

## STUDY REPORTS

1. Title: Mosquito Fauna of Thailand

Principal Investigators: Bruce A. Harrison, CPT, MSC  
Y.M. Huang, Ph.D.  
E.L. Peyton  
Rampa Rattananarithikul  
John E. Scanlon, Ph.D.

Assistant Investigators: Prajim Boonyakanist  
Sumeth Chunchulcherm  
Kol Mongkolpanya  
Larp Panthusiri

### OBJECTIVES

To collect, identify, catalog and redescribe all of the mosquito species of Thailand. Information is also assembled on the distribution, larval habitats and other aspects of the bionomics of the various species. The eventual goal is the production of monographs on the mosquitoes of the area, together with keys, handbooks and other identification aids for the use of workers in public health and associated fields and the later inclusion of this material into larger monographic works on the mosquitoes of Southeast Asia. Since the final monographs will not be completed for several years, periodic papers with keys, descriptions and distribution of important groups will be made available as soon as practical. The immediate objective is to make available as much information as possible on the Anopheles and species of other genera which are known or suspected to be vectors of disease. The training of competent Thai and, more recently, U.S. military personnel in Southeast Asia in the identification and bionomics of the mosquito fauna of Thailand is also a major concern. Another objective is the building of a reference collection at SMRL of all the mosquitoes known to occur in Thailand to provide readily accessible study material to newly assigned SMRL personnel and others who may have the need for rapid familiarization with the mosquitoes of this region.

### DESCRIPTION

Mosquitoes are collected from many areas of Thailand in connection with various studies on arthropod-borne viruses and malaria. Additional collections of a specialized nature are made to obtain correlated series of larvae, pupae and adults for illustration and other detailed studies. These have consisted mainly of collections of the immature stages from forested or undeveloped areas; these immature stages are reared individually, as far as is possible, in order to recover a correlated series of cast skins and adults. All of the reared material is later identified and processed at SMRL in Bangkok. After processing, the material is transferred to the Southeast Asia Mosquito Project at the Smithsonian Institution, for confirmation, description and eventual inclusion in final monographs.

### PROGRESS

During the year 1,284 mosquito collections were made in 18 provinces of Thailand. These collections resulted in 14,726 pinned adults, 8,693 slide mounts of larvae, larval skins and pupal skins and 1,186 slide mounts of male and female terminalia.

Aedes: Taxonomic research on this genus was concentrated during this period on species of the subgenus Stegomyia which contains such important vector species as Aedes aegypti, A. albopictus and A. scutellaris. The majority of the collections of Aedes (Stegomyia) mosquitoes were of larval stages—both from natural habitats and from water filled bamboo oviposition cups set out at collection sites.

The following 14 species of Aedes (Stegomyia) were collected during this period: A. aegypti, A. albolineatus, A. albopictus, A. annandalei, A. craggi, A. desmotes, A. gardnerii imitator, A. novalbopictus, A. pseudalbopictus, A. subalbopictus, A. vittatus, species in the A. mediopunctatus and A. scutellaris complexes and a possible new species, near A. unilineatus. Table 1 lists the methods utilized in collecting these species, while Table 2 summarizes the larval habitats where these species were found.

Bamboo oviposition cup collections were made in Lampang, Chon Buri, Nakhon Sawan, Surat Thani and Nonthaburi provinces during this report period. These cups were left in situ an average of 7 days, then returned dry to Bangkok, where water was added to hatch any eggs contained therein. Larvae thus obtained were reared to maturity, and the larval and pupal skins, and adults were preserved. Table 3 lists the Aedes (Stegomyia) species collected by this method in the first 3 provinces, according to the habitat in which the bamboo cups were set.

Aedes aegypti was found in bamboo cups set out in Chon Buri and Nakhon Sawan provinces, and some of these cups were located as far as 100 meters away from the nearest houses. In Lampang Province larvae of this species were found in a small hole in a log and in a hollow log. Both of these natural collection sites were over 100 meters from the nearest house. Finally, A. aegypti larvae were found in a water jar at an abandoned radar site on the top of a mountain in Nakhon Sawan province well over 1000 meters from the nearest house. All the above observations suggest either that feral populations of A. aegypti may exist in Thailand or that the flight range of this mosquito may be greater than is commonly accepted.

