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Biodiversity of Family Chironomidae (Diptera) in Srebarna Lake (North-East Bulgaria) and Genome Instability of Some Species from Genus Chironomus Meigen, 1803

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Abstract. The biodiversity of the family Chironomidae, Diptera in Lake Srebarna - a lake of natural origin, a Biosphere Reserve and a Ramsar Site of International Importance was studied. The conducted study revealed high concentrations of phosphates and some heavy metals (Cu, Pb, Zn, Mn, Fe) in the water. High concentrations of heavy metals (Cd, Cu, Pb, Mn) in the sediment were also found. Through the detailed analysis of the external morphology of the larvae and the species-specific cytogenetic markers of the polytene chromosomes of the larvae, we established a total of 16 genera and 11 species. Ten genera and eight species were new to the fauna of the lake. For the first time, we reported malformations in the larvae of some genera *Endochironomus*, *Chironomus* and *Glyptotendipes* (0.84÷2.04%) and species (*Endochironomus tendens* - 0. 29%). Genomic instability realized through somatic structural chromosomal aberrations in the polytene chromosomes of the four species of the genus *Chironomus* was found. Based on these aberrations, the Somatic index (S) was calculated (*C. nuditasis*, S-3.25; *C. annularius*, S-5.75; *C. balatonicus*, S-7.5 *C. pallidivittatus*, S-4.50). In addition, inherited chromosome aberrations have been observed, which were important for the adaptation of species to specific living conditions. The reasons of genomic instability and the importance of *Chironomus* species for determining the degree of pollution of aquatic ecosystems were discussed.

Key words: Srebarna Lake, Chironomidae, biodiversity, trace metals, polytene chromosomes, genome instability.

Introduction

Srebarna Lake is declared as a Ramsar Site of International Importance and a Biosphere Reserve, situated in the flood terrace of the Danube River in the North-East part of Bulgaria. In 1949 the lake was disconnected from the Danube River by building of a dyke and the main source of fresh water remained the surface run-off and ground waters. Due to the critical ecological conditions, later was restored the channel which connected the lake to the Danube. Nowadays the water inflow is regulated by two floodgates.

It is important to emphasize the role of benthic organisms and in particular the larvae of non-biting aquatic midges (Chironomidae, Diptera) in the function and structure of aquatic ecosystems. In freshwater habitats, they are very important

and applied to monitor the environmental health and water quality. Chironomid larvae spend their development in sediment surface, where they remain exposed to different pollutants and carry sediment-associated contaminants to higher trophic levels in the food web (AL-SHAMI *et al.*, 2013).

Before the reconstruction of the connection with the Danube River, MICHAILOVA (1998) determined cytotaxonomically 11 species of family Chironomidae, which belonged to 6 genera: *Chironomus* Meigen, 1803, *Cryptochironomus* Kieffer, 1918, *Endochironomus* Kieffer, 1918, *Glyptotendipes* Kieffer, 1913, *Cricotopus* van der Wulp, 1874 and *Parakiefferiela* Thienemann, 1936.

After the restoration of the connection with the Danube River, intensive studies of the benthic organisms in the lake have begun. The first study of KOVACHEV *et al.* (1995) did not report any benthic macroinvertebrates in the lake. Later, in a period of 15 years (from 1997 to 2012) 18 species from 11 genera, determined by external morphology of the larvae were registered in the lake area including peripheral pools (UZUNOV *et al.*, 2001; VARADINOVA *et al.*, 2011; 2012; VIDINOVA *et al.*, 2016).

It is very important in the study of biodiversity of the Chironomidae species, together with the analysis of the external morphological features of the larva, to be used additional signs. These external features show either great variability in a species or are indistinguishable between species. Nowadays, the specific cytogenetic markers of the polytene chromosomes from the salivary glands of the larvae are used as an additional taxonomic sign for the precise determination of Chironomid species (KEYL, 1962; MICHAILOVA, 1989; KIKNADZE *et al.*, 1991; 2016). Also, these chromosomes are a good candidate for evaluating the genotoxic effect of different mutagenic factors in the environment (MICHAILOVA *et al.*, 2012; 2015). These authors suggested many somatic chromosome rearrangements in the salivary gland chromosomes (heterozygous inversions, deletions, deficiencies, amplifications) as biomarkers for detecting damages in the environment under different pollutants.

Having in mind the significance of Chironomid larvae for aquatic ecosystems, the purpose of our study is to determine their biodiversity in Lake Srebarna by analyzing the external morphological features and species - specific banding patterns of the salivary gland chromosomes of the larvae in order to determine the genera and species of the family. In addition is shown the genome response of some *Chironomus* species to pollutants established in the lake.

Material and Methods

Study area. Srebarna Lake is a lake of natural origin. The lake area includes free-standing water surrounded by reed and bulrush and little ponds in-between. We collected larvae material from Pristan, Kamaka, Dragajka, Central free-standing water, Chervenka and South floodgate. The first four sites are also used in the routine monitoring of the lake. The sites Kamaka and Chervenka are considered as peripheral pools. The GPS (Global Position System) coordinates of the sampling sites are as follows: Pristan - 44.102411°, 27.064064°; Kamaka - 44.106481°, 27.081678°; Dragajka - 44.111583°, 27.075106°; Central free-standing water - 44.107700°, 27.071867°; Chervenka - 44.110469°, 27.062489; South floodgate - 44.121104°, 27.081487°. The samples were collected from the bottom of the lake as well as from submerged and emergent vegetation. For cytogenetic analysis, the larvae were collected from site Pristan, which is situated closer to the village and is used as a boat pier.

