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Review

The *Anopheles culicifacies* and *An. subpictus* species complexes in Sri Lanka and their implications for malaria control in the country

Sinnathamby Noble Surendran[1*] and Ranjan Ramasamy[2]

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Abstract: *Anopheles culicifacies*, the major vector of malaria in Sri Lanka, and *An. subpictus*, a secondary vector, exist as species complexes in the country. Among the globally reported five sibling species (A-E) of the *An. culicifacies* complex, only species B and E have been detected in Sri Lanka. However, all four sibling species (A-D) of the *An. subpictus* complex present globally are found in Sri Lanka. This article reviews the data on the characteristics of the sibling species of these two main malaria vectors in Sri Lanka and methods for differentiating them, and highlights the importance of understanding the bio-ecological variations among the sibling species in order to develop an effective vector control program in the country. It is proposed that *An. culicifacies* species E may have evolved from species B in Sri Lanka and then spread to South India. The development of DNA probes suitable for differentiating the sibling species of *An. culicifacies* and *An. subpictus* in field studies is identified as a particular priority for future work.

Key words: *Anopheles culicifacies*, *Anopheles subpictus*, DNA probes, malaria, sibling species, species complex, vector, Sri Lanka

INTRODUCTION

Malaria has caused several epidemics during the past century in Sri Lanka and shows an unstable and cyclic transmission pattern in the island nation [1, 2]. The annual incidence of malaria in the country over the past decade has decreased from a high of 211,691 in 1998 to 670 in 2008 (Fig. 1) due to effective malaria control measures. *Plasmodium vivax* continues to be the most prevalent malaria parasite in the country. *Plasmodium falciparum* shows a variable prevalence (19.1% and 3.7% of total incidence in 1991 and 2007 respectively) that increases during epidemics, and it is also increasingly resistant to chloroquine [3, 4]. The spraying of indoor residual insecticides to control the anopheline vector remains a major component of the national anti-malaria control program promoted by the Minis-

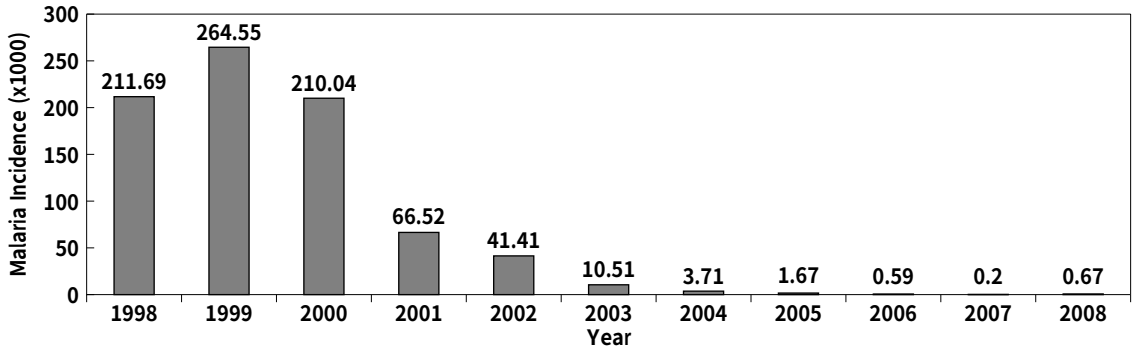

Fig. 1. Malaria incidence in the period 1998-2008 due to all species of malaria parasites in Sri Lanka (adapted from data of the Anti Malaria Campaign, Ministry of Health, Jaffna)

[1] Department of Zoology, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka
[2] Institute of Medicine, University of Brunei Darussalam, Gadong, Brunei Darussalam
*Corresponding Author:
Department of Zoology, Faculty of Science, University of Jaffna, Jaffna 40000, Sri Lanka.
E-mail: noble@jfn.ac.lk
Tel: +94-21-222-5925,
Fax: +94-21-222-2685.

try of Health. Despite the recent reduction in malaria incidence, the malaria control program continues to account for a large part of the national health budget of the country. There is also a potential for resurgence of the disease should the vector control measures be scaled down, similar to what occurred in 1964-1969 after DDT spraying was drastically reduced in 1963 due to the detection of only 7 indigenous cases of malaria in the country [4].

