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POLYTENE CHROMOSOMES ARRANGEMENT OF *CHIRONOMUS* AND *POLYPEDILUM* MIDGE LARVAE

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ABSTRACT

Chironomus and *Polypedilum* are among important genera in Chironomidae family. Both genera are abundance and very diverse in aquatic ecosystem. *Chironomus* and *Polypedilum* are dominant and frequently found during the sampling processes. The objectives of this study were to establish the standard polytene chromosome arrangement of *Chironomus* and *Polypedilum* and to distinguish variable polytene chromosome arrangement of both genera. This study was based on cytogenetic approach which revealed the species-specific characteristics in each polytene chromosome arms. The results also showed that polytene chromosomes of *Chironomus* and *Polypedilum* have some similarities and differences in polytene chromosome arms. Both genera have four polytene chromosomes which comprise of three long combined arms and one short arm. There were also several distinct forms of polytene chromosome observed from both genera. The Nuclear Organizing Region (NOR) structure was found in chromosome I of *Chironomus* while BR structure was found in chromosome I of *Polypedilum*. There is a

rearrangement pattern found in chromosome II and two BR structures in chromosome IV of *Chironomus* while two large puffing forms found in chromosome III and BR structure, rearrangement pattern and NOR found in chromosome IV of *Polypedilum*. Cytotaxonomic study revealed the cryptic structure of polytene chromosomes arrangement has provided important source of indentificating the genus or spesies name. This study indicates that chromosome arrangement could aid in revealing *Chironomus* and *Polypedilum* diversity.

Keywords: *Chironomus*, *Polypedilum*, polytene, chromosomes, Balbiani ring

ABSTRAK

Chironomus dan *Polypedilum* merupakan antara genera terpenting dalam Chironomidae famili. Genera ini mempunyai kelimpahan dan kepelbagaian yang sangat tinggi dalam ekosistem akuatik serta kerap dijumpai semasa proses persampelan dijalankan. Objektif untuk kajian ini adalah untuk menghasilkan susunan piawai kromosom politen ke atas genera ini dan membuat perbezaan variasi susunan kromosom politen untuk kedua-dua genera. Hasil menunjukkan susunan kromosom politen *Chironomus* dan *Polypedilum* mempunyai beberapa persamaan dan kelainan. Kedua-dua genera ini mempunyai empat lengan kromosom politen iaitu melibatkan tiga lengan panjang dan satu lengan pendek. Perbezaan antara genera ini ialah ciri jelas iaitu struktur NOR bagi genus *Chironomus* kerap dijumpai pada kromosom I manakala struktur BR pula lebih kerap dijumpai pada kromosom I bagi genus *Polypedilum*. Susunan piawai *Chironomus* menunjukkan terdapat satu struktur penyusunan semula dijumpai pada kromosom II dan dua struktur BR dijumpai pada kromosom IV manakala susunan piawai *Polypedilum* menujukkan terdapat dua struktur gelembung besar pada kromosom III dan struktur BR, struktur penyusunan semula serta NOR dijumpai pada kromosom IV. Kajian Sitotaksonomi ini telah mendedahkan struktur kriptik dan aneh di dalam susunan kromosom politen di mana ia menjadi sumber penting dalam mengenalpasti nama genus atau spesies. Kajian ini juga menunjukkan

susunan kromosom politen dapat membantu dalam pendedahan kepelbagaian *Chironomus* dan *Polypedilum*.

Kata kunci: *Chironomus*, *Polypedilum*, kromosom, politen, cincin Balbiani.

INTRODUCTION

Chironomidae, also called chironomids are holometobolous insect which undergoing a complete metamorphosis. There are four stages of chironomids' life cycle. They are involving eggs, larvae, pupae and adult stages. The habitat of three lower stages of chironomids is in bentic of aquatic ecosystem while adults have ability to fly and survive in terrestrial. Chironomids are widely disperse and abundance in benthic communities. Chironomids are important food sources to larger predatory invertebrates (Benke et al. 2001). *Chironomus* and *Polypedilum* are well known genera of Chironomidae family. However, many species of chironomids are morphologically indistinguishable at the larval stage (Michailova 1989). This study revealed the species-specific characteristics in each polytene chromosome arms. It also was applied for easier identification and classification of chironomids. This study is limited and less implemented and conducted in Malaysia. This approach was applied for easier identification and classification of chironomid. However, this approach is limited and less implemented in Malaysia.

