

Cytogenetic Studies of *Glomeris* (Diplopoda: Glomeridae)

Elżbieta WARCHAŁOWSKA-ŚLIWA, Anna MARYAŃSKA-NADACHOWSKA and Grzegorz KANIA

Accepted January 27, 2004

WARCHAŁOWSKA-ŚLIWA E., MARYAŃSKA-NADACHOWSKA A., KANIA G. 2004. Cytogenetic studies of *Glomeris* (Diplopoda: Glomeridae). Folia biol. (Kraków) 52: 67-71.

Karyotypes and meiosis of *Glomeris hexasticha* and *G. connexa* (Diplopoda: Glomeridae) from Poland were described using C-heterochromatin distribution and observations of the location of NORs. These species were characterized by $2n \geq 16$ and the XY sex determination system. Differences were found in the amount of C-heterochromatin in X and Y chromosomes between the studied species. In *G. hexasticha*, supernumerary B chromosomes were described.

Key words: Diplopoda, Glomeridae, C-banding patterns, NOR, meiosis, B chromosomes, insect cytogenetics.

Elżbieta WARCHAŁOWSKA-ŚLIWA, Anna MARYAŃSKA-NADACHOWSKA, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.

E-mail: warchalowska@isez.pan.krakow.pl
maryanska@isez.pan.krakow.pl

Grzegorz KANIA, Department of Biology & Parasitology, Medical University, Radziwiłłowska 11, 20-080 Lublin, Poland.

E-mail: gakania@panaceum.am.lublin.pl

Millipedes (Diplopoda), constitute one of the largest classes within the Arthropoda, comprising about 10 000 species described in the world fauna (HOPKIN & READ 1992). Pill millipedes of the genus *Glomeris*, family Glomeridae, are represented by 8 species in Poland (WYTWER 1997) and 15 species in Central Europe (HOESS 2000). The two species studied in this paper, *Glomeris hexasticha* and *G. connexa*, live in the litter of mixed, leafy and coniferous forests in lowlands and mountains (STOJAŁOWSKA & STARĘGA 1974).

A description of karyotype variation, particularly in differences in chromosome number and morphology, allows for a description of the rate and mode of karyotype evolution. Karyotypes of some species belonging to various families have been described from Asia (ACHAR 1983, 1984, 1986, 1987; ACHAR & CHOWDAIAH 1979, 1980; CHOWDAIAH 1966 a,b,c, 1967, 1969; CHOWDAIAH & KANAKA 1974, 1979; TANABE 1992), Brazil (FONTANETTI 1987, 1990, 1991, 1996 a,b,c 1998, 2000; FONTANETTI *et al.* 2002), and Europe (VITTURI *et al.* 1997). The karyotype *Glomeris annulata* and *Glomeris marginata*, has been described for the Glomeridae family (BESSIERE 1948).

This paper provides information on the chromosome number, the C-banding pattern in meiotic

and mitotic chromosomes, the behavior of the nucleolus and NORs during spermatogenesis as well as notes on B chromosomes in *Glomeris hexasticha* Brandt, 1833 and *Glomeris connexa* C. L. Koch, 1847.

Material and Methods

Specimens of *G. hexasticha* were collected in 2000 and 2001 from Zemborzyce in the Lublin area, and *G. connexa* was collected in 2002 near Rogoźno in the Łęczna-Włodawa Lake District.

Cytological analysis of two species from Poland was performed for twenty five adult males and four females of *G. hexasticha* as well as ten males of *G. connexa*.

The testes and ovarioles were excised, incubated in hypotonic solution (0.9% sodium citrate for 20 min), fixed in ethanol:acetic acid (3:1), and squashed. Air dried preparations were then stained with C-banding by treatment with 0.2.N HCL for 20 min, immersed in a saturated solution of Ba(OH)₂ at 60°C for 5 min, rinsed in water, immersed in 2xSSC at 60°C for 60 min., rinsed, air dried, and stained with 2% Giemsa. A general spermatogenetic analysis was carried out using silver staining methods which permitted observation

of the nucleolar organizer regions (NORs). Usually, 3–4 day slides were treated with silver nitrate solution with gelatine and incubated at 60°C for about 10 minutes.

