RESEARCH

Open Access

An analysis on the divergence of *Chironomid* spp. based on the study of 18S rRNA and polytene chromosome organization in the species revealing the role of environment on speciation

Sanjay Kumar Dey^{1,2,3*} , Swapna Bhaduri^{1†} and Trilochan Midya¹

Abstract

Background: Nine species of *Chironomus* evolved throughout the world were measured for their divergence with regard to their DNA sequences concerning 18S rRNA since it is conserved for a specific species. With the advancement of the field of molecular evolution, cytogenetics requires further correlation between molecular architecture and morphological features of a species to compare amongst others to decipher their role in speciation. Therefore, divergence of DNA sequences of the *Chironomus* were compared with differences in the polytene chromosome features of most of the species under this investigation to evaluate underlying correlation among them, if any, to finally establish a novel method of molecular classification broadly applicable in cytogenetics studies.

Results: When *Chironomus javanus* Kieffer was considered as a reference organism, an in silico pair-wise alignment of sequences for the 18S rRNA gene regions of the other eight different species of the same genus exhibited nucleotide sequence homology ranging from 67 to 98%. This divergence of the species under consideration might be due to environmental impact causing alteration of nitrogenous bases probably due to mismatch pairing in DNA replication. This may be suggested as a cause of evolution of species in nature. A concomitant study on the polytene chromosome band patterns of majority of these species belonging to this series also indicated a divergence ranging from 10% to 30%.

(Continued on next page)

* Correspondence: sanjaydey@south.du.ac.in

[†]Sanjay Kumar Dey and Swapna Bhaduri contributed equally to this work.

¹Department of Zoology and Molecular Biology & Genetics, Presidency University, Kolkata 700073, India

²Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ 08854, USA

Full list of author information is available at the end of the article

© The Author(s). 2021 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

(Continued from previous page)

Conclusions: Sequence analysis based on 18S rRNA of nine species of *Chironomus* under this investigation shows a similarity in the polytene chromosome organization in most of the Chironomid species of the series. Hence, molecular divergence in the species is consistent with cytological difference among Chironomid species. Therefore, molecular data based on 18S rRNA and cytological characters based on the polytene chromosome features of the Chironomid species may be useful for their taxonomical recognition. Moreover, variations concerning two aspects of this study may be correlated to their environmental distinctions.

Keywords: *Chironomus*, Polytene chromosome, 18S rRNA, Pair-wise alignment, Conserved sites, Biological indicator

Background

The Chironomids belong to the insect order Diptera and they represent the family Chironomidae (Ashburner, 1970; Maitra, 2001). The members of the family are small fly like mosquitoes. Chironomid flies are known as 'blind mosquitoes', but they are neither blind and nor mosquitoes. The dipteran insects belonging to the genus *Chironomus* are considered as excellent biological indicators (El-Shenawy, Ahmed, Ismail, & Abo-Ghaila, 2010; Lotfi, Ahmed, El-Shatoury, & Hanora, 2016; Midya, Bhaduri, Sarkar, & Ghosal, 2013; Sarkar, Bhaduri, Ghosh, & Midya, 2011; Vermeulen, 1995; Warwick, 1985) inhabiting almost all ecological zones. They cause little harm to human life or other animals of interest. Known harmful impact of these flies refers to a variety of nuisance problems, health hazards, and human diseases.

Chironomids respond to a wide range of sensitivities to environmental parameters such as dissolved oxygen, acidity or alkalinity, salinity, water current, food, temperature, humidity, depth, pollution by organic wastes as well as by inorganic contaminants like heavy metals.

Based on their morphological analysis many species of the genus could be recognized by the taxonomists. However, in the current state of progression of biological studies, the morphotaxonomical analysis needs a correlation with molecular analysis of the species. When the data obtained through morphometric analysis, cytological analysis, and molecular analysis are clubbed together to designate a species, those may be of great help for using one organism for many biological investigations. In this regard, the species of *Chironomus* being ubiquitous in inhabiting various ecological habitats in the environment may be studied in terms of their molecular organization.

In the higher eukaryotes, the 18S rRNA being conserved by organization in the living arena, is considered by many investigators for using this molecule in taxonomical analysis (Gunderina, Golygina, & Broshkov, 2015). A number of researchers have analyzed the molecular organization of the 18S rRNA of many

Chironomus spp. (Degelmann, Royer, & Hollenberg, 1979; Gunderina et al., 2015; Herrero, Planelló, & Morcillo, 2016; Schmidt, Godwin, Keyl, & Israelewski, 1982).

