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GROUPING OF DIFFERENT MOSQUITO SPECIES ON THE BASES OF LARVAL HABITATS

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Morphological and molecular characteristics do not provide information on habitat characteristics of members of a particular taxonomic group. Due to habitat adaptations in the course of evolution, it is common for the closely related species to flourish in different habitats. To get information about habitat characteristics of insect species especially disease vectors is utmost important to control their populations under limits. So, in this scenario, present day mosquito ecologists start to classify mosquito species on the bases of their habitat characteristics. Therefore immature mosquitoes were collected from different habitat types and data were recorded for quantitative as well as qualitative parameters that were later used to group the species on the bases of habitats by using the cluster analysis. Twenty two mosquito species collected were grouped in three groups while the two forms *Ae. pseudotaeniatus* and *An. nigerrimus* have been represented separate as they do not share the habitat with any of the species. Two anopheline species viz. *An. stephensi* and *An. subpictus* have been found to occur in the same group while dengue vector species, *Ae. albopictus* and *Ae. aegypti* have been seen in separate groups.
Keywords: Mosquito, cluster analysis, agro-ecological zones, Punjab

INTRODUCTION

Classification of living organisms is generally based on apparent internal or external morphological characters. The systems of classification are supported by evolutionary evidences or paleontological records. Now a day, the existing systems are also supported by modern techniques of PCR and DNA fingerprinting. Morphological and molecular characteristics do not provide the information about habitat characteristics of members of a particular taxonomic group. Due to habitat adaptations in the course of evolution, it is common for the closely related species to flourish in different habitats. To get information about habitat characteristics of insect species especially disease vectors is utmost important to control their populations under limits. So, in this scenario, present day mosquito ecologists start to classify mosquito species on the bases of their habitat characteristics (Almiron and Brewer, 1996; Schafer and Lundstrom, 2001; Devi and Jauhari, 2007). For this purpose, cluster analysis are being used to make groups of species with dendrograms being constructed on the bases of similarity matrices. These dendrograms present an obvious picture for habitat preferences of the disease vector mosquitoes.

Pakistani Punjab has a vast seasonal and ecological diversity. Due to increase in urbanization resulting from fast growing population (Naeem-Ullah and Akram, 2009), change in climatic conditions has fesulted in recurring epidemics of mosquito borne diseases like dengue fever has taken place. It is therefore necessary to know about ecological factors that govern population fluctuations, for controlling the vector and diseases. In the Punjab, small scale studies have been carried out in the past for the said purpose (Aslamkhan and Salman, 1969; Reisen, 1978; Reisen and Boreham, 1979; Reisen and Milby, 1986) which need to be revised as they do not provide in depth studies (Herrel et al., 2001; Mukhtar et al., 2003). Present studies were therefore carried out from 2006-08 to cover the entire canvas of the Punjab, Pakistan from different agro-ecological zones during the winter, summer and rainy seasons (Rengel 2004 and Mohiuddin 2007).

MATERIALS AND METHODS

Immature mosquitoes were collected with the help of standard dipper from different habitats in (six) agro-ecological zones of the Punjab (Anonymous, 2004). Habitat types like wastewater near houses, catch basins (tap catch basin, rain catch basin), seepage pools, roadside pools, irrigation channels, rice area (Suleman et al., 1993), parks, ground waters, rock pools (Suleman and Khan, 1993), irrigated fields, wetlands, fish farm (Herrel et al., 2001), tires, tree holes (Akram and Lee, 2004), sewerage drain (Pramanik et al., 2007) etc. were explored while capturing the larvae. Biotic characteristics of the

collection sites were recorded that included predators, non-predators, land cover like plants, shrubs and grasses present in and / or around the collecting site along with onsite recording of abiotic parameters like Dissolved Oxygen (DO), Electric Conductivity (EC), pH (Akram and Lee, 2004), Total Dissolved Salts (TDS) (Adebote et al., 2008). Meteorological characteristics like temperature (Reisen et al., 1981) and Relative Humidity (RH) of the air (Akram and Lee, 2004) were also recorded during sampling.

