



## ANATOMICAL CHARACTERIZATION, ON THE BASIS OF GENITAL ORGANS OF THREE COSMOPOLITAN SPECIES OF *DROSOPHILA* COLLECTED FROM LAHORE, PAKISTAN

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### ABSTRACT

The *Drosophila* genus has played a very important role as the best eukaryotic research organisms to understand various kinds of biological phenomena. Pakistan is very rich in insect fauna; despite the fact that only a small number has been reported. Around 150 *Drosophila* species have been explored from India while only 20 have yet been reported from Pakistan. Only a few laboratories in Pakistan are using *Drosophila* as a research organism. Three species named *Drosophila melanogaster*, *Drosophila busckii* and *Drosophila immigrans* have been described on the basis of anatomy of genital organs and some external morphological features. These species are cosmopolitan and have been previously reported from a few localities of Pakistan. The characterization was done from cultures placed in the genetics laboratory at Quaid-i-Azam University, Islamabad, Pakistan. Isofemale cultures were raised from *Drosophila* species collected during the period of November 1990 to October 1991 from different localities of Lahore. The methodology involved the microdissection of 2-3 mm small sized flies under a dissecting microscope to separate the genital organs of males (Phallic organ and periphallallic organ) and egg-guide of females. Study of these organs is very useful in systematics of this genus of dipteran flies. The approach used in this investigation provided a very remarkable way for the identification of, expectedly, more than a hundred unexplored species of *Drosophila* from different localities of Pakistan.

**Keywords:** *Drosophila melanogaster*, *D. busckii*, *D. immigrans*, Phallic organ, Periphallallic organ, egg-guide, Lahore, Pakistan

### INTRODUCTION

Since earlier than the last 100 years, *Drosophila* has remained the best model organism for study of various biological phenomena. At present there are around 22,000 transgenic *Drosophila* lines, offering wide a range of research on gene expression profiling in modern studies of genomics, metabolomics and bioinformatics (Dietzl *et al.*, 2007). Biologists across the globe utilizing this remarkably efficient organism in their biological research but unfortunately, in Pakistan very few laboratories are benefitting from the use of *Drosophila* as a research organism. Among the species described during the present study, *Drosophila melanogaster* (Meigen, 1830), and *Drosophila immigrans* (Sturtevant, 1942) has been reported from, Islamabad, Lahore, Rabwah, KPK, Karachi, Murree, Barian, Nathia Gali, while *Drosophila busckii* (Coquillett, 1901) has been reported from Rabwah and Lahore. Overall only 20 species have yet been reported in total (Tahir *et al.*, 2016).

The objective of this study is to identify different species on the basis of genital anatomy of organism, being the most convenient way of identification. The insight for presently described investigation is to promote In-vitro propagation of *Drosophila* in native laboratories to use in teaching and research projects. Sturtevant (1919) was the first to show that the male genital apparatus offers constant and diagnostically valid species differences especially among insects. The practical importance of these structures, as mentioned by Gupta (1973), has also been fully realized by many other previous systematists such as Hsu (1949), Stalker (1953), Okada (1955), Spassky (1957), Takada (1966), Parshad and Paika (1964) and Wheeler and Takada (1971). Thus, considering the importance of the study of the genital structures in systematics, phallic and periphallallic organs of three *Drosophila* species viz. *D. melanogaster*, *D. immigrans* and *D. busckii*, were examined in detail during this study.

