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Culex (Culex) gaugleri, a new species (Diptera: Culicidae) from India

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Abstract

The present study describes *Culex (Culex) gaugleri* sp. nov. (Diptera: Culicidae) from Kodaikanal, Tamil Nadu, India based on morphological characters and the DNA sequences, after comparing with other closely related species. The presence of banded and spotted wing classified *Culex gaugleri* sp. nov. in the *Mimeticus* subgroup of the genus, *Culex* Linnaeus. The diagnostic characters of *C. gaugleri* sp. nov. are include the furcation of vein M on the wing without a pale spot and the anterior surface of all tibia without the longitudinal pale stripes. Further, phyletic relationship based on the mitochondrial cytochrome oxidase I (COI) gene indicates that *C. gaugleri* sp. nov. is closer to *Culex jacksoni* Edwards, 1934 compared to other species of the *Mimeticus* subgroup, *i.e., Culex mimuloides* Barraud, 1924, *Culex mimeticus* Noè, 1899 and *Culex tsengi* Lien, 1968. Similarly, mitochondrial 16s rRNA phylogeny includes both *Cx. mimeticus* and *Cx. gaugleri* in the same clade and separates from other mosquito species. These evidences suggest that *Cx. gaugleri* sp. nov. is a distinct species. Morphometric data generated on various attributes such as band length in wings are also significant in differentiating from other closely related species. Further studies on other life stages including eggs, larvae and pupae and vector bionomics are suggested.

Keywords: Molecular Phylogeny, Mosquito, New Species, Species Prevalence, Vectors

Introduction

Mosquitoes cause over 500 million cases of vector-borne disease annually (Aziz *et al.*, 2017; Doss *et al.*, 2017; Group, 2017). *Culex* mosquitoes are important vectors of human and animal diseases caused by nematodes and arboviruses such as filaria, West Nile virus, Japanese encephalitis etc (WHO, 2020). The information on the prevalence, distribution and biology of a mosquito species is crucial for the effective vector management and reduction of pathogen transmission risk. To grow *au fait* in mosquito biology the first step is, however, to inventorize the extant taxa under a known vector group of mosquitoes such as the genus *Culex* Linnaeus.

The genus *Culex* is highly diverse in morphology, categorizing in several subgroups including *Gelidus*, *Bitaeniorhynchus*, *Sitiens*, *Vishnui*, *Barraudi* and *Mimeticus* (Barraud, 1934; Sirivanakarn, 1976). The *Mimeticus* subgroup is comprised of 14 species that can be diagnosed from the presence of pale spots in wings in the genus *Culex* (Harbach, 2017; Sirivanakarn, 1976, Karlekar *et al.*, 2020; Somboon *et al.*, 2021a, b). Due to morphological complexity, this subgroup was subsequently divided into *mimeticus* and *mimulus* complexes using pale spot pattern (Sirivanakarn, 1976). However, molecular phylogenetics and DNA barcoding have been effectively used to identify closely related species, resolve complexes and understand the evolutionary pathways (Kumar *et al.*, 2007; Hemmerter *et al.*, 2007; Minard *et al.*, 2017). Recently, *Culex katezari* Karlekar, Andrew & Deshpande, 2020, *Culex bhutanensis* (Somboon & Harbach, 2020) and *Culex longitubus* Somboon, Namgay & Harbach, 2021 were reported from the same *Mimeticus* subgroup (Karlekar *et al.*, 2020; Somboon *et al.*, 2021a, b) using morphological and molecular analyses.

In the present study, we report a new species, *Cx. gaugleri* sp. nov. based on morphometric description and have explained the phylogenetic relationship with closely related species using mitochondrial gene sequences for COI and 16s rRNA. The results show that the unique morphometric features allude toward a new species, *gaugleri* sp. nov. which is phylogenetically closer to *Culex mimeticus* Noè, 1899 in the *Mimeticus* subgroup.

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Materials and Methods

Mosquito Collection, Rearing and Identification

Larvae of *Culex* sp. nov. were collected from water bodies including ditches, pools and water channels near the villages of Kodaikanal regions, Tamil Nadu, India located at a high altitude ranging from 1,500 – 2,000 m. Larvae were laboratory-reared under standard conditions of 26 ± 1°C, 65 ± 5% RH and 12:12 h light-dark photoperiod. Dried Brewer's yeast (30 mg/l) was provided as food (Suman *et al.*, 2011). Pupae were transferred to adult cages (12 cm x 12 cm x 12cm) for adult emergence, and adults were provided 10% sugar solution *ad libitum* for feeding. Adults were anaesthetized and pinned for dry preservation. The taxonomic keys of Sirivanakarn (1976), Barraud (1934) and Rattanarithikul *et al.* (2005) were used for species identification. The terminology for morphological features of adults and wing venation system were followed to Rattanarithikul (1982) and Sirivanakarn (1976), respectively.

Morphology and Morphometric Analysis

Female mosquitoes were photographed using Leica M205A montage stereomicroscope and measured for morphometric studies with Leica Application Suite (LAS) V 4.5.0 software package. The length was measured from occiput to pedicel for the head, labellum to base for proboscis, pedicel to the apex of palpus for maxillary palpus, and anterior pronotum to scutellum for the thorax. Abdomen length was measured dorsally from segment II to VIII. Legs were measured from basal end to knee spot for the femur, from femora-tibio joint and tibio-tarsal joint for tibia, from tibio-tarsus joint to the terminal end of tarsomere V except for claws for tarsus and from basal to the apex of their pale spots for tarsomeres. Wing length was measured from alula to distal part of wing excluding fringe scales. Each wing vein was measured from its base to the apical end. All measurements are represented in mean ± standard error (S.E.) unless otherwise noted.

