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## Chromosomes of the *Chironomus javanus* Kieffer, 1924, from Manipur, India

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### Abstract

The exploration of chironomids from Manipur was a successful one. Many of them are abundantly found in the every aquatic habitats and it is not easy to identify. The larval identification was also very difficult as many of them are identical in species of different genera. Here in the present study we are reporting *Chironomus javanus* Kieffer. It was collected from two habitats-sewage drainage and a lake. Some larvae were fixed in fixative to study the salivary polytene chromosomes with acetocarmine-squashed method. There was no differences in the morphology and karyotypes from two spots. The characteristics that separate out this from rest of other species is in having 7 (seven) teeth in the premandible besides well separated and while the adults are yellow-green in colour. The karyotype consists of 8 chromosomes with pseudo-thummi combination. There is much to be study to understand the underlying mechanism to understand the speciation in the genera. The chironomids of Manipur are not fully explored and many are yet to be named and described. The morphological features as well as the cytology of the *C. javanus* of Manipur is quite different from the rest of the world and much is needed to study.

**Keywords:** Manipur, *C. javanus*, drainage, lake, polytene chromosomes

### Introduction

The non-biting aquatic midges of the family Chironomidae (Order: Diptera) include hundreds of species; the larvae inhabit almost all types of inland waters, such as ponds, lakes, swamps, rivers, sewage ditches, fish ponds and rice fields <sup>[1, 2, 3]</sup>. From India, 313 species in 59 genera under 4 subfamilies had been described <sup>[4]</sup>, and only six species of the genus from the eastern Himalaya of India. From Manipur, the number of species reported are very low at the moment comprising of eight species under four genera (unpublished data). The practical utility of the insect in natural environment include heavy metal tracing <sup>[5]</sup> and other bio indicators <sup>[6, 7, 8, 9, 10, 11]</sup>. 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 <sup>[12]</sup>. The insect had been used as materials used in classroom for polytene chromosomes.

The exploration of the insect fauna from lakes/pats and other parts of Manipur is far from initiation. In this endeavor the chironomids from Lousipat were collected and fixed in the fixative. The identification of the species was done on the basis of the larvae, pupa and adults. It is again very important as many patlakes are vanishing due to heavy sedimentation, the organisms will be minimizing their distribution or extinct in near future above all these the global climate change that hindered the distribution of the organisms. Hence present study was taken up. In the present study reports the *Chironomus javanus* from the two habitats from Manipur. Many works are yet to study from different parts of Manipur in future. The chironomids of Manipur are not fully explored and many are yet to be named and described. The morphological features as well as the cytology of the *C. javanus* of Manipur is quite different from the rest of the world and much is needed to study.

### Materials and Methods

The larvae along with water were collected from two spots first from Iroishemba Mayai Leiai (drain) (E 24.808533, N 93.892862), and from field converted part of Lousipat, a lake (E 24.5515809, N 93.98702339) between 15 to 17 June, 2021. Three scoops of the mud and larvae were separately collected to ascertain the species diversity. The Iroishemba drain was somewhat clean with the domestic waste bottom and was not connected with. The field in the Lousipat was clear.

## Morphological studies

### Larvae

The larvae were fixed in the 1:3 glacial acetic acid and methanol by volume for 24 hours and transferred to 70% ethanol for further storage and fresh preparation 70% ethanol treatment was omitted. The material used for polytene studies, head, and tail of the same were also made temporary mount on the same slide. The head and tail features were used for larval identification.

### Cytological studies

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 three minutes and dipped in fixative for 40 seconds and one drop of lactic acid for one minute or after dissolving the salivary cells. Cover with cover slide and observed under 10X objective lens of a compound microscope and photographs were taken using 100X objective lens with help MI 9 prime mobile camera from the eye piece. The identification of the larvae, pupal exuviae and adults was done according to Martin [13, 14, 15]. The individual chromosomes were named according to Keyl [16] and Martin [14] and personal communications).

