BIONOMICS STUDIES OF *MANSONIA* MOSQUITOES INHABITING THE PEAT SWAMP FOREST

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Abstract. The present study was conducted in the years 2000-2002 to determine the bionomics of *Mansonia* mosquitoes, vectors of nocturnally subperiodic *Brugia malayi*, inhabiting the peat swamp forest, "Phru Toh Daeng", Narathiwat Province, Thailand. Fifty-four species of mosquitoes belonging to 12 genera were added, for the first time, to the list of animal fauna in the peat swamp forest. *Mansonia* mosquitoes were the most abundant (60-70%) by all collection methods and occurred throughout the year with a high biting density (10.5-57.8 bites per person-hour). *Ma. bonneae* was most prevalent (47.5%) and fed on a variety of animal hosts, including domestic cats, cows, monkeys, and man with a maximum biting density of 24.3 bites per person-hour in October. The infective bites were found for the first time in *Ma. annulata* collected at Ban Toh Daeng (13 00-14 00 hours) and also *Ma. bonneae* at forest shade (16 00-17 00 hours) and in a village (20 00-21 00 hours) with rates of 0.6, 1.1 and 1.0%, respectively.The biting activities of these two species occurred in both the day and night time, with two lower peaks at 10 00 hours (18.5 bites per person-hour) and 13 00-15 00 (8.5-10.0 bites per person-hour) hours, but the highest peak was 19 00-21 00 hours (31.5-33.0 bites per person-hour) The biting activity patterns corresponded with the periodicity found in man and domestic cats and may play an important role in either transmission or maintenance of the filarial parasites in the peat swamp forest. The relative role of *Ma. bonneae* and *Ma. uniformis* in different environmental settings (primary swamp forest and open swamp) on the transmission of nocturnally subperiodic *B. malayi* merits further study.

INTRODUCTION

Mosquito species of the genus *Mansonia*, subgenus *Mansonioides*, are vectors of lymphatic filariasis in Thailand. Six species in this subgenus have been shown to be natural vectors of lymphatic filariasis (WHO, 1984). In Thailand, *Ma. bonneae, Ma. indiana* and *Ma. uniformis* were firstly incriminated as vectors of brugian filariasis by Harinasuta *et al* (1970). Since filariasis transmission and epidemiology can be locally specific, vector ecology in different environments should be investigated. Differences in the species and strains of the mosquitoes and differences in the strains of the parasites can affect mosquito susceptibility to infection (Edeson and Wilson, 1964). In spite of the epidemiological importance of *Mansonia* mosquitoes in Narathiwat Province, few observations have been carried out regarding their bionomics, although data on biting densities has been collected by field workers from the Division of Filariasis Control, Ministry of Public Health.

Considerable information exists regarding the bionomics of Thai mosquitoes (Iyengar and Menon, 1956; Thurman, 1959, 1963; Bram, 1967; Harrison and Scanlon, 1975; Huang, 1979; Gass *et al*, 1982), but little is known of the ecology of mosquitoes inhabiting peat swamp forests. The peat swamp in Thailand is estimated to occupy approximately 64,000 hectares (Phlengklai, 1989), of which 26,600 hectares are in Narathiwat Province (Vijarnson and Pinichapong, 1987). Geographically, the study area was rather homogeneous, occupied by an

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extensive area of peat swamp forest with some variation in density of trees and vegetation. The average annual rainfall was 2,560 mm with 171 rainy days; relative humidity and average temperature were 77-83% and 28ºC, respectively (Phlengklai, 1989).

This study aimed to determine the mosquito fauna of the peat swamp forest and the bionomics of the vector species in relation to brugian filariasis, such as seasonal variation, biting behavior, and vector incrimination.

MATERIALS AND METHODS

Study areas

The present study was carried out in a peat swamp forest, Sirindhorn Research and Nature Study Center located in Su-ngai Kolok District, Narathiwat Province, Thailand (6º 4'N, 101º 58' E). In 1991, the Royal Forest Department declared approximately 50,000 acres (20,234 hectares), or 66.8% of the total Phru Toh Daeng area, as a wildlife conservation zone in honor of Her Royal Highness Princess Maha Chakri Sirindhorn. The research center was established to be a field station for research study, and a recreation place for people to learn and understand the peat swamp forest by observation along the study trail. Topographically, the peat swamp forest is low-lying flatland and year-round water logged. Rain-fed water drains out of the forest floor very slowly. The peat water is brownish in color resulting from decomposition of tannic substances disintegrated from dead plants in water with a pH of 4.5-6.0. This peat swamp forest has a high level of biodiversity with a recorded 470 plant species and 335 animal species, including birds (196), mammals (62), reptiles (50), amphibians (17), and rare animals (10). The plant community is made up of large trees, shrubs, vines and various short-lived herbaceous flora. There is no record of mosquito fauna and mosquito vectors inhabiting the peat swamp forest.

