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Dr. Raj Kumar Singh
National Institute of Malaria Research, Sector-8, Dwarka, New Delhi-77, India.
E-mail: singhriku@yahoo.co.in
Tel: +91-9911164342

Mr. Gaurav Kumar
National Institute of Malaria Research, Sector-8, Dwarka, New Delhi-77, India.
E-mail: gauravnimr@gmail.com
Tel: +91-9811542216

Dr. Pradeep Kumar Mittal
National Institute of Malaria Research, Sector-8, Dwarka, New Delhi-77, India.
Email: pk_mittal52@yahoo.co.in
Tel: +91-9868964637

Dr. Ramesh Chand Dhiman
National Institute of Malaria Research, Sector-8, Dwarka, New Delhi-77, India.
E-mail: r.c.dhiman@gmail.com
Tel: +91-9871535858
Tel: 011-25307100

Correspondence:
Dr. Ramesh Chand Dhiman
National Institute of Malaria Research, Sector-8, Dwarka, New Delhi-77, India
E-mail: r.c.dhiman@gmail.com
Tel: +91-9871535858
Tel: 011-25307100

Bionomics and vector potential of *Anopheles subpictus* as a malaria vector in India: An overview

Raj Kumar Singh, Gaurav Kumar, Pradeep Kumar Mittal, Ramesh Chand Dhiman

ABSTRACT

Anopheles subpictus has been recognised as an important vector of malaria in Sri Lanka and some other countries like Malaysia and Maldives. It has been found to play an important role in malaria transmission as a secondary vector in certain parts of Odisha and coastal areas of south India. *An. subpictus* is a widely distributed mosquito species that breeds in a variety of fresh as well as saline water habitats. The species is a complex of four sibling species provisionally designated as: sibling species A, B, C and D, but the role of these sibling species in malaria transmission is not clearly known. As there is limited research work available on this species in India, it was thought prudent to review the bionomics and the role of *An. subpictus* in malaria transmission in Indian context. Further studies are required on the bionomics of *An. subpictus* and its role in malaria transmission in other parts of the country under the influence of changing ecological conditions.

Keywords: *Anopheles subpictus*; Distribution; India; Malaria; Species complex; Vector

1. Introduction

Malaria is one of the major communicable disease causing high mortality and morbidity among population in India. About one million malaria positive cases with 519 deaths were reported during the year 2012, out of which about half the cases were of *Plasmodium falciparum* (*Pf*) [1]. This disease is transmitted by biting of the female anopheline mosquito species. Out of 58 *Anopheles* species found in India, the six species *viz*., *Anopheles culicifacies*, *An. stephensi*, *An. minimus*, *An. sundaicus*, *An. fluviatilis* and *An. dirus* are major vectors of malaria and three species *viz*., *An. annularis*, *An. philipinensis* (*nivipus*) and *An. varuna* are minor vectors of malaria [2, 3].

Another species *viz*., *An. subpictus* has also been incriminated as a malaria vector in India but its role in transmission has not been established in the country. *An. subpictus* was first described by an Italian scientist Grassi in 1899 [2]. *An. subpictus* is widely distributed and found in abundance in the Oriental region. It is found to the west of India in Afghanistan, Pakistan and Iran and to the east in New Guinea and in Marina's islands. It is also found in Sri Lanka in south and China in north of India. In India, it is found throughout the mainland [2].

An. subpictus has been incriminated as a vector of malaria in Maldives Islands, Celebes, South Java, Portuguese Timor and Malaysia [4]. It is a secondary vector of malaria in Sri Lanka [5]. While in India, the role of *An. subpictus* as a vector of malaria is obscure. But in view of the ongoing process of climate change; *An. subpictus*, may also act as a primary vector of malaria [6]. Keeping in view the above facts, this review has been prepared on the basis of work reported in India as there is limited research work available on this species in India.

2. Biology

The species *An. subpictus* is a complex of four sibling species A, B, C and D in India. Egg float, ridge number, larval mesothoracic seta 4-M, pupal seta 7-I and the female palpi have species specific diagnostic value in the *An. subpictus* complex. Degree of interspecific divergence in these morphological characters differentiates them more than usual for sibling species [7].

