Identifications of the *Culex pipiens* Complex in Thailand, Based on the Study of Male Terminalia D/V Ratios

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ABSTRACT: Mosquitoes belonging to the *Culex pipiens* complex were collected from several localities in Thailand and identified by using D/V ratios of male terminalia. Most of the mosquitoes were *Cx. quinquefasciatus*, having D/V ratios between 0.1 - 0.6, mean 0.35 ± 0.07. Among the mosquitoes from Klong Tauy, Phuket and Chiang Mai, 9/262 had D/V ratios of 0.7 - 0.9, which may be extreme variations of *Cx. quinquefasciatus* or may be the variety *pallens* of *Culex pipiens*.

INTRODUCTION

The *Culex pipiens* complex is subdivided into *Cx. pipiens pipiens*, *Cx. pipiens quinquefasciatus (fatigans)*, *Cx. pipiens australicus*, *Cx. pipiens* var. *molestus*, *Cx. pipiens* var. *pallens* and *Cx. globocoxitus* (1). Sirivanakarn changed the status of some of the above names, e.g., *quinquefasciatus* was elevated to species status and *pallens* was elevated from variety status to subspecies (2).

All over Thailand, *Cx. quinquefasciatus* is a pest and local vector of Japanese B. encephalitis and carrier of Chikungunya infection (3). It could be an experimental vector of periodic Bancroftian filariasis but to a lesser degree for the subperiodic form (4). *Cx. quinquefasciatus* control or eradication is difficult because the breeding places are rapidly increasing with urbanization and because of the occurrence of insecticide resistance (5, 6). To cope with the *Culex pipiens* complex problems, it is first necessary to identify the different members of the species complex. *Cx. p. pallens* and *Cx. p.* var. *molestus* have not been previously reported in Thailand (3). This study was aimed at revealing variations of male terminalia, as described by Sasa (5) in order to distinguish forms of the *Cx. pipiens* complex collected from various localities of Thailand.

MATERIALS AND METHODS

Mosquito collection

Culex pipiens complex larvae were collected from different provinces in Thailand, reared and maintained in the laboratory in the Bangkok School of Tropical Medicine. Adult males aged 4 to 5 days were used for measuring D/V ratios of male terminalia.

Preparation of male terminalia

Male terminalia were prepared by cutting off the last abdominal segment of adult males, aged 4-5 days, slide-mounting each in Gater's medium, and leaving it for two or three days until the exoskeleton was clear. The specimens having dorsal and ventral arms spread properly were chosen for identification and measurement. The specimens were assessed by measuring the distances between tips of the dorsal and ventral pairs of arms. The ratios of spans between dorsal arms and ventral arms were calculated and recorded. About one hundred terminalia were used from each locality.

RESULTS

The D/V ratios of male terminalia of these *Culex* are given in Tables 1 and 2, showing a range of 0.1 - 0.9, with mean 0.35 ± 0.07. In most cases the ratios were 0.2 - 0.5, which would be typical of *Cx. quinquefasciatus*. Most variations were observed in the samples from Klong Tauy, Phuket and Chiang Mai, where some ratios were 0.7 or more. These forms comprised 9.52%, 1% and 2% of the populations of Klong Tauy, Phuket and Chiang Mai, respectively (Table 2).

DISCUSSION

The D/V ratios of male terminalia of the *Culex pipiens* complex in Thailand mostly coincide with *Cx. quinquefasciatus* as shown in Table 1 and 2. Sasa and Shirasaka showed that in Southeast Asia and Japan the *Culex pipiens* complex could roughly be classified into three forms: *fatigans* (now being referred to *quinquefasciatus*), distributed in Southern Asia, and the South Amami Islands (tropical and subtropical) was anautogenous, nonstenogamous and had the smallest value of D/V ratio (0.3); *pallens*, from the Japanese mainland (temperate), also anautogenous and with varying D/V ratios (0.4 - 0.9) greater than for *quinquefasciatus*; and *molestus*, mainly from underground waters in large cities of the Japanese mainland (temperate), autogenous and stenogamous, with the largest D/V ratio (1.2) (7, 8).

