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# Anopheline Diversity: Morphological and Molecular Variation of *An. subpictus* in Rural and Urban Areas of West Bengal

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A systematic survey (March 2012- Feb 2013) of Anopheline population in rural (Mogra) and urban (Dumdum) areas of West Bengal has been conducted to know the present population load of *Anopheles subpictus*. A preliminary attempt has been made to assess the potential morphological (wing) as well as molecular variation of *Anopheles subpictus* by using ITS-2 sequence primer. A considerable sequence variation is observed between the rural and urban population of West Bengal.

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**Keyword:** *An. subpictus*, Population load, Wing Variation, ITS-2 sequence.

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### 1. Introduction

The cytological and molecular variation is a unique feature in the genus *Anopheles*. However, the rate of mutation in *Anopheles* is lower magnitude than that of *Drosophila*<sup>[1,2]</sup>. Malaria is one of the most prominent and problematic infectious as well as major parasitic and vector borne diseases in the world<sup>[3]</sup>. India harbour about 58 morphologically identified species of *Anopheles*. It has been reported that about 13 Anopheline species are available in Kolkata and sub urban areas of West Bengal<sup>[4]</sup>. The abundance of this vector mosquitoes varies with the seasons and available habitat<sup>[5, 6]</sup>. Among these *Anopheles* vector mosquitoes *Anopheles subpictus* is considered as an emerging vector of malaria in some parts of Indian sub continent<sup>[7, 8]</sup>. A preliminary attempt has been made to know the present population status of the Anopheline mosquitoes in urban (Dumdum) and rural (Mogra) areas of West Bengal. As it is a ubiquitous and suspected vector of malaria, a

special emphasis has been taken to know the morphological and molecular variation of the species in the above mentioned areas.

### 2. Materials and Methods:

#### A. Collection and Morphological Identifications of Mosquitoes:

Adult *Anopheles* mosquitoes were collected from two different malaria prone areas of West Bengal viz. Mogra (rural) and Dumdum (urban). Collection was made in early morning (6-8 am) from different biotopes like cattle sheds & human dwellings (near to cattle shed) by using manual aspirator. Adult mosquitoes were morphologically identified using classical keys<sup>[9, 10]</sup>.

#### B. DNA Isolation:

DNA was isolated from individual adult mosquitoes by phenol chloroform extraction by following protocols of [11, 12] and standardized in the laboratory.

### C. PCR amplification:

The ITS2 region of r DNA was amplified using the specific forward and reverse primer (FP, RP) consisting of 20-21 base oligomers having the sequence 5' TGTGAACTGCAGGACACACAT-3' (Code46JB) and 5'-TGTGCTTAAATTCAGGGGGT-3' (code 47JB) respectively (13). A PCR master mix was prepared by mixing 10X PCR buffer, dNTP mix (100mM each), MgCl<sub>2</sub>, Taq polymerase (3 units/μl) double distilled water and template DNA. The thermal cycling conditions were: initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 30 sec/ 1 min, annealing at 50-60 °C for 1 min, extension at 72°C for 2-5 min and final extension at 72°C for 10 min. The PCR product and standard DNA ladder were electrophoresed in 2% agarose gel and visualized with ethidium bromide.

**D. DNA Sequencing:** The ITS-2 regions from some of the collected specimens were amplified according to the condition described above. The DNA of these samples was sequenced taking at least three mosquitoes and aligned with Clustal W multiple sequence alignment algorithms ([www.ebi.ac.uk/clustalw/](http://www.ebi.ac.uk/clustalw/)).

