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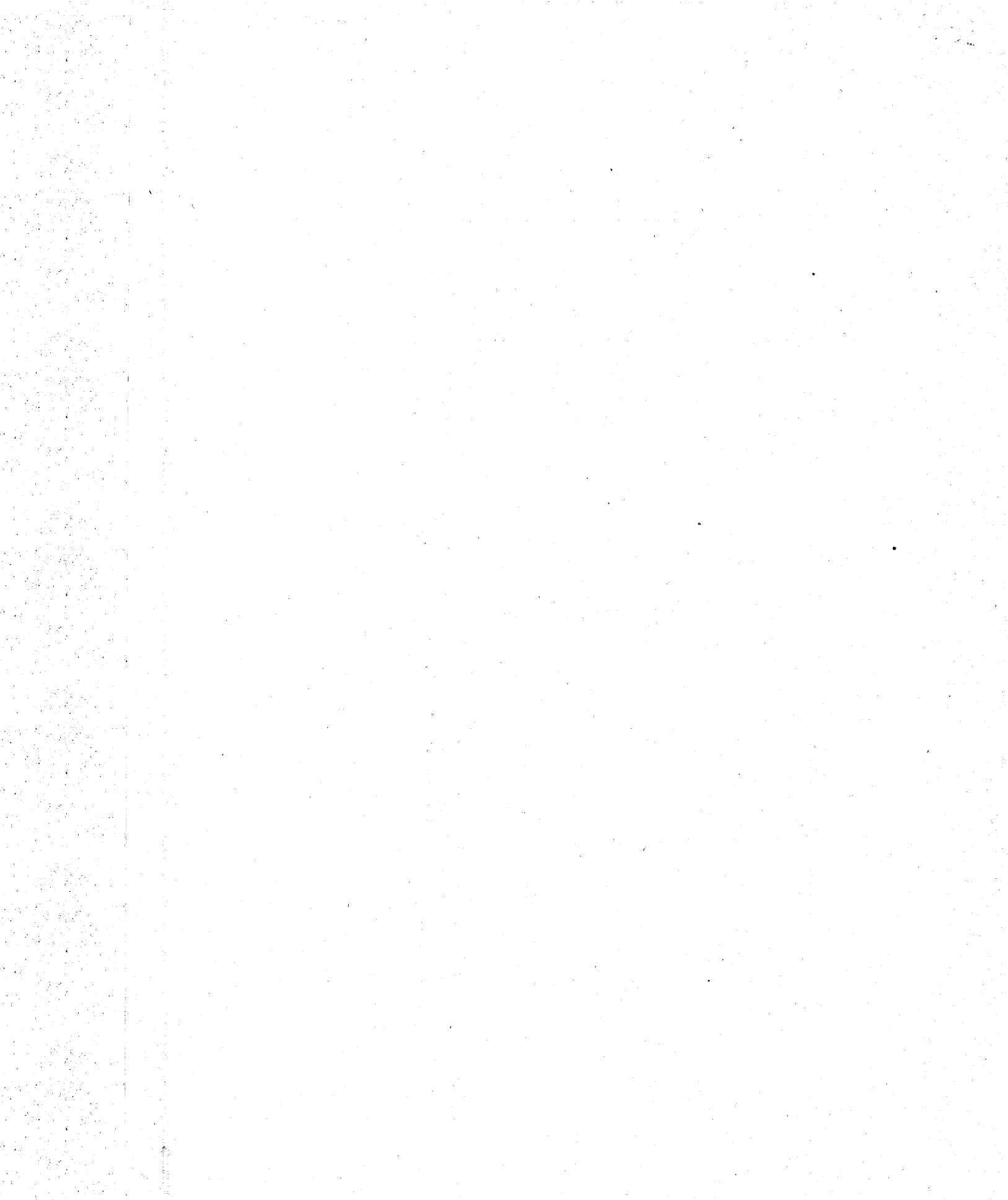
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## NOTES ON PHILIPPINE MOSQUITOES, XIV

### The Larval Instars of *Anopheles*

By F. E. BAISAS

*Of the Malaria Control Section, Bureau of Health*

This is but a preliminary to a more comprehensive study which is expected to include at least the most common Philippine anophelines. First instar larvæ of ten species have so far been examined, of which three (*barbirostris*, *hyrcanus lesteri* and *hyrcanus nigerrimus*) belong to the *Myzorhynchus* series of subgenus *Anopheles*; and seven (*minimus flavirostris*, *filipinæ*, *subpictus indefinitus*, *vagus limosus*, *kochi*, *annularis* and *philippinensis*) represent the four groups (*Myzomyia*, *Pseudomyzomyia*, *Neomyzomyia* and *Neocellia*) of subgenus *Myzomyia* in the Philippines. Species belonging to the *Anopheles* series of subgenus *Anopheles*, being largely sylvan, are not easily available from points within a few hours' trip from Manila; hence, adults of these for egg-laying and breeding have not been obtained.

