species and it has rendered important clues regarding the evolutionary changes of the groups. The meaning of the heterochromatic fraction is one of the key points of current cytogenetical analyses (GUERRA, 1988).




Figs. 1, 2. Gonopods of *Urostreptus atrobrunneus* sp. nov.: 1, oral view; 2, caudal view. Bar = 1mm.




Figs. 3-8. Meiosis of *Urostreptus atrobrunneus* sp. nov.: 3–5, 7, staining with Giemsa. 6, 8, C-banding technique. Arrows in 3, 4 = associations between chromosomes; arrows in 5-8 = heteromorphic pair; X, Y = sexual pair. Bar = 10 µm.

Uncountable functions have been attributed to constitutive heterochromatin, including the protection of euchromatic regions (Hsu, 1975). However, the main functions credited to heterochromatin are the determination of the three-dimensional structure of the interphase nucleus and its possible effect, direct or indirect, on gene expression (Hilliker & Appels, 1980). In millipedes, several other studies will be needed in order to understand the role of constitutive heterochromatin in the karyotype, since most of the species studied up to now present over 50% of their genome composed of this type of chromatin.

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