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Genome instability of *Chironomus riparius* Mg. and *Chironomus piger* Strenzke (Diptera, Chironomidae)

Ilkova[1] Julia, Thomas Hankeln[2], Erwin R. Schmidt[2], Paraskeva Michailova[1*], Ninel Petrova[4], Gabriella Sella[3] and Keith White[5]

[1] Institute of Zoology, Bulgarian Academy of Sciences, Boulv. Tzar Osvoboditel 1, Sofia 1000, Bulgaria.
[2] Johannes Gutenberg Universität, Institut für Molekulargenetik, J.J. Becherweg 32, D-55099 Mainz, Germany.
[3] Department of Animal and Human Biology, University of Turin, Via Accademia Albertina 13, 10123 Turin, Italy.
[4] Institute of Zoology, Russian Academy of Sciences, ul. Universitetskaya 1, 199034 St. Petersburg, Russia.
[5] Faculty of Life Sciences, University of Manchester, Manchester, UK.

Abstract — Intra and interspecific variation was evaluated in two Bulgarian populations (Pancharevo and Kokalijane) of the two sibling and homosequential species *Chironomus riparius* Mg. and *Chironomus piger* Strenzke, by analyzing structural and functional alterations in salivary gland polytene chromosomes. In both species genome instability was demonstrated, which was expressed by structural and functional somatic chromosomal alterations. In the *C. riparius* population from Pancharevo, living in sediments containing high concentrations of Cu, Pb and Zn, salivary gland cells containing somatic rearrangements appeared at a significantly higher frequency (51.92%) than in the Kokalijane *C. piger* population, living in heavy metal-unpolluted sediments (21.3%). In the *C. riparius* population, somatic aberrations were distributed at different points along the chromosomes while in the *C. piger* population, somatic rearrangements were concentrated in the pericentromeric regions of chromosomes CD, EF and in proximal part of arms D and F. At the cytological level using FISH analysis, both species can be identified also by the different location of tandem-repetitive minisatellite DNA clusters of Alu and Hinf families, and insertion sites of the LINE retrotransposon NLRCTh1. While location of the former is fixed, NLRCth1 appeared to have both fixed and inter-individually variable positions in both species. On average, 19.0 ± 9.5 insertions of NLRCth1 per individual were observed in *C. riparius* and 5.57 ± 2.09 in *C. piger.* In both species locations of minisatellite DNA clusters, NLRCth1 retrotransposons and aberration breakpoints concentrated in proximal regions of chromosomes, and the majority of the breakpoints were located in sections containing blocks of repetitive DNA clusters.

Key words: *Chironomus*, chromosomal aberrations, insertion sites, minisatellite, NLRCth1 retrotransposon, polytene chromosomes, repetitive DNA.

INTRODUCTION

Chironomus piger and *Chironomus riparius* (syn. *Chironomus thummi*) are closely related, morphologically nearly indistinguishable species (Keyl and Strenzke 1956). Being homosequential, they are differentiated at the cytological level only by the amount of DNA and appearance of the constitutive heterochromatin (Keyl 1965a; Michailova and Petrova 2004). Until recently, both species were considered as nearly monomorphic chromosomally (Michailova 1989). However, Michailova *et al.* (1996; 1998; 2000) and Sella *et al.* (2004) found that nearly two hundreds types of structural and functional somatic chromosome aberrations were present in five hundred *C. riparius* larvae from trace metal polluted sites. Bovero *et al.* (2002) hypothesized that in *C. riparius* genome instability by spontaneous or induced chromosomal breaks occurs more frequently in sections containing blocks of repetitive DNA composed of AT-rich minisatellite DNA (Schaefer and Schmidt 1981; Schmidt 1984; Hankeln *et al.* 1994), or copies of transposable elements (Wobus *et al.* 1990; Blinov *et al.* 1993). The sibling species *C. piger* contains far lower numbers of repetitive DNA clusters (Schaefer and Schmidt 1981; Hankeln *et al.* 1994), and its genome size is 30% lower than that of *C. riparius* (Keyl 1965b).

* Corresponding author: phone: (359) 2 988 5115; fax (359) 2 988 28 97; e-mail: michailova@zoology.bas.bg

Currently, very limited information is available on the genome instability of natural populations of *C. piger* (POLUKONOVA *et al*. 1996). HÄGELE (1984) found that chromosomes of both species showed spontaneous somatic aberrations in 10% of hybrids from crosses between males of *C. riparius* and females of *C. piger,* pointing at some degree of destabilization of the genome under hybridization conditions. GUNDERINA and AIMANOVA (1998) found that γ-ray treatments induced many breaks in the centromere regions of *C. riparius* larvae, but none in centromere regions of *C. piger.*

The aim of this study is to examine the degree of genome instability of *C. piger* and *C. riparius* collected from two locations in Bulgaria, which are characterized by different levels of trace metal pollution in their sediments. We analyzed type, localization and frequency of somatic alterations along the salivary gland chromosomes of both species. We also examined the location of two general types of repetitive DNA, i.e tandem-repetitive minisatellite clusters (Alu and Hinf elements) and a LINE-type retrotransposon (NLRCth1; BLINOV *et al*. 1993; ZAMPICININI *et al.* 2004), along the chromosomes of each species, by means of fluorescent in situ hybridization (FISH). We hypothesized that the comparatively larger amount of heterochromatin and repetitive DNA within the *C. riparius* genome may correlate with a propensity towards elevated levels of chromosomal rearrangements, as compared to *C. piger*.

