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# The Dominant Mosquito Vectors of Human Malaria in India

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Additional information is available at the end of the chapter

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## 1. Introduction

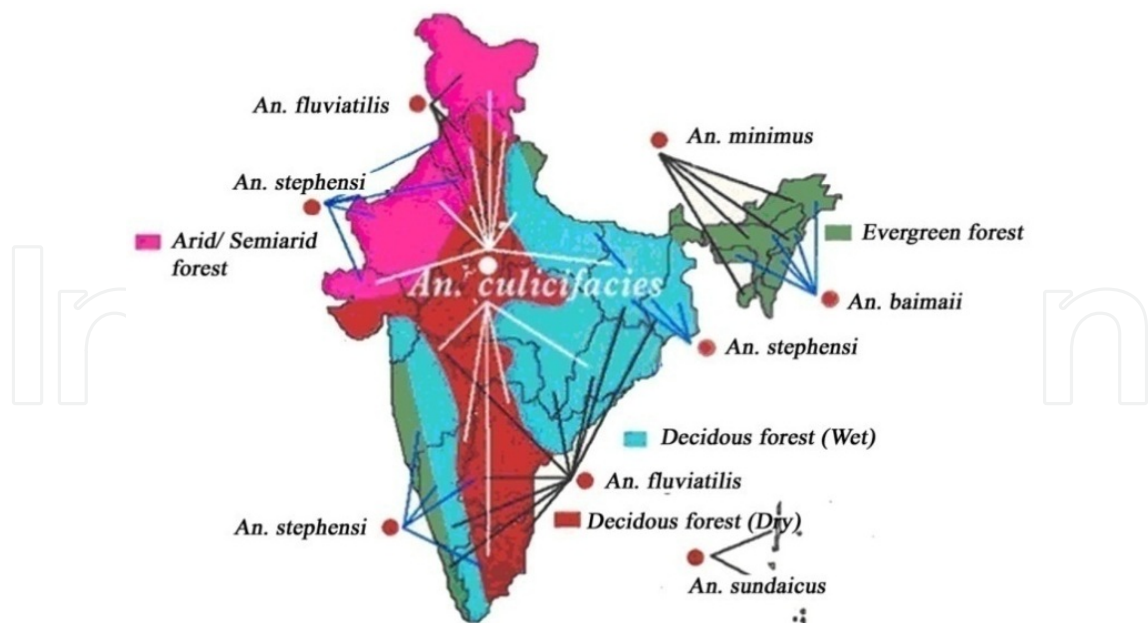
In India malaria endemicity is characterized by diverse ecology and multiple disease vector species [1]. In the Southeast Asian region, India alone contributes to nearly 80% of malaria cases with the largest population of the world living at risk of malaria. In 2011, India reported 1.3 million confirmed malaria cases and 753 attributable deaths, but estimated cases and deaths are 10 to 20 times more [2,3]. Of the two *Plasmodium* prevalent in India, *Plasmodium falciparum* incidence has not declined significantly although *P. vivax* has resulting in the rising trend of the former parasite to presently contributing ~50% of the reported cases. Distribution and spread of chloroquine resistance and emergence of multi-drug resistant strains may have contributed to this phenomenon [4]. Even though transmission intensities across India are low-to-moderate, disease remains geographically entrenched in poor marginalized population groups particularly living in remote/ forest fringe/ tribal belts of eastern, central and north-eastern states for contributing >65% of malarial episodes [5,6].

Mosquito fauna is rich in the tropical climate with numerous and diverse breeding resources [7]. Of 58 anophelines in India, only six taxa are major malaria vectors with regional distribution (Figure 1). *Anopheles culicifacies* s.l. is the vector of rural malaria in the country and generates about 65% of cases annually. *An. fluviatilis* s.l. is found in the plains and foothills breeding in streams contributing 15% of malaria cases, *An. minimus* breeds in streams of foothills of the northeast, *An. dirus* s.l. is found in jungles of northeastern states, *An. sundai-cus* is found in Andaman and Nicobar islands and breeds in brackish water, and *An. stephensi* is the well known vector species of urban malaria. All these mosquito species except *An. stephensi* have been characterized as species complexes with number of morphologically indistinguishable sibling species which vary for their role in malaria transmission [8].

India is experiencing rapid ecological changes owing to population explosion, urbanization, development projects, deforestation and human migration affecting mosquito ecology and disease transmission. In the recent past, significant progress has been made in understanding the genetics and bionomics of the disease vectors, and in the development of newer control tools to strengthen primary healthcare services specific to India [9-14]. In this chapter we shall restrict systematic review on dominant *Anopheles* vectors of human malaria and their current bionomics to help develop malaria-risk maps for strengthening malaria control for sustainable interventions with ultimate goal of malaria elimination.

2. *Anopheles (Cellia) culicifacies* Giles species complex

*Anopheles culicifacies* s.l. is widely distributed in India and has been recorded in all mainland zones including Kashmir and high elevations in the Himalayas (up to 3000 meters) except islands of Andaman & Nicobar and Lakshadweep [7,8,11]. It is the most important vector in plains of rural India contributing 60-70% of reported cases annually [15]. Success stories in malaria control during 1950-1960, and malaria resurgence in the 1970s deal primarily with the control of *An. culicifacies* s.l. Biology and genetics of *An. culicifacies* has been extensively studied in India [16-17], and presently characterized to be a species complex with five informally designated species A, B, C, D and E. These five sibling species are spread across India with distinct biological characteristics and role in malaria transmission (Table 1).



**Figure 1.** Map of India showing distribution of major malaria vectors in relation to physiogeographic regions encompassing evergreen tropical forest (wet zone receiving rainfall >200 cm), deciduous wet forest (monsoon forests receiving rainfall 100-200 cm), deciduous dry forest (scrub forest receiving rainfall 50-100 cm), and desert forest (arid and semi-arid area receiving rainfall <50 cm) annually.

