Oceanological and Hydrobiological Studies
International Journal of Oceanography and Hydrobiology

Volume 42, Issue 2

ISSN 1730-413X
eISSN 1897-3191

(164–172)
2013

VERSITA

DOI: 10.2478/s13545-013-0070-2
Original research paper

Received: *April 30, 2012*
Accepted: *September 26, 2012*

Environmental agents in Lake Łuknajno (Poland) affecting the genome of *Chironomus melanotus* Keyl, 1961 (Diptera, Chironomidae) – a new species of Polish fauna

Izabela Jabłońska-Barna[1,*], Ewa Szarek-Gwiazda[2], Paraskeva Michailova[3]

[1]*Department of Applied Ecology, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, ul. M. Oczapowskiego 5, 10-719 Olsztyn, Poland*
[2]*Institute of Nature Conservation, Polish Academy of Sciences al. Adama Mickiewicza 33, 31-120 Krakow, Poland*
[3]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel boulv. 1000 Sofia, Bulgaria*

Key words: Diptera, Chironomidae, *Chironomus melanotus*, chromosome rearrangements, *Chara* sp.

Abstract

Chironomus melanotus Keyl, a new species of Polish fauna, is described on the basis of cytogenetic characteristics. It belongs to the cytocomplex *thummi* with the chromosome set 2n = 8, chromosome arm combinations AB CD EF G and species-specific karyotype markers.

Two types (somatic and inherited) of structural chromosome rearrangements in salivary gland chromosomes were identified in the species and somatic rearrangements (heterozygous inversions, deficiencies, deletions – Somatic index – 0.54) were observed for the first time in this species. In addition to those in the mosaic state, some genome alterations - trisomy and "B" chromosome, as well as larval malformations (10.27%) were detected for a first time. The malformations and somatic structural and genome aberrations may have been caused by different stress agents in the environment. Thus, we suggest that the high spectrum of somatic rearrangements observed in *C. melanotus* may indicate the existence of pollution (elevated Cd and Pb concentrations) in Lake Łuknajno (the study area) and perhaps trace metals and different chemicals produced by the *Chara* species.

INTRODUCTION

The main task of our society is to protect the environment and to find methods of detecting and assessing the impact of contaminants in ecosystems. The biological approaches have a number of advantages that make them particular suitable for assessing the dynamic process in the ecosystems (Walker et al. 1998). Several end points have been employed in monitoring studies using chironomid species, such as species occurrence, mouthpart deformities and somatic genome alterations expressed in the larval salivary gland chromosomes (Sæther 1975, Bhattacharyay et al. 2005, Michailova et al. 2011).

In this paper, *Chironomus melanotus* Keyl, 1961 was analyzed cytotaxonomically, a new species in the Polish fauna, collected from Lake Łuknajno.

The larvae of this species were found in the *Chara* patches, which are widely distributed macrophytes in this lake. We also performed a detailed analysis of the salivary gland chromosomes of the species and their alterations under environmental agents (such as heavy metals and allelopathy) in Lake Łuknajno.

STUDY AREA

Lake Łuknajno (53°49'N, 21°38'E) is situated in the northeastern part of Poland in the Great Masurian Lakes Region. It is a shallow lake (mean depth – 0.6 m; maximum depth – 3.0 m) with a

* Corresponding author: *ijpb@uwm.edu.pl*

surface area of 623 ha. Due to its natural values, it has been protected as a Nature Reserve since 1937 and a UNESCO Biosphere Reserve since 1977. The immediate lake watershed, dominated by sandy, permeable soils, includes arable lands (55%), pastures and meadows (26%) and forests (18%) (Kufel, Kufel 1997). The lake is surrounded by a wide belt of emergent plants. Łuknajno is a meso-eutrophic, hard water reservoir. Bottom sediments (calcareous gyttja) are highly mineralized and poor in mineral forms of nutrients (Kufel, Kufel 1997). The taxonomic structure of the benthic fauna was described by Jabłońska-Barna (2007) and the cytotaxonomic characteristics of some *Chironomus* species, the main group of macrozoobenthos, was presented by Michailova and Jabłońska-Barna (2008), Jabłońska-Barna and Michailova (2009). To date, there has been no description of the taxonomic structure of species connected with submerged macrophytes, which cover about 50% of the lake bottom. Submerged vegetation is dominated by *Chara* species, which are able to produce some allelochemicals (sulfur, polyphenols, etc.) in the surroundings. The associations of Characeae occupied nearly 90% of the overgrown bottom area (Królikowska 1997). In the lake, seven species of *Chara* were recorded with the dominance of *Chara aculeolata* and *C. tomentosa*. Four species remain green all year (Królikowska 1997).

MATERIALS AND METHODS

The living material and sediments were sampled simultaneously from littoral of Lake Łuknajno in September 2011. Sediment samples (layer 0-10 cm) were collected from 3 sites. They were sieved through a 0.2 mm mesh. Three subsamples from each station were digested with 65% HNO_3 using a Speed Wave (Berghof) microwave. The heavy metal concentrations in the solutions were analyzed using a Varian (Spectra AA–20) atomic absorption spectrophotometer. Standard Reference Material (NCS DC 73308) was used to estimate analytical accuracy for sediment samples. The measured concentrations and certified concentrations of analytical standards were as follows (in $\mu g\ g^{-1}$): Cd (1.25 ±0.05, 1.12 ±0.08, respectively), Pb (28.5 ±0.5, 27 ±2), Cu (21.6 ±1.4, 22.6 ±1.3), Zn (43.2 ±0.5, 46 ±4), Mn (994 ±10, 1010 ±29), and Ni (29 ±0.9, 30 ±2).