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## MATERIALS AND METHODS

*Drosophila* flies were captured by putting traps in different localities of Lahore and brought to the Genetics lab in Quaid-i-Azam University Islamabad, Pakistan during the period of November 1990 to October 1991. Isofemale cultures were made by transferring single mature female fly captured from wild in vial containing culture medium. Different isofemale cultures of apparently similar species were examined for reproductive isolation. The species were maintained in incubator at 25°C as distinct laboratory cultures. In order to study external morphology and genital structures, ten of the male and female *Drosophila* flies from laboratory cultures were over-etherized and kept for 12 to 18 hours, in 10% solution of KOH (potassium hydroxide). Flies were washed with tap water. Mirco-dissections were done by placing single fly on microscopic slide facing ventral side upwards, in a drop of saline solution. Fine needles were used to separate organs from lower abdominal side of fly. The separated organs were mounted on Canada balsam on clean slide, and edges of coverslips were sealed. Periphallalic and phallic organs were characterized according to schematic diagrams (Fig. 1). Specimens were photographed by using oil immersion at magnification of 1000X. Dissections were done using 20X and 40X objectives, under the dissecting microscope (Leitz, Wetzlar, German) and photomicrographs were taken by compound microscope with inserted camera (Nikon; E 400, Japan)

## RESULTS AND DISCUSSION

### *Drosophila melanogaster*

*Drosophila melanogaster* Meigen, 1830; is a cosmopolitan species belongs to subgenus *Sophophora* Sturtevant, 1939; species group *melanogaster* and species subgroup *melanogaster* Hsu, 1949.

### External Morphology (Male & Female)

Arista with 5 branches above and 3 below in addition to the terminal fork. Palpus with more than one prominent seta. Second orbital half the size of other two orbitals. Acrostichal hair in eight regular rows. In male, last two tergites completely black, other tergites except first with a board, uninterrupted, black band on their caudal margin. In female, first tergites yellow, all other tergites with an uninterrupted black band on their caudal margins. Sex-comb present on metatarsal segment of forelegs of male with 10-12 similar, densely packed black teeth.

### Periphallic Organs

Genital arch concave, upper posterior margin with 7-9 bristles, toe with tuft of about 20 small bristles and 3 relatively large bristles arranged in a transverse row. Caudal margin of genital arch with a triangular flap bearing 5 short, stout bristles. Cerci oval, separated from genital arch, with more than 45 small bristles. Clasper single, narrow and incurved with a row of 4-5 teeth on upper portion, lower portion of clasper with about 18 irregularly arranged teeth and 1-2 long incurved bristles (Fig. 2)

### Phallic Organs

Aedeagus apparently narrowing at apex and with short serration. Anterior paramere small T-shaped and apically with a few sensilla. Posterior paramere pectinate at the base, apically with quadrate flag-like flap, dorsobasally with a long branch and several conical processes at apex. Novasternum with strong lateral processes, shallow median notch and rather short submedian spines. Ventral fragma nearly quadrate. Basal apodeme extends the ventral fragma (Fig. 3)

### Comments

The presence of uninterrupted black bands on the abdomen, the separation of cerci from genital arch, prominence of preapicals on all tibiae, eggs with two filaments and absence of prescutellars, warrant the inclusion of the species in the subgenus *sophophora* (Okada, 1956; Gupta, 1974). The characters such as the presence of sex-comb,Periphallic organs with well-developed genital arch, clasper with teeth, phallic organs with anterior and posterior parameres, long coiled ventral receptacle and spiral testes, qualify this species for inclusion in the *melanogaster* species-group (Bock and Wheeler, 1972). The characters of the species such as palpus with more than one prominent setae, clasper with somewhat irregularly arranged teeth, and posterior paramere with flag-like appendages and pectinate aedeagus are evidence of inclusion in *melanogaster* species subgroup, Hsu, 1949. On the basis of distinct external morphology and anatomy of genital organs the species resemble *D. melanogaster* as described by Okada (1956). *D. melanogaster* has a very closely resembled species named *Drosophila simulus* both are completely reproductively isolated and revealed differences in detailed comparative analysis (Gibert *et al.*, 2004).