MATERIALS AND METHODS

Nineteen larvae of *C. riparius* from a livestock drinking trough at Pancharevo, known to be heavy metal polluted (SELLA *et al.* 2004), and 27 larvae of *C. piger* from Kokalijane on the River Vadena, near Sofia (Bulgaria), were fixed in alcohol-glacial acetic acid (3:1). Chromosome preparations and phase identification of IVth larval stage were done according to MICHAILOVA (1989) and WÜLKER and GÖTZ (1968) respectively. Differential "C" banding methodology according to MICHAILOVA (1987) was used in order to identify each species. Data concerning structural and functional chromosome rearrangements in *C. riparius* have been taken from PETROVA *et al.* (2004). Since *C. piger* has the same chromosomal banding pattern of *C. riparius*, for detailed cytogenetical analysis in the former species we employed the standard chromosome maps by HÄGELE (1970) and KIKNADZE *et al.* (1991). In both species, levels of puffing of the Balbiani rings (BRc and BRb) and the nucleolar organizer (NOR) regions of chromosome G were scored according to BEERMANN (1971) from a sample of 208 salivary gland cells from *C. riparius* and 379 cells from *C. piger*. Fluorescent in-situ hybridization of probes of the NLRCth1 retrotransposon and the Alu and Hinf minisatellite DNA clusters was performed following the protocols of HANKELN *et al.* (1994) and SCHMIDT (1992). Four preparations of *C. riparius* and seven of *C. piger* respectively were analyzed for location of NLRCth1 retrotransposon copies and three of both species for location of Alu and Hinf clusters.

Somatic chromosomal aberrations in our analysis are defined as aberrations, which appear in only one cell or a clonal cell lineage within a salivary gland. "Common breakpoints" of chromosome rearrangements are defined according to BOVERO *et al.* (2002) as those occurring in more than one larva. The retrotransposon copies occurred in fixed and variable states. Fixed insertions are those which appeared as labeled in all cells of all studied individuals. Variable insertions are those, which occurred either in homozygous or heterozygous state not in all individuals.

The chromosome arms of both species were divided into proximal and distal regions according to BOVERO *et al.* (2002). We checked whether frequencies of rearrangements and repetitive DNA clusters were randomly distributed in the distal and proximal regions by comparing their frequency in the two regions by the G-test. Correlation between frequencies of repetitive DNA clusters and breakpoints in distal and proximal regions was estimated by the Spearman correlation coefficient.

Data on the concentration of trace metals in Pancharevo sediment are taken from SELLA *et al.* (2004). Concentration of the trace metals Al, Cr, Cu, Fe, Mn, Pb and Zn at Kokaljiane were measured according to MICHAILOVA *et al.* (2003). Sediment data of FORSTNER and SALOMONS (1980) from unpolluted sites were taken as a reference.

RESULTS

Concentration of trace metals in Pancharevo and Kokalijane sediments - According to the review of FORSTNER and SALOMONS (1980) concentrations of Pb, Cu and Zn in Pancharevo sediment are respectively 27, 2 and 2 times higher than in fossil sediments. In contrast, heavy metal concentrations in Kokalijane sediments are indicative of an unpolluted site.

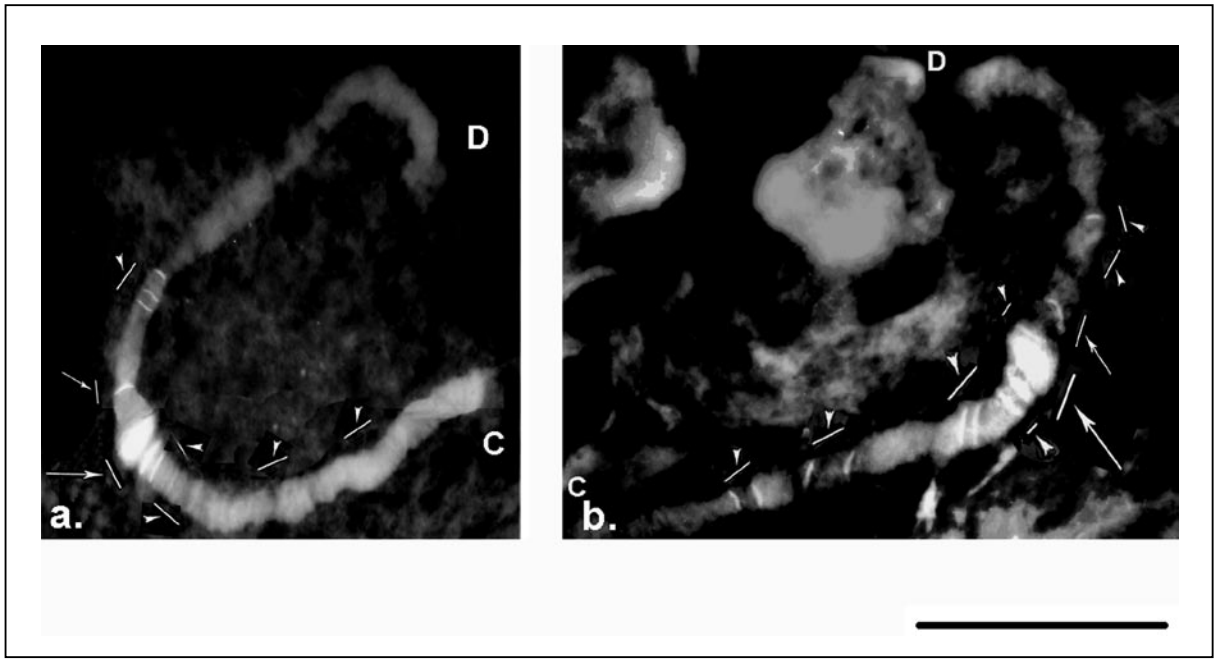

Fig. 1 — *C. riparius*. NLRCth1 fixed and variable FISH signals in Chromosome CD of two individuals. Short arrow: variable signal, long arrow: fixed signal. **a**. In arm C one variable signal (C2h) in the pericentromeric region; the other three short arrows indicate 6 NLRCth1 sites which are unoccupied in this individual and occupied in individual of figure 1b. In arm D, two fixed signals (in the centromere and at site C4e) and three variable signals (C6g, C6i, D1d). **b**. in Arm C, seven variable signals (C2h, C1j, C1f, B2a, B3h, B5a, B5m). In arm D, two variable signals (D1d and C4c). Signals at sites C6g and C6i, present in the individual of fig.1a, here are absent.

Cytogenetic characteristics of C. riparius and C. piger - Both species have a chromosome set of 2n = 8 that belongs to the *"thummi"* cytocomplex, with chromosome arm combinations AB, CD, EF, G. Three BRs are present (BRa, BRb, BRc), one of which (BRa) is active only in few cells of the salivary glands of both species. BRs and NOR are localized in chromosome G. Both species were distinguished by the appearance of a species-specific pattern of the constitutive heterochromatin. In *C. piger* constitutive heterochromatin is localized in the centromere region only, while in *C. riparius* there are additional intercalary heterochromatin sites.

