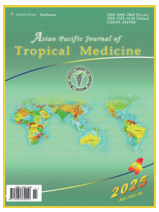

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First report of *Culex* (*Lophoceraomyia*) *cinctellus* in Sri Lanka: Evidence from morphological and molecular analysis

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ABSTRACT

Objective: To confirm the presence of *Culex* (*Cx.*) (*Lophoceraomyia*) *cinctellus* in Sri Lanka using morphological and molecular evidence.

Methods: From October 2019 to April 2020, mosquito surveillance was conducted fort-nightly in the Banduragoda Public Health Inspector area. Larvae were collected using standard siphoning methods, while adults were sampled using Cattle Baited Trap, Gravid Traps, Light Traps, Bird-Baited Traps, Dog Baited Traps, and diurnal human landing collections. Specimens were transported to the Entomology Laboratory at the Medical Research Institute for identification. Morphological identification was performed using standard taxonomic keys. Molecular confirmation was achieved through DNA sequencing of mosquito head and thoracic regions, followed by sequence analysis using NCBI BLAST and Geneious software (version 7.1.3).

Results: Adults of *Cx. cinctellus* were identified in Bird-Baited Traps and human bait collections. Unique morphological characteristics, including well-developed pulvilli, wing vein 1A ending before the apex of cross vein mcu, basal transverse pale bands on abdominal terga, and two labial basal setae on the proboscis, confirmed species identity. Morphometric measurements included mean thoracic length (0.58±0.02) mm, thoracic width (0.63±0.02) mm, abdominal length (2.15±0.03) mm, abdominal width (0.61±0.01) mm, and wing length (2.91±0.02) mm. Molecular analysis corroborated the morphological identification, affirming the species as *Cx. cinctellus*. *COI* sequences of the collected specimen (452 bp) were confirmed as *Cx. cinctellus* for sequence identity by BLAST and BOLD analysis. These sequences were subsequently deposited in GenBank under the accession number OR225623.1.

Conclusions: This study documents the first occurrence of *Cx. cinctellus* in Sri Lanka, highlighting the need to enhance

Summary

Question: Is *Culex* (*Cx.*) (*Lophoceraomyia*) *cinctellus* present in Sri Lanka?

Findings: Adults of *Cx. cinctellus* were reported from bird-baited traps and human bait collections within the Banduragoda Public Health Inspector area in the Gampaha District. Well-developed pulvilli, a wing vein 1A ending before the apex of the cross vein mcu, basal transverse pale bands on abdominal terga, and two labial basal setae on the proboscis, along with *COI* sequences (452 bp), confirmed the newly discovered species as *Cx. cinctellus*.

Meaning: It is necessary to conduct further investigations into the population dynamics and vector ecology of *Cx. cinctellus*.

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entomological surveillance to monitor its dispersal and population dynamics.

KEYWORDS: *Culex cinctellus*; Entomological surveillance; Morphological identification; Molecular confirmation; Mosquito checklist Sri Lanka

1. Introduction

The occurrence of unexpected outbreaks caused by arthropod-borne diseases is increasing rapidly across the globe, mainly due to human-induced changes in ecological landscapes[1]. A variety of arthropods, such as mosquitoes, ticks and sandflies tend to act as vectors, transmitting pathogens to vertebrate hosts. Vector-borne diseases have been one of the major health concerns in many countries, including Sri Lanka for decades. From time to time, Sri Lanka has been threatened by outbreaks of mosquito-borne diseases, such as malaria, chikungunya, Japanese B-encephalitis, yellow fever, filariasis and dengue[2].

Therefore, Sri Lanka has a strong entomological surveillance system, dating back to 1930s, which has evolved based on the changing epidemiology of vector borne diseases in the country[3]. The government institutions such as Anti-Malaria Campaign, National Dengue Control Unit, Medical Research Institute and the Anti Filariasis Campaign are engaged in controlling mosquito borne diseases in the country through their well-established public health networks[2]. In addition, various research groups stationed at national universities and other research institutions in Sri Lanka are conducting entomological surveillance to update and fill knowledge gaps on vector ecology and distribution patterns of medically important mosquitoes in the country.

