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From the Editors' Desk

Dear Colleagues,

We are immensely pleased to present you the Volume 3, Issue 2, of *Journal of Medical Arthropodology & Public Health*, published by the SOCIETY OF MEDICAL ARTHROPODOLOGY (www.soma16.org), dedicated to the spirit of 'serving science and society', now bejewelled with the ISSN: 2583-6455 (Online).

Journal of Medical Arthropodology & Public Health aims to spark the principle of the inextricable triad of 'invention, innovation and discovery', and promote interdisciplinary collaboration by providing a forum for research across all scientific disciplines that tackles important, emerging topics under the umbrella of medical arthropodology, particularly vector-borne diseases, and many of the world's grand challenges. The torrent of *Journal of Medical Arthropodology & Public Health – a vade mecum* - is continuing to cascade its way forward to bring you once again results of the most thoughtful scientific works on the Indian soil, just as in the past, the Volume 3, No. 2 (December 1, 2023) is right there in your hands, on time. It has been our efforts to bring diversity in our research papers drawn from various disciplines of medical arthropodology. Therefore, you find in the current issue a variety of exciting research papers. These papers however represent only a fraction of the vast and unexplored spectrum of disciplines within the unfathomable folds of the integrated science of medical arthropodology and public health which are to come to surface gradually and periodically in the future issues! Thus, we are endeavouring hard to publish on the pages of the *Journal of Medical Arthropodology & Public Health* – a broad-scope, open access-cum-print journal papers on both basic and applied research that has a positive impact on

translation of sophisticated data-based scientific studies into usable products by the end-user – the research papers that truly serve the science and society. We will accomplish this task through consilience. Our aim at the *Journal of Medical Arthropodology & Public Health* is to maximize the global visibility and impact of your published articles.

The *Journal of Medical Arthropodology & Public Health* is for all those men and women who are interested in scientific discovery, and in its industrial, commercial, and social consequences. It will explore, interpret and report the results of human endeavour set in the context of science and society. Through the *Journal of Medical Arthropodology & Public Health* scientists will be motivated to think beyond their discipline and believe that collaborative science and interdisciplinary ideas can advance national policies related to the control of vector-borne diseases, on one hand, and bring other biomedical concerns under thorough scanning and surveillance, on the other. This transformation is essential to inspire new thinking, besides exploring new horizons in the biology of medically important arthropods and paving pathways to consolidate new ideas toward their control through a process ensuring way to new and revolutionary ideas!

Soliciting your continued support and patronage in our comprehensive evolution both as the journal and the authors, we remain as heretofore,

Yours cordially,

Prof. Dr B.K. Tyagi & Dr Rina Tilak

December 1, 2023 Chief Editor Executive Editor

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Contents

Journal of Medical Arthropodology & Public Health
(Volume 3 • Number 2)

Perspective

IS IT *AEDES ALBOPICTUS* (SKUSE, 1894) OR *AEDES ALBOPICTUS* (SKUSE, 1895)? A SERIOUS TAXONOMIC CONUNDRUM IN MODERN LITERATURE RESOLVED FOR FUTURE CITATION

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INTRODUCTION

It is ironical that a globally well-referenced mosquito, *Aedes albopictus*, or Asian Tiger Mosquito, is being treated taxonomically in the literature more often than not in a wrong manner, in the first place by citing an incorrect nominal nomenclature concerning the year of its author's description! Whereas, *Ae. albopictus* was factually described by Skuse in 1894, thus giving the correct nomenclature as ***Aedes albopictus* (Skuse, 1894)**[1], in the contemporary literature

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the species is repeatedly quoted as *Aedes albopictus* Skuse, 1895 or *Aedes albopictus* Skuse, 1894 [1895] (see examples 1-3 below). It is, therefore, considered commensurate to bring forth the correct reference related to the species and rectify the problem for the benefit of future research studies. I have made all possible searches into this problem and describe below a systematic analysis of the data available to rest any confusion related to the species' nomenclature.

Fig. 1. *Aedes albopictus* (Skuse, 1894) – The Asian Tiger Mosquito and a deadly vector of dengue, chikungunya, yellow fever and zika viruses worldwide.

Bengal province has ever been an abode to faunal and floral wealth, and, as far as Culicidae is concerned during the British regime[2], it possibly was, due to its approximation to the infamous marshy lands and thick forests of Sunderbans, overwhelmingly infested by mosquitoes of varieties many of which involved in the transmission of several human and animal diseases. To sustain Bengal's natural richness and encourage Oriental studies for the aid of Britishers' rule in India and many other Asiatic nations, Sir William Jones, a British lawyer and Orientalist

founded the world-famous Asiatic Society of Bengal in Calcutta (now Kolkata) on Jan. 15, 1784. Soon, it became evident that to preserve vast animal and plant life in the region, there was an inevitable necessity to establish a repository that could house all the 'preserved' material to continue to transcend knowledge from the past! Therefore, the world-famous Indian Museum was founded in 1814 at the cradle of the Asiatic Society of Bengal (at the present building of the Asiatic Society, 1 Park Street, Kolkata). Indian Museum is the earliest and the largest multipurpose Museum not only in the Indian subcontinent but also in the Asia-Pacific region of the world, where the Types of many mosquito species are still preserved.

During the 16th and 19th centuries, malaria research climaxed, and some countries such as Britain, France, Italy, Russia and United States paced up their findings neck-to-neck to garner priority of research, further ignited by the prospects of the Nobel Prize the money under which, apart from the honour and familiarity associated with it, virtually took the 'malaria-mosquito war' to an unprecedented lofty pitch[3,4]. After Manson (1878)[5] postulated a link between transmission of human lymphatic filariasis and the mosquito *Culex pipiens* and sermonised – "Follow the Flagellum" – to young Ronald Ross, serving as a British Medical Officer in India of a possible link of malaria with mosquitoes, a whirlwind of investigative research on mosquitoes prompted around the world. Ross (1897)[6] and Grassi (1899)[7] fiercely logged their horns on the discovery of the inextricable relationship of malaria with the mosquito, and the battle was won by Ross[3]. After the discovery at the end of the 19th century that malaria was transmitted by mosquitoes, the British Empire in England promoted mosquito taxonomy as a necessary step in controlling mosquitoes. With this support, Theobald compiled his knowledge about the world mosquitoes and put up for the first time a thoroughly classified mosquito in his five-volume series '*A Monograph of the Culicidae or Mosquitoes of the World*' (1901–1910) for the Colonial Office and the Royal Society[8-15]. To complete his marathon series of monographs he visited the Indian Museum to study the types deposited there[7].

It is noteworthy here that Theobald (1901), who put up his mosquito classification in his five-volume series 'A Monograph of the Culicidae', introduced the genus Stegomyia, with a brief description of *Stegomyia fasciata* (Fabricius). More than 60 years later, this was formally recognized by the International

Commission on Zoological Nomenclature (1964): *"The generic name Stegomyia Theobald, 1901 (gender: feminine), type-species, by designation by Neveu-Lemaire, 1902, Culex fasciatus Fabricius, 1805, is hereby placed on the Official List of Generic Names in Zoology."* The species *Stegomyia fasciata* (Fabricius) was a logotype, i.e., determined from a written description in the absence of both a specimen and an illustration. The ICZN, therefore, has had to further clarify, "*It has been suggested that those unfamiliar with the nomenclatural procedure may form the impression that the above declaration prejudices the use of the name aegypti in such combinations as Aedes aegypti* (Linnaeus) *or Aedes* (Stegomyia) *aegypti* (Linnaeus). *This is not the case. It remains perfectly proper to employ the name in these combinations or any others that further taxonomic study may render desirable.*"

In as far as *Aedes albopictus* is concerned, it was Mr E.C. Cotes, who first took charge of the Entomological Section at the Indian Museum, Calcutta, in 1884, had got his hands on such three specimens which he found exciting and sent to Dr Frederick A. A. Skuse, an Entomologist at the Australian Museum. These mosquitoes were a great nuisance in Calcutta and must be authentically identified beforehand. Skuse, however, found that the specimens were closely allied to *Culex nostoscriptus* Sk., from New South Wales, and *Cx. bancrofti* Sk., from Queensland, but the silvery ornamentation of the thorax in the latter was of an elaborate pattern. Therefore, Skuse (1894), after considering all possible similarities among allied species, erected a new species, *Culex albopictus* out of Cotes' three specimens (all females). The Type (female) was deposited in the Sydney Museum by Skuse (*vide* Barraud, 1928)[16]. Much later, after the genus Aedes was coined *Culex albopictus* was christened *Aedes albopictus* (Skuse) in 1894. Phylogenetically, *Aedes albopictus* was first placed under the genus *Aedes*, subgenus *Stegomyia*, group *Scutellaris*, and subgroup *Albopictus* (Order Diptera, Suborder Nematocera, Family Culicidae)[17,18].

It is believed that *Aedes albopictus* initially occurred in the tropical forest of Southeast Asia, where many closely related species are now known to coexist, such as, for example, *Aedes aegypti*, another medically important Stegomyia and popularly known as the Yellow Fever Mosquito, thought to have been introduced into Asia from Africa. These two species are the most important vectors of dengue, chikungunya and zika worldwide across today. Additionally, *Ae. albopictus* is a

vector for the animal-borne dirofilariasis. Currently, *Ae. albopictus* is found across the globe and is regarded as the fastest spreading vector mosquito in the world. Recently, *Ae. albopictus* is advocated to be a species complex comprising as many as 11 sympatric cryptic species[19,20].

As mentioned above, more and more authors and libraries were recently noticed referring to the species under discussion in various different patterns, particularly concerning the year of the discovering author which, in my opinion, needs to be set right with proper documentation so that a scientifically factual event would not be distorted and mislead future generations of brooding scientists. Some confounding and confusing examples are representatively reproduced below to highlight the amount of distortion of the species' authority's year.

SPECIES | HOMOTYPIC SYNONYM

Culex albopictus Skuse, 1894

Published in: Skuse, F.A.A. 1894. The banded mosquito of Bengal. Indian Museum Notes 3(5)[1895]: 20. [1894.11.14] source: Systema Dipterorum

Synonym of *Aedes albopictus* (Skuse, 1894)

(i) Example 1.

Description	Skuse's (died 1896) original description of the Asian tiger mosquito *Aedes albopictus*, then called *Culex albopictus*: F. A. A. Skuse (1894) [1895] The banded mosquito of Bengal. *Indian Museum Notes* **3(5)**:20
Date	1894/95
Source	Armed Forces Pest Management Board, slightly modified
Author	Frederick Askew SKUSE

(i) Example 2.

Parasitology Research (2018) 117:453–460
https://doi.org/10.1007/s00436-017-5721-6

ORIGINAL PAPER

CrossMark

Aedes albopictus **(Skuse, 1895) (Diptera: Culicidae) in Greece: 13 years of living with the Asian tiger mosquito**

E. Badieritakis[1] · D. Papachristos[1] · D. Latinopoulos[2] · A. Stefopoulou[1] · A. Kolimenakis[3] · K. Bithas[3] · E. Patsoula[4] · S. Beleri[4] · D. Maselou[1] · G. Balatsos[1] · A. Michaelakis[1]


See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/352936883

Ecology and Distribution of the Invasive Mosquito Species Aedes albopictus (Skuse, 1895) in the South of the European Part of Russia

Article in Russian Journal of Biological Invasions · April 2021

DOI: 10.1134/S2075111721020041

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Fig. 3a,b. Example 3.

I have completely read the journal *Indian Museum Notes* (Vol. 3, No. 5, Year 1894) and found the paper titled, "*The banded mosquito of Bengal*" printed on page 20, *hook, line and sinker*, further duly substantiated by the PAHO (1995). Therefore, the correct citation of the said research paper is

Skuse, F.A.A. 1894. The banded mosquito of Bengal. Indian Mus. Notes 3(5): 20.

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Original Article

A REVIEW OF THE BIOLOGY AND ECOLOGY OF *CULICOIDES* VECTORS (DIPTERA: CERATOPOGONIDAE) ABUNDANT IN INDIA

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Date of submission : 27th Sept., 2023

Date of acceptance : 12th Nov., 2023

ABSTRACT

The medico-veterinary importance of the biting midges *Culicoides* (Diptera: Ceratopogonidae) lies in the fact that they vector a multitude of arboviruses, protozoa, and nematodes among livestock, wild ruminants as well as humans. Bluetongue (BT) is a non-contagious viral disease causing morbidity and mortality in affected wild ruminants and livestock. Frequent outbreaks of this disease have caused substantial economic losses, particularly in the southern states of India.

BT's controlling strategy is confined to developing vaccines in disease-prone states and has overlooked these potentially neglected virus-transmitting agents. In India, the majority of studies are seroprevalence-based and largely overlooked the

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significance of knowledge about the biology and ecology of these vectors. Among 84 species reported from India, seven are designated as bluetongue virus (BTV) vectors. An information regarding biosystematics and bionomics of these vector species, i.e., *C. peregrinus* Kieffer, *C. oxystoma* Kieffer, *C. actoni* Smith, *C. brevitarsis* Kieffer, *C. fulvus* Sen & Das Gupta, *C. imicola* Kieffer, and *C. orientalis* Macfie will not only provide a better insight for their control but also render a comprehensive idea of their epidemiologically significant vector competence and vectorial capacity. This review stitches together the information generated on biology and ecology of *Culicoides*, the neglected vectors prevalent in India.

