Research Article

Life Cycle, Bio-ecology and DNA Barcoding of mosquitoes *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse)

A N Anoopkumar¹, Sreedev Puthur², Paulson Varghese³, Sharrel Rebello⁴, Embalil Mathachan Aneesh⁵

**Abstract**

Mosquito borne diseases remain as the world’s most severe insect-borne disease with excessive rates of morbidity and mortality. Mosquitoes transmit various severe diseases such as dengue, malaria, filariasis, viral encephalitis, chickungunya and zika virus infections causing millions of deaths worldwide; and no part of the world is liberated from mosquito borne diseases. *Aedes aegypti* and *Aedes albopictus* represent the two important mosquito vectors for dengue virus transmission in America and Asia. According to the Integrated Disease Surveillance Project (ISDP), 8888 confirmed dengue infections were reported from January 1st to June 30th 2017 in Kerala state, India with 409 confirmed dengue infections reported from Thrissur district of Kerala including Irinjalakuda Municipality (the area of this study). Additionally 15 confirmed and 56 suspected dengue fever deaths were also reported from Kerala state, India. Current epidemics of dengue and severe mosquito borne diseases from Kerala have exposed the need for more comprehensive understanding of the mosquito species types, their vectorial capacity and the habitat characteristics that offer them for proper breeding environment in the study area. The present study also explored the applicability of CO1-based DNA bar coding as an alternative approach to identify mosquito species such as *A. aegypti* and *A. albopictus*.

**Keywords:** *Aedes aegypti, Aedes albopictus, CO1 barcoding, vectors*

**Introduction**

Mosquitoes are significant groups of arthropods that inhabit aquatic habitats. They are probably adverse arthropods, which transmits wide range of pathogens that cause drastic deadly diseases such as human malaria, dengue, filariasis and viral encephalitis[1]. Zika virus, another mosquito borne infection was first reported in Brazil by Pan American Health Organization in 2015 and since then the virus has spread tremendously all around America. Zika infection begins with mild symptoms lasting up to a week after being bitten by vector mosquito; but an infection during pregnancy may cause certain neurological anomalies like microcephaly and several other brain defects[2]. Thus control of mosquitoes becomes the need of the hour to prevent wide epidemic infections. However, it is difficult to control and prevent severe consequences created by mosquito species[3].

Currently a total of 3549 recognized mosquito species belonging to subfamily Culicinae are reported all over the world[4];

¹Department of Zoology, Christ College Irinjalakkuda, University of Calicut.

²,⁴,⁵Communicable Disease Research Laboratory, Department of Zoology, St. Josephs College, Irinjalakkuda.

³Trivandrum Medical College.

**Correspondence:** Dr. Embalil Mathachan Aneesh, Communicable Disease Research Laboratory, Department of Zoology, St. Josephs College, Irinjalakkuda.

**E-mail Id:** aneeshembalil@gmail.com

**Orcid Id:** http://orcid.org/0000-0001-8140-3888

**How to cite this article:** Anoopkumar A.N, Puthur S, Varghese P et al. Life Cycle, Bio-ecology and DNA Barcoding of mosquitoes *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). *J Commun Dis* 2017; 49 (3): 32-41.