Polytene chromosomes are present in several dipteran tissues, including the salivary glands, malpighian tubules, and the epithelium of the midgut and hindgut (Staiber & Benke 1985; Michailova 1989). There are several distinct characteristics which aid in classifying the group of chromosome arrangement. They are nucleolar organizing region (NOR), Balbiani rings (BR), formation of chromosome rearrangement, puffing of transcription process and the position of centromere. The centromere structures are difficult to appear if the banding patterns are non-heterochromatic. The characteristics of chromosome arrangement of each individual were compared to the cluster and form groups in *Chironomus* classification.

MATERIALS AND METHODS

Samples collection and Culture

Chironomids were collected from the sampling sites along Sg. Langat. The collected mass eggs and larvae were cultured in the laboratory. The larvae were cultured until the fourth instar stage. Sampling procedures were done along the the downstream of the river. Midge larvae were cultured in aquarium of 9 cm x 6 cm size.the mass egg samples were cultured separately from other samples to avoid mixing of samples.

Identification of Chironomids

Midge larvae were identified morphologically up to the genus level. The identification of larvae was done by referring to the key of Wiederholm (1983) based on characters of the head capsule.

Dissection of Chironomids

Dissection of salivary gland was done on fourth instar of midge larvae of *Chironomus* under the microscope. Method of dissecting the midge larvae was referring to the method used by Saxena et al. (1985). A few drops of 45% acetic acid were put to kill the larvae and to avoid the samples from drying and damage. Then, the head capsule was cut and salivary gland was gently removed from the body. A total of 6-10 drops of aceto-orcein were applied onto the salivary gland for staining process. Then, the salivary glands were squashed gently by thumb finger, and the slide was left 30 minutes for the absorption process. The polytene chromosomes were observed by using compound microscope, Olympus BX41 and the images were captured at 400X magnification using camera ClorView8, Olympus U-CMAD3.

Mapping of polytene chromosomes

Mapping of polytene chromosomes was done and referred to the previous studies on chironomids (Devai et al. 1989; Porter & Martin 2011; Michailova 2010; Saxena et al. 1985). The arms of polytene chromosomes were drawn by using the tablet pen and the positions of specific characteristics were mapped. The arms of polytene

chromosomes were labeled as A, B, C, D, E, F and G. The whole polytene chromosomes were divided into several major divisions and three sub-divisions (a-c) (Saxena et al. 1985).