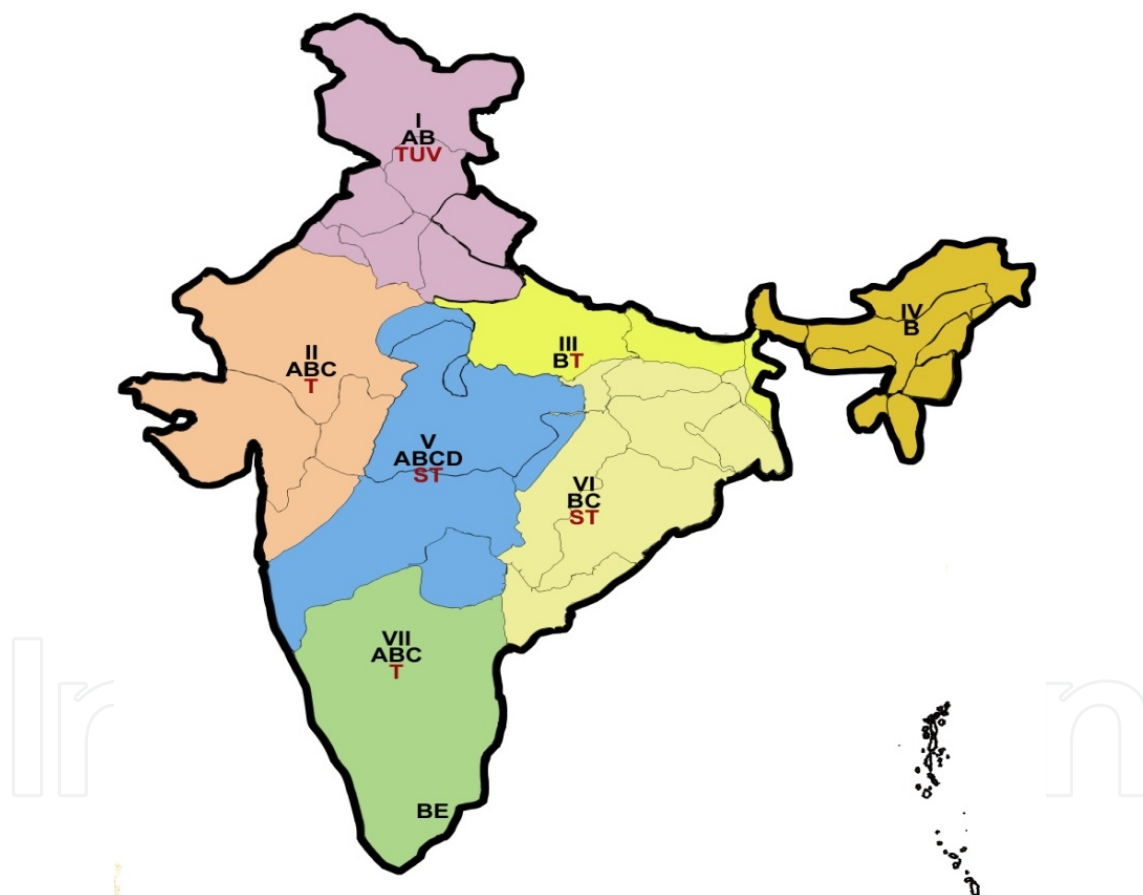
Sibling species were initially characterized by species specific diagnostic fixed paracentric inversions readable in polytene chromosomes suggestive of pre-mating barriers in field populations [18-24], and further substantiated by number of techniques including post-zygotic isolation mechanisms in laboratory conditions [25], mitotic karyotype Y- chromosome polymorphism [26-28], gene enzyme variation [29], cuticular hydrocarbon profiles [30], and species specific DNA probes [31]. Recently, PCR-based diagnostic assays were developed for sequencing 28S-D3 domain [32], ITS2-PCR-RFLP [33], rDNA ITS2 region [34], which grouped *An. culicifacies* sibling species into two distinct groups namely Group I (species A/D) and Group II (species B/C/E). In another assay from COII region, A/D specific primers distinguished species A and D, and B/C/E specific primers distinguished B, C and E [35]. More recently, a multiplex PCR-based diagnostic assay using D2 domain of 28S rDNA has been reported which can consistently and accurately discriminate members of the species complex forming two unambiguous monophyly clades of species A/D (Group I) and species B/C and E (Group 2) which were supported by strong bootstrap values [36].

Characteristic	Sibling species				
	A	B	C	D	E**
Inversion genotype	X <sup>+</sup> a <sup>+</sup> b <sup>+</sup> ; 2+g <sup>1</sup> +h <sup>1</sup> ; +i <sup>1</sup> /i <sup>1</sup>	Xab; 2g <sup>1</sup> +h <sup>1</sup>	Xab; 2+g <sup>1</sup> h <sup>1</sup>	X <sup>+</sup> a <sup>+</sup> b <sup>+</sup> ; 2i <sup>1</sup> +h <sup>1</sup>	Xab; 2g <sup>1</sup> +h <sup>1</sup> ;
Anthropophilic Index (%)	0-4	0-1	0-3	0-1	80
Biting activity (Peak biting activity)	All night (2200-2300 h)	All night (2200-2300 h)	All night (1800-2100 h)	Till midnight (1800-2100 h)	No data
Vector potential	Moderate	Poor	Moderate	Moderate	High
Sporozoite infection rate (%)	0.51	0.04	0.3	0.4	20
Breeding preferences	Rainwater, clean irrigation water	Riverine ecology	Rainwater, clean irrigation water	Rainwater, clean irrigation water	Riverine ecology
Rate of development of resistance					
DDT	Slow (9-10 yr)	Fast (4-5 years)	Fast (4-5 years)	No data	No data
Malathion	Slow (9-10 yr)	Medium (6-7 years)	Fast (4-5 years)	No data	No data
Pyrethroids	No data	Medium (6-7 years)	Medium (6-7 years)	No data	No data

\*Source Reference No. 16, 37. \*\*In Rameshwaram island of Tamilnadu

**Table 1.** Inversion genotype and biological characteristics of *Anopheles culicifacies* sibling species complex in India\*

The distribution, relative abundance and predominance of sibling species (but not exclusive) is given in Figure 2. Among its sibling species, species B is the most predominant throughout the country and occurs sympatrically in most areas with predominance of species A in the north and species B in the south [37]. In eastern Uttar Pradesh, north Bihar and northeastern states, species B is either predominant or the only prevalent species. Species B and C are sympatric in western and eastern India. Species D is sympatric with species A and B in northwestern region, and with species A, B and C in central southern India. Species E is sympatric with species B in southern Tamilnadu including Rameshwaram islands. The proportions of sibling species, however, varied in different geographical zones and seasons, e.g., in Alwar (state of Rajasthan), species B proportions increased in post-monsoon months; whereas proportions of species D remained the same throughout the year and density of species C remained very low [38].



**Figure 2.** Map of India showing geographical distribution of predominant sibling species of *Anopheles culicifacies* complex (A,B,C,D,E) and *An. fluviatilis* complex (S,T,U, form V), and stratification (Divisions I–VII) for suggested vector control options. For control of *An. culicifacies* malaria vectors in Division I & III: No routine vector control is necessary except for treatment of imported cases of malaria; Division II: Insecticide spraying based on susceptibility status of *An. culicifacies* species A or C; Division IV: DDT spraying to continue; Division V–VII: Insecticide spraying based on susceptibility status of *An. culicifacies* species C. For control of *An. fluviatilis* malaria vectors, even though DDT remains the insecticide of choice, in areas where it is sympatric with *An. culicifacies*, insecticide spraying used for control of latter should be applied. Source Reference No. 37.



