

THE ANOPHELES (CELLIA) LEUCOSPHYRUS
DONITZ 1901 GROUP IN THAILAND

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INTRODUCTION

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The distribution of members of the *Anopheles leucosphyrus* group was discussed in detail by Reid (1949) and by Colless (1956, 1957). These authors dealt chiefly with the forms found on the Malayan Peninsula and in Borneo, but Colless included distribution records for all known members of the group. This material was later summarized by Chow (1961) in a mimeographed publication of the World Health Organization for use by workers in the public health field, primarily because of the rapidly growing number of observations that one member of the group (*A. b. balabacensis* Baisas) is an important malaria vector in many areas of Southeast Asia. Extensive observations on *b. balabacensis* in Thailand were reported by Scanlon and Sandhinand (1965), who noted that records of the Royal Thai Ministry of Public Health indicated that two other members of the group, *balabacensis introlatus* Colless and *riparis macarthuri* Colless, had been found in southern Thailand near the Malayan border. Specimens of these were not available at the time, but additional collections by members of the Department of Medical Entomology, U.S. Army Medical Component, Southeast Asia Treaty Organization (SEATO) have shed further light on the distribution of members of the *leucosphyrus* group in Thailand, particularly in the Malayan border area. The term Malaya is used here to refer to the peninsular portion of the country now known as Malaysia. The larger political entity of Malaysia also includes the territories of Sabah, Brunei and Sarawak on the island of Borneo.

Interest in *A. balabacensis* and other members of the *leucosphyrus* group has also been heightened by the emergence of strains of *Plasmodium falciparum* which exhibit varying degrees of resistance to treatment by the most commonly used synthetic anti-malarial drugs. In many of the areas of SE Asia where this phenomenon has been observed *balabacensis* is the most important vector. In addition, several mem-

bers of the *leucosphyrus* group have been implicated as natural or laboratory vectors of simian malaria parasites, and recently Chin *et al.* (1965) reported on a naturally acquired human infection with the simian parasite, *P. knowlesi*, in a jungle area of Malaya.

The species discussed here are members of the Series *Neomyzomyia* Christophers, of the subgenus *Cellia* Theobald. Two other *Neomyzomyia* species are known from Thailand, *A. kochi* Dönitz and *A. tessellatus* Theobald. The members of the *leucosphyrus* group are all easily recognized by the presence of a broad white band covering the apex of the hind tibia and the base of the first hind tarsomere. No other *Anopheles* species in SE Asia exhibit this character. Identification of species and other forms within the group is far more difficult. A full discussion of relationships within the group and the probable evolutionary relationships among the forms may be found in Colless (1956). The species of the *leucosphyrus* group may be regarded as a series of sibling species in the sense of Mayr *et al.* (1953) in which morphological distinctions are perhaps somewhat more readily made than is the case with members of the *Anopheles hyrcanus* (Pallas) species group in southeast Asia.

Unless otherwise stated all of the specimens discussed in this review are in the collections of the United States National Museum.

Anopheles balabacensis balabacensis BAISAS 1936

This is by far the most abundant and widely distributed member of the group in Thailand. Scanlon and Sandhinand (1965) noted that it had been reported from many localities in the country and continued collection by SEATO and Thai Ministry of Health personnel has reinforced this impression. *A. b. balabacensis* is somewhat secretive in its habits as regards adult resting and larval habitat, and it tends to be spotty in distribution even in what appear to be suitable areas. Furthermore, the females usually feed late at night and they may be missed unless night-

