Indian Journal of Entomology 85(3): 767-769 (2023) DoI. No.: 10.55446/IJE.2021.384

CHIRONOMIDS DIVERSITY OF MANIPUR

Bhubaneshwari Devi Moirangthem[1] and Dhananjoy Chingangbam Singh[2]*

[1]Laboratory of Entomology, P G Department of Zoology
D M College of Science, Imphal 795001, Manipur, India
[2]Department of Zoology, Manipur University, Canchipur, Imphal 795003, Manipur, India
Email: dhananjoych@gmail.com (corresponding author)

ABSTRACT

Chironomids are Diptera and these are good bioindicators of aquatic habitats, and serve as food of aquatic as well as the lower terrestrial animals. The diversity of these were assessed from the Dhanamanjuri University Campus, which revealed three genera and one unidentified genus viz., *Kiefferulus calligaster*, *Chironomus plumosus*, *Chironomus circumdatus* and one morphotype closely resembling Yama (Yama type). The chromosome compliments of these were observed to be with 2n=8 (*K. calligaster*- AB, CD, EF, G; *C. plumosus*- AB, CD, EF, G; *C. circumdatus*- BF, CD, AE, G and Yama type- AE, BF, CD, G). The differences were in the translocation both in NOR and chromosomes. *Kiefferulus calligaster*, *C. plumosus*, Yama type and *C. circumdatus* were observed coexisting in cemented drainage, with Yama type being predominant in the pond; the latter was mainly collected from more contaminated ditches with domestic waste. The polytene chromosomes were species-specific and could be utilized to identify the species in the larval forms.

Key words: Manipur, Chironomidae, diversity, *Kiefferulus calligaster*, *Chironomus plumosus*, *Chironomus circumdatus*, polytene chromosomes, karyotypes, habitats, ponds, sewage drains

Manipur, the easternmost part of India is a small hilly state, is part of Indo-Burma hotspots of the world (Myers et al., 2000). Chironomids are the most widely distributed and one of the most abundant insects in freshwater in their larval and pupal stage. The chironomids are decomposers and play an important role in the environment. Chironomids can develop in polluted waters such as waste stabilization ponds where they become a dominant macroinvertebrate (Broza et al., 2000) so they are regarded as bioindicators. Chironomids are used in the fields of genetics, cytogenetics, and as food for the fish and amphibian (Sharifian Fard et al., 2014). These also serve as bioindicators of the depredating habitats or pollution (Lucian et al., 2008). Some reports indicate that these are scavengers of toxic substances so not advisable for human consumption (Sharifian Fard et al., 2014). Cytologically the chironomids are arranged descending as AB (1st), CD (2nd), EF (3rd) and G (4th) and specific NOR bearing chromosome is the general rule. From India, 313 chironomid species in 59 genera under 4 subfamilies had been described (Chaudhuri et al., 2001), and only six species are known from the eastern Himalaya of India, and there are no reports from Manipur. This study is an attempt to identify the chironomids and explore their polytene chromosomes and species-specific polymorphism.

MATREIAL AND METHODS

The larvae along with water were collected from two spots located in the front drainage of the Zoology department- spot 1 (E 240 82'08.77", N 930 94'11.87"), spot 2 (E 240 82'07.89", N 930 93'94.16.59") and one spot 3 from the pond (E 240 82'18.20", N 930 93'94.19.89") on the 16th June 2021. Three scoops of the mud and larvae were separately collected. The drain was somewhat clean with the leafy bottom. The larvae were fixed in the 1:3 glacial acidic acid and methanol by volume for 24 hr and transferred to 70% ethanol, while for fresh preparation 70% ethanol was omitted. The material used for polytene studies, head, and tail of the same were made as temporary mount on the same slide. The head and tail features were used for larval identification. The polytene chromosome preparations were done according to Porter and Martin (1977) with slight modifications. The salivary glands were dissected in fixative and stained with 4% acetocarmine solution for 3 min and dipped in fixative for 40 sec and one drop of lactic acid for 1 min or after dissolving the salivary cells. It was observed under coverslip in 10x objective of a compound microscope and photographs were taken using 100x objective with MI 9 prime mobile camera from the eyepiece. The identification of the larvae was done according to Martin (2013, 2016, 2019).