### *Drosophila busckii* Coquilett, in 1901

*Drosophila busckii* is a cosmopolitan species belongs to subgenus *Dorsilopha* Sturtevant, 1942

### External morphology (Male & Female)

Arista possesses five branches above and 2 below, beside the terminal fork. Pulpus with 3-4 prominent setae and many small bristles. Three black stripes on mesonotum, central stripe forking posteriorly. Abdominal tergites with interrupted black bands, all bands are interrupted at mid-dorsal line, bands of each side are themselves deeply notched in middle position and there is a small black spot on the ventral side of the abdomen on both sides of these bands (Fig. 4)

### Periphallic Organs

Genital arch well developed, pubescent, slightly narrowing below, with about 5 bristles at toe, upper posterior margins with 8-9 bristles. Cerci thickly bristled pubescent and contiguous to genital arch. Clasper single with 13-14 primary teeth arranged in a row. There are three marginal bristles and one distal tooth (Fig. 5)

### Phallic Organs

Aedeagus proximally narrowing, and apically with four pointed processes, two inner ones being longer. Anterior paramere fuse to novasternum, with tuft of bristles at tip. Posterior paramere absent. Novasternum pubiscent with two pairs of incurved sub median spines. Ventral fragma narrowly

triangular (Fig. 6)

### Comments

The character such as the presence of preapicals on the hind tibia only, novasternum with two pairs of long sub median spines, the longest axis of eye exceedingly oblique to body axis and black egg- guide teeth, qualify this species for inclusion is the subgenus *Dorsilopha* (Sturtevant, 1942; Okada 1956; Gupta 1974). So far, four species have been explored in sub-genus *Dorsilopha* (Bächli, 2010). The phylogeny of this subgenus has been remained uncertain for long period of time (Van-der-Linde and Houle, 2008). During the previous decade this genus has got defined position been placed in between the subgenus *Drosophila* and *Sophophora* (Robe *et al.*, 2010; Van-der-Linde *et al.*, 2010). *D. busckii* was the only one known member of the subgenus *Dorsilopha*, until 1990 with quite distinct markings on mesonotum (Strickberger, 1962).

### *Drosophila immigrans* Sturtevant, 1921

*Drosophila immigrans* is cosmopolitan species belongs to subgenus *Drosophila* Fallen, 1823; species-group *immigrans* Sturtevant, 1942.

The features of *Drosophila immigrans* as were initially described by Sturtevant (1942) and Okada (1955, 1956, 1988), with respect to several characters such as abdominal banding pattern, number of spines on fore femur, the periphallallic and phallic organs and egg-guides.

### External Morphology (Male & Female)

Arista with 6 branches on the above side and 3 on lower side, in addition to the terminal fork. Palpus three prominent setae and 9-10 small bristles. Second orbital is about one third of the other two orbitals. In males dark brown medially interrupted bands on second to fifth tergites. While in the last tergites interruption of bands usually obscure due to blackish abdominal ground color, while in females all the tergites except first have brownish medially interrupted bands.

### Periphallic organs

Genital arch broader above, narrowing below, upper posterior margin pubescent and without any bristle, lower margin with 6-7 long bristles arranged in a transverse row on the margin. Cerci not contiguous to genital arch with more than 35 short and long bristles. Clasper single, much longer than broad, 15-16 primary teeth arranged in a concave row and 5-6 bristles present along with the row of primary teeth (Fig. 7)

### Phallic organs

Aedeagus apically abruptly pointed in lateral aspect and obtuse in ventral view, subapically it has two jointed long flaps which are fused to posterior paramere. Anterior paramere fused to novasternum and apically with fine sensilla. Novasternum notched in middle and possess a pair of short sub median spine. Ventral fragma quadrate, much longer than broad. Basal apodeme short with long ventral rod (Fig. 8)

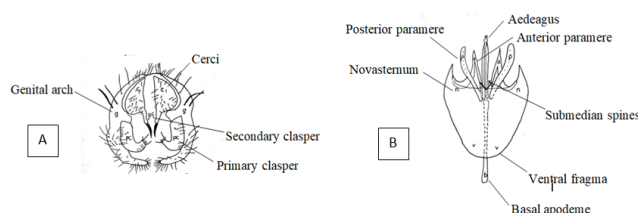
### Comments

The presence of interrupted bands on the abdomen, absence of prescutellars, and eggs with four filaments, warrant the inclusion of the species in the subgenus *Drosophila* (Okada, 1956; Gupta, 1974). Characters of the species such as the cerci

being separated from epandrium, presence of short stout spines on inner side of fore femur and absence of sinuous bow in phallic organs; warrant its inclusion in the *immigrans* species-group (Okada, 1956). Detailed structure of phallic, periphallallic organ and presence of 9-10 stout femur comb spinules are identification markers of *Drosophila immigrans* Sturtevant, 1921.