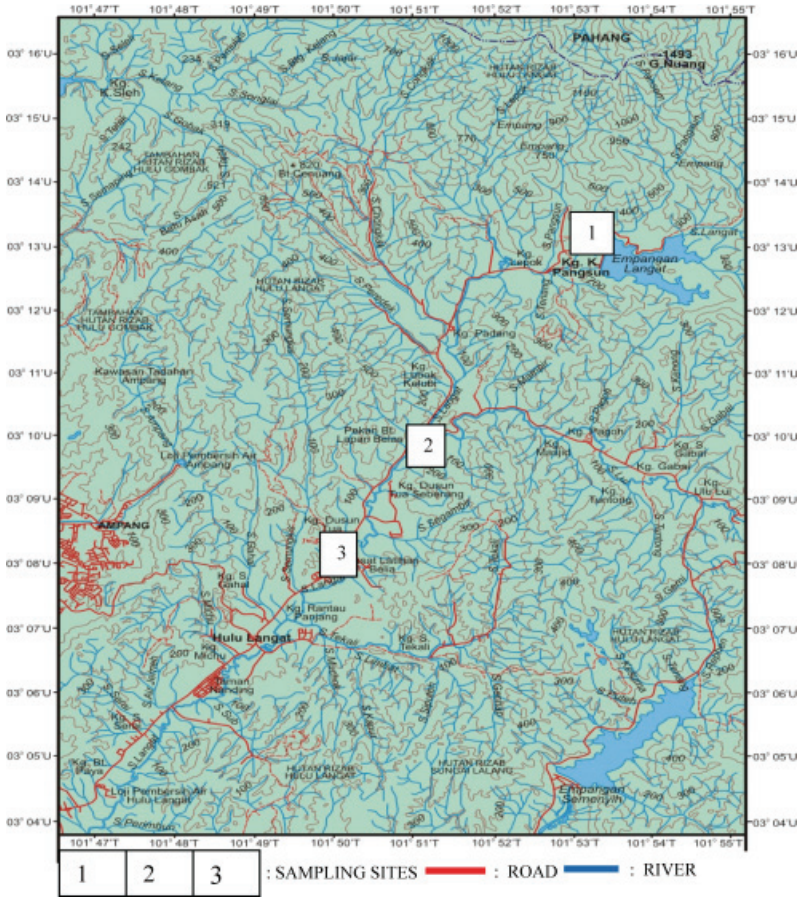

Fig. 1. Map and sampling sites along Sg. Langat, Selangor.

