

## Biosystematics of *Culex vishnui* and *Culex pseudovishnui* based on ecobehavioural pattern

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**Abstract.** *Culex vishnui* group consists of 3 species of mosquitoes viz *Culex vishnui*, *Culex pseudovishnui* and *Culex tritaeniorhynchus*. It was found that out of 16 species of mosquitoes collected in 3 years from two different biotopes, 8.28 and 24.89% of *Culex vishnui* mosquitoes were collected from human habitations and cowsheds respectively. The corresponding data of *Culex pseudovishnui* were 1.80 and 5.66% respectively. Out of 12274 mosquitoes belonging to 17 species collected off man-baits in one year, *Culex vishnui* constituted 34.6% while the latter species encountered 9.04%. When the collection was made by placing man-baits and cow-baits side by side, the attraction ratio (man : cow) of *Culex vishnui* was found to be 1:1.9 and that of *Culex pseudovishnui* was 1:2.6. Bloodmeal analysis of these two species of mosquitoes revealed that *Culex vishnui* was more inclined to human blood (21.5%) than that of *Culex pseudovishnui* (16.14%). *Culex vishnui* and *Culex pseudovishnui* were both susceptible to DDT, dieldrin and malathion, but the  $LT_{50}$  value was slightly higher in the former species than the latter. *Culex vishnui* utilised its breeding spots like paddy fields, ponds, ditches and burrow-pits more effectively in almost all the months of a year than *Culex pseudovishnui*. The density of the larvae of *Culex pseudovishnui* was found to be lower than that of *Culex vishnui*. Japanese encephalitis virus was isolated only from *Culex vishnui* in West Bengal.

**Keywords.** Biosystematics; *Culex vishnui*; *Culex pseudovishnui*; ecobehavioural pattern.

### 1. Introduction

The activity of Japanese encephalitis (JE) in India is known to occur in the southern part since the 50's (NIV 1980). During the period 1955–56, altogether 17 strains of JE virus have been isolated from *Culex vishnui* group in south India (Hati 1981). However, until 1969, the taxonomic status of *Cx. vishnui* group was not very clear. It was Reuben (1969) who first demonstrated that this group consists actually of 3 species viz *Cx. vishnui*, *Cx. pseudovishnui* and *Cx. tritaeniorhynchus*. Later on, several investigators (Rajagopalan and Panicker 1978; Soman *et al* 1980; Mishra *et al* 1984) reported that in the rural areas of India *Cx. vishnui* group is being represented by 3 distinct species as mentioned by Reuben. *Cx. vishnui* and *Cx. pseudovishnui* are prevalent in the rural areas of West Bengal (Soman *et al* 1976; Mahadev *et al* 1978). Uptil now *Cx. vishnui* has been the only member of the *Cx. vishnui* group, incriminated as the vector of JE in the state of West Bengal (Chakraborty *et al* 1980). The present study was conducted in order to find out whether the ecobehavioural pattern of *Cx. vishnui* and *Cx. pseudovishnui* also corroborate the idea of Reuben (1969).

### 2. Materials and methods

#### 2.1 Study area

A typical village, named Nudipur in the district of Burdwan, West Bengal state, 80 km from Calcutta was selected as the central field station. Another village, named

Korea, in the same district 15 km from Nudipur village was selected only for bait preference study. No insecticide had been sprayed in those two villages for the last 20 years.

## 2.2 Study period

The period of study extended from December 1980 to November 1983.

## 2.3 Collection of mosquitoes

Aspirators and battery operated torches were used for collection of adult mosquitoes. Mosquitoes were collected thrice a week during one hour in the morning (0600 AM–0700 AM) from cow sheds and human habitations. For the collection of mosquitoes, the methods of Holstein (1954) have been adopted with suitable modifications.

Mosquitoes (female) were collected off man-baits overnight from 1800–0600 h weekly once for one year following the method of Hati *et al* (1981) with necessary modifications.

Mosquitoes (female) were collected from man-baits and animal baits placed side by side from 1800–0600 h twice a month throughout the year. The method of Hati *et al* (1981) was employed for this comparative study of bait preference with necessary modifications.

