Biology & control of *Anopheles culicifacies* Giles 1901

Malaria is a major public health problem in India. The epidemiology of malaria is complex on account of multiple vectors, vast and varied terrain, and a number of contextual determinants[1-3]. In Southeast Asia Region of the World Health Organization, India alone contributes nearly 80 per cent malaria cases, and about 95 per cent of the population is estimated to be living at risk of malaria with large concentration of cases in forests and in the hilly and inaccessible terrains[4]. In 2013, the National Vector Borne Disease Control Programme (NVBDCP) reported 0.88 million microscopically confirmed malaria cases and 440 deaths, and each year these figures are on the decline, although scientific institutions have repeatedly highlighted gross under-reporting of malaria morbidity and mortality[4-7]. *Plasmodium vivax* and *P. falciparum* are the two main malaria parasites that produce nearly equal proportion of cases (50:50), with a few cases of *P. malariae* from certain parts of Odisha (formerly Orissa), and sporadic reports of *P. ovale*. There are six major mosquito vectors in India (*Anopheles culicifacies, An. fluviatilis, An. stephensi, An. minimus, An. dirus,* and *An. sundaicus*), and all taxa except *An. stephensi* are species complex[8]. Among these, *An. culicifacies sensu lato* is the most abundant vector. It is widely distributed in rural and peri-urban India. *An. culicifacies* has played a major role in perennial malaria transmission in forested areas (*e.g.* Madhya Pradesh), and this vector species has penetrated deforested areas of Northeast[9-12]. *An. culicifacies* is responsible for epidemic malaria in its range of distribution and may cause intense malaria transmission in an estimated 75 million tribal population. *An. culicifacies* is multiple resistant to insecticides, and its control alone costs about 80 per cent of budget year marked for malaria control of the NVBDCP of the Government of India[4]. Furthermore, *An. culicifacies* comprises five sibling species with varying responses to insecticides and transmission potential[13]. Resurgence of malaria in late 1960s in India and adjoining countries of South Asia was largely

the result of failure in the control of *An. culicifacies*[14,15]. The present review provides comprehensive information on *An. culicifacies*, the most important malaria vector in India and the neighbouring countries.

Taxonomy & distribution

An. culicifacies Giles 1901 belongs to subgenus *Cellia* and series Myzomyia. Three synonyms of *An. culicifacies* are reported from India. These are the *indica* Theobald, 1901 of Chennai (formerly Madras); *listonii* Giles, 1901 from Ellichpur, Maharashtra, and *punjabensis* James, 1911 from Punjab[16]. It is in the last three decades that cytogenetic research has revealed the presence of five sibling species of *An. culicifacies* provisionally designated as A, B, C, D and E[17]. These sibling species differ in their biology, vectorial capacity and response to malaria control interventions. *An. culicifacies s.l.* is widely distributed in arid and semi-arid zones ranging from Afghanistan, Bangladesh, Cambodia, Southern China, Eretria, India, Iran, Laos, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam and Yemen (Fig. 1).

Seasonal prevalence

An. culicifacies s.l. is among the most widely distributed mosquito species in India and occurs in all mainland zones including Kashmir and high elevation in the Himalayas excluding islands of Andaman & Nicobar and Lakshadweep (Fig. 2). It is predominantly a vector of unstable malaria in the entire semi-arid and arid zones. Populations of *An. culicifacies* are low to scanty at higher altitudes and may transmit malaria under favourable conditions, *e.g.* global warming may bring some ecotones under transmission. It has been reported from 1000 to 2000 meters above mean sea level in Nilgiris hills and Kashmir, respectively[18-21]. This mosquito thrives well in plains receiving fair amount of rainfall; heavy rains in various parts of the country (*e.g.* Maharashtra, Uttrakhand), droughts and floods (*e.g.* Uttar Pradesh, Bihar), and excessive cold and hot weather in the plains are changing the ecology leading to re-distribution of vector densities capable of malaria transmission. Generally, *An. culicifacies* populations peak in monsoon and post-monsoon months, and from negligible numbers attain enormously high densities within 4-6 wk resulting in focal to regional epidemics[9-11, 22]. It is an important vector of epidemic malaria throughout its range of distribution, although it is a rare species in the Western Ghats.