Genome instability in C. riparius and C. piger: structural chromosome rearrangements and functional activity of BRs and NOR - In *C. piger* we identified one inherited and 16 different types of somatic rearrangements (Table 1), which are mainly in arms D and F. In *C. riparius* 29 different types of somatic heterozygous inversions, distributed in all arms were observed (PETROVA et al. 2004). Homozygous somatic deletions of BRc and BRc + BRb affected chromosome G of both species, with higher frequency in *C. riparius* (16.8%) compared to *C. piger* (7.39%). In *C. piger* one case of a deletion of BRb was also observed. The chromosome G was converted into a "pompon" chromosome (MICHAILOVA *et al.* 1998) in 5.8% of cells of *C. riparius* and in 3.9% of cells of *C. piger* (Fig. 2a, b).

The frequency of somatic aberrations observed in the *C. riparius* genome (51.9%) was significantly higher than that observed in *C. piger* (21.3%) ($G = 61.923$, $df = 1$, $P<0.001$). Six out of a total of 92 aberration break points (60 in *C. riparius* and 32 in *C. piger*) were shared by both species. One third of breakpoints were considered as common *sensu* BOVERO *et al.* (2002) in both species.

The high level of activity of NOR (++/++) was significantly more frequent in *C. riparius* than in *C. piger,* while the intermediate state (+/+) was significantly more frequent in *C. piger* compared to *C. riparius* (Fig. 3a). The intermediate level of activity (+/+) of BRc/BRb was significantly more frequent in *C. riparius* than in *C. piger* while the (+/-) and (-/-) activity levels of BRc/BRb were significantly more frequent in *C. piger* compared to *C. riparius* (Fig. 3b) ($P<0.05$).

Table 1 — Somatic aberrations in *C.piger* from Bulgaria (Kokaljane)

n - individuals 27
n_1 - cells 379

Aberration	Arm	Localization	number individ. (n)	%	number cells (n_1)	%
Het. inv. paracentric	B	E2g – E2m	1	3.70	1	0.26
Pericentric inversion	AB	D1k – D3a	10	37.03	20	5.28
Het. inv. paracentric	C	B5a – B5i	2	7.40	2	0.52
Het. inv. paracentric	D	D1d – D3a	1	3.70	1	0.26
		D2a – D2g	3	11.11	3	0.79
		C4a – C4g	1	3.70	1	0.26
		C5a – C5f	1	3.70	1	0.26
Pericentric inversion	CD	C3a – C3i	1	3.70	1	0.26
Het. inv. paracentric	E	A3e – A4e	1	3.70	1	0.26
Het. inv. paracentric	F	B3a – B3o	3	11.11	6	1.58
		B3b – B3h	2	7.40	2	0.52
		B3d – B3k	2	7.40	7	1.85
Pericentric inversion	EF	B2f – B2o	1	3.70	1	0.26
Het. Inv.	G	A1b – A1e	3	11.11	5	1.32
Deletion	G	BRc	3	11.11	5	1.32
Deletion	G	BRc+BRb	10	37,07	23	6.07
Deletion	G	BRb	1	3.70	2	0.52

Localization of Alu and Hinf clusters and NLRCth1 copies in C. riparius and C. piger - In the *C. riparius* population from Pancharevo we established 34 hybridization signals of Hinf and 22 of Alu minisatellite clusters in all chromosomes apart from chromosome G (Fig.4). In the *C. piger* population from Kokalijane 38 sites were recorded for Hinf and only one for Alu clusters (Fig.5). All the Hinf and Alu insertion sites were fixed in both species. Fifteen Hinf clusters were shared by both species.

The retrotransposon NLRCth1 appeared to have both fixed and variable positions in both species. Fifteen out of 76 of NLRCth1 signals were fixed in *C. riparius* chromosomes and sixteen out of 39 in *C. piger.* On average, 19.0 ± 9.5 insertions per individual were observed in *C. riparius* and 5.57 ± 2.09 in *C. piger*.

Fixed NLRCth1 signals were recorded for both species in the centromeric and pericentromeric regions of the chromosomes AB, CD and EF and in a few additional sites (Fig. 4 and Fig. 5). In *C. riparius* such fixed insertion sites occurred in arm A (D1d), in arm B (D3d, D3h, E3e), in arm C (C2l), in arm D (C4e), in arm E (B2h), in arm F (B2m) and in chromosome G (A2b). In *C. piger* they were found in arm A (the pericentromeric and centromeric regions D2a, D2b, D2c, D2d), in arm B (D3a, D3h), in arm C (the pericentromeric and centromeric regions C2l, C3c, C3g, C3h), in arm D (C4a, C4b, C4e) and in arm E (the pericentromeric and centromeric regions B2j and B2k). Species-specific fixed sites are eight in *C. piger* and seven in *C. riparius*. Twelve sites are shared by both species (in arm A, band D1d, in arm B bands D3d, D3h, E1c, F3b, in arm C bands B5a, C2l, C3c, in arm D band C4e, in arm E bands B1r and B2k, in arm G band A2b). Six of them are fixed sites in both species; three are fixed in *C. riparius*