Short Title: Bio-ecology of *Culicoides* vectors

Keywords: biology, BTV vectors, *Culicoides*, ecology, taxonomy

INTRODUCTION

Members of the *Culicoides* Latreille, 1809 (Diptera: Ceratopogonidae) are the smallest (1-3 mm) nematoceran haematophagous midges which are implicated as the world players in the epidemiology of more than 50 arboviruses of veterinary and public health importance such as bluetongue virus (BTV), African horse sickness virus (AHSV), epizootic hemorrhagic disease virus (EHDV); protozoa such as *Haemoproteus* sp., *Leucocytozoon* sp., *Hepatocystis* sp., *Leishmania* spp., *Crithidia* sp. and filarial nematodes including *Onchocerca gibsoni*, *O. cervicalis*, *Dipetalonema reconditum*, *Mansonella perstans* and *M. ozzardi* of livestock, wild ruminants, birds as well as humans[1,2,3,4,5,6]. Bluetongue virus (BTV) belonging to the genus *Orbivirus* of the family Reoviridae[7]. In Bluetongue disease (BTD) epidemiology, 23 serotypes of BTV have so far been reported from India, and records of several outbreaks among various union territories, especially in the southern states, have led to substantial economic losses[8]. Species within the subgenera mainly transmitting bluetongue virus (BTV) in the Indian scenario include: *Avaritia* (*C. actoni* Smith, *C. brevitarsis* Kieffer, *C. orientalis* Macfie, *C. fulvus* Sen & Das Gupta and *C. imicola* Kieffer), *Hoffmania* (*C. peregrinus* Kieffer) and *Remmia* (*C. oxystoma* Kieffer)[9,10]. Previous studies suggested the prevalence of those seven putative vectors within BTD-prone areas[9,11]. The prevalence of BTV serotypes in different states of India has been already summarized[7]. Following BTV

serotypes have been isolated from *C. oxystoma* (serotype-1 and 16), while *C. peregrinus* (serotype-23) associated with Indian livestock farms of Gujarat and Tamil Nadu, respectively[12,13,14]. BTV serotype-21 was isolated from *C. fulvus* and *C. orientalis*,[15] while BTV serotype-1, four was detected from *C. imicola*[16, 17]. BTV serotype-1 was also identified from *C. brevitarsis*[18]. Besides, vectoring BTV, *C. peregrinus* is also a vector of the ephemeral fever virus in cattle[2]. *Culicoides oxystoma* are the vectors of various (i) arboviruses viz., *Orbivirus*: AHSV, EHDV, Chuzan virus (CHUV), D'Aguilar virus (DAGV), Ibaraki virus (IBAV); *Orthobunyavirus*: Akabane virus (AKAV), Aino virus (AINOV); (ii) *Onchocerca gibsoni*, the causative agent of filaria of cattle in Malaya, (iii) *Leucocytozoon* sp., an intracellular haemosporidian blood parasite, and (iv) *Leishmania* (*Mundinia*) *martiniquensis*, *L.* (*M.*) *orientalis* and *Crithidia* species[1,5,6,12,19]. *Culicoides imicola* is reported as potential vectors of AHSV, Schmallenberg virus (SBV)[20,21], and *Culicoides brevitarsis* is a significant vector of AKAV, DAGV, ephemeral fever virus, and Ngaingan virus affecting livestock[2]. Microfilariae of *Onchocerca gibsoni* were found in wild-caught *C. actoni* and *C. orientalis* females in Malaysia[2]. *Leucocytozoon* sp. was also detected from *C. fulvus* collected from Phatthalung Province, Southern Thailand[5]. Despite various records of serotypes and disease-causing agents, gathering information regarding the putative vector species prevalent in India is urgently warranted (Fig.1).

This article reviews various aspects of biosystematics and bionomics, especially ecology, taxonomy, and biology of seven neglected vectors of *Culicoides* abundant in India. This baseline information will facilitate the development of effective vector control strategies.

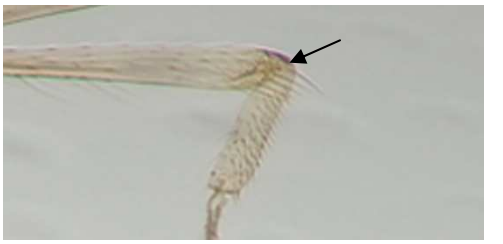
1. Cu fork with proximal pale streak ..... *C. peregrinus*
   Cu fork without proximal pale streak 2

C. peregrinus

C. fulvus

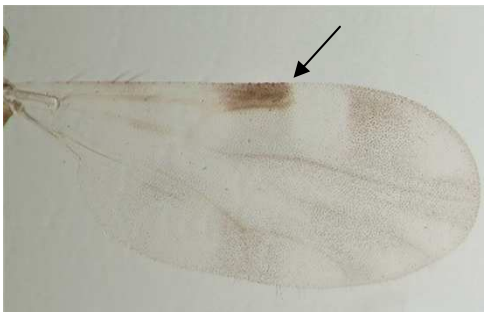
2. Eyes separated; hind tibial comb with 4 spines ..... *C. oxystoma*
   Eyes contiguous; hind tibial comb with 5 spines 3


C. oxystoma; (a) Eyes separated, and (b) hind tibial comb with 4 spines

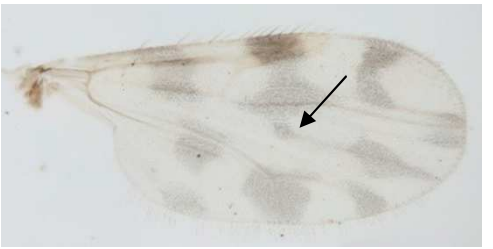
C. fulvus; (a) Eyes contiguous, and (b) hind tibial comb with 5 spines

3. Eyes with interommatidial hairs; poststigmatic pale spot covering 2nd radial cell distally ..... *C. actoni*
   Eyes without interommatidial hairs; poststigmatic pale spot covering distal half of 2nd radial cell 4

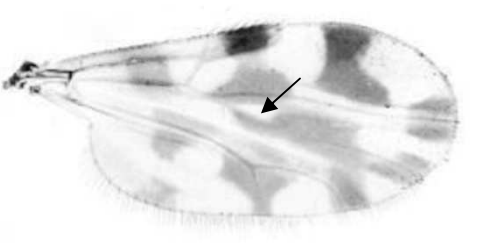



C. actoni *C. fulvus*

4. Proximal pale spot in cell m_1 straddling through vein M_2 5
   Proximal pale spot in cell m_1 not straddling through vein M_2 6

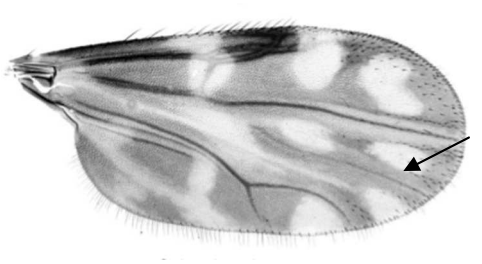


C. fulvus



*C. imicola**

5. Distal dark spot on vein M_1 broad .....*C. orientalis*
   Distal dark spot on vein M_1 narrow ..... *C. fulvus*



C. orientalis

C. fulvus

6. Distal pale spot in cell r_3 quadrate .....*C. imicola*
   Distal pale spot in cell r_3 oval .....*C. brevitarsis*

*C. imicola**

C. brevitarsis

Fig. 1. Key to the adults of vector species of India (*Source:* Economic *Avaritia* key of G. Bellis).

TAXONOMY

Traditional adult *Culicoides* (Figure 1a) taxonomy is phenetic, primarily based on wing spots along with other morphometric characteristics such as antennal ratio, localization of sensilla coeloconica (SCo) on antennal flagellomeres, proboscis/ head ratio, palpal ratio, number of mandibular teeth, hind tibial spines, banding pattern of leg, length and breadth of wing, costal ratio, infuscation of halter and parts of genitalia[2]. Among species, this often leads to misidentification due to minute differences. Many of these require taxonomic validation by comparing with the types in light of modern terminologies. The two vector species, i.e., *C. peregrinus* and *C. oxystoma*, were first recorded from Puri's coastal location and Calcutta, respectively[22]. *Culicoides fulvus*, *C. orientalis*, *C. peregrinus*, *C. alatus* Das Gupta and Ghosh (synonym of *C. oxystoma*), *C. pattoni* Kieffer (synonym of *C. oxystoma*), *C. actoni* and other species were morphologically identified and collected from various regions of India (West Bengal, Assam, Madhya Pradesh, Madras, Coimbatore, Bihar, Bombay, Dharwar, Orissa)[23]. *Culicoides pseudoturgidus* Das Gupta was collected from Calcutta and adjoining areas[24]. Distribution of seven vectors recorded from India is summarized in Table 1.

Table 1. A list of distribution and pathogens transmitted by vectors of India

Subgenus	Vector Species	Distribution	Disease Pathogen*
Avaritia Fox	*Culicoides actoni* Smith	West Bengal, Bihar, Odisha, Assam, Madhya Pradesh, Tamil Nadu, Kerala, Karnataka, Maharashtra	Virus: Bluetongue virus Nematode: *Onchocerca gibsoni*
	Culicoides brevitarsis Kieffer	West Bengal, Tamil Nadu, Karnataka	Virus: Bluetongue virus, epizootic haemorrhagic disease virus, D'Aguilar virus, Aino virus, Akabane virus, ephemeral fever virus, Ngaingan virus
	Culicoides fulvus Sen & Das Gupta	West Bengal, Tamil Nadu	Virus: Bluetongue virus

Subgenus	Vector Species	Distribution	Disease Pathogen*
	Culicoides imicola Kieffer	West Bengal, Tamil Nadu, Karnataka, Kerala, Maharashtra	Virus: Bluetongue virus, African horse sickness virus, Schmallenberg virus
	Culicoides orientalis Macfie	West Bengal, Sikkim, Karnataka	Virus: Bluetongue virus, Nematode: *Onchocerca gibsoni*
Hoffmania Fox	*Culicoides peregrinus* Kieffer	West Bengal, Odisha, Assam, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra	Virus: Bluetongue virus, ephemeral fever virus Protozoa: *Leishmania (Mundinia) martiniquensis*
Remmia Glukhova	*Culicoides oxystoma* Kieffer	West Bengal, Bihar, Odisha, Assam, Gujarat, Himachal Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra	Virus: Bluetongue virus, African horse sickness virus, epizootic hemorrhagic disease virus, Chuzan virus, D'Aguilar virus, Ibaraki virus, Akabane virus, Aino virus Nematode: *Onchocerca gibsoni* Protozoa: *Leucocytozoon* sp., *Leishmania (Mundinia) martiniquensis*, L. (*M.*) *orientalis* and *Crithidia* spp.

*Name of the pathogens transmitted by these seven vectors reported from worldwide

Later on, synonyms of *C. imicola*, *C. actoni*, *C. brevitarsis,* and *C. orientalis* were recognized as follows: *C. pseudoturgidus*, *C. minutes* Sen and Das Gupta*, C. imperceptus* Das Gupta, *C. superfulvus* Das Gupta, *C. nayabazari* Das Gupta respectively[10]. A checklist of 27 species was published initially, which was later increased to 73 species based on an updated annotated checklist[25,26]. The list containing 79 *Culicoides* spp. collected within India, was further taxonomically resolved to 11 subgenera, five species groups, and three unplaced species[27]. Till now, 84 species belonging to 12 subgenera, five species groups (*clavipalpis*, *ornatus*, *saundersi*, *shermani*, *shortti*), and four species belonging to 3 unplaced groups have been documented from India[10,28]. An identification key based on adults

of the Indian *Culicoides* spp. was provided[29]. This article also represented an identification key of seven vector species of India. The present status of the Schultzei group is messy as records suggested most of the Asian literature until 1960 misidentified *C. oxystoma* as *C. schultzei* Enderlein[2]. Specific primers of *C. actoni* and *C. oxystoma* have been developed that may be helpful in rapid identification among pooled samples[30]. The integrative taxonomic approach may address some of the problems faced in morphological taxonomy. So, reinterpretation of phylogenetic relationships will be needed to follow significant discrepancies in the placement and identification of cryptic species.

Adult taxonomy needs to be revised to resolve issues of species delimitation and become more challenging as many vector species belong to complexes of morphologically similar species that may be taken up by studying the immatures of these midges. For this reason, the immature taxonomy of this genus needs to be studied better. The structural elaboration of immature stages, i.e., eggs, larval instars, and pupae of *C. peregrinus*, was elucidated by a Scanning Electron Microscope[31]. In order to develop egg taxonomy and to enumerate species-specific characteristics, a description of eggs of *C. fulvus* and a redescription of eggs of *C. oxystoma*, along with a key based on the structure of eggs, were provided[32]. Likewise, the ultrastructure of the egg surface of *C. actoni*, *C. imicola*, and *C. brevitarsis* was elaborated[33,34,35]. The structure of the larva and pupa of *C. brevitarsis* was also depicted[36]. Notwithstanding these developments, the morphological features of immature *C. orientalis* and *C. oxystoma* are yet to be worked out. Therefore, immature taxonomy of proven vectors and abundant midges leads to more extension of works that may be useful in understanding their feeding habits and breeding habitats.

ECOLOGY

Several biting midges were collected by using UV traps from 11 different livestock farms of cattle, buffalo, sheep, and goats in rural and urban districts of Bangalore. It was noted that *C. imicola* and *C. oxystoma* as the most predominant species[4]. Prevalence of *C. peregrinus, C. actoni*, *C. oxystoma,* and *C. imicola* occurred in the livestock farms of Marathwada[37]. Adult midges were collected by a UV LED light trap fabricated in collaboration with the University Science Instrumentation Center at the University of Burdwan. UV LED traps attracted more

adult midges, followed by blue and green light-based traps[38]. A high relative abundance of *C. oxystoma* followed by *C. peregrinus*, *C. fulvus*, and other non-vector species was recorded from West Bengal[39]. Later, several *Culicoides* spp. were collected from goat and sheep pens of Jharkhand, where *C. peregrinus* was abundant, followed by *C. imicola*[40]. Other midge collection methods included a mouth aspirator, sticky trap, and emergence trap[41].

The preferred time of feeding of *C. peregrinus* and *C. oxystoma* on cattle was found to be early morning, and the preferential landing of these vectors on hosts was mainly restricted to the sites of neck and hump of the cattle. *Culicoides actoni* and *C. fulvus* were observed to prefer landing initially on cattle, followed by sheep and goats in Adisaptagram, West Bengal[42]. *Culicoides orientalis* preferred to feed on the dorsal parts of cattle rather than ventral[2]. Earlier studies identified blood meal sources of these midges by precipitin test; further, a DNA-based approach has been applied to detect this[43]. Reports suggested that *C. peregrinus* is strongly zoophilic and a general feeder[2]. *Culicoides oxystoma* fed the blood of cows, buffalo, sheep, and humans[43]. Blood meal analysis records detected positive *C. actoni* for cow, buffalo, chicken, horse, and human blood, *C. brevitarsis* for red-collared dove and human blood, *C. fulvus* for cow, buffalo, chicken, goat, and sheep blood, *C. imicola* for cow, buffalo, horse, donkey, human blood, and *C. orientalis* for cow blood[5,43,44,45,46,47,48]. This increases the chances of zoonotic pathogen transmission among their hosts. Resting sites of adult *Culicoides* spp. from cattle-sheds were studied in West Bengal[49].

