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LARVAL HABITATS OF MALARIA VECTORS AND OTHER *ANOPHELES* MOSQUITOES AROUND A TRANSMISSION FOCUS IN NORTHWESTERN THAILAND

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ABSTRACT. We sampled 199 bodies of water for *Anopheles* larvae around a malaria-endemic village near Mae Sot, Thailand, over 2 years. *Anopheles dirus* species A and D occurred in 54 small, well-shaded, stream and temporary ground pools. The larval habitat of species D is reported for the first time. *Anopheles minimus* species A occurred in 8 samples from slow-moving streams, in 8 from rice fields, and in 2 from ground pools. *Anopheles pseudowillmori* occurred in one sample from a ground pool, in 3 from streams, and in 9 from rice fields.

INTRODUCTION

One method of malaria control is elimination of *Anopheles* larvae by application of chemical or biological toxicants or by source reduction through removal of their habitat. Although source reduction is often the least expensive and most environmentally acceptable control strategy, its feasibility is determined by accessibility and consistency of habitats.

The *Anopheles* (*Cellia*) *dirus* complex, which in Thailand comprises 5 sibling species (Peyton 1990), includes the major vectors of malaria parasites in much of Southeast Asia where forests exist (Scanlon and Sandinand 1965, Rosenberg 1982, Rosenberg et al. 1990). This is true for Ban Tham Seu, a Karen hill tribe village in western Thailand where both species A and D of *An. dirus* are the major vectors (Green et al. 1991). We report here the larval habitat types for these 2 species as well as for 2 secondary vectors and another 17 species of *Anopheles* found in the area.

MATERIALS AND METHODS

Ban Tham Seu is a Karen (Sgaw) village of about 200 people, 12 km ESE of Mae Sot (16°41'N, 98°41'E), near the Myanmar border with Thailand (Fig. 1). The village is compact and is located at about 200 m above sea level in the deciduous woodland of the eastern watershed of the Moei River, which drains westward into the Salween River. It is about 5 km due east of a second Karen village, Ban Pha De, which lies on the eastern edge of the Moei River erosion plain. Forest is confined to the watershed itself, elsewhere having been eliminated for agricultural purposes around the erosion plain. Much of the area between Ban Tham Seu and Ban Pha De has been cleared and is used now for cash-crop production, with some reforestation with teak. These trees are now about 3 m high and have developed a continuous canopy. The region has distinct wet (May–October) and dry (November–April) seasons. The illustrations (Figs. 1–3) are modified from the topographic map series L 7017, 4742 III and aerial photographs LPR-PK, PK-120 No. 31168-81, scale 1: 15,000, from the Geographical Survey of Thailand.

Two larval surveys during August 1989 and October 1990 were made around Ban Tham Seu (Figs. 1–3); a single survey was made at an area of wet rice cultivation near Ban Pha De and Mae Tao during 1990. Classification of larval habitats follows the method used by Belkin (1962).

All possible bodies of water were sampled even though *Anopheles* rarely colonize natural containers, such as plant axils or stumps. Due to the morphological similarity of *An. dirus* species A and D, identification was made using DNA probes specific for each species (Panyim et al. 1988) and enzyme electrophoresis (Green et al. 1991). Species C of *Anopheles* (*Cel.*) *minimus* commonly can be distinguished from species A of *An. minimus* by the presence of a presector pale spot (Green et al. 1990). When specimens collected during 3½ years of sampling were identified by electrophoresis (Green et al. 1990), species C had presector pale spots (n = 5) and all species A lacked presector pale spots (n = 50).

RESULTS AND DISCUSSION

A total of 199 bodies of water were sampled: 146 were ground waters and 53 were natural

