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The effect of long-term contamination by heavy metals on community and genome alterations of Chironomidae (Diptera) in a stream with mine drainage water (southern Poland)

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Key words: Chironomidae, community, genome alteration, heavy metals

Abstract

This paper reports on studies of the effect of heavy metals on the Chironomidae that inhabit the Matylda stream, which has been contaminated for about 100 years by discharge water from a zinc and lead ore mine. Stream sediment was strongly polluted by Cd, Pb, Cu and Zn. These metals did not affect the Chironomidae community but strongly changed the genome system of the Chironomidae species that inhabited the sediment. The salivary gland chromosomes of six species belonging to the two genera *Chironomus* and *Prodiamesa* are analyzed. In all species the somatic index is calculated on the basis of somatic chromosome alterations. *Chironomus riparius* has the highest numbers of somatic alterations and the highest somatic index - 9.67. The smallest chromosome G carries the key structures known as "Balbiani rings", which play an important role in species development. This chromosome is very sensitive in the genome of the most studied species. The high sensitivity of the *C. riparius* genome is discussed in light of its DNA organization. The results show a high response of the salivary gland chromosomes to heavy metal pollution, and this makes them a valuable indicator in the assessment of water quality and detection of mutagenic agents in the aquatic environment.

INTRODUCTION

Contamination of the environment is one of the serious problems in the world - it influences biodiversity, and the structure and functioning of ecosystems. Heavy metals are especially dangerous for biota and human beings, and have drawn major attention due to their persistent toxic effect and their ability to accumulate within compartments in the environment (Lagrana et al. 2011). They are bound to organic and inorganic particles which may settle at the bottom of streams, rivers and lakes. When they exceed threshold concentrations they can have very harmful effects on individual organisms, populations, communities and ecosystems. In aquatic ecosystems heavy metals can influence invertebrate development (Martinez et al. 2004), abundance and structure of the population (Gower et al. 2006), as well as influencing invertebrate morphology (Martinez et al. 2009) and some genetic and physiological systems (Florea, Büsselberg 2006; Michailova et al. 2012a, b).

Chironomids are benthic insects and can reflect aquatic environmental degradations. Their larval stage is especially important. They make up 25-100% of the macroinvertabrates in different aquatic ecosystems (Kownacki 2011). They play an important role in purification processes due to their ability to accumulate different pollutants in their tissues and later to transfer them to a higher level of biological

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organization (Warwick 1988). Because they are abundant in the aquatic ecosystem, it is useful to follow the effect of contaminants on their biodiversity. On the other hand, some studies done by Michailova (2012 a,b) showed that the genome of the individual chironomid species is also very sensitive, making it a good biomarker for stress agents in the environment. This approach can help to understand the effect of chronic heavy metal contamination on the genetic systems of species that have inhabited a contaminated area for decades or even centuries. Concentrations of heavy metals in stream or river sediments receiving discharge from ore mining and processing are a thousand times higher than in the local geochemical background (Aleksander-Kwaterczak, Ciszewski 2012).

This study concentrated on two aims:

1. To estimate the influence of high heavy metal concentrations on the Chironomidae community.
2. To analyze the genome response of widely distributed Chironomidae species.

Chironomidae from a stream in the industrial region of southern Poland were studied. This stream has received discharges from ore mining for over one hundred years. The genome response was estimated by studying somatic alterations in the salivary gland chromosomes of chironomid species from this stream.

Materials And Methods

Study area

The study area was the Matylda stream (Fig. 1), which for about a 100 years received mine water from the Matylda ore mine (Upper Silesia, southern Poland). The Matylda Zn-Pb mine in Chrzanów operated from 1850 to 1973. It was closed due to the depletion of the metal ores. Fine-grained sediments contaminated with Cd, Zn and Pb were transported along with the discharged mine waters and accumulated in the inundated valley bottom, stream, fish ponds and associated wetlands. Since 1973, the Matylda stream has naturally drained a small catchment basin (11.5 km^2) (Ciszewski et al. 2011; Aleksander-Kwaterczak, Ciszewski 2012). The consequence of the long-term discharges from the Matylda Zn-Pb ore mine are high concentrations of Cd, Zn, Pb in the alluvial soils of the Matylda valley (up to 100 μg g^{-1}, 24%, and 4%, respectively) (Aleksander-Kwaterczak, Ciszewski 2012) and in the sediments of the Matylda stream and the fish ponds supplied with those waters (up to 135 μg g^{-1}, 2.1%, and 1.4%, respectively) (Ciszewski et al. 2011).