Among the 23 anopheline species that have been recorded so far in Sri Lanka [5], *Anopheles culicifacies* Giles *sensu lato* is clearly established as the major vector of malaria throughout the country [6, 7]. However, *Anopheles subpictus* Grassi *sensu lato* has been implicated as an important secondary vector in many parts of the island [7-9]. In addition to these two vector species, studies using enzyme-linked immunosorbent assays or dissection of mosquitoes show infection with malaria parasites in field populations of *An. aconitus* Dönitz, *An. annularis* van der Wulp, *An. nigerrimus* Giles, *An. pallidus* Theobald, *An. tessellatus* Theobald, *An. vagus* Dönitz, *An. varuna* Iyengar, *An. barbirostris* van der Wulp and *An. peditaeniatus* Leicester [1, 7-10]. Localized changes in the prevalence of different vector species sometimes occur consequent to ecological changes that accompany the rapid developmental changes in Sri Lanka. For example, *An. annularis*, which breeds in the newly constructed irrigation canals in North-Central Sri Lanka, functioned locally as the most important malaria vector with a very high entomological inoculation rate of 0.12 infective bites per hour during a peak transmission season [10].

Both *An. culicifacies* and *An. subpictus*, the two best characterized malaria vectors in Sri Lanka, exist as species complexes (discussed in detail below). The characterization of species complexes and their members (sibling species) is important to understand the dynamics of disease transmission and to control malaria, since sibling species are reproductively isolated evolutionary units with distinct gene pools and biological traits that determine their vectorial status [11]. This article reviews current knowledge on the sibling species of *An. culicifacies* and *An. subpictus* in Sri Lanka. It also discusses why a detailed knowledge of the bionomics of sibling species is important for the development of appropriate vector control strategies and outlines the existing gaps in knowledge for this purpose.

SIBLING SPECIES AND SPECIES COMPLEXES

Mayr [12] in 1942 coined the word sibling species to denote morphologically similar and reproductively isolated populations within a taxon. Other terms such as cryptic species [13] or isomorphic species [14] have also been used to refer to sibling species. The concept of a species complex was also proposed after the discovery of the first such species complex in Europe, the *Anopheles maculipennis* species complex, by Hackett [15]. It has since been demonstrated that malaria vectors often occur as species complexes and that sympatric populations show behavioral and ecological differences that can influence their role as vectors [16, 17].

Sibling species of different Anopheline species complexes are known to exhibit variations in seasonal prevalence, resting behavior, feeding and breeding preference, and susceptibility to parasites and insecticides in other countries [18, 19]. The presence of two or more uncharacterized sibling species in a particular locality may therefore conceal the real transmission pattern of malaria and lead to sub-optimal vector control programs. Furthermore, changes in the density of vector populations and variations in the disease transmission pattern may result from the differential survival of sympatric sibling species in the aftermath of insecticide application. Therefore, failure to identify the actual vector species in a locality can result in a waste of resources if vector control measures unknowingly target a non-vector species in a species complex [18-20].

ANOPHELES CULICIFACIES COMPLEX

Anopheles culicifacies Giles *s.l.* has a wide distribution that extends from Ethiopia, Yemen and Iran in the west *via* Afghanistan, Pakistan, India, Bangladesh, Burma and Thailand, to Laos and Vietnam in the east. To the north it is found in Nepal and southern China, and in the south in Sri Lanka [19, 20]. This major vector species is widely distributed and present as a species complex comprising five members i.e. A, B, C, D and E, on the Indian subcontinent [19-21]. While species B is widespread in many Asian and South Asian countries such as Iran, Cambodia, China and Thailand [22], species A, C and D are mainly confined to northern India [20, 22]. Species E is the new addition to this complex and has been reported in southern India [21, 22]. This species was first reported in 1999 in the southern Indian island of Rameshwaram, which is located approximately 32 km to the west of northern Sri Lanka and implicated as a major vector of malaria [21] (Fig.2). The available data on the worldwide distribution of the different sibling species of *An. culicifacies* Giles *s.l* are summarized in Fig 3. The different members of this complex show different bio-ecological characteristics, and species A, C, D and E are reported to be efficient vectors of malaria on the sub-