Results

The two analyzed species have a chromosome complement consisting of $2n=16$ and the XY (in the male) and XX (in the female) sex determination mechanism. Among seven pairs of autosomes, all chromosomes are meta- or submetacentric. In spermatogonial metaphase, it is apparent that in *G. hexasticha* the X chromosome is metacentric and larger than the medium sized metacentric Y (Fig. 1). However, in *G. connexa*, chromosomes X and Y are similar in size. The X chromosome is metacentric, whereas the Y is submetacentric or subacrocentric (Fig. 2).

C-heterochromatin in both species is characterised by the presence of only paracentromeric C-bands in all chromosomes (Figs 1 & 2).

The first two pairs of autosomes and the X chromosome of *G. hexasticha* show thick paracentromeric C-bands. These bands are doubled. Thin paracentromeric C-bands were found in five medium/small pairs of autosomes and also in the Y chromosome. Differences in amount of C-bands in one of the small pairs in most males were observed (Fig. 2).

The C-bands of *G. connexa* are characterised by the presence of blocks similar in size in all autosomes. The X chromosome has double C-bands, whereas the Y shows only a single C-band in the paracentromeric region (Fig. 2).

The meiotic behavior of chromosomes in males of both species is similar. The diffuse stage during the early first meiotic prophase, extending to early diplotene, is clearly visible. The heterochromatin detected by C-banding is highly condensed, and the number of C-bands agrees with the chromosome number in the karyotype (Figs 3 & 4). During diplotene, chromosomes remain partially dispersed, while positively heterochromatic elements with highly condensed heterochromatin are placed in the central parts of autosomes and sex chromosomes (Figs 5 & 6). One, two, or three nucleoli are attached to the X and Y, and two (or sometimes one) small autosomes are observed in both species. Spermatocyte diakinesis and metaphase I (MI) (Figs 7 & 8) include seven autosomal bivalents and the XY pair. Bivalents show one or two chiasmata in terminal or subterminal positions. In diakinesis of *G. hexasticha*, size differences in C-bands of X and Y chromosomes are present (Fig. 7).

Spermatogonial prometaphase shows two nucleoli attached to three or four chromosomes. Ag-

stained areas of NORs are present in early spermatogonial metaphase and in the meiotic prophase up to the pachytene (early diplotene) stage. In diplotene the X, the Y, and one or two chromosomes of the small pair contain nucleolar remnants attached near the centromeric region. From anaphase I up to metaphase II, no Ag-positive NOR was observed. The silver pattern of *G. hexasticha* sometimes includes an unpaired autosome, a NOR polymorphism may occur. This is connected with C-band polymorphism in one of the small bivalents sometimes observed in this species (Fig. 9 a-d).

