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Phenotypic diversity in *Culex vishnui* Theobald (Culicidae: Diptera)

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Abstract

Samples of *Culex vishnui* Theobald randomly collected from different water bodies in Chandigarh and surrounding parts of Punjab and Haryana states reveal significant morphological differences which may be minor or quite apparent. These differences relate to the distribution and colouration of scales on different parts of body including head, legs and abdomen. The scales present on proboscis and maxillary palp are arranged in a few bands or some of them are scattered on general surface. Their colour varies from pale to dark brown. The shape of such bands on abdominal terga also varies from transverse to triangular bands. The male genitalia is also highly variable presenting difference in number of finger like processes on phallosome along with the length of mesal spine and sternal spine. Possible causes for the appearance of alternate phenotypes have been discussed.

Keywords: Mosquito, *Culex vishnui*, taxonomy, Intraspecific variations.

1. Introduction

Culex vishnui Theobald 1901 belongs to subgenus *Culex* of Genus *Culex*. In India this genus is represented by seven subgenera i.e. *Barraudius*, *Culex*, *Culiciomyia*, *Eumelanomyia*, *Lophoceraomyia*, *Oculeomyia* and *Maillotia* [1]. Among these, the members of subgenus *Culex* are most dominant and are the vectors of various diseases like filariasis, elephantiasis and Japanese encephalitis [2, 3]. This subgenus includes species which show a high degree of variability in their morphology. The species belonging to subgenus *Culex* exhibit resemblance with one another to such an extent that sometimes it becomes difficult to identify them morphologically. Moreover, so many interspecific as well as intraspecific variations exist in subgenus *Culex* [4, 5]. Accordingly, several groups, subgroups and complexes have been recognized in subgenus *Culex* in order to give a right location to its component species [2, 3, 6]. Harbach [3] has described two groups i.e. *pipiens* and *sitiens* under subgenus *Culex*. Under *sitiens* group, three subgroups i.e. *vishnui*, *sitiens* and *mimeticus* subgroups have been mentioned. Out of these, *Culex vishnui* subgroup is most important which comprises three species viz., *Cx. tritaeniorhynchus* Giles, 1901 [7], *Cx. vishnui* Theobald, 1901 [8] and *Cx. pseudovishnui* Colless, 1957 [9]. All these species are the vectors of the deadly viral disease Japanese Encephalitis. Out of these, *Cx. vishnui* and *Cx. pseudovishnui* show so much resemblance that sometimes it becomes difficult to identify them morphologically. Moreover, both these species show a lot of intraspecific variations which also lead to the overlapping of characters in these two species. A lot of attention has also been paid to study the interspecific variations met with in *Culex* species [3, 6, 10], but very few observations have been made on the intraspecific variations among different species [6, 11].

Hence, during present investigations the efforts have been made to study the intraspecific variations in *Cx. vishnui* in randomly collected samples from Chandigarh and its adjoining areas, so as to make a clear distinction among the members of *Cx. vishnui* and *Cx. pseudovishnui* as till now no such study has been carried out in this area.

2. Materials and Methods

For present investigations, the area selected for the collection of mosquitoes was Chandigarh and adjoining areas of Punjab, Haryana and from Himachal Pradesh. Geographically, this area extends from 74°E to 77°E and 29°N to 32°N. In this area, as far as 19 sites of Chandigarh and 26 sites of Punjab and Haryana were investigated.

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As *Cx. vishnui* is mainly found in rainy seasons, therefore the collections were made during the months of July, August, September, October and November.

Five rounds of surveys each year (from 2008 to 2012) were conducted in above mentioned sites. For recording the variations almost 1000 specimens of *Culex vishnu* were investigated.

The adults were collected either with hand nets or aspirators from various resting places (cattle sheds, human dwellings, mixed dwellings etc.) and breeding places (ponds, pools, puddles, submerged water plants and vegetation around water bodies). These adults were individually examined under a stereo zoom binocular microscope for generic separation. The species were identified by following the keys given by Barraud [11], Sirivanakarn [6], Harbach [3] and Reuben, *et al.* [10]. The descriptions of external morphology including genitalia were recorded.

3. Observations

The normal characters of *Culex vishnui* mentioned below are the ones which have been described by the author in present study.

