MALARIAL MOSQUITO SPECIES COMPLEX: A GENERAL REVIEW

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Article Importance: Malaria is a mosquito born disease caused by parasite of the genus Plasmodium. Nearly three billion people are at risk of malaria worldwide. Malaria, a disease of antiquity that has proved to be a formidable deterrent to the cultural and socio-economic progress of humans in the tropical, sub-tropical and monsoon prone zones of the world. It is not easy to control malaria by controlling protozoan but is easy to control vector through various vector control programme but the success of any vector control programme relies on knowledge of vector species and their bionomics. So my present review paper focus on species complex of *Anopheles* especially the primary vectors of malaria. As their study may be helpful in vector control as these species complex are differ in their biological characteristics like vectorial potential, host preference, resting behavior and response to insecticides that may be vital for malaria control.

ABSTRACT : The success of any vector control programme relies on knowledge of vector species and their bionomics, which is complicated due to the fact that among the six recognized primary malaria vectors in India, all except *An. stephensi* are species complexes .There are growing evidences that the members of species complexes differ significantly in biological characteristics that are vital for malaria control point of view such as vectorial potential, host-preference, resting behaviour and response to insecticides. Culicifacies and Fluviatilis complexes are the most studied vector from India, which are togetherly responsible for a majority of malaria cases. This review paper gives an update on recent advances in the field of vector biology, particularly recognition of sibling species, methods for their identification, differential bionomics of members of species complexes.

Keywords: *Anopheles,* complex, species.

INTRODUCTION: In India malaria spread due to diverse ecology and multiple disease vector species. In the Southeast Asian region, India contributes to nearly 80% of malaria cases with the largest population of the world living at risk of malaria. Mosquito fauna is rich in the tropical climate with various and diverse breeding resources. Of 58 anophelines in India, only are primary malaria vectors with their regional distribution. *Anopheles culicifacies* is the vector of rural malaria in the country and generates about 70 % of cases every year. *An. fluviatilis* is found in the plains and foothills and breeds in streams contributing 15% of malaria cases, *An. minimus* breeds in streams of foothills of the northeast part of the country, *An. dirus* is established in jungles of northeastern states, *An. sundaicus* is found in Andaman and Nicobar

islands and breeds in brackish water this makes it different from other species, and *An. stephenesi* is the renowned vector species of urban malaria. India is experiences quick ecological changes owing to population explosion, urbanization, development projects, deforestation and human migration affecting mosquito ecology and disease transmission. Besides these species, some are of local importance, viz. *An. philippinensis-nivipes*, *An. varuna*, *An. annularis* and *An. jeyporiensis*. Current knowledge on the vectors and their accurate role in malaria transmission is incomplete due to the fact that all the major malaria vectors, except *An. stephensi*, are complexes of more than one biological species which are morphologically indistinguishable and these are called as sibling or cryptic species. Studies on their bionomics, distribution, role in malaria transmission are important due to growing evidences that these cryptic species may differ significantly in biological characteristics especially those which are of importance from malaria control point of view, such as, response to insecticides, vectorial competence, host-preference and resting behaviour. The correct identification of malaria vector in vector-control programme is another issue which is critical to the success of the programme. This becomes more important in the case of closely related species and members of species complexes. Sibling species in most of the vectors have been recognized through chromosomal studies or biochemical methods. Cyto-taxonomic methods based on ovarian polytene chromosome are often difficult to be carried out, need expertise and are applicable to semi-gravid individuals, which constitute a small proportion of the population. Molecular methods are generally used for recognition of sibling species; however, such tools have been extensively used for species diagnosis. A PCR-based species-diagnostic assay is relatively easy to be performed and is applicable for all stages and sex of mosquitoes, but such a technique however simple, is available in research laboratories only. During the past two decades there has been a momentous progress in the direction of recognizing new sibling species in vector systems and development of methods for their identification, understanding of their bionomics and establishment of their role in malaria transmission.

An. culicifacies Complex

Anopheles culicifacies entrenched peninsular India. It is the main cause of 70% of the malaria. It is the main vector of malaria in rural plains areas and peri-urban areas. It is found in a variety of natural and man-made breeding sites. It is highly zoophilic because of the presence of a high density of cattle relative to that of humans limits its vectorial capacity. It invade from India eastward into Nepal, Bangladesh, Thailand, Myanmar, Chin, Veitnam, Cambodia, westward into Afganistan , Pakistan, Iran, Yemen, Ethiopia, and southward into Sri Lanka [1]. In India culicifacies has been found throughout the country including Kashmir and high elevations of the Himalayas except Andaman and Nicobar Islands. It is abundant in the plains but is found in lesser amount in the eastern parts of the country. In the monsoon and post monsoon months its number is less in the hilly areas of Western Ghats, deserts of Rajasthan, northern Gujarat, Kutch and Punjab[2].

