

MORPHOLOGICAL CHARACTERS OF *CULEX PSEUDOVISHNUI* LARVAE FROM MALAYSIA, JAPAN AND FORMOSA

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INTRODUCTION

In 1957, Colless described a new species, *Culex pseudovishnui* based on specimens from Singapore, and pointed out that the species appears to be pan-Oriental and has in the past been most frequently mis-identified as *Culex vishnui*. Members of the "vishnui" complex are of Public Health interest as one of the species in this group, *Culex tritaeniorhynchus* is an important vector of Japanese B. Encephalitis over wide areas of Southeast Asia. In 1968, Lien described a new species, *Culex neovishnui* based on specimens from Formosa, differing from *Culex pseudovishnui* in the larval characters. According to Lien (1968), in *Culex neovishnui*, the prothoracic hairs 4-P (hereafter 4-P) are usually bifid, rarely single, and there is only one pair of lateral hair tufts on the siphon (hereafter LH), while in *Culex pseudovishnui*, 4-P are usually multiple-branched, rarely bifid, and there are two pairs of LH. The larvae of both are easily identified by the characters mentioned above, but the adults are not easily distinguishable.

Since that time, Japanese specimens, previously mis-identified as *Culex vishnui*, have been identified as *Culex pseudovishnui* by some Japanese workers (Wada 1969, Kamimura 1969, etc.) and as *Culex neovishnui*

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by others (Miyagi 1970, 1971, Tanaka 1971, etc.).

In 1970, Matsuo compared in detail larvae from Japan with larvae from Formosa and pointed out that both larvae are identical and can be differentiated from larvae from Singapore by LH and 4-P. There is, however, no evidence whether the differences of the characters between specimens from Japan and Formosa and specimens from Singapore represent independent species or are geographical variations within a single species. Therefore, larvae from Japan will be considered as *Culex pseudovishnui* until this problem can be resolved.

The morphological characters of specimens from various countries in this region was studied by the authors. In this study, LH and 4-P etc. of larvae from Malaysia (hereafter m-larvae) are described and compared with larvae from Japan (hereafter j-larvae) and larvae from Formosa (hereafter f-larvae).

MATERIALS AND METHODS

Larvae from Malaysia were studied by examining whole 4th instar larvae, skins of larvae collected from the field in Malaysia in 1970, and skins of 3rd and 4th instar larvae raised individually from egg rafts, which were deposited by females collected in cowsheds in Malaysia in 1970. The f-larvae examined were the 4th instar larvae from egg rafts deposited by females collected

from Formosa that were kindly supplied by Lien of the Medical Ecology Department, United States Naval Medical Research Unit No. 2 in Formosa. The j-larvae examined were the 4th instar larvae collected in the field and reared from egg rafts deposited by females collected in cowsheds, in Honshu and Shikoku in Japan from 1958 to 1968.

RESULTS AND DISCUSSION

The results of the study of the number of pairs of LH are summarized in Table 1. Of the 68 m-larvae collected in field, 43 have 2 pairs of LH (63.2%), 13 have 1 pair (19.1%) and 12 have 1.5 pairs (17.6%). It became apparent that larvae with 1 pair of LH and larvae with 2 pairs of LH are both present, and from the present data it appears that the latter is dominant in Malaysia. It is remarkable that the larvae with 1.5 pairs of LH which are rare in Japan are frequent in Malaysia. The data may indicate that 1.5 pairs of LH is intermediate between 1 and 2 pairs of LH. On the other hand, the following tests may indicate that 1 pair of LH is most probably a fixed character. In m-larvae reared from the egg rafts, A and B, the majority have 1 pair of LH, while 1.5 pairs and 2 pairs are rare.

