

Purchased by the
National Institutes of Health
for official use

Reprinted from AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 3, No. 3, May, 1954
Printed in U.S.A.

MORPHOLOGIC VARIATIONS OF LARVAE OF THE *SCUTELLARIS*
GROUP OF *AEDES* (DIPTERA, CULICIDAE) IN POLYNESIA

LEON ROSEN¹ AND LLOYD E. ROZEBOOM²

*Institut de Recherches Médicales de l'Océanie Française (Pacific Tropic Diseases Project),
Papeete, Tahiti and the Department of Parasitology, School of Hygiene and Public Health,
Johns Hopkins University, Baltimore, Maryland*

The *scutellaris* group of *Aedes* (Stegomyia), which ranges from the eastern part of the Australasian Region to the eastern part of the Oriental Region, includes species which have been incriminated as important vectors of human filariasis and dengue. This complex now contains 17 species, all of which are similar morphologically. The various species are differentiated primarily by the structure of the male terminalia, although in certain species the coloration characters of the adults are of use.

Larvae of only one species, *Aedes horrescens* Edwards, have been considered as disinctive (Edwards, 1935). This species was originally distinguished from other members of the *scutellaris* group by the prominent stellate setae of the larvae, which made them appear hairy (hence the name *horrescens*). These hairy larvae have been found primarily in tree holes (Paine, 1943; Lever, 1944 and 1945) in contrast to most species of the *scutellaris* group which utilize a wide variety of small containers as breeding sites.

Since the work of Bahr (1912), members of the *scutellaris* complex have been recognized as important vectors of nonperiodic filariasis in Polynesia. At the present time, four species of this group are known to occur in endemic areas of nonperiodic *Wuchereria bancrofti*.

Aedes polynesiensis Marks is a widespread species in Polynesia and a proven vector of *W. bancrofti*.

Aedes tongae Edwards is a suspected but unproven vector of *W. bancrofti*. It is the only species of the *scutellaris* group known to occur in the Tonga Islands (an endemic area of *W. bancrofti*).

Aedes horrescens Edwards is reported to be an unsuitable host for *W. bancrofti* (Manson-Bahr and Muggleton, 1952). It is known only from the Fiji Islands.

Aedes pseudoscutellaris Theobald is also known only from the Fiji Islands. The

¹ Senior Assistant Surgeon (R), National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases, Bethesda, Maryland.

² Associate Professor of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.

We are indebted to Dr. P. F. Mattingly of the British Museum for specimens of *A. horrescens*; to Dr. H. S. Leeson of the London School of Tropical Medicine for specimens of *A. pseudoscutellaris*; to Lt. L. A. Jachowski, U.S.N., for specimens from the Samoa Islands; to Mr. Isimeli M. Rakai for specimens from the Fiji Islands; to Dr. H. K. Beye for specimens from the Tonga Islands; and to Dr. A. Stone of the U. S. National Museum for helpful advice and assistance in the course of this study.

This work was supported in part by a grant-in-aid to the Johns Hopkins University from the Division of Research Grants, National Institutes of Health, U. S. Public Health Service.

ability of this mosquito to serve as a host of *W. bancrofti* is unknown since *A. polynesiensis*, which also occurs in Fiji, has only recently been recognized as a distinct species (Marks, 1951), and hence it is not known with which species of the *scutellaris* group previous workers in Fiji had dealt. *A. polynesiensis* is known to be a vector of *W. bancrofti* because it is the only species recognized from the Samoa Islands where it has been incriminated, under various synonyms, by several epidemiologic investigations (see Byrd *et al.*, 1945).

Early in the course of studies on filariasis in the Society Islands, the senior author encountered two types of larvae of the *scutellaris* group, a common form conforming to the description of *A. polynesiensis*, and a less common hairy variety, which was found primarily in tree holes, conforming in most respects to the description of *A. horrescens*. Meanwhile, Lt. Leo A. Jachowski, U. S. N., during the course of his studies on filariasis, also encountered hairy larvae of the *scutellaris* group on several occasions in the Samoa Islands (1951).

