2. Vector Biology and Control

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2.1. Mosquito biodiversity in mangrove forest ecosystem (A. R. Rajavel, V. P. Soniya, R. Natarajan & K. Vaidyanathan; IM 9634 VBC; Duration : 7.5 years, Jun 1997 – Dec 2004)

The mangrove forests of Chorao in Goa, Vikhroli in Mumbai, Kundapur in Karnataka and Kannur in Kerala on the western coast and Muthupet/Maravakadu in Tamil Nadu and Coringa in Andhra Pradesh on the eastern coast of India were surveyed for mosquito species diversity in the year 2004. Characteristics of these mangroves were given in the previous annual reports. A total of 37 species (including 2 undetermined), belonging to 13 genera and 19 subgenera, were recorded in these mangrove forests (Table 2.1). Swamp pools, crab holes and tree holes were the larval habitats. *Aedes albopictus, Culex sitiens, Uranotaenia atra* and *Verrallina lugubris* were the common species occurring in most of these mangroves. Of the 37 species recorded, *Ae. albopictus*, *Anopheles subpictus*, *Cx. sitiens*, *Cx. tritaeniorhynchus*, *Cx. malayi*, *Ochlerotatus wardi*, *Ur. atra* and *Ve. lugubris* occurred on both the eastern and western coasts. The number of species recorded in different mangroves was, 12 in Chorao, 9 in Vikhroli, 26 in Kundapur, 6 in Kannur, 9 in Muthupet/Maravakadu and 7 in Coringa. While 6 to 12 species only were obtained in most of the mangroves, the higher number of species in Kundapur was due to the occurrence of several species of genus *Culex* and *Mansonia* obtained as adults in this area where the mangroves are confluent with the adjacent agro-ecosystem.

2.2. Biodiversity of mosquitoes in the Eastern hill ranges of India (A.R. Rajavel, R. Natarajan and K. Vaidyanathan; IM 0301 VBC; Duration: 3 years, Jan 2005 – Dec 2007)

Following the preliminary surveys in Noamundi district of Jharkhand and Keonjhar district of Orissa reported in 2003, surveys for mosquito biodiversity were made during September-October 2004 in the forested hilly terrain of these districts. Collections were done in Badakomarda (22° 11′N; 85° 31′E), Poorthidigia (22° 09′N; 85° 33′E), and Murga (22°06′N; 85°28′E) in Noamundi district and Mamalapusi (21° 33′N; 85° 28′E), Saharpur (21° 35′N; 85° 24′E), and Badagosda (21° 35′N; 85° 25′E) in Keonjhar district.

Larvae were collected from several habitats including stream, paddy field, pond, bamboo, tree hole, crab hole, leaf axil and well. Adult collections included indoor resting in human dwelling and cattle shed, out door resting, light trap catches and biting adults. Altitudes at which collections were made ranged from 1200 to 1450 ft in Badakomarda, 1300 to 1550 ft in Poorthidigia, 1600 to 1800 ft in Murga, 1700 to 2000 ft in Mamalapusi, 1800 to 2000 ft in Saharpur and 1800 to 1900 ft in Badagosda. In Noamundi, 24 species belonging to 7 genera and 12 sub genera were recorded, while in Keonjhar, 37 species (including 4 undetermined species) in 10 genera and 12 subgenera were recorded (Table 2.2). Together with the following 9 species, *Aedes trimaculata, Anopheles aitkeni, Armigeres magnus, Culex murrelli, Cx. peytoni, Malaya genurostris, Ochlerotatus albolateralis, Oc. khazani,* and

Orthopodomyia anopheloides recorded during the present collection, the total number of species in this region of the Eastern hills is 84, belonging to 13 genera and 23 subgenera.

Table 2.1. Mosquito species recorded in different mangrove regions during 2004 (X denotes presence)

Genus	Subgenus	Species	Chorao	Vikhroli	Kundapur	Kannur	Muthupet/ Maravakadu	Coringa
Aedes	Cancraedes	cancricomes						X
	Diceromyia	iyengari			X			
		reginae					X	X
	Fredwardsius	vittatus			X			
	Lorrainea	fumidus						X
	Stegomyia	albopictus	X	X	X	X	X	
		novalbopictus	X	X				
Anopheles	Cellia	jamesi			X	X		
		subpictus	X	X			X	
Armigeres	Armigeres	aurolineatus			X			
		subalbatus	X		X	X		
Culex	Culex	bitaeniorhynchus			X			
		cornutus			X			
		gelidus	X		X			
		pseudovishnui			X			
		quinquefasciatus			X			
		sitiens	X	X	X		X	X
		tritaeniorhynchus			X	X	X	X
	Eumelanomyia	malayi		X	X		X	
	Lophoceraomyia	infantulus			X			
		minor			X			
		uniformis			X			
		undt.	X					
		undt.		X				
Heizmannia	Heizmannia	chandi			X			
Lutzia	Metalutzia	fuscana			X			
Mansonia	Mansonoides	indiana			X			
		uniformis			X			
Ochlerotatus	Finlaya	cogilli			X			
	Rhinoskusea	portonovoensis					X	
		wardi		X	X			X
Orthopodomyia		anopheloides	X					
Toxorhynchites	Toxorhynchites	splendens	X					
Tripteroides	Rachionotomyia	affinis			X			
Uranotaenia	Pseudoficalbia	atra	X	X	X	X	X	
		stricklandi	X					
Verrallina	Verrallina	lugubris	X	X	X	X	X	X

Table 2.2. Mosquito species recorded in Noamundi and Keonjhar during September-October 2004 (X mark denotes presence)