Based on the ten species examined, the first instar larvae of the *Myzorhynchus* series differ from those of subgenus *Myzomyia* in four distinct and easily visible characters. These are:

	<i>Myzorhynchus</i> series	Subgenus <i>Myzomyia</i>
(1) Frontal hairs (Nos. 5, 6 and 7).	Each with two to six branches.	All single.
(2) Sub-antennal hair (No. 12).	Branched dichotomously.	Split into only two branches.
(3) Secondary comb teeth.	Each with a long point free from fringes.	Tip rounded, fringed; point short if present.
(4) Leaflet (hair No. 1) of thorax and abdomen.	Relatively broader but shorter; present also on abdominal segment I; that on metathorax usually composed of two or more leaflets.	Longer, pointed; absent on abdominal segment I; that on metathorax always single.

There are other but less apparent differences: Prothoracic hair 14, hair 11 of the meso- and meta-thorax, and 1 and 2 of abdominal segment I are present in the *Myzorhynchus* series, absent in subgenus *Myzomyia*; the antennal hair (11) is rela-

tively long, its tip nearly extending to the apex of the antennal shaft in the *Myzorhynchus*, very much shorter and arises very close to the base of the antenna in subgenus *Myzomyia*. Hair 13 and 17 of the head are comparatively longer and usually split into two or three branches in *Myzorhynchus*, shorter and single in *Myzomyia*.

Though quite difficult because first instar larvæ are small and the parts of diagnostic value are minute, these ten species can be differentiated from each other. However, details of the differences and descriptions of the instars will be presented when further studies are completed. Only *Anopheles hyrcanus nigerrimus* and *A. minimus flavirostris* are dealt with in some details in this paper.

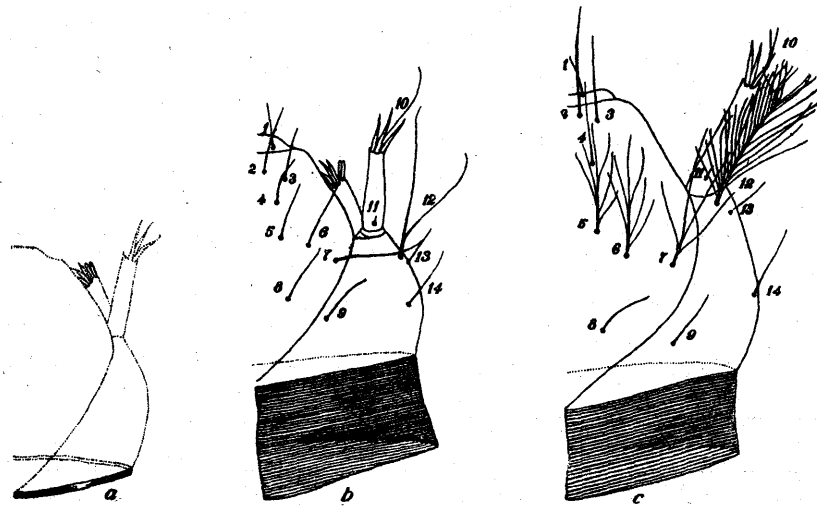


FIG. 1. Larval head of *Anopheles minimus flavirostris*.

- (a) First instar just after emergence from egg shell. Note narrow collar.
- (b) First instar when fully developed. Note wide collar.
- (c) Second instar. Note changes in frontal and sub-antennal hairs. Compare with Plate I.

A first instar larva of either *Myzorhynchus* or *Myzomyia* after emergence from the egg shell possesses a relatively broad head with narrow collar—comparable in shape to that of the mature larva. The broad collar is developed after emergence, which may take, in the laboratory, from 24 hours to several days. There is apparently no change in the shape of the head, the elongation noted in fully developed first instar larva being due to the addition of the broad collar (see Fig. 1, a and b).

But there is an increase in size not only of the head but of the entire larva when it attains full development in the first instar.

For practical purposes the first instar larva may be recognized by the following characters:

- (1) The presence of the egg-breaker on the head.
- (2) Two pairs of outstanding hairs on the mesothorax, each pair arising from very prominent tubercle.
- (3) Hair 1 of the thorax and abdomen, and also prothoracic hair 2 and metathoracic hair 3 represented by leaflets.
- (4) Abdominal segment III, similar to I and II, has two long lateral hairs (Nos. 6 and 7).
- (5) Two sets of comb teeth on abdominal segment VIII, the primary and the secondary.
- (6) All hairs of segment X single.
- (7) Fan on X represented by a group of scales or teeth.
- (8) Several hairs absent, most notable being prothoracic O, mesothoracic 2, metathoracic 6, and abdominal O, 10, and 12.