Physical and chemical analysis of water and sediment. Alkalinity (pH), conductivity, water temperature and dissolved oxygen/oxygen saturation was measured *in situ* according to the following standards:

BSS (Bulgarian State Standard) ISO (International Organization for Standardization) 10523:2012, BSS EN 27888:2000, BSS 17.1.4.01:1997 and BSS/EN/ ISO 5814:2012. The analysis of the nitrogen and phosphorus forms - NH_4-N, NO_2-N, NO_3-N, PO_4-P was performed *in lab* according to BSS EN ISO 11732:2006, BSS EN ISO 13395:2001, and BSS EN ISO 15681:2005. The concentrations of heavy metals (Cu, Pb, Zn, Mn, Cd, Fe) from water and sediment are according to UMPMR (Updated Management Plan of Managed Reserve) "Srebarna" (2015), Annex 7.5.2.3. As reference data for heavy metals in water we used those appointed by Regulation N-4/23.09.2014 and Directive 2013/39/EU, and for heavy metals in sediments – data, given by FÖRSTNER & SALOMONS (1980).

Chironomid larvae were sampled in July of the years 2014 and 2017. The samples were collected using a hand-net (0.25x0.25 m²) and the sample from the Central free-standing water – an Eckman's grab (181 cm²) according to ISO 10870:2012.

The larvae used for the analysis of the genera were fixed in a dilution of 4% formaldehyde. Permanent slides of external morphology of the larvae were done according to SAETHER (1979). Their identification by external morphology was done according to WIEDERHOLM (1983); SCHMIDT (1993); BITUŠÍK & HAMERLÍK (2014).

The larvae for cytogenetic analysis were fixed in alcohol: acetic acid (3:1). Slides of salivary gland chromosomes were done according to MICHAILOVA (1989), applying acet-orcein method. For species identification were used the polytene chromosome markers by MICHAILOVA (1989) and KIKNADZE *et al.* (2016). Chromosome maps, presented by KIKNADZE *et al.* (1991; 2016) for *Chironomus nuditarsis* Keyl, 1961, *C. annularius* Meigen, 1818, *C. balatonicus* Devai, Wuelker & Scholl, 1983 and *C. pallidivittatus* Edwards, 1929 were applied for localizing the chromosome aberrations and establishment of the genome instability. Two types of these rearrangements were considered: somatic and inherited. Somatic aberrations occurred in few cells of the individual and affected very small region of the polytene chromosomes. On these aberrations the Somatic index (S) was calculated (SELLA *et al.*, 2004). Inherited aberrations affected all cells of the individual and appeared in a large region of the polytene chromosomes. The frequency of the aberrations was presented in percentage. Also, some alterations in functional activity of key structures: Balbiani rings (BRs) and Nucleolar Organizers (NOR) were shown.

Malformations, registered in the species and genera were calculated as a percentage of the number of tribe Chironomini, calculated to 1 m².

Results

Physical and chemical parameters of the water and sediment

The values of pH varied between 7.5 and 8.35 indicating slightly alkaline water, typical for the eutrophic basins. Temperature of the water was typical for the summer season (24.7°C – 26.3°C). The values of conductivity varied between 412 and 431 µSi/cm, indicating slightly hard water. The oxygen concentration and saturation were lower at the sites Pristan, Kamaka, Chervenka and South floodgate (1.58÷4 mg/l) and were higher in the Central free-standing water (6.8 mg/l) and Dragajka (4.8 mg/l).

The concentrations of ammonium, nitrite (0÷0.04 mg/l) and nitrate nitrogen (1.5÷2 mg/l) were very low. The concentrations of phosphates were elevated at the Central free-standing water (0.12 mg/l) and at site Chervenka (0.09 mg/l).

All concentrations of heavy metals measured in water (Fig. 1) exceeded from 3 to 10 times the Average Annual Values, defined in Regulation N-4/23.09.2014 and Directive 2013/39/EU as the highest concentrations were those of copper and iron. The concentrations of Cu (Pristan and Dragajka), Pb (Dragajka) and Mn (Pristan, Dragajka, Central free-standing water)

measured in sediment samples exceeded less than one time, cadmium exceeded - 3 times the reference data (according to FÖRSTNER & SALOMONS, 1980), while the concentrations of Zn and Fe didn't exceed these data (Fig. 2).

Chironomid biodiversity

Table 1 summarized the genera and species determined in the lake Srebarna by external morphology of the larva and specific markers of their polytene chromosomes respectively. During our study, 16 genera were established and 11 species were identified by species - specific banding patterns of the polytene chromosomes (Table 1). Ten genera and 8 species of the genera *Chironomus, Endochironomus*, *Glyptotendipes, Kiefferulus* Goetghebuer, 1922, *Polypedilum* Kieffer, 1912 were new for the lake fauna.

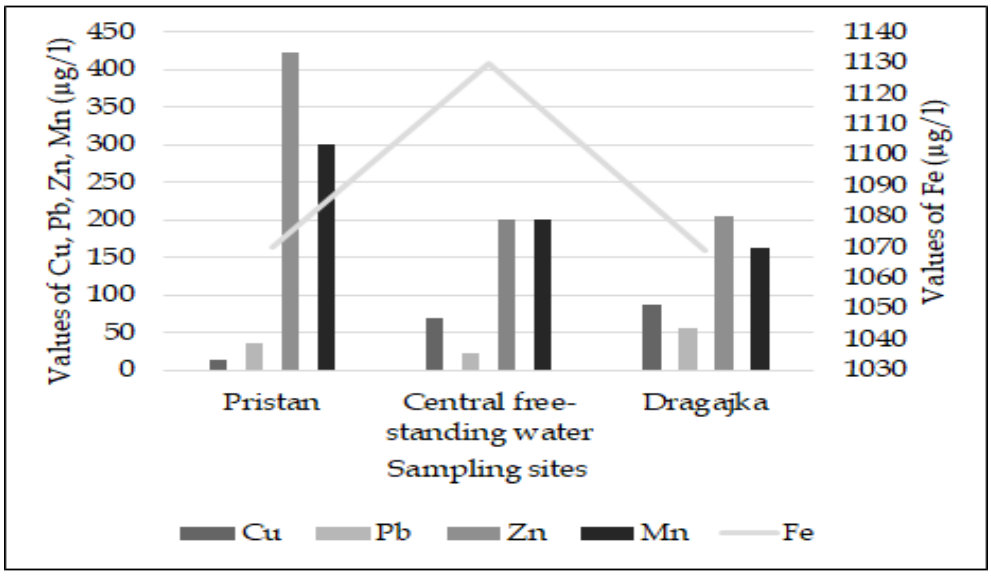

Fig. 1. Concentrations of heavy metals in water of Lake Srebarna.