**Digital Object Identifier (DOI):** 10.24321/0019.5138.201719

**ISSN:** 0019-5138

© ADR Journals 2017. All Rights Reserved.
with more than hundred species of mosquitoes capable of disease transmission to humans and other animals\textsuperscript{[1]}. One-third of this subfamily is represented by the Genus \textit{Aedes} (Suleman \textit{et al}. 1996) of which members of \textit{A. aegypti} and \textit{A. albopictus} representing the two important mosquito vectors for dengue virus transmission in America and Asia\textsuperscript{[20]}. Urbanization significantly made modifications in the \textit{Aedes} mosquito ecology by certain environmental changes\textsuperscript{[21]}. The mosquito borne diseases such as dengue, zika, and yellow fever are primarily transmitted to the humans by the mosquito species \textit{A. aegypti} and vector control is significant to restrain the transmission of these drastic viral diseases\textsuperscript{[22]}. Studies indicate that 390 million people in the world face dengue infections per year with 96 million infections confirmed clinically\textsuperscript{[23]}. Additionally, the rapid spread of mosquito borne diseases is vitalized by global freight transportation and international travel\textsuperscript{[8, 10]}. \textit{A. albopictus} are vigorous throughout the year in tropical and subtropical habitats. Over winter they lay eggs on the sides of water-filled containers such as bird baths, flowerpots, animal watering dishes, natural holes in vegetation and tires\textsuperscript{[24]}. Destruction of these mosquito breeding habitat is an efficient method for mosquito vector control\textsuperscript{[12]}. For the proper development, the larvae and pupae of mosquitoes necessitate an aquatic environment with standing or flowing water. Larvae of the majority mosquito species generally filter out and feed organic matter, other microorganisms from water\textsuperscript{[13]}. Heterotrophic microorganisms such as bacteria, fungi and protozoans from detritous surfaces or containers are significant for the larval diet\textsuperscript{[14]}. Freshwater swamps, rice fields, borrow pits, marshes, puddles, water-filled tracks, ditches, gulleys and drains are tremendous source of mosquito larval habitats. Wide range of ‘natural container- habitats’ such as, rock-pools, water-filled bamboo stumps, tree holes filled with water, leaf axis in banana, snail shell and coconut husks offer enough requirements for mosquito larval habitat. Man-made container habitats including water storage jars, tin cans, motor vehicle tyres and discarded kitchen utensils also contribute space for breeding of mosquitoes\textsuperscript{[15]}. After the larvae have accomplished their fourth larval molt they develop into pupae (called tumblers). Pupae don’t require food and be alive for 1-3 days before the adult form. Male adult mosquitoes primarily feed nectar from plants to get sugar while the female mosquitoes imbibe the blood meal to generate viable eggs\textsuperscript{[11]}. Female mosquitoes usually nourish every 3-5days. \textit{A. albopictus} females are diurnal feeders; they not only give preference to attack large mammals but also imbibe blood meals from birds\textsuperscript{[16]}. A conventional method to identify mosquito species is morphological identification, which is critically based on external characters and they may differ for different species. Taxonomic keys such as Bram, Harrison and Scanlon, Rattanarithikul are significantly used for morphological identification of individual mosquito species\textsuperscript{[17-22]}, Apart from the requirement of much expertise, morphological identification is exceedingly time-consuming and imperfect identification may result when significant morphological features such as bristles and scales are impaired\textsuperscript{[23]}. Prompt and perfect species identification with immense accuracy can be achieved through molecular approach\textsuperscript{[24]}. DNA barcoding has been encouraged as a consistent method for the species identification of both invertebrate and vertebrate taxa\textsuperscript{[25]}. DNA based molecular techniques and approaches for species identification, molecular phylogeny, and genetic diversity of mosquito acquire attention in recent years\textsuperscript{[26-31]}. The present study aimed to evaluate the molecular diversity, vectorial capacity and habitat characteristics of \textit{Aedes} mosquito species in the study area. **Materials and Methods** **Sample collection** Samples of \textit{A. aegypti} and \textit{A. albopictus} were collected from Irinjalakkuda municipality which belongs to Thrissur district of Kerala state, India (10.33 N 76.23 E) (Fig. 1). The study area is rich in diverse topography and provides habitat for different kinds of mosquito species. Different spots were randomly selected with an intention to cover entire topography of the study area.
Mosquito larval collections were carried out from different habitats including both natural and artificial using dippers (12cm diameter and of 300 ml capacity), pippets and plankton nets. Dippers and plankton nets were used in open sources for sampling while pippets were used in tree holes. Collected larvae were carefully transferred alive to the insectary and allowed to emerge as adults. Identification of the collected specimen was authenticated at CDRL, St. Joseph’s College, Irinjalakuda.

