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DIFFERENTIATION OF SECOND AND THIRD STAGE LARVAE OF CALIFORNIA CULEX

(DIPTERA: CULICIDAE)¹

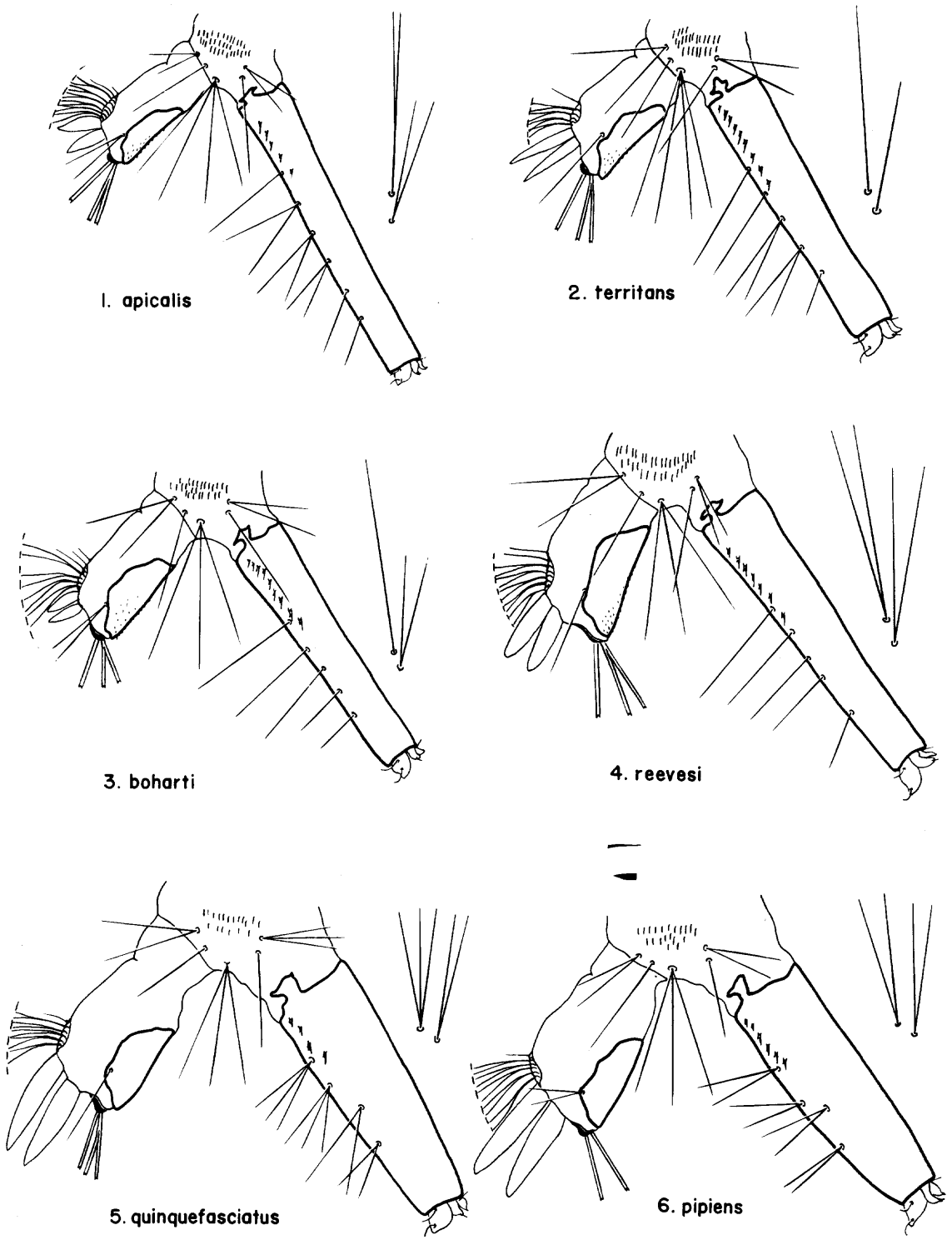
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Species identification on the basis of the fully grown larva has reached a state of perfection in mosquitoes hardly equalled by any other group of insects of comparable size. However, the substantial field of literature on larval taxonomy has included little on the earlier larval stages even though these are frequently collected in the field. It is not uncommon to find a mixture of larvae of all stages in a single pool. As climate has a variable influence on oviposition and eclosion in different species, such a mixture may contain fourth stage larvae of one species, third and second stages of another, and second and first stages of two more. Routine practice usually results in identification of the oldest larvae only.

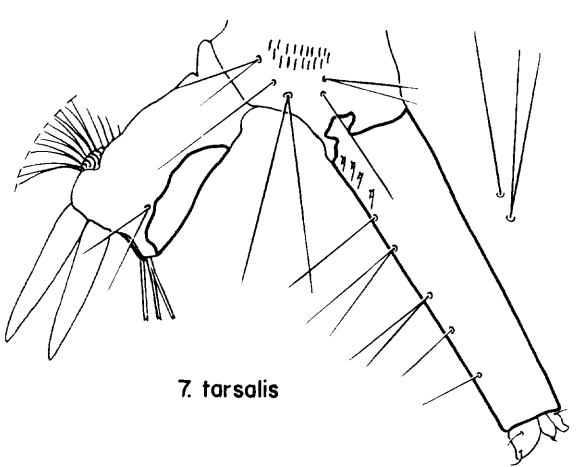
In a previous paper (Bohart, 1954), California species of *Aedes* have been compared in the first larval stage. Unfortunately, first stage *Culex* do not appear to have enough constant characters to permit easy differentiation. On the other hand, the second and third stages are reasonably distinctive. As these have not been described previously for most of the 12 California species, we have attempted a key and a short diagnosis of each stage. Obviously, the observed limits of hair branching, number of comb scales, and the like is conditioned by the material available for study, so this has been indicated in each case.