RESULTS AND DISCUSSION

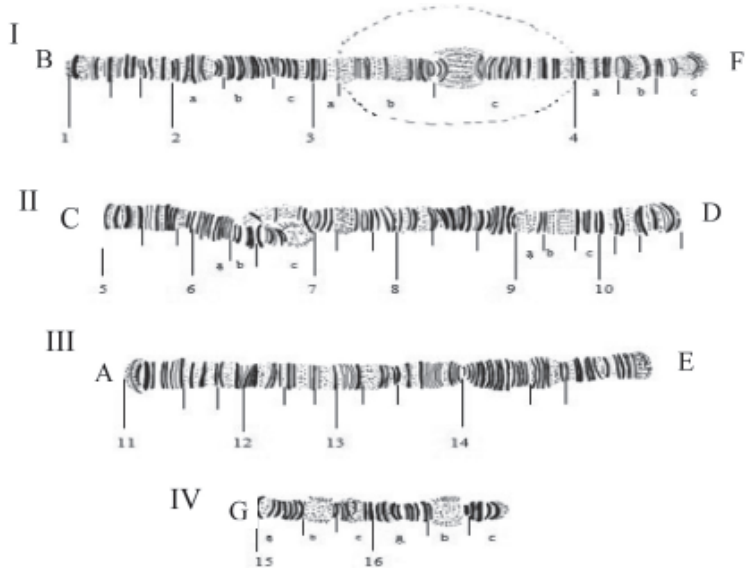

Fig. 2. Standard polytene chromosome arrangement of *Chironomus*

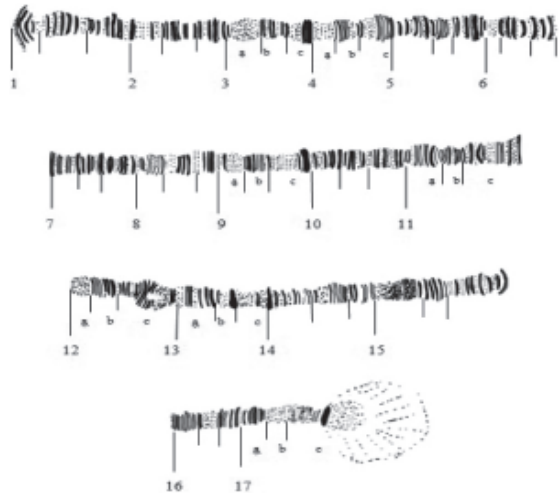

Fig. 3. Arrangement of polytene chromosome of *Chironomus* - Group 1

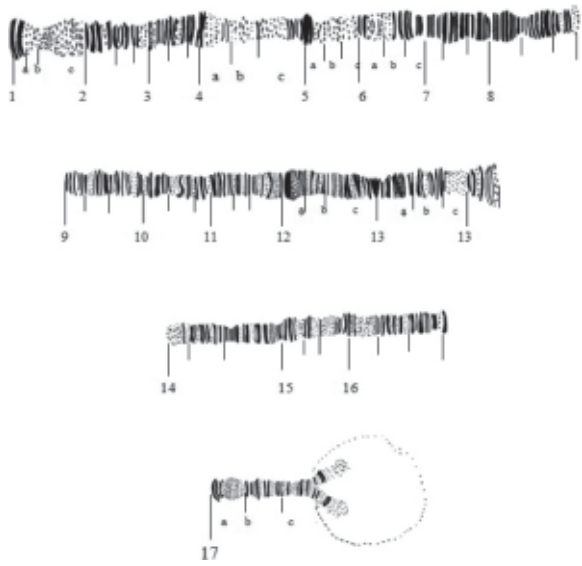

Fig. 4. Arrangement of polytene chromosome of *Chironomus* - Group 2

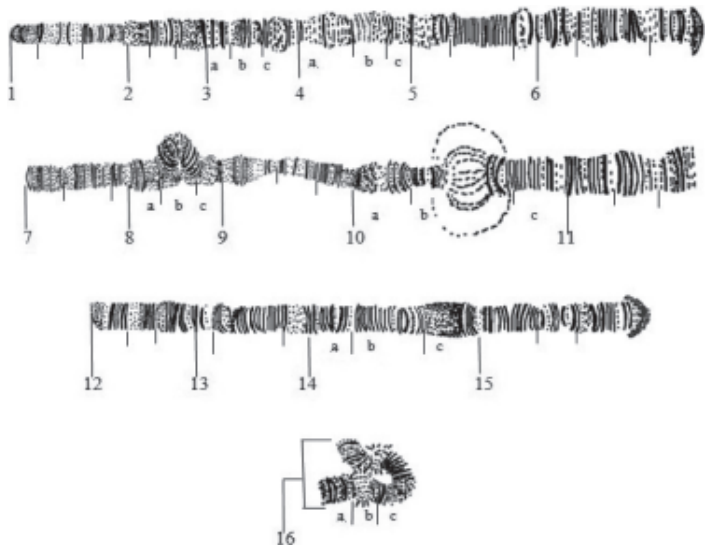

Fig. 5. Arrangement of polytene chromosome of *Chironomus* - Group 3

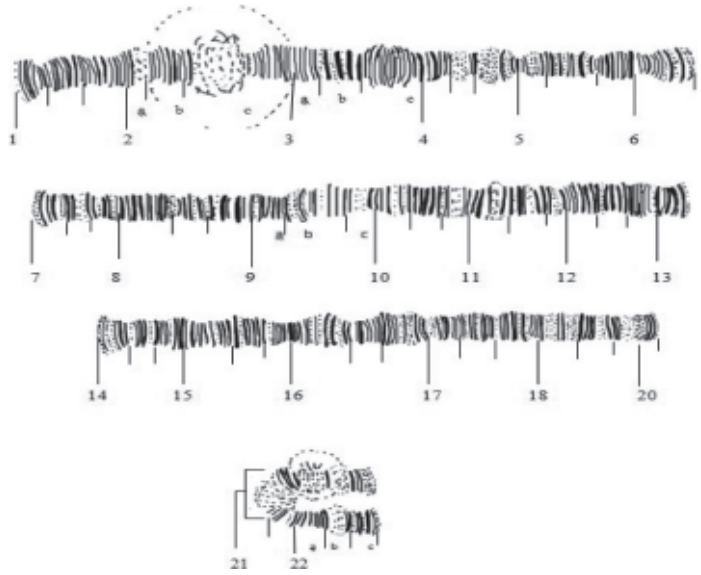

Fig. 6. Arrangement of polytene chromosome of *Chironomus* - Group 4

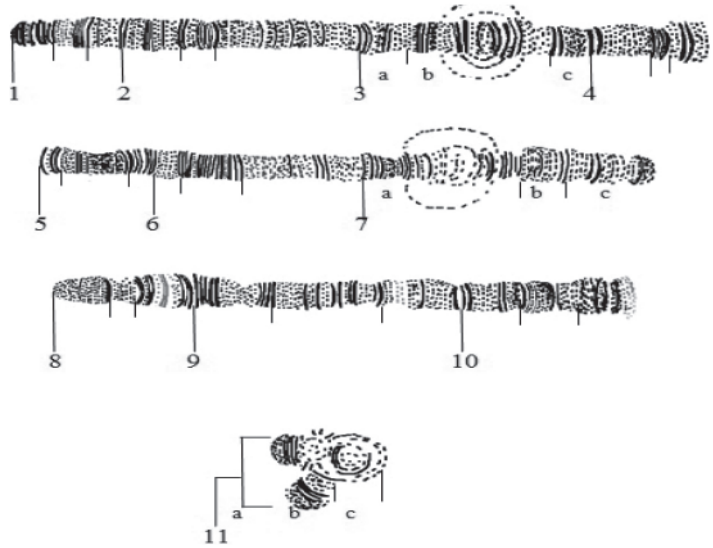

Fig. 7. Arrangement of polytene chromosome of *Chironomus* - Group 5

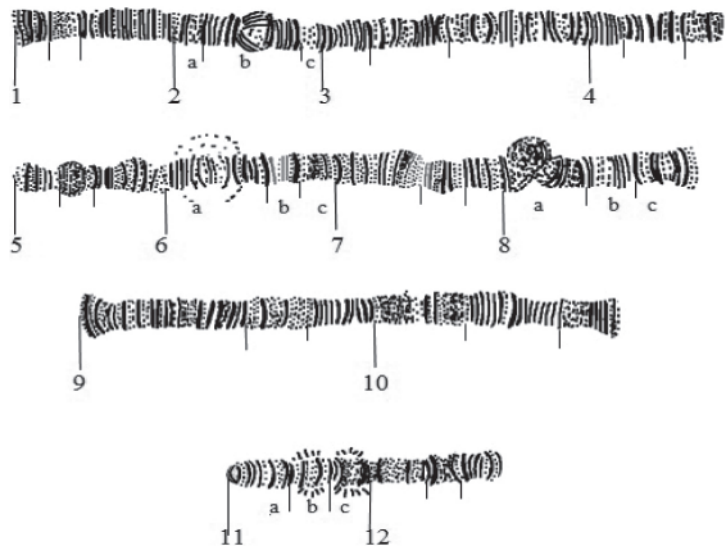

Fig. 8. Arrangement of polytene chromosome of *Chironomus* - Group 6

