ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 18, Issue, 02, pp.36293-36296, February, 2026
DOI: https://doi.org/10.24941/ijcr.50215.02.2026

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF GENETIC VARIABILITY AMONG MOSQUITO SPECIES IN JALGAON DISTRICT OF MAHARASHTRA USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

[1]Bhalchandra S. Pawar, [1]Savita Kalwale and [2,*]Prakash S. Lohar

[1]Department of Zoology, D.N. Mahavidyalaya, Faizpur 425503 Dist Jalgaon, MS
[2]SSVPS Smt. P. B. Bagal Arts, Commerce and Science College, Dondaicha 425408 Dist. Dhule MS

ARTICLE INFO

Article History:
Received 25th November, 2025
Received in revised form
20th December, 2025
Accepted 18th January, 2026
Published online 27th February, 2026

Keywords:
Jalgaon, Mosquito Species,
RAPD, Phylogenetic tree,
Genetic Variability.

**Corresponding author:*
Prakash S. Lohar

ABSTRACT

Background: DNA-based molecular markers have been proposed as an excellent tool for identifying genetic diversity, species characterization and authentication of insect species. Randomly amplified polymorphic DNA (RAPD) markers were used effectively for the characterization of different mosquito species collected from 15 tehsils in Jalgaon district of Maharashtra. **Methods:** Morphological identification was performed using taxonomic keys. Genetic analyses included isolation of DNA), PCR amplification, and using decamer primers. RAPD-PCR reaction used to verify the reproducibility of scored polymorphic bands. Phylogenetic tree constructed based on RAPD data. **Results:** RAPD profiling showed a 96% molecular variance among the RAPD loci developed by within species and only 4% variance among different species (total *df*=239, FST = 0.028, $p > 0.001$). The primers gave a range of 18-26% polymorphic bands that revealed 64.5% polymorphism within the mosquito population. The molecular variance analysis (AMOVA), the genetic distance between populations was significant (RAPD:phiST = 0.086; $P < 0.001$). Cluster analysis of genetic similarity data grouped the population in four major clusters. **Conclusion:** RAPD technique had indeed revealed the genetic diversity among the four mosquito population and can significantly aid in identification of individual species.

Citation: Bhalchandra S. Pawar et al. 2026. "Evaluation Of Genetic Variability Among Mosquito Species In Jalgaon District of Maharashtra Using Random Amplified Polymorphic DNA (RAPD) Markers". ***International Journal of Current Research, 18, (02), 36293-36296.***

INTRODUCTION

DNA-based molecular markers have a great significance in determining genetic diversity, regional variation, and phylogeny relationship in insect species authentication (Zomuanpuii *et al*., 2024). From this perspective, many Anopheles species were successfully characterized using randomly amplified polymorphic DNA markers (Chaudhry, 2011). The clarification of genetic diversity in the species' wild populations would be made possible by RAPD analysis. The method yields amplified fragments that differ between species; taxa that are more distantly linked have more divergent fragment distributions, whilst those that are closely related have similar ones (Wilkerso *et al*.,1993; Zhao *et al.*, 2012). In a heterozygote, amplification products from the same alleles vary in length and can be identified by the presence or lack of bands in the RAPD profile, which contains significant phylogenetic information (Espinasa and Borowsky, 1998). RAPD helps in species identification, assessing differences between mosquito populations and their genetic relatedness (Casas-Martinez *et al.*, 2023; Pobmi *et al*.,2024; Eiras *et al*., 2024). RAPD (Randomly Amplified Polymorphic DNA) markers are PCR-based instruments that amplify random DNA segments using short, arbitrary primers, revealing genetic variations (polymorphisms) through differences in DNA banding patterns on gels. This makes them important for quick, low-cost DNA fingerprinting, evaluating genetic diversity, creating genetic maps, researching evolutionary relationships, and identifying varieties in plants, animals, and microbes without prior sequence knowledge. (Mbwana *et al*., 2006; Dhakane *et al.*, 2021). DNA-based molecular markers have a great significance in determining genetic diversity, regional variation, and phylogeny relationship in insect species authentication (Zomuanpuii *et al*., 2024). From this perspective, many Anopheles species were successfully characterized using randomly amplified polymorphic DNA markers (Sharma *et al.*, 2024). Clarification of genetic diversity in the species' wild populations would be made possible by RAPD analysis. RAPD helps in species identification, assessing differences between mosquito populations and their genetic relatedness (Casas-Martinez *et al.*, 2023; Pobmi *et al*.,2024; Eiras *et al*.,2024). The genus Culex includes many Indian mosquito species, notably *Culex quinquefasciatus* (a common domestic mosquito and filariasis vector), *Culex tritaeniorhynchus*, *Culex vishnui*, and *Culex pseudovishnui* (important Japanese encephalitis vectors), along with *Culex bitaeniorhynchus*, *Culex gelidus,* and *Culex fuscocephala,* among others found in various habitats across India (Panda and Barik, 2022). Based on

