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Structural Maps of the Polytene Chromosomes of the Fruit Flies *Bactrocera zonata* and *Zeugodacus tau* (Diptera: Tephritidae) †

Farzana Yesmin [1,*], Md Nazim Uddin [1,2] and Md. Hasanuzzaman [1]

[1] Cytology and Biocontrol Research (CBR), Institute of Food and Radiation Biology (IFRB), Bangladesh Atomic Energy Commission, GPO Box 3787, Dhaka 1000, Bangladesh
[2] Department of Zoology, Darsana Govt. College, Chuadanga, Bangladesh
* Correspondence: farzana.baec@gmail.com; farzanayesmin75@yahoo.com
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Abstract: Structural polytene chromosome maps of the two Tephritid (Diptera) fruit fly species were studied. *Bactrocera zonata* originates in South and South-East Asia. It attacks more than 50 host plants, several of which are commercial species- guava, mango, peach, apricot, fig and citrus. The probable risk of its introduction to a new country is aided by travel and trade, and is influenced by changes in climate and land use. The EPPO classified it as A1 Pests, suggesting for regulation as a quarantine pest. *Zeugodacus tau* is widespread in Asia and Australia. It attacks 34 hosts from 9 families, major commercial hosts- melon, cucumber, pumpkin, luffa, sapodilla, bitter gourd and guava. The aim of the study was to identify polytene chromosomes from the 3rd instar larval salivary glands and to construct structural polytene maps based on the banding patterns of the polytene arms. In both species, five polytene chromosomes (10 polytene arms) corresponding to the five autosomes of the mitotic chromosomes and a heterochromatic mass were observed in polytene nuclei. Heterochromatic mass is a granular network and supposed that it corresponds to sex chromosomes. Polytene maps were constructed by dividing each of chromosome arms into twenty sections. The tips of each chromosome arm, prominent landmarks, characteristic banding patterns and puffs were recognized that reflected the fundamental differences in the chromosomes. The structural maps presented here allow future identification and location of chromosomal rearrangements or structural differences within other tephritid species.

Keywords: Tephritid fruit fly; Agricultural pest; salivary gland; polytene chromosomes; structural map; banding patterns

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1. Introduction

Fruit flies (Diptera: Tephritidae) are widely distributed, among them *Bactrocera* and *Zeugodacus* are very significant in pest category [1]. Twenty nine species of Tephritidae have been recorded in Bangladesh [2], where the peach fruit fly *Bactrocera zonata* (Saunders) is a major pest of cultivated mango orchard and the pumpkin fruit fly, *Zeugodacus tau* (Walker) attacks 34 hosts from 9 families including Curcurbitaceae, Fabaceae, Loganiaceae, Moraceae, Myrtaceae, Oleaceae, Sapotaceae and Vitaceae. *Z. tau* was formerly known as *Bactrocera tau* (*Dacus tau*), now *Zeugodacus* is declared as genus [3,4]. Both the species are widespread in Asia and Australia. Adult females of these pests prefer to lay eggs in soft fruits and fleshy vegetables by piercing with their ovipositor, resulting in serious damage and enhance secondary infection by bacteria in agriculture sectors. Cytogenetic studies of the Dipteran insects have greatly been simplified by the presence of polytene chromosomes [5]. It provides an excellent genetic tool for chromosomal studies in

order to examine chromosome structure, their functions, gene activity, phylogenetic relationship and to demark the members of species complexes [6,7].

Taking into account of the above, we studied structural polytene chromosome maps in the polytene nuclei of *B. zonata* and *Z. tau* that would enrich the cytogenetic knowledge of these important pest species. In our previous paper [8], five long chromosomes with their identifying tips and well defined characteristics were reported in each polytene arms of *B. zonata*. Here, we construct and describe the structural polytene maps of *B. zonata* and *Z. tau* based on the banding patterns of their polytene chromosome arms.

