

---

## **IDENTIFICATION OF MOSQUITO SPECIES (DIPTERA: CULICIDAE) IN SOUTHEASTERN AUSTRALIA USING MOLECULAR TECHNIQUES**

**Anshra Hasmi<sup>1</sup>, Dr. Amit Sharma<sup>2</sup>**

**<sup>1</sup>Research Scholar, <sup>2</sup>Associate Professor**

**Department of Zoology, Shri Venkateshwara University, Uttar Pradesh, India**

### **Abstract**

A contemporary species identification approach, DNA barcoding, can be employed to tell between visually various organisms, and it is particularly helpful when utilising little quantities of initial data from incomplete pieces or adolescent ages. There needs to be a collection of mosquitoes genetic polymorphisms for the use of DNA barcoding in a surveillance operation. One hundred and eleven specimens, comprising 29 species, six tribes, and 12 taxa, have been analyzed for Cytochrome Oxidase I (COI) sequences; 17 of these creatures have never had a barcode applied. Four members of both the 29 species that were originally misidentified since they were challenging to distinguish morphological characters are Larvae palpalis, Macleaya macmillani, and then an unidentified life forms that was earlier known as Tripteroidesatripes, proving the efficiency of Molecular techniques in these situations. Employing COI, the Cx. hand - carved subtype wasn't able to be morphologically distinguished even though the majority of species did (reciprocally monophyletic). The conspecific and congeneric p-distances were on average 0.8 percent and 7.6 percent. The research utilised a single Constants melanogaster egg that was confiscated at an international terminal to show how well Dna fingerprinting works to differentiate exotic larvae from native mosquitoes. By eliminating the need to raise specimens to adulthood and then employ DNA barcoding, researchers were able to demonstrate the technique's usefulness in antiviral monitoring. The "Chigger bites of Dominion" group has released the DNA sequences produced throughout this investigation. upon that Genome of Life Registry in order to aid the Victorian Avian influenza Infection Prevention Plan and some other national and global mosquito monitoring projects (BOLD).

**Keywords:** *DNA barcoding, species distributions, and the Culicidae family*

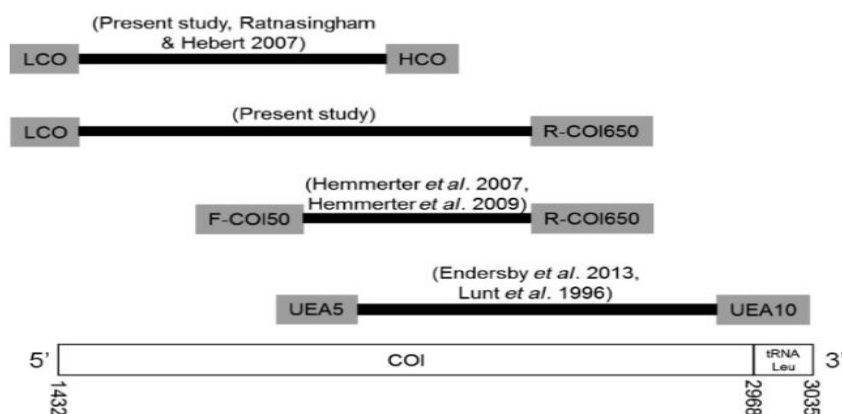
### **Introduction**

Rapid and precise identification of important species is required for vector surveillance. Australia is home to over 300 species of mosquitoes, many of which are capable of transmitting diseases that poses a risk to public health of people and animals (Ehlersm and Alsemgeest 2011). Stegomyiaalbopicta, and the now plant, and St. melanogaster, a tropical Australian plant) constitute a significant public health danger because of the wide range of diseases they can spread. Australian surveillance methods for mosquito identification use dichotomous keys to identify specimens based on morphology. When attempting to identify damaged specimens or separate morphologically identical species, the traditional method

might be troublesome since it takes a long time, requires specialized knowledge, and is difficult to use. DNA barcoding is an additional technique of identifying that may be able to overcome these existing drawbacks in the near future.

In order to accurately identify species, DNA Short DNA sequences, which vary less within individuals than both species, are used for sequence analysis. Since the beginning of molecular studies, only one genera of indigenous Australian bugs has really been examined (Dyson et al. 2009; Puslednik et al. 2012; Endersby et al. 2013; Ali et al. 2013; Mccarthy et al. 1998, 2007; Hemmerter et al. 2007). By showcasing how DNA barcoding may be used to better determine geographical ranges and taxonomic gene pool, these research have demonstrated. Because the genetic areas employed as DNA barcodes vary widely, the results obtained are difficult to compare with those of other mosquito species in Australia.