The index of geoaccumulation was calculated according to Müller (1981).

Larvae of *C. melanotus* were collected from the patches of *Chara* sp. and fixed in alcohol/acetic acid (3:1) – a suitable fixation for cytogenetic analysis. We examined larvae of IVth instar, phase 6-7 (Wülker, Gotz 1968), as this stage has well-banded polytene chromosomes.

Preparations of polytene chromosomes were obtained from squashes of salivary gland cells stained with aceto-orcein (Michailova 1989). *C. melanotus* was identified cytotaxonomically by specific markers of polytene chromosomes. The numbering of chromosomes follows Hirvenoja and Michailova (1991). Arms A, E, F were divided following Keyl (1962). The percentage of the cells with chromosome alterations (paracentric/pericentric inversions, deficiencies, deletions, polyploidy) was assessed. Chromosome aberrations were divided into two groups: somatic (affecting a few cells of an individual) and inherited (all cells of an individual) (Sella et al. 2004) and the somatic index was calculated following the described method (op. cit.).

Each specimen, together with chromosome preparation, larval capsule and body, was slide-mounted in Euparal. The description of larval morphology follows Sæther (1980).

The material studied is kept in the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia and in the Faculty of Environmental Protection and Fisheries, University of Warmia and Mazury in Olsztyn, Poland.

RESULTS

Physicochemical parameters of the sediment

Sediment of Lake Łuknajno was characterized by 7.5-7.8 pH and a low amount (ca. 5%) of organic matter (expressed LOI). The concentrations of Cu, Zn, Mn, and Ni in the sediment were low, Pb was slightly elevated, while those of Cd were elevated (Table 1). The values of the index geoaccumulation showed that the sediment was unpolluted by Cu, Zn, Ni, and Mn, slightly polluted by Pb and strongly polluted by Cd (Table 1).

Characteristics of salivary gland chromosomes

2n = 8. The species belongs to the cytocomplex thummi, with chromosome arm combinations: AB CD EF G. Chromosomes have large heterochromatinized centromere regions. Chromosomes AB, CD are metacentric, chromosome EF – submetacentric and chromosome

Table 1

Heavy metal concentrations (mean and SD, in μg g^{-1}) in the sediment of Lake Łuknajno and in sediments (mean concentrations) of different lakes in Poland.

	Cd	Pb	Cu	Zn	Mn	Ni	References
Łuknajno Lake	3.6 ±0.1	60.7 ±2.3	11.3 ±1.1	37.8 ±1.1	322.6 ±5.4	26.3 ±0.8	
Igeo	3.0[c]	1.0[b]	-2.6[a]	-1.9[a]	-2.0[a]	-2.0[a]	
PEL	3.53	91.3	197	315		36	Smith et al. 1996
SDF	0.2	16	25	105	406	51	Förstner, Salomons 1980
11 lakes in WNP*	4.5	52.3	15.2	86.2	538	17.1	Sobczyński, Siepak 2001
Maśluchowskie Lake	-	37.2	25.6	61.7	172.3	-	Solecki, Chibowski 2000
Swarzędzkie Lake	7.4	110	89	719	640	44	Szyper et al. 1994
Pond in Bolesław	3.7-9.4	290-682	70-128	1673-2168	175-336	23.1-31.0	Michailova et al. 2012

*WPN – Wielkopolski National Park

Igeo calculated according to Müller (1981): a – unpolluted sediment, b – slightly polluted, c – strongly polluted.

G − telocentric. One nucleolar organizer is located in chromosome G.

Chromosome AB

Arm A: It is not distinguished from *Chironomus plumosus* (Linnaeus), described by Keyl (1962) as well as arm A of *C. melanotus* from Germany (Keyl 1961, 1962) and Finland (Hirvenoja, Michailova 1991). It has the following chromosome band sequences (Fig. 1a):

1-2a-c - 10 - 11 - 12 - 3- 2k-h- 4d -5 – 7 – 8 – 9 - 2d-g - 4c-a - 13-19 C (centromere)

Arm B: It has the same band sequences as the specimens from Germany (Keyl 1961) and Finland (Hirvenoja, Michailova 1991). However, the dark bands of the specimens from Poland are thicker and the band sequences are (Fig. 1a):

20 - 21- 22 - 23 - 24 - 25 - 26 - 27 - 28

Chromosome CD

This chromosome has a large heterochromatin centromere region and thicker dark bands compared to the specimens from Germany (Fig. 1b)

Arm C: Band sequences as in specimens from Germany (Keyl 1961) and Finland (Hirvenoja, Michailova 1991), except for region 5-7 which is always active (Fig. 1b).

1 - 2 - 3 - 4 - 5 - 6 - 7 - 8

Arm D: Band sequences as in specimens from Germany (Keyl 1961) and Finland (Hirvenoja, Michailova 1991) (Fig.1b).

