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Ultrastructure of Wing Scales in Adult Mammophilic Vector *Culex* (*Culex*) *quinquefasciatus* (Say) in a Semi-arid Zone

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ABSTRACT

Wing scales are considered a significant taxonomic character in the family Culicidae. Scanning Electron Microscopy (SEM) of wing scales has been studied. The anatomy, morphology and distribution of wing scales of *Culex* (*Culex*) *quinquefasciatus* Say were studied using scanning electron microscopy (SEM). This new role of scales has been described here to segregate it from other species of subgenera Culicidae. Also, we have added some vital variatious in larval characters such as setae and its disposition during development of larvae in the laboratory as well as been recorded in their actual niche. There appears to be a need to record these larval variations, which are regularly occurring, but has not been recorded the in semi-arid zone of India. The fourth instar of *Cx. quinquefasciatus* Say has been examined in the compound microscope. The chaetotaxy variations on segments VII, VIII, and X have been observed. Whereas, on the X segment seta 2-X and 3-X with 2-6 branches were recorded. These observations have been compared with the work of Sirivankaran and White (1978) descriptions, where differences have been distinctly tabled. The role of wing scales in morphology cannot be ignored in any species specific and site-specific study in a semi-arid zone which can improve upon the taxonomy of Culicidae itself, when studied under higher magnifications. Reported variations of species could play a role in management of new diseases in this area. This ultimately can be an establishment factor for a relationship with epidemics with environmental parasitology and systematics of vectors.

Key words: *Cx. quinquefasciatus,* ultrastructure, wing scales, dorsal tufts, environmental parasitology.

INTRODUCTION

The mosquito species of the genus *Culex* are globally important vectors of human diseases (Allen *et al*., 2001). A *Culex* (*Culex*) *quinquefasciatus* Say is a widely dispersed domestic mosquito and vector for several diseases that affect wildlife and humans (Belkin, 1977; Ahumada *et al*., 2004), including dog heartworm (*Dirofilaria immitis*) avian malaria (*Plasmodium relictum*) avian pox (*Poxvirus avian*) and West Nile virus (Gubler, 1966; Warner, 1968; van Riper *et al*., 1986, 2002). *Cx. quinquefasciatus* Say is a strongly mammopophilic species, feeding extensively on

cattle, pigs and birds, but only occasionally on man, and are thus important in maintaining reservoirs of the virus in nature (Pant, 1979). A pictorial key of the Culicidae is also now available (Huang, 2001). Also, the taxonomic study of immature sages of mosquito is an essential for generating entomological data and suggesting appropriate control strategies with its correct identification to prevent the establishment of the exotic species of the *Culex* (*Culex*) in India.

A comparative analysis of the ventral brush and its attachment to segment X of fourth instar in the tribe Aedini provided important information for defining taxa at the species group and higher taxonomic level (Reinert, 2002). Most studies provide only limited details regarding variation in *Culex* species, their habitat and its environment (Aditya *et al*., 2006). The changing patterns of some of the diseases caused by pathogens vectored by *Culex* species are directly influenced by the variable climate in India (Bhattacharya *et al.,* 2006). This, probably, could be a major drawback in view of the importance of ecological factors in assessing and understanding the larval variation of species. Moreover, the wings of mosquitoes exhibit ridges or furrows, sometimes with thickening veins. The vestiges of the vein have suffered evolutionary loss or lines of stress or flexion in the membrane, are generally inconspicuous and, unless corresponding to an important element of standard venation, are mostly ignored by taxonomists (Colles, 1979).

The present study enumerates wing vein scale patterns and reveals some of the morphological variations in the fourth instar of *Cx*. *quinquefasciatus* Say.

MATERIALS AND METHODS

The *Culex quinquefasciatus* larvae were collected from various localities including urban, rural and semi-urban regions of Agra (27°10'N, 78°05'E) in India during a three- year period. We followed the terminology and morphological characters of Harbach & Knight (1980, 1982). The samples were collected between 6:00-8:00 am from different water containers, like waste plastic, tin containers, water in the roadsides burrow, ditches and flooded pastures and brought to the laboratory. The preparation and preservation of slides were according to Rattanarithikul (1982). The freeze drying methods were used for scanning electron microscopy of Harbach & Harrison (1983). We prefered to freeze dry 10 specimens collected from rearing. Each reared specimen was anesthetized with ethyl acetate and then placed into a petri dish. This petri dish was held over ice to slow down autolytic enzyme activity. After all the specimens were anesthetized, and the petri dish was placed into a freeze drying apparatus which had been precooled to -30 °C. Rapid freezing was essential, for it results in the formation

of small ice crystals, causing less damage to membranes and tissue. After the specimens were frozen, they were vacuum dried for a period of 2-3 days. The larvae were reared in a laboratory following standard methods (Gerberg, 1979). Drawings were then constructed under compound microscope with Camera Lucida and measured at 12X and 40X magnifications using a Motic Microscope (B1 series).

