

# Morphometric Variations in Mysore City Populations of *Culex quinquefasciatus* (Say) Larvae

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**Abstract** The posterior-segment morphology of *Culex quinquefasciatus* fourth-instars of the three populations from Mysore city were observed for morphological variations. Two field populations, i.e., from Manasagangotri and Chamundi hill, and one laboratory population were considered. Eight morphological parameters regarding to siphon, saddle, and number of comb-scales and pecten-teeth of 115 larvae from each population were measured. MANOVA-ANOVA denoted that the three populations were significantly different from each other with regard to all the parameters ( $p < 0.005$ ). Pair-wise comparison revealed that each population was distinct in one or the other parameter. The DA generated two canonical functions, both being highly significant; where the Function 1 explained 66.2%, and the Function 2 explained 33.8% variations within the data, and indicated that the siphon index and the siphon length and width were the most distinguishing parameters. Classification of groups results found that  $\approx 75\%$  of each population was distinct from each other. Hence, the results validate the existence of three populations of *C. quinquefasciatus* larvae in Mysore city.

**Keywords** *Culex quinquefasciatus* Larvae, Variations, Morphometry, Mysore City

subjected to various selection pressures and environmental changes, which bring about phenotypic variations; nevertheless, they are measured seldom. The meaning of word morphometry is the measurement of morphological characters of an organism and accounting the variations, arising in certain species-specific morphological traits, also enabling us to differentiate populations of an organism. In the discipline of Vector Biology and Ecology, morphometric studies have aided to ascertain differences in the transmission and vector competence [8]. These variations in an organism reflect upon the environmental changes in the phenotype; however, more is involved than environment. They also have evolutionary consequences, e.g., differentiation of *C. pipiens* from *C. quinquefasciatus* [9] based on the structure of male genitalia by morphometrics. As these phenotypic variations have relation to both environment and genetic background, its study can help medical entomologists to detect local populations with potentially important characters [10]. Present study was aimed to document the ranges of parameters for *C. quinquefasciatus* larval populations from Mysore city; and to identify variations existing in them, hypothesizing that within a short distance of  $\approx 6$  km, variations do exist.

## 1. Introduction

*C. quinquefasciatus* (Say, 1823) (Diptera: Culicidae) is commonly known as the southern-house mosquito [1] gained medical importance across the world as a potential vector transmitting  $\approx 90\%$  of filarial infections caused by *Wuchereria bancrofti* in Asian countries such as India [2]. Globally, over 120 million people are infected; with 20% of the population remain in peril of contracting it [3]. In India, 20 states are endemic for filariasis, including Karnataka [4] where eight districts are the foci [5-7]. *C. quinquefasciatus* adults are highly anthropophilic and are abundant throughout the year, as its larvae mature in forlorn environments—the befouled waters, which most of the time exists due to anthropogenic activities. These insects are

## 2. Materials and Methods

### 2.1. Procurement of the Larvae

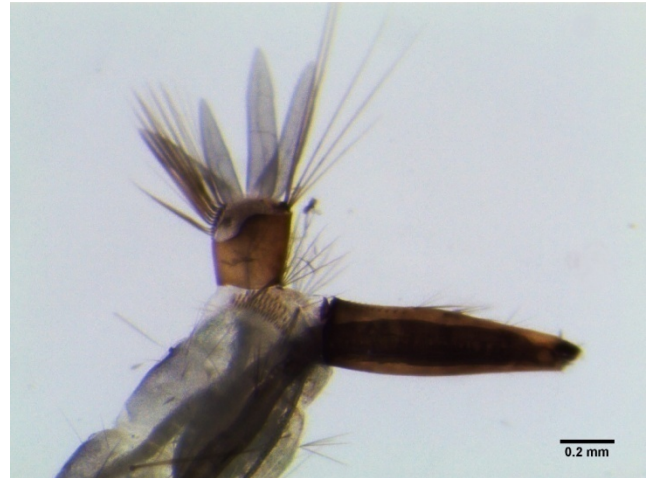
The city of Mysore belongs to Karnataka state of the peninsular India ( $12^{\circ} 18' 0''$  N,  $76^{\circ} 39' 0''$  E); and it is non-endemic for lymphatic filariasis. The fourth-instar larvae were procured from the three different areas of Mysore city, namely, Vector Biology laboratory (Dept. of Zoology, Manasagangotri), cesspit located in Sapodilla plantation of Manasagangotri, and from the cesspit having moderately-polluted-water in Chamundi hills; with the help of a 350 ml capacity enamel dipper, during the months of January and March, 2013. The laboratory and the Managangotri population were less than 1 km apart, but isolated; the two field populations were approximately 6 km