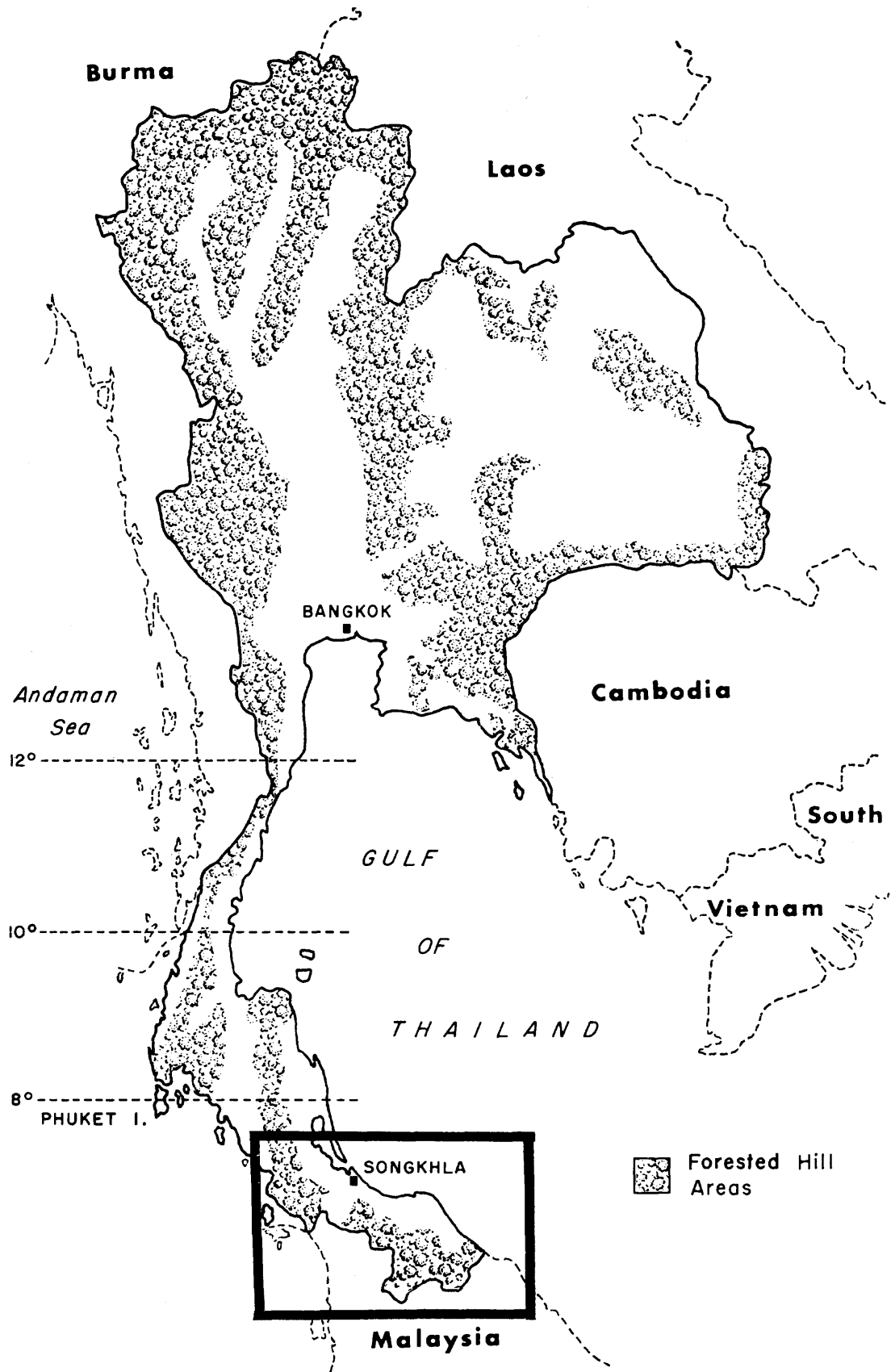


Figure 1 – Map of Thailand showing distribution of monsoon and evergreen forest areas. Rectangle indicates approximate area covered by Figure 2.

