THE LARVAL STAGES AND THE BIOLOGY OF THE MOSQUITO, ORTHOPODOMYIA ALBA BAKER (DIPTERA: CULICIDÆ)[1]

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Introduction

The writers have been studying tree hole breeding mosquitoes for the past several years, and during this period hundreds of collections have been made from many localities in Texas. *Orthopodomyia alba* Baker is one of the most interesting of these tree hole species, since until recently it was considered to be rare. This species was described in 1936 from Ithaca, New York (Baker 1936) and since that time it has been collected at only a relatively few localities. In most areas, larval collections have usually yielded only a few specimens, but Ross (1947) reported a colony in Illinois that had persisted for several years. This same year, many larvæ were discovered near Austin, Texas (Breland, 1947a; 1947b), and since then numerous specimens have been collected here and at other localities of the state.

Jenkins and Carpenter (1946) have shown that no satisfactory features are known by which the adults of *O. alba* and *Orthopodomyia signifera* (Coq.) can be distinguished. Consequently, positive identification of *O. alba* should at present be based upon the larvæ which are distinct. This species, based upon larval determinations, is now known to occur in one or more localities in Alabama, Illinois, Kentucky, Louisiana, Mississippi, Missouri, New York, North Carolina and Texas (Jenkins and Carpenter 1946; Breland 1947a). The writers have collected *O. alba* near the following localities in Texas: Austin, Bartlett, Helotes, Junction, Marble Falls, San Antonio and Sheffield. All work to date indicates that although the species may be relatively common within a limited area, it is very sparsely distributed over its

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range. Even in areas that have been searched intensively over a period of several years, larvæ have been recovered from only a small percentage of the tree holes investigated. Once a colony has become established, however, one may often recover larvæ repeatedly over a period of several years.

THE PRESENT STUDY

The objectives of the present study were to observe the reactions of *Orthopodomyia alba* under field and laboratory conditions, and to find features by which the various larval instars could be distinguished. During the course of the investigation hundreds of collections were made from tree holes. Many tree holes have been checked or collected from once to several times per week during all months of the year. Collections were made from tree holes containing rain water when these could be found, but during long dry spells, tree holes were filled with water from streams. The use of this type of water had no apparent effect upon the breeding habits of the mosquitoes, and this method was used to refill cavities after each collection, if all the water was removed.

Attempts to obtain egg deposition in the laboratory have so far been unsuccessful. This was tried on severol occasions at laboratory temperatures which averaged near 90° F., and in a constant temperature room, used for another purpose, which was kept at approximately 70° F. Humidity was maintained by the use of moist towels over the breeding cages, and tree hole water was supplied in various types of containers. It seems probable that the temperatures involved were either too high or too low, although factors other than temperatures may have been involved.

Larvæ which were used for the instar study were collected in various stages of development, and placed in individual staining dishes in the laboratory. Tree hole water was used which was diluted somewhat with pond water if it was too dark for good observation. It is well known that first instar mosquito larvæ can be distinguished by the presence of egg bursters on the head (EGB, Plate I, fig. 1). Many first instar larvæ of *O. alba* were recovered after it was discovered that this stage often occurs a few days after stream water was added to a dry cavity, or to one

from which all the old water had been removed. The instars after the first were determined by the recovery of larval skins after ecdysis in the individual staining dishes.

Larval studies were made from living and freshly killed larvæ, larval skins, preserved and permanently mounted specimens. Examination of freshly killed larvæ of the first instar especially is important, since this stage often becomes distorted if placed on permanent slides. The conclusions to be presented relative to the larval instars are based upon an examination of more than 300 specimens. Most of the first three instars were collected in the region of Austin, Texas although a few specimens were procured from other localities. Many fourth instars were collected from other areas in Texas as noted above.

GENERAL BIOLOGY

Jenkins and Carpenter (1946) state that little is known of the biology of *O. alba*. The adults observed in the present study were not active in breeding cages, but tended to seek dark corners of the cages and to remain there even though attempts were made to dislodge them. On one occasion, several adults accidently escaped from a cage and instead of flying away, most of them alighted upon one of the writers and attempted to hide in folds of his clothing. The adults have been observed in the breeding cages apparently feeding on a sugar solution, but none attempted to bite the arms of the writers, although they were given optunity on numerous occasions.