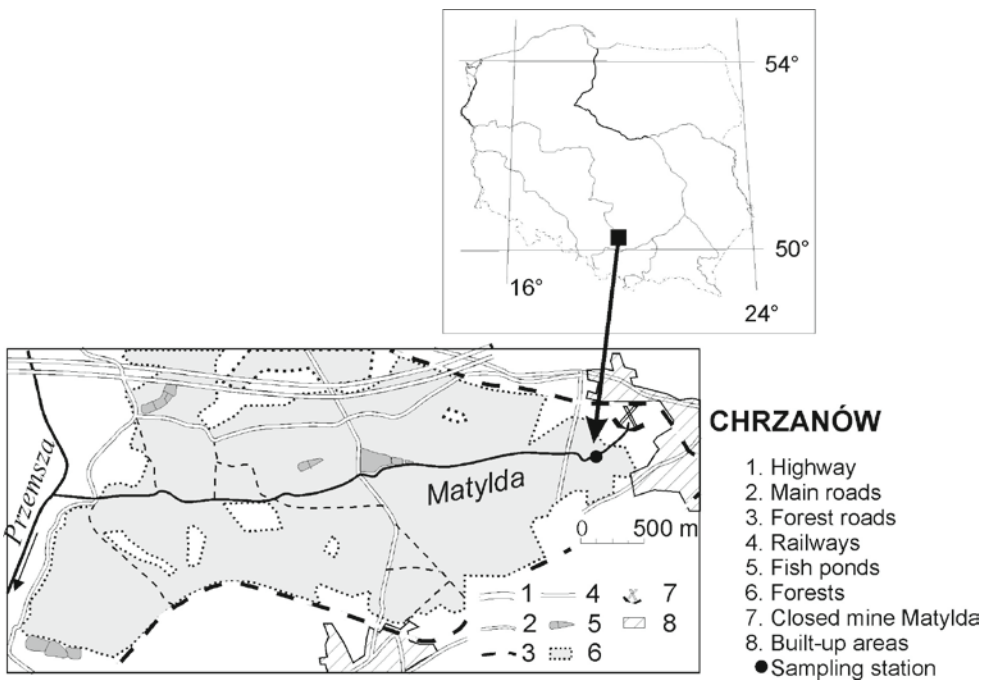

Fig. 1. Location of the sampling station on the Matylda stream

Sampling

Samples of sediments and chironomid larvae were collected from the Matylda stream, about 1.2 km below the closed mine (50°8'12" N, 19°22'17" E), in July and August 2011. Each time 5 samples of benthic fauna were collected using a hand net covered with a 0.3-mm mesh gauze. Chironomidae larvae were also picked up for cytogenetic studies. The Chironomidae larvae used for the cytogenetic analysis were washed and fixed in alcohol and acetic acid at a ratio of 3:1. Samples of benthic animals were formalin-fixed. In the laboratory, only Chironomidae larvae were removed from the benthic samples.

Physicochemical analysis of sediment samples

Organic matter content in the sediment samples was analyzed by the ignition of a sediment sample at 550°C for 2 hrs (expressed as Loss of Ignition - LOI). For the analyses of heavy metals in the sediment sample, a silt-clay fraction (0.063 mm) was wet-separated. For the analysis of total metal (Cd, Pb, Zn, and Cu) concentrations, sediment samples were digested with 10 cm^3 of 65% HNO_3 and 2 cm^3 of 30% H_2O_2 in Teflon bombs using a microwave digestion technique. To study the fractionation of heavy metals in the sediments the operationally defined BCR (Community Bureau of Reference of the European Commission, now the Standards, Measuring and Testing Programme) procedure was used (Larner et al. 2006). This procedure allows a determination of four operationally defined phases of metals, i.e. (F1) exchangeable and bound to carbonates, (F2) reducible, bound to iron and manganese oxides, (F3) oxidisable, bound to organic and sulphide compounds and (F4) residual. The concentrations of metals in solutions were determined with an inductively coupled plasma-mass spectrometer (Perkin Elmer ELAN 6100). Metal analyses were performed according to the standard certified analytical quality control procedure (PN-EN ISO 17294-1:2007).

Cytogenetical methods and analysis

The species, the number of studied larvae and cells can be seen in Table 1. The salivary gland chromosomes were prepared using Michailova's method (1989). Species identification from the genera *Chironomus* and *Prodiamesa* and the localization of different types of chromosome aberrations were done using chromosome maps made by Michailova (1989) and Kiknadze et al. (1991). Somatic aberrations were considered following Lagadic and Caquet (1998) and Michailova et al. (2012b).