Therefore, in the present study, a comparison of the molecular data obtained from several species of *Chironomus* was considered to measure the degree of divergence of 18S rRNA. Concomitant with this, a comparison of cytological features of several Chironomid species has been carried out to find out their homology and divergence. Both the molecular and cytological analyses could show a correlation deciphering related features based on which the role of environmental impact on divergence among the *Chironomus* species may be suggested.

Methods

Sequence-based molecular analysis of 18S rRNA genes of *Chironomid* spp.

Nine species of *Chironomus* were considered for homology analysis for their 18S rRNA based on scientific reports of their worldwide ubiquitous distribution differing in ecological conditions. The species taken under consideration in the present study were *Chironomus javanus* Kieffer; *Chironomus xanthus* Rempel, 1939; *Chironomus transvaalensis* Kieffer; *Chironomus (Lobochironomus) dorsalis* Meigen, 1818; *Chironomus riparius* Meigen, 1804; *Chironomus matures* Johannsen, 1908; *Chironomus maddeni* sp.; *Chironomus duplex* sp. and *Chironomus crassiforceps* Kieffer, 1916. The 18S rRNA sequences of the studied species were reported by a number of investigators (Martin, Blinov, Alieva, & Hirabayashi, 2007). Experimentally obtained latest nucleotide sequence data for 18S rRNA genes of all of these nine species were retrieved from the NCBI website (<https://www.ncbi.nlm.nih.gov/>) for their pair-wise alignment using standard methods as briefly described below (Dey, Ganguli, Basu, Roy, & Datta 2010). Sequences were searched using the terms "18S rRNA" and "Chironomus" under the gene category of the NCBI website resulting in 18S rRNA gene sequences for a diverse Chironomid species from which the aforesaid nine species including *Chironomus javanus* were selected for

Fig. 1 Graphical presentation of % homology of DNA sequences (for 18S rRNA gene) of eight different species of *Chironomus* with *C. javanus*

the present analysis. Accession numbers and nucleotide sequences of these retrieved data are listed in the Supplementary Data 1.

The above-mentioned nucleotide sequences for this rRNA gene as obtained for different species under investigation were considered for pair-wise alignment taking the sequence of *C. javanus* as the reference one. Clustal Omega online server (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) was used to conduct the pairwise alignment of the retrieved nucleotide sequences of the 18S rRNA genes of the nine *Chironomus* species under study. Retrieved sequences in FASTA format were utilized to calculate percent sequence similarity and divergence, if any using standard protocols (Dey et al., 2010; Dey, Ganguli, Roy, & Basu, 2011).

Collection and rearing of *Chironomus javanus*

To investigate whether the obtained nucleotide sequence divergence of the said Chironomid spp. shows similar pattern in terms of cytological features, their polytene chromosomes were analyzed and compared taking *Chironomus javanus* as a reference species. The egg masses laid by females of *Chironomus javanus* were collected from outskirts of Kolkata, West Bengal, India. These egg samples were randomly collected throughout the year and from different water bodies to ensure sufficient sample size and remove time dependent bias, if any. They were made to hatch in the laboratory and the larvae developed from the eggs were reared in the culture trays with sufficient sterilized pond (or waterbody) water over the soil so that water submerge base of the culture trays at least by 1/2 inch. The larvae were

Table 1 Polytene chromosome features in different species of *Chironomus* (Figs. 2, 3, 4, 5, 6, 7, and 8)

Name of the species	Number of bands (major) arm wise								BR ^a total	NOR ^b total	Number of waists, arm wise								Centromere total
	A [^]	B [^]	C [^]	D [^]	E [^]	F [^]	G [^]	total			A [^]	B [^]	C [^]	D [^]	E [^]	F [^]	G [^]	total	
<i>C. javanus</i>	57	62	64	58	49	67	40	397	3	2	2	2	3	3	4	4	2	20	4
<i>C. dorsalis</i>	54	84	78	92	47	82	32	469	3	1	1	1	2	2	2	2	2	12	6
<i>C. riparius</i>	50	66	59	64	36	53	26	354	2	1	4	3	4	5		2	1	19	4
<i>C. matusus</i>	57	73	51	49	39	59	41	369	3	1	5	3	3	2	2	3	2	20	4
<i>C. maddenii</i>	56	55	36	42	66	46	42	343	1	1	3	4	4	1	1	2	2	17	4
<i>C. duplex</i>	56	66	67	76	55	61	38	419	1	1	2	2	4	2	3	4	2	19	4

^a BR: Balbiani Ring

^b NOR: Nucleolar Organizer

[^]A-G represent corresponding arms of polytene chromosome

Fig. 2 Arm A of the polytene chromosomes of six species of *Chironomus*. **a** *C. javanus*. **b** *C. dorsalis*. **c** *C. riparius*. **d** *C. matus*. **e** *C. maddenii*. **f** *C. duplex*

allowed to grow for about three weeks to achieve the penultimate fourth instar stage. The penultimate fourth instar larvae were taken from the culture trays to observe their polytene chromosomes in the salivary gland cells using below mentioned protocol.