Larvae of Aedes (Stegomyia) craggi were collected in Lampang and Chiang Rai provinces from bamboo stumps and holes in logs. This species was originally described from the Indian subcontinent and these collections are the first recorded for this species from Thailand.

During this period, Aedes (Stegomyia) scutellaris was collected in Nonthaburi, Phuket and Prachuap Khiri Khan provinces. This species was reported in Thailand for the first time in the previous report. The populations in Nonthaburi and Prachuap Khiri Khan represent the first collections of this species on the mainland of Thailand. The collections from Nonthaburi were made approximately 45 kilometers north of the Gulf of Siam, at distances no greater than 700 meters from the Chao Phraya river. In Prachuap Khiri Khan this species was found from 200 meters to approximately 10 kilometers from the Gulf and at elevations of from 60 meters to 550 meters above sea level. Larvae of this species have been found in pandanus axils, rock pools, bamboo oviposition cups, holes in stumps, and in large and small clay jars. Other mosquito species found in association with A. scutellaris larvae thus far include Aedes (S.) aegypti, A. (S.) albolineatus, A. (S.) albopictus, A. (S.) subalbopictus, A. (S.) vittatus, A. (F.) aureostriatus and Anopheles sintonoides. Female Aedes (S.) scutellaris were also collected biting man between 1000 and 1600 hours in Prachuap Khiri Khan, together with females of A. (S.) albopictus, A. (S.) gardnerii imitator and A. (Ochl.) vigilax.

A possible new species of Stegomyia, near Aedes unilineatus, was collected during the year from Chon Buri and Nakhon Sawan provinces. Several female specimens of this species were collected in Lopburi province 3—4 years ago. Collections this year resulted in adequate series of males, females, larval skins with associated rearing data. Eggs of this species were collected from bamboo oviposition cups set on the ground and as high as 2 meters in trees. Collections of larvae of this species were also made from a bamboo stump and from split bamboo. Eggs of A. (S.) aegypti and A. (S.) albopictus were found in bamboo cups in association with this species, while larvae of A. (S.) albopictus, A. (S.) annandalei and

Tripteroides aranoies were collected together with this species from the natural bamboo sites. Although several diurnal man biting collections were made in areas where larvae of this species were found, no adults were collected.

One female Aedes (Paraedes) ostentatio was collected biting man in Nakhon Sawan province. Only the female of this species is known and it has not been previously recorded biting man in Thailand.

Anopheles: During this period over 17,000 anophelines were collected, comprising forty one species. A summary of these collections is given in Table 4.

Studies of the progeny of An. subpictus and An. subpictus var. indefinitus in Malaya (Reid, 1966)\* resulted in the raising of indefinitus to specific level. Anopheles indefinitus is now considered the predominate fresh water form, while An. subpictus is most commonly found in brackish water. Specimens of indefinitus with associated larval and pupal skins were collected this past year from Lampang and Prachuap Khiri Khan provinces.

A possible new species in the An. hyrcanus group was collected in Phuket province during the year. This species is represented by two females and one male with associated larval and pupal skins.

The Anopheles minimus complex in Thailand contains seven species (An. aconitus, An. culicifacies, An. filipinae, An. fluviatilis, An. minimus, An. pampanae, and An. varuna) all of which are morphologically very similar. Two of the species, An. minimus and An. aconitus, are vectors of malaria in Thailand. All of the other members, except An. pampanai, have been incriminated as vectors of malaria elsewhere in the Orient. Because of their similarity in appearance and frequent variations within species, of the external characters used in identification, separation of members of this complex is often difficult.

During this report period a study of the progeny of members of this complex was begun to determine the extent of morphological variation existing amongst sibling offspring of females of this complex. In addition, squash preparations of salivary gland chromosomes from 4th stage larvae of species of this complex have been prepared for comparison of specific gene loci.

During the year a total of 230 An. aconitus, 189 An. culicifacies, and 1, 164 An. minimus adults with associated larval and/or pupal skins were collected. No specimens of An. fluviatilis or An. pampanai were collected; and only a single specimen of An. filipinae was collected during this period.