Determination of Chironomidae

Along with chromosome preparations from every individual of the genera *Chironomus* and *Prodiamesa*, a preparation of the external morphology of the larva was done. Apart from the genera *Chironomus* and *Prodiamesa*, all other species were identified according to the Wiederholm (1983) and Lengton and Visser (2003) keys. The taxonomy of Chironomidae corresponded to Fauna Europea (http://www.faunaeur.org).

Statistical analysis

Chromosome aberrations occurring in only a few cells were considered somatic aberrations (Sella et al. 2004) and were presented as percentages. On the basis of the observed somatic chromosome

Table 1

The cytogenetically studied species, number of individuals, cells, the percentage of their alterations and Somatic index

Species	NUMBER OF					S index
	studied individuals	individuals with aberrations	studied cells	cells with aberrations (%)	somatic chromosome rearrangements	
Chironomus riparius	15	15	428	253 (59.11)	145	9.67
Chironomus piger	8	8	196	81 (41.33)	58	7.25
Chironomus melanotus	3	3	109	30 (27.52)	10	3.33
Chironomus luridus	11	5	135	20 (14.81)	11	1.0
Prodiamesa bureschi	9	8	255	31 (12.16)	15	1.7
Prodiamesa olivacea	5	4	98	16 (16.33)	8	1.6

rearrangements, a Somatic index (S) was calculated (Sella et al. 2004) for each species. The somatic structural chromosome alterations occurring in different species of the same genus were compared by a contingency G test (Sokal, Rohlf 1995). Their percentages are given based on the average abundance of each Chironomidae taxa.

RESULTS

Physicochemical analysis of sediment

The sediment of the Matylda stream is characterized by neutral pH (7.6), various amounts of organic matter (express as LOI, 3.7-23.2%) and high concentrations of Cd, Pb, Cu and Zn (up to 112, 5022, 208.8 µg g^{-1} and 1.96% respectively, Table 2).

The results of the sequential extraction of the sediment indicated different associations of the metals with the target fraction in the sediment of the Matylda stream (Fig. 2). Most Zn and Cd was bound to F1 (exchangeable and carbonates) and F2 (iron and manganese oxides), while a smaller amount was associated with F3 (organic and sulphide compounds) and F4 (residual). A great portion of Pb was associated with F3 and a considerable portion was bound to F2. Similarly, the majority of Cu was associated with F3 and a smaller amount to F4 (residual). Very small portions of Pb and Cu were bound to F1 and F2. These results indicate that a great portion of the Cd, Pb and Zn occurred in potentially mobile phases.

Table 2

Contents of organic matter and heavy metals in the sediment of the Matylda stream

Parameter	Range	SFF[a]	SEL[b]
LOI (%)	3.7-23.2		
Cd (µg g^{-1})	94.9-112.0	0.3	10
Pb (µg g^{-1})	4994-5022	30	250
Cu (µg g^{-1})	155.3-208.8	51	110
Zn (%)	3.19-1.96	0.0115	0.82

[a] SFF – Sediment fossil river (Förstner, Salomons 1980)
[b] SEL – Severe effect level (Persaud et al. 1993)

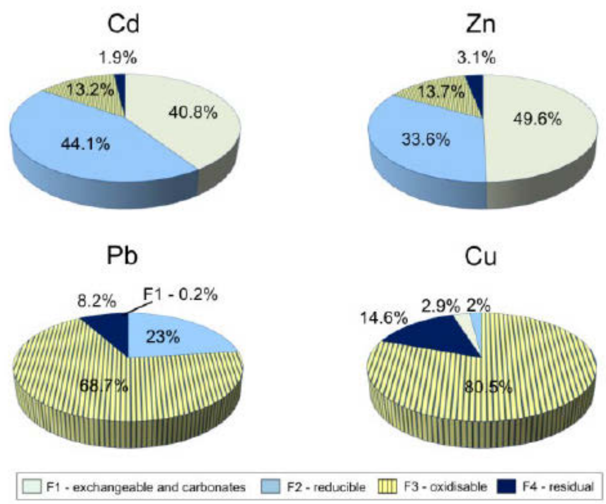

Fig. 2. Binding forms of the heavy metals found in the sediment of the Matylda stream

Cytogenetic characteristics

Six species of the Chironomidae were determined cytotaxonomically: four from the genus *Chironomus* Meigen and two from the genus *Prodiamesa* Kieffer.