In general the same structures used in the fourth stage are of value in the second and third even though they may be less developed. There is an obvious tendency for hairs to become more branched at each molt. For instance, a hair may be single in the first stage, double in the second, triple in the third, and many-branched in the fourth. Similarly the sclerotization of the siphon and anal segment increase progressively. In all of the Californian species the fully grown larva has the anal ring or saddle complete. In the earlier stages the position of the lateral hair of the anal segment relative to the saddle has taxonomic significance. The number of comb scales becomes greater with age, and the average differs between species. For example, in our material the average numbers of scales in the comb patch of the first to fourth stages of *Culex stigmatosoma* are 9, 17, 29, and 36. In *erythrothorax* the comparable figures are 12, 38, 67, and 81. Another feature of interest is the degree of branching of the inner caudal seta of the anal segment. This is single in both second and third stages of *restuans*; usually single in the second and double or triple in the third in *erythrothorax*, *stigmatosoma*, *thriambus*, and *pipiens*; and often or usually double in the second and multiple in the third in the rest of the species. A possible indication of phylogenetic significance is the presence in the subgenera *Neoculex* and *Melano-*

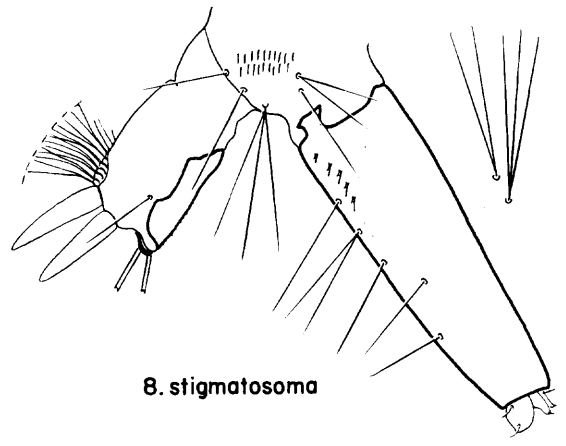
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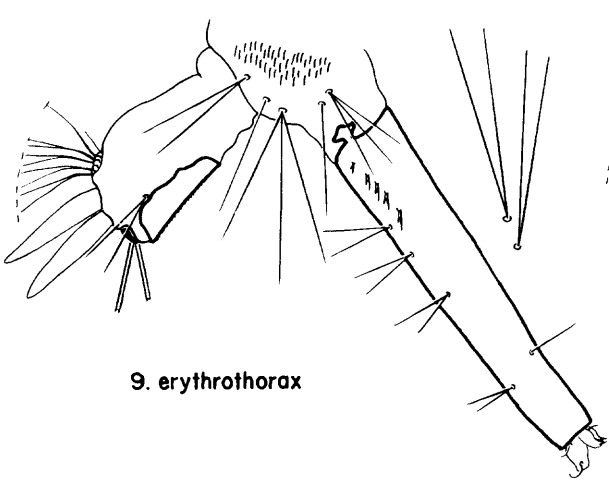
FIGS. 1-6, second stage larvae of *Culex*, terminal segments and head hairs on left side of body.



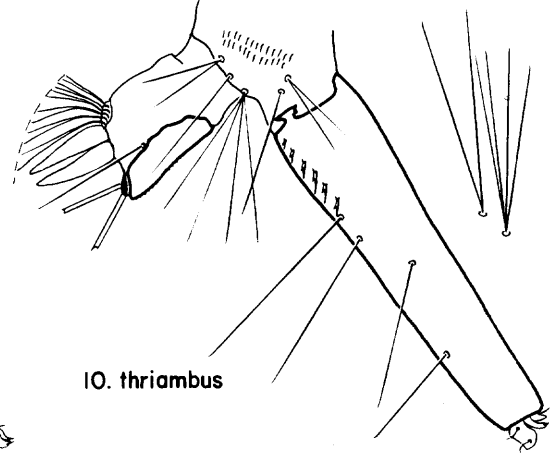
7. tarsalis



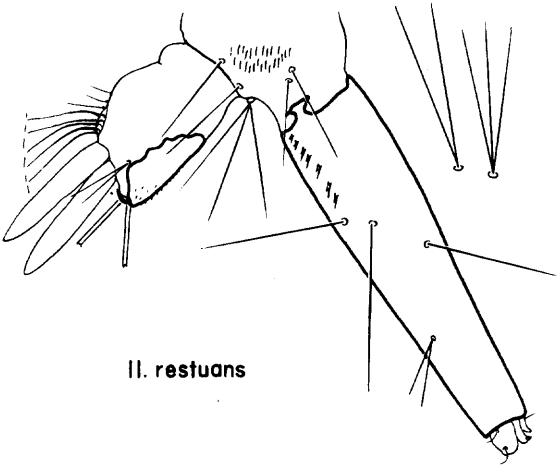
8. stigmatosoma



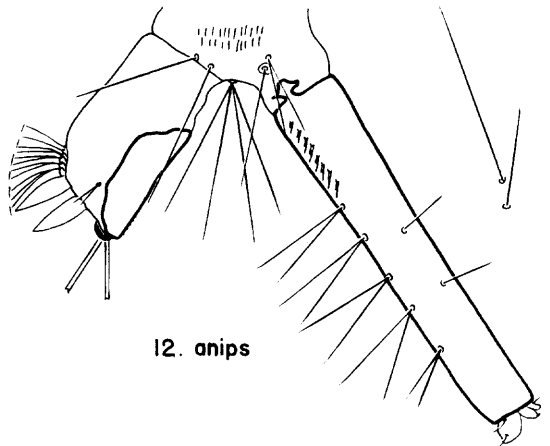
9. erythrothorax



10. thriambus



11. restuans



12. anips

FIGS. 7-12, second stage larvae of *Culex*, terminal segments and head hairs on left side of body.

conion of stout, black clypeal spines instead of the usually pale, attenuate ones in the typical subgenus *Culex*.

KEY TO SECOND AND THIRD STAGE LARVAE
OF CALIFORNIA *CULEX*

1. Clypeal spine stout to tip; usually black throughout. 2
Clypeal spine attenuate toward apex, often pale. 6
2. Siphon with two pairs of subdorsal hairs, inner caudal seta single, second pentad hair inserted on a sclerotized plate. **anips**
Siphon without subdorsal hairs, inner caudal seta rarely unbranched; second pentad hair not inserted on a plate. 3
3. Siphon, excluding acus and valves, at least four times as long as saddle of anal segment; pigmentation of abdominal segment IV about equal to that of III and V (best seen in living or freshly killed specimens). **apicalis**
Siphon, excluding acus and valves, less than four times as long as saddle of anal segment. 4
4. Upper and lower head hairs (5 and 6) single; pigmentation of abdominal segment IV about equal to that of III and V. **territans**
Upper head hair (5) double; pigmentation of abdominal segment IV light as compared with that of III and V. 5
5. Lower head hair (6) single. **boharti**
Lower head hair (6) double. **reevesi**
6. Not more than four pairs of siphon hairs, basal three long, strong, out of line, and almost always single; inner caudal seta single, at least in second stage. 7
With five to six pairs of siphon hairs or with three basal ones not long, strong and single. 8
7. Antennal tuft inserted near middle of antenna, mental plate teeth each three or more times as high as wide. **restuans**
Antennal tuft inserted near distal two-thirds of antenna, mental plate teeth less than three times as high as wide. **thriambus**
8. Lateral hair of anal segment plainly inserted on saddle; siphon with four pairs of usually multiple tufts which are not in line. **pipiens** and **quinquefasciatus**
Lateral hair of anal segment inserted tangent to or near saddle. 9
9. Siphon without sublateral hairs. **tarsalis**
Siphon with one or two hairs out of line sublaterally. 10
10. Comb of fewer than 35 scales (usually fewer than 20 in the second stage and fewer than 30 in the third). **stigmatosoma**
Comb of more than 35 scales (more than 35 in the second stage and usually more than 50 in the third). **erythrothorax**

