

## DIVERSITY IN EXTERNAL MORPHOLOGY, SEX COMB AND DEVELOPMENTAL STAGES OF THREE *DROSOPHILA* SPECIES OF MELANOGASTER GROUP

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### Abstract

Samples of *Drosophila* species were collected from some selected sites (*viz.*, Dohar Upazila of Dhaka Division, Hironpoint of the Sundarbans, Satchori of Sylhet Division) of Bangladesh and these were cultured in the laboratory to study developmental stages, external morphological traits and sex comb. They were identified as *D. melanogaster*, *D. ananassae* and *D. bipectinata*. The sex comb teeth number and arrangement were distinct for all three species. *D. melanogaster* had one row of 10 - 11 teeth in the sex comb. *D. ananassae* had eight rows of teeth in the sex comb possessing 32 - 33 teeth in total. *D. bipectinata* had 2 - 3 rows of teeth in the sex comb and total teeth number varied from 11 to 15. Among three species, sex comb teeth number of *D. bipectinata* varied most. Eleven different patterns (n=20) in sex comb were observed in *D. bipectinata* indicating significant diversity within species. Variation in pupation sites among three species was also observed in the laboratory condition. *D. bipectinata* pupated on the food medium, whereas other two species pupated on the vial wall. Thus, diversity among these three species of *Drosophila* was documented which could be utilized as bio-indicator in future.

### Introduction

*Drosophila* species are commonly known as fruit fly or vinegar fly<sup>(1)</sup>. It is mainly used as a model organism in biomedical research. *Drosophila* spp. has been used for the study of human genetic diseases, mechanisms of development, cell biology, neurobiology, behavior, physiology and evolution<sup>(2)</sup>. About 75% of known human disease genes have a recognizable match in the genome of fruit flies. It has been reported that 548 genes of *Drosophila* are related to human disease causing genes<sup>(3)</sup>. Besides, chromosomal diversity is found to decrease in *Drosophila* due to global warming<sup>(4)</sup>. It can also be a pollution indicator since the fruits (whether grown organically or inorganically) they feed on have impact on their fertility, longevity, activity and stress resistance<sup>(5)</sup>. Thus, diversity among the species of *Drosophila* might indicate overall environmental condition of a region.

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In Bangladesh, a very few research work on *Drosophila* have been reported so far. Ten *Drosophila* species were recorded from Bangladesh<sup>(6-7)</sup>. Since then, no other report could be retrieved. So, it has become a priority task to collect, identify and record the *Drosophila* species found in this region for any future reference required to monitor the environmental impact on biological systems.

Morphological identification of *Drosophila* species is based on body coloration, sex comb teeth number and arrangement, bristle numbers and location, genital region, and developmental stages<sup>(8)</sup>. Among all the characteristics, sex comb is a male-specific morphological structure that plays major role in species-specific mating, and thus in species identification. The male sex comb is an array of specialized bristles on the forelegs. It is highly variable secondary sexual trait of the *melanogaster* species groups of *Drosophila*<sup>(9-10)</sup>.

Change in the fly environment affects development immediately. So, egg-laying, larval instars and behavior, pupa and pupation site preferences are also important traits of study. Therefore, in the present study, the collected samples of *Drosophila* species were maintained in the laboratory rearing to study development, and external morphological traits with special emphasis on sex comb teeth number and patterns.

### Methods and Materials

To collect *Drosophila* from nature, a catch box of plastic jar with partially cut lid was made. Yeasted food was used as bait. When flies entered the plastic jar, they were trapped by covering the open portion of the jar by the lid. The lid was fitted with a food vial beforehand. Thus, flies were collected in the food vials where they were cultured and maintained for subsequent experiments. Standard fly food media (semolina, yeast, sugar, glucose and agar) was used in this case. The flies were reared in room temperature ( $25 \pm 2^\circ\text{C}$ ) and humidity could not be recorded. *Drosophila* flies were collected from nine different locations. Total three species strains collected from Dohar, Dhaka Division ( $23^\circ 33' 42.7''\text{N}$   $90^\circ 08' 49.5''\text{E}$ ), Hiron Point, Sundarbans ( $21^\circ 48' 54''\text{N}$   $89^\circ 27' 43''\text{E}$ ), Satchori, Sylhet ( $24^\circ 07' 12''\text{N}$   $91^\circ 27' 03''\text{E}$ ) were maintained in the laboratory for the study.