FIELD OBSERVATIONS IN THE SOCIETY ISLANDS

As a part of the assessment of the importance of imagoes associated with the hairy type of larvae in the transmission of *W. bancrofti* in the Society Islands, an attempt was made to establish a laboratory colony of these mosquitoes in order to facilitate infection experiments. Approximately 165 fourth instar larvae of the hairy type were collected and examined individually to be certain that all were typical. Adult females which developed from these larvae were allowed to feed on an individual having microfilariae of *W. bancrofti* in his peripheral blood and were then given the opportunity to lay eggs. After these eggs had hatched, the resulting larvae were reared on a diet of powdered bread crumbs. When these larvae had developed to the fourth instar they were examined and, surprisingly, they resembled not the hairy larvae of the preceding generation, but rather, the non-hairy larvae of *A. polynesiensis*.

The development of *W. bancrofti* in those mosquitoes which had fed on the infected donor was similar both qualitatively and quantitatively to that which occurred in mosquitoes reared from non-hairy larvae (Rosen, 1951).

It was also observed that when young hairy larvae (late second or early third instar) were removed from their natural habitat and reared in the laboratory on powdered bread crumbs, they became relatively less hairy by the time they attained the fourth instar.

Further field observations revealed that some tree holes contained larvae intermediate between the hairy and the non-hairy types. Moreover, a single tree hole sometimes contained a range of larvae from typical hairy to typical non-hairy types. Furthermore, a tree hole containing only hairy larvae, might, a few weeks later, contain only non-hairy larvae. In general, it seemed as if hairy larvae were found most frequently in tree holes which contained a large amount of debris. Occasionally, hairy larvae were found in other containers, such as rock holes, in which the larval environment seemed similar to that in tree holes.

The preceding observations suggested that the hairiness of certain larvae of the *scutellaris* group in the Society Islands was the result of a peculiar larval

environment occurring most frequently in tree holes. This possibility was further explored by the following experiment. Water and debris were collected from a tree hole which harbored numerous hairy larvae in all stages of development. All mosquito larvae were removed and the remaining contents of the tree hole were placed in three enamel pans. Both debris and water from the tree hole were placed in each of two pans, and only water (which had been filtered through ordinary filter paper) was placed in the third. Tap water and brewer's yeast were placed in a fourth pan. About 50 mature eggs which had been laid by adults reared from non-hairy larvae were placed in each of the pans except one of the two containing both debris and water from the tree hole. The pan in which no eggs were placed was used as a control to be certain that no unhatched eggs, which might have been laid in the tree hole in nature, remained in the debris. All pans were placed in a bobbinet cage to prevent adult mosquitoes from laying eggs in them.

Almost all eggs placed in the three pans hatched. However, the larvae in the two pans containing tree hole contents grew slowly, as compared with those in the tap water and yeast, and eventually many of them died. This seemed to be due to a lack of larval food. On the fourth day after hatching, larvae in the pan with both debris and water from the tree hole were definitely identifiable as of the hairy type. Daily examination of larvae in all three pans was continued until the ninth day after hatching. At no time were larvae of the hairy type found either in the pan with tap water and yeast or in the pan with filtered tree hole water. No larvae were found in the control pan with both debris and water from the tree hole. After nine days, about 50 eggs from the same lot as those referred to above were placed in this control pan along with a small amount of brewer's yeast. The eggs hatched and the larvae grew rapidly but no indication of hairiness could be found.