Perhaps the most detailed study of larval instars undertaken in the past was that by Miss Cora A. Smith (1914), followed by that of Dr. W. D. Lang (1920).<sup>1</sup> Miss Smith deals with only one species, *A. punctipennis*; while Dr. Lang discusses three: *claviger* (*his bifurcatus*<sup>2</sup>), *maculipennis* and *plumbeus*. These four species belong to the *Anopheles* series of subgenus *Anopheles*. As shown in the discussions and illustrations (Smith—p. 9, and Pl. I, Figs. 2 and 3; Lang—pp. 55–56; Figs. 98, 99, and 99a) the frontal hairs are simple in the first instar larvæ of these four species, but Lang states that one or more in *maculipennis* and *claviger* may be forked. Smith refers to the frontal hairs as the “six bristles across the dorsal surface of the head” while Lang calls them “post-antennal.” It is quite interesting to note that among North American species of the *Anopheles* series, hair 12 of the first instar larvæ seems diagnostic for at least certain species.

But more complete in details and each instar fully discussed and illustrated is the work of Dr. H. S. Hurlbut (1938) on *A. walkeri*. When this, our own paper, was already in the press, we received on May 31, 1947, through the courtesy of Dr. W. V. King, a copy each of Doctor Hurlbut's 1938 and 1941 papers. His interpretation of the missing hairs in the first instar is somewhat different from our own. According to his communication, dated 30 April, 1947, he “made a more or less detailed

<sup>1</sup> A copy of Dr. W. D. Lang's “Handbook of British Mosquitoes” was very kindly lent to us by Dr. Alan Stone of the U. S. National Museum.

<sup>2</sup> The synonym is by Dr. F. W. Edwards (1933, p. 38.)

study of all the instars of *A. quadrimaculatus*, *A. punctipennis*, and *A. crucians* in 1940 but did not publish it." However, in his 1941 paper, he gives a key differentiation of first instar larvæ of *walkeri*, *punctipennis*, *quadrimaculatus* and *crucians*.

The system of hair notation used here is that by Martini (1923), and Root (1924) as modified by Puri (1931). There are difficulties in homologizing the hairs of the first instar with those of the fourth, particularly of the mesothorax where some hairs undergo great changes in sizes and positions from the first to the second instar. Opinions will likely differ also regarding the hairs that are not present in the first instar. Our interpretation is indicated in Plates I and II. By this, hair 11 seems the one present on abdominal segment VIII instead of 10 as given by Puri and other workers. Differences in hairs of the *Myzorhynchus* and *Myzomyia* are also shown in these plates.

Some objections may be raised against the scheme of Puri since it does not take into consideration the sequence of hairs according to the order of their appearance in the different larval instars. If "ontogeny repeats phylogeny" then it follows that the hairs of the first instar larva represent the most primitive and should therefore be given the lowest consecutive notations. Those hairs that appear only on the second and subsequent instars should be given the next (higher) designations. According to this, the head which has only nineteen hairs in the first instar, should have these hairs numbered consecutively from 1 to 19. That which is designated as No. 16 (on the maxillary palp) by Puri in the mature larva should be number 20 because it appears only on the third and last instars.

Yet the order of appearance cannot be strictly adhered to in numbering the hairs of the thorax. The hairs that are absent on the prothorax are evidently not identical with those which are absent on the meso- and meta-thorax. Puri (1931) himself thinks that hair 2 of the mesothorax and 6 of the metathorax are absent in the first instar. Our own examinations show that in addition O of the prothorax, 11 and 14 of the mesothorax, and 11 of the meta-thorax are also absent in subgenus *Myzomyia*. For this subgenus, therefore, there are fourteen hairs on the prothorax, eleven on the mesothorax, and eleven on the meta-thorax. But because hair 11 is present on both the meso- and the meta-pleural plates of the *Myzorhynchus*, the hair counts for this are fourteen, twelve, and twelve for the pro- meso- and metathorax respectively. The corresponding counts given by Puri (1931, p. 48) are fourteen, thirteen, and twelve.

From the first to the second instar, the hairs of the thorax undergo a variety of changes not equalled by those on any other part of the larva, although when considered individually the transformation from scales or teeth to long, branched hairs of the fan on abdominal segment X is perhaps the most remarkable.