Preparation of polytene chromosomes of *Chironomus javanus* and their cytological comparison with other Chironomid spp.

A comparison of the polytene chromosome arms of six species belonging to the above-mentioned series was also made to find out the homology or similarity between the species. For polytene chromosome morphology comparison, the species taken under consideration were *Chironomus javanus* Kieffer; *Chironomus dorsalis* Meigen, 1818; *Chironomus riparius* Meigen, 1804; *Chironomus matus* Johannsen, 1908; *Chironomus maddenii* sp. and *Chironomus duplex* sp.

For this analysis, a comparison was made pair-wise also taking *C. javanus* as the reference organism as mentioned above. Polytene chromosomes of *C. javanus* as obtained from natural habitats of West Bengal, India, and reared as above, was characterized as per the formula given by Keyl (1957, 1962), Devai, Miskolczi, and Wülker, (1989) and Kerkis, Kiknadze, Filippova, and Gunderina, (1989).

Polytene chromosome from *C. javanus* were prepared from the salivary gland cells and stained with 2% aceto-orcein using standard protocols (Hollenberg, 1976; Zacharopoulou, 1987). However, the chromosomes were studied under high power objective for determining its cytological features. The polytene chromosome features of the other *Chironomus* spp. used in this study have been taken from the works of some other investigators (Kiknadze, Michailova, Istomina, Golygina, Int, & Krastanov, 2006; Kiknadze, Broshkov, Istomina, Gunderina, &

Fig. 3 Arm B of the polytene chromosomes of six species of *Chironomus*. **a** *C. javanus*. **b** *C. dorsalis*. **c** *C. riparius*. **d** *C. matus*. **e** *C. maddenii*. **f** *C. duplex*

Fig. 4 Arm C of the polytene chromosomes of six species of *Chironomus*. **a** *C. javanus*. **b** *C. dorsalis*. **c** *C. riparius*. **d** *C. matures*. **e** *C. maddeni*. **f** *C. duplex*

Vallenduuk, 2008; Martin, 1971; Martin et al., 2007; Yamamoto, 1977). The polytene chromosomes exhibiting major bands, Balbiani rings, constricted sites (waists), centromeric region, and NOR along each of the chromosome arms were taken into consideration for the comparative analysis.

Both the data obtained from molecular analysis and cytological analyses were assessed for the degree of divergence of these species of *Chironomus* under consideration using Microsoft Excel software (Niglas, 2007).

Generation of dendrogram for the nine Chironomid spp. under study

A dendrogram or phylogram was generated on the basis of divergence and least distances between 18S rRNA

nucleotide sequences of the above mentioned nine Chironomid species from the mini-matrices calculated based on sequence dissimilarity matrices. For this analysis, 18S rRNA nucleotide sequences of all nine Chironomid spp. were first aligned together and % identity was calculated. % dissimilarity was then calculated by subtracting the similarity values from 100%. Using similar methods, 18S rRNA nucleotide sequences of individual Chironomid species under study were then analyzed and % dissimilarity was calculated to draw the dendrogram thereby identifying the nearest neighbors with evolutionary relevance. GraphPad Prism and Microsoft Excel software were used for these calculations and representations (Motulsky, 2007; Niglas, 2007).

Fig. 5 Arm D of the polytene chromosomes of six species of *Chironomus*. **a** *C. javanus*. **b** *C. dorsalis*. **c** *C. riparius*. **d** *C. matures*. **e** *C. maddeni*. **f** *C. duplex*

Fig. 6 Arm E of the polytene chromosomes of six species of *Chironomus*. **a** *C. javanus*. **b** *C. dorsalis*. **c** *C. riparius*. **d** *C. matus*. **e** *C. madden*. **f** *C. duplex*