Two forms of Anopheles varuna have been referred to in the literature. This species was originally described (Iyengar, 1924)\*\* as having a dark scaled proboscis, while subsequent publications by this author and others refer to An. varuna as having the apical half of the proboscis pale as in An. aconitus. A total of 27 females of the former type An. varuna were collected in the central area of Thailand during the year. Five of these females produced offspring, some of which, when reared to maturity, were typical An. minimus while others were varuna forms or intermediate forms with small humeral spots on the wing. Of 121 sibling broods obtained from typical An. minimus females, only 2 broods contained varuna-like females (Table 5). It is concluded, therefore, that the varuna-like forms in central Thailand are most probably variations of An. minimus.

The presence of a humeral pale spot on the wing in An. minimus as well as An. aconitus is inconstant in specimens collected thus far. In any given collection of wild-caught or reared adults approximately 10% of An. minimus males lack this character, while the percentages of male and female An. aconitus which lack this character run even higher. Anopheles aconitus was originally described as having a pale humeral wing spot (Donitz, 1902),\*\*\* but approximately 80-90% of An. aconitus from central Thailand lack this spot. This spot was present in about half the specimens from southern Thailand (Nakhon Sri Thammarat), and the

\* J. Med. Ent. 3:327

\*\* Ind. J. Med. Res. 12:23

\*\*\* Z. Hyg. infek Kr. 41:70

occurrence of 2 humeral spots on the wings of An. aconitus increased in frequency. Six specimens out of 256 An. aconitus collected there exhibited this character, while only 1 such specimen has been seen in more than 1,000 specimens from central Thailand.

The occurrence of a fringe spot at the apex of wing vein 1A is much more common in An. minimus than originally suspected. During this period it was found that as many as 10% of the specimens from certain areas in Saraburi province exhibit this character, thus making distinction from An. aconitus, which normally has this character, much more difficult.

During this period the preparation of chromosome smears from the salivary glands of 4th stage Anopheles larvae was initiated. The staining procedure used is that described by Welshons and Russell (1959).<sup>\*</sup> All chromosome preparations are shipped to the University of Maryland for study and the preparation of chromosome maps. A total of 109 slides prepared from An. aconitus and An. minimus larvae were shipped during the year.

Mansonia and Coquillettidia: During this period research continued on the distribution, ethology, larval habitats and colonization of mosquitoes of these two genera, which include vectors of human filariasis in Southeast Asia. A total of 2,613 specimens were collected from 5 provinces of Thailand during 1968-69.

Mansonia bonnea and Coquillettidia nigrosignata are reported here for the first time in SMRL collections from Thailand. Mansonia bonnea was collected in Phuket and Ranong provinces, always in association with M. dives. Both collections were biting man. A single specimen of Coquillettidia nigrosignata was collected biting man in Nakhon Sri Thammarat province.

During this period five different foods (ground-up commercial hamster food, ground-up guinea pig feces, 50% liver powder-50% yeast powder, cultured Paramecium spp. and hay infusion) were tested for their suitability in rearing field-collected larvae. Each food was tested separately with two aquatic plants, Eichornia sp. and Pistia sp., and blocks of styrofoam, as larval air sources. Field-collected 3rd and 4th stage larvae of M. annulifera, M. indiana and M. uniformis were used in these experiments. Hamster food and the liver and yeast mixture (1 gm per 2000 ml water, in each case) gave the best results (85% and 75% survival, respectively) when associated with the plant Eichornia. Only the hamster food yielded favorable results (65% survival) when associated with Pistia. When the amount of food was raised to 2 gm per 2000 ml of water the liver-yeast mixture and ground guinea pig feces yielded the best results (80% and 70% survival respectively) when associated with the plant Eichornia. Unsatisfactory results were obtained when this food concentration was used with Pistia. Styrofoam blocks failed to give favorable results in either of these experiments.