Nos. 3 and 4 of the mesothorax of the first instar larva are grouped together and arise from a very prominent tubercle. Similarly 5 and 6 are also grouped together and arise from another very prominent tubercle. The two tubercles are close to each other, bulge out considerably on the dorso-lateral border of the mesothorax thereby exaggerating the breadth of the thorax at that point. These four hairs are rather stout, each with a few conspicuous frayings, 4, 5, and 6 very long, subequal, but 3 is only about half or less in length. In the second instar 3 and 4 becomes slender single hairs, fairly long and subequal, but relatively much shorter than 4 of the first instar. They are placed close to each other but with separate small tubercles. Nos. 5 and 6 become widely separated and much shorter than 3 or 4.

Miss Smith<sup>1</sup> (1914, p. 10) mentions "—the disappearance of two pairs of long hairs" on the thorax which occurs in the third stage larva of *punctipennis*. This seems to refer to the mesothoracic hairs 3 to 6. If so, *punctipennis* would be quite different in having this "disappearance" in the third instar instead of in the second instar as noted in all Philippine anophelines examined.

Changes toward different directions and of unequal degree are shown by the thoracic leaflets. In the first instar prothoracic 1 and 2, mesothoracic 1, and metathoracic 1 and 3 are in the form of flattened leaflets. Among the *Myzorhynchus*, metathoracic 1 is often composed of two or more leaflets—thus showing the earliest indication of a true palmate hair. Certain anophelines not found in the Philippines have prothoracic 1 as well as 1 of the meso- and the meta-thorax transformed into normal palmate hairs. But for the local species, only that of the meta-thorax becomes a palmate hair in subgenus *Anopheles* and in a few of subgenus *Myzomyia*. In others of the *Myzomyia* it turns either to rudimentary palmate or to the usual slender hair. Prothoracic 1 becomes a normal hair differing, however, according to species or group of species. In *hyrcanus lesteri* and *hyrcanus*

<sup>1</sup> Dr. Hurlbut, in his letter of April 30, 1947, states; "With reference to *Anopheles punctipennis*, I believe it will interest you to know that the changes in the setal pattern from instar to instar follow *A. walkeri* very closely. The long dorsal hairs of the mesothorax disappear in the second instar as you have indicated for the Philippine anophelines."

*nigerrimus* it becomes a short hair which is usually single but may be split into two or more short branches; many-branched, stout, and rather long in *minimus flavirostris*, *filipinae*, *annularis* and *philippinensis*; weaker and with less branches in some



FIG. 2. Some hairs of second instar larvae. Nos. 1 to 7 = respectively: Prothoracic hairs 1, 2, 3; mesothoracic hairs 1, 2; metathoracic hair 1; and hair 1 of abdominal segment I of *Anopheles minimus flavirostris*. Nos. 8 to 14 = the equivalent respectively of 1 to 7, but of *Anopheles hyrcanus nigerrimus*.

other species. Prothoracic 2 invariably becomes much longer and with more branches than 1; while mesothoracic 1 develops into still more stout, highly pinnate hair. In contrast, metathoracic 4 retrogresses into a weak slender hair in all species.

Keiler (1915, pp. 166-173), as cited by Puri (1931, p. 37), thinks that the pleural hair groups of the thorax represent the last remains of the larval legs in Diptera. Siribaed (1936), based on his studies of a large number of American mosquitoes, assumes that originally each of the pleural groups was composed of four long hairs as in *Dixa*. If the foregoing suppositions are correct, retrogression of hair 11 on the meso- and meta-thorax of subgenus *Myzomyia* must have been already complete even during those primitive evolutionary ages represented by the first instar larva in mosquitoes, since in that stage as well as in the second, this hair is absent. But because it appears in the third and fourth instars and because in *Myzorhynchus* it is present on all segments of the thorax in the first instar and increases in length in each succeeding instar, it appears doubtful whether this is actually a case of retrogression. It would seem more like development, apparently more so when viewed in the light of other hairs which also appear only on the third and fourth instars.

The commonly considered more primitive subgenus *Anopheles* shows specializations which appear, in certain respects, to be of higher degree or else attained at a much earlier period than those in subgenus *Myzomyia*. Of such is the retention in subgenus *Myzomyia* of the wide distance between the inner clypeal hairs in all its larval stages, whilst in subgenus *Anopheles* particularly in the *Myzorhynchus* series these become closely approximated in the second and subsequent instars. Evidently earlier in period because present even in the first instar is the development of branches which are numerous and dichotomous in hair 12 of the head, much fewer in frontal hairs (5 to 7), and mere splitting into two in certain other hairs of the head and abdomen of the *Myzorhynchus*. Hair 12 of the head in subgenus *Myzomyia* is merely split sub-basally into two, while all the other hairs of the entire larva are normally simple; branchings of the kind found in the first instar of subgenus *Anopheles* are present only in the second and later instars of subgenus *Myzomyia*.