No eggs of *O. alba* were recovered and consequently nothing is known regarding the egg deposition of this species. *O. signifera* has been reported to deposit its eggs in a cavity just above the water line (Howard, Dyar and Knab Vol. 4, 1917) and also upon the water surface itself (Horsfall 1937). The habits of *O. alba* may be similar.

In this area, most of the cavities in which tree hole mosquitoes normally breed may be dry for several weeks or months during the summer. Debris from these dry cavities was collected and flooded with water in an effort to determine whether or not eggs of *O. alba* were present under these conditions. More than one hundred such samples from several localities have been so

treated. To date no *O. alba* have been found, although numerous larvæ of *Aedes triseriatus* Say and *Aedes zoosophus* D. & K. (formerly called *A. alleni* Turner) have been recovered in many samples. In many instances dry tree holes from which material had been taken were filled with stream water and then checked at intervals for young larvæ. In no case were *O. alba* larvæ found less than a week after the cavities had been filled. However, a week to ten days after such procedure, first instar larvæ of *O. alba* have been recovered in several instances. Such observations indicate that *O. alba* survives periods of drouth as adults.

BREEDING HABITAT AND ASSOCIATED SPECIES: *Orthopodomyia alba* has been reported from several species of trees including "elm" (Baker, 1936), silver maple, pecan and sweet gum (Jenkins and Carpenter 1946). The writers collected the species from elm (*Ulmus crassifolia* Nutt.) and Texas oak (*Quercus texana* Buckl.), but never from artificial containers as has been reported previously (Jenkins and Carpenter 1946).

There is some evidence that *O. alba* in this area is more limited in the type of cavity in which it will normally breed than are some of the other species of tree hole mosquitoes. For several months, records were kept of the type of cavity from which this species was recovered, and there seems to be a definite tendency for the mosquito to deposit its eggs in holes with a relatively small external opening. Out of twenty-seven cavities in which larvæ occurred, twenty-one had an opening of 3 inches or less in diameter and many of these were barely large enough to insert the one-half inch rubber tube used in collecting the mosquitoes. The other openings were four to five inches in diameter with only a single one more than five inches wide. It may be that the size or nature of the available cavities is an important factor that limits the distribution of *O. alba* in a given area.

So far as could be determined there has never previously been a large series of pH readings made upon the water occurring in tree holes; although Seaman (1945) reports that the average pH of a few samples from sycamore trees in which he found *Aedes varipalpus* Coquillett in California was 8.04. A large series of samples in which *O. alba* was found and tested by the writers varied from 7.6 to 8.4.

Before 1947 *O. alba* had always been reported as being associated with *O. signifera* (Jenkins and Carpenter 1946). In 1947 one of the writers (Breland 1947a) noted some collections in the absence of *O. signifera.* Since that time the species has often been found in the absence of *O. signifera,* and in some cases *O. alba* has been the only species present at the time of the collection. At one time or another however, *O. alba* has been found associated with several other tree hole breeding species. These include *Orthopodomyia signifera, Aedes triseriatus, Aedes zoosophus* and *Toxorhynchites rutilis septentrionalis* (Dyar and Knab). This last mosquito, formerly designated as *Megarhinus septentrionalis* D & K, has recently had its name changed twice (Stone 1948; Jenkins 1949).

In view of the fact that the amount of water found in cavities is often limited, attempts were made to rear larvæ in other types of water. A sufficient number of experiments were not performed to allow positive conclusions, but indications are that tree hole water alone or pond water to which a few drops of tree hole water is added at intervals are the best rearing media. High mortality resulted from using only pond water plus small amounts of brewer's yeast.

RESISTANCE TO FREEZING AND DRYING: It is generally accepted that *O. alba* passes the winter as larvæ over a large part of its range (Baker 1936; Matheson 1944). There is considerable evidence, however, that in this area the mosquitoes survive the winter as adults. Although numerous collections have been made during the winter months, no larvæ have been found between November and April. Dry material collected during this period and flooded with water also failed to yield larvæ of this species.

Over fifty larvæ including second, third and fourth instars were used to determine the resistance of the larvæ to low temperatures. The specimens were frozen in a refrigerator in tree hole water and before thawing, were allowed to remain frozen for periods of from two hours to two days. No larvæ survived this treatment. These observations indicate that the larvæ here are physiologically different from those in the northern parts of their range, since Baker (1936) found that the second and third instar larvæ of *O. alba* survived after having been frozen in solid ice for a week.

