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Karyo-morphometric studies of *Chironomus circumdatus* from Manipur, India

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Abstract
The present investigation was conducted to study the karyo-morphometric characteristics of *Chironomus circumdatus* from Manipur during July 2016. The specimens of the *Chironomus* were collected from sewage drainage located along the road. The larvae were reared in the water from the site till the adults were collected and reared again for the eggs. The study was carried out using the eggs, larvae, pupal exuviae and adults. The confirmation was mainly done from the male adult fly after treating with KOH. The results showed that superior volsella was D type curved at tip in the species of *Chironomus circumdatus*. The polytene chromosomes were four (i.e. 2n=8) with the pseudothummi-cytocomplex combination AE, BF, CD, G. Nucleoli in arm B and C, with a small one also present sub-terminal in arm G. Not much variation could be observed in the specimens from Manipur both in morphology and polytene chromosomes from the rest of the world. Further studies of the *Chironomus* from Manipur will be much be an interesting regarding the organisms, distribution, evolutionary and adaptation.

Keywords: Manipur, *Chironomus circumdatus,* Northeast India, biodiversity hotspots

1. Introduction

Manipur is one of the tiny hilly states in the North-Eastern (NE) region of India. The state is one of the Indo-Burma Hotspots of the world [1]. A high endemism for including in the World hottest hotspots categories for this region might be due to the edge effect of the two continents: India and Euro-Asian plates that collided in the early Eocene epoch 50 to 55 million years ago [2, 3], besides other plausible reasons. Still many endemic species has been reporting from the region. Above all the invertebrates and fish are not included in the study for determining the hotspots. In order to fill some of the lacunae of biodiversity assessment of Manipur, the present studies have been taken up.

Chironomids or non-biting midges (Diptera: Chironomidae) are being utilising in the practical classes from undergraduate to post graduate studies in Manipur, but systematic studies had not been studied in the state so far. So an attempt is made to systematically arrange the chironomids for proper understanding of the polytene chromosomes and species specific polymorphism. From India 313 species in 59 genera under 4 subfamilies had been described [4] and only six species of the genus from eastern Himalaya of India. From Manipur the present study will be first of its kind in studying the through systematic exploration of the Chironomids.

Among the benthic macroinvertebrates, Chironomidae are noteworthy for their wide geographical distribution and abundance [5]. They occupy a wide range of ecological niches due to their physiological plasticity, and their role in nutrient cycling and energy flow in aquatic ecosystems [6]. The biological characteristics of chironomids enable their use in different types of studies, such as population dynamics [7], ecological interactions [7, 8], taxonomy [9], as indicators of environmental impact [10] and as test-organisms in toxicity bioassays [11].

Chironomus circumdatus was described by Kieffer [12] from Formosa (Taiwan), but redescribed by many people including Chaudhuri *et al*., [13], Kumar and Gupta [14]; Tripathi *et al*., [15] etc. The giant chromosomes, polytene chromosomes of Dipteran particularly the Chironomids are good for general studies as well as the research studies. They are usually utilised for species identification as well as bio indicator of the water pollution [10, 11]. The Morphometry is a widely used tool in the determination of insect instars [16]. The dimensions most commonly used to determine the number of larval instars is the width [17, 18] or the length of the head capsule from ventral view [19, 20].

From Manipur taxonomical studies on *Chironomus* are meagre and it is high time for their identification before too late through polytene chromosomes and morphology for species exploration of the fascinating Chironomid insect.

2. Materials and Methods

The larvae along with the sewage water were collected from front drainage of Canchi Indane (E 24^0 79'04.443", N 93^0 91'57.197") near Regular High School, Tiddim Ground, Airport Road on the 29th July, 2016. The sewage drain was somewhat clean with sandy bottom. The pupa exuviae were first seen and while scooping them many larvae were collected. They were reared at room temperature roughly 34^0 C, some of the adults came out on 3rd August and laid eggs on 7th August, 2016.