DNA Isolation, Amplification and Gene Sequencing

The left fore- and mid-legs of *C. gaugleri* sp. nov. were excised for Genomic DNA and processed with Macherey-Nagel DNA nucleospin purification kit (Macherey-Nagel, GmbH & Co., KG, Duren, Germany) following the manufacturer's protocol with slight modification in lysis and digestion temperatures. Cytochrome oxidase I and 16s rRNA genes were considered for amplification. For PCR of COI gene, the primers were F-LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') (Folmer *et al.*, 1994) and R-COI650 (5'-TAG CAG AAG TAA AAT AAG CTC G- 3') (Hemmerter *et al.*, 2007). COI-PCR reactions were performed in a 25 µL volume containing 2 µL of DNA, 12.5µL of a PCR mixture with dNTP, $MgCl_2$ and Taq DNA polymerase, 1.5µL of each primer and 7.5µL nuclease-free water. The amplification of COI gene was performed using an initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 30 s, 45°C for 45 s and 72°C for 45 s. The final extension was conducted at 72°C for 5 min. 16s rRNA amplification was performed using primers- Forward (5'-CGC CTG TTT ATC AAA AAC AT-3') and Reverse (5'-CTC CGG TTT GAA CTC AGA TC-3') (Shouche and Patole, 2000) using initial denaturation at 94°C for 4 min, followed by 40 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. the final extension was conducted at 72°C for 10 min. Reagents were purchased from Himedia Inc., India and primers were synthesized from Sigma-Aldrich Inc., India.

The PCR product was confirmed in 1 % agarose gel and conducted Sanger sequencing on Applied Biosystems units (Biokart Solutions Inc. Pvt. Ltd, Bangalore, India). The analysed sequences were submitted to GenBank.

Phyletic Analysis using COI and 16s rRNA Gene Sequences

Using Bioedit V 7.2.5 (Hall, 1999), the forward and reverse sequences of COI and 16s rRNA genes of *Cx. gaugleri* sp. nov. were aligned and compared with other mosquito species sequences obtained from GeneBank (https://www.ncbi.nlm.nih.gov/genbank/). For COI phylogenetic analysis, sequences such as *Cx. mimeticus*- MK402796, MK402806, LC054532, KJ012101, MW476159, KF406801, near *Cx. tsengi*- MW476160, MW476154, *Culex jacksoni* Edwards, 1934- MW476157, near *Cx. tianpingensis*- MW476150, MW476151, MW476156, *Cx. bhutanensis*- MW476155, MW476149, MW476147, MW476152, MW476153, *Cx. longitubus*- MW476151, MW476150, MW476156, *Culex quinquefasciatus* Say, 1823- MF172299, *Culex mimuloides* Barraud, 1924- EU259294, *Culex mimulus* Edwards, 1915- MG774467, KF564751, KF564749, KF564748, KF564747, *Culex orientalis* Edwards, 1921- LC054469, LC054468, AB690841, LC054467, LC054470, LC054471, MW476163, *Culex murrelli* Lien, 1968- MW476161, MW476162, and for 16s rRNA, *Cx. mimeticus*- EF593021,

Anopheles stephensi Liston, 1901- AF034467, *Culex vishnui* Theobald, 1901- EF593018, *Culex tritaeniorhynchus* Giles, 1901- AF034469, *Culex bitaeniorhynchus* Giles, 1901- EF593019, *Cx. quiquefasciatus*- EU711092 were used. All sequences were aligned and trimmed to get homologous regions of COI and 16s rRNA genes using MEGA V 10.1.8 (Kumar, 2018). Phylogenetic trees for COI and 16s rRNA were constructed using the maximum likelihood statistical method based on Kimura-2-parameter substitution model (Kimura, 1980), Nearest-Neighbor-interchange heuristic method taking bootstrapped 1000. Pairwise genetic distance was estimated using Kimura-2-parameter substitution model with 1000 bootstrap replications.

Results

Taxonomy

Order DIPTERA Linnaeus, 1758
Family CULICIDAE Meigen, 1818
Subfamily CULICINAE Meigen, 1818
Tribe **Culicini** Meigen, 1818
Genus ***Culex*** Linnaeus, 1758

Culex (Culex) gaugleri sp. nov. Suman (Figure 1, 2, 3, 4)

Type material: Holotype: 1 female: India, Tamil Nadu, Kodaikanal hills, 10°13'23"N 77°20'48"E, 3-iii-2019, collected D.S. Suman (21927/H6). Paratype: 1 Female: same locality data as holotype (21928/H6).

Type locality: India, Tamil Nadu, Kodaikanal hills.

LSID: urn:lsid:zoobank.org:pub:E5656E2F-4F5A-44BB-B109-D3A11776DC69

Figure 1. *Culex gaugleri* sp. nov. (holotype, female) a lateral view of entire mosquito.

Type depository: The type material is deposited in Diptera section, Zoological Survey of India, Kolkata (NZSI).