Genus *Chironomus*

On the basis of chromosome arm combinations due to homozygous translocations, the species of this genus are grouped in different cytocomplexes (Keyl 1962). Two cytocomplexes were established in the study area:

<u>Cytocomplex "thummi" with chromosome arm combinations AB, CD, EF, G</u>

Chronomus riparius **Meigen.** The chromosome set is 2n = 8. Chromosomes AB, CD are metacentric, EF - submetacentric and G – acrocentric. Chromosome G has three Balbiani rings: BRa, BRb, BRc and one Nucleolar Organizer (NOR) (Fig. 3). BRa can be seen in a few cells only.

Chironomus piger **Strenzke.** The chromosome set is 2n = 8. It is a species homosequental to *C. riparius* (both species have the same band pattern along the polytene chromosomes) and can be distinguished by the appearance and localization of heterochromatin and repetitive DNA elements (Keyl 1965, Michailova et al. 2009).

Chironomus melanotus Keyl. The chromosome set is 2n = 8. Chromosomes AB, CD, EF, G have large heterochromatinized centromere regions. Chromosomes AB, CD – metacentric, EF – submetacentric and chromosome G - telocentric. One (NOR) and two (BRs) are localized in chromosome G (Fig. 4).

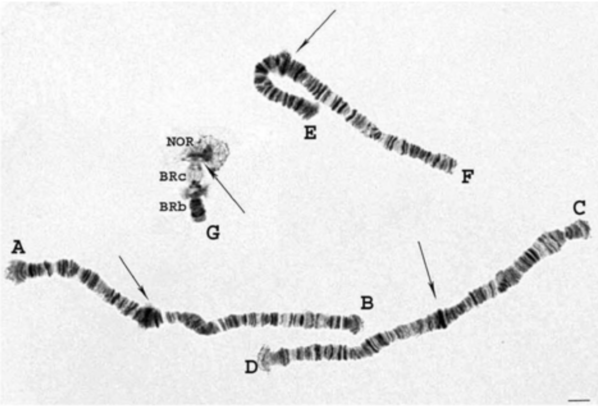

Fig. 3. Polytene chromosomes of *Chironomus riparius*: chromosomes AB, CD, EF and G; BR-Balbiani ring; NOR – Nucleolar Organizer; arrow-centromere region. Bar 10 µm

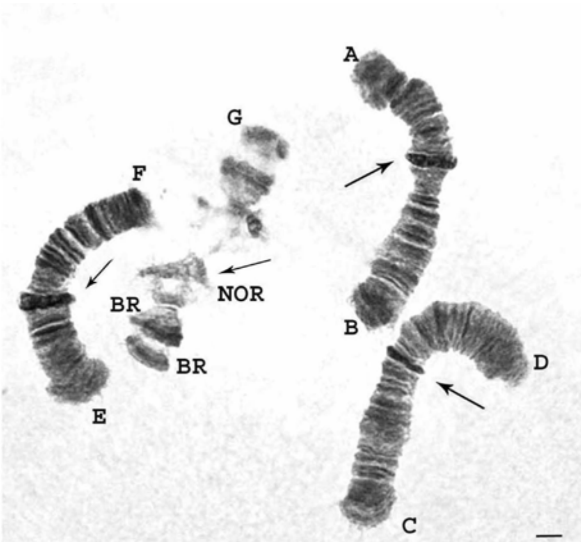

Fig. 4. Polytene chromosomes of *Chironomus melanotus* – chromosomes AB, CD, EF and G; Abbreviations and bar as in Fig. 3

<u>**Cytocomplex “pseudothummi” with chromosome arm combinations: AE BF CD G**</u>

Chironomus luridus Strenzke. This has the chromosome set 2n = 8. Chromosomes CD and BF are metacentric, chromosome AE – submetacentric, and chromosome G is acrocentric. Chromosome G has one NOR and three BRs (Fig. 5).

Genus *Prodiamesa* Kieffer

Two sibling species were found: *Prodiamesa bureschi* Michailova and *Prodiamesa olivacea* Meigen with the chromosome set 2n = 6 and chromosome arm combinations AB, CD, EF plus an additional compact chromosome, so called “B” chromosome. These chromosomes are not homologous to those of genus *Chironomus*, they are indicated conditionally. Chromosomes AB and CD are metacentric, the chromosome EF is submetacentric and “B” is a very compact heterochromatin body (Fig. 6). Both species can be distinguished by fixed homozygous inversions in chromosome AB and the band sequences in chromosomes CD and EF (Michailova 1989).