2.1 Morphological studies

Larvae: The larvae were fixed in the 1:3 glacial acidic acid and methanol by volume for 24 hours and transferred to 70% ethanol for further storage and for fresh preparation 70% ethanol treatment were 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 the larval identification [21].

2.2 Adults: 1.5 ml of phenol + 1.5 of absolute alcohol were mixed and the adults were dipped into it and wait for 2 hours at room temperature. The individual was transfer to a clean slide from the phenol dehydrated alcohol and respective organs like male genitalia, plumose antennae etc. were dissected and before all these the wings were spread to get a beautiful of well spread wings. Finally put DPX and mount with a slight light pressing (courtesy to Niladri Hazra hod@zoo.buruniv.ac. personal communication).

2.3 Cytological studies: The polytene chromosome preparations were done according to [22] 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.

The identification of the larvae, pupal exuviae and adults was done according to Martin [22, 23] (oriental *chironomus* species, web version, *Chironomus circumdatus* Kieffer, 1916 and personal communications).

3. Results

3.1 Eggs: They were laid in a spiral mass embedded in gelatinous cylinder attached to the bottom of the beaker of about 1200 eggs (Fig. 1 A). The eggs were oval and red in colour (Fig. 1B).

3.2 Larvae: Larvae were medium *plumosus* type with the average length of 12.25 mm from 10 IV instar larvae (Fig. 1 C). 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 (Fig. 1 G), ventromental plate separated medially by at least the width of the median teeth of the mentum (Fig. 1 H), pectin epipharyngis with exactly 15 teeth (Fig.1 I), premandible is with inner tooth about twice the width of the outer (Fig. 1 J), 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. Antenna with basal segment is 2 times as long (35 micron) as wide (17 micron); A2/A1 is about 0.42; A4/A3 is about 1.6, AR is about 2.11. Distance between antennal bases was greater than that of the between the S4 setae (Fig. 1F).

3.3 Pupal exuviae: Exuviate brown in colour (Fig.2 A), Frontal tubercle with a subapical seta (Fig.2 B), Caudolateral spur with 2 spines (Fig. C) as against 2 to 4 spines (Martin, 2016). Body about 6.6 - 7.7 mm (male) and 6.5 - 7.6 mm (female). The thoracic horn is prominent (Fig, 2 D) and the anal fringe (Fig. 2 E).

3.4 Adults-Male: AR about 3.12 - 3.8. Frontal tubercles about 25 - 43 µm long, 10 - 17 µm wide. Palpal proportions (µm) 56: 54: 213:218: 334. Clypeal setae - 17 - 34. Thorax greenish, scutal stripes conspicuous with dark brown margins; scutellum pale yellow, postnotum dark brown. Thoracic setae: acrostichals - 13 - 18; dorsocentrals - 18 - 27; prealar - 5 - 6; scutellar - 8 - 14 in anterior row, 13 - 26 in posterior row (higher numbers have an intermediate row of 11 - 12 setae). Wing length: 2.72 - 3.04 mm; wing width 0.67 - 0.74 mm. VR about 1.02 - 1.05. Wings without darkening of the cross vein (Fig. 3 A to D). 25 -27 setae in squamal fringe. Legs pale, with darkening at distal ends, also on distal half of Ti4 and all of Ta5. Abdominal segments pale, but with increasing central dark oval patch, so that tergites V -VIII are virtually all dark. Anal point narrow; 1-16 setae on tergite IX. Superior Volsella of the D-type (Fig. 3E). Basal setae on IV was ramose.

Table 1: The comparative studies on male legs of Imphal (present paper) with the data from Martin (2016).