## 2.4 Bloodmeal analysis

Fully fed females of *Cx. vishnui* and *Cx. pseudovishnui*, collected from human habitations and cow sheds, were selected for bloodmeal analysis so that no difficulty would be encountered in conducting the test. The stomach contents of the females were dissected with a pair of needles and the abdominal contents were squeezed on to the filter paper. The test was performed in wells prepared on glass slide with agar gel by modified Ouchterlony gel diffusion method (Ouchterlony and Nilson 1973) as adapted by Collins *et al* (1983). The antisera used in the analysis were prepared by the laboratory of the Serologist and Chemical Examiner to the Government of India, Calcutta. The samples were run against antihuman, antiovine, antiporcine and antiavian sera.

## 2.5 Insecticide susceptibility tests

Insecticide susceptibility tests were carried out (December 1982 to November 1983) using WHO kits, following WHO (1970) techniques.

## 2.6 Collection of mosquito larvae

Out of 3 methods of collection of mosquito larvae, viz dipping, netting and pipetting advocated by WHO (1975), the first method was adopted in the present study. From the fields, ponds, ditches and burrowpits, collection of larvae was attempted twice a month for one year.

### 3. Results

#### 3.1 Wild caught

Altogether 34155 mosquitoes belonging to 16 species were collected from two different biotopes in 3 years, of which *Cx. vishnui* topped the list (33.17%) while *Cx. pseudovishnui* occupied fifth position, which constituted only 7.46%; 8.28 and 24.89% of *Cx. vishnui* mosquitoes were encountered in human habitations and cowsheds respectively. The corresponding data of *Cx. pseudovishnui* were 1.80 and 5.66% respectively (table 1). The per man-hour collection of *Cx. vishnui* was found to be highest in the rainy season (15.59) and lowest during the winter (10.34). The highest and lowest per man-hour collections of *Cx. pseudovishnui* were obtained in the rainy season (3.73) and summer (2.03) respectively. A strain of JE virus was isolated from a pool of *Cx. vishnui* mosquitoes, collected from human habitations in September, 1982 (table 1) (figure 1).

**Table 1.** Comparative study of ecobehavioural pattern of *Cx. vishnui* and *Cx. pseudovishnui* using several set parameters.

	<i>Cx. vishnui</i>	<i>Cx. pseudovishnui</i>
<i>Wild caught (%)</i>		
Human habitation	8.28	1.80
Cowshed	24.89	5.66
Total	33.17	7.46
<i>Per man-hour collection</i>		
Summer	13.40	2.03
Rainy season	15.59	3.73
Winter	10.34	3.08
Collected off man-bait (%)	34.6	9.04
Collected off man and cow bait (%)	27.90	8.84
Man: Cow ratio	1:1.9	1:2.6
<i>Bloodmeal analysis</i> (% positive for human blood)		
Human habitation	15.9	12.7
Cowshed	5.5	3.5
Total	21.5	16.14
<i>Insecticide susceptibility test</i> LT <sub>50</sub> values (min)		
DDT	37.05	32.32
Dieldrin	25.80	23.55
Malathion	7.25	6.25
<i>Larval density</i> (larvae/dip)		
Fields	1.93	0.62
Ponds	0.51	0.16
Ditches	0.25	0.12
Burrowpits	0.31	0.24
Detection of JE virus	+	-