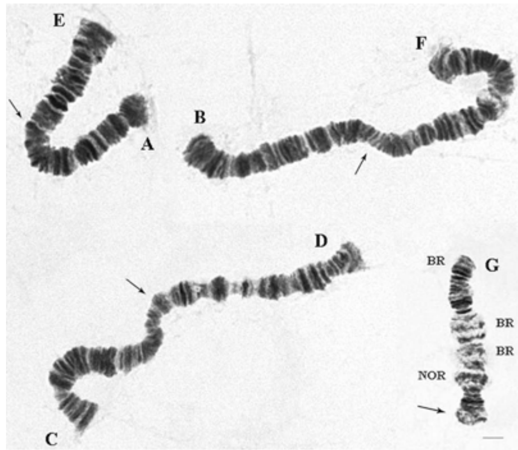

Fig. 5. Polytene chromosomes of *Chironomus luridus*: chromosomes AE, BF, CD and G; Abbreviations and bar as in Fig. 3

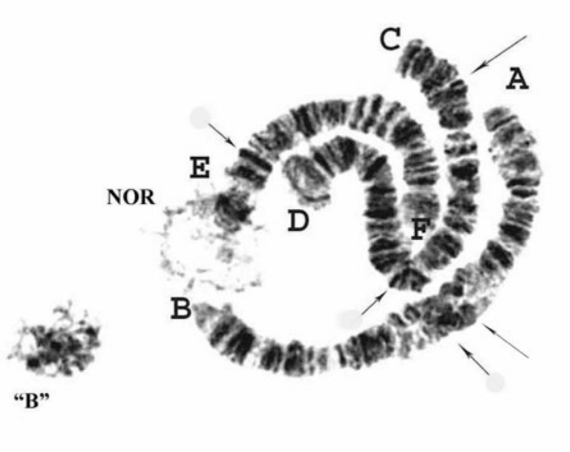

Fig. 6. Polytene chromosomes of *Prodiamesa bureschi*: chromosomes AB, CD, EF and “B”; NOR – Nucleolar Organizer; “B” – chromosome. Somatic pericentric inversion in chromosome AB and somatic paracentric inversion in arm C indicated by large arrow. Small arrow- centromere region. Bar 10 µm

Larval morphological deformities and somatic chromosome alterations

The fourth instar larvae of species of the two genera *Chironomus* and *Prodiamesa* collected from the trace metal polluted Matylda stream were investigated for their morphological features and for the response of the salivary gland polytene chromosomes.

Deformities in the larvae of the studied species

Only one malformation was found. It was discovered in one specimen of *P. bureschi* and it affected the mentum of the mouthparts.

Somatic chromosome alterations

All studied individuals of *C. riparius*, *C. piger*, and *C. melanotus* had somatic alterations in their salivary gland chromosomes, while in the other three species, *C. luridus*, *P. olivacea* and *P. bureschi* such rearrangements were only observed in a few individuals (Table 1).

Somatic rearrangements included para- and pericentric inversions, deficiencies, amplifications and deletions, which were distributed in all chromosomes of the species. In the genus *Chironomus* the highest number of somatic rearrangements was detected in the *C. riparius* genome (Fig. 7a), followed by the *C. piger*, *C. luridus* and *C. melanotus* genomes (Figs. 7b, c, d) which reflect their somatic index (Table 1, Fig. 8). Also, the percentages of aberrant cells were the highest in *C. riparius* followed by *C. piger*. The number of aberrant cells was significantly higher in *C. riparius* than in *C. piger* (G = 17.068, df = 1, P<0.001), *C. melanotus* (G = 35.393, df = 1, P<0.001) and *C. luridus* (G = 87.822, df = 1, P<0.001). It is worth underlining that chromosome G of *C. riparius* and *C. piger* is very sensitive. It showed somatic heterozygous inversions and homozygous deletions. Due to these deletions the important key structures BRc and BRb were lost and chromosome G was converted to a "pompon" chromosome (Figs. 7e, f, g). These aberrations occurred to a significantly greater extent in *C. riparius* than in *C. piger* (G = 5.246, df = 1, P<0.05).