Larval ecology

An. culicifacies s.l. is a prolific breeder and its preferred habitats are numerous and varied[16,18]. Irrigation channels, seepage, unused wells, field channels, waste irrigation water, hoof marks and cart tracks, *etc*. are some the preferred breeding places. *An. culicifacies*
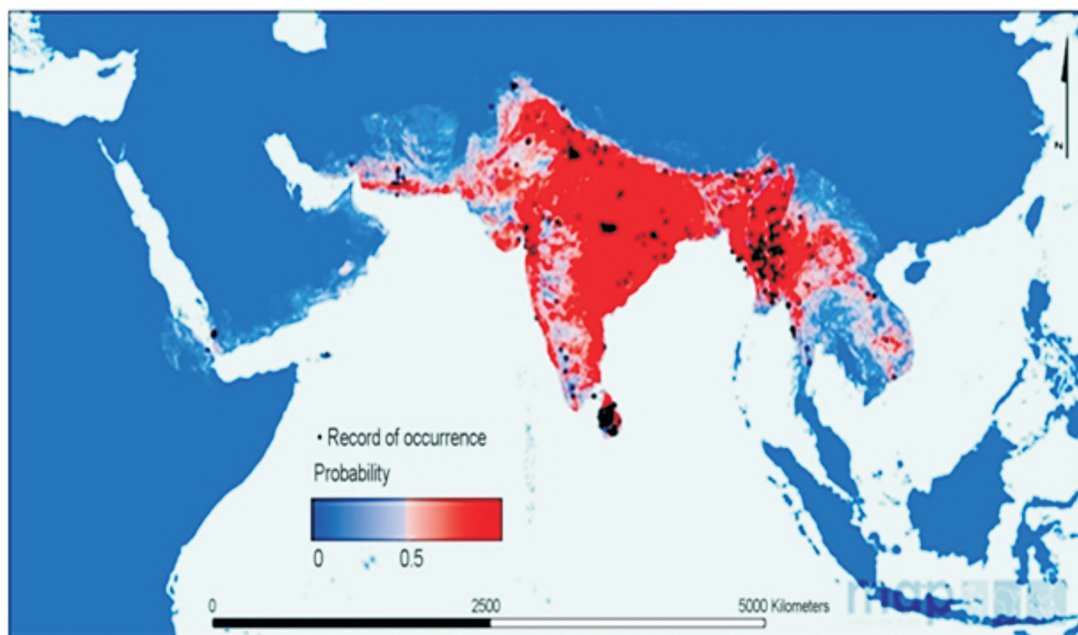

Fig. 1. Distribution map of *An*. (*Cellia*) *culicifacies* species complex in the world. Red and blue color depicts the high and low probability of occurrence of this species. Black dots display the sites of data collected (*Source*: *http://www.map.ox.ac.uk/client_media/medialibrary/2011/10/vector_Asia-Pacific_paper.pdf*).
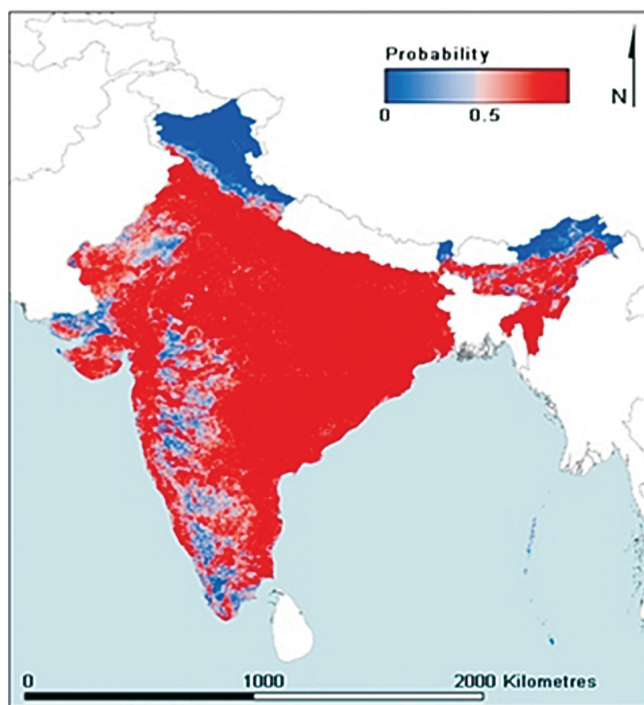
Fig. 2. Predictive map of the occurrence of *An.* (*Cellia*) *culicifacies* in India. Red and blue color depicts the high and low probability of its occurrence. (*Source*: *http://www.maps.ox.ac.uk/browse-resources/vector-occurrence/culicifacies/IND/*).