BIOLOGY

Culicoides spp. inhabit a wide range of biotopes, but the breeding habitat of few species has been known. The larval habitat of *C. actoni* was not found despite extensive searching, but later, it was reported that they breed in rotting native fruits[2,50]. *Culicoides orientalis* was reared from 2-3 weeks old manure piles[2]. Breeding sites include banana vegetation, and soil samples of fringes of ponds for *C. alatus*, *C. turgidus* Sen and Das Gupta, and *C. peregrinus*[51]. *Culicoides peregrinus* is also common in ricepaddies and puddles, while *C. oxystoma* was recorded from unspecialized aquatic and semi-aquatic sites, including margins of streams, lakes, drains, ponds, and puddles containing little organic matter and rich in oxygen[2,52]. An earlier attempt was made to rear *C. oxystoma* from exposed mud

on the margins of muddy pools and wells[53,54,55]. Pupae of this species were collected from the margins of small muddy pools, and adult females emerged, but details of the rearing procedure were not mentioned[54]. The breeding habitat of this species was recognized, so the life stages of this species were retrieved from mud and slime taken from the sides of drains or small streamlets and developed into adults[1]. Rearing of this species was done from pupae isolated from the substrate at the margins of water bodies[55]. The screening of larvae and pupae was performed from the soil of the intertidal zone of the Ganga estuary, Sagar Island, and collected adults, followed by rearing[56]. It was reared from their habitat, where it was found along with the larvae of *C. peregrinus* (Figure 1b), *C. guttifer* de Meijere, and *C. huffi* Causey[2]. Previously, many researchers tried to rear *C. oxystoma* frequently, but they have yet to report this vital vector species' life history traits and rearing parameters in laboratory settings.

Laboratory colonies of vector species are essential for a better understanding their vectorial capacity and competence. Standardizing larval food, rearing, captive mating, and artificial blood feeding in laboratory conditions is essential. Globally, only 23 species were attempted to rear under laboratory conditions, of which only two colonies, i.e., *C. sonorensis* Wirth & Jones and *C. nubeculosus* (Meigen), are extant[57,58,59,60,61,62]. Laboratory rearing of *C. peregrinus* and *C. schultzei* were performed[58]. Later on, the biology of *C. peregrinus* was worked on in detail, including fecundity, rearing, and life history parameters. It showed the highest adult emergence possible when inoculating substrate from their habitat into rearing plates[59]. The overwintering of *C. brevitarsis* was noted after the influence of temperature on the development and rearing of this species was also noticed[63,64]. Larvae and pupae were found on the cow pat; this species is anautogenous[2]. Life history parameters of *C. imicola* depending on various temperatures were also observed[65]. The bacterial communities among *C. imicola* populations are shaped by various biotic and abiotic factors[66]. Along with this, metagenomic analysis of microbial communities associated with the life history of *C. peregrinus* and identification of fungal communities from the fourth instar of this vector species were recorded[67]. The haemolytic bacteria, i.e., *Bacillus pumilus* (CU1A and CU1B) and one blood-utilizing bacterium, *Bacillus licheniformis* (CU2B) were isolated and identified from wild-caught *C. peregrinus* and *C. oxystoma* and suggested a possible role in shortening of blood digestion period[68]. Further isolation, biochemical characterization, and antibiotic sensitivity of haemolytic bacterial

strains across life history documented that 13 bacterial strains were beta haemolytic while only one was alpha haemolytic bacteria[69]. Only specific strains of culturable bacteria and effective antibiotics can be used for further applications in managing vector species by paratransgenesis techniques[69]. It was noticed that adults and juveniles of *Menemerus bivittatus* and juveniles of *Marpissa* sp. also feed on engorged adults of *C. peregrinus* and *C. oxystoma* and suggested that the spiders may serve as biological control agents of these vector species[70].

CONCLUSION

Studying intraspecific variation among vector species is urgently needed because of Chitradurga's (Karnataka) recent BT disease outbreak. For this reason, proper identification and documentation of Indian *Culicoides* spp., an entomological survey covering our country's physiographic regions, and an effective dry and wet repository are urgently required. Along with this, the study of type specimens and validation of existing *C. schultzei* were recorded by several researchers from India. Species complexes and associated knowledge gaps may be taken up by practicing integrative taxonomy. Despite several host-specific observations, vector-centric dispersal, host range expansion and biology-based study will be needed to develop effective management strategies. Besides categorizing and identifying larval microhabitats, standardizing larval food for rearing, blood feeding, and captive mating are pivotal to developing a thriving laboratory colony and further strengthening vector research.

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Review article

IS *PLASMODIUM RELICTUM* A THREAT TO AVIAN DIVERSITY IN INDIA? TIME FOR A REALITY CHECK

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ABSTRACT

Plasmodium relictum is the most widely prevalent avian malaria parasite, infecting over 400 species of birds. It is relatively non-pathogenic in most cases, but, it may be lethal to the naïve species which have not evolved resistance to the parasites. Hitherto, there have been no reports of *P.relictum* avian malaria outbreaks in India.

However, the possibility of the emergence of virulent strains of *P. relictum* is not unlikely. This exploratory review is an attempt to assess and evaluate if *P. relictum* is a threat to Indian avian biodiversity.The pSGS1 and pGRW4 lineages

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of *P.relictum* are the most virulent lineages associated with the pathogenesis and decline in naïve bird populations. The parasite is highly prevalent in tropical and subtropical parts of the world. The absence of reports regarding the outbreak or emergence of *P. relictum* is possibly indicative of an enzootic circulation. However, considering the evolving nature and dynamism of host-parasite interaction, changes in environmental and ecological drivers of this disease may facilitate the emergence of virulent lineages.

The effect of the emergence of virulent lineages could result in a decline in bird populations in India that eventually may lead to disruption of ecological homeostasis as well as affecting the agro-economic sectors. Therefore, sustained surveillance and monitoring of bird populations in the different geo-climatic zones of India are necessary to detect the presence or emergence of the virulent lineages of *P. relictum,* if any.

Short Title: *Plasmodium relictum* and avian diversity

Keywords: Avian malaria, *P. relictum*, Genetic lineages, Avian conservation, India

INTRODUCTION

Plasmodium parasites infect a diverse range of hosts, including humans, non-human primates, bats, rodents, reptiles, and birds.[1,2] Apart from a few minor differences, all these species share a nearly identical life cycle, with an asexual replicative stage in the vertebrate host and a sexual stage in a blood-sucking culicid mosquito (Diptera: Culicidae).[3] *Plasmodium* are widespread in wild birds globally. Till now fewer than 60 *Plasmodium* species that infect birds have been described morphologically.[4-6] Among these morphologically distinct species, *Plasmodium relictum* is a widely prevalent and most common pathogen for avian malaria, infecting over 400 species of birds with a global distribution. Primary vectors of *Plasmodium relictum* are *Culex quinquefasciatus* and *Culex pipiens,*[7] and the passerine birds are the primary hosts of most of the lineages of this parasite. Although the susceptibility of passerine birds to *P. relictum* varies depending on the bird species.[7] Under most circumstances, *P. relictum* is relatively non-pathogenic. However, it may be lethal to the species which have not evolved resistance due to the parasites, and naïve birds often suffer from severe disease and mortality during

infection.[8] The GRW4 lineage of *P. relictum* is responsible for severe disease and mortality associated with the decline and extinction of several bird species on the islands of Hawaii.[9] The pSGS1 and pGRW4 lineage of *P .relictum* are the most prevalent among different cytochrome *b* lineages, causing infection in several wild bird species.[9] However, the severity of the infection varies among the avian species.[9] The broad host range, coupled with a wide range of vectors might facilitate the invasion of *P. relictum* in newer areas.[7,10] Hitherto, there are no reports of any major outbreak of *P. relictum* avian malaria in India. In this context, this exploratory review is an attempt to assess and evaluate if *P. relictum* is a threat to Indian avian biodiversity. It will help to understand the current situation of *P. relictum* malaria in India and possible future threats, if any.

METHODOLOGY

Using electronic databases such as PubMed/Medline, Google Scholar, Wiley Online Library, and Semantic Scholar in addition to a manual search in Google Scholar with relevant keywords, a narrative review was carried out. The journal papers that were sought and used, both peer-reviewed and not, were written in English. These databases cover a wide range of topics related to *Plasmodium relictum*, avian malaria, mosquito biology, ecology, and other related fields. Using the pertinent keywords that could be found in the paper titles and/or abstracts, the initial search was done on PubMed. The method of searching was also applied to other databases. Every conclusion and observation made in this review about the subject matter is supported by published data, which is cited in the references.

LINEAGES OF *PLASMODIUM RELICTUM*

Hitherto, there are five closely related lineages of *P. relictum* identified to be circulating globally with prevalence in the different bio-geographical regions among several hosts.[7] Among 29 mitochondrial cytochrome b lineages of genus *Plasmodium*, the five closely related lineages pSGS1, pGRW4, pGRW11, pLZFUS01, and pPHCOL01 belong to *Plasmodium relictum* (Table 1). [7] The reported lineage of *P. relictum* (pPHCOL01) was new. This lineage is clustered with other morphological lineages of *P. relictum* (pSGS1, pGRW4, pGRW11, pLZFUS01), supporting the close phylogenetic relationships among them. Genetic differences among five lineages of *P. relictum* varied between 0.2% (minimum, the

lineages pSGS1 and pGRW11) and 3% (maximum, the lineages pSGS1 and pLZFUS01).[7] The discovery of considerable geographically structured genetic variation in MSP1 across different cyt b lineages of the *P. relictum* morphospecies suggests that different cyt b lineages of *P. relictum* should not be considered as one panmictic population.[9] Among the five lineages, pSGS1 and pGRW4 are the most widespread. [9]

pSGS1 is the cosmopolitan lineage of the *P. relictum,* and numerous bird and mosquito species are susceptible to this infection.[11] Hitherto, the pSGS1 lineage has been reported to be most widespread among all *P. relictum* lineages geographically. The lineage pSGS1, predominantly infects birds in tropical Africa, most of Europe north of the Polar circle, and in Asia east to South Korea, but is strikingly absent in North America.[10,12,13] In recent studies, it has been found that SGS1 is transmitted among several different species in New Zealand and was also found in several resident bird species in Peru.[14]

pGRW4 gets actively transmitted in countries with warm climates and temperate regions of the New World.[11] The main transmission area of PGRW4 is tropical Africa, North America, and several Oceanic islands including Hawaii (Beadell et al., 2006).[10] This lineage is not transmitted in Europe.[15] The possible reason for the absence of GRW4 in Europe can be due to the absence of *Culex quinquefasciatus,* which is the primary vector of pGRW4.[16] However, unlike SGS1, GRW4 has only a single case of confirmed transmission in the temperate regions of the Old World,[17] except Japan, where oocysts and sporozoites of GRW4 were detected in mosquito vectors. GRW4 is frequently observed in adults of migratory bird species with tropical wintering ranges.[5,18]

Table 1: Different genetic lineages of *Plasmodium relictum,* their distribution and Host range

Lineage	Distribution		Host (Bird Order)	Main findings	References
	Zoogeographic region	Country			
pSGS1	Palaearctic, Afrotropic, Neotropic, Indo-Malay,	Kenya. Nigeria, Italy, Turkey,	Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes,	pSGS1 lineage is cosmopolitan and is most widespread	Palinauskas *et al.*, 2008; [19] Hellgren *et al.*, 2015; [9]

Lineage	Distribution		Host (Bird Order)	Main findings	References
	Zoogeographic region	**Country**			
	Australasian	Bulgaria, Spain, Lithuania, Japan, South Africa, Peru, New Zealand, Scandinavia	Galliformes, Gruiformes, Passeriformes, Procellariiformes, Sphenisciformes, Strigiformes, Trochiliformes	among birds.	Valkitūnas *et al.*, 2018. [7]
pGRW4	Palaearctic, Afrotropic, Nearctic, Neotropic, Indo-Malay, Australasian, Oceanic	Kenya. Nigeria, Italy, Turkey, Bulgaria, Spain, Lithuania, Japan, South Africa, Zambia, USA, Bermuda, Hawaii, Argentina, Brazil, New Zealand	Passeriformes, Ciconiiformes, Psittaciformes	pGRW4 is most widespread geographically and has been the reason behind extinction of endemic birds in Hawaii. However, it has not been found in many bird orders as compared to pSGS1.	Atkinson *et al.*, 1994; [20] Hellgren *et al.*, 2015; [9] Valkiūnas *et al.*, 2018 [7]
pGRW11	Palaearctic, Afrotropic, Australasian,	Italy, Bulgaria, Spain, Lithuania, Japan, South Africa	Charadriiformes, Galliformes, Passeriformes	pGRW11 has not been recorded in New world yet. It appears to be restricted to the temperate regions with	Hellgren *et al.*, 2015; [9] Valkiūnas *et al.*, 2018 [7]

Lineage	Distribution		Host (Bird Order)	Main findings	References
	Zoogeographic region	Country			
				some exceptions.	
pLZFUS01	Palaearctic, Afrotropic, Nearctic, Indo-Malay	NA	Passeriformes	Possible distribution is in the temperate regions of Europe.	Valkiūnas *et al.*, 2018 [7]
pPHCOL01	Palaearctic	NA	Passeriformes	Possible distribution is in the temperate regions of Europe.	Valkiūnas *et al.*, 2018 [7]

HOST SPECIFIC BEHAVIOUR OF *P. RELICTUM*

P. relictum is the most common agent of avian malaria, which got reported to infect over 300 bird species belonging to 11 orders. [4,21] Susceptibility of these birds to the infection of *P. relictum* is markedly different. The prevalence of *P. relictum* varies according to the host species and geographical region, but it can attain extremely high levels, particularly in passerine birds. Five known genetic lineages of *P. relictum* viz. pSGS1, pGRW4, pGRW11, pLZFUS01, and pPHCOL01 are prevalent among Passeriformes birds.[7] Genetic lineages pSGS1 is the host generalist ranging from more than 11 avian orders. Apart from Passeriformes birds, pSGS1 is prevalent among bird order Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes, Galliformes, Gruiformes, Procellariiformes, Sphenisciformes, Strigiformes, Trochiliformes.[7] On the other hand, the most virulent lineage pGRW4 is prevalent among Ciconiiformes, Psittaciformes orders apart from the Passeriformes birds.[9]