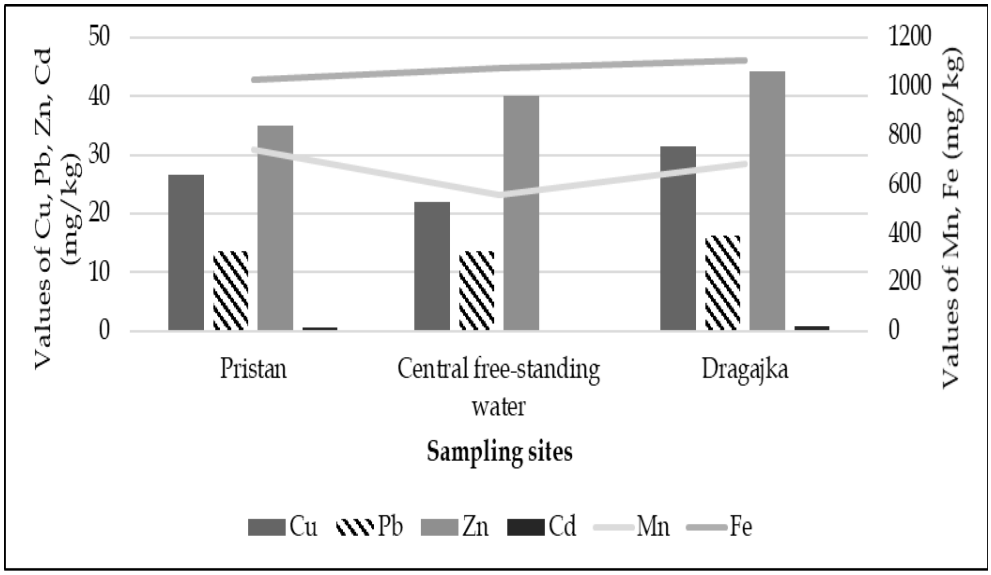

Fig. 2. Concentrations of heavy metals in sediment of Lake Srebarna.

Table 1. Genera and species of the Srebarna Lake (* - new genera, determined by external morphology of larva, ** - new species, determined cytotaxonomically, x – registered species in the lake, P – registered only at a peripheral pool), column A – data after MICHAILOVA, 1998; column B – data after UZUNOV *et al.*, 2001; VARADINOVA *et al.*, 2011; 2012; VIDINOVA *et al.*, 2016.

Genera and species	This study	Number of studied larvae	A	B
Tanypodinae				
**Ablabesmyia* Johannsen, 1905	x	35		
**Anatopynia* Johannsen, 1905	x	12		
**Guttipelopia* Fittkau, 1962	x	7		
**Monopelopia* Fittkau, 1962	x	20		
**Tanypus* Meigen, 1803	x	1		
**Telmatopelopia* Fittkau, 1962	x	6		
Prodiamesinae				
P. olivacea (Meigen, 1818)				x/P
Orthocladiinae				
Corynoneura Winnertz, 1846			x	
Cricotopus Kieffer, 1909	x	25		x
C. algarum (Kieffer, 1911)				x
C. ornatus (Meigen, 1818)			x	x
C. sylvestris (Fabricius, 1794)			x	x
C. trifascia Edwards, 1929			x	
Eukiefferiella gracei (Edwards, 1929)				x/P
E. cf. similis Goetghebuer, 1939				x
Parakiefferiella bathophila (Kieffer, 1912)			x	x
Psectrocladius ishimicus Chernovskij, 1949			x	
Tvetenia Kieffer, 1922				x
Chironominae				
Tanytarsini				
**Paratanytarsus* Thienemann & Bause, 1913	x	19		
Tanytarsus van der Wulp, 1874	x	1		
T. gregarius Kieffer, 1909				x/P
Chironomini				
Camptochironomus Kieffer, 1918			x	
Chironomus Meigen, 1803	x	8		x
C. annularius Meigen, 1818	x	6	x	x/ P
***C. balatonicus* Devai, Wuelker & Scholl, 1983	x	3		
***C. nuditarsis* Keyl, 1961	x	4		
***C. pallidivittatus* Edwards, 1929	x	2		
***C. parathummi* Keyl, 1961	x	1		
C. plumosus (Linnaeus, 1758)			x	x
C. riparius (Meigen, 1804)			x	x
Cryptochironomus Kieffer, 1918				x
C. defectus (Kieffer, 1913)			x	x
Dicrotendipes nervosus (Stæger, 1839)	x	2		x
Endochironomus Kieffer, 1918	x	93	x	x
***E. tendens* (Fabricius, 1775)	x	14		

Glyptotendipes Kieffer, 1913	x	53		
G. glaucus (Meigen, 1818)	x	2	x	x
G. cauliginellus (Kieffer, 1913)			x	x
G. pallens (Meigen, 1804)				x
***G. paripes* (Edwards, 1929)	x	6		
**Kiefferulus* Goetghebuer, 1922	x	49		
***Kiefferulus tendipediformis* (Goetghebuer, 1922)	x	4		
**Parachironomus* Lenz, 1921	x	29		
**Polypedilum* Kieffer, 1912	x	49		
***P. sordens* (van der Wulp, 1875)	x	12		

Morphological deformities

In some species we observed malformations which affected only mentum: *Endochironomus tendens* (0.29%), *Chironomus sp.* (0.84%) and *Glyptotendipes sp.* (0.58%) and mentum and mandibles: *Endochironomus sp.* (2.04%).