## Results

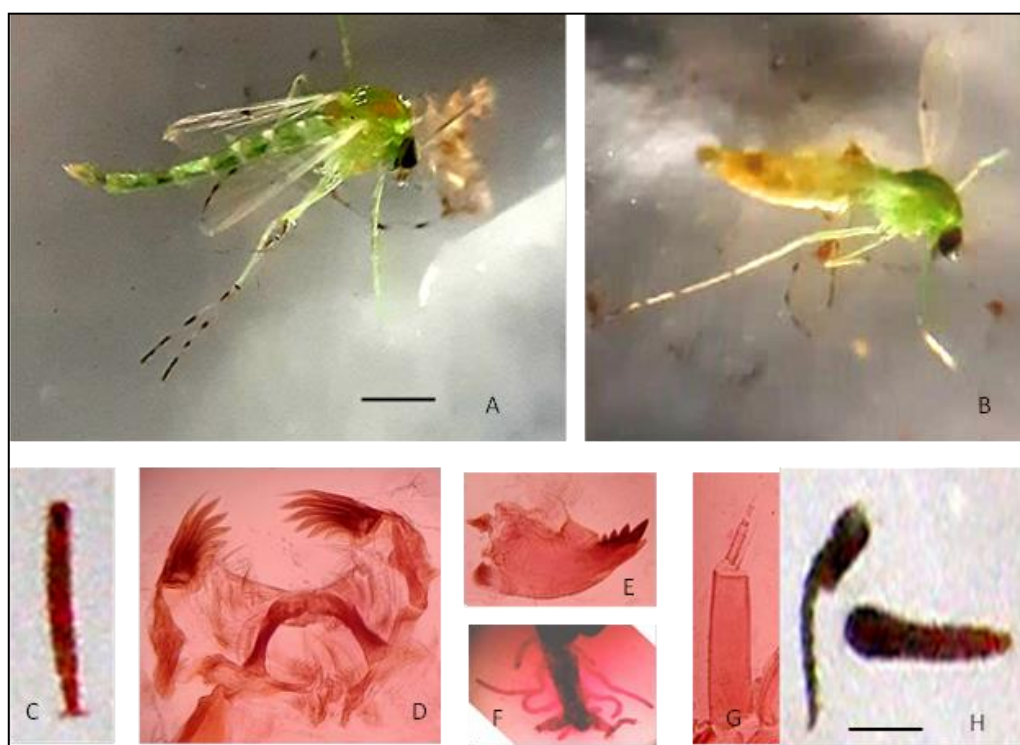
The characteristics features of the species *C. javanus* were seen in the specimens collected from the two study sites

### Male (n=4)

Mean body length, 5.6 mm. Body colouration largely greenish yellow; antennal shaft brown, hair grey; ground colour of scutum greenish yellow; stripes reddish brown, scutellum yellow, postnotum brown, halteres yellow; abdominal tergites I-VII greenish yellow without dark masks, abdominal tergite VIII and hypopygium brown; wings marked with black spots at the periphery and middle, r-m darker than the remainder of veins; in the forelegs, femur, tibia and tarsus I nearly white, both ends of tarsi II and III dark brown and their middle parts white, tarsi IV and V entirely dark brown; in the middle and hind legs, femora yellowish brown, tibiae and proximal 4/5 of tarsi I white, distal end of tarsi I and both ends of tarsi II and III dark brown, middle of tarsi II and III yellowish brown, tarsi IV and V entirely dark brown. Frontal tubercles well developed. Acrostichal setae of scutum usually absent. Scutal setae 10–16 in a single transverse row. Fore tarsus III longer than fore tarsus II (Fig. 1 A).

### Female (n=5)

All characters, in general, similar to the male, with usual sexual differences and the presence of 20–26 acrostichal setae on scutum (Fig. 1 B).



**Fig 1:** The different stages of *Chironomus javanus*, male adult (A), female (B), Larva (C), the premandible with seven teeth (D), mandible (E), anal tubules (F), antenna (G) and pupa (H). Bar represents 0.5 cm.