The inner and outer clypeal hairs of *pseudobarbistrotris* and *pseudosinensis* of subgenus *Anopheles* on one hand, and of *annularis* and *philippinensis* of subgenus *Myzomyia* on the other show parallel development. Branches of these hairs appear in increasing number from the second to the fourth instar, although the degree is less on the outer but more on the inner clypeals of *annularis* and *philippinensis* than those of *pseudobarbistrotris* and *pseudosinensis*.

Among other marked changes that occur from the first to the second instar are the transformation of hair 12 of the head in subgenus *Myzomyia* from bifurcate to very highly pinnate with more than twenty branches; the increase in size of such as 4 and 5 of the prothorax, 8 of the mesothorax, and 5 and 7 of the metathorax; the reduction of hair 7 on abdominal segment III; the complete disappearance of the secondary comb on VIII<sup>2</sup> and the transformation of the fan on X from scales or teeth to long hairs with long branches. All these changes are not equalled by those that occur in other instars.

Abdominal segment I of subgenus *Myzomyia* does not possess, in the first instar, 1, 2, and 8, and in common with the other segments, also 10 and 12. It is not apparent why 1 is not represented on I even among those species which have developed palmate hair on this segment in the fourth instar. But in the second instar of such species, particularly in *minimus flavirostris* and *filipinae*, palmate I is well represented. For these two species the palmate hair on I of the second instar is composed of six or more unpigmented well-spread leaflets; but in *annularis* and *philippinensis* palmate I may be a single flattened leaflet, often of two or three such leaflets. *Vagus limosus* which has rudimentary palmate hair on I of the mature larva has also this as a single flattened leaflet in the second instar.

Segment I of the abdomen in *Myzorhynchus* has 1 and 2 in the first instar, but 8 together with 10 and 12 (which are also absent on segments II to VIII) is wanting. Nos. 8, 10, and 12 of I as well as 10 and 12 of the other segments and O of II to VIII seem to be developments of the third instar and carried over to the fourth. In some individuals, perhaps in some species, O seems to appear only on the fourth instar. But *nigrerrimus* has what seems to be O on VIII even in the first instar.

Abdominal hair 4 of the first instar in all species is invariably long specially on the middle segments where it may equal or exceed the lateral hairs (6 and 7) in length. Spicules are present on the antennal shaft of *Myzorhynchus*, none in most species and very few in others of subgenus *Myzomyia*. The tergal plates are not indicated in the first instar larva of all species, but these are well represented in the second instar.

Some of the hairs of the first instar are very minute or else represented merely by sockets. Hair 19 of the head is but a

<sup>2</sup> King and Bradley (1941, p. 63) think this secondary comb probably represents the true comb scales, while the primary comb probably corresponds to the pecten in culicines.