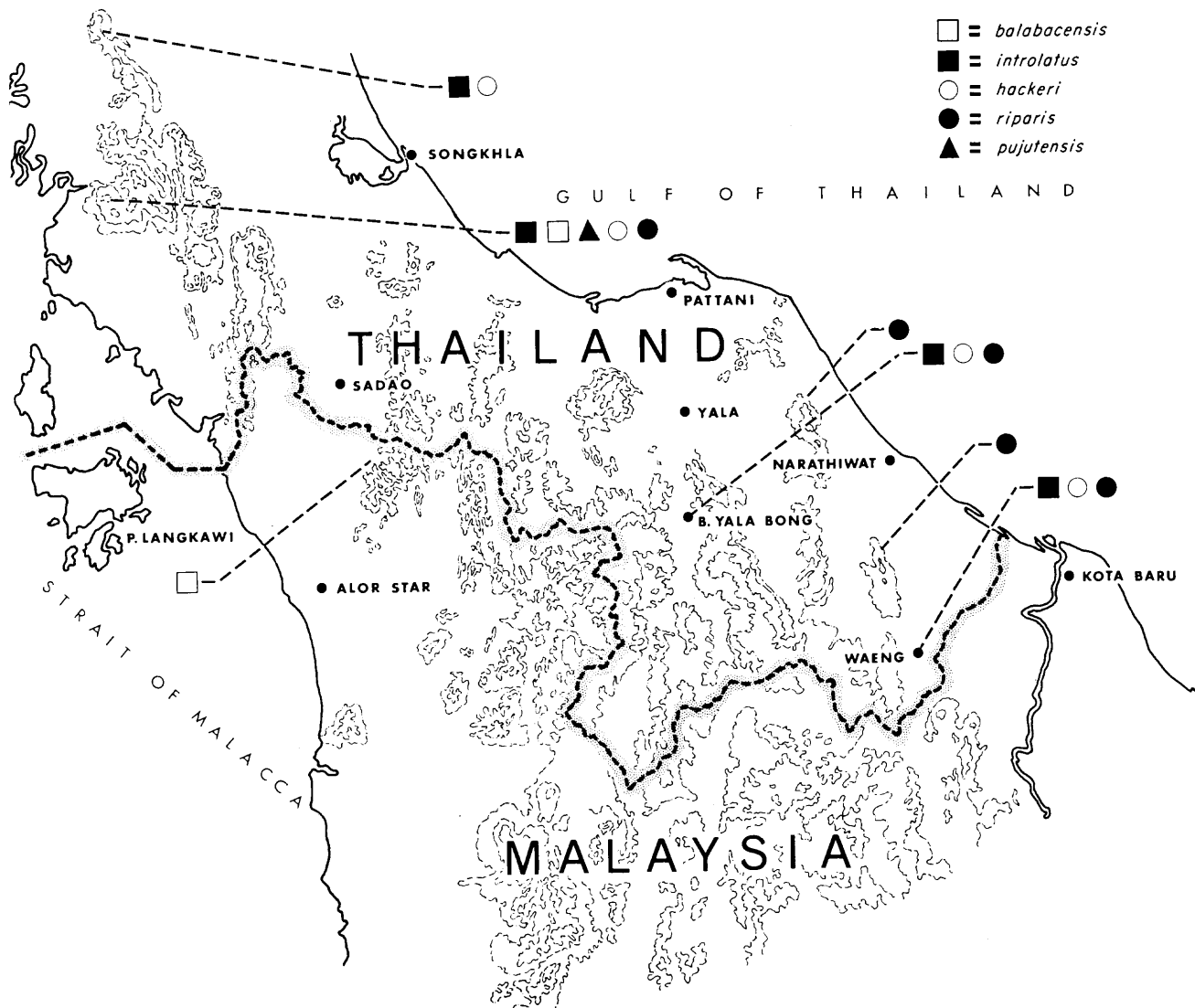


Figure 2 — Sites in Southern Thailand and Northern Malaya from which species of the *Anopheles leucosphyrus* group have been examined.

long collections are made. However, the general statement of Scanlon and Sandhinand appears to hold true—that one may expect to find *b. balabacensis* in any place in Thailand, north of the most southern Provinces, where forested hills or mountains are found. The area in figure 1 indicated as forested may be taken as a reasonably accurate guide to the distribution of the nominate form of *balabacensis* in Thailand.

The very large populations of *b. balabacensis* reported by Scanlon and Sandhinand (1965) for Cholburi Province in 1964 have not been encountered elsewhere in Thailand. Nor have such large numbers been found again in Cholburi. The site of the 1964 studies has been further deforested, and has received several heavy DDT treatments, and it is assumed

that these factors combined to reduce the population. It does not appear, however, that very large populations of *A. balabacensis* are required to sustain malaria transmission in forested areas. In 1965 and 1966 small teams from SEATO, WRAIR and cooperating agencies conducted studies on forest *Anopheles* in the Pak Thong Chai District, in Nakorn Ratchisima Province, approximately 100 miles northeast of Bangkok. In the 1966 experiments approximately 6 percent of the *A. balabacensis* females examined were carrying sporozoites. During each year two members of the teams sustained *falciparum* malaria attacks (despite prophylaxis) while in the area or shortly after leaving it. One striking feature of each of these experiences was the small number of *balabacensis*

captured (approximately 1-3 per man, per night) and the complete or almost complete absence of other *Anopheles* biting man at the time. It would appear that *balabacensis* is an extremely efficient malaria vector under these circumstances. Similar high sporozoite and malaria attack rates have been reported by SEATO and Thai Ministry of Health investigators in other areas of Thailand, but usually other known or suspected vectors, especially *A. minimus*, were also present in the areas studied.