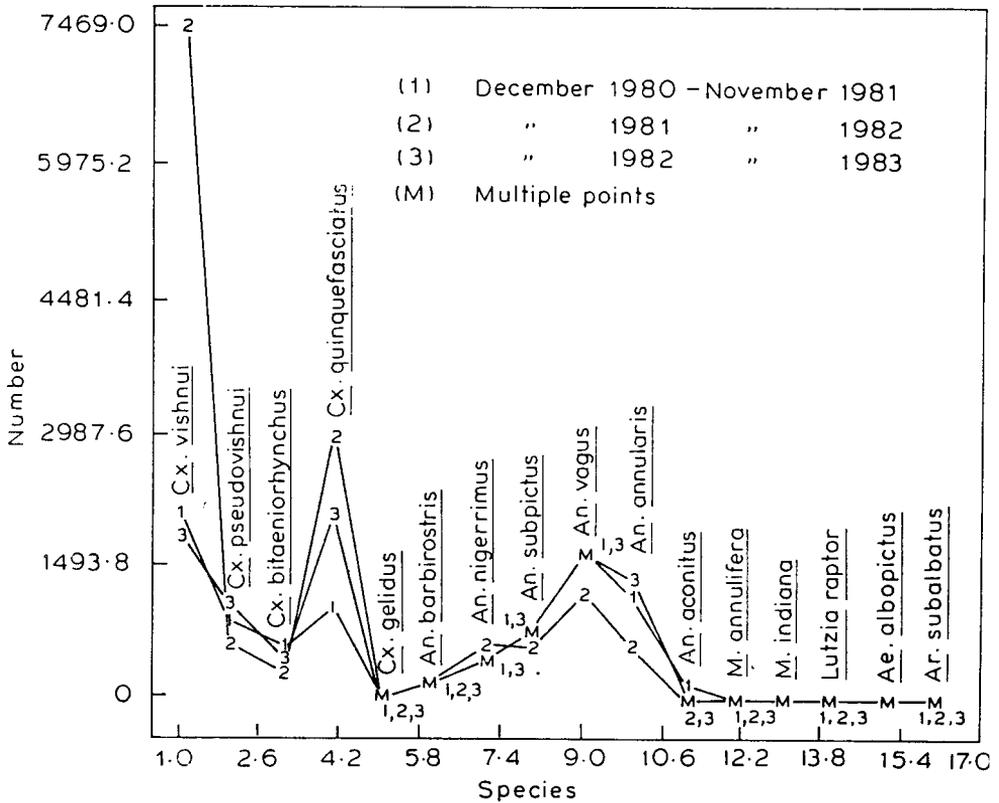


Figure 1. Composition of mosquito fauna of Nudipur village, Burdwan district in different biotopes in 3 consecutive years.

### 3.2 Man-bait

As a whole, 12274 mosquitoes (female) comprising of 17 species were collected off man-baits during the 12 months study period. Altogether 4247 *Cx. vishnui* and 1110 *Cx. pseudovishnui* were collected, comprising of 34.6 and 9.04% of the total respectively (table 1).

### 3.3 Bait preference

A total of 20375 mosquitoes (female) belonging to 12 species were attracted to human and cow-baits. *Cx. vishnui* and *Cx. pseudovishnui* occupied the first (27.90%) and the fifth (8.84%) positions respectively. The attraction ratio (man:cow) of *Cx. vishnui* and *Cx. pseudovishnui* was 1:1.9 and 1:2.6 respectively (table 1) (figure 2).

### 3.4 Bloodmeal analysis

Out of 563 *Cx. vishnui* mosquitoes tested, 121 (21.5%) were found to be positive for human blood (table 1), 357 (63.4%) for bovine blood, 59 (10.5%) for porcine blood

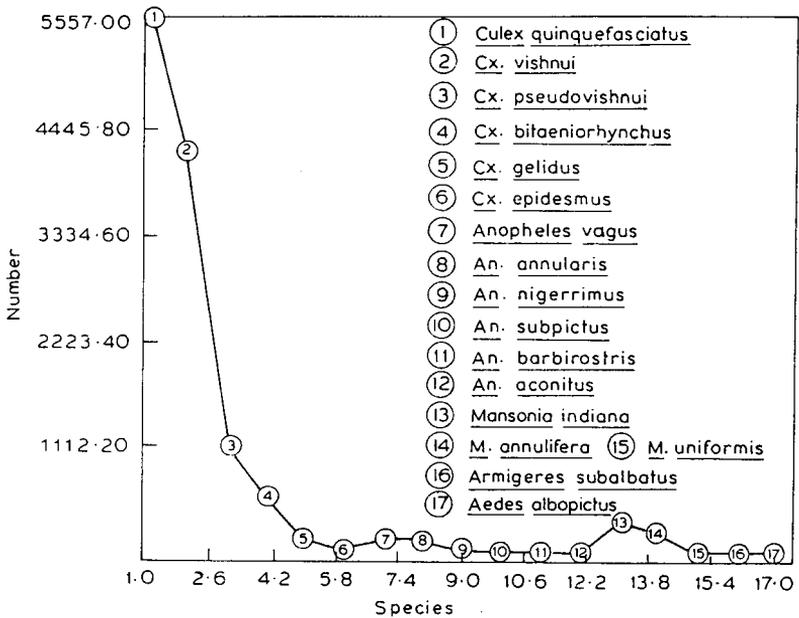


Figure 2. Composition of 17 species of mosquitoes caught off human baits (December 1980–November 1981).