Cytogenetic characteristics of the studied Chironomus species

Species of genus *Chironomus*: *C. nuditarsis, C. annularius* and *C. balatonicus* cytogenetically belong to cytocomplex thummi (KEYL, 1962) with chromosome arm combinations: AB CD EF G. Chromosome G carries the Balbiani Rings (BRs) and Nucleolar Organizer (NOR) in all above mentioned species. In the karyotype of *C. annularius* additionally NORs are localized in arms A, C, and E. Also, one Balbiani ring functions in arm B in these species. Chromosomes AB and CD of the species are metacentric, while chromosome EF is subemetacentric (Fig. 3). The chromosome G is telocentric in *C. nuditarsis* (Fig. 3) and *C. annularius.* In *C. balatonicus* it is acrocentric. *C. pallidivittatus* belongs to *Camptochironomus* complex (KEYL, 1962) with chromosome arms combinations: AB, CF, DE and G. Arm A has a NOR, arm G carries three BRs. Chromosomes AB, CF are metacentric, chromosome DE is submetacentric and chromosome G is telocentric.

Genome instability in the above Chironomus species

In the studied 4 species of genus *Chironomus* (*C. nuditarsis, C. annularius, C. balatonicus, C. pallidivittatus*) were observed two types of chromosome aberrations: inherited and somatic.

Inherited aberrations

C. nuditarsis: in the studied material two inherited aberrations were established: heterozygous inversions in arms: A - A1/A2 (75%) and B - B1/B2 (50%) as well as a dark knob. (DK) at the end of chromosome G, which appeared in homo (Fig. 4a) and heterozygous state (25%). *C. annularius:* several heterozygous inversions localized in arms A - A1/2 (50%), B - B1/2 (25%), D- (D1/D2) (25%) and F - F1/F2 (50%) were found. In studied individuals the dominant is the homozygous inversions in arm A - A2/A2, section 13ab-12cbq. *C. pallidivittatus*: two inherited heterozygous inversions were recognized. They affected chromosome arms D - D1/D2 (50%) and E, region 13-14 (50%). In all studied cells BR_3 showed a slight functional activity. *C. balatonicus:* several chromosome rearrangements were detected - homozygous inversions in arms C (50%) and B (50%), heterozygous translocation between AB and CD chromosome (Fig. 4b) and heterozygous inversions in arm A (A1/A2) (50%) (Fig. 4b). BRs showed a slight functional activity.

Somatic aberrations

The genome instability of the studied *Chironomus* species *C. nuditasis, C. annularius, C. balatonicus* (Fig. 4c) and *C. pallidivittatus* (Fig. 4d) is realized by somatic para - and pericentric heterozygous inversions, deficiency, an appearance of a dark knob (in somatic state) and a slight functional activity or complete loss of BRs. Their frequency and localization can be seen in Table 2.

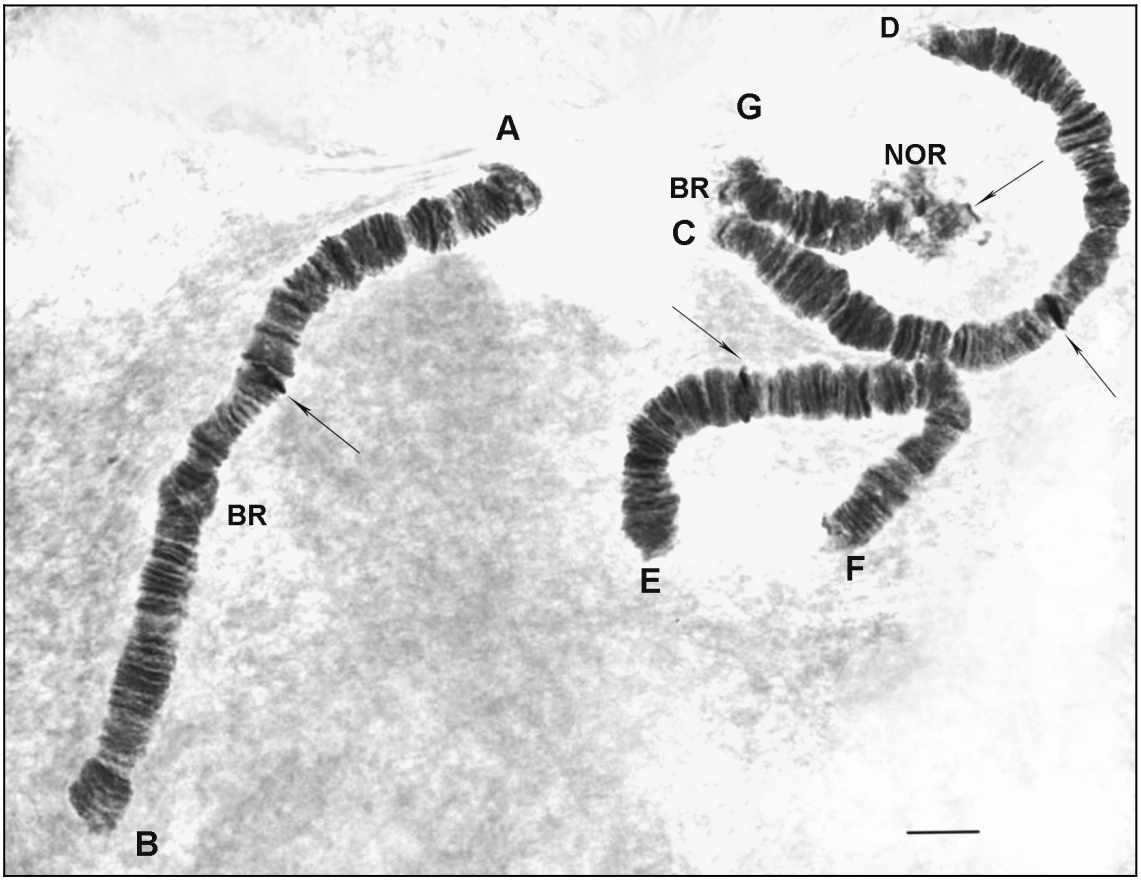

Fig. 3. Polytene chromosomes of *C. nuditarsis.*
Arrow indicates the centromere regions of the chromosomes. Bar – 10 μm.