As with most other complexes of anopheline mosquitoes, rearrangements in the polytene chromosome banding sequence are crucial to the interpretation of species relationship in the *An. subpictus* complex. The four members of this complex have been described as possessing characteristic paracentric fixed inversions on the X-chromosome *viz*., species A ($X+^{a}$, $+^{b}$), species B (Xa, b), species C (Xa, $+^{b}$) and species D ($X+^{a}$, b). Members of the complex also differ in breeding site, feeding preference and seasonal abundance [7-9]. Based on homozygous inversion in the X chromosome involving subzones 2A–4C, Chaudhry and Soni [10] separated *An. subpictus* allopatric populations (one breeding in rain water and another breeding in brackish water). With the introduction of the techniques of molecular taxonomy, PCR may be used for chromosomal and genomic characterization of these 4 species [11]. To supplement data of polytene chromosome studies, Sharma [12] began by carrying out RAPD-PCR-based characterization of the genomic DNA of 2 different populations of species B of *An. subpictus* using only 3 random primers and isolated several bands of variable base pair lengths in which intraspecific differences could be detected in the form of presence, absence, population-specific, and species-specific bands.

3. Vector incrimination

3.1 Field studies

Studies on the vector status of *An. subpictus* started in earlier years of nineteenth century. Prior to 1933, several studies conducted in different parts of India were unable to get any infection in *An. subpictus*. But Strickland *et al*. [13] succeed to get 2 gland positive mosquitoes out of 10452 dissected. Later on Russel and Jacob [14] and Russel *et al*. [15] got both gut and gland positive for *Plasmodium* from Ennore Nellore area of Tamil Nadu. Russel and Rao [16] also got naturally infected *An. subpictus* from Tamil Nadu, whereas Neogy and Sen [17] and Das *et al*. [18] failed to get any infection in field collected *An. subpictus*. There has been renewed interest in *An. subpictus*, because of reports of persistent malaria transmission in coastal areas of Tamil Nadu [19]. *An. subpictus* was reported as a suspected vector in Lakshadweep islands [20]. In an outbreak of malaria in a few coastal villages near Pondicherry, inconclusive evidence was found for *An. subpictus* as a vector, but further studies showed that out of 6133 dissected *An. subpictus* mosquitoes, 52 had oocysts (0.085%) and 4 had sporozoites (0.07%), (VCRC unpublished reports). Panicker *et al*. [4] incriminated *An. subpictus* from coastal villages of south India[4]. Kulkarni [21] was the first to incriminate *An. subpictus* from north central India. Chatterjee and Chandra [22] and Kumari *et al*. [23] found *Plasmodium* positive *An. subpictus* mosquitoes from eastern India (Figure1). In an unpublished report from Ranchi, *Pf* positive *An. subpictus* mosquito has been detected among field collected samples (Table 1).