Diagnosis: This new species contains the following diagnostic features that can be used to distinguish from other species of the *Mimeticus* Subgroup: (1) absence of pale spot at the furcation of M vein, (2) the furcation points of vein R_{2+3} and M are at the same levels, and (3) absence of anterior tibial longitudinal pale stripe on fore-, mid- and hind legs. The presence of a completely dark area at vein M furcation explicitly differentiates *Cx. gaugleri* sp. nov. from the species having prominent pale spots on the furcation of vein M *i.e. Cx. jacksoni Cx. mimeticus, Culex diengensis* Brug, 1931, *Cx. mimuloides,*

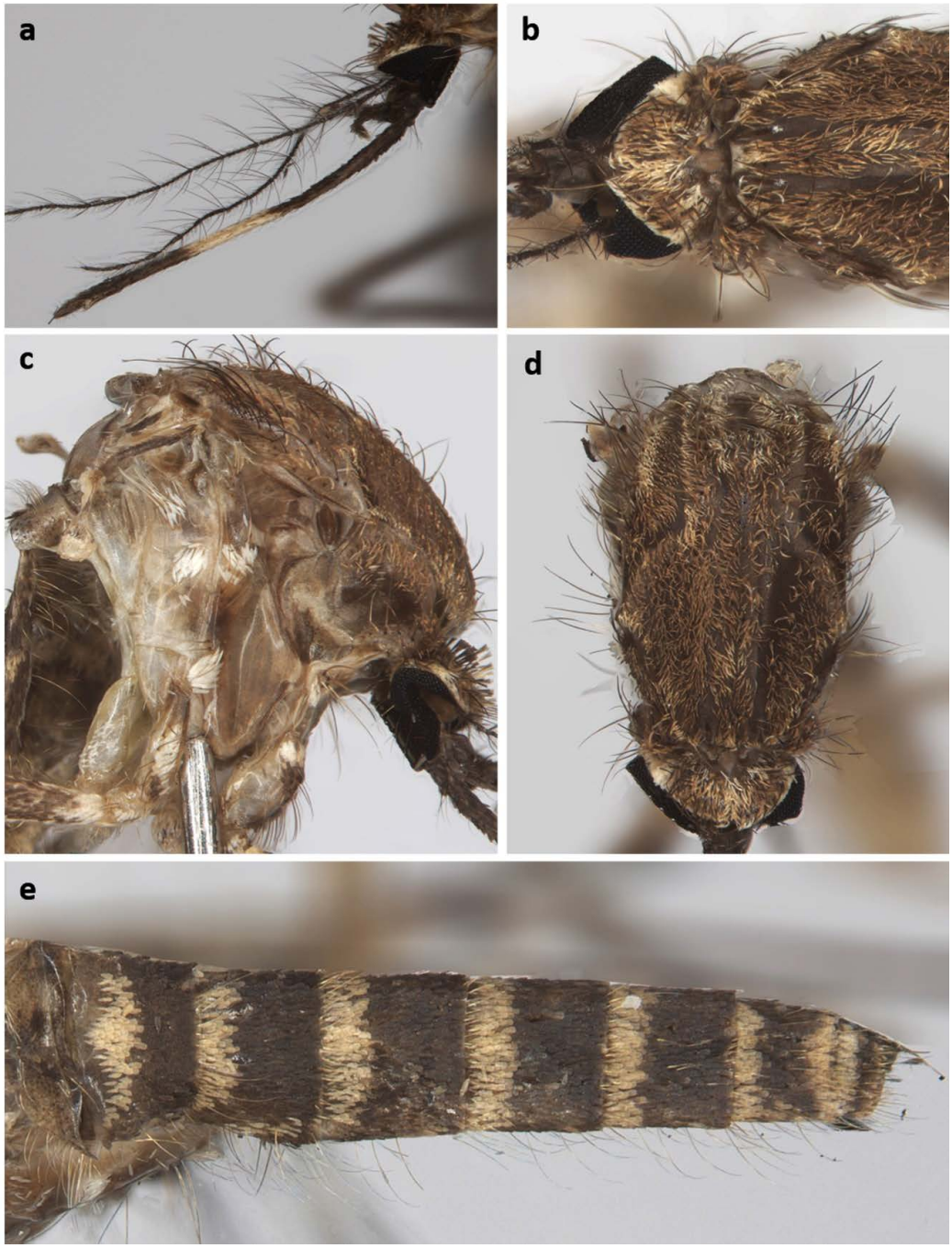

Figure 2. *Culex gaugleri* sp. nov. (holotype, female): **a)** proboscis and maxillary palpi, **b)** head, **c)** lateral thorax, **d)** scutum, and **e)** dorsal abdomen.

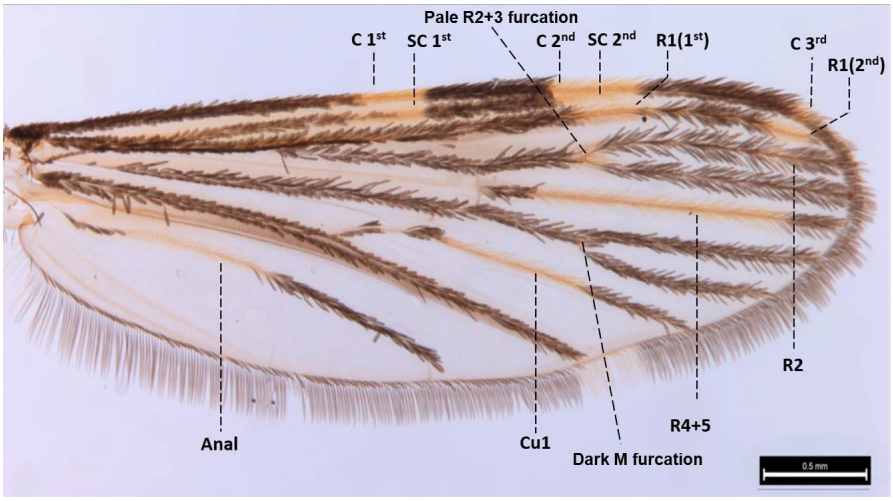

Figure 3. *Culex gaugleri* sp. nov. (holotype, female), wing with pale spots representation on different veins and dark spot at M-vein.