### Larvae (n=25)

Body, 12–13 mm long, blood red in coloration. Two pairs of eyes present. Head, yellowish with dark postoccipital margin. Pecten epipharyngis simple, consisting of 15 teeth of almost equal lengths. Premandibles with 7 teeth (Fig. 1 D) the most distinguishing feature of the species. Ventromentum plates, fan-shaped, separated medially by approx. 1/3 width of mentum plate. Mentum with the central trifid tooth set below the 1st laterals, and the c2 teeth markedly separated from c1

tooth (type III) and pointed towards it; 4th laterals at most slightly reduced (type I). Mandible (Fig. 1 D) with third inner tooth darkened and completely separated (type IIIB), with three spines on inner margin, and about 12 - 13 striae at the base. Antenna (b, below) with the basal segment about 4 times as long as wide (Fig. 1 G). Anterior parapods with numerous pale claws, generally simple, but larger ones with accessory spikes near tips; posterior parapods with 16 dark brown claws, larger ones with 1 or 2 pointed spikes.

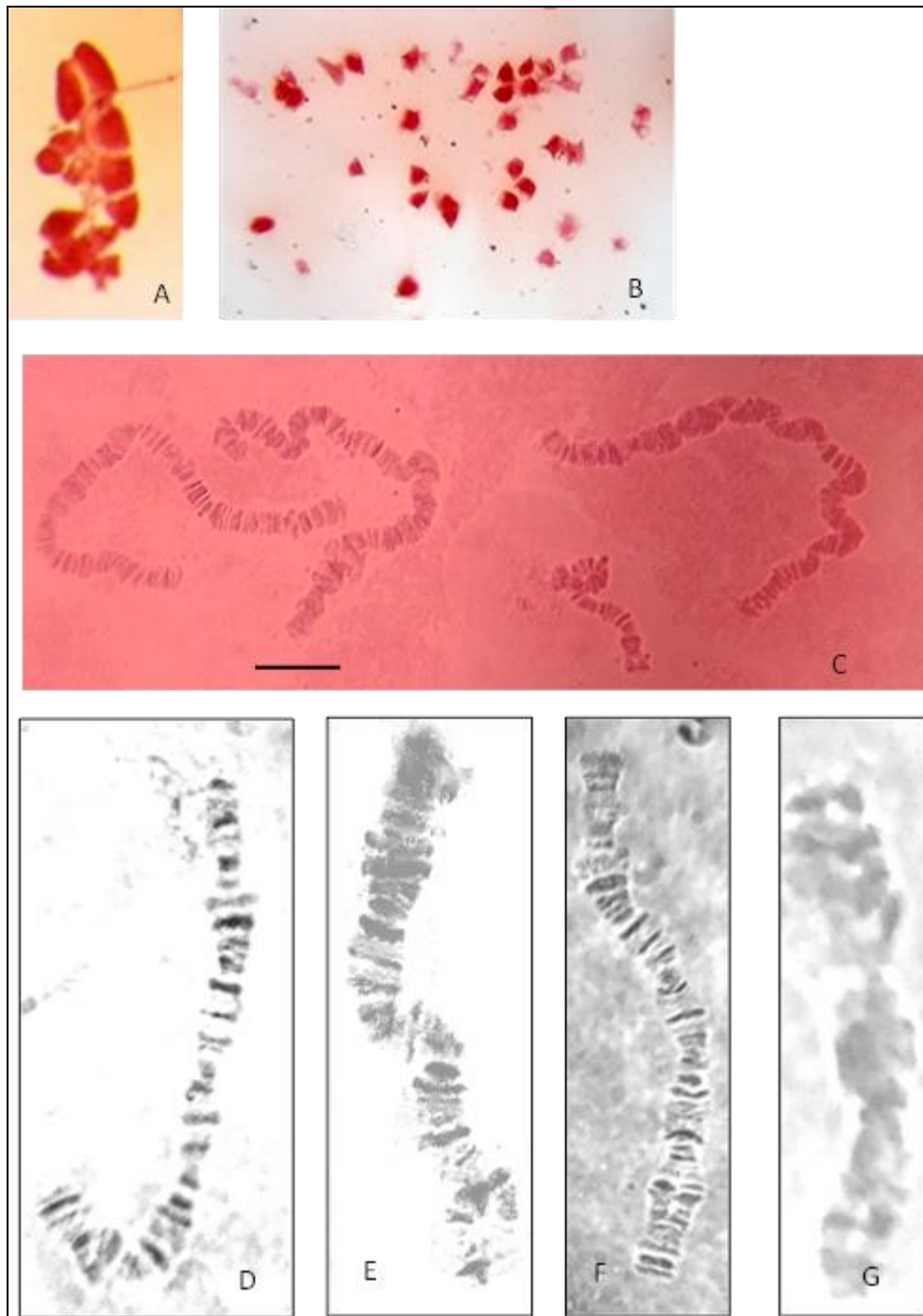
Abdominal segment VII with a pair of caudolateral processes extending up to the middle of segment VIII. Three pairs of ventral tubules present on abdominal segment VIII (Fig. 1 F): the anterior most is shortest while the middle one was the longest of the three, extending beyond caudal extremity of body, and the posterior pair coils ventrally. Anal tubules relatively large, longer than half the length of the posterior parapods (Fig. 1 C).