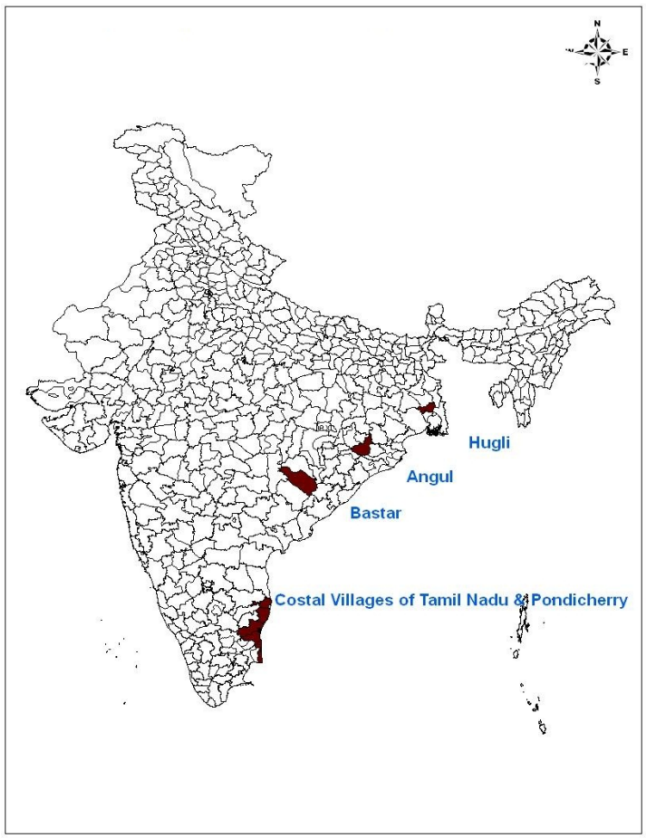

Fig 1: Reported vector incrimination of *An. subpictus* in India

Table 1: Natural infection in *Anopheles subpictus* mosquitoes

Locality/ state	No. dissected	Gut positive	Gland positive	Total positive	Reference	Reference no.
Darjeeling terai (West Bengal)	10452	0 (0%)	2 (0.019%)	2 (0.019%)	Strickland *et al. (*1933)	13
Ennore Nellore (Tamil Nadu)	4897	2 (0.04)	1 (0.02%)	3 (0.061%)	Russel and Jacob (1939)	14
Ennore Nellore (Tamil Nadu)	8381	4 (0.04%))	1 (0.012%)	5 (0.06%)	Russel *et al.* (1939)	15
Pattukottai (Tamil Nadu)	13277	1 (0.007%)	1 (0.007%)	2 (0.353%)	Russel and Rao (1940)	16
Pondicherry & Tamil Nadu	3752	45 (1.2%)	2 (0.053%)	47 (1.25%)	Panicker *et al.* (1981)	4
Bastar (Madhya Pradesh)	12107	0 (0%)	3 (0.025%)	3 (0.025%)	Kulkarni (1983)	21
Tarakeshwar (West Bengal)	621	0 (0%)	2 (0.32%)	2 (0.32%)	Chatterjee and Chandra (2000)	22
Angul (Odisha)	1158	-	6 (0.52%)	6 (0.52%)	Kumari *et al.* (2009)	23

3.2 Laboratory work

Efforts were also made to get this mosquito infected with parasite in laboratory. First experimental infection of *Plasmodium* by *An. subpictus* was done by Strickland and Roy [24] but he was able to get only gut positive mosquitoes. Later, Russel and Mohan [25] and Roy [26] succeed for the first time to achieve infection both in gut and gland. *An. subpictus* was less susceptible to human malaria infection as compared to main malaria vectors, *An. culicifacies* and *An. stephensi* [27, 28]. In a comparative study of *An. subpictus* from fresh and brackish water; Sahu [29] observed that the mean susceptibility rate among fed mosquitoes was 6.1% and 17.4% from the fresh and brackish water respectively (Table 2).

4. Breeding habitats and its relationship with vegetation

An. subpictus breeds in a variety of breeding habitats which includes wells, burrow pits, channels, lake margins, ponds, cemented tanks, ground pools, fallow and freshly flooded rice fields and cisterns etc [2]. It can breed both in fresh or brackish water, particularly species B, which is mainly reported to breed in brackish waters predominate in coastal areas. Reuben *et al*. [30] conducted an experiment on salinity tolerance of sibling species. They showed that species A was found in habitats with salinity ranging from 0.004 to 0.734% NaCl while species B was not recorded at below 0.40% salinity. They also observed that both sibling species were able to withstand pollution as judged by low dissolved oxygen content. It has also been reported to breed in stagnant and flowing water, whether clean or polluted. It is perhaps the most ubiquitous of all species regarding its breeding places [2]. *An. subpictus* breeds predominantly in association with *Spirogyra*, *Pistia* and water hyacinth [31, 32]. One of the aquatic plant *Chara*, often regarded as inimical to the breeding of anopheline larvae, did not inhibit growth of *An. subpictus* [33]. *An. subpictus* was the most abundant species which bred in association with most of the vegetation. The largest number of larvae of *An. subpictus* was found in association to *Trachelomonas*, while it had poor association with *Eichhornia* and *Nymphea* [34]. In one of the study carried out in central Gujarat, larvae of *An. subpictus* showed the most frequent association with filamentous green algae (80.83%) such as *Spirogyra* followed by water hyacinth (76.11%) [35]. Overall it can breed in association with a variety of aquatic plants but thick vegetation has been reported to inhibit its growth [36].