Results

Latest available NCBI data revealed that 18S rRNA gene (partial) of *Chironomus javanus* contains 782 base pairs, *Chironomus xanthus* contains 806 base pairs, *Chironomus transvaalensis* contains 752 base pairs, *Chironomus dorsalis* contains 620 base pairs, *Chironomus riparius* contains 941 base pairs, *Chironomus matus* contains 752 base pairs, *Chironomus madden* contains 964 base pairs, *Chironomus duplex* contains 696 base pairs and *Chironomus crassiforceps* contains 862 base pairs as shown in method section above. Comparison of the sequences of *C. javanus* and *C. xanthus* indicated 97% homology (Fig. 1 and Supplementary Figure 1). Therefore, the two species diverged by 3%. Similar comparison between *C. javanus* and *C. transvaalensis* showed 81%

homology and therefore they are 19% diverged from each other, *C. javanus* and *C. dorsalis* showed 68% homology and so they diverged by 32%, *C. javanus* and *C. riparius* showed 98% homology and so they possess 2% divergence, *C. javanus* and *C. matus* showed 98% homology and so they have 2% divergence, *C. javanus* and *C. madden* showed about 67% homology, *C. javanus* and *C. duplex* showed homology value nearing 67%, *C. javanus* and *C. crassiforceps* showed 98% homology and so they have 2% divergence (Fig. 1).

Hence, with regard to the homology of the sequences among these species indicated a nearness relation in the order as *Chironomus javanus*, *Chironomus crassiforceps*, *Chironomus matus*, *Chironomus riparius*, *Chironomus xanthus*, *Chironomus transvaalensis*, *Chironomus*

Fig. 7 Arm F of the polytene chromosomes of six species of *Chironomus*. **a** *C. javanus*. **b** *C. dorsalis*. **c** *C. riparius*. **d** *C. mature*. **e** *C. madden*. **f** *C. duplex*

dorsalis, *Chironomus maddeni*, and *Chironomus duplex* (Figs. 1 and 11).

The polytene chromosome features from different arms of the chromosomes as obtained from six different species considered in this investigation have been shown in Table 1 below. Along with this table, the figures of the polytene chromosome arms of different species are showing there polytene chromosome features under consideration (Figs. 2, 3, 4, 5, 6, 7, and 8).

Pair-wise comparison of the polytene chromosome features as found in different species was performed with those obtained from *C. javanus* obtained in our laboratory. Comparison of the polytene chromosome features was mainly based on band number (major), Balbiani Ring (BR), Nucleolar Organizer (Lotfi et al., 2016), number of waists and centromeric heterochromatin region. The data obtained in relation to the above-noted polytene chromosome features of different species under investigation are shown in Table 2.

Therefore, the divergence of each of these species from *C. javanus* with regard to their polytene chromosome parameters may be indicated as under (Table 3, Fig. 9).

Discussion

In most of the species of genus *Chironomus* there are three large metacentric chromosomes and one small acrocentric chromosome (Figs. 2, 3, 4, 5, 6, 7, 8, and 10). *Chironomus* spp. has usually eight chromosomes in diploid set having been differentiated into seven different

arms viz. A, B, C, D, E, F, and G (Figs. 2, 3, 4, 5, 6, 7, 8, and 10) (Keyl, 1962).

The larvae of the flies are aquatic and the adult Chironomids are terrestrial. The larvae form a link between terrestrial and aquatic animals (Beck & Beck, 1969; Beck & Beck Jr, 1969; Dendy & Sublette, 1959; Lellak, 1953; Michailova, Petrova, Ramella, Sella, Todorova, & Zelano, 1996; Michailova, Sella, & Petrova, 2012; Saether, 1971; Sublette, 1970).

Polytene chromosomes appear in the interphase nucleus of the somatic cells of *Chironomus* and their larval salivary glands which are the principal sites for locating the polytene chromosomes (Ashburner, 1970; Aziz, Akrawi, & Nassori, 1991; Bhattacharyay, Sadhu, Mazumdar, & Chaudhuri, 2005; Michailova et al., 1996, 2012; Midya, Sarkar, & Bhaduri, 2012).

These macromolecular elements in the living organisms create hope among the taxonomists for characterizing species at the molecular level. The DNA segment promoting the synthesis of 18S rRNA of the ribosome has been found to be a conserved region in the DNA. Many investigators have tried to characterize the 18S rRNA gene of different species of *Chironomus* to reveal the variation in the nucleotide sequence usable for taxonomic categorization of the species (Gunderina et al., 2015; Martin et al., 2007; Michailova, Petrova, Bovero, Cavicchioli, Ramella, & Sella, 2000).