Insect rearing

A. aegypti and A. albopictus larave were reared in plastic trays (36x 24x7) containing dechlorinated water. The larvae were fed on powdered dog biscuits and yeast in the ratio of 3:1. The insectary was maintained at 27± 2°C and 75–85% relative humidity, with 12:12 light and dark photoperiod cycle for larval growth. Pupae were transferred to a bowl containing dechlorinated water and were maintained in insectary (30x30x30) where adults emerged. Adult mosquitoes were fed with water soaked grapes. On day 6, an immobilized young chick was kept inside the cage to provide blood meal for female mosquitoes. Plastic bowl (12 cm diameter) containing dechlorinated water with a lining of partially immersed filter paper was kept inside the cage to allow the female mosquitoes to lay their eggs.

DNA isolation, PCR amplification and sequencing

CTAB method was used to isolate Genomic DNA from insects (Hunt, 1997). The mitochondrial gene of the mosquito species was amplified by CO1 PCR with forward primer 5’-TATTATTAGACAAGAATCTGGTAAA-3’ and reverse primer: 5’-AGGAAATGTTGA GGGAAGAAAGTAA-3’. PCR amplification was performed in 25μl volumes containing 15ng of DNA, 30 pmol of each primer, Taq DNA polymerase reaction buffer with MgCl₂, 2.5 mM dNTPs and 1 unit of Taq DNA polymerase mixed in the PCR buffer (Sigma). Amplification parameters in the Mastercycler were as follows: An initial denaturaton of 95°C for 2 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds. Annealing and extension were performed at 54°C for 30 seconds and 72°C for 1 minute respectively. The final extension was accomplished at 72°C for 10 minute. PCR product was purified with Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare), according to the manufactures instructions and recommendations. Sequencing was performed using Big Dye Terminator cycle sequencing chemistry for ABI Bioprism (Applied Biosystems). The sequences generated were deposited in the NCBI gene bank data base (http://www.ncbi.nlm.nih.gov).

Data analysis

Significant variation in the birth rate and duration of development of A. aegypti and A. albopictus was illustrated as pie diagram using IBM SPSS Version 24.

Phylogenetic analysis

Neighbor-Joining method was used to investigate the evolutionary relationships[32] The sum of branch length of tree is equal to 1.23192720. Maximum Composite Likelihood method was used to calculate evolutionary distance[33]. Eight nucleotide sequences were involved in analysis. 1st+2nd+3rd+Noncoding positions were included. Position showing gaps and missing data were excluded. A total of 371 positions were appeared in the final data set. Evolutionary analyses were performed through the software MEGA6 (Molecular Evolutionary Genetics Analysis, version 3.1)[34]. Species discrimination using DNA barcode was performed by comparing the nucleotide sequences on NCBI gene bank data base.

Results

Life cycle and bio-ecology of A. aegypti and A. albopictus

The data obtained on the vectorial capacity of the collected mosquito species were compiled in table I. Continuous observations were made for both the species in respect of their fecundity and other ecological aspects for three generations. Thus a comparative analysis on the life cycle (Table II and Fig.2) of A. aegypti and A. albopictus was possible. The results from the present study revealed certain variation in birth rate and duration of development (in terms of number of days). A. aegypti took 28.5 days to complete its life cycle while A. albopictus use only 22.5 days as depicted in fig 3. Both of them took 8.5 days to attain pupal stage; and there was no variation observed in this aspect. However, there was significant difference in the life expectancy of adult mosquitoes of A. aegypti and A. albopictus which was calculated to be twenty and fourteen respectively. This variation was evident from the stage of number of eggs obtained from A. aegypti (=114) and A. albopictus (= 65) as shown in table III. However, the percentage of egg hatching (85.9 and 86.32) and percentage of adult emergence (80 and 81.5) remained almost same for A. aegypti and A. albopictus.
Vector status of *Aedes aegypti* and *Aedes albopictus*

| **Aedes aegypti** | **Aedes albopictus** |
|-------------------|---------------------|
| A. aegypti is the formost Zika vector\[^{45}\]. | Capable of transmitting Zika virus\[^{45}\]. |
| Dengue vector \[^{46}\]. | Dengue vector (Beebe et al. 2005), Viral pathogens including chikungunya, several filarial Nematodes namely Dirofilaria immitis\[^{47}\]. |
| Bites mainly humans (anthropophilic)\[^{45}\]. | Primarily attack wild and domestic animals (zoophilic) but also humans\[^{45}\]. |
| Typically feeds several times per cycle of egg production\[^{41}\]. | Usually feeds single per cycle of egg production\[^{41}\]. |
| Adapts well to human urban settlements\[^{41}\]. | Inhabits rural and urban areas\[^{41}\]. |