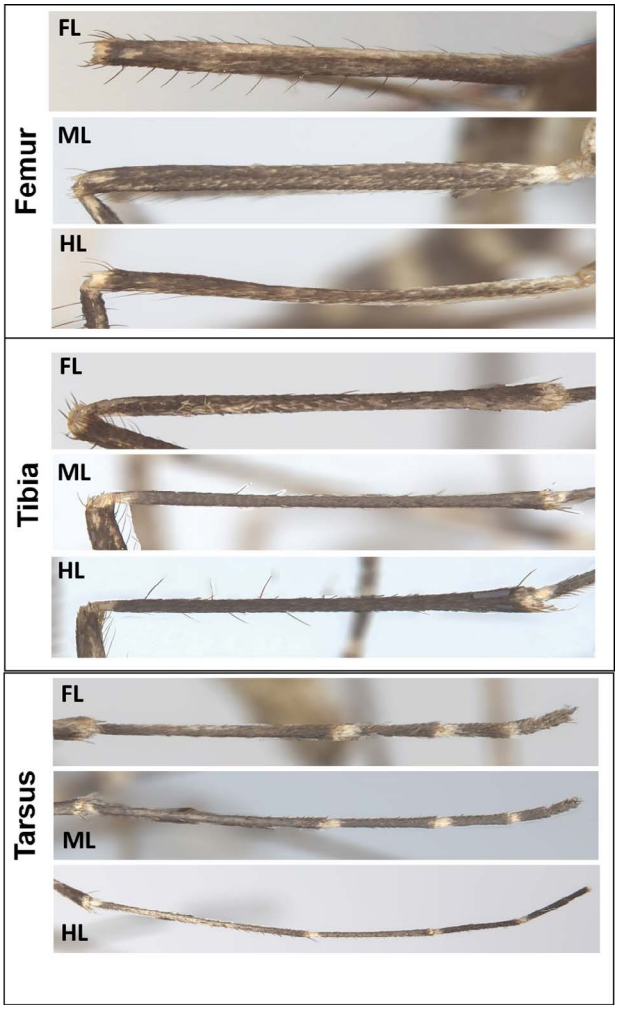

Figure 4. *Culex gaugleri* sp. nov. (female, holotype), legs. Anterior view. Abbreviations: FL - Fore leg, ML – Mid leg, HL – Hind leg.

Cx. mimulus, Cx. orientalis, *Culex tsengi* Lien, 1968 and *Cx. katezari*. Besides, the occurrence of R2 pale spot in the apical wing region of *Cx. gaugleri* sp. nov. differs from *Cx. tsengi* and *Culex propinquus* Colless, 1955. The furcation points at same level in vein R_{2+3} and M of *Cx. gaugleri* sp. nov. differentiate from *Cx. mimuloides*. The absence of anterior tibial longitudinal pale stripe in *Cx. gaugleri* sp. nov. differs from *Cx. mimeticus* and *Cx. mimuloides* (pale stripe on the hind tibia) and *Cx. jacksoni, Cx. tsengi* and *Cx. mimuloides* (mid tibia). Basal pale bands on abdominal terga from segment II-VI of *Cx. gaugleri* sp. nov. differ from *Culex fasyi* Baisas, 1938 having apical pale bands on terga from segment III-VI.

Description (holotype, female): *Culex gaugleri* sp. nov. females were large in size and slender, brownish with a slightly golden scale appearance with pale white scaling at anterior and mid-proboscis, maxillary palps, vertex, lateral thorax, abdominal segments, legs and wings in different formations.

Head: (Figure 2a, b). 0.33±0.00 mm long. Vertex with numerous pale and white erect scales distinguishable from the top view. Maxillary palpus (0.34±0.01 mm long) dark brown with white pale scaled tip. Proboscis (2.25±0.15 mm long) round slender and with median broad pale ring (0.50±0.00 mm) located anteriorly from the centre. Antenna 1.91±0.32 mm long, entirely dark brown in colour.

Thorax: (Figure 2c, d). 1.71±0.07 mm long. Scutum integument ornamented with numerous narrow golden-

brown and white scales on a dark brown surface. Acrostichal, supraalar, prescutelar and scutellum areas with narrow white scale patches. Long setae: 8-10 post-pronotal, 8 prealar knob, 4 upper mesokatepisternum, 2 lower mesokatepisternum, 12 antepronotal, 22 scutellum, 25-28 prescutellar, 10-12 acrostichal, 15-18 dorso-central and 10 upper mesepimeron. Prespiracular and lower mesepimeron setae absent. Pleuron light brown. Golden-yellowish scales similar to scutum scales present on antepronotum and postpronotum. White spatulate scale patches ornamented on upper mesokatepisternum, anterior mesepimeron, adjacent of lower mesokatepisternum, proepisternum, and upper region of procoxa, mesocoxa and metacoxa.

Wing: (Figure 3)**.** Elongated, 3.96 ± 0.13 mm long, 1.03 ± 0.07 mm wide. Veins with white-pale spots. Vein C with 1st, 2nd, 3rd costal pale spots present at sectorial, subcostal and apical areas respectively. 1st Costal pale spot at the middle of vein C along with 1st subcostal pale spot. 1st Costal pale spot larger than 1st subcostal pale spot. Second costal pale spot at 0.75 of vein C parallel to 2nd subcostal pale spot and 1st R1 pale spot. Third costal pale spot at apex of vein C. A tiny pale spot present at 0.7 towards apical end of vein R2. Furcation of R_{2+3} and M present at same level with pale spotting pattern of furcation R_{2+3} only. Vein R_{4+5} extensively pale spotted (basal 0.15 to apical 0.8) and similar to the length between humeral end of 2nd costal and humeral end of 3rd costal pale spot. Vein R, rs, M, M_{1+2} and M_{3+4} dark and without pale spot. Pale spot of vein Cu1 ranges from basal 0.1 to apical about 0.6 parallel to apical end of 1st costal pale band and furcation of vein R_{2+3}. Vein Cu and Cu2 without pale spot. Anal vein pale spot covers between basal 0.15 to apical 0.51. A pale fringe spot located ahead of vein Cu2 end. The lengths of various veins and associated pale spot are summarized in Table 1.