Additional collections of adults and immature stages have been made by SEATO personnel in many areas of Thailand, from near sea level to as high as 4,800 feet on a mountain near the Cambodian border. Larvae have been found in many small water collections under shade. Elephant footprints appear to be a favored habitat, but any water-holding depression in the soil is a potential breeding site, provided it receives sufficient shade. With very few exceptions, however, the larval habitats seen have been shallow temporary pools that are frequently refreshed by rain. The species has not been found in grassy pools or in larger semi-permanent pools with one exception discussed below. This form does not occur as frequently in rock pools as some of the other forms in the group. Scanlon and Sandhinand (1965) described an unusual larval site in sapphire pits in southeastern Thailand, and collections in 1965 and 1966 confirmed the continued high larval production in this niche.

The females are primarily exophilic, although they enter fairly open shelters to feed. Small numbers were collected in September 1966 at Pak Thong Chai in a Landrover in which a four man collecting team had taken shelter from extremely heavy rains. Females began entering the vehicle at approximately 11 p.m., apparently having flown through the rain from the surrounding jungle. This late night feeding pattern was observed in most collecting sites in Thailand, but SEATO personnel have occasionally found local populations where the females came to human bait much earlier in the evening. At times this early feeding pattern was very striking and similar patterns have been reported from several areas in Vietnam. No reason for this difference in feeding and activity pattern has been detected as yet.

Anopheles balabacensis is known to feed readily on monkeys in Thailand (Scanlon and Sandhinand, 1965) and in Cambodia (Eyles *et al.*, 1964). Eyles and his co-workers failed to infect any of seven rhesus monkeys inoculated with sporozoites of *balabacensis* origin in Cambodia. Cheong *et al.* (1965) later reported the isolation of *Plasmodium cynomolgi* and *P. inui* from *balabacensis* collected in Perlis, Malaya, near the Thailand border. Recently Gould *et al.* (1966) infected splenectomized gibbons (*Hulobates lar*) in Thailand by inoculation of triturated *balabacensis* which had been permitted to feed on human *Plasmodium falciparum* patients some two weeks earlier. The possibility exists that *balabacensis* is also a natural vector of simian malaria in Thailand, but much additional work will be needed to establish the point. It is an excellent laboratory vector of several species of human and simian malaria (Coatney, 1966).

Several hundred adults and an equal number of immature specimens were examined during this review. No attempt will be made to list these, but a few localities in the far southern part of Thailand are indicated in figure 2. The locality in northern Malaya indicated for *balabacensis* is the approximate site where British Commonwealth troops encountered drug resistant *falciparum* malaria in 1963 (Montgomery and Eyles, 1963) and where Cheong *et al.* (1965) isolated simian parasites. There is no doubt that the specimens of *balabacensis* reported from that area were the nominate subspecies, as they were identified by workers who were very familiar with *balabacensis* and *introlatus*. This northwestern corner of Malaysia probably marks the southernmost extension of the range of *balabacensis* on the Malayan peninsula.

Anopheles balabacensis introlatus COLLESS 1957

Colless (1957) assigned this name to the form which he had previously called *A. leucosphyrus* "Kepong Form." At that time *b. introlatus* was known only from the vicinity of the type locality in Malaya. Subsequently it has been reported from many areas of Malaya, apparently never in large numbers (Warren *et al.* 1963). It was first found in southern Thailand by collectors of the Royal Thai Ministry of Public Health in 1960 or 1961 according to unpublished reports. Localities from which SEATO collectors have reported *A. b. introlatus* are indicated in figure 2. Extensive collections elsewhere in Thailand failed to yield the species, except for a single male with associated larval and pupal skins, collected in Ranong Province, above 10° N. latitude which is tentatively identified as *b. introlatus*. Aside from this single specimen, all of the *b. introlatus* collected were from that portion of Thailand south of approximately 8° N. latitude. From the relatively few collections at hand it appears that the nominate subspecies may extend farther south on the west side of the Malayan Peninsula, while *b. introlatus* extends farther north on the eastern side. Several collections from the Waeng District of Narathiwat Province included adults which could not be placed in either subspecies with confidence. Most specimens for which associated larval and pupal skins were available were identified as *b. introlatus*, but a significant percentage were intermediate. These collections appeared to fit the criteria of an intermediate population as outlined by Mayr *et al.* (1953) and lend further weight to Colless's (1957) decision to regard *introlatus* as a subspecies of *balabacensis*. Additional collecting will be needed to define precisely the line of demarcation of the two subspecies. This is a matter of some practical interest, since *introlatus* has never been implicated as a vector of human malaria.