Mosquitoes species collected were grouped according to similar breeding habitats (Almiron and Brewer, 1996 and Devi and Jauhari, 2007). Both qualitative(micro habitat, bottom, water quality, standing / flowing, color, light / shade, flora, predator, non-predator and habitat types in rural / urban) and quantitative (area size, DO, EC, TDS, pH, Temperature and RH) characters were used during this analysis. All these characters were subdivided into groups and everyone was considered as one parameter. Season and agro-ecological zones were also included. Codes of 1 / 0 (present / absent) were assigned to all these. A table of data was constructed in which all collected species of mosquito were placed as rows and all above mentioned environmental characteristics in (subcategorized 130) columns. Another table was constructed to show the presence of mosquito species in different habitat types in rural and urban areas. A basic data sheet was prepared on the basis of this codified data. This data sheet was employed to draw out the similarity for all collected species. The Dice (Crisci & Lopez Armengol 1983) association of coefficients was used to calculate similarity for all groups of species and later these species were grouped through means of similarity using cluster analysis. The groups thus formed was shown in a dendrogram.

RESULTS

On the basis of coefficients of association presented in the similarity matrix (Table 1), the larvae belonging to different mosquito species have been arranged into three groups with two species treated as separate. This arrangement has been presented in the form of a dendrogram (Figure 1). According to the dendrogram, Group A consists of *Culex quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. malayi*, *Anopheles stephensi*, *An. subpictus*, *Aedes albopictus* and *Ae. vittatus*. Whereas, *Verrallina yusafi*, *Ae. lineatopennis*, *Ve. indica*, *Ae. unilineatus*, *Ae. aegypti*, *Cx. minutissimus*, *Ae. caspius*, *Ae. subalbatus*, *Cx. bitaeniorhynchus*, *Coquillettidia crassipes*, *Cx. fuscocephala* and *Mimomyia chamberlaini* are included in Group B. It is the largest group according to the dendrogram. Group C consists of *Ae. w-albus* and *Lutzia fuscanus*. Two separate species that are not included in any group are *Ae. pseudotaeniatus* and *An. nigerrimus*.

According to the similarity matrix (Table 1), the highest association coefficient is 0.90 which is present between *Cx. quinquefasciatus* and *Cx. Tritaenio-rhynchus* therefore they are grouped together in A. On contrary, the lowest coefficient of association 0.39 has been calculated between *An. nigerrimus* and three species: *Ae. vittatus*, *Cx. pseudovishnui* and *Cx. quinquefasciatus*.

Species in Group A were found in all seasons of the year and from all agro-ecological zones with some exceptions at individual level. In this group, three distinct subgroups are also obvious (Figure 1). Least number of species were collected from Wet Mountains, only *Cx. quinquefasciatus*, *Ae. albopictus* and *Ae. vittatus* were found there. Some species of the group were found from whole elevation range of the survey area such as *Cx. quinquefasciatus* and to some extent *Ae. albopictus* and *Ae. vittatus*. All members of the group were inhabitants of tires and water reservoirs other than tires. *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Ae. albopictus* and *Ae. vittatus* were also found in tree-holes. Later two species were also collected from cans. Species in this group were found from habitats having mud, concrete, plastic, wood and rubber at bottom, although anophelines sub-cluster (*An. stephensi* and *An. subpictus*) were not found from plastic and wood indicating that they do not prefer cans or tree holes for lay eggs. All species of the group under consideration were found from all types of area sizes recorded during present study with the exceptions of aedine sub-cluster (*Ae. albopictus* and *Ae. vittatus*). They were not present in areas larger than 1000 m^2. First three species of the group were found from all water qualities but remaining five were not collected from clear foul water although they were present in other water qualities like clear, turbid and turbid foul. All members of the group were captured from standing as well as running water with the exception of *An. stephensi* and *Ae. vittatus*. They were never collected from flowing water. Aedine sub-cluster of this group was not found from the habitats where typha, water hyacinth and water lettuce covered the water and anophelines sub-group was not found from habitats having crops and water lettuce. This group occupies all quartiles of quantitative parameters (DO, EC, TDS, pH, temperature and RH) with the exception