apart with a rise in elevation by 300 m. The larvae were bought back to the laboratory, and were transferred to clean water with the help of a sieve and a Pasteur pipette immediately segregating the fourth instars. The larvae were identified using the taxonomic keys [11,12]. The laboratory population was maintained in an ambient temperature of  $26 \pm 2^\circ \text{C}$  for nearly eight years, and was fed with mixture of Yeast and dog biscuit in the ratio of 2:1 respectively. Freshly prepared 70% alcohol was used to kill the larvae prior to the observation.

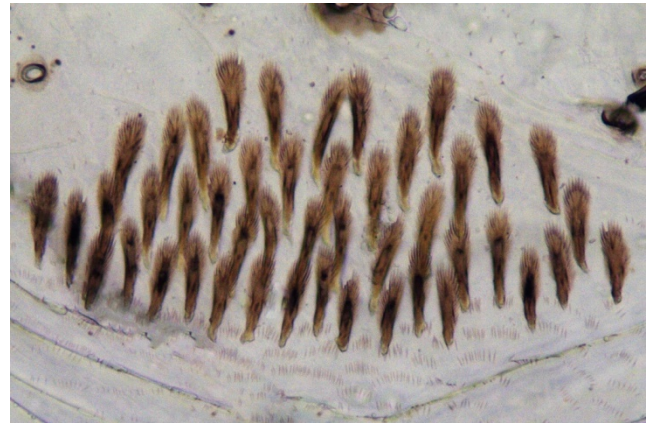
Siphon length and width, saddle length and width, and number of comb-scales and pecten-teeth were the morphological parameters considered for the observation. The measurements of siphon and saddle were taken using Motic® SFC-28 series microscope equipped with linear ocular micro-meter, Erma® with a calibration factor of 0.025 mm (their indices were taken as well). Later, the posterior-segments were carefully dissected using LABOMED® Luxeo 4Z Stereozoom microscope and cover-slips were placed. Number of comb-scales and pecten-teeth were counted under Lawrence and Mayo® XSZ-N207 under high magnification. To avoid the discrepancy, in certain times, the slides were photographed using Olympus® BX41 microscope with a Progres CT3 (Jenoptik®) digital camera system (Figure 1-4). A total of 345 larvae were measured in this manner.



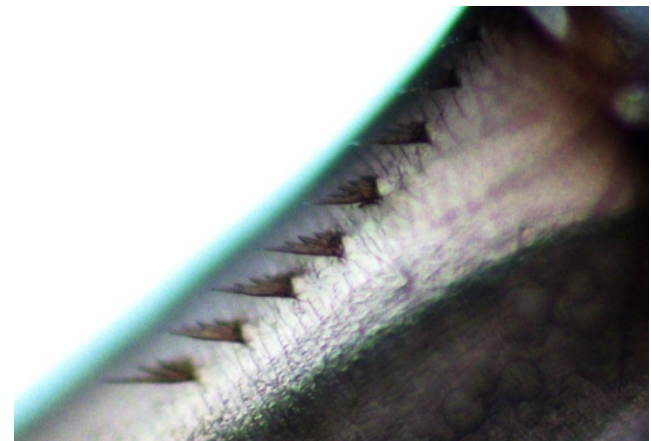
**Figure 1.** Morphology of *C. quinquefasciatus* larvae.