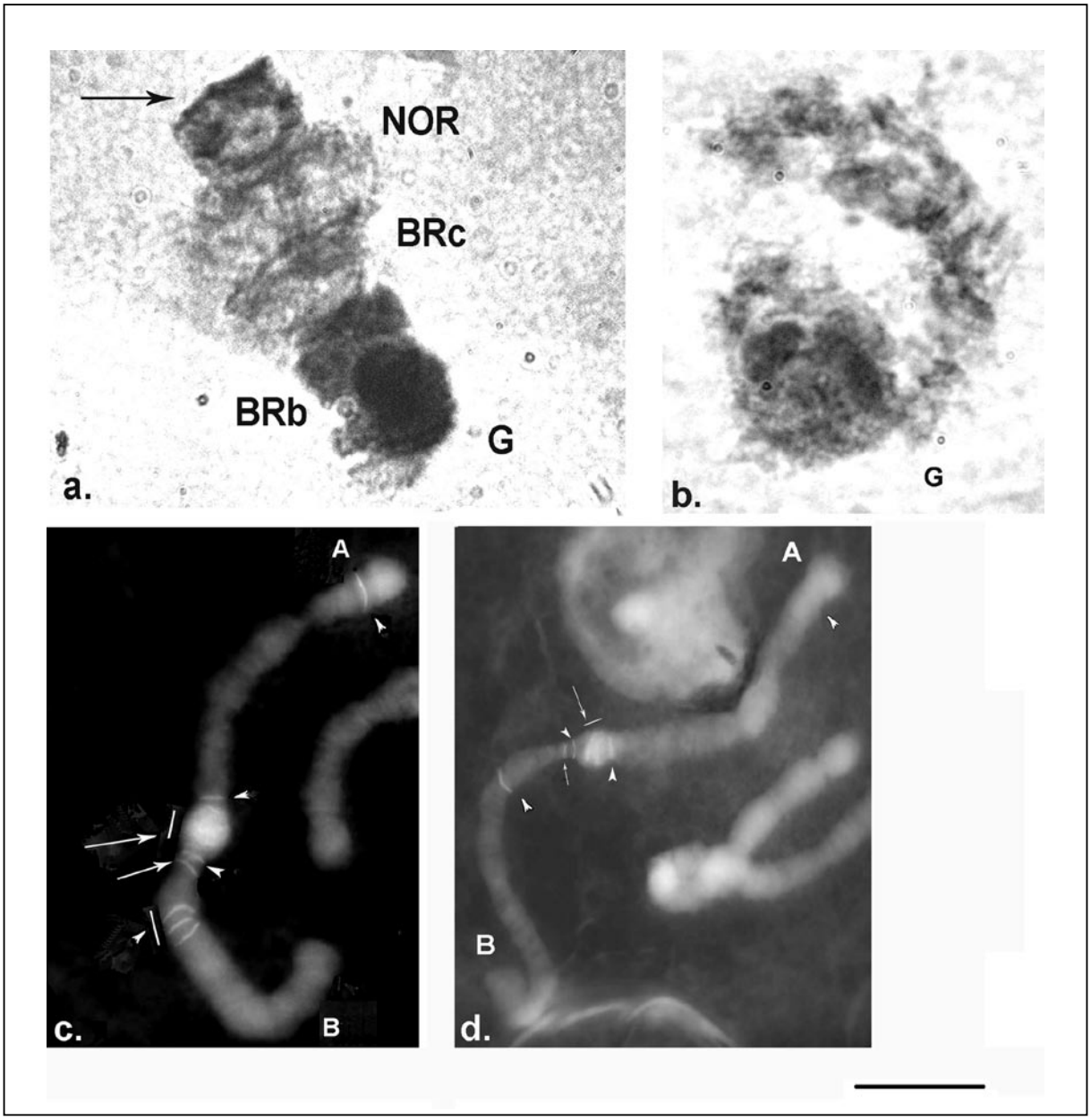

Fig. 2 — *C.piger*. **a**. Standard chromosome G. NOR: nucleolar organizer; BRb and BRc: Balbiani rings; **b**. "Pompon" chromosome G with deletions of Balbiani rings BRc and BRb. **c**. Chromosome AB with centromeric and pericentromeric fixed and variable NLRCth1 signals. In arm A two variable signals (A2h and D1d) can be seen. In arm B four variable signals (D3d, E1c, E3i, E3l) are visible. **d**. Another chromosome AB from the same individual of fig.2c. In arm A the signal of the variable site D1d can be seen, while the signal of the variable site A2h is very faint and not clearly visible. In arm B two only out of four variable signals (D3d and E3i) are visible.

and variable in *C. piger* (sites D1d in arm A, D3d in arm B and A2b in arm G).

All the other signals appeared to be variable and were much more frequent in *C. riparius* than in *C. piger*. In the four *C. riparius* larvae 11 variable insertions occurred at the same sites in at least two larvae (on arm A at the site C4c, on arm B at the site E1a, on arm C at the sites C2h (Fig. 1a), C1j, B2a, B3h, B5m (Fig. 1b) and on arm D at the sites C5a, C6g, C6i and D1d (Fig. 1a)). All the other variable signals were seen in only one larva and often not in all cells, indicating additional somatic instability. In the *C. piger* sample, half of the variable insertions occurred in the examined cells