previous studies, presence of 4 species respectively denoted as C1 to C4 viz., *Culex quinquefasciatus, Cx.tritaeniorhynchus, Cx.vishnui* and *Cx. psedovishnui* in different tehsils of Jalgaon district has been confirmed. While, The genus Mansonia consists of mosquitoes known for their specialized larval adaptation they attach to aquatic plants to extract oxygen Mansonia species play a significant role in lymphatic filariasis transmission, particularly in coastal and wetland regions of India. The key Mansonia species in India, crucial for transmitting Brugian filariasis, include *Mansonia annulifera* (most significant vector), *M. uniformis*, and *M. indiana,* found in endemic regions like Kerala, breeding in aquatic plants like *Pistia stratiotes* and *Eichhornia crassipes***,** with distinct biting behaviors (indoor/outdoor) and seasonal activity peaking in monsoon/post-monsoon (Rani *et al*.,2025). The genus Mansonia were found in Jalgaon district with 2 species respectively denoted as M1 to M2 viz., *M. annulifera* and *M.uniformis* respectively. RAPD is used to evaluate the genetic relationship among all four mosquito genera and its species found in Jalgaon district can help to develop vector borne disease control strategies. The lack of research in this field specifically related to identification of mosquito species using RAPD technique in Jalgaon district, it was worth to undertake work to determine their prevalence and distribution pattern that will help to adopt vector management strategies in affected region.

Based on the previous survey of 15 tehsils in Jalgaon district during study period, it was noted that the percentage of Anopheles, Aedes, Culex and Mansonia was 55, 37,7.9 and 0.01 respectively. In the present study, the genus Anopheles was represented by 8 species respectively denoted as A1 to A8 viz., *Anopheles culicifacies, An.subpictus An. annularis,An. barbirostris, An.quadrimaculatus, An. vagus*, *An. Gigas*and rarely found *An.* stephensi .The genus Aedes included 4 species denoted as D1 to D4 viz., *Aedesaegypti, Ae. albolateralis, Ae. albopictus* and *Ae.vittatus.* The genus Culex revealed by the presence of 4 species respectively denoted as C1 to C4 viz.,*Culex quinquefasciatus, Cx.tritaeniorhynchus, Cx.vishnui*and *Cx. P sedovishnui*. Whereas, the genus Mansonia marked their presence with 2 species respectively denoted as M1 to M2 viz., namely, *M. annulifera* and *M.uniformis.* All four mosquito genera and its species were processed for the RAPD analysis and their intergeneric and interspecific relatedness were evaluated.

MATERIALS AND METHODS

Individual mosquito species belonging to Anopheles, Aedes, Culex and Mansonia were captured separately from 15 tehsils in Jalgaon district during the study period of December 2024 to November 2025. Adult mosquitoes were captured outdoors using CDC light traps and by aspiration of mosquitoes at rest. Morphological identification was performed using taxonomic keys. Genetic analyses included isolation of DNA using method described by Barik *et al* (2013), PCR amplification, and the 10 microliter PCR amplification products were subjected to electrophoresis in a 1% agarose(Bangalore genei) stained with ethidium bromide in 1X TBE buffer to check to quantity and quality of DNA. The extracted DNA samples were then stored at -20°C till their further use. These DNAs were used as templates in a PCR based search for producing RAPD markers.

Screening of primers and PCR amplification: Twelve random oligonucleotide decamer primers (Chromus Biotech Pvt. Ltd Bangalore, India) produced reproducible polymorphic bands. 10-15ng/µl of genomic DNA were amplified in a 25 µl reaction mixture containing 2.5µl of 10XTaq polymerase buffer (10mM TrisHCl, pH 7.5 and 50mM KCl), 0.2mM dNTPs, 1.5U Taq DNA Polymerase, 3mM MgCl2, 0.6mg/ml BSA and 11.5 pmole of random primer. The samples were subjected to 40 cycles of initial denaturation at 94°C for 4 min, 94°C for 1 min (denaturation) followed by 37°C for 2min (annealing), 72°C for 2min (extension),and finally 72°C for 10 minutes. A reaction mixture without DNA template was used as negative control. Ten microliters of the PCR product was loaded in 1.5% agarose gel, 1XTAE pH 8.3 and ethidium bromide (0.5µg/ml final concentration). The gel was run at 100V for 1h and visualized with UV transilluminator and photographed using gel documentation system (BioEra, Pune). PCR amplicon was estimated using low range DNA ruler (Genei Lab, India). Out of 12, three primers were selected for further study since they produced clear and good bands (Table 1). RAPD-PCR reaction was repeated thrice for mosquito species of four genera i.e., Anopheles, Aedes, Culex and Mansonia in order to verify the reproducibility of scored polymorphic bands.