MORPHOLOGIC CHARACTERISTICS OF LARVAE AND ASSOCIATED ADULTS

Morphologic study of larvae of the *scutellaris* group was continued at the Johns Hopkins University, School of Hygiene and Public Health. The material examined included series of hairy and non-hairy larvae and associated adults from the Society Islands; several series of hairy and non-hairy larvae and associated adults from the Samoa Islands; several series of hairy and non-hairy larvae from the Fiji Islands (one series of hairy larvae was associated with adults); a single series of larvae and associated adults from the Tonga Islands; larvae and associated adults from the laboratory colony of *A. pseudoscutellaris* maintained at the London School of Tropical Medicine and Hygiene (see Marks, 1951); and a male and several larvae of the series of *A. horrescens* from which the type material of this species had been chosen.

It was found that the male terminalia of adults associated with both the hairy and the non-hairy types of larvae from both the Society Islands and the Samoa Islands were identical and that they conformed to the published descriptions and illustrations of *A. polynesiensis* (Farner and Bohart, 1945). Twelve collections containing 75 larvae, mostly of the hairy variety, were examined from the Society Islands. There were 12 hairy and 3 non-hairy larval skins which were

individually associated with *A. polynesiensis* males and 4 *A. polynesiensis* males which had been bred out from hairy larvae collected with other hairy larvae which were preserved. Six collections containing 74 larvae, mostly of the hairy variety, were examined from the Samoa Islands. There were 16 non-hairy, one hairy, and one intermediate type larval skins which were individually associated with *A. polynesiensis* males. In addition, there were mass associations of 62 *A. polynesiensis* males with non-hairy larvae and 9 males of this species with hairy larvae.