The 'Universal' or Species presently use the 'Folmer' domain of such gene results Cyt Hydroxylase I (COI) as their primary flag. The Beacon of Life Network (BOLD), a global internet collection for Genotyping information, has designated this area as the standard marker (Ratnasingham and Hebert 2007). While this region is the most commonly used for mosquito barcoding studies, other sections of COI have been used in other investigations (Fig. 1). Mitochondrial and nuclear genes can be utilized to differentiate species, and often more than one marker is employed (Lin and Danforth 2004). Researchers have used a wide range of nuclear markers, including elongation factor 1 alpha (EF1A), In studies on mosquito sequence analysis, zinc tip and external transcriptome interval province 2 (ITS2) is described by Ferris et al. (2007), Hamid et al. (2009), Hemmerter et al. (2009), and Puslednik et al. (2012).). If more than one locus is employed, it is possible to make distinctions between strongly connected species and subspecies and groupings that could not be likely to be evolutionarily discriminated if just one genomic area would be used (Foster et al. 2013; Jiang et al. 2014).



**figure 1 Different mosquito DNA barcoding studies have used different primer positions inside the COI. Hardy et al. (2014), GenBank accession number: NC 025473, provided mitochondrial gene locations.**

As part of a mosquito surveillance program, proper identifications of mosquitoes must first be made in order to apply DNA barcoding effectively. An Australian mosquito monitoring program previously discovered 36 endemic species (Gallagher et al. 2015 secret information); yet, only ten of all these creatures have publicly available COI alleles in Iom and Blue, and therefore only twelve species (25%) are sourced from Australia (read 05.11.15). Its identification method will be made useful by molecular marker mosquito vectors acquired in temperate central Australia, laying the groundwork for the development of a significantly bigger catalogue of anopheles segments from throughout Australia and adding to the collection of source arthropod patterns easily accessible globally. Although DNA barcoding is presently underused, vectors may be recognised more precisely through the establishment of a geographically focused code bank.

Gene composition can also be learned from the study of mosquito species that have been barcoded. Recent years have seen a succession of contested modifications in mosquito taxonomy, with the Aedini tribe receiving more alterations than any other Costlier et al. 2009; Persists and Harbach 2005). Several of these taxa comprise monophyletic and individuals form populations, according to genomic research. Modern categorization of vectors, however, mainly depends on cosmetic similarities, leading to vast genus groupings that inaccurately reflect the real variety (Harbach 2007). Genealogical approaches, which are thought to be largely free of subjectivity in morphological feature identification, have the potential to discover the existence of previously unknown species complexes (e.g., Hemmerter et al. 2007). As a result, the accuracy of a monitoring program depends on barcoding as a technique of identifying mosquitoes.

As part of this research, they aimed to enhance the effectiveness of current vector surveillance systems by creating a COI DNA barcode library for 26 species of endemic Australian mosquitoes collected in temperate southeastern Australia. Images and collection details from the "Mosquito of Commonwealth" (MOAV) initiative, which expanded the Mosquitoes of both the Earth mission to include temperate eastern Victoria, supported the DNA barcode library. DNA barcoding was also used to identify a mosquito egg that had been seized at an airport. The findings show that a bigger COI fragment can be used to overlap with data from prior research that have explored various COI regions and analyze the linkages between mosquito species and their genus composition.

## **Conclusions**

An Australian mosquito barcode library based on DNA barcoding has been established as an effective method for detecting and preventing vector-borne diseases. With the help of this barcode library, vector surveillance programs around the world will be able to use DNA barcoding as an additional means of identification. DNA barcoding can be used for both monitoring and biosecurity purposes because it can identify species at every life stage, including eggs. Additional genetic markers should be added to this technology in order to

strengthen its discriminatory power in future applications. Large samples (i.e. bulk samples) of mosquitoes might be quickly and accurately identified using DNA barcoding and next-generation sequencing, reducing the processing time required for species identification dramatically (McCormack et al. 2013). DNA barcoding is a vital aspect of vector monitoring because of its accuracy and versatility. As more barcode libraries and resources are generated, its importance will only increase.

