Culex katezari, A new species of mimeticus mosquito (Diptera: Culicidae) from the forest of Gadchiroli region of central India

Sanjay Karlekar, Raymond Andrew and Mukund Deshpande

Abstract
The Mimeticus subgroup of Culex is well distinguished by the presence of pale spots on the wings. In this study the presence of pale spots on newly found mosquito was compared morphologically and genetic confirmation was made by insect 16SrRNA gene sequencing by BLAST with all the species belongs to mimeticus subgroup of Culex, i.e. C. mimeticus C. mimus, C. mimaloides, C. fascifusficus, C. orientalis C. jacksoni, C. diengensis, C. fasyi, C. confused, C. suborientalis, C. solitarius, C. propinquus, C. neomimulus, C. mureli, C. kangi, C. tsengi, and C. tianpingensis. The result reveals that the species is newly identified. The species was from the Katezari village of Gadchiroli district of Mahaarashtra state, and has been named accordingly.

Keywords: Mimeticus, 16srRNA gene, BLAST and Culex katezari

1. Introduction
Among all insects, diversity of mosquitoes is of greater importance in terms of public health. These highly adaptable insects continue to coexist with man and transmit diseases to more than 700 million people annually [1-2]. Because of their medical importance, it is essential to make an inventory to upgrade the status of the diversity and behavioral resilience at different geographical loci [3-4]. Barraud (1934) stated that “many mosquito species await discovery in India,” and the number of new mosquito records gradually increased mostly from the north eastern region of India [5]. According to the most recent classification of mosquitoes, the family Culicidae includes two subfamilies, 11 tribes, 113 genera and 3583 species in the world [6]. The genus Culex is not only one of the most dominant in terms of number of species and individuals but is also one of the most complex and difficult genera among the mosquitoes of Southeast Asia [5]. The Mimeticus subgroup of Culex is characterized by their spotted wings. The species of the subgroup are likely to be encountered along the foothill of villages [7]. The Mimeticus subgroup was originally established by Edwards to include seven species: mimeticus, mimus, mimaloides, fuscifusficus, orientalis, jaksoni and diengensis were based particularly on the character of the pale spotting of the wings [8]. Later, three forms; fasyi, confused and suborientalis were added from the Philippines [9], while in 1938, C. solitarius was described by Bonne-Wepster from Netherland [10]. Colless (1955) [11] described a new species under the mimeticus group C. propinquus from Malaya. Lien (1968) [12] described four more new species: neomimulus, mureli, kangi and tsengi from Taiwan later on Chen (1981) [13] described C. tianpingensis from Taiwan, thus bringing the total to 17 species in this subgroup.

2. Material and Methods
A regular entomological surveillance was undertaken by zonal entomology team of the office of Assistant Director Health Services (Malaria) Nagpur, to evaluate the mosquito density and vector species status in the forest of Gadchiroli region of central India. Under this survey, mosquitoes were also collected from the outskirts of Katezari village located in the forest of Gadchiroli district. Adults and immature forms were collected from and around stagnant very slow flowing ditches rich in organic matter. Immature stages of mosquitoes were collected from the ditches with decaying plant leaves and other organic matters by dipping methods and adult mosquitoes were collected using aspirator and flash light from both indoor and outdoor


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resting habitats during morning hours (06:00–08:00) as per WHO guidelines [14]. The collected larvae were reared in the laboratory for the emergence of adult. Standard taxonomic keys and catalogues were used to identify the emerged mosquitoes [8-13]. For the unidentified specimens, the right wings of five female mosquitoes were treated to micro technique procedures to make a permanent in situ mount. The pale spots on the right wing of female specimens were studied by sketching Camera Lucida diagram and by photomicrography. The different pattern of pale spots of known species were compared and analyzed to confirm the status of the species. The conformation of the new mosquito species was undertaken by one of the authors (MMD) at the Department of Health Services, Office of the Assistant Director Health Services (M) Pune.

3. Observation and Discussion
3.1 Material
The holotype and male paratype are deposited in 70% alcohol at the Research cum Training Center of Filaria (National Vector Borne Disease Control Program), Public Health Department, Maharashtra state, Nagpur. The generalized pattern of pale spots on the wings of Mimeticus mosquito females on each position of the wing venation 1-20a is followed as per mosquito taxonomists [6-11]. The specimens were zoomed in SX16 Olympus stereo microscope and captured by Canon IXUS 132 14.2MP point and shoot camera. All the images were processed by CorelDraw 12.