None of the specimens collected in Thailand by SEATO were from human or animal biting collections, but Wharton *et al.* (1964) indicate that *b. introlatus* is attracted to human and monkey bait in Malaya. Two species of simian malaria parasites have been isolated from *b. introlatus* in Malaya, *P. cynomolgi* and *P. fieldi* (Wharton *et al.*, 1964).

Immature stages were collected from the same general types of habitat as the nominate subspecies. Larvae were also found a number of times in the rocky stream pools more characteristic of *A. riparis*.

Anopheles hackeri EDWARDS 1921

This species was described from Malaya and also recorded from Borneo and doubtfully from Sumatra (Colless, 1955). The distribution in Thailand is given in figure 2. All of the SEATO collections have been of immature specimens, and the habits of the adults in Thailand are not known. In Malaya most studies on the species have been conducted in the coastal areas where the larvae and adults are strongly tied to a Nipah palm habitat (Reid and Weitz, 1961). However, Warren *et al.* (1964) note that the primary hill forest of central Malaya was originally thought to be the habitat of the species, the larvae being found in fallen bamboos, tree holes and ground pools. In the SEATO collections larvae were taken from a tree hole near ground level, rock holes and ground pools. Specimens from Thailand were compared with others from coastal areas of Malaya but no significant differences were detected.

A. hackeri has not been collected biting man in Thailand, and there is evidence from Malaya (Reid and Weitz, 1961) that it feeds preferentially on monkeys, rarely on man. It has been found naturally infected with sporozoites rather frequently in Malaya, and five species of simian parasites have been isolated from *hackeri* (Wharton *et al.* 1964).

Anopheles pujutensis COLLESS 1948

This is another of the species which appears to reach the northern limit of its distribution in the Thailand-Malayan border area. Colless (1955) reported it from Borneo, Malaya and Sumatra. Reid and Weitz (1961), Warren *et al.* (1963) and Wharton *et al.* (1964) gave additional data on the species in Malaya in connection with studies on simian malaria. The single confirmed Thai record is from Songkhla Province (Figure 2). Since this locality is so far north of the border and the known collection localities in Malaya it seems quite likely that it will be encountered farther south in Thailand in the future (subsequent examination of records indicates that *pujutensis* also was collected once in the larval stage in the Waeng District of Narathiwat Province). Reid and Weitz (1961) found that *pujutensis* was strongly attracted to monkeys in Selangor, Malaya, and showed serological evidence of biting man. Wharton *et al.* (1964) reported similar results with precipitin tests. No adults were captured in Thailand. Reid and Weitz (1961) and Warren *et al.* (1963) reported sporozoites in wild caught *pujutensis* in Malaya, but these were probably of simian origin. The precise role of *pujutensis* as a vector of simian malaria, however, is still in doubt. The serological evidence of feeding on man should probably be interpreted with caution, and the failure to capture the species in net traps with human bait may be more revealing. *A. pujutensis* appears to be fairly rare wherever encountered. The Thailand specimens were immatures, taken from stream side rock pools with *b. balabacensis* and *riparis macarthuri*. Similar sites were described by Warren *et al.* (1964) in Malaya.

Anopheles riparis macarthuri COLLESS 1956

Colless (1955) described subspecies *macarthuri* for the form of *Anopheles riparis* found in Sarawak. Differences from the type form from the Philippines

were mostly of a statistical nature, and Reid (1966) has expressed some doubt that the Malayan form deserves subspecific status. The reared adults from Southern Thailand were extremely variable, and on adult characters alone would be difficult to assign to either subspecies. However, the larvae and pupae were closer to *r. macarthuri* and the pattern of distribution of other species in the group lend weight to the maintenance of subspecific status for it. All of the Thailand specimens were taken in the immature stages and no observations were made on the adult habits. The adults appear to be quite secretive, as Wharton *et al.* (1964) found that while the larvae of *macarthuri* were the most common of the *leucosphyrus* group in the Malayan hill forest, only two adults were attracted to monkey bait. They stated that these were the first adults ever captured.

According to Warren *et al.* (1963) one of two adults of *A. r. macarthuri* taken from monkey bait yielded sporozoites which failed to infect monkeys on inoculation. It is likely however that this member of the *leucosphyrus* group is also a vector of simian malaria.

A. r. macarthuri larvae were most commonly taken from rock holes, stream-side pools and animal footprints along hill streams under forest cover. They appeared to be particularly abundant in areas near waterfalls.