Notes on variation in chaetotaxy as well as the source and number of specimens examined are given below. Head hairs 5 and 6 are designated upper and lower head hairs respectively. The five lateral hair tufts in a semi-circle below the comb patch are called the pentad hairs and the branches of each from dorsal to ventral are indicated in the pentad formula. The inner caudal seta is abbreviated to ics.

Culex (Melanoconion) anips Dyar

(Fig. 12)

Instar II: Head hairs single, 19–22 comb scales, pentad formula 1–1–4–1–2, siphonal tufts

mostly double but sometimes single or triple, ics single.

Material: 2 in second stage, near Descanso, Baja California, Mexico.

Culex (Neoculex) apicalis Adams

(Fig. 1)

Instar II: Upper and lower head hairs usually double but lower are commonly single from middle to base, 27–36 comb scales (usually 32–33), pentad formula usually 2–1–4–1–2, siphonal tufts single or double, ics single or occasionally double.

Instar III: Upper and lower head hairs double, lower are usually single at base, 44–76 comb scales (usually 54–64), pentad formula from 2–1–2–1–2 to 4–1–8–1–3, siphonal tufts occasionally single but usually double or triple, ics double or rarely triple.

Material: 32 in second stage and 27 in third stage from Green Valley, Solano Co., Calif.; Ojai, Ventura Co., Calif.; Gaviota Pass, Santa Barbara Co., Calif., Oak Creek Canyon, Coconino Co., Arizona (type locality).

Culex (Neoculex) boharti Brookman and Reeves

(Fig. 3)

Instar II: Upper head hairs double, lower head hairs single, 20–35 comb scales (usually 26–35), pentad formula usually 2–1–3–1–2 but varying to 3–1–4–1–2, siphonal tufts single or rarely double, ics double.

Instar III: Upper head hairs double or rarely single, lower head hairs single, 40–62 comb scales, pentad formula 2–1–3–1–2 to 3–1–6–1–3, siphonal tufts mostly double but sometimes single or triple, ics double or sometimes triple.

Material: 39 in second stage and 28 in third stage from Trinity, Marin, Napa, Solano, Santa Barbara, and Ventura Counties, California.

Culex (Neoculex) reevesi Wirth

(Fig. 4)

Instar II: Head hairs double, 24–47 comb scales (usually 27–38), pentad formula usually 2–1–3–1–2 but varying from 1–1–2–1–1 to 3–1–3–1–3, siphonal tufts single or sometimes double, ics usually double but rarely single or triple.

Instar III: Head hairs double, upper ones rarely triple, 49–73 comb scales, pentad formula 2–1–4–1–2 to 4–1–7–1–3, siphonal tufts usually double but sometimes single or triple, distal tuft often single, ics double or triple.

Material: 26 in second stage and 20 in third stage from Black Lake, San Luis Obispo Co., Calif.

Culex (Neoculex) territans Walker

(Fig. 2)

Instar II: Head hairs single, 21–29 comb scales, pentad formula 2–1–3–1–1 to 2–1–4–1–2,

siphonal tufts single or double, ics single or double.

Instar III: Head hairs single, 44-55 comb scales, pentad formula 2-1-4-1-2 to 3-1-6-1-3, siphonal tufts double or triple, ics double.

Material: 6 second stage and 5 third stage larvae from Weaverville, Trinity Co., Calif.; and Canyon Dam, Plumas Co., Calif.

Culex (Culex) erythrothorax Dyar

(Fig. 9)

Instar II: Head hairs double, 36-43 comb scales, pentad formula 2-1-2-1-2 to 3-1-4-1-3, siphonal tufts single or double, ics single.

Instar III: Upper head hair triple to quintuple but usually triple, lower head hair usually double but sometimes triple, 48-91 comb scales (usually 57-73), pentad formula 2-1-3-1-3 to 5-1-6-1-4, siphonal tufts usually double but sometimes single or triple, ics usually double but sometimes single or triple.

Material: 11 in second stage and 30 in third stage from Merced, Ventura, and Orange Counties, Calif.; and Descanso, Baja California, Mexico.

Culex (Culex) pipiens Linnaeus

(Fig. 6)

Instar II: Head hairs double or sometimes triple, 16-26 comb scales, pentad formula 2-1-3-1-2 or more rarely 1-1-3-1-1, siphonal tufts double or sometimes triple, ics single or rarely double.

Instar III: Head hairs triple or quadruple, rarely more, 36-52 comb scales, pentad formula 2-1-4-1-2 to 4-1-6-1-4, siphonal tufts mostly double but sometimes single or triple, ics single or double.

Material: 13 in second stage and 13 in third stage from Davis, Yolo Co., Calif.

Culex (Culex) quinquefasciatus Say

(Fig. 5)

Instar II: Head hairs double or triple, rarely quadruple, 16-23 comb scales, pentad formula usually 2-1-3-1-2 but varying to 2-1-4-1-3, siphonal tufts double or triple, rarely single, ics single or double.

Instar III: Head hairs triple or quadruple, rarely quintuple, 31-46 comb scales, pentad formula 3-1-4-1-3 to 4-1-6-1-4, siphonal tufts double to sextuple, ics double or rarely single.

Material: 27 in second stage and 27 in third stage from Manteca, San Joaquin Co., Calif.; and Springville and Visalia, Tulare Co., Calif.

Culex (Culex) restuans Theobald

(Fig. 11)

Instar II: Head hairs double or triple, 20-27 comb scales, pentad formula 1-1-3-1-1 to 2-1-3-1-2, siphonal tufts single except distal one which is sometimes double, ics single.

