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RESEARCH COMMUNICATION

First record of the presence of *Aedes* (Phagomyia) *cogilli* (Edwards, 1922) in Sri Lanka

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Abstract: Sri Lanka is known for its biological diversity with a high richness in insect diversity. So far, 159 mosquito species representing 19 genera have been reported. *Phagomyia gubernatori* is the only species identified under subgenus *Phagomyia* in Sri Lanka. This study reports the presence of *Aedes (Phagomyia) cogilli* for the first time in Sri Lanka. During weekly field visits from October to November 2018, larval samples were collected from canals in the Jaffna municipal area in Jaffna District (N 9° 39' 32" E 80° 3' 23" and N 9° 39' 35" E 80° 3' 25") and reared at the insectary. Emerged adults were identified with available identification keys. DNA was extracted from the individual adults and a portion of the *mitochondrial cytochrome c oxidase subunit I* (*COI*) gene was amplified using Polymerase Chain Reaction (PCR). The amplified fragments of four individuals were sequenced in both directions. In addition to morphological confirmation, the presence of *Ae. cogilli* was also supported with the DNA sequence of *cytochrome c oxidase subunit I*. Insecticide susceptibility status of adult mosquitoes to the insecticides Deltamethrin, Permethrin, Malathion, Propoxur, and DDT were performed according to the World Health Organization procedures. The findings showed the presence of *Ae. cogilli* in Sri Lanka and that it had developed resistance to insecticides, suggesting further investigation on the species.

Keywords: *Ae. cogilli, COI*, diversity, Sri Lanka.

INTRODUCTION

Sri Lanka is an island of 65, 610 km^2 land area and known for its richness in biological diversity with high insect diversity (Wijesekara & Wijesinghe, 2003; Weerakoon & Wijesundara, 2012). Continuation of the pioneering work of Green (1901) followed by Christophers (1933) and Barraud (1934), the first comprehensive list of mosquito species, describing 125 species of 14 genera, was published by Carter in 1950 and later updated by Jayasekera and Chelliah (1981). Amerasinghe (1991) described 141 species under 16 genera and recently Gunathilaka (2018) reviewed the Sri Lankan mosquito checklist describing 159 species belonging to 19 genera. Among the 19 genera, the medically important *Aedes* genera composed of 18 subgenera, which includes *Phagomyia* Theobald. The only reported species under subgenus *Phagomyia* in Sri Lanka is *Phagomyia gubernatori* Giles (Barraud, 1934). During the mosquito vector surveys in Northern Sri Lanka, mosquitoes belonging to *Aedes* (*Phagomyia*) *cogilli* (Edwards, 1922) were identified. *Ae. cogilli* has been reported in India, Nepal and Pakistan (Richard *et al*., 1993; Ashfaq *et al*., 2014; Bhattacharyya *et al*., 2014). This species apparently quite rare and originally known to be from India is one of several tree hole species which have been recorded so far. The collection, identification and molecular characterisation of *Ae. cogilli* are reported in this paper for the first time from Sri Lanka.

METHODOLOGY

Collection and identification of *Aedes* mosquitoes

During the routine mosquito larval collection from October to November, 2018, *Aedes* mosquitoes were collected from two sites (Figure 1; N 9° 39' 32" E 80°

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3’ 23” and N 9° 39’ 35” E 80° 3’ 25”) in the periphery of the Jaffna municipality. Samples were collected and reared in the insectary facility at the Medical and Molecular Entomology Laboratory of Department of Zoology, University of Jaffna under standard laboratory conditions (Jude *et al*., 2012). Emergent *Aedes* adults were identified using available taxonomic keys (Barraud, 1934; Stojanovich & Scot, 1965).