RESULTS

The wing veins were generally found to be covered with two types of scales: First scales were broad. It had a size of 100.45 µm 20.7 µm (Fig. 1). The distance between the two scales were recorded 15 µm vertically and 20 µm longitudinally. The area had 3-6 scales per 50µm^2 have been found. These wing scales were comprised of 12 veins. Whereas in the second type of the wing scales, they were geometrically narrow with a frequency of 3-6 scales per 50µm^2 (Fig. 2). They are the means of the two size classes of 20 specimens. Here we are describing there variation of means as such. These wing scales comprised of 9 veins. The length recorded was 100µm in length and 20 µm in width. Significantly, the distance between the two wing scales was 10.9µm vertically and 40µm longitudinally. The two scale type found in same region of the wing of the same specimen. These are not due to preservation but are genetic type of variation or mutations found in all the specimens studied. Also, the wing was covered with small setae, which have also been described for the first time and were at 4,000 X when recorded under SEM (Fig. 3). These were generally found to be scattered on the wings. The length was about 10 µm and with pointed distal end. The distance between two setae was about 2.5 µm, and their frequency 5 per 10-µm^2, which is the typical of the species, being described and recorded here for the study.

The setae on the larval skins and fourth instar larvae were compared with the work Sirivankaran and White (Table 1). Abdomen: segment VII setae 1-VII with 3-4 (3) branches, 2-VII single and well developed, 3-VII single, 4, 5-VII absent, 6-VII dendritic, 7-VII single, 8, 9-VII with 1-5 (2) branches, 10, 12 -VII single, 13-VII with 2-4 (3) branches. Setae 1-VIII with 4-8 (6) branches, 2-VIII with 3-6 (4) branches, 3-VIII with 6-10 (8) branches, 4-VIII single and 5-VIII with 3-6 (5) branches. Setae 1-X single, 2-X with 2-4 (3) branches, 3-X with 2-6 (4) branches, 4-X with 6-8 pair of setae (Fig. 4). The habitat -linked chaetotaxy has been found 100 % in dwellings, 91.7% in permanent ponds, 90% in the botanical garden of DEI, 85.8% in roadside ditches, 75% in cement tanks and temporary ponds (Table 2, Fig. 5). In the study, humidity was found to be correlated with the number of variations in specimens (Fig. 6) (Deposited in the Zoology Department Museum, No. EPL- CL 01).

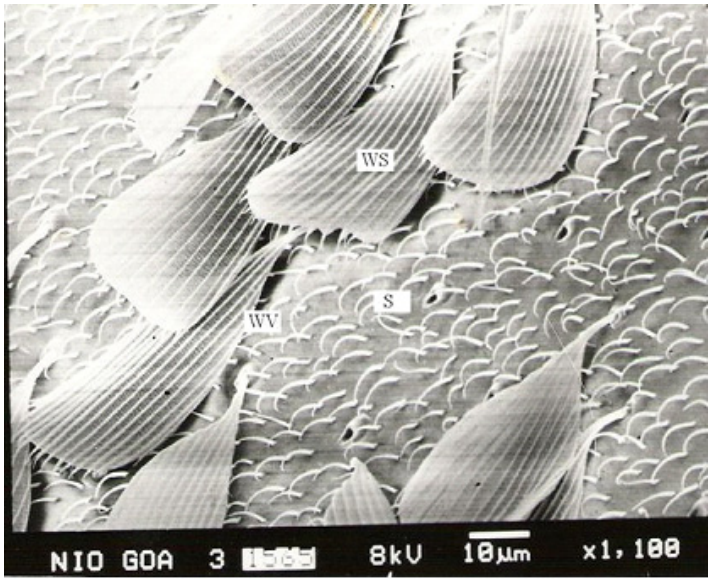

Fig. 1. Broad wing scales of *Cx. quinquefasciatus* Say scattered on wing with fine and smooth scales, WS- wing scale, WV- wing vein, S- seta.