**Figure 2.** Posterior-segment morphology of *C. quinquefasciatus* larvae.



**Figure 3.** Spatulate comb-scales of *C. quinquefasciatus* larvae.



**Figure 4.** Array of pecten-teeth of *C. quinquefasciatus* larvae.

## 2.2. Analysis of the Data

The statistical analyses for the data were carried out in IBM SPSS Statistics for Windows, Version 21.0, following the instructions given by Field [13]. The three samples having an equal sample size ( $N = 115$ ) were analyzed for eight morphological parameters regarding—descriptive statistics, and for normal distribution by observing

scatter-plots, for analysis of variance (ANOVA), multivariate analysis of variance (MANOVA) and discriminant analysis (DA) at  $p < 0.005$ . The descriptive statistics gave the range for the parameters of the three different samples; ANOVA indicated the parameters which were significantly different for the populations, MANOVA tested our hypothesis that all the three groups are significantly different from each other cumulatively for the eight parameters in a linear combination. Further, exploratory data analysis included Post-Hoc test with Bonferroni's adjustments, to find out which two groups were significantly different from each other, and DA, in order to find out the most differentiating parameter of the populations.

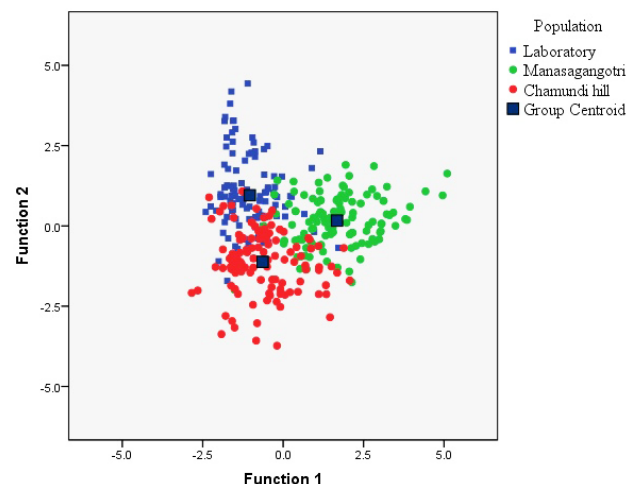
### 3. Results

Table 1 conveyed that the three Mysore city populations of *C. quinquefasciatus* larvae were distinct from each other, by the results of descriptive statics and ANOVA for the parameters. The range for the parameters obtained from the Table 1, were consolidated as 'Mysore city population' and were compared with one available description about *C. quinquefasciatus* larvae of Oriental regions (including India) [12]; there were deviations with regard to: Siphon length (0.73–1.1 mm), saddle length (0.25–0.38 mm), comb-scales (30–52), and pecten-teeth (3–13). Siphon index, however, was found to be mostly within the range (2.5–4.5); especially those from Chamundi hill agreed upon previous observations.

The MANOVA elicited the significant effect of areas on the morphological parameters by considering Pillai's trace,  $F(16, 672) = 43.27$ ,  $p < 0.005$ . Post-Hoc test, utilizing Bonferroni's adjustment to scrutinize the pair-wise comparison, revealed that all the three populations were discernible from each other by one or the other morphological parameter ( $p < 0.0001$ )—Manasagangotri population by their number of comb-scales and siphon index, Chamundi hill population by their number of pecten-teeth and siphon length, and the laboratory population by their saddle length and width. Nevertheless, saddle index was significantly different only between the laboratory and Manasagangotri population; but the siphon width was discrete for all the three populations. No significant difference was found between—the laboratory population and Chamundi hill population regarding: Number of comb-scales ( $p = 0.238$ ), siphon index ( $p = 0.976$ ), and saddle index ( $p = 0.015$ ); the laboratory and Manasagangotri population regarding: Number of pecten-teeth ( $p = 0.269$ ) and siphon length ( $p = 0.222$ ); furthermore, Manasagangotri and Chamundi hill population in their saddle length ( $p = 1.00$ ), saddle width ( $p = 0.003$ ),

and saddle index ( $p = 0.015$ ).