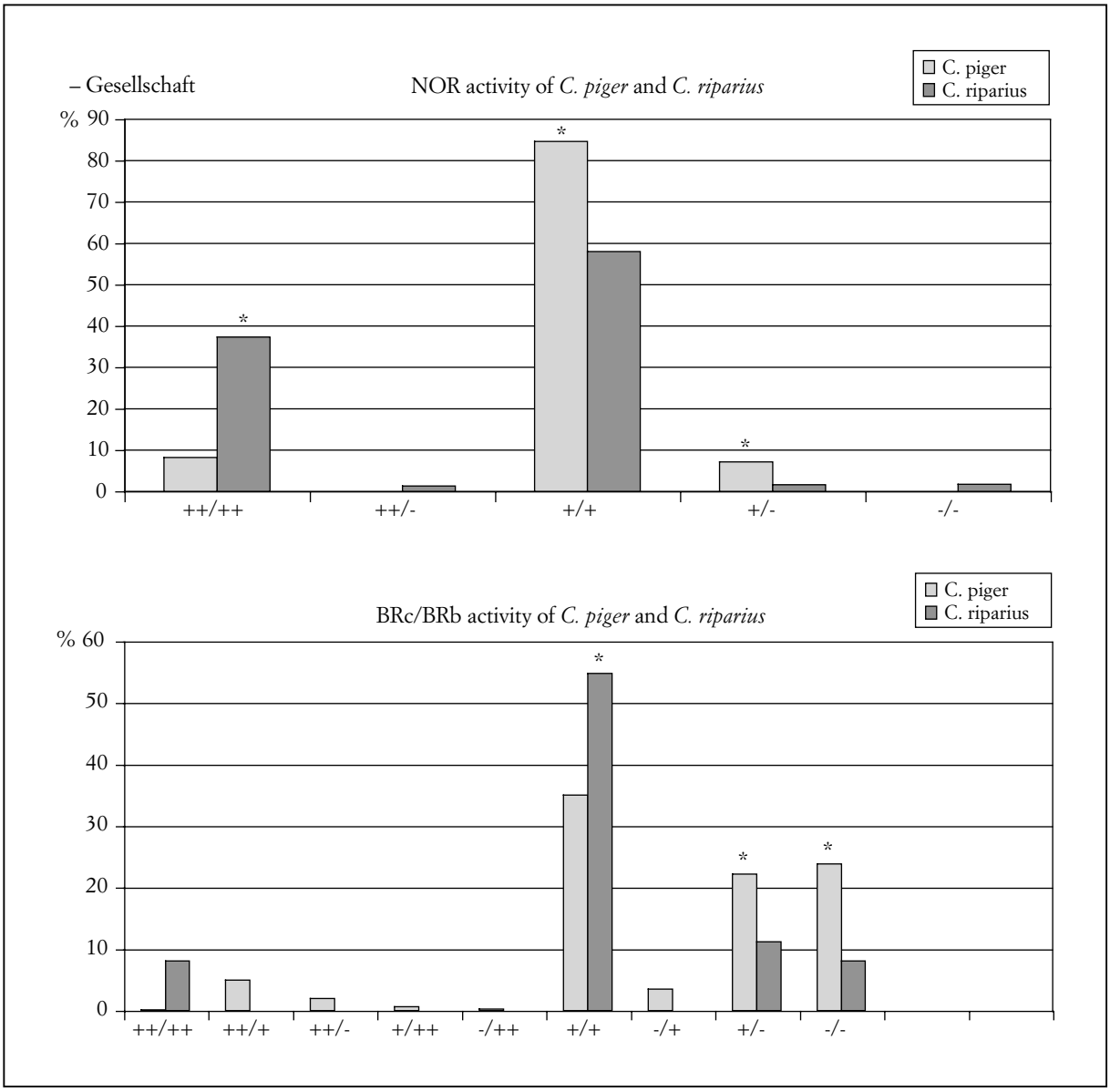

Fig. 3. **a** — Activity of NOR and BRc/BRb –, Activity of NOR in *C. riparius* and *C. Piger*. ++/++: high activity of NOR of both homologues; ++/-: high activity (++) in one homologue, no activity (-) in other homologue; +/+: intermediate activity of both homologues; +/-: intermediate (+) activity in one homologue, no activity (-) in other homologue; -/-: no activity of both homologues; *: significant difference in activity of the homologues between both studied species (P<0.05).
The activity of both homologues (++/++) of *C. riparius* is significant higher in comparison with those of *C. piger*; intermediate activity (+/+) of both homologues of *C. piger* is significant higher in comparison with *C. riparius*; the state (+/-) of both homologues of *C. piger* is significant higher than those of *C. riparius*;
b — Activity of BRc/BRb in *C. riparius* and *C. Piger*: ++/++: the high activity of BRc and BRb of both studied species; ++/+: the high activity of BRc and intermediate activity of BRb of both studied species; ++/-: the high activity of BRc and no activity of BRb of both species; +/++: the intermediate activity of BRc and high activity of BRb of both species; -/++: no activity of BRc and high activity of BRb of both species; +/+: intermediate activity of BRc and BRb of both species; -/+: no activity of BRc and intermediate activity of BRb of both species; +/-: the intermediate activity of BRc and no activity of BRb of both species; -/-: no activity of BRc and BRb of both species; * significant difference between the two species (P<0.05): the state of BRc/BRb (+/+) is significant higher in *C. riparius* in comparison with those of *C. piger.*
The states of BRc/BRb (+/-) and BRc/BRb (-/-) are significant higher in *C. piger* in comparison with those of *C. riparius*.