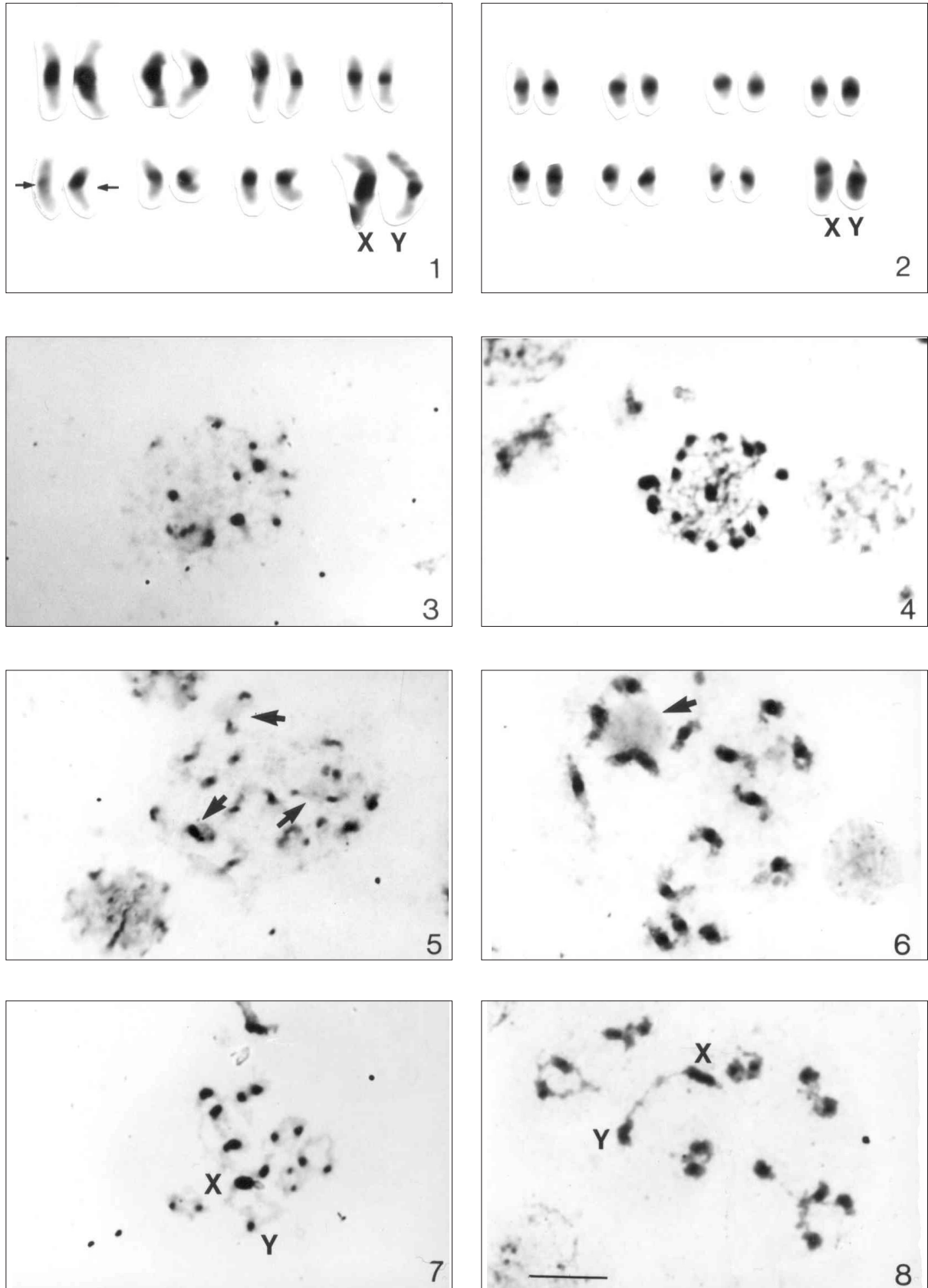
Three (out of twenty five) males of *G. hexasticha* show supernumerary Bs. They are banded and represent the smallest elements in the karyotype. They are mitotically and meiotically unstable as their numbers vary from 1 to 3 among cells within individuals. When two Bs are present, they remain as two univalents (25%) or as a bivalent (37%), when three Bs are observed they form either a bivalent and one univalent (7%) or three univalents (7%) in metaphase I, respectively (Fig. 10 a-c).