Table 2: Laboratory infection in *Anopheles subpictus* mosquitoes

Locality/ state	No. dissected	Gut positive	Gland positive	Total positive	Reference	Reference no.
-	221	6	-	-	Strickland and Roy (1936)	24
South east madras	67	-	1	2.30%	Russel and Mohan (1939)	25
Salt Lake Kolkata	77	22	5	27	Roy (1943)	26
Uttar Pradesh	-	-	-	31%	Nanda *et al.* (1987)	28
Malkangiri, (Odisha)	245 (fresh water)	-	-	6.10%	Sahu (1998)	29
	224 (brackish water)			17		

5. Distribution

In India, *An. subpictus* is the most common species occurring in all the mainland zones. It is found up to 1900 metres height. Though its numbers are small, it occurs in many parts of Himalayas. *An. subpictus* gradually declines in abundance proceeding eastwards in India [2]. Based on the published reports it may be understood that this mosquito species is present almost all over India (Figure 2) [37-76].

6. Longevity

Longevity of the vector species is one of the prime parameter which determines the vectorial potential of a mosquito species. If the mosquito does not live long enough to the infective stage (extrinsic incubation period), it cannot act as a vector. Only few laboratory experiments have been carried out to determine the longevity of *An. subpictus*. It is shown to survive comparable to that of major vectors, *An. culicifacies* and *An. stephensi*. A great number of females lived up to two weeks at 30 ^{0}C and 70% RH (relative humidity) but low humidity was injurious to its life [77-78]. Panicker and Rajgopalan [79] in his laboratory experiments have shown that longevity ranged from 2-21 days and fifty percent of mosquitoes live more than 7 days. It has been reported from different parts of the country that in nature it may live up to 7-14 days which is sufficient for parasites to attain the infective stage [21, 26, 29, 80] (Table 3).

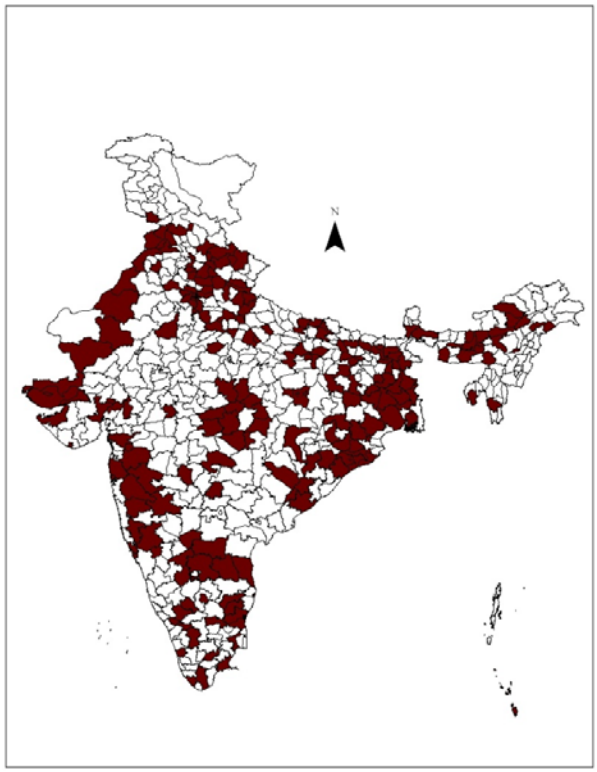

Fig 2: Reported distribution of *An. subpictus* in India

Table 3: Longevity of *Anopheles subpictus*

Locality/state	Longevity (Days)	Reference	Reference no.
Karnal (Haryana)	14 at 30 ^{o}C and 70% RH	Mehta (1934)	78
Salt Lake, Kolkata (West Bengal)	12.5	Roy (1943)	26
Bastar (Madhya Pradesh)	2 weeks	Kulkarni (1983)	21
Pondicherry	2-21	Panicker and Rajgopalan (1984)	79
Malkangiri, Odisha	7-8 days	Sahu (1998)	29
Tarakeshwar (West Bengal)	12 days (3 gonotrophic cycles)	Chatterjee and Chandra (2000)	22
Chilka lake, Odisha	8 days (2 gonotrophic cycles)	Dash *et al.* (2000)	80