Genus	Subgenus	Species	Noamundi	Keonjhar
Aedes	Aedimorphus	trimaculata		X
	Fredwardsius	vittatus	X	
	Stegomyia	albopictus	X	X
		unilineatus		X
Anopheles	Anopheles	aitkeni		X
		barbirostris	X	
		peditaeniatus	X	
	Cellia	annularis	X	
		culicifacies	X	X
		fluviatilis	X	X
		maculatus		X
		minimus		X
		pallidus	X	
		subpictus	X	X
		tessellatus		X
		theobaldi		X
		vagus		X
		varuna	X	X
Armigeres	Leicesteria	magnus	X	X
Culex	Culex	bitaeniorhynchus	X	X
		fuscocephala		X
		murrelli	X	X
		pseudovishnui	X	
		quinquefasciatus	X	
		vishnui	X	
	Culiciomyia	pallidothorax	X	
	Eumelonomyia	brevipalpis		X
		malayi	X	
		sp. undt.		X
	Lophoceraomyia	infantulus	X	X
		minor		X
		minutissimus	X	
		peytoni		X
		sp. undt.		X
		sp. undt.		X
Heizmannia	Heizmannia	chandi	X	X
Lutzia	Metalutzia	fuscana	X	
		halifaxii	X	
Malaya		genurostris		X
Ochlerotatus	Finlaya	albolateralis		X
		gubernatoris	X	X
		khazani		X
Orthopodomyia		anopheloides		X
Tripteroides	Rachionotomyia	affinis		X
		aranoides		X
Uranotaenia	Pseudoficalbia	bicolor		X
		luteola		X
		recondita		X
		sp. undt.		X
	Uranotaenia	longirostris		X

2.3. Vector Control Research Centre Mosquito Museum (VCRCMM) – Reference collection and bar codes of mosquito species (A.R. Rajavel, V.P. Sonia, R. Natarajan and K. Vaidyanathan; IM 0301VBCa; Duration: 11 years, Jan 1998 – Dec 2008)

As on date, there does not exist a comprehensive reference collection of mosquito species in the country. The available reference specimens are scattered in various institutions and are not properly catalogued and preserved. Lack of such a facility is reflected in the limited mosquito taxonomic work that has been carried out in India. The need for a comprehensive reference collection of mosquitoes in the country has been recognized for a long time.

An initiative was therefore taken to establish a centralized collection of mosquito species prevalent in India that would serve as reference material for taxonomic studies and training in mosquito identification. This initiative resulted in the development of the Vector Control Research Centre Mosquito Museum (VCRCMM). Mosquito collections made as part of biodiversity studies in the mangrove forests and in other ecotypes of India were identified, curated and catalogued for this purpose. The current collection in the VCRCMM includes 14,800 adult specimens of which 8,426 are individually pinned and labeled, while the rest are held in stock vials. It also includes 791 male genitalia and 257 female genitalia preparations and 1,124 larvae, 815 larval exuviae, and 444 pupal exuviae on microscope slides.

About 105 species have associated dissected genitalia and 86 species have associated larval and pupal exuviae on microscope slides. Mosquitoes of 19 genera, 37 subgenera and 181 species are represented in the collection. A list of the species is given in Table 2.3. The specimens currently housed in the mosquito museum represent about 50% of the known mosquito species of India. It also forms the single largest collection in terms of specimens and species to be maintained at a specified location in the country. This museum facility could serve as a repository for the deposition of mosquito specimens.

With the advent of molecular systematics the trend now is to develop molecular methods for species identification. Though molecular applications do not represent a substitute for morphological study, because these techniques by themselves cannot be used to resolve formal taxonomical problems, molecular characterization of species in terms of 'bar codes' is increasingly becoming an important component of global bio-diversity studies and development of such a database would be of additional value to the reference collection. Therefore, 'DNA bar-coding of mosquito species' was initiated. Sequences of 45 species of mosquitoes, accurately identified and including collection details (Table 2.3) have been developed and deposited in the GenBank along with citation to the voucher specimens deposited in the VCRCMM.

2.4. Reappearance of Anopheles minimus in Singhbum hills, East-Central India (P. Jambulingam, S. S. Sahu & A.M. Manonmani; DIF 0303 VBC/PD; Duration: 6 months, Mar 2004 – Sep 2004)

Keonjhar district in Orissa State has been endemic for malaria for many decades, with more than 95% of the cases due to *P. falciparum* infection. During 1997-2002, malaria incidence in the district showed an increasing trend with the Annual Parasite Incidence ranging from 31.8 to 36.3%. On the request of the district administration of Keonjhar, a situation analysis was carried out by the VCRC in August 2001. During the survey, among other vector species, *An. minimus* was also recorded. This species had been absent for nearly 45 years since the launching of the Malaria Eradication Programme and was believed to have disappeared from this part of India.

The recording of *An. minimus* prompted us to carry out four more surveys during 2003-2004, one in summer, one in the rainy and two in the cold seasons, for confirmation. Day time indoor