Different developmental patterns of the same lineages are often evident in birds. The variation of biological properties (parasitemia dynamics, blood pathology, prepatent period) in different isolates of the same lineage might be greater than the variation in different lineages during development in the same species of birds.[7] It remains unclear why geographical isolates of the same lineages

of *P. relictum* behave differently in the same species of birds. The differences might be due to clonal intra-lineage genetic diversity.[9] However, current lineage information is limited in predictions about relationships in host-parasite associations.[7]

VECTORS AND POSSIBLE VECTORS OF *PLASMODIUM RELICTUM*:

Plasmodium relictum primarily gets transmitted by the *Culex pipiens* complex mosquitoes globally.[22] The two most widespread primary vectors of this parasite are *Culex quinquefasciatus* and *Culex pipiens* (Table 2).[7,16] *Culex quinquefasciatus* is the primary vector of genetic lineage pGRW4, while *Culex pipiens* are the primary vectors for genetic lineage pSGS1 of *P. relictum*.[7,23] Apart from that, *Cx. tarsalis* and *Cx. stigmatasoma* is also the designated natural vector of this parasite.[23] *Cx. tarsalis* is the well-known vector for the genetic lineage pGRW11, circulating the parasite in Europe.[9,11,24] *Culex pipiens pallens* is also a vector for transmitting the pathogen throughout European and African countries.[9] It is interesting to note that, mosquitoes belonging to the genus *Aedes and Wyeomyia viz. Aedes albopictus and Wyeomyia mitchellii* respectively were found susceptible to the pGRW4 parasite, and the sporogony got completed in both of these mosquito species. However, the prevalence of the parasite was significantly less in comparison to the primary vector *Cx. quinquefasciatus*.[23] These mosquitoes may contribute to the *P. relictum* epidemiology as possible complementary or secondary vectors; however, the assumption has not been proven yet. In experimental setups, *P. relictum* develops in at least 26 species of mosquitoes belonging to genus *Culex, Aedes, Culiseta,* and *Anopheles*, but only a few of these studies confirmed the presence of sporozoites in the salivary glands of the mosquitoes.[23]

Table 2. Primary and secondary/ maintenance vectors of *Plasmodium relictum*

Vectors	Distribution	Lineages	Main findings	Reference
Culex *quinquefasciatus*	Globally distributed, especially throughout tropical and subtropical	GRW4, GRW11,	Primary vector for the pGRW4 lineage. May also act as the vector for pGRW11.	Vézilier *et al.*, 2010; [22] Chagas *et al.*, 2021; [16] Valkiūnas *et al.*, 2018 [7]

Vectors	Distribution	Lineages	Main findings	Reference
	region.			
Culex pipiens	Switzerland, Northan hemishphere	pSGS1, pGRW4, pGRW11	Primary vector for the pSGS1 genetic lineage. The complete sporogony of pGRW4 has been evident in experimental setups.	Chagas *et al.*, 2021; [16] Valkiūnas *et al.*, 2018; [7] Platonova and Palinauskas, 2021 [25]
Culex tarsalis	Present throughout the European region	pGRW11	Natural vector for the pGRW11 lineage	LaPointe *et al.*, 2012; [23] Valkiūnas *et al.*, 2018 [7]
Aedes albopictus	Global distribution across all continent	pLZFUS01	Found susceptible in experimental setup	LaPointe *et al.*, 2005; [26]
Wyeomyia mitchellii	African and European regions	pGRW4	Found susceptible in experimental setup	LaPointe *et al.* ,2012, [23]
Culex theileri	Trans-Himalayan and European region	pSGS1	Isolated from the wild caught mosquitoes	Ferraguti *et al.*, 2013 [27]
Cx. Sasai	Mostly prevalent in Asian region	pSGS1	Isolated from the wild caught mosquitoes	Kim *et al.*, 2009 [28]

ENVIRONMENTAL AND CLIMATIC FACTORS INVOLVED IN TRANSMISSION OF *P. RELICTUM*

For malaria parasites, the ambient temperature is one of the influencing factors for development.[29,30] Avian malaria *P. relictum* optimally develops within vectors at 27°C, and temperatures below 20°C inhibited or strongly delayed sporozoite development.[25] For the genetic lineage pGRW4, the complete sporogony was seen at the mean temperature of 19°C, with the fluctuation ranging from 24°C to 14°C, in an experimental setup.[31] Another group of researchers found that pGRW4

sporogony can get completed at a temperature ranging from 23 °C to 7°C. However, patterns of sporogonic development were different.[25] Lower temperatures lengthens the development duration of oocysts and sporozoites,[25] thus validating the temperature sensitivity of the *P. relictum*. Apprehensions have been raised by a group of researchers that delay in sporogony may contribute as a limiting factor to the expansion of *P. relictum* in Northern Europe.[25]Therefore, climatic factors in the tropical and sub-tropical regions can be favourable for the life cycle of *P. relictum, like many other haemosporidian parasites. This increases the possibility of native birds in these regions becoming hosts for the infection of P. relictum.*

DISCUSSION

Plasmodium relictum is one of the most widely distributed avian haemosporidian parasites circulating among a large number of hosts throughout the world in the form of five different lineages.[32,33] One of the main reasons behind this wide geographical distribution and host range is the invasive nature of *P. relictum,* it got cataloged on the IUCN list of 100 world's worst invasive species.[32] For virulence and invasive nature, the lineage pGRW4 of *P. relictum* is well known in avian malariology. After the introduction of pGRW4 lineage in the Hawaiian Islands, the pathogen caused lethal malaria in birds leading to the extinction of many endemic species,[11] thus, indicating the effect of invasive pGRW4 on naïve isolated bird populations or populations in isolated islands. Historically lineages pSGS1, pGRW4, and pGRW11 of *P. relictum* are prevalent in migratory birds globally, primarily belonging to orders Galliformes, Columbiformes, and Passeriformes.[9] Previously pSGS1 was reported in non-migratory birds of Africa endemic to regions climatically similar to the Mediterranean region of Europe,[9] thus designating climatic factors as a driver for the disease transmission. Moreover, it was experimentally shown by several authors that many physical and biological factors such as environmental temperature, humidity, and the simultaneous presence of vertebrate hosts, vector, and parasite species in the same place affect the sporogony of *P. relictum* parasites.[34,35] Apart from that, the breeding behavior and blood-sucking behavior of mosquitoes are also influenced by warmer climates.[36] Therefore, global warming and other climatic factors are may be important drivers for a possible host range expansion of different lineages of *P. relictum* in the migratory, non-migratory, and/or endemic bird populations. The anthropogenic factors such as urbanization, deforestation, globalization, and land-

use change have changed the environmental conditions in the habitats of the vectors and hosts for several diseases,[37] which may create novel habitats for the hosts of *P. relictum*. The anthropogenic activities may facilitate the geographical expansion of known vectors of the disease, such as *Culex quinquefasciatus*.[23] Changes in the host and vector distribution may eventually lead to the emergence of *P. relictum* avian malaria in newer geographic areas. However, further experimental and field-based data are required to validate this assumption.

Hitherto recognized genetic lineages of *P. relictum*, pSGS1, pGRW4, pGRW11, pLZFUS01, and pPHCOL01, markedly vary in their host range, geographical distribution, and other biological characteristics.[7] However, concomitant infection of different lineages while circulating among wild birds is well known.[19,34] The interaction of these parasites can be positive or negative, and they may affect the development of one another.[38] This association may result in competition, as seen in the case of pSGS1, which is widespread throughout Europe and possibly suppresses the tropical lineages.[19,34] Protozoan evolution as a consequence of interspecific competition and eventual generation or sorting of newer lineages due to selection pressure has been evident.[39] Similar possibilities for *P. relictum* may not get ignored. Wild birds having co-infection may act as a mixing pot for several lineages leading to inter-lineage competition and subsequent generation of newer lineages under selection pressure. However, this hypothesis needs to be consolidated through further research supported by experimental data.

TIME FOR A REALITY CHECK ABOUT THE PRESENCE OF VIRULENT LINEAGES OF *P. RELICTUM* IN INDIA

In 1889, Sir Ronald Ross, in his pioneer research on the transmission of malaria in Kolkata, India used Passeriformes birds viz. sparrows, larks, and crows as model organisms showing that Grey mosquitoes (possibly *Culex quinquefasciatus*) can transmit the pathogen among these birds.[40] The Passeriformes birds, known as the most recognized host for *P. relictum,* are abundant in India.[11] Patra *et al.*, 2020, observed that *P. relictum* was present in about 20% of wild bird samples in north-east India in their investigation, with prevalence in common hoopoe, red vented bulbul, house sparrow, and egrets.[41] The primary vector of several lineages of *P. relictum*, the *Culex quinquefasciatus* mosquitoes are also present in almost all terrines of India, acting as a bridge vector for several diseases in urban, peri-urban,

and rural habitats owing to its adaptability in diverse ecological niches.[42] Apart from that, other *Culex pipiens* complex mosquitoes are prevalent in the tropical regions of India, which are effective vectors for multiple genetic lineages of *P. relictum*.[11,23] It has been demonstrated by several authors that the temperature and humidity in the tropical and sub-tropical regions are highly conducive to the sporogony of *P. relictum*.[11,24] So far, there are no reported cases of any major outbreak of *P. relictum* in Indian avifauna. Owing to the mosquitogenic environment coupled with the abundance of hosts or possible hosts and favorable ecological conditions in India, the parasite may remain enzootic, circulating perennially among different hosts and with vectors. As host-parasite interaction is a dynamic phenomenon, the involvement of young non-immune nestlings in the transmission dynamics of *P. relictum,* together with a seasonal peak in vector abundance, may transform the infection from enzootic to epizootic. During the high pathogen load, the parasite may tangentially spill over to naïve populations. However, sustained surveillance, especially the favorable climatic condition for the vector and parasite i.e. spring and summer season, is required to substantiate this hypothesis.

Having the migratory birds as the primary hosts has particular relevance in the disease transmission of *P. relictum*. Evidence of avian malaria parasites is being carried by migratory birds from wintering areas with warm climates to the breeding grounds is abundant.[12] Several researchers earlier demonstrated the presence of several tropical *Plasmodium sp*. in the European long-distance migrating birds returning from South Asia and Africa,[12,43,44] and some of these parasites, such as pGRW4, were highly virulent to their hosts in the European region.[34,45] Invasive parasites could be highly virulent to naïve and isolated endemic bird populations which have not co-evolved with the pathogen. [46,47] India is a migratory corridor for many bird species around the world. Therefore, regular monitoring of migratory bird populations is necessary for early detection of *P. relictum* invasion and their subsequent effect on native bird populations of India, if any.

Different birds get affected by *P. relictum* differently.[7] Even the same lineage of the pathogen sometimes affects the same species of hosts in varied manners.[7] The reason behind this pattern of infection could be the dynamism of host-parasite interaction, an evolutionary phenomenon. As an ongoing evolutionary process, the generation of newer lineages or variants and their subsequent emergence is highly

possible. The emergence of novel parasites may cause severe pathologies in naïve hosts leading to a potential decline in wildlife populations.[48,49] Each bird plays an important ecological role in the ecosystem's functioning. The poultry birds belonging to the order Anseriformes, Galliformes are highly susceptible to *P. relictum*[7] thereby, indicating a potential threat to the agro-economy and poultry sectors also.

During our literature review on *P. relictum* parasite for this article, it got noticed that very scanty work has been done from the Indian perspective on the disease. Hence, sustained surveillance and monitoring of bird populations in India are required to detect the prevalence of pathogenic lineages of *P. relictum* if any. *P. relictum* transmission is largely dependent on environmental factors. The distribution of different lineages also varies according to climatic factors.[11,24,34,35] India is a diverse country concerning geo-climatic situations. Therefore, considerations should also be made regarding the prevalence of different lineages for geo-climatic zones. The use of sentinel bird models for a better understanding of the current situation may be beneficial in this regard. That would help formulate area-wise strategies of conservation medicine for the susceptible bird populations.

CONCLUSION

Plasmodium relictum is unique among the protozoans belonging to the genus *Plasmodium* owing to an exceptionally broad host range for being cosmopolitan and host generalist. The pSGS1 and pGRW4 lineages of *P. relictum* are the most virulent lineages associated with the pathogenesis and even decline in isolated bird populations naïve to this parasite, though their effect varies according to hosts and environmental conditions. The parasite is highly prevalent in tropical and subtropical parts of the world, as their temperature-sensitive and vector-specific sporogony gets supported by the tropical climatic and ecological factors. With a favorable ecological and environmental situation, *P. relictum* is expected to be widespread in Indian avifauna; however, scanty research has been done with a limited understanding of their distribution in bird populations from an Indian perspective.

The absence of reports regarding the outbreak or emergence of *P. relictum* is possibly indicative of an enzootic circulation of the parasite in wild birds. However, considering the evolving nature and dynamism of host-parasite interaction, changes

in environmental and ecological drivers of this disease may facilitate the emergence of virulent lineages. The subsequent effect of the emergence of virulent lineages could result in a decline in bird populations in India that eventually may lead to disruption of ecological homeostasis as well as affecting the agro-economic sectors. Therefore, sustained surveillance and monitoring of bird populations in the different geo-climatic zone of India are necessary to detect the presence or emergence of the virulent lineages of *P. relictum,* if any. That would be helpful in formulating area-wise strategies for avian conservation based on the principles of conservation medicine.

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Review Article

MALARIA DRUG RESISTANCE IN INDIA: CURRENT STATUS ANDFUTURE PERSPECTIVES

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ABSTRACT

Malaria persists as a significant public health challenge in India, with an annual reporting of millions of cases. One of the most formidable challenges in malaria control is the emergence and spread of drug-resistant strains of Plasmodium parasite. The prevalence of chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) resistance across the country necessitated a strategic shift to artemisinin-based combination therapies (ACTs) as the first-line of treatment for

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Plasmodium falciparum malaria infection. Extensive investigations have been undertaken since the initial documentation of drug resistance, particularly in response to alarming reports of failure in ACT failures in recent years. The period from 2018 to 2023 witnessed a surge in publication addressing drug resistance, and thus we have selectively highlighted a pivotal study that uncovered resistance in case where it was previously not reported. Notably, resistance to artemisinin has manifested predominantly in the northeastern regions, underscoring the dynamic nature of drug resistance. The complicating factor of glucose-6-phosphate dehydrogenase (G6PD) deficiency, particularly in the context of use for P. vivax infections, adds further complexity. India has responded proactively by implementing rigorous surveillance and monitoring mechanisms, collecting data from various regions to promptly detect changes in resistance patterns and treatment efficacy. Research and development initiatives have been intensified, emphasizing the exploration of novel anti-malarial drugs and innovative approaches to combat evolving drug resistance.