A review of papers, in which the number of pairs of LH are studied in comparative

detail, shows that larvae with 1 pair of LH are described from Japan (Matsuo, 1970; Miyagi, 1970), Ryukyu Islands (Miyagi, 1971), Formosa (Lien, 1968; Matsuo, 1970), Luzon Island, Mindanao Island (Miyagi, 1971) and Thailand (Bram, 1967), and by contrast larvae with 2 pairs of LH are described from Palawan Island (Miyagi, 1971), Singapore (Lien, 1968) where larvae with 1.5 and 2.5 pairs of LH are not frequently found and Thailand where larvae with 1 pair and 3 pairs of LH are found. It appears that the two forms, with regard to the number of pair of LH, have a distinct geographical distribution with an overlap in Malaysia and nearby Thailand.

The results of the study of the number of branches of 4-P are summarized in Table 2. The m-larvae from the field are divided into three groups based on the number of pairs of LH, 1 pair, 1.5 pair and 2 pair groups, and the number of 4-P with the branches of each are summarized. The 4-P of the larvae belonging to the 1 pair group are bifid to 10-branched (average: 5.0 branches). Among them, 10 4-P are bifid and identical in j- and f-larvae. The 4-P of the 2 pair group are bifid to 13-branched (average: 6.1 branches), a larger number than in the 1 pair group. The 1.5 pair group have 2 to 20-branched 4-P. The m-larvae reared from the egg rafts, A and B, have 1 to 6-branched

Table 1
Number of pairs of lateral hair tufts on the siphon.

Locality	Malaysia			Japan	Formosa
	Field*	A**	B**		
No. examined	68	26	118	265	34
No. pairs 1.0	13	25	114	263	34
1.5	12	1	3	2	0
2.0	43	0	1	0	0

* Specimens collected in the field.

** Specimens reared from individual egg rafts, A and B; used in all tables, except tables 3 and 4.

Table 2
Number of branches of the prothoracic hair 4-P.

Locality	Malaysia			A	B	Japan	Formosa
	1 pair*	1.5 pairs*	2 pairs*				
No. examined	24	22	75	50	183	614	65
No. branches							
1				1	5	134	2
2	10	4	11	21	109	472	63
3		1	5	9	48	8	
4		6	11	14	21		
5	3	4	8	4			
6	3	1	10	1			
7	1		7				
8	5	3	6				
9		1	7				
10	2		2				
11			3				
12			2				
13			3				
14							
15		1					
16							
17							
18							
19							
20		1					
Average	5.0	5.9	6.1	3.6	2.5	1.8	2.0

* Showing the number of pairs of lateral hair tufts of larvae collected from the field in tables 2, 5, 6, 7 and 8.

4-P (average: 3.6) and 1 to 4-branched 4-P (average: 2.5), respectively.

For the purpose of examining the development of the number of branches of 4-P at successive instars of larvae, 30 young larvae from egg rafts A and B were reared individually and the number of branches on the 3rd and 4th instar larval skins was examined (Tables 3 and 4). The number of branches was 1 to 3 (average: 1.6) on the 3rd instar larva and 1 to 6 (average: 2.7) on the 4th instar larva. In the majority of larvae the number of branches becomes larger as the larvae grow up from the 3rd instar to the 4th instar, while the remainder have the same number of branches of 4-P between these instars. The length of 4-P is shorter as the number of branches gets larger. The bifid 4-P are about 1/2 to 1 times as long as

the antenna, the trifid 4-P are less than 2/3 the length of the bifid 4-P and 4-P with more than 10 branches are less than 1/5 the length of the trifid 4-P and more delicate. While the 4-P of j- and f-larvae have a small number of branches, 1 to 3, usually bifid, the single 4-P are 2/3 to 1 times as long as the antenna and the bifid and trifid 4-P are shorter than the single 4-P.

From these results, it is suggested that m-larvae are an integration of the characters of LH and 4-P. That is : (1) the larva with 1 pair of LH and a small number of branches of 4-P, are identical to j- and f-larvae and to the larvae from Mindanao Island, (2) the larva with 1 pair of LH and multiple-branched 4-P, are identical to the larvae from Luzon Island, (3) the larva with 2 pairs of LH and a small number of branches

Table 3
Number of branches of 4-P in the 3rd
and 4th instar larvae.