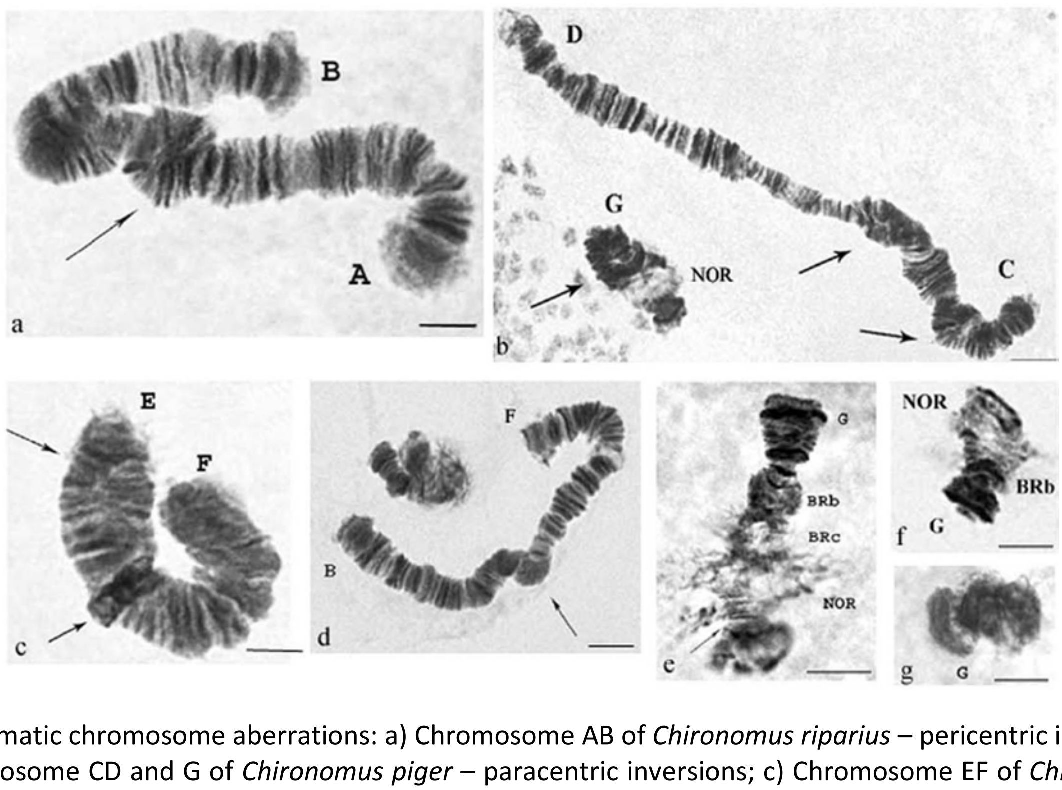

Fig. 7. Somatic chromosome aberrations: a) Chromosome AB of *Chironomus riparius* – pericentric inversion; b) Chromosome CD and G of *Chironomus piger* – paracentric inversions; c) Chromosome EF of *Chironomus melanotus* – paracentric inversion; d) Chromosome BF of *Chironomus luridus* – pericentric inversion; e) Chromosome G of *Chironomus riparius* – standard; f) Chromosome G of *Chironomus piger* – deletion of BRc; g) Chromosome G of *Chironomus riparius* – deletion of BRb and BRc; Large arrow – somatic aberrations; Small arrow – centromere region; Bar 10 µm

However, there were no significant differences between heterozygous inversions of chromosome G in both species (G = 1.768, df = 1, P>0.1).

In the genus *Prodiamesa*, *P. olivacea* had the highest percentage of aberrant cells (Table 1). There were no significant differences in aberrant cells between *P. bureschi* and *P. olivacea* (G = 1.015, df = 1, P>0.1). Both species in this genus had almost the same somatic index (Table 1, Fig. 8). Also, in both species there were ectopic conjugations between different chromosomes. In *P. bureschi* in almost all cells there were conjugations between the telomeres of the chromosomes or between the telomere of chromosome AB and the centromere of chromosome CD (7.06%). In *P. olivacea* the conjugations were between the centromeres of chromosomes AB, CD and EF (48.99%). In *P. bureschi* there were inherited heterozygous inversions as well as somatic alterations (Fig. 6) in two individuals.

Biodiversity of Chironomidae

In the Matylda stream 17 taxa of Chironomidae were found (Table 3). The dominant taxa were *Psectrotanypus various* (Fabricius) and *Prodiamesa olivacea* (Meigen), ubiquitous species which favor sediments of small, nutrient-rich standing or slowly flowing water bodies. They were accompanied by numerous species of the genus *Chironomus*.

Discussion

The concentrations of Cd, Pb, Cu, and Zn in the sediment of the Matylda stream were typical of sediments affected by metal mining (Byrne et al. 2010) and several times higher than in unpolluted ones (Förstner, Salomons 1980) (Table 2). Concentrations of Cd were higher (ca. 11 times), Pb (ca. 20 times), Cu (ca. 2 times) and Zn (ca. 4 times) than the severe effect level (SEL, Table 2) (Persaud et al. 1993), above which adverse effects on the majority of sediment dwelling organisms are expected.