Short title: Malaria drug-resistance

Keywords: Malaria, Drug resistance, ACT, Humanized mice, Kelch (K-13)-propeller domain

INTRODUCTION

Malaria has consistently posed a significant public health challenge in India, with an annual reporting of millions of cases[1]. Despite considerable strides in mitigating the impact of this disease, the emergence of drug-resistant strains of the *Plasmodium* parasite remains an enduring challenge[1]. This review article provides an in-depth analysis of the status of malaria drug resistance in India spanning the period from 2018 to 2023. It examines the dynamic evolution of resistance, its repercussions on treatment strategies, and the proactive measures implemented to address this critical issue. It is important to note that the mechanism and targets may vary among different antimalarial drugs, thus here we have provided the brief information about their mechanism and associated targets (Table 1).

Table 1. The mechanism of antimalarial drugs and their site of action (adapted and modified from[2])

Antimalarial drug	Mechanism of action	Associated target
4-aminoquinolines (CQ, AQ,PQ)	Inhibit heme polymerase, preventing the detoxification of heme to hemozoin in the parasite's food vacuole	Heme polymerase in food vacuole
Artemisinin and its derivatives (artesunate, artemether)	Generate free radicals in the presence of iron, damaging the parasite's proteins and structure (by alkylation of proteins and lipids)	Iron-dependent protein in the parasites (in endoplasmic reticulum-ER and vesicular structure)
Naphthoquinones (Atovaquone)	Inhibits the mitochondrial electron transport chain (ETC), disrupting the parasite's energy metabolism	Mitochondrial ETC
Quinine	Disrupts the parasite's ability to degrade hemoglobin, leading to the accumulation of toxic heme	Hemoglobin degradation process
Quinoline-methanol (Mefloquine)	Interfere with the function of parasite's heme detoxification, leading to the accumulation of toxic heme	Heme detoxification process
Antifolates (Glycosylamines: Pyrimethamine and cycloguanil)	Inhibition of dihydrofolate reductase (DHFR)	Cytosol
Antifolates (Sulfonamide/Sulfones: Sulfadoxine)	Inhibition of dihydropteroate reductase (DHPS)	Cytosol
Antibiotics Doxycycline and Clindamycine	Inhibit protein synthesis in the parasite by binding to the 30S ribosomal subunit and inhibit protein translation inside the apicoplast	30S ribosomal subunit Inside the apicoplast

THE CHANGING LANDSCAPE OF DRUG RESISTANCE

In the context of malaria endemicity in India, the emergence of resistance to antimalarial drugs, such as CQ and SP, has emerged as a major concern. One of the primary treatments for uncomplicated malaria in India, CQ faced its first reports of

resistance in 1970s, initially in the Northeastern states[3]. This resistance subsequently spread to other regions, including Odisha, Tripura, and Assam. By the 1990s, widespread resistance was observed across the country, particularly in *P.falciparum*, the most severe malaria parasite species[3]. This necessitated the exploration of alternative drugs to address the growing ineffectiveness of CQ.

(A) *Plasmodium vivax* Surveyor

(B) Sulphadoxine pyrimethamine (SP) Molecular Surveyor

Fig. 1. The drug resistance status against (A) P.vivax and, (B) SP molecular survey displays the prevalence of molecular markers associated with resistance to SP found in *P. falciparum* in India (*Source*: Infectious Diseases Data Observatory (IDDO) (2015): The ACT Partner Drug Molecular Surveyor. Infectious Diseases Data Observatory. (Interactive Resource: https://www.wwarn.org/tracking-resistance/act-partner-drug-molecular-surveyor)

SP, a combinational drug crucial for intermittent preventive therapy in pregnant women and malaria treatment, also encounter resistance in India[3, 4]. Initial reports of SP resistance echoed the patterns observed with CQ, first emerging in the Northeastern states and gradually spreading to Meghalaya, Mizoram, Nagaland, and beyond[3]. The rise in SP resistance posed challenges for its utilization in both malaria treatment and prevention strategies[4].

To counter the escalating resistance to CQ and SP, the National Vector Borne Disease Control Programme (NVBDCP) in India underwent a critical revision of its national drug policy and treatment guidelines[5]. The updated guidelines recommended the adoption of ACT as the new first-line treatment for uncomplicated malaria caused by *P. falciparum*[5].

Drug resistance status for ACT partner drugs in India

Fig. 2. The drug resistance status against the ACT Partner drugs in India found in *P. falciparum pfmdr1* and *pfcrt* gene (*Source*: Infectious Diseases Data Observatory (IDDO) (2015): The ACT Partner Drug Molecular Surveyor. Infectious Diseases Data Observatory. (Interactive Resource: https://www.wwarn.org/tracking-resistance/act-partner-drug-molecular-surveyor)

From 2018 to 2023, notable changes occurred in India's malaria drug resistance landscape. Historically, CQ and SP were widely used for malaria treatment [6]. However, the escalating of resistance to these drugs was particularly in the northeastern and eastern regions[6] (Fig. 1).

Consequently, there was a shift to ACTs as the first-line treatment. However, despite the initial efficacy of ACTs, indication of artemisinin resistance surfaced in various region of India. This resistance, predominantly observed in the northeastern states, posed a significant challenge to the effectiveness of one of the most potent malaria treatments (Fig. 2)[1, 7, 8]

Several factors contribute to this situation, such as excessive and inappropriate use of antimalarial drugs, poor quality drugs and, the circulation of counterfeit drugs. Malaria drug resistance manifest in two primary forms: the resistance to artemisinin derivatives, representing the gravest concern as it can result treatment failure and mortality, and resistance to partner drugs. Partner drugs are employed in conjunction with artemisinin derivatives to enhance efficacy and thwart the emergence of resistance[8, 9].

THE G6PD DEFICIENCY CONUNDRUM

An additional challenge in the malaria eradication efforts in India stems from the prevalence of Glucose-6-phosphate dehydrogenase (G6PD) deficiency within some specific Indian populations[10, 11]. G6PD deficiency amplifies the risk of hemolysis when administering certain anti-malarial drugs, such as primaquine. This deficiency complicates the treatment of *P. vivax* infections; where primaquine is crucial for eliminating dormant liver-stage parasites[12]. Consequently, healthcare providers have adopted a cautious approach by incorporating G6PD testing as a prerequisite before primaquine administation[13]. It's noteworthy that the widespread accessibility of G6PD testing remains limited, posing a potential obstacle to the optimum utilization of primaquine[14].

In this direction, a cross-sectional, multi-centric study was carried out to investigate the prevalence and molecular diversity of G6PD deficiency in India[15]. Including more than 20,000 individuals form 15 states, focusing on malaria-endemic regions, samples were collected between 2015 and 2017 underwent several screening methods. Initial screening for common Indian mutations was followed by

DNA sequencing, revealing 12 mutations causing G6PD deficiency, with G6PD Orissa and G6PD Mediterranean as predominant variants[15]. Prevalence varied (0.8%-6.3%) across states, with G6PD Orissa and G6PD Mediterranean present in most states, except Karnataka and Meghalaya where G6PD Namoru and G6PD Mahidol variants were observed, respectively. A novel mutation, c.544C G, was identified in Madhya Pradesh[15]. Further, enzyme modeling suggested its impact on G6PD protein stability, advocating for further structural characterization using molecular dynamics. Results highlight genetic heterogeneity influenced by migration and historical factors. The study underscores the importance of G6PD screening in malaria endemic areas, emphasizing appropriate anti-malarial therapy and dosage management for deficient individuals[15].

Of late, Dixit *et al*., carried out a study focusing on evaluating the prevalence of haemoglobinopathies and G6PD deficiency among Particularly Vulnerable Tribal Groups (PVTGs) in malaria-endemic regions of Odisha, India[16]. The study was conducted from July 2018 to February 2019 using two-stage sampling method. The study's findings revealed a significant prevalence of G6PD deficiency among PVTGs, ranging from 1.1% to 10.4% across different tribes[16]. Notably, 57.4% of individuals with G6PD deficiency were concurrently positive for malaria. Despite the World Health Organization's (WHO) recommendation for G6PD testing before PQ administration in malaria treatment, the study highlighted the infrequent practice of such testing in Odisha[16]. The nexus between G6PD deficiency and malaria is pivotal, especially in malaria elimination initiatives incorporating PQ for both *P. falciparum* and *P. vivax.* In summary, the study underscores the prevalence of G6PD deficiency and its correlation with malaria within PVTGs in Odisha. It stresses the necessity of G6PD testing before administering PQ for malaria treatment and advocates for the implementation of screening programs and counseling, particularly for genetic disorders like G6PD deficiency, among PVTGs.

CURRENT STATUS OF DRUG RESISTANCE IN INDIA

In the last decade, the escalation of drug resistance assessments and surveillance in India has been notably propelled by advancements in the healthcare sector. The reporting of the contemporary landscape of drug resistance, coupled with the identification of novel genes associated with resistance, has further intensified these efforts. Consequently, since 2018, our exclusive focus has centered on the most pivotal and illuminating research pertaining to drug resistance in India.

The first-line treatment for blood stage infection of *P. vivax* is CQ; however, instances of CQ resistance have been documented in India. One monitoring approach involves analyzing single nucleotide polymorphisms (SNPs) in relevant gene markers to trace CQ-mediated resistance in diverse regions[17]. In this context, a study was undertaken in the highly endemic area of Mangaluru city in South Western Coastal region of India. The investigation aimed to assess the prevalence of SNPs in *P.vivax* orthologs of *P. falciparum* CQ-resistant and multi-drug resistant genes (*pvcrt*-o and *pvmdr*-1, respectively) and *pvmdr*-1 copy number variations. The study's outcome revealed a K10 insertion, with the remainder exhibiting a wild-type sequence[17]. This marked the initial identification of a K10 insertion in *P. vivax* isolates from India. While the current findings indicate that the pvcrt-o and pvmdr-1 gene variants linked to *P. vivax* CQ resistance are less frequent in Mangaluru, they suggest the emergence of a resistance trend. Nonetheless, further *in vitro* studies are imperative to corroborate drug susceptibility in the region[17].

A subsequent study by Anatabotla and colleagues focused on investigating gene polymorphisms associated with drug resistance of *P. vivax* across four different regions in India namely, Puducherry, Mangaluru, Cuttack, and Jodhpur [18]. Notably, this study marked the first investigation into drug resistance gene screening in *P. vivax*, particularly from Puducherry and Jodhpur. Results indicated a high prevalence of CQ resistance in *P. vivax* isolates in the Southeastern and Southwestern coastal regions of India and East India. The study identified new mutations in the molecular markers associated with CQ resistance in the *pvcrt*-o and *pvmdr*-1 genes, corroborating earlier findings [18]. Despite the observed resistance, further *in vitro* studies are essential to confirm drug susceptibility in the region.

Over the past six decades, the treatment of *P. Vivax* has relied on CQ and antifolate medications like pyrimethamine and sulfadoxine[19]. However, due to co-infection with *P. falciparum*, *P. vivax* has been exposed to antifolate drugs through misdiagnosis or inappropriate treatment, exerting selection pressure on the parasite to adapt[20]. Consequently, a study was conducted to assess antimalarial drug resistance among both complicated and uncomplicated *P. vivax* cases in Chandigarh, North India. The study revealed concerning polymorphism in the genes associated with antimalarial drug resistance, underscoring the need for continued scruntiy[21]. The report emphasizes the imperative for India to formulate a well-

defined antimalarial medication strategy. The foundational molecular data from this study on CQ and antifolate drug resistance can contribute significantly to the development of future malaria treatment approaches in India.

Similarly, a study conducted in the same city aimed to investigate the prevalence of *P. falciparum* resistance to SP, a commonly used antimalarial drug[22]. The research revealed that nearly all isolates (98.2%) exhibited at least one mutation in genes associated with SP resistance. The most prevalent mutations were found in the pfdhfr and pfdhps genes, with all patients having been treated with SP between 2014-2016[22]. Despite the high prevalence of SP resistance mutations, no mutations in the K13 gene, liked to artemisinin resistance, were identified. The study suggests an imperative expanded molecular marker monitoring and clinical trials on alternative first-line antimalarials in India due to the observed spread and intensification of SP resistance.

Even with ACT treatment, multiple studies identified drug resistance, leading to investigations into gene mutations and their correlation with parasite clearance. The first report on genetic mutations associated with artemisinin resistance in malaria parasites in Eastern India identified the G625R polymorphism in the *pfkelch*13 gene, linked to artemisinin resistance. The polymorphism was associated with increased parasite clearance half-life (PCHL) and higher ring-stage survival rates[23]. Although the F441I polymorphism was not directly linked to treatment failure, it was associated with greater PCHL, consistent with a prior study conducted in northeastern India[23]. The study emphasized the urgency of updating malaria treatment guidelines and exploring new therapies for artemisinin-resistant malaria in Eastern India.

Moreover, given that Tripura shares borders s with Southeast Asia, a focal point for drug-resistant malaria, vigilance over the prevalence of drug-resistant malaria strains becomes paramount. Consequently, a recent study was conducted in the Eastern region of Tripura to elucidate the drug resistance and genetic diversity of *P. falciparum* parasites. The findings reveal that 87% of *P. falciparum* isolates exhibit triple mutations at codons M74I, N75E, and K76T in the *Pfcrt* gene, indicating significant resistance to CQ [24]. Notably, no polymorphism was detected in the PfK13 propeller. The results underscore a substantial presence of the resistant *Pfcrt* gene in Tripura, aligning with the region's treatment approach. Additionally, 53.85% of the isolates manifest polyclonal infections, signifying multiple parasite

infections within the same host. This observation implies a persistent high frequency of the *Pfcrt* gene mutation associated with CQ resistance, even after the removal of the drug from national treatment guidelines. The outcomes prompt inquires into the efficacy of the existing ACT in the region and raise concerns about informal use of CQ for uncomplicated *P. falciparum* malaria.

