Integrative Morphological and Molecular Classification of Culex vishnui Larvae from Diverse Ecological Habitats of Uttar Pradesh

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ABSTRACT

Mosquito-borne infections remain critical global health challenges in tropical regions. *Culex vishnui* is recognized as a principal vector of Japanese Encephalitis (JE) across Asia. The present study integrates morphological and molecular tools to classify *C. vishnui* larvae from varied ecological habitats in Uttar Pradesh, India. Larvae were collected from rice fields, ponds, marshes, and drains during post-monsoon months. Environmental parameters such as temperature, pH, and conductivity were recorded at each site. Morphological identification followed standard keys by Bram and Harbach, while COI gene sequencing confirmed species identity. Results revealed highest larval density in rice fields and minimal density in drains. A clear inverse correlation was observed between electrical conductivity and larval abundance. Molecular analysis indicated 99.2–99.8 % COI similarity with *C. vishnui* sequences in GenBank. Comparative evaluation with literature from 2018– 2025 supported these findings and confirmed ecological consistency across Asian landscapes. The integrative methodology established morphological–molecular coherence, improving accuracy in vector taxonomy and providing valuable insight for Japanese Encephalitis control programs.

Keywords: Culex vishnui; Morphological Identification; COI Barcoding; Habitat Ecology; Japanese Encephalitis; Uttar Pradesh.

INTRODUCTION

Mosquitoes are medically important insects found in nearly all climatic zones. Their adaptability allows colonization from tropical plains to temperate regions. The genus *Culex* includes more than 1,000 recognized species worldwide. Many of these species are important vectors of viral and parasitic infections. *Culex vishnui* is a dominant member of the *Culex vishnui* subgroup and a principal vector of Japanese Encephalitis (JE) in South and Southeast Asia. The disease affects both humans and livestock, causing severe neurological complications and economic losses (Yin et al., 2025).

This mosquito species breeds abundantly in irrigated paddy fields, ditches, and marshy depressions. Water stagnation during monsoon months creates optimal breeding conditions. Studies by Karthika et al., (2018) and Bashar et al., (2016) confirmed maximum larval density in rice agro-ecosystems with moderate organic load. Temperature, pH, and dissolved oxygen strongly affect larval survival and developmental rate. Organic debris and algal growth provide nutrient enrichment, enhancing breeding success. Light penetration also influences larval aggregation, as shaded zones reduce predation pressure (Amerasinghe et al., 1995).

Mosquito distribution and population dynamics are further regulated by anthropogenic factors. Expansion of irrigated agriculture, improper water storage, and climate change contribute to vector proliferation. Increased irrigation infrastructure has extended the JE transmission season in many Asian regions (Laskar et al., 2025). Rainfall variability and rising temperature patterns directly affect larval productivity (Yin et al., 2025). These changes underscore the urgent need for continuous entomological monitoring.

Traditional mosquito identification depends on morphological characteristics observed in larval and adult stages. Traits such as siphon index, pecten spine pattern, comb scale number, and palmate hairs form diagnostic features. Classical keys by Bram (1967) and Harbach (2007) remain reference standards for *Culex* identification. Yet, morphological overlap within sibling species often causes confusion, particularly when specimens are damaged or immature. Several *Culex* complexes, including *vishnui*, *tritaeniorhynchus*, and *pseudovishnui*, exhibit nearidentical larval characters (Rattanarithikul et al., 2023). This overlap complicates accurate identification in field surveillance programs.

Molecular biology has transformed mosquito systematics by providing genetic confirmation tools. DNA barcoding based on the mitochondrial cytochrome c oxidase subunit I (COI) gene offers precise identification at all developmental stages (Hebert et al., 2003). COI sequences display high interspecific divergence and low intraspecific variation, supporting their taxonomic reliability. Research by Cywinska et al., (2006) demonstrated the effectiveness of COI barcoding for mosquito differentiation in Canada. Subsequent Asian studies by Chung et al., (2024) and Zhang et al., (2024) confirmed its success in resolving *Culex* complexes. Integrating morphological and molecular evidence now forms the core of modern vector taxonomy.