2. Materials and Methods

Colonies of *B. zonata* and *Z. tau* have been maintained routinely at the Cytology and Biocontrol Research (CBR), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka. Lab conditions- 25 ± 2 °C RT, 65–70% RH and photoperiod 14:10h. A mixture of sugar and yeast extract (3:1) was provided as artificial adult diet and soaked cotton with normal tap water as drinking water. For larval samples, eggs were collected using banana and sweet gourd for *B. zonata* and *Z. tau*, respectively. Healthy third instar larvae of *B. zonata* and *Z. tau* were used for the salivary gland polytene chromosome preparations following the method of Zacharopoulou et al. [9] and Yesmin [10]. Chromosome preparations were examined using Olympus microscope (CX41) and Carl Zeiss (AXIO Lab A1) and well-spread polytene nuclei were photographed under 400x.

3. Results

Both species contains five polytene chromosomes (10 polytene arms) corresponding to the five autosomes of the mitotic chromosomes. Mitotic metaphase chromosomes were reported in our previous study [11]. Polytene chromosome arms with clear banding patterns were used to construct the structural polytene maps. It was done by dividing each of the chromosome arms into twenty sections with a total of hundred sections. In each polytene nucleus, chromosome arms were identified and numbered based on the methods of other tephritid species [5,8]. Efforts were done to show the clearly identified banding patterns of the corresponding sections in the polytene chromosome arms of *B. zonata* and *Z. tau* (Figures 1–6).

3.1. Structural polytene chromosome maps of Bactrocera zonata

Chromosome 2 (Sections 1–20, Figure 1): 1- Flattened tip with two dark bands; 2- Three thin bands with faint region & one thick band; 3- Six thick bands; 4- Four thick dark bands with faint region at both ends; 5- Three dark bands with two thin bands; 6- Three dark bands with large faint region & two deep bands; 7- Five faint bands closed to two dark thin bands; 8- Two faint bands, one thin dark band and one faint band followed by dark band; 9- Two dark defused bands; 10- One thin band with faint region on both sides with two dark bands at each end; 11- Three dark bands with large faint region; 12- Two dark bands with faint region each side; 13- Three thick dark bands; 14- Three thin bands; 15- Dark thick band with faint region; 16- Large faint region closed with dark bands; 17- Five dark thick bands; 18- Faint region closed to thin dark bands at both ends; 19- Three thick bands; 20- Two dark bands along with faint region.

Chromosome 3 (Sections 21–40, Figure 1): 21- Compressed tip with one dotted band & one thin band; 22- Three thick dark bands; 23- Three thick dark defused bands with faint region each side; 24; Four thin dark bands; 25- Two faint bands, one thin dark band closed with thick dark bands; 26- Three dark bands, one faint band and then two dark bands; 27- Large faint region with two dark thick bands; 28- Two thin dark bands closed with faint region at both end; 29-One thick dark band, faint band with one thin dark band, faint region with two thick dark bands; 30- Two thin dark bands with faint region at each side; 31- four thin dark bands including one thick dark band; 32- Three dark bands and two thin dark bands joint with faint region; 33- two thin dark bands, followed by a constriction in the middle with one fused band; 34- Four dark bands with faint region each side; 35-

Five thin bands with faint region at left; 36- one dark band with two thick dark bands; 37- One dark band followed by one dark band with two thin bands and a faint region; 38- Two faint bands with two thick bands; 39- One thick dark band and two thin dark bands with faint region each side; 40- Three thin bands with one dark band.