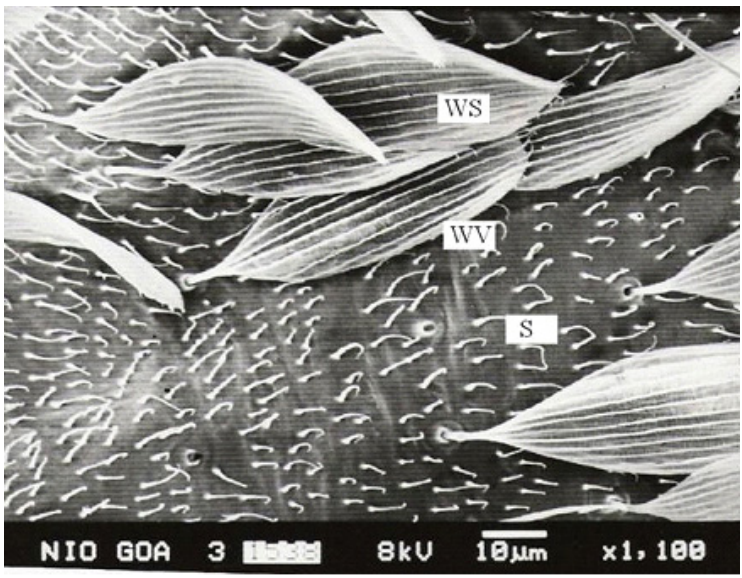

Fig. 2. Narrow wing scales with sharp apex of *Cx. quinquefasciatus* Say on the wing, WS- wing scale, WV- wing vein, S- seta.

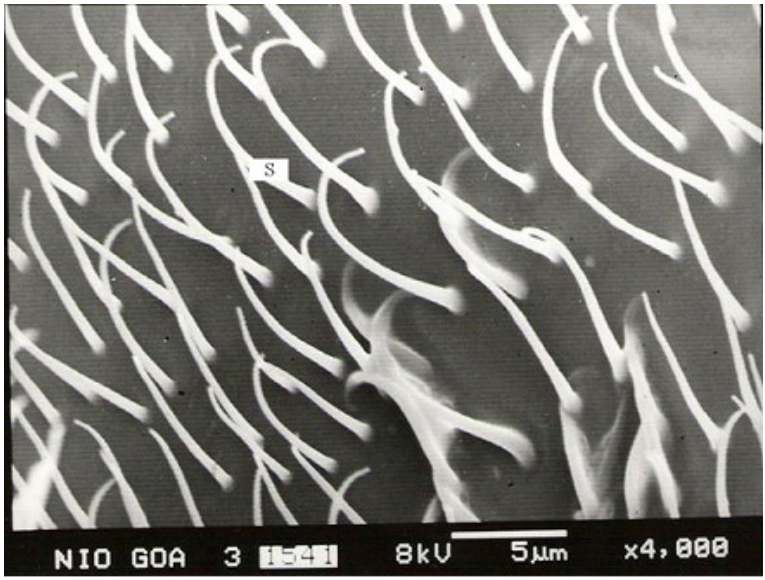

Fig. 3. Fine and smooth wing setae (S) of *Cx. quinquefasciatus* Say undetectable under compound microscope.