Discussion

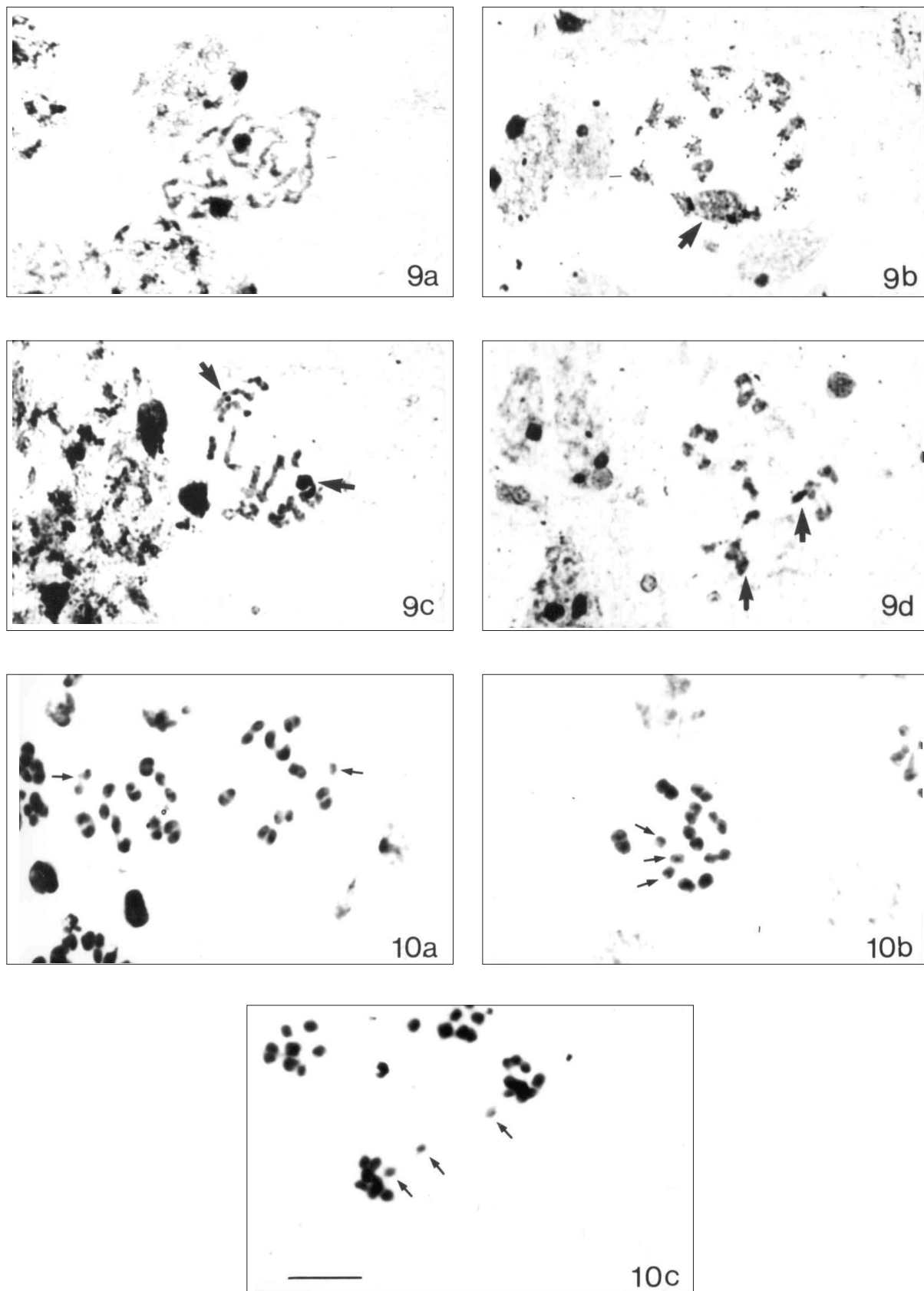
Published data show that only 0.1% of Diplopoda species have been cytogenetically studied (FONTANETTI *et al.* 2002). In Diplopoda, chromosome numbers range from $2n=12$ to 30, and the XY (males) and XX (females) type of sex determination occurs in the majority of species studied (ACHAR 1987; FONTANETTI *et al.* 2002; WHITE 1979). The $2n=16$ and the XY sex chromosome mechanism, observed in two species of *Glomeris* described in this paper, has been found in eight other millipede species belonging to five families (ACHAR 1987; CHOWDAIAH & KANAKA 1979; FONTANETTI 1996a, 1998, 2000; SOKOLOFF 1914; TANABE 1992).

The majority of the analysed species of Diplopoda are characterized by little or no heteromorphism in chromosomes of the sex pair after standard staining (ACHAR 1983; FONTANETTI 1991, 1996a,b, 1998, 2000; TANABE 1992). However, the use of C-banding techniques in *G. connexa*, having X and Y chromosomes of the same size, provided a possibility for identification of both sex chromosomes.

Formerly, the C-banding and Ag-staining techniques, fluorochromes, and FISH methods, allowing precise identification of particular chromosomes in Diplopoda, were used only in one Indian species (ACHAR & CHOWDAIAH 1980) and two species from Italy (VITTURI *et al.* 1997). The patterns of C-heterochromatin distribution in *G. hexasticha* and *G. connexa* were similar and restricted only to the centromeric region. However, the size



Figs 1-8. Figs 1, 3, 5, 7 *G. hexasticha*. Figs 2, 4, 6, 8 *G. connexa*; Figs 1-2. C-banded karyotype of mitotic metaphase of male. Differences in size between the homologue C-bands in one of small pair were observed (Fig. 1 – arrows); Figs 3-4. Diffuse stage; the number of C-bands are connected with chromosome numbers. The differences in size of C-banding between two species are visible; Figs 5-6. Diplotene, chromosome undergo partial dispersion. Nucleolus containing X, Y chromosomes and one or two chromosomes from small bivalent are visible (arrows). Figs 7-8. Metaphase I with seven bivalents and XY chromosomes. Bar = 10 μ m.