Attempts at colonization of these mosquitoes were made utilizing the most promising food and plant combinations from the previous experiments. Species involved in these attempts were wild-caught M. annulifera, M. indiana, M. uniformis and Coq. crassipes. These females were placed in oviposition containers, containing small Pistia plants. A total of 12,547 M. annulifera, 52,184 M. indiana and 47,419 M. uniformis eggs were deposited in clusters on the underside of the Pistia leaves. Hatch rates obtained were 59.1% (7,413) for M. annulifera, 51.6% (26,935) for M. indiana and 58.1% (27,570) for M. uniformis. A total of 1,231 Coq. crassipes eggs were deposited in rafts, and 52.1% (641) of these hatched. The effects of sunlight, temperature, types of substrate, water, food, and plants were all tested, yet very few F<sub>1</sub> adults were reared. More than sufficient numbers of larvae hatched to initiate colonies, but the majority of larvae died before reaching the third stage. As noted earlier, as high as 85% survival was obtained when wild-collected 3rd and 4th stage larvae were used. It is presumed the reason for failure in the colonization attempts begun with eggs is due to some unknown factor involving the 1st and 2nd instars. It was observed that the first two instars are much more active than the two later instars, which are more or less sessile, remaining attached to the plant rootlets the majority of the time. These observations suggest a different diet is possibly needed for the early instars.

<sup>\*</sup>Proc. Nat. Acad. Sci. 45:560

Table 1. Species and sources of Aedes (Stegomyia) mosquitoes collected during 1968-69.

Species	Adults Biting Humans	Resting Adults	Larval Habitats	Bamboo Oviposition Cups
<i>aegypti</i>	×	×	×	×
<i>albolineatus</i>			×	×
<i>albopictus</i>	×	×	×	×
<i>annandalei</i>	×		×	×
<i>craggi</i>			×	
<i>desmotes</i>	×			×
<i>gardnerii imitator</i>	×		×	×
mediopunctatus complex	×		×	
<i>noalbopictus</i>			×	×
<i>pseudalbopictus</i>	(*)	×	×	×
scutellaris complex	×		×	×
<i>subalbopictus</i>	×		×	×
<i>vittatus</i>			×	×
sp. near <i>unilineatus</i>			×	×

(\*) — Females of albopictus and pseudalbopictus are very difficult to separate.





Table 4. Species and sources of Anopheles collected during 1968-69.

Subgenus & Species	Biting		Co <sub>2</sub> -Net Trap	Larval
	Man	Cow		
(Anopheles) aitkenii group				+
.. argyropus	+			+
.. barbirostris	+	+	+	+
.. barbumbrosus				+
.. bengalensis				+
.. campestris	+	+	+	+
.. crawfordi	+	+		+
.. hodgkini		+		+
.. hyrcanus gr. sp. n.				+
.. indiensis	+		+	+
.. insulaeflorum				+
.. lesteri	+			
.. montanus				+
.. nigerrimus	+	+	+	+
.. palmatus				+
.. peditaeniatus	+	+	+	+
.. pursati	+	+	+	+
.. roperi				+
.. sinensis	+	+	+	+
.. sintonoides			+	+
(Cellia) aconitus	+	+	+	+
.. annularis	+	+	+	+
.. balabacensis	+		+	+
.. .. introlatus				+
.. culcifacies		+		+
.. indefinitus				+

Table 4 (Cont'd)

Subgenus & Species		Biting		Co <sub>2</sub> -Net Trap	Larval
		Man	Cow		
(Cellia)	jamesli	+	+	+	+
"	karwari	+	+	+	+
"	kochi	+	+		+
"	maculatus	+	+	+	+
"	minimus	+	+	+	+
"	nivipes	+	+	+	+
"	philippinensis	+	+	+	+
"	pujutensis				+
"	ramseyi	+	+		+
"	riparis macarthuri				+
"	splendidus	+	+	+	+
"	subpictus	+	+	+	+
"	sundaicus	+			+
"	tessellatus	+	+	+	+
"	vagus	+	+	+	+

Table 5. Appearance of progeny from "minimus" and "varuna" forms of Anopheles minimus.

#	Female parent form	Total No. Offspring	Form of progeny					
			"minimus"		intermediate*		"varuna"	
			♀	♂	♀	♂	♀	♂
1	"varuna"	2	2	—	—	—	—	—
2	"varuna"	2	1	—	1	—	—	—
3	"varuna"	9	1	3	—	2	1	2
4	"varuna"	10	4	—	4	1	—	1
5	"varuna"	17	4	2	3	5	1	2
6	"minimus"	5	1	—	2	—	1	1
7	"minimus**"	8	1	—	—	—	2	5
	Total	53	14	5	10	8	5	11

\* — intermediates = specimens with very small humeral spots on wing

\*\* — this specimen was an intermediate