

MOSQUITO SURVEY AND TAXONOMIC STUDIES

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OBJECTIVE : To elucidate the mosquito fauna of Thailand and Southeast Asia, with primary emphasis on the identification of diagnostic characters for the separation of vector species and groups containing vectors of human pathogens.

BACKGROUND : This is a continuation of efforts begun in the early 1960's and currently conducted in partial collaboration with the Medical Entomology Project (MEP), Smithsonian Institution, Washington, D.C. A brief review of the history of these efforts was presented in the 1977-78 annual report (1).

METHODS : Surveys for target species and species groups are conducted at selected sites and habitats throughout Thailand. These surveys involve the collection of immature and adult mosquitoes, with emphasis placed on reared adults with associated larval and pupal skins, and on progeny adults (with associated larval and pupal skins) reared from eggs oviposited by known wild collected females. Specimens are curated and pinned or slide mounted for further study by investigators in the laboratory, or shipped for study to MEP or other world recognized authorities. Studies primarily consist of an analysis of intra-interspecific variations to identify useful characters for separating the species. Useful diagnostic characters, new species records, and new taxa found in Thailand are prepared for publication and described in scientific journals.

RESULTS : Major efforts continued during this period to assist in the monographic revisionary study of the Leucosphyrus Complex of *Anopheles (Cellia)* by Mr. E.L. Peyton at the Medical Entomology Project (MEP), Smithsonian Institution, Washington, D.C. These efforts were directed primarily toward supportive projects such as field studies on behavioral patterns and the definition of habitats and distributions, colonization efforts (see Cytogenetic and Cross Mating Studies -

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this annual report) and laboratory studies to identify morphological characters of value for species identification.

Field studies in different areas of Thailand have provided additional evidence that the distribution of the Leucosphyrus Complex is closely associated with the distribution of evergreen wet forests in this country. Although *Anopheles dirus* may be found in areas with deciduous forest, it is most abundant in areas with evergreen wet forests growing on clay soils. Other members of the complex in Thailand have not been found in areas lacking evergreen wet forest. Members of this complex were not collected during trips to : (1) Mae Hong Son Province where deciduous, dipterocarp and pine forests are predominant; and (2) high elevations (1,000 m plus) in Chiang Mai Province. Large populations of members of this complex, however, were found at elevations from 90-700 m in evergreen wet forest areas in Kanchanaburi Province and in evergreen wet forest at the base of Phu Kradung Mountain, Loei Province. This association with evergreen wet forests also is supported by the presence of an *An. dirus*-like member of the complex on the west coastal area of India. This area of India, the "Malabar Strip", has a wet tropical climate with dense forests (where not disturbed) and a unique mosquito fauna.

Colony specimens of 4 members of the Leucosphyrus Complex (*dirus*, *balabacensis* Perlis form, *takasagoensis* and Fraser's Hill form) were dissected and measurements were made of the spermatheca, wing length and proboscis length. Significant spermatheca size differences have been detected in certain members of the Gambiae Complex of *Anopheles* (2), however, no significant differences were detected in the sizes of the spermathecae of the 4 species/strains in this study. Wing length and proboscis length differences were detected, with *takasagoensis* and Fraser's Hill form females being consistently larger than those of *dirus* and *balabacensis* Perlis form. Whether these differences are a reflection of colonization selection pressure or natural size are conjectural.

Studies comparing the affects of rearing progeny immatures of colonized *An. dirus* and *An. balabacensis* Perlis form at different water temperatures, on pupal branching and adult wing spot characters, have identified potential wing differences in these 2 species. Pupal branching and wing spot measurements for both species were significantly different (in most cases) when comparisons were made between specimens of the same progeny broods reared at hot versus control, cold versus control and hot versus cold temperatures. The 3 different water temperatures were: hot ($\bar{x} = 27.9^{\circ}\text{C}$), control ($\bar{x} = 25.0^{\circ}\text{C}$) and cold ($\bar{x} = 21.4^{\circ}\text{C}$). These water temperatures are encountered in larval habitats in Thailand. Such environmentally induced, nongenetic variations (3) are probably responsible for the wide range of variation previously encountered in the wing characters of the Myzomyia Series of *Anopheles* (*Cellia*) in Thailand (4), and currently being encountered in the Leucosphyrus Complex (5). Analysis of data from this study will continue.

A large number of progeny adults with associated immature skins were reared from individual mothers during this period from 2 colonies of the Fraser's Hill form, a very poorly known member of the Leucosphyrus Complex. In addition, adults with associated immature skins were reared from a very small colony of

An dirus-like member of the Complex originating on the west coastal area of India. These 2 species or strains still require additional study to determine their positions in the complex. There is strong preliminary evidence that the Fraser's Hill form is distinct and deserves species status. Large numbers of reared specimens of these 2 species/strains have been shipped to the Medical Entomology Project (MEP), Smithsonian Institution, Washington, D.C., for further study.

A morphological analysis of the Subpictus Group of the Pyretophorous Series, *Anopheles (Cellia)*, in Thailand was completed during this period (6). This study identified additional adult, pupal and larval characters of value for identifying the 3 species currently recognized in Thailand (*indefinitus*, *subpictus* and *vagus*). Keys were prepared and a manuscript is in preparation.