3.2 Etymology
The species is named after the Village Katezari from where it was collected.

3.3 Culex katezarri
Holotype female ♀: India, Maharashtra State, Gadchiroli District, village Katezari (20.10° N, 80.0° E). (Fig 1 and 2) Paratype male ♂: India, Maharashtra State, Gadchiroli District (20.10° N, 80.0° E) (Fig 3).

Fig 1: (aka Soottari (Fonalo A. Lateral view (Captured by Canon IXUS 132 14.2MP point and shoot camera) B. Lateral view (zoomed in SX16 Olympus and captured by Canon IXUS 132 14.2MP point and shoot camera) C. Donal view (Captured by Canon IXUS 132 14.2MP point and shoot camera) D. Dorsal view (zoomed in SX16 Olympus and captured by Canon IXUS 132 14.2MP point and shoot camera)

Fig 2: Orkx kairari (Female) A. Ventral view (zoomed in SX16 Olympus and captured by Cancel IXUS 132 14.2MP point and shoot camera) B. Wing (zoomed in SX16 Olympus and captured by Canon IXUS 132 14.2MP point and shoot camera)
Fig 3: Culex kalrari (Male) A. Dorsal view (Captured by Canon IXUS 132 14.2MP point and shoot camera) B. Head (zoomed in SD (16 Olympus and captured by Canon IXUS 132 14.2MP point and shoot camera)

Fig 4: Generalized positton of pale spots on %mg veins in eufee numetteus subgroup of species.

Fig 5: Position of pale spots on u ins veins of Cuter katcati.

Fig 6: Insect 16sRNA gene sequencing. By BLAST 3.4 Wings of Culex katezari (female)

The generalized pattern of pale spots on the wings of Mimeticus mosquito females on each position of the wing venation 1-20a is categorized and described by various mosquito taxonomists [6-11]. There are three pale spots (C1, R 2), Cu (3) on humeral area, seven pale spots (C (4), Sc (5), R1 (6), Rs (7), M (8), Cu (9), An (10) on sectoral area, eight pale spots (C (11), R1 (12), R2-R3 (13), R4+5 (14), M1-M2 (15), Cu1 (16), Cu2 (17), Cu2 (17a) on subcostal area, four pale spots (C (18), R1 (19), R2 (20), R2 (20a)) on apical area (Fig. 4).

In Culex katezari the wing shows presence of pale spots Cu 3 on Humeral area, C (4), Sc (5), R1 (6), Cu (9), An (10) on sectoral area, C (11), R1 (12), R 2-R3 (13), M1-M2 (15), Cu1 (16), Cu (17a) on subcostal area and C (18), R1 (19), R2 (20) on apical area (Fig. 5). The genetic data obtained by insect
16SrRNA gene sequencing (By BLAST) is summarized (Fig. 6). Pale spotting pattern in the humeral and sectoral areas were excellent characters for diagnosis of Mimeticus subgroup [18]. The extent of the pale spots on the wings of all the mimeticus group of Culex, i.e. C. mimeticus C. minuloides, C. fusciicurucus, C. orientalis C. jacksoni, C. diengensis, C. fasyi, C. confuses, C. suborientalis, C. solitarius, C. propinquus, C. neonimulus, C. mureli, C. kangi, C. tsengi, and C. tianpingensis, was compared with the new species. The results of the pale spots on each postion 1-20a, as shown in schematic diagram are summarized, as described by various workers [8-17].

The differentiation of the wing spots of Culex katezari and the known species of mimeticus subgroup is described below (Table 1):

**Culex mimeticus**: - Differentiates with Culex katezari by the presence of pale spots on R4-5 (14) and absence of pale spots on Cu (3), R1 (6), and Cu (9), position.

**Culex minuloides [Culex confuses]** [18]: - Differentiates with Culex katezari by the presence of pale spots on M (8), R 4-5 (14), and absence of pale spots on Cu (3), R2-3 (13) and Cu (17a), position.

**Culex mureli**: - Differentiates with Culex katezari by the presence of pale spots on R4-5 (14) and absence of pale spots on Cu (3), R1 (6), Cu (9) and M1 (9), position.