larvae have also been recorded in varying densities in pools, fallow fields, mining pits, river-bed pools, seaside marsh, turf pools, river edges, perennial streams, tanks, and artificial containers. The most suitable places for the breeding of *An. culicifacies* are small rocky pools and pits of perennial stream with clear water or with perceptible flow without shade and growth of any vegetation or macroscopic algae; and the least suitable places have highly turbid, stagnant and/or brackish water with good growth of vegetation including floating, sub-merged or vertical vegetation, growth of blue green algae and rich plankton; and the intermediate level of preference is found in fields, and channels with flowing water. *An. culicifacies* preferentially breeds in sunlit water collections. It breeds profusely in the freshly dug out pits, ornamental waters, unused swimming pools, and rain filled borrow pits along side of railway tracks. Although *An. culicifacies* is believed to breed in fresh water bodies, but it has adapted to lay eggs and undergo pre-imaginal development in saline/brackish water[23].

Sibling species complex

An. culicifacies s.l. was first colonized in the laboratory by the National Institute of Malaria Research (formerly Malaria Research Centre) in 1977[24]. Initially considered to comprise biological races it has now been characterized as species complex with five informally designated species A, B, C, D and E[13,17]. Techniques are now available to identify these sibling species within the taxon *Anopheles culicifacies s.l.* (Table I). Among these, the use of diagnostic fixed readable inversions in the ovarian nurse cell's polytene chromosomes unequivocally helps to identify different sibling species. Initially, two sibling species A and B were identified near Delhi; one with standard arrangement in polytene X-chromosome (X $+^a+^b$) designated as 'A' and the other with two paracentric inversions 'a' and 'b' with chromosome arrangement (Xab) designated as 'B', without any evidence of heterozygotes in natural populations[25]. Subsequently, examination of polytene chromosomes of populations from other regions of India revealed presence of two fixed inversions, *i.e.* g^1 and h^1 in chromosome arm 2 in species B, and two distinct populations with chromosome arrangement

Sibling species	Polytene chromosome inversion genotype	Mitotic karyotype Y-chromosome	LDH enzyme alleles	Cuticular hydrocarbon profile	Species specific DNA probes	PCR-RFLP	ASPCR
A	X$+^a+^b$; $2+g^1+h^1$; $+i^1/i^1$	Submetacentric	Fast	Identified	Yes	Yes	Yes
B	Xab; $2g^1+h^1$	Acrocentric, Submetacentric	Slow	Identified	Yes	Yes	Yes
C	Xab; $2+g^1h^1$	Acrocentric, Submetacentric	Slow	Identified	As B	As B	Yes
D	X$+^a+^b$; $2i^1+h^1$	Submetacentric	Fast	Not done	Not tested	As A	Yes
E	Xab; $2g^1+h^1$	Submetacentric	Slow	Not done	Not tested	Yes	Yes

Table I. Techniques applied in the identification of *An. culicifacies* sibling species

Source: Ref. 17 (reproduced with permission). PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; ASPCR, allele specific polymerase chain reaction; LDH, lactate dehydrogenase