Legs: (Figure 4)**.** *Fore leg:* 7.08 mm long covered mainly with dark brown scales. Fore femora 1.91 mm long with dark brown scales scattering pale scale distribution anteriorly, apical pale spots present. Fore tibia 2.25 mm

Table 1. Morphometric for the length of wing veins and pale spot of *Culex gaugleri* sp. nov. All values represent the mean ± standard error

Vein	Length (mm ± SE)	Pale spot	Length (mm±SE)
Costa	3.82 ± 0.07	Costal 1st	0.30 ± 0.04
		Costal 2nd	0.33 ± 0.08
		Costal 3rd	0.09 ± 0.003
Subcosta	2.68 ± 0.11	Subcostal 1st	0.31 ± 0.02
		Subcostal 2nd	0.16 ± 0.07
Radius	1.25 ± 0.001	-	-
R1	2.51 ± 0.10	R1 (1st)	0.25 ± 0.04
		R1(2nd)	0.22 ± 0.01
R2	1.22 ± 0.02	R2	0.17 ± 0.06
R3	1.24 ± 0.04	-	-
Rs	0.89 ± 0.08	-	-
R_{2+3}	0.50 ± 0.01	-	-
R_{4+5}	1.68 ± 0.05	R_{4+5}	1.16 ± 0.03
r-m	0.14 ± 0.006	-	-
Media	2.47 ± 0.04	-	-
M_{1+2}	1.14 ± 0.02	-	-
M_{3+4}	0.92 ± 0.02	-	-
m-cu	0.42 ± 0.01	-	-
Cu1	1.25 ± 0.04	Cu1	0.66 ± 0.04
Cu+Cu2	2.62 ± 0.06	-	-
Anal vein	1.94 ± 0.09	Anal	0.64 ± 0.04

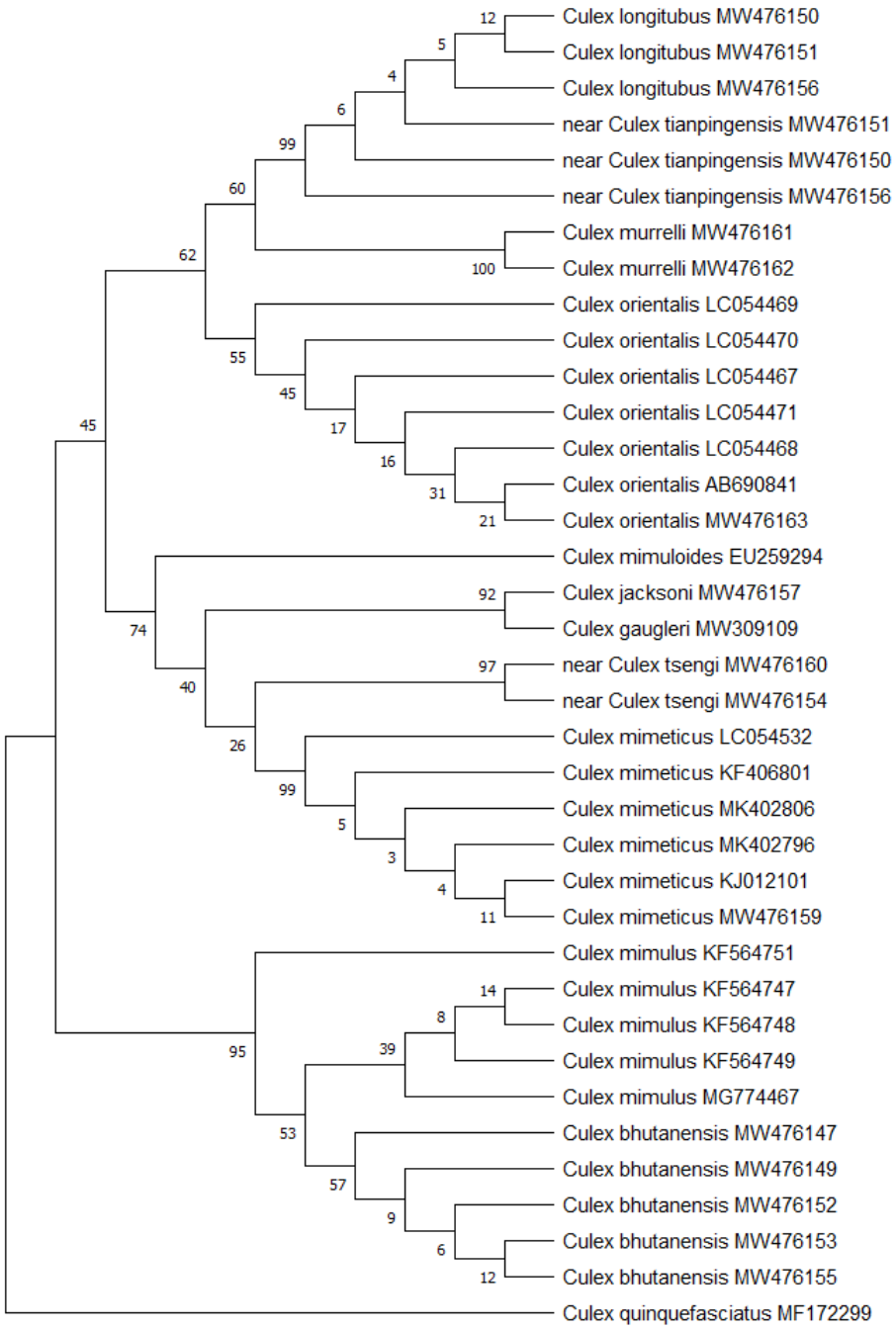

Figure 5. Phylogenetic tree of *Culex gaugleri* sp. nov. with other mosquitoes using mitochondrial cytochrome oxidase I (COI) gene sequences constructed with maximum likelihood method (1000 boot straps).

long covered with entirely dark scales with no pale stripe. Tibio-tarsus joint with pale spot. Tarsus 2.90 mm long. Tarsomere - I: 1.38 mm, II: 0.49 mm, III: 0.32 mm, IV: 0.13 mm, V: 0.13 mm long and I-IV entirely dark brown with basal pale band. *Mid leg:* 7.97 mm long. Femur 1.93 mm long dark brown with basal and apical pale spots. Tibia (2.41 mm long) without a longitudinal pale stripe. Tarsus (3.62 mm long) with tarsomere I-IV with basal pale band.