**Culex kangi**: - Differentiates with Culex katezari by the presence of pale spots on Rs (7), M (8), two breaks on Cu 9, An 10, R4-5 (14), four breaks on Cu2 (17) and absence of pale spots on Cu (3), M1-M2 (15), Cu2 (17a), R2 (20) position.

**Culex tsengi**: - Differentiates with Culex katezari by the presence of pale spots on Rs (7), M (8), R4-5 (14) and absence of pale spots on Cu (3), Cu (9) and Cu (17a) position.

**Culex confuses**: - Differentiates with Culex katezari by the presence of pale spots on Cu (9), M1 (15) and M1-M2 (15) position.

**Culex orientalis**: - Differentiates with Culex katezari by the presence of pale spots on C (1), R (2), M (8), R4-5 (14), Cu2 (17) position and absence on C (18) position.

**Culex diengensis**: - Differentiates with Culex katezari by the presence of pale spots on R4-5 (14) and absence of pale spots on Cu (3), R1 (6), Cu (9) position.

**Culex fasyi**: - Differentiates with Culex katezari by the presence of pale spot on R4-5 (14), R2 (20a) and absence of pale spots on Cu (3), C (4), R1 (6), Cu (9), C (11), R 1 (12) and M1-2 (15) position.

**Culex suborientalis**: - No evidence available (nomen dubium) [18].

**Culex solidaries**: - Differentiates with Culex katezari by the presence of pale spots on R4-5 (14) and absence of pale spots on Cu (3), R1 (6), Cu (9) position.

**Culex propinquus**: - Differentiates with Culex katezari by the presence of pale spots on Rs (7), M (8), two breaks on Cu 9, An 10, R4-5 (14), four breaks on Cu2 (17) and absence of pale spots on Cu (3), M1-M2 (15), Cu2 (17a), R2 (20) position.

**Culex neonimulus**: - Differentiates with Culex katezari by the presence of pale spots on Rs (7), M (8), R4-5 (14) and absence of pale spots on Cu (3), Cu (9) and Cu (17a) position.

**Culex mureli**: - Differentiates with Culex katezari by the presence of pale spots on R4-5 (14) and absence of pale spots on Cu (3), Cu (9) and M1-M2 (15) position.

**Culex tsengi**: - Differentiates with Culex katezari by the presence of pale spots on Rs (7), M (8), R4-5 (14) and absence of pale spots on Cu (3), R1 (6), Cu (9) and Cu2 (17a) position.

**Culex orientalis**: - Differentiates with Culex katezari by the presence of pale spots on R (2), Rs (7), M (8), R4-5 (14), Cu2 (17) and absence of pale spot on Cu (9) position. The mimeticus subgroup of genus Culex is characterized by their spotted wings. The wing spot pattern of Culex katezari is so dissimilar from other mimeticus mosquitoes that there seems no doubt that Culex katezari is a distinct species of the mimeticus subgroup.

**Table 1**: Comparative account of the wing spot of Culex katezari with the known species of mimeticus subgroup. (* Indicates position of pale spot).

<table>
<thead>
<tr>
<th>Species of culex mimeticus subgroup</th>
<th>Humeral area</th>
<th>Sectoral area</th>
<th>Subcostal area</th>
<th>Apical area</th>
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<tr>
<td>C. mimeticus</td>
<td>C (1)</td>
<td>R (2)</td>
<td>Cu (3)</td>
<td>C (4)</td>
</tr>
<tr>
<td>C. mureli</td>
<td>C (4)</td>
<td>R (5)</td>
<td>C (6)</td>
<td>C (7)</td>
</tr>
<tr>
<td>C. propinquus</td>
<td>C (11)</td>
<td>R (3)</td>
<td>C (12)</td>
<td>C (13)</td>
</tr>
<tr>
<td>C. orientalis</td>
<td>C (10)</td>
<td>R (4)</td>
<td>C (11)</td>
<td>C (12)</td>
</tr>
<tr>
<td>C. kangi</td>
<td>C (19)</td>
<td>R (5)</td>
<td>C (20)</td>
<td>C (21)</td>
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<tr>
<td>C. tsengi</td>
<td>C (20)</td>
<td>R (6)</td>
<td>C (21)</td>
<td>C (22)</td>
</tr>
<tr>
<td>C. tianpinge -nsis</td>
<td>C (21)</td>
<td>R (7)</td>
<td>C (22)</td>
<td>C (23)</td>
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</tbody>
</table>

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