### 3. Result

Faunestic survey (Table-1) reveals that five species of Anopheles mosquitoes viz *An subpictus*, *An vagus*, *An barbirostris*, *An culicifacies* & *An annularis* are prevalent in the rural (Mogra) & urban (Dumdum) areas of West Bengal. Table-2 showed the seasonal prevalence

of the Anopheline mosquitoes in the above mentioned two areas. Some variations in the morphology (wing) of *An. subpictus* (Fig.3a, b and Table 3) have been observed in our survey. In rural areas the scales are thinly oriented in the margin of the wing. The Fig 1a and b show the frequency of five available Anopheles species population in the rural (Mogra) and urban (Dumdum) areas of West Bengal during 2012-13. The comparative prevalence of these different species in the above mentioned areas are displayed in Fig.2. Studies have been extended not only on the morphological variations (wing) but also in the molecular level i.e the sequence variation in spacer ITS-2 of *An. subpictus* in the above mentioned two areas (Fig.4a,b). ITS-2 sequence in rural population was 686 bp and 641 bp in urban one. All the sequence were G: C rich (Table-4).

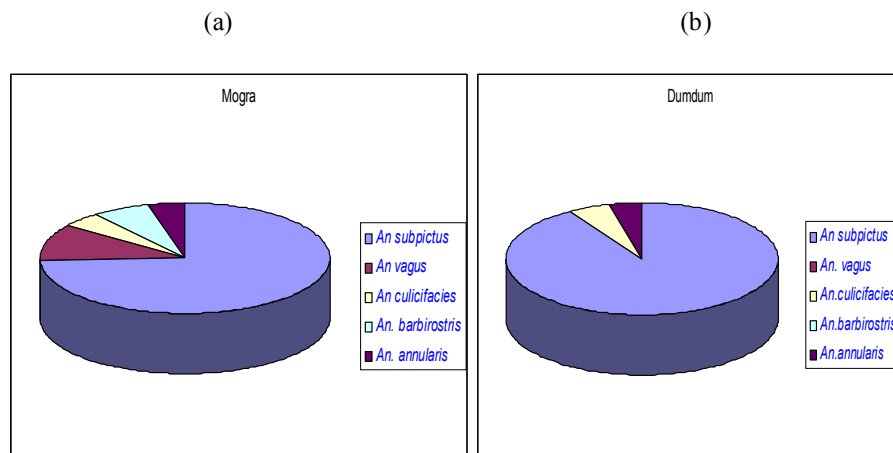
**Table 1:** Type of Anopheles found in Mogra and Dumdum

Type of Anopheline Species	Mogra	Dumdum
<i>An.subpictus</i>	105	92
<i>An.vagus</i>	15	–
<i>An.culicifacies</i>	6	5
<i>An.barbirostris</i>	9	–
<i>An.annularis</i>	6	4

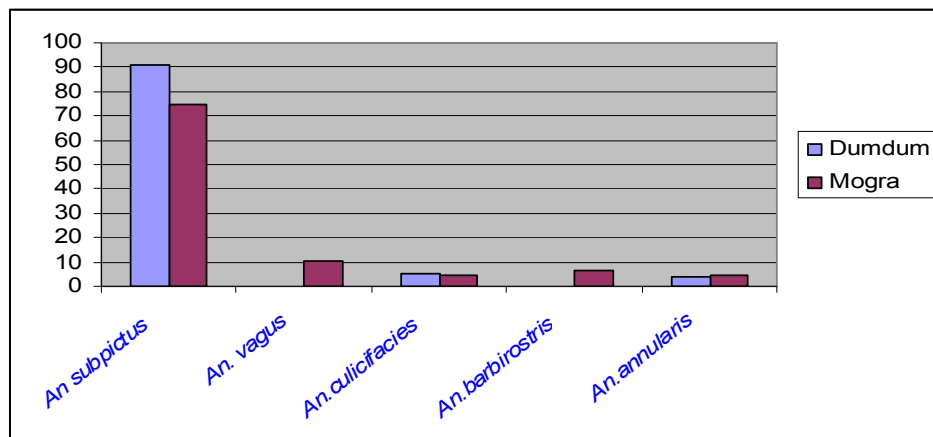
**Table 2:** Seasonal prevalence of Anopheline mosquito in Rural and Urban area