7. Man hour Density

Man hour density (MHD) is the total number of mosquitoes collected by a person in one hour duration. It is an important parameter in entomological investigations to find out the density of any mosquito species. MHDs of *An. subpictus* from different parts of India reported by several authors have been summarized in Table 4. Bhatia *et al.* [81] reported MHD of *An. subpictus* ranging from 0 to 98 based on season and highest MHD reported in the month of August while studying the anopheline prevalence near Delhi. Neogy and Sen [82] observed 12.9 MHD of *An. subpictus* in a malaria outbreak study in rural Bengal. Sharma *et al.* [83] studied malaria transmission in Delhi and observed MHD ranged from 0 to 132.50 depending on the season. Ghosh *et al.* [84] observed very low MHD of 0.05% in rural west Bengal. In Bastar, Kulkarni [21]

reported 66 to 84 per man hour density from June to August. A wide range of MHD for *An. subpictus* has been observed from Daman, Assam, Madhya Pradesh, Chhattisgarh, Maharashtra, West Bengal and Uttar Pradesh [39, 85-90].

Table 4: Man Hour Density of *Anopheles subpictus*

Locality/state	MHD	Reference	Reference no.
Gorakhpur (Uttar Pradesh)	21.1	Samuel *et al* (2009)	90
Purulia (West Bengal)	9.23	Chandra (2008)	89
Pune (Maharashtra)	20	Tilak *et al* (2008)	38
Raipur (Chhattisgarh)	1.31 to 35.40	Baghel *et al* (2008)	88
Korea (Chhattisgarh)	17.5	Mishra and Chand (2004)	87
Chhindwara (Chhattisgarh)	2.7	Singh *et al* (2003)	86
Tmulpur (Assam)	0.7	Das *et al* (1997)	85
Daman	10.39	Khamre and Kaliwal (1988)	39
Hooghly (West Bengal)	0.05	Ghosh *et al* (1985))	84
Delhi	0 to 132.50	Sharma *et al* (1985)	83
Bastar (Madhya Pradesh)	66.0 to 84.0	Kulkarni (1983)	21
Burdwan (West Bengal)	12.9	Neogy and Sen (1962)	17
Delhi	0 to 98.0	Bhatia *et al* (1958)	81

8. Host preferences

Host preference is important in terms of Anthropophilic Index (AI), which is the proportion of freshly fed mosquitoes found to contain human blood. This index is an essential attribute in epidemiology of vector-borne diseases and also, needed to assess the vectorial capacity of a vector [91]. *An. subpictus* feeds predominantly on cattle and other domestic animals [92]. Very low AI has been reported for *An. subpictus* [93]. Contradictory to these studies, a high AI; 25, 35.6, 33.3 and 31.05 was reported from West Bengal, Madhya Pradesh, Delhi and Odisha respectively [21, 23, 26, 94]. In one study from Odisha, Sahu [29] observed 9% positivity for human antiserum in mosquitoes blood meal samples collected from fresh water area; while 44.65% from brackish water area. Chatterjee and Chandra [22] got 41% human blood positive mosquitoes out of 480 indoor resting *An. subpictus*. Kumar *et al*. [94] observed overall AI 16.93% irrespective of season in Delhi. The highest AI recorded during the June (33.33%) and July (26.8%) months of summer season. Thus AI of *An. subpictus* ranges from very low to 44.6 % in India (Table 5).