Xab; $2g^1+h^1$ and that with Xab; $2+g^1h^1$ were identified which were considered reproductively isolated. The new population with Xab; $2+g^1h^1$ chromosome arrangement was designated as species 'C'[26]. Additionally, examination of a few more populations from north India revealed polymorphism for yet another inversion 'i' in chromosome 2 of species A which was observed to be fixed in populations from south India. The evidence of deficiency of heterozygotes in northern and central Indian populations for inversion 'i' and total absence of heterozygotes in populations of species A with $X+^a+^b$; $2+g^1+h^1$ and with $X+^a+^b$; $2i^1+h^1$ chromosome arrangement in southern populations provided cytogenetic evidence for species 'D' ($X+^a+^b$; $2i^1+h^1$)[27-29].

In addition to sibling species A, B, C and D characterized by analyses of polytene chromosome arrangements, sibling species E was identified by Y-chromosome polymorphism in sibling species B populations from Rameshwaram island of Tamil Nadu, south India[30]. Population with acrocentric Y- chromosome was retained as B while that of submetacentric Y-chromosome karyotype and high sporozoite infectivity was designated as new species E. Both sibling species B and E are homosequential for polytene chromosomes and occur in sympatry but apparently reproductively isolated by pre-mating isolating mechanisms.

Populations of *An. culicifacies* in range of its distribution other than India have also been examined for sibling species characterization, *e.g.* in Yeman and Iran, species A was identified[31,32], and both A and B are reported occurring sympatrically in Pakistan, of which species A was incriminated[33]. In Sri Lanka, based on polytene chromosome analysis and DNA probes only species B was identified[34,35], which later confirmed to comprise another sibling species E, the one that was responsible for malaria transmission with high sporozoite infectivity [36]. In Thailand, both A and B were identified[37].

The pre-mating reproductive isolation between sibling species was further substantiated by post-mating isolation mechanisms marked by unidirectional fertility/ hybrid male sterility/ reduced fertility/ atrophied reproductive organs in reciprocal crosses between species A/B, and A/C. Reciprocal crosses between species B and C, however, produced fertile F1 hybrid males and females[33,38,39]. In addition to these methods, these siblings could also be identified with reasonable accuracy by mitotic karyotype Y- chromosome

Fig. 3. Map of India showing geographical distribution of *An. culicifacies* complex (sibling species A, B, C, D, and E), and stratification (Divisions I-VII) for vector control options. (*Source* : Ref. 57).

polymorphism[40-43], electrophoretic variation in lactate dehydrogenase enzyme for fast and slow allele (LdhF & LdhS)[44], cuticular hydrocarbon profiles[45], and highly repetitive DNA sequences[46]. PCR-based diagnostic assays have been developed for sequencing 28S-D3 domain[47], ITS2-PCR-RFLP (restriction fragment length polymorphism)[48], rDNA-ITS2-PCR[49], 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 two step PCR assay based on sequence difference within the COII region, A/D specific primers distinguished species A and D, and B/C/E specific primers distinguished B, C and E[50], however, this assay could not be used to distinguish species B and E in Sri Lanka[51]. An efficient and less expensive 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) were supported by strong bootstrap values[52].

Sibling species distribution and sympatricity

Populations of *An. culicifacies* of diverse origin have been studied for distribution and abundance of its sibling species, A, B, C, D and E in India[17]. Among these, species B is the most predominant spread throughout the country and occurs sympatrically with

A or C or D (Fig. 3). Species A and B are sympatric in north and south India with predominance of species A in the north and species B in the south. In eastern Uttar Pradesh, north Bihar and northeastern States, species B is either predominant or the only prevalent species. Species B and C are predominant in the western and eastern regions. Species D is sympatric with species A and B in northwestern region, and with species A, B and C in central India and a few areas in southern India. Species E is sympatric with species B in southern Tamil Nadu including Rameshwaram islands and Sri Lanka, and there are reports of expansion of distribution range of species A, D and E in Odisha and that of species E in Madhya Pradesh but these investigations lack the component of chromosome analyses for diagnostic confirmation[53-55]. The proportions of sibling species, however, varied in different geographical zones and seasons, *e.g.* in Delhi where species A and B are sympatric, A was predominant throughout the year but proportions of B increased in post-monsoon months[56]. In Alwar (Rajasthan), amongst sibling species A, B, C and D, species B increased in post-monsoon months, proportions of D remained the same throughout the year and densities of species C remained very low[57].