DNA isolation, PCR amplification and sequencing

DNA was extracted from individual adult mosquitoes that were morphologically identified as *Ae. cogilli* using the DNeasy Blood & Tissue Kit (Qiagen, California, USA) following manufacturer’s instructions. The extracted DNA was subjected to Polymerase Chain Reaction (PCR) amplification of a portion of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene using the primer pair LCO1490 (Forward - 5'-GGT CAA CAA ATC ATA AAG ATATTG G-3') and HCO2198 (Reverse-5'-TAA ACTT CAG GGT GAC CAA AAA ATC A-3') (Folmer *et al*., 1994). Each 25 µL PCR reaction consisted of GoTaq® Green Master Mix (Promega, USA), 2 mM $MgCl_2$, 1 µL (100 pmol/µL) of each primer and 5 µL of DNA. The samples were heated at 94 °C for 5 min before 30 cycles of amplification at 94 °C for 30 sec, 45 °C for 30 sec, and 72 °C for 30 sec followed by a final extension at 72 °C for 7 min. The PCR products were purified using QIAquick® PCR Purification Kit (QIAGEN). Purified PCR products of four individual samples were sequenced in both directions at Macrogen Inc., South Korea. The sequences were edited in Finch TV (Geospiza, USA) and aligned with ClustalW in MEGA 5.0 software and the resultant sequences were compared with available sequences in the Barcode of Life Data Systems (BOLD) and GenBank sequence database of the National Center for Biotechnology Information (NCBI) (Tamura *et al*., 2011).

Phylogenetic analysis

Sri Lankan *Ae. cogilli* mitochondrial *COI* sequence was aligned along with other sequences retrieved from GenBank for *Ae. aegypti* (MK265729.1, KF406397.1, MG004699.1), *Ae. albopictus* (KY352245.1, MK736660.1, KR817732.1, MN909291.1, LC431230.1), *Ae. vittatus* (AY834246.1, MF429950.1) and *Ae. cogilli* (MF537655.1, KF406623.1) using ClustalW2 in MEGA, version 5 (Tamura *et al*., 2011). Phylogenetic relationships among those species were inferred using the maximum likelihood (ML) method. The substitution model selection was also performed in MEGA5 based on

(a) (b)

Figure 1: *Aedes cogilli* sample collection sites in Jaffna, Sri Lanka: (a) open drain and (b) discarded plastic water holding containers

the lowest Bayesian Information Criterion (BIC) value. The Tamura 3-parameter with gamma distribution model for the *COI* sequence dataset was selected. Bootstrap (Felsenstein, 1985) supports were based on 1000 re-sampled datasets. GenBank sequence for *Mansonia bonneae* (MK033673.1) was used as out-group species.

WHO insecticide susceptibility test

Standard World Health Organization procedures (WHO, 2016) were followed to determine the insecticide susceptibility status of adult female mosquitoes that were morphologically identified as *Ae. cogilli*. Non-blood-fed 3 – 4 days old adult female mosquitoes were tested with the WHO discriminating dosages of 0.03% Deltamethrin and 0.25 % Permethrin (pyrethroids), 0.8 % Malathion (organophosphate), 0.1 % Propoxur (carbamate) and 4 % DDT (organochlorine) using WHO bioassay test kits. Two to three batches of 10 – 20 mosquitoes were exposed to insecticide impregnated paper for 60 min. Minimum of 75 mosquitoes per replicate were tested per insecticide. After exposure females were transferred into holding tubes and kept for 24 h and fed on 10 % sugar solution. After 24 h of recovery period, mortality was counted, and percentage mortality was calculated and adjusted using Abbot's formula if the control mortalities were less than 20 %. The WHO criteria was used to define a population susceptible (≥ 98 % mortality), suspected of resistance (90 –97 % mortality) and resistant (< 90 % mortality) (Abbot, 1925; WHO, 2016).

RESULTS AND DISCUSSION

Mosquitoes were morphologically identified as *Aedes* (*Phagomyia*) *cogilli.* The characteristic medium size black and white species have a head without the median white line and eye margins with narrow border of white scales (Figure 2 Ai and Aii). Further reported characteristics are; the white patch on front of mesonotum which is large, round and more silky; smaller prealar white patches; scutellum with mid lobe densely covered with flat white scales, and flat black scales on lateral lobes (Barraud, 1934). The voucher samples of *Ae. cogilli* are kept in the Insectary Museum of Department of Zoology, University of Jaffna.