Table 1. Vector status of *Aedes aegypti* and *Aedes albopictus*

Table 2. Characteristics of common mosquito vectors *Aedes aegypti* and *Aedes albopictus*

| Characteristics of common mosquito vectors *Aedes aegypti* and *Aedes albopictus* | **Aedes aegypti** | **Aedes albopictus** |
|-------------------------------|-------------------|---------------------|
| **A. Eggs**                  |                   |                     |
| • Eggs are laid on damp surfaces in areas\[^{48}\]. |                   | • Water-holding containers such as tyres, animal watering dishes, birdbaths, flowerpots and natural holes in vegetation are the excellent space for egg laying\[^{11}\]. |
| • Commonly eggs are laid at varying distances above the water line\[^{48}\]. |                   | • Eggs are typically elongate and / or ovoid in shape and are smooth\[^{49}\]. |
| • Eggs are smooth, long, and ovoid in shape\[^{49}\]. |                   | • Eggs do not encompass floats and micropylar collars\[^{50}\]. |
| • Eggs are usually one millimeter long and encompass the presence of very prominent micropylar collars\[^{50}\]. |                   | • Eggs are black and having a length of 0.5 mm\[^{11}\]. |
| **B. Larvae**                |                   |                     |
| • The larvae pass through four instars. And Temperature significantly influences larval development\[^{49}\]. |                   | • Larvae are vigorous feeders\[^{49}\]. |
| • Males develop quicker than females\[^{51}\]. |                   | • They feed particulate organic matter from water\[^{51}\]. |
| **C. Pupae**                 |                   |                     |
| • After the fourth larval molt, it enters the pupal stage\[^{52}\]. |                   | • Pupae are active\[^{51}\]. |
| • pupae may react to stimuli\[^{49}\]. |                   | • They do not require food but can move about\[^{51}\]. |
| **D. Adult**                 |                   |                     |
| • Adult having just about 4 to 7 mm\[^{52}\]. |                   | • Adult having approximately 2.0 to 10.0 mm. males are on average 20% smaller than females\[^{51}\]. |
| • Adults posses white scales on the dorsal (top) surface of the thorax\[^{51}\]. |                   | • Adult posses bold black shiny scales and distinct silver white scales on the palpus and tarsi\[^{11}\]. |
| • The abdomen is usually dark brown to black and also showed white scales\[^{52}\]. |                   | • Each tarsal segment bear white basal scale and legs are black in color\[^{51}\]. |
| • Males are smaller than females\[^{49}\]. |                   | • Males are approximately 20% smaller than females\[^{51}\]. |
| • Males encompass\[^{49}\]. |                   | • The abdomen narrows into a point is the characteristic of the genus *Aedes*\[^{51}\]. |
Figure 2. Aedes Mosquito Development Cycle

(a) Aedes aegypti: Development in days

(b) Aedes albopictus: Development in days

Figure 3. Development in days (a) Aedes aegypti (b) Aedes albopictus
The study indicated that manmade materials were predominantly found to be a habitat for mosquitoes rather than natural counterparts. Tree hole, cemented tanks, stream pools, tyres, bowls and containers are the six different kinds of habitats populated by both the species *A. aegypti* and *A. albopictus* (Table IV). Out of the 14 habitats, 57% of mosquitoes prefer artificial means of habitat selection. Analysis on different habitats occupied by mosquitoes revealed that out of the 11 habitats of *A. aegypti* was only four were natural habitats. Similarly only four of the 9 habitats used by *A. albopictus* were natural habitats. Out of the total 14 habitats populated by mosquito species *A. aegypti* and *A. albopictus*, six were inhabited by both the species.