The present study dealt with the molecular features concerning the 18S rRNA gene sequences of nine chironomid species and the cytological features concerning

Fig. 8 Arm G of the polytene chromosomes of six species of *Chironomus*. **a** *C. javanus*. **b** *C. dorsalis*. **c** *C. riparius*. **d** *C. matures*. **e** *C. maddeni*. **f** *C. duplex*

Table 2 Comparative analysis of the polytene chromosomes of five different species with *C. javanus* based on the major features of the chromosomes (Figs. 2, 3, 4, 5, 6, 7, and 8)

Feature	C. <i>javanus</i>	C. <i>dorsalis</i>				C. <i>riparius</i>				C. <i>matures</i>				C. <i>maddenii</i>				C. <i>duplex</i>			
		Actual count	Diff. with C. <i>javanus</i>	% diff. with C. <i>javanus</i>	Actual count	Diff. with C. <i>javanus</i>	% diff. with C. <i>javanus</i>	Actual count	Diff. with C. <i>javanus</i>	% diff. with C. <i>javanus</i>	Actual count	Diff. with C. <i>javanus</i>	% diff. with C. <i>javanus</i>	Actual count	Diff. with C. <i>javanus</i>	% diff. with C. <i>javanus</i>	Actual count	Diff. with C. <i>javanus</i>	% diff. with C. <i>javanus</i>		
Band no. (major)	397	469	72	18	354	43	11	369	28	7	343	54	136	419	22	5.5					
BR ^a	3	3	0	0	2	1	33	3	0	0	1	2	67	1	2	67					
NOR ^b	2	1	1	50	1	1	50	1	1	50	1	1	50	1	1	50					
Waists ^c	20	12	8	40	19	1	5	20	0	0	17	3	15	19	1	5					
Centro-mere	4	6	2	50	4	0	0	4	0	0	4	0	0	4	0	0					
		Total		158	Total		99	Total	57		Total		1456	Total		127.5					
Value of divergence		158/5 ± 2.62			99/5 ± 1.99			57/5 ± 1.25			1456/5 ± 2.57			127.5/5 ± 0.85							
		=311.6 ± 2.62			=19.8 ± 1.99			=11.4 ± 1.25			=29.19 ± 2.57			=25.5 ± 0.85							

^a BR: Balbiani Ring^b NOR: Nucleolar Organizer^c waists=constricted regions

Table 3 Degree of divergence of five *Chironomus* species from *C. javanus*

Sl. no.	Name of the species	Divergence ^a (%) from <i>C. javanus</i>
1	<i>C. matuses</i>	11.4 ± 1.25
2	<i>C. riparius</i>	19.8 ± 1.99
3	<i>C. duplex</i>	25.5 ± 0.85
4	<i>C. maddeni</i>	29.19 ± 2.57
5	<i>C. dorsalis</i>	31.6 ± 2.62

^aThe data are expressed as means ± standard error of mean

the polytene chromosome organization of majority of these species. A comparative analysis of the two aspects, i.e., molecular sequence and cytological characteristics as polytene chromosome bands, constrictions, Balbiani Rings were made in order to quantify their importance in speciation of Chironomids.

In this comparison and for easy analysis, *C. javanus* has been taken as the reference one and a comparison at random among the species have been done. For the sake of simplicity, a pair wise alignment of the sequence of *C. javanus* and that of any of the other considered species namely, *Chironomus xanthus*, *Chironomus transvaalensis*, *Chironomus dorsalis*, *Chironomus riparius*, *Chironomus matuses*, *Chironomus maddeni*, *Chironomus duplex*, and *Chironomus crassiforceps* was made to measure the range of their divergence in the organization of 18S rRNA gene (Supplementary Figure 1). The comparative molecular analysis of the species in

terms of their polytene chromosome features as the number of bands (major), BR, NOR, waists, and centromere along each of the chromosome showed that the percentage of divergence from *C. javanus* ranged from a low of 11.4 ± 1.25 in *C. matuses* to a high of 31.6 ± 2.62 in *C. dorsalis* (Table 3).

The flies of different species of *Chironomus* are identical in appearance but in minute details they differ greatly for achieving a distinct status of a species. This divergence may also be noted by cytological study at the chromosomal level because this species differs by morphological feature as a result of their genetic distinction (Gunderina et al., 2015; Martin, 1971).

It is therefore clear that divergence of the species or molecular evolution of Chironomid runs parallel along with the morphological features of the polytene chromosome organization.

The dendrogram based on the percentage of dissimilarity between different species examined indicated that G-H and C-B are closer to each other than other species (Fig. 11, Supplementary Figure 1).