In 2018, Gunathilaka[2] has updated the checklist of mosquito species found in Sri Lanka after 27 years from the last such record. This inventory includes 159 species of mosquitoes (including sibling species) listed under 19 genera (Diptera: Culicidae), namely; *Anopheles* Meigen, *Aedeomiya* Theobald, *Aedes* Meigen, *Verrallina* Theobald, *Armigeres* Theobald, *Heizmannia* Ludlow, *Culex* Linnaeus, *Lutzia* Theobald, *Ficalbia* Theobald, *Mimomyia* Theobald, *Hodgesia* Theobald, *Coquillettidia* Dyar, *Mansonia* Blanchard, *Orthopodomyia* Theobald, *Malaya* Leicester, *Topomyia* Leicester, *Tripteroides* Giles, *Toxorhynchites* Theobald, and *Uranotaenia* Lynch Arribalzaga. Among the 159 species listed, 23 species are considered endemic to Sri Lanka.

A recent preliminary surveillance conducted in the Banduragoda Public Health Inspector area in Mirigama Medical officer of Health (MOH) area, in the District of Gampaha, Western Province, Sri Lanka, has reported the presence of *Cx. cinctellus*, for the first time in Sri Lanka. The genus *Culex*, which includes many species of significant epidemiological importance, has been widely studied due to its role in transmitting pathogens such as arboviruses and filarial parasites[4]. However, species-level identification of *Culex* mosquitoes often remains challenging due to morphological similarities among closely related species. The integration of molecular techniques with morphological identification has significantly improved accuracy in species classification, enabling the identification of cryptic or lesser-known species.

Cx. cinctellus is a lesser-known mosquito species belonging to the subgenus *Lophoceraomyia* within the genus *Culex*. It is primarily recognized in Southeast Asia and parts of the Indian subcontinent, where it inhabits a variety of ecological niches[5]. While its role in pathogen transmission is not well documented, *Cx. cinctellus* has been sporadically reported in entomological surveys, indicating its presence in both rural and urban environments. Morphologically, this species is characterized by unique features such as the wing vein 1A ending before the apex of the cross vein mcu, the absence of pale scale patches on the mesokatepisternum and mesepimeron, and the presence of basal transverse pale bands on the abdominal terga[5].

These distinctive traits, combined with molecular identification using *COI* gene sequences, are essential for differentiating it from closely related species such as *Cx. quinquefasciatus*[6]. Although *Cx. cinctellus* is not widely studied, its inclusion in biodiversity assessments contributes to a deeper understanding of mosquito fauna and its potential implications for vector ecology and public health. Therefore, the current study was conducted to validate the presence of *Cx. cinctellus*, based on the morphological and molecular evidence. This finding is of significant importance for the vector control entities in Sri Lanka to strengthen their entomological surveillance activities and epidemic management measures.

2. Methods

2.1. Study sites

Entomological surveillance activities were conducted at fortnight intervals from October 2019 to April 2020 in the Banduragoda (7°15'04.630 8" N and 80°08'48.232 8" E) Public Health Inspector area in the Mirigama MOH area, in the District of Gampaha, Western Province, Sri Lanka. The Gampaha district of Sri Lanka covers an area of 1 387 km^2. It has 2 574 324 people, emerging as the highest residential population in Sri Lanka. The annual rainfall is about 2 500 mm, which is received mainly during two monsoonal

periods: from April to June and October to December. The District of Gampaha comprises 16 MOH areas[7].

2.2. Entomological surveillance

Adult mosquito specimens were collected from Cattle Baited Traps (CBT), Gravid Traps (GT), Light Traps (LT), Bird-Baited Traps (BBT), Dog Baited Traps (DBT) and Human Landing (HL) collection methods. The collected specimens were transported to the Entomology Laboratory of the Medical Research Institute, Sri Lanka. The collected live adult mosquitoes from the adult sampling collections were identified to species level, under the Dissecting Stereo Microscope (Olympus Optical Co. Ltd., Tokyo) using standard morphological keys[8].

2.3. DNA extraction

The head and thoracic regions of mosquitoes were removed using clean, sterile forceps. Genomic DNA was extracted using DNeasy blood and tissue® DNA extraction kit (Qiagen, Hilden, Germany)[9], according to the procedure supplied by manufacturer. The extracted DNA was stored at -20 ℃ until further analysis.

2.4. Polymerase chain reaction and DNA sequencing

DNA barcoding of mosquitos were performed targeting mitochondrial cytochrome oxidase subunit 1 (*COI*) gene (A 735 bp region) using the forward primer (5'-GGATTTGGAAATTGATTAGTTCCTT-3') and reverse primer (3'AAAAATTTTAATTCCAGTTGGAACAGC5')[10]. Polymerase Chain Reactions (PCR) were performed in a GeneAmp® PCR System 9700. The 50 μL PCR reaction consisted of 15 ng of extracted DNA, 1.5 mM $MgCl_2$ (Promega, USA), 0.2 mM dNTPs (Promega, USA), 1× reaction buffer (Promega, USA), 1.5 U Taq DNA polymerase (Promega, USA), and 0.3 μM of each primer.