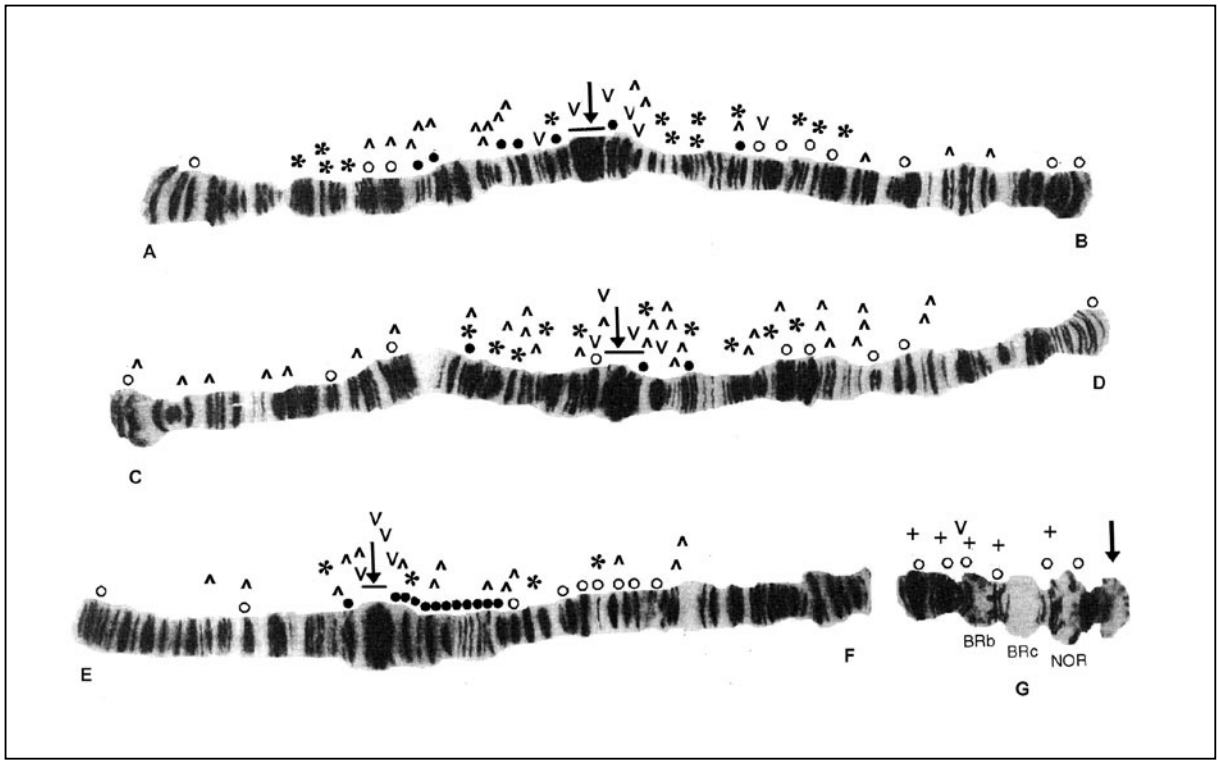

Fig. 4 — Standard chromosome map of *C. riparius* (by KIKNADZE *et al*. 1991): the localization of retrotransposon NLRCth1 appeared in two states along the chromosomes of *C. riparius* – fixed and variable. V: indicated the fixed signals of NLRCth1 along the chromosomes; ^: indicated the variable signals of NLRCth1 along the chromosomes. "Common" breakpoints – these are the breakpoints of aberrations which occurred more than once in larvae of the studied population. They are indicated along the chromosomes by "*". • — indicated Alu clusters. Alu is the repetitive DNA clusters; o — indicated Hinf clusters. Hinf is the repetitive DNA clusters; +: break points of the observed deletions in the studied population.

of more than one individual (sites D1d (Fig. 2c, d) on arm A; sites D2e, D2f, D3d, E1c, E3i, E3l and F3b on arm B; site B5a on arm C; site B1r on arm E; sites A2b and E2e of arm G)). In this species too, the remaining variable signals could be seen only in a single individual and often not in all cells of the same salivary gland.

Repetitive DNA clusters, including NLRCth1 loci, (G test, G = 10.457, P<0.001) as well as breakpoints (G test, G = 12.32, P<0.001) were not randomly distributed in *C. piger*. They concentrated in the proximal part of the chromosomes. The same tendency was observed in *C. riparius* (for breakpoints: G = 9.144, P<0.001; for repetitive DNA clusters: G = 40.145, P<0.001). In both species the breakpoints of deletions on chromosome G occurred at sites A2b and B/C at sites where the Hinf clusters were localized (Fig.s 4 and 5).

The association between proximal or distal location of inversion breakpoints and sites of NLRCth1, and Alu and Hinf clusters was significant for both *C. piger* (Spearman correlation, $r_s = 0.584$, $P<0.05$), and *C. riparius* (Spearman correlation, $r_s = 0.615$, $P<0.05$).

DISCUSSION

C. riparius and *C. piger* are sibling species which have been shown to be significantly different with regard to the amount and distribution of tandem repetitive elements (SCHAEFER and SCHMIDT 1981; SCHMIDT 1984). The results obtained in this study show that both species can be identified by the location of specific and easily established molecular markers, specifically the Alu and Hinf minisatellite clusters. The locations of Hinf and Alu repeats in the *C. riparius* genome are very stable since larvae from Pancharevo have the same number of Hinf and Alu loci as larvae from Santena (Italy) and Varna (Bulgaria) popula-

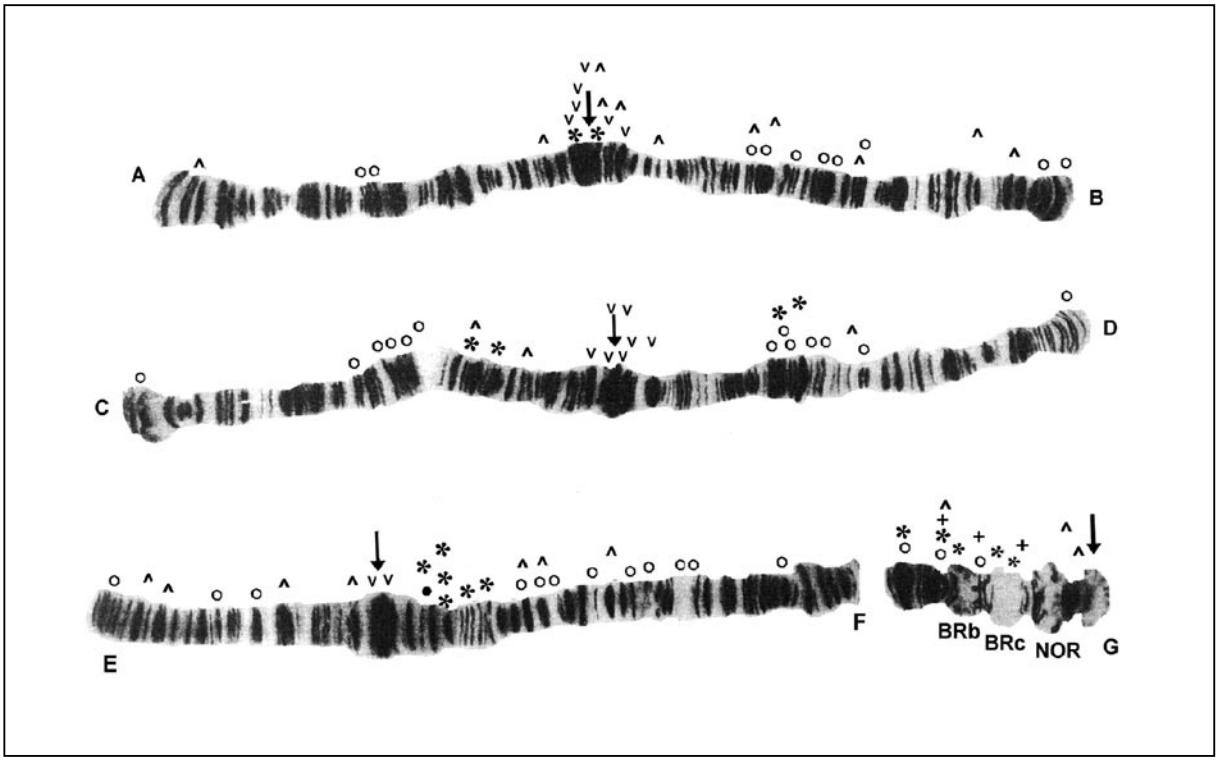

Fig 5 — Standard Chromosome map of *C. piger* (by KIKNADZE *et al.* 1991). Localization of retrotransposon NLRCth1 appeared in two states along the chromosomes of *C. piger* – fixed and variable. V: indicated the fixed signals of NLRCth1 along the chromosomes; ^: indicated the variable signals of NLRCth1 along the chromosomes. *: indicated "common" break points of inversions along the chromosomes; •: indicated Alu clusters. Alu is the repetitive DNA clusters; o: indicated Hinf clusters. Hinf is the repetitive DNA clusters; +: break points of the observed deletions in the studied population.