Conclusions

A pair wise alignment of the 18S rRNA gene sequences of nine Chironomid species showed that the divergence of the species or molecular evolution of Chironomid runs parallel along with the morphological features of the polytene chromosome organization.

Fig. 9 Graphical representation of the degree of divergence (%) of five species of *Chironomus* from *C. javanus*, based on their polytene chromosome features

Fig. 10 Four polytene chromosomes of *C. javanus* (Kieffer). The chromosome I is a combination of arms B and F while the chromosome II is a combination of arms A and E. The chromosome III is a combination of arms C and D and the chromosome IV represents the arm G. Arrows represent centromeres

Fig. 11 Dendrogram (unrooted) showing % distance of the nine species of Chironomids under study. The scale on the left is a distance measure. **A** *Chironomus javanus*. **B** *Chironomus xanthus*. **C** *Chironomus transvaalensis*. **D** *Chironomus dorsalis*. **E** *Chironomus riparius*. **F** *Chironomus maturas*. **G** *Chironomus maddenii*; **H**: *Chironomus duplex*; and **I**: *Chironomus crassiforceps*

Abbreviations

BR: Balbiani Ring; *C. javanus*: *Chironomus javanus* Kieffer; *C. xanthus*: *Chironomus xanthus* Rempel, 1939; *C. transvaalensis*: *Chironomus transvaalensis* Kieffer; *C. dorsalis*: *Chironomus (Lobochironomus) dorsalis* Meigen, 1818; *C. riparius*: *Chironomus riparius* Meigen, 1804; *C. matures*: *Chironomus matures* Johannsen, 1908; *C. maddenii*: *Chironomus maddenii* sp.; *C. duplex*: *Chironomus duplex* sp.; *C. crassiforceps*: *Chironomus crassiforceps* Kieffer, 1916; NOR: Nucleolar Organizer; 18S rRNA: 18S ribosomal RNA

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s41936-021-00215-0>.

Additional file 1: Supplementary Data 1. Accession numbers and nucleotide sequences for each of the 18S rRNA genes, of the nine Chironomid spp. for the current study. **Supplementary Figure 1.** Pairwise alignment of 18S rRNA gene sequences of nine species of Chironomid.

Acknowledgements

The authors are thankful to the Department of Zoology and Molecular Biology & Genetics, Presidency University, Kolkata, India, for providing facilities to carry out the present investigation. The authors also acknowledge the anonymous reviewers of the manuscript for their constructive suggestions to improve it extensively.

Authors' contributions

TM, SB, and SKD designed the study, drafted, and edited the manuscript. SKD performed the pair-wise alignment studies of the 18S rRNA sequences of Chironomid spp. SB cultured the *C. javanus* and compared its polytene chromosome organization along with SKD and TM. All authors have read and approved the manuscript.

Funding

Authors are thankful to the Ministry of Environment and Forests, Government of India, for the Grant (No. 19-16/2007-RE) to TM for necessary funding required for the current work.

Availability of data and materials

All data related to the current study are included in the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests for this work.

Author details

¹Department of Zoology and Molecular Biology & Genetics, Presidency University, Kolkata 700073, India. ²Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ 08854, USA. ³Dr. B.R. Ambedkar Center for Biomedical Research, Delhi University, Delhi 110007, India.