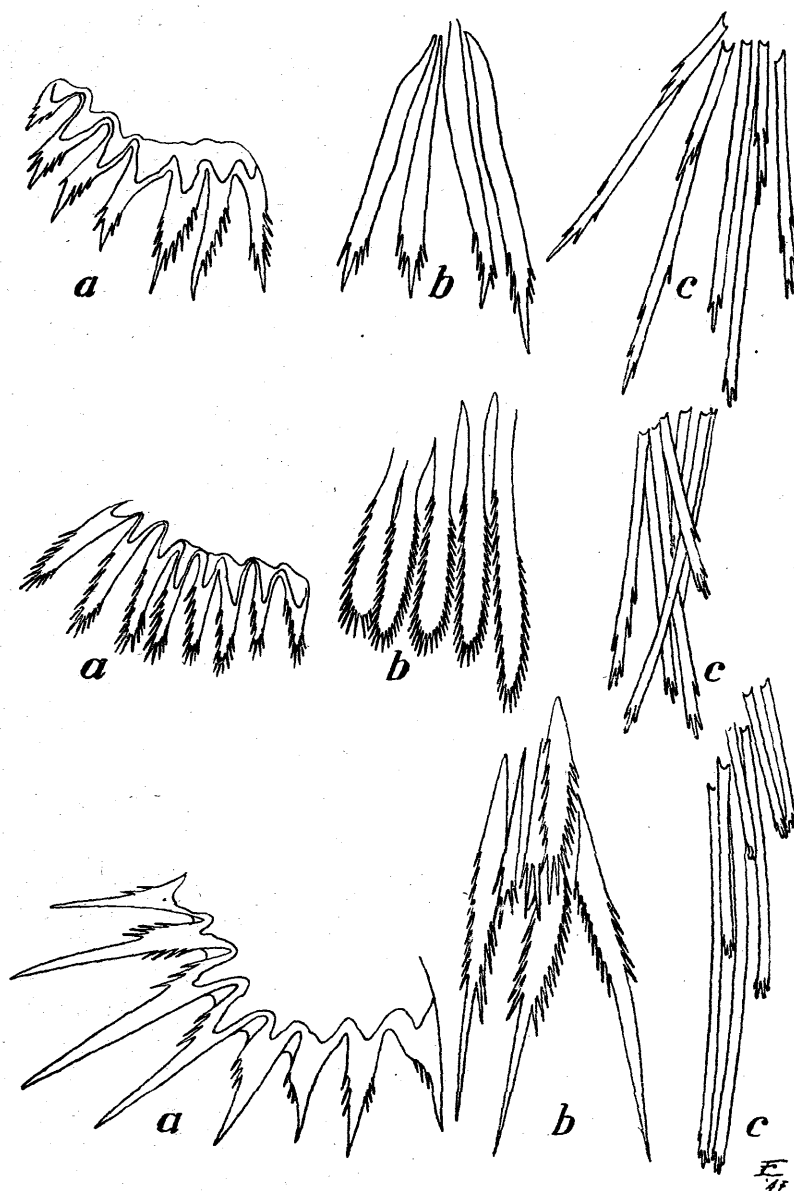


FIG. 3. *a* = Primary comb of first instar larva.  
*b* = Secondary comb of first instar larva.  
*c* = Teeth representing fan of abdominal segment X in first instar larva.

Top: *Anopheles annularis*

Middle: *Anopheles minimum flavirostris*

Bottom: *Anopheles hyrcanus nigerrimus*

socket in all species examined except in *barbistrotris* where a very short relatively stout hair is present. Most if not all of the fossate and scoop hairs are also indicated by sockets in subgenus *Myzomyia*, but these are present as very minute hairs in *Myzorhynchus*. The so-called sensory pit is present on the ventral side of the head, and the position of hair 2 on abdominal segment VI is directly posterior to 1, just as it is in the mature larva. *Subpictus indefinitus* and *vagus limosus* are recognizable by the comparatively coarse spicules they have on the head. Such spicules are absent in most species, finer in others. All, however, have the numerous fine spicules on the under-surface of the thorax and abdomen (see Plates I and II). The spicules on abdominal segment X are the coarsest, particularly those toward the posterior border where, in certain species, the spicules may be trifid or bifid. Quite unlike other species, first instar *kochi* has a few but conspicuous branches on its inner clypeal hairs, which renders it readily separable from the others. The first to report this was Stanton (1914, p. 130 and Fig. 1).

The primary and the secondary comb teeth, and the fan are the most useful in the classification of first instar larvæ. Three types are shown in Fig. 3. It seems, however, that the peculiarities of the secondary comb teeth are somewhat variable in certain species (*annularis*, for example), though the variation does not seem to nullify the specific value of these teeth.

The arrangement and characteristics of the different hairs of the second instar larva are about the same as those on the fourth, although of course the branched hairs have less branches, and the palmate hairs usually with less leaflets. The leaflets in all species seen conform to the primitive type such as those of *Myzorhynchus*, i. e., lanceolate, with or without a few serrations but without "shoulder" and filament. The leaflets of those on II to VIII of the second instar are more or less pigmented. Feathering is well indicated on the lateral hairs (6 and 7) of abdominal segments I and II and on some of the long thoracic hairs. The secondary comb disappears entirely. The number of teeth on the primary comb is still low, about half a dozen or more, but these are pigmented and the serrations are readily visible.

A better understanding of the first instar larva, and the differences between *Myzorhynchus* and subgenus *Myzomyia* may be gained by referring to Plates I and II. The first illustrates the dorsal and ventral aspects of *hyrcanus nigerrimus*, while the second is for *minimus flavirostris*. The drawings were all done by the artist of the Malaria Section, Mr. E. Enriquez.