tions (BOVERO *et al*. 2002). This may reflect a lack of extensive transposition activity of these tandem repeat classes.

We also established that both species can be differentiated by nine and six fixed insertion sites of the NLRCth1 retrotransposon in *C. piger* and *C. riparius,* respectively. Insertion site variability of NLRCth1 retrotransposon has been observed with the FISH technique not only in the populations of *C. piger* from Kokalijane and *C. riparius* from Pancharevo, but also in a *C. riparius* Russian population by BLINOV *et al*. (1993). Using the PCR-based Transposon Insertion Display (TID) method of KORSWAGEN *et al.* (1996), a high level of insertion variability was also found in a sample of 14 *C. riparius* larvae from Pancharevo reordered by ZAMPICININI *et al.* (2004). Several single copy insertions, i.e. insertions in only one out of 80 individuals from 6 different populations were observed. According to WRIGHT *et al.* (2001), such single copy insertions can be regarded as an indication of extant transposition activity.

Some variable insertion sites appeared in a homozygous or heterozygous condition, but not in all the examined cells of the same individual, indicating somatic instability. We note, however, that the apparent absence of an insertion site in some cells of the same salivary gland could occasionally depend on partially damaged polytene chromosomes or on different efficiencies of probe hybridization in salivary gland cells.

The rather high frequency of somatic chromosome rearrangements observed in *C. riparius* larvae from the heavy metal polluted Pancharevo pond supports the hypothesis advanced by SELLA *et al.* (2004) that there is a relationship between the level of trace metal pollution in a sediment and the spectrum of somatic rearrangements of larvae living in it. Deletions in chromosome G leading to a "pompon"-like structure of this chromosome are a frequently observed aberration, and such "pompon" chromosome appearance in *C. riparius* has been proposed as a cytogenetic biomarker of genotoxicity (MICHAILOVA *et al.* 1989). A detailed

comparison of the number of breakpoints in Pancharevo larvae of *C. riparius* with those of other polluted localities (SELLA *et al.* 2004) revealed that one third of them were common break points which might correspond to chromosomal "weak spots" on the *C. riparius* genome.

Although the Kokalijane sediments are not polluted with trace metals, in the Kokalijane population of *C. piger* we also found a number of somatic rearrangements, including deletions in chromosome G. Since somatic rearrangements are often associated with the presence of genotoxic agents in the environment (LAGADIC and CAQUET 1998), we advance the hypothesis that they could have been induced by some anthropogenic agent, other than trace metals, present in water or sediments of this station.

As stated by KING in his seminal book (1993), frequency and type of chromosomal rearrangements depend on the structure and organization of DNA sequences within the genome. While somatic inversions in *C. riparius* occur in all chromosomes, those in the *C. piger* genome are concentrated mainly in pericentromeric regions and some sites on arms D and F. Such a difference between the two species may depend either on the different level of pollution of the two stations or, more likely, on the markedly different organization of repetitive DNA genome fractions, i.e. the higher number of copies of dispersed repetitive sequences throughout the *C. riparius* genome compared to that of *C. piger*. This finding can explain the fact that a significant correlation was found between common breakpoints and repetitive DNA chromosomal locations of both *C. piger* and *C. riparius.*

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REFERENCES

BEERMANN W., 1971 — *Effect of L- Amanite on Puffing and Intranucleolar RNA Synthesis in Chironomus Salivary glands.* Chromosoma, 34: 152-164.

BLINOV A.G., SHOBANOV Y.V., BOGOEV S.S., FILIPOVA M.A. and DOCHENKO A.P., 1993 — *Chironomus thummi genome contains a non-LTR retrotransposon.* Mol.Gen.Genet., 237: 412-420.

BOVERO S., HANKELN T., MICHAILOVA P., SCHMIDT E. and SELLA G., 2002 — *Nonrandom chromosomal distribution of spontaneous breakpoints and satellite DNA clusters in two geographically distant populations of Chironomus riparius (Diptera, Chironomidae).* Genetica, 115: 273-281.

CARR M., SOLOWAY J., ROBINSON Th. and BROOKFIELD J., 2002 – *Mechanisms regulating the copy numbers of six LTR retrotransposons in the genome of Drosophila melanogaste*r. Chromosoma, 110: 511-518.

FORSTNER U. and SALOMONS W., 1980 — *Trace metal Analysis in Polluted Sediments*. Environ. Techn Lett., 1: 494-517.

GUNDERINA L.I. and AIMANOVA K.G., 1998 — *Genetic consequences of exposure to γ-radiation in Chironomus thummi: aberrations of polytene chromosomes*. Russ. J. Genet. 34: 273-280.

HÄGELE K., 1970 — *DNS–Replikationmuster der Speicheldrüsenchromosomen von Chironomiden*. Chromosoma, 31: 91-138.

HÄGELE K., 1984 — *Different hybrid effect in reciprocal crosses between Chironomus thummi and Chironomus th. piger including spontaneous chromosome aberrations and sterility*. Genetica, 63: 105-111.

HANKELN T., ROHWEDDER A., WEICH B. and SCHMIDT E., 1994 — *Transposition of minisatellite sequences during evolution of Chironomid midges*. Genome, 37: 542-549.

KEYL H., 1965a — *A demonstrable local and geometric increase in the chromosomal DNA of Chironomus*. Experientia, 21:191-193.