The PCR reaction conditions were as follows: an initial denaturation of 95 ℃ for 5 minutes, followed by 5 cycles of denaturation at 94 ℃ for 40 seconds, annealing at 45 ℃ for 1 minute and extension at 72 for 1 minute. The amplification reaction was continued for another 35 cycles of denaturation at 94 ℃ for 40 seconds, annealing at 51 ℃ for 1 minute and extension at 72 ℃ for 1 minute, followed by a final extension at 72 ℃ for 10 minutes[10]. The resulting PCR products were visualized in 1.5% agarose gels stained with ethidium bromide. The PCR products were purified using Wizard® SV Gel and PCR Clean-up System (Promega, USA) and were sequenced at a commercial sequencing facility (Macrogen, Korea). The sequences were analysed for sequence identity using the Basic Local Alignment Search Tool analysis and by using 'Identification Request' function in BOLD[11].

2.5. Construction of phylogenetic tree

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. The bootstrap consensus tree inferred from 1 000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. This analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 920 positions in the final dataset. Evolutionary analyses were conducted in MEGA11[12].

2.6. Ethics approval and consent to participate

Ethical clearance for the study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka (Reference No.: P/17/02/2019; Date: 7 March 2019). All procedures followed during this study adhered to the ethical guidelines of Declaration of Helsinki.

3. Results

3.1. Entomological monitoring

A total of 11 565 mosquitoes (Diptera: Culicidae) were collected from the study areas by all entomological techniques (BBT, CBT, DBT, GT, HL and LT) performed from October 2019 to April 2020, as shown in Table 1. Morphological identification confirmed the abundance of 15 genera and 50 different mosquito species in the study area (Table 1). Among the collected mosquitoes, *Culex* genera denoted the highest abundance accounting for 71.9% (n=8 312), followed by *Armigeres* (12.2%, n=1 408). The adult mosquitoes that could not initially be identified were subsequently confirmed morphologically as *Cx. cinctellus*.

3.2. Morphological identification

The adult females were identified as *Cx. cinctellus* based on the distinctive morphological characters. Morphological features such as wing with vein 1A ending before the apex of cross vein mcu (Supplementary Figure 1), thoracic pleura without pale scale patches on mesokatepisternum and mesepimeron (Supplementary Figure 2), basal transverse pale bands in abdominal terga (Supplementary

Table 1. Total number of mosquitoes collected from the study area using various field techniques (BBT, CBT, DBT, GT, HL, and LT) during the study period (October 2019 to April 2020).