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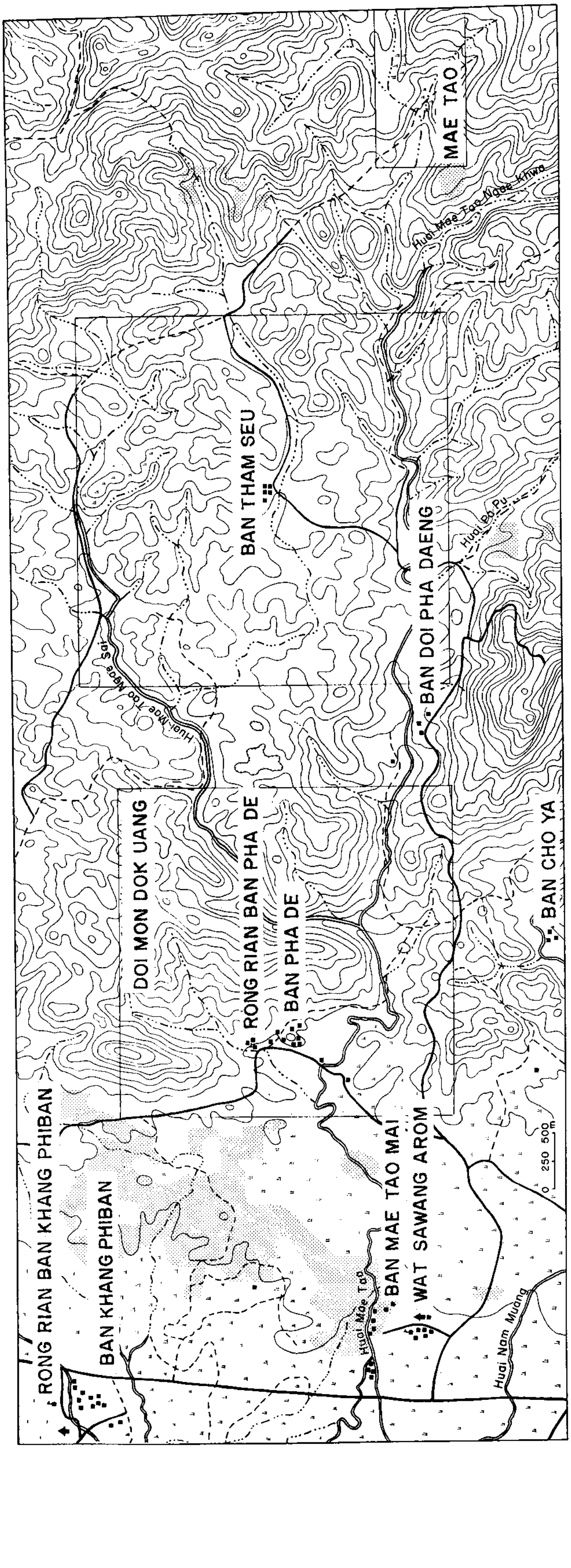

Fig. 1. Topographic map of the villages Ban Tham Seu, Ban Pha De, and Mae Tao.