Data Analysis and dendrogram: A plot showing the distance migrated by each band on X axis versus the natural logarithms (*ln*) of the size of DNA ladder bands (Promega) revealed on the horizontal agarose gel on Y axis to get a linear standard curve and equation of the trendline of the curve in order to apply all the band distance values (X) to the equation which resulted in *ln*size of the targeted band and to get the value of size of each band by inverse natural logarithm**.** RAPD bands are scored for presence ”1” and absence ”0”. Only clear, consistent and polymorphic bands are usually used to create a binary matrix for future statistical analyses (Wilkerson *et al*.,1993: Bardakci, 2001; Zhao and Wu, 2012). To evaluate the genetic variability among species and populations graphically, tree diagram was constructed and combined among the species of genus Anopheles, Aedes, Culex and Mansonia. Cluster analysis created a tree diagram, also known as a *dendrogram* or *phenogram* that contained the results of hierarchical clustering of mosquito species was done with help of online D-UPGMA program.

RESULT AND DISCUSSION

A total of 1470 Anopheles mosquitoes belonging to 8 species (A1 to A8), a total of 648 Aedes mosquitoes belonging to 4 species (D1 to D4), a total of 246 *Culex* mosquitoes belonging to 4 species (C1 to C4) and a total of 42 Mansonia mosquitoes belonging to 2 species (M1 and M2) were captured separately from 15 tehsils in Jalgaon district during the study period of December 2024 to November 2025.

Table 1. Properties of 3 primers selected for RAPD-PCR profiling of mosquito species collected from Jalgaon district in Maharashtra)

Primer number	Sequence of a primer	GC%	Tm value
Primer 1	5’CGCACTGCCG 3’	70	60
Primer 2	5’AGGCTCGATA 3’	60	55
Primer 3	5’TGCAGCTCCT 3’	60	55

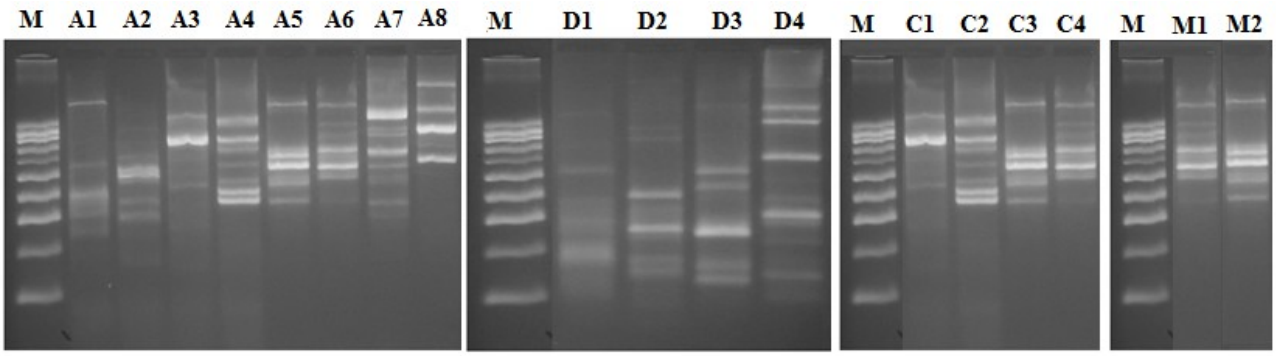

Figure 1. RAPD-PCR banding pattern of genomic DNA of Anopheles spp.(A1 to A8), Aedes spp (D1 to D4), Culex spp.(C1 to C4) and Mansonia spp (M1 & M2)