Anopheline Sp	Seasons							
	Summer (March-May)		Monsoon (June-August)		Post Monsoon (Sept-Nov)		Winter (Dec-Feb)	
	Mogra	Dumdum	Mogra	Dumdum	Mogra	Dumdum	Mogra	Dumdum
<i>An.subpictus</i>	64	33	26	28	12	29	3	2
<i>An.annularis</i>					6	4		
<i>An.vagus</i>	15							
<i>An.culicifacies</i>			6			5		
<i>An. barbirostris</i>					9			

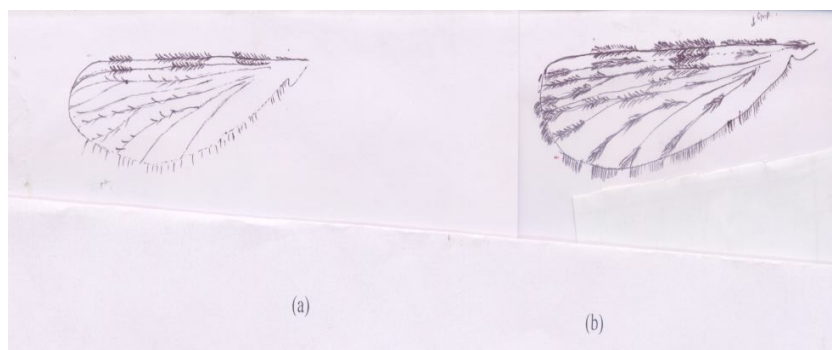
**Fig. 1:** Frequencies of different Anopheles in (a) rural (Mogra) and (b) urban (Dumdum)



**Fig. 2:** Graphical representation of percentage of different Anopheline populations in both the rural and urban areas.



**Fig:3 (a & B)** Wing variation of *An. Subpictus* from rural (Mogra) and urban (Dumdum) areas.



**Table 3:** Wing Variation in *An. subpictus* collected from two different place

Sub	Typical wing	Kol (Dumdum)	Mogra
Fringe spot(2a-2b)	Present	Absent	Absent
Fringe spot (4a- 4b)	Present	Present	Absent
Scaling pattern of wing	Thickly oriented	Thickly oriented	Thinly oriented.

**Fig: 4(a):** ITS2 sequence of *Anopheles subpictus* collected from Mogra (rural)

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>b_F_5949-2_P1, Raw Sequence (686 bp)
GTGGTATTGCTGACGCATATGGCGCATCGGACGTTTCAACCCGA
CCGATGCACACATCCT
TGAGTGCCTACTAGGTACTGAGAGATTCTATAAATTGACTACA
GACGGCGCCACAAACG
GGCTGACGGCCATCCGTCGTCGGCGTGC GACTGTGCAGCAT
GGCGTGCTCGGGTCTCG
GCGTGGACCCTGGGCGCTGAAAGTGGACACTGTTGGCGGCA
CCTGCGCGTGTGCTCTC
AGTGTGACGTATGGTGAGGGTAGTGTCAAATCGCACGGTTCG
ACAACAAGCGTACCCTG
GAGTTGGTGCAATCGGATGCCTACTACCATGGGCGGAGCCGG
CGTGCATTCAACTCG
ACGTCCTGTATCAACCCGATGCCAACTGGTTGGTGGTGCCGGC
GCAGACAGGACACTGA
ATCGATCTTGGTGGTACAACCCACATGTGGGTGATCAAGGAGG
GGGTGTAAGTGTGGGAG
GGACACGAGGGTGGCGCCGACGCACACGCCGGCACTACCCCC
ACGTCCCTCGTTGCGTG
TATTGCGGGTGAATCATAGAGTGATCTGTTGAATAGTGGGTTG
AACTGGGTATGAAAAA
AGTTACCAAAAAAGCTGACCACACTCCAGTAGGCCCTTCCATG
ATGTGTGACTAGATGTG
GGACTACACCCAGAATTTAAGCATAA.
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**Fig 4(b):**ITS2 sequence of *Anopheles subpictus* collected from Dumdum (urban)