No. branches	3rd instar larva	4th instar larva
1	31	2
2	25	31
3	5	15
4		11
5		1
6		1
Average	1.6	2.7

Table 4
Patterns of development in number
of branches of 4-P.

3rd instar larva	4th instar larva
1	1 2 3 4
2	2 3 4 5
3	3 6

of 4-P, are identical to some larvae from Singapore and Palawan Island and (4) the larva with 2 pairs of LH and multiple-branched 4-P, are identical to some larvae from Singapore. All four types are present in Malaysia. Besides these types, it is noticed that 1.5 pairs of LH are frequently found there. According to Lien (1968), the first form is *Culex neovishnui*, the fourth

form is *Culex pseudovishnui* and the larvae with 1.5 pairs of LH can not be identified either as the former or as the latter. The present data indicate clearly that the number of pairs of LH and the number of branches of 4-P are geographically different. At present, however, there is no evidence whether these differences represent independent species or *pseudovishnui*. In order to resolve this taxonomic problem, it will be necessary to carry out a variation test of 1.5 and 2 pairs of LH among larvae from a single female, and a cross breeding experiment among the forms mentioned above. So, at present, we will identify the various forms in LH and 4-P as *Culex pseudovishnui*.

The majority of m-, j- and f-larvae have about 4 to 9 long sharp comb scales in more or less, regular, single or double rows, and occasionally some short teeth, 1/5 to 1/3 the length of the long ones, mixed with the long teeth. The number of comb scales and pecten teeth, the number of pairs of ventral hair tufts (hereafter VH), the number of branches of the basal ventral hair tufts (hereafter 1-VH) and LH, do not show any remarkable differences among m-, j- and f-larvae as seen in Tables 5 and 6. The results of the study of the siphon length are summarized in Table 7. The average of the siphon length in m-larvae is 1.0 mm to 1.4 mm and tends to be a little shorter than in j-larvae. The siphon index in m-larvae is 4.8 to 7.8 (average: 5.3 to 6.9) and the majority is within the index of j-larvae. The siphon-saddle index of m-larvae is 3.0 to 4.8 (average: 3.4 to 4.0), and falls within the range of the index of j- and f-larvae. The position of 1-VH, LH and the most distal pecten teeth on the siphon are shown in Table 8. The positions were indicated by the following method. The siphon length (hereafter SL), the distance between the base of the siphon and the insertion of 1-VH (hereafter B-1VH), the distance between

Table 5
Number of comb scales and pecten teeth, and number of pairs of ventral hair tufts on the siphon (VH).

Locality	No. comb scales			No. pecten teeth			No. pairs of VH		
	No. exam.	Range	Ave.	No. exam.	Range	Ave.	No. exam.	Range	Ave.
Malaysia									
1 p.	26	4-8	6.0	12	8-12	10.6	12	5.0-7.0	5.9
1.5 p.	24	5-8	6.3	18	8-12	9.9	12	5.0-7.0	5.8
2 p.	84	5-10	6.5	52	6-13	10.1	42	4.5-6.5	5.6
A	50	5-8	5.8	50	7-13	9.4	25	5.0-6.5	5.9
B	159	5-15	8.2	188	8-13	10.8	94	5.0-7.0	6.1
Japan	306	5-16	7.8	290	6-14	10.3	140	4.0-6.5	5.7
Formosa	80	6-20	9.9	56	8-15	10.9	28	5.0-7.0	5.9

Table 6
Number of branches of lateral hair tufts (LH) and basal ventral hair tufts (1-VH).