3.1. *Cx. vishnui* Adult

Proboscis: 1.4mm; Fore Femur: 1.4mm; Wings: 2.4mm; Abdomen; 2.4mm

3.1.1. Head: Head integument brown, vertex with whitish to golden narrow scales, mixed with very few brown scales; erect forked scales light yellow or golden in centre and dark brown at periphery (Plate-1, Fig. A, B); lateral broad decumbent scales dirty white or grey (Plate-1, Fig. B).

3.1.1.1. Proboscis: Proboscis with median band of pale scales, area distal and proximal to median band with dark brown scales (Plate-2, Fig. A).

3.1.1.2. Maxillary palp: Maxillary palp with 3 basal bands of white, brown or yellow scales on segment 2, 4 and 5 and one in the middle of 3rd segment (Plate-3, Fig. A).

3.1.2. Thorax: Mesonotal integument light brown, with pale, light yellow to golden scales. Scales on anterior promontory and supraalar region comparatively lighter than that of scutal region. Prescutellar area with short, white sometimes dirty white scales and long and dark brown setae. Scutellum with white narrow scales, 6 setae on the median lobe, 5 setae on the lateral lobe.

3.1.2.1. Legs: Fore and mid femora covered with dark brown scales that are more darker towards the tip (Plate-4, Fig. A). Hind femur with stripe of dark scales dorsally speckled with few pale scales, ventrally all the three femora covered with white scales. Tarsomeres may or may not be with scattered pale scales, and without or with poorly defined basal or apical bands of pale scales (Plate-4, Fig. D).

3.1.3. Abdomen: Abdominal terga covered with brown scales. Tergum I with median patch of pale scales, terga II-VII with transverse basal bands of pale scales, rest of the terga covered with brown scales. Transverse band of pale scales on tergum VII extends laterally along whole of the tergal plate. (Plate-5, Fig. A). Abdominal sterna with uniformly scattered pale/ dirty white scales throughout (Plate-5, Fig. D).

3.1.4. Male genitalia

3.1.4.1. Phallosome (Plate-36, Fig. E) – Inner division of lateral plate with apical tergal crown of 4 strong finger-like processes on each side, curved and projecting upwards, first process being longest and rest gradually shorter. 3rd process shorter (0.487mm) than the 4th (0.58mm) and it is approximately equal to the length of mesal spine (0.485mm) of outer division. Finger like processes longer than apical sternal spiculate portion, margin of which irregular not well defined. Outer division with strong sternal spine extends from apex to the base with average length of 0.625mm. Lateral basal process narrow, extends outwards.

3.2. Morphological variations in different parts of *Cx. vishnui* from different localities (Table 1).

Table 1: Details of Morphological variations along with the typical characters in *Cx. vishnui* from different localities.

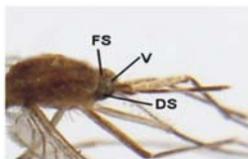
S. No.	Body Part	Charactor/Variations	Localities		
			Chandigarh	Punjab	Haryana
1.	Head				
	Forked scales on Head				
	Typical	Light Yellow or golden in centre and dark brown at periphery (Plate-1, Fig. A, B)	+	+	+
	Variations:	1. Grey or dirty white forked scales (Plate-1, Fig. C)	+	-	-
		2. Forked Scales dark brown at periphery and white in center (Plate-1, Fig. D).	-	-	+
	Decumbent Scales				
	Typical:	Scales Dirty white or grey (Plate-1, Fig. B).	+	+	+
	Variations:	1. Scales brown in colour. (Plate-1, Fig. E)	-	+	-
	Proboscis:				
	Typical	Median band of pale scales (Plate-2, Fig. A).	+	+	+
	Variations:	1. Very broad median band (Plate-2, Fig. B)	+	-	-
		2. Two bands on the proboscis (Plate-2, Fig. C).	+	-	-
3. Band accompanied by a small accessory pale spot (Plate-2, Fig. D).		-	+	-	
4. Band with 3 accessory patches of pale scales (Plate-2, Fig. E).		-	+	-	
5. Faint band with the speckling of pale scales (Plate-2, Fig. F).		-	-	+	