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>k_F_5949-3_P1,Raw Sequence (641 bp)
TAGATGTGCTTGACGCATATGGCGCATCGGACGTTTCAACCCGA
CCGATGCACACATCCT
TGAGTGCCTACTAGGTACTGAAAGATTCTATAAATTGACTACA
GACGGCGCCACAAACG
GGCTGACGGCCATCCGTCGTCGGCGTGC GACTGTGCAGCAT
GGCGTGCTCGGGTCTCG
GCGTGGACCCTGGGCGCTGAAAGTGGACACTGTTGGCGGCA
CCTGCGCGTGTGCTCTC
AGTGTGACGTATGGTGAGGGTAGTGTCAAATCGCACGGTTCG
ACAACAAGCGTACCCTG
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GAGTTTGGTGCAATCGGATGCCTACTACCATGGGCGGAGCCGG
CGTGCATTCAACTCG
ACGTCCTGTATCAACCCGATGCCAACTGGTTGGTGGTGCCGGC
GCAGACAGGACACTGA
ATCGATCTTGGTGGTACAACCCACATGTGGGTAGTAAGTAGGT
GGTCGAAGTGTGCAAG
GGACAACCCGATGCCCGCGATGGCCGTGCCGGCGCTCACACC
ACACTCCTTGCTAGGTC
GCTTGTGGGGGTAAACGCGTGTGATCTGTACACATACCTGTTG
AGCTGTGCGTTGAGCA
CATTAGGATGAGAGTTGACAAATCCAGTCCACCTTCCAGT
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**Table 4:** ITS2 sequence length and GC content of Mogra (rural) and Kolkata (urban) population.

Population	Base pair length	GC Content
Mogra	686	55.90%
Dumdum	641	57%

#### 4. Discussion

The present investigation reveals that *An. subpictus* is a predominant Anopheline species in the rural (Mogra) & urban (Dumdum) areas of West Bengal. Several lines of data [14, 15, 2, 16] also highlighted the prevalence of the above mentioned Anopheline mosquitoes in the urban and sub-urban areas of West Bengal. *An. subpictus* is also reported as a potent malarial vector in some areas of this state [17]. Therefore in our study the prevalence of five Anopheline mosquitoes (rural-Mogra & urban-Dumdum) has been presented graphically (fig-2). Table-2 also showed that the population of *Anopheles subpictus* is more during the month of March - May.

Previous investigation also indicated [15] that preponderance of incidence of *An. subpictus* some way reflects competition, co-existence among the Anopheles, fitness in selection & co-adaptation in domestic and natural population. At present in our survey, the population load of Anopheles is carried out by the population volume of *An. subpictus* both in rural and urban areas of West Bengal for their ecological as well as genetical fitness.

Earlier investigation <sup>[15]</sup> showed that palp and proboscis variation are more prevalent in urban areas. On the other hand wing variations have mainly been observed in rural areas. The present investigation (Table-3 and Fig3 a,b) also reveals the wing variations (scale orientation) in rural and urban populations.

When population may expand positively from small size to larger one some of the non adaptive characters present in the original gene pool may have chance to increase in proportion. Simultaneously some other adaptive characters are also favoured and encouraged by selection during population expansion <sup>[15]</sup>. In the present investigation wing variation (orientation of less scale in the margin of the wing) may be such characters. Our data also corroborate with the earlier result<sup>[15]</sup>. Besides the studies on faunestic survey & morphological (wing) variation, our investigation has also been extended on ITS-2 sequence variation of *An. subpictus*. The data also revealed that GC content of ITS2 region of *An. subpictus* in the rural and urban areas were 55.9% and 57%respectively. Several lines of data <sup>[18, 19(communicated)]</sup> also reported that GC content of ITS-2 (50%-56%) of Anopheles is consistent with spacer composition. Tandem repeats were lacking in the two populations. The tetramer TGCA has restriction site for a restriction enzymeHpyCH4IV <sup>[20]</sup>. Present studies also suggest that molecular variation through ITS-2 sequence variation between the said populations is a type intragenomic mechanism which promotes morphological as well as molecular (sequence) variation of this species due to constantly changing environment <sup>[18]</sup>. It can be concluded that morphological, as well as molecular variations are considerable in between the urban and rural areas of West Bengal.

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