External morphology, sex comb arrangement and developmental stages of all the species strains were observed and studied under a stereomicroscope (Leica ZOOM 2000). For the analysis of sex comb, both stereo and compound microscopes were utilized. To ascertain variations in sex comb, at least 20 individual flies were studied from each fly species. To observe developmental stages, adult flies were allowed to lay eggs on egg-laying plates (made of agar, apple juice, sucrose and water). First instar larvae were collected from egg-laying plates after 24 hrs and transferred to standard food media for their subsequent development up to adult. Some images of Hiron Point strain (HP) was captured by using a 3D camera (JVC digital camera KY-F75U, 3-CCD). All other images

were taken by using a hand-held digital camera mounted on the eyepiece of the microscope. *Drosophila* species were identified using published morphological keys<sup>(8)</sup>.

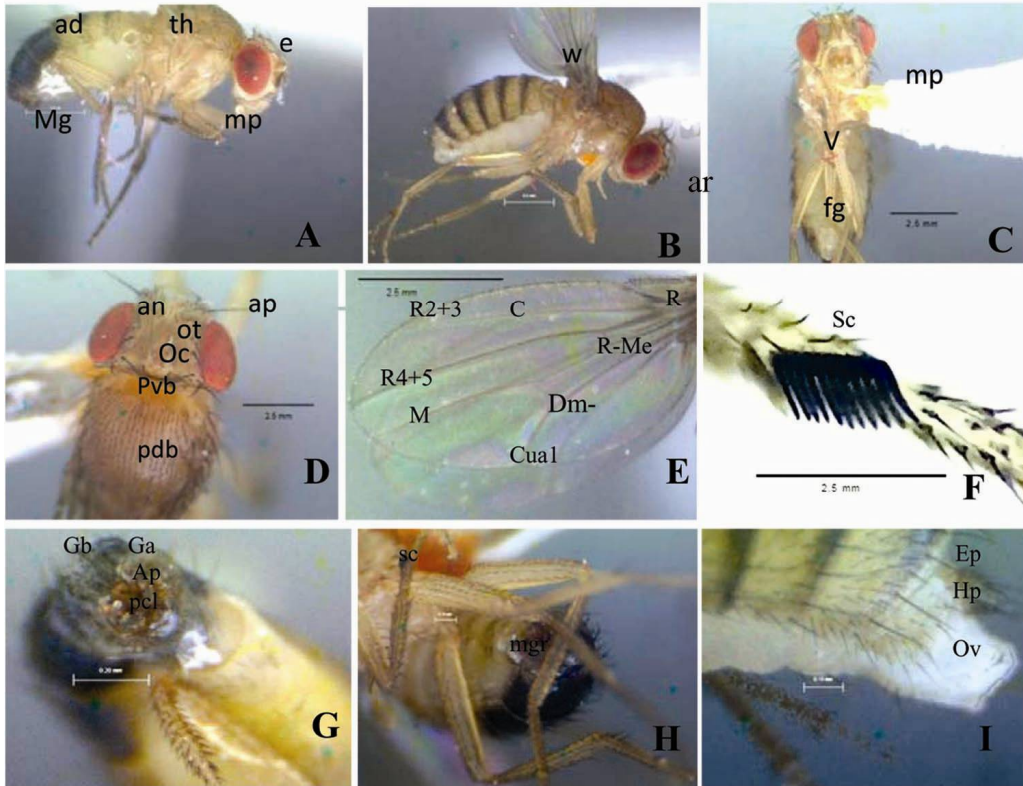
## Results and Discussion

Among the collected strains in the present study, only three have been characterized and identified as *D. ananassae* (SC), *D. bipectinata* (DR) and *D. melanogaster* (HP) (Fig. 1, Table 1). All these species belong to the melanogaster group. Among them, *D. ananassae* and *D. bipectinata* are closer belonging to the ananassae subgroup of melanogaster group. Variations in external morphological phenotypes (body shape, size and color, abdominal region and sex comb) observed among these species have been presented in the Fig. 1 and Table 1. More species of *Drosophila* have been reported from Bangladesh previously. Ten *Drosophila* species have been reported from Bangladesh, which are *D. kikkawai*, *D. ananassae*, *D. repleta*, *D. takahashii*, *D. pseudoananassae*, *D. bipectinata*, *D. melanogaster*, *D. buskii*, *D. latifshahi* and *Scaptodrosophila meijerei*<sup>(6-7)</sup>. The three species in question were also listed in the previous reports<sup>(6)</sup>.

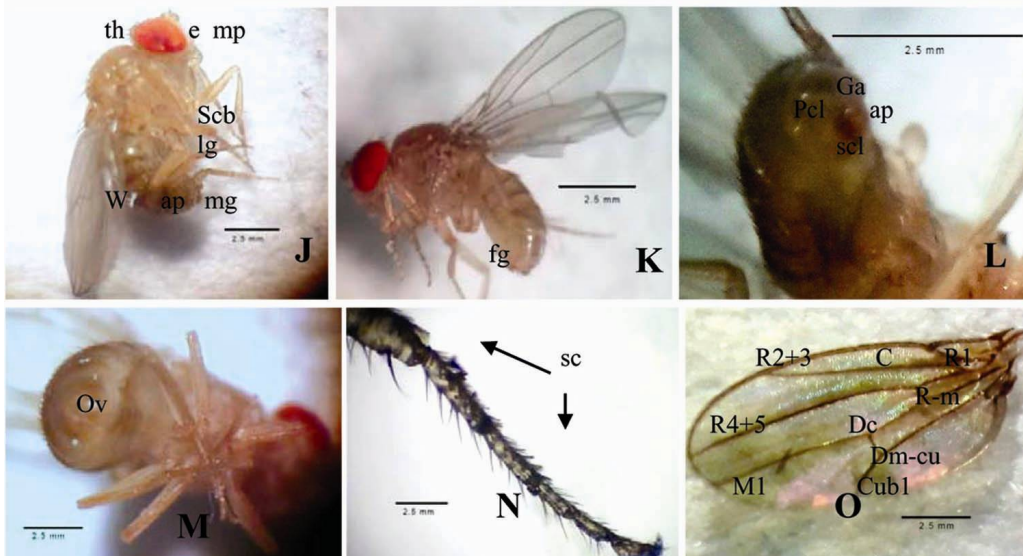
**Table 1. Distinguishing characteristics of *D. melanogaster*, *D. ananassae* and *D. bipectinata*.**

Characteristics	<i>D. melanogaster</i>	<i>D. ananassae</i>	<i>D. bipectinata</i>
Body length	2.5 - 3 mm	> 3 mm	< 3 mm
Body color	Yellow brown	Yellow light brown	Pale brown
Male abdomen	A distinct black patch present at the abdomen	Unbroken abdominal pigmentation present	Transverse black rings present across the abdomen.
Third instar larva length	<4.5 mm	<4.5 mm	4.5 - 5 mm
Third instar larval crawling on the wall of the vials	Yes	Less than <i>D. melanogaster</i>	No or rare
Row and sex comb teeth number (SCTN)	One row and SCTN 10 - 11	8 rows and SCTN 32-33	2 - 3 rows and SCTN 11 - 15
Oviposition on egg laying plate	On the surface of agar medium	On the surface of agar medium	Into the egg-laying agar medium
Show negative geotaxis behavior (data not shown)	More than <i>D. ananassae</i> and <i>D. bipectinata</i>	Less than <i>D. melanogaster</i> and more than <i>D. bipectinata</i>	Less than <i>D. melanogaster</i> and <i>D. ananassae</i>
Cold shock tolerance	More than <i>D. ananassae</i> and <i>D. bipectinata</i>	Less than <i>D. melanogaster</i> and <i>D. bipectinata</i>	Less than <i>D. melanogaster</i> but more than <i>D. ananassae</i>
Pupation takes place	Mostly above the food on the vials wall	Both on the vial wall and on food medium	Mostly on the food medium