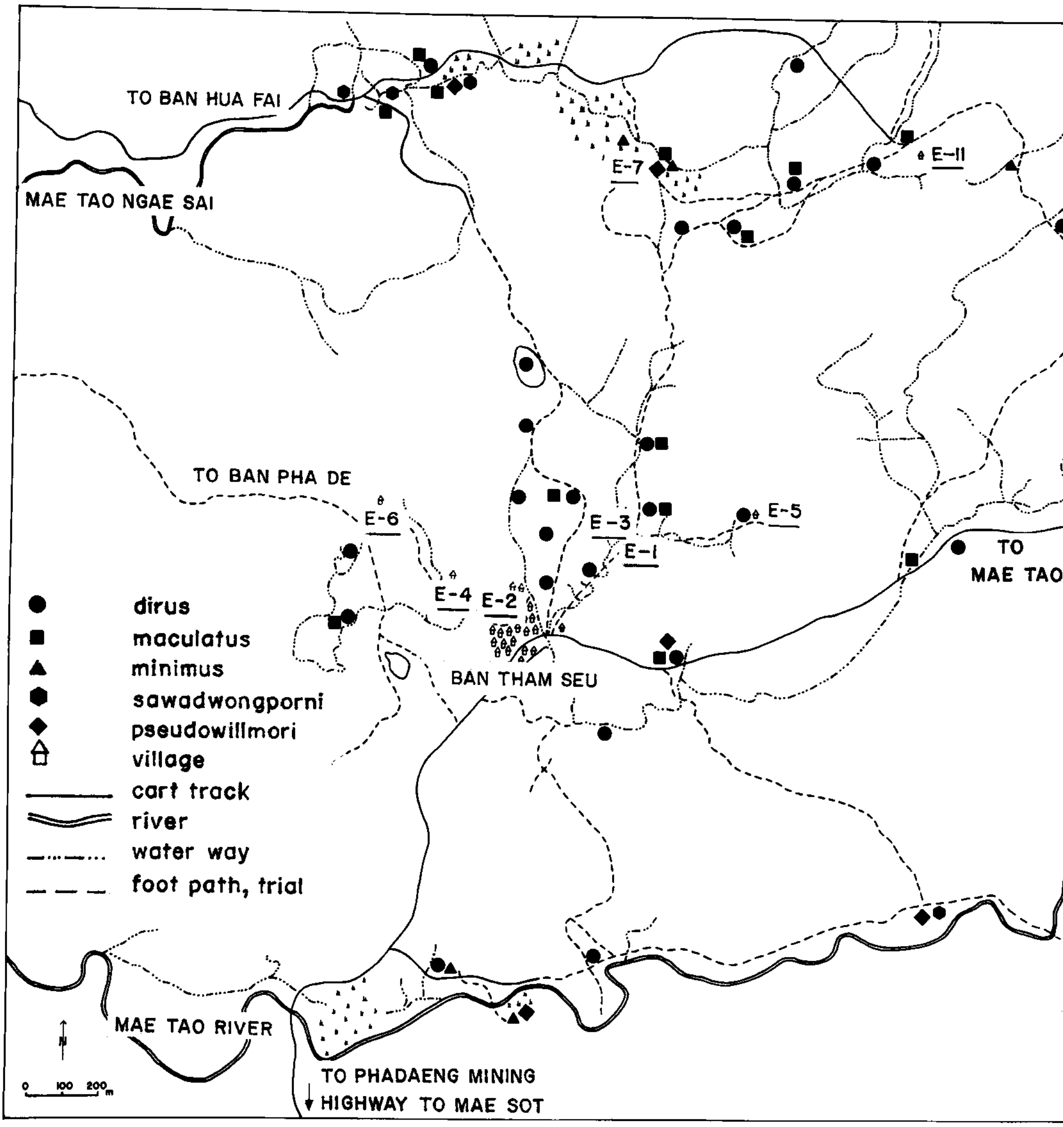

Fig. 2. Map of Ban Tham Seu showing sites of malaria vectors larvae collected, including members of the Maculatus Group.

containers (e.g., bamboo stumps, tree holes and stumps, plant axils). All ground water sites contained *Anopheles* larvae, as did one tree stump. The numbers and types of water bodies in which important *Anopheles* species were found are shown in Table 1. The geographic distributions of larval habitats of all vectors around Ban Tham Seu, together with nonvectors taxonomically related to *Anopheles* (*Cel.*) *pseudowillmori* (Theobald), are shown in Fig. 2. The distributions of species A and D of *An. dirus* are shown in Fig. 3. *Anopheles pseudowillmori,* a member of the Maculatus complex was found earlier to be a vector of human malaria parasites at our study site (Green et al. 1991). Three other members of this complex, *Anopheles* (*Cel.*) *maculatus* Theobald, *An.* (*Cel.*) *dravidicus* Christophers, and *An.* (*Cel.*) *sawadwongporni* Rattanarithikul and Green, were found biting man at the same site but were consistently negative for malaria parasites.

In this study *An. minimus* species A was found 18 times in flooded rice fields or stream margins; this species was collected 2 times from ground pools in dry rice fields, next to the streams. Species found together with *An. mini-*