No.	Species	BBT	CBT	DBT	GT	HL	LT	Total
1	*Culex* (*Culex*) *tritaeniorhynchus*	0	4 406	1	0	7	0	4 414
2	*Culex* (*Culex*) *quinquefasciatus*	0	2	2	98	84	3	189
3	*Culex* (*Culex*) *gelidus*	0	2 782	0	0	33	1	2 816
4	*Culex* (*Culex*) *fuscocephala*	0	607	1	0	34	0	642
5	*Culex* (*Culiciomyia*) *pallidothorax*	6	1	0	7	0	0	14
6	*Culex* (*Culex*) *hutchinsoni*	0	3	0	0	0	0	3
7	*Culex* (*Culex*) *pseudovishnui*	0	52	0	0	0	0	52
8	*Culex* (*Oculeomyia*) *bitaeniorhynchus*	4	18	0	0	0	0	22
9	*Culex* (*Oculeomyia*) *infula*	16	2	0	0	0	0	18
10	*Culex* (*Culiciomyia*) *nigropunctatus*	11	107	0	0	0	0	118
11	*Culex* (*Lophoceraomyia*) *minutissimus*	13	3	0	0	1	0	17
12	*Culex* (*Lophoceraomyia*) *cinctellus*	3	0	0	0	4	0	7
13	*Culex* (*Eumelnomyia*) *brevipalpis*	0	0	0	0	0	0	0
14	*Lutzia* (*Metalutzia*) *vorax*	0	2	0	0	0	0	2
15	*Lutzia* (*Metalutzia*) *fuscanus*	0	11	0	0	0	0	11
16	*Ficalbia minima*	0	1	0	0	0	0	1
17	*Anopheles* (*Cellia*) *tessellates*	0	443	0	0	5	0	448
18	*Anopheles* (*Cellia*) *vagus*	0	286	0	0	3	2	291
19	*Anopheles* (*Cellia*) *kawari*	0	8	0	0	0	0	8
20	*Anopheles* (*Cellia*) *jamesii*	0	97	0	0	0	0	97
21	*Anopheles* (*Cellia*) *pseudo jamesii*	0	2	0	0	0	0	2
22	*Anopheles* (*Cellia*) *culicifacies*	0	4	0	0	0	0	4
23	*Anopheles* (*Cellia*) *aconitus*	0	1	0	0	0	0	1
24	*Anopheles* (*Anopheles*) *nigerrimus*	0	93	0	0	0	0	93
25	*Anopheles* (*Anopheles*) *barbirostris*	0	259	0	0	1	0	260
26	*Anopheles* (*Anopheles*) *peditaeniatus*	0	139	0	0	0	0	139
27	*Mansonia* (*Mansonioides*) *uniformis*	0	275	0	0	62	1	338
28	*Mansonia* (*Mansonioides*) *indiana*	0	10	0	0	0	0	10
29	*Toxorhynchites* (*Toxorhynchites*) *splendens*	0	0	0	0	0	0	0
30	*Coquillettidia* (*Coquillettidia*) *crassipes*	2	1	0	0	0	2	5
31	*Armigeres* (*Armigeres*) *subalbatus*	0	1 232	23	0	149	4	1 408
32	*Armigeres* (*Armigeres*) *aurolineatus*	0	0	0	0	0	0	0
33	*Aedes* (*Stegomyia*) *krombini*	0	0	0	0	0	0	0
34	*Aedes* (*Stegomyia*) *albopictus*	0	16	4	0	15	1	36
35	*Aedes* (*Aedes*) *vexsansvexsan*	0	3	0	0	0	0	3
36	*Aedes* (*Neomelaniconion*) *lineatopennis*	0	5	0	0	0	0	5
37	*Aedes* (*Aedes*) *vittatus*	0	5	0	0	2	0	7
38	*Aedes* (*Stegomyia*) *walbus*	0	1	0	0	0	0	1
39	*Aedes* (*Aedimorphus*) *pipersalatus*	0	39	0	0	0	0	39
40	*Aedes* (*Aedimorphus*) *pallidostriatus*	0	1	0	0	0	0	1
41	*Aedes* (*Finlaya*) *greenii*	0	2	0	0	0	0	2
42	*Aedes* (*Aedes*) *jamesii*	0	2	0	0	0	0	2
43	*Aedes* spp.	0	3	0	0	0	0	3
44	*Malaya genurostris*	0	0	0	0	0	0	0
45	*Orthopodomyia* (*Orthopodomyia*) *anopheloides*	0	0	0	0	0	0	0
46	*Orthopodomyia* (*Orthopodomyia*) *flavithorax*	0	0	0	0	1	0	1
47	*Tripteroides* (*Tripteroides*) spp.		0	2	0	0	0	0
48	*Heizmannia* (*Heizmannia*) *greenii*	0	0	0	0	0	0	0
49	*Uranotaenia* (*Pseudoficalbia*) *srilankansis*	0	1	0	0	0	2	3
50	*Mimomyia* (*Etorleptiomyia*) *luzonensis*	0	30	0	0	0	0	30
	Total	55	10 957	31	105	401	16	11 565

Note: BBT: Bird-Baited Traps; CBT: Cattle Baited Traps; DBT: Dog Baited Traps; GT: Gravid Traps; HL: Human Landing; LT: Light Traps.

Figure 3), well develop pulvilli (Supplementary Figure 4) and two labial basal setae in proboscis (Supplementary Figure 5) were observed from the adult mosquitoes. These characteristics helped to exclude any possible misidentification with the morphologically closely related *Cx. quinquefasciatus*.

Females of *Cx. cinctellus* reported a mean thoracic length of (0.58±0.02) mm, thoracic width of (0.63±0.02) mm. The average abdominal length and width were (2.15±0.03) mm and (0.61±0.01) mm, respectively, along with a wing length of (2.91±0.02) mm (Table 2). In case of the male mosquitoes, a mean thoracic length of (0.51±0.01) mm was reported, along with a mean thoracic width of (0.57±0.02) mm. Further, average abdominal length and width of the male *Cx. cinctellus* mosquitoes were (1.98±0.02) mm and (0.53±0.01) mm, respectively (Table 2).

Table 2. Morphometric parameters of adult *Culex cinctellus* mosquitoes collected during the study, with mean values and standard deviations.