| Habitat preference of different *Aedes* species in the study area |
|---------------------------------------------------------------|
| Habitat | *Aedes aegypti* | *Aedes albopictus* |
| --- | --- | --- |
| Tree hole | + | + |
| Cemented tanks | + | + |
| Stream pools | + | + |
| Mud pot | + | - |
| Plastic containers | + | - |
| Tyers | + | + |
| Plant pot | + | - |
| Nuts | + | - |
| Tin | - | + |
| Bowls | + | + |
| Coconut shell | + | - |
| Leaf axils | - | + |
| Bamboo | - | + |
| Containers | + | + |

**Table 3. Fecundity of *Aedes aegypti* and *Aedes albopictus***

| Fecundity of *Aedes aegypti* and *Aedes albopictus* | *Aedes aegypti* | *Aedes albopictus* |
| --- | --- | --- |
| No. of Eggs | 114 | 65 |
| Hatching | 98 | 56 |
| Percentage of hatching | 85.9 | 86.32 |
| 4th instar larvae | 95 | 54 |
| Pupae | 93 | 53 |
| Adult | 92 | 53 |
| Percentage of adult emergence | 80 | 81.5 |

**CO1-based DNA barcoding and phylogenetic analysis**

The morphological features of the test specimens were matched with molecular characterization while comparing the CO1 genes with NCBI genbank. Thus it confirms that the test specimens were *A. aegypti* and *A. albopictus*. CO1-based phylogenetic analyses (Table V) showed that *A. aegypti* strain was congregated with *A. aegypti* KT313648.1 (Fig. 4a) and *A. albopictus* strain was clustered with *A. albopictus* KP896552.1 (Fig. 4b).
Table 5. Accession id with species retrieved from phylogenetic analysis

| Number | Species              | GenBank ID      |
|--------|----------------------|-----------------|
| 1      | Aedes aegypti        | Fu Aedes ae     |
| 2      | Aedes aegypti        | KT313648.1      |
| 3      | Aedes cinereus       | KM457571.1      |
| 4      | Aedes albopictus     | KP896552.1      |
| 5      | Aedes albopictus     | KT313648.1      |
| 6      | Aedes albopictus     | KT313648.1      |
| 7      | Aedes albopictus     | KT313648.1      |
| 8      | Aedes albopictus     | KT313648.1      |
| 9      | Aedes albopictus     | KT313648.1      |
| 10     | Aedes albopictus     | KT313648.1      |
| 11     | Aedes albopictus     | KT313648.1      |
| 12     | Aedes albopictus     | KT313648.1      |
| 13     | Aedes albopictus     | KT313648.1      |
| 14     | Aedes albopictus     | KT313648.1      |
| 15     | Aedes albopictus     | KT313648.1      |

Discussion

*A. aegypti* is well adapted to live urban areas and they preferentially feed humans[35]. Agro industrial modifications of habitats of mosquitoes including climatic discrepancy significantly influence outbreak of mosquito borne diseases. Hence the disease situation can be tackled by improving the knowledge about the mosquito vectors and mosquito.
borne-diseases. The information should explain the risk of diseases transmitted, and planning of control measures to support disease control programs. In the light of their economic importance, the present study was made to monitor and assess the mosquito species types in the study area, their vectorial capacity and the habitat characteristics that offer them proper breeding environment. The present study reported the life expectancy of adult *A. aegypti* and *A. albopictus* ranged from 22.5 to 28.5 days. This report goes in accordance to findings of Maricopa in 2006[36], who found the above mosquito species utilized around two weeks to a month depending upon environmental condition to complete its life cycle.

A number of *Aedes* mosquito species are responsible for the transmission of arboviral diseases such as dengue and yellow fever[6, 37, 38]. Mosquito-borne diseases critically posses economic impact, including defeat in commercial; and all part of the world were infected by vector-borne diseases[39]. The incidence of dengue has grown dramatically worldwide. The World Health Organization reported that 50–100 million people infected by dengue fever every year[40, 41].

