A 02

The squash blot Technique; a reliable technique to identify immature stages of *Anopheles culicifacies*

Anopheles culicifacies is considered to be the most important vector of malaria in Sri Lanka. Continuous vector surveillance provides information on the transmission of

malaria and helps in the control of the disease. Correct identification of A. culicifacies by screening immature stages of anopheline mosquitoes is an important aspect of vector surveillance.

Sixteen species of immature stages of anophelines were collected from various malaria endemic areas. Standard taxonomic keys as well as DNA probes were used for the identification of mature larvae.

Mature larvae of A. culicifacies were identified by their specific chaetotaxonomic characters such as presence of one simple metathoracic pleural hair, moderately developed thoracic palmate hairs, and strong and dark roots of shoulder hairs. All mosquito larvae identified by taxonomic characters were also screened by squash blot hybridization with radio labeled and biotin labeled DNA probe Rp234 which is specific for A.. culicifacies. Radioactive DNA probes were prepared by random priming using ^{32} P-dCTP and detected by autoradiography. Non-radioactive DNA probes were prepared by labelling with biotin using nick translation method and detected by colorimetrically.

A strong positive hybridization signal was given by all larvae identified as A. culicifacies by taxonomic features while those of other mosquito species gave a negative hybrization signal under stringent filter washing conditions (0.1 x SSC, 0.5% SDS, at 60 ^{O}C).

This squash blot technique enables a single investigator to accurately immature stages (third and Fourth instar larvae) collected from the field and the sample unidentified or misidentified by chaetotaxonomy due to the damage of taxonomic features.