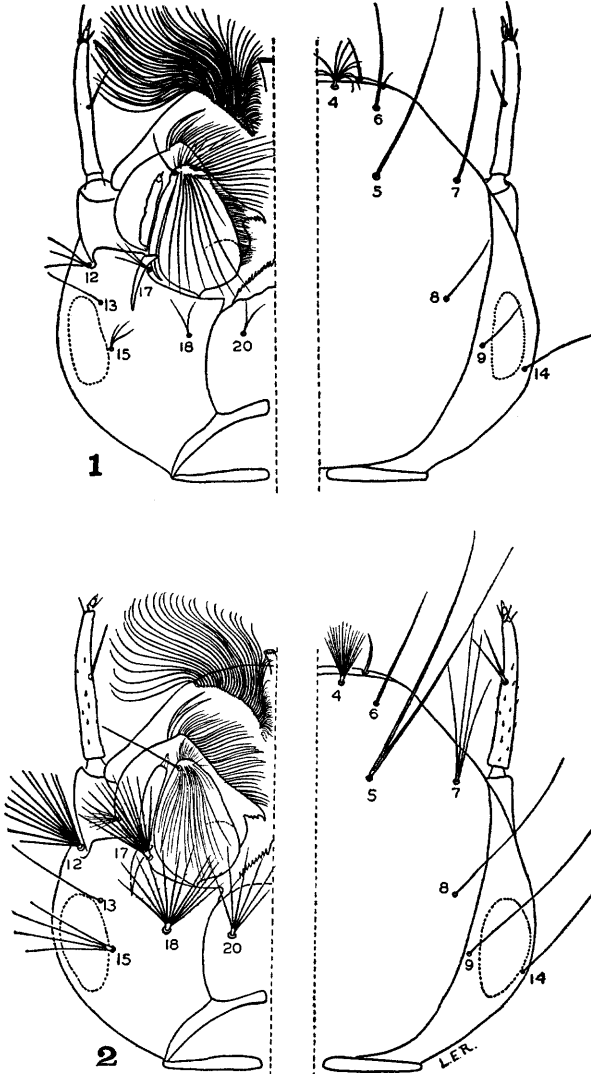


FIG. 1. *Aedes polynesiensis*, head of non-hairy larva

FIG. 2. *Aedes polynesiensis*, head of hairy larva

The male terminalia and scutal markings of 6 adults (3 males and 3 females) associated with a series of 9 hairy larvae from the Fiji Islands were as described for *A. pseudoscutellaris* (Marks, 1951). Unfortunately, the adults were not individually associated with larval skins; however, they were bred out from hairy larvae taken from the same tree hole at the same time as the 9 larvae which were preserved. The identification of *A. pseudoscutellaris* was based on the presence of a line of pale scales on the anterolateral margins of the scutum and on the presence of a row of specialized setae on the basal lobe of the male terminalia (Fig. 10). Another series of hairy larvae (11 specimens) from the Fiji Islands was also tentatively identified as *A. pseudoscutellaris* on the basis of the ratio between the length of the lower and the upper pairs of anal papillae (Marks, 1951). This ratio is higher (i.e. closer to one) in *A. pseudoscutellaris* than in *A. polynesiensis*,

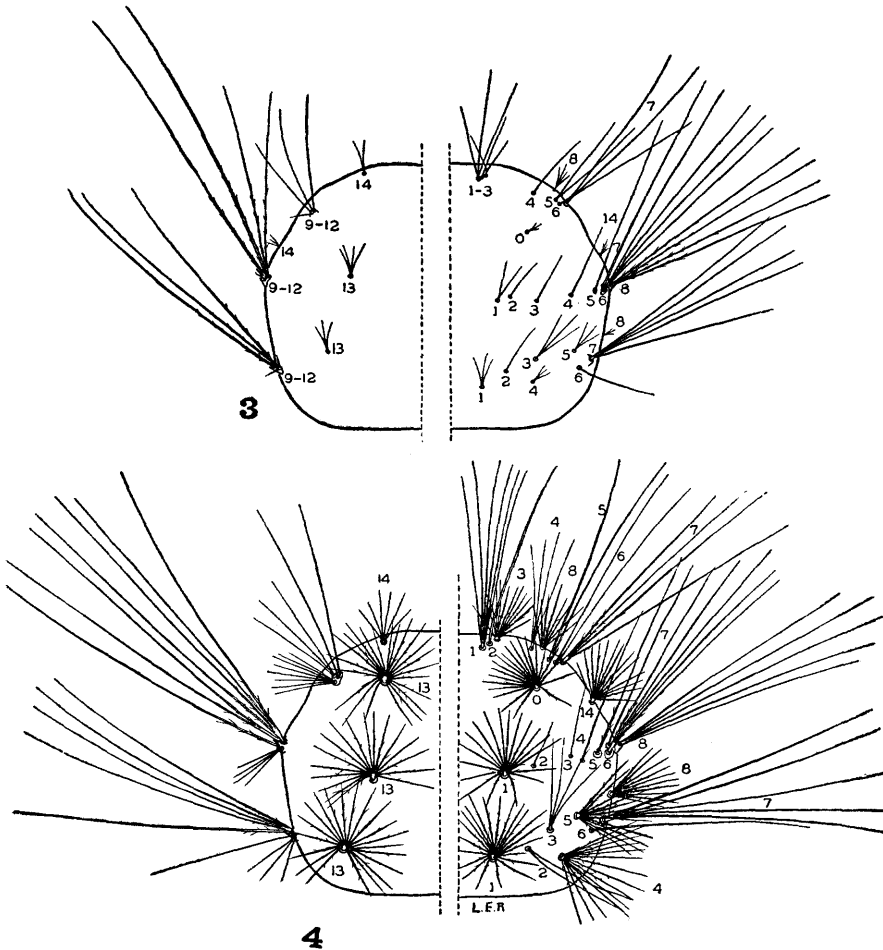


FIG. 3. *Aedes polynesiensis*, thorax of non-hairy larva
 FIG. 4. *Aedes polynesiensis*, thorax of hairy larva

and is also higher than that described for the larvae of *A. horrescens* (Edwards, 1935). Larvae from the colony of *A. pseudoscutellaris* from the London School of Hygiene and Tropical Medicine (10 specimens were studied in detail) were of the non-hairy variety, as was another series of larvae from the Fiji Islands (14 specimens) tentatively identified as *A. pseudoscutellaris* on the basis of the anal papillae.