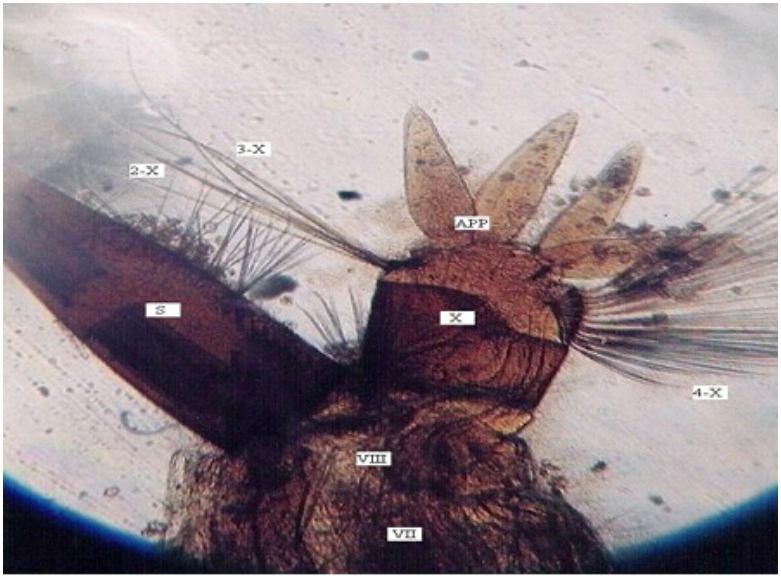

Fig. 4. Abdominal segment of Cx. *quinquefasciatus* Say: VII, VIII, X segment, S-siphon, APP- anal papilla, 2-X and 3-X- dorsal tufts, 4-X-Ventral brush.

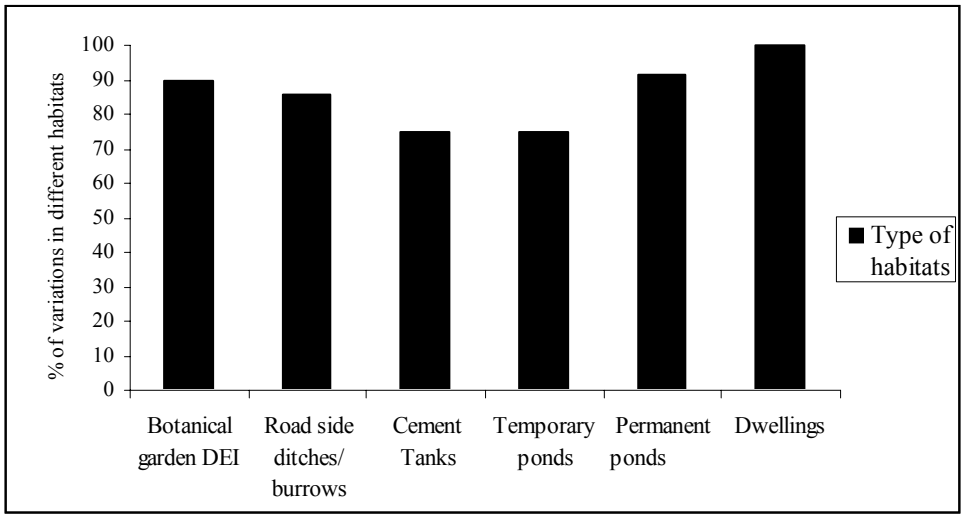

Fig. 5. Percentages of Chaetotaxy variations of *Cx. quinquefasciatus* Say of different habitats.

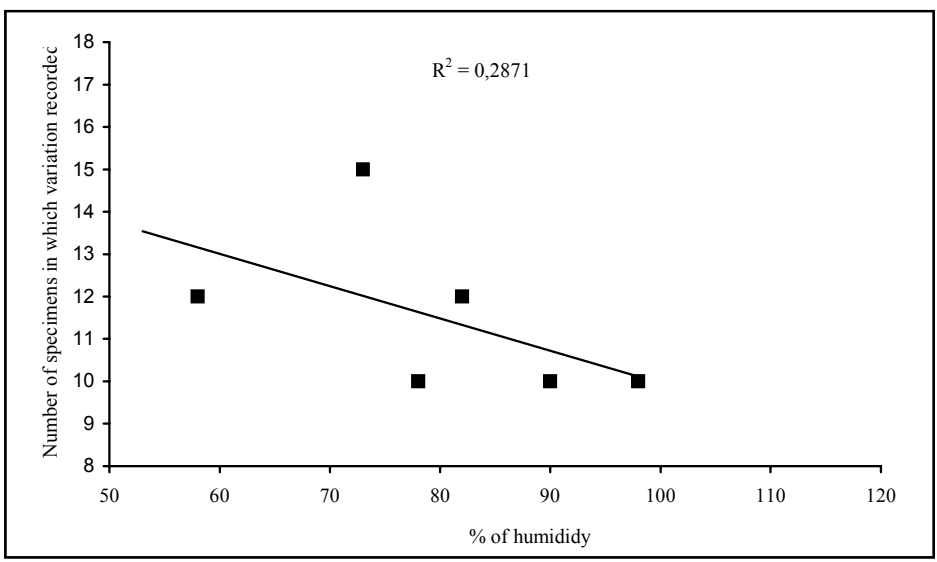

Fig. 6. Correlation between number of specimens in which variations were recorded and humidity.