Instar III: Head hairs triple to quadruple, rarely quintuple, 43-49 comb scales, pentad formula 2-1-5-1-2 to 3-1-6-1-3, siphonal tufts usually single except for distal one which is often double and may be triple, ics single.

Material: 11 in second stage and 5 in third stage from Ojai, Ventura Co., Calif.; Peters Canyon, Orange Co., Calif.; and Descanso, Baja California, Mexico.

Culex (Culex) stigmatosoma Dyar

(Fig. 8)

Instar II: Head hairs double or triple, 14-22 comb scales, pentad formula 1-1-2-1-1 to 3-1-4-1-3, siphonal tufts single or double, rarely triple, ics usually single, rarely double.

Instar III: Upper head hair triple to quintuple, lower head hair double to quadruple, 22-40 comb scales, pentad formula 3-1-5-1-2 to 5-1-7-1-3, siphonal tufts single to sextuple, ics double or triple.

Material: 21 second stage and 76 third stage from Yolo and Solano Counties, Calif.

Culex (Culex) tarsalis Coquillett

(Fig. 7)

Instar II: Head hairs single to triple, upper head hair most often double, lower head hair usually single, 12-22 comb scales, pentad formula 1-1-2-1-1 to 3-1-4-1-2, siphonal tufts single or double, ics usually double, sometimes single.

Instar III: Upper head hair double to quadruple, lower head hair double or triple, 21-40 comb scales, pentad formula 3-1-4-1-2 to 4-1-6-1-3, siphonal tufts double to quadruple, rarely quintuple, ics double or triple, rarely quadruple.

Material: 35 in second stage and 41 in third stage from Butte, Napa, Yolo, Solano, and Kern Counties, Calif.

Culex (Culex) thriambus Dyar

(Fig. 10)

Instar II: Upper head hair triple to quintuple, lower head hair double or triple, 21-33 comb scales, pentad formula 2-1-3-1-2 to 4-1-5-1-3, siphonal tufts single, rarely double, ics single.

Instar III: Upper head hair quadruple to sextuple, lower head hair triple to quadruple, 43-55 comb scales, pentad formula 3-1-5-1-2 to 5-1-7-1-3, siphonal tufts single except distal one which is sometimes double, ics double, rarely single or triple.

Material: 25 in second stage and 20 in third stage from Ojai, Ventura Co., Calif.; Snelling, Merced Co., Calif.; and Descanso, Baja California, Mexico.

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THE DEVELOPMENT OF THE SALIVARY GLANDS IN ANOPHELES ALBIMANUS WIEDEMANN (DIPTERA, CULICIDAE)¹

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A great amount of detailed information exists on the development of various organs in immature insects and on the differentiation of imaginal discs especially among the Diptera (Bodenstein, 1953). Except for a paper by Thompson (1905), it is altogether surprising to find that for mosquitoes there is apparently no description of the growth of the larval salivary glands or of the origin and development of the imaginal glands. Even the anatomy of the larval glands has received scant attention (Imms, 1907, Salem, 1931, Frizzi, 1947, Schildmacher, 1950), and this mostly in connection with other problems.

The present paper describes the anatomy of the pre-imaginal glands of *Anopheles albimanus* and gives detailed information on how the larval and adult salivary glands develop.⁴ Brief anatomical comparisons are made between the larval glands of *Anopheles*, *Culex*, and *Aedes*.

MATERIALS AND METHODS

The mosquitoes used in this study were reared in an insectary held at $22 \pm 2^\circ\text{C}$, with a relative humidity ranging from 50% to 70%. The larvae were fed daily on dogfood and yeast. Adults were given 5% sugar solution and blood.

Since the duration of the various stages varied considerably, all studies were made on individually reared specimens of known history and precise chronological ages.

Salivary glands were examined in living intact larvae, in freshly dissected whole mounts from each stage in 0.85% NaCl (Jensen, 1955), either without staining or after staining with acetocarmine, or with Berger's modification (1938) of the Feulgen reaction. Material from each stage was fixed in Carnoy, Bouin, or formalin, then sectioned at 6 to 8 microns, and stained with Delafield's or Heidenhain's hematoxylin.

Measurements on whole glands and associated structures were made at magnifications of 210 to 430, and nuclei were measured at 980.

RESULTS

1. *Morphology of the larval glands.*—The salivary glands of *Anopheles albimanus* are paired

organs which lie in the thorax on either side of the alimentary canal; they extend as far as the metathorax, where in most species portions of them are clearly visible directly through the cuticle. Each gland has two well-defined parts: a large terminal sac or distal portion (fig. 1, TS), and a smaller, spherical or globular anterior or proximal part (AS). The two portions are connected by a short neck or isthmus. When the glands are dissected out in 0.85% NaCl the anterior sac retains its spherical form, but the terminal part tends to collapse into an irregular shape as shown in figure 2. The glands are ensheathed by a fine basement membrane.

The terminal portion is composed of 50 to 60 cells which have large, round, centrally located nuclei, prominent nucleoli, and well-defined

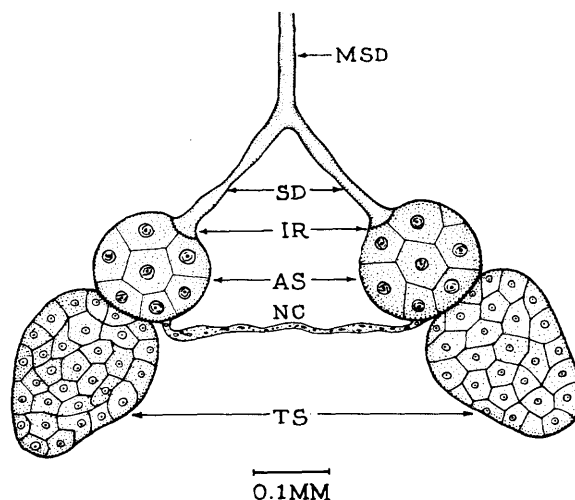


FIG. 1. The larval salivary glands of *Anopheles albimanus* showing the median salivary duct (MSD), the side ducts (SD), the anterior sac (AS), the ventral nephrocyte chain (NC), and the terminal sac (TS). The ring of tissue from which the adult glands differentiate is indicated at IR. The drawing is semi-diagrammatic.