According to the Integrated Disease Surveillance Project (ISDP), 8888 confirmed dengue infections were reported form January 1st to June 30th 2017 in Kerala state, India (Table VI). Of which 409 confirmed dengue infections were reported from Thrissur district, Kerala (Table VI). 15 confirmed dengue fever deaths were also observed from Thiruvananthapuram (6), Kollam (5), Palakkad (1), Kozikkode (3) districts of Kerala state, India[42].

| Sl No. | Month | Dengue fever confirmed | Death |
|-------|-------|------------------------|-------|
| 1     | January | 435                    | -     |
| 2     | February | 302                    | 1     |
| 3     | March   | 463                    | -     |
| 4     | April   | 1066                   | 1     |
| 5     | May     | 2477                   | 7     |
| 6     | June    | 4145                   | 6     |
| Total |        | 8888                   | 15    |

Immature mosquitoes use a variety of aquatic habitats such as ponds, ditches, streams, marshes, temporary and permanent pools, plant containers (leaves, tree holes, and bamboo nodes), artificial containers (tires, cans, flower vases, bird feeders) habitats[43, 44]. Similarly, the important habitats chosen by *Aedes* species in the study area includes tree hole, cemented tanks, stream pools, fountains, ditches, mud pot, plastic containers, tyres, plant pot, rocky pool, pods, nuts, tin, flower bracts, interriodes, latex collecting containers, bowls, duck weed ponds, coconut shell, leaf axils, temporary pools, fishponds, bamboo and containers (Table II). The present study reported that 57% of “mosquitoes prefer artificial means of habitat selection”. These results drastically alarming the health care systems. *A. aegypti* increases arbovirus transmission in urban areas while *A. albopictus* in rural areas[40].

In addition, we also explored the applicability of advanced DNA-based molecular approaches such as DNA barcoding for taxonomical identification of mosquito species. Comparison with data in Genbank revealed that the test DNA sequences were close sequence matches against *A. albopictus* and *A. aegypti*. The phylogenetic investigation was achieved through MEGA 6. The results from MEGA 6 authenticated that *A. albopictus* strain was congregated with *A. albopictus* KP896552.1. While *A. aegypti* strain was clustered with *A. aegypti* KT313648. Similarly, Abigail Chan and co workers in 2016 used DNA barcoding as an alternative, universally applicable method to support the existing methods for mosquito identification. Conclusively, the presence of diverse habitats (including artificial and natural) and the progressing history of disease outbreak by *Aedes* species in the study area inspire an intensification of the vector surveillance activities.

**Acknowledgements**

We thank Principal, St. Joseph’s College, Irinjalakuda for the laboratory facilities provided. The authors thank University Grants Commission, Government of India (F.NO.42-609/2013 SR), Kerala State Council for Science, Technology and Environment (KSCSTE) (No.27/SRSLS/2013/CSTE).