Pupa (n=12) 5.9 mm. Grey, frontal tubercles 0.10-0.11 long and 0.06-0.07 mm in diameter, subapical seta 0.09-0.10 long, i.e. about as long as the tubercles. Respiratory base about 0.11-0.14 wide. 2 pairs of precorneal setae. Abdomen with

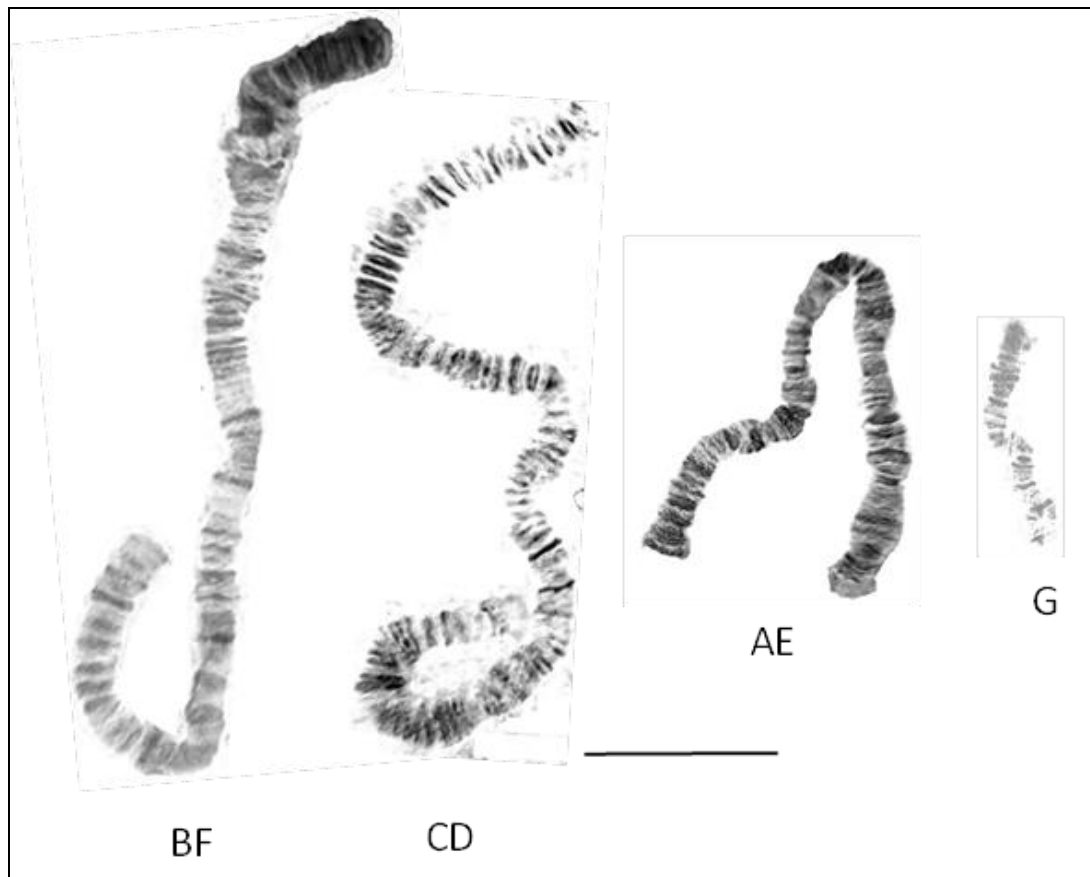
Pedes spurii A caudolateral on segments IV-VI, Pedes spurii B basolateral on segment I and caudolateral on segment II, which also bears a caudal row of about 69 hooks (Fig. 1 H).