chromosomes (fig. 4). The anterior portion is made up of 12 to 15 cells which are much larger than those in the distal part, and its huge, round, central nuclei have giant, banded chromosomes (fig. 3). In *A. quadrimaculatus* the banding is visible in live whole mounted larvae. In general, the cells of both portions are roughly polygonal in

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⁴Based on the Senior Author's Master's Thesis (1953), on deposit in the George Washington University Library.

shape. Cells in the proximal part of the gland are thick and bulge inwards, making the lumen small; at times cells in the distal part may also bulge slightly, but usually they are thin and flattened, leaving a large lumen. A compact band of very minute cells (fig. 1, IR, and fig. 5) encircles the anteriormost part of the gland where it empties into the salivary duct (fig. 1, SD). A slender syncytial chain of tissue extends between the two salivary glands, the ends being attached near the junction of the proximal and distal portions as shown in figure 1 (NC). This strand, when seen in freshly dissected material, contains many relatively large, pale greenish inclusions, and has seven or eight round nuclei in each portion of the chain. When larvae are

fed ammonia carmine this tissue becomes filled with bright red granules like the pericardial cells. These cells were termed ventral nephrocytes by Metalnikov (1902), who first discovered them in *Culex*.

An examination of the salivary glands of *Anopheles quadrimaculatus*, *freeborni*, *aztecus*, and *stephensi* showed them to be quite similar to those of *albimanus*. When live, whole-mounted *Anopheles* larvae are viewed ventrally, two salivary ducts can be seen to enter the head and join to form a single median duct (fig. 1, MSD). This common duct runs ventral to the subesophageal ganglia and passes up the labium to empty on the antero-dorsal aspect of the prementum directly in front of the pharynx as described by

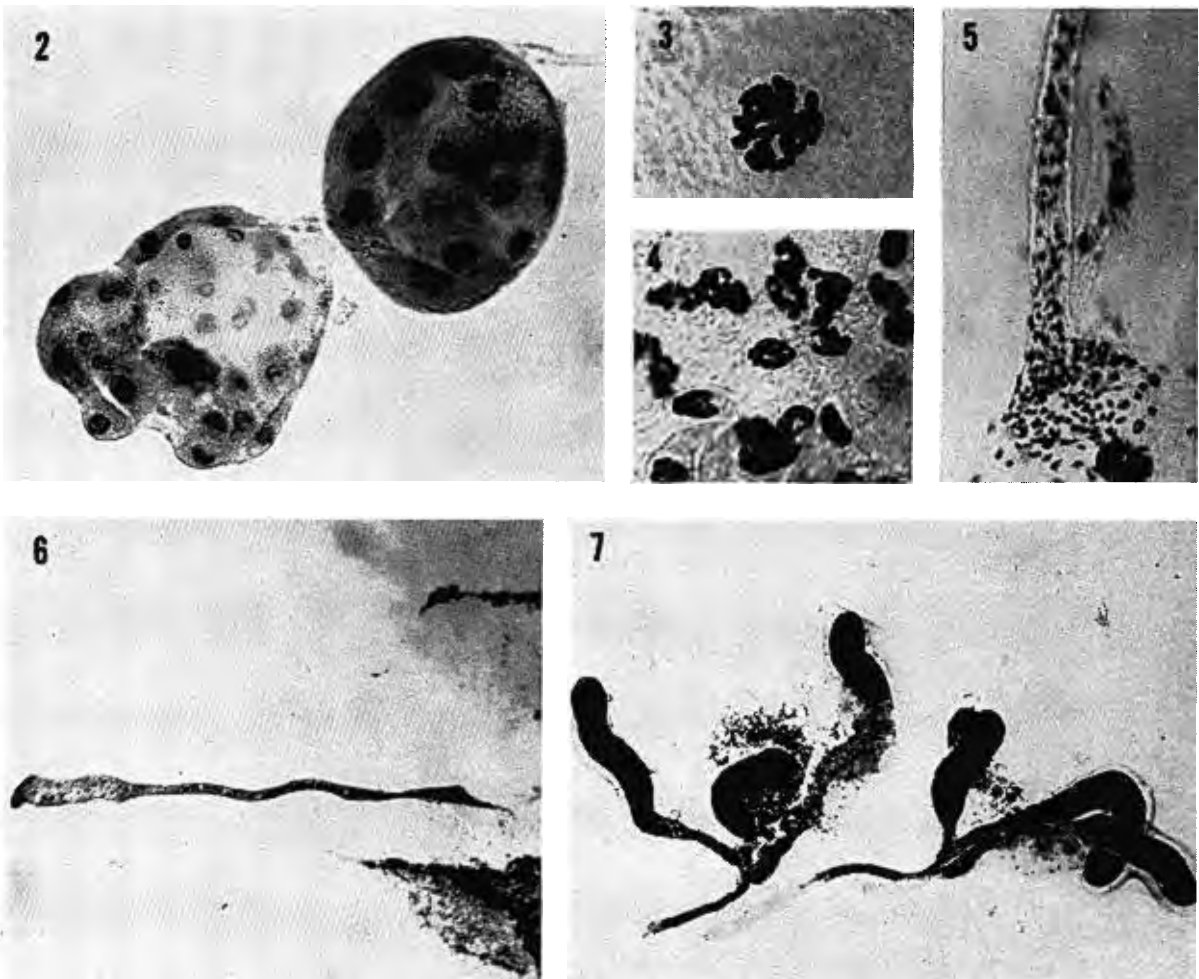


PLATE I, FIG. 2. A single salivary gland from a second stage *Anopheles albimanus* larva. Note the dark staining anterior and light staining posterior sacs. Feulgen stain. $\times 568$.

FIG. 3. Typical large nucleus in anterior sac. Fourth stage larva. Feulgen stain. $\times 870$.

FIG. 4. Typical nuclei in posterior sac. Fourth stage larva. Feulgen stain. $\times 870$.

FIG. 5. Imaginal ring cells at the base of the slender salivary duct in fourth stage larva. Note absence of taenial rings in the duct. Feulgen stain. $\times 870$.

FIG. 6. Developing imaginal salivary gland in 20-hour old male pupa. Feulgen stain. $\times 210$.

FIG. 7. Definitive imaginal salivary glands in 24-hour old female. Delafield's hematoxylin. $\times 75$.

Farnsworth (1947). The salivary ducts in larvae do not have taenidial rings. Neither muscles nor nerves were found to be associated with the pre-imaginal salivary glands or their ducts in *Anopheles*. The glands also lack tracheae.