Table 5: Anthropophilic index of *Anopheles subpictus*

Locality/state	Anthropophilic index (%)	Reference	Reference no.
South eastern India	3.1	Russel and Jacob (1939)	14
Salt Lake, Kolkata	25	Roy (1943)	26
NA	9.2	Bruce Chwatt *et al* (1966)	93
Pondicherry and Tamil Nadu	14.5	Panicker *et al* (1981)	4
Bastar (Madhya Pradesh)	35.6	Kulkarni (1983)	21
Malkangiri (Odisha)	9(fresh water) and 44.6 (brackish water)	Sahu (1998)	29
Tarakeshwar (West Bengal)	41	Chatterjee and Chandra (2000)	22
Delhi	16.93	Kumar *et al* (2002)	94
Angul (Odisha)	31	Kumari *et al* (2009)	23

9. Flight range, swarming and mating

An. subpictus is generally regarded as a strong flier better than *An. culicifacies*, but reliable information does not exist in India [2]. Nagpal and Sharma [37] reported its flight range from 1.5-6 km [37]. The swarming and mating has been described by Rao *et al*. [95] in the Chilka lake areas of Odisha. The swarms consisted of as many as 5,000 specimens comprising both *An. subpictus* and *An. sundaicus*. The swarms were about 3 meters long with 1 meter width and 0.3 meter height. Roy [96] observed that it could not readily mate in cages of 2x2x1 feet, while *An. annularis* and *An. stephensi* did so. Panicker and Bai [97] observed that a beam of dim light from a torch stimulated their swarming activity when they were kept in 2x2x2 feet cage. Thus based on these observations, they stimulated a twilight condition by reducing the intensity of

light. Several mating pairs were observed in this dim light.

10. Insecticide susceptibility status

An. subpictus was the first anopheline species to be reported resistant to DDT [98]. It has been reported resistant to organochlorine insecticide, DDT from various parts of India [98-105]. Resistance against another organochlorine compound, dieldrin, has been detected from Rajasthan and Pondicherry [103-105]. In one of the studies carried out in arid and semi arid parts of India, resistance to organophosphate malathion and tolerance to synthetic pyrethroid was also reported [106]. In the aquatic stages of this species, tolerance to DDT, chlorpyriphos, fenthion, malathion, bifenthrin and carbofuran has been detected [105-106]. Since *An. subpictus* is emerging as a key vector, these reports are of great concern to the vector control programme. Use of organochlorine insecticides may be discontinued for the effective control of this vector species. As, extensive studies on the insecticide susceptibility status are lacking and there is need for more work on this important aspect as a preparedness.

11. Conclusion

An. subpictus is the most abundant anopheline species in most parts of India which can breed in almost every type of breeding habitat. It comprises of a complex of four biological species in India. Species A, C and D occur in fresh water habitats of inland areas, while species B occurs in brackish water. Most of the studies report it as a zoophilic species but a few studies reported AI up to 41%. It seems that under certain climatic conditions it can live long enough to gain infective stage. Studies in Sri Lanka showed that it is now acting as a main vector. This represent that it might be a vector under certain conditions. The percent of sporozoite positivity ranged from 0.053 to 0.52% which is comparable to other primary established vectors. Under laboratory conditions 31% sporozoite rate was recorded. Laboratory studies including the wild population dissection and experimental infection indicate that *An. subpictus* can be a potential vector and the term emerging vector has been given to this species [107]. Due to ecological changes some species of mosquitoes acting as a non-vector species may act as vector. Global warming consequent to increasing emissions of carbon dioxide is leading to the melting of polar ice and a rise in sea level. As a consequence, an increasing number of surface water bodies will become more saline in coastal areas of many countries. These will provide fertile breeding grounds for more salt tolerant anopheline vectors of malaria parasites and this poses a risk of increasing malaria transmission in coastal areas. Such an effect is likely to be compounded by increasing temperatures generally inducing a spread of the malaria vectors into greater latitudes in the temperate zones. The ability of *An. subpictus* to breed in brackish water poses a problem for India. Since 1990, there is sharp rising trend in temperature and variations in rainfall are also projected in India [108].

The climatic change has been projected to alter distribution of disease vectors spatially and temporally [109]. For malaria transmission, impact of different temperature range on the duration of sporogony has been well documented [110]. In view of above mentioned review, it is imperative to revisit the issue for determining the role of *An. subpictus* as a vector of malaria both under natural and experimental conditions in a changing climatic scenario.

An. subpictus occurs as a species complex that is identifiable with morphological characters within complex. Keeping in view of its vectorial status, there is a need for the development of a rapid and precise method for the sibling species identification. This must be based on molecular methods. Again there is also a need of study of biological characteristics of these sibling species so that effective mosquito control methods may be applied for malaria control.

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