These concentrations do not affect the Chironomidae diversity nor the external morphology of the larvae. Only one specimen of *P. bureschi* had deformities.

The composition and structure of the Chironomidae community occurring in the Matylda stream was characteristic for small, nutrient-rich, standing or slowly flowing water bodies (Fittkau 1962; Fittkau, Roback 1983). Therefore, no clear effect of metals on Chironomidae communities was found. Winner et al. (1980) found that Chironomidae larvae are tolerant to high heavy metal concentrations. They comprised 75 to 86% of all insects collected from the stations most grossly polluted by heavy metals and less than 10% of the insect communities at the least polluted stations. According to Winner et al.'s (1980) hypothesis, habitats which have been heavily polluted by heavy metals are dominated by Chironomidae, moderately polluted habitats are dominated by Chironomidae and caddisflies, and minimally polluted or unpolluted

Table 3

Percentage share of Chironomidae taxa in the Matylda stream

CHIRONOMIDAE	%
TANYPODINAE	
Procladius (*Holotanypus*) sp. (l)	0.7
Macropelopia nebulosa (Meigen 1804) (p, l)	9.3
Psectrotanypus varius (Fabricius 1787) (p, l)	30.2
Tanypodinae (juv.)	3.5
PRODIAMESINAE	
Prodiamesa olivacea (Meigen 1818) (l)	37.3
Prodiamesa bureschi Michailova 1977 (l)	2.8
ORTHOCLADIINAE	
Cricotopus (Isocladius) sylvestris gr (l)	1.3
CHIRONOMINAE	
Chironomus (Chironomus) anthracinus Zetterstedt 1860 (cytogen.)	X
Chironomus (Chironomus) riparius Meigen 1804 (cytogen.)	X
Chironomus (Chironomus) piger Strenzke 1956 (cytogen.)	X
Chironomus (Chironomus) uliginosus Keyl 1960 (cytogen.)	X
Chironomus (Chironomus) luridus Strenzke 1959 (cytogen.)	X
Chironomus (Chironomus) melanotus Keyl 1961 (cytogen.)	X
Chironomus spp. (l)	9.2
Polypedilum (Pentapedilum) sp. (l)	0.4
Micropsectra sp. (l)	3.4
Tanytarsini (juv.)	1.4

l – larvae, p – pupae, cytogen. – cytogenetically determinated, juv. – juvenile stage, X – present in stream, cytogenetically determinated

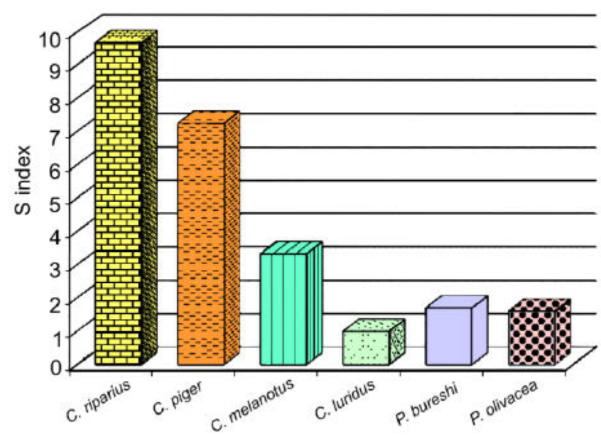

Fig. 8. Somatic index

habitats are dominated by caddisflies and mayflies. Clements et al. (1992) also observed an increased abundance of Chironomidae taxa in the Clinch River in Virginia, which is severely polluted with heavy metals. Along-term study of the impact of heavy metals originating from a Zn and Pb mine on the macroinvertebrate community showed an increasing abundance of Chironomidae (Armitage et al. 2007). Similar results were obtained by Canfield et al. (2009) and Michailova et al. (2012a). They didn't find any influence of high concentrations of heavy metals on the macroinvertebrates which inhabited strongly contaminated sediments.

Metals in the Matylda stream were mainly bound to exchangeable and carbonates (F1), amorphous ferromanganese oxyhydrates (F2), and organic and sulphide compounds (F3) which are potentially available to the biota. The most mobile and bioavailable metals in the sediment of the Matylda stream were Cd and Zn, of which a considerable portion was bound to F1. A considerable amount of Cd, Pb, and Zn bound to F2 in the Matylda stream may also be available to biota under reductive conditions in the sediment, while great amounts of the Cu and Pb bound to F3 will be released as a consequence of organic matter decay (Calmano et al. 2005). Bervoets et al. (1997) showed that metal concentrations in Chironomidae larvae were positively related to the easily reducible metal fraction.