KEYL H.G., 1965b — *Duplikationen von Untereinheiten der chromosomalen DNS während der Evolution von Chironomus thummi*. Chromosoma, 17: 139-180

KEYL H. and STRENZKE K., 1956 — *Taxonomie und Cytologie von zwei Unterarten der Art Chironomus thummi*. Z. Naturforsch., 116: 727-735.

KIKNADZE I.I., SHILOVA A., KERKIS I., SHOBANOV N., ZELENZOV N., GREBENJUK F., ISTOMINA A. and PRASOLOV B., 1991 — *Karyotype and morphology of larvae in Chironomini*. Atlas. Novosibirsk, 117 pp..

KING M., 1993 — *Species Evolution: the role of chromosome change*. Cambridge University Press, Cambridge, UK.

KORSWAGEN H., DURBIN R., SMITS M. and PLASTERK R., 1996 — *Transposon Tc 1-derived, sequence tagged sites in Caenorhabditis elegans as markers for gene mapping*. Proc.Natl. Acad. Sci. U.S.A., 93: 14680-14685.

LAGADIC L. and CAQUET T., 1998 — *Invertebrates in Testing of Environmental Chemicals: Are they alternatives?* Environmental Health Perspectives, 106(2): 593-603.

MICHAILOVA P., 1987 — *"C" banding in polytene chromosomes of species of the group "plumosus" (Diptera,Chironomidae)*. Genetica, 74: 41-51.

MICHAILOVA P., 1989 — *The polytene chromosomes and their significance to the systematics of the fam-*

ily Chironomidae, Diptera. Acta Zool.Fennica. 186: 107.

MICHAILOVA P., PETROVA N., RAMELLA L., SELLA G., TODOROVA J. and ZELANO V., 1996 — *Cytogenetic characteristics of a population of Chironomus riparius Meigen 1804 (Diptera, Chironomidae) from a polluted Po river station*. Genetica, 98: 161-178.

MICHAILOVA P., PETROVA N., BOVERO S., CAVICCHIOLI O., RAMELLA L. and SELLA G., 2000 — *Effect of environmental pollution on the chromosome variability of Chironomus riparius Meigen 1804 (Diptera, Chironomidae) larvae from two Piedmont stations*. Genetica, 108: 171-180.

MICHAILOVA P., PETROVA N., SELLA G., RAMELLA L. and BOVERO S., 1998 — *Structural-functional rearrangements in chromosome G in Chironomus riparius Meigen (Diptera, Chironomidae) collected from a heavy metal polluted area near Turin, Italy*. Environmental Pollution, 103: 127-135.

MICHAILOVA, P., ILKOVA J. and WHITE K.N., 2003 — *Functional and structural rearrangements of salivary gland polytene chromosomes of Chironomus riparius Mg. (Diptera, Chironomidae) in response to freshly neutralized aluminium*. Environmental Pollution, 123: 193-207.

MICHAILOVA P. and PETROVA N., 2004 — *Natural hybridization in Insects, Diptera (Model group — family Chironomidae)*. In: Bakardzhieva N. and Michailova P. (Eds.) "Evolution and Ecology, 2004", US Bulgaria, Sofia, 9-19.

MICHAILOVA P., ILKOVA J., KERR R and WHITE K.N., 2006 — *The Effect of Aqueous Aluminium on the Structure and Function of the Polytene Chromosomes in Chironomus species*. in *16th International Chironomid Symposium. Abstracts*. Funchal, Madeira.

PETROVA N., MICHAILOVA P. and ILKOVA J., 2004 — *Comparative Cytogenetic Variation of the Salivary gland Polytene Chromosomes in Chironomus riparius Mg. (Diptera, Chironomidae) from two Polluted Biotopes in Bulgaria and Russia*. Russian Journal of Genetics, 40(1): 40-48.

POLUKONOVA R., BELJANINA S. and DUROVA N., 1996 — *Differential diagnosis of Ch. piger Strenzke and C. riparius Meigen.* In: Shobanov N.A. and Zintchenko T.D. (Eds.) "Ecology, Evolution and Systematics of Chironomid", Tolijti, Borok, RAS, 109-115.

SCHAEFER J. and SCHMIDT E., 1981 — *Different repetition frequencies of a 120 base-pair DNA element and its arrangement in Chironomus thummi thummi and C. piger*. Chromosoma, 84: 61-66.

SANDMEYER S., HANSEN L. and CHALKER D., 1990 — *Integration specificity of retrotransposons and retroviruses*. Annu. Rev. Genet., 24: 491-518.

SCHMIDT E., 1984 — *Cluster and interspersed repetitive DNA sequence family of Chironomus*. J. Mol. Biol., 178: 1-15.

SCHMIDT E., 1992 — *Multicolor in situ hybridization – a helpful technique for precise gene localization*. Sci. Tech. Inform. Serv. X, 3: 80-84.

SELLA G., BOVERO S., GINEPRO M., MICHAILOVA P., PETROVA N., ROBOTTI C. and ZELANO V., 2004 — *Inherited and somatic cytogenetic variability in Palearctic populations of Chironomus riparius Meigen 1804 (Diptera, Chironomidae)*. Genome, 47: 332-344.

WRIGHT S.I., LE Q.H., SCHOEN D.J. and BUREAU T.E., 2001 — *Population dynamics of an Ac-like transposable element in self- and cross-pollinating Arabidopsis*. Genetics, 158: 1279-1288.

WOBUS U., BAUMLEIN H., BOGACHEV S., BORISEVICH I., PANITZ R. and KOLESNIKOV N., 1990 — *A new transposable element in Chironomus thummi*. Mol. Gen. Genet., 222: 311-316.

WÜLKER W. and GŐTZ M., 1968 — *The Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von Chironomidenlarven*. Zeitschr. Morph.Tiere, 62: 363-382.

ZAMPICININI, G., BLINOV A., CERVELLA P,. GURYEV V., and SELLA G., 2004 — *Insertional polymorphism of a non-LTR mobile element (NLRCth1) in european populations of Chironomus riparius (Diptera, Chironomidae) as detected by Transposon Insertion Display*. Genome, 47: 1154-1163.

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