Fourth instar larvæ were found to be quite resistant to low temperatures as long as the insects were not completely frozen. These included larvæ inside cubes of ice, the centers of which were still liquid, and some that were exposed to alternate periods of cold and laboratory temperatures. Low temperatures ranged from 32.5° F to 36° F. One group was kept in a refrigerator for two weeks at temperatures of from 32 to 33° F. When removed the insects pupated shortly and emerged with a mortality of only some 10 per cent.

It has been noted previously on the basis of limited observations (Breland 1947a) that the larvæ and pupæ of *O. alba* can survive for a time in the absence of free water. Large numbers of larvæ and pupæ were, on several occasions, placed in staining dishes on moist blotting paper upon which there was no free water. A drop or so of water was added at intervals to keep the paper moist. Larvæ died in less than forty-eight hours if the tops were left off the dishes even though the blotting paper was kept moist. When the dishes were kept covered, larvæ survived for as much as ninety-six hours. No larvæ were observed to shed their skins during the treatment. A single specimen pupated but died shortly thereafter. It seems probable that the relatively short survival time of the larvæ in the uncovered dishes was due to the drying of the integument on one side.

Pupæ demonstrated a surprising ability to attain the adult stage under these conditions. Percentage of emergence of a series of test groups varied from fifty to one hundred per cent.

LARVAL INSTARS

The descriptions of each larval instar, as previously indicated, are based upon a study of large numbers of permanently mounted, freshly killed larvæ and larval skins. There is considerable variation among the various instars in the number of branches of some of the hairs and in the number and arrangement of certain other structures. Often the number of structures or hair branches will be different on opposite sides of the body. However, those features present in the first instar are easily correlated with comparable structures in later instars. Most structures are labeled in the drawings of the first instar (Plate IX)

while those that are added in later instars are labeled on the drawing of the first stage in which they appear. The comb scales increase in size in older stages although the drawings of these structures of different instars are not drawn to scale. Many of the head hairs increase in number of branches between the first and fourth instars as indicated in the illustrations. However, these changes are not sufficiently abrupt to be of diagnostic value in distinguishing between the various stages.

FIRST INSTAR LARVA: (Plate IX), Fourth instar larvæ of *O. alba* are well known for their hairy appearance and even in the first instar this condition is somewhat anticipated. Several hairs on the head and posterior abdominal segments are two to three branched and some of them have barbs distinguishable under high power (e.g. 880 ×). In all other species of first instar larvæ previously examined by the writers these hairs have been single. From dorsal view the egg burster appears as a transverse heavily chitinized bar or ridge slightly wider in the center (EGB, Fig. 1). It appears to lie in a partial depression the bounds of which are more evident posteriorly. From a lateral view it may be seen that the structure comes to a point near the center. No ventral brush occurs and the dorsal brush consists of two long hairs on each side. There is a single irregular row of comb scales (CS, Fig. 3). The individual comb scale is elongate with a series of teeth distally (Fig. 2). The dorsal plate or saddle (DP, Fig. 3) is represented by a small chitinized cap, while the siphon is chitinized distally. In general, the specimens examined conformed with the key features given by Dodge (1946) for the identification of first instar larvæ to genus.

Since no eggs were discovered, the exact length of the first instar could not be determined. However, on several occasions, eggs were apparently present in water when collected since first instar larvæ appeared later. The first instar stage of larvæ obtained in this way varied from two to four days.

SECOND INSTAR LARVA: (Plate X). A ventral brush appears in this stage with posterior hairs of two or three branches, (VB, Fig. 5), while the egg burster is absent. These features alone are sufficient to distinguish the second from the first instar. The dorsal brush now consists of a single lower caudal hair on each

side (LC, Fig. 5) and an upper caudal hair on each side of four or five branches (UC, Fig. 5). The chitinized portion of the siphon has increased in extent and the subventral tuft is attached either just within the edge of the chitin or slightly proximal to it. The comb scales occur as overlapping rows; the individual scales vary in size and shape and are somewhat similar to those in later instars (Plate XIII, figs. 10 and 13).

Several hairs on the head have additional branches and most of them are barbed.

A few second instar larvæ stayed in this instar as long as five and one-half days, but most specimens under observation moulted within approximately three days.