All member sibling species of the *An. culicifacies* complex are predominantly zoophilic except species E, and rest indoors in human dwellings and cattle sheds [39]. All are night biting species with different peak biting activity (Table 1). The main strategy for malaria control in areas of *An. culicifacies* distribution is by indoor spraying of residual insecticides chosen based on their susceptibility status in the given region. Presently, *An. culicifacies* has developed resistance to most insecticides in use including malathion (except certain areas) leaving the only option of pyrethroid use for which there are already reports of increased tolerance [40-45]. Molecular characterization revealed a low frequency of the *kdr* allele (mostly in heterozygous condition) in field populations that were resistant to DDT and pyrethroids [46,47]. Based on the geographical distribution of sibling species, the country is now stratified into seven divisions for benefit of prioritizing control options, e.g., for division I and III, no routine control interventions are required, whereas for divisions II, IV - VII, insecticide spraying is necessary based on susceptibility status against the dominant vector species (Figure 2).

*An. culicifacies* is indeed a prolific breeder and breeding sites are numerous including river-bed pools, rain water collections (Figure 3), streams, rice-fields, seepage water, borrow pits, irrigation channels, etc [7,11]. It has been incriminated by detection of gut and salivary gland infections by numerous independent investigators across its range of distribution throughout India [7]. Further studies using immunoradiometric analysis revealed that sibling species A, C, D and E are vectors of *Plasmodium vivax* and *P. falciparum* malaria, and species B is non-vector or poor vector [48]. Among these, species E was observed to be highly anthropophilic in Rameswaram islands of Tamilnadu [49]. These observations were further supported by comparative reproductive fitness for which sibling species B was observed to be less fit than species A and C of the complex as well as susceptibility to malaria sporogony [50-52].



**Figure 3.** Breeding habitats of *Anopheles culicifacies* (left – rain water pools; right – river bed pools). Courtesy: N. Nanda and R. Namgay.

However, more information on distribution and bionomics of species E is deemed necessary to substantiate its distribution range and role in malaria transmission in India. In addition, understanding population structure of *An. culicifacies* in adjoining countries is also warranted for effective interventions to check spread of drug-resistant malaria across borders. Additional data on crossing experiments between sibling species to demonstrate

post-zygotic isolation and existence of possible morphological differences would help name the individual species formally similar to other well defined species complexes of *An. dirus* and that of *An. maculatus* [8,10]. *An. culicifacies* is indeed a fast invading species in areas hitherto with low density (deforested pockets in Northeast India), and its control has become a formidable challenge with its sibling species developing multiple resistance including pyrethroids (42-45). Regional control strategy would require monitoring the insecticide susceptibility status periodically for any given area that qualifies for residual spraying for effective control of *An. culicifacies* malaria vectors.

3. *Anopheles (Cellia) fluviatilis* James species complex

*Anopheles fluviatilis* s.l. is widespread in mainland India and is considered to be an important vector in hills and foothills contributing ~15% of reported cases annually [1]. It has been extensively studied and recognized a species complex comprising three sibling species, i.e., S, T, U and a form ‘V’ based on cytotaxonomic study for fixed chromosomal inversions readable in the polytene chromosomes arm 2 [7-11,53]; differentiation of S and T, however, not possible due to diagnostic inversion polymorphism but can be characterized by distinct biological characteristics and regional distribution (Table 2). Earlier reports of existence of X and Y sibling species in *An. fluviatilis* based on rDNA-ITS2 polymerase chain reaction assay subsequently correlated X with sibling species S, and Y with T based on chromosomal data [54,55]. To substantiate these observations, robust molecular techniques now have been developed which distinguish sibling species S, T and U unequivocally based on differences in nucleotide sequences within the D3 domain of 28S rDNA [56]. However, contrary to observations of Garros et al [57] and Chen et al [58] on conspecificity of *An. fluviatilis* species S with *An. harrisoni* (species C of *An. minimus*), Indian population of these two species were observed to be distantly related and did not merit synonymy based on pair-wise distance and phylogenetic inferences using ITS2 sequences [59].

Sibling Species**	Inversion genotypes on Chromosome arm 2	Mosquito densities (per person hour)	Feeding preference	Preferred adult habitat	Prevalence	
					Ecotype	Endemicity
S	+q'+r'	Low to Moderate (1-40)	Anthropophilic	Human dwellings	Hilly forests & foothills	Hyperendemic
T	q'+r'	High (up to 200)	Almost totally zoophilic	Cattle sheds	Foothills & plains	Hypo - mesoendemic
U	+q'r'					

\*Source Reference No. 37, \*\*Distribution, bionomics and biology of new sibling form ‘V’ is being investigated

Table 2. Inversion genotype and biological characteristics of *Anopheles fluviatilis* sibling species complex in India\*

Sibling species S is highly anthropophilic and responsible for maintaining hyperendemic malaria predominantly in state of Odisha (formerly Orissa), eastern India [60]. It prefers to rest indoor human dwellings and have been incriminated and proven to be an efficient vector in areas of its distribution [61,62]. Sibling species T is widely distributed but is largely zoophilic and rests in cattle sheds [63]. Sibling U holds similar characteristics but has limited distribution range presently restricted to northern India. Chen et al [58] documented three haplotypes in species T (designated T1, T2, Y) with its distribution in India, Nepal, Pakistan and Iran implicating the existence of additional taxa within the *An. fluviatilis* species complex provisionally designated as 'V form' in India, and the same has recently been recorded in district Hardwar, Uttarakhand state of North India [63]. Both sibling species T and U are held very close with similar biological characteristics and there exists possibility of hybridization in some areas. Even though both siblings species are poor vectors but have shown inherent ability to support normal sporogony in laboratory feeding experiments [64].

Preferred breeding habitats are seepage water streams with perceptible flow of water, river margins, irrigation channels, shallow wells, terraced rice fields along foothills etc [7,11,65]. Peak biting activity occurs between 20:00 to 24:00 hours but it may vary in different seasons and locations. Both *An. fluviatilis* species S and *An. minimus* share similar resting and breeding habitats and are efficient vectors in their respective zones of distribution [66]. Both are subject to misidentification due to morphological variation to the extent that the earlier records of prevalence and seasonal abundance of *An. fluviatilis* in northeast India have now been proven to be hypermelanic variant of *An. minimus* s.s. by molecular assays [67].

For control of *An. fluviatilis*, the choice of insecticide should be based on the susceptibility status of prevalent sibling of *An. culicifacies* in endemic areas where species of both complexes share similar indoor resting behavior and sympatric distribution records (Figure 1). More investigations are, however, warranted for precise distribution of different sibling species of this complex especially in areas hitherto unexplored, particularly 'form V' and its role in malaria transmission. Similar to *An. culicifacies* species complex, there is dearth of data for morphological differentiation and crossing experiments to distinguish member sibling species enabling binomial nomenclature.