Locality	No. branches of LH			No. branches of 1-VH		
	No. exam.	Range	Ave.	No. exam.	Range	Ave.
Malaysia						
1 p.	23	2-4	3.4	11	3-5	4.4
1.5 p.	36	1-4	3.1	17	3-5	4.2
2 p.	165	2-5	3.2	47	2-5	4.2
A	41	2-4	3.3	43	3-5	4.3
B	180	1-5	3.3	160	2-6	4.3
Japan	591	1-5	3.2	278	2-6	4.4
Formosa	72	3-5	4.0	54	3-7	5.0

Table 7
Values of siphon length, siphon index and siphon-saddle index.

Locality	Siphon length (mm)			Siphon index			Siphon-saddle index		
	No. exam.	Range	Ave.	No. exam.	Range	Ave.	No. exam.	Range	Ave.
Malaysia									
1 p.	13	1.0-1.6	1.3	6	5.4-7.5	6.6	13	3.3-4.5	3.9
1.5 p.	12	1.2-1.5	1.4	9	5.5-7.0	6.4	12	3.6-4.3	4.0
2 p.	43	1.1-1.6	1.4	26	5.0-7.8	6.9	43	3.4-4.8	4.0
A	25	0.9-1.0	1.0	25	4.8-5.8	5.3	25	3.0-3.7	3.4
B	94	0.9-1.2	1.1	94	5.3-6.6	5.8	94	3.1-3.9	3.5
Japan	259	1.0-1.9	1.5	259	5.0-9.7	6.0	259	3.2-4.5	3.9
Formosa	40	1.1-1.3	1.2	40	4.9-6.4	5.5	40	3.2-3.9	3.5

Table 8
Position of the basal ventral hair tufts (1-VH), the lateral hair tufts (LH)
and the distal pecten teeth on the siphon.

Locality	1-VH			LH			Pecten		
	No. exam.	Range (%)	Ave. (%)	No. exam.	Range (%)	Ave. (%)	No. exam.	Range (%)	Ave. (%)
Malaysia									
1 p.	6	39 - 52	43	3	62 - 68	66	6	33 - 39	36
1.5 p.	9	41 - 60	51	9	55 - 71	63	9	23 - 43	35
2 p.	24	37 - 62	49	25	50 - 71	60	26	30 - 45	37
A	48	28 - 42	37	51	65 - 78	69	50	23 - 33	28
B	186	30 - 45	36	190	55 - 76	66	188	23 - 32	28
Japan	275	25 - 46	38	289	66 - 85	76	298	17 - 34	26
Formosa	52	30 - 43	40	52	69 - 85	78	52	26 - 34	30

Position indicates the percentage of the siphon length and the distances between the base of the siphon and the basal hair tufts, the lateral hair tufts and the distal pecten teeth.

the base of siphon and the insertion of LH (hereafter B-LH) and the distance between the base of the siphon and the insertion of the distal pecten teeth (hereafter B-P) were measured. Then the per cent of SL and B-1VH, B-LH and B-P indicate the position of 1-VH, LH and the distal pecten teeth on the siphon. The position of 1-VH and pecten in m-larvae collected from the fields tends to be at a more or less proximal portion of the siphon than in j- and f-larvae. The position of LH in m-, j- and f-larvae is 50 to 71, 66 to 85 and 69 to 85, respectively. With reference to the characters, except for LH and 4-P, there were no remarkable differences between larvae from Malaysia and larvae from Japan and Formosa.

SUMMARY

Culex pseudovishnui larvae from Malaysia were compared with larvae from Japan and Formosa with reference to the characters, the number of pairs of the LH and VH, the number of branches of the LH, 4-P and 1-VH, the number of the comb scales and pecten teeth, the siphon length, the siphon index, the siphon-saddle index and the

position of 1-VH, LH and the distal pecten teeth on the siphon.

With reference to the above characters, except for the number of pairs of LH and the number of branches of 4-P, there were no remarkable differences between larvae from Malaysia and larvae from Japan and Formosa.