Bionomical characteristics

Five sibling species spread across India have distinct biological characteristics and role in malaria transmission (Table II). All members of *An. culicifacies* complex except E were predominantly zoophilic[13]. Species A had relatively high anthropophilc index (0-4%) compared to species B and D (0-1%), species C had intermediate level of anthropophilic index (0-3%), and species E, had the highest anthropophilic index (80%). All member species largely rest indoors human dwellings preferentially on roof ceilings after feeding on cattle, but also rests outdoors[18]. All are night biting species with different peak biting activity. The biting activity of A, B and C was observed all through the night except for D for which there was no biting after midnight. The peak biting activity of species A and B occurred between 2200 till 2300 h whereas for species C, it was seasonal; in April it occurred between 1800 till 2100 h and shifted to second quarter of the night in December[17].

An. culicifacies s.l. has been incriminated by detection of gut and salivary gland infections by numerous independent investigators across its range of distribution throughout India[16,18]. However, the seasonal infection rate varied from moderate to high in northwestern States, low to moderate in Deccan plateau,

low rates in Gangetic plains and east central region, and very low rates in northeastern States[18]. The sporozoite infectivity rates also varied among sibling species. Immunoradiometric based investigations revealed that sibling species A, C and D are vectors of *P. vivax* and *P. falciparum* (including drug resistant strains), and cumulative sporozoite infection rates were recorded 0.51, 0.3 and 0.4 per cent, respectively[58,59]. Species B is a non-vector or poor vector evidenced by the low prevalence of malaria where species B is predominantly prevalent such as in eastern districts of Uttar Pradesh and southern Indian States[60]. Species E is the most efficient vector and maintains endemic to epidemic malaria. 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 parasite development[61]. Species A is susceptible to sporogony marked by higher oocyst and sporozoite rate than C and B; in species B parasite development is inhibited by oocyst encapsulation mechanism[62,63]. Variations to development of insecticide resistance have also been reported among sibling species but with slower development of resistance to DDT in species A than species B and C, and faster development of resistance to malathion in C than B and at slower rate in A than B (Table II)[64,65].

Vector control

Indoor residual spraying (IRS) is the main stay for vector control in the country. For control of *An. culicifacies,* currently three rounds of spraying of malathion (25%WP, 2 g/m^2) are undertaken in DDT resistant areas. In areas with double resistance, *i.e.* DDT and malathion, two rounds of synthetic pyrethroid insecticides, *i.e.* deltamethrin (2.5%, 20 mg/m^2)*,* or cyfluthrin (10% WP) or lambdacyhalothrin (10% WP) or alphacypermethrin (5% WP) or bifenthrin (10% WP) each are sprayed as 25 mg/m^2. Malathion and/ or synthetic pyrethroids are also sprayed to control epidemic malaria in complex emergency situations. In urban and industrial areas 5 per cent malathion thermal fogging is undertaken to contain build up of vector population.