***Drosophila melanogaster***



***D. ananassae***



(Contd.)

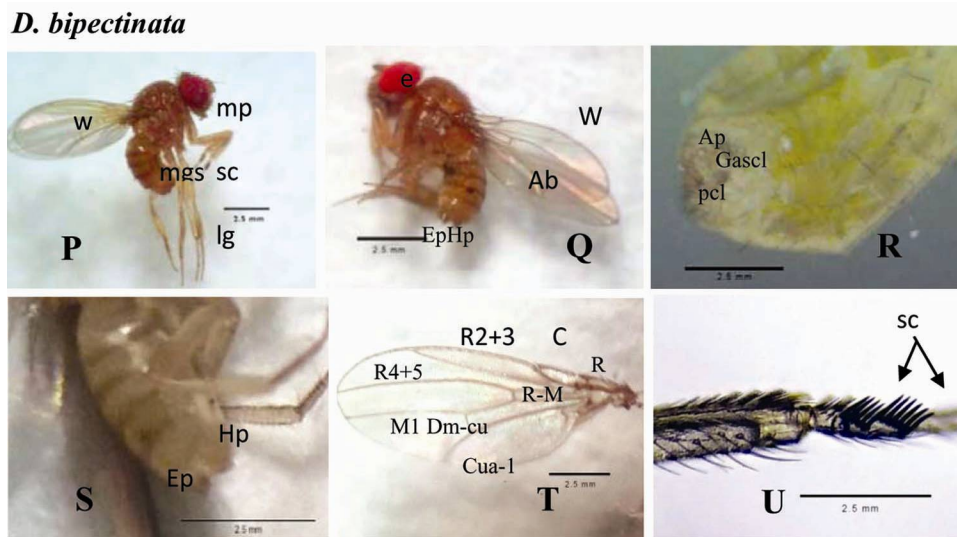


Fig. 1. Morphological characteristics of three *Drosophila* species - *D. melanogaster* (A= Male, B = Female, C = Ventral view of female, D = Head, E = Wing venation, F = Sex comb arrangement, G & H = Male genital region, I = Female genital region); *D. ananassae* (J = Male, k = Female, L = Male genital region, M = Female genital region, N = Sex comb arrangement, O = Wing venation); and *D. bipectinata* (P = Male, Q = Female, R = Male genital region, S = Female genital region, T = Wing venation, U = Sex comb). ad = Abdomen, th = Thorax, e = Compound eye, mg = Male genital, mg r = Male genital region, fg = Female genital, lg = Legs, w = Wings, ar = Arista, mp = Mouth parts, v = Ventral view, an = Antenna, ap = Arista plumase, ot = Ocellar triangle, oc = Ocellus, pvb = Post vertical bristle, pdb = Posterior dorso-central bristles, R2+3 = Radius2+3, R4+5 = Radius 4+5, M = Media1, C = Costa, R = Radius, Cual = Cubitus anale1, Dm-Cu = Distal medial-cubital, R-Me = Radial-media, Sc = Sex comb, Gb = Genital bristles, Ga = Genital arch, Ap = Anal plate, Pcl = Primary clasper, Scl = Secondary clasper, Ep = Epiproct, Hp = Hypoproct, Ov = Oviscapt, Scb = Sex comb bristles.