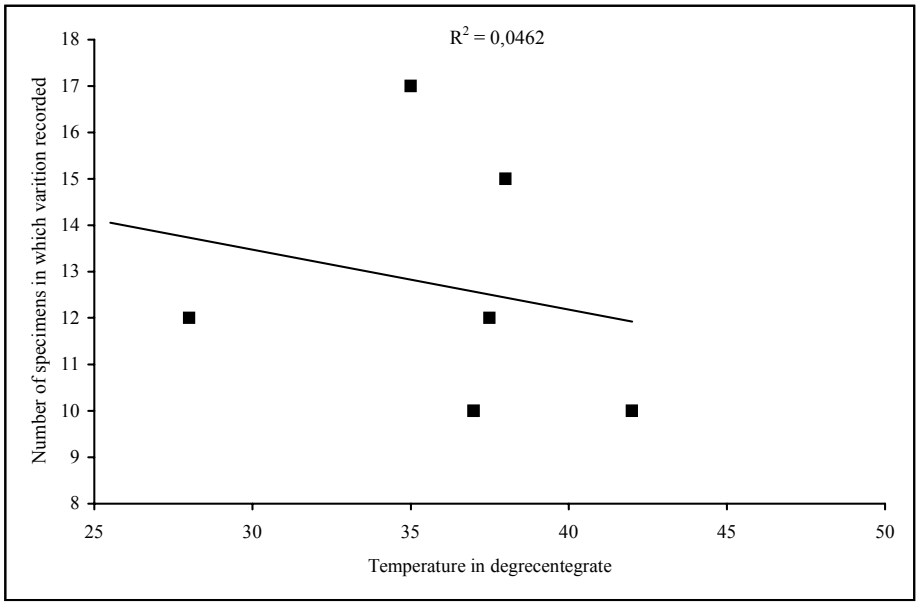

Fig. 7. Correlation between number of specimens in which variations were recorded and temperature.

DISCUSSION

The classification of mosquito genera, subgenera, and species is based on the structure and position of setae in larva and on the wing scales of adults. *Hodgesia* is a very small mosquito possessing scales on the outer ½ of the wing. Similarly, *Megarhinis albipes* wing was with scanty dark scales, forked long with broad scales (Barraud, 1934). To assist field workers in identifying mosquitoes in Africa, a pictorial key to male and female adults is now available (Huang, 2001). Whereas, a few of an additional characters have been necessarily added to facilitate the identification. This key can provide morphology of scales and setae. In the present study we have applied a scanning electron microscopy (SEM) technique to install some new taxonomic characters. So far it has not been detailed out under the higher magnifications. The magnifications have been increased from 1, 100X to 4, 000X, to elaborate the description, pattern and geometrical details of the scales. Also, this study in turn could provide better taxonomic characters. Onyeka (1982) in his study also revealed the taxonomic value of pre- alar scales of *Culex* (*Culex*) *pipiens* L. and *Culex* (*Culex*) *torrentium* Maritini, where the pre-alar scales in *Cx. pipiens* adult were reported and raised unreliability of this character as a diagnostic adult female. The presence and

Table 1. Comparisons of the morphological variations of fourth instar larvae of *Cx. quinquefasciatus* Say recorded by Sirivankaran and White (1978) with the present investigation.

Seta no.	Sirivankaran and White				Present investigation			
	VII	VIII	X	Siphon	VII	VIII	X	Siphon
0	1	-	-	-	1	-	-	-
1	3-4 branches	5-6 branches	single	9 branches, barbed	3,4 branches	4-8 branches	single	6-8 branches
2	-	single	1 short, 1 single	single	single, well developed	3-6 branches	2-4 branches	single
3	single	7-8 branches	single		single	6-8 branches	2-6 branches	
4	1,2	single	6 pair		-	single	6-8 pair	
5	-	4 branches			-	3-6 branches	-	
6	-	-	-		dendritic	-	-	
7	single	-	-		1,2	-	-	
8	-	-	-		2	-	-	
9	-	-	-		2	-	-	
10	single	-	-		single	-	-	
11	-	-	-		-	-	-	
12	single	-	-		single	-	-	
13	3 branches	-	-		2-4 branches	-	-	

Table.2. Larval variability observed in different types of habitat in *Cx. quinquefasciatus* Say from 2004-2006 at Agra.