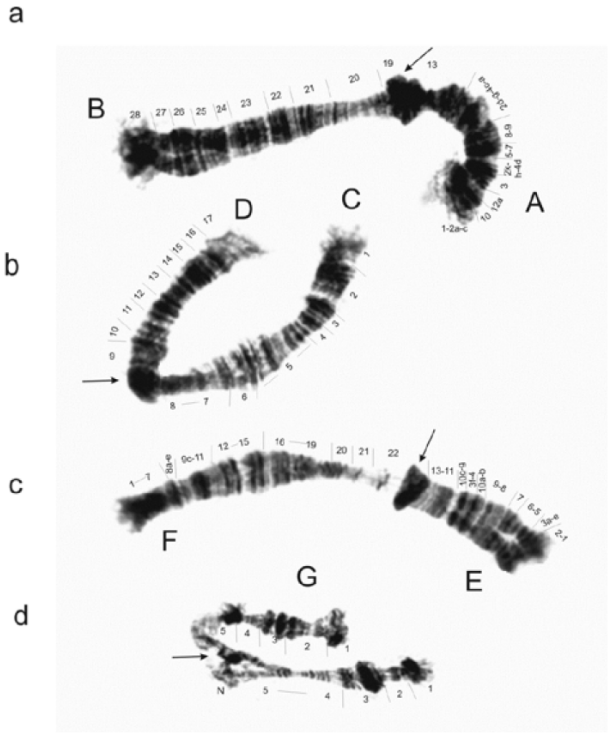

Fig. 1. Salivary gland chromosomes of *Chironomus melanotus*

a. Chromosome AB – Numbering of the chromosome arms is according to Keyl (1962) and Hirvenoja, Michailova (1991). An arrow indicates the position of the centromere region;

b. Chromosome CD − Numbering of the chromosome arms is according to Hirvenoja, Michailova (1991). An arrow indicates the position of the centromere region;

c. Chromosome EF − Numbering of the chromosome arms is according to Keyl (1962) and Hirvenoja, Michailova (1991). An arrow indicates the position of the centromere region;

d. Chromosome G − Numbering of the chromosome arms is according to Hirvenoja, Michailova (1991). An arrow indicates the position of the centromere region; N − Nucleolar Organizer.

Chromosome EF

Arm E: Band sequences as in specimens from Germany (Keyl 1961) and Finland (Hirvenoja, Michailova 1991).
The bands sequences coincided with those of *C. plumosus* (Fig. 1c)

1 - 2 - 3a-e - 5 - 6 - 7 - 8 - 9 - 10ab - 4 - 3 - 10c-g - 12 - 13

Arm F: Band sequences are the same as those from Germany (Keyl 1961) and Finland (Hirvenoja, Michailova 1991) (Fig. 1c).

23 - 22 - 21 - 20 - 19 - 16 - 15 - 12 - 11 - 9c - 8 cb - 7- 1

Arm G: Both homologues are always unpaired. The nucleolar organizer is located at the end of the arm. The centromere region of one homologous of chromosome G is very often at the telomere, while in the other, it is before the telomere: one homologous is acrocentric and the other is telocentric (Fig. 1d).

Larval morphology

Dark red body, length up to 18 mm, *plumosus* type (a pair of very short lateral tubules and 2 pairs of ventral tubules longer than the posterior parapodia). Head yellow with dark gula. SI simple, about 60 μm long, with hairs on one side, apical hairs on both sides. SII simple, about 100 μm long.

Premandible pale, with 2 teeth (Fig. 2a). The ventral tooth thinner and slightly shorter than the dorsal tooth. Pecten epipharyngis with 12-14 teeth (Fig. 2a).

Mandible. Apical tooth, 1st and 2nd inner teeth dark, 3rd inner tooth and dorsal tooth pale. Subdental setae simple, about 20 μm long. First and second internal setae simple, 3rd and 4th branched (Fig. 2b).

Antenna about 215 μm long. Length proportions of segments: 10:8:2:2:1. The antennal blade reaching 5th segment. RO located in the proximal part (one-third of the length) of the first segment (Fig. 2c)

Mentum dark with trifid median tooth (43-50 μm wide); shorter relative to nearest lateral tooth; 6 pairs of lateral teeth; 1st and 2nd very close to each other; 4th smaller than 2 neighboring teeth (Fig. 2d). Lateral teeth decreasing from 1 to 4; 5th tooth higher than 4th and 6th. The inner surface of ventromental plates is serrated (Fig. 2e).

Certain chromosome alterations can be detected in every studied individual. However, 10 individuals have malformations in the submentum and pecten epipharyngis.

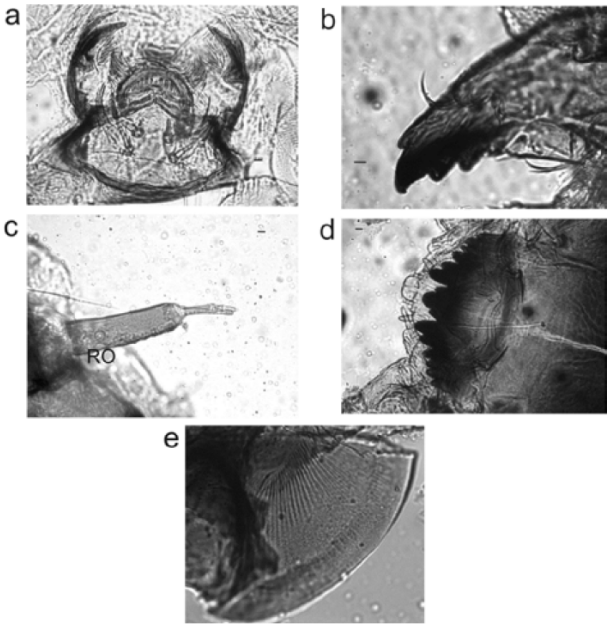

Fig. 2. Morphology of larval mouthparts: a) Premandible and pecten epipharyngis, b) Mandible, c) Antenna (RO) Ring Organ, d) Mentum, e) Ventromental plate.