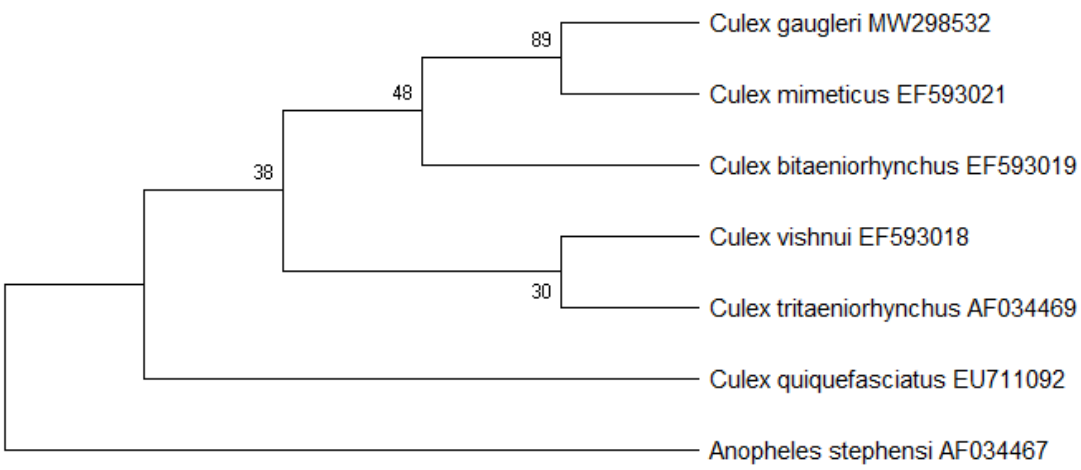

Figure 6. Phylogenetic tree of *Culex gaugleri* sp. nov. with other mosquitoes using mitochondrial 16s rRNA gene sequences constructed with maximum likelihood method (1000 boot straps).

Tarsomere – I: 1.60 mm, II: 0.66 mm, III: 0.40 mm, IV: 0.22 mm, and V: 0.19 mm long. *Hind leg:* 10.34 mm long. Hind leg femur 2.36 mm long with dark anterior surface with scattered pale scales. Apical pale spots present. Hind tibia 2.38 mm long entirely dark without any longitudinal pale stripe. Tarsus 5.60 mm long. Tarsomere I with pale stripe anteriorly. Tarsomere II-IV entirely dark without pale stripe. All tarsomere with basal pale bands except tarsomere V. Tarsomere I: 2.32 mm, II: 1.22 mm, III: 0.90 mm, IV: 0.45 mm, V: 0.23 mm long.

Abdomen: (Figure 2e). 2.94 mm long. Abdominal terga II-VI covered with broad basal pale bands. Segment VII covered with basal and apical pale band. Segment VIII with basal pale band. Pale scales on basal pale band of segment I slightly extending forward in median triangle. Centre region of each dorsal band consisted of denser pale scales than edges.

Male and Immature stages: Unknown.

Etymology: The new species is named after Prof. Randy Gaugler of Rutgers University, NJ, USA, in recognition of his significant contributions to vector biology and management.

Comments: The *Mimeticus* subgroup belongs subgenus *Culex* of genus *Culex*. The *Mimeticus* Subgroup mosquitoes can be distinguished from other *Culex* mosquitoes with the presence of pale spots and band on the veins of the wing.

Distribution: Kodaikanal hills, Tamil Nadu, India.

Bionomics: All the specimens were collected as larvae from the water pools collected near the village. Nothing is known about the resting and biting behaviour of the adult females of the species.

Phylogenetic analysis Using COI and 16s rRNA Gene Sequences

For COI, 678 bp sequence was obtained and submitted to GenBank repository (accession no. MW309109). The sequence included 31.12 % A, 38.49 % T, 15.04 % G, and 15.33 % C nucleotides. The sequence of the 16s rRNA gene was 496 bp long and comprised of 36.69 % A, 38.50 % T, 15.72 % G, and 9.07 % C nucleotides (NCBI accession no MW298532).

The phylogenetic tree analysis based on COI gene sequences resulted in multiple clusters of *Culex* species. The members of *mimeticus* species complex formed an upper cluster in which *Cx. gaugleri* sp. nov. formed a clade with *Cx. jacksoni* whereas near *Cx. tsengi* formed a separate clade and shows a close relationship with *Cx. mimeticus*. *Culex mimulus* formed separate clades and near *Cx. tianpingensis* form a cluster with *Cx. murrelli* (Figure 5). The phylogenetic tree based on 16s rRNA gene sequences of *Cx. gaugleri* sp. nov. showed a close relationship with *Cx. mimeticus* whereas *Cx. vishnui* showed clade with *Cx. tritaeniorhynchus* (Figure 6).