and 26 (4.6%) for avian blood. The corresponding figures for *Cx. pseudovishnui* were 93 (16.14%), 404 (70.1%), 60 (10.4%) and 19 (3.3%) respectively out of 576 tested.

### 3.5 Insecticide susceptibility test

Altogether 100 wild caught *Cx. vishnui* mosquitoes were taken for each insecticide test in each exposure. In 15 min, 30 min, 1 h and 2 h exposure to DDT (4%), the mortality of the species was 8, 42, 78 and 100 respectively. The mortality of *Cx. vishnui* in 15 min, 30 min and 1 h exposure to dieldrin (0.4%) was 14, 64 and 100 respectively. It was observed that in a 15 min exposure to malathion (5%), 100% *Cx. vishnui* were killed within 24 h. With the same exposure period for each insecticide, the mortality figures of *Cx. pseudovishnui* were 12, 46, 90 and 100 in DDT; 18, 74 and 100 in dieldrin and 38, 82 and 100 in malathion respectively.  $LT_{50}$  in min of adult *Cx. vishnui* to DDT, dieldrin and malathion were 37.05, 25.80 and 7.25 respectively. The corresponding data for *Cx. pseudovishnui* were 32.32, 23.55 and 6.25 respectively (table 1) (figure 3).

### 3.6 Breeding sites and larval density

Out of 6 species of mosquito larvae viz *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. bitaeniorhynchus*, *An. barbirostris*, *An. nigerrimus* and *An. subpictus* collected from the fields, ponds, ditches and burrow-pits, *Cx. vishnui* larvae were obtained in all the 4 breeding sites in almost all the months throughout the seasons. While the monthwise distribution of *Cx. pseudovishnui* larvae was found to be limited (present for only 7 months

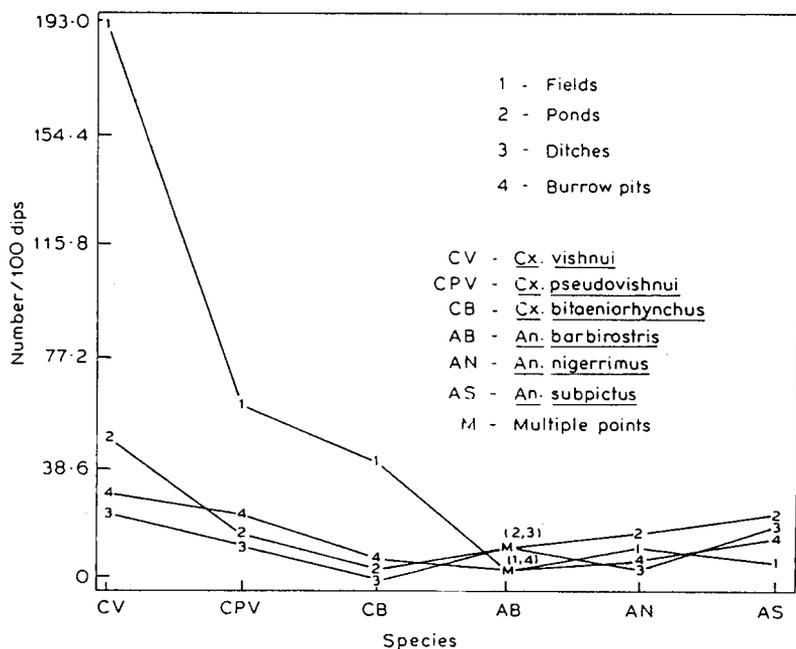


Figure 3. Total collection of different species of mosquito larvae from fields, ponds, ditches and burrowpits (April 1982–March 1983).

in the fields, 6 months in ditches and ponds and 5 months in burrowpits). Yearly collection of *Cx. vishnui* larvae per dip in the fields, ponds, ditches and burrowpits were 1.93, 0.51, 0.25 and 0.31 respectively. The corresponding data for *Cx. pseudovishnui* were 0.62, 0.16, 0.12 and 0.24 respectively (table 1).