Study of sex comb patterns showed clear differences among three *Drosophila* spp. strains. In *D. melanogaster*, number of sex comb row is one (*i.e.* bearing one row of teeth) (Fig. 1F). It's sex comb is confined to only first tarsomere of the foreleg. The number of sex comb teeth (SCTN) did not vary much, either 10 or 11. First tarsal segment is reported to have a large sex comb bearing 11 black teeth arranged in one row<sup>(6)</sup>. Though variation is there, individuals with 11 black teeth have also been found in the present study. On the other hand, more variability in sex comb teeth number (e.g. 7 - 14<sup>(11)</sup> and 7 - 13<sup>(12)</sup> in *D. melanogaster* was reported previously.

In *D. ananassae*, eight rows of sex comb present. Total SCTN detected in *D. ananassae* was 32 - 33 (Fig. 2B). Out of 20 samples analyzed, only two patterns of sex comb teeth arrangement were found. The variability found in the present study is much less than that reported in previous studies<sup>(11-12)</sup>. SCTN in *D. ananassae* varies from 22 - 35<sup>(12)</sup> to 31-35<sup>(13)</sup>. The teeth number found in the present study falls within the range of that found in the previous studies.

In *D. bipectinata* male, first tarsomere has 4-6 teeth in the proximal comb, and 5-8 teeth in the distal comb. On the other hand, second tarsomere bears 0-2 in the proximal comb and 0-1 in the distal comb. Eleven sex comb teeth arrangement patterns (out of 20 samples) have been detected in *D. bipectinata* and sex comb teeth number ranges from 10 to 15 (Fig. 2C-O and Table 2). Thus, sex comb teeth number and arrangement varied most in *D. bipectinata* compared to other two species of melanogaster group. The males of *D. becptinata* have two obliquely placed sex combs on metatarsal segment, upper comb with