THIRD INSTAR LARVA: (Plate XI). The point of attachment of the subventral tuft is perhaps the easiest feature by which the third instar may be distinguished from the second. In the second instar this hair is attached to the distal edge of the chitinized area of the siphon or just distal to it (Plate X, Fig. 5). The siphon of the third instar is almost as completely chitinized as in the fourth stage and the subventral tuft is attached well within this chitinized portion (Plate XI, fig. 7). Additional branches have been added in the third stage to the upper caudal hair and to the individual hairs of the ventral brush. The former has seven to ten branches while the posterior ventral brush hairs have four to six. Representative comb scales are illustrated in Plate XIII, figures 11 and 14.

The length of the third instar varied from two to seven days for most specimens but a few remained in this stage for two to three weeks. Mortality was high in those larvæ that were third instar for more than seven days and it is thus believed that a period of more than a week for this stage is abnormal.

FOURTH INSTAR LARVA: (Plate XII). A chitinized lateral plate near the base of the anal segment appears for the first time in the fourth instar (LP, Plate XII, fig. 9). The presence of this plate alone is thus assurance that the stage is fourth instar. Each upper caudal hair is ten to fourteen-branched while the hairs of the posterior ventral brush have seven to twelve branches.

The dorsal plate or saddle of the larvæ of *O. alba* has previously been found to vary in specimens collected in the vicinity

of Austin, Texas (Breland 1947b). In some of these larvæ the plate completely surrounded the anal segment while in others it was incomplete ventrally. This same situation has been found in other localities.

The lateral hair of the anal segment is usually attached just posterior to the dorsal plate or saddle, but is attached to the posterior edge of the plate in a few specimens. Comparatively speaking it is a more delicate hair, has fewer branches in general and is less conspicuously barbed than in younger instars. This is a rather unusual situation since other hairs noted by the writers become more conspicuous and/or add branches in older instars if they change to a noticeable degree.

The sutural and supraorbital hairs (SU and SO, Plate IX, fig. 1), are sparsely barbed in an occasional specimen, visible under high power.

The length of the fourth instar has been found to vary from four to twenty-three days. More than seventy per cent of the larvæ under observation pupated within seven days to two weeks after the fourth instar was attained. As was true for third instar larvæ, mortality was much greater in those larvæ with the longest developmental period. All that exceeded three weeks in this stage died without pupating or during the process. Pupation was a very critical process in the life cycle of the larvæ under observation and more than ninety per cent of fourth instar larval loss occurred at this time.

STATUS OF *O. Alba*

There has been considerable discussion relative to the status of *Orthopodomyia alba*. To date no constant differences have been noted between the adults and the suggestion has been made that *O. alba* may be simply a genetic variant of *O. signifera* (Jenkins and Carpenter 1946). This suggestion was apparently based principally upon the fact that up until that time *O. alba* had always been collected in association with *O. signifera*.

The writers however, believe that *O. alba* should continue to be given full specific rank. This conclusion is based upon several facts. First, larvæ of *O. alba* have often been collected in numbers not associated with *O. signifera*. Second, no larvæ inter-

mediate between the two types have ever been seen out of hundreds of both species collected from several localities. All have been easy to distinguish, indicating that interbreeding in nature does not occur. Third, the larvæ of the two species may be distinguished beginning with the first instar. That the first instars are distinct has been indicated by Dodge (1946); the present study shows that second and third stages are also different. Fourth, *O. alba* seems to be more limited than *O. signifera* in its choice of breeding habitat suggesting different physiological reactions in the two groups. *O. signifera* may be found in almost any type of tree cavity that contains water, while *O. alba* seems to prefer a cavity with a small external opening.

Incomplete studies by the writers, of adults reared from known larvæ indicate that slight differences between the adults of *O. alba* and *O. signifera* do exist. The results of this work will be published later. However, even though constant adult differences are not found, this should not be sufficient reason to reduce *O. alba* to subspecific rank. The inheritance of an organism expressed in its immature stages should be given as much weight as adult features. This has long been recognized by many workers in mosquito taxonomy and today there are many species that can be more easily distinguished in the larval than in the adult stage. In addition there are others which are distinct as larvæ, but which to date cannot be distinguished as adult females (e.g. *Aedes tormentor* D & K and *Aedes atlanticus* D & K).