Figs 9-10. Fig. 9 a-d. *G. connexa*: a – pachytene, b – diplotene, c – metaphase spermatogonial, d – metaphase I with NORs (arrows). Fig. 10 a-c. *G. connexa* – B chromosomes (arrows). Bar = 10 μ m.

and shape of C-blocks were different in these species. In *G. connexa*, centromeric bands were almost identical in all autosomes. This corresponds with the observation of ACHAR & CHOWDAIAH (1980) on *Carlogonus acifer*.

The post-pachytene diffuse stage observed in prophase of *Glomeris* and other species of Diplopoda (ACHAR 1984, 1985, 1986; ACHAR & CHOWDAIAH 1979; CHOWDAIAH & KANAKA 1979; FONTANETTI 1990, 1996 a,b,c; SHARMA & HANDA 1974) is also common in beetles and coincides with the diplotene growth period (SMITH & VIRKI 1978; ROZEK 1988).

Supernumerary chromosomes were observed in Diplopoda for the first time in the Brazilian species *Plusioporus setiger* from the order Spirostrepida (FONTANETTI 1998), however, its size and morphology were not described. The B chromosome, observed in *G. hexasticha*, was mitotically and meiotically unstable. This leads to the production of gametes with different numbers of Bs in the same individual. The origin of the B is not clear in *Glomeris*. The B was a little smaller in size than the smallest autosomes and had the same condensation after diffuse stage as in autosomes and sex chromosomes. Numerous hypotheses on the origin of B chromosomes based on cytological analysis of morphology, C-heterochromatin distribution, and natural polymorphism have been suggested (for a review see CAMACHO *et al.* 2000).

The cytological data presented in this paper for the genus *Glomeris* contribute to the knowledge of the evolution and cytotaxonomy of the family Glomeridae and create a basis for further studies of other species of this European genus.