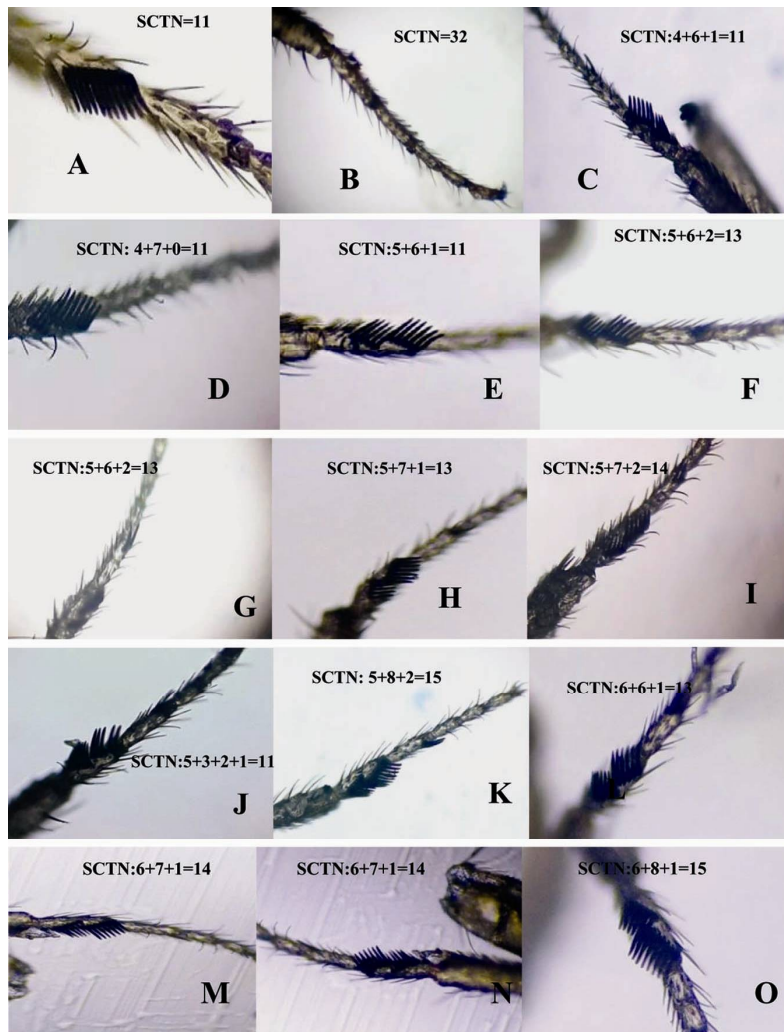


Fig. 2. Sex comb teeth arrangement patterns in three *Drosophila* species. (A) *D. melanogaster*, (B) *D. ananassae*, and (C-O) *D. bipectinata*. The sex comb teeth number (SCTN) formula indicates the number of teeth in the proximal comb of first tarsomere, distal comb of first tarsomere, and proximal comb of second tarsomere, respectively.

**Table 2.** Sex comb teeth number and arrangement in *D. bipectinata* (n = 20).

Sex comb patterns	First tarsomere		Second tarsomere		Total SCTN	Claws No.
	Proximal comb	Distal comb	Proximal comb	Distal comb		
1	4	6	1	0	11	1
2	4	7	0	0	11	1
3	5	6	1	0	12	1
4	5	6	2	0	13	1
5	5	7	1	0	13	1
6	5	7	2	0	14	1
7	5	3	2	1	11	1
8	5	8	2	0	15	1
9	6	6	1	0	13	1
10	6	7	1	0	14	1
11	6	8	1	0	15	1

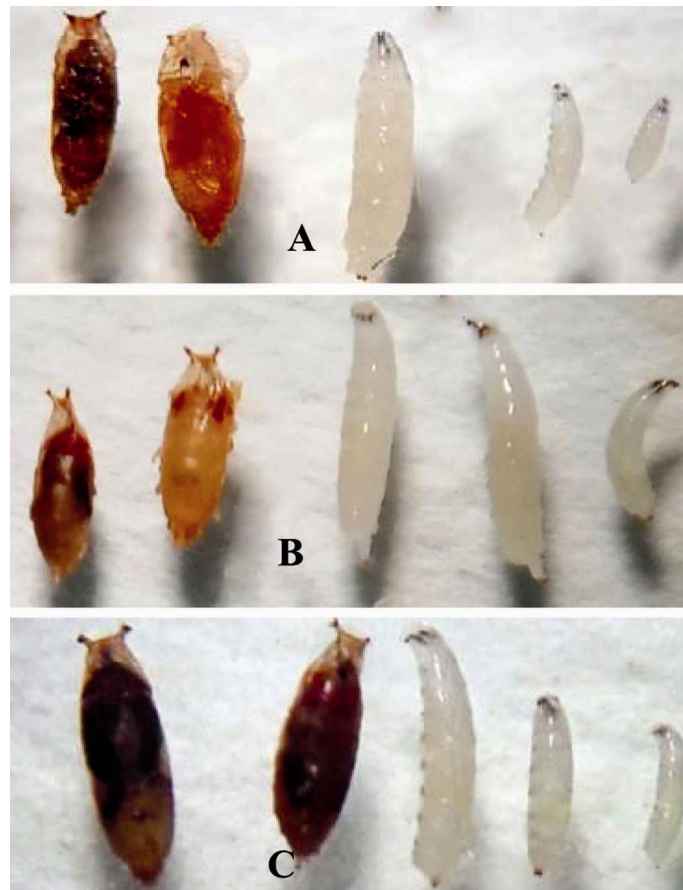


Fig. 3. Comparison among developmental stages of three *Drosophila* spp. A, B and C represents developmental stages of *D. melanogaster*, *D. ananassae* and *D. bipectinata*, respectively.