The larval salivary glands in *Culex pipiens* differ considerably from those in *Anopheles*. In *Culex* the proximal portion is elongated and cylindrical and made up of small cells, while the terminal sac is globular and made up of much larger cells. A conspicuous muscle is attached at the end of the terminal sac in *Culex*. In *Aedes aegypti* the salivary glands are cylindrical for three-fourths of their length and then become dilated towards the distal end. In *Aedes atropalpus* the entire salivary gland is elongated and cylindrical.

2. *Growth of the larval glands.*—Glands removed from newly hatched larvae are very minute, measuring about 60 microns in length.

pupation steadily decrease in size. The ventral nephrocyte chain, on the other hand, reaches its maximum size in the pupal stage (fig. 8) and its growth is linear through the fourth stage.

The amount that different portions of the salivary gland complex grew up through the third larval stage was calculated to obtain day to day as well as stage to stage growth rates (table 1). The differences between the growth rates of anterior and posterior sacs are not statistically significant. The nephrocyte chain, which is not destroyed during the early hours after pupation, has a significantly different growth rate from that of the salivary glands themselves (table 1). During larval life the anterior sac increases about 7 times in size, the posterior part of the gland increases roughly 5 times, and the salivary ducts enlarge 3 to 4 times. The ventral nephrocyte chain approximately doubles in width during larval life.

TABLE I
GROWTH RATIOS FOR DIFFERENT PORTIONS OF THE LARVAL SALIVARY
GLANDS OF *Anopheles albimanus*, ALONG WITH THE ASSOCIATED
VENTRAL NEPHROCYTE CHAIN

PORTION MEASURED	GROWTH RATIOS THROUGH THIRD STADIUM	
	Day to Day Rate (geometric mean)	Stage to Stage Rate (geometric mean) ³
Anterior sac		
length.....	1.22	1.82
width.....	1.22	1.82
nuclei.....	1.23	1.86
Posterior sac		
length.....	1.18	1.64
width.....	1.18	1.64
nuclei.....	1.21	1.77
Salivary duct width.....	1.15	1.52
Ventral nephrocyte chain....	1.04	1.12

The anterior part is poorly developed and barely distinguishable from the salivary duct; even after staining it is difficult to distinguish its nuclei. The terminal sac is larger, has very distinct nuclei, and its lumen contains droplets. By the second day of the first stage, the anterior sac has become well-defined and its nuclei clearly distinguishable and of nearly the same size as those in the terminal sac. Banding of the chromosomes first becomes evident in late third stage larvae.

As with other dipterous larvae, the number of cells in the salivary glands of *Anopheles* was found to remain essentially constant throughout larval life, so that growth occurs solely through increases in cell size. This growth is shown graphically in figure 8. The log dimensions of the various portions are seen to be approximately linear with respect to age through the third stage. The glands reach their maximum size about the third or fourth day of the fourth stage and with

3. *Metamorphic events.*—While the salivary glands of the larva are growing in size, a series of less conspicuous but important events have been occurring in the cells destined to form the salivary glands of the adult. A ring of 12 to 15 minute cells at the anterior end of each larval gland becomes first clearly visible in the late first instar. The number of cells in this ring approximately doubles in each stadium until at last more than a hundred cells are found therein in fourth instars (fig. 5). They are tightly packed, very tiny cells with oval nuclei except for a few at the distal end which are larger and have round nuclei. Mitotic divisions were not observed in the imaginal ring cells.

When pupation occurs, the nuclei in the terminal sac of the larval gland degenerate rapidly and the whole portion diminishes in size (fig. 8). Nuclei in the anterior part of the larval gland degenerate less rapidly. Four to eight hours after pupation, the glands become