#### 4. Discussion

Ecological data have always been involved in taxonomy in a broadway and are also now increasingly important and precise. Species at any one time and place are ecologically incompatible (Simpson 1969). Mayr (1958) demonstrated and adequately illustrated the taxonomic usefulness of behavioural data.

*Cx. vishnui* mosquitoes constituted 8.28 and 24.89% of the total catch in human habitations and cowsheds respectively whereas *Cx. pseudovishnui* encountered in those habitats was only 1.80% and 5.66% respectively. The data show that in each biotope, density of *Cx. vishnui* population was significantly higher than *Cx. pseudovishnui* in this area. The per man-hour collection of *Cx. vishnui* was found to be the highest in rainy season and lowest in winter, while *Cx. pseudovishnui* was obtained in greater numbers in the rainy season and lowest collection was evident in summer. This finding suggests that *Cx. vishnui* and *Cx. pseudovishnui* populations differ not only quantitatively with each other but also seasonally; although in the rainy season a higher catch was recorded for both the species. Mosquito population, in general, find the rainy season favourable for breeding owing to the availability of more natural breeding grounds.

Significantly more *Cx. vishnui* mosquitoes were attracted to human beings than *Cx. pseudovishnui*. The density ratio of these two culicine species was found to be 1:3.8 (*Cx. pseudovishnui*:*Cx. vishnui*).

When animal baits (cow) were placed near human baits, a significantly greater number of *Cx. vishnui* mosquitoes were caught off man baits in comparison to *Cx. pseudovishnui*. The attraction ratio (man:cow) of *Cx. vishnui* and *Cx. pseudovishnui* was found to be 1:1.9 and 1:2.6 respectively. This observation suggests that the latter species have high predilection for animal baits than the former. But, in both the situations (i.e. in man-bait and bait preference studies) *Cx. vishnui* was significantly found to be attracted to man-baits than its counterpart, *Cx. pseudovishnui*. This observation agrees with the findings of Self *et al* (1975) in Indonesia.

Bloodmeal analysis of these two species of mosquitoes has also revealed that *Cx. vishnui* was more inclined to human blood than *Cx. pseudovishnui*.

The criteria for interpreting the results on the susceptibility status against DDT are arbitrarily adopted for explaining the results of single susceptibility test with mosquito populations collected in the field as mentioned by Ismail and Phinichpongse (1980) and they are basically on those recommended by Davidson and Zahar (1973), with some modifications for field application in West Bengal. Using the above criteria it was found that *Cx. vishnui* and *Cx. pseudovishnui* were both susceptible to DDT, dieldrin and malathion; but differed in their  $LT_{50}$  values. The  $LT_{50}$  value was found to be higher in *Cx. vishnui* than in *Cx. pseudovishnui*.

*Cx. vishnui* could effectively utilise the different breeding sites such as paddy fields, ponds, ditches and burrowpits in almost all the months throughout the seasons, while the monthwise distribution of *Cx. pseudovishnui* was found to be limited. In all the 4 biotopes the former species topped the list as far as the density (larvae/dip) was concerned. In the fields and burrowpits *Cx. pseudovishnui* occupied the second position while in the ponds and ditches the position was found to be third and fourth respectively. The largest number of *Cx. vishnui* larvae was evident in the fields than in the other 3 breeding sites. Similar observation was made by Reuben (1971) in south India. Uptil now *Cx. vishnui* has been the only member of the *Cx. vishnui* group, incriminated as the vector of JE in the state of West Bengal.

All the observations stated above on ecobehavioural pattern of the two culicine species, suggest that *Cx. vishnui* and *Cx. pseudovishnui* are ecologically incompatible in this area and this finding reinforces the idea of Reuben (1969).

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