The selected village, Ban Toh Daeng, which has a population of 129, is 1.5 km from the research center. In 1986, in the Annual Report of the Division of Filariasis Control, the highest microfilarial infection rate in this region was reported as 8.25% (Ministry of Public Health, 1986). The average annual temperature is 27.6 ºC, with 77-83% relative humidity, and an average annual rainfall of 2,560 mm with 171 rainy days. The heaviest part of the rainy season, causing yearly flooding, is from November-December and the driest period is from February-April.

Human biting and landing rates

The collection stations were set at Ban Toh Daeng and Sirindhorn Research and Nature Study Center. Previously, the seasonal prevalence of *Mansonia* mosquitoes was determined in the swamp-fringed areas in Khosit Subdistrict by the Division of Filariasis Control (Ministry of Public Health, 1999). This was used as a guideline to set an appropriate sampling period (April-October) and time for the present study.

Guptavanij *et al* (1973) reported that the ratio of outdoor/indoor collections of *Mansonia* mosquitoes in Narathiwat Province was 1.6:1, therefore, outdoor collections were performed in the present study. To determine monthly density of *Mansonia* species, mosquito collections were conducted outdoors between 18 00-22 00 hours for two consecutive nights per month at Ban Toh Daeng from April-October 2000. It was not possible to extend the sampling period into the following year because of heavy flooding in the collection sites.

To determine mosquito biting cycles, human biting and landing counts were made hourly for 50 minutes for two groups, each with two persons, for three consecutive nights per month in September and October 2000. The captures were performed at 06 00-17 00 hours in the forest shade near the walking trail at the research center, and at 13 00-23 00 hours at Ban Toh Daeng.

Mosquito identification and dissection for filarial parasites were performed at a field laboratory at the Pikun Thong Development Study Center. All mosquito specimens were identified as to species using a standard dissecting microscope using taxonomic keys (Thurman, 1959; Wharton, 1962; Peyton and Scanlon, 1966; Bram, 1967; Harrison and Scanlon, 1975; Huang, 1979; Rattanarithikul, 1982). Dissection for filarial parasites was performed on the head

and thorax portions of *Mansonia* mosquitoes as described by Leemingsawat *et al* (1987).

RESULTS

Seasonal abundance of *Mansonia* sp

A total of 1,361 mosquitoes belonging to 19 species were collected at Ban Toh Daeng, of which 1,192 were *Mansonia* mosquitoes: *Ma. annulata*, *Ma. annulifera, Ma. bonneae*, *Ma. dives, Ma. indiana*, and *Ma. uniformis*. The remainder were *Aedes albopictus, Ae. caecus, Anopheles letifer, An. nigerrimus, Armigeres subalbatus, Coquillettidia crassipes, Cq. nigrosignata, Culex bitaeniorhynchus, Cx. gelidus, Cx. pseudosinensis, Cx. sinensis, Cx. tritaeniorhynchus,* and *Cx. vishnui*. The most abundant species was *Ma. bonneae* (47.5%) followed by *Ma. annulata* (32.8%), *Ma. uniformis* (9.8%), *Ma. indiana* (6.0%), and *Ma. dives* (3.9%).

Wharton (1962) published a detailed study of the biology of *Mansonia* mosquitoes in relation to filariasis in Malaysia. He reported that *Ma. annulata* and *Ma. bonneae* bred in dense swamp forests where larvae were attached to the pneumatophores of trees, while *Ma. indiana* and *Ma. uniformis* inhabited open swamp forests where the immature stages attached to the roots of floating aquatic plants, such as water lettuces (*Pistia stratiotes*) and water hyacinth (*Eicchornia crassipes*). Our findings for *Ma. annulata, Ma. bonneae*, *Ma. dives, Ma. indiana* and *Ma. uniformis* are shown in Fig 1a and 1b. The seasonal patterns for *Ma. annulata* and *Ma. bonneae* were similar with bimodal peaks; a small peak in May-August and a big peak after August. *Ma. uniformis* and *Ma. indiana* both had a unimodal peak in April-October. The biting of *Ma. bonneae* occurred in high numbers throughout the study period, ranging from 4.3 bites per person-hour in April to 24.3 bites per person-hour in October. *Ma. indiana* had lower rates (0.2-5.7 bites per person-hour). *Ma. annulata* bit people at the highest rate of 31.7 bites per person-hour in October but no bites in April and August. The biting of *Ma. uniformis* was fluctuated, with the highest rate of 11.5 bites per person-hour in July. *Ma. dives* displayed a low biting activity with the highest biting peak of 3.0 bites per person-hour in May. The average biting rate of the five major *Mansonia* species was 25.2 bites per person-hour, reaching a maximum of 57.8 bites per person-hour in October. Overall, *Mansonia* mosquito activity increased gradually from April to October with the first peak (28.5 bites person-hour) in July followed by a greater peak in October (Fig 1). *Ma. uniformis* and *Ma. indiana* were the main species comprising the highest biting density in April-August, while *Ma. annulata, Ma. bonneae* and *Ma. dives* formed the bigger subsequent peak in August-December.