	Imphal	Martin	Imphal	Martin	Imphal	Martin	Imphal	Martin	Imphal	Martin	Imphal	Martin	Imphal	Martin
	Fe	Fe	Ti	Ti	Ta1	Ta1	Ta2	Ta2	Ta3	Ta3	Ta4	Ta4	Ta5	Ta5
PI	1130	1145	1010	1060	1640	1610	850	835	740	745	630	645	330	325
PII	1250	1230	1110	1110	690	705	390	395	280	295	170	165	140	135
PIII	1080	1350	1230	1350	990	1000	540	560	430	475	260	250	160	170

3.5 Female

Wing length 2.8 mm. Antennal proportions (µm): 80: 190: 120: 120: 130: 280. Frontal tubercle short and stout, 24 µm long and 17 µm wide. Palpal proportions (segs. 2-5) (mm): 60: 240: 250: 540 (Fig. 3 G to J).

3.6 Cytology: There were four prominent well stained polytene chromosomes in *Chironomus circumdatus* in Manipur as reported confirming 2n=8. The polytene chromosome were 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 was having two prominent Balbiani rings (br) (Fig. 4) [24].

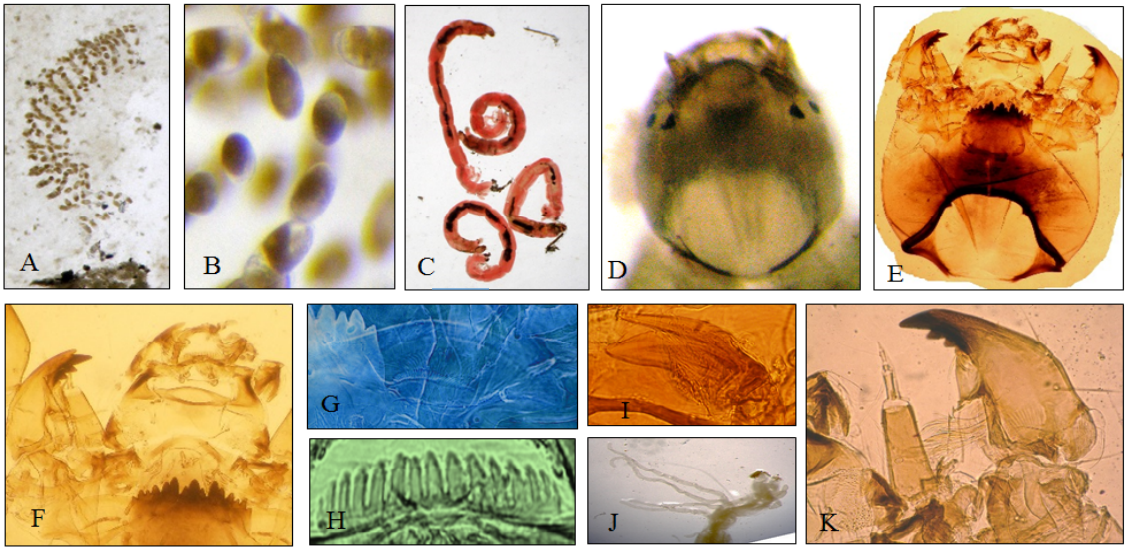

Fig 1: The life stages from eggs to IV instar larva of *Chironomus circumdatus* and morphometric features of immature larval: spiral mass of eggs (A), 10 individual eggs (B), IV instar larvae (C), dorsal view of IV instar larval head with three coloured strands and darkening anterior (D), ventral view of D (E), ventral view of the larvae mantum and other parts of IV instar larva (F), Ventromentum plate (G), Pecten epipharyngis with 15 teeth (H), premandible (I), ventral tubules in which posterior pair longer than the anterior pair (J), and antenna and mandible K). Bar represent 10 micronmeter.