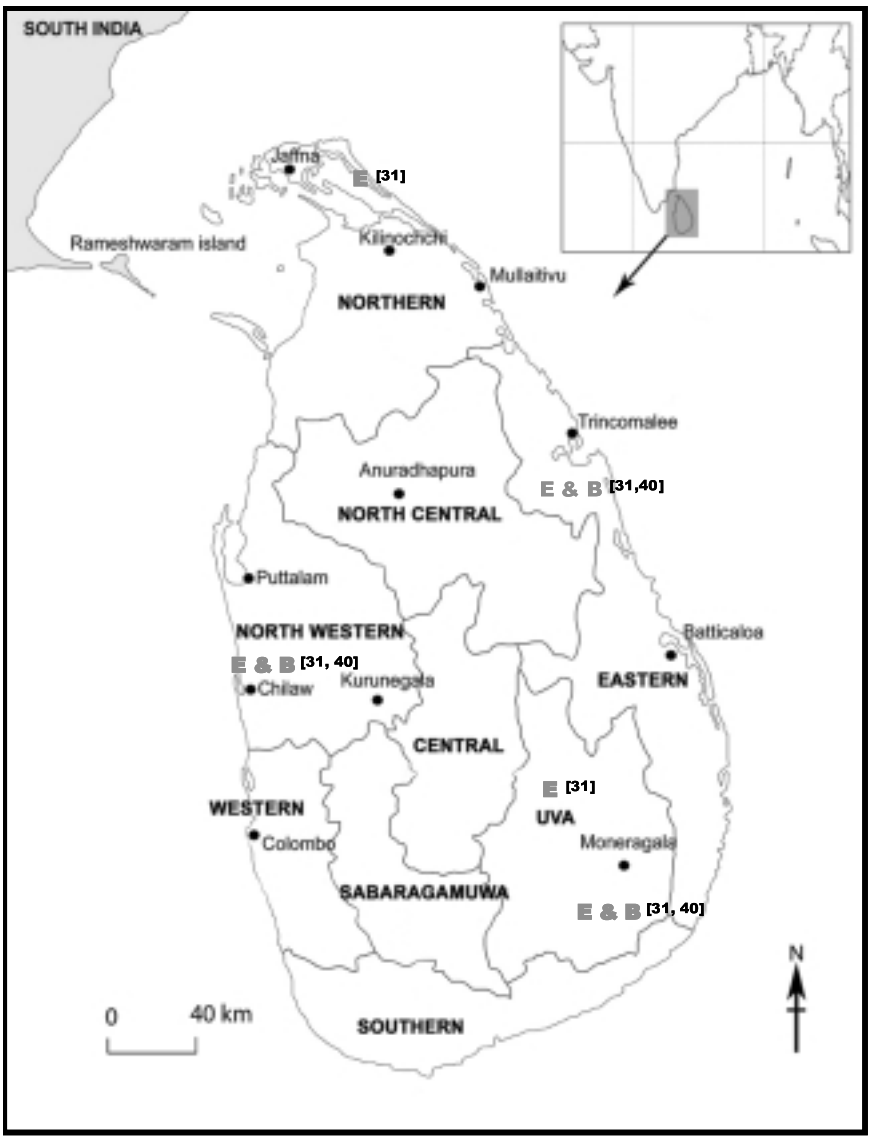

Fig. 2. Map showing the distribution of sibling species of the *Anopheles culicifacies* complex have been detected, the provinces of Sri Lanka, major cities in different districts of the provinces and the relative location of Rameshwaram Island in the south Indian state of Tamil Nadu.

continent [20-22]. Molecular and cytological characteristics including polytene chromosome banding patterns [23], mitotic chromosome structures [24], cuticular hydrocarbon profiles [25] and isoenzyme variation [26], and differences in DNA sequences [27, 28] have been used to distinguish some members of this taxon in India and elsewhere.

The presence and differential identification of* An. culicifacies *species B and E in Sri Lanka

Until recently, only species B, which is a poor vector of malaria in India, was reported to be present in Sri Lanka through an identification procedure based on polytene chromosome banding patterns [29]. A study based on a species-specific DNA probe showed the absence of species A in Sri Lanka [30]. This puzzle was subsequently resolved through the identification of vector species E, based on mitotic Y chromosome variation, in Sri Lanka [31]. Species B and E share the same diagnostic banding pattern in polytene chromosome but differ in the mitotic Y chromosome structure, the former being acrocentric and the latter metacentric [21]. Therefore the differentiation of field-caught species B and E females depends on the examination of mitotic chromosomes of their F1 progeny, which is a resource and time-intensive procedure not suitable for identification on a large scale. A PCR assay based on sequence variations in the internal transcribed spacer 2 (ITS2) of the ribosomal DNA