References

- ACHAR K. P. 1983. Karyological studies in nine species of Indian Diplopoda (Myriapoda). *Nucleolus* **26**: 191-197.
- ACHAR K. P. 1984. Analysis of male meiosis in five species of Indian Diplopoda (Myriapoda). *Caryologia* **37**: 373-386.
- ACHAR K. P. 1986. Analysis of male meiosis in seven species of Indian Diplopoda (Myriapoda). *Caryologia* **39**: 145-155.
- ACHAR K. P. 1987. Chromosomal evolution in Diplopoda (Myriapoda: Arthropoda). *Caryologia* **40**: 145-155.
- ACHAR K. P., CHOWDAIAH B. N. 1979. The use of air drying in the study of diplopod chromosomes. (In: Myriapod Biology, Camatini M. ed., Academic Press, London): 21-23.
- ACHAR K. P., CHOWDAIAH B. N. 1980. The use of C-banding technique in the chromosome studies of millipede species *Carlogonus acifer*. *Caryologia* **33**: 185-191.
- BESSIERE Cl. 1948. La spermatogenese de quelques Myriapodes. *Arch. Zool. Exp. Gen.* **85**: 149-236.
- CAMACHO J. P. M., SHARBEL T. F., BEUKEBOOM L. W. 2000. B chromosome evolution. *Philos. Trans. R. Soc. Biol. Sci.* **335**: 163-178.
- CHOWDAIAH B. N. 1966a. Cytological investigations of some Indian Diplopoda - I (Myriapoda). *Nature* **210**: 847.
- CHOWDAIAH B. N. 1966b. Cytological studies of some Indian Diplopoda - I (Myriapoda). *Cytologia* **31**: 294-301.
- CHOWDAIAH B. N. 1996c. Chromosome studies in two species of pill-milipedes (Diplopoda-Myriapoda). *Caryologia* **19**: 135-141.
- CHOWDAIAH B. N. 1967. A note on the abnormal sex chromosome behavior in the male of *Thyropygous* species. *Curr. Sci.* **36**: 105-106.
- CHOWDAIAH B. N. 1969. A note of the occurrence of chromatid bridges in the male of *Anthrosphaera* sp., a pill-millipede. *Curr. Sci.* **38**: 169-170.
- CHOWDAIAH B. N., KANAKA R. 1974. Cytological studies in six species of pill-milipedes (Diplopoda-Myriapoda). *Caryologia* **27**: 55-64.
- CHOWDAIAH B. N., KANAKA R. 1979. Chromosome cytology of seven species of Indian Diplopoda (Myriapoda). (In: Myriapod Biology, Camatini M. ed. Academic Press, London): 9-20.
- FONTANETTI C. S. 1987. Estudo citotaxonomico em especies brasileiras de Diplopoda. Masters Thesis, Instituto de Biociencias, UNESP.
- FONTANETTI C. S. 1990. Meiotic prophase in Diplopoda. *Rev. Brasil. Genet.* **13**: 697-703.
- FONTANETTI C. S. 1991. Karyotypes of some Brazilian diplopods. *Rev. Brasil. Genet.* **14**: 645-651.
- FONTANETTI C. S. 1996a. Description of a new species and the karyotype of the cavernicolous millipedes *Pseudonannolene strinatii* Mauries, 1974 (Diplopoda, Pseudonannolenida, Pseudonannolenidae). *Rev. Brasil. Zool.* **13**: 419-426.
- FONTANETTI C. S. 1996b. The use of cytogenetics to certify a diplopoda species (Pseudonannolenida, Pseudonannolenidae). *Rev. Brasil. Biol.* **56**: 775-781.
- FONTANETTI C. S. 1996c. Karyotypes of a termitophilic species of Diplopoda (Polydesmida, Chelodesmidae). *Brazilian J. Genetics.* **19**: 593-595.
- FONTANETTI C. S. 1998. Chromosome numbers of some Brazilian species of Diplopods (Diplopoda, Arthropoda). *Cytologia* **63**: 149-154.
- FONTANETTI C. S. 2000. Description and chromosome number of a species *Pseudonannolene silvestri* (Arthropoda, Diplopoda, Pseudonannolenidae). *Rev. Brasil. Zool.* **17**: 187-191.
- FONTANETTI C. S., CAMPOS K. A., PRADO R. A., DA SILVA SOUZA T. 2002. Cytogenetic studies in Diplopoda. *Cytologia* **67**: 253-260.
- HOESS R. 2000. Bestimmungsschlüssel für die Glomeris-Arten Mitteleuropas und angrenzender Gebiete (Diplopoda: Glomeridae). *Jahrb. Naturhist. Mus. Bern.* **13**: 3-20.
- HOPKIN S. P., READ H. J. 1992. *The Biology of Millipedes*. Oxford University Press.
- ROZEK M. 1988. Karyotype in four species of *Amara* (Coleoptera, Carabidae). *Caryologia* **41**: 1-8.
- SHARMA G. P., HANDA S. M. 1974. Comparative cariological studies on *Gonoplectus malayus* and *Phyllogonostreptus nigrolabiatus* (Diplopoda, Myriapoda). *Cytologia* **39**: 673-680.
- SMITH S. G., VIRKKI N. 1978. *Animal Cytogenetics*. 3. Insecta. 5. Coleoptera. Gebruder Birntraeger, Berlin, Stuttgart.
- SOKOLOFF J. 1914. Über die Spermatogenese bei *Polyxenus* sp. *Zool. Anz.* **44**: 558-566.
- STOJAŁOWSKA W., STAREGA W. 1974. Diplopoda of Poland. *Catalogus faunae Poloniae*. Institute of Zoology, Warszawa **14**: 1-71.
- TANABE T. 1992. Karyotypes of four Xystodesmid millipedes from Japan. *Acta Arach.* **41**: 87-90.
- VITTURI R., COLOMBA M. S., CAPUTO V., SPARACIO I., BARBIERI R. 1997. High heterochromatin content in somatic chromosomes of two unrelated species Diplopoda (Myriapoda). *Chromosome Res.* **5**: 407-412.
- WHITE M. J. D. 1979. The present status of Myriapod Cytogenetics. (In: Myriapod Biology. M. Camatini ed. Academic Press, London): 3-8.
- WYTWER J. 1997. Diplopoda (In: Checklist of Animals of Poland. J. Razowski ed. Polish Academy of Sciences, Institute of Systematics and Evolution of Animals) **IV**: 268-272.