Two sibling species A and B of *Anopheles culicifacies* were discovered by Green and Miles in 1980 from ovarian polytene X-chromosomes in half gravid females. Later on C[3] and D[4] were also discovered. All four species i.e. A, B, C and D can be differentiate by paracentric inversions on the X-chromosome and chromosome-2 and finally species E was discovered by mitotic karyotyping and sporozoite positivity[5] . They differ in several biological characteristics, i.e in their distribution pattern, vectorial competence, seasonal prevalence[1], response to insecticides and host preference[6]. *An. culicifacies* species B is a non-vector and is found exclusively found in the eastern states of India, while in other areas it is found to be sympatric with species A, C, D or E. Species A is dominant throughout the year and a raise in the proportion of species B was observed in the post-monsoon months in villages surrounding Delhi, Haryana and Uttar Pradesh[1]. Species B and C together found in forest and plain areas of Sundergarh, Orissa, but here B is predominant throughout the year.

***An. fluviatilis* Complex**

The *An. fluviatilis* is entrenched from Yemen to Formosa (Taiwan)[7] its presence beyond east of northeastern states of India is doubtful. Earlier report of the presence of *An. fluviatilis* (species U) from Assam, India is doubtful. Recently, using DNA data it was found that the morphologically identified *An. fluvatilis* from Assam are the morphological variants of sympatric species *An. minimus s.s. Anopheles fluviatilis* is recognized as a species complex comprising at least three sibling species – species S, T and U[8]. Existence of two additional taxa within the *An. fluviatilis* complex is found i.e. one in Iran and another in India, which are temporarly designated as *An. fluviatilis* forms V and X, respectively, were distinguished based on variant ITS2 sequences[9]. However, it was also found that species X is identical to species S[10]. Sibling species of Fluviatilis Complex was recognized on the basis of fixed inversions in polytene chromosome[11]. However, it is difficult to differentiate between species S and T by chromosomal method in areas where q1 inversion, a marker for differentiation between species S and T, is polymorphic.

It has been proposed that, to distinguish such species (S and T) biological characteristics (such as host preference) may be taken as criteria[3]. A molecular method based on 28S rDNA has also been developed for the differentiation of all members of morphologically identical *An. fluviatilis*[12]. All the three species of the Fluviatilis Complex are found in India. Species T is most extensively distributed in India and also found in Iran[13]. Species S seems to be forest species and is dominant in Orissa, India, where it is the main malaria vector. Differences in biological characteristics have been reported among members of the Fluviatilis Complex. Species S has been predicted as highly efficient malaria vector and is predominantly anthropophagic, whereas species T and U are recognized as non-vectors and are almost exclusively zoophagic[14]. It was observed that species T which does not act as vector in nature is highly susceptible to malaria sporogony in laboratory[7]. This is possibly because of its zoophagic nature making it a nonvector, but potentially act as vector where man : cattle ratio is high.

***An. Minimus* Complex**

The distribution of *An. minimus* extends from Uttar Pradesh down to the northeastern tip of Andhra Pradesh in India, across the Indochina– Malay peninsular countries down to the Thai–Malay border and north across the People's Republic of China (upto 30"N latitude) to Taiwan and the Ryukyu islands. This species has been reported to have disappeared from the Terai region of Uttar Pradesh (now in Uttaranchal)[3] because of the introduction of DDT. In Singhbhum hills of east-central India, where this species has been missing for nearly 45 years, has now reappeared[15]. *A. minimus* is recognized as species complex comprising at least three sibling species A, C and E3. Species A has now officially been recognized as *An. minimus s.s.* Until now no chromosomal method is available for the identification of members of *An. minimus*. However, *An. minimus s.s.* and species C can be differentiated by the octanol dehydrogenase (*odh*) analysis[16] and ITS2-based PCR–RFLP[17] or ASPCR[18]. In India, so far only *An. minimus s.s.* has been found in northeastern[19], Singhbhum hills[20] and Jalpaiguri, West Bengal. In India *An. minimus s.s.* is extremely anthropophagic and is an efficient malaria vector mainly in northeastern states[21]. Among *An. minimus* complex, species C has been reported to be exophagic and zoophilic as compared to species A[22]. *An. fluviatilis* and *An. minimus* are closely related species[23]. These two species are morphologically identical and *An. minimus* has been misidentified as *An. fluviatilis*[24, 25] probably due to variation in palpal ornamentation which has been described as 'hypermelanic form' of *An. minimus*[13]. Some considered *An. fluviatilis* S, an important malaria vector in India, as a synonym of *An. minimus* C[9, 23, 26] because of the homology of small region of rDNA sequence data and similarity in biological characteristics[9]. But later after sequencing of d2–d3 domain of 28S rDNA, ITS2 and cytochrome oxidase II revealed that *An. fluviatilis* S and *An. minimus* C independent species[10].