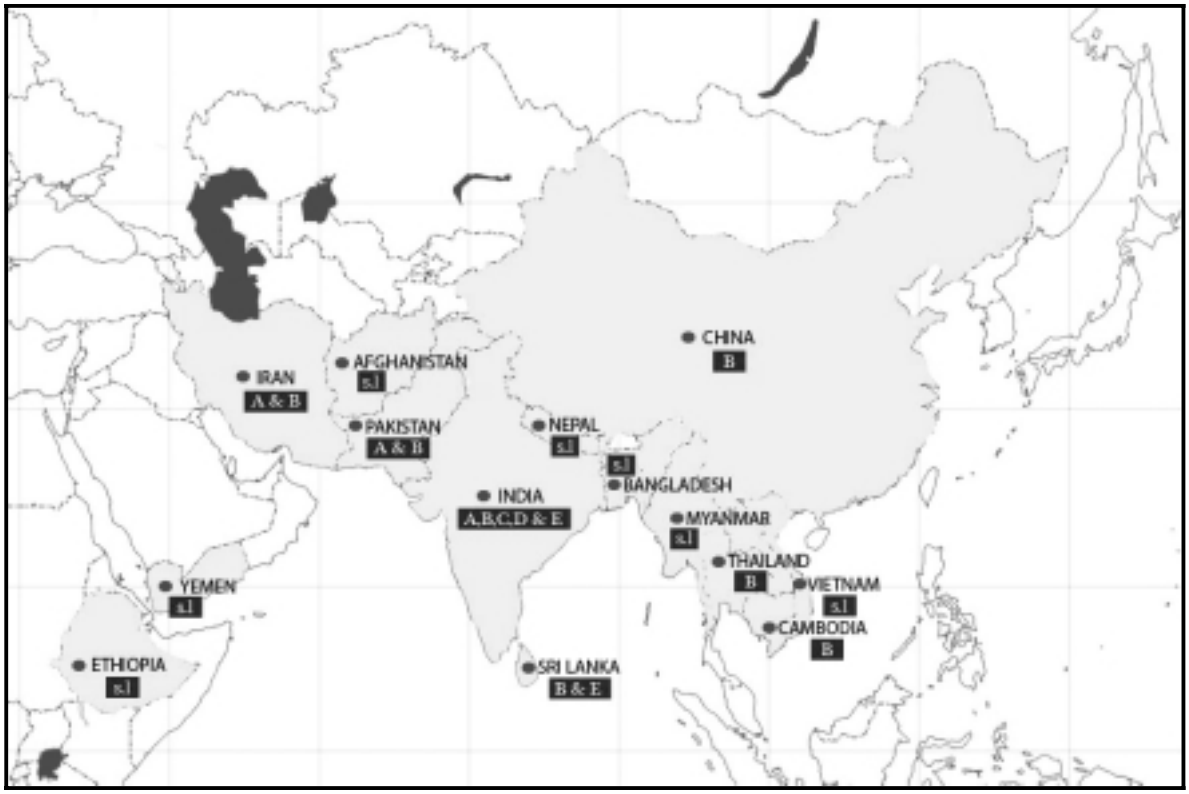

Fig. 3. Map showing the reported detection of members of the *Anopheles culicifacies* species complex in different countries

A, B, C, D, E and s.l. refer to sibling species A, B, C, D, E and *An. culicifacies sensu lato* respectively

(rDNA) and mitochondrial cytochrome oxidase II (COII) subunit is reported to differentiate among all five members of the *An. culicifacies* taxon in India [28]. However, a preliminary molecular analysis for the reported sequence variation in the ITS2 and COII regions for Indian B and E, deposited in the GenBank database, showed that there were no corresponding sequence differences for Sri Lankan species B and E [32]. Therefore, the published ITS2 and COII DNA-based technique used in India [28] may not differentiate species B and E in Sri Lanka, and therefore different molecular techniques need to be developed for this purpose.

The recent detection of species E in Sri Lanka may have implications for the evolution of the *An. culicifacies* species complex. Based on similarities in the polytene chromosome diagnostic banding sequences, species A and D are more closely related to each other than to the group of species B, C and E, which in turn are more closely related to each other [20, 21]. This cytogenetic evidence is also supported by reports based on sequence differences in 28S-D3 rDNA domains [27] and ITS2 rDNA region [28] that all five sibling species can be classified into two groups i.e. A/D and B/C/E. Considering the wider prevalence of species B in Asia, it is conceivable that species A and B originally evolved from a common ancestor, while species D diverged from species A and species C from species B at a later period. However, the origin and spread of species E remain unclear. Considering the geographic locations where species E is reported to be present [21, 31] and the long history of malaria epidemics in Sri Lanka [1], it is possible that the vector species E (Section 3.3 below) first evolved from the poor vector species B in Sri Lanka and then later spread to Rameshwaram Island in the south Indian state of Tamil Nadu. The divergence of species B and E may have occurred relatively recently during the period of known human settlements. Therefore, the south Indian species E may show greater differences in DNA sequences to Indian species B than to Sri Lankan species B. A detailed phylogenetic study at the molecular level may establish the origin and spread of species E.

Prevalence, longevity and fecundity of* An. culicifacies *species B and E in Sri Lanka

Although species B and E of Sri Lanka appear to share considerable DNA sequence identity, there is evidence to suggest that they show bio-ecological variations (Table 1).

Table 1: Bio-ecological characteristics of species B and E of the *Anopheles culicifacies* complex in Sri Lanka.