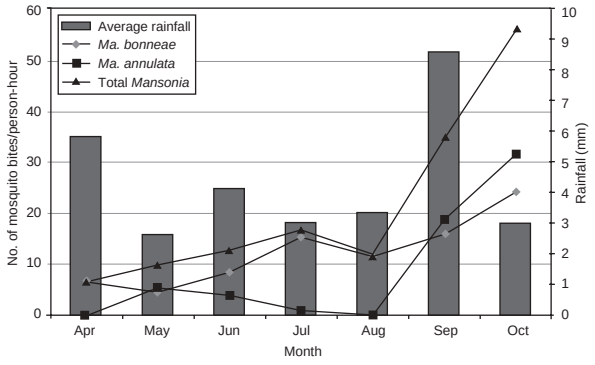

Fig 1a

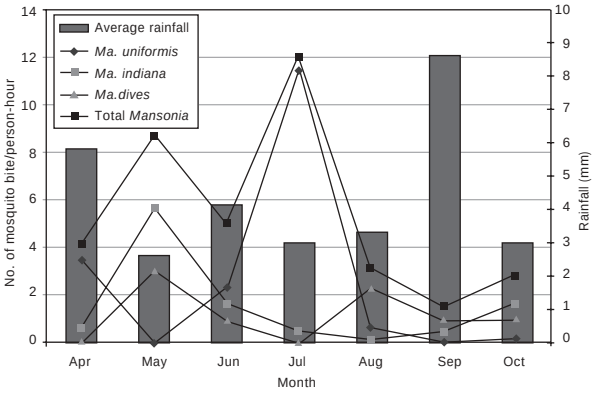

Fig 1b

Fig 1–Monthly variation in man-biting rates (total no. of mosquitoes collected per person-hour) of *Mansonia* mosquitoes and rainfall patterns at Ban Toh Daeng, Puyo Subdistrict (a. *Ma. bonneae Ma. annulata*; b. *Ma. uniformis, Ma. indiana, Ma.dives*).

Biting cycles of *Mansonia* mosquitoes

Biting cycles of *Mansonia* mosquitoes were determined by landing rates in the forest shade

of the Sirindhorn Research and Nature Study Center and at Ban Toh Daeng village. There were 22 species of mosquitoes collected during the biting cycle study. These were: *Ae. albopictus, Ae. caecus, Ae. niveus* subgroup*, An. letifer, An. nigerrimus, Ar. kuchingensis, Ar. subalbatus, Ar. theobaldi, Cq. crassipes, Cq. nigrosignata, Cx. bitaeniorhynchus, Cx. gelidus, Cx. pseudosinensis, Cx. sinensis, Cx. tritaeniorhynchus, Cx. vishnui, Heizmannia reidi, Ma. annulata, Ma. bonneae, Ma. dives, Ma. indiana* and *Ma. uniformis.* Four of 17 species (*Ae. niveus* subgroup, *Ar. kuchingensis*, *Ar. theobaldi* and *Hz. reidi*) representing forest inhabiting mosquitoes were captured only in the forest, while 6 of 19 species (*An. nigerrimus, Cx. bitaeniorhynchus, Cx. gelidus, Cx. sinensis, Cx. tritaeniorhynchus* and *Cx. vishnui*) representing domestic rural mosquitoes were collected only from the village. *Ma. annulata* and *Ma. bonneae* were the most abundant species collected from both sites, accounting for 49.0% and 46.8%, respectively of all *Mansonia* collected (570) at Ban Toh Daeng, and 52.2% and 32.6%, respectively, of all *Mansonia* collected (301) at the Sirindhorn Research Center. More mosquitoes were collected at the village than at the Research Center.