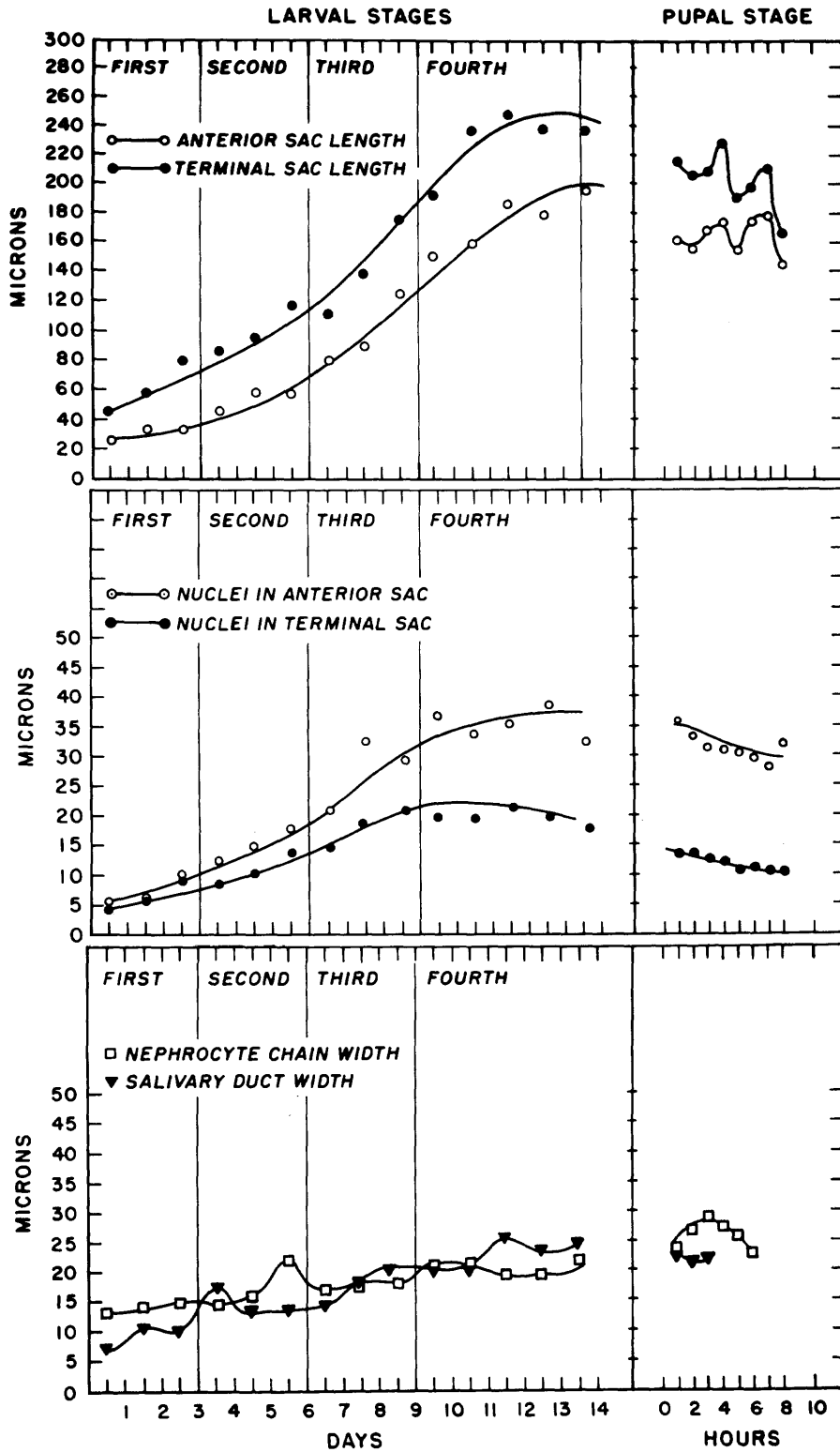


FIG. 8. The growth of different portions of the larval salivary glands and the ventral nephrocytes in *Anopheles albimanus*. Each point is the average of measurements taken from 10 to 25 larvae.

increasingly vacuolated, and their nuclei are found at the outer edges of the cells. Histolyzing glands were seen up to 20 hours of pupal life, hemocytes taking no visible part in their degeneration.

As these changes take place, the imaginal glands begin to differentiate. Approximately five hours after pupation, the imaginal ring in the male is separated from the larval glands in dissected material, and appears as a tiny one-lobed structure dilated at its distal end, apparently having its full complement of cells at this time (fig. 6). In the female pupa the imaginal ring differentiates into three finger-like prongs amidst the degenerating larval gland cells. At about the seventh or eighth hour of pupal life minute three-lobed glands separate from the larval glands in dissected material. The cells in the differentiating glands of the female at this developmental stage seem definitely fewer than those visible later on in the definitive adult.

Differentiation is completed chiefly during the early hours after pupation. The glands then become greatly elongated as the cells increase in size. The imaginal glands do not attain their full size and the middle acinus in the female does not completely differentiate until shortly after the emergence of the adult (fig. 7). Shortly before emergence, the lateral acini of the glands contain droplets.

DISCUSSION

The development of the salivary glands and the ventral nephrocytes in *Anopheles albimanus* beautifully illustrates three well-known concepts of growth and differentiation among the Diptera. (1) The larval glands grow through continuous and essentially linear increase in cell size, but the cells do not multiply. These glands reach their maximum size shortly before pupation and are rapidly destroyed during the early hours of metamorphosis. (2) The imaginal salivary gland discs grow during larval life primarily through cell multiplication. Although mitotic divisions were not observed in any of our preparations,⁵ we assume that they do occur and suspect that all of the cells in the ring undergo mitosis rapidly and at the same time. If this speculation is correct the growth of these discs during larval life is a cyclic phenomenon. (3) Tissues like the ventral nephrocyte chain, which do not undergo profound metamorphosis, grow more slowly during the larval stages and so reach their maximum size in post-larval life, quite unlike strictly larval tissues.

Some interesting comparisons can be made between the morphology and the development of the salivary glands in *Anopheles* and *Drosophila*. In making this comparison we have used the

detailed account of *Drosophila* salivary glands as given by Bodenstern (1950).

In *Drosophila* larvae the salivary ducts, which have taenidia, open into the pharynx itself, while in *Anopheles* the ducts have no taenidia⁶ (as first shown by Imms, 1907) and empty in front of the opening into the pharynx. In *Drosophila virilis* the salivary glands are made up of 115 cells whereas in *Anopheles* the glands are composed of notably fewer cells (72 to 75). In *Drosophila* larvae the glands grow down as far as the second abdominal segment, while in *Anopheles* they are strictly confined to the thorax. The salivary glands of *Drosophila* appear as simple elongated sacs until the third instar (Bodenstern's stage 6), at which time anterior and posterior zones are recognizable. In *Anopheles* larvae, on the other hand, the salivary glands have two clearly defined regions from the first stage onwards. In the salivary gland of *Drosophila*, Bodenstern states, ". . . there is never any sharp separation of anterior and posterior cells." In *Anopheles*, however, cells in the anterior and posterior portions are distinctly different as to size and nuclei and there is no intermediate zone of graduation. According to Bodenstern, "the anterior cells grow more slowly than the posterior cells" in *Drosophila*. In *Anopheles* no statistically significant differences between the growth rates of anterior and posterior cells could be demonstrated up through the third stage. In *Drosophila* the imaginal salivary gland ring appears first in the second instar (30 hour old larvae), while in *Anopheles* the ring is visible in the late first instar. In both *Drosophila* and *Anopheles* the posterior portion of the salivary gland degenerates before the anterior part during the early stages of metamorphosis.

The larval glands are probably more or less continuously secretory even in the absence of food for they do not diminish in size in *Anopheles* starved for several days.

It would be very interesting to know if both portions of the salivary glands in *Anopheles* larvae have distinctly different functions. The larger nuclei of the anterior sac suggest the possibility that this is the actively secreting portion. Perhaps the posterior sac is a reservoir. The anatomical differences noted between the salivary glands of the different species may provide a means of determining the answers to such questions.

SUMMARY

1. The paired salivary glands of *Anopheles albimanus* larvae have a spherical anterior portion of 12 to 15 polygonal cells with large nuclei and a larger terminal sac of 50 to 60 cells with smaller nuclei. No muscles, nerves, or tracheae were

⁵Ross (1939) also failed to detect mitotic divisions in the imaginal salivary gland ring in *Drosophila*.

⁶*Chaoborus* larvae also lack taenidial rings in their salivary ducts (Debauche, 1933).

found associated with these glands. A slender chain of ventral nephrocytes extends between the paired glands.

2. The larval glands grow only by increases in cell size. The growth rates of the different portions are essentially the same. The maximum size of the glands is reached shortly before pupation, after which they rapidly histolyze, the posterior portion degenerating before the anterior.

3. The imaginal salivary glands originate from a small ring of cells first visible at the anteriormost end of the larval glands in the first instar. The number of cells in this ring approximately doubles in each larval stage. As the larval glands begin to degenerate in the pupa, the ring cells differentiate into one-lobed glands in the male and three-lobed glands in the female.