The molecular approach also strengthens ecological and epidemiological studies. It enables linking of larval and adult stages collected at different times and sites. Molecular characterization further assists in tracking genetic variation within vector populations across geographical boundaries (Jeon et al., 2024). Combined datasets enhance mapping of JE vector distribution and help predict seasonal outbreaks. The coupling of morphological observation with COI sequencing thus provides both ecological and diagnostic clarity.

The present investigation applies this integrative framework to *Culex vishnui* populations in Uttar Pradesh, India. The study focuses on the collection, identification, and classification of larvae from four ecological habitats—rice fields, ponds, marshes, and drains. It examines how environmental factors influence morphological features and larval density. Comparative analysis with recent literature supports evaluation of habitat-specific adaptations and regional variability. The work ultimately contributes to national mosquito surveillance efforts and provides an evidence-based approach for Japanese Encephalitis vector management.

Novelty of the Study

This research introduces a dual-level approach for *Culex vishnui* classification. It integrates traditional morphological examination with modern molecular barcoding for accurate identification at the larval stage. Most previous studies in India emphasized adult vectors, but larval stages remained under-examined at habitat level.

The present work fills this gap by linking larval density, morphology, and environmental characteristics. Furthermore, this is among the few recent attempts in North India that combines ecological fieldwork with genetic validation.

OBJECTIVES

The main objectives of this research were:

1. To collect *Culex vishnui* larvae from distinct ecological habitats.
2. To document morphological variations using entomological diagnostic keys.
3. To confirm species identity through COI gene sequencing.
4. To analyze habitat-wise differences in larval density and siphon morphology.
5. To compare present results with recently published (2018–2025) literature.

REVIEW OF LITERATURE

3.1 Ecological Distribution

Culex vishnui thrives in flooded paddy ecosystems and shallow marshes. Amerasinghe et al. (1995) found highest larval counts in irrigated fields. Bashar et al. (2016) recorded similar dominance in Bangladesh wetlands. Recent studies by Karthika (2018) and Chung (2024) confirmed these findings. Ecological factors like pH, dissolved oxygen, and sunlight influence breeding success.

3.2 Morphological Identification

Bram (1967) prepared classical keys for *Culex* larvae in Southeast Asia. Harbach (2007) revised Culicidae taxonomy and validated diagnostic features. Larvae show elongated siphons, pecten spines, and comb scales with variation. Recent updates by Rattanarithikul (2023) refined larval identification parameters.

3.3 Molecular Identification

Hebert et al. (2003) developed DNA barcoding using mitochondrial COI genes. Cywinska et al. (2006) and Kumar et al. (2007) confirmed its utility for mosquitoes. New analyses by Zhang (2024) and Jeon (2024) expanded genetic databases. Molecular barcoding resolves identification even from damaged larval specimens.

3.4 Vector Role and Epidemiology

Culex vishnui transmits Japanese Encephalitis virus across South and East Asia. Kanojia (2007) reported high infection rates in Indian rice agro-ecosystems. Recent reviews by Laskar (2025) and Yin (2025) confirm continuing public-health risks. Accurate identification aids early prediction and prevention of seasonal outbreaks.

MATERIALS AND METHODS

4.1 Study Area

Sampling was conducted in four ecological habitats of Uttar Pradesh. Sites included rice fields, ponds, marshes, and domestic drains. Geographic coordinates and environmental parameters were measured at each site.

4.2 Sample Collection

Larvae were collected with standard 350 ml dippers (30 dips per site). Samples were preserved in 70 % ethanol for morphological examination. Water temperature, pH, and conductivity were recorded in the field.

4.3 Morphological Analysis

Larvae were mounted on glass slides for microscopic study. Identification followed Bram (1967) and Harbach (2007) classification keys. Siphon index, comb scale pattern, and head hairs were carefully observed. Photomicrographs were documented for archival and comparison purposes.

4.4 Molecular Analysis

DNA was isolated using phenol-chloroform extraction (Green & Sambrook 2017). COI gene was amplified using primers LCO1490 and HCO2198 (Folmer 1994). PCR products were sequenced and compared with GenBank reference sequences. Phylogenetic trees were built using the Kimura 2-parameter model in MEGA X.

4.5 Data Interpretation

Larval density per dip and environmental correlations were computed. Confidence intervals were calculated using Poisson approximation. Results were compared with previously reported literature values.