Chromosome variability

Two types of chromosome rearrangements were detected in the studied population: the inherited type, which affected all cells of an individual and the somatic one, which appeared in few cells of the studied individuals. The somatic index is 0.54.
Inherited aberrations: a complex heterozygous inversion (13.5%) was found in arm D (Fig. 3a).
Somatic aberrations: paracentric inversions, deficiencies and deletions were detected: they occurred with low frequency and affected several bands of the chromosome arms A, B, C, D, E, F. For the frequency and location, see Table 2 and Fig. 3b, c, d. Homozygous deletions were observed only in chromosome G, which converted this chromosome into a small heterochromatin body (Fig. 3d).

Together with chromosome alterations, genome aberrations realized by "B" chromosome are detected (Fig. 4a). A trisomy in chromosome arms A, B, C, E, F, G was observed in 6 individuals. The presence of three homologues was observed in chromosome arms in different specimens, which was more evident in the distal regions of chromosome arms. In one individual, a trisomy could be observed either for one arm or for two arms. It was more evident in the

Table 2

Chromosome rearrangements in *Chironomus melanotus.*

Type of aberration	Arm A	Arm B	Arm C	Arm D	Arm E	Arm F	Arm G
Inherited het. inversion	-	-	-	3 ind. (15.79%)	-	-	-
Somatic het. inversion	1 ind. (1 cell)	1 ind. (1 cell)	1 ind. (1 cell)	2 ind. (2 cells)	1 ind. (1 cell)	1 ind. (1 cell)	-
Somatic het. deficiency	-	2 ind. (2 cells)	-	1 ind. (1 cell)	2 ind. (2cells)	-	-
Somatic deletion	-	-	-	-	-	-	1 ind. (4 cells)

Number of studied individuals - 19

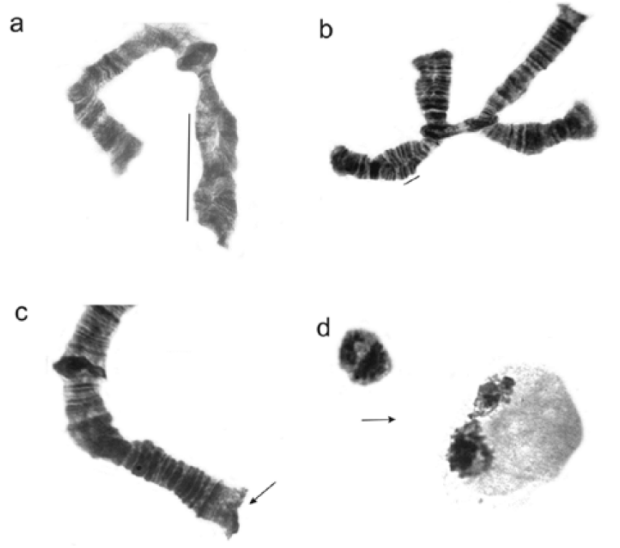

Fig. 3. Chromosome rearrangements: a) Inherited heterozygous inversion in chromosome arm D; b) Somatic heterozygous inversion in arm F, section 16-19; c) Somatic heterozygous deficiency in arm D, section 17; d) Homozygous deletion in arm G, section 2-4.

distal region of arm E (Fig. 4b) or in the middle of arm B.

There were also other interesting results. In every studied individual in the mosaic state, an ectopic pairing between centromere regions was observed: centromeres of AB and CD, AB and EF, AB, CD and EF, AB and G etc. Two or three of these centromeres may join and form the so-called "pseudochromocenter". In addition, an ectopic pairing of telomeres was detected. Ectopic pairing can be found either by conjugation of two centromeres or by a bridge connecting two centromeres or a centromere with a telomere. In 5 individuals in the mosaic state, the telomeres of chromosomes AB CD EF appeared in active grain structure. An asynapsis in some chromosome arms (A, E, F) appeared in all the individuals studied.

In the mosaic state, the centromere regions of chromosomes CD and EF were in the heterozygous state in four individuals (Fig. 5).