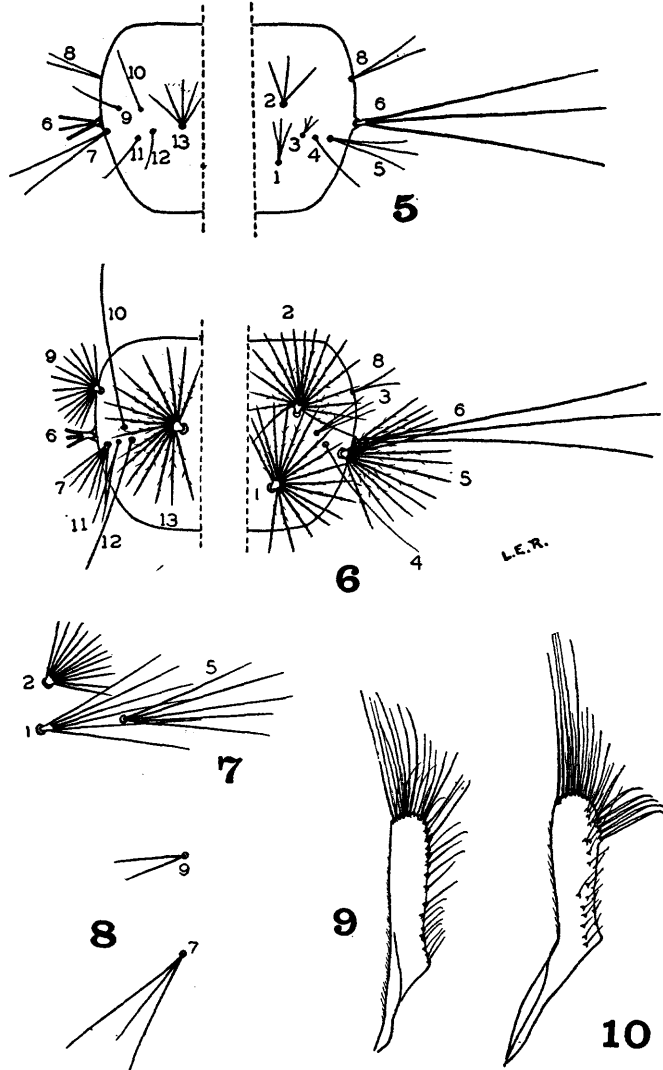


FIG. 5. *Aedes polynesiensis*, second abdominal segment of non-hairy larva

FIG. 6. *Aedes polynesiensis*, second abdominal segment of hairy larva

FIGS. 7 AND 8. *Aedes polynesiensis*, variations in certain setae of the second abdominal segment of hairy larvae.

FIG. 9. *Aedes polynesiensis*, basal lobe (lateral view) of male terminalia

FIG. 10. *Aedes pseudoscutellaris*, basal lobe (lateral view) of male terminalia

The single series of larvae (21 specimens) from the Tonga Islands was non-hairy and the male terminalia of the associated adults were as described and illustrated for *A. tongae* (Farner and Bohart, 1945).

Eight larvae and one male from the type locality of *A. horrescens* (Taveuni, Fiji Islands) were obtained through the British Museum. These specimens had been collected in 1933 and 1934 by R. W. Paine, and according to Mattingly (1953) the 1934 collection is the one from which the type material was bred and is also the source of the single male which we have examined.