#### 4. *Anopheles (Cellia) minimus* Theobald species complex

*Anopheles minimus* s.l. is considered to be the predominant malaria vector in the oriental region [68]. It is a major vector in sub-Himalayan foothills of eastern and northeastern region of India. In the pre-DDT era (1940s), it was extensively studied in Assam and Bengal for its bionomics and control, and it was widely incriminated across its range of distribution [69-74]. With the advent of DDT and large scale application for residual spraying to control, *An. minimus* disappeared from Terai of Uttarakhand (formerly Uttar Pradesh), eastern Odisha, northeastern states and Nepal [75,76]. Subsequently besides *An. dirus* s.l., *An. philippinensis* was implicated in malaria transmission in northeastern region of India [77]. However, return of malaria required containment of persistent transmission and spread of drug-resistant malaria.



Towards this objective, systematic investigations were initiated *denovo* during 1980s to incriminate vectors of malaria and to ascertain their relative importance [78,79].

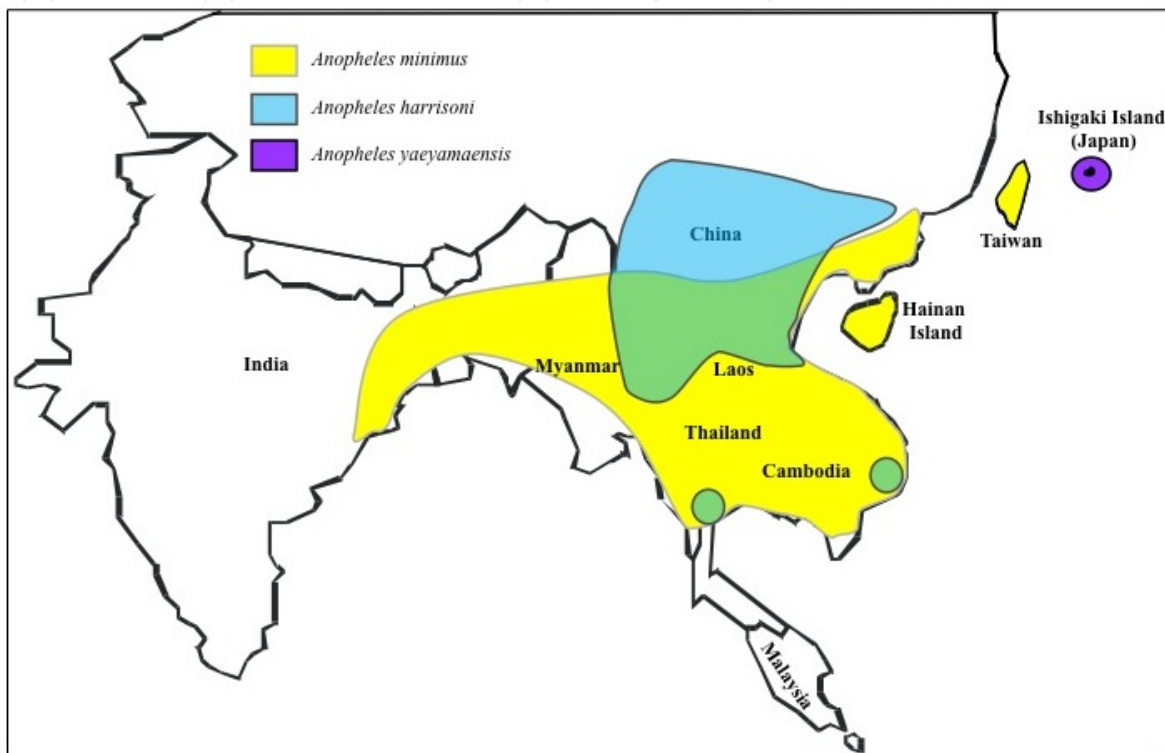
Consequently, systematic studies by independent investigators revealed the reappearance of *An. minimus* in vast areas of northeast. *An. minimus* was re-incriminated in almost all states of the northeast India except in Terai area of Uttarakhand (North India) where it did not return [80-86]. It is only recently that *An. minimus* has been reported to have resurfaced in Odisha (eastern India) after a lapse of 45 years and were observed to be abundant sharing *An. fluviatilis* habitats, and both vectors were incriminated [87,88]. It is presently the most efficient vector in foothill valley areas of northeastern states accounting for nearly 50% reported cases in the region annually, and responsible for focal disease outbreaks characterized by high rise in cases and attributable deaths [89-94]. *An. minimus* is the predominant vector in rice-growing foothill valley areas, and it supplements transmission in forest fringe areas (adjoining to undisturbed forest reserve) predominated by *An. baimaii* [95].

Ever since initial recognition of *An. minimus* as species complex for its three morphological forms [96] and subsequent characterization by population genetic evidence for two isomorphic species [97], *An. minimus s.l.* has been identified to a species complex comprising three formally named species, *An. minimus s.s.* (species A), *An. harrisoni* Harbach & Manguin (species C), and *An. yaeyamaensis* Somboon & Harbach (species E) with distinct bionomical characteristics and distribution [98-101]. The natural distribution range of these species is given in Figure 4. Even though based on classical taxonomy, three designated species are difficult to distinguish due to overlapping morphological characters, yet these can be identified reliably by number of molecular assays [102-107].

Based on DNA sequences of internal transcribed spacer 2 (ITS2) and D3 domain of 28S rDNA (28S-D3) of morphologically identified *An. minimus s.l.* across Indian states of Assam, Arunachal Pradesh, Meghalaya and Nagaland [108] and that of Odisha [87], it has now been clearly established that these populations are indeed *An. minimus* (species A), whereas *An. harrisoni* and *An. yaeyamaensis* are not recorded from India. Correct identification of *An. minimus* is further complicated by the existence of morphological variants which closely resemble *An. varuna* and *An. fluviatilis s.l.*, and these species share similar distribution range and habitats. In northeast India, morphologically identified populations of *An. fluviatilis s.l.* (formerly designated species U based on polytene chromosome banding pattern) have now been genetically characterized as the hypermelanic seasonal variant of *An. minimus* prevalent during cooler months [67]. The ITS2 and 28S-D3 rDNA sequences of morphologically identified *An. fluviatilis* populations of from Assam were observed homologous to that of *An. minimus s.s.* and different from that of any member of the *An. fluviatilis* complex.

*An. minimus* is primarily an endophilic and endophagic species with a strong predilection for human host for blood meal [85]. It is a perennial species with seasonal peak density during April to August (wet season), and is the most predominant collection in human bait landing catches (13.7 per person/night) with peak biting activity during 01:00–04:00 hours. It has been incriminated in all months of the year (sporozoite infection rate 3.31%) but relative abundance and entomologic inoculation rates (EIRs) vary across malaria endemic districts [85,109]. The relative abundance and risk of malaria is high in localities

near to breeding habitat (<1km) suggestive of poor flight range (Figure 5). *An. minimus* breeding were primarily recorded in perennial seepage water foothill streams with grassy margins in all seasons but occasionally recorded in paddy field water pools with perceptible flow of water [110].