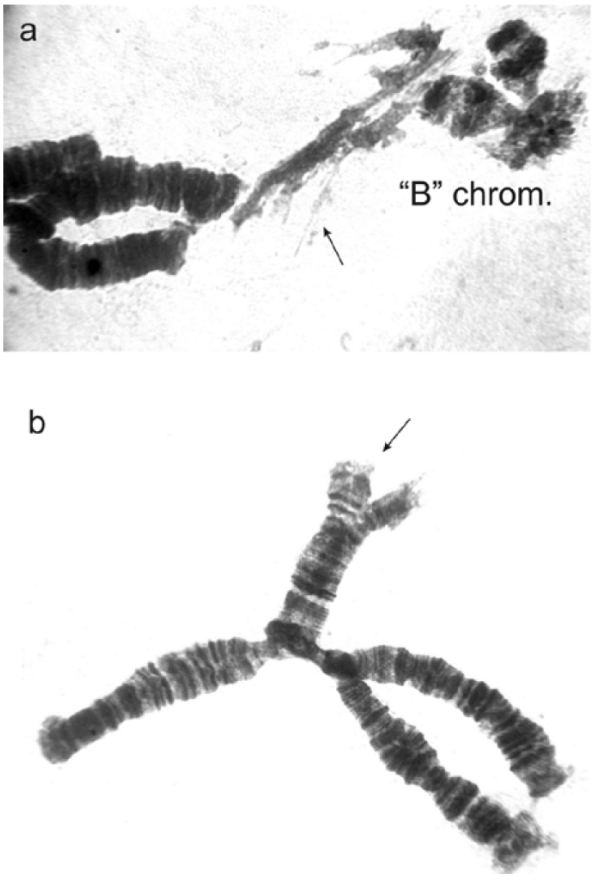

Fig. 4. a) "B" chromosome – looks like a heterochromatin structure; b) Trisomy in chromosome arm E and ectopic pairing between centromere regions of chromosomes EF and CD.

Certain chromosome alterations were detected in every studied individual. Specimens without alterations were not observed. Some malformations were detected: 7 on the submentum and 3 on the epipharyngis (altogether 10.27%). Malformations on the submentum are realized by a fusion of medium (Fig. 6) or lateral teeth, as well as by the absence of the medium teeth. Malformations affecting the pecten epipharyngis appeared as double teeth or some teeth are not developed.

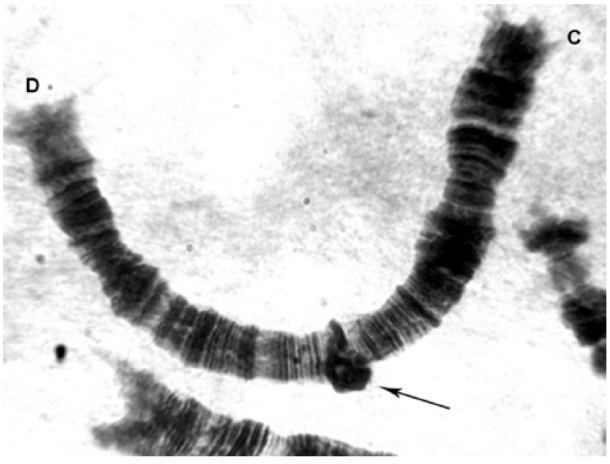

Fig. 5. The centromere region in chromosome CD in the heterozygous state, indicated by an arrow.

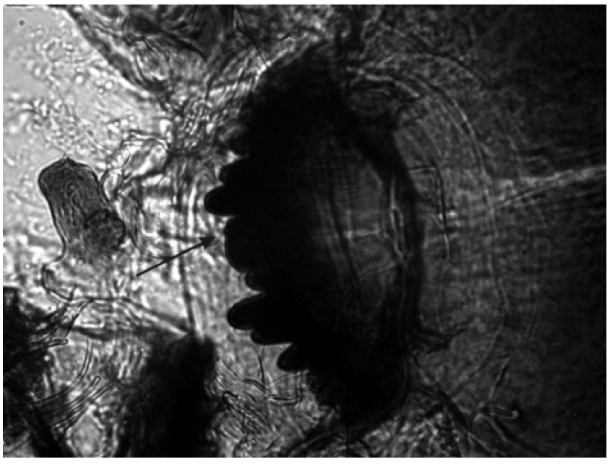

Fig. 6. Fusion of teeth in the middle part of the submentum. An arrow indicates the malformation.

DISCUSSION

The sediment analysis of some trace elements in Lake Łuknajno showed that the concentrations of Cu, Zn, Mn, and Ni in the sediment were similar or lower than those detected in unpolluted lakes of Poland (Solecki, Chibowski 2000; Sobczyński, Siepak 2001), and were considerably lower compared to polluted water bodies situated near cities (Szyper et al. 1994) or within industrial regions (Michailova et al. 2012). Only the concentration of Cd in the sediment of Lake Łuknajno was similar to the "probable effect level" PEL (Smith et al. 1996) above which its adverse effect on organisms is expected to occur frequently. The detected elevated concentration of Cd and Pb in the sediment of Lake Łuknajno was probably related to fertilizers present in soil, because the lake is strongly affected by its agricultural watershed (Kufel, Kufel 1997). Very often, nitrogen fertilizers may contain high concentrations of Cd (0.05-8.5 μg g^{-1}), while phosphate fertilizers may contain Cd (0.1-170 μg g^{-1}) and other heavy metals (Pb 7-225, 1-300 Cu, Zn, 50-1450, Mn 40-2000 μg g^{-1}) (Kabata-Pendias, Pendias 1999).