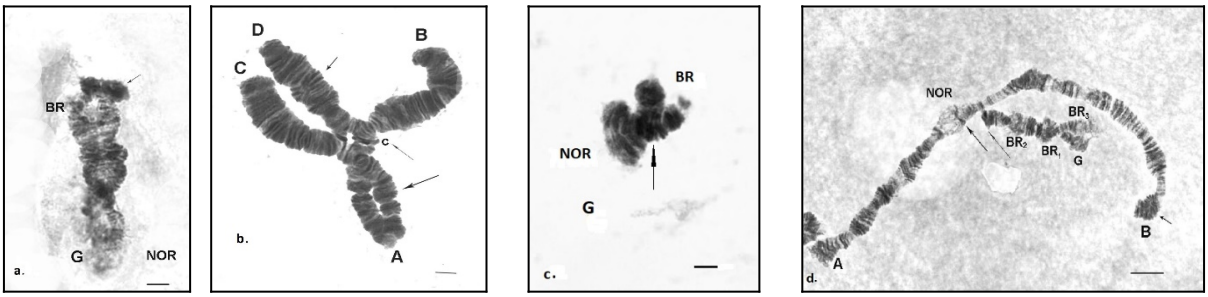

Fig. 4. Chromosome aberrations, bar – 10 μm.
a. Chromosome G of *C. nuditarsis* with a dark knob (DK). A small arrow shows the localization of DK. BR – Balbiani Ring, NOR – Nucleolar Organizer.
b. Translocation between chromosomes AB and CD of *C. balatonicus*. A small arrow indicates the somatic heterozygous inversion in arm D. A large arrow shows the inherited aberration in arm A. Arrow C indicates the centromere region.
c. Chromosome G of *C. balatonicus;* Arrow shows the somatic aberration – a homozygous deletion, BR – Balbiani Ring, NOR – Nucleolar Organizer.
d. Chromosomes AB and G of *C. pallidivittatus*.; A small arrow shows the localization of the somatic aberration – heterozygous inversion in arm B; A large arrow shows the centromere region. BR_3 - Balbiani ring 3 with a slight functional activity, NOR – Nucleolar Organizer.

Table 2. Somatic aberrations in polytene chromosomes of *Chironomus* species, collected from Lake Srebarna N. number; Het.inv. - heterozygous inversion; Hom. del. – homozygous deletion.

Aberr. and localization/ N. of cells			
Chromosome AB	Chromosome CD	Chromosome EF	Chromosome G
***C. nuditarsis*, cytocomplex – thummi** (arm combination AB CD EF G), **N. of st. ind./cells: 4/148; N. of cells with aberr.: 20; N.of somatic aberr.: 13; Somatic index (S): 3.25**			
Het.inv.A -13a/ 1 cell	Pericent. het.inv.CD/ 1 cell	Het.inv. E 13q/ 1cell	Het.inv. in the middle/2cells
Pericent.het.inv.AB/ 2 cells	Deficiency D/ 1 cell	Het.inv.F-18a/ 2 cells	Dark knob/ 2 cells
	Het.band D/ 1cell	Het.inv.F-18e/ 1cell	Dark knob het.state/ 1 cell
		Pericent.inv.EF/ 4cells	
***C. annularius*, cytocomplex – thummi** (arm combination AB CD EF G), **N. of st. ind./cells: 4/135; N. of cells with aberr.: 22; N. of somatic aberr.: 11; Somatic index (S): 5.75**			
Het.inv.A2 2h-3i/ 1 cell	Het.inv. C 12c/ 1 cell	Het.inv.F 6/ 1 cell	Het.inv. in the middle/3cells
Het.inv.A2 8-7/ 1 cell	Het.inv. C 13/ 1 cell	Het.inv. F 12/ 1 cell	
Het.inv.B in the middle/7 cells	Het.inv. D 18d-a/ 2 cells		
Het.inv. near the telomere/2cells			
Pericent.inv.AB/ 2 cells			
***C. balatonicus*, cytocomplex - thummi** (arm combination AB CD EF G), **N. of st. ind./ cells: 2/86; N. of cells with aberr.: 22; N. of somatic aberr.: 15; Somatic index (S): 7.5**			
Het. inv. B 15ab/ 1 cell	Het. inv. C 15-16/ 2 cells	Het. inv. E 3-4/ 1 cell	Hom. del./ 1 cell
Het. inv. B 21-25/ 2 cells	Het. inv. C 16-17/ 2 cells	Het. inv. E 6-9/ 2 cells	Het. del./ 1 cell
	Het. inv. C 23-25/ 1 cell	Het. inv. F 12-14/ 2 cells	
	Het. inv. D 4-9/ 2 cells	Het. inv. F 13/ 1 cell	
	Het. inv. D 7-2a/ 2 cells	Het. inv. F 13-14/ 1 cell	
	Het. state of the centromere		
***C. pallidivittatus*, cytocomplex - camptochironomus** (AB CF DE G), **N. of st. ind./cells: 2/73; N. of cells with aberr.: 10; N.of somatic aberr.: 9; Somatic index (S): 4.5**			
Het.inv. B 3- 4/ 1 cell	Het.inv. C 3/ 1 cell	Het.inv. E13-14/1 cell	Het.inv. 4/ 2 cell
Het.inv. B 9/ 1 cell		Het.inv. F12-13/ 1cell	Het.inv. 5/ 1 cell
Het.inv. C 3/ 1 cell		Het.inv. F 16/ 1 cell	
		Het.inv.F18-17/ 1 cell	