**Figure 4.** Distribution map of member species of the *Anopheles minimus* complex in Southeast Asia based on molecular identification (Courtesy: Dr. S. Manguin). *An. minimus* has wide distribution extending from East India to Northeast and eastwards to China including Taiwan, and occurs in sympatry with *An. harrisoni* over large areas in southern China, Vietnam, Laos and Thailand. *An. yaeyamaensis* is restricted to Ishigaki island of Ryukyu Archipelago of Japan.



**Figure 5.** Breeding and resting habitats of *Anopheles minimus* (left- seepage water foothill streams are preferred breeding habitat; right – mud house with thatched roofing located often adjacent to breeding resource is the ideal resting habitat for which relative risk of malaria is high).

*An. minimus* is susceptible to DDT despite decades of insecticide residual spraying (IRS) by virtue of its physiological resistance and high behavioral plasticity [93]. It avoids resting indoors and instead establishes extra-domiciliary transmission only to return to original habitat after 10 to 12 week post-spray. With the introduction of pyrethroid coated/ incorporated long-lasting insecticidal nets (LLINs) and enhanced population coverage in high-risk areas, the populations of *An. minimus* are once again fast diminishing particularly in broken forest reserve erstwhile domains of this anthropophilic species [111-113]. The niche thus vacated is being accessed by *An. culicifacies* populations which are tolerant to multiple insecticides posing a new challenge for effective vector control and associated transmission (unpublished observations).

It is suggested that in areas with *An. minimus* and *An. fluviatilis* sympatric populations, viz., Odisha and West Bengal, there is need to apply integrated vector management for sustainable interventions [114,115]. Given the adaptability of *An. minimus* to varied environments, there is continued need to monitor its bionomical characteristics in the changing ecological context due to rapid socio-economic development and diminishing malaria transmission in erstwhile areas of high receptivity [116]. Additional data are warranted for analyses of mitotic karyotypes, polytene chromosome maps and cross-breeding experiments which may of diagnostic importance. Equally important would be to understand the population dynamics of member species of the *An. minimus* complex in the adjoining countries of Myanmar, Bangladesh and Bhutan for developing cross-border initiative to institute appropriate interventions to contain drug-resistant malaria.

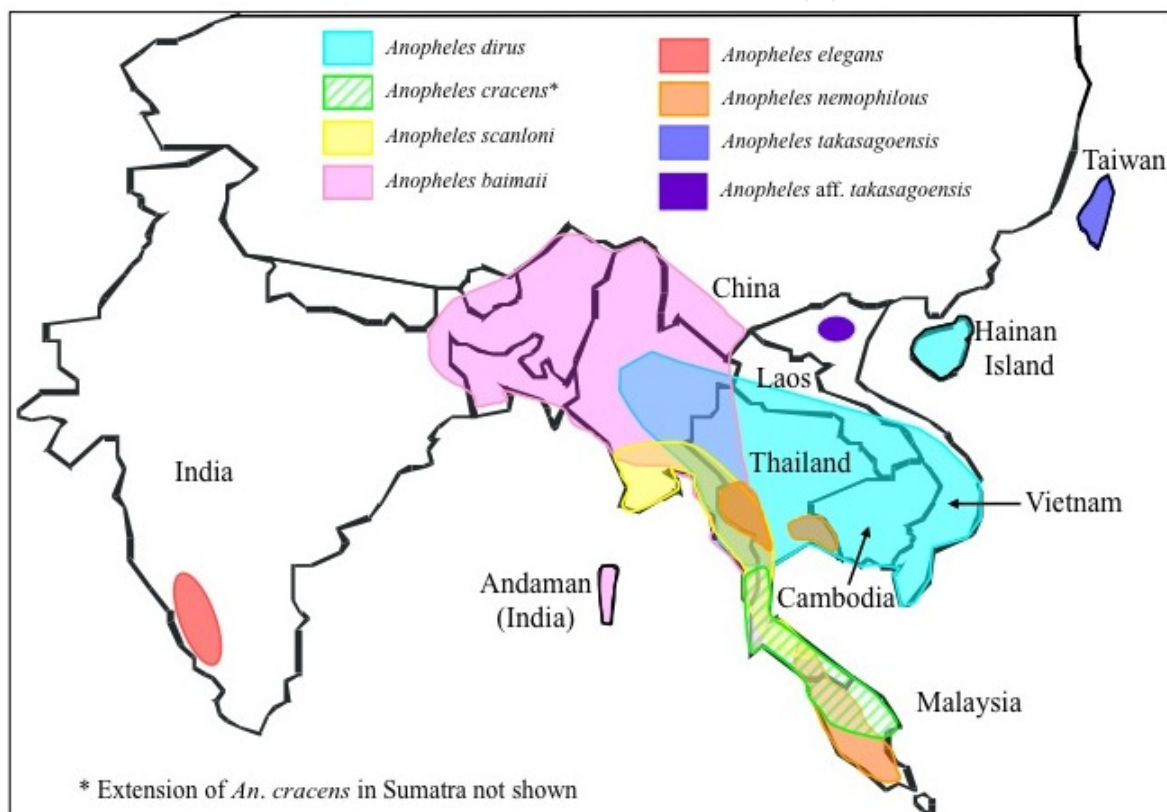
## 5. *Anopheles (Cellia) dirus* Peyton & Harrison species complex

*Anopheles dirus* s.l. comprises eight sibling species, seven of which have been formally named, i.e., *An. dirus* s.s. (species A), *An. cracens* (species B), *An. scanloni* (species C), *An. baimaii* (species D), *An. elegans* (species E), *An. nemophilous* (species F), *An. takasagoensis*, and a cryptic species tentatively designated as *An. aff. takasagoensis* (Figure 6). Each of the seven named species has morphological description [117], distribution range and have varied epidemiological significance in Southeast Asia [10,118], whereas the eighth species, reported in northern Vietnam, is morphological similar but phylogenetically distant from both *An. dirus* and *An. takasagoensis* [119]. All these sibling species except *An. aff. takasagoensis* have been well characterized by a number of techniques including cross-mating experiments, karyotypic studies, polytene chromosome banding patterns, gene enzyme variation, DNA probes and egg morphology [8,10,120-122]. In addition, PCR assays have been developed based on ITS2 sequences and SCAR (sequence characterized amplified region) based PCR which distinguishes five of its member species unambiguously [123,124]. Further investigations, however, are warranted to characterize *An. aff. takasagoensis* to formally name this as valid species of the *An. dirus* species complex.