**Conflict of Interest:** None

**References**

1. Rasool, V., S. Rasool, and S. Mushtaq, *Viral encephalitis and its management through advanced molecular
diagnostic methods: a review. Clinical pediatrics 2014. 53(2): p. 118-120.
2. Plourde, A.R. and E.M. Bloch, A literature review of Zika virus. Emerging infectious diseases 2016. 22(7): p. 1185.
3. Anesh, E. and V. Vijayan, Laboratory selection of carbofuran tolerant line of Culex quinquefasciatus Say, the filarial vector at Mysore. J Commun Dis 2010. 42(3): p. 201-07.
4. Harbach, R.E. and I.J. Kitching, The phylogeny of Anophelinae revisited: inferences about the origin and classification of Anopheles (Diptera: Culicidae). Zoologica Scripta 2016. 45(1): p. 34-47.
5. Freitas, M., et al., Novel DNA extraction assay for molecular identification of Aedes spp eggs. Genetics and Molecular Research 2014. 13(4): p. 8776-8782.
6. Li, Y., et al., Urbanization increases Aedes albopictus larval habitats and accelerates mosquito development and survivorship. PLoS neglected tropical diseases, 2014. 8(11): p. e3301.
7. Souza, V.M. Identifying Aedes aegypti Mosquitoes by Sensors and One-Class Classifiers. in Iberoamerican Congress on Pattern Recognition 2016. Springer.
8. Bhatt, S., et al., The global distribution and burden of dengue. Nature 2013. 496(7446): p. 504.
9. Straetemans, M. and E.C.G.o.V.-R.f.C.V.T.i. Europe, Vector-related risk mapping of the introduction and establishment of Aedes albopictus in Europe. Euro surveillance: bulletin Européen sur les maladies transmissibles= European communicable disease bulletin 2008. 13(7).
10. Medlock, J.M., et al., A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector-borne and zoonotic diseases 2012. 12(6): p. 435-447.
11. Hawley, W.A., The biology of Aedes albopictus. Journal of the American Mosquito Control Association. Supplement, 1988. 1: p. 1-39.
12. Dame, D. and T. Fasulo, Mosquitoes. Public Health Pesticide Applicator Training Manual 2003.
13. Rueda, L.M., Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission 2004, Walter Reed Army Inst Of Research Washington Dc Department Of Entomology.
14. Merritt, R., R. Dadd, and E. Walker, Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annual review of entomology 1992. 37(1): p. 349-374.
15. Service, M., Medical entomology for students 2008: Cambridge University Press.
16. Savage, H., et al., Host-feeding patterns of Aedes albopictus (Diptera: Culicidae) at a temperate North American site. Journal of Medical Entomology 1993. 30(1): p. 27-34.
17. Bram, R.A., Contributions to the mosquito fauna of southeast Asia-II. The genus Culex in Thailand (Diptera: Culicidae) 1967, SMITHSONIAN INSTITUTION WASHINGTON DC.
18. Harrison, B.A. and J.E. Scanlon, Medical Entomology Studies-II. The Subgenus Anopheles in Thailand (Diptera: Culicidae)(Contributions of the American Entomological Institute. Volume 12, Number 1) 1975, WALTER REED ARMY INST OF RESEARCH WASHINGTON DC DEPARTMENT OF ENTOMOLOGY.
19. Rattanarithikul, R., et al., Illustrated keys to the mosquitoes of Thailand I. Background; geographic distribution; lists of genera, subgenera, and species; and a key to the genera. Southeast Asian Journal of Tropical Medicine and Public Health, 2005. 36(S1): p. 1.
20. Panthusiri, P., ILLUSTRATED KEYS TO THE MOSQUITOES OF THAILAND IV. ANOPELLES. The Southeast Asian Journal of Tropical Medicine and Public Health 2006. 37: p. 2.
21. Rattanarithikul, R., et al., Illustrated keys to the mosquitoes of Thailand V. Genera Orthopodomyia, Kimia, Malaya, Topomyia, Tripteroides, and Toxorhynchites. The Southeast Asian Journal of tropical medicine and public health 2007. 38: p. 1-65.
22. Panthusiri, P., ILLUSTRATED KEYS TO THE MOSQUITOES OF THAILAND VI. TRIBE AEDINI. The Southeast Asian Journal of Tropical Medicine and Public Health 2010. 41: p. 1.
23. Jinbo, U., T. Kato, and M. Ito, Current progress in DNA barcoding and future implications for entomology. Entomological Science 2011. 14(2): p. 107-124.
24. Cywinska, A., F. Hunter, and P.D. Hebert, Identifying Canadian mosquito species through DNA barcodes. Medical and veterinary entomology 2006. 20(4): p. 413-424.
25. Hebert, P.D., A. Cywinska, and S.L. Ball, Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B: Biological Sciences 2003. 270(1512): p. 313-321.
26. Manonmani, A., et al., rDNA-ITS2 polymerase chain reaction assay for the sibling species of Anopheles fluviatilis. Acta tropica 2001. 78(1): p. 3-9.