Received: 11 April 2020 Accepted: 10 March 2021

Published online: 06 April 2021

References

- Ashburner, M. (1970). Function and structure of polytene chromosomes during insect development. *Advances in Insect Physiology*, Elsevier, 7, 1–95. [https://doi.org/10.1016/S0065-2806\(08\)60240-4](https://doi.org/10.1016/S0065-2806(08)60240-4).
- Aziz, J., Akrawi, N., & Nassori, G. (1991). The effect of chronic toxicity of copper on the activity of Balbiani rings and nucleolar organizing region in the salivary gland chromosomes of *Chironomus ninevah* larvae. *Environmental Pollution*, 69(2–3), 125–130. [https://doi.org/10.1016/0269-7491\(91\)90138-M](https://doi.org/10.1016/0269-7491(91)90138-M).
- Beck, E. C., & Beck Jr., W. M. (1969). The chironomidae of Florida: II. The Nuisance Species. *Florida Entomologist*, 1–11.
- Beck, E. C., & Beck, W. (1969). *Chironomidae (Diptera) of Florida. III. The Harnischia complex (Chironominae)*. Fla State Mus Bull Biol Sci.
- Bhattacharyay, G., Sadhu, A., Mazumdar, A., & Chaudhuri, P. (2005). Antennal deformities of chironomid larvae and their use in biomonitoring of heavy metal pollutants in the river Damodar of West Bengal, India. *Environmental Monitoring and Assessment*, 108(1–3), 67–84. <https://doi.org/10.1007/s10661-005-3963-8>.
- Degelmann, A., Royer, H.-D., & Hollenberg, C. P. (1979). The organization of the ribosomal RNA genes of *Chironomus tentans* and some closely related species. *Chromosoma*, 71(3), 263–281. <https://doi.org/10.1007/BF00287136>.
- Dendy, J., & Sublette, J. E. (1959). The Chironomidae (= Tendiipedidae: Diptera) of Alabama with descriptions of six new species. *Annals of the Entomological Society of America*, 52(5), 506–519. <https://doi.org/10.1093/aesa/52.5.506>.
- Devai, G., Miskolczi, M., & Wülker, W. (1989). Standardization of chromosome arms B, C and D in *Chironomus* (Diptera, Chironomidae). *Acta Biologica Debrecina Oecologica Hungarica*, 2(1), 79–92.
- Dey, S. K., Ganguli, S., Basu, P., Roy, P., & Datta, A. (2010). Lysine richness in human snurps possible sites for electrophilic attacks. *Bioinformation*, 4(9), 409–411. <https://doi.org/10.6026/97320630004409>.
- Dey, S. K., Ganguli, S., Roy, P., & Basu, P. (2011). Pseudoknots in human snRNPs. *International Journal of Bioinformatic Research*, 3(1), 194–199.
- El-Shenawy, N., Ahmed, R. S., Ismail, F., & Abo-Ghaila, A. (2010). Use of *Chironomus calipterus* (Diptera: Chironomidae) as a Bioindicator in El-Tall El-Keber, Egypt. *Journal of Fisheries and Aquatic Science*, 5(2), 94–105. <https://doi.org/10.3923/jfas.2010.94.105>.
- Gunderina, L., Golygina, V., & Broshkov, A. (2015). Chromosomal organization of the ribosomal RNA genes in the genus *Chironomus* (Diptera, Chironomidae). *Comparative Cytogenetics*, 9(2), 201–220. <https://doi.org/10.3897/CompCytogen.v9i2.9055>.
- Herrero, Ó., Planelló, R., & Morcillo, G. (2016). The ribosome biogenesis pathway as an early target of benzyl butyl phthalate (BBP) toxicity in *Chironomus riparius* larvae. *Chemosphere*, 144, 1874–1884. <https://doi.org/10.1016/j.chemosphere.2015.10.051>.
- Hollenberg, C. (1976). Proportionate representation of rDNA and Balbiani ring DNA in polytene chromosomes of *Chironomus tentans*. *Chromosoma*, 57(2), 185–197. <https://doi.org/10.1007/BF00292917>.
- Kerkis, I., Kiknadze, I., Filippova, M., & Gunderina, L. (1989). Cytogenetic differentiation of the *Chironomus* species of the plumosus group. *Acta Biologica Debrecina Supplementum Oecologica Hungarica Fasc.*, 2, 103–114.
- Keyl, H. (1957). Karyotypes of *Chironomus thummi*. I. Diagram of the salivary chromosomes of *Chironomus thummi thummi* and cytological differentiation of the subspecies *Chironomus thummi thummi* and *Chironomus thummi piger*. *Chromosoma*, 8(6), 739–756.
- Keyl, H.-G. (1962). Chromosomenevolution bei *Chironomus*. *Chromosoma*, 13(4), 464–514. <https://doi.org/10.1007/BF00327342>.
- Kiknadze, I., Broshkov, A., Istomina, A., Gunderina, L., & Vallenduuk, H. (2008). Geographic variability of the polytene chromosome banding sequence of non-biting midge *Chironomus pseudothummi* Str. (Diptera, Chironomidae). *Cell and Tissue Biology*, 2(4), 417–427. <https://doi.org/10.1134/S1990519X08040123>.
- Kiknadze, I., Michailova, P., Istomina, A., Golygina, V., Int, L. P., & Krastanov, B. (2006). The chromosomal polymorphism and divergence of populations in *Chironomus nudatarsis* Str. (Diptera, Chironomidae). *Tsitologiya*, 48(7), 595–609.
- Lellak, J. (1953). The Chironomidae and other bottom fauna of some stagnant waters in the central Elbe (Labe) region. *Rozprawy Československé Akademie Věd, Řada MPV*, 63, 69–144.
- Lotfi, M., Ahmed, R. S., El-Shatoury, S. A., & Hanora, A. (2016). In situ morphological abnormalities in the mouthparts of *Chironomus transvaalensis* (nonbiting midges) stressing their role as bioindicators. *Journal of Entomology and Zoology Studies*, 4(4), 1299–1305.
- Maitra, B. (2001). *Cytotaxonomic categorization of a few species of Chironomus Miegen and Kiefferulus Goetghebuer (Diptera : Chironomidae)*. Ph. D. Thesis, Burdwan University.
- Martin, J. (1971). A review of the genus *Chironomus* (Diptera, chironomidae). IV. The karyosystematics of the australis group in Australia. *Chromosoma*, 35(4), 418–430. <https://doi.org/10.1007/BF02451448>.
- Martin, J., Blinov, A., Alieva, E., & Hirabayashi, K. (2007). A molecular phylogenetic investigation of the genera closely related to *Chironomus* Meigen (Diptera:

- Chironomidae). In *Contributions to the systematics and ecology of aquatic Diptera-A tribute to Ole A. Sæther*, (pp. 193–203). The Caddis Press.
- Michailova, P., Petrova, N., Bovero, S., Cavicchioli, O., Ramella, L., & Sella, G. (2000). Effect of environmental pollution on the chromosomal variability of *Chironomus riparius*. *Genetica*, 108(2), 171–180. <https://doi.org/10.1023/A:1004172019131>.
- 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(2), 161–178. <https://doi.org/10.1007/BF00121364>.
- Michailova, P., Sella, G., & Petrova, N. (2012). Chironomids (Diptera) and their salivary gland chromosomes as indicators of trace-metal genotoxicity. *The Italian Journal of Zoology*, 79(2), 218–230. <https://doi.org/10.1080/11250003.2011.622084>.
- Midya, T., Bhaduri, S., Sarkar, P., & Ghosal, S. K. (2013). A model on the structure and organization of the polytene chromosome based on the study on *Chironomus striatipennis* kieffer (diptera: chironomidae). *The Bioscan: An International Quarterly Journal of Life Sciences*, 8(1), 21–24.
- Midya, T., Sarkar, P., & Bhaduri, S. (2012). Effect of lead as heavy metal in aquatic habitat to promote polymorphism of polytene chromosomes in *Chironomus striatipennis* Kieffer (Diptera: Chironomidae). *The Ecoscan. Special(1)*: 173–178.
- Motulsky, H. (2007). Prism 5 statistics guide, 2007. *GraphPad Software*, 31(1), 39–42.
- Niglas, K. (2007). Media review: microsoft office excel spreadsheet software. *Journal of Mixed Methods Research*, 1(3), 297–299. <https://doi.org/10.1177/1558689807301250>.
- Sæther, O. A. (1971). Nomenclature and phylogeny of the genus *Harnischia* (Diptera: Chironomidae). *The Canadian Entomologist*, 103(3), 347–362. <https://doi.org/10.4039/Ent103347-3>.
- Sarkar, P., Bhaduri, S., Ghosh, C., & Midya, T. (2011). The species of *Chironomus* as biosensor in detecting environmental pollution: a study on *Chironomus striatipennis* Kieffer (Diptera: Chironomidae). *The Ecoscan. special issue 1*: 363–368.
- Schmidt, E. R., Godwin, E., Keyl, H.-G., & Israelewski, N. (1982). Cloning and analysis of ribosomal DNA of *Chironomus thummi* piger and *Chironomus thummi* thummi. *Chromosoma*, 87(4), 389–407. <https://doi.org/10.1007/BF00327181>.
- Sublette, J. E. (1970). Type specimens of Chironomidae (Diptera) in the Illinois Natural History Survey Collection, Urbana. *Journal of the Kansas Entomological Society*, 44–95.
- Vermeulen, A. C. (1995). Elaborating chironomid deformities as bioindicators of toxic sediment stress: the potential application of mixture toxicity concepts. *Annales Zoologici Fennici*, JSTOR.
- Warwick, W. (1985). Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meigen. *Canadian Journal of Fisheries and Aquatic Sciences*, 42(12), 1881–1914. <https://doi.org/10.1139/f85-236>.
- Yamamoto, K. D. (1977). *A comparison of Salivary gland chromosomes of Chironomus larvae of acid polluted strip-mines lakes*. MS Thesis, Sonthern Illionois University.
- Zacharopoulou, A. (1987). Cytogenetic analysis of mitotic and salivary gland chromosomes in the medfly *Ceratitis capitata*. *Genome*, 29(1), 67–71. <https://doi.org/10.1139/g87-011>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)