1. Al- Hussaini, M. T. , Muhammed Ali A. K., and Al- Rubae H. M.. 2013. PCR based identification of *Culex pipiens* complex (Diptera: Culicidae) collected in Al- Najaf governorate. *Mag. Al- Kufa Uni. Biol.* 5:177–186.
2. Ballard, J. ,Puslednik L., Wolff J., and Russell R.. 2009. Variation under nature: a sesquicentennial DNA barcoding perspective. *Chiang Mai J. Sci.* 36:188–199.
3. Čandek, K. , and Kuntner M.. 2015. DNA barcoding gap: reliable species identification over morphological and geographical scales. *Mol. Ecol. Resour.* 15:268–277.
4. Chan, A. , Chiang L. P., Hapuarachchi H. C., Tan C. H., Pang S. C., Lee R., et al. 2014. DNA barcoding: complementing morphological identification of mosquito species in Singapore. *Parasit. Vectors* 7:569.
5. Cywinska, A. , Hunter F. F., and Hebert P. D. N.. 2006. Identifying Canadian mosquito species through DNA barcodes. *Med. Vet. Entomol.* 20:413–424
6. Dobrotworsky, N. V. 1965. *The mosquitoes of Victoria*. Melbourne University Press, London.
7. Ehlersm, G. , and Alsemgeest D.. 2011. *Common mosquitoes of north Queensland: identification and biology of adult mosquitoes*. Mosquito Control Association of Australia, Kirwan, QLD, Australia.
8. Endersby, N. M. , White V. L., Chan J., Hurst T., Rašić G., Miller A., et al. 2013. Evidence of cryptic genetic lineages within *Aedes notoscriptus* (Skuse). *Infect. Genet. Evol.* 18:191–201.
9. Foley, D. H. , Bryan J. H., Yeates D., and Saul A.. 1998. Evolution and systematics of *Anopheles*: insights from a molecular phylogeny of Australasian mosquitoes. *Mol. Phylogenet. Evol.* 9:262–275.
10. Foley, D. H. , Wilkerson R. C., Cooper R. D., Volovsek M. E., and Bryan J. H.. 2007. A molecular phylogeny of *Anopheles annulipes* (Diptera: Culicidae) sensulato: the most species- rich anopheline complex. *Mol. Phylogenet. Evol.* 43:283–297.

11. Folmer, O. , Black M., Hoeh W., Lutz R., and Vrijenhoek R.. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.* 3:294–299.
12. Foster, P. G. ,Bergo E. S., Bourke B. P., Oliveira T. M., Nagaki S. S., Sant'Ana D. C., et al. 2013. Phylogenetic analysis and DNA- based species confirmation in *Anopheles (Nyssorhynchus)*. *PLoS One* 8:e54063
13. Harbach, R. E. 2007. The Culicidae (Diptera): a review of taxonomy, classification and phylogeny Pp. 591–688. *Linnaeus tercentenary: progress in invertebrate taxonomy*. Magnolia Press, Auckland.
14. Harbach, R. , Harrison B., and Gad A.. 1984. *Culex (Culex) Molestus* Forskål (Diptera: Culicidae): neotype designation, description, variation, and taxonomic status. *Proc. Entomol. Soc. Wash.* 86:521–542.
15. Hardy, C. M. , Court L. N., Morgan M. J., and Webb C. E.. 2014. The complete mitochondrial DNA genomes for two lineages of *Aedes notoscriptus* (Diptera: Culicidae). *Mitochondrial DNA*.
16. Hasan, A. U. ,Suguri S., Ahmed S. M., Fujimoto C., Harada M., Rahman S. M., et al. 2009. Molecular phylogeography of *Culex quinquefasciatus* mosquitoes in central Bangladesh. *Acta Trop.* 112:106–114.
17. Hemmerter, S. ,Šlapeta J., van den Hurk A. F., Cooper R. D., Whelan P. I., Russell R. C., et al. 2007. A curious coincidence: mosquito biodiversity and the limits of the Japanese encephalitis virus in Australasia. *BMC Evol. Biol.* 7:100.
18. Hemmerter, S. ,Šlapeta J., and Beebe N. W.. 2009. Resolving genetic diversity in Australasian *Culex* mosquitoes: incongruence between the mitochondrial cytochrome c oxidase I and nuclear acetylcholine esterase 2. *Mol. Phylogenet. Evol.* 50:317–325.
19. Jansen, C. C. ,Hemmerter S., van den Hurk A. F., Whelan P. I., and Beebe N. W.. 2013. Morphological versus molecular identification of *Culex annulirostris* Skuse and *Culex palpalis* Taylor: key members of the *Culex sitiens* (Diptera: Culicidae) subgroup in Australasia. *Aust. J. Entomol.* 52:356–362.
20. Jiang, F. ,Jin Q., Liang L., Zhang A. B., and Li Z. H.. 2014. Existence of species complex largely reduced barcoding success for invasive species of Tephritidae: a case study in *Bactrocera* spp. *Mol. Ecol. Resour.* 14:1114–1128.
21. Kassim, N. F. A. , Webb C. E., Wang Q., and Russell R. C.. 2013. Australian distribution, genetic status and seasonal abundance of the exotic mosquito *Culex molestus* (Forskål) (Diptera: Culicidae). *Aust. J. Entomol.* 52:185–198.