Characteristics	*An. culicifacies*	
	Species B	**Species E**
Prevalence[31]	Sympatric	Sympatric
Breeding sites[40]	Rock pools, sand pools, quarries	Rock pools, sand pools, quarries, wells, irrigation channels
Resting and feeding places[31,33]	Indoor and outdoor	Indoor and outdoor
Vector potential[34]	Poor vector or non-vector	Vector of *P. vivax* and *P. falciparum*
Longevity[34]	Tendency to survive less than 3 ovipositions	Tendency to survive more than 3 ovipositions
Insecticide resistance[34]	Resistant to DDT Less resistant to malathion Susceptible to λ-cyhalothrin and deltamethrin	Resistant to DDT More resistant to malathion Susceptible to λ-cyhalothrin and deltamethrin

While species E appears to be numerically dominant in many parts of the country, both species are sympatric in all parts of Sri Lanka studied [31]. The collection of more species E than species B from cattle baited huts (a technique used to collect indoor feeding and resting mosquitoes), indicated that species E preferred to feed and rest indoors [31]. While this suggests that species E is anthropophagic, there is no definitive data yet on the relative anthrophagicity and zoophagicity of species B and E, making this an area for further research.

Limited studies on fecundity suggested that the mean fecundity of species E tended to be higher than that of species B [33, 34]. Investigations on longevity suggested that species E tended to survive longer than species B with nearly 16% of the field-caught species E surviving long enough to support the extrinsic developmental cycle of *Plasmodium vivax* and *P. falciparum* [34]. The causes for the possible greater longevity of species E compared to species B are presently unknown. Increased longevity is an important factor that increases the rate of malaria transmission in the MacDonald model of disease transmission dynamics [35], and it has to be taken into consideration in adopting appropriate vector control measures.

Differential infectivity of human malaria parasites to* An. culicifacies *species B and E in Sri Lanka

Studies on the relative infectivity of human malaria parasites to species B and E in Sri Lanka showed that, while species E could support the extrinsic cycle of *P. vivax* and *P. falciparum*, no species B isolated in Sri Lanka could be infected with either parasite through feeding on infected blood [34]. These observations are consistent with the poor vector capability of species B reported in India [18] and Pakistan [36]. Therefore species E, and not species B, is the major malaria vector in Sri Lanka.

Differential insecticide susceptibility of* An. culicifacies *species B and E in Sri Lanka

The members of *Anopheles culicifacies* complex show differential susceptibility to common insecticides in India. Species A, B and C are reported to have developed resistance to DDT and malathion and species B, C and E to pyrethroids in India [37, 38]. In Sri Lanka, species B and E are completely susceptible to the pyrethroids λ-cyhalothrin and deltamethrin, and totally resistant to DDT in standard tests [34]. However, species E was found to be significantly more resistant to malathion than species B [34]. Specific causes for the different degrees for malathion resistance in species B and E have not been investigated, largely because it has not yet been possible to establish colonies of the two species or to obtain large enough numbers from field collections.

The greater resistance of species E to malathion had important implications for malaria control in some parts of the island. Malathion was used almost exclusively until April 2003 in the malaria endemic districts of Kilinochchi and Mullaitivu in the northern province of Sri Lanka (Fig. 2) for malaria control, while its use had been terminated elsewhere. When findings on the presence of species B and E and differential insecticide susceptibility became known to the Department of Health, malathion was replaced by pyrethroids for malaria control operations in the northern province in 2003. This change in strategy may have partly contributed to the dramatic reduction in malaria incidence subsequent to 2003, particularly in the northern province (from 1,829 cases in 2002 to 45 in 2004 and subsequently only 04 in 2008) (personal communication from Anti-Malaria Campaign, Jaffna).

A recent study based on biochemical analysis aiming at determining resistance mechanisms among *An. culicifacies s.l.* populations collected from various parts of the

Table 2: Bio-ecological characteristics of sibling species of the *Anopheles subpictus* complex in Sri Lanka

Characteristics	*An. subpictus*			
	Species A	**Species B**	**Species C**	**Species D**
Prevalence[44,45,47]	Sympatric	Sympatric	Sympatric	Sympatric
Breeding sites[44-47]	Inland	Coastal	Inland	Inland
Resting and feeding places[48]	Indoor	Outdoor	Indoor	Indoor and outdoor
Peak abundance[47]	Not known	January, April, December and July	November and January	Not known
Vector potential[52]	Not known	Not known	Vector	Not known
Insecticide resistance[54]	Not known	Resistant to DDT and susceptible to malathion in Jaffna distinct	Susceptible to malathion in Jaffna district	Resistant to DDT and susceptible to malathion in Jaffna district