4. The anatomy of the salivary glands in *Anopheles albimanus* is briefly compared with several other *Anopheles*, and with *Culex* and *Aedes* larvae.

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EXTERNAL MORPHOLOGICAL DESCRIPTION OF THE LARVA OF EXOMALOPSIS CHIONURA COCKERELL, INCLUDING A COMPARISON WITH OTHER ANTHOPHORIDS^{1, 2}

(HYMENOPTERA: APOIDEA)

JEROME G. ROZEN, JR.³

Included in this paper is a description of both the mature larva and the first instar of *Exomalopsis (Anthophorula) chionura* Cockerell. This represents the first relatively complete account of the mature larva of any member of the Exomalopsinae

and is of special significance because it tends to clarify the relationship of this subfamily with others of the Anthophoridae. This relationship has been open to question until now (Michener, 1944, 1953).

The early stage larva is dealt with because it is different in appearance from the last and consequently may possess characters that would further refine the understanding of the phylogenetic affinities within the Anthophoridae. Unfortunately, too few young larvae of other subfamilies are available at the present time for this purpose, although there is sufficient material to clearly show that pronounced differences exist between the first instars of some of the groups.

The material employed in this study was

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³I would like to express my appreciation to Professors E. Gorton Linsley and J. W. MacSwain for allowing me to examine the various stage larvae of *Melitoma euglossoides* Lepelletier, *Ptilothrix sumichrasti* (Cresson), *Nomada opacella* Timberlake, and *Melissodes timberlakei* Cockerell.

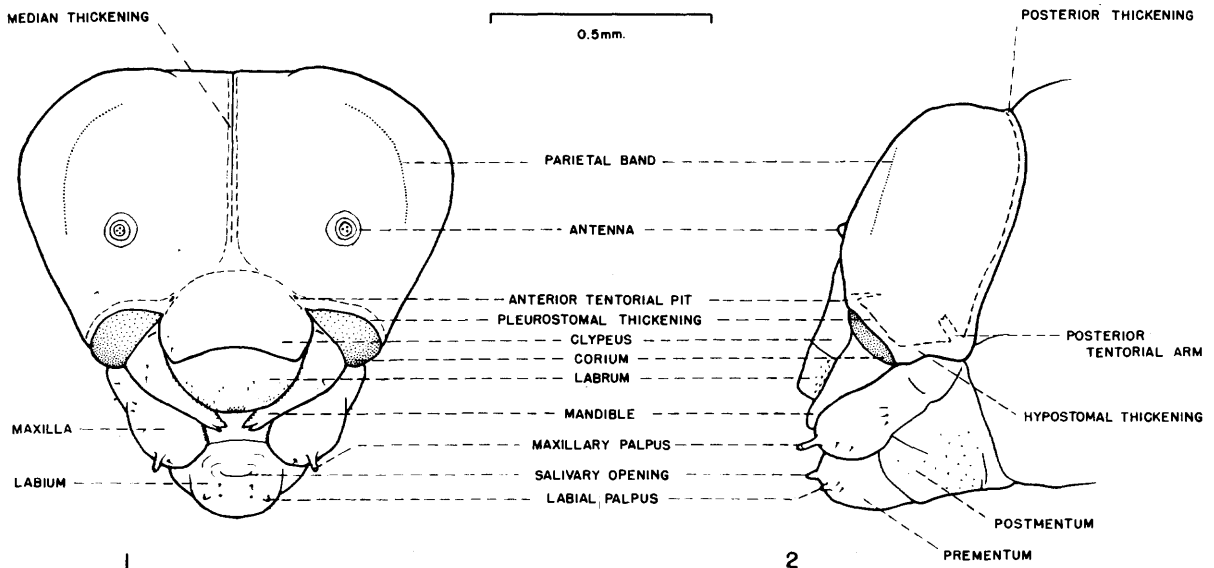
collected at Alpine Lake, Marin County, California, on August 12, 1955, by C. D. MacNeill and J. G. Rozen. It has been deposited in the collection of the California Insect Survey, University of California, Berkeley.

The study techniques and morphological terminology are those of Michener (1953). However, because the first instar is very delicate, both the head capsule and body were cleared in warm lactophenol and then examined in a droplet of the same agent. For purposes of comparison, the form of Michener's description has been adopted with only slight change.

MATURE LARVA

The following description refers to both predefecating and postdefecating (hibernating) individuals. As has been previously discussed

thickening moderately well developed; hypostomal thickening more pronounced; epistomal thickening and corresponding suture moderately well developed laterad of anterior tentorial pits; median portion of thickening scarcely visible, being broad, thin, and ill-defined; corresponding suture obliterated. Longitudinal median thickening of head capsule pronounced, accompanied by more or less evident suture (epicranial); thickening becoming weaker toward epistomal thickening and broadly joining it; cleavage lines absent. Parietal band weak. Antennae feebly convex, each bearing distinct papilla which is at most as long as its diameter; papilla usually with three sensilla though occasionally with four. Labral tubercles weak, scarcely noticeable; labrum feebly emarginate apically (not noticeable on drawing), spiculate laterally, strongly so on epipharyngeal



Exomalopsis chionura Cockerell

FIG. 1, frontal view of head of mature larva. FIG. 2, same, lateral view.

(Rozen, 1954: 203), the two forms are markedly dissimilar in appearance, although both represent the same instar. The differences pertain solely to the shape of the post-cephalic region of the body and are not associated with such structures as the cranium, mouthparts, or spiracles. As explained elsewhere (Rozen and MacNeill, manuscript) there is also a pronounced difference between the two forms with respect to behavior and physiology.

Description of Mature Larva

(Figs. 1-5, 8-10)

Head: Capsule and mouthparts with few widely scattered, minute setae. Posterior tentorial arms strong, arising from marginal thickening; anterior arms weaker. Marginal and pleurostomal

surface. Mandibles seen from above and below broad basally, narrow apically; apex bidentate, with upper tooth somewhat exceeding lower; upper margin broadly serrate apically, lower broadly bidentate (or perhaps tridentate on some); mandibular cusp, as seen from inside, oblique, forming pronounced inner subapical concavity; cusp strongly dentate; teeth also present on inner upper surface of mandible where they appear elongate, hair-like (lack of alveoli and the presence of a broad base, as revealed by microscopic examination under oil immersion, clearly demonstrate that these are not setae). Maxillae with palpi slightly subapical; maxillary apices bent mesad though not nearly as much as in the emphorines and anthophorines (this character is more obvious when seen in different view from