The DA presented two discriminant functions, the Function 1 (F1) explained 66.2% of the variance (canonical  $R^2 = 0.58$ ) and the Function 2 (F2) explained 33.8% (canonical  $R^2 = 0.42$ ). In combination, these discriminant functions significantly differentiated the populations,  $\Lambda = 0.236$ ,  $\chi^2(16) = 489$ ,  $p < 0.005$ . Even after removing the F1, the F2, however, contributed significantly to differentiate the populations,  $\Lambda = 0.576$ ,  $\chi^2(7) = 187$ ,  $p < 0.005$ . The correlations between the parameters and the discriminant functions revealed the factor loading for the eight parameters: in the F1—siphon index ( $r = -0.872$ ), siphon width ( $r = 0.737$ ), saddle width ( $r = 0.364$ ) had strong correlation, whereas, number of comb-scales and saddle index had weak correlation ( $r < 0.300$ ), in the F2—siphon length ( $r = -0.768$ ) and number of pecten-teeth ( $r = -0.516$ ) had strong correlation, and saddle length ( $r = -0.283$ ) had weak correlation. Thus, the F1 was highly defined by siphon width, later followed in the order of siphon index, saddle width, saddle index, and number of pecten-teeth; major contributors for the F2 were in the order of siphon index, saddle width, saddle index, siphon width, and number of comb-scales. The discriminant function plot (Figure 2) corroborated that the F1 discriminated the laboratory population from Manasagangotri population, as well as, the two field populations; F2 discriminated the laboratory population from Chamundi hill population. The cross-validated result for the classification of group membership was found to be  $\approx 80\%$  correct for all the populations, with all the populations being  $\approx 75\%$  distinct from each other. The laboratory and Chamundi hill population both shared  $\approx 14.5\%$  similarity; while, Manasagangotri population shared  $\approx 10\%$  similarity with Chamundi hill population and  $\approx 4\%$  with the laboratory population.



**Figure 5.** Canonical discriminant function for the three populations of *C. quinquefasciatus* larvae.

**Table 1.** Descriptive statistics and ANOVA for the three Mysore city populations of *C. quinquefasciatus* larvae.

Morphological parameters	POPULATION															p-value*
	Laboratory					Manasagangothri					Chamundi hill					
	Mean	Std. Dev.	Min.	Max.	Range	Mean	Std. Dev.	Min.	Max.	Range	Mean	Std. Dev.	Min.	Max.	Range	
Number of comb-scales	42.3739	4.28064	31.00	52.00	21.00	39.1826	4.40809	30.00	51.00	21.00	41.3739	4.23117	31.00	52.00	21.00	0.000
Number of pecten-teeth	8.6348	1.44082	5.00	13.00	8.00	8.9565	1.44119	3.00	12.00	9.00	10.1304	1.41745	7.00	13.00	6.00	0.000
Siphon length in mm	0.8824	0.06762	0.73	1.03	0.30	0.8685	0.05381	0.75	1.03	0.28	0.9689	0.05407	0.85	1.10	0.25	0.000
Siphon width in mm	0.2354	0.01813	0.20	0.28	0.08	0.2785	0.01652	0.25	0.30	0.05	0.2611	0.01819	0.23	0.30	0.08	0.000
Saddle length in mm	0.2991	0.02144	0.25	0.35	0.10	0.3139	0.01849	0.28	0.35	0.08	0.3126	0.02104	0.25	0.38	0.13	0.000
Saddle width in mm	0.2926	0.02341	0.25	0.35	0.10	0.3187	0.01869	0.28	0.35	0.08	0.3096	0.02083	0.28	0.35	0.08	0.000
Siphon index	3.7605	0.30635	2.90	4.50	1.60	3.1273	0.24161	2.50	4.00	1.50	3.7245	0.28010	3.17	4.44	1.28	0.000
Saddle index	1.0253	0.07094	0.91	1.20	0.29	0.9873	0.06711	0.85	1.09	0.24	1.0112	0.05338	0.83	1.18	0.35	0.000