Molecular characterisation based on *COI* sequence analysis based on sequences derived from four individual specimens of *Ae. cogilli* mosquitoes resulted in a single haplotype of 635 bp sequence, which was deposited in GenBank (GenBank Accession Number: MK209633).

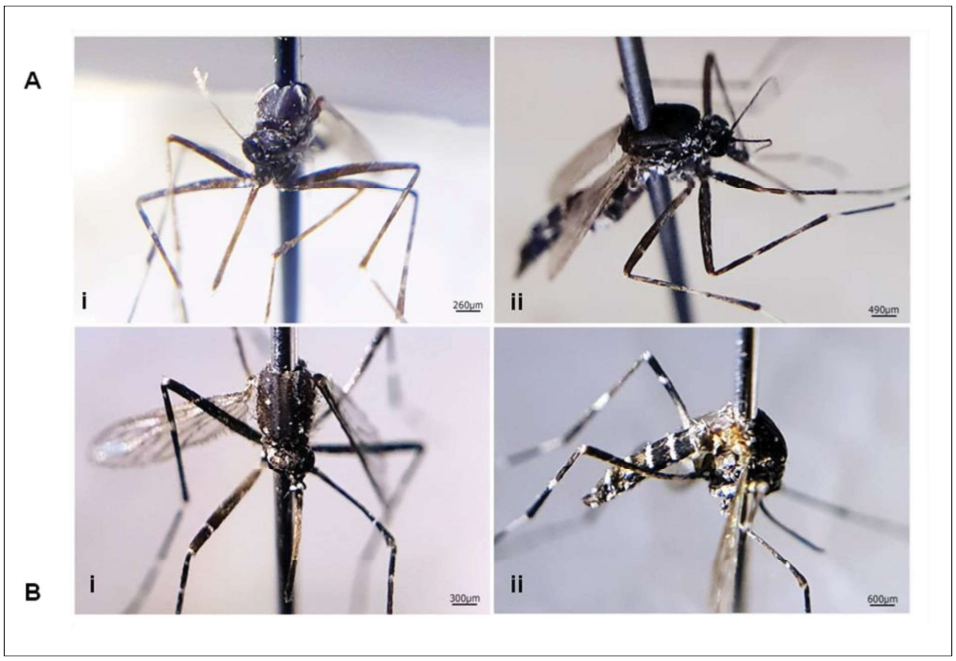

Figure 2: Prominent morphological description of *Ae. cogilli* in comparison with *Ae. vittatus*. (A) *Ae. cogilli* female frontal (i) and lateral view (ii); (B) *Ae. vittatus* female frontal (i) and lateral view (ii)

The BOLD system and BLAST comparison in GenBank revealed 100 % and 99 % identity with *Aedes* (*Phagomyia*) *cogilli,* respectively. Phylogenetic analysis revealed that *Ae. vittatus* and *Ae. cogilli* are genetically very close to each other (Figure 3). A recent study from Spain showed that both *Ae. vittatus*, a potential vector of arbovirus including dengue virus, and *Ae. cogilli* are genetically very close but morphologically different to one another; the white dots in the scutum of *Ae. vittatus* (Figure 2 Bi and Bii) are missing in *Ae. cogilli* (Fernandez *et al*., 2018). Although *Ae. cogilli* is reported to be a tree hole species (Richard *et al*., 1993), it has undergone adaptation to develop in an urban environment in Sri Lanka.

The *Ae. cogilli* samples showed mean percentage susceptibility of 98.83 (± 6.29), 5.9 (± 6.0), 97.5 (± 2.5), 94.1 (± 5.2) and 73.3 (± 1.4) to 0.03 % Deltamethrin, 4 % DDT, 0.8% Malathion, 0.25 % Permethrin and 0.1 % Propoxur, respectively. According to WHO criteria the populations are resistan (< 90 % mortality) to DDT and Propoxur while possibly resistant (mortality 90–97 %) to Deltamethrin and Permethrin (WHO, 2016). The results showed that the mosquito populations have long been adapted to the urban environment to breed in polluted water and thus have developed resistance to common insecticides.