SUMMARY

1. The four larval instars of *Orthopodomyia alba* Baker have been described. Eggs have not been discovered.
2. Distinctions between the various stages were determined by rearing larvæ individually and by a study of freshly killed larvæ, larval skins and permanently mounted specimens.
3. The known biology of the species has been summarized.
4. The writers believe that *O. alba* should continue to be regarded as a distinct species rather than as a subspecies or variety. Reasons for this conclusion include the absence of intergrades in larvæ between this species and *O. signifera;* the fact that *O. alba* has often been collected alone; suggested differences

in selection of a breeding habitat and possible differences in the adults of the two groups.

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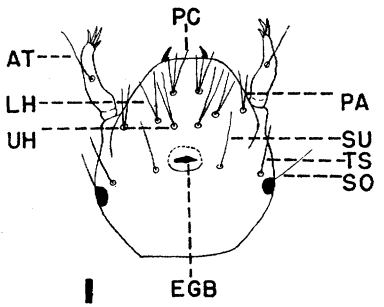
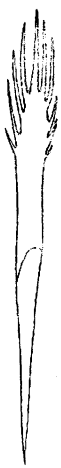
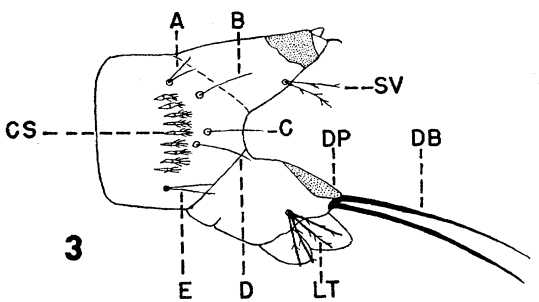




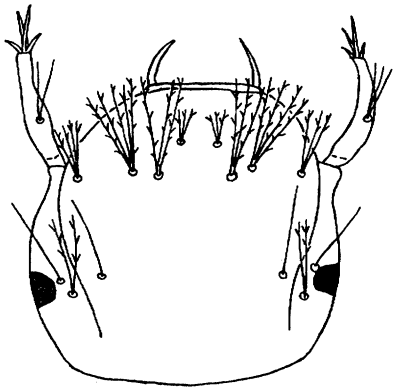


EXPLANATION OF PLATE IX

Orthopodomyia alba Baker, first instar larva. Fig. 1, Head. Fig. 2, Representative comb scale. Fig. 3, Posterior abdominal segments.

Abbreviations

A—hair A (Siphonal hair).
At—antennal hair.
B—hair B.
C—hair C (subsiphonal hair).
CS—comb scales.
D—hair D.
DB—dorsal brush.
DP—dorsal plate or saddle.
E—hair E (anal hair).
EGB—egg burster.
LH—lower head hair.
LT—lateral hair, anal segment.
PA—preantennal hair.
PC—postclypeal hair.
SO—supraorbital hair.
SU—sutural hair.
SV—Subventral tuft (siphonal hair).
TS—trans-sutural hair.
UH—upper head hair.



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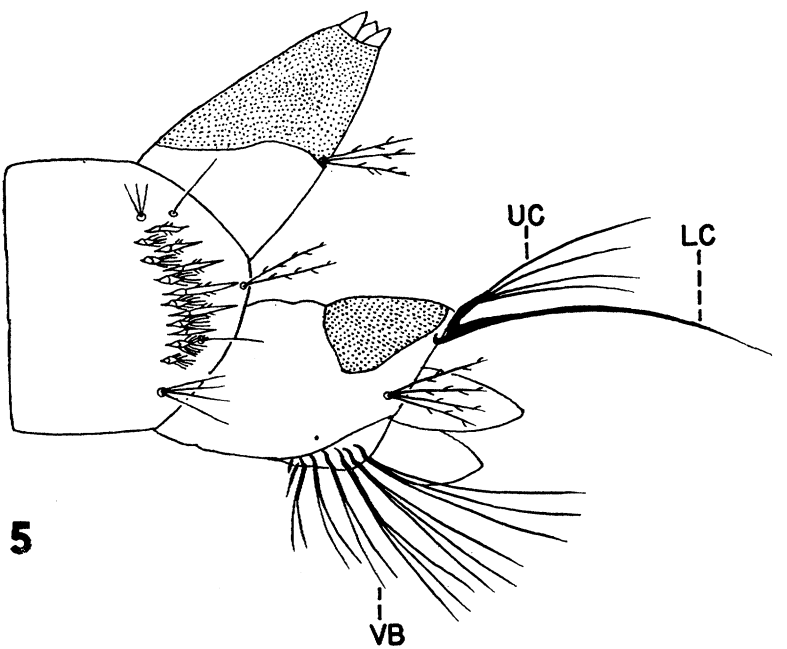