Among these member species, only *An. baimaii* and *An. elegans* are prevalent in India with distinct distribution range and epidemiological significance [8]. *An. baimaii* is widely abundant



in northeastern states and is an efficient vector of human malaria contributing the remaining 50% of reported cases in the region annually [1]. It has been widely incriminated across northeastern states (sporozoite infection rate 1.9%) and its neighboring countries associated with transmission of drug-resistant malaria [125-132]. In earlier records what was initially described as *An. balabacensis balabacensis* and later *An. dirus* (species D) in India are now referred as *An. baimaii* for all purposes. *An. baimaii* is very closely related to *An. dirus*, populations of both species are of significance in understanding evolution and history of expansion in geological time scale [133,134].



**Figure 6.** Distribution map of member species of the *Anopheles dirus* complex in Southeast Asia (Courtesy: Dr. S. Man-guin). *An. dirus* has a wide distribution in eastern Asia including Myanmar, Thailand, Cambodia, Laos, Vietnam and Hainan Island. *An. cracens* occurs in southern Thailand, peninsular Malaysia and Sumatra (Indonesia). *An. scanloni* distribution is restricted along border of southern Myanmar and western Thailand. *An. baimaii* distribution extends from southwest China to northeast India through western Thailand, Myanmar, Bangladesh and Andaman Islands (India). *An. elegans* distribution is restricted to hilly forests of southwestern India. *An. nemophilous* has a patchy distribution along Thai-Malaya peninsula and Thai border with Myanmar and Cambodia. *An. takasagoensis* is restricted to Taiwan and *An. aff. takasagoensis* has recently been reported from northern Vietnam.

*An. baimaii* is a forest dweller and actively transmits malaria during monsoons in forest fringe population groups particularly along inter-state and inter-country border areas (Figure 7). It is a hygrophilic species (flight range <1km) and demonstrates phenomenon of 'horizontal' pulsation, i.e., population expansion from 'mother foci' in deep forests to periphery during monsoons (June–October) and then retracting to 'mother foci' in dry seasons (November–

March) accounting for its high and low prevalence in respective season, and 'vertical' pulsation for its ability to feed on alternate host to humans in the changing environmental conditions [135]. It is a highly anthropophilic species for its predilection to human host and bites throughout night both indoors and outdoors (36.1 bites/person/night) with peak infective biting activity during second quartile (21:00–24:00) of the night hours [136,137]. The relative risk of infective bite, however, was estimated to be much greater in the post-monsoon season. It is largely an exophilic species and breeds in a variety of habitats in forest including small transient pools, elephant foot prints [138]. It is highly susceptible to all residual insecticides but avoids contact with sprayed surfaces making vector control a difficult proposition [139].



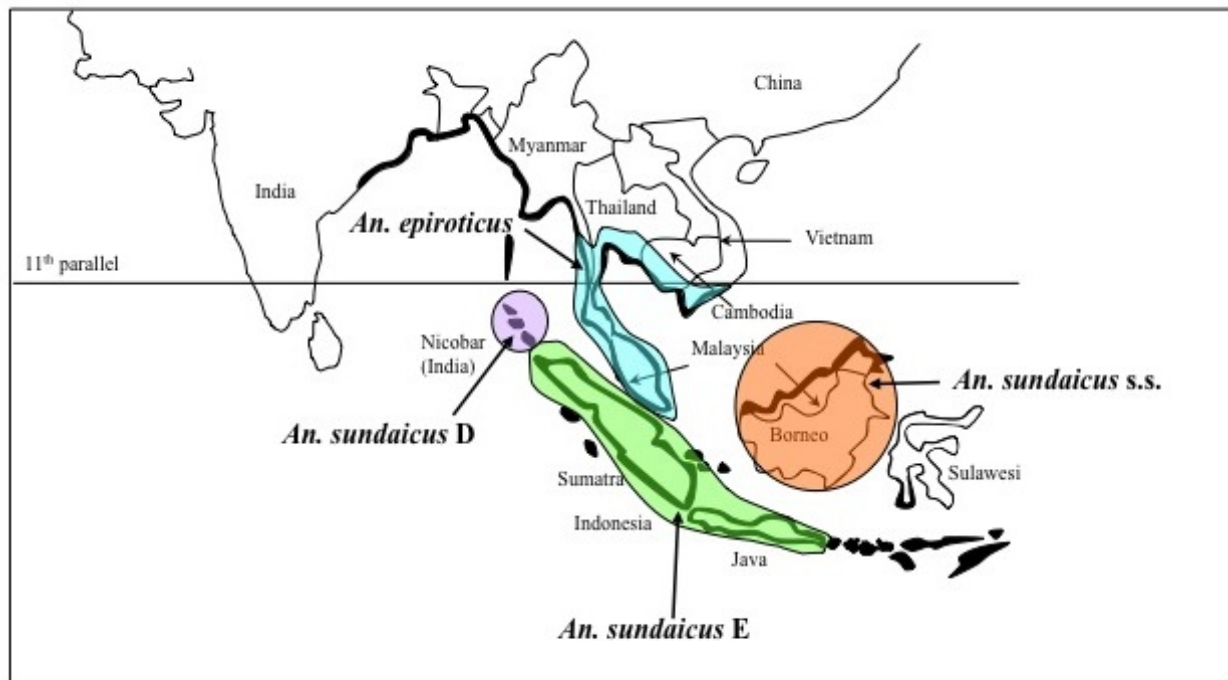
**Figure 7.** A typical housing structure receptive for *Anopheles baimaii* transmitted malaria located along Indo-Bangladesh border in northeast India

Even though populations of *An. baimaii* from northeast India had high genetic diversity, these populations were genetically distinct from those of the adjoining countries of Bangladesh, Myanmar and Thailand suggesting significant barrier to gene flow [140]. However, there was no significant genetic differentiation between populations of northeast (except for population in the Barail hill range of northeast), thus be considered one entity for implementation of control interventions [141]. Yet owing to continued deforestation and possible disruption of gene flow between populations, there is possibility of existence of another taxon tentatively designated as 'species x' which call for additional investigations. *An. baimaii* is also known to inhabit forests of Andaman and Nicobar islands but there is dearth of data on population genetic structure and role in malaria transmission. *An. elegans* is exclusively found in south-western India but there is no evidence of its role in malaria transmission [8].



## 6. *Anopheles (Cellia) sundaicus* (Rodewaldt) species complex

*Anopheles sundaicus* s.l. is an important vector of malaria throughout its range of distribution in the oriental region (Figure 8). It is currently a complex of four species, i.e., *An. sundaicus* s.s., *An. epiroticus* Linton & Harbach (formerly species A), *An. sundaicus* species D and *An. sundaicus* species E [8,10,13,14,142,143]. In India, it has disappeared from the mainland eastern coastal belt of West Bengal and Orissa except small focus in the Kutch area of Gujarat [144], and is widely prevalent in Andaman and Nicobar islands populations of which have been characterized to be cytotype species D [145-147]. It is largely a brackish water species and breeds in a variety of habitats including swamps, salt water lagoons, creeks, pits along embankments but breeding in fresh water collections has also been recorded. Molecular characterization of cytotype D, however, did not reveal any difference between fresh water and brackish water populations but were different from *An. epiroticus* of Vietnam and *An. sundaicus* s.s from Borneo, Malaysia [148].