Maxillary Palp				
Typical:	Three basal bands of white, brown or yellow scales and one in the middle of 3 rd segment (Plate-3, Fig. A).	+	+	+
Variations:	1. Only 2 bands of pale scales (Plate-3, Fig. B).	+	-	-
	2. Faint bands with speckling of pale scales (Plate-3, Fig. C).	+	-	-
	3. Presence of three bands on maxillary palp (Plate-3, Fig. D).	-	+	-
	4. 4 bands with 1 st and 2 nd bands very broad (Plate-3, Fig. E).	-	-	+
	5. 4 bands; 2 nd and 3 rd band broad and confluent with each other (Plate-3, Fig. F).	-	-	+
Colour of Scales on Legs.				
Typical:	Scales on fore and mid femora are brown or dark brown (Plate-4, Fig. A); tarsomeres covered entirely with dark scales (Plate-4, Fig. D).	+	+	+
Variation:	1. Brown or dark brown scales are mixed with light coloured scales (Plate-4, Fig. B).	-	+	-
	2. Fore and mid Femora with pure white scales (Plate-4, Fig. C); tarsomeres with basal and apical pale bands (Plate-4, Fig. E).	-	-	+
Abdomen				
Pattern of pale scales on abdominal terga.				
Typical	Pale Scales present in the form of transverse bands (Plate-5, Fig. A).	+	+	+
Variations	1. Pale scales extend anteriorly in the middle (Plate-5, Fig. B).	+	-	-
	2. Pale scales present in the form of triangular bands (Plate-5, Fig. C).	+	-	-
Pattern of pale scales on abdominal Sterna.				
Typical	Scales on the abdominal sterna all pale (Plate-5, Fig. D).	+	+	+
Variation:	Scales on sterna are present in the form of transverse bands of pale scales (Plate-5, Fig. E).	+	-	-
Male Genitalia				
Typical	Details mentioned in Table 2	+	+	+
Variations	Details mentioned in Table 2	Variant I,II, III IV	Variant II, III, IV, V	Variant I

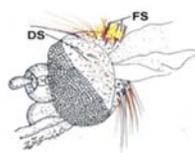
+: Character/Variation Present

_: Character/Variation Absent

PLATE-1
Culex (Culex) vishnui Theobald

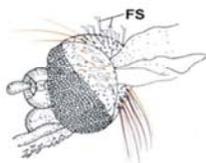


A: Head

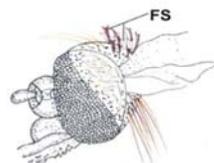


B: Typical head (Decumbent Scales(DS) dirty white grey, erect Forked Scales (FS) light yellow or golden in centre and dark brown at periphery

Variations in the colour of forked scales on Head

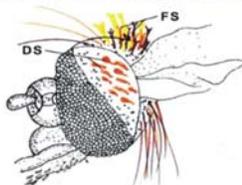


C: Head with grey or dirty white forked scales



D: Erect Forked Scales (FS) dark brown at periphery and white scales in center

Variations in the colour of Decumbent scales on Head



E: Decumbent Scales Brown

PLATE-2
Culex (Culex) vishnui Theobald



A: Proboscis Typical



Variations in the pattern of Pale scale Bands on Proboscis



B: Very Broad Band



C: Two bands



D: Band with accessory patch of pale scales



E: Band with three accessory patches of pale scales



F: Band not clear speckled posteriorly

PLATE- 3
Culex (Culex) vishnui Theobald



A: Maxillary palp Typical

Variations in the pattern of Pale scale Bands on Maxillary Palpi



B: Only 1st or 2nd band seen



C: Bands not clear



D: Three Bands



E: 1st and 2nd band very broad



F: 2nd and 3rd band very broad

PLATE- 4
Culex (Culex) vishnui Theobald
Fore and Mid Femora



A: Fore & mid femora with dark brown scales

Variations in the pattern of scale on Fore and Mid Femora



B Fore & Mid femora with mixture of pale and dark brown scales

C: Fore & Mid femora with white scales

Tarsomeres



D: Tarsomeres with out basal and apical bands (Typical)

Variations in the pattern of Pale scale Bands on Tarsomeres



E: Tarsomeres with basal and apical bands

PLATE- 5
Culex (Culex) vishnui Theobald
Abdomen (Dorsal View)



A: Abdominal terga with transverse pale bands (Typical)

Variations in the pattern of Pale scale bands on Abdominal tergites



B: Transverse pale bands extend along median line



C: Triangular Bands of pale scales
Abdomen (Ventral View)