The larvae were indistinguishable from hairy larvae of *A. polynesiensis* (they were distinguishable from *A. pseudoscutellaris* on the basis of the anal papillae

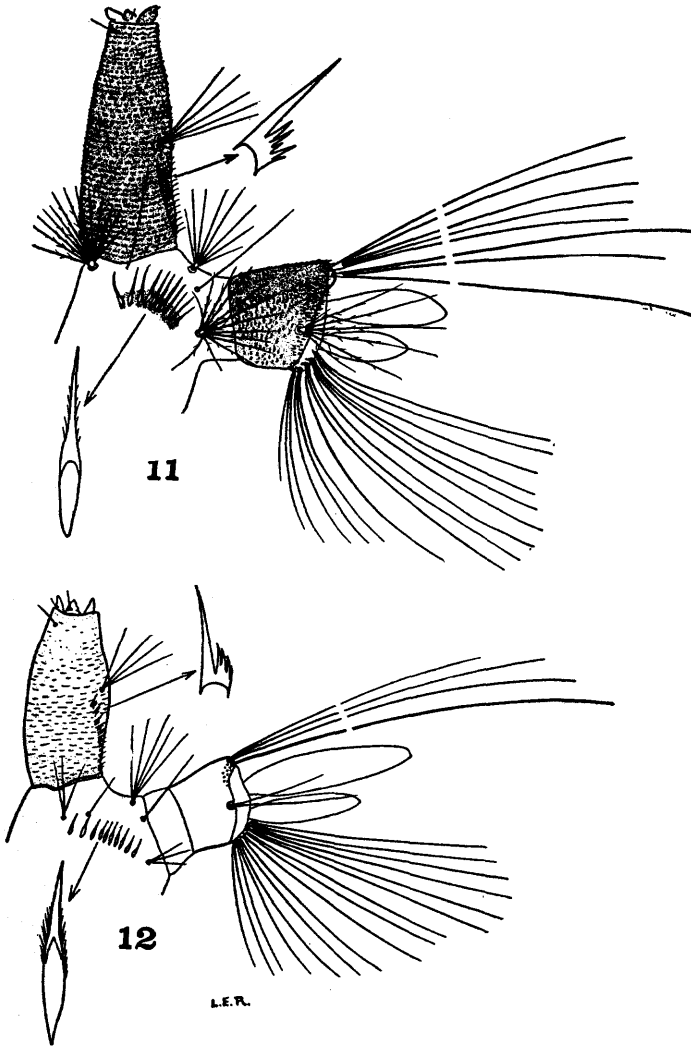


FIG. 11. *Aedes polynesiensis*, end of abdomen of non-hairy larva

FIG. 12. *Aedes polynesiensis*, end of abdomen of hairy larva

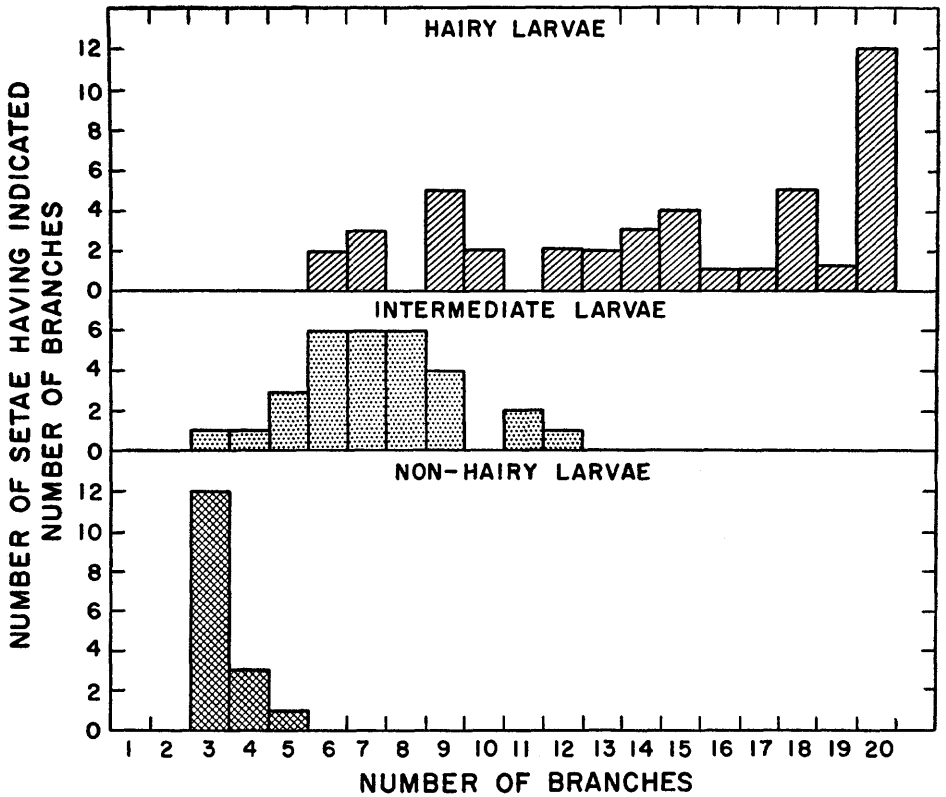


FIG. 13. Frequency distribution of the number of branches of abdominal seta 2 of three collections of fourth instar larvae of *Aedes polynesiensis*.

as mentioned above). The terminalia of the single male were distinctly different from those of the other Polynesian species of the *scutellaris* group and were as illustrated for *A. horrescens* by Stone and Farnier (1945).

The differences between the extreme hairy and non-hairy larvae of *A. polynesiensis* are illustrated in Figures 1-8, 11, and 12. Table 1 indicates quantitatively the wide range of variation which was observed in the branching of certain selected setae of the larvae of *A. polynesiensis* and *A. pseudoscutellaris*. Each of the series of larvae described in Table 1 represents a single larval collection from a single breeding site. In making these collections, no attempt was made to obtain a random sample of all larvae present; rather, in the case of hairy larvae, the hairiest specimens were usually selected. Figure 13 presents data in greater detail for a single seta. As shown in Figures 1-8, 11, and 12, the differences between larvae of the same species was by no