In black, the known distribution of the Sundaicus complex

**Figure 8.** Distribution map of the four member species of the *Anopheles sundaicus* complex in Southeast Asia (Courtesy: Dr. S. Manguin). *An. sundaicus* s.s. is distributed along the coast of Borneo. *An. epiroticus* occurs in coastal brackish water sites extending from southern Vietnam to peninsular Malaysia. *An. sundaicus* species E occurs in Sumatra and Java (Indonesia). *An. sundaicus* species D distribution is restricted to Andaman and Nicobar islands in India.

In Andaman and Nicobar islands, *An. sundaicus* is predominantly zoophilic except for indoor resting populations in human dwellings which had a significantly higher predilection for human host [149]. The relative abundance is reported to be higher in monsoon and post-monsoon months, populations of which rest both indoors and outdoors [149,150]. Biting activity occurred all through

the night but peak biting was during 21:00 till 04:00 hours. The species is susceptible to DDT and malathion. It is possible that given the richness of fauna of evergreen equatorial forest in the Andaman and Nicobar group of islands, additional sibling species of the *An. sundaicus* complex do exist with distinct bionomical characteristics, thus additional investigations are warranted for formulating appropriate control interventions [151].

## 7. *Anopheles (Cellia) stephensi* Liston – A complex of variants

*Anopheles stephensi* is an important vector of urban malaria and has been widely incriminated in most metropolitan cities by detection of gland and gut infections [7]. It is not considered a species complex but instead comprises three ecological variants, i.e., 'type form', 'intermediate form' and variety '*mysorensis*' characterized by egg morphometrics [152-154]. The 'type form' is an efficient vector of malaria in urban areas, and the variety '*mysorensis*' is largely zoophilic and has no role in malaria transmission [155-157]. The 'intermediate form' is typically recorded in rural and peri-urban localities but its role in malaria transmission is not known. The existence of ecological variants is further evidenced by Y-chromosome variation [158], spiracular index [159], and frequencies of inversion polymorphism in urban and rural populations in range of its distribution [160,161]. However, results of cross-mating experiments were variable ranging from infertility to reduced fertility [162,163] as opposed to full compatibility between populations [152].

*An. stephensi* is prevalent throughout the year but most abundant during months of rainfall (June–August) which coincides with the transmission period. In urban areas, it is generally endophilic and endophagic and breeds in domestic containers, building construction sites, overhead tanks, underground cement tanks, and evaporator coolers [155,164]. It is largely the 'type form' that is responsible for malaria outbreaks in urban areas related to construction projects and associated tropical aggregation of labor from malaria endemic areas. It is a thermophilic species and has longer flight range, and maintains a high degree of contact with human population [151]. In rural areas it is predominantly a zoophilic species and rests outdoors in cattle sheds, barracks, poorly constructed houses, and breeds in fresh water ponds, stream beds, seepage canals, wells etc. Peak biting activity is recorded between 22:00 to 24:00 hours but varies seasonally in different localities [7,165]. It is an invasive species and enters new towns and settlements.

The species is resistant to multiple insecticides but indoor residual spraying is not used for control. Instead recommended control measures are (i) source reduction, (ii) minor engineering interventions (iii) anti-larval methods including chemical and biological larvicides, (iv) application of larvivorous fish, i.e., guppy and gambusia, (v) aerosol space spraying for control of adult vector populations, (vi) legislative bylaws for preventing mosquito breeding [2]. In the face of rapid urbanization, unplanned growth and mushrooming of urban slums, rationed water supply and unsafe water storage practices; urban malaria is a growing problem presently accounting for >10% reported malaria cases in the country [166]. Overall, malaria cases in the rural and urban areas are grossly underestimated due to scanty surveillance and unreliable laboratory services.

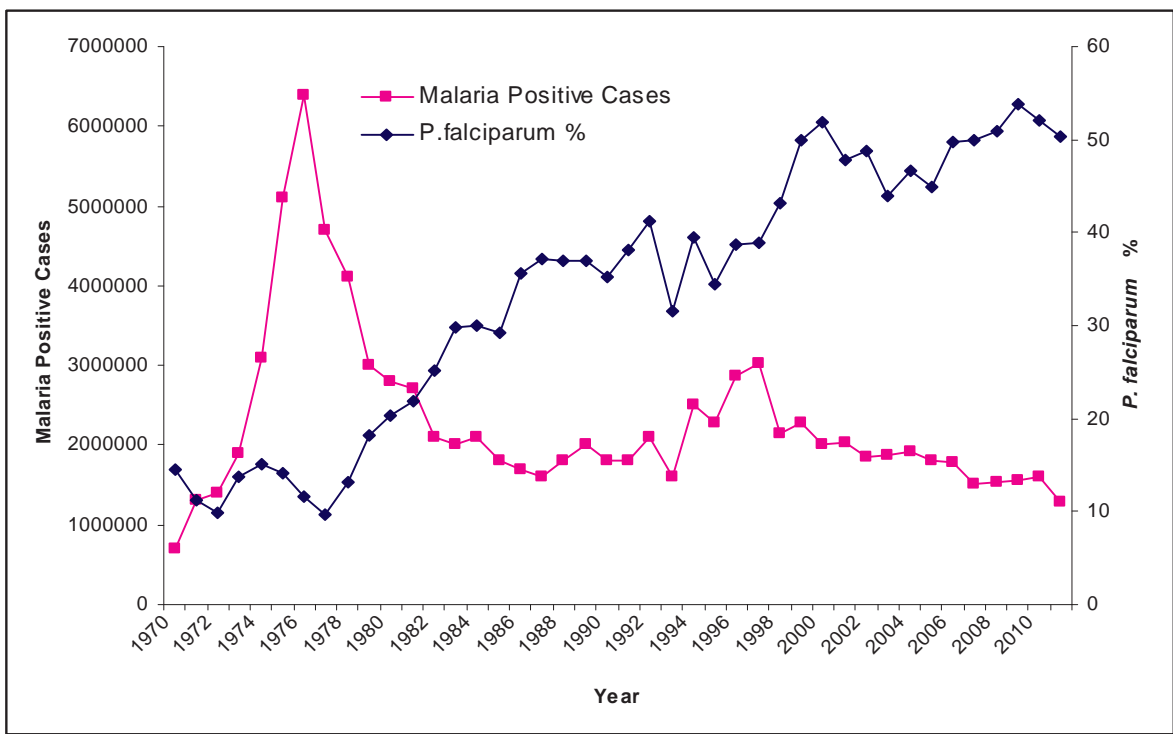
## 8. Prospects of vector control and research priorities

India has about a billion population at risk of malaria and accounts for the highest disease burden in Southeast Asia for estimated loss of disability adjusted life years [3,6]. Malaria transmission is complex due to multi-species co-existence and variable species dominance and bionomical characteristics [13,14]. Although, transmission trends seem to be declining (Figure 9), National Vector Borne Disease Vector Control Programme (NVBDCP) is faced with new emerging challenges. Some of these are (i) multiple insecticide resistance against target disease vector mosquito species, (ii) emerging multi-drug resistance and steadily rising proportions of *P. falciparum*, (iii) shortage of antimalarial drugs and insecticides, and (iv) human resource attrition of skilled personnel to meet the future challenges.