country suggested that there may be different resistance mechanisms to the various common insecticides. For example, all *An. culicifacies s.l.* populations were resistant to malathion except for a population from the Kurunegala district (Fig. 2), where no carboxylesterase activity was detected in the mosquitoes [39]. The study included *An. culicifacies s.l.* populations from five malarious districts (Puttalam, Anuradhapura, Kurunegala, Trincomalee and Moneragala) of the country (Fig. 2) and showed a heterogeneous pattern of resistance to common insecticides such as DDT (all populations resistant), malathion (all except Kurunegala population resistant), fenitrothion (Kurunegala and Puttalam populations were susceptible) and deltamethrin (Anuradhapura, Kurunegala and Puttalam populations were susceptible) [39]. A plausible explanation for the complexity of the resistance variation is the presence of sibling species with different resting and breeding preferences [39]. Indoor resting sibling species are under greater selection pressure due to indoor residual spray (IRS) and may develop resistance more rapidly than outdoor resting species. Therefore, more studies on the feeding and resting behaviour and the biochemical nature of resistance to insecticides in female adult *An. culicifacies* sibling species B and E in different districts of Sri Lanka are warranted.

Breeding habitats of An. culicifacies *species B and E in Sri Lanka*

Investigations on larval breeding sites suggest that species E is able to exploit a wide range of breeding habitats with different limnological characteristics [40]. This may be an adaptive variation in species E. A high density of species E was also found in open bound wells (wells with cemented outer coating) and unbound wells (wells without cemented outer coating) used for domestic purposes, and in irrigation channels [40]. The presence of species E in rock pools in quarries with turbid water supports the report of van der Hoek et al. [41] that the physical quality of water may not play a significant role in the development of immature stages of *An. culicifacies s.l.* Species B was isolated from quarries created for domestic or economic purposes as well as rock pools and sand pools in the river margins. However, none of the collected water samples contained both species B and E larvae, suggesting the existence of local inter-species competition or niche segregation due to unknown environmental factors.

The egg laying preferences of species B and E that differ in their vector potential may reduce the efficacy of a vector control measure formulated without any prior knowledge of the relative preponderance of the two populations in different habitats. Therefore, a more extensive study to investigate the possible differential preferences of species B and E for breeding sites and larval habitats would be a prerequisite for designing effective vector control programs.

ANOPHELES SUBPICTUS COMPLEX

Anopheles subpictus s.l. is relatively important among the many secondary vectors of malaria in Sri Lanka, since its role in the transmission of *P. vivax* and *P. falciparum* has been reported from many parts of the country [1]. A study carried out in northern Jaffna district during the peak transmission period indicates the role of *An. subpictus s.l.* in the local transmission of malaria. Among 882 salivary gland and 967 midgut dissections, 1 mosquito was found with sporozoites in the salivary gland and 3 mosquitoes with oocysts in the midgut, while no sporozoites or oocysts were detected from the 94 dissected adults of *An. culicifacies*