Table 1 Similarity matrix calculated for grouping of mosquito species using the Dice Method

Ani	1.00																								
Ast	0.42	1.00																							
Asu	0.45	0.86	1.00																						
Aps	0.46	0.45	0.48	1.00																					
Avi	0.39	0.78	0.75	0.53	1.00																				
Alt	0.54	0.56	0.60	0.62	0.55	1.00																			
Aca	0.49	0.71	0.68	0.58	0.70	0.65	1.00																		
Aae	0.53	0.80	0.81	0.55	0.78	0.63	0.78	1.00																	
Aal	0.40	0.75	0.75	0.51	0.87	0.60	0.70	0.80	1.00																
Aun	0.53	0.64	0.68	0.62	0.68	0.78	0.76	0.71	0.70	1.00															
Awa	0.43	0.49	0.53	0.55	0.51	0.65	0.65	0.61	0.50	0.77	1.00														
Asb	0.43	0.66	0.66	0.64	0.76	0.63	0.74	0.76	0.75	0.75	0.62	1.00													
Vyu	0.57	0.53	0.57	0.62	0.57	0.80	0.70	0.69	0.62	0.76	0.73	0.69	1.00												
Cfu	0.47	0.74	0.78	0.57	0.73	0.64	0.73	0.78	0.75	0.73	0.59	0.62	0.64	1.00											
Cps	0.39	0.86	0.85	0.43	0.76	0.55	0.65	0.77	0.80	0.59	0.45	0.64	0.53	0.70	1.00										
Cqu	0.39	0.80	0.81	0.41	0.78	0.49	0.61	0.72	0.82	0.58	0.41	0.64	0.50	0.67	0.88	1.00									
Ctr	0.41	0.80	0.82	0.41	0.79	0.52	0.64	0.75	0.85	0.61	0.44	0.64	0.53	0.70	0.88	0.90	1.00								
Cma	0.47	0.82	0.85	0.46	0.79	0.58	0.70	0.80	0.83	0.69	0.51	0.68	0.58	0.77	0.87	0.84	0.84	1.00							
Cmi	0.61	0.70	0.74	0.56	0.68	0.69	0.78	0.83	0.72	0.78	0.70	0.74	0.71	0.71	0.68	0.66	0.69	0.77	1.00						
Cbi	0.64	0.64	0.64	0.66	0.63	0.68	0.74	0.76	0.62	0.72	0.66	0.65	0.71	0.74	0.58	0.54	0.56	0.65	0.78	1.00					
Lfu	0.51	0.60	0.59	0.50	0.53	0.57	0.61	0.67	0.54	0.62	0.65	0.52	0.62	0.70	0.51	0.47	0.49	0.55	0.61	0.66	1.00				
Mch	0.52	0.68	0.72	0.60	0.67	0.69	0.69	0.73	0.70	0.70	0.58	0.68	0.65	0.77	0.65	0.61	0.64	0.70	0.78	0.72	0.65	1.00			
Ccr	0.57	0.61	0.65	0.62	0.63	0.71	0.75	0.70	0.66	0.72	0.67	0.66	0.76	0.71	0.59	0.55	0.59	0.63	0.74	0.80	0.62	0.76	1.00		
Tin	0.51	0.69	0.74	0.55	0.73	0.74	0.79	0.75	0.76	0.87	0.68	0.77	0.74	0.73	0.67	0.66	0.69	0.75	0.83	0.72	0.62	0.75	0.75	1.00	