RESULTS AND DISCUSSION

The data given in Fig. 1, reveal that *Kiefferulus* was dominant in the drainages while Yama type was predominant in the pond. These were found attached to leaves, while *Chironomus plumosus* and *C. circumdatus* were attached to stones or on cemented walls of drainage in their igloo-type mud. Larvae of the Yama type were freely swimming or feeding on the bottom of pond and drainage. Larva of *K. calligaster* (Kieffer) showed a light red small (8-10mm) plumosus-type with ventral and lateral tubules. Mentum with fourth laterals was not at the level to fifth laterals (type I), sixth laterals vertical; c2 teeth of the central tooth not well separated, ventromental plate separated medially by at least the width of the median teeth of the mentum, pectin epipharyngis with 15 teeth, premandible is with inner tooth about twice the width of the outer, posterior pair of ventral tubules longer than anterior pair with length and coiled. Lateral tubules well developed (Fig. 2 A, E). *K. calligaster* is common in the Indian subcontinent. The larvae of *C. plumosus* were characterized with well-developed mentum and anal tubules (Fig. 2 B, F). Larvae of *C. circumdatus* were medium plumosus type and 12.25 mm long from 10 IV instar larvae. Mentum with fourth laterals reduced to about the level of fifth laterals (type II), sixth laterals pointed slightly outwards; c2 teeth of the central tooth well separated, ventromental plate separated medially by at least the width of the median teeth of the mentum, pectin epipharyngis with exactly 15 teeth, premandible is with inner tooth about twice the width of the outer, posterior pair of ventral tubules longer than anterior pair with length of 1.95 mm of the anterior while posterior is 3.0 mm and coiled. Lateral tubules well developed (Fig. 2 C, G). A small (7-10 mm) Yama-type with no ventral or lateral tubules, anal tubules in a 'star'-arrangement, posterior prolegs about 4x long than width at base was also observed. Gula and frontoclypeus slightly dark to dark, sometimes with slight darkening outside the frontoclypeus. This may be a difference between the two localities. Clypeal aperture wider at mid-point with a curved ventral border, about 2.5x longer than width at widest point (Fig. 2 D, H). Notable for an unusual larval type (yama-type). This might belong to a new subgenus (http://www.chironomidae.net/Martin/SEAChironfile/SEADSC1.htm).

The diploid number of *K. caligaster* was 8 (2n=8) with three long chromosomes and very short chromosomes. The second chromosome had nucleolus at the one-third of the chromosome. The chromosomes were thick and could be used to test in the laboratory. *K. calligaster* belongs to the cytocomplex "thummi" with chromosome arm combinations: AB CD EF G. (Fig. 2 I); *C. plumosus* had (2n=8) consists of three long chromosomes (chromosomes AB and CD- metacentric, chromosome EF – submetacentric) and one short (chromosome G – acrocentric) (Fig. 1, A-D). It has three Balbiani rings (BRs): one in chromosome arm B and two in chromosome G. The nucleolar organizer (NOR) is localized also in chromosome G; *C. plumosus* belongs to the cytocomplex 'thummi' with chromosome arm combinations: AB CD EF G. The centromere regions of the chromosomes are expressed by thin dark bands in the studied populations (Fig. 2 J). There were four prominent well stained polytene chromosomes in *Chironomus circumdatus*. The polytene chromosomes are typical pseudothummi -cytocomplex combination of BF, CD, AE, and G. The first (B), second (C) and shortest (G) chromosomes were having nucleoli (n). The shortest chromosome is having two prominent Balbiani rings (br) (Fig. 2 K) (courtesy Jon Martin).