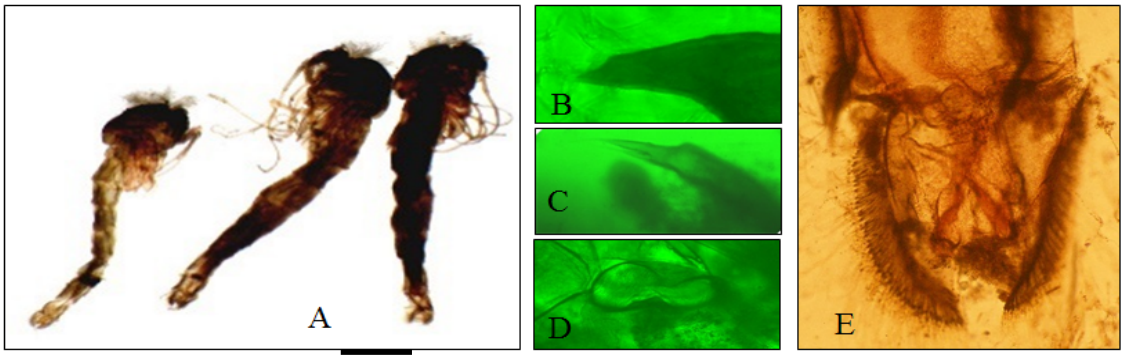

Fig 2: Pupal exuviate of *Chironomus circumdatus* and morphometric features of exuviate (A), Frontal tubercle in lateral view (B), Caudolateral spur (C), Thoracic horn (D) and Ventral view of anal fringe of exuviate (E). Bar represent 10 micronmeter.

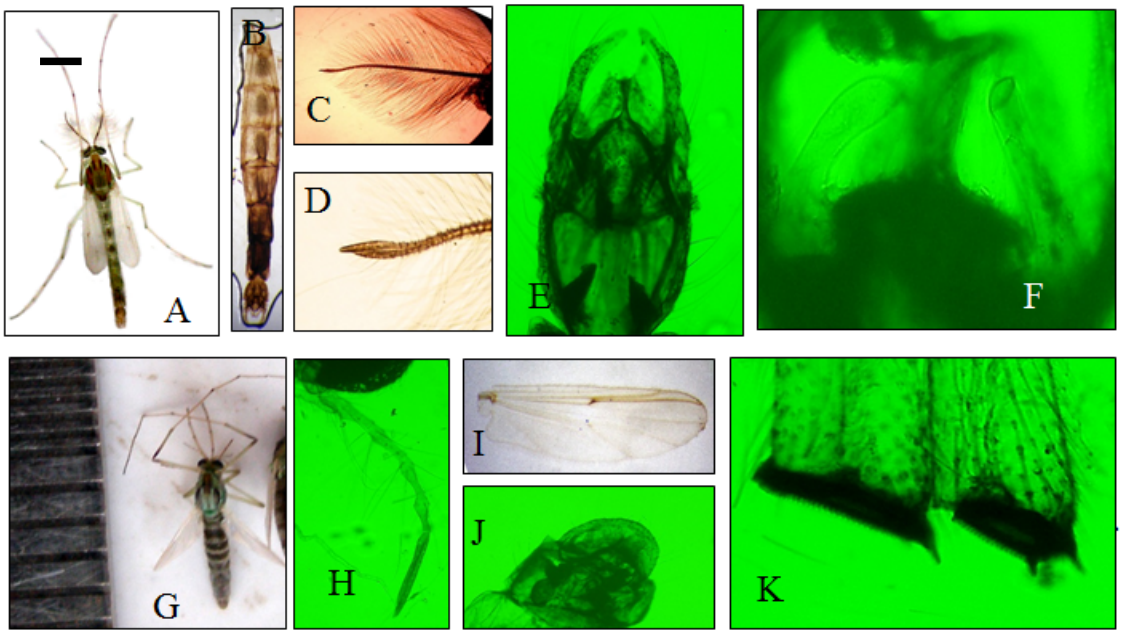

Fig 3: Adult *Chironomus circumdatus* and their correspondinfg features: male (A), dorsal view of adult (B), male antenna (C), apical view of male antenna (D), Ventral view apical end of male adult (E), superior volsella of male (F), fel\male adult (G), antenna of female (H), wing of female (I), female anal point (J), lateral view of tibial comb and tibial spur (K). Bar represent 10 micronmeter.