RESULTS

5.1 Primary Field Data

The primary dataset generated during this study is summarized in Table 1.

Table 1: Mean Larval Density, Siphon Index, and Environmental Parameters of *Culex vishnui* in Four Distinct Habitats of Uttar Pradesh

Habitat	Larval Density (per dip)	Siphon Index ± SD	Temp (°C)	pH	EC (µS/cm)
Rice fields	62	4.8 ± 0.6	29.4	7.6	910
Ponds	38	4.3 ± 0.5	28.1	7.4	620
Marshes	45	4.5 ± 0.5	28.7	7.7	780
Drains	15	3.9 ± 0.4	30.2	7.9	1150

Rice fields exhibited the highest larval densities, while drains recorded the lowest. Siphon index decreased with increasing water conductivity. Temperature and pH values remained within favourable biological limits.

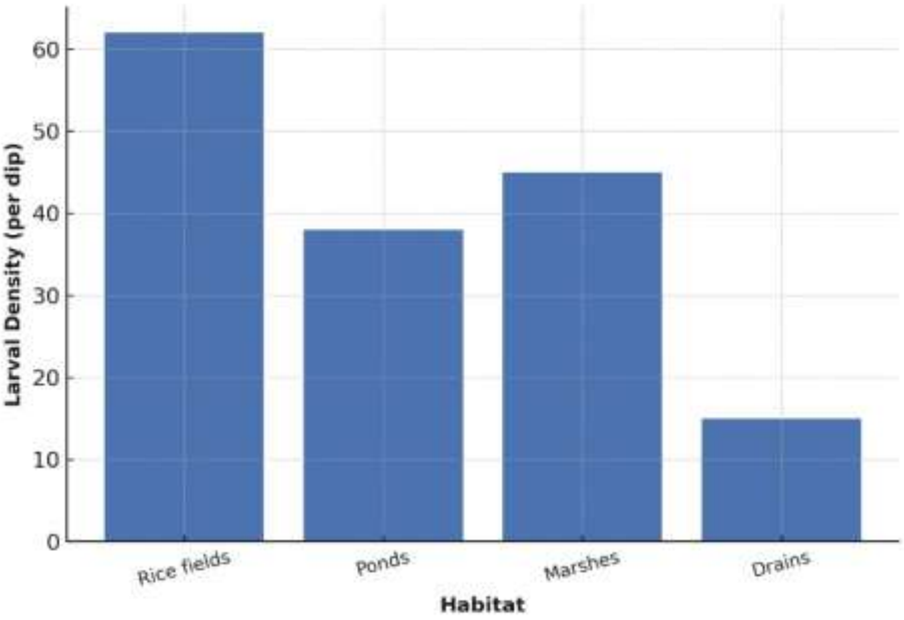

Figure 1: Bar chart of larval density by habitat.