Chironomus circumdatus was described by Kieffer (1916) from Formosa (Taiwan); other works include those of Chaudhuri et al. (1992), Kumar and Gupta (1990; cytological aspects), Tripathi et al. (2004). Kumar and Gupta (1990) reported altogether seven inversions comprising six paracentric and one pericentric. The present study reveals that *C. circumdatus* is the common *Chironomus* sp. in Manipur inhabiting fairly uncontaminated aquatic medium. On the basis of homozygous reciprocal translocations species of the genus may have the chromosome arm combination: AB, CD, EF, G or AE, BF, CD, G. Species having the same chromosome arm combination may be united into complexes (Keyl, 1962). So, the karyotype based on polytene chromosomes plays an integrating role in the systematics of the Chironomidae. In these complexes species are differentiated on the basis of homozygous inversions (Keyl 1962). Four relatively long thin polytene chromosomes were observed apparently with the pseudothummi-cytocomplex combinationm AE, BF, CD, G. Arm G with a terminal nucleolus and a BR about

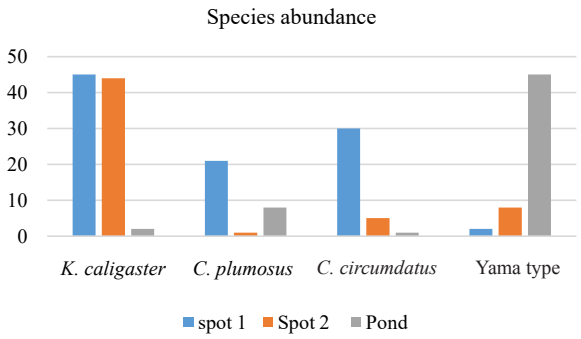

Fig. 1. Comparative species diversity

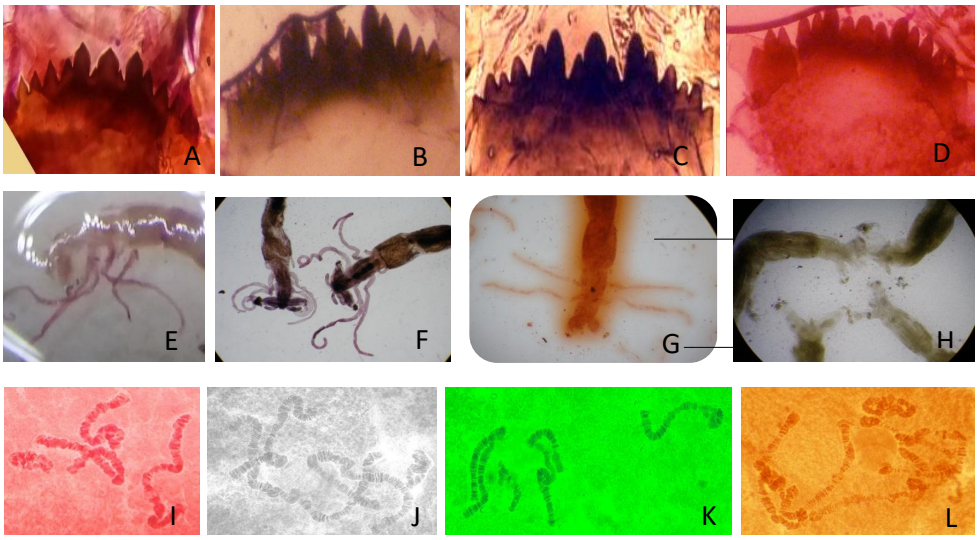

Fig. 2. Mentum, anal tubules and salivary gland polytene chromosomes in larval form of *K. caligaster* (A, E, I), *C. plumosu*s (B, F, J), *C. circumdatus* (C, G, K) and Yama type (D, H, L) respectively. Bar represents 0.5 mm.

a third from the other end (Fig. 2 L). Sublette and Martin (1980) described a new genus Yama, closely related to *Chironomus* with special features of relatively long, rather tanypodine-like posterior prolegs along with a star arrangement of anal tubules (Martin and Chingangbam, 2016). Since then this type is placed as Yama type.