The understanding of genetics of *An. culicifacies* species complex for its sibling species composition, distribution and behavioural characteristics has helped develop stratification for optimizing control efforts and saving costs (Fig. 3). Taking cognizance of the biological differences of sibling species, the rural India is stratified into seven divisions (excluding urban

Table II. Bionomical characteristics of *An. culicifacies* sibling species and role in malaria transmission in India

Bionomical characteristic	Sibling species				
	A	B	C	D	E
Anthropophilic index (%)	0-4	0-1	0-3	0-1	80
Biting activity	All night	All night	All night	Till midnight	No data
(Peak biting activity in h)	(2200-2300)	(2200-2300)	(1800-2100)	(1800-2100)	
Vector potential	Moderate	Poor	Moderate	Moderate	High
Sporozoite infection rate (%)	0.51	0.04	0.3	0.4	4.6
Breeding preferences	Rainwater, clean irrigation water	Riverine ecology	Rainwater, clean irrigation water	Rainwater, clean irrigation water	Riverine ecology
Insecticide resistance					
DDT	Slow (9-10 yr)	Fast (4-5 yr)	Fast (4-5 yr)	No data	No data
Malathion	Slow (9-10 yr)	Medium (6-7 yr)	Fast (4-5 yr)	No data	No data
Pyrethroid	No data	Medium (6-7 yr)	Medium (6-7 yr)	No data	No data

Source: Ref. 8 (reproduced with permission)

metropolitan cities) for benefit of prioritizing vector control options (Table III)[53-57].

No specific strategy has been proposed for species D for its limited distribution and occurrence in low numbers often in sympatricity with other sibling species. In areas with predominance of species E like Rameshwaram Island and Odisha existing interventions should continue. In areas where A, C, and D are sympatric, the choice of insecticide should be guided by the susceptibility status of the predominant sibling species. In addition to stratification, it is strongly advised to develop stratification at the district/ block level to economize field operations. For example, in Shankargarh block of Allahabad district, Uttar Pradesh, the prevalence of malaria had direct correlation with cumulative abundance of sibling species A (64%) plus C (11%)[66]. In general, it is proposed to integrate bioenvironmental methods; larvivorous fish (Guppy and Gambusia) in particular which have been applied successfully in different ecological terrains for control of vector breeding in malaria endemic States Gujarat, Maharashtra and Karnataka[67-70].

Challenges in malaria control

India has reported appreciable decline in malaria cases from two million in 2001 to less than one million in 2013 and notable decline in malaria deaths[4]. However, there is a steady rise in *P. falciparum* proportions with large concentration of cases in tribal populations/forest belts, hilly and difficult/ inaccessible areas. Malaria transmission is perennial affecting all age groups and with serious consequences to high risk groups, particularly with heavy death toll in pregnant mothers and infants[4,7,71]. *An. culicifacies* transmitted malaria is highly uneven, maintains unstable to intermediate stability and brings periodical epidemics. This vector is responsible for malaria resurgence in India, Pakistan and Sri Lanka, although malaria is at the verge of elimination in Sri Lanka[14-15,72-74].

An. culicifacies maintains endemic malaria throughout rural India and its control has become a formidable task consuming huge resources year after year. Malaria control is particularly problematic in forested and degraded forests as in some areas spraying is difficult and remains unsupervised. Malaria transmission in forests is usually prolonged; for example, *An. culicifacies* was incriminated for over 10 months in a year in Balaghat area in Madhya Pradesh[75,76]. *An. culicifacies s.l.* has grown multi-resistant to DDT, HCH (hexachlorocyclohexane) and malathion, in most parts of the country and in some States increased resistance to pyrethroids[77-80]. Molecular characterization revealed a low frequency of the *kdr* allele (mostly in heterozygous condition) in field populations that were resistant to DDT and pyrethroids[81,82]. This species is invasive and expanding its territory by penetrating degraded forests of northeastern States of the country, formerly domain of *An. minimus* and *An. dirus*[11,12]. In addition, global

Table III. Distribution of *An. culicifacies* sibling species and stratification for vector control options in India