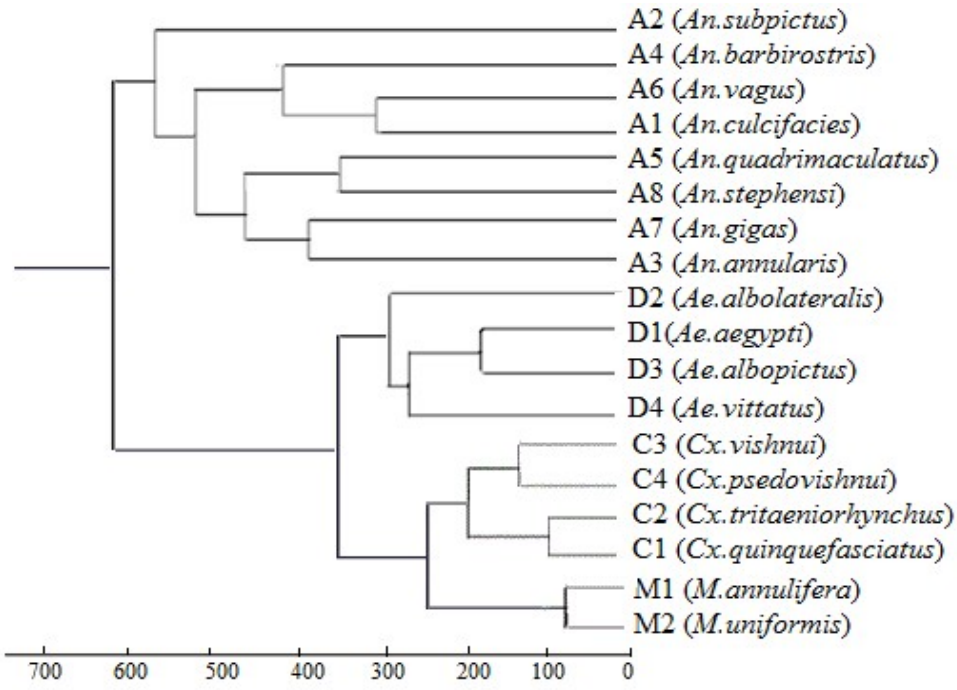

Figure 2. Dendrogram showing phylogenetic relation between four different genera of mosquito found in Jalgaon district

After analysis of DNA, RAPD Patterns of polymorphism and monomorphism generated using three primers separately in A1 to A8 species of Anopheles, D1 to D4 species of Aedes, C1 to C4 species of Culex and Mansonia species indicated by M1 and M2 (Figure 1). Based RAPD data, the dendrogram constructed and phylogenetic relationship revealed among four mosquito genera Anopheles, Aedes, Culex and Mansonia found in Jalgaon district of Maharashtra with branch lengths showing evolutionary divergence—the longer the branch, the greater the genetic distance from common ancestors (Figure 2). Anopheles forms a distinct, early-diverging clade, meaning it is genetically more distant from the other genera. Aedes and Culex are more closely related to each other than either is to Anopheles. Mansonia is the most genetically distant, branching off earliest and acting almost like an out group in this tree. The 8 species of Anopheles were found divided into 4 clades. The genus Anopheles having species *An. annularis* and *An.gigus* together formed a clade, which then found connected to the second clade comprised the *An. stephensi* and *An. quadrimaculatus.* The third clad consisted of 2 another species of *An. calcifacies* and *An.vagus*, which was found associated the mosquito *An.barbirostris.* Both subgroups containing 7 species of the genus Anopheles clustered together *An.subpictus* showing genetic relatedness among all the species. In another clade of genus Aedes, 2 species namely, *Ae.aegypti* and *Ae. albopictus* clustered together, which was then clustered with *Ae. vittatus* and finally clustered with *Ae.albolateralis*. The analysis showed a 96% molecular variance among the RAPD loci developed by within species and only 4% variance among different species (total *df*=239, FST = 0.028, p > 0.001). The primers gave a range of 18-26% polymorphic bands that revealed 64.5% polymorphism within the population. The molecular variance analysis (AMOVA), the genetic distance between populations was significant (RAPD:phiST = 0.086; P < 0.001). *Culex quinquefasciatus* is the predominant mosquito species in the Jalgaon district of Maharashtra, representing up to 77.9% of mosquito collections as compared to other 3 species of genus Culex. It prefers breeding in organically polluted water bodies, which are commonly found in residential areas, making it a major urban pest and a primary vector for diseases such as lymphatic filariasis. *Cx. vishnui, Cx. tritaeniorhynchus* and *Cx. psedovishnui* mainly breed in paddy fields. Mansonia species are found thriving in areas with aquatic vegetation. In case of Mansonia species, M1 (*Mansonia annulifera*) and M2 (*Mansonia uniformis*) cluster very closely together, indicating they are highly similar and share a recent common ancestor. Poonja (2024) reported resembling results related to number of

mosquito species in Kalyan city in the Maharashtra with *Anopheles* genus dominated by eight species, *Culex* five, *Aedes* three, *Armigeres* two, and *Mansonia* represented by only one species. Among 19 species, the population of *Cx.quinquefasciatus* was most abundant in the populated zone followed by *Ar. Subalbatus* and *Ar. abturbans. subpictus*.