Chironomidae inhabiting the Matylda stream sediment were directly exposed to high concentrations of Cd, Pb, Zn and Cu, which can mark their toxic effect on the polytene chromosome. It is known that the genotoxicity of cadmium may involve either indirect or direct interaction with DNA and produce cytogenetic damage, as a consequence of DNA strand breaks (Waalkes, Misra 1996), which we observed in all studied benthic chironomid species. Also, Pb ions induced either single or double DNA breaks or inhibited the DNA replication or repair process (Yousef et al. 2010) influencing the calcium regulated process. Because we detected a high spectrum of somatic chromosome alterations in all these species, it could be assumed that these alterations were induced by high concentrations of the above-mentioned metals. Somatic rearrangements are associated with genotoxic agents in the environment (Lagadic, Caquet 1998; Michailova et al. 2012b). However, future experimental studies with different single and combined heavy metal concentrations can confirm this idea.

The studied *Chironomus* species made their "home" tubes in the contaminated Matylda stream sediment. The BRs structures in the polytene chromosomes play a very important role in the formation of these "homes". This is where there is the synthesis of the high molecular weight proteins, which are involved in the process of tube formation (Wieslander 1994). These structures are located in the smallest chromosome G of most benthic species, which in *C. riparius* and *C. piger* is very sensitive to stress agents in the environment. Due to chromosome rearrangements – deletions of BRb, BRc, or BRba and BRc separately- this chromosome was converted into a "pompon" chromosome. Under the action of heavy metal contaminants, the normal function of chromosome G was hampered. As a consequence, the normal development of the species is destroyed because the deletions of the BRs prevent the synthesis of the proteins for tube building. These types of alterations of *C. riparius* and *C. piger* were detected in many other water ecosystems characterized by trace metal pollutants (Michailova et al. 2012a, b). Sarkar et al. (2011) also established a high somatic polymorphism in chromosome G of *Chironomus striatipennis* (Kieffer) and explained it by the high sensitivity of this chromosome to environmental stress. In the same species a high frequency of asynapsis in this chromosome was observed by Midya et al. (2012). They considered this event to be a response to pollution in the environment, which might affect gene expression in the chromosome and lead to asynapsis. However, it would be good in the future to carry out special molecular studies of the gene organization of this chromosome to understand in detail which genes are affected. Due to the high sensitivity of chromosome G, we suggest that it can be used as an indicator of the pollution level of the aquatic environment and the best tool for monitoring aquatic pollution.

The studied species show species-specific responses to contaminants of the environment. The *C. riparius* genome has very high sensitivity expressed by the highest number of somatic alterations and the highest value of the Somatic index. This species-specific response depends on the DNA organization of each species. King (1993) stated that the frequency of chromosome rearrangements depends on structure and DNA organization. His idea is very well reflected in the results obtained from two homosequental sibling species, *C. riparius* and *C. piger*, in the stream. They are distinguished by DNA organization; *C. piger* contains lower numbers of repetitive DNA clusters and its genome is 30% smaller than that of *C. riparius* (Hankeln et al. 1994, Michailova et al. 2009). From this and other studies (Ilkova et al. 2007, Michailova et al. 2009) it has been shown that its genome is less sensitive than that of *C. riparius*. Bovero et al. (2002) and Ilkova et al. (2013) hypothesized that in *C. riparius* somatic rearrangements occurred more frequently in the sites of polytene chromosomes rich in repetitive DNA or transposable elements and they called these sites „hot spots". Both species inhabiting the same stream have different genome responses to the same environmental stress agents. This can be interpreted as differences in their DNA organization. The higher number of repetitive DNA clusters and transposable elements (Ilkova et al. 2013) in *C. riparius* could explain the higher sensitivity of the *C. riparius* genome.

In conclusion, we can emphasize that long-term high contamination of stream sediment with Cd, Pb, Cu and Zn do not affect the diversity of Chironomidae but affect the genome of a species which is very sensitive. The results obtained show that the polytene chromosomes and somatic rearrangements established in them are a good indicator of the genotoxicity of these heavy metals and can be used as additional parameters in a water quality monitoring program, especially in industrial regions. They have been proven to be sensitive and reliable tools in the detection of mutagenic activity in the aquatic environment. Taking into account the importance of Chironomidae in aquatic ecosystems, information concerning the genome instability of these species can be valuable for environmental risk assessment.

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