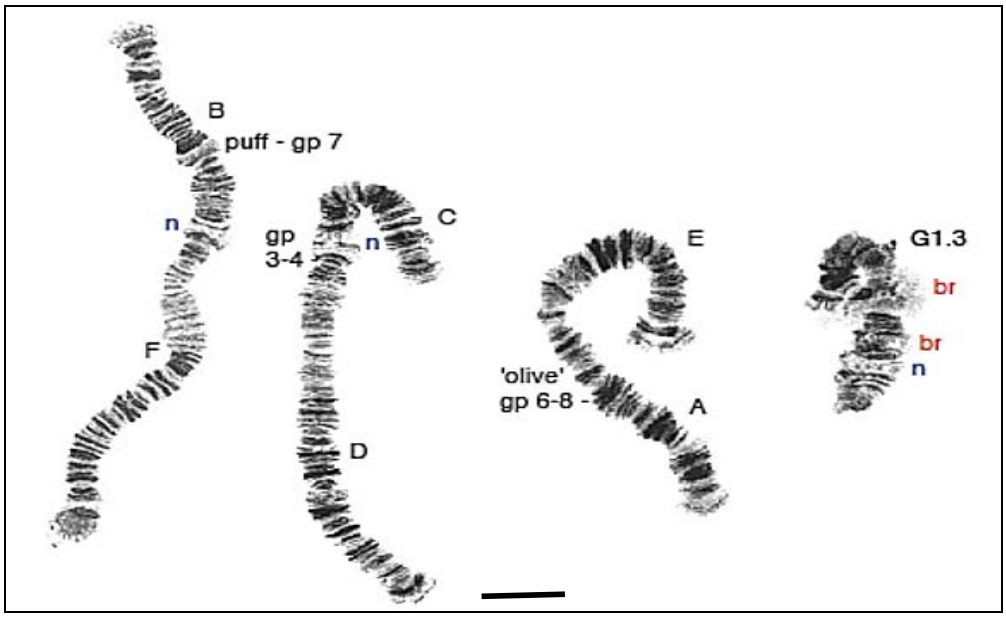

Fig 4: The four polytene chromosomes of *Chironomus circumdatus.* The typical pseudothummi -cytocomplex combination of BF, CD, AE, G. The first, second and shortest chromosomes are having nucleoli (n). The shortest chromosome is having two prominent Balbiani rings (br) (courtesy to Jon Martin). Bar represent 10 micronmeter.