27. Singh, O., et al., Differentiation of members of the Anopheles fluviatilis species complex by an allele-specific polymerase chain reaction based on 28S ribosomal DNA sequences. The American journal of tropical medicine and hygiene 2004. 70(1): p. 27-32.
28. Kang, D. and C. Sim, Identification of Culex complex species using SNP markers based on high-resolution melting analysis. Molecular ecology resources 2013. 13(3): p. 369-376.
29. Pfeiler, E., et al., Genetic diversity and population genetics of mosquitoes (Diptera: Culicidae: Culex spp.) from the Sonoran Desert of North America. The Scientific World Journal 2013. 2013.
30. Low, V., et al., Mitochondrial DNA analyses reveal low genetic diversity in Culex quinquefasciatus from residential areas in Malaysia. Medical and veterinary entomology 2014. 28(2): p. 157-168.
31. Shepard, J.J., T.G. Andreadis, and C.R. Vossbrinck, Molecular phylogeny and evolutionary relationships among mosquitoes (Diptera: Culicidae) from the northeastern United States based on small subunit ribosomal DNA (18S rDNA) sequences. Journal of medical entomology 2006. 43(3): p. 443-454.
32. Saitou, N. and M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution 1987. 4(4): p. 406-425.
33. Tamura, K., M. Nei, and S. Kumar, Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America 2004. 101(30): p. 11030-11035.
34. Tamura, K., et al., MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 2013. 30(12): p. 2725-2729.
35. Medeiros-Sousa, A.R., et al., Diversity and abundance of mosquitoes (Diptera: Culicidae) in an urban park: Larval habitats and temporal variation. Acta tropica 2015. 150: p. 200-209.
36. Maricopa, Life cycle and information on Aedes aegypti mosquitoes 2008.
37. Gubler, D.J., Dengue, urbanization and globalization: the unholy trinity of the 21st century. Tropical medicine and health 2011. 39(4SUPPLEMENT): p. S3-S11.
38. Murray, C.J., et al., Disability-adjusted life years (DALYS) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The lancet, 2012. 380(9859): p. 2197-2223.
39. Fradin, M.S. and J.F. Day, Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine 2002. 347(1): p. 13-18
40. Van den Berg, H., R. Velayudhan, and M. Ejov, Regional framework for surveillance and control of invasive mosquito vectors and Re-emerging vector-borne diseases 2014–2020. World Health Organization, 2013. 26.
41. Organization, W.H., Yellow fever: rapid field entomological assessment during yellow fever outbreaks in Africa: handbook: methodological field approaches for scientists with a basic background in entomology 2014.
42. ISDP, Integrated Disease Surveillance Project Kerala State. 2017. Directorate of Health service, District wise daily report (24-06-2017, 28-06-2107).
43. Laird, M., The natural history of larval mosquito habitats 1988: Academic Press Ltd.
44. Rueda, L.M., Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. Hydrobiologia 2008. 595(1): p. 477-487.
45. Organization, W.H., Zika virus, microcephaly and Guillain-Barré syndrome situation report 2016.
46. Ashfaq, M., et al., Analyzing mosquito (Diptera: Culicidae) diversity in Pakistan by DNA barcoding. PLoS One, 2014. 9(5): p. e97268.
47. Scholte, E.-J., W. Takken, and B.G. Knols, Infection of adult Aedes aegypti and Ae. albopictus mosquitoes with the entomopathogenic fungus Metarhizium anisopliae. Acta tropica 2007. 102(3): p. 151-158.
48. Jia, P., et al., How does the dengue vector mosquito Aedes albopictus respond to global warming? Parasites & vectors 2017. 10(1): p. 140.
49. Zettel, C. and P. Kaufman, Yellow fever mosquito Aedes aegypti (Linnaeus)(Insecta: Diptera: Culicidae) 2012. Citeseer.
50. Bova, J., S. Paulson, and G. Paulson, Morphological Differentiation of the Eggs of North American Container-Inhabiting Aedes Mosquitoes. Journal of the American Mosquito Control Association 2016. 32(3): p. 244-246.
51. Rios, L. and J.E. Maruniak, Asian Tiger Mosquito, Aedes albopictus (Skuse)(Insecta: Diptera: Culicidae) 2004, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
52. Farajollahi, A. and D.C. Price, A rapid identification guide for larvae of the most common North American container-inhabiting Aedes species of medical importance. Journal of the American Mosquito Control Association 2013. 29(3): p. 203-221.

Date of Submission: 2017-08-11
Date of Acceptance: 2017-09-07