Discussion

The ecological situation in the lake can be assessed as favorable according to the data of physical and chemical parameters of the water and nitrogen forms in it, as the chemical state was varying between "good" and "very good". According to a proposed working scale for assessment of the condition of wetlands by physicochemical quality elements (IVANOVA *et al.*, 2017), the values of conductivity, ammonium and nitrite nitrogen showed very good chemical state and those of pH and nitrate nitrogen – a good one. The dissolved oxygen concentration at sites Chervenka and Dragajka was moderate, while sites Pristan, Kamaka and South floodgate had very bad chemical state. At those sites the aquatic vegetation was dense and probably causing a depletion of the oxygen in the water. On the other hand, the concentrations of phosphates at Central free-standing water were bad and at Chervenka – moderate (IVANOVA *et al.*, 2017). A tendency of phosphates increasing was observed as it was mentioned by HIEBAUM *et al.* (2012) since 2007. It is interesting to emphasize that in comparison to the data of HIEBAUM *et al.* (2012) heavy metal concentration in water were higher from less than 1 time to 17 times. Especially Pb and Mn, which were increased 17 and 14 times.

The concentrations of Fe, Cu and Cd in sediment, compared to a previous period (HIEBAUM *et al.*, 2012) had an increase of less than 1 time, 2 times and 6 times respectively. On the other hand, Mn, Zn and Pb concentrations had a decrease from less than 1 time to 2 times.

Considering the newly established genera and species in the lake, it was confirmed the importance of the habitat e.g. aquatic vegetation for their diversity. Also VARADINOVA *et al.* (2011) and BORISOVA *et al.* (2014) pointed the local habitats (modified by the flooding regime) and the peripheral zones of the lake, mostly covered with aquatic vegetation as the main factors for the development and high diversity of the bottom macroinvertebrate fauna. For the first time in the present study were established the malformations in the mouth structures of the larvae in Srebarna Lake. The registered increasing of all heavy metals and phosphates in water and copper, cadmium and iron in sediment could influence the appearance of the malformations (ODUME *et al.*, 2012; ILKOVA *et al.*, 2018). However, they could emerge also under the influence of other factors of the environment (like seasonal temperature) as well (SERVIA *et al.*, 2000).

Many data indicated that Chironomid genome is more sensitive to the contaminations in the aquatic ecosystems than the external morphology (MICHAILOVA *et al.*, 2012; 2015). The cytogenetic analysis in this study revealed inherited and somatic aberrations in the polytene chromosomes of Chironomus species. The established inherited heterozygous inversions in *C. nuditarsis* were announced by KIKNADZE *et al.* (2006) for the Bulgarian population as well, however, in different frequency. The dark knob in chromosome G have been found in Siberian and different European populations (KIKNADZE *et al.*, 2006) and appeared after local amplification of DNA. The heterozygous inversions, found in polytene chromosomes of *C. annularius* were mentioned in other Palearctic populations as well (KIKNADZE *et al.*, 2012).

In the studied individuals of *C. annularius*, the homozygous inversion in arm A was dominant. It is interesting to note that the same homozygous inversion with a high frequency was found in trace metal polluted Chehlo River in south Poland (MICHAILOVA *et al.*, 2018). As in Chehlo River, the functional activity of NORs in arm A, E was disturbed. They occurred either in heterozygous state or completely depressed (37.04%). The heterozygous inversion that affected chromosome E, region 13-14 of *C. pallidivittatus* was announced for the first time. The homozygous inversions, established in arms C and B of the polytene chromosomes of *C. balatonicus* were

announced also in larvae, exposed to a mutagenic environmental pollution (PETROVA, 2013) and in different European populations (KIKNADZE *et al.*, 2016). They were involved in so called polymorphic system (standard homozygous type of the karyotype through heterozygotization transformed into another homozygous type) (MICHAILOVA, 2015). These inversions are varying with a selective priority in different ecologic-climatic conditions. In the studied area in these arms, priority, with high frequency appeared the homozygous inversions in comparison with the standard. The observed heterozygous translocation between AB and CD chromosome was the first one, observed for this species. Also, KIKNADZE *et al.* (2016) announced of heterozygous translocation between AB and G and EF and G. Translocation between A and E announced also PETROVA (2013) in radioactive Chernobyl region. It is important to underline that heterozygous inversions in arm A (A1/A2) is very common in different populations of the species along its range. Most of the inherited aberrations were found in other Palearctic populations, however with different frequency, because these aberrations have different adaptive role in geographical distant regions with specific climatic conditions.

Polluted sediments induced great damages of the genome of the studied *Chironomus* species: *C. nuditasis*, *C. annularius*, *C. balatonicus* and *C. pallidivittatus*. The data of the established somatic aberrations are in agreement with MICHAILOVA *et al.* (2012; 2015) who found that polluted sediments by heavy metals and some organic toxicants caused significant chromosome damages to chironomid's polytene chromosomes. So, the polytene chromosomes are a reliable and sensitive tool in the determination of the genotoxic effect of polluted sediments.

In the study, we found that different species, other than *C. riparius*, *C. piger* Strenzke, 1956, C. *bernensis* Kloetzli, 1973 and *C. annularius* (MICHAILOVA *et al.*, 2012; 2016; 2018) of genus *Chironomus* have great sensitivity of the genome and are good candidates for evaluating the mutagenic effect of different agents. However, as a result of our study, it is well seen that different species inhabiting the same lake have different genome response to the same environment stress agents. It is known that frequency and type of chromosomal rearrangements depend on the structure and organization of DNA sequences (KING, 1993). Our previous study (MICHAILOVA *et al.*, 2009) showed very well the role of transposable elements in genome instability and their different amount and localization in different species (SELLA *et al.*, 2004).