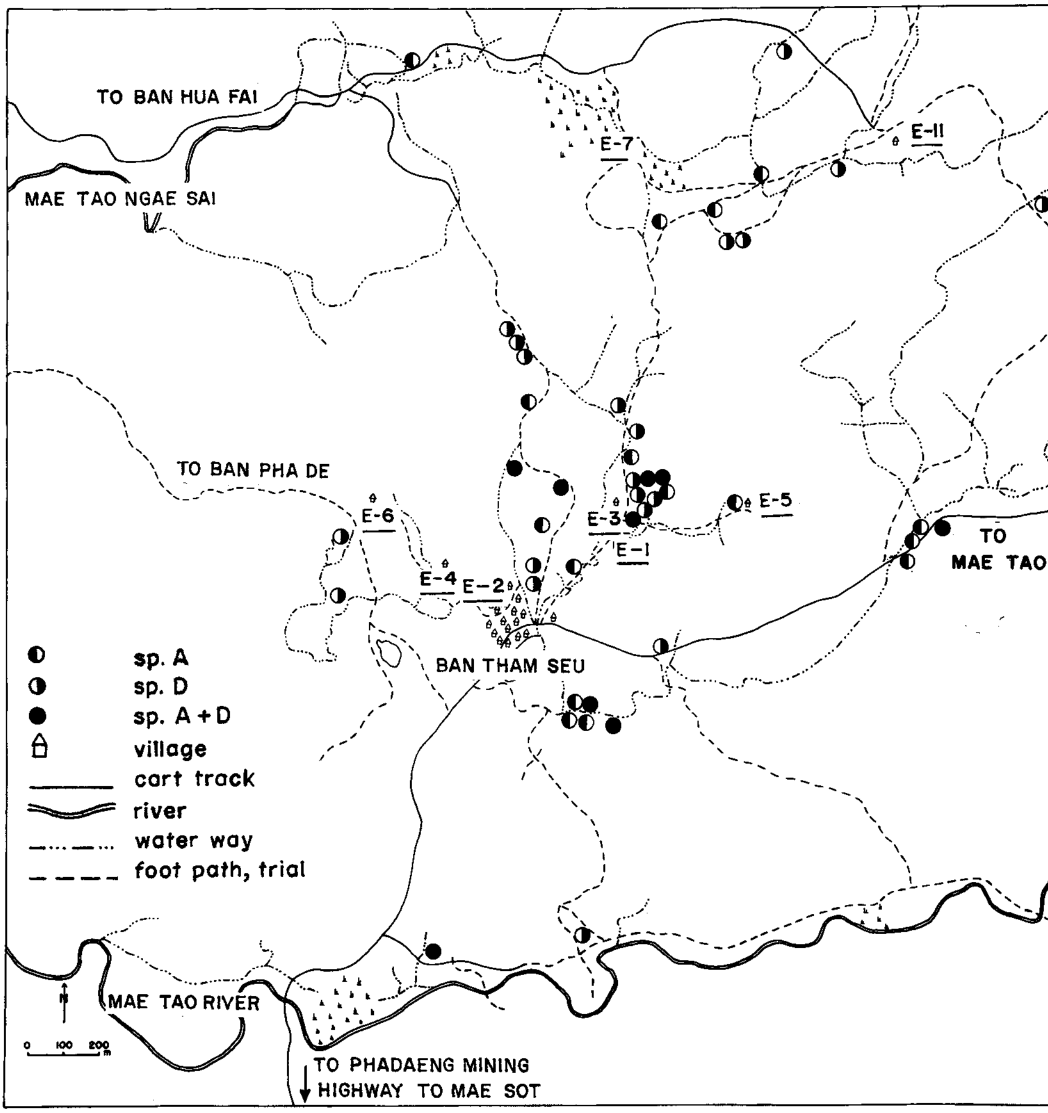

Fig. 3. Map of Ban Tham Seu showing the distribution of bodies of water where *Anopheles dirus* species A and D were collected.

mus included *Anopheles* (*Cel.*) *aconitus* Doenitz, *An.* (*Cel.*) *annularis* Van der Wulp, *An.* (*Cel.*) *kochi* Doenitz, *An. pseudowillmori, An.* (*Cel.*) *tessellatus* Theobald, *An.* (*Cel.*) *vagus* Doenitz, *An.* (*Anopheles*) *barbirostris* Van Der Wulp, *An.* (*Ano.*) *bengalensis* Puri, and *An.* (*Ano.*) *peditaeniatus* (Leicester).

The larval habitats of *An. dirus* D, which appear to be similar or identical to those of *An. dirus* A, are reported here for the first time. The major habitats were tracks made by domestic elephants, wheels tracks and cart tracks along paths or road, animal wallows, seepage pools or pools left by floods, pools in drying stream beds, and margins of streams where water was shallow and stagnant or very slow running. Most of the pools were less than 2 m in width and less than 30 cm in depth and under partial to heavy shade, although some were exposed to the sun. A single sample (9 larvae) of *An. dirus* A was collected from a cut tree stump, about 1 m in height, 50 cm in diameter, and 15 cm in depth containing muddy water over leaves and plant debris. The ratios of the number of sites positive for either species A or D of *An. dirus* to the total number of sites sampled around Ban Tham Seu (45:157)

Table 1. Larval habitats containing malaria vectors and closely related species around the villages of Ban Tham Seu (TS) and Ban Pha De (PD) and the wet rice land at Mae Tao (MT), Thailand.