Table 2.3. Mosquito species in museum

Genus	Subgenus	Species
Anopheles	Anopheles	aitkeni, annandalei, barbirostris, gigas, nigerrimus, peditaeniatus, sinensis, umbrosus
	Cellia	aconitus, annularis, culicifacies, fluviatilis, jamesi, jeyporiensis, karwari, kochi, maculatus, minimus, moghulensis, pallidus, philippinensis, pseudojamesi, splendidus, stephensi, subpictus, sundaicus, tessellatus, theobaldi, vagus, varuna
Aedeomyia	Aedeomyia	oatasticta
Aedes	Aedimorphus	alboscutellatus, caecus, jamesi, lowisii, pallidostriatus, pipersalatus, syntheticus, vexans
	Cancraedes	cancricomes, simplex
	Christophersiomyia	annulirostris, thomsoni
	Diceromyia	iyengari, periskelatus, ramachandrai, reginae
	Fredwardsius	vittatus
	Lorrainea	amesii, fumidus
	Neomelaniconion	lineatopennis
	Stegomyia	aegypti, albopictus, annandalei, edwardsi, imitator, krombeini, malayensis, novalbopictus, pseudoalbopictus, seampi, unilineatus, w-albus
Armigeres	Armigeres	kesseli, kuchingensis, subalbatus, theobaldi
	Leicesteria	flavus, longipalpis, magnus
Coquillettidia	Coquillettidia	crassipes
Culex	Culex	barraudi, bitaeniorhynchus, cornutus, epidesmus, edwardsi, fuscocephala, gelidus, hutchinsoni, infula, luzonensis, mimuloides, mimulus, murrelli, perexiguus, perplexus, pseudovishnui, quinquefasciatus, sinensis, sitiens, tritaeniorhynchus, vishnui, whitei, whitmorei
	Culiciomyia	bailyi, fragilis, nigropunctatus, pallidothorax, spathifurca
	Eumelanomyia	brevipalpis, foliatus, khazani, hinglungensis, malayi
	Lophoceraomyia	bengalensis, infantulus, mammilifer, minor, minutissimus, pilifemoralis, rubithoracis, uniformis, wilfredi
Culiseta	Culiseta	indica
Ficalbia		minima
Heizmannia	Heizmannia	chengi, covelli, chandi, himalayensis, indica
Hodgesia		bailyi
Lutzia	Metalutzia	fuscana, halifaxi
Malaya		genurostris, jacobsoni
Mansonia	Mansonioides	annulifera, indiana, uniformis
Mimomyia	Etorleptiomyia	luzonensis
	Mimomyia	chamberlaini, hybrida
Ochlerotatus	Bruceharrisonius	greeni
	Finlaya	albolateralis, assamensis, chrysolineatus, cogilli, gubernatoris, harveyi, khazani, lophoventralis, niveus, pseudotaeniatus, pulchriventer
	Kenknightia	dissimilis
	Mucidus	scatophagoides
	Ochlerotatus	caspius
	Rhinoskusea	longirostris, portonovoensis, wardi
Orthopodomyia		andamanensis, anopheloides, flavicosta
Toxorhynchites	Toxorhynchites	splendens
Tripteroides	Rachionotomyia	affinis, aranoides, serratus
	Tripteroides	indicus
Uranotaenia	Pseudoficalbia	atra, bicolor, luteola, maculipleura, nivipleura, obscura, recondita, unguiculata
	Uranotaenia	annandalei, campestris, christophersi, edwardsi, longirostris, macfarlanei, orientalis, testacea
Verrallina	Harbachius	yusafi
	Neomacleaya	andamanensis, cauta, indica, pseudomediofasciata, unca
	Verrallina	butleri, lugubris

resting collections in human dwellings and cattlesheds, CDC light traps catches in human dwellings and cattlesheds and man landing collections between 1800-2100 hrs were carried out and the catches comprised a total of 3858 anophelines belonging to 18 species, including *An. fluviatilis* (24.4%), *An. culicifacies* (6.3%) and *An. minimus* (3.5%).

In total, 135 *An. minimus* were collected, 115 from hand catches in human dwellings and 20 from man landing catches. 98 were obtained during the rainy season and 37 during the cold season in five hilltop villages. In summer, the species could not be collected. A total of 24 *An. minimus* was dissected and no specimen harboring sporozoite was found.

Sequencing of four adult specimens showed that they were species A of the *An. minimus* complex. The D3 sequences were identical to those deposited by Chen et al. (2002) for the same species of this complex (Acc. No. AJ 459420). *An. minimus*, in this survey, was collected from villages situated in hill ranges and surrounded by forest. Since malaria incidence is showing an increasing trend in this area in recent years, further studies are needed to understand the extent of distribution of *An. minimus,* its bionomics and role in malaria transmission in this region.

2.5. Morphological and molecular characterization of Anopheles minimus and An. fluviatilis (A.M.Manonmani, A.R.Rajavel, R.Natarajan & S.S.Sahu; IM 0403 VBC/PD; Duration: 3 years, Aug 2004 – Jul 2007)

The identification of *Anopheles fluviatilis* and *An. minimus,* important vectors of malaria, is problematic due to occasional overlapping of morphological characters in the adults, requiring confirmation from associated larval and pupal exuviae. Hence, the present study was taken up to understand the frequency of such overlapping characters in F1 progenies, and to simultaneously sequence various regions of the rDNA and design DNA probes for distinguishing the two species.

The objectives of the project are (i) to study the frequency of variations in morphological characters and determine stable characters for differentiating the two species; (ii) to sequence the various regions of the ribosomal DNA in morphological variants and look for differences if any; and (iii) to design/modify the DNA probes for distinguishing the two species.

During 2004, samples of *An. fluviatilis* and *An. minimus* collected from Keonjhar district of Orissa were used for standardizing the PCR assays. The sequences of the D3 domain of the 28S gene and the Internal Transcribed Spacer 2 regions of the ribosomal DNA of these two species showed that the populations of *An. fluviatilis* and *An. minimus* occurring in Keonjhar district of Orissa, belong, respectively, to species S and species A (Table 2.4). The sequences have been deposited in gene bank and Accession numbers have been obtained (Acc. Nos. AY 841147-841148).

As proposed in this study, morphological variants, feral females and their progeny have to be studied to confirm whether these differences in the sequences are constant and stable.

2.6. DNA bar coding of mosquito species (Diptera : Culicidae) - A molecular approach to the mosquito biodiversity in India (N Pradeep Kumar, A.R. Rajavel, R. Natarajan and P. Jambulingam; IM 0405 VBC/PD; Duration : 4 years, Apr 2004 – Mar 2008)

The objectives of the project are (i) to construct species specific "DNA bar codes" for different mosquito species prevalent in India; (ii) to analyze the phylogenetic relationships and devise a classification engine for the systematics of mosquitoes in India; and (iii) to study the intra-specific relationships of important vector species of mosquitoes.