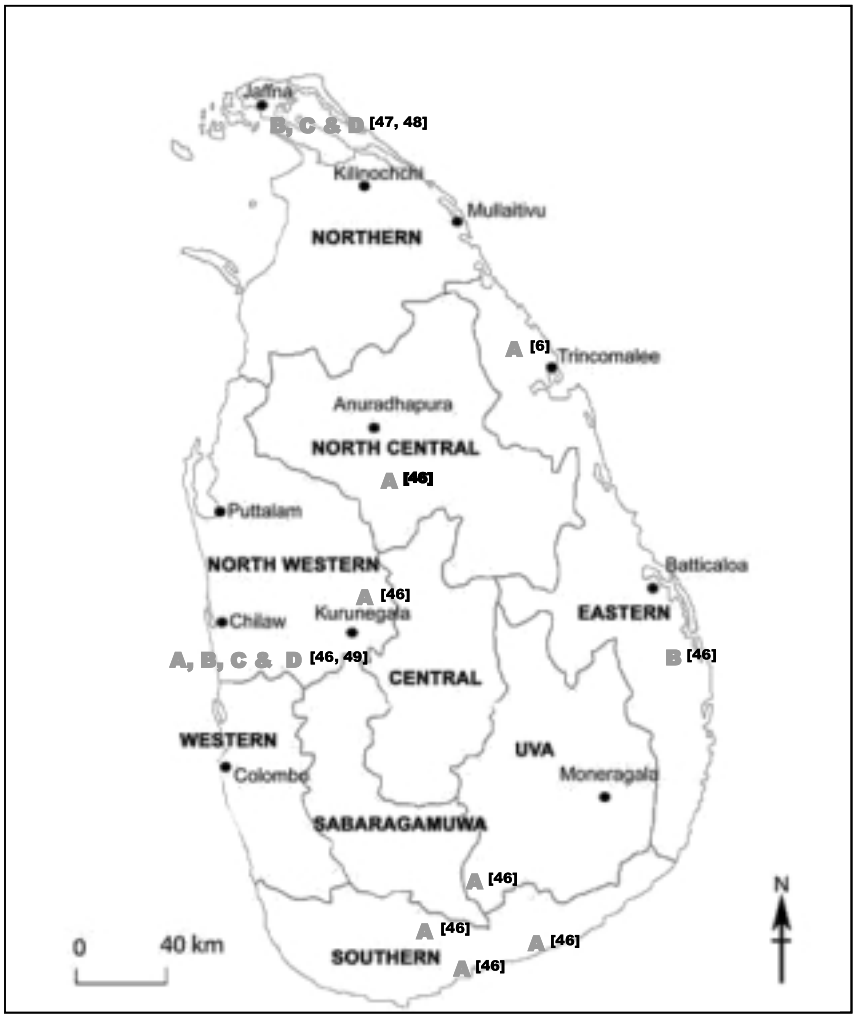

Fig. 4. Map showing areas where the different sibling species of the *Anopheles subpictus* complex have been detected, the provinces of Sri Lanka and the major cities in different districts of the provinces/

[42].

Differential prevalence and bionomics of* An. *subpictus* *subspecies in Sri Lanka

Anopheles subpictus is widely distributed on the Indian subcontinent as well as from Papua New Guinea in the east to Iran in the west of the subcontinent [43, 44]). Although its role in malaria transmission is established in Indonesia and Malaysia ([45], *An. subpictus* plays a minor role in malaria transmission in many parts of the Indian subcontinent and other parts of Southeast Asia. The taxon *An. subpictus* is reported to exist as a species complex comprising four members A, B, C and D in south India [20]. All four members can be distinguished by polytene chromosome banding patterns [20, 43] and stage specific morphometric characteristics [43]. Initially, a study based on a single inversion in the X arm of the polytene chromosome revealed the presence of species A and B in Sri Lanka [46]. Later studies based on morphometric diagnostic characteristics revealed the presence of all four sibling species in the country (Fig.4) [47-49]. The members of the complex are reported to show different bio-ecological characteristics (Table 2), many of which are of importance in vector control.

While species B, C and D were detected in the Jaffna district of the northern province [47, 48], the presence of all four members was reported from the north western province of the country [49, 50]. Species B was predominant on the east coast of the Jaffna district and the west coast of the Chilaw district in the north western province. Species C was predominant in inland localities of Jaffna and Chilaw districts [46-49]. The density of species A and D in the coastal areas was found to be low in comparison with the

inland areas [49, 50]. A similar situation prevails in nearby India where species B is reported to be a coastal species with greater tolerance to salinity in larval breeding sites than the inland sibling species of the complex [51].

Among the siblings, species B prefers to feed and rest outdoors while species A and C prefer indoors [50]. However, species D does not show any significant difference in feeding and resting preference [48]. Peak abundance of sibling species C inland has been observed in November and January, while species B is abundant in January, December, April and July in coastal areas of the Chilaw district [49]. Although indoor resting siblings seem to prefer feeding on humans (anthropophagic) and similarly outdoor resting sibling species to prefer feeding on animals (zoophagic), the feeding preferences of the different *An. subpictus* sibling species remain to be definitively established.

Susceptibility to parasites and insecticides of* An. subpictus *subspecies in Sri Lanka

In India, species B, which is associated with coastal areas [51], was incriminated as a malaria vector [52, 53]. Although *An. subpictus s.l.* has been incriminated as a vector of *P. vivax* and *P. falciparum* on many occasions [1], there is no confirmatory information on the involvement of sibling species. A limited study from north western province (Chilaw district) identified a single species C mosquito with sporozoites [54]. Hence there is a need to establish the relative potential of the different sibling species of *An. subpictus* to transmit malaria in Sri Lanka.

In general, *An. subpictus s.l.* populations are reported to be highly resistant to DDT and malathion throughout the country. A detailed analysis based on resistance data reported from many parts of Sri Lanka during the period 1991-2003 shows a stratified (coastal and inland) resistance pattern for *An. subpictus s.l.* that may be attributable to the distribution of sibling species [55]. A study carried out in 1996 revealed that the coastal population of *An. subpictus s.l.* was more susceptible to malathion even though these areas were widely covered by IRS with malathion [46]. A lower selection pressure may be attributed to feeding preference, as the coastal predominant species B tends to feed and rest outdoors [49]. A study in the Jaffna district revealed that species B, C and D were highly susceptible to malathion and that species B and D were highly resistant to DDT [56]. The results of a recent study based on biochemical analysis for resistance mechanisms of *An. subpictus s.l.* populations collected from different malarious areas (the districts of Puttalam, Kurunegala, Anuradhapura, Trincomalee and Moneragala) revealed the presence of heterogeneity of resistance to common insecticides except DDT and malathion to which all the populations are resistant [39]. For example, Trincomalee populations are resistant while Moneragala populations are only intermediately resistant to permethrin. Carbamate resistance was detected in paddy-field breeding (inland) *An. subpictus* [39] as carbamates are used only for agricultural purposes, and not for IRS, in Sri Lanka. These findings are consistent with the prevalence of sibling species with different genetic properties and consequent differential susceptibility to common insecticides [39]. However, the greater susceptibility to malathion shown by *An. subpictus* population of Jaffna district may be attributable to infrequent spraying in the Jaffna district due to the decades-long civil war and resulting lower selection pressure. A detailed study on the susceptibility and resistance mechanisms in the different sibling species in the *An. subpictus* complex can help elucidate the causes of the observed variations and prove useful for the adoption of appropriate vector control measures.