OTHER SPECIES OF THE *leucosphyrus* GROUP

During this study specimens of most of the other members of the *leucosphyrus* group in the USNM collection were examined in order to verify the identification of the forms found in Thailand. These included: *A. leucosphyrus* Dönitz (Malaya), *A. cristatus* King and Baisas, 1936 (Mindanao, Philippines), *A. riparis* King and Baisas, 1936 (Samar and Mindanao, Philippines), *A. balabacensis* Colless, 1957 (Luzon, Philippines) and *A. elegans* James, 1903 (Karwar, India). With the exception of the somewhat doubtful status of the form of *riparis* found in Thailand, none of these members of the *leucosphyrus* group occurred in the Thailand collections.

DISCUSSION

Anopheles b. balabacensis is the only member of the *leucosphyrus* group found in Thailand north of the area close to the Malayan border. It is also the only member of the group found in the neighboring countries of mainland SE Asia north of Malaya. The reasons for this homogeneity, as opposed to the multiplicity of forms in South Thailand and Malaysia, may be found in the rainfall patterns in the respective areas and the resultant differences in vegetational cover and hydrography. Reid (1950) has commented in detail on several *Anopheles* species, including *A. minimus* Theobald, 1901, *A. annularis* Van der Wulp, 1884, and *A. ramsayi* Covell, 1927, which occur widely in mainland SE Asia but disappear south of the general area of the Thailand-Malaya border. As can be seen in figure 2, the mountains in this region run in a general north-south direction, and there does not appear to be any geographical barrier to the movement of species in the area. According to Reid (1950) the same discontinuity in distribution has been noted in butterflies, rep-

tiles and amphibia. These more recent collections from Thailand again emphasize the strength of this barrier. This is a matter of some importance to Malaya, since *A. b. balabacensis* is the most important malaria vector in the species group, and its spread southward into Malaya could have serious consequences for the malaria eradication program just beginning there.

An examination of the climatological data for the area (Ohman, 1965) indicates that the 150 inch isohyet roughly approximates the southern limit of *A. b. balabacensis*. Reid (1950) also points out that the distribution of rainfall within the year may also be extremely important. In Malaya proper the rainfall is distributed fairly evenly throughout the year, with one or two periods of somewhat higher rainfall. In most of Thailand and the other countries of mainland SE Asia there is generally a distinct single monsoon period each year, with a marked contrast between wet and dry seasons.

Anopheles b. balabacensis seems to extend somewhat further into Malaya on the western side of the peninsula than on the eastern, (fig. 2). If confirmed by further collecting in the area this distribution may be due to the effect of the November and December rains from the China Sea which strike the eastern side of the peninsula, but contribute little rain to the western side. The latter region has a rainfall pattern similar to areas further north in Thailand, with rains from May to September and a prolonged dry period from October to May.

The border area is a particularly interesting one from the standpoint of zoogeography, and deserves much additional attention. Work in the area is however, somewhat hampered by the lack of developed roads and transportation, particularly during the wetter time of the year. Present plans of the government of Thailand for opening up forested areas to cultivation should make the task somewhat easier, and the settling of large numbers of farmers in previously virgin forest make it all the more important to define the distribution of the various members of the *leucosphyrus* group and their status as malaria vectors in the border region.

SUMMARY

Anopheles balabacensis balabacensis is the most abundant and important member of the *Anopheles leucosphyrus* group in most of Thailand. It is found predominantly in the hill forests and is a potent vector of human malaria. In the far southern Provinces of Thailand it is replaced by *A. balabacensis introlatus*, and the latter subspecies extends southward into Malaya. In the vicinity of the border populations of *A. balabacensis* occur which cannot readily be assigned to either subspecies, but the nominate subspecies extends slightly into Malaya on the western side of the peninsula.

Three other members of the *leucosphyrus* group have now been found in the border area and somewhat northward into Thailand. These are: *A. hackeri*, *A. pujutensis* and *A. riparis macarthuri*. These seem to be absent above approximately 8° N. latitude in Thailand, probably because of the transition from a rain forest environment to a monsoon environment

with a prolonged dry period which occurs in the rest of Thailand.

Anopheles balabacensis balabacensis is the only species listed which has been implicated in the transmission of human malaria. However, all of the species probably serve as vectors of simian malaria. Further investigation is needed on the distribution of the *leucosphyrus* group in the Thailand-Malaya border area, particularly from the aspect of determination of the precise climatic factors which influence this distribution.

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