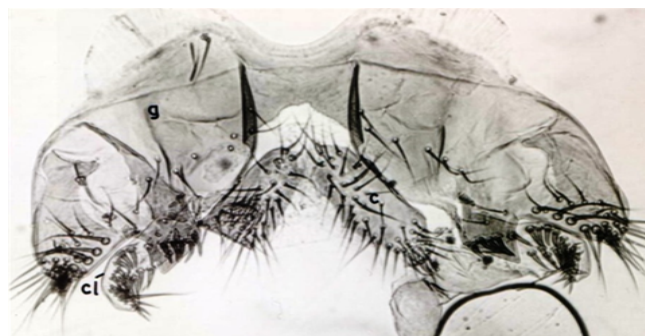
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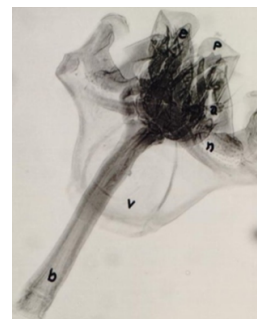
**Fig. 1**

Schematic representation of *Drosophila* periphallallic (A) and Phallic (B) organs.



**Fig. 2**

Periphallic organ of *D. melanogaster* (c cerci, cl. clasper, g. genital arch).



**Fig. 3**

Phallic organ of *D. melanogaster* (a: anterior paramere, b: basal apodeme, e. aedeagus, n: Novasternum, p: posterior paramere, v: ventral fragma).





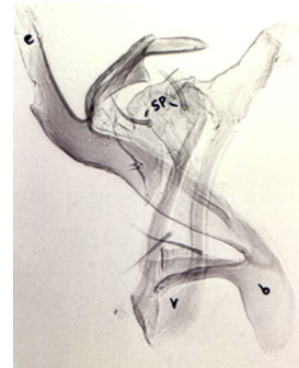
**Fig. 4**  
External morphology of *D. busckii*. Black stripes on thorax as identification marks.



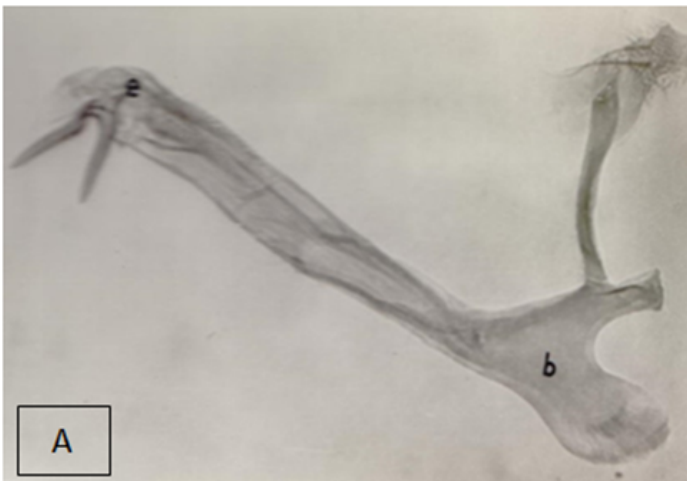
**Fig. 7**  
Peripheral organ of *D. immigrans* (c. cerci, cl. Clasper and g. genital arch).



**Fig. 5**  
Peripheral organ of *D. busckii* (c. cerci and cl. Clasper).



**Fig. 8**  
Phallic organ of *D. immigrans*. (b. basal apodeme, e. aedeagus, sp. Sub median spine and v. ventral fragma).



**Fig. 6**  
Phallic organs of *D. busckii* (A) Aedeagus apically with four pointed processes and (B) Novasternum with 2 pairs of sub median spines (b. basal apodeme, e. aedeagus sp. Sub median spine and v. ventral fragma).

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