For the first time, species-specific DNA bar codes were constructed for some mosquito species prevalent in India. Mosquitoes collected from different states of India, viz., Pondicherry, Andhra Pradesh, Karnataka, Goa, Orissa and

Table 2.4. DNA sequence of the rDNA-D3 and ITS2 region of An. fluviatilis & An. minimus

	Nucleotide positions	
	rDNA-D3 region	rDNA-ITS2 region
	7 7 9 1	1 1 2 1 1 1 2 3 3 3 3 3 3 3 3 3 3 3 3
	7 8 3 6	8 9 1 7 7 7 0 2 4 4 4 5 5 5 5 5 5 6 6
	2	6 7 9 0 6 1 2 6 1 2 3 4 5 6 8 4 5
An. fluviatilis S	A C T T	A T T T C C G – C A A T A G A T G C A C
An. minimus A	G A G C	C A C G T C T A G G C G C A T G A A T A

Jharkhand, towards updating the mosquito fauna in India, were used for this study. Adult and larval collections were done in the field. Larvae were individually reared to adult stages and associated larval and pupal skins were mounted. Adults which emerged were used for DNA extraction. Corresponding larval and pupal skins mounted were deposited in the museum as reference material. Also, the genitalia was mounted and deposited in the museum when male specimens were used for DNA extraction. Wherever adult collections were carried out, females were isolated for oviposition and F1 generation adults were used for DNA extraction. In this case larval, pupal skins as well as F1 generation female were deposited with the museum. All these voucher specimens were provided museum identification numbers. Identification of species was done based on standard taxonomic keys.

The extracted DNA from individual specimens was purified and about 500 base-pairs of the 5' region of Cytochrome c Oxidase subunit I (COI) gene of the mitochondrial genome were amplified using conserved insect DNA primers. The PCR fragments were purified and custom sequenced. These sequences were aligned using 'ClustalW' and were subjected to molecular evolutionary analytical tools such as 'DnaSP4' and 'MEGA2'. A genetic distance (Kimura 2 Parameter) of 0.03 was considered the threshold level of nucleotide diversity for con-specificity. The phylogenetic analysis using different approaches of these data is in progress to understand the evolutionary trends of the family Culicidae.

DNA sequences of COI were elucidated for 53 individual mosquitoes, which belonged to 45 haplotypes, corresponding to 45 identified species of mosquitoes, belonging to 15 genera. These sequences, which form the DNA bar codes for these species, were deposited with the GenBank. The details of the species and their respective GenBank accession numbers are provided in Table 2.5.

The study is in progress so as to include all the species of Culicidae prevalent in India and also involve multiple specimens for each species.

2.7. Relative contribution of rainwater harvesting systems (RWHS) towards proliferation of mosquito vectors – A comparative study in Cuddalore and Pondicherry towns (T. Mariappan, R. Srinivasan and P. Jambulingam; IM 0402 VBC; Duration: 2 years, Jun 2004 – May 2006).

Rainwater harvesting system (RWHS) is mandatory for all buildings in Tamil Nadu, both public and private, as per the order “Tamil Nadu Municipal Laws Ordinance-2003”. The Government has specified standard designs for constructing RWHS in households to harvest rainwater. Despite the specifications, many inhabitants have designed the RWHS to suit their convenience leading to deviations from the standard. A preliminary investigation carried out to assess the mosquito breeding potential of such RWHS showed that 21% of them supported *Aedes* mosquito breeding due to faulty construction. Therefore, a systematic investigation was carried out in two urban areas *viz*., Cuddalore, where

Table 2.5. Details of GenBank submissions of DNA bar code sequences for mosquito species prevalent in India

Sl No.	Museum ID No.	Genera (Subgenera)	Species	Locality	Collection details GPS data	GenBank Accession Number
1	A11459	Anopheles (Cellia)	*stephensi*	Pondicherry	11°59.6'N; 79°51.1'E	AY729980
2	A11512	"	*culicifacies*	Jharkhand	22°09.3'N; 85°33.7'E	AY917198
3	A11510	"	*minimus*	Jharkhand	21°33.3'N; 85°28.7'E	AY917196
4	A11505	"	*fluviatilis*	Orissa	21°33.3'N; 85°28.7'E	AY917202
5	A11511	"	*annularis*	Jharkhand	22°09.3'N; 85°33.7'E	AY917197
6	A11438	"	*subpictus*	Pondicherry	12°07.6'N; 79°36.7'E	AY729970
7	A11606	"	*subpictus*	Orissa	21° 33.4'N; 85°28.7'E	AY917203
8	A11440	"	*jamesii*	Pondicherry	12°07.5'N; 79°36.7'E	AY729972
9	A11442	"	*pallidus*	Pondicherry	11°55.7'N; 79°45.7'E	AY729974
10	A11509	"	*pallidus*	Jharkhand	22°09.3'N; 85°33.7'E	AY917212
11	A11500	"	*vagus*	Pondicherry	11°50.0'N; 79°44.0'E	AY834247
12	A11508	"	*splendidus*	Jharkhand	22°11.6'N; 85°31.5'E	AY917207
13	A10486	Anopheles (Anopheles)	*aitkenii*	Jharkhand	21° 35.9'N;85°25.0'E	AY917209
14	A11462	"	*barbirostris*	Pondicherry	11° 53.3'N;79°41.0'E	AY729982
15	A11448	Culex (Culex)	*quinquefasciatus*	Pondicherry	11°47.5'N; 79°46.4'E	AY729977
16	A11443	"	*tritaeniorhynchus*	Pondicherry	11°49.0'N; 79°46.0'E	AY729975
17	A11444	"	*tritaeniorhynchus*	Pondicherry	11°47.3'N; 79°48.0'E	AY917206
18	A11502	"	*tritaeniorhynchus*	Pondicherry	11°53.3'N; 79°41.0'E	AY834249
19	A11441	"	*vishnui*	Pondicherry	11°56.0'N; 79°46.0'E	AY729973
20	A11530	"	*vishnui*	Pondicherry	12°00.4'N; 79°41.0'E	AY917214
21	A11501	"	*psuedovishnui*	Pondicherry	11°53.3'N; 79°41.0'E	AY834248
22	A11532	"	*psuedovishnui*	Pondicherry	11°53.5'N; 79°40.0'E	AY917215
23	A11470	"	*infula*	Pondicherry	11°53.4'N; 79°43.0'E	AY729983
24	A11428	"	*gelidus*	Pondicherry	11°57.0'N; 79°45.4'E	AY729965
25	A11435	"	*murrelli*	Pondicherry	11°57.0'N; 79°45.4'E	AY729968
26	A11430	Culex (Lophoceromyia)	*infantulus*	Pondicherry	11°57.0'N; 79°45.4'E	AY729966
27	A11461	Culex (Lophoceromyia)	*rubithoracis*	Pondicherry	11°53.3'N; 79°41.0'E	AY729981
28	A10490	"	*minor*	Jharkhand	21°35.2'N; 85°25.3'E	AY917211
29	A11445	Culex (Culiciomyia)	*nigropunctatus*	Pondicherry	11°57.0'N; 79°45.4'E	AY729976
30	A11478	Culex (Lutzia)	*fuscanus*	Pondicherry	11°57.1'N; 79°48.0'E	AY729985
31	A11491	Aedes (Stegomyia)	*aegypti*	Pondicherry	11°57.1'N; 79°48.6'E	AY729987
32	A11475	"	*albopictus*	Pondicherry	11°57.1'N; 79°48.0'E	AY729984
33	A10211	"	*albopictus*	Karnataka	12°52.2'N; 74°52.1'E	AY834241
34	A11529	Aedes (Aedimorphus)	*vexans*	Pondicherry	11°51.0'N; 79°48.3'E	AY917213
35	A11499	Aedes (Fredwardsius)	*vittatus*	Karnataka	11°58.5'N; 79°47.5'E	AY834246
36	A11451	Aedes (Lorrainea)	*fumidus*	Andhra Pradesh	16°53.0'N; 82°15.1'E	AY729978
37	A10120	Ochlerotatus (Finlaya)	*cogilli*	Karnataka	12°52.2'N; 74°52.1'E	AY834240
38	A11490	Armigeres (Armigeres)	*subalbatus*	Pondicherry	11°49.1'N; 79°47.1'E	AY729986
39	A9802	"	*subalbatus*	Karnataka	12°46.0'N; 74°52.0'E	AY834244
40	A11439	Mansonia (Mansonioides)	*annulifera*	Pondicherry	11°49.0'N; 79°46.0'E	AY729971
41	A11494	"	*uniformis*	Pondicherry	11°53.1'N; 79°49.0'E	AY729988
42	A10197	Heizmannia (Mattinglyia)	*discrepans*	Karnataka	12°56.2'N; 75°09.0'E	AY834242
43	A10454	Heizmannia (Heizmannia)	*chandi*	Karnataka	21°36.0'N; 85°25.0'E	AY917208
44	A11421	Uranotaenia(Pseudoficalbia)	*atra*	Goa	15°25.0'N; 73°55.0'E	AY917201
45	A10451	"	*bicolor*	Orissa	21°33.5'N; 85°29.0'E	AY917205
46	A10551	"	*recondita*	Jharkhand	21°36.0'N; 85°25.0'E	AY917199