Ani. (An. nigerrimus), Ast. (An. stephensi), Asu. (An. subpictus), Aps. (Ae. pseudotaeniatus), Avi. (Ae. vittatus), Alt. (Ae. lineatopennis), Aca. (Ae. caspius), Aae. (Ae. aegypti), Aal.(Ae. albopictus), Aun. (Ae. unilineatus), , Awa. (Ae. w-albus), Asb. (Ar. subalbatus), Vyu. (Ve. yusafi), Cfu.(Cx. fuscocephala), Cps. (Cx. pseudovishnui), Cqu. (Cx.quinquefasciatus), Ctr. (Cx. tritaeniorhynchus), Cma. (Cx. malayi), Cmi. (Cx. minutissimus), Cbi. (Cx. bitaeniorhynchus), Lfu.(Lt. fuscanus), Mch. (Mi. chamberlaini), Ccr. (Cq. crassipes), Tin. (Ve. indica).

Figure 1

Dendrogram

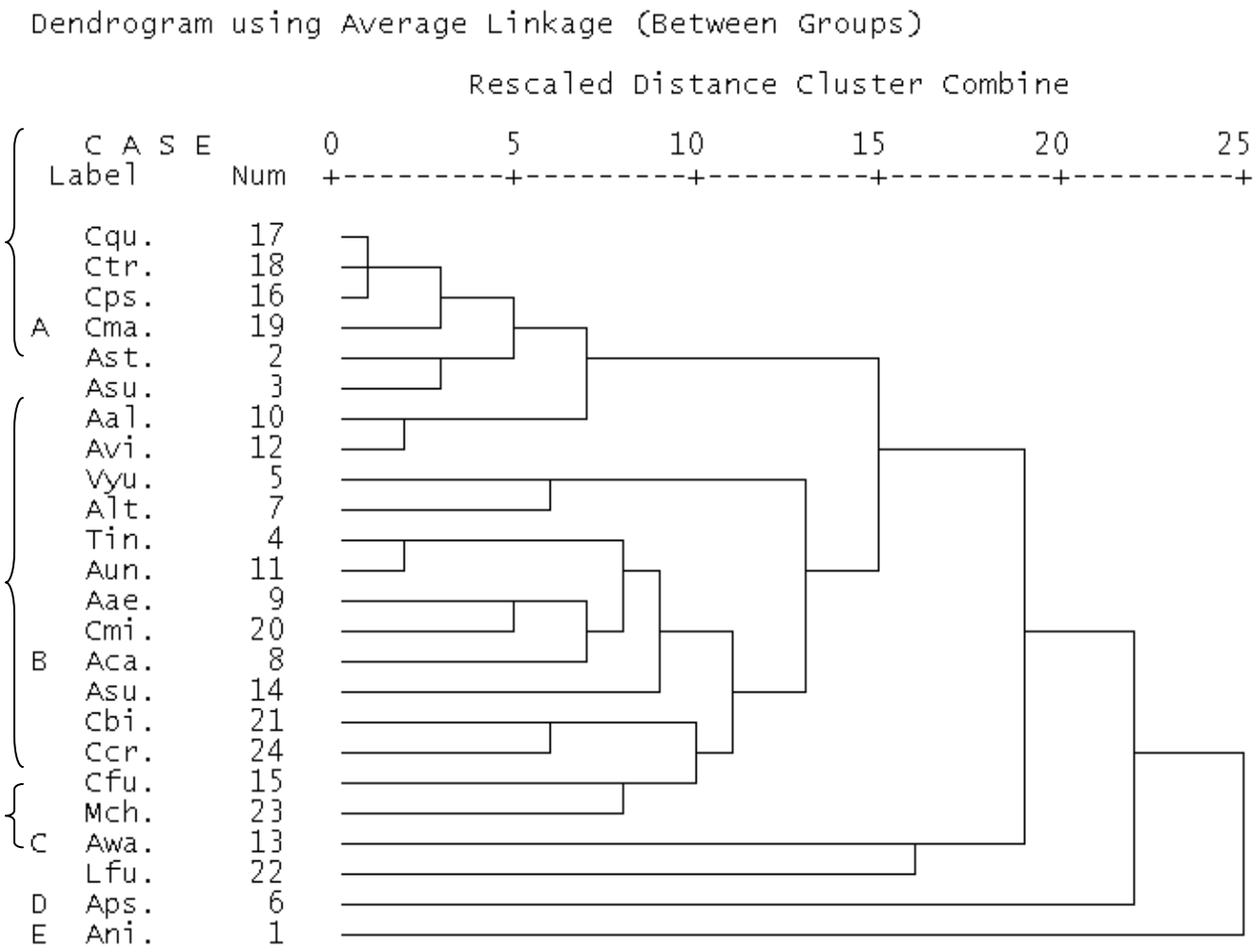

Table 2 Habitats Types in Rural (*) and Urban (x) Areas Contributing in Cluster Analysis

Group	A												B									C		D	E
Habitats? Species?	Cqu	Ctr	Cps	Cma	Ast	Asu	Aal	Avi	Vyu	Alt	Tin	Aun	Aae	Cmi	Aca	Asb	Cbi	Ccr	Cfu	Mch	Awa	Lfu	Aps	Ani
Farm House		x					x																	
Near Agriculture Field	*x	*x	*		*	*		x														*		
Rice Area	*	*	*				*						*		*		x							x
Livestock Farm	*x	*				*x													*					
Poultry Farm						*														*				
Fish Farm					*																			
Watercourse	*x	*	*				*		*	*														
Tap Catch Basin	*	x	x			x		*																
Seepage Pool	*x	*	*	*	*	*					*	*		*	*	*					*			
Roadside Pool	*x	*x	*	*		x	*x		*		*x			*		*		*x		x				
Wetland			*		*	*							*											
Irrigated Field	*	*																						
Wastewater Near Houses	*x	*x	x	x	*x	x																		
Stagnant Pool	*x	*x	*x	*x	x	x	*						x				x		x	*				
Drain	*x	*x	*x	*x	x	x													x					