Morphometric parameter	Sex	Measurement, mm
Thorax length	Female	0.58±0.02
	Male	0.51± 0.01
Thoracic width	Female	0.63±0.02
	Male	0.57±0.02
Abdominal length	Female	2.15±0.03
	Male	1.98±0.02
Abdominal width	Female	0.61±0.01
	Male	0.53±0.01
Wing length	Female	2.91±0.02
	Male	2.46±0.02
Wing width	Female	0.28±0.01
	Male	0.24±0.01

Data were expressed as mean±SD.

Figure 1. Phylogenetic tree constructed using *COI* sequences of *Culex cinctellus* (accession number OR225623.1). The tree was built using the MEGA software, highlighting the genetic relationships with closely related species. Bootstrap values are indicated at the branch nodes.

3.3. Genetic confirmation

COI sequences of the collected specimen (452 bp) were confirmed as *Cx. cinctellus* for sequence identity using the Basic Local Alignment Search Tool and BOLD analysis. These sequences were subsequently deposited in GenBank under the accession number OR225623.1.

3.4. Phylogenetic analysis

The Tamura 3-parameter model with a gamma distribution was selected for analyzing the *COI* sequence dataset, reflecting the most suitable model based on the Bayesian Information Criterion. This indicates that the dataset accounts for variable mutation rates across sites. *Mansonia* (*Mn.*) *bonneae* (GenBank sequence MK033673.1) was used as an outgroup, effectively rooting the phylogenetic tree. This establishes a baseline to compare evolutionary divergence and helps in inferring the direction of relationships. *Cx. cinctellus* (voucher Mirigama) clusters closely with other sequences identified as *Cx. cinctellus*. The genetic similarity and close grouping indicate minimal divergence within this species across the collected samples (Figure 1). The robust bootstrap support (based on 1 000 replicates) confirms the reliability of this grouping. A high bootstrap value suggests strong evidence for the close genetic relationship among these sequences. The grouping pattern suggests that the sampled populations of *Cx. cinctellus* are likely from the same or closely related genetic pool, with limited variation in the *COI* gene region. This could imply a restricted gene flow between geographically distributed populations or high conservation of the *COI* region within this species. The clear separation of *Mn. bonneae* as an outgroup reinforces the distinct evolutionary lineage of *Cx. cinctellus*. This contrast highlights genetic divergence between the studied species and the outgroup.

4. Discussion

The identification of *Cx. cinctellus* in Sri Lanka validates the hypothesis that mosquito species diversity in the region is likely underestimated due to gaps in surveillance and identification methodologies. Both morphological and molecular evidence strongly support this new finding. However, the absence of breeding or larval stages in this study raises critical questions regarding whether *Cx. cinctellus* has recently been introduced to the region or if its habitats remain undetected. This highlights the need for further investigation into its habitat preferences and reproductive behavior, which are essential for understanding its ecological role and potential as a vector.

Historically, the first list of mosquitoes in Sri Lanka was published by Green in 1901[13], documenting 20 species, of which 18 are still valid today. Subsequent studies by Theobald[14] in 1905 and 1910, followed by Christophers[15] and Barraud's[16] work, expanded the list to 92 species. By 1950, Carter[17] compiled a database of 125 mosquito species across 14 genera. Chow *et al.*[18] and Jayasekera & Chelliah[19] later modified this list, which was updated up to 140 mosquito species belonging to 16 genera by Amerasinghe in 1991[20]. The most recent checklist from 2018 includes 159 species across 19 genera, reflecting ongoing entomological surveillance in the country, particularly driven by the need to control mosquito-borne diseases[21]. This long history of mosquito research underscores the importance of continuing surveillance, particularly in light of emerging species like *Cx. cinctellus*.

To the best of our knowledge, this is the first report of *Cx. cinctellus* in Sri Lanka, supported by both morphological characteristics and *COI* gene-based DNA barcoding. This new addition necessitates an update to the national mosquito checklist. The discovery of this species highlights the ongoing efforts in Sri Lanka to expand mosquito surveillance and improve identification techniques, both morphological and molecular. As research advances, this will likely result in further additions to the mosquito species catalog.

The *Cx. cinctellus* specimens were collected from a rural area in the Gampaha district. The highest number of adult mosquitoes was captured through human landing collections (n=4), followed by BBT (n=3). While entomological surveys conducted in the Mirigama area confirmed the presence of *Cx. cinctellus*, no breeding sites or larvae have been identified in the country as of yet. This gap in knowledge underscores the need for further investigation into the distribution, habitat types, and breeding behavior of this species. Strengthening entomological surveillance in Sri Lanka is essential to fill these knowledge gaps and monitor the potential spread of *Cx. cinctellus*.