Importance of developing molecular diagnostic techniques for differentiating* An. subpictus *subspecies in Sri Lanka

Preliminary molecular analysis of sibling species based on sequences similarity in the ITS2 region showed that species A and C can be differentiated from B and D [57]. The differential identification techniques presently used are stage-specific, being dependent on identifying banding patterns in polytene chromosomes of adult female ovaries (applicable therefore only to a proportion of field caught females) and morphometric characteristics of egg and larval stages (a resource and time intensive procedure). Hence a reliable molecular technique for differentiating all members in the complex, as is the case for distinguishing between species B and E of *An. culicifacies*, would be highly advantageous for field studies.

CONCLUSIONS

The available evidence suggests the presence of sibling species with different eco-biological traits in the *An. culicifacies* and *An. subpictus* complexes of Sri Lanka. The data also suggest that sibling species differ in their ability to transmit both falciparum and vivax malaria, and in their sensitivity to commonly used insecticides. Therefore knowing the distribution of the different sibling species in different parts of the country is an important prerequisite for formulation of effective malaria control measures based on vector control. Other biological properties of the different sibling species need to be more firmly established as these also has an impact on the adoption of appropriate vector control measures.

The lack of a simple and inexpensive method to differentiate the sibling species in field studies is a major disadvantage for research. This is particularly so in the case of *An. culicifacies*. Ideally, the method applied should not be specific to a particular stage in the life cycle or sex of the mosquitoes and should be applicable to the analysis of a large number of samples at the same time. Such differentiating tools should also be tested and shown applicable to Sri Lankan specimens in view of the reported differences between Sri Lankan and Indian specimens [32]. Therefore, priority should be given to research leading to the development of a suitable method, e.g. the development of a DNA probe based on identified genomic differences between the sibling species.

In order to distinguish sibling species based on DNA variation, mitochondrial DNA (mtDNA) genes and nuclear ribosomal DNA (rDNA) have been useful targets because they can reflect the effects of restricted gene flow among populations due to reproductive isolation [58]. The mtDNA sequence variations are useful because of their haploid nature, maternal transmission, and lack of recombination. The rDNA is organized with tandemly repeated gene copies and possesses the same sequence at a locus within an individual and within reproductively isolated populations. Since there is a single X-linked rDNA locus in anopheline mosquitoes, the inter-genic spacer regions of the rDNA accumulate species-specific sequence differences [58]. Several DNA-based identification techniques have been developed, including hybridization assays based on species-specific repeat sequences, and diagnostic PCR amplified fragments produced either using random primers or primers based on known species-specific sequences [59, 60]. Random fragmentation of total DNA has also been employed for the identification of isomorphic species [61]. The random amplified polymorphic DNA markers and species specific restriction fragment length polymorphisms in rDNA have been employed to differentiate members of different *Anopheles* complexes [61-66].

For field studies, species-specific DNA probes (incorporating suitable labels for subsequent enzymatic or fluorescence detection) that hybridize with mosquito DNA squash blots on nylon membranes would be ideal to screen a large number of samples at room temperature with minimum cost and technical involvement [67]. Since genome sequencing is now becoming increasingly easier, it is feasible to sequence relevant segments of the genomes of the sibling species to identify sequence differences that can be used to develop DNA probes. The development of an appropriate molecular tool for distinguishing sibling species of *An. culicifacies* and *An. subpictus* will permit more detailed studies on entomological parameters such as longevity, fecundity, vectorial capacity, biting rhythm, feeding and resting preferences, breeding habitats and susceptibility to insecticides of each member of the two complexes. The findings from such studies will, in turn, yield a better understanding of the dynamics of malaria transmission in endemic localities. Apart from the two major malaria vector species, identified secondary vectors such as *An. annularis*, *An. barbirostris* and *An. maculates* also exist as species complexes [20], and similar considerations (albeit less important from a malaria control point of view) apply to distinguishing the sibling species and studying entomological parameters of their sibling species. Since many other *Anopheles* vector species exits as species complexes, the considerations presented here are of general global relevance for malaria vector control.

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