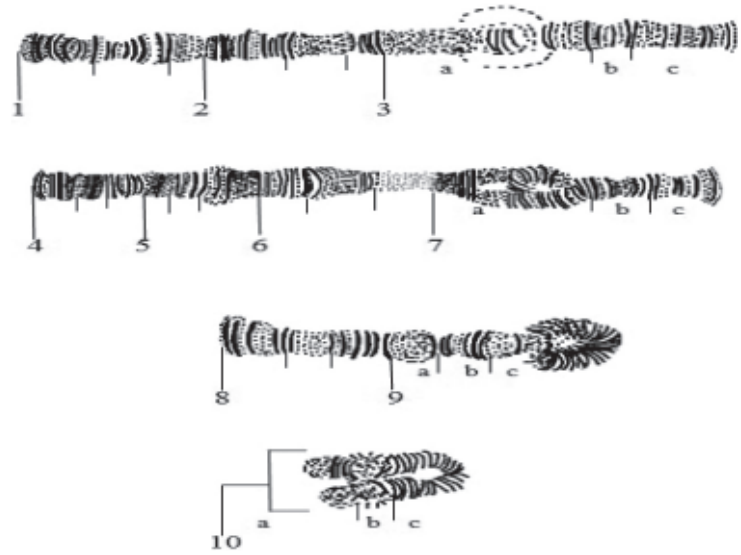

Fig. 9. Arrangement of polytene chromosome of *Chironomus* - Group 7

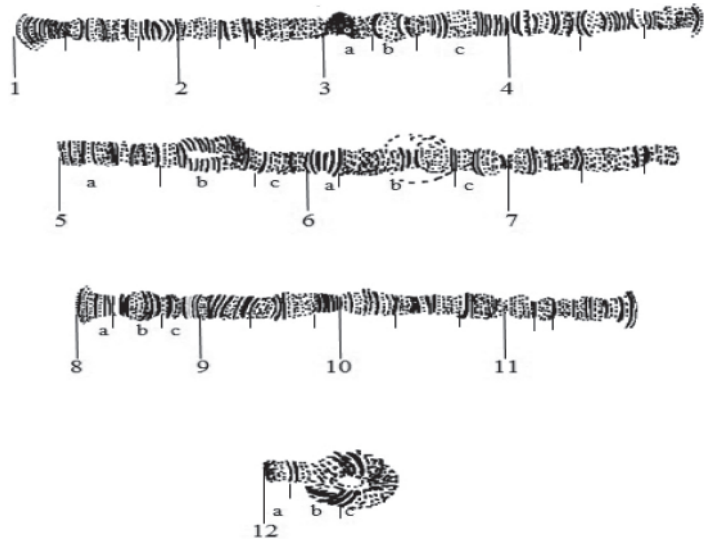

Fig. 10. Arrangement of polytene chromosome of *Chironomus* - Group 8

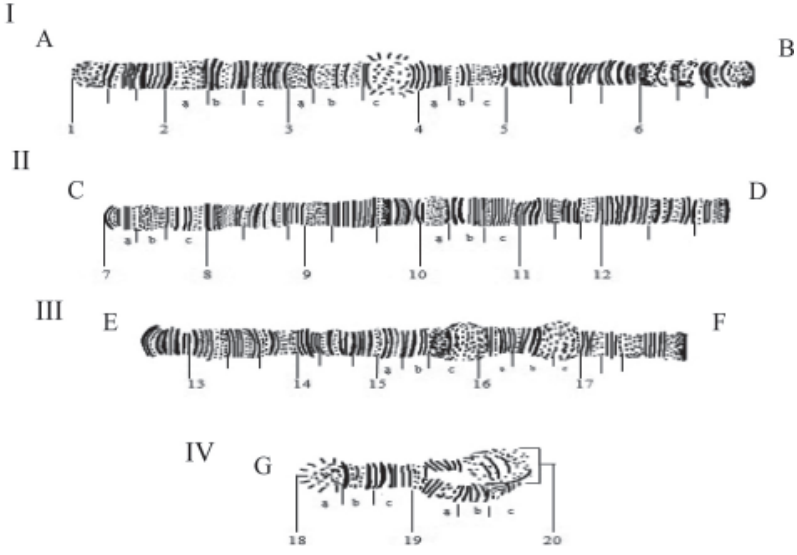

Fig. 11. Standard polytene chromosome arrangement of *Polypedilum*

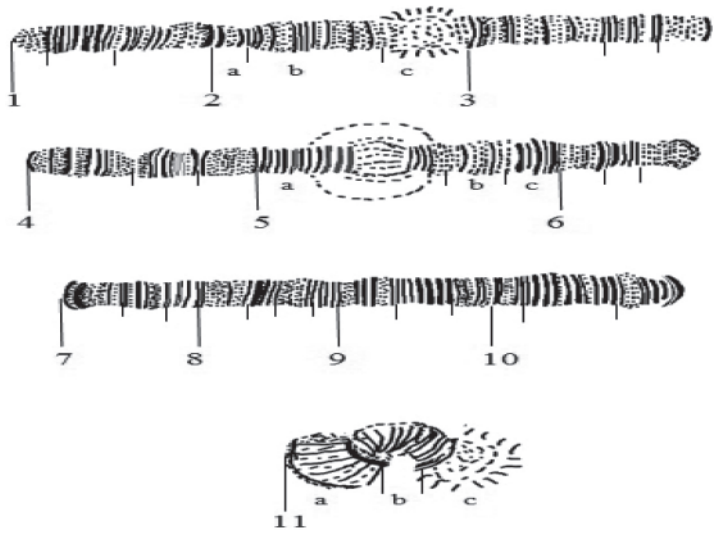

Fig. 12. Arrangement of polytene chromosome of *Polypedilum* - Group 1

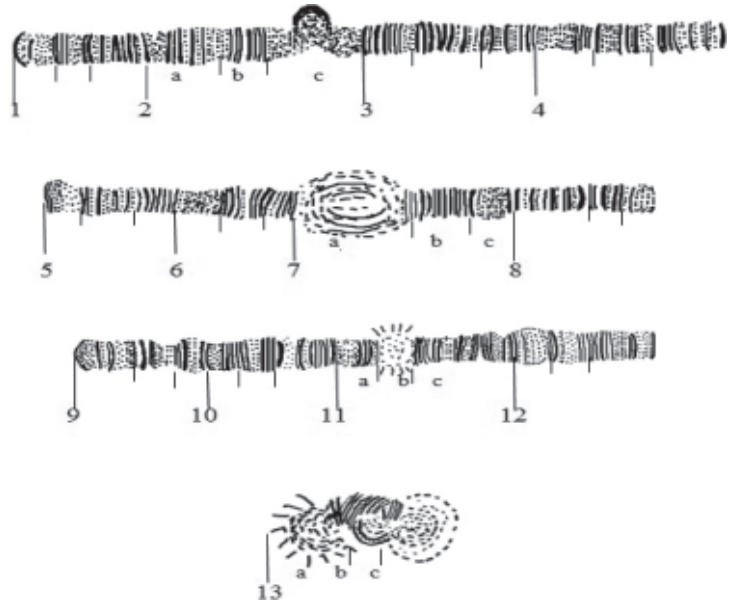

Fig. 13. Arrangement of polytene chromosome of *Polypedilum* - Group 2

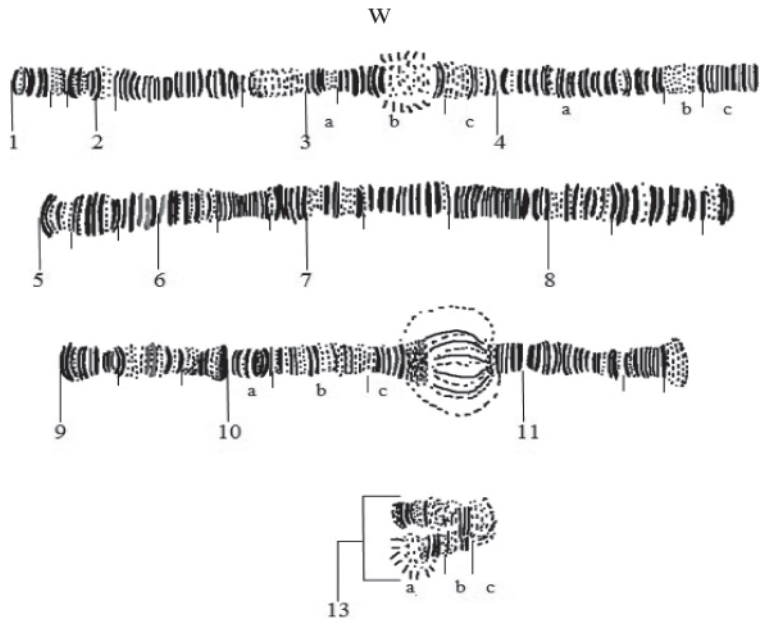

Fig. 14. Arrangement of polytene chromosome of *Polypedilum* - Group 3

All collected samples were cultured until the emergence of fourth instar stage of larvae. The size of the larvae at this stage is suitable to be dissected because the salivary gland can be removed easily. The polytene chromosome patterns were drawn by using tablet pen. Hence, the polytene chromosome structures and arrangement can be mapped easily. Since the cytotaxonomic study was applied, the identification of different genera has strongly supported the morphology. Cytotaxonomic study or cytogenetics study revealed the cryptic structure of polytene chromosomes arrangement and provided important source in classifying the genus or spesies name. The specific characteristics of polytene chromosomes in the salivary gland have promising in taxonomic diagnosis of chironomid species (Michailova 2001).

The large NOR structures indicate the process of transcription that responsible in synthesis of preribosomal RNA. NOR also known as large puff structure can be observed clearly and it is located inside

the nucleolus. NOR also responsible to give some informations in taxonomic classification (Martin 2010, Michailova 1989). There is another distinct structure in chromosome arm and it is frequently can be found which is known as BR. BR structure is a large chromosome puff that located at the shortest chromosome arm or chromosome IV. Both genera have the same total number of four polytene chromosomes. They are involving three long chromosome combined arms and one short arm. Chromosome I and II are called metacentric while chromosome III and IV are known as submetacentric and acrocentric chromosome respectively.