Overall patterns of biting activity for *Mansonia* mosquitoes collected at the Sirindhorn Research Center and Ban Toh Daeng are shown in Fig 2. Biting of *Mansonia* mosquitoes in the forest at the Research Center occurred throughout the collection period (06 00-17 00 hours) with different biting patterns and lower densities when compared to those from the Ban Toh Daeng collections. Biting activity of *Ma. annulata* was bimodal in pattern and commenced at the highest rate recorded of 18.5 bites per person-hour at 10 00 hours, immediately after 09 00 hours, with a smaller biting peak (8.0-8.5 bites per person-hour) between 12 00 and 17 00 hours (Fig 2a). Biting activity of *Ma. bonneae* was also bimodal in pattern with peaks between 06 00 and 11 00 hours, and at 15 00 hours (Fig 2a). *Ma. dives, Ma. indiana* and *Ma. uniformis* had very low biting densities (Fig 2a). The biting activity for *Mansonia* mosquitoes collected in Ban Toh Daeng is shown in Fig 2b. *Ma. annulata* and *Ma. bonneae* were caught biting after 1700 hours,

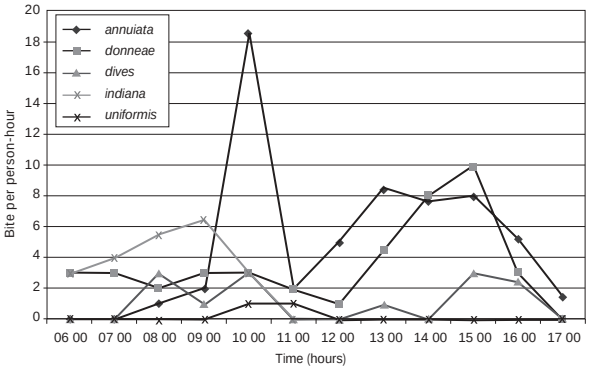

Fig 2a

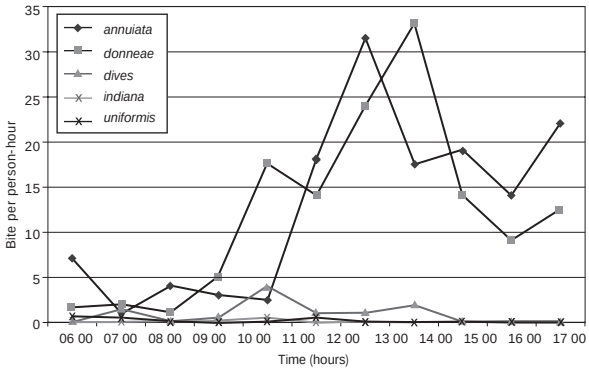

Fig 2b

Fig 2–Average biting activities of *Mansonia* mosquitoes captured on landing human bait at the Sirindhorn Research and Nature Study Center (a), and Ban Toh Daeng (b).

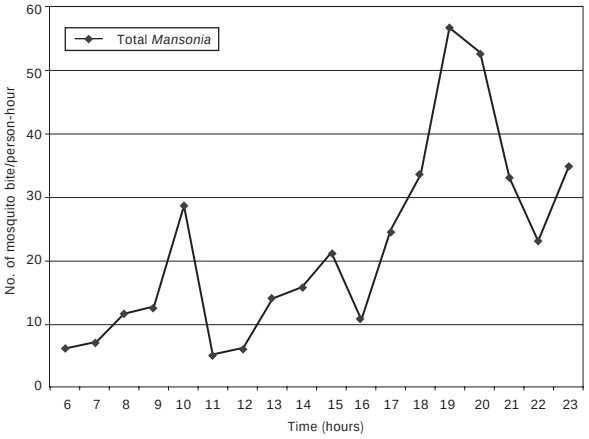

Fig 3–Overall biting activity of *Mansonia* mosquitoes at 06 00-23 00 hours, combining biting cycles from both collecting stations.

with the highest peaks at 19 00 hours (31.5 bites per person-hour) and at 20 00 hours (24.0 bites per person-hour), respectively.

Combining biting activities of *Ma. annulata* and *Ma. bonneae* for both stations, there were two peaks during the daytime (at 08 00-11 00 hours followed by a smaller peak at 13 00-16 00 hours) and one prolonged peak at night (17 00-23 00 hours) (Fig 3). *Ma. annulata* and *Ma. bonneae* were caught biting after 17 00 hours, with the highest peaks at 19 00 hours (31.5 bites per person-hour) and at 20 00 hours (24.0 bites per person-hour), respectively.