means limited to the particular setae chosen for illustration in Table 1 and Figure 13. In general, setae of the hairy larvae were thicker, longer, and had more branches than did the corresponding setae in the non-hairy larvae, although some setae varied more than others. In addition, other parts of the integument of the hairy larvae, such as the siphon and anal saddle, were

TABLE 1

The variation in the number of branches of certain setae of the fourth instar larvae of *A. polynesiensis* and *A. pseudoscutellaris*

SPECIES	LOCALITY	MESOTHORACIC-1			ABDOMINAL*-2			LATERAL HAIR OF ANAL SADDLE		
		M.N.B.	R.N.B.	N.S.E.	M.N.B.	R.N.B.	N.S.E.	M.N.B.	R.N.B.	N.S.E.
<i>A. polynesiensis</i>	Tahiti†	3.0	2-4	16	3.3	3-5	16	2.1	2-3	16
	Samoa	3.2	2-4	17	3.3	3-4	12	2.0	2	1
	Samoa	3.2	2-5	17	2.9	2-4	18	3.0	3	3
	Tahiti	3.2	2-5	15	4.5	3-7	14	2.8	2-4	15
	Samoa	3.3	2-6	17	4.9	2-7	20	3.2	2-5	15
	Tahiti	3.0	2-4	7	3.7	3-5	6	3.0	2-4	7
	Tahiti	3.6	2-4	12	4.0	3-6	9	3.1	2-5	9
	Tahiti	6.7	3-9	7	5.4	4-7	8	3.8	3-4	4
	Tahiti	6.2	4-7	11	11.3	8-16	14	4.4	3-7	14
	Tahiti	5.2	3-9	29	7.3	3-12	30	3.4	2-5	29
	Tahiti	4.9	3-9	10	8.0	5-11	11	3.2	2-5	12
	Tahiti	7.9	4-15	8	8.4	4-15	11	3.9	3-6	11
	Samoa	7.1	2-12	10	11.7	2-20	13	3.0	2-4	10
	Samoa	10.9	3-25±	42	15.8	6-25±	43	4.6	2-9	42
Samoa	19.5	4-25±	25	20.9	6-25±	24	6.4	3-12	27	
<i>A. pseudoscutellaris</i>	Fiji	2.9	2-5	20	2.7	2-5	20	2.2	2-3	19
	Fiji	3.2	2-5	20	2.7	2-5	27	2.3	2-3	13
	Fiji	5.1	3-6	16	9.1	6-15	20	4.2	3-6	21
	Fiji	6.7	3-9	11	9.2	6-13	15	4.9	4-8	17

M.N.B. Mean number of branches.