Division	Distribution of sibling species and vector control options
I	No routine vector control measures are required except for chemotherapy of imported malaria. It includes States of Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Delhi and parts of north-western Uttar Pradesh and Rajasthan. Sibling species A and B are sympatric; species B is predominant in northern region whereas in canal irrigated areas species A is in the majority.
II	Insecticide spray should be based on susceptibility status of species A or C. It includes States of Gujarat and districts of north-western Rajasthan, western Madhya Pradesh and north-western Maharashtra. Species B and C are widely prevalent except in irrigated areas where A is predominant. *An. culicifacies* is reported resistant to DDT and malathion.
III	No routine vector control measures are required except for chemotherapy of imported malaria. It includes districts of eastern and southern Uttar Pradesh, and districts of northern Bihar. In this division, species B is the only prevalent species. This division is less receptive to malaria transmission and measures such as minor engineering works for source reduction, use of biological control agents can be exploited for sustainable control.
IV	Indoor residual spraying (IRS) and existing interventions including insecticide-treated nets/ long-lasting insecticidal nets should continue. It includes seven States of northeastern region, districts of northern Bengal and State of Sikkim. In this division, *An. minimus* and *An. baimaii* are the two predominant vectors species; both are highly susceptible to DDT. *P. falciparum* is the predominant infection. Species B is the only member species of *An. culicifacies* but has very little role in malaria transmission.
V	Insecticide spray based on susceptibility status of species A or C should be applied. It includes most districts of Madhya Pradesh, north-western Maharashtra, northern Andhra Pradesh and districts of southern Uttar Pradesh. Transmission is intense and *P. falciparum* is the predominant infection. *An. culicifacies* is resistant to DDT and malathion, thus susceptibility status should be monitored for choice of insecticide targeting the most predominant sibling species. There are reports of invasion of species E in this zone warranting constant vigil and intensive control interventions to avert epidemics[55].
VI	Insecticide spray based on susceptibility status of species C and use of insecticide-treated nets/ long-lasting insecticidal nets are recommended. It includes State of Odisha (formerly Orissa), Chhattisgarh, Jharkhand, districts of southern Bihar, north-eastern districts of Andhra Pradesh. However, with recent reports of invasion of sibling species A, D and E, there is need for monitoring relative abundance for planning intensification of control interventions against A and E[53,54].
VII	Insecticide spray and wherever feasible larvivorous fish should be applied. It includes districts of southern Andhra Pradesh, States of Karnataka, Kerala and Tamil Nadu. This division needs to be further stratified for distribution range of species A, B, C and E; the latter presently recorded from Rameshwaram island of Tamil Nadu. The control measures should be judiciously intensified against species A and E. The application of larvivorous fish has been a successful strategy in the control of breeding in wells.

Source: Refs 53-57.

warming, climate change, vector ecology, population migration, deforestation, developmental projects, and poor infrastructure have led to the opportunities for vector proliferation and increased malaria receptivity in the country[83,84].

The main strategy for malaria control in rural India continues to be indoor residual spraying (IRS) of insecticide based on the vector susceptibility status. It is the need of time to develop approaches for management of insecticide resistance for increased duration of its efficacy against target vector species by insecticide rotation, mosaic application, and integrating bio-environmental approaches[85-88]. IRS has become less effective and operationally difficult on account of poor acceptance by communities[89]. Instead of IRS,

implementation of insecticide-treated netting materials / long-lasting insecticidal nets (LLINs) and biological control may produce substantial transmission reduction in malaria transmitted by *An. culicifacies*[90,91]. Most LLINs, however, employ pyrethroid insecticide against which already incipient resistance has been reported. There is an urgent need to develop innovative strategies against resistant *An. culicifacies* populations through research and development in key areas identified from field operations[92-95].

Priority areas of research

An. culicifacies is the best studied mosquito species for its sibling species identification, range of distribution and relationship with malaria transmission in India. Yet, study of chromosomes (ovarian polytene chromosomes

for A, B, C, D, and mitotic chromosomes for B and E) is still the only technique that can distinguish sibling species A, B, C, D and E. No other probe distinguished each species unequivocally. PCR-based diagnostic assays distinguished only two distinct groups namely group I (species A/D) and group II (species B/C/E). Efforts to distinguish species of each group A/D and that of B/C/E based on PCR assays are still far from perfect and could not be used reliably[51].