Sl No.	Museum ID No.	Genera (Subgenera)	Species	Locality	Collection details GPS data	GenBank Accession Number
47	A11497	Verrallina (Neomaclaya)	*indica*	Pondicherry	11^{0}58.5'N; 79^{0}47.5'E	AY834245
48	A11434	Ficalbia	*minima*	Pondicherry	11^{0}48.0'N; 79^{0}46.0'E	AY729967
49	A11437	Aedomyia	*catasticta*	Pondicherry	12^{0}07.2'N; 79^{0}36.3'E	AY729969
50	A11458	Mimomyia (Mimomyia)	*chamberlaini*	Pondicherry	12^{0}04.4'N; 79^{0}53.0'E	AY729979
51	A10579	Orthopodomyia	*anopheloides*	Jharkhand	21^{0}35.6'N; 85^{0}25.2'E	AY917200
52	A10487	Tripteroides (Rachionotomyia)	*aranoides*	Jharkhand	21^{0}36.0'N; 85^{0}25.0'E	AY917210
53	A10450	Malaya	*genurostris*	Orissa	21^{0}35.1'N; 85^{0}25.3'E	AY917204

RWHS has been implemented at household level, and Pondicherry, where no such system is introduced, to assess the contribution of RWHS to mosquito vector proliferation in relation to other habitats.

A cross sectional survey was made during June-August 2004 and a total of 792 and 753 houses in Cuddalore and Pondicherry respectively were sampled employing a stratified systematic sampling technique. All the potential habitats including RWHS in and around each household were encountered and examined for mosquito breeding. Mosquito immatures were sampled using dipper in cement tanks and bucket in wells; while absolute count was made in all other habitats.

In Cuddalore, RWHS has been installed in 651 households (82.19%), while in Pondicherry none of the households had such structures. Of the 651 RWHS recorded 99.5% were meant for recharging the ground water, where rainwater was let into either well or percolation pit. The rest were used for storage in cement tank or metal drum. About 58.95% of RWHS were constructed adopting the standard specifications and the remaining (41.05%) had defects. Mosquito breeding was observed in 16.1% of the RWHS. Of the 2983 adult mosquitoes comprising eight species, obtained from pupae and larvae collected from both RWHS and other habitats in Cuddalore, 1593 were contributed by RWHS; in Pondicherry, 1384 adults were obtained from immatures collected from peri-domestic habitats. Mosquito species recorded from various habitats during the survey are given in Table 2.6.