R.N.B. Range number of branches.

N.S.E. Number of setae examined.

* Second abdominal segment.

† Society Islands.

also more heavily pigmented than were corresponding parts of the non-hairy larvae.

CONCLUSIONS

At least two species of the *scutellaris* group, *A. polynesiensis* and *A. pseudoscutellaris*, other than *A. horrescens*, can have hairy larvae under certain environmental conditions. *A. horrescens* remains as a distinct species, not because of the hairiness of the larvae by which it was detected and for which it was named, but rather because of the characteristics of the male terminalia.

Although the environmental factor responsible for the hairiness of larvae of *A. polynesiensis* was not elucidated, in the Society Islands it apparently occurs most commonly in tree holes, though not in all tree holes, or a majority of tree holes, or even in the same tree hole at all times. In the Samoa Islands, Jachowski (1951) encountered hairy larvae in various types of artificial containers; all of these, however, contained a considerable amount of debris. As demonstrated in

the experiments cited above, the factor seems to be associated with the debris in the breeding site but evidently is not solely the result of the physical presence of this debris.

Since Manson-Bahr and Muggleton (1952), in reporting that *A. horrescens* was an unsuitable host for *W. bancrofti*, based their identification of the mosquitoes on the hairiness of the larvae, and since it has now been shown that the other two species of the *scutellaris* group which are known to occur in the Fiji Islands can also have hairy larvae, it is not clear with which species they dealt.

REFERENCES

- BAHR, P. H., 1912. Filariasis and elephantiasis in Fiji, Suppl. No. 1, *J. London School Trop. Med.* pp. 192, Witherby, London.
- BYRD, E. E., ST. AMANT, L. S., AND BROMBERG, L., 1945. Studies on filariasis in the Samoan area, *U. S. Naval Med. Bull.* **44**: 1-20.
- EDWARDS, F. W., 1935. Mosquito Notes—XII, *Bull. Entomol. Res.* **26**: 127-136.
- FARNER, D. S., AND BOHART, R. M., 1945. A preliminary revision of the *scutellaris* group of the genus *Aedes*, *U. S. Naval Med. Bull.* **44**: 37-53.
- JACHOWSKI, L. A., 1951. Personal communication.
- LEVER, R. J. A. W., 1944. On the breeding places of some local mosquitoes, *Agric. J.*, Fiji **15**: 47-48.
- LEVER, R. J. A. W., 1945. Entomological Notes: 2. Local distribution of the mosquito *Aedes scutellaris* Wlk. *horrescens* Edw. *Agric. J.*, Fiji **16**: 47.
- MANSON-BAHR, P., AND MUGGLETON, W. J., 1952. Further research on filariasis in Fiji, *Trans. Roy. Soc. Trop. Med. and Hyg.* **46**: 301-326.
- MARKS, E. N., 1951. The vector of filariasis in Polynesia: a change in nomenclature, *Ann. Trop. Med. Parasitol.* **45**: 137-140.
- MATTINGLY, P. F., 1953. Personal communication.
- PAINE, R. W., 1943. An introduction to the mosquitoes of Fiji, *Bull. No. 22*, Dept. of Agriculture, Fiji. pp. 35.
- ROSEN, L., 1951. Unpublished data—to be published.
- STONE, A., AND FARNER, D. S., 1945. Further notes on the *Aedes scutellaris* group (Diptera, Culicidae), *Proc. Biol. Soc. Wash.* **58**: 155-162.