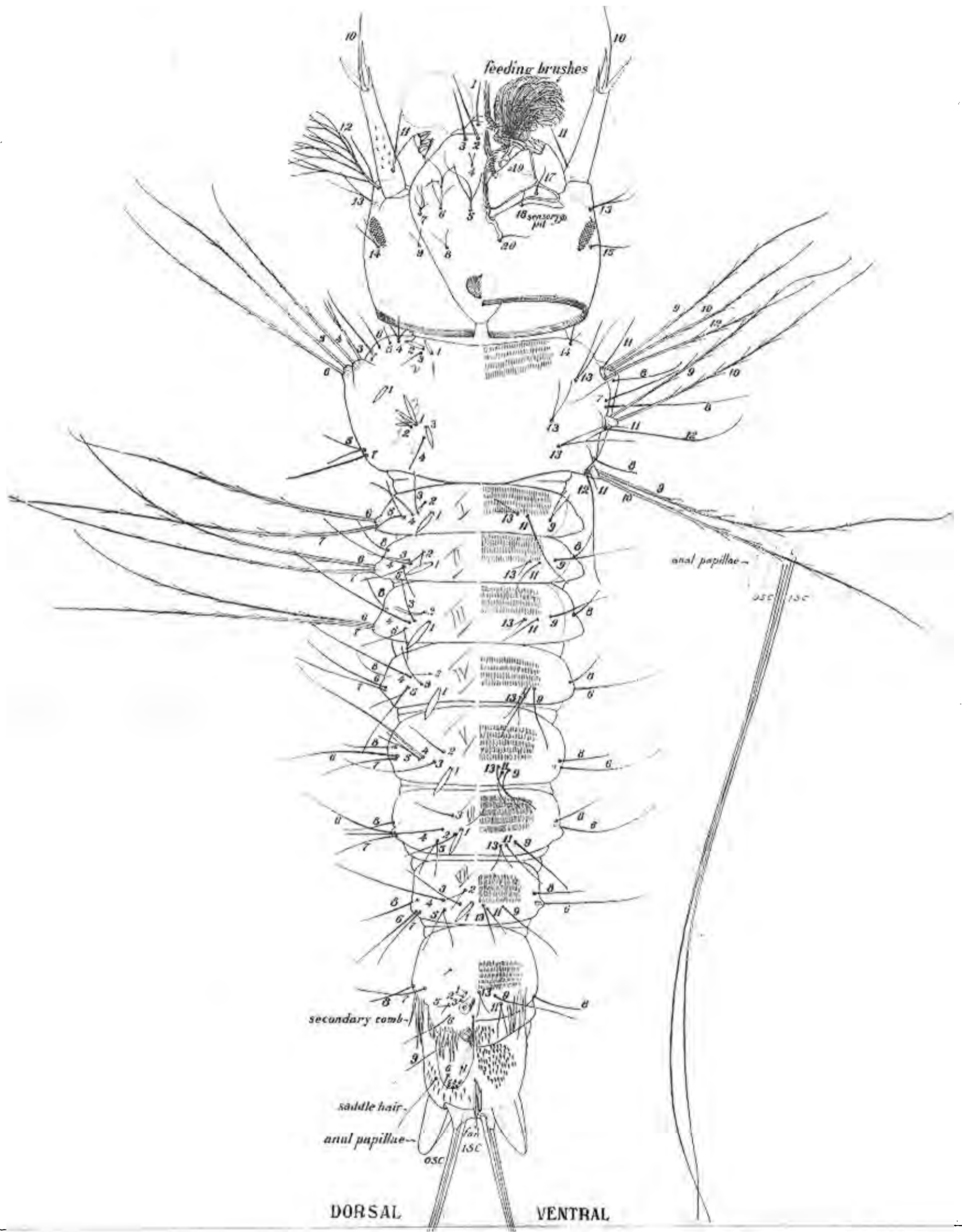


PLATE I. Dorsal and ventral aspects of first instar larva of *Anopheles hyrcanus nigerrimus*.