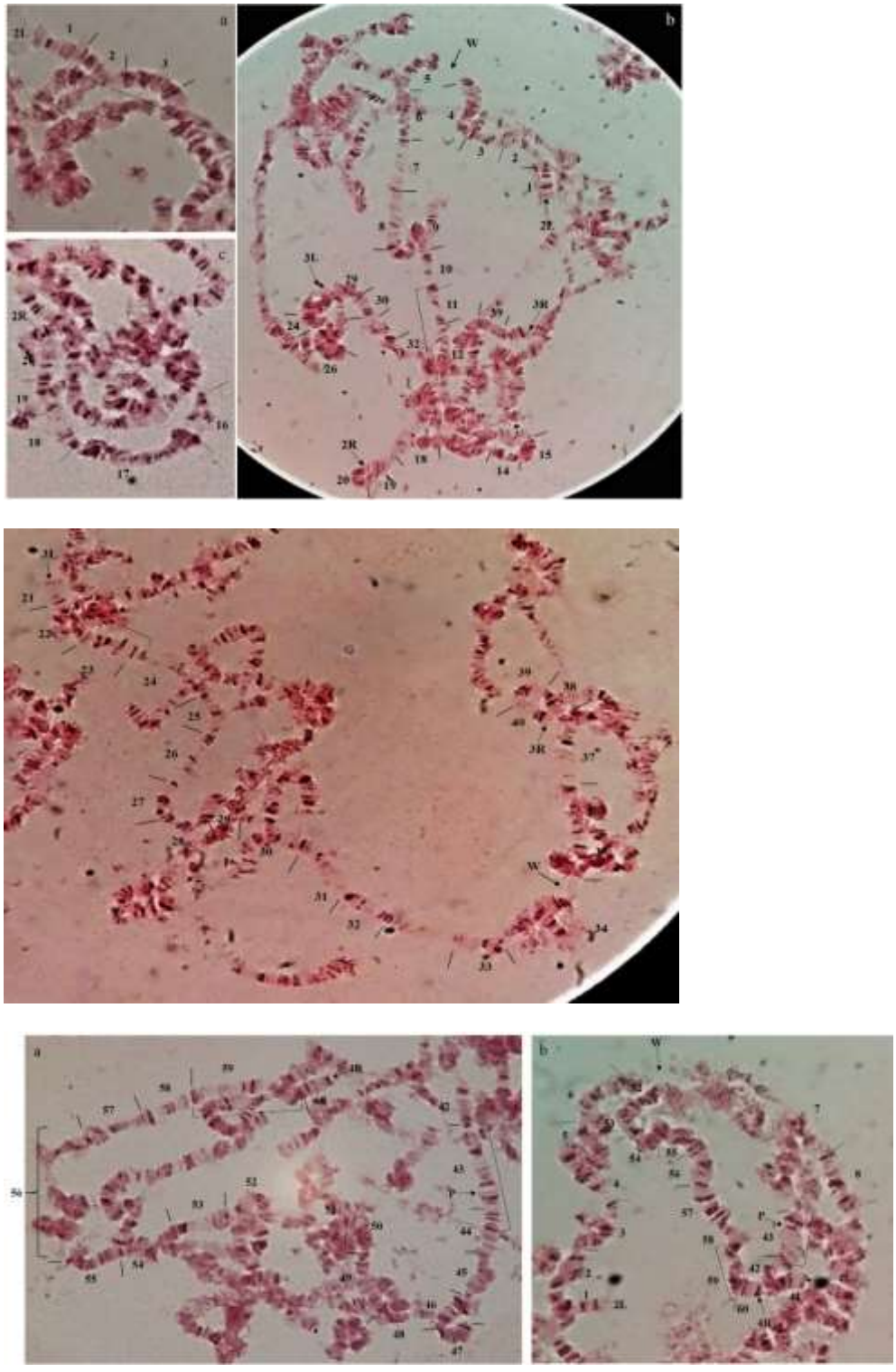

Figure 1. Map of salivary gland polytene chromosomes (2–4) of *Bactrocera zonata*; W- Weak point; L- Left arm; R- Right arm; brackets indicate important landmarks; Top: (**a**) Left arm partial (**b**) Full arm (**c**) Right arm partial; Bottom: (**a,b**) Full arm.

Chromosome 4 (Sections 41–60, Figure 1): 41- Tip with one dark band followed by two deep bands; 42- Two dark bands in the middle of large faint region with one dark band; 43- Small puff with thin band in the middle composed of two thick bands, one thin band and one thick band; 44- three thick bands with two thin bands; 45- Two dark thin bands with three faint bands; 46- One large dark band with faint region; 47- One dark band along with large faint region; 48- Three dark bands triangular shaped with one dark band; 49- Three dark bands; 50- Two dark bands with faint region and one thin dark band; 51- Two dark bands with faint region and two thin dark bands; 52- Three dark thick bands; 53- One thin band, two dark thick bands followed by large faint region; 54- Two thick dark bands attached with two thick dark bands; 55- Three thin dark bands with faint region; 56- Two thick bands with faint region each side; 57- series of six thin dark bands; 58- Faint region with two dark bands each side; 59- Dark region with three dotted bands and two dark bands; 60- Tip with one thin band followed by one thick dark band.