Conclusions

During the present study were registered 10 new genera and 8 new species for the lake fauna. For the first time the malformations of larvae were detected - important signs for changes in the environment. Different species of genus *Chironomus*, which show a genome response, depending on the DNA structure and organization, can be used as an indicator of genotoxic concentrations of pollutants in aquatic ecosystems. Due to the great resolution of the polytene chromosomes, they are very useful in applied investigations on environmental quality. The somatic cytogenetic damages plus functional alterations are particularly suitable as biomarkers as these cytogenetic changes are easily identified and provide early warning signals of adverse long effects in organisms. Analysis of cytogenetic responses is therefore potentially a powerful tool in preventing the long-term effects of anthropogenic stress at the populations and community level.

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References

AL-SHAMI S. A., M. R. CHE SALMAH, S. A. MOHD NOR, A. H. AHMAD, N. A. MOHD ISHADI, H. DIENG. 2013. Genotoxicity in *Chironomus kiiensis* (Chironomidae: Diptera) after exposure to polluted sediments from rivers of North Peninsular Malaysia: Implication for ecotoxicological monitoring. - *River Research Applications*, 29(9): 1195-1204. [DOI]

BITUŠÍK P., L. HAMERLIK. 2014. *Identification key for Chironomidae larvae of Slovakia. Part 2. Tanypodinae*. Belianum, Vydavateľstvo Univerzity Mateja Bela v Banskej Bystrici, Slovakia, 96 p. (In Slovak).

BORISOVA P., E. VARADINOVA, M. KERAKOVA, S. KAZAKOV, S. STOICHEV, Y. UZUNOV, L. PEHLIVANOV. 2014. Seasonal changes in benthic communities of the Srebarna Lake (Northeast Bulgaria): habitat perspective. - *Acta Zoologica Bulgarica*, 66(2): 239-245.

EC. 2013. Directive 2013/39/EU amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. - *Official Journal of the European Union*, L 266: 1-17. Available at: [eur-lex.europa.eu].

FÖRSTNER U., W. SALOMONS. 1980. Trace metal analysis in polluted sediments. Part I: Assessment of Sources and Intensities. - *Environmental Technology Letters*, 1: 494-505. [DOI].

HIEBAUM G., V. TSAVKOVA, R. CHRISTOVA, V. VASSILEV. 2012. Hydrochemistry and water quality. - In: UZUNOV Y., B.B. GEORGIEV, E. VARADINOVA, N. IVANOVA, L. PEHLIVANOV, V. VASILEV (Editors). *Ecosystems of the Biosphere Reserve Srebarna Lake.* Sofia. "Professor Marin Drinov" Academic Publishing House, pp. 197-212.

ILKOVA J., P. MICHAILOVA, E. SZAREK-GWIAZDA, A. KOWNACKI, D. CISZEWSKI. 2018. *Prodiamesa olivacea* Meigen and *Prodiamesa bureshi* Michailova (Diptera, Chironomidae, Prodiamesinae) as a candidate for assessing the genotoxicity of trace metals in fluvial sediments. – *Environmental Monitoring and Assessment*, 190 (9): 542. [DOI]

IVANOVA N., V. GEORGIEV, CH. GUSEV, S. TSONEVA, E. VARADINOVA, R. FIKOVA, R. HRISTOVA. 2017. Assessment of the condition of inland wetlands. - In: Ivanova N. (Editor). *Mapping and Assessment of Ecosystem Services of Wetlands in Bulgaria*. Sofia. IBER-BAS, pp. 35-57.

KEYL H.-G. 1962. Chromosomenevolution bei Chironomus. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. - *Chromosoma, 13*: 464–514. [DOI].

KIKNADZE, I.I., A.I. SHILOVA, I.E. KERKIS, N.A. SHOBANOV, N.I. ZELENTSOV, L.P. GREBENIUK, A.G. ISTOMINA, V.A. PRASOLOV. 1991. *Karyotypes and morphology of larvae of tribe Chironomini.* Atlas. Publishing House "Nauka", Novosibirsk. (In Russian).

KIKNADZE I.I., P. MICHAILOVA, A. ISTOMINA, V. GOLYGINA, L. INT. PANIS, B. KRASTANOV. 2006. The chromosomal polymorphism and divergence of populations in *Chironomus nuditarsis* Str. (Diptera, Chironomidae). - *Tsitologiia*, 48, 7: 595-609. (In Russian)

KIKNADZE I.I., A. ISTOMINA, V. GOLYGINA. 2012. The karyotype and chromosome polymorphism of the Holarctic species *Chironomus annularius* sensu Strenzke, 1959, (Diptera, Chironomidae). - *Euroasian Entomological Journal*, 11(2): 95-114.

KIKNADZE I., A. ISTOMINA A., V. GOLYGINA, L. GUNDERINA. 2016. *Karyotypes of Palearctic and Holarctic species of the genus Chironomus.* Russian Academy of Sciences, Siberian Branch, Federal Research Center, Institute of Cytology

and Genetics. Novosibirsk. Academic publishing House "GEO".

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

KOVACHEV S., T. MICHEV, V. VELEV, M. STOYNEVA, M. BARDAROVA, N. MIKHOV, M. MARINOV. 1995. Implementation of the program for monitoring activities in Srebarna Biosphere Reserve. Sofia. Intermediate report for the Ministry of Environment (October 1994 – February 1995), March 1995. (In Bulgarian).

MICHAILOVA P. 1989. The polytene chromosomes and their significance to the systematic of the family Chironomidae, Diptera. - *Acta zoologica fennica,* 186: 1–107.

MICHAILOVA P. 1998. Order Diptera. Family Chironomidae. - In: Michev T., B. B. Georgiev, A. Petrova, M. Stoyneva (Eds.): *Biodiversity of the Srebama Biosphere Reserve. Checklist and Bibliography.* Sofia. EW/Pensoft, pp. 67-68.

MICHAILOVA P. 2015. Genomic changes in speciation of the family Chironomidae, Diptera. - *Journal of BioScience and Biotechnology*, 4(1): 1-8.

MICHAILOVA P., J. ILKOVA, T. HANKELN, E. SCHMIDT, A. SELVAGGI, G. ZAMPICININI, G. SELLA. 2009. Somatic breakpoints, distribution of repetitive DNA and non-LTR retrotransposon insertion sites in the chromosomes of *Chironomus piger* Strenzke (Diptera, Chironomidae). – *Genetica,* 135: 137–148. [DOI].

MICHAILOVA P., G. SELLA, N. PETROVA. 2012. Chironomids (Diptera) and their salivary gland chromosomes as indicators of trace metal genotoxicology. - *Italian Journal of Zoology*, 79(2): 218–230. [DOI].

MICHAILOVA P., J. ILKOVA, A. DEAN, K. WHITE. 2015. Cytogenetic index and functional genome alterations in *Chironomus piger* Strenzke (Diptera, Chironomidae) in the assessment of sediment pollution: A case study of Bulgarian and UK rivers. - *Ecotoxicology and Environment Safety*, 111: 220-227. [DOI].

MICHAILOVA P., J. ILKOVA, K. WHITE. 2016. Implications of genome alterations in *Chironomus bernensis* Klotzli (Diptera) for assessment of trace metal pollution in two Bulgarian rivers. - *River Research Applications,* 32: 914-924. [DOI].

MICHAILOVA P., J. ILKOVA, E. SZAREK-GWIAZDA, A. KOWNACKI, D. CISZEWSKI. 2018. Genome instability in *Chironomus annularius* sensu Strenzke (Diptera, Chironomidae): A biomarker for assessment of the heavy metal contaminants in Poland. - *Journal of Limnology,* 77(1) [DOI].

ODUME O.N., W.J. MULLER, C.G. PALMER, F.O. ARIMORO. 2012. Mentum deformities in Chironomidae communities as indicators of anthropogenic impacts in Swartkops River. – *Physics and Chemistry of the Earth,* 50-52: 140-148. [DOI].

PETROVA N. 2013. *Polytene Chromosomes of the larvae of Chironomids (Diptera, Chironomidae) and their response to mutagenic environmental pollution (Chernobyl Disaster)*. St. Petersburg. Zoological Institute. Russian academy of Sciences.

Regulation N-4/23.09.2014 for characterizing of surface waters. - *State Gazette,* 79, 23.09.2014.

SAETHER O. 1979. Chironomids communities as water quality indicators. - *Holarctic Ecology*, 2: 65-74. [DOI].

SCHMIDT P. 1993. *A Key to the larval Chironomidae and their instars from Austrian Danube region streams and rivers.* Part I: Diamesinae, Prodiamesinae and Orthocladiinae. Federal Institute for Water Quality, Wien–Kaisermühlen. Wasser und Abwasser, Supplement 3.

SELLA G., S. BOVERO, M. GINEPRO, P. MICHAILOVA, N. PETROVA, R. ROBOTTI, V. ZELANO. 2004. Inherited and somatic cytogenetic variability in Palaearctic populations of *Chironomus riparius* Meigen (Diptera, Chironomidae). - *Genome* 47: 332–244. [DOI].

SERVIA M., F. COBO, M. GONZALEZ. 2000. Seasonal and interannual variations in the frequency and severity of Dieformities in Larvae of *Chironomus riparius* (Meigen, 1804) and *Prodiamesa olivacea* (Meigen, 1818) (Diptera, Chironomidae) collected in a polluted site. - *Environment Monitoring and Assessment, 64*: 617–626. [DOI].

UMPMR "Srebarna", Updated Management Plan of Managed Reserve "Srebarna". 2015. MOEW, RIEW-Ruse, Operational Program "Environment". (In Bulgarian)

UZUNOV Y., V. TZAVKOVA, I. TODOROV, E. VARADINOVA. 2001. The macrozoobenthic fauna of the Biosphere reserve Srebarna Lake in North-Eastren Bulgaria. – *Lauterbornia,* 40: 43-51.

VARADINOVA E., L. PEHLIVANOV, S. STOICHEV, Y. UZUNOV. 2011. Recovering and succession of the species diversity of macrozoobenthos in the Srebarna Biosphere Reserve (North-East Bulgaria). - *Acta zoologica bulgarica*, 63 (1): 85-95.

VARADINOVA E., P. BORISOVA, L. PEHLIVANOV, Y. UZUZNOV. 2012. Macroinvertebrate communities of the Srebarna Lake Biosphere Reserve: Species diversity, abundance and modeling of the ecological status. - In: Uzunov Y., B.B. Georgiev, E. Varadinova, N. Ivanova, L. Pehlivanov, V. Vasilev (Eds.). *Ecosystems of the Biosphere Reserve Srebarna Lake*. Sofia, Professor Marin Drinov Academic Publishing House, pp. 93-115.

VIDINOVA Y., V. TYUFEKCHIEVA, E. VARADINOVA, S. STOICHEV, L. KENDEROV, I. DEDOV, Y. UZUNOV. 2016. Taxonomic list of benthic macroinvertebrate communities of inland standing water bodies in Bulgaria. – *Acta zoologica bulgarica*, 68 (2): 147-158.

WIEDEHOLM T. 1983. *Chironomidae of the Holarctic Region: keys and diagnosis.* Part I. Larvae. Borgströms Tryckeri AB, Motala. Entomologica Scandinavica. Suppl. 19.).

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