ACKNOWLEDGEMENTS

The authors thank the Head of Department Zoology, Manipur University and Dhanamanjuri College of Science, Imphal for providing facilities. The authors are grateful to Superman, the Choukidar of D. M. College of Science, Imphal and his son for helping in collection of the material. Jon Martin, Genetics, Genomics and Development, School of Biosciences, The University of Melbourne, Vic 3010, Australia is acknowledged for his contributions in studying the species and guidance.

REFERENCES

Broza M, M Halpern, M Inbar. 2000. Non-biting midges (Diptera; Chironomidae) in waste stabilization ponds: an intensifying nuisance in Israel. Water Science and Technology 42: 71-74.

Chaudhuri P K, Das S K, Sublette J E. 1992. Indian species of genus *Chironomus* Meigen (Diptera: Chironomidae). Zoologische Jahrbucher Systematik 119: 1-51.

Chaudhuri P K, Niladri Hazra, J R B Alfred. 2001. A checklist of chironomid midges (Diptera: Chironomidae) of the Indian subcontinent. Oriental Insects 35: 1, 335-372.

Keyl. 1962. Chromosomenevolution bei *Chironomus*. II. Chromosomenumbauten und phyogenetische Beziehungen der Arten. Chromosoma 13: 496-541.

Kieffer J J. 1916. Tendipedides (Chironomides) de Formose conservés au Muséum National Hangrois de Budapest et détérmines par J. J. KIEFFER. - Annales historico-naturales Musei nationalis Hungarici 14: 81-121.

Kumar A, Gupta J P. 1990. Cytogenetic studies of *Chironomus circumdatus* from India (Diptera: hironomidae). Genetica 82: 157-163.

Lucian Gavrilă, Liliana Burlibaşa, Maria Daniela Uşurelu, Irina Radu, Laura Monica Magdalena, Aurel Ardelean, Marian Cărăbaş. 2008. Chromosomal rearrangements in *Chironomus* sp. As genosensors for monitoring environmental pollution. Roumanian Biotechnological Letters 13 (5): 3962-3969.

Martin Jon. 2013. Oriental *Chironomus* V. Morphology and cytology of Oriental (Indomalayan Realm) *Chironomus* species.

Martin Jon. 2016. Oriental *Chironomus* Species, web version, *Chironomus circumdatus* Kieffer, 1916, and personal communication.

Martin J. Dhananjoy Ch. 2016. An additional larval type in the genus *Chironomus*-the Yama type. Chironomus Journal of Chironomidae Research 29: 38.

Myers Norman, Russell A. Mittermeier, Cristina G. Mittermeier, Gustavo A. B. da Fonseca, Jennifer Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 852-858.

Ningthoujam P S, C S Dubey, S. Guillot, A S Fagion, D P Shukla. 2012. Origin and serpentinization of ultramafic rocks of Manipur Ophiolite Complex in the Indo-Myanmar subduction zone, Northeast India. Journal of Asian Earth Sciences 50: 128-140.

Porter D L, Martin J. 1977. The cytology of *Polypedilum nubifer* (Diptera, Chironomidae). Carylogia 30: 41-62.

Sharifian Fard Mojdeh, Frank Pasmans, Connie Adriaensen, Gijs Du Laing, Geert Paul Jules Janssens, A Martel. 2014. Chironomidae Bloodworms Larvae as Aquatic Amphibian Food. Zoo Biology 9999: 1-7.

Sublette, J E, Martin, J1. 1980. *Yama tahitiens* n. gen., n. sp. from Tahiti (Diptera: Chironomidae). Pan Pacific Entomologist 56: 221-237.

Tripathi N K, Sharma, O P, Khanna P. 2004. Studies on the polytene chromosomes of *Chironomus circumdatus* (Diptera: Chironomidae) from Jammu region. Proceedings of the National Academy of Sciences India, Section B, Biological Sciences 74: 1-6.

(Manuscript Received: July, 2021; Revised: December, 2021;
Accepted: December, 2021; Online Published: April, 2022)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e21172