Vector infection rates with brugian filariasis in the peat swamp forest

Ma. bonneae and *Ma. annulata* were incriminated as vectors of *Brugia malayi* in the present study. In September, infected *Ma. bonneae* were found at both the Sirindhorn Research Center and Ban Toh Daeng village, with the infective rates of 1.1% (1/90 mosquitoes dissected) and 1.0% (1/97 mosquitoes dissected), respectively. *Ma. annulata* was also collected from the village with an infection rate of 0.6% (1/157 mosquitoes dissected). In addition, *Ma. bonneae* collected from the village in October was found infected with stages I and II filarial larvae (1.8% infection rate; 3/170 mosquitoes dissected). The infective mosquitoes were collected at 13 00-14 00 hours for *Ma. annulata*, and during 16 00-17 00 hours and 20 00-21 00 hours for *Ma. bonneae*.

DISCUSSION

Primary peat swamp forest has particularly rich mosquito fauna, 22 species belonging to seven genera were obtained during a brief survey period. In the previous studies on subperiodic *B. malayi* in southern Thailand, 39 species representing six genera of mosquitoes were collected by means of human-bait catches in Narathiwat Province (Guptavanij *et al*, 1978), and 43 mosquito species representing five genera in Chumphon Province (Guptavanij *et al*, 1971). Data on the presence and relative abundance of *Mansonia* mosquitoes could provide valuable information on species that is more likely to be infected by the parasites in a natural setting.

The peak numbers of *Mansonia* mosquitoes occurred in October and January, following the early and late monsoon season. This was different from the single big peak, from July to December, recorded in Tanjong Karang, Malaysia (Chiang *et al*, 1984). Tanjong Karang, however, has steady rainfall throughout the peak mosquito period. Chang *et al* (1991) reported that there was no clear correlation between the biting densities of *Ma. bonneae, Ma. dives* and *Ma. uniformis* and total rainfall in a coastal swamp area in Sarawak, while Sudomo *et al* (1984) reported that the densities of *Ma. annulata* and *Ma. dives* in Sumatra, Indonesia were significantly correlated to rainfall. In Narathiwat, evidence from the present study certainly indicates that although the area is wet all year round, rainfall patterns may well determine the abundance of *Mansonia* mosquitoes.

Ma. bonneae and *Ma. annulata* were the vectors of subperiodic form of *B. malayi* in the primary peat swamp forest. The infected filarial parasites were detected by dissection in both *Ma. bonneae* and *Ma. annulata* collected in the afternoon. A recent study by Kanjanopas *et al* (2001) reported that all filarial parasites found in *Mansonia* mosquitoes in Puyo Subdistrict were confirmed as *B. malayi* by polymerase chain reaction (PCR). The microfilariae found in domestic cats in Narathiwat have been previously confirmed as *B. malayi* (Kanjanopas *et al*, 2001; Chansiri *et al,* 2002). It was assumed that the infective filarial parasites found in this study were also *B. malayi.*

Guptavanij *et al* (1971) studied subperiodic *B. malayi* in Chumphon Province and dissected 1,367 *Ma. annulata* for filarial parasites. Only one mosquito was infected by the second stage larvae. In 1978, Guptavanij *et al* found that two *Ma. bonneae* and one *Ma. dives* from 2,242 *Mansonia* mosquitoes in Narathiwat were infective with subperiodic *B. malayi*, and none of the *Ma. annulata* were infected or infective. Gass *et al* (1983) considered *Ma. annulata* only as a potential vector. This study, therefore, was the first report to incriminate *Ma. annulata* as a vector of subperiodic *B. malayi*. Infection with filarial worms in *Ma. bonneae* and *Ma. annulata* was

observed in September before their peaks in October with infection rates of 1.1% (at the Research Center) and 1.0% (at Ban Toh Daeng) for *Ma. bonneae*, and 0.6% for *Ma. annulata*. The infection rates are higher than those reported by Guptavanij *et al* (1978) which were 0.18% for *Ma. bonneae* and 0.20% for *Ma. dives.*

The predominant *Mansonia* species which attacked man at daytime and night time were *Ma. bonneae and Ma. annulata*. Their biting densities were very high, reaching 50 bites per person-hour at peak periods in Ban Toh Daeng village and 25 bites per person-hour in the forest of the peat swamp forest. Daytime-biting occurred during 09 00-11 00 hours and 14 00-16 00 hours and night time-biting occurred from 18 00 through 23 00 hours. These biting times appear to coincide with the nocturnal subperiodicity of *B. malayi*, which peaks in the peripheral blood at 06 00 hours (03 00-10 00 hours) and 20 00 hours (16 00-02 00 hours) as reported by Guptavanij and Harinasuta (1977) and the nocturnally periodic type detected in domestic cats at 21 00 hours and 24 00 hours detected by Phantana *et al* (1995). The biting patterns of these two species possibly play an important role in the transmission and maintenance of nocturnal subperiodic *B. malayi* in the peat swamp forest.

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