Gutter			x																					
Garbage	*x		x																			x		
Rock pool		*					*	*								*								
Broken Boat	*	x		x			*	*x			*	*				*								
Lakes		*x					*			*	*	*												
Parks	*x	*x	*x	x	*		x	*x											x					
Rain Catch Basin	*x	*x	*x	x	x	x	*x	*x					*x	x	x	*x				*			*	

of aedine sub-group that was not present in lower limits or higher limits or both for these quantitative characteristics. Most of the members of this group were not recorded from pH below 6.5 and temperature below 11 ^{o}C. Larvae of the species in Group A were captured almost from all habitat types ranging from rural to urban with the exception at individual species level. No species was found from gutter in the rural areas (Table 2).

According to the dendrogram, (Figure 1), Group B is the largest one which is further sub-divided into small sub-units. All species of this group were recorded during rainy season and many were recorded in summer with the exception of *Ae. yusafi*, *Ae. lineatopennis*, *Ve. indica* and *Ae. unilineatus*. No member of the group was recorded from Suleiman Piedmont. Only *Cq. crassipes*, *Ae. aegypti* and *Ar. subalbatus* were recorded from Sandy Desert A (Cholistan), Sandy Desert B (Thal) and Wet Mountains, respectively (one from each mentioned zone). Most were recorded from Northern Irrigated Plains with the exceptions of *Cx. bitaeniorhynchus* and *Cq. crassipes*. Although the members of the group were recorded from all elevation except first (<100 m) but majority were found at 100 – 200 m above sea level.