Table 2.6. Mosquitoes emerged from immatures collected from various habitats during RWHS survey

Species (%)	Cuddalore		Pondicherry	
	RWHS	Other habitats	RWHS*	Other habitats
Aedes aegypti	74.7	73.1	-	87.2
Ae. albopictus	11.2	5.7	-	7.8
Anopheles stephensi	7.7	6.9	-	1.2
Culex quiquefasciatus	2.8	9.9	-	3.2
Cx. tritaeniorhynchus	1.4	1.8	-	0.0
Cx. vishnui	1.2	1.1	-	0.0
Armigeres subalbatus	0.8	1.4	-	0.6
An. hyrcanus gr.	0.2	0.1	-	0.0
Cx.(L) fuscanus	0.0	0.02	-	0.0

* No rain-water harvesting system recorded in Pondicherry,

2.8. Laboratory evaluation of NeemAzal T/S 1.2% EC for its larvicidal and insect growth regulating activity against three vector species, Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti (K. Gunasekaran and C. M. R. Reddy; SP 0202 VBC; Duration: 11 months, Feb 2003 – Dec 2004)

Larvicidal and insect growth regulating activity of NeemAzal T/S 1.2% EC was studied in the laboratory respectively against early 4th and early 3rd instar larvae of *An stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. Among the three species tested, *An.stephensi* was highly susceptible to the NeemAzal T/S as indicated by the least LC_{50} and LC_{90} values (1.9 & 2.8 ppm). LC_{50} against *Ae.aegypti* and *Cx.quinquefasciatus* was four and eight times higher.

The formulation produced an overall mortality or inhibition of emergence of 90% (when 3rd instar larvae were treated) at 0.046, 0.208 and 0.866 ppm in the three vector species, *An.stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* respectively. The corresponding EI_{50} values were 0.006, 0.048 and 0.249 ppm (Table 2.7).

The concentration at which the NeemAzal T/S caused 90% EI in *Cx.quinquefasciatus* was four to ten times higher when compared to Fenoxycarb, diflubenzuron and methoprene, the other common insect growth regulators (IGR).

The morphogenetic abnormalities induced by NeemAzal T/S fell into five categories viz., enlarged distended fourth instars, dechitinized extended pupae, pupae and adult intermediate (partially emerged adult), partially exuviated adult with part of the abdomen contained within the pupal case and adult attached to pupal case by legs. The percentage of dead specimens of any stage showing morphogenetic abnormalities was the maximum in *Cx.quinquefasciatus* (14.4%; n=2113) followed by *Ae. aegypti.*

2.9. Small and medium scale field evaluation of an IGR, Novaluron 10% EC against Culex quinquefasciatus in Pondicherry (P. Jambulingam, C. Sadanandane and N. Nithiyanandan; EM 0203 VBC; Duration: 1 year, Apr 2003 – Mar 2004)

Novaluron 10% EC, a new acylurea compound and chitin synthesis inhibitor, was evaluated under World Health Organisation Pesticide Evaluation Scheme (phase II & phase III) against *Culex quinquefasciatus* in Pondicherry. The objective was to determine the efficacy, residual effect and field application dosage of the IGR. The insect growth regulator (IGR) was tested at three dosages viz., 1, 5 & 10 mg/m² in cesspits, drains, and abandoned wells and the results were given in VCRC Annual Report 2003. During the reporting year, medium scale evaluation (phase III) was carried out using the field application dosages selected in phase II trial.

The results of phase III trial were in agreement with that of small-scale trial. The IGR was found effective, producing 80 -100% Emergence Inhibition (EI) for 11 - 14 days in cesspits at 1mg/m² (Fig. 2.1a) and for 14 -19 days at 10 mg/m² (Fig. 2.1b). In the abandoned wells, application of Novaluron at 5 mg/m² was effective (80 -100% EI) for a period of 37 - 60 days (Fig. 2.1c). The study showed that Novaluron 10% EC can be used for the control of *Cx. quinquefasciatus,* at the application rate of 1 mg/m² at 10 days interval in cesspits, at 5 mg/m² at 60 days interval in abandoned wells and at 10 mg/m² at fortnightly interval in street

Table 2.7. EI_{50} and EI_{90} of NeemAzal T/S against the three mosquito vectors

Species tested	Emergence Inhibition (ppm)			
	EI_{50}	95 % CL	EI_{90}	95% CL
An.stephensi	0.0060	0.0053 – 0.0069	0.0460	0.0362 – 0.0583
Cx.quinquefasciatus	0.0482	0.0440 – 0.0528	0.2083	0.1697 - 0.2556
Ae.aegypti	0.2494	0.2308 – 0.2696	0.8657	0.7406 – 1.0118

drains. At the lowest dosage, 1mg/m^2, the IGR was effective in suppressing adult emergence at least for a week in cesspits and street drains and for a month in disused wells.

2.10. Phase II evaluation of granular (WG) formulation (2%) of an IGR, diflubenzuron, in comparison to its wettable powder (WP) formulation (25%), against Culex quinquefasciatus in Pondicherry (C. Sadanandane, P. S. Boopathi Doss and P. Jambulingam; EM 0401 VBC; Duration: 10 months, Jul 2004 – Apr 2005)

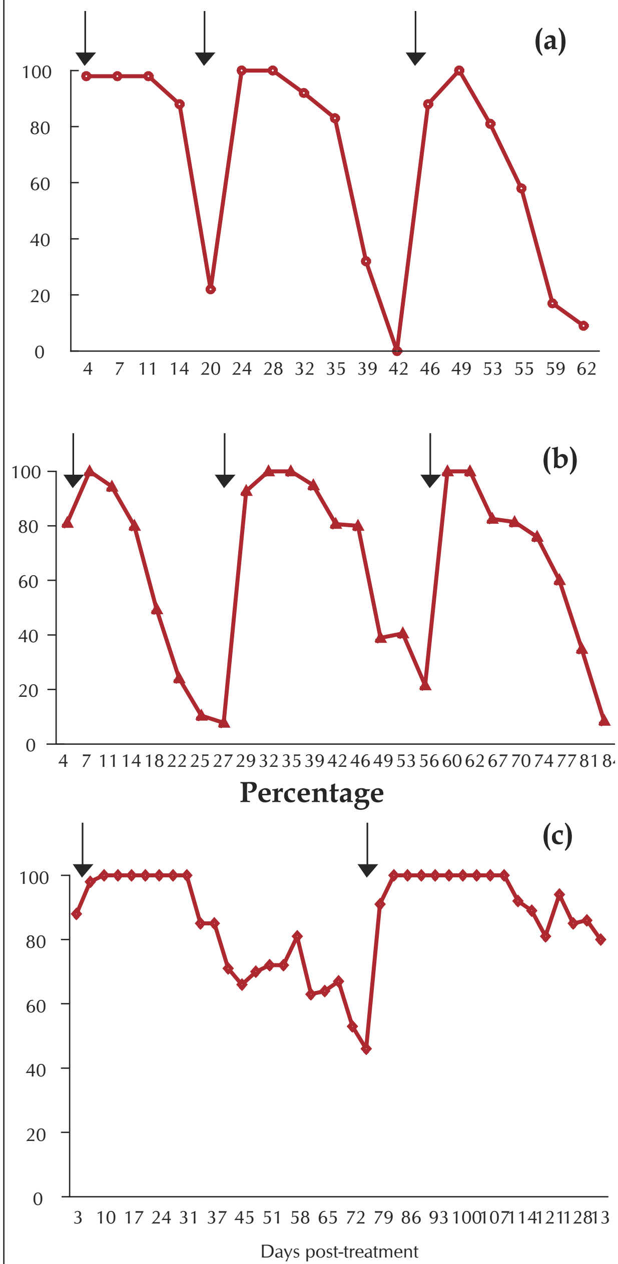
Diflubenzuron 2% GR, a new formulation was, evaluated against *Culex quinquefasciatus* under World Health Organization Pesticide Evaluation Scheme (WHOPES) (phase II trial) in comparison to the WP (25%) formulation, which is widely used for mosquito control. The objective of the trial was to determine efficacy, residual effect and field application dosages for the two formulations.

