First record of the presence of *Aedes (Phagomyia) cogilli* (Edwards, 1922) in Sri Lanka: Naturally adapted to develop in an urban environment

Tibutius TP Jayadas, Vaikunthavasan Thiruchenthooran, Annathurai Tharsan, Kokila Sivabalakrishnan, Sharanga Santhirasekaram, Kalingarajah Karvannan and Sinnathamby N Surendran

Abstract

Sri Lanka is known for its biological diversity with high richness in insect diversity. So far 159 mosquito species representing 19 genera have been reported. *Phagomyia gubernatorii* is the only species identified under subgenus *Phagomyia* in Sri Lanka. For the first time *Aedes (Phagomyia) cogilli* has been recorded from Sri Lanka. In addition to morphological confirmation, the presence of *Ae. cogilli* is supported with molecular characterization. Findings also show that *Ae. cogilli* had developed resistance to common insecticides and developed adaptations to urban environment, raising health and environmental concerns.

Keywords: Mosquitoes, species, Sri Lanka, morphology

1. Introduction

Sri Lanka is an island nation of 65,610 km² land area and known for its richness in biological diversity with high insect diversity [1, 2]. Continuation of the pioneering work of Green in 1901 followed by Christophers in 1933 and Barraud in 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 in 1981 [3-7]. Amerasinghe in 1991 described 140 species under 16 genera and recently Gunathilaka reviewed the Sri Lankan mosquito checklist describing 159 species belonging to 19 genera [8, 9]. 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 gubernatorii* Giles [5]. During the mosquito vector surveys in Northern Sri Lanka mosquitoes belonging to *Aedes (Phagomyia) cogilli* were identified [10]. The collection, identification and molecular characterization of *Aedes cogilli* are reported for the first time from Sri Lanka.

2. Materials and Methods

2.1 *Aedes* collection and identification

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° 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 department of zoology, university of Jaffna as described previously [11]. Emergent *Aedes* adults were identified using available taxonomic keys [5, 9, 12].

2.2 DNA isolation, PCR amplification and sequencing

DNA from individuals morphologically identified as *Ae. cogilli* was extracted 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 (COI) gene using the primer pair LCO1490 (5’-GGT CAA CAA ATC ATA AAG ATATTG G-3’) and HCO2198 (5’-TAA ACTT CAG GGT GAC CAA AAA ATC A-3’) [13]. Each 25 μl PCR reaction consisted of GoTaq® Green Master Mix (Promega, USA), 2 mM MgCl₂, 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 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 635 bp sequence was compared with available sequences in the Barcode of Life Data Systems (BOLD) and GenBank sequence database of the National Center for Biotechnology Information (NCBI) [14].

Fig 1: Aedes cogilli samples collections sites in Jaffna, Sri Lanka.

2.3 WHO insecticide susceptibility test
As a separate experiment, the standard World Health Organization (WHO) procedures were followed to determine the insecticide susceptibility status of adult mosquitoes morphologically identified as Aedes cogilli [15]. Non-blood-fed adult female mosquitoes of 3 - 4 days old 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 per rearing conditions. After exposure females were transferred into holding tubes and kept for 24 hrs and fed on 10% sugar solution. After 24 hrs of recovery period, mortality was counted 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 for resistance (90 –98% mortality) and resistant (< 90% mortality) [15, 16].

3. Results and Discussion
Mosquitoes were morphologically identified as Aedes (Phagomyia) cogilli (Figure 2). Molecular characterization of four Aedes cogilli mosquitoes resulted in a single haplotypes of 635 bp sequence which is deposited in GenBank (GenBank Accession Number: MK209633). The BOLD system and BLAST comparison in GenBank revealed 100% and 99% identity with Aedes (Phagomyia) cogilli.

Fig 2: (A). Aedes cogilli female frontal (i) and later view (ii). (B). Aedes vittatus female (i) frontal and (ii) later view.

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 resistance (<90% mortality) to DDT and Propoxur while possibly (mortality 90-95%) resistant to 0.03% Deltamethrin, 0.8% Malathion and 0.25% Permethrin [15].

Medical importance of Ae. cogilli is not been reported so far as Ae. cogilli is regarded as a sylvatic species mainly breeding in tree holes and hollow bamboos, but in this study Ae. cogilli was collected in the canals of the Jaffna Municipality area, which is an urban area in Jaffna district [5]. The results show 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.

We, for the first time, morphologically and molecularly confirm the presence of Ae. cogilli in Sri Lanka, which is known to be present in India [17]. A recent study from Spain revealed that both Ae. vittatus, a potential vector of arbovirus including dengue virus, and Ae. cogilli are genetically very closer to each other but morphologically different to one another (Figure. 2) [5, 9, 12, 18]. Molecular based identification using barcoding region of the mitochondrial DNA alone is not
useful, therefore both molecular and morphological characterization are required to confirm the species identification.

4. Conclusion
The identification of *Ae. cogilli* in Jaffna Municipal area, adding a new species to mosquito biodiversity of Sri Lanka. Beside the close resemblance of the *Ae. cogilli* to *Ae. vittatus*, the adaptation to develop in the urban environment and resistance to common insecticide alarming the health and environmental concerns.

5. Acknowledgement
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6. References