S. No	Types of habitat	No. of specimens observed	Percentage of variations
1	Botanical garden DEI	50	90%
2	Road side ditches	35	85.8%
3	Cement Tanks	40	75%
4	Temporary ponds	20	75%
5	Permanent ponds	60	91.7%
6	Dwellings	40	100%

absence of only pre-alar scales were considered. However, in the present study the highest magnification by scanning electron microscopy revealed major dispositional differences and also the number of wing scale veins could be used significantly.

It is significant now that the scales have been considered as credentials for the taxonomic character of *Culex*. It has not been detailed so far and described under SEM technique. The narrow wing scale of *Melanoconion ocellatus* comprises with the 6 wing scale veins (Sirivankaran, 1982). Similarly, in C*hrysonotum* the clavate wing scales have been 5 wing scale veins and in S*pissipes* with 9 wing scale veins in the wing scale. In the present study, *Cx. quinquefasciatus* Say which was recorded under SEM technique. The basic differences in wing scale disposition, morphology, and the types are recorded, which is a distinct variation from the previous, disclosed species of the subgenus. Therefore, we can conclude that an accurate taxonomy of species and biological data now becomes important. Many new diseases and viral fevers are emerging which need information of larval biology to target larval control measures effectively (Ebsworth *et al.*, 2001). Whereas, Reinert (2002) has provided a comparative anatomical analysis of the ventral brush and its attachment to the segment X of the fourth instar. It was then conducted for the genera and subgenera in the tribe Aedini. Moreover, he reported four types and recognized eighteen sub types in the tribe Aedini. We are yet to record conclusively the exact larval identification characters as described by Sirivankaran and White (1978). Also, the variations in species were revealed in the state Nebraska (USA), of the both floodwater and in the standing water larva due to the differences in topography, vegetation, and climate (Janousek & Kramer, 1999).

In this present study we could only reveal a few of the significant larval character variation in the species, which is a new information to a known species with interfacing changing environmental conditions along with adult wing scale characters. At present we do not consider it as a rarely found variation. This we recorded from all sites of the selected regions. The keys of larval identification are based on the number and morphology of setae on each segment (Dodge, 1966). A single seta changes its position or number, and it warrants an immediate modification in the identification keys or larval descriptions. Similarly, in identification key of *Ae*. (*Stegomyia*) *albopictus* the median hair 1-VII was four (Huang, 1972). Whereas, Lamche and Whelan have reported the hair 1-VII three with a variability of larval identification character of the median hair (Lamche & Whelen, 2003). Also, we could observed in the present study, a correlation with the humidity (Fig.6). Whereas in case of the temperature it was found not perform a direct correlation.We have recently reported the microscopic variations in *Anopheles stephensi* Liston at Agra. Moreover, we have also recorded the species variations in *Lutzia*, *Armigeres*, *Stegomyia*, *Culex* and *Anopheles* from 2003-2006 in our field survey study. Recently, a new species of *Stegomyia* have been reported and the two new species were also identified from the genus of *Armigeres* and *Lutzia*. It validates the significantly changing environmental parameters and its role on mosquito diversity. This diversity could be the formation of a new species in semi-arid zone of India and hence require further study on its systematics. Hence, to dispose of the degree of unreliability and in sibling species identification, the setae clustering and geometry of the scales of the wing might help if it could be detailed under higher magnifications. These scales are detectable under SEM study presently which might help in further differentiating species. Moreover, a field survey with molecular sequencing details can be an essential validation. We now need also to find out the mosquito diversity in mitochondrial DNA (mDNA) in the semi-arid region of India in further cladistical stress also.

The present study, therefore, distinctly depicts that these wing scales of mosquito adult could provide many new taxonomic characters. It can be explored further in establishing wing scale characters as a valid variation in the architecture of the wing. It has not been described earlier that the environmental factors can also be reflect larval variations. This study helps to better our understanding of variation of *Cx*. *quinquefasciatus* Say in a semi-arid zone. A cladistical analysis could be initiated now to support mDNA studies and to evaluate evolution of genus *Culex*.

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