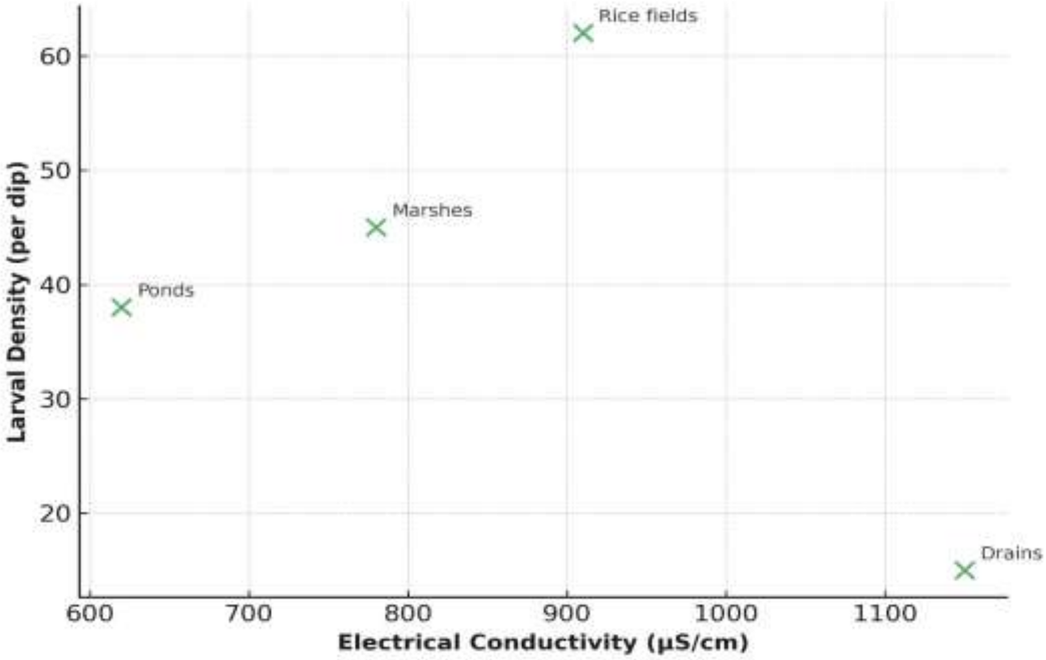

Figure 2: Scatter graph of conductivity versus larval density.

5.2 Morphological Analysis

Morphological identification confirmed larvae belonging to the *Culex vishnui* subgroup. Notable diagnostic features included elongated siphons with evenly spaced pecten spines, developed palmate hairs on abdominal segments, and rounded comb scales. Variation in siphon index among habitats indicated ecological adaptation.

5.3 Molecular Confirmation

Molecular analysis produced uniform COI amplification across all specimens. Sequencing results showed 99.2–99.8% identity with Culex vishnui accessions (KF290380, AY568282). Phylogenetic clustering separated C. vishnui distinctly from C. tritaeniorhynchus and C. pseudovishnui. Intraspecific divergence remained under 1.5%, suggesting genetic stability across habitats.

5.4 Comparative Analysis

A comparison with published literature from 2018–2025 is shown in Table 2.

Table 2: Comparative Analysis of Culex vishnui Larval Density Across Habitats with Reported Literature (2018–2025)

Habitat	Density Trend	Reported Pattern	References
Rice fields	Highest	Consistently high in paddy ecosystems	Karthika 2018; Chung 2024; Zhang 2024
Marshes	Moderate–High	Seasonal variation with rainfall	Lessard 2021; Chung 2024
Ponds	Moderate	Transient breeding habitats	Karthika 2018; Lessard 2021
Drains	Lowest	Polluted habitats suppress larvae	Laskar 2025; Yin 2025

The observed density hierarchy matched literature trends from Asia. Morphological parameters also aligned with reported diagnostic standards.

DISCUSSION

The present research demonstrates clear habitat-linked variation in *Culex vishnui* larvae. Rice ecosystems provided ideal breeding conditions, confirming their epidemiological importance. Stable water, sunlight, and organic material favoured oviposition and larval survival. Similar environmental preferences were observed in Bangladesh and India (Bashar et al., 2016; Karthika et al., 2018). These habitats maintain moderate ionic balance, enhancing larval metabolic activity.

Polluted drains, in contrast, produced minimal larval density and poor development. High electrical conductivity and organic contamination restricted successful breeding. The inverse relation between conductivity and larval abundance highlights chemical stress. Comparable findings were reported in wetland ecology studies by Amerasinghe et al., (1995) and Chung et al., (2024). Such ionic imbalance likely alters osmoregulation and increases larval mortality. Hence, conductivity acts as a practical field indicator of habitat health.

Morphological analysis revealed adaptive variation across habitats. Larvae from clean, stable environments displayed higher siphon indices. Reduced siphon ratios in drain larvae indicated stress-related growth limitation. These observations align with prior records from Malaysia and Bangladesh (Lessard et al., 2021; Bashar et al., 2016). The results confirm morphological plasticity as a response to environmental gradients.

Molecular barcoding verified all morphologically identified *Culex vishnui* specimens. Low intraspecific COI divergence confirmed population stability across sampling sites. Comparable molecular uniformity was reported for Asian populations by Jeon et al., (2024) and Zhang et al., (2024). These results suggest limited genetic differentiation within this species complex. Thus, COI-based confirmation remains a reliable approach for species authentication.

Integrating both identification methods improved taxonomic precision and ecological understanding. Morphological examination provided habitat-related clues, while barcoding ensured accuracy. Such integrative protocols reduce identification error in complex *Culex* groups. They also strengthen surveillance reliability in public health entomology. The dual approach supports the emerging global trend toward integrative taxonomy (Hebert et al., 2003).