22. Kearse, M. , Moir R., Wilson A., Stones- Havas S., Cheung M., Sturrock S., et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649.
23. Kumar, N. P. ,Rajavel A. R., Natarajan R., and Jambulingam P.. 2007. DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 44:1–7.
24. Laurito, M. , de Oliveira T. M. P., Almirón W. R., and Sallum M. A. M.. 2013. COI barcode versus morphological identification of *Culex (Culex)* (Diptera: Culicidae) species: a case study using samples from Argentina and Brazil. *Mem. Inst. Oswaldo Cruz* 108(Suppl 1):110–122.
25. Lee, Y. , Seifert S. N., Nieman C. C., McAbee R. D., Goodell P., Fryxell R. T., et al. 2012. High degree of single nucleotide polymorphisms in California *Culex pipiens* (Diptera: Culicidae) sensulato. *J. Med. Entomol.* 49:299–306.
26. Lin, C.- P. , and Danforth B. N.. 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. *Mol. Phylogenet. Evol.* 30:686–702.
27. Lunt, D. H. , Zhang D. X., Szymura J. M., and Hewitt G. M.. 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Mol. Biol.* 5:153–165.
28. McCormack, J. E. ,Hird S. M., Zellmer A. J., Carstens B. C., and Brumfield R. T.. 2013. Applications of next- generation sequencing to phylogeography and phylogenetics. *Mol. Phylogenet. Evol.* 66:526–538.
29. Puslednik, L. , Russell R. C., and Ballard J. W. O.. 2012. Phylogeography of the medically important mosquito *Aedes (Ochlerotatus) vigilax* (Diptera: Culicidae) in Australasia. *J. Biogeogr.* 39:1333–1346.
30. Ratnasingham, S. , and Hebert P. D. N.. 2007. bold: the Barcode of Life Data System. *Mol. Ecol. Notes*, 7:355–364.
31. Reinert, J. F. , and Harbach R. E.. 2005. Generic and subgeneric status of Aedine mosquito species (Diptera: Culicidae: Aedini) occurring in the Australasian region. *Zootaxa* 887:1–10.
32. Reinert, J. F. ,Harbach R. E., and Kitching I. J.. 2009. Phylogeny and classification of tribe Aedini (Diptera: Culicidae). *Zool. J. Linn. Soc.* 157:700–794.
33. Richards, S. L. , Anderson S. L., and Alto B. W.. 2012. Vector competence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) for dengue virus in the Florida Keys. *J. Med. Entomol.* 49:942–946.

34. Russell, R. C. 1996. *A colour photo atlas of mosquitoes of southeastern Australia*. The Department of Medical Entomology, Westmead Hospital and the University of Sydney, Sydney, Australia.
35. Russell, R. C. 2012. A review of the status and significance of the species within the *Culex pipiens* group in Australia. *J. Am. Mosq. Control Assoc.* 28:24–27.
36. Smith, J. L. , and Fonseca D. M.. 2004. Rapid assays for identification of members of the *Culex (Culex) pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). *Am. J. Trop. Med. Hyg.* 70:339–345.
37. Tamura, K. , Dudley J., Nei M., and Kumar S.. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24:1596–1599.
38. Versteirt, V. , Nagy Z. T., Roelants P., Denis L., Breman F. C., Damiens D., et al. 2015. Identification of Belgian mosquito species (Diptera: Culicidae) by DNA barcoding. *Mol. Ecol. Resour.* 15:449–457.
39. Wang, G. , Li C., Guo X., Xing D., Dong Y., Wang Z., et al. 2012. Identifying the main mosquito species in China based on DNA barcoding. *PLoS One* 7:e47051.
40. Wilkerson, R. C. , Linton Y. M., Fonseca D. M., Schultz T. R., Price D. C., and Strickman D. A.. 2015. Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PLoS One* 10:e0133602.