In the majority of India, the first-line treatment for uncomplicated *P. falciparum* malaria is a combination of artesunate plus sulfadoxine-pyrimethamine (ASP), except for six provinces in the northeast where treatment failure rates are elevated[25]. Prior to the introduction of ASP, a study conducted in Ujjain, central India, in 2009 and 2010 revealed an incidence of mutations associated with enhanced medication tolerance, though not overt resistance, at 9% for pyrimethamine and >80% for sulfadoxine. Subsequently, a study conducted 3 to 4 years earlier, finding no significant increase in mutations frequency[26]. While double mutations were prevalent in most samples, triple mutations were rare, and certain samples displayed a quadruple mutation, hinting at the potential development of more resistant haplotypes. Comparative analysis with other Indian regions indicated variations in mutation prevalence, suggesting geographical differences in the evolving if drug resistance[26]. Despite signs of accumulating drug resistance mutations, the study affirmed the continued effectiveness of ASP at the research site[26]. Notably, the absence of identified alterations in the kelch-13 propeller domain implies that parasites in Madhya Pradesh, India remain responsive to combination therapy based on artemisinin.

Following the implementation of ACT, a study was conducted in 2020-21 to assess the prevalence and distribution of genetic markers associated with resistance in *P. falciparum* in Pune district, Maharashtra, India. Molecular markers were examined using PCR sequencing (amplifying the DNA region of interest prior to Sanger sequencing), revealing that *pfcrt* K76T mutation, linked to CQ resistance, was present in 78% of the samples[27]. However, mutations associated with artemisinin resistance (C580Y and R539T in pfkelch13) were not identified in any of the isolates. The study concludes that drug-resistant *P. falciparum* is becoming more prevalent in the Pune district, emphasizing the need for ongoing monitoring. Despite the absence of the typical genotype for artemisinin resistance, the prevalence of mutations in both pfdhfr and pfdhps, along with the quadruple mutant, underscores the important of evaluating the effectiveness of SP as a partner

drug for artemisinin in the treatment of *P. falciparum*[27]. It is noteworthy that the study's limitation includes a small sample size and the use of PCR sequencing techniques instead of next-generation sequencing methods.

In a parallel study conducted in Odisha, India, between 2018 and 2020, focused on molecular surveillance of anti-malarial drug resistance genes, findings indicated that the prevalence of mutations associated with resistance to artemisinin derivatives was relatively low (1.4%-5.7%). However, the prevalence of mutations linked to resistance to partner drugs (CQ, SP and ACT) was higher (10.0%-30.0%)[28]. The study also noted a relatively low occurrence of multiple mutations in the same gene (0%-2.9%), suggesting that the development of multi-drug resistance in *P. falciparum* is still in its early stages in Odisha, India[28]. Despite these findings, the report highlighted the continued effectiveness of ACTs in treating malaria in the state of Odisha. However, the increasing frequency of mutations associated with drug resistance in combination with other medications raises concerns.

Subsequently, in 2022, a report assessed the efficacy of treating SP resistant genetic variations in eastern India. The evaluation spanned periods before, during, and six to eight years after the implementation of the new pharmaceutical regime in Kolkata and Purulia districts in West Bengal, India, between 2008 and 2017[29]. The results revealed an overall high prevalence of dhfr and dhps polymorphisms associated with SP resistance against *P.falciparum*. Notably, Purulia exhibited a higher frequency of the triple and quadruple mutants compared to Kolkata, where isolates displayed quintuple and quadruple mutant haplotypes[29]. The study also analyzed treatment outcomes for *P. falciparum* infected patients treated with SP, demonstrating its effectiveness in 61.7% of isolates from Kolkata and 72.1% of isolates from Purulia[29]. Moreover, the study's analysis of the relationship between the IC_{50} of SP suggests the ineffectiveness drug in treating *P. falciparum* infections in this region of India. These findings underscore the necessity for diverse antimalarial medication combinations in areas with high SP resistance and emphasize the ongoing importance of molecular surveillance for developing effective malaria control strategies.

Recently, a comprehensive study was published, undertaking a genetic profiling analysis of drug resistance in *P. falciparum* and *P. vivax* spanning over three decades from, 1993 to 2019 [30]. This investigation delved into the distribution and incidence of molecular markers associated with drug resistance in these

parasites, providing valuable insights for the formulation of effective malaria management strategies. The study specifically focused on mono-infections with *P. falciparum* or *P. vivax*, revealing the highest proportions of mixed infections in Karnataka (64.7%) and Madhya Pradesh (44.4%). Various drug resistance genes, including *pfcrt*, *pfmdr*1, *pfdhfr, pfdhps, pvmdr1, and pvdhfr*, were scrutinized in the study. The research brought to light regional and temporal variations in the occurrence of drug resistance indicators.[30]. For example, in *P. falciparum* isolates from most locations, there was a noticeable decrease over time in frequency of the *pfcrt* K76T mutation linked to CQ resistance. However, certain localities exhibited an increase in the incidence of the mefloquine-resistant *pfmdr*1 N86Y mutation[30]. In the analysis of *P. vivax* isolates, the study observed a declined over time in the prevalence of the CQ-resistant *pvmdr*-1 Y976F mutation in most area. Nevertheless, some regions showed an increase in the frequency of the sulfadoxine-resistant *pvdhfr* S58R mutation[30]. Overall, these findings underscore the importance of continuous monitoring and characterization of *P. falciparum* and *P. vivax* populations, serving as a proxy for evaluating the effectiveness of anti-malarial medications in India. This is particularly crucial in light of the autonomous evolution of drug resistance to artemisinin, as observed in Africa recently[31].

Simultaneously, another study delved into the examination of drug resistance genes in *P. falciparum* from Kolkata, West Bengal, India, aiming to enhance our understanding of drug resistance and virulence factors[32]. A principal component analysis (PCA) was employed to explore the interrelatedness among the Kolkata samples and a global endemic region dataset, encompassing 2570 genomes from 15 countries. The PCA analysis revealed distinct cluster corresponding to Indian isolates standing out prominently from others worldwide[32]. Furthermore, researchers conducted a phylogenetic analysis on 15 representative samples from each country, reinforcing India's unique position relative to other isolates. The genetic composition of the *P. falciparum* isolates from India was found to differ from those in Southeast Asia and Africa, yet shared more similarities with African isolates, include a high frequency of mutations linked to antigenic variation genes[32]. These results suggest that the current first-line treatment for malaria, ACT, may face challenge in effectively treating malaria cases due to unique alterations identified in the Indian isolates[32]. No known mutations associated with artemisinin resistance were found in the *PfKelch*13 gene; however, new mutations were discovered in the ubiquitination and vesicular transport genes, along with novel

mutation in the *PfKelch*13 gene reported to support artemisinin resistance in the early stages of ACT resistance. The study underscores the necessity for a comprehensive understanding of the malaria parasite genome across diverse endemic regions, particularly in areas where drug resistance has emerged[32]. Analyzing samples from these regions could provide insights into the dynamics of host-pathogen interactions, specific genomic traits, and potential markers of treatment resistance.

Considering the comprehensive data released nationwide, it is imperative to address the persistent drug resistance issue with utmost seriousness promptly. Failure to do so could pose significant challenges for our diverse nation in combating this formidable disease.

FUTURE PERSPECTIVES

The endeavour to combat drug resistance in malaria within the Indian context offers numerous promising avenues for future consideration, demanding a comprehensive and integrated approach. To effectively tackle malaria drug resistance in India, it is crucial to adopt strategies that encompass advanced surveillance, dedicated research efforts, active community engagement, strengthened healthcare infrastructure, and enhanced global collaboration[33]. Embracing these strategies can bring India closer to the objective of successfully addressing drug-resistant malaria and alleviating the associated disease burden on its population[34]. Key points that can contribute to controlling the challenging scenarios of drug resistance are outlined below.

Enhanced Surveillance and Data Sharing: Investment in state-of-the-art surveillance tools and data sharing platforms is crucial for real-time monitoring of drug resistance patterns. A comprehensive nationwide surveillance system, integrating clinical, genomic, and epidemiological data, offers valuable insights into the dynamic resistance landscape. Encouraging collaboration with international partners for data sharing and comparative analysis is essential[35].

Research and Development of New Anti-Malarials: Prioritizing the development of novel anti-malarial drugs is paramount. Continuous investment in research to identify and test new compounds, alongside exploring alternative therapeutic strategies such as immunotherapies or vaccines, is critical. Collaborations between the public and private sectors can expedite the process

of bringing new drugs to market. Omics approaches, utilizing high-throughput technology and data mining, can be employed to identify crucial proteins and molecular pathways in the parasite's life cycle, shedding light on emerging drug resistances in India[36].

Effect of Pharmacokinetics of antimalarial drugs: The pharmacokinetic profile of antimalarial drugs holds paramount significance in delineating their therapeutic efficacy, safety, and dosing requirement. This discourse encapsulates pivotal facets pertaining to the impact of pharmacokinetics on antimalarial drugs drawing attention to nuanced pharmacological intricacies[37] as it can affect their efficacy and toxicity. Pharmacokinetics studies on CQ reveal notable interindividual variability in CQ and monochloroquine concentration. This variability is implicated in influencing parasitological treatment outcomes, with lower blood/plasma concentrations observed in cases of treatment failure compared to sensitive treatment outcomes[37]. Furthermore, this variability may correlate with heightened CQ toxicity, particularly retinopathy. While malaria infection itself appears to have minimal impact on CQ pharmacokinetics, exceptions include a higher C_{max} in Thai patients with malaria post-intravenous infusion of chloroquine diphosphate (15 mg base/kg body weight)[37]. Notably, the binding of CQ to plasma proteins remains unaltered during malaria infection. The pharmacokinetics of sulfadoxine and pyrimethamine in SP exhibit complex, nonlinear patterns influenced by factors such as age, weight, pregnancy, and malaria infection. Pregnant women exhibit an increased clearance of sulfadoxine and decreased clearance of pyrimethamine[37]. Artemisinin derivatives, a key component of ACT, experience altered pharmacokinetics influenced by age, weight, and malaria infection. Rapid absorption and metabolism characterize these derivatives, resulting in a short half-life. The pharmacokinetics of partner drugs in ACT is also subject to factors such as age, weight, and pregnancy. For instance, lumefantrine clearance decreases in pregnant women, while piperaquine clearance increases[37]. In summary comprehending the intricacies of antimalarial drug pharmacokinetics is imperative for optimizing therapeutic strategies and mitigating the risk of adverse effects. This understanding provides a foundation for refining treatment protocols and advancing the development of antimalarial interventions.

Usage of Engineered Bacteria-A Biosynthetic Platform: The application of genetically modified bacteria for the synthesis of antimalarial compounds and their derivatives represents a highly promising avenue in the realm of malaria treatment and preventions[38, 39]. This innovative approach harnesses the potential of synthetic biology and genetic engineering to engineer microorganisms capable of adeptly producing these crucial molecules. An exemplary instance entails the synthesis of artemisinin, a pivotal component in numerous antimalarial medications, employing genetically modified yeast strains. Researchers achieved the transfer of genes accountable for artemisinin biosynthesis from the sweet wormwood plant (Artemisia annua) into yeast cells[39]. This genetic manipulation enables the efficient production of artemisinin on a significant scale within a controlled and scalable environment.

Targeted Interventions: Tailoring interventions based on resistance patterns is essential. This involves refining treatment protocols in regions with diverse resistance profiles and ensuring robust and timely drug supply chains for effective resistance management.

Community Engagement and Education: Community awareness and engagement play a pivotal role in preventing malaria and combating drug resistance. Education programs can advocate for the use of insecticide-treated bed nets, early diagnosis, and the importance of completing full treatment courses. Empowering local communities to actively participate in malaria control efforts can have a lasting impact[40].

Healthcare Infrastructure Strengthening: Strategic investing in healthcare infrastructure, especially in remote and underserved areas, is crucial. Enhanced access to healthcare facilities and diagnostic tools ensures early detection and treatment, reducing the likelihood of resistance development[41].

Poor Drug Quality: In tropical areas factors such as suboptimal storage conditions, substandard manufacturing practices, and the widespread distribution of counterfeit drugs contribute to the diminished effectiveness of medications, due to the poor drug quality, fostering the development of drug-resistant parasites. To mitigate this pressing issue, implementation of robust regulatory frameworks, enhanced supply chain management, and targeted public awareness campaigns are indeed. These measures are indispensable in ensuring

the production and dissemination of high-quality drugs in tropical regions, thereby fostering a proactive approach in the battle against drug resistance.

Policy Frameworks and Regulation: Strengthening regulatory mechanisms for drug use, ensuring quality control, and developing robust policies to combat counterfeit drugs are essential for preventing resistance[1].

International Collaboration: Continued collaboration with international organizations, research institutions, and neighboring countries is essential for knowledge sharing, exchanging experiences, and pooling resources. Global cooperation remains instrumental in addressing this trans-boundary issue.

Climate Change Adaptation: Recognizing the impact of climate change on the distribution of malaria vectors, adapting control strategies to changing environmental conditions is imperative. Implementing climate-resilient interventions can help sustain progress in malaria control[42-44].

Role of Humanized mice in Drug Resistance Research

The exploration of humanized mice research holds significant promise in the context of addressing malaria drug resistance in India, constituting an emerging and valuable area of investigation[45-47]. Humanized mice, genetically modified to incorporate human cells or tissues, enables researchers to replicate specific elements of the human immune system[48]. In the fight against malaria drug resistance in India, humanized mice research stands poised to expedite drug development, offer crucial insights into drug resistance mechanisms, and contribute to the development of more effective and targeted anti-malarial strategies[45]. Serving as a crucial link between laboratory research and clinical applications, this approach provides a safer and more efficient avenue for drug discovery and evaluation.

This technology holds significant promise for advancing the exploration of malaria drug resistance through various avenues:

(i) ***In Vivo* Drug Testing:** Humanized mice serve as a sophisticated model for the *in vivo* evolution of potential anti-malaria drugs. This model system, closely recapitulating human physiological conditions, enables a more nuanced assessment of drug efficacy and resistance patterns, enhancing the translational relevance of preclinical findings[45].