The phylogenetic analysis revealed a clear genetic structure within *Cx. cinctellus* populations, with the Tamura 3-parameter model and gamma distribution effectively capturing variability in mutation rates, consistent with established methodologies for mitochondrial DNA analysis[12]. The clustering of *Cx. cinctellus* (voucher Mirigama) with other sequences of the same species, supported by high bootstrap values, indicates strong genetic homogeneity, likely due to restricted gene flow or conservation of the *COI* gene region, as observed in other mosquito species[22]. The use of *Mn. bonneae* as an outgroup provided a robust phylogenetic baseline, confirming the evolutionary distinctiveness of *Cx. cinctellus* and aligning with previous studies that emphasize the utility of outgroups for rooting and evolutionary inference[23]. These findings underscore the potential of *COI* sequences in delineating species boundaries and elucidating genetic relationships within and between mosquito species, a critical factor for vector management and evolutionary studies[24].

Geographically, *Cx. cinctellus* has been documented across South and Southeast Asia, including India, Pakistan, Nepal, Myanmar, Thailand, Vietnam, and Indonesia. This mosquito species is typically found in rural and peri-urban landscapes, often near aquatic habitats such as rice fields, irrigation canals, and stagnant water bodies, which serve as breeding grounds. It has also been identified in forested areas and human settlements, demonstrating its adaptability to diverse ecological conditions[4,5]. Studies from India and Thailand suggest that *Cx. cinctellus* may play a role in transmitting arboviruses and filarial parasites, although its vectorial capacity requires further investigation. The species shows a preference for low-altitude areas but has also been found at higher elevations, indicating ecological versatility. Population density is influenced by seasonal variations, with peaks observed during or after monsoon periods, which correlate with increased availability of breeding sites[25].

In Sri Lanka, filariasis remains a public health concern, despite the success of mass drug administration programs that have significantly reduced infection rates. However, the threat of re-emergence persists due to a variety of ecological and socio-economic factors[26]. As *Cx. cinctellus* has been identified in areas such as Gampaha, which are at risk for filariasis, the species could be of medical interest as a potential vector. The presence of this mosquito species in human landing collections increases the likelihood of it contributing to disease transmission. Continued vigilance and enhanced surveillance are necessary to monitor its spread and assess its role in filariasis transmission cycles. Additionally, the findings underscore the importance of integrated vector management and sustained public health campaigns to prevent the resurgence of filariasis in Sri Lanka.

The detection of *Cx. cinctellus* in the Gampaha district, an area with endemic filariasis, underscores its potential as a vector of concern. While the species' vectorial capacity remains unverified, its presence in human landing collections suggests an affinity for human hosts, which raises the possibility of it playing a role in the transmission of filarial parasites[27]. Further studies are needed to investigate its role in the transmission cycles of filariasis and other vector-borne diseases[28]. This highlights the need for strengthened entomological surveillance to monitor vector populations and identify new vectors of public health importance[29].

Given the potential implications of *Cx. cinctellus* as a vector, this study advocates for a proactive approach in vector control policy. Strengthening collaborations between research institutions, public health entities such as the Anti-Filariasis Campaign, Anti Malaria Campaign and National Dengue Control Unit, in Sri Lanka and local mosquito control programs can help optimize resource allocation for entomological surveillance[21]. Additionally, capacity-building initiatives focused on advanced identification techniques and training for field staff would enhance the effectiveness of mosquito management programs.

While this study provides the first confirmed record of *Cx. cinctellus* in Sri Lanka, it is important to note that the research relied primarily on adult mosquito collections, which limits our understanding of the species' full ecological profile. Future research should prioritize surveys of larval habitats and conduct longitudinal studies to assess population dynamics. Evaluating the susceptibility of *Cx. cinctellus* to filarial parasites through laboratory infection studies will be crucial for clarifying its role in disease transmission. Additionally, expanding the geographical scope of surveillance efforts will help assess the distribution and potential for further spread of this species across the island.

In conclusion, this study reports the first confirmed presence of *Cx. cinctellus* in Sri Lanka, marking a significant addition to the island's mosquito fauna. Although no records exist regarding its disease transmission capabilities, this finding emphasizes the need for extensive mosquito surveillance to further investigate its biology and potential as a vector. As the threat of vector-borne diseases remains a public health concern, strengthening entomological surveillance and research into mosquito species like *Cx. cinctellus* will be essential for ensuring the effectiveness of control programs and preventing future outbreaks.

Conflict of interest statement

The authors declare that they have no competing interests.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. The generated sequence is available in the GenBank database under the accession number OR225623.1.