Most charophytes require hard waters with relatively high alkalinity (Kufel, Kufel 2002), such as in Lake Łuknajno (Kufel, Kufel 1997). In such conditions, the external cell wall in numerous species of *Chara* is covered by calcium carbonate incrustation of varied thickness. The degree of calcification increases with age of a plant (Siong, Asaeda 2009). Two processes are involved in the accumulation of trace metals from the water by *Chara*: (1) surface adsorption on the calcium carbonate deposit and (2) biological absorption (Beaugelin-Seiller et al. 1995). Carbonate incrustation plays a key role in the accumulation of elements such as Cd, Zn and Mn (Siong, Asaeda 2009; Urbaniak 2006, 2010). However, the knowledge of these phenomena is limited. Experimental studies have shown that the amount of Cd bound to the carbonate fraction in *Chara* samples may reach 73% (Siong, Asaeda 2009). Other studies (Lucas, Smith 1973) showed pH changes on the surface of *Chara corallina* cells during illumination and darkness. Therefore, a partial release of metals from the carbonate incrustation is possible. Changes in metal concentrations near the stem of *Chara* are important because the Chironomidae larvae for cytogenetically studies were found there. In general, *Chara* can accumulate heavy metals in large concentrations even in a small polluted aquatic ecosystem (Lacerda et al. 1992; Siong, Asaeda 2009; Clabeaux 2011). A thick marl bottom sediment layer frequently occurs below charophyte meadows, because the biomass of this species is concentrated at the bottom of the lake (Lee 1989). The sediment of Lake Łuknajno was also composed of calcareous gyttja (Kufel, Ozimek 1994) and had a low content of organic matter, as evidenced by the present study. Charophyte calcite may be important in the long-term storage of calcium (Ca) and other elements in the sediment during plant senescence and decomposition (Siong, Asaeda 2009).

Although *Chara* species produce different allelochemicals (sulfur, polyacetylenes, polyphenols,

oxygenated fatty acids) (Gross 1999), which affect plants and invertebrates found in the lake, *Chara* species are known to exert allelopathic effects on aquatic species and on planktonic algae (Antoni et al. 1980). The polytene chromosomes of the species *C. melanotus*, whose larvae have been found in patches of *Chara*, are very sensitive to some agents in the environment and show great genome instability. A high spectrum of somatic chromosome rearrangements has been found for the first time in this species. Lagadic and Caquet (1998), Sella et al. (2004), Michailova et al. (2009) and Michailova (2011) demonstrated that somatic alterations can be used as a biomarker for assessing the genotoxic effect of some environmental agents, especially trace metals, and they might be induced by stress agents in the environment. A high spectrum of somatic rearrangements was also reported for *C. riparius*, *C. piger*, *C. plumosus* and *C. bernensis* collected from trace metal polluted aquatic sites in different European populations (Michailova et al. 1996; Sella et al. 2004; Michailova et al. 2009).

A very rare event – a trisomy, which affected almost all chromosome arms of the species – was found in the studied species for the first time together with somatic chromosome rearrangements. Furthermore, the additional compact heterochromatin "B" chromosome observed in the population is new for the species. We can demonstrate for the first time that the *Chara* species might have also some effect on another group of organisms (insects – the larva stage of Chironomidae, a group very important for the aquatic ecosystem). The larvae of the studied species have been found on *Chara* and they developed the normal and rich fourth larva stage. However, the chemical compounds in the *Chara* species may have a strong effect on the presence of such a high spectrum of somatic aberrations in the salivary gland chromosomes. It is a well-known fact that somatic aberrations indicate the genotoxicity of different chemicals, trace metals, pesticides, etc. (Michailova 2011). It is quite possible that allelochemicals in *Chara* do not inhibit the growth of Chironomid larvae but, together with some trace metals (Cd and Pb), have some genotoxic effect expressed by a wide spectrum of somatic chromosome alterations, including a supernumerary "B" chromosome and a very rare phenomenon – a trisomy. Such a wide spectrum of somatic aberrations has been never reported in this species from other Palaearctic populations (Wülker 1973; Hirvenoja, Michailova 1991). Additionally, we observed a high level of morphological deformations in the mouth part of the larvae. The occurrence of deformed mouth parts in populations from uncontaminated sites is generally less than 1%. Higher percentage (5-25%) is considered to be an indicator of strongly polluted sites (Wiederholm 1984). The occurrence of head capsule deformations of 49% classifies the habitat as "Toxic Fair" in terms of water quality in situations of organic (Lenat 1993) and heavy metal contaminations (Al-Shami et al. 2010). Michailova et al. (2000, 2009) found that the Chironomid genome is more sensitive to trace metal pollution than the larval external morphology. The high frequency of malformations established in *C. melanotus* from Lake Łuknajno, together with high sensitivity of its genome, might be caused by chemicals produced by some macrophytes, such as *Chara*. Once again, we would like to stress that larvae of *C. melanotus* developed in *Chara* patches, whose chemical compounds may have induced such a high level of genome and phenotype alterations. This idea is supported by the finding that substances isolated from *Chara* species have toxic and cytotoxic effects on other organisms (Berger, Schagel 2004). Aquatic macrophytes have long been suspected of suppressing the phytoplankton growth through the excretion of chemical substances (Hutchinson 1975). It was found that some macrophytes were able to release polyphenols in the surrounding water and suppress some growth of cyanobacteria and green algae (Gross, Sutfeld 1994). The effect of *Chara* species on different green algae and algal community structures is particularly interesting, which indicated a species-specific response to chemical compounds of the *Chara* species (van Nes et al. 2002).

The increased spectrum of chromosome rearrangements may have been caused by toxic substances, such as a combination of heavy metals and allelochemicals produced by the *Chara* species. A common molecular mechanism in the toxicity of many xenobiotics may be caused by the production of so-called free radicals, which may result in a condition known as 'oxidative stress' (Stohs and Bagchi 1995). The free radicals are formed in the cells by ionizing radiation (by different chemicals) and are considered to be an important factor in the generation of free radicals and the formation of DNA damage in target cells.

Further research is required with different concentrations of Cd and allelochemicals extracted from *Chara* to determine which chemical substances induce genome instability in *C. melanotus*.