(ii) **Study of Drug Resistance Mechanisms:** The deliberate infection of humanized mice with drug-resistant strains of the malaria parasite offers a unique opportunity to dissect the underlying mechanisms of resistance evolution. A comprehensive understanding of these mechanisms facilitates the development of targeted and efficacious strategies to counteract resistance in human population[49].

(iii) **Testing Novel Therapies:** Leveraging humanized mice as a testing platform facilitates the rigorous evaluation of novel therapeutic modalities, encompassing new drug candidates, combination therapies, and immunotherapeutic approaches. The controlled experimental environment ensures the systematic exploration of safety and efficacy parameters[50].

(iv) **Immunological Studies:** The utilization of humanized mice allows for detailed immunological investigations, shedding light on the intricacies of the human immune response to malaria infection and drug interventions. Such insights are pivotal for advancing the development of immune-mediated interventions and prophylactic vaccines[51].

(v) **Personalized Medicine:** Envisioning a role in personalized medicine, humanized mice could be instrumental in tailoring malaria treatment. By incorporating patient-derived samples into customized mouse models, researchers gain a platform to assess individualized responses to anti-malarial drugs, thereby optimizing therapeutic regimens.

(vi) **Testing Drug Combinations:** Given the frequent reliance on drug combinations in malaria treatment to forestall resistance, humanized mice provide a systematic mean to evaluate the effectiveness of diverse drug combinations. This approach aids in discerning the most efficacious and sustainable strategies for malaria treatment.

(vii) **Investigating Host-Parasite Interactions:** Humanized mice models offer a nuanced exploration of the intricate interactions between the human host and the malaria parasite. Such insights are indispensable for devising interventions aimed at disrupting malaria transmission and impeding disease progression[45].

(viii) **Ethical Considerations:** The application of humanized mice in research provides an ethically viable alternative to conventional human clinical trials during preliminary drug testing phases. This methodology assists in prioritizing promising drug candidates for subsequent human trials.

CONCLUSION

The period spanning 2018 to 2023 witnessed a noteworthy evolution in the landscape of Malaria drug resistance in India, marked notably by the emergence of artemisinin resistance as a prominent concern. In response to this pivotal threat, India undertook a comprehensive and multifaceted approach. Strategic adaptations include the modification of treatment protocols through the incorporation of ACT treatment, augmented monitoring endeavors, and substantial investment in the research and development of innovative anti-malarial drugs. Despite persistent challenges, such as G6PD deficiency, the nation has approached them judiciously. The ongoing battle against malaria drug resistance underscores the imperative of sustained vigilance, dedicated research initiatives, and collaborative efforts on the international stage in the endeavor to eradicate this formidable disease. In totality, while malaria drug resistance remains a significant threat, India's unwavering commitment to comprehending, monitoring, and addressing this challenge exemplifies a resolute effort to safeguard the health and well-being of its populace. Through steadfast adherence to inclusive strategies encompassing surveillance, research, and collaboration, India is poised to surmount the enduring challenge of malaria drug resistance and advance towards a future devoid of malaria within its borders.

Conflict of Interest: All authors declare no financial or non-financial competing interests.

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Author contributions *Conceptualization; NT, RKT; resources & information collection: NR and NT; writing—original draft preparation: NR and, RKT; writing—review and editing: NT, RKT*

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Scientist's Bio-bibliography

DR P.K. DAS — AN OUTSTANDING MEDICAL ENTOMOLOGIST

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Part I

BIOGRAPHY

"Where the mind is without fear and the head is held high;
Where knowledge is free;
Where the world has not been broken up into fragments
by narrow domestic walls;
Where words come out from the depth of truth;
Where tireless striving stretches its arms towards perfection;
Where the clear stream of reason has not lost its way into
the dreary desert sand of dead habit;

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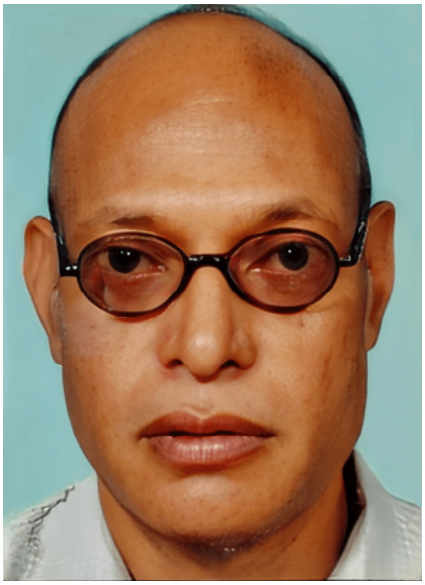
Where the mind is led forward by thee into ever-widening
thought and action ….
Into that heaven of freedom, my Father, let my country awake."

— Rabindranath Tagore



These ever inspiring words by '*Gurudev*' Rabindranath Tagore most fittingly describe the life of Dr Pradeep Kumar Das, a completely inexhaustible medical entomologist and innovative vector-borne disease control specialist. Dr Das is one of those rare and thinking scientists in the vast arena of medical arthropodology who by their energetic character not only practically educate the budding researchers in habits of industry, but by the example of diligence and perseverance which they set before them, largely influence the scientific activity in all directions and contribute in a great degree to evolve the core character of research, i.e., invention, innovation and discovery. Self-educated with these attributions ingrained in his persona through dint of perseverance, focused industry, indefatigable energy and upholding rightful and illuminating paths of action, Dr Das soon emerged as a champion bestowed with exceptionally reinvigorating and deep knowledge of nearly all the conceivable consilience in the realms of vector-borne disease control, particularly medical entomology. Having been himself a Director of the internationally renowned ICMR-Vector Control Research Centre (1995-2005), he has left behind a legacy of innovative research among the budding medical entomologists in the country. A septuagenarian, in his late seventies, Dr Das is still standing tall and radiant, and helping youngsters in their research.

Dr Pradeep Kumar Das was born on 29th December, 1947 in Ranchi, Jharkhand State, India. He completed M.Sc. with specialization in Cell Biology from University of Gorakhpur in 1970. He obtained Ph.D. in Zoology (Cytogenetics) from University of Kalyani in 1974. After completing Ph.D. he Joined the WHO

ICMR's Genetic Control of Mosquitoes Unit (GCMU). When the above project abruptly aborted in 1975, the government of India shifted the trained personnel in two newly established research centers, namely, Malaria Research Centre (MRC; now rechristened as National Institute of Malaria Research) in Delhi and Vector Control Research Centre (VCRC) in Puducherry. Dr Das moved to VCRC.

Fortune has often been blamed for her blindness, but fortune is not so blind as men are. Those who look into practical life will find that fortune is usually on the side of the industrious, as the winds and waves are on the side of the best navigators. In pursuit of even the highest branches of human inquiry the commoner qualities are found the most useful – such as common sense, attention, application and perseverance. Dr Das carefully cultivated within him these attributes and applied them with full force in all his scientific pursuits – *hook, line and sinker!* His highly chequered career graph presents an example of the fact that hard work never goes waste; having himself risen from a humble position of a Research Assistant to the most coveted rank of Director, Vector Control Research Centre, through sheer dint of perseverance, focused attention and dedication, within a span of two decades. His life also portrays that for the production of any great result in life, the common highway of steady industry and application, and not the boldest of the occasional sparks of brilliance, is the only safe and sure road to travel. Sedulous attention and painstaking industry always marked his life!

During his early days at the VCRC, Dr Das worked relentlessly on malaria and filariasis, the latter in particular. His penchant for knowing everything about this disease soon caught the eye of his the then Director, VCRC, Dr P.K. Rajagopalan who was himself a brilliant medical entomologist as much as an astute administrator. He found in Dr Das a great future leader, and decided to train him abroad in some of the best institutions in the fields of tropical medicine and hygiene. Consequently, on winning a WHO fellowship in 1982, he was sent to University of Sussex, London School of Tropical Medicine, University of New Castle, Imperial College of Science and Technology, and Liverpool School of Tropical Medicine, all in the UK, for training on various aspects of vector-borne disease control and soon thereafter to the Centers for Disease Control (CDC), Atlanta, USA for handling patents, setting up surveillance and monitoring systems for epidemiological intelligence, application of GIS and spatial analytical tools in infectious disease control and environmental risk assessment. Besides, in 1983, he

was trained in programme evaluation at the World Health Organization, Geneva. Equipped with extensive knowledge, he rejoined at the VCRC as an Assistant Director to steer a novel ICMR-funded research project on human lymphatic filariasis control in Pondicherry (=Puducherry) through environmental methods, with a minimum use of insecticides, in 1982. It was during the course of this project when I had joined the VCRC, Puducherry, as a Senior Research Officer, in 1984, and was attached to the Insecticide Division of which Dr Das was the Head. As a keen observer I found in Dr Das an undying desire to learn more by himself, in the true spirit of 'Self Help' – the greatest of all human virtues! The success of the Pondicherry Lymphatic Filariasis Control Project had many ramifications; one of which was the world began to notice a transformed Dr P.K. Das, now an emerging authority on lymphatic filariasis control through integrated vector management (IVM).

In 1995 when he took over the helm of VCRC as its fourth Director, he guided the VCRC to become a major stronghold in medical entomology and innovative research on vector-borne diseases (VBDs), though lymphatic filariasis continued to be his forte! The VCRC had already become a hub for training for national and international enthusiasts in medical entomology and control strategies for VBDs and he gave a new dimension to his Center, by offering research training and consultancy on vectors and vector-borne disease management. Growing *au fait* in diseases such as malaria, filariasis, Japanese encephalitis, dengue and chikungunya etc. transmitted by arthropods of public health importance, the VCRC under his leadership soon became a force to reckon with in the realms of VBDs as the World Health Organization recognized VCRC as a "Collaborating Centre for Research and Training in Lymphatic filariasis and Integrated Vector Management." Around the same time, in 2000, the Ministry of Health and Family Welfare, Government of India, recognized the Centre as one of the Institutes of Excellence in India for Courses in Health Training.

Dr Das initiated many novel research programmes, especially in thrust research areas of (i) Epidemiology and ecological modeling for developing site specific strategy for controlling vector-borne diseases, and (ii) Planning and implementation of Integrated Vector Management and community-based mass drug administration for interruption of lymphatic filariasis transmission. His staunch guidance on research teaching and consultancy services for more than 30 years provided

substantial inputs for global programme to eliminate lymphatic filariasis, malaria and other neglected tropical diseases. This massive effort helped him to publish more than 200 research publications, in addition to many book-chapters.

His scientific interests are manifold. An ecologist, a conservationist and a Nature lover , his present interests include. sustainable development, environment and health. He has been serving as an expert on VBDs on several national and international bodies. He is also a Fellow or Member of a large number of International and Nation scientific associations such as, for example, Society for Vector Ecology, Malayasian Society of Parasitology & Tropical Medicine, National Geographic Society (USA), Indian Science Congress, Indian Red Cross Society, Computer Society of India, Environmental Society, Pondicherry, Indian Association for Communicable Diseases, Bombay, Association for advancement of Entomology, Trivandrum, Indian Society for malaria and other communicable Diseases, New Delhi, and Indian society of Parasitology.

A widely traveled scientist Dr Das has presented his research work in a large number of national and international symposia, conferences or congresses (181 #). Known for his spontaneous lectures Dr Das speaks his mind fearlessly regardless of a crowd-feeling, although he is always highly affable, vivacious and willing to befriend at first sight. Due to these factors he is always a well sought after speaker in scientific conferences.

After superannuating on retirement as the Director, VCRC, in 2005, Dr Das accomplished what he is best at; he took up his long cherished desire to develop an eco-village near Puducherry! Dr Das is an inexhaustible environment scientist, sailing smoothly in his late seventies, but even now he can amaze many a budding scientists with his agility and briskness in action, which remind me of the famous lines of Robert Lee Frost:

"Woods are lovely, dark and deep,
but I have promises to keep,
and miles to go, before I sleep,
and miles to go before I sleep."

Part II

List of Publications by Dr. P.K. Das

1. **Das, P.K.** (1973). Pattern of RNA synthesis and amino acid accumulation in the antigen sensitize spleen of mice. *Indian J. Zool.* 1(2): 77-80.
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Book Review

BIOLOGY, DIAGNOSIS AND MANAGEMENT OF INDIAN PESTIFEROUS BLACKFLIES

(By: Vijay Veer)

Dr Vas Dav

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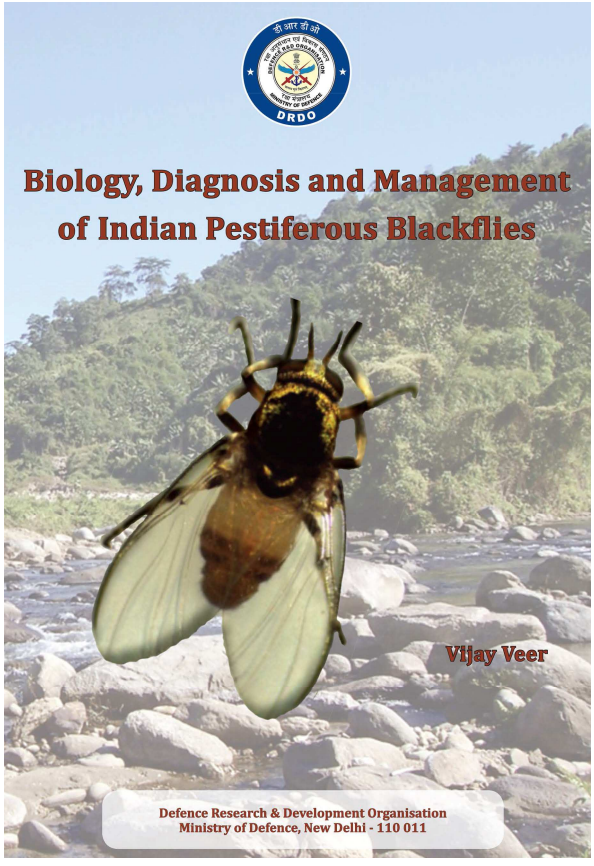
I encountered blackflies several times while collecting/surveying mosquito fauna or carrying out experiments in North-East India and got bitten by them. They are locally known as '*Dim Dam'* fly and considered serious pest insects especially in Arunachal Pradesh. I am more than happy to write a review on much awaited monograph by Defence Research & Development Organization (DRDO) on, "Biology, Diagnosis and Management of Indian Pestiferous Blackflies' by Dr. Vijay Veer (Former Director, Defense Research Laboratory, Tezpur, Assam) is indeed an illustrious volume on Blackflies (Simuliidae: Diptera), much known for their notoriety for being ferocious biter, an insect pest of medical and veterinary importance. Blackflies are a big nuisance (next only to ubiquitous mosquitoes) particularly in the foothills of Himalayas (fast flowing rivers provide ideal ecology for proliferation) more so in the north-eastern states of India affecting humans and agricultural produce adversely. Blackflies are arthropods lood-sucking humans and agricultural produce adversely. Blackflies are blood-sucking arthropods and known vector of Onchocerciasis (river blindness presently endemic in Africa, and known vector of Onchocerciasis (river blindness presently endemic in Africa, South American countries and Yamen) and several other pathogens in the tropics, but very

little is known on their biology, distribution, systematics, and management with special reference to India. This volume fulfills the longstanding demand, providing up-to-date information on various aspects of biology and economic significance of Blackflies in this part of the world. The monograph encompasses 10 chapters each well composed giving an illustrated account on allied aspects such as taxonomy (molecular systematics), biodiversity and distribution, bionomics and control supported by glossary for the benefit of students and young researchers. The book helps to identify gaps of information and future thrust areas to prioritize research on this important insect pest having implications affecting human as well as animal health in this modern age of 'One Health' perspective.