D/V ratios of 0.7 - 0.9 shown in Table 2 for the mosquitoes from Klong Tauy, Phuket and Chiang Mai coincided with *Cx. p. pallens* or *Cx. p. pipiens*. Alternatively these specimens might represent extreme variations within the strains of *Cx. quinquefasciatus* or be the results of crosses between tropical and temperate species of *Culex pipiens* complex, e.g., *quinquefasciatus* and *pallens*. Sirivanakarn, who inlcuded *pallens* as subspecies of *pipiens*, noted that he has never found *Cx. p. pallens* extend into Southeast Asia and other parts of the Oriental region (2). It is most probably restricted to the cold temperate climate similar to that of the typical *pipiens* of Northern Europe in the Western palearctic (2). Klong Tauy, is the Bangkok seaport area where there are frequent communications between Thailand and other countries. Six mosquitoes had D/V ratios higher than for *Cx. quinquefasciatus* and they could possibly be *Cx. pallens*. Selective breeding biological and biochemical analysis of diagnostic

allozymes studies of mosquitoes from these populations could eventually confirm whether or not they represent a separate subspecies.

Harinasuta et al., reported that *Cx. p. pallens*, *Cx. quinquefasciatus* and *Cx. p.* var. *molestus* experimentally harboured 27.7%, 6.4% and 0.9%, respectively, of infective stage of subperiodic *W. bancrofti* from Kanchanaburi, Thailand (9). Thirty percent of Japanese *Cx. p. pallens* could also be experimentally infected with *B. malayi* (10).

The introduction of *Cx. p. pallens* into Thailand from elsewhere, e.g., Japan and China, is possible as Phuket and Klong Tauy are ports visited by ships from such places. Chiang Mai is located in the Northern part of Thailand where mosquitoes could be introduced from South China. Further adaptations of temperate strains to tropical climate, or their seeking suitable microclimatic conditions, could give chances of *Cx. p. pallens* survival in Thailand.

The possibility of Bancroftian filariasis occurring in Bangkok was indicated by Sucharit and Harinasuta (4). In areas such as Southern Thailand, where *Cx. quinquefasciatus* and *Cx. p. pallens* breed, the immigration of infected people from other endemic areas might contribute a risk of introducing this disease.

The present evidence of D/V ratios suggesting the presence of *Cx. p. pallens* as well as *Cx. quinquefasciatus* in Thailand will complicate studies on the vector status of this group of mosquitoes in Southeast Asia.

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TABLE I. D/V ratios of *Culex pipiens* complex males from various provinces of Thailand.

Provinces	Mean of D/V ratios	Range of D/V ratios
Klongton, Bangkok	0.30 ± 0.06	0.20 - 0.50
Klong Tauy, Bangkok	0.46 ± 0.226	0.29 - 0.94
Kanchanaburi	0.37 ± 0.034	0.28 - 0.48
Aranyaprathet, Prachin Buri	0.38 ± 0.057	0.24 - 0.63
Udon Thani	0.35 ± 0.052	0.25 - 0.48
Ranong	0.32 ± 0.04	0.21 - 0.44
Chumphorn	0.33 ± 0.036	0.24 - 0.45
Phuket	0.37 ± 0.095	0.25 - 0.84
Chiang Mai	0.35 ± 0.103	0.19 - 0.91
Mean	0.35 ± 0.07	0.19 - 0.94

TABLE 2. Distribution of D/V and DV/D ratios of *Culex pipiens* complex males from various provinces of Thailand.

Provinces	No. of mosquitoes having D/V and DV/D ratios of								
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	4.5	2.0	1.166	0.75	0.5	0.33	0.214	0.125	0.055
Klongton, Bangkok		49	42	8	1				
Klong Tuay, Bangkok			9	30	15	3	2	3	1
Kanchanaburi		10	59	31					
Aranyaprathet, Prachin Buri		4	61	30	4	1			
Udon Thani		16	59	25					
Ranong		33	60	7					
Chumphorn		16	77	7					
Phuket		18	43	28	7	2		1	
Chiang Mai	2	19	56	17	3	1		1	1
Total	2	165	466	183	30	7	2	5	2