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Characterization of the sibling species status of *Anopheles culicifacies* breeding in polluted water bodies in Trincomalee District of Sri Lanka

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Anopheles culicifacies, the major vector of malaria in Sri Lanka, is known to breed in clean and clear water. However, recent findings have confirmed breeding in waste water containing drains. However, no study has been conducted to identify whether it is vector or non vector siblings. Therefore, the objective of the study was to identify the sibling species status of *An. culicifacies* breeding in waste water containing drains. *An. culicifacies* adult samples (Reared from larvae) were obtained from the Padavisiripura Entomological team attached to Tropical and Environmental Diseases and Health Associates (TEDHA) Malaria Elimination Program in the Trincomalee District. The collected mosquito specimens were processed for the extraction of genomic DNA individually. The PCR amplifications were carried out using different primer combinations for differentiating species A from D, species B from C, species B from E, and species B, C, and E from each other. The results obtained from the gel electrophoresis were compared with the marker, and band sizes of 359 bp, 248 bp, 95 + 248 bp, 166 + 359 bp and 178 + 248 bp were used to identify the sibling species A, B, C, D and E respectively. The molecular biological identification of the field caught *An. culicifacies* samples indicated that only 6.25% (1/16) represented sibling species B. About 93.75% (15/16) of the samples were *An. culicifacies* sibling species E. According to the results, the majority of the species belongs to sibling species E which is considered as the vector sibling species of *An. culicifacies*. This is the first time that *An. culicifacies* E breeding in waste water was confirmed by a molecular method. However, malaria control programs focus on rural communities as a result of bio-ecology of *Anopheles* mosquitoes. Therefore, unusual breeding habitats such as waste water collections may mislead the current vector controlling programs. These results reconfirm that *An. culicifacies* has adapted to breed in water bodies including waste water collections. Since a majority of them belong to sibling E, which is considered as the vector, this may adversely affect the current malaria elimination program. Therefore, new strategies should be adopted to control malaria vector breeding in these unusual breeding habitats under the current malaria elimination program in Sri Lanka.

Keywords: *Anopheles culicifacies*, malaria, vector, sibling

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