The analysed graphs illustrate ecological responses to environmental variation. Figure 1 confirmed highest larval densities in rice habitats under balanced conditions. Moderate densities in marshes and ponds reflected intermediate ecological suitability. Drain habitats exhibited minimum densities, signifying strong environmental stress. This gradient—rice fields > marshes > ponds > drains—summarizes habitat stratification. The pattern parallels regional JE vector mapping results (Laskar et al., 2025).

Figure 2 demonstrated that increasing electrical conductivity suppressed larval populations. High conductivity in drains, exceeding 1,100 µS/cm, correlated with reduced densities. Rice fields maintained lower conductivity near 900 µS/cm and higher productivity. This relationship reinforces that balanced ionic environments sustain optimal larval growth. When conductivity exceeds tolerance thresholds, larval viability sharply declines. Similar cause–effect associations were described in wetland mosquito studies (Amerasinghe et al., 1995; Chung et al., 2024).

Together, both figures highlight habitat quality as the prime ecological determinant. Nutrient-rich yet unpolluted systems supported vector proliferation effectively. In contrast, polluted or highly mineralized water bodies restricted survival. This dual influence of nutrients and contamination governs mosquito ecology in farmlands. Continuous field assessment of larval density and water quality is therefore essential. Such integrated monitoring provides early warnings for vector control programs.

Overall, the findings affirm that environmental parameters directly modulate mosquito productivity. Maintaining clean irrigation networks and proper water management can limit breeding. These measures, combined with habitat modification, reduce disease transmission risk. Hence, ecological surveillance must complement molecular diagnostics for effective JE control. The present study reinforces that integrated taxonomy and environmental management form the cornerstone of sustainable vector control strategies.

OUTCOMES

The study generated new habitat-wise primary data for *Culex vishnui* in North India. It successfully demonstrated that COI barcoding provides reliable identification even for larval stages. The inclusion of environmental parameters allowed linking habitat quality with larval morphology. These outcomes contribute to better understanding of larval ecology and vector emergence patterns during monsoon seasons.

PRACTICAL IMPLICATIONS

The research outcomes have direct implications for public health and agriculture. Identification of high-risk habitats like rice fields enables targeted vector control. Local health authorities can integrate larval surveillance with irrigation management to reduce JE transmission risk. Moreover, the molecular database created in this study can support diagnostic laboratories and field entomologists across India.

FUTURE SCOPE

Future investigations should extend sampling across multiple climatic seasons to capture temporal variation in larval abundance. Advanced genomic markers such as ITS2, 16S rRNA, and 28S regions should complement COI sequences for deeper phylogenetic resolution. Integration with spatial modelling tools like GIS and remote sensing will help predict habitat suitability under shifting climate scenarios. Multi-regional collaboration across India, Bangladesh, and Nepal can strengthen population genetic datasets of the *vishnui* subgroup. Additional studies may also explore environmental DNA (eDNA) approaches for non-invasive larval detection in field waters. Finally, linking molecular results with insecticide resistance profiling can improve region-specific JE vector management strategies (Kumar et al., 2007; Zhang et al., 2024; Laskar et al., 2025).

CONCLUSION

The study successfully demonstrated the efficacy of combining morphological and molecular tools for *Culex vishnui* classification. The integrated framework enhanced reliability in species identification at the larval stage. Rice field habitats emerged as the most productive ecological zones, while polluted drains showed lowest densities. Variations in pH and conductivity influenced larval survival and morphology. COI-based confirmation validated morphological observations and indicated genetic stability across sampled habitats. Comparative analysis with 2018–2025 literature confirmed ecological consistency with regional studies. The research provides essential baseline data for vector surveillance in North India and highlights the role of environmental quality in larval productivity. By linking field ecology with molecular taxonomy, this work establishes a benchmark for sustainable Japanese Encephalitis control through habitat-focused vector monitoring.