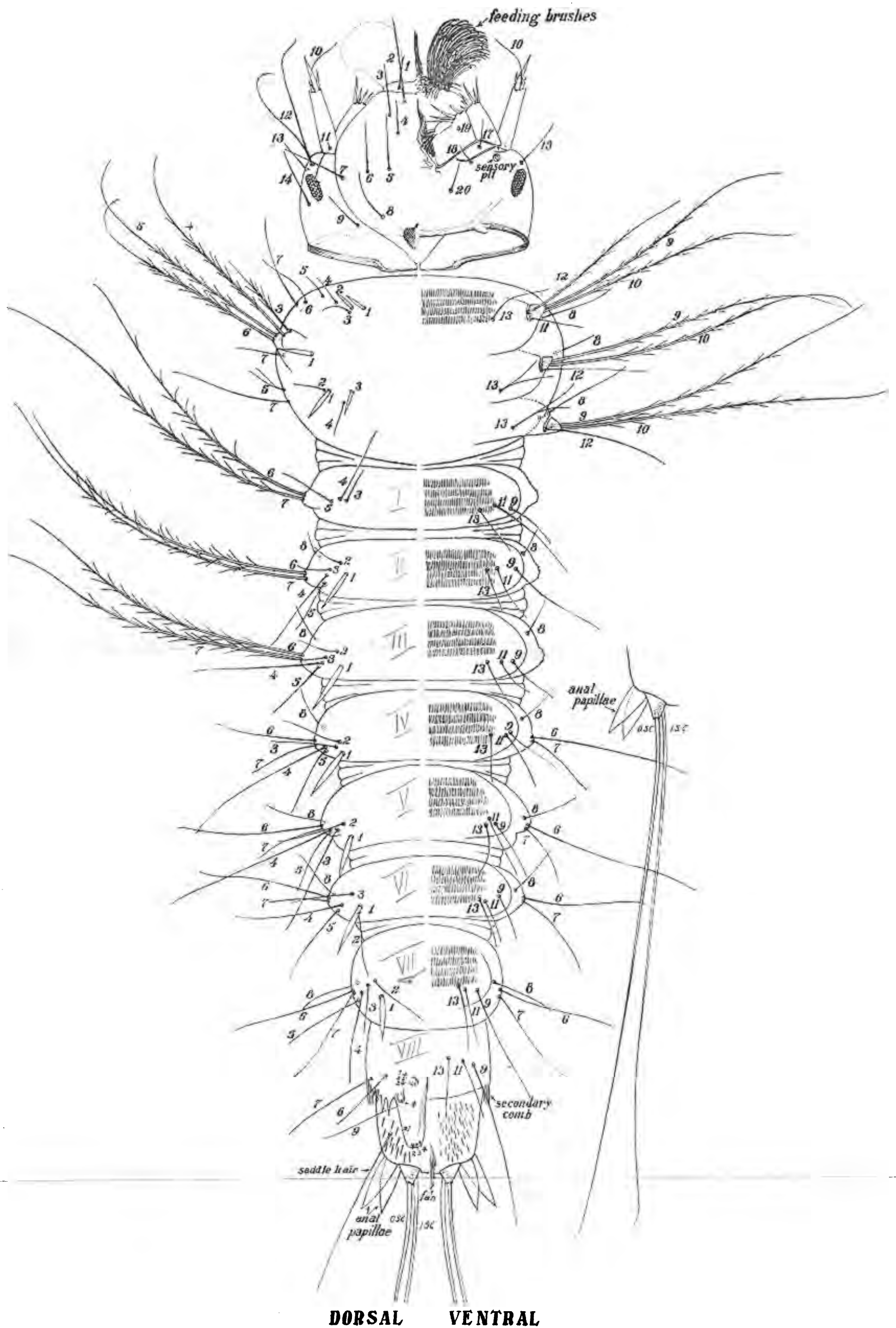


PLATE II. Dorsal and ventral aspects of first instar larva of *Anopheles minimus flavirostris*.

The original camera lucida sketches of the comb teeth and the fan were through the oil immersion lens. They are somewhat reduced in the reproduction here.

The author is indebted to many people who assisted in this work; in particular to Mrs. A. U. Pagayon who is (in the rather crude but expressive term) the "beast of burden" in malaria and mosquito work undertaken through these many years in Malaria Laboratory at Manila. To Mr. C. Urbino and Mr. C. Calingo and others of the Malaria Field Laboratory at Tala; and to Mr. M. Serrano, Mr. F. de Guia, Mr. I. Puhawan, Mr. F. V. Dantis, Miss R. Sanchez, and Mrs. F. Umatan—all of the staff in the Manila laboratory.

#### REFERENCES

1. CRISTOPHERS, S. R.  
The Fauna of British India. Diptera. Family Culicidae. Tribe Anophelini 4 (1933) 36.
2. HURLBUT, H. S.  
A study of the larval chaetotaxy of *Anopheles walkeri* Theobald. Amer. Jour. Hyg. 28 (1938) 149-173.  
First instar characters for distinguishing the common inland species of anophelines of Eastern United States. Amer. Jour. Hyg. 34 (1941) 47-48.  
Personal communication, 30 April, 1947; received at Manila, May 31, 1947.
3. KING, W. V. and BRADLEY, G. H.  
General Morphology of *Anopheles* and Classification of the Nearctic Species. A symposium on Human Malaria (1941) 63-70.
4. LANG, W. D.  
A Handbook of British Mosquitoes. (1920) 50-57.
5. MARTINI, E.  
Über einige für das system bedeutungswolle Merkmale der Slechmucker. Zoöl. Jahrb. 46 (1923) 517-590.
6. PURI, I. M.  
Larvae of Anopheline Mosquitoes, with full Description of those of the Indian species. Ind. Med. Res. Mem. No. 21 (1931) 1-225.
7. ROOT, F. M.  
The Larval Pilotaxy of *Anopheles quadrimaculatus* and *A. punctipennis*. Amer. Jour. Hyg. 4 (1924) 710-724.
8. ROZEBOOM, L. E. and KNIGHT K. L.  
The *Punctulatus* Complex of *Anopheles*. Jour. Parasit 32 (1946) 95-131.
9. SMITH, Miss CORA A.  
The Development of *Anopheles punctipennis*, Say. Psyche 21 (1914) 1-19.
10. STANTON, A. T.  
The *Anopheles* of Malaya—Part I. Bull. Ent. Res. 4 (1913) 129-133. Part II. *Ibid* 5 (1914) 129-132. The Larvae of Malayan *Anopheles*. *Ibid* 6 (1915) 158-172.