For the first time, the presence of *Ae. cogilli* in Sri Lanka was confirmed using both morphological and molecular characterisation. Presence of *Ae. cogillii* in India, Nepal and Pakistan has already been reported (Richard *et al*., 1993; Ashfaq *et al*., 2014; Bhattacharyya *et al*., 2014). Medical importance of *Ae. cogilli* has not been reported so far as *Ae. cogilli* is regarded as a sylvatic species mainly breeding in tree holes and hollow bamboos (Barraud, 1934; Richard *et al*., 1993). However, the preset study reveals that *Ae. cogilli* has developed resistance to insecticides.

CONCLUSION

First report of the presence of *Ae. cogilli*, a tree hole species in urban environment and its associated insecticide resistance and genetic similarity to potential arbovirus vector *Ae. vittatus* warrants further investigation of this species.

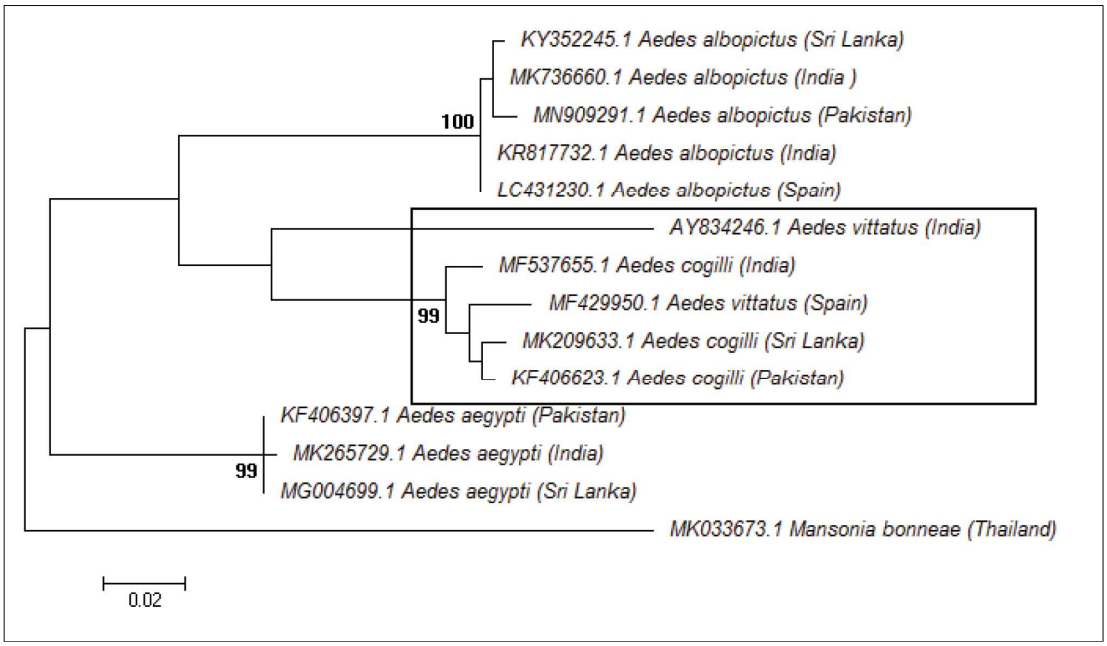

Figure 3: Phylogenetic tree based on *COI* sequence dataset (346 nt positions) constructed using the maximum likelihood method using Tamura 3-parameter with gamma distribution model; bootstrap values > 60 % are shown. The sequences used for analysis include samples from Jaffna (GenBank: MK209633) and other GenBank entries. *Mansonia bonneae* (GenBank: MK033673) was used as the outgroup. Phylogenetic position of *Ae. vittatus* and *Ae. cogilli* is shown within the rectangular box.

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