The formulations were tested at four application rates, 25, 50, 75 & 100 g/ha, in cesspits and street drains in and around Pondicherry. Larval and pupal density and adult emergence were monitored in these habitats using dippers and emergence traps on alternate days before (2 weeks) and after application of IGR. The dosage at which >80% larval and pupal mortality or emergence inhibition was observed for a longer duration was selected as the field dosage for each habitat.

In cesspits, the 2% WG formulation was effective yielding > 80% EI for 10 days at all application rates tested, while the 25% WP was effective for 7 days at 25 and 50 g/ha and for 10 days at 75 and 100 g/ha. In drains, the WG formulation caused >80% EI for 7 days at 100g/ha and 4 days at 25, 50 and 75 g/ha. The WP formulation yielded >80% EI for 7 days at all dosages tested. Evaluation in disused wells is in progress.

Fig. 2.1. Emergence inhibition of Cx.quinquefasciatus adults in Novaluron 10% EC

Treated (a) cesspits (1 mg/m2) , (b) drains (10 mg/m2) and (c) wells (5mg/m2).



2.11. Phase II evaluation (experimental hut) of lambdacyhalothrin 10% CS in `comparison to lambdacyhalothrin 10% WP for residual spraying against malaria vectors (P. Jambulingam, K. Gunasekaran and S. S. Sahu; EM 0301 VBC; Duration: 1 year, Mar 2004 – Apr 2005)

As a part of development of newer insecticides or formulations against malaria vectors, the two formulations of lambdacyhalothrin were tested (under WHOPES) at two dosages, 20 and 30 mg/m^2, against *An. fluviatilis* in Malkangiri district of Orissa State. Each dosage was applied in four experimental huts built in the study villages. Another four huts were used for comparison. To attract mosquitoes into the experimental huts overnight, one volunteer from the same village slept in each of the huts from 20.00 hours to 06.00 hours. Informed consent was obtained from these volunteers. The next morning mosquitoes were collected from the room and verandah of the hut. The efficacy of the formulations was determined in terms of reduction of entry rate, exit rate, blood feeding rate and mortality rate of the major malaria vector, *An. fluviatilis*.

A total of 62 collections were carried out in each of the twenty experimental huts, 4 prior to treatment and 58 post-treatment. Among the 17 anopheles species collected during the study period, *An. fluviatilis* was the predominant species forming about 67.3% of the total collection. *An. culicifacies,* the secondary vector, constituted 13.8% of the collections.

Prior to treatment, the entry rates of *An. fluviatilis* into the experimental huts ranged from 29.1 to 46.1 per hut and the groups were comparable ($F = 0.293$, $df = 4$, $P<0.05$) with respect to the entry rates. During post-treatment, the number of *An. fluviatilis* which entered into the untreated huts was 613 per hut. The overall reduction of the entry of *An. fluviatilis* during post-treatment period ranged from 89.0 to 96.7% in the treated huts. The reduction of entry rates in the huts treated at 30mg/m^2 WP, 20mg/m^2 CS and 30mg/m^2 CS was significantly higher than that into the huts treated at 20mg/m^2 WP.

The exit rates of *An. fluviatilis* recorded from the huts of different experimental groups ranged from 1.2 to 6.5% prior to the treatment and were comparable ($p>0.05$). During the post-treatment period, the exit rate from the untreated huts was 25.2% and from the treated huts it was significantly ($p<0.05$) higher, ranging from 77.8 to 86.0. There was no significant ($P> 0.05$) difference in the exit rates between the treatment groups.

After the treatment, the feeding rates of *An. fluviatilis* were significantly ($P<0.05$) lowered in the treated huts compared to that in the untreated huts and the same ranged from 70.4 to 77.8%. However, there was no significant difference in the feeding rates between the treatment groups ($p>0.05$).

The mortality rate in the untreated huts was 0.3% during post-treatment while the same was 46.6%, 47.8%, 52.7% and 74.8% in the huts treated at 20mg/m^2 WP, 30mg/m^2 WP, 20mg/m^2 CS and 30mg/m^2 CS respectively. The mortality of *An. fluviatilis* in the huts treated at 30mg/m^2 CS was significantly ($P>0.05$) higher than that in the huts treated at other dosages.

Interview of the spray man revealed no other side effect except for complaint of slight headache, which lasted for about 30 minutes. There was no complaint of any sort from the volunteers who slept in the experimental huts. The volunteers observed that Lambadacyhalothrin formulations had no odour or stain on surfaces. They also perceived the benefit of reduction in the mosquito nuisance in the treated huts.