	Localities			Habitats[1]				
Anopheles species	TS	PD	MT	1	2	3	4	5
sawadwongporni	5	0	0	1	2	1	1	0
dirus sp. A	16	2	1	5	5	8	0	1
dirus sp. D	20	3	0	13	6	4	0	0
dirus spp. A + D	9	3	0	5	1	6	0	0
minimus sp. A	12	2	4	0	2	8	8	0
maculatus	20	2	2	8	7	5	4	0
pseudowillmori	6	1	6	0	1	3	9	0
Total water bodies	157	25	17	42	31	41	17	53

[1] Types of bodies of water: 1 = very temporary bodies of water likely to dry out in dry spells during the rainy season, footprints, wheel tracks; 2 = pools likely to remain as long as the rainy season, not associated with streams, wallows; 3 = bodies of water associated with streams, seepages, pools; 4 = rice fields; 5 = natural containers, bamboo stumps, leaf axils, puddles in rocks, tree stumps, etc.

and Ban Pha De (8:25) were similar ($\chi^2 = 0.06$; df = 1; $P > 0.05$). These ratios do not differ significantly from those at Mae Tao (1:17; $\chi^2 = 1.95$; df = 1; $P > 0.05$). The greater number of bodies of water positive for species D was unexpected based on the relative abundance of the 2 species in human bait collections where a ratio of species A to species D of 2:1 occurred consistently each month over 3 rainy seasons (Rattanarithikul et al., unpublished data; Green et al. 1990). Two possible explanations for this difference in larval and adult abundance are: 1) species A concentrates eggs in fewer bodies of water, or 2) species A is more anthropophilic than species D.

During the dry season, *An. dirus* was found in streams, along the banks of pools left after streams flood, or in other seepage areas. When the rainy season started, larval habitats increased, particularly in small pools in the soil of paths and roads trodden by men and animals [Wilkinson et al. (1978) as *Anopheles balabacensis* Baisas]. The occurrence of both species in temporary habitats, the survivability of their eggs in soil, and their tendency to oviposit on damp soil in the wet season (Wilkinson et al. 1978) suggest that *An. dirus* could be expected to occur around Ban Tham Seu throughout the year. The relationships between species A and D of *An. dirus* are particularly interesting. Although both species can occur together, species D seems more likely to occur in very temporary bodies of water (Habitat 1, Table 1) than species A.

Further studies of the microhabitat differences of species A and D of *An. dirus* would provide useful information on how the larval stages of the 2 species share resources. Whether or not differences in the microhabitats of these species can be identified, prospects for vector control by means of source reduction of their larval habitats seem to be extremely poor because both species use small, widely dispersed bodies of water (Fig. 3).

In contrast to *An. dirus* and *An. maculatus*, which prefer to lay their eggs in temporary habitats, the strategy of *An. minimus* and *An. pseudowillmori* seems to be to oviposit in more stable (semipermanent) habitats in rice fields and streams. Prospects for control of immature stages of *An. minimus* and *An. pseudowillmori* would seem to be greater than that for species that utilize many varied and temporary habitats.

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REFERENCES CITED

Belkin, J. N. 1962. The mosquitoes of the South Pacific, Volume 1. Univ. Calif. Press, Berkeley and Los Angeles.

Green, C. A., R. F. Gass, L. E. Munstermann and V. Baimai. 1990. Population genetic evidence for two species in *Anopheles minimus* in Thailand. Med. Vet. Entomol. 4:25–34.

Green, C. A., R. Rattanarithikul, S. Pongparit, P. Sawadwongporn and V. Baimai. 1991. A newly-recognized vector of human malarial parasites in the Oriental Region, *Anopheles (Cellia) pseudowillmori* (Theobald, 1910). Trans. R. Soc. Trop. Med. Hyg. 85:35–36.

Panyim, S., S. Yasothornsrikul, V. Baimai, R. Rosenberg, R. G. Andre and C. A. Green. 1988. Identification of isomorphic malaria vectors using a DNA probe. Am. J. Trop. Med. Hyg. 38:47–49.

Peyton, E. L. 1990. A new classification for the Leucosphyrus Group of *Anopheles (Cellia)*. Mosq. Syst. (1989) 21:197–205.

Rosenberg, R. 1982. Forest malaria in Bangladesh.—III. Breeding habitats of *Anopheles dirus.* Am. J. Trop. Med. Hyg. 31:192–201.

Rosenberg, R., R. G. Andre and L. Somchit. 1990. Highly efficient dry season transmission of malaria in Thailand. Trans. R. Soc. Trop. Med. Hyg. 89:22–28.

Scanlon, J. E. and U. Sandinand. 1965. The distribution and biology of *Anopheles balabacensis* in Thailand (Diptera: Culicidae). J. Med. Entomol. 2: 61–69.

Wilkinson, R. N., D. J. Gould, P. Boonyakanist and H. E. Segal. 1978. Observations on *Anopheles balabacensis* (Diptera: Culicidae) in Thailand. J. Med. Entomol. 14:666–671.