D: Scales on Sterna pale

Variations in the pattern of Pale scale bands on Abdominal Sternites



E: Bands of light and Dark scales on sterna

3.4. Variations in male genitalia (plate-6):

These variations are tabulate below:

PLATE- 6
Culex (Culex) vishnui Theobald



A: Lateral Plate of Phallosome (40X)

Variations in Lateral Plate of Phallosome of Male Genitalia



B: Variation I (40X)



C: Variation II (40X)



D: Variation III (40X)



E: Variation IV (40X)



F: Variation V (40X)

Table 2: Variations in Lateral plate of Phallosome of male genitalia.

Structures	Typical <i>Cx. vishnui</i> (Plate-6, Fig. A)	Variant I (Plate-6, Fig. B)	Variant II (Plate-6, Fig. C)	Variant III (Plate-6, Fig. D)	Variant IV (Plate-6, Fig. E)	Variant V (Plate-6, Fig. F)
Finger like processes (FLP) on Phallosome	4	4	4	4	3	4
3 rd Process	0.487±0.023 mm	0.537±0.103 mm	0.712 ±0.125 mm	0.714±0.057mm	0.730±0.073mm	0.632±0.028 mm
4th Process	0.58±0.010 mm	0.567±0.033 mm	0.759 ±0.245 mm	0.520±0.011mm	--	0.744±0.104mm
Mesal Spine	0.485±0.120 mm	0.472±0.122 mm	0.552 ±0.045 mm	0.267±0.020mm	0.517±0.032mm	0.589±0.034mm
Sternal Spine	0.625±0.054 mm	0.966±0.055mm	0.792 ±0.027 mm	1.035±0.122mm	0.740±0.017mm	0.729±0.087mm

*These measurements are the average of 20 readings each.

It follows from the data that variations in male genitalia include number and size of finger like processes (FLP) on the inner plate of phallosome, size of mesal and sternal spine is almost equal to that of the 3rd process of phallosome in most of the specimen. These intraspecific variations in male genitalia have already been reported by author in its previous communication ^[12].

4. Discussion

Previous workers such as Barraud ^[11], Sirivanakarn ^[6] and Reuben *et al* ^[10] have also observed high degree of variability in *Cx. vishnui*. Bram ^[2] has declared the whole subgenus *Culex* containing variable forms. Moreover, in addition to the variations mentioned in this report, the difference pertaining to the colour of mesonotal scaling and pattern of speckling on the hind femor have been reported by Barraud ^[11] and Sirivanakarn ^[6]. Another variation relating to the extent of speckling on wings has been recorded by Reuben *et al* ^[10].

It follows from the above account that high degree of variability has been noticed in *Cx. vishnui*. The variations relate to the colour of scales on different body parts. As far as the nature of variability is concerned, it is definitely not a case of genetic polymorphism as there is no constancy and continuity in the morphological changes in different individuals. These appear to be the result of polyphenism (environmental polymorphism). The changes of minor significance may be due to 'reaction norm' caused by gradually changing environment and not the appearance of significant alternative phenotypes as noticed in some other insects ^[13]. In fact, there is hardly any information on the cause of appearance of variations in mosquito species. The only worker who has commented on such a phenomenon in a mosquito, *Wyeomyia smithii*, is Walker ^[14]. He has proposed a new hypothesis, assigning the variations in mosquitoes due to the joint existence of polymorphism, polyphenism and stochastic polymorphism. Accordingly, the applications of molecular technique might ultimately solve the problem of occurrence of variability in mosquito species.

It is worth mentioning that Mayr ^[15] has clearly defined the scope of terms polymorphism and polyphenism whereas polymorphism includes the expression of distinct genotypes into concrete phenotypes which constitute a permanent stock. Polyphenism relates to the formation of alternative phenotypes caused by the effect of varying environments on the same genotype. Stochastic mode of morph formation implies the inclusion of only a part of the population adaptable to the future predicted environment ^[14].

5. Conclusion

The given observations clearly show that the samples of *Cx.*

vishnui collected from Chandigarh, Punjab and Haryana show a large number of variations pertaining to colouration of scales and their arrangement on different parts of body. It appears that these variations are the result of gradually changing environmental cues pertaining to temperature, pH and mineral content of both land and different water bodies.

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