REFERENCES

Al-Shami, S., Rawi, C.S.M., Nor, S.A.M., Ahmad, A.H. & Arshad A. (2010). Morphological deformities in Chironomus spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of anthropogenic and environmental stresses on three rivers in the Juru River System, Penang, Malaysia. *Environ Entomol.*, 39(1): 210-22.

Anthoni, U., Christiphersen, C., Madsen, J.O., Wium-Andersen, S. & Jacobsen N. (1980). Biologically active sulphur compounds from the green alga Chara globularis. *Phytochem.*, 19: 694-95.

Beaugelin – Seiller, R.K., Baudin, J. & Casellas C. (1995). Experimental study of the effect of various factors on the uptake of ^{60}Co by freshwater mosses. *Arch. Environ. Contam. Toxicol.*, 28: 125-133.

Berger, J.& Schagel M. (2004). Allelopathic activity of Characeae. *Biologia*, Bratislava, 59(1): 9-15.

Bhattacharyay, M., Gibson, D. E., & Doty, D. H. (2005). The effects of flexibility in employee skills, employee behaviors, and human resource practices on firm performance. *Journal of Management*, 31: 622-640.

Clabeaux, B.L. (2011). *Potential use of charophytes in the phytoremediation of cadmium-contaminated soils.* Unpublished doctoral dissertation, State University of New York at Buffalo, USA, 202 pp.

Förstner, U. & Salomons W. (1980). Trace metal analysis in polluted sediments. *Environ. Techn. Letters*, 1: 494-517.

Gross, E.M. (1999). Allelopathy in benthic and littoral areas: case studies on allelochemicals from benthic cyanobacteria and submersed macrophytes In Inderjit Boca Raton (Eds.): *Principles and Practices in Plant Ecology,* Fla., CRC Press, pp. 179-99.

Gross, E.M. & Sutfeld R. (1994). Polyphenols with algicidal activity in the submerged macrophyte Myriophyllum spicatum L. *Acta Hortic.*, 381:710–16.

Hirvenoja, M. & Michailova P. (1991). Chironomus melanotus Keyl and its cytotaxonomical and ecological relations to Ch. riihimakiensis Wülker (Diptera, Chironomidae). *Spixiana*, München, 14(3): 309-20.

Hutchinson, G.E. (1975). *A Treatise on Limnology. Vol. III. Limnological Botany*. New York: Wiley, 660 pp.

Jabłońska-Barna, I. (2007). Macroinvertebrate benthic communities in the macrophyte-dominated Lake Łuknajno (Northeastern Poland*). Oceanol. Hydrobiol. Stud.*, 36, sup.4: 29-38.

Jabłońska–Barna, I. & Michailova P. (2009). Cytogenetic characteristics of species of the Chironomus genus (Diptera, Chironomidae) from Lake Łuknajno Biosphere Reserve (northwest Poland). *Oceanol. Hydrobiol. Stud.*, 38 (2): 77-81.

Kabata-Pendias, A. & Pendias H. (1999). *Biogeochemistry of trace elements*. Warszawa: PWN (in Polish), 398 pp.

Keyl, H.G. (1961). Chromosomenevolution bei Chironomus. I. Strukturabwandlungen an Speicheldruesen-Chromosome. *Chromosoma*, 12: 26-47.

Keyl, H. (1962). Chromosomeevolution bei Chironomus, II Chromosomenumbauten und phylogenetische Beziehungen der Arten. *Chromosoma*, 13: 464-514 (in German)

Królikowska, J. (1997). Eutrophication processes in a shallow, macrophyte-dominated lake - species differentiation, biomass and the distribution of submerged macrophytes in Lake Łuknajno (Poland). *Hydrobiol.*, 342/343: 411-16.

Kufel, I. & Kufel L. (1997). Eutrophication processes in a shallow, macrophyte-dominated lake -nutrient loading to and flow through Lake Łuknajno (Poland). *Hydrobiol.*, 342/343: 387-94.

Kufel, L. & Kufel I. (2002). Chara beds acting as nutrient sinks in shallow lakes—a review. *Aquatic Botany*, 72: 249–260.

Kufel, L.& Ozimek T. (1994). Can Chara control phosphorus cycling in Lake Luknajno (Poland)?. *Hydrobiol.*, 275/276: 277-283.

Lacerda, L.D., Fernandez, M.A., Calazans, C.F. & Tanizaki K.F. (1992). Bioavailability of heavy metals in sediments of two coastal lagoons in Rio de Janeiro, Brazil. *Hydrobiol.*, 228: 65–70.

Lagadic, L. & Caquet T. H. (1998). Invertebrates in testing of Environmental Chemicals: Are they Alternatives? *Environ Health Persp.*,106(2): 593–613.

Lee, R.E. (1989). *Phycology, 2nd ed.* Cambridge: Cambridge University Press, 645.

Lenat, D.R. (1993). Using mentum deformities of Chironomus larvae to evaluate the effects of toxicity and organic loading to streams. *Journal of the North American Benthological Society*, 12: 265-69.

Lucas, W. J. & Smith F. A. (1973). The formation of alkaline and acid regions at the surface of Chara corallina cells. *Journal of Experimental Botany*, 24: 1-14.

Michailova, P. (1989). The polytene chromosomes and their significance to the systematics of the family Chironomidae, Diptera. *Acta Zoologica Fennica*, 186: 1-107.

Michailova, P. (2011). Rearrangements in Chironomidae (Diptera), genomes induced by various environmental stress factors. *Russian Journal of Genetics: Applied Research,* Vol. 1(1):10–20.