### Cytology

The salivary polytene consisted of 4 free without any chromocenters and heavily banded (Fig. 2 C). G was with one nucleolus and with at least three Balbiani rings. There were transcriptional differences in the banding of in different plates (Fig. 2 D-G). The arm combinations was BF, CD, AE, and G at the moment (Fig. 3).



**Fig 2:** Salivary gland of *Chironomus javanus*, and the polytene chromosomes in the salivary nuclei. Salivary gland (A), well separated nuclei of the gland (B), polytene chromosome complement (C), the different transcriptional activity in G chromosomes (D-G). Bar represents 10  $\mu$ .



**Fig 2:** Karyotype of polytene chromosomes of *Chironomus javanus*, with pseudo thummi arm combination of BF, CD, AE, and G. Bar represents 10  $\mu$

### Discussion

This species, *Chironomus javanus* was first described by Kieffer [17] from Buitenzorg, Java; later Johannsen [18] described specimens from Lake Ranau, Sumatra. Specimens from various regions have subsequently been described by many authors, such as by Tokunaga [19] (1964) from a number of Micronesian Islands, Hashimoto *et al.* [20] from Thailand, Sasa and Hasegawa [21] from Okinawa, Hashimoto [22] from Shizuoka, Kikuchi *et al.* [23] (1985) from rice paddies of Tokushima, Hasegawa and Sasa [24] from Ryuku Island, Japan, Chaudhuri and Chattopadhyay [25] from rice paddies of West Bengal, India, Kikuchi and Sasa [26] from the Lake Toba area, Sumatra, Indonesia, and Sasa and Ogata [27] from the Kurobe municipal sewage treatment plant, Japan. The present study reports the species from field and drainage meaning the predominant preference is the field but could be found in the drainage that might be connected to field or flow down from field (as in our case).

The larva is most readily recognized by the unusual premandible, which has 6 (as illustrated by Chaudhuri *et al.* [28] for Indian specimens) or 7 (as illustrated below) teeth rather than the usual two. However, specimens have been described from Malaysia and Singapore (e.g. Karuankaran [29]) where the premandible has a normal premandible with only two teeth (Oriental *Chironomus* Species, web version, 1916, <http://www.chironomidae.net/Martin/SEACHironfile/OrientalChironomusv0319.pdf> and personal communications). The present study reinforced the special features of 7 teeth of this species. On the basis this feature Yamamoto [30] has suggested that this species should be in a separate subgenus as most of the species of the *Chironomus* has 2 teeth. The yellow greenish and spots on the wings of the adults also support this

suggestion. Much are to be learnt from this species.

The cytological features of the species are also very variable as the previous report was the thummi-cytoplex combination, AB, CD, EF, G. Subterminal nucleolus in arm G, with prominent BR about one third from the other end. Dr. Midya has an alternative species from India identified as *C. javanus* [14]. The present study reports is the pseudo thummi arm combination of BF, CD, AE and G. the shortest chromosome bears a big nucleolus which is quite different from previous reports. So it can be concluded that the chromosome translocation arms and NOR translocation also enhance the speciation in the genus of *Chironomus* as reported. To ascertain the exact chromosome combination further studies with different habitats and huge number of specimens are recommended for the future.

### Conclusion

The chironomids of Manipur are not fully explored and many are yet to be named and described. The morphological features as well as the cytology of the *C. javanus* of Manipur is quite different from the rest of the world and much is needed to study.

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