Dr Vijay Veer, 309 pp., ISBN 978-93-94166-08-0, priced Rs. 1500/-, US $35, UK £30, Year 2022

The author has expressed concern of introduction of newer infections (formerly non-endemic) such as 'Onchocerciasis' evidenced by lone case detected in Assam, north-east India related to eye-infection by *Onchocerca volvulus,* a filarial worm (the causative agent of river blindness) with increased trade and travel, and highlighted the need for additional taxonomic surveys in areas 'hitherto unexplored' particularly western Himalayas as well as need for updated taxonomic classification and development of guidelines for control and management tools/trapping devices.

The subject which remained grossly neglect in Indian Science deserves its place for prioritizing further research to have firsthand knowledge on faunistic diversity,

distribution of species across Indian landscape, sibling-species composition and vector potential enabling effective pest management. This compendium would be of immense value to defense establishments, medical and veterinary entomologists, young researchers/ academicians providing updated information and good reference material (certainly a valued addition in the library) as well as to programme and policy managers helping device interventional strategies.

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Acknowledgment to the Reviewers

The Editors are thankful to the Referees and the Members of Editorial Board (vide infra) for their continued support and guidance in finalizing the manuscripts for the Vol. 4 (Nos. 1 & 2) published on June 1 and December 1, 2023. We sincerely continue to solicit their guidance and support in our future ventures as well.

1. Dr Siraj A Khan,
2. Dr Abhijit Mazumdar,
3. Dr Vijay Veer,
4. Dr BK Tyagi,
5. Dr PK Sumodan,
6. Dr S Anbalagan,
7. Dr Rajiv Tyagi,
8 Dr Rajnikant Dixit, and
9. Prof Lalita Gupta

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Suggestions to Authors

Introduction

The Journal invites Original articles, Short communications, Editorials, Review articles, and other type of scientific information in the field of Medical Arthropodology & Public Health from prospective authors worldwide. At present, the journal does not take any charge for submission, processing, publication of manuscripts, and copy/supply of pdf version of the research paper published.

Manuscripts will be accepted for publication with the understanding that the submission (entire contents or in part) have not been published and will not be published elsewhere. Submissions received for consideration will be acknowledged. Manuscripts will be initially reviewed by the Editorial team for suitability of content. Manuscripts satisfying criterion of quality would be processed for formal review by blinded peer reviewers for originality, scientific content, methodology, quality, importance and suitability for publication in the journal. Reviewer comments will be forwarded to the corresponding author for response, revision and resubmission within a specified timeframe. Manuscripts accepted for publication will be edited for grammar, punctuation and format. Final proofs will be sent to the corresponding author for corrections and resubmission by email. Articles once rejected will not be entertained ordinarily for reconsideration in future. The decision of the Editorial Board will be considered final for all purposes. Decisions of rejection may not reflect upon the quality of research submitted and merely a statement of current needs of the journal.

Articles resubmitted after the specified period has expired will be considered as new submissions at the discretion of the Chief Editor or the Executive Editor.

Authorship

All individuals listed as authors should qualify for authorship. An 'author' is someone who has made substantive intellectual contributions to a published study. The lead author should be confident of his/her co-authors' competence and integrity. Co-authors who do NOT meet the criteria for authorship should not be listed as authors, however they should be acknowledged.

Article categories

The following categories of articles are accepted for publication in the journal. The authors should select the category that best describes their paper. If the paper does not qualify in any of these categories, please contact the Editorial Office.

Original Articles: These are submissions from research workers engaged in the field of Medical Arthropodology & Public Health. Articles pertaining to the field of current topics/path breaking research and those of general interest to medico-arthropodologists and public health specialists will be published on priority.

All studies should have been approved by the Institutional/local Ethics committee.

Responsibility for correctness of data, statistical analysis and interpretations wherever applicable will lie entirely with the authors.

Format – Abstract (Structured) & Keywords; Introduction; Material & Methods; Results; Discussion; Conclusion.

Review Article or Update Article: These will be on invitation from senior faculty and experts in the field who have published quality original research articles in the same field. Prospective authors are requested to contact the Chief Editor or the Executive Editor for prior approval of their topic.

Short Communication: Any research study or finding of interest which does not qualify for a full length original study.

Perspective: Opinion articles written by senior faculty/scientists, experts in the field and policy makers.

Budding Researcher's section: Preliminary or original fresh findings of Postgraduate / Doctoral/Post Doc students can be submitted for publication in this section.

[Format for the entire above category except for Original articles – unstructured abstract with key words; Introduction; Materials and Methods (if applicable); Results (if applicable); Discussion.]

Letter to the Editor: These should be brief with constructive criticism of published articles, supported with additional data and information, sources etc. A short title referring to the recently published article along with a covering letter should be submitted. Current interesting topics or news can also be considered for Letter to Editor.

Others: This includes Editorials and Perspectives which are solicited by the Editorial Board.

Size of manuscript

The Table below provides guidelines regarding maximum permissible size of text as well as number of Tables, Figures and References. Non-adherence of the manuscript to the specifications is likely to result in rejection at the discretion of the editorial team.

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Perspective	2000	-	10
Original Article	3500	8	35
Review/Update Article	4500	8	45
Budding Researcher's Section	3500	8	35
Letter to Editor	500	2	3

Manuscripts submitted to the ***Journal of Medical Arthropodology & Public Health*** (*J Med Arthropodol & Public Health*) should not have been published previously or be under simultaneous consideration for publication by any other journal. Any violation of this will lead to a retraction of the published article by the Journal and any other actions as deemed necessary by the Editorial Board. All manuscripts including invited articles will be peer-reviewed. Accepted articles will be edited to the Journal's style. Accepted submissions will become the permanent

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Participation solely in the acquisition of funding, for the collection of data or data entry, and general routine supervision does not justify authorship. The order of authorship should be a joint decision of all the co-authors. Once submitted, the order will not be changed without written consent of all the co-authors, and acceptance by the Executive Editor, *J Med Arthropodol & Public Health*.

Intellectual contribution

The contribution of each author is to be mentioned, on the Author Certificate in all multi-author research papers under the following headings: Study Concept, Drafting & Manuscript Revision, Statistical Analysis, Study Supervision.

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Articles in English only will be accepted for publication.

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The manuscripts will be submitted in electronic form (later after announcement through the Journals website: http://www.soma16.org) and should be accompanied by (1) Manuscript with author details including email ID (2) Tables (3) Figures with legends (4) Ethical Clearance (5) Authors' originally signed and scanned Certificate (6) Duly signed Copy Transfer Certificate. The files should be uploaded separately in the order mentioned.

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The title page should have the following: Title (in capitals), author(s) names with highest degree, affiliation, email ID with footnotes as a, b, c, d, short title, word count (excluding abstract and references), number of Tables and Figures, corresponding author with address, email ID and mobile number.

Abstract

Structured abstract arranged into the headings: Background, Methods, Results and Conclusion. No abbreviations should be used in the abstract. Give not more than 6 keywords. Abstract is not required for Letters to the Editor.

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The main text should have the following headers – Introduction, Material and Methods, Results and Discussion. Authors should maintain individuality of each section. All tables, figures and references should be cited in the text. The Journal discourages use of any abbreviations which are not authorized or accepted internationally. Full form of all abbreviations must be mentioned in the first instance barring standard units of measurement.

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The Responsibility of accuracy of the references rests exclusively with the authors. References should be in Vancouver style (i.e., numeral) as described in examples. Relevant, important and recent references should be included in the submissions. The references should be indicated in the text by Arabic numerals superscripted with word or punctuation. The manuscript should include all references cited in the text. The reference should list all authors, surname followed by initials when six or less; when seven or more, mention only first three authors followed by et al. Full stops should not be used in abbreviations of journal names.

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(ii) *Organization as Author –*

National Vector Borne Disease Control Programme. Dengue/Dengue Haemorrhagic Fever. Delhi: National Vector Borne Disease Control Programme; c2005-2018. Available from:

http://www.nvbdcp.gov.in/dengue5.html, accessed on March 26, 2018.

(iii) *Epub ahead of print with DOI –*

Slamon D, Neven P, Chia S, et al. Overall survival with ribociclib plus fulvestrant in advanced breast cancer. N Engl J Med. 2019; published online Dec 22. DOI:10.1056/NEJMoa1911149.

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Tables should be typed serially numbered in Arabic numerals (e.g. Table 1, Table 2) with a short title specifying the contents. Horizontal lines should not be used in the body of the table except between a column heading and its sub-headings. Vertical lines should not be used in the table. Tables should be concise and restricted to a page in length and should be self-explanatory. The data presented should not be duplicated in the text.

Figures/Photographs/ Illustrations

Colour images may be submitted but must be in good quality of production. Illustrations should have at least 300 × 300 dpi resolutions and be clear enough. Photographs/illustrations may be submitted as 'JPEG', or 'TIFF' files. Line art drawing must have a minimum resolution of 1200 dpi. Borrowed photographs or

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Any submission involving human subjects should have been conducted with informed consent by the subjects and of approval by the institutional ethics committee.

Keywords

Not more than 3-6 Keywords in alphabetical order should be mentioned in the abstract section of the following article categories: Review Articles, Original Articles and Short Communications.

No keywords are required for Editorials, Perspectives, and Letters to the Editor.

Acknowledgements

All individuals who were directly involved with the study but do not qualify to be authors should be acknowledged. Consent should however be taken from these

individuals prior to including their names. People who have provided only secretarial, clerical or technical help and whose contribution was limited to their routine job profile should not be included in the acknowledgement.

The Chief Editor shall have the final decision-making power to accept or reject a submission and also reserve the right to adjust the style to certain standards of uniformity and suitability of the journal.

All correspondence regarding manuscripts and inquiries, if any, should be made to the Executive Editor Dr Rina Tilak [***email:*** rinatilak@hotmail.com], with a cc. to Prof. Dr B.K. Tyagi [***email:*** abktyagi@gmail.com].

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Request for contributing manuscripts for the next issue scheduled to be published on December 1, 2023

The power of scientific research lies in its ability to transform people's lives. Helping colleagues, peers and the wider general public to get a better understanding of your research and the impact that it can have on society is a win for everyone involved. Promoting your research helps it reach a wider audience in the arena of your research interests, which could lead to future collaboration and further academic opportunities, such as invites to conferences and commissioned articles.

Journal of Medical Arthropodology & Public Health, an open access journal, is an official organ of SOCIETY OF MEDICAL ARTHROPODOLOGY (SOMA; www.soma16.org), published semiannually on June 1 and December 1. It is general policy that all manuscripts are critically peer-reviewed. It focuses on medically important arthropods' parasitological and pathological significance in public and veterinary health as well as their hazardous, pestilent and/or vectorial behaviour to mediate a large number of deadly and/or debilitating diseases, besides poisonous bites and stings, allergies etc., on one hand, and their pharmacological, nutritional, medicinal, biotechnological and bioengineering utilities for direct human benefit, on the other.

The *Journal of Medical Arthropodology & Public Health* is for all those dedicated researchers who are interested in scientific discovery, and in its industrial, commercial and social consequences. It will report, explore and interpret the results of human endeavour set in the context of science and society. Through its focused, and yet diverse, coverage of scientists will be motivated to think beyond their discipline and believe that collaborative science and interdisciplinary ideas can advance national policies related to the control of vector-borne diseases, on one hand, and bring other biomedical significance under discussion, on the other, to inspire new

thinking. The Journal aims to explore new horizons in the biology of medically important arthropods and pave pathways to consolidate new ideas toward their control.

Vol. 3, No. 2, of *Journal of Medical Arthropodology & Public Health* (December 1, 2023) was published on date. We take this opportunity to request the scientific fraternity having research interests in medically important arthropods (e.g., insects, arachnids, centipedes, millipedes, crustaceans etc.) to submit their research manuscripts for consideration of publication in the Vol. 4, No. 1 (June 1, 2024) on or before April 31, 2024. For SUGGESTIONS TO AUTHORS please see either this issue or the SOMA website, www.soma16.org.

Expecting your kind understanding and cooperation in this regard, and looking forward to receiving your manuscripts before very long, we remain, with best wishes,

Cordially yours,

Prof. Dr B.K. Tyagi **&** **Dr Rina Tilak**
Chief Editor Exec. Editor

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DECLARATION

1. Name of the periodical	**:**	**Journal of Medical Arthropodology & Public Health** **ISSN: 2583-6455 (Online)**
2. Published by the Society	**:**	SOCIETY OF MEDICAL ARTHROPODOLOGY
3. Registration Number	**:**	103 of 2017 (Registrar of societies, Hyderabad; dt. Mar 1, 2017)
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5. Address of Publication	**:**	Department of Zoology, University College of Science, Osmania University Hyderabad, Telangana-500007 India
6. Frequency	**:**	Semiannual (Commencing w.e.f. June 1, 2021)
7. Language	**:**	English
8. Date of Declaration	**:**	22nd May, 2023

We solemnly declare that above information is true to the best of our knowledge.

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