Figure 2. Map of salivary gland polytene chromosome 5 of *Bactrocera zonata*; P—Puff; L—Left arm; R—Right arm; brackets indicate important landmarks; a, b = Full arm.

Figure 3. Map of salivary gland polytene chromosome 6 of *Bactrocera zonata*; P- Puff; L- Left arm; R- Right arm; brackets indicate important landmarks; a = Right arm, b, c = Left arm.

Chromosome 5 (Sections 61–80, Figure 2): 61- Curve tip with one dark band followed by two thick bands; 62- four thick bands, faint region and one dark band; 63- four dark bands with different thickness; 64- Faint region with three thick dark bands; 65- Two dark bands with faint region each side; 66- Region with one thin band; 67- One dark thick band with faint region and one thin band; 68- Band not clear; 69- Two dark bands; 70- Two dark thick bands with faint region; 71- Three dark thick bands; 72- Two dark bands with faint region; 73- Four thick dark bands; 74- Four thick bands followed by a puff; 75- Two dark bands along with faint region each side; 76- Four dark bands as triangular shaped with faint region; 77- Two dark bands with faint region each side; 78- Small puff surrounded by one dark band at both sides; 79- Two dark bands just after the flattened end; 80- Tip with three dotted bands.

Chromosome 6 (Sections 81-100, Figure 3): 81- Flattened tip with two thick bands; 82- Small puff with one thick band at both side; 83- Five thin bands; 84- One dark band followed by faint region with one thin band in the middle; 85- three prominent thick bands; 86- Four dark thick bands; 87- Three thin bands with a large faint region; 88- Row with five thick bands; 89- Faint region with three thick bands; 90- Two dark bands with faint region; 91- Three thin bands with one dark thick band; 92- Four thin bands; 93- Two dark bands with faint region; 94- One dark band with three thin bands; 95- Three dark bands with large faint region; 96- Constriction with six thin bands; 97- Six dark thick bands; 98- Two dark bands with faint region both sides; 99- Three thin bands closed with one dark band at each end; 100- One dark band attached with large defused band.

3.2 Structural polytene chromosomes maps of Zeugodacus tau

Chromosome 2: (Sections 1–20, Figure 4): 1- Convex tip with four bands, one dark band, two thin bands and then one dark band; 2- three dark bands; 3- Six bands with three dark bands; 4- Four thin band with faint region, then two thin bands; 5- Six regular thin bands with thick band both side; 6- Two dark bands with faint region; 7- Three thin bands with one dark band and a faint region with one thin band at middle; 8- Two thin bands with faint region; 9- Two thin bands with faint region and two dark bands; 10- Four thin bands; 11- Five bands with two dark bands; 12- Two dark bands with faint region each side; 13- Two thin bands with faint region and one thin band; 14- Five dark bands; 15- Faint region closed to thick band each side; 16- One dark band with faint region containing dark band in the middle; 17- Five thin bands; 18- One dark band, three thin bands and two dark thick bands; 19- Three thick bands with faint region; 20- One dark band with three thin bands.

Chromosome 3: (Sections 21-40, Figure 4): 21- Tip with two thin faint bands and one dark band; 22- Two close dark bands and five thin bands; 23- Small puff with one faint band in the middle with thick band each side; 24- Narrow region with three dark bands and faint region and two dark bands; 25- Five thin bands including one thick band in the middle; 26- Five thin bands closed to one dark band each side; 27- One thin band, faint region with dotted band and one thick bands followed to two dark bands; 28- Four thick bands with faint region each side; 29- Eight thin dark bands; 30- Faint region with six dark bands and one dark band; 31- Three dark bands with two thin bands; 32- Faint region with two thick bands and two thin bands; 33- four dark bands; 34- Two thin bands with two thick bands and one dark band; 35- One dark band; 36- Two dark bands with four thin bands; 37- A series of six thick bands; 38- Faint region with a dark band; 39- three dark bands; 40- Thin band with dotted band.