\*Significant at  $p$ -value < 0.005

## 4. Discussion

*C. quinquefasciatus* being a cosmopolitan vector, has adapted itself to unceasing changes of human activities. As observed in the present study, the range for the parameters of Mysore city population is apparently deviating from the observations made by Sirivanakarn [12] for Oriental regions (including India). This necessitates further investigations to revise the range for various attributes of *C. quinquefasciatus* populations, if necessary; as it has been over three decades, since the last observation was made. Also, it suggests that *C. quinquefasciatus* is a dynamic species with inherent phenotypic plasticity, which enabled them to adapt to the new environmental conditions influenced by various anthropogenic activities.

The results validated the existence of three populations of *C. quinquefasciatus* larvae in Mysore city; possibly, due to important environmental conditions, such as nutrition, climate and isolation. With regard to latter, the laboratory population exhibited significant variations with respect to saddle length and saddle width than other two populations. These variations may be due to conditioned environment maintained for the laboratory populations. Genetic analyses can be conducted to check for the variations among the populations to know further whether it is genetically distinct from the other two populations [14]. The two field populations were separated by a distance of 6 km, with a difference in elevation of  $\approx 300$  m, as the flight dispersal range of *C. quinquefasciatus* is not usually greater than 1 km and its propensity to remain close to its breeding site [15,16], implies that the Chamundi hill population might have been subjected to restricted breeding. The variations might have been occurred due to the effect of altitude, which greatly modifies the climate; similar has been observed for *C. theileri* adults [17]. However, it requires further verification by raising the field populations in controlled laboratory conditions [18].

The former studies on *C. pipiens* larvae have denoted that the average range of siphon index has direct relationship with larval habitat's water quality, with decrease in the value of siphon index in increased pollution levels [19,20]. The same was found to be true for *C. quinquefasciatus* larvae, as classification results of DA put forth that the laboratory and Chamundi hill populations shared overall similarity of  $\approx 14.5\%$ , with both of their siphon lengths and siphon indices being greater than Manasagangotri population, due to its breeding site, the cesspit of Manasagangotri, which was highly polluted. Further, to affirm the above, it was observed that the laboratory population had larger siphon index than the two field populations, due to clear fresh-water habitat in which they were grown. These findings furnish the information that great geographic distance is not really necessary to modify the phenotype of *C. quinquefasciatus* larvae; and purports that morphological changes can be rapid when different environmental conditions occur [21].

The available literatures regarding morphometry and ecology of *C. quinquefasciatus* is sparse even though they cause baleful filariasis in various parts of India. These

morphological variations are also been known to be related with differential vectorial capacities among natural populations of *C. quinquefasciatus* [14] and their inherent plasticity. However, they do not infer to the gene flow, nor do they estimate the flow of migrants [10]. Thus, variations arising in them are due to intricate coalesced interactions between their genetical make-up and environment; these have to be monitored in order to mitigate such outbreaks. Even then recent studies are centred on isoenzyme variations; they are expensive and time-consuming, as well as, less informative; so studies should be based more on morphometrics [22] as they can determine the fate of distinct variations acquired by several populations throughout evolution, in a way to enable the biologists to explore further [23].

## Acknowledgements

This project was carried out for the partial fulfilment of Masters in Zoology. We appreciate the Chairman of the Department of Studies in Zoology, University of Mysore, for providing congenial workspace to carry out this study; amicable research-fellows of the Vector Biology laboratory for their valuable guidance to understand the study better; and finally, our dear friends Suhas and Vijay, who helped us out of goodwill to procure the larvae.

## Conflict of Interests

The authors declare that no conflicting interests exist.

## Authors' Contributions

Mr. Anirudh R. Acharya and Ms. Jhansi Lakshmi Magisetty, both have equally contributed to the preparation of the manuscript, and they agree to share the authorship. Prof. Vijayan V. A. supervised the work and amended the manuscript.

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