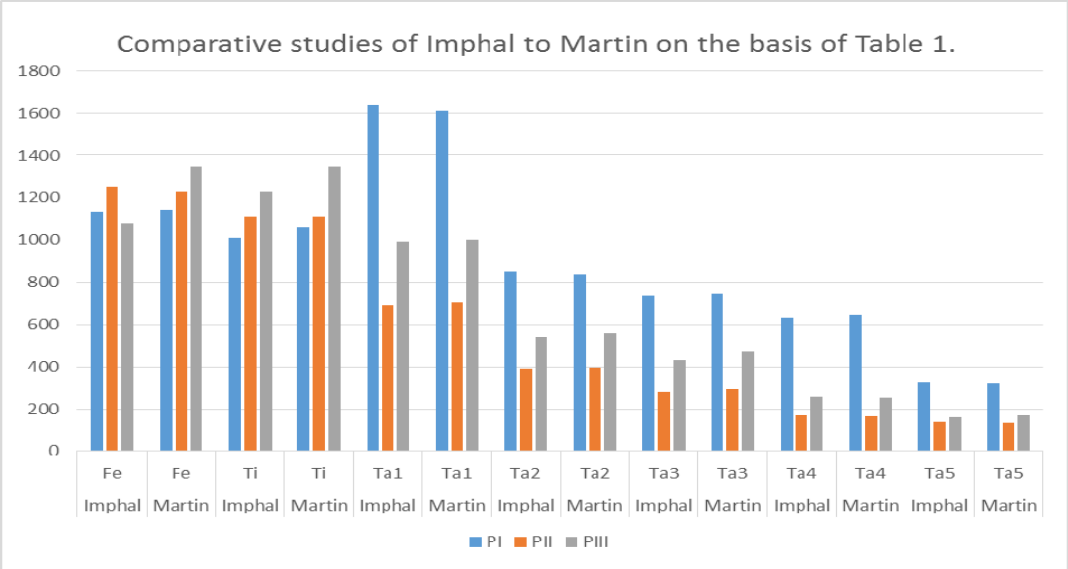

Fig 4: The bar graph displaying the comparison between the present material (Imphal) and Martin regarding the legs-PI, PII and PIII of male individuals.

4. Discussion

Chironomids are the most widely distributed and frequently one of the most abundant insect in freshwater. Chironomids species can tolerate and develop in polluted waters such as waste stabilization ponds where they become a dominant macro-invertebrate [25].

The studies of chironomids in the fields of genetics, cytogenetics, other perspective of the insects as food for the fish and amphibian [26] for their highly nutritive protein, fats, carbohydrates and bioactive substances and as bio-indicators of the depredating habitats or pollution [27]. Some reports confirmed that they are scavengers of toxic substances so not advisable for the human consumption [28]. Chironomid larvae (the life history stage that is exposed to aquatic contaminants) have giant (polytene) salivary gland chromosomes with a well-characterized band structure that permits precise cytogenetic analysis and a clear definition of cytogenetic damage [29]. *Chironomus circumdatus* was described by Kieffer (1916) [5] from Formosa (Taiwan). The present karyotype is in accordance with Kumar and Gupta [14]; Tripathi *et al*., [15], Pramual *et al*. [30] being the pseudothummi cytocomplex with AE CD BF G karyotype. The species is thoroughly studied cytologically by Kumar and Gupta [7]. They reported altogether seven inversions comprising six paracentric and one pericentric are detected in the Indian populations. Suggesting that the paracentric inversion is harmless as reported by White [31]. Some of the deviations of present study are highlighted in the Fig. 3, Pramual *et al*. [30] reported three Balbiani rings on G chromosomes and nucleolus is at far end of the chromosome, but in the present study the nucleolus is very close to the two Balbiani rings. All these deviations might be speculated to pericentric inversion. So in future studies on chromosomes polymorphism gathered from many specimens from more study sites are

recommended. The present study shows that *C. circumdatus* is one of the *Chironomus* species in Manipur inhabiting in fairly uncontaminated aquatic medium.

Chironomus circumdatus has been found in Korea, Japan, Thailand, India and Micronesia [32]. Morphologically the species are well described by Martin [23, 24] and present studies is also confirmed to be *Chironomus circumdatus* from studies of eggs, larvae, pupal exuviae and male and female adult flies. One very minor deviation from the reported so far is only smaller in size as shown in Fig. 4 as comparison to Martin's data [24]. The deviation might be due data handling and measurements or existing in grown level is yet to be ascertained. In future study huge number of specimens, incorporating the diverse study sites and compared with the different genera and species will yield much plausible results.

5. Conclusion

The chironomids of Manipur are not fully explored and many are yet to be named and described. *Chironomus circumdatus* (Kieffer, 1916) is cosmopolitan and no differences could be found in Manipur species in both morphology and polytene chromosomes from the rest of the world. The morphology and larval features are quite agreed with the previously identified features. The species has four polytene chromosomes (2n=8). The polytene chromosomes are pseudothummi cytocomplex type with quite easily distinguishable arms. In future study huge number of specimens, incorporating the diverse study sites and compared with the different genera and species will yield much plausible results.

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