Fig. 2 and Fig. 11. show the standard maps of polytene chromosome arrangement in *Chironomus* and *Polypedilum* respectively. There are common and distinct characteristics are NOR, BR, puffing and rearrangement patterns in both polytene chromosome arms for both genera. The standard mappings of polytene chromosome arrangement were produced because these patterns of arrangement are frequently can be found in many individuals from *Chironomus* and *Polypedilum*. However, these characteristics and patterns of polytene chromosomes arms arrangement from several other individuals were found to have differently arranged and located at different position compared to the standard patterns. Thus, the individuals that have the differences of polytene chromosome arrangement patterns were placed in different group from the standard arrangement.

There are total of eight groups of different polytene chromosome arrangements were identified to have different arrangement from the standard. Figs 3 - 10 are the different patterns of polytene chromosome arrangement for *Chironomus* while Figs. 12 - 14 are the different patterns of polytene chromosome arrangement for *Polypedilum* were mapped. The differences were compared from the standard map according to atleast one different pattern and distinct characteristic that located at different position. The common structure such as NOR is frequently found in the longest chromosome arm of *Chironomus,* the rearrangement pattern usually appear in chromosome II and BR structures commonly located at chromosome IV. However, in the other groups, these structures were arranged at different positions. The same way in differentiating the polytene chromosome arrangement of *Polypedilum* was taken. BR structure

is the most common structure found in the longest arm of chromosome I, large puffing structures can be found in chromosome III and a rearrangement pattern structure usually appears in the shortest arm of *Polypedilum*.

Asian *Chironomus* usually have the combination of *pseudothummi* arrangement of polytene chromosomes. *Pseudothummi* is the combination of polytene chromosomes arms formed when the pairing and endomitosis process occurred. The combination of the arms are Chromosome I, II, III, and IV = BF, CD, AE, and G respectively. However, combination of polytene chromosome arms in *Polypedilum* is not specifically proved. By referring to Michailova (1989), combination of polytene chromosome arms has the arrangement of AB, CD, EF, and G. Besides, the polytene chromosomes of both genera are highly heterochromatic. However, the centromere form is invisible and cannot be observed.

There are several comparisons according to the similarities and differences of the polytene chromosome arrangement of *Chironomus* and *Polypedilum* in each standard map. The longest polytene chromosome arm of *Chironomus* has a dictinct character of NOR at unit 3c while chromosome I of *Polypedilum* have a distinct character of BR at unit 3c. The structure of NOR is commonly found in each indivual of Chironomus sample as well as BR structure is commonly found in each individual of Polypedilum of this chromosome. Chromosome II of *Chironomus* has a distinct character of chromosome rearrangement at unit 6c but there is no distinct character in chromosome II of *Polypedilum*. However, there is no distinct character appear in chromosome III of *Chironomus* but, two large forms of puffing in chromosome III of *Polypedilum* can be observed. Lastly, the fourth chromosome of both genera has their own distinct characteristics and forms. *Chromosome* IV of *Chironomus* has two forms of BR at unit 15b and 16b while chromosome IV of *Polypedilum* has BR structure at unit 18a, a rearrangement pattern at unit 19a, 19b and 19 c, and there is a NOR structure appear at end of unit 20. These structures contribute to the folded form of chromosome IV in *Polypedilum*. The other groups can be considered to have different characteristics and they maybe come from the different kind of species. If further researches are taken place, the investigation of the species can be analysed.

CONCLUSION

Cytotaxonomic study revealed that cryptic structure of polytene chromosomes arrangement has provided important source of identification of the genus or spesies. The standard maps of *Chironomus* and *Polypedilum* polytene chromosom were established. Varieties of polytene chromosome of *Chironomus* and *Polypedilum* were successfully established. Different groups of both genera were distinguished according to the mapping of polytene chromosome arrangement in cytogenetics study. This study also indicates that chromosome arrangement could aid in revealing *Chironomus* and *Polypedilum* diversity.

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