5-8 teeth, lower one with 6-9 teeth and 1-2 teeth on distal part of second tarsal segment of the first leg<sup>(14)</sup>. In *D. bipectinata* male, first tarsomere has proximal comb teeth no (4-7) and distal comb teeth no (6-10) and second tarsomere proximal comb teeth no 1-2 and no distal comb teeth<sup>(15)</sup>. Present findings of 5-7 proximal comb teeth and 5-8 distal comb teeth and 1-2 second tarsomere proximal comb teeth are in conformity with previous reports<sup>(14-15)</sup>.

Developmental stage (egg, larva and pupa) images of three *Drosophila* species strains are presented in the Fig. 3. Maturation time of *D. bipectinata* from egg to adult was longer than that of both *D. melanogaster* and *D. ananassae*. No difference in egg shape and size could be detected among three *Drosophila* species (Fig. 3). *D. melanogaster* egg length is reported to be 0.51 mm and width 0.18 mm and *D. ananassae* egg length 0.49 mm width 0.18 mm, i.e. they are not significantly different between these two species<sup>(16)</sup>.

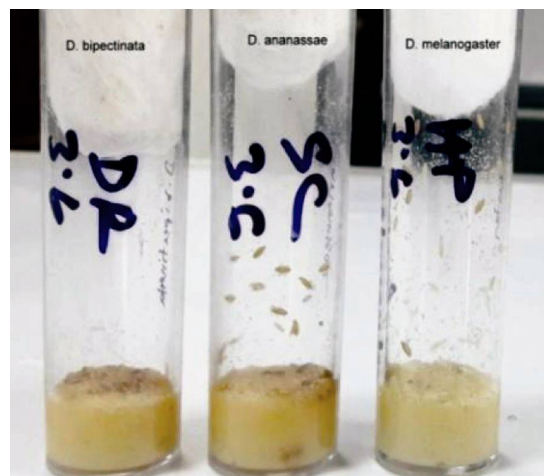


Fig. 4. Pupation height of three *Drosophila* species.

However, egg-laying site (oviposition site of the egg-laying female) differed in *D. melanogaster* and *D. ananassae* which laid eggs on the surface of the medium, while *D. bipectinata* laid eggs into the food medium (egg inserted into the medium). Each egg has two anterior dorsal appendages which are respiratory filament and one micropylar cone associated with an exochorionic surface of the larvae (First and second instars). The body length, shape and color of three *Drosophila* species were almost similar. However, the third instar larva of *D. bipectinata* was relatively larger than that of *D. melanogaster* and *D. ananassae*. Third instar larva of all but *D. bipectinata* crawls to the wall of the vial for a drier area to pupate. It preferred to stay on the food medium. No difference could be detected in pupa color, shape and size, but pupation sites differed. *D. bipectinata* pupation took place on the surface of the food medium, not on the wall of the vial. On the other hand, pupation in *D. melanogaster* and *D. ananassae* took place on the wall of the



vial (Fig. 4). Differences in the pupation site among the three species have been observed when they were kept in the same condition with the same density, light and temperature. The same result was also reported in *D. melanogaster* which preferred to pupate on the vial walls<sup>(17)</sup>. However, the pupation site of *D. bipectinata* on the food surface is a finding of the present study.

### Conclusion

Only three closely related *Drosophila* species have been studied here. Though the species are closely related to each other and belong to the same melanogaster subgroup, they have significant variation in some aspects. Diversity in sex comb teeth number and arrangement is a prominent one. Even, there is within-species diversity in terms of sex comb teeth number as found in *D. bipectinata* (Fig. 2C-O). Since the flies live in and around human habitat, the impact of environmental change on the fly life could be a reflection of the same on human. Thus, within and among species diversity in *Drosophila* could be utilized as a bio-indicator. Present work has provided baseline data that will be valuable in evaluating the influence of climate changes on biological system in future.

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