The present distribution of sibling species requires further research for understating population genetics structure in high risk areas of malaria. Recent findings revealed invasion by *An. culicifacies* sibling species A, D and E in addition to prevalent B and C in Odisha[54], and species 'E' in Madhya Pradesh[55]. Similarly, in Assam (northeast India), species A is also observed to occur besides species B (Nanda N, personal communication). Additional investigations on distribution and bionomical characteristics of species E including insecticide susceptibility status, biting time to delimit its role in malaria transmission would be desirable in problem areas.

The crossing experiments between species A and B revealed post-mating barriers marked by unidirectional hybrid male sterility/ reduced fertility/ atrophied reproductive organs. Similar observations are deemed necessary to establish the extent of post-zygotic isolation by crossing experiments between other member sibling species. The inter-cross fertility data and the existence of possible morphological differences between sibling species A, B, C, D and E should be accorded priority to designate binomial nomenclature similar to other anopheline species vector taxa[96-98].

An. culicifacies is highly adaptive, holds enormous behavioural plasticity and a fast invading species in areas hitherto found with low vector density, *e.g.* in deforested pockets in eastern and northeast India and breeding in altered ecological habitats[23,99-101], and believed to be undergoing significant genetic differentiation with obvious implications in its control[102]. There is a need to understand species-specific bionomics and insecticide susceptibility status before undertaking IRS for the control of *An. culicifacies* transmitted malaria.

The primary emphasis in the control of *An. culicifacies* should be on the innovative integrated methods that are cost-effective, safe and sustainable. It should, therefore, be realized that insecticides should be used sparingly and IRS when it becomes absolutely essential, *e.g.* epidemic situations. The routine vector control should rely on the bioenvironmental/ integrated vector management methods. Field research should be undertaken on malaria control in various ecotypes such as the rural malaria, forest malaria, irrigation malaria, peri-urban malaria, industrial malaria, border malaria, coastal malaria by implementation of integrated vector management (IVM) methods involving the communities. The minimum area for the demonstration of new technologies should be a Primary Health Centre/ Community Health Centre and extended to cover a district. This would require mapping of malaria risk areas for *An. culicifacies* sibling species distribution, potential mosquito breeding habitats, site of contracting infection and control of outdoor malaria transmission. Insecticide incorporated plastic sheeting and insecticide treated hammock should be field tested in areas not responding to treated bed nets/LLINs[103,104].

Along with bionomics of *An. culicifacies,* socio-economic research should continue on human settlements, housing structure, vocations, sleeping habits, migration pattern, and community participation in vector control. Intensive information, education and communication (IEC) for various target groups, health system strengthening and capacity building including technology transfer to the control programme should constitute a continuing activity of the NVBDCP.

Conclusions

In the past decades, a wealth of data on the biology and control of *An. culicifacies* has been generated. *An. culicifacies* populations build up during monsoon and post monsoon season, and bring periodical focal outbreaks and epidemics throughout its range of distribution. Recent studies utilizing the molecular techniques for identification of sibling species complex have helped in understanding bionomics and role of each sibling species in malaria transmission. *An. culicifacies* is an invasive species and its further spread is supported by the changing environmental determinants. Therefore, entomological surveillance should be integral to malaria surveillance. *An. culicifacies* rapidly develops multiple insecticide resistance, and *inter alia* it is playing an increasing role in the transmission of *P. vivax* and *P. falciparum*, and spread of multi-drug resistant malaria. Research leading to newer evidence-based interventions, community based integrated vector management strategies should be strengthened in tackling the emerging challenges in the control of *An. culicifacies* transmitted malaria. The priority research areas in vector control may include developing malaria-risk maps, evidence based focused and selective vector control, tackling problems in vector control that

arise in the field, protection from contracting malaria in the settlements in forests including inaccessible areas, personal protection measures for individuals and communities, reduction in malaria receptivity legislative measures and biological control, vector surveillance, monitoring insecticide resistance and developing countermeasures, protecting target areas from vector invasion, cross-border synchronized vector control, and establishing priority in vector control to eliminate drug-resistant malaria foci.

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