Discussion

Approximately 3583 mosquito species are reported globally (http://mosquito-taxonomic-inventory.info/) with India native to 404 species in 50 genera (Tyagi *et al.*, 2015). The present study provides a taxonomic diagnosis of *Cx. gaugleri* sp. nov. with morphological, morphometric and phylogenetic analysis. The subgenus *Culex* of genus *Culex* contains 26 species from India that includes six species of *Mimeticus* subgroup *i.e. Cx. mimeticus, Cx. jacksoni, Cx. mimulus, Cx. murrelli, Cx. mimuloides*, and *Cx. katezari* (Tyagi *et al.*, 2015; Karlekar *et al.*, 2020). *Mimeticus* subgroup species are well demarcated from other *Culex* mosquitoes with the presence of pale spots on wings and banded proboscis. Morphologically, the absence of pale spots on vein C, R, Cu (humeral area) and vein R1 (sectorial area) positioned *Cx. gaugleri* sp. nov. in *mimeticus* complex rather than the *mimulus* complex of *Mimeticus* subgroup (Matsuo *et al.*, 1974; Sirivanakarn, 1976). In the case of *mimulus* complex species *i.e. Culex murrelli*, *Cx. orientalis* and *Cx. propinquus,* a pale spot found on vein R1 in sectorial area which is absent in *Cx. gaugleri* sp. nov. Recently, *Cx. katezari* and *Cx. bhutanensis,* and *Cx. longitubus* new species were reported in *mimulus* complex having R1 and extending pale spot on vein Cu1 (Karlekar *et al.*, 2020; Somboon *et al.*, 2021a, b). However, *Cx. katezari* contains a distinct dark area on vein R_{4+5} in comparison to *Cx. gaugleri* sp. nov.

The distribution of white or pale scales in the form of spots or stripes of *Culex* mosquitoes is significant in species differentiation (Matsuo *et al.*, 1974; Sirivanakarn, 1976; Rattanarithikul *et al.*, 2005). Based on descriptions provided by Sirivanakarn (1976), the presence of a completely dark area at vein M furcation explicitly differentiates *Cx. gaugleri* sp. nov. from the species having prominent pale spots on the furcation of vein M *i.e. Cx. mimeticus, Cx. diengensis, Cx. mimuloides, Cx. mimulus, Cx. orientalis*, *Cx. tsengi* and *Cx. katezari*. Sirivanakarn (1976) described that *Cx. jacksoni* contains dark brownish scales on scutum, speckled anterior surface of forefemur, mid tibia with longitudinal pale stripe anteriorly, 3rd coastal spot of wing sometimes involves only apical portion of vein R_1 and furcation of vein M usually having pale scales. However, Lien (1968) has shown the presence of pale spots on both furcations at M and R_{2+3} veins of *Cx. kangi* (synonymized as *Cx. jacksoni* by Sirivanakarn (1976) that distinguish *Cx. kangi* from *Cx. gaugleri* sp. nov. which needs further investigation on these species. Besides, *Cx. gaugleri* sp. nov. characterized with pale scales on scutum, tibiae without longitudinal pale stripe and occurrence of 3rd costal spot along with apical portion of vein R_1 and R_2 distinctly separate from *Cx. jacksoni, Cx. tsengi* and *Cx. propinquus* (Sirivanakarn, 1976).

The levels of furcation points and spot length are important in species identification in *mimeticus* complex (Sirivanakarn, 1976). The furcation points of vein R_{2+3} and M are at different levels in *Cx. mimuloides,* whereas, the furcation is observed at the same level in *Cx. gaugleri* sp. nov. Our study observed that the length of R_{4+5} pale spot (0.1 to 0.8) in *Cx. gaugleri* sp. nov. which is different from *Cx. mimeticus* (0.4 to 0.75) and other species of the group (Sirivanakarn, 1976).

The tibial spots or stripes are significantly important to differentiate species of *Mimeticus* subgroup (Sirivanakarn, 1976). *Culex gaugleri* sp. nov. does not contain any tibial longitudinal pale stripe anteriorly differs from other members that show pale stripe on the hind tibia (*Cx. mimeticus* and *Cx. mimuloides)* and mid tibia (*Cx. jacksoni, Cx. tsengi* and *Cx. mimuloides*) (Sirivanakarn, 1976). Additionally, basal pale bands on abdominal terga from segment II-VI of *Cx. gaugleri* sp. nov. differ from *Cx. fasyi* having apical pale bands on terga from segment III-VI. These morphological features distinguish *Cx. gaugleri* sp. nov. from other species.

Mitochondrial genes-based phylogenetic analysis is significant in species delimitation, identification and assessing evolutionary paths for mosquitoes and other insects (Shouche and Patole, 2000; Chan *et al.*, 2014; Minard *et al.*, 2017). Fewer COI and 16s rRNA gene sequences of *Mimeticus* subgroup are available (Minard *et al.*, 2017; Ashfaq *et al.*, 2014; Chan *et al.*, 2014; Maekawa *et al.*, 2016). Our study shows that the COI gene sequence of *Cx. gaugleri* sp. nov. formed a distinct cluster of the *Mimeticus* Subgroup species and developed a close relation with *mimeticus* species complex having *Cx. jacksoni* in the same clade. While the other species of *Mimeticus* subgroup *i.e. Cx. murrelli* and *Cx. orientalis* formed a separate cluster from *Cx. gaugleri* sp. nov. It was interesting to see the integrity of the clades or clusters of different *Cx. mimeticus, Cx. mimulus* and *Cx. orientalis* despite having intra-specific variations. The present study showed that *Cx. gaugleri* sp. nov. phylogenetically closed with *Cx. mimeticus* in comparison to other mosquito

species using 16s rRNA genes. This established *Cx. gaugleri* sp. nov. as a separate species.