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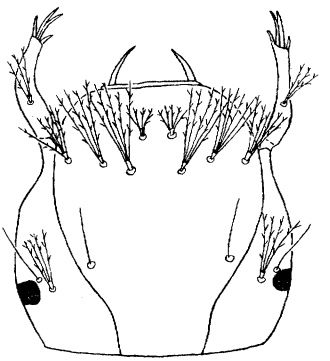
EXPLANATION OF PLATE X

Orthopodomyia alba Baker, second instar larva. Fig. 4. Head. Fig. 5. Posterior abdominal segments.

Abbreviations

LC—lower caudal hair of dorsal brush.
UC—upper caudal hair of dorsal brush.
VB—ventral brush.



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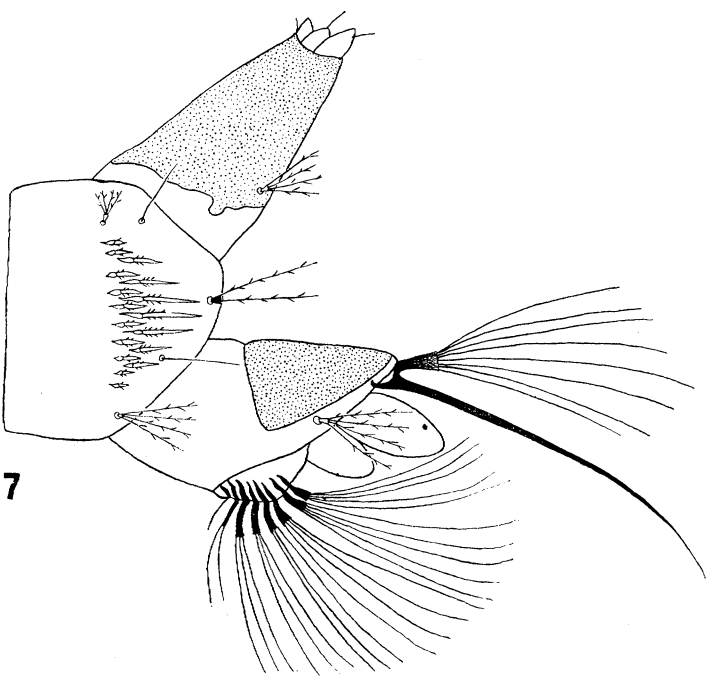

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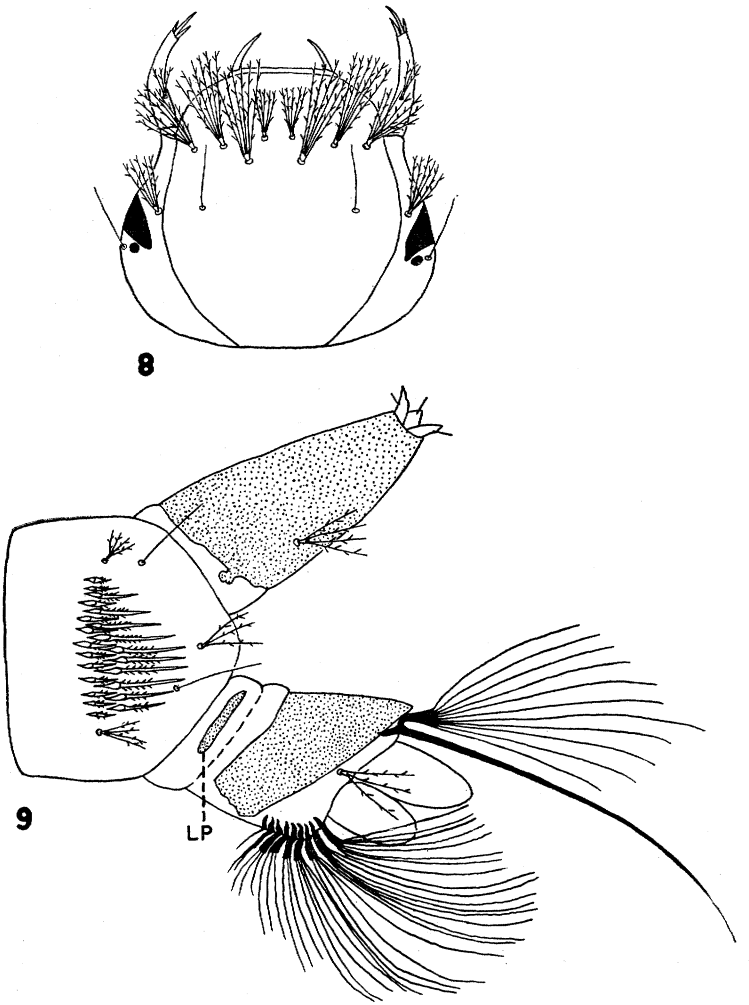
EXPLANATION OF PLATE XI