Conclusion: It is to conclude that the dendrogram clearly separates Mansonia from Culex mosquitoes, while also showing well-defined subgroups within the Culex genus, reflecting their evolutionary relationships. Genus-level separation is strong, validating current taxonomic classification. Vector status often correlates with genetic clustering**,** especially in Culex and Aedes. Anopheles shows the greatest internal diversity**,** consistent with its ancient lineage. These results provide supporting data for a better understanding of the distribution of mosquito vectors in Jalgaon district.

REFERENCES

Bardakci, F. Random Amplified Polymorphic DNA (RAPD) Markers. Turkish J. Biol. 2001:25:185-196.

Barik S.K., R. K.Hazra, M. R.Prusty, A.Rath& S.K. Kar. A simple, rapid and very efficient protocol for DNA isolation from mosquito species. Protocol Exchange. doi:10.1038/protex.2013.007

Casas-Martínez M, Orozco-Bonilla A, Muñoz-Reyes M, et al. A new tent trap for monitoring the daily activity of *Aedes aegypti* and *Aedes albopictus* . J. of Vector Ecol. 2023:.38:277–88.

Chaudhry, N.S. The Genetic Structure of Anophelines (Culicidae: Diptera): RAPD Versus Allozymes. Proc. Zool.Soc. 2011:64(1): 8–16.

Dhakane R, K.Ahirrao, A. Shinde, Janardhan K. DNA-based identification and control of disease spreading mosquito species: A review.J. of Applied Biology & Biotechnology 2021: 9(1), pp. 124-134.

Eiras AE,Buhagiar TS, Ritchie SA. Development of the gravid ***Aedes*** *trap for the capture of adult female container-exploiting mosquitoes (Diptera: Culicidae). J. Med Entomol. 2024:.51:200–9*

Espinasa, L. and Borowsky, R. Evolutionary Divergence of AP-PCR (RAPD) Patterns. Mol. Biol. Evol. 1998:15(4):408-414.

Mbwana J, Bölin I, LyamuyaE, Mhalu F, Lagergård T. Molecular characterization of *Haemophilus ducreyi* isolates from different geographical locations. J Clin.Microbiol. 2006:.44(1):132-7.

Panda D. and T. K. Barik. Molecular characterization and genetic divergence of seven Culex mosquito (Diptera: Culicidae) species using Mt COI gene from Odisha State, India. J. of Basic and Applied Zoology.2022: 83:41-53

Poonja M. Study of seasonal and spatial variation of mosquito species in Kalyan, Maharashtra. Intern' J.of Entomology Res. 2024:9, (I): 90-95

Rani, V., Kumar, A. and Arya, H. Understanding Mosquito Biodiversity in India: Key Genera, Ecology, and Disease Transmission – A Review. J. of Science Innovations and Nature of Earth. 2025:5. 85-90.

Sharma SK, Nanda N, Dua VK, Joshi H, Subbarao SK, Sharma VP.Studies on the bionomics of *Anopheles fluviatilissensulato* and the sibling species composition in the foothills of Shiwalik range (Uttar Pradesh), India. Southeast Asian J. Trop Med Public Health.2022:26:566-72.

Wilkerson, R.C., Parsons, T.J., Albrightt, D.G., Klein, T.A. and Braun, M.J. Random amplified polymorphic DNA (RAPD) markers readily distinguish cryptic mosquito species (Diptera: Culicidae: *Anopheles*). Insect Mol. Biol. 1993:1(4):205-211.

Zhao, Y.E. and Wu, L.P. RAPD–SCAR marker and genetic relationship analysis of three *Demodex* species (Acari: Demodicidae). Parasitol. Res. 2012:110(6):2395-2402.

Zomuanpuii R., Guruswami G. And N.S. Kumar. Random Amplified Polymorphic DNA (RAPD) reveals genetic diversity among *Anopheles* (Diptera: Culicidae) species. Science and Technology Journal, 2024: 2(1): 36-44

****