Anopheles stephensi is a sub-tropical species and entrenched throughout the Middle East and South Asia region and is considered an important vector in India, Pakistan and Iran. Till now there is no description of sibling species in *An. stephensi*; however two races, 'type form' and '*var. mysoriensis*' have been classified based on egg-dimension and number of ridges present on the floats[27,28]. The 'type form' is predominant in urban area and is a malaria vector whereas *var. mysorensis* , is inhabitant of rural areas, is not a vector.[29] An another has been reported i.e. 'intermediate'. All these three forms are found in India[29] and Iran[30] According to recent observation 'type form' and '*var. mysoriensis*' these two forms are ecological adapted to different ecological niche. The *var mysorensis* has lower spiracular index as compared to 'type form' and hence more adapted to dry climate. *An. stephensi* is mainly a zoophilic species but considerable variability in human blood index (HBI) has been reported. In Kolkata, an urban area, as high as 100% HBI has been reported[31]. The precise breeding sites in urban areas are: building-construction sites, wells, garden ponds, cisterns, overhead tanks, ground level cement tanks, water coolers, etc. In rural areas it commonly breeds in a variety of breeding sites such as streams and channels, tanks and ponds, seepages and wells. This is chiefly an urban vector. Since a majority of breeding sites for *An. stephensi*

in urban areas are man-made and limited, so it is possible to control their breeding by community involvement, biological control and enforcement of legislative measures.

***An. Dirus* Complex**

The *An. dirus* is mainly found in the forest and forest-fringe area and its members are vectors in India, Bangladesh, Myanmar and Thailand. It is a complex of seven sibling species designated as *Anopheles dirus s.s.* (species A), *Anopheles cracens* (species B), *Anopheles scanloni* (species C), *Anopheles baimaii* (species D), *Anopheles elegans* (species E), *Anopheles nemophilous* (species F) and *An. takasagoensi*[32]. In India, only two species, *An. baimaii* and *An. elegans* are found. The species D is highly anthropophagic[33] and an efficient malaria vector, is found in north-eastern states and the latter in Shimoga hills of Karnataka, the vectorial status of which is unknown. The presence of *An. baimaii* in northeastern states of India has been confirmed by ITS2-rDNA sequencing and ASPCR[19]. An ITS2-based ASPCR is available for distinguishing five members of the complex, *An. dirus, An. cracens, An. scanloni, An. Baimaii* and *An. nemophilous*[34].

***An. sundaicus* Complex**

An. sundaicus is a malaria vector of coastal areas in Southeast Asian region. It extends from northeastern India to southern Vietnam, south to the Nicobar, Andaman, and Indonesian islands[35]**.** In India it was reported from West Bengal, Orissa, the coastal areas of Andhra Pradesh and Andamans[36], but its presence is now restricted to Andaman and Nicobar Islands[37] and Kutch of Gujarat state[38]. It preferentially breeds in saline/brackish water, though it has been reported to breed in freshwater also. There has been three sibling species of *An. sundaicus* reported in Thailand and Indonesia on the basis of chromosomal and biochemical (isozyme) evidences and designated as species A, B and C. Species *An. sundaicus s. s.* has been formally designated from Malaysia[39]. Species A has been formally designated as *An. epiroticus*[40] . Recently a new cytotype D from Car Nicobar island has been reported[41]. Being allopatric, specific status of cytotype D has not been assigned as sibling species. Molecular characterization of this cytotype using ITS2 region discovered no difference in population from brackish and freshwater habitats, but is different from *An. sundaicus* A of Vietnam and *An. sundaicus s.s.* of Malaysia[42]. *An. sundaicus* has adaptability to breed in wide salinity conditions from freshwater to brackish water[35].

CONCLUSION:

During the past decade, there has been considerable progress in development of molecular techniques in identification of sibling species of the dominant mosquito vector taxa, under- standing their bionomical characteristics and role in malaria transmission in India. All the primary vectors of malaria in India except *stephensi* are rported with sibling species. An. stephensi has been reported with three type of variants which are distinguishable because of the presence of ridges present on the eggs. Out of the reported sibling species few of them are vectors and not all. Knowledge about the sibling species help us to know about their biological characteristics i.e. their breeding site, their resting behavior, etc. which greatly help us in

vector control management. Ecological changes driven by deforestation, human migration and unmanaged urbanization have amplified the densities of human hosts and vector breeding sites in some malarious region. Knowledge of the breeding habits of the sibling species can assist in designing optimal vector control strategies. Knowledge on the feeding preferences of mosquito larvae may guide the development and use of biocontrol measures.

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