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Authors' contributions

PR, GK, NJ and WR: Conducted field surveillance and laboratory experiments, supported data analysis; LU: Wrote the manuscript and supervised the study; TR: Designed and supervised the research, wrote the manuscript; TK: Performed the molecular analysis and reviewed the manuscript. All authors read and approved the final manuscript.

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Publisher's note

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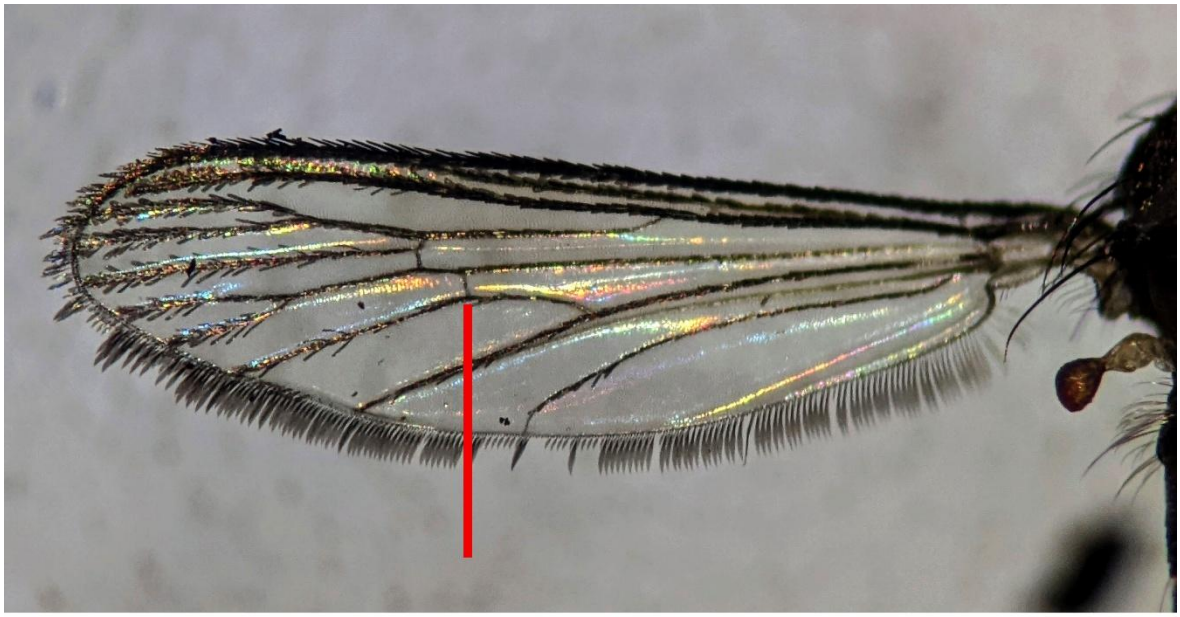
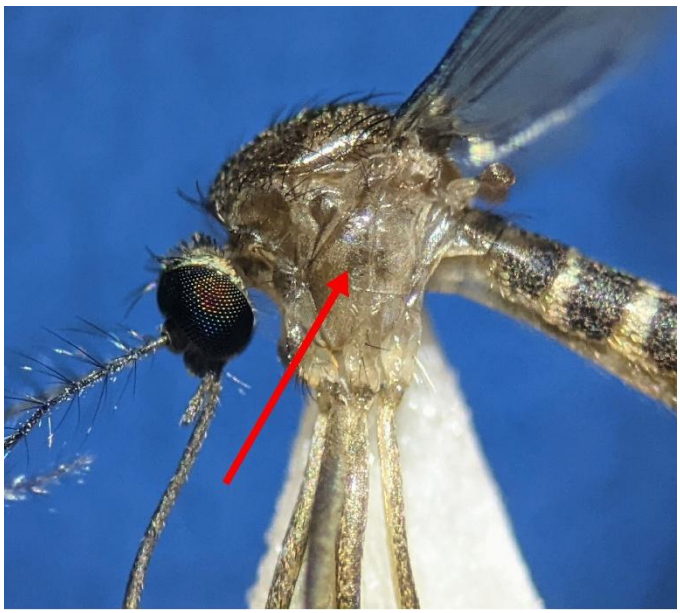
Supplementary Files

Figure Legends:

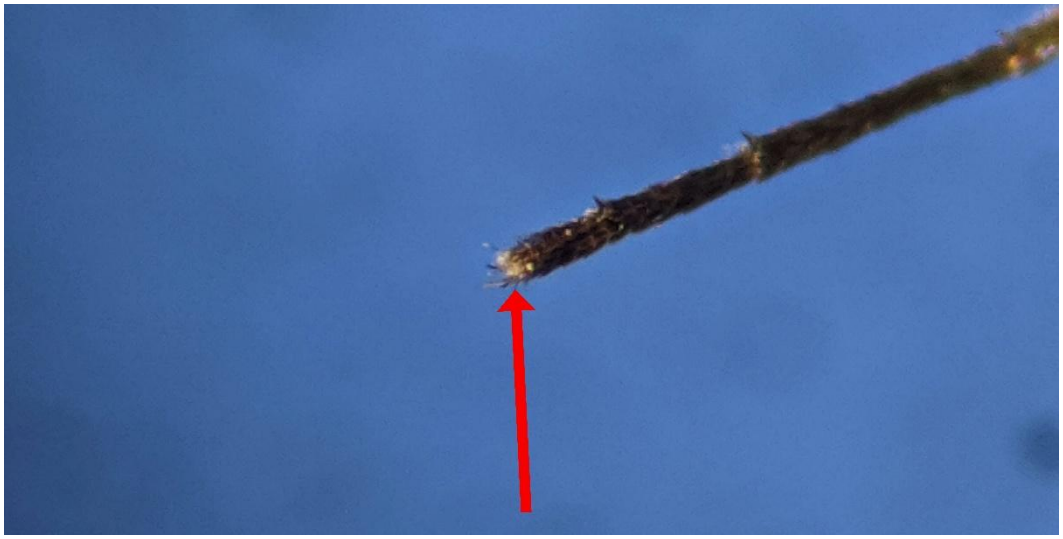


Supplementary Figure 1. Wing vein 1A of *Culex cinctellus* terminates before the apex of cross vein mcu, illustrating a distinguishing morphological feature.

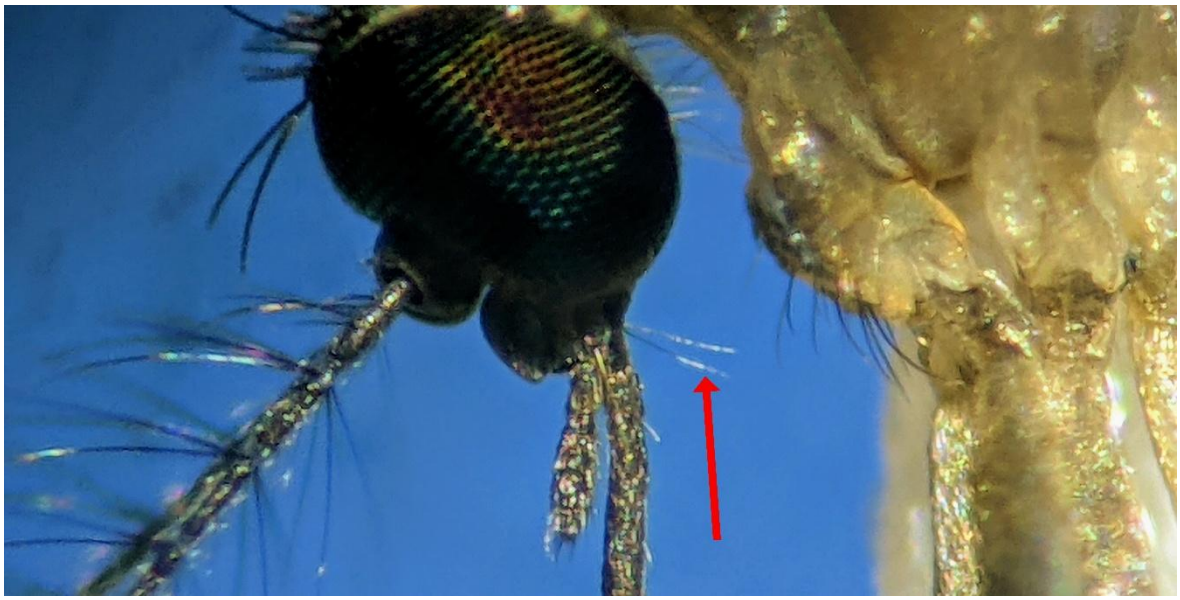


Supplementary Figure 2. Thoracic pleura of *Culex cinctellus*, showing the absence of pale scale patches on the mesokatepisternum and mesepimeron.

Supplementary Figure 3. Abdominal terga of *Culex cinctellus*, characterized by distinct basal pale bands, a key identification feature.



Supplementary Figure 4. Hind leg of *Culex cinctellus* displaying well-developed pulvilli, a morphological trait used for species differentiation.



Supplementary Figure 5. Proboscis of *Culex cinctellus* with two prominent labial basal setae, an essential diagnostic characteristic for identification.