2.12. Acceptability of long lasting treated nets (LLTN). (K. Gunasekaran, K. N. Vijayakumar, S. S. Sahu & P. K. Das; EM 0201 VBC; Duration: 19 months, May 2003 - Dec 2004)

The aim of the study was to determine factors related to acceptance and use of long lasting permethrin treated net (LLTN) in a rural society and to assess it's washing practices and willingness to buy LLTN. Two districts viz., Malkangiri and Koraput in Orissa State, India, were selected for the study. In each district, two groups of villages, high and moderate/low malaria endemic, were included. In the villages of Malkangiri district, ITMN programme was already ongoing while in the villages of Koraput district there was no such programme. The study design including net distribution has already been communicated in the Annual Report 2003. The results summarized in this report are the outcome of the first evaluation conducted at the end of the first peak of transmission following distribution of nets.

Scheduled tribes formed the predominant group (53%-89%) of the target population and about 50% had no formal education. Malaria was ranked as the most common health problem. Though the population has a relatively lower literacy rate, the proportion of respondents who associated malaria with mosquito bites was considerably high. This might be due to their exposure to malaria and its control programmes for many years. There were, however, some misconceptions about the transmission of malaria.

The majority of people in the two districts consider malaria a serious problem, mainly due to income loss, expenditure towards treatment and severe sufferings. Some people did not regard malaria as a serious problem and one of the reasons attributed for their perception was that the use of LLTN had prevented the occurrence of malaria fever.

Seeking treatment, including for malaria, from the traditional healers was the most common practice of the tribes, especially for their children. Only a few people used to approach hospitals/ medical doctors for treatment. Financial constraints and non-availability of health facilities prevent the tribes from seeking modern treatment. Introduction of ITMN or LLTN, or both, had a visible impact amongst the people and most of them perceived that sleeping inside mosquito nets could reduce the occurrence of malaria. In general the villagers, particularly the tribes, practice traditional methods to protect themselves against mosquito bites and only a small proportion uses modern methods. But, after the introduction of ITMN or LLTN, there was a change in people's behaviour. The villagers who were depending on traditional measures switched over to nets for protection against mosquito bites. Such behavioural change showed the acceptability of nets. Since, the majority was aware that mosquito bites cause malaria and use of nets could protect them against mosquito bites, people were ready to buy a net when it was offered by the government at a subsidized price. In the villages where the ITMN programme was not launched, only a few owned other nets, purchased from the market. The majority, who did not have other nets, said that they could not afford to buy from the market. Though it was affordable for some people they did not know where to buy it or there was no store selling it. Nylon net was the one commonly available with the people; no one had a polyethylene net. However, this would not reflect the preference of the people to a particular type of net, as it was mainly depending on the availability.

The villagers, as soon as ITMN or LLTN was distributed, quickly adapted themselves to the use of nets. A higher percentage of children <5 years and pregnant women, who are vulnerable groups, used to sleep under nets. Usage of net by children was relatively higher during the rainy season, followed by the cold season. Due to lower mosquito density and high night-time temperatures, the use rate of nets during summer was relatively low. In the villages where there was no ITMN programme and people were not accustomed to use nets, they managed to hang their LLTN and used them effectively. About 73%-85% of the LLTN distributed were found hanging inside the houses, indicating that those nets were under use. People used to wash the nets either monthly or bimonthly. In case of LLTN, since its distribution (i.e. during the period of six months), the majority of recipients washed it once or twice. Soap and water were commonly used for washing ITMN or LLTN.

Regular users of LLTN were in considerable proportion. High night-time temperatures was the main reason attributed in cases of irregular use. Some people experienced good ventilation inside LLTN due to its larger hole-size and stated that they could sleep under the net even during summer. In the villages where there was no ITMN and the LLTN was the only net distributed, >90% of the respondents perceived the benefit of reduced mosquito bites. In the ITMN villages about 80% perceived such benefit. Also, the participants in the qualitative surveys said that reduced mosquito bites and decline in malaria incidence were their common observations after using LLTN. Wrinkle-free durable quality, attractive blue colour, good ventilation inside the net, bigger size, warmth inside the net during cold season, were the other merits of the LLTN as perceived by the users. Entry of mosquitoes through the larger holes, net sizes being too large to tie inside houses and insufficient length for taller persons were considered as demerits of LLTN.

Both quantitative and qualitative surveys revealed one controversial feature of LLTN, the (mesh) hole size. Not only for irregular use, but also for the overall lower use rate during summer, less mosquito density and high night-time temperatures were attributed as the reasons. In this context, a certain proportion of people appreciated the larger hole size of LLTN, which facilitates ventilation inside the net, thereby tempting them to sleep inside LLTN even during summer. On the other hand, some people, especially in the villages where they had used ITMN too, considered the larger size of the holes in LLTN as a demerit as these holes allowed easy entry of mosquitoes inside the net. Otherwise, the merits, the observations made after using LLTN, the likeable factors in LLTN, the reasons for which the respondents were prompted to advise other members in the households as well as the community to use LLTN, the causes for the preference for LLTN and the reasons for willingness to buy LLTN were almost uniformly the same.

The perceived benefits after use appeared to have prepared the people mentally to buy LLTN. Relatively, a higher percentage of people in the villages where there was ITMN programme, was willing to buy LLTN, perhaps because of their experience of purchasing ITMN. Besides affordability, some people stated that the LLTN already supplied to them was sufficient for their families and hence it was not necessary for them to buy more nets. Below Rs. 100/- was the affordable price quoted by most of the respondents for one LLTN. The price quoted by the participants in the group discussion and in-depth interviews varied between Rs. 10 and Rs. 150. Many people were interested in buying LLTN at a subsidized price, as they could not afford the market price. Most preferred cash payment, while a few mentioned payments in instalments. People also desired that the nets should be made available after they had harvested and sold some of their crops, so that they would have money to buy them.

The second evaluation, initiated in November 2004 in order to know the users' perceptions of the effectiveness of LLTN over a period of time and after several washes, is in progress.