Orthopodomyia alba Baker, third instar larva. Fig. 6. Head. Fig. 7. Posterior abdominal segments.

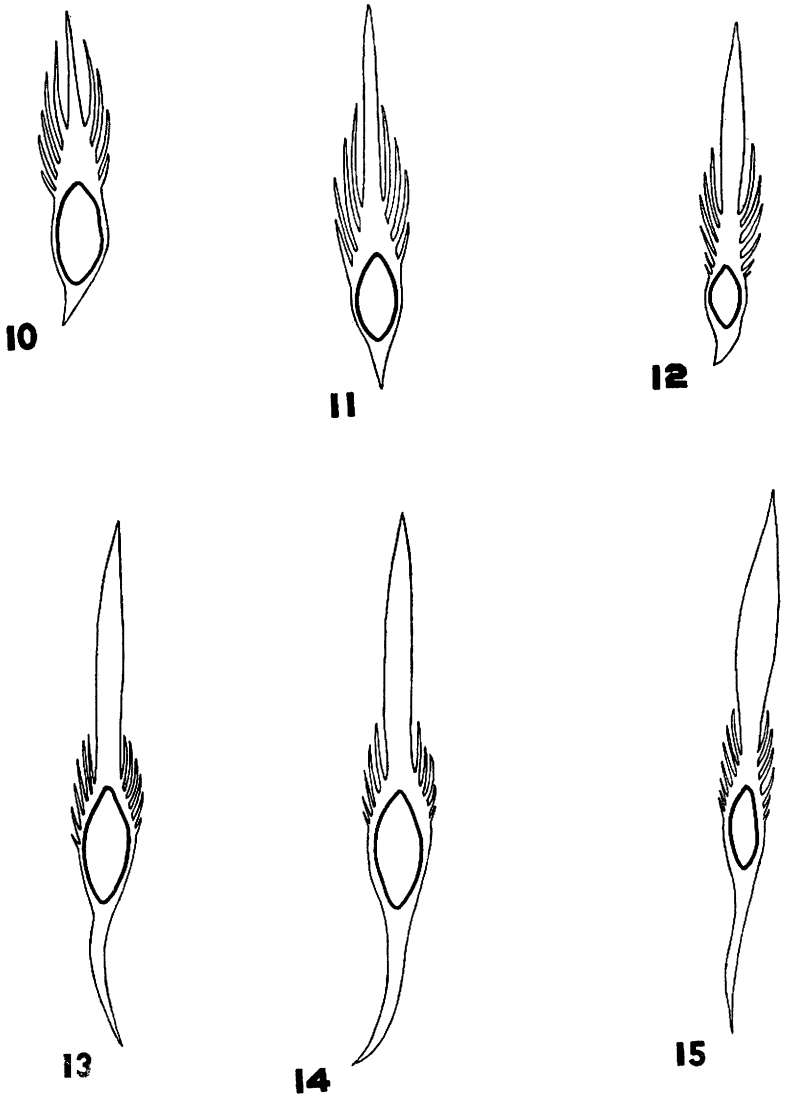


EXPLANATION OF PLATE XII

Orthopodomyia alba Baker, fourth instar larva. Fig. 8. Head. Fig. 9. Posterior abdominal segments.

Abbreviations

LP—lateral plate of the anal segment.



EXPLANATION OF PLATE XIII

Representