Quest Journals
Journal of Research in Environmental and Earth Sciences
Volume 8 ~ Issue 2 (2022) pp: 43-49
ISSN(Online) :2348-2532
www.questjournals.org

Research Paper

New genomic approach for assessing pollution of aquatic ecosystems (Review)

*[1]Michailova, Paraskeva;[1]Ilkova, Julia;[2]White, Keith
[1]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia,Bulgaria.*
[2]*Department of Earth and Environmental Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT, UK.*
Corresponding Author:**Michailova**, P

ABSTRACT: *The paper examine the role of Chironomidae, an important group of invertebrate in assessing pollution of aquatic ecosystems. Chironomid larvae account for at least 50% of the macro-invertebrate species in freshwater ecosystems. They also play a key role in the purification of standing and running waters because they are directly exposed to the effect of the contaminants and are able to accumulate in their tissues pollutants which later are transferred to fish and wildfowl and, hence, to humans. Chironomid larvae possess good banded salivary gland chromosomes which make Chironomids prospective subjects for cytogenetic monitoring of environmental stress. The paper presents two indices: Somatic and Cytogenetic, that have been used successfully to assess the degree of pollution of freshwater bodies and species responses. These indices are calculated on the basis of somatic aberrations as biomarkers, identified in the polytene chromosomes of model Chironomid species.Closely related species of Chironomidae show species-specific responses of the genome through a number of somatic rearrangements related to changes in DNA structure and organization. Somatic chromosomal aberrations,used for calculating the indices, are most often located in specific site, so called "hot spot" of the polytene chromosomes..*
KEYWORDS: *Chironomidae, polytene chromosomes, somatic and cytogenetic indices, transposable elements, species-specific response*

I. POLLUTION AND THE ROLE OF INVERTEBRATES AS MONITORS OF ECOLOGICAL CONTAMINANTS

Contamination of the environment is one of the most serious problems facing the world. Large numbers of species have disappeared, the structure and function of the ecosystems have changed and biodiversity has decreased. A key task of humanity is therefore to protect the environment and improve quality of life. Central to environmental protection is the development of methods and approaches for detecting and assessing the impact of contaminants on ecosystems.

Biological approaches have a number of advantages that make them partitcularly suitable for assessing antropogenic ecosystem change (Walker *et al.*, 1998). Biological communities provide a direct means of studying the impact of contaminants as they are exposed to and involved in the movement and transformation of pollutants. Invertebrates have been extensively evaluated as monitors of ecological contaminants at the individual, population and community level (Lagadic & Caquet, 1998). The development of bioassays using invertebrates has been stimulated by both the biological and toxicological characteristics of these organisms. In particular, many species can be maintained under controlled conditions while their specificity in response to different stress agents is an advantage in toxicological stuides. Lagadic & Caquet (1998) emphasized that they can allow prediction of effects at population and community levels and could therefore be used as early warning indicators of both deterioration and recovery of ecosystems. By their position at different levels of the food web, invertebrates play functional key role in ecosystems. Invertebrates can be found in a very wide range of habitats, they often have intimate contact with the substrate. Many have a limited ability to move large distances and in this way they cannot escape exposure to pollutants. Studies on aquatic invertebrates provided evidence to support the existence of causal links between individual responses and changes at population and community level (Lagadic & Caquet, 1998).

I.1. FAMILY CHIRONOMIDAE, DIPTERA FOR ASSESSING POLLUTION IN THE AQUATIC ENVIRONMENT

One very important group of invertebrates is the Dipera, family Chironomidae and they have many advantages in the assessment of environmental stress. Chironomid larvae are a widely distributed, abundant group of species in aquatic ecosystems and have an important role in these ecosystems. Chironomid larvae account for at least 50% of the macro-invertebrate species in freshwater ecosystems. Individual larva represents a basic unit of a biological community as they integrate into single whole responses (molecular, cellular and organismal) to environmental stress at the lower level of biological organization and being at the base of the food web will impact on the higher levels of organization (population, communities and ecosystems) (Warwick, 1990).

From the perspective of assessing the pollution by biomarkers as an early warning system signalling potentially compromised situations (Conti, 2008), studies of benthic larval stages of Chironomidae have been considered as a suitable bio-monitoring model for eco-toxicological tests (Jannssen de Bisthoven *et al*., 1992) and are included in both the Extended Biotic Index (De Pauw *et al.*,1992) and the Annex V.1.2.6 of the EC Water Framework Directive. Chironomids can also be used for defining the trophic state of lakes (Saether, 1979). They play a key role in the food chain transfer of contaminants of standing and running waters because they are directly exposed to such substances and are hence able to accumulate in their tissues pollutants which later are transferred to fish and wildfowl and, hence, to humans. Chironomid larvae are thus a primary link in the food chain, from sediment to the higher trophic levels. They are very sensitivite to a range of environmental factors including temperature, pH, food, freezing, salinity, oxygen and pollutants. Studies have demonstrated that metal accumulation in Chironomidae can be used as an integrated measure of metal bioavailability and to predict the ecological effects of metal toxicity on macroinvertebrate communities (De Jonge *et al*., 2012). Faria *et al.* (2008) used larval development and growth as endpoints to biomonitor water quality and to assess the biological recovery of metal contaminated freshwater ecosystems in mine waste polluted areas that are subject of restoration measures. Larval growth and development were markedly inhibited in a stream that received acid drainage from a tungsten mine (Faria *et al.*, 2008). In-situ bioassays using *Chironomus riparius* Mg. larvae are a suitable tool to monitor restoration efficacy after a prolonged period metallic sediment contamination (Faria *et al.*, 2008).The external morphology of the larvae: mandibles, antenna, submentum and epypharings are very sensitive to the pollutans in the environment (Warwick, 1990) and the frequency of much mouthpart deformities in Chironomids correlate with environmental pollution (Rawal *et al*., 2019). Some studies evaluated the potential for mentum deformity in chironomids as an indicator endpoint of anthropogenic contamination level in freshwaters (Goretti *et al*., 2020). They considered that morphological deformities possess a number of advantages for assessing and detecting the impact of pollution in aquatic ecosystems. A modified toxic score index (MTSI) was recommended in the bioassessment of water and sediment quality using the mentum deformities of *Chironomus* spp. larvae from aquatic ecosystems receiving sewage, agricultural, or industrial discharges (Al-Shami *et al.,* 2011).

However, Beermann *et al.* (2018) highlights the potential of DNA-based approaches when studying environmental impacts, especially for ecologically important taxon such as Chironomidae and in the context of multiple stressors as there are many studies indicating that the Chironomid genome is rather sensitive to contaminatants in aquatic ecosystems (Sella *et al.*, 2004; Michailova *et al*., 2012; Petrova & Michailova, 2021).

Chironomid larvae possess large and clearly banded salivary gland chromosomes which make this insect prospective subject for cytogenetic monitoring of environmental stress and hence as an early warning of contamination. According to Conti (2008) biomarkers are divided in two groups: general and specific. General biomarkers are those responses indicate a general stress effect on the organisms in the ecosystems; specific biomarkers are the molecular and biochemical responses detectable in organisms as a consequence of exposure to a particular class of contaminants (Conti, 2008). Somatic chromosome aberrations in Chironomids salivary gland chromosomes can be used as specific biomarkers in tracing the mutagenic effects of a number of stress agents both in the field and under lab conditions (Michailova *et al.*, 2012; Petrova & Michailova, 2021).

Having in mind the advantages of Chironomids as biomonitors, we used this knowledge to examine the effect of several factors in the environment, in particular the impact of trace heavy metals pollutants on the structure and functional organization of the polytene chromosomes with the aim of establishing their suitability as biomarkers and the development of methods for detecting and assessing the impact of contaminants in aquatic ecosystems and the consequent toxic effects.

II. TWO INDICES FOR ASSESSING THE DEGREE OF POLLUTION IN FRESH WATER ECOSYSTEMS

One very good biomarker at the cytogenetic level are somatic aberrations which affect a few (4-6) cells of the salivary glands, specifically inversions, deletions, duplications and deficiencies (Michailova *et al*., 2012; Petrova & Michailova, 2021). Using somatic chromosome aberrations, we developed two original and integrative indices for quantifying the genome response to freshwater pollution.We propose two indices based on, respectively, somatic and cytogenetic changes. The Somatic Index (S) was calculated as the ratio of number of different somatic aberrations relative to the number of individuals studied and hence showed the response of the species to a pollutant (Sella *et al.*, 2004). The Cytogenetic Index (C) was estimated as the ratio of the mean percentage of somatic aberrations per individual in the control to that at each polluted station and detects the degree of pollutation (Ilkova *et al.*, 2014). Three levels of pollution was established based on the cytogenetic indices: slight pollution with a cytogenetic index near to the control (0.9–0.8), moderate pollution (0.7–0.5) and high pollution (<0.5). To estimate the level of sediment contamination by heavy metals the geoaccumulation index (Igeo) was calculated using the Mùller (1981) equation. According to values of this index, Mùller (1981) described seven categories of sediment contamination from unpolluted (class 0; Igeo≤0) to extremely contaminated sediment (category 6; Igeo≥5). Studies of different species of Chironomids in the field and in the laboratory indicate the applicability of these methods for assessing pollution of aquatic ecosystems (Table 1). The Somatic index of the species presented in Table 1 results from a wide spectrum of somatic aberrations induced by trace metal pollution of the aquatic basisns and shows the degree of chromosome instability in contaminated environment. It is also apparent from Table 1 that a high level of pollution at all the studied sites is revealed by Cytogenetic index. As can be seen from the Table 1, in both species: *Prodiamesa olivacea* Mg.and *P.bureshi* Michailova, no somatic aberrations were found in the controls, and hence did not allow the calculation of a Somatic and Cytogenetic index. However, it should be emphasized that in both species from unpolluted regions a high frequency of contacts (ectopic pairing) between different chromosomes and parts of different chromosomes where detected (Michailova *et al*., 2003b). These contacts are very important for the normal functioning of the cell and the three dimensional structure of the nucleolus (Zhimilev, 1996) and therefore are likely to result in toxic impacts on the whole organism.

From our laboratory experiments (Michailova *et al.*, 2001; 2003a) and studies in the field (Sella *et al*, 2004; Michailova *et al.*, 2012; 2015b; Ilkova *et al.*, 2014; Petrova & Michailova, 2021) we concluded that trace metal ions are highly genotoxic to Chironomid species. In all the sites shown in Table 1 the concentrations of trace metals were much higher than the reference (unpolluted) sites. The data obtained for different species indicate a relationship between the metal concentrations and the degree of genotoxicity as shown by the two indices (Table 1). As Conti (2008) underlined, many contaminants are able to modify the genetic material and it is therefore extremely important to use biomarkers to assist in the prevention of stressor that are harmful both to humans and ecosystems. The various types of aberrations used to determine the two cytogenetic indices will likely have an adverse impact on the species. It is therefore very important to identfy which cellular functions are affected by the chromosomal aberrations resulting from environmental stress. A single biomarker alone might not be sufficient to monitor the environmental quality. In general, it is necessary to have a complex of biomarkers allowing the evaluation of the physiological integrity of the species present in a given ecosystem (Conti, 2008). Using both indices based on established somatic aberrations increases the chance for a more detailed analysis of the impact of environmental pollution on the species. Cytogenetical tests can also be used to evaluate the damage done to the test species while information on the degree of pollution is important for the protection and preservation of the environment. Therefore both indices represent a sensitive and reliable methodological approach in the assessment of the impact of contaminants in sediments containing chironomid larvae.

Table 1: Somatic and Cytogenetic index in Chironomid species from sediments with concentrations of trace metals higher than control (unpolluted) sites.

Species	**Site /year**	**Somatic index (S)**	**Cytogenetic index (C)**	**Source**
Chironomus riparius	Maritsa River, Bulgaria (2009)	1	0.0968	Ilkova *et al.* 2014
	Chaya River, Bulgaria (2009)	1.57	0.0904	
	Chaya River, Bulgaria(2010)	3.25	0.0648	
	Chaya River, Bulgaria (2013)	11.66	0.0635	
	Plovdiv farm, Bulgaria (2010)	2.28	0.0742	
	Control, Bulgaria (2013)	0.5	1.00	
Chironomus piger	ChayaRiver-Katunitsa, Bulgaria (site 1) (2013)	4	0.07	Michailova *et al.* 2015b
	Chaya River- Kemera, Bulgaria (site 2) (2013)	3.64	0.06	
	Northern Afon Goch, UK (site 3) (2013)	1.92	0.10	
	Southern Afon Goch, UK (site 4) (2013)	3	0.13	
	Southern Afon Goch, UK (site 5) (2013)	3.5	0.13	
	Control - Balchik botanical garden, Bulgaria (2013)	0.26	1.00	
Chironomus bernensis	Chaya River – Kemera, Bulgaria (site 1) (2013)	1.93	0.33	Michailova *et al.* 2016
	Chaya River – Katunitsa, Bulgaria (site 2) (2013)	2.61	0.34	
	Maritsa River – Milevo, Bulgaria (site 3) (2013)	1.79	0.27	
	Maritsa River- Parvomay, Bulgaria (site 4) (2013)	2.36	0,37	
	Maritsa River – Dimitrovgrad, Bulgaria (site 5) (2013)	2.39	0.34	
	Control, Dendrarium, Bulgaria (2013)	1.67	1.00	
Chironomus annularius	Chechlo River, Poland, (Site 1)(2016)	1.5	0.38	Michailova *et al.* 2018;
	Chechlo River, Poland, (Site 2)(2016)	1.48	0.163	

	Chechlo River, Poland, (Site 3) (2016)	2.23	0.193	Ilkova *et al.* 2017;
	Chechlo River, Poland (2016)	2.00	-	
	Control - Saraya (Pazardzhik), Bulgaria (2002)	1.08	1.00	
Prodiamesa bureshi	Biała Przemsza River, Poland (2016)	1.63	-	Ilkova *et* al. 2017, 2018; Michailova *et al*. 2003b
	Biała Przemsza River, Poland (2016)	1.2	-	
	Biała Przemsza River, Poland (2016)	1.71	-	
	Control - Old Mill Brook, Derbyshire UK (2000) - no somatic aberrations	-	-	
Prodiamesa olivacea	Biała Przemsza River, Poland (2016)	7	-	Ilkova *et al.* 2017, 2018;
	Biała Przemsza River, Poland (2016)	1.24	-	
	Biała Przemsza River, Poland (2016)	0.69	-	Michailova *et al.* 2003b
	Control - Glossop Brook, Derbyshire UK (2000) - no somatic aberrations	-	-	

III. SPECIES-SPECIFIC RESPONSE TO CONTAMINANTS IN THE ENVIRONMENT

Another interesting phenomenon that we have identified is a species-specific response to heavy metal contaminants. Closely related Chironomid species show different genomic response to trace metals depending on their DNA structure and organization. For instance, closely related and morphologically nearly indistinguishable *Chironomus riparius* Mg. and *Chironomus piger* Strenzke (Keyl & Strenzke, 1956) are sibling and homosequential (have one and the same banding patterns) species differentiated at the cytological level by their amount of DNA, by the localization of some retrotransposones (NLRCth1, CTRT1, TFB1), and by the amount and localization of the the repetitive DNA elements (Alu and Hinf) (Michailova *et al*., 2012; Ilkova *et al*., 2014). In *Chironomus piger*, a sibling species of *C.riparius*, the pericentromere regions contain far lower numbers of satellite DNA clusters and, overall, much fewer copies of repetitive sequences (Schaefer & Schmidt, 1981). Both species have striking differences in the number of C heterochromatin bands, which in the *C. piger* genome are localized in centromere regions only, while *C.riparius* possesses numerous C-bands including in the centromere regions (Hägele, 1977; Michailova *et al*., 2015a). Also, these closely related species differ in the organization of the heterochromatin (Michailova *et al*., 2015a). The difference in the organization of DNA in the two species reflect their sensitivity to the pollutants in the environment. For a long time, both species were considered as nearly monomorphic (Michailova, 1989). Later, however, Michailova *et al.* (1996; 1998; 2000) and Sella *et al.* (2004) observed more than 200 structural somatic chromosomic aberrations in *C. riparius* larvae from heavy metal polluted stations. These aberrations were distributed at different points in all arms of the polytene chromosomes of *C.riparius* (Petrova *et al.*, 2004; Ilkova *et al*., 2007). In *C.piger* they were concentrated in pericentromeric regions of the chromosomes CD and EF and proximal parts of arms D and F. It was shown that significantly more somatic aberrations occurred in the *C.riparius* genome compared to *C.piger* ($G = 61.923, df = 1, P<0.001$) (Ilkova *et al* ., 2007). Different genome responses as shown by somatic structure chromosome rearrangements, that from the basis for calculating the somatic and cytogenetic indices, are also found in another sibling species of the genus *Prodiamesa*: *P.olivacea* Mg. and *P.bureshi* Michailova, collected from polluted rivers in Poland (Ilkova *et al*., 2017; 2018). More cells containing aberrations were observed in *P. olivacea* than in *P. bureshi*.

Of particular interest is the behavior of chromosome G of these sibling species. In trace metal polluted sites (Michailova *et.al.*, 2012; 2015b; Ilkova *et al*., 2014; Petrova & Michailova, 2021) as well as in laboratory studies (Michailova *et al*., 2001; 2003a) with specific trace metals, chromosome G of both sibling species were converted into a “pompon” chromosome due to the deletion of the very important key structures in this chromosome, the so called Balbiani Rings (BRs) (Figure 1). This aberration can be easily identified by those who are not familiar with the polytene chromosome banding pattern of Chironomids and hence the appearance of “pompon” chromosome can be used as a good genomic biomarker in the routine assessment of environmental stress.

Together with somatic structural chromosome aberrations, other very important indicators of environmental stress are changes in the functional activity of two key structures, the Balbiani Rings (BRs) and Nucleolar Organizer Region (NOR). Between one and three BRs are present (depending on the species) and contain genes coding for the high-molecular weight silk proteins that are necessary for the production of the tubes in which the larvae live. They are sites of intensive transcription, which can be detected by the formation of large puffs. The NOR is essential for cellular maintenance and ribosomal production machinery, which is highly conserved through evolution. The level of activity of the BRs and NOR was significantly lower in laboratory exposed larvae or those from the polluted sites (Michailova *et al.*, 2012; 2015b; Petrova & Michailova, 2021) compared to chironomids from unpolluted waters. These key cell structures therefore may play a pivotal role in monitoring and responding to stress in the environment and can be identified by every one who is not familiar with the polyetene chromosomes.

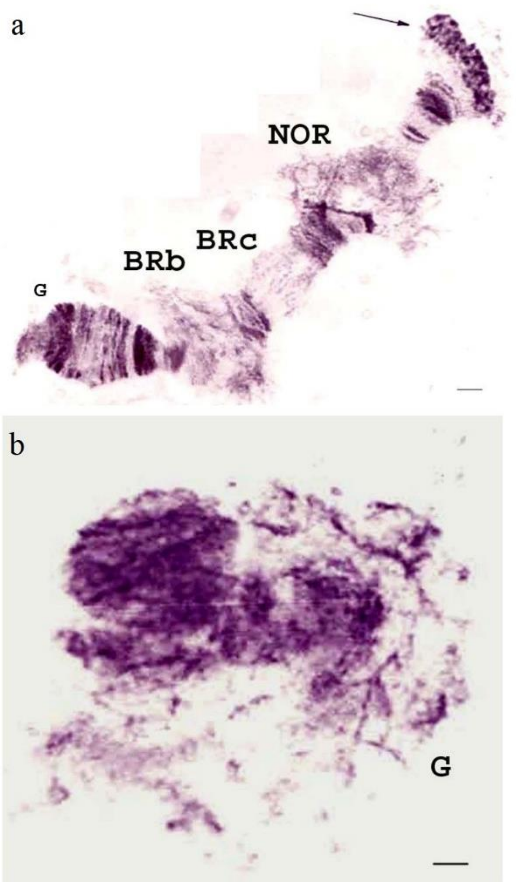

Figure 1: Chromosome G of *Chironomus riparius* Mg.
a. Standard band structure with two Balbiani rings (BRb, BRc) and Nucleolar Organizer Region (NOR); arrow – centromere region. Bar: 10 µm.
b. "Pompon" chromosomes formed due to deletions of the BRs and part of the chromosome. Bar: 10 µm.

IV. "HOT SPOTS" IN THE POLYTENE CHROMOSOMES

Most somatic chromosome rearrangements used in both the above indices are not randomly distributed. They occur more frequently in specific sites on the chromosome, called by us "hot spots", and contain satellite DNA and/or transposable elements (Hankeln *et al*., 1994; Bovero *et al*., 2002; Michailova *et al*., 2009). The insertion sites of the transposable elements (NLRCth1, CTRT1) in the *C. riparius* genome, detected by FISH were higher than those of *C. piger* genome (Michailova *et al*., 2007; 2009; Ilkova *et al*., 2013). The higher number of copies of dispersed repetitive sequences in *C.riparius* genome compared to *C.piger* indicate a greater sensitivity of the *C.riparius* genome to pollutants. In *C.riparius*, the somatic aberrations affected all chromosomes, while in *C.piger* they are concentrated mainly in pericentromeric regions, and at a few sites, of two arms. This confirmed Kale's *et al*. (2005) suggestion that stress agents in the environment activate transposable element mobility in the genome and influence genome instability, resulting in numerous somatic chromosome rearrangements.

It is therefore apparently that structural aberrations and functional alterations (i.e.a decrease in the activity of BRs and NOR) are particularly suitable biomonitoring tools in that they provide early warning signals of adverse long-term effects of pollution in the population. Moreover cytogenetic changes area more sensitive indicator of pollution than is the external morphology of the larvae.

V. CONCLUSION

It is clear that the cytogenetic response of chironomids, specifically somatic aberrations which can be quantified to produce a somatic and cytogenetic index provides a potentially powerful environmental monitoring tool which can aid in preventing long-term effects of anthropogenic stress at the population and community level. Both indices could also provide a reliable and effective biomarkers tool to assess the degree of genotoxicity of a range of toxicants. We conclude that the somatic and cytogenetic indices could be included in the suite of bio assessment methods.

Future research should be aimed at defining precisely the mechanisms involved in the specific phenotype and genotype changes of Chironmid species from polluted sites.

REFERENCES

[1]. Al-Shami, S.A., Salmah, M.R., Hassan, A.A. and Azizah, M.N. (2011). Evaluation of mentum deformities of *Chironomus* spp. (Chironomidae: Diptera) larvae using modified toxic score index (MTSI) to assess the environmental stress in Juru River Basin, Penang, Malaysia. *Environmental Monitoring and Assessment*,**177**: 233-244.

[2]. Beermann, A.J., Zizka, V., Elbrecht, V., Baranov, V. and Leese, F. (2018). DNA metabarcoding reveals the complex and hidden responses of chironomids to multiple stressors. *Environmental Sciences Europe*,**30** (26):1-15.

[3]. Bovero, S., Hankeln, T., Michailova, P., Schmidt, E. and Sella, G. (2002). Nonrandom chromosomal distribution of spontaneous breakpoints and satellite DNA clusters in two geographically distant populations of *Chironomus riparius* (Diptera, Chironomidae). *Genetica*, **115**(3): 273 –281.

[4]. Conti, M. E. (2008). Biomarkers for environmental monitoring. *WIT Transactions on State of the Art in Science and Engineering*,**30**: 25-45.

[5]. De Jonge, M., Belpaire, C., Geeraerts, C., De Cooman, W., Blust R. and Bervoets, L. (2012). Ecological impact assessment of sediment remediation in a metal-contaminated low land river using translocated zebra mussels and resident macroinvertebrates. *Environmental Pollution*,**171**: 99-108.

[6]. De Pauw, N.P., Ghetti, P., Manzeni, F. and Saggiari, R. (1992). Biological assessment methods for running water. In: *River water quality- Ecological assessment and control. Brussels: European Communities*, 217-248.

[7]. Faria, M.S., Lopes, R.J., Malcato J., Nogueira, A.J.A and Soares, A.M.V.M. (2008). In situ bioassays with *Chironomus riparius* larvae to biomonitor metal pollution in rivers and to evaluate the efficiency of restoration measures in mine areas. *Environmental Pollution*,**151**(1): 213-221.

[8]. Goretti, E., Pallottini, M., Pagliarini, S., Catasti, M., La Porta, G., Selvaggi, R., Gaino, E.. Di Giulio, A.M. and Ali, A. (2020). Use of Larval Morphological Deformities in *Chironomus plumosus* (Chironomidae: Diptera) as an Indicator of Freshwater Environmental Contamination (Lake Trasimeno, Italy). *Water*, **12**(1): 1-17.

[9]. Hägele, K. (1977). Differential staining of polytene chromosome bands in *Chironomus* by Giemsa banding methods. *Chromosoma*, **59**: 207-216.

[10]. Hankeln, T., Rohwedder, A., Weich, B. and Schmidt, E. (1994). Transposition of minisatellite sequences during evolution of Chironomid midges. *Genome*, **37**: 543-549.

[11]. Ilkova, J., Hankeln, T., Schmidt, E., Michailova, P., Sella, G., Zampicinini, GP., Petrova, N. and White, K. (2007). Genome instability of *Chironomus riparius* Mg. and *Chironomus piger* Strenzke (Diptera, Chironomidae). *Caryologia*, **60**(4): 299-308.

[12]. Ilkova, J., Cervella, P., Zampicinini, G.P., Sella, G. and Michailova, P. (2013). Chromosomal breakpoints and transposable-element-insertion sites in salivary gland chromosomes of *Chironomus riparius* Meigen (Diptera, Chironomidae) from trace metal polluted stations. *Acta Zoologica Bulgarica*, **65**(1): 63-79.

[13]. Ilkova, J., Michailova, P., Thomas, A. and White, K. (2014). Genome instability of *Chironomus riparius* Mg. (Diptera,Chironomidae) from polluted water basins in Bulgaria. *Ecologica Balkanica*, **5**: 1–8.

[14]. Ilkova, J., Michailova, P., Szarek-Gwiazda, E., Kownacki, A. and Ciszewski, D. (2017). The response of genome of the Chironomidae (Diptera) to heavy metals pollution in two Rivers of southern Poland. *Acta Zoologica Bulgarica*, Suppl. **8**: 9-15.

[15]. Ilkova, J., Michailova, P., Szarek-Gwiazda, E., Kownacki, A. and Ciszewski, D. (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** (542): 2-12.

[16]. Jannsse De Bistoven, L.G., Timmermans, K.R. and Ollevier, F. (1992). The Concentration of cadmium, lead, copper and zinc in *Chironomus riparius* larvae with deformed versus normal menta. *Hydrobiologia*, **239**: 141–149.

[17]. Kale, S., Moor, L., P. Deininger, P. and Engel, A. (2005). Heavy metals stimulate human LINE-1 retrotransposition. *International Journal of Environmental Research and Public Health*, **2**(1): 14-23.

[18]. Keyl, K. and Strenzke, K. (1956). Taxonomie und Cytologie von zwei subspecies der Art *Chironomus thummi.* Zeitschr. *Naturforsch.*, **116**: 727-735.

[19]. Lagadic, L. and Caquet, Th. (1998). Invertebrates in Testing of Environmental Chemicals: Are they Alternatives? 13th Meeting of the Scientiic Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC). *Environmental Health Perspectives*, 106, Suppl.**2**: 593–661.

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

[21]. Michailova, P., Petrova, P., Ramella, L., Sella, G., Todorova, J. and Zelano, V. (1996). Cytogenetic characteristics of *Chironomus riparius* Meigen, 1804 (Diptera, Chironomidae) from a polluted Po river station. *Genetica*, **98**: 161-178.

[22]. Michailova, P., Petrova, N., Sella, G., Ramella, L. and Bovero, S. (1998). Structure and functional alterations in chromosome G of *Chironomus riparius* Mg. from a heavy metal polluted area near Turin, Italy. *Environmental Pollution*, **103**: 127–134.

[23]. Michailova, P.,Petrova, N., Bovero, S., Cavicciolі, O., Ramella, L. and Sella, G. (2000). Effect of environmental pollution on the chromosomal variability of *Chironomus riparius* Meigen (Diptera, Chironomidae) larvae from two Piedmont stations. *Genetica*, **108**: 171-180.

[24]. Michailova, P., Ilkova, J., Petrova, N. and White, K. (2001). Rearrangements in the salivary gland chromosomes of *Chironomus riparius* Mg. (Diptera, Chironomidae) following exposure to lead. *Caryologia*, **54**(4): 349-363.

[25]. Michailova, P., Ilkova, J. and White, K. (2003a). Functional and structural rearrangements of salivary gland polytene chromosomes of *Chironomus riparius* Mg. (Diptera, Chironomidae) in response of freshly neutralised aluminium. *Environmental Pollution*, **123**:193-207.

[26]. Michailova, P., Ilkova, J. And White, K. (2003b). Cytogenetic alterations in *Prodiamesa* species (Diptera, Chironomidae) from different polluted regions. Folia Biologica (Krakow), **51**(1-2): 69-79.