Larvae from Malaysia are an integration of the character of LH and 4-P. That is : (1) the larva with 1 pair of LH and a small number of branches of 4-P, identical in larvae from Japan and Formosa, (2) the larva with 1 pair of LH and multiple-branched 4-P, (3) the larva with 2 pairs of LH and a small number of branches of 4-P and (4) the larva with 2 pairs of LH and multiple-branched 4-P are all present in Malaysia. Besides them, the larvae with 1.5 pairs of LH which are rare in Japan are found frequently there.

According to Lien (1968), the first form is *Culex neovishnui*, the fourth form is *Culex pseudovishnui* and the 1.5 pairs of LH can not be identified either as the former or

the latter. The present results may indicate that 1.5 pairs of LH is intermediate between 1 pair and 2 pairs of LH; on the other hand, 1 pair of LH is probable a fixed character. At present, there is no evidence whether these differences represent independent species or are geographical variations within a single species, *Culex pseudovishnui*. In order to resolve this taxonomic problem, it will be necessary to carry out further experiments, including cross breeding experiments and variation tests of 1.5 and 2 pairs of LH among larvae from the one female. So, for the present, we shall treat all the forms in LH and 4-P as *Culex pseudovishnui*.

REFERENCES

- BARRAUD, P.J., (1937). The fauna of British India including Ceylon and Burma. Diptera 5, family Culicidae, tribe Megarhinini and Culicini. *Taylor and Francis*, London.
- BRAM, R.A., (1967). Contributions to the mosquito fauna of Southeast Asia. II. The Genus *Culex* in Thailand (Diptera : Culicidae). *Contributions of the Amer. Entom. Inst.*, 2 : 1.
- COLLESS, D.H., (1957). Notes on the Culicine mosquitoes of Singapore II. The *Culex vishnui* group (Diptera, Culicidae), with descriptions of two new species. *Ann. Trop. Med. Parasit.*, 51 : 87.
- KAMIMURA, K., (1968). The distribution and habit of the medically important mosquitoes of Japan. *Jap. J. Sanit. Zool.*, 19 : 15.
- LACASSE, W.J. and YAMAGUCHI, S., (1950). Mosquito fauna of Japan and Korea. *Off. Surgeon, 8th U.S. Army, Tokyo*.
- LIEN, J.C., (1968). New species of mosquitoes from Taiwan (Diptera : Culicidae) Part V. Three new subspecies of *Aedes* and seven new species of *Culex*. *Trop. Med.*, 10 : 217.
- MATSUO, K., (1970). Taxonomic studies on *Culex tritaeniorhynchus* and *Culex pseudovishnui*. 10. Prothoracic hairs 4-P etc. of larvae of "Shirohashi-ieka" in Japanese from Japan and Taiwan. *Sanitary Injurious Insect*, 13 : 21.
- MATSUO, K. and RAMALINGAM, S., (1971). On *Culex tritaeniorhynchus* and *Culex pseudovishnui*. X. Morphological characters of *Culex pseudovishnui* larvae from Malaysia. *The 23rd Annual Meeting of Japanese Sanitary Zoology*.
- MIYAGI, I. and KHA, S., (1970). Notes on *Culex (Culex) neovishnui* Lien, 1968 from the Ryukyus and Japan proper (Diptera : Culicidae). *Trop. Med.*, 12 : 71
- MIYAGI, I., (1971). *Culex vishnui* group from Philippines. *The 23rd Annual Meeting of Japanese Sanitary Zoology*.
- TANAKA, K., (1971). Mosquitoes of the Ryukyu Islands. *The 23rd Annual Meeting of Japanese Sanitary Zoology*.
- WADA, Y., (1969). Ecological studies of *Culex tritaeniorhynchus summosus* (Diptera : Culicidae) II. Observation on the resting behavior before and after taking blood meal. *Jap. J. Sanit. Zool.*, 20 : 8.