Michailova, P. & Jabłońska-Barna I. (2008). Chromosome polymorphism of *Camptochironomus tentans* (Fabricius 1805) (Dietera: Chironomidae) from Łuknajno (Mazurian Lakeland), Poland. *Acta Zool. Bulg.*, 60(2): 155-63.

Michailova, P., Petrova, N., Bovero, S., Sella, G. & Ramella L. (2000). Effect of environmental pollution on the chromosomal variability of Chironomus riparius Meigen 1804 (Diptera, Chironomidae) larvae from two Piedmont stations. *Genetica*, 108: 171-80.

Michailova, P., Petrova, N., Ramella, L., Sella, G., Todorova J. & Zelano V. (1996). Cytogenetic characteristics of a population of Chironomus riparius Meigen 1804 (Diptera, Chironomidae) from a polluted Po River station. *Genetica* 98:161–78

Michailova, P., Sella, G. & Petrova N. (2011). Chironomids (Diptera) and their salivary gland chromosomes as indicators of trace – metal genotoxicity. *Italian J. of Zoology*, DOI: 10.1080/11250003.2011.622084.

Michailova, P., Szarek–Gwiazda E. & Kownacki A. (2009). Effect of contaminants on the genome of some species of genus Chironomus (Chironomidae, Diptera) live in sediments of Dunajec River and Czorsztyn Reservoir. *Water, Air and Soil Pollution,* 202: 245-58.

Michailova, P., Warchałowska-Śliwa, E., Szarek-Gwiazda, E. & Kownacki A. (2012). Does biodiversity of macroinvertebrates and genome response of Chironomidae larvae (Diptera) reflect heavy metal pollution in a small pond?. *Environ. Monit. Assess.*, 184(1): 1-14.

Müller, G. (1981). Die Schwermetallbelastung der Sedimente des Neckars und seiner Nebenflüsse. Eine Bestandaufnahme. *Chemiker-Zeitung*, 6: 157-64.

Sæther, O.A. (1975). Nearctic and Palaearctic *Heterotrissocladius* (Diptera: Chironomidae). *Bulletin of the Fisheries Research Board of Canada*, 193, 1–67.

Sæther, O.A. (1980). Glossary of Chironomid morphology terminology (Diptera: Chironomidae). *Entomol. Scand.*, Suppl. 14:1-51.

Sella, G., Bovero, S., Ginepro, M., Michailova, P., Petrova, N., Robotti, C. & Zelano V. (2004). Inherited and somatic cytogenetic variability in Palearctic populations of Chironomus riparius Meigen 1804 (Diptera, Chironomidae). *Genome*, 47: 332-344.

Siong, K. & Asaeda, T. (2009). Calcite encrustation in macro-algae Chara and its implication to the formation of carbonate-bound cadmium. *Journal of Hazardous Materials*, 167: 1237–1241.

Smith, S.L., MacDonald, D.D., Keenlyside, K.A., Ingersoll, C.G. & Field J. (1996). A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. *J. Great Lakes Res.*, 22: 624-638

Sobczyński, T. & Siepak J. (2001). Accumulation of biogenic compounds and speciation analysis of metals in bottom deposits from the Wielkopolski National Park lakes. *Zesz. Nauk. Wydz. Bud. Inż. Środ.*, 20: 265–290.

Solecki, J. & Chibowski S. (2000). Examination of trace amounts of some heavy metals in bottom sediments of selected lakes of South-Eastern Poland. *Pol. J. Environ. Stud.*, 9: 203-208.

Stohs, S. & Bagchi D. (1995). Oxidative mechanisms in the toxicity of metal – ions. *Free Radic.Biol.Mede.*, 18, 321-336.

Szyper, H., Goldyn, R. & Romanowicz W. (1994). Lake Swarzędzkie and its influence upon the water. In R. Goldyn (Eds.) *Protection of the water of the catchment area of the River Cybina.* Poznańskie Towarzystwo Przyjaciół Nauk, Prace Komisji Biologicznej, 74, pp. 59-73.

Urbaniak, J. (2006). Zn accumulation by two species of Chara (Charophyta). *Cryptogamie*, *Algologie* 27 (4): 451-459.

Urbaniak, J. (2010). Manganese accumulation by two species of Chara. *Oceanol. Hydrobiol. Stud.*, 39: 145-154.

Van Nes, E.H., Scheffer, M., van den Berg, M.S.& Coops H. (2002). Aquatic macrophytes: restore, eradicate or is there a compromise? *Aquatic Botany,* 72: 387–403.

Walker, C., Kaiser, K., Klein, W., Lagadic, L., Peakall, D., Sheffield, St., Soldan & Th. M. Yasuno. 1998. Environmental Health Perspectives. In *Alternative Testing Methodologies for Ecotoxicity*, 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): 106 (2): 441-457.

Wiederholm, T. (1984). Incidence of deformed chironomid larvae (Diptera: Chironomidae) in Swedish lakes. *Hydrobiol.*, 109: 243 – 249.

Wülker, W. (1973). Revision der Gattung Chironomus Meig. III. Europäische Arten des thummi-Komplexes. *Arch. Hydrobiol.*, 72(3): 356–74 (in German).

Wülker, W.& Götz, P. (1968). Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von Chironomus-Larven (Dipt.). *Z. Morphol. Tiere,* 62: 363-388.