Chromosome 4: (Sections 41–60, Figure 4): 41- Tip with two dark bands and two thin bands; 42- Six thin bands; 43- Eight thin band with a constriction; 44- Dark band with two thin bands and three thick bands; 45- Faint region with two thin bands in the middle; 46- four dark bands; 47- Two dark bands with two thin bands at middle with faint region and dark band; 48- Thick band with thin band and three dark bands; 49- two dark bands in the middle; 50- Three dark bands; 51- A series of five dark bands; 52- Four dark bands; 53- Faint region with two thin and two dark bands; 54- Three thin bands with two dark bands; 55- Two thin bands together with faint region with three dark thick bands; 56- three thin

bands; 57- Three thick bands with thin band and thick band; 58- Four dark bands; 59- diffused bands with two dark bands; 60- Faint zone with two dark bands.

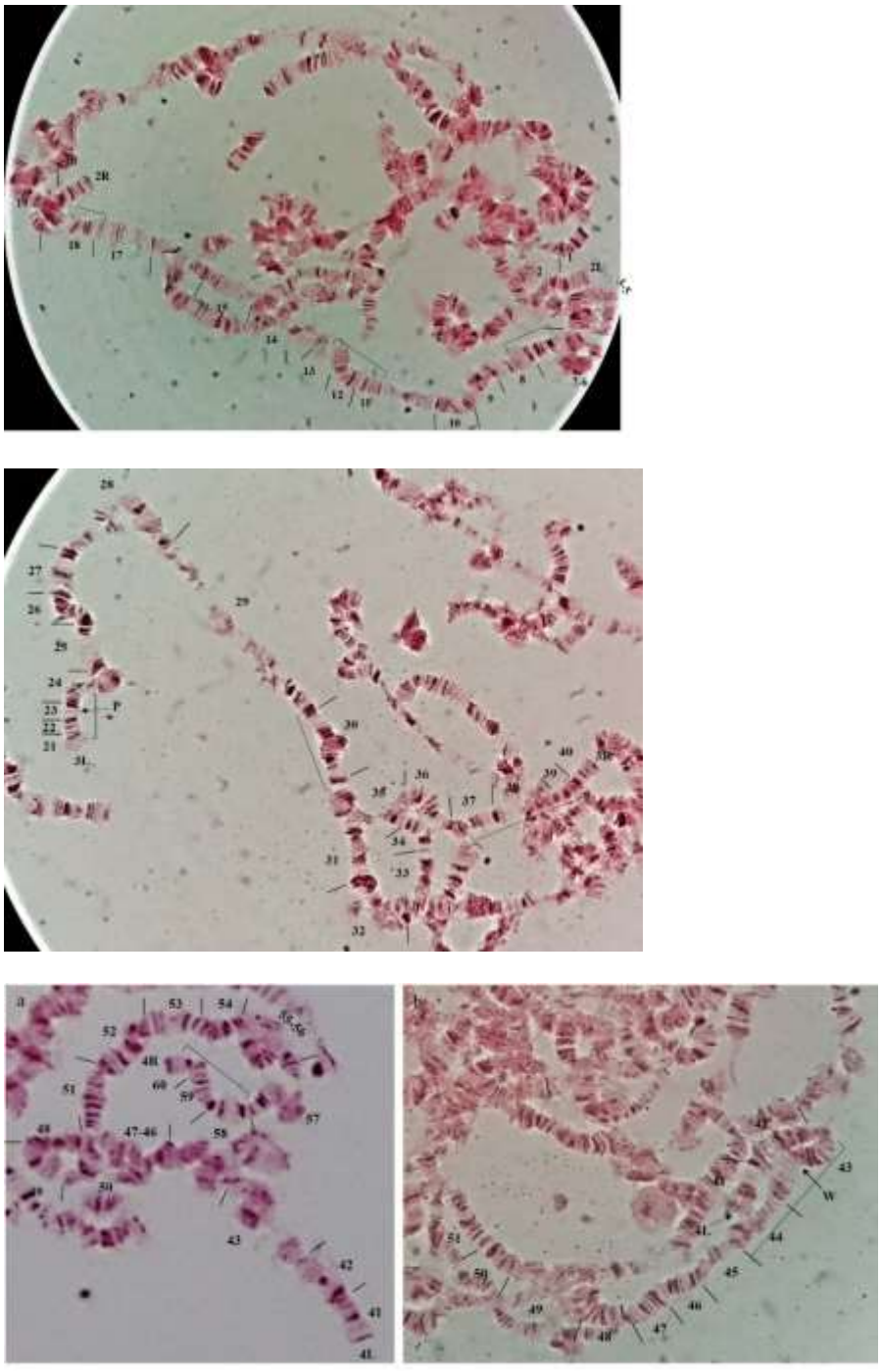

Figure 4. Map of salivary gland polytene chromosomes (2–4) of *Zeugodacus tau*; P—Puff; W—Weak point; L—Left arm; R—Right arm; bracket indicate important landmark; a = Full arm, b = Left arm.

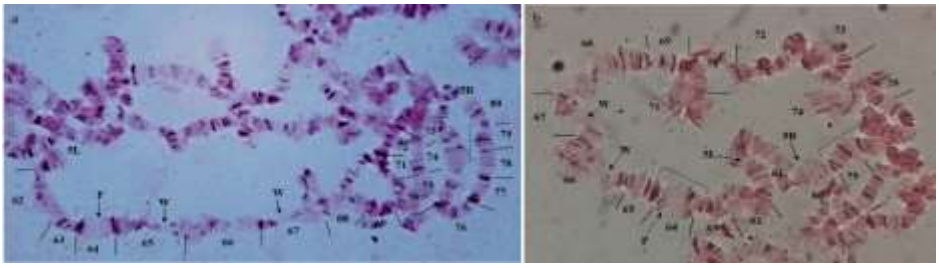

Figure 5. Map of salivary gland polytene chromosome 5 of *Zeugodacus tau*; P—Puff; W—Weak point; L—Left arm; R—Right arm; brackets indicate important landmarks; a, b = Full arm.

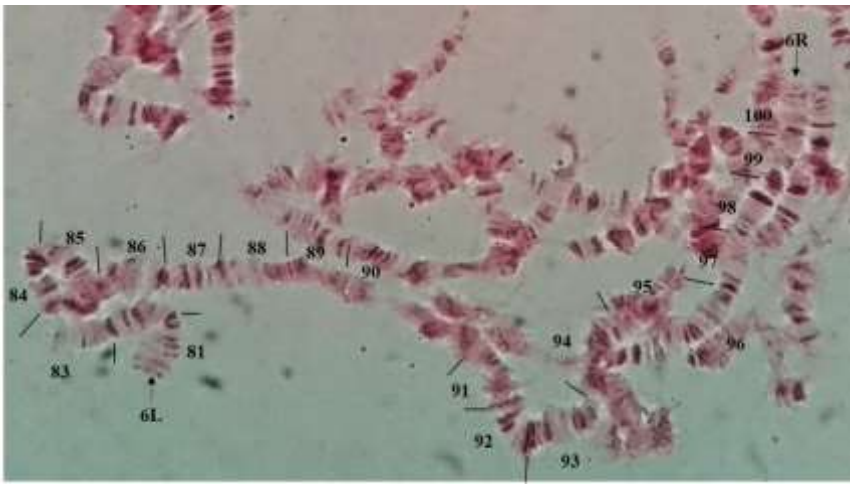

Figure 6. Map of salivary gland polytene chromosome 6 of *Zeugodacus tau*; L—Left arm; R—Right arm.