All of the species were collected from water reservoirs. *Ae. aegypti*, *Cx. minutissimus* and *Ar. subalbatus* were also found from tires with later from cans as well. None of the species was present in tree-holes. Same is the case with bottom of the habitat that no species were collected from the habitats having wood at bottom. Majority of species inhabited large water reservoirs (100 m^2 to 1000 m^2). *Ve. yusafi* was found only from large sized areas (100 m^2 – 1000 m^2) while *Cq. crassipes* was found only from medium sized area (10 m^2 – 100 m^2). As far as water quality of sampling site is concerned, majority of these species were collected from turbid water with the exception of *Ae. caspius*, that was present in clean and turbid foul waters. *Ae. aegypti* was collected from clear, turbid and turbid foul sampling sites and not from clear foul. All were recorded from standing water but *Ve. yusafi*, *Ae. lineatopennis*, *Cq. crassipes*, *Cx. fuscocephala* and *Mi. chamberlaini* were recorded from both standing and flowing water. Majority of the members of this group were present in exposed and exposed shady habitats while *Cx. minutissimus* and *Cx. fuscocephala* were found only from exposed shady environment. Most of the species were present with (except *Ve. yusafi* and *Cx. fuscocephala*) and / or without (except *Cx. bitaeniorhynchus*) algae however all were present in waters having no typha except *Cx. fuscocephala* that was found with or without typha. All were present in habitats having grasses in it rather a few were not present if grass was absent like *Ve. yusafi*, *Ae. lineatopennis* and *Cx. bitaeniorhynchus*. Majority were collected from habitats having no crops in it. All species in this group were collected from environments with and /or without debris. Only one, *Cx. fuscocephala* was found in habitats having water hyacinth while none was present in waters having water lettuce. All species were found to have different level of association with predators and non-predator species.

Most members of the group were captured with low to medium range of quantitative parameters of the aquatic habitats. For example, majority were present within the first three quartiles of DO (<3.6 – 10.8 mg/L) and first quartile of EC (<4380 µS/m) and TDS (<2163 ppm). All were recorded from pH ranging from 6.5 to 9.3 with some exceptions i.e., *Ve. indica*, *Cx. bitaeniorhynchus* and *Cx. fuscocephala* were also recorded from pH above 9.3. None was recorded at temperatures below 11 ^{o}C and only five *Ve. indica*, *Ae. aegypti*, *Ar. subalbatus*, *Cx. fuscocephala* and *Mi. chamberlaini* were present in temperatures between 11 ^{o}C to 22 ^{o}C. Most were found from temperatures above 33 ^{o}C. As far as RH is concerned, most were captured with atmospheric RH between 25 to 75 %. *Ae. aegypti* and *Cq. crassipes* were also found below 25 % RH while *Ae. lineatopennis* and *Mi. chamberlaini* were present at RH above 75 %.

No species from this group was collected from agriculture field, fish farm, tap catch basin, irrigated field, wastewater near houses, drain, gutter, garbage and park from rural environments. Mostly were recorded from seepage pool and roadside pool. *Ae. aegypti* was recorded from rice area, wetland and rain catch basin from rural habitats. In urban environments, most of them were collected from rain catch basin. Other location types in urban environments where mosquitoes were recorded include rice area, roadside pool, stagnant pool, drain and park. *Ae. aegypti* was collected from stagnant pool and rain catch basin from urban areas.

According to the dendrogram, Group C consists of only two species, *Ae. w-albus* from tribe Aedini and *Lt. fuscanus* from tribe Culicini. They share many ecological characters but also at the same time, they differ in some. Both of them were found from only one agro-ecological zone i.e., Northern Irrigated Plains at a same group of elevation above sea level (100 – 200 m). They were not collected from tires, tree-holes and cans but only from water reservoirs having mud at

bottom and area size between 100 – 1000 m^2 (large size), although *Lt. fuscanus* was found from area size larger than 1000 m^2, too. As for as, water quality of their habitat was concerned, *Ae. w-albus* was found in clear water while *Lt. fuscanus* was found to be the inhabitant of turbid foul water. But both were found from only standing water and not from flowing in the exposed shady type of habitats. *Ae. w-albus* was found from habitats having no algae, typha, crop, debris, water hyacinth and water lettuce except grass. On the other hand, *Lt. fuscanus* was found in habitats with or without algae, typha, with grass and debris and without crop, water hyacinth and water lettuce. They both were present in the absence and presence of predators and non-predators. As for as the quantitative characters are concerned, both forms were found in water reservoirs having DO =< 3.6 mg/L, EC =< 4380 µS/m (although *Lt. fuscanus* was present in higher EC and TDS values), TDS =< 2163 ppm, pH between 7.91 to 9.3 and RH = 25 – 50 %. Temperature ranges for both forms were different. *Ae. w-albus* was found at temperature > 33 ^{o}C and *Lt. fuscanus* was present between 11 to 33 ^{o}C. Former was captured from seepage pools in rural environments and the later was caught from water course near agriculture field in rural environment and garbage from urban areas (Table 2). Finally the two forms *Ae. pseudotaeniatus* and *An. nigerrimus* have been represented separately in dendrogram as they do not share the habitat with any of the species.

DISCUSSION

On the basis of ecological characteristics, groups of larval populations of different mosquito species were organized by some earlier workers like Almiron and Brewer (1996) and Devi and Jauhari (2007). In these studies, cluster analysis was used to make groups and in the present work, the same method was used to construct dendrogram to show groups of larval mosquitoes of the Punjab, Pakistan on the basis of their habitats. Almiron and Brewer (1996) recorded 19 species (Operative Taxonomic Units) into four groups and Devi and Jauhari (2007) proposed three groups and two separate species. In present study, 24 species of mosquito immature are organized under three distinct groups and two separate species. In the study conducted by Devi and Jauhari (2007), *An. subpictus* was not included in any group and mentioned as a separate entity, but under present study, the species is included into Group A and share similar habitat characteristics as *An. stephensi*, *Cx. malayi* and some other culicine and aedine species (Figure 1). In this group, *Cx. tritaeniorhynchus* is also included. Reisen et al., 1981 reported positive larval association between *An. subpictus* and *Cx. tritaeniorhynchus*. Group A also describes some similarities in habitat characteristics between *An. subpictus* and *Cx. quinquefasciatus* under present study. These results are not in agreement with those recorded by Reisen et al., 1981 who reported negative larval association between these species and also with those reported by Devi and Jauhari (2007) who did not included these species in similar group as in present study. Under current work, *Cx. quinquefasciatus* showed much similarity in habitat characteristics with *Cx. tritaeniorhynchus* and *Cx. pseudovishnui*. These results are in agreement with those obtained by Reisen et al., 1981who mentioned positive larval association among these species. *Ae. albopictus* was also present in Group A during present captures with *Cx. quinquefasciatus*, *An. stephensi*, *An. subpictus*, *Ae. vittatus* and other species. These results are partially in agreement with those obtained by Devi and Jauhari 2007, who included *An. stephensi* and *Ae. albopictus* in same group as in present study and *Ae. albopictus*, *An. subpictus* and *Cx. quinquefasciatus* into separate groups which is different from current work. *Ae. albopictus* and *An. nigerrimus* are included in different groups according to dendrogram in current work and it is not in agreement with the results obtained by Devi and Jauhari 2007, who included these species in the same group in their dendrogram. *An. stephensi* is present in same group with *Ae. albopictus* and *Cx. pseudovishnui* as presented by Devi and Jauhari 2007, who reported these species in the same group. They recorded *Cx. vishnui* instead of *Cx. pseudovishnui*, but Reisen et al., 1981 reported negative larval association of *An. stephensi* with *Cx. pseudovishnui* and *Cx. tritaeniorhynchus* that is not in agreement with the current findings. Under present work, *An. stephensi* is not present in the same group with *An. nigerrimus* and *Lt. fuscanus* and it is supported by the findings of Reisen et al., 1981 who reported negative association of *An. stephensi* with both above mentioned species.

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