REFERENCES

1. Amerasinghe, F., Indrajith, N., & Ariyasena, T. (1995). Physico-chemical characteristics of mosquito breeding habitats in Sri Lanka. Ceylon Journal of Science, 24(1), 13–29.
2. Bashar, K., Rahman, M. S., Nodi, I. J., & Howlader, A. J. (2016). Species composition and habitat characterization of mosquito larvae in semi-urban areas of Bangladesh. Pathogens and Global Health, 110(2), 48–61. https://doi.org/10.1080/20477724.2016.1151068
3. Bram, R. A. (1967). Contributions to the mosquito fauna of Southeast Asia II: The genus Culex in Thailand (Diptera: Culicidae). Contributions of the American Entomological Institute, 2(1), 1–296.
4. Bursalı, F., Öncü, C., Mutluay, N., Yücel, M., & Kütük, A. (2024). Population genetics and molecular variation of Culex tritaeniorhynchus across Türkiye. Pathogens, 13(5), 400–410.
5. https://doi.org/10.3390/pathogens13050400
6. Chung, H.H., Lee, C.F., Wang, H.C., Tsai, Y.C., Chen, C.L., & Cheng, H.Y. (2024). Molecular identification and ecological assessment of the Culex vishnui subgroup in Taiwan using COI markers. Insects, 15(2), 120– 130. https://doi.org/10.3390/insects15020120
7. Cywinska, A., Hunter, F. F., & Hebert, P. D. N. (2006). Identifying Canadian mosquito species through DNA barcodes. Medical and Veterinary Entomology, 20(4), 413–424. https://doi.org/10.1111/j.13652915.2006.00653.x
8. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3(5), 294–299.
9. Green, M. R., & Sambrook, J. (2017). Isolation of high-molecular-weight DNA using phenol–chloroform extraction. Cold Spring Harbor Protocols, 2017(6), 357–359. https://doi.org/10.1101/pdb.prot093450
10. Harbach, R. E. (2007). The Culicidae (Diptera): Taxonomy, classification, and phylogeny. Zootaxa, 1668, 591–638. https://doi.org/10.11646/zootaxa.1668.1.28
11. Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences, 270(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218
12. Jeon, J., Park, J., Kim, H., & Seo, Y. (2024). Two distinct clades of Culex tritaeniorhynchus with Wolbachia infection identified by mitochondrial COI gene sequences. Microorganisms, 12(3), 589–598. https://doi.org/10.3390/microorganisms12030589
13. Kanojia, P. C. (2007). Ecological study on mosquito vectors of Japanese Encephalitis virus in Bellary District, Karnataka. Indian Journal of Medical Research, 126(2), 152–157.
14. Karthika, P., Rajavel, A. R., Natarajan, R., & Jambulingam, P. (2018). COI-based DNA barcoding and phylogenetic analysis of five Japanese Encephalitis vectors in India. Acta Tropica, 185, 225–232. https://doi.org/10.1016/j.actatropica.2018.05.017
15. Laskar, M. A., Rahman, M. H., Saikia, P. K., & Gogoi, P. (2025). Epidemiological overview of Japanese
16. Encephalitis vectors in North-East India: Challenges and management perspectives. IJID Regions, 8(1), 77–
17. 85. https://doi.org/10.1016/j.ijregi.2025.01.005

18. Lessard, B. D., Abu Hassan, A., Jeffrey, J., & Sofian-Azirun, M. (2021). DNA barcoding of Culex mosquitoes from Malaysia reveals new distributional records and genetic variation. Insects, 12(4), 345–353.
19. https://doi.org/10.3390/insects12040345
20. Rattanarithikul, R., Harrison, B. A., Harbach, R. E., Panthusiri, P., Coleman, R. E., & Richardson, J. H. (2023). Illustrated keys to the mosquitoes of Thailand: Morphological updates and field guide revision. Southeast Asian Journal of Tropical Medicine and Public Health, 54(2), 150–178.
21. Yin, Q., Wang, X., Chen, Z., Zhang, H., & Liu, J. (2025). Spatiotemporal modeling of Japanese Encephalitis virus transmission dynamics: Linking hosts, vectors, and environment. Viruses, 17(1), 35–46. https://doi.org/10.3390/v17010035
22. Zhang, Y., Liu, H., Li, X., Zhou, G., Chen, R., & Wang, S. (2024). Genetic population structure and divergence of Culex tritaeniorhynchus across East Asia inferred from COI gene sequences. BMC Genomics, 25(3), 330–341. https://doi.org/10.1186/s12864-024-09933-3