[27]. Michailova, P., Ilkova, J., Petrova, N., Selvaggi, A., Zampicinini, G. P. and Sella, G. (2007). Relationship between chromosome rearrangements and repetitive DNA clusters in *Chironomus riparius* Mg. (Diptera, Chironomidae) from anthropogenic polluted Palaearctic regions. *Comparative Cytogenetics*, **1**(1): 45-49.

[28]. Michailova, P.,Ilkova, J., Hankeln,T., Schmidt, E., Selvaggi, A., Zampicinini, G.P. and Sella, G. (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.

[29]. Michailova, P., Sella, G. and Petrova, N. (2012). Chironomids (Diptera) and their salivary gland chromosomes as indicators of trace metal genotoxicology. *Italian Journal of Zoology*, **79**: 218-230.

[30]. Michailova, P., Ilkova, J., Mariyanska-Nadachowska, A. and Warchalowska-Sliwa, E. (2015a). Characterization of heterochromatin in the salivary gland chromosomes of *Chironomus riparius* Meigen and *Chironomus piger* Strenzke (Diptera, Chironomidae) by differential and fluorochrome staining. *Folia Biologica*, **63**(2): 108-117.

[31]. Michailova, P., Ilkova, J., Dean, A.P. and White, K. N. (2015b). 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.

[32]. Michailova, P., Ilkova, J. and White, K. (2016). Implications of Genome alterations in *Chironomus bernensis* Klotzli (Diptera) for assessment of trace metal pollution in two Bulgarian rivers. *River Research and Applications,* **32**: 914-924.

[33]. Michailova, P., Ilkova, J., Szarek-Gwiazda, E., Kownacki, A. and Ciszewski, D. (2018). Genome instability in *Chironomus annularius* sensu Strenzke (Diptera, Chironomidae) – a biomarker for the assessment of heavy metal contaminants in Poland. *Journal of Limnology*, **7**(s1): 15-24

[34]. Müller, G. (1981). Die Schwermetallbelastung der Sedimente des Neckars und seiner Nebenflüsse: eine Bestandaufnahme. *Chemiker-Zeitung,* **6**: 157-164.

[35]. Petrova, N.A., Michailova, P. and Ilkova, J. (2004). Comparative Cytogenetic variation of the salivary gland Polytene Chromosomes in *Chironomus riparius* Mg. 1804 (Diptera, Chironomidae) from two Polluted Biotopes of Bulgaria and Russia. *Russian Journal of Genetics*, **40**(1): 40-48.

[36]. Petrova, N. and Michailova, P. (2021). The use of salivary gland chromosomes of Chironomids (Diptera,Chironomidae) for assessing the pollution of aquatic ecosystems. *Euroasian Entomological Journal*, **20**(1): 38-48. (In Russian).

[37]. Rawal, D., Prajapat, G. and Verma, H. (2019). Status of Chironomids (Diptera) as Potential Bioindicators. *International Journal of Advanced Scientific Research and Management*, **4**(1): 154-156.

[38]. Saether O.A. (1979). Chironomid communities as water quality indicators. *Holarctic Ecology*, **2**: 65-74.

[39]. Schaefer, J. and Schmidt, E. (1981). Different repetition frequencies of a 120 base-pair DNA element and its arrangement in *Chironomus thummi thummi* and *Chironomus thummi piger. Chromosoma*, **84**: 61-66.

[40]. Sella, G., Bovero, S., Ginepro, M., Michailova, P., Petrova, N., Robottica, A. and Zelano, V. (2004). Inherited and somatic cytogenetic variability in Palaearctic populations of *Chironomus riparius* Meigen (Diptera, Chironomidae). *Genome*, **4**(7): 332–344.

[41]. Walker, C., Kaiser, K., Klein, W., Lagadic, L., Peakall, D., Sheield, St., Soldan, H. and Yasuno, M. (1998). 13th Meeting of the Scientiic Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Alternative Testing Methodologies for Ecotoxicity. *Environmental Health Perspectives*, **106**(2): 441-457.

[42]. Warwick, W. F. (1990). The use of morphological deformities in Chironomid larvae for biological effects monitoring. *Inland Waters Directorate Scientific Publications*, **173**: 1-34.

[43]. Zhimulev, I. (1996). Morphology and Structure of Polytene Chromosomes. Advances in Genetics, **34**, 489 pp.