Chromosome 5: (Sections 61–80, Figure 5): 61- Tip with two thin bands and two dark bands; 62- Two thin bands and two thick bands; 63- Two thin bands and three dark thick bands; 64- Prominent puff closed to dark band each side; 65- Constriction with two dark bands followed to faint zone and two thin bands; 66- Two thin bands, one dark band and one thin band; 67- Three thin bands with dark band at each end; 68- Four thin bands and three dark bands; 69- Five dark bands; 70- Faint region with two thick bands each side; 71- Four thin bands with dark band each side; 72- Five dark bands; 73- Two dark bands with thin band and thick band; 74- Puff with thin bands and two dark bands; 75- Two thick bands with thin band and thick band; 76- Three thin bands accompanied by two thick bands and faint region with thick dark band; 77- Four dark narrow band as triangular shape with faint region and dark band; 78- Two thin faint bands, one thin band and two faint thin bands closed to thick band each side; 79- Small puff with dark band in the middle followed to thick band and thin band including two prominent dark bands with a constriction; 80- Oval end with five dotted bands with a thin band and two thick bands.
Chromosome 6: (Sections 81–100, Figure 6): 81- Tip with five dotted bands accompanied by two dark bands in the constriction; 82- Small puff with faint thin band in the middle closed to thick band at both sides; 83- Three thin bands, one thick band, a faint region with three thin bands; 84- Three dark narrow bands as triangular shaped, then dark band and faint region with two thick bands; 85- Faint region with thin band in the middle with two thick bands; 86- Four thin bands; 87- Four dark bands with faint region; 88- five thick dark bands; 89- Thin band followed to three thick bands; 90- Faint region with four dark bands; 91- Two dark bands with thin and thick band; 92- Five dark thick bands; 93- Three thin bands followed to faint region with three dark bands; 94- Dark region with five bands; 95- Two dark bands with faint region and three narrow bands as triangular shaped; 96- Three thick bands with three faint thin bands and two dark bands; 97- Faint region with four

thin bands; 98- Three dark bands; 99- Two thin dark bands with large faint region; 100- One dark band with four thin bands.

3.3. Heterochromatic mass (Figure 7)

A granular network of heterochromatic mass was observed in the polytene nuclei of *B. zonata* and *Z. tau*, corresponding to the sex chromosomes. This mas was represented as non-polytenized underreplicated X or Y chromosome in polytene nucleus.

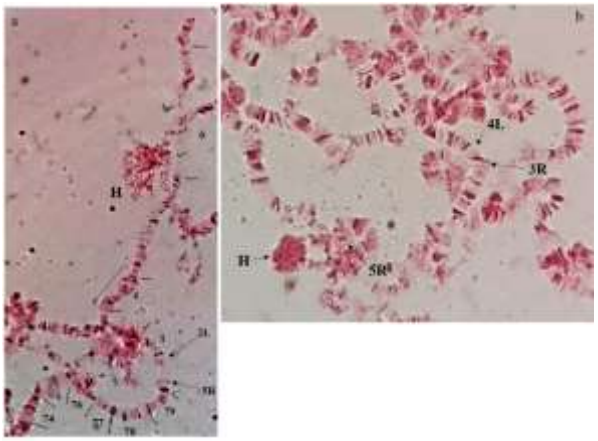

Figure 7. H = Heterochromatic Mass. (**a**) *Bactrocera zonata* (**b**) *Zeugodacus tau.*

4. Discussion

Bactrocera zonata and *Zeugodacus tau* have chromosome set 2n = 10 + XX/XY. The mitotic metaphase chromosomes of them consists of five pairs of autosomes and one pair of heteromorphic XX/XY sex chromosomes [11]. Five long polytene chromosomes (i.e., 10 polytene arms) are corresponding to the five autosomes of metaphase chromosomes. Sex chromosomes are not polytenized that is they do not form polytene elements rather make a granular network known as heterochromatic mass in polytene nuclei. These results are in accordance with previous reports for other Tephritid genera- *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus* and *Rhagoletis* [5,9,12–20]. To date, polytene maps are available for different species of *Ceratitis*, *Anastrepha*, *Bactrocera* (now *Zeugodacus*), *Dacus* and *Rhagoletis* [21]. Also, near about 270 species of Drosophilids have polytene maps; photographic maps based on electron microscopy by Sorsa [22] are still in use in *Drosophila* [23]. Furthermore, polytenized systems provide an excellent element for studying chromosomal aberrations, any structural rearrangements as well as correspondent alteration in somatic synapsis.

5. Conclusions

The presented structural chromosome maps of *Bactrocera zonata* and *Zeugodacus tau* can serve as a basis for further cytogenetic research of this and related pest species.

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