Indoor residual spraying (IRS) for vector control has become less effective and operationally difficult proposition [9,94]. In addition, ecological driven changes, population migration across borders, deforestation, developmental projects, and poor infrastructure have led to the opportunities for vector proliferation and increased malaria receptivity. Due to poor community acceptance for IRS and spray coverage of target population groups [167], India has embarked upon large scale implementation of Insecticide-treated netting materials / long-lasting insecticidal nets (LLINs) prioritizing high-risk population in malaria endemic states/districts. Disease transmission trends are declining in beneficiary population groups (formerly intractable high-risk areas); hence it is the right time to seize the opportunity for up scaling LLIN based intervention coupled with appropriate drug policy in place to combat the malaria illness and preventing spread of drug-resistant malaria [112,113,168-170]. It is worrisome, however, that the LLINs presently in use employ only pyrethroids, and *An. culicifacies* that is multi-resistant, is fast invading new territories making a malaria control a complex enterprise. What would be tantamount to vector control is the management of insecticide resistance for increased duration of its efficacy against target disease vector species by strategic application, insecticide rotation and mosaic application, and integrating bio-environmental approaches which should all be considered [171,172]. These approaches combined with environment management methods which are situation-specific and community-based would yield long term dividend for sustainable vector control [173,174]. Among alternate methods of vector control, large scale application of larvivorous fish, i.e., *Poecilia reticulata* and *Gambusia affinis* have been proven to be effective against *An. culicifacies* transmitted malaria in South Indian state of Karnataka [175,176], and inspired by the success story as role model, other malaria endemic states are also contemplating incorporating this method as component of the integrated approach for vector control [177].

Besides dominant proven vector species, sporadic gut/ gland infections have also been recorded in *An. maculatus* s.l., *An. annularis* s.l., *An. nivipes/philippinensis*, and *An. subpictus* s.l. substantiated by variable levels of anthropophily and detection of circumsporozoite proteins [8,69,77,109,178,179]. These mosquito species, however, are considered of lesser significance for their role in malaria transmission except in areas reporting diminishing population densities of dominant vector species. Among these, *An. maculatus*, has been investigated in depth for spatial distribution and molecular characterization of its member species for possible

role in malaria transmission specific to northeast India [180]. Of the nine formally named species of *An. maculatus* complex [181], six species namely, *An. pseudowillmori* and *An. maculatus* (most abundant), and *An. willmori*, *An. sawadwongporni*, *An. rampae*, *An. dravidicus* (restricted distribution) have been recorded to exist in northeast region; none of these, however, found positive for human malaria parasite [180]. Of the five species in the *Anopheles annularis* group of mosquito species, *An. annularis*, *An. nivipes*, *An. philippinensis* and *An. pallidus* are widely prevalent in India. Among these, *An. annularis* comprises two cryptic species provisionally designated as species A and B with variable distribution records [182]. It has been incriminated in certain localities but it is a predominantly zoophilic species [183]. *An. nivipes* and *An. philippinensis* are morphologically very similar, yet can be characterized by cytogenetic and molecular techniques [184-186]. Both are also predominantly zoophilic. *An. subpictus* that is widely abundant in mainland India has been characterized to be complex of four sibling species provisionally designated as A, B, C and D identified by distinctive morphology, species specific diagnostic inversion genotypes and breeding characteristics [8,187]. It has been incriminated in coastal villages of South India, Central India, and Sri Lanka but additional investigations are warranted for distribution of individual sibling species and role in malaria transmission [188-190].



**Figure 9.** Malaria cases in India (1970-2011) recorded by the Directorate of National Vector Borne Disease Control Programme (NVBDCP). Cases started rising in 1970, reporting 6.45 million cases in 1976 and following the implementation of the Modified Plan of Operation in 1977, malaria cases declined but mainly *Plasmodium vivax* malaria due to its sensitivity to chloroquine. Beginning 2005 with increased allocation of resources for strengthening interventions, cases are gradually declining. *Plasmodium falciparum* proportions, however, that was about 10% in 1977, has risen to about 50% and the parasite has become mono to multi-drug resistant (data source: NVBDCP).

In moving forward for achieving ambitious goal of malaria elimination in feasible districts/states, lot more needs to be accomplished in understanding vector bionomics in the altered ecology. The future priority area should include developing malaria-risk maps for focused interventions, ecological succession of disease vector species, monitoring insecticide resistance, cross-border initiative with neighboring countries for data sharing and coordinated control efforts, development of evidence-based newer tools for vector control, strengthening health systems for improved surveillance and monitoring, and universal access to malaria treatment and prevention which would help meeting the Millennium Development Goal in reducing malaria morbidity and mortality by 2015 [191-193].

## 9. Conclusions

During the past decade, there has been significant progress in development of molecular techniques in identification of sibling species of the dominant mosquito vector taxa, understanding their bionomical characteristics and role in malaria transmission in India. Among these, for *An. culicifacies* and *An. fluviatilis* which account for nearly 80% of malaria cases, vector control strategy has been formulated for judicious application of insecticide and saving operational costs. In the changing ecological context, *An. culicifacies* that is fast invading new territories is reportedly developing resistance to multiple insecticides including pyrethroids and inter-alia rising proportions and spread of multi-drug resistant *P. falciparum* malaria are some of the major concerns which call for continued research efforts for newer interventions that are evidence-based, community oriented and sustainable. Future priority area of research in vector control should include developing malaria-risk maps for focused interventions, monitoring insecticide resistance, cross-border initiative with neighboring countries for data sharing and coordinated control efforts for achieving substantial transmission reduction, and help check spread of drug-resistant malaria.

## Abbreviations used

DDT: Dichloro-diphenyl-trichloroethane; rDNA: Ribosomal deoxyribonucleic acid; ITS2: Internal Transcribed Spacer 2; PCR: Polymerase Chain Reaction; RFLP: Restricted Fragment Length Polymorphism; CO II: Cytochrome Oxidase II; IRS: Indoor Residual Spray; LLIN: Long-lasting Insecticidal Net; MPO: Modified Plan of Operation; NVBDCP: National Vector Borne Disease Control Programme.

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