The status of *Anopheles nivipes* as a separate species from *Anopheles philippinensis* was definitely established during this period by Cytogenetic and Cross Mating studies (see that section - this annual report). Prior to these studies, *An. nivipes* has been recognized as distinct by our researchers based only on morphological and distributional evidence (7). *Anopheles nivipes* is much more common and widely distributed in Thailand than *An. philippinensis* and must be considered a suspected vector of human malaria parasites. The Thailand Malaria Division considers *An. philippinensis* a suspected vector. Furthermore, the Malaria Division has not recognized *An. nivipes* as occurring in Thailand, but almost certainly has been identifying this species as *An. philippinensis*. The possibility also exists that what is now recognized as *An. nivipes* in Thailand may in actuality be 2 cytospecies. Although distinctive cytogenetic differences have been detected for 2 types of *An. nivipes*, no external morphological differences have been found. Work is continuing on these problems.

The question of *Anopheles maculatus* as a single species or a complex of several species, received considerable attention during this period. Last year 2 Thailand strains of *An. maculatus* were reported colonized (7) and cross mating experiments during this period using these 2 strains and the Kuala Lumpur strain revealed these 3 strains to be completely compatible. However, cytogenetic studies of polytene chromosomes from specimens of *An. maculatus* from other widely separated areas in Thailand have revealed very distinct homozygous chromosomal arrangements on several chromosome arms. These cytogenetically distinct arrangements are sympatric, without evidence of heterozygous individuals. Accordingly, these data strongly suggest the presence of at least 3 distinct cytospecies in the Maculatus Complex in Thailand. Cross mating studies for these 3 cytospecies have not been carried out. The presence of several distinct cytogenetic species under the name *An. maculatus* would provide a logical solution to the disparity previously noted in the vector abilities of *An. maculatus* from different areas of S.E. Asia (8, 9), and also in different regions of Thailand.

Excellent progress was made on the taxonomic revision of the Kochi Group of *Aedes (Finlaya)* during this period. The study has been expanded to include the 11 species currently recognized in the Southeast Asian Subregion. This includes *Aedes poicilius*, the primary vector of *Wuchereria bancrofti* in many parts of the Philippines. Descriptions and keys have been made for most of the species and numerous adult, male genitalia, pupal and larval characters have

been found which will assist in the differentiation of the species. Illustrations have been completed for most of the species. Two new species have been identified and the types of only 2 more species need to be examined to confirm their status. Characters have been found which will also differentiate all of the Southeast Asian members from the Austral-Asian and South Pacific members of the Kochi Group.

Over the last 3 years a number of mosquito species have been collected in various areas of Thailand which are new records for Thailand. Last year, the first records of *Anopheles leucosphyrus* in Thailand were reported (7). Specimens of *Anopheles gigas baileyi* and *Anopheles lindesayi* (near *cameronensis*) were collected during 1978 and 1981 at high elevations in Chiang Mai Province and are reported here for the first time. Additional new species records for Thailand include: *Aedes (Finlaya) pulcheriventer*, *Armigeres moultoni*, *Culex* sp. nr. *bokorensis*, *Culex edwardsi*, *Culex jacksoni* and *Culex tsengi*. New species in several genera were also discovered during this period and will be described later.

A number of infrequently encountered or rare species were also collected in Thailand during the last year, these include: *Anopheles culicifacies* larvae in Udon Thani Province; *Anopheles kyondawensis* (one larva) in Kanchanaburi Province; *Anopheles pampanai* larvae in Loei and Udon Thani provinces; *Anopheles riparis macarthuri* larvae in Phangnga Province; *Anopheles stephensi* adult females biting man and buffaloes in Mae Hong Son Province; and *Aedes (Isoaedes) cavaticus* larvae in Sri Sawat District, Kanchanaburi Province. This last species is recently described (10), and previously known only from a cave at 200 m elevation in Sai Yok District, Kanchanaburi. The present record is also from a cave but approximately 80-90 km distance from the original (type) locality, and at 780 m elevation. Specimens (larvae, pupae and adults) of *Anopheles varuna* were seen from Bo Phloi District, Kanchanaburi Province. These last specimens were kindly provided by Mr. Suchart Phatipongse, a graduate student at the Faculty of Tropical Medicine, Mahidol University, Bangkok. Confirmed specimens of *varuna* have been seen from 3 provinces in Thailand (Chiang Mai, Kanchanaburi and Lampang).

The monographic study (4) "The Myzomyia Series of *Anopheles (Cellia)* in Thailand, with emphasis on intra-interspecific variations (Diptera: Culicidae)" was published in November, 1980. A paper (11) elevating *Anopheles takasagoensis* Morishita, to full species status was also published during that month. This species, *takasagoensis*, was previously called *An. balabacensis* Taiwan form.

A large manuscript, "A guide to the genera of mosquitoes of Thailand, with illustrated keys, biological notes and preservation and mounting techniques" has been completed and submitted for clearance. This manuscript was prepared for students and public health workers, and contains adult, pupal, larval and male genitalia keys, with 27 full-page plates for the 18 genera occurring in Thailand.

Personnel of the department gave lectures and provided laboratory training for graduate students in a Culicidology course offered by Chulalongkorn

University, Bangkok. Laboratory training was also provided for several graduate students from Mahidol University, Bangkok.

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