Conclusions

We describe a new species, *Culex (Culex) gaugleri* sp. nov. from the Kodaikanal hills, Tamil Nadu, India. This species belongs to the *Mimeticus* subgroup of the *Culex* genus and can be differentiated with morphological features and DNA barcoding. The phylogenetic relationship confirms the taxonomic position of the species. Further studies are suggested on the bionomics of *Cx. gaugleri* sp. nov. as it was prevalent near human populations and may play a role in pathogen transmission.

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References

Ashfaq, M., Hebert, P.D., Mirza, J.H., Khan, A.M., Zafar, Y. and Mirza, M.S. 2014. Analyzing mosquito (Diptera: culicidae) diversity in Pakistan by DNA barcoding. *PLoS ONE,* e97268.

Aziz, H., Zia, A., Anwer, A., Aziz, M., Fatima, S. and Faheem, M. 2017. Zika virus: Global health challenge, threat and current situation. *Journal of Medical Virology,* **89**(6):943-951.

Barraud, PJ., 1934. *The fauna of British India, including Ceylon and Burma. Diptera. Volume V. Family Culicidae. Tribes Megarhinini and Culicini*. Taylor and Francis, London: 1-510pp.

Chan, A., Chiang, L.P., Hapuarachchi, H.C., Tan, C.H., Pang, S.C., Lee, R., Lee, K.S., Ng, L.C. and Phua, S.G.L. 2014. DNA barcoding: complementing morphological identification of mosquito species in Singapore. *Parasites & Vectors*, **7**(569):014-0569.

Doss, C.G., Siva, R., Christopher, B.P., Chakraborty, C. and Zhu, H. 2017. Zika: How safe is India? *Infectious Diseases of Poverty,* **6**:37.

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. *Molecular Marine Biology and Biotechnology,* **3**(5):294-299.

Group, S.Z.S. 2017. Outbreak of Zika virus infection in Singapore: an epidemiological, entomological, virological, and clinical analysis. *Lancet Infectious Diseases,* **17**:813-821.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series,* **41**:95-98.

Harbach, R. 2017. Mosquito Taxonomic Inventory. Electronic version accessed on 06.11.2020. http://mosquito-taxonomic-inventory.info/sites/mosquito-taxonomic-inventory.info/files/Culex%20classification_15.pdf.

Hemmerter, S., Šlapeta, J., van den Hurk, A.F., Cooper, R.D., Whelan, P.I., Russell, R.C., Johansen, C.A. and Beebe, N.W. 2007. A curious coincidence: mosquito biodiversity and the limits of the Japanese encephalitis virus in Australasia. *BMC Evolutionary Biology,* **7**(1):100.

Karlekar, S., Andrew, R. and Deshpande, M. 2020. Culex katezari, A new species of mimeticus mosquito (Diptera: Culicidae) from the forest of Gadchiroli region of central India. *International Journal of Mosquito Research,* **7**(6):54-58.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution,* **16**(2):111-20.

Kumar, N.P., Rajavel, A.R., Natarajan, R. and Jambulingam, P. 2007. DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, **44**(1):1-7.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution,* **35**:1547-1549.

Maekawa, Y., Ogawa, K., Komagata, O. and Tsuda, Y. 2016. DNA barcoding for molecular identification of Japanese mosquitoes. *Medical Entomology and Zoology,* **67**:183-98.

Matsuo, K., Lien, J.C., Kunou, I. and Ito, S. 1974. Morphological studies of the mimeticus group of Genus *Culex* (Diptera: Culicidae): I. Pale spots on wing and wing venation. *Medical Entomology and Zoology,* **25**(1):51-56.

Minard, G. and Tran, F.H. 2017. Identification of sympatric cryptic species of Aedes albopictus subgroup in Vietnam: new perspectives in phylosymbiosis of insect vector. *Parasites & Vectors,* **10**(1):1-14.

Rattanarithikul, R. 1982. A guide to the genera of mosquitoes (Diptera: Culicidae) of Thailand with illustrated keys, biological notes and preservation and mounting techniques. *Mosquito Systematics,* **14**(3):139–208.

Rattanarithikul, R., Harrison, B.A., Panthusiri, P. and Coleman, R.E. 2005. 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,* **1**:1-80.

Shouche, Y.S. and Patole, M.S. 2000. Sequence analysis of mitochondrial 16S ribosomal RNA gene fragment from seven mosquito species. *Journal of Biosciences,* **25**(4):361-366.

Sirivanakarn, S. 1976. Medical entomology studies – III. A revision of the subgenus *Culex* in the Oriental Region (Diptera: Culicidae). *Contributions of the American Entomological Institute,* **12**(2).

Somboon, P., Phanitchakun, T., Saingamsook, J., Yoshihide, M., Namgay, R. and Harbach, R.E. 2021a. Culex bhutanensis, a new species of the Mimeticus Subgroup of the nominotypical subgenus of the genus Culex Linnaeus (Diptera: Culicidae) from Bhutan. *Acta Tropica,* **217**:105868.

Somboon, P., Phanitchakun, T., Saingamsook, J., Namgay, R. and Harbach, R.E. 2021b. Culex (Culex) longitubus, A New Species of the Mimeticus Subgroup (Diptera: Culicidae) From Bhutan. *Journal of medical entomology,* 1-10.

Suman, D., Tikar, S., Mendki, M., Agrawal, O., Parashar, B. and Prakash, S. 2011. Variations in life tables of geographically isolated strains of the mosquito *Culex quinquefasciatus*. *Medical and Veterinary Entomology,* **25**(3):276-288.

Tyagi, B., Munirathinam, A. and Venkatesh, A. 2015. A catalogue of Indian mosquitoes. *International Journal of Mosquito Research*, **50**:50-97.

WHO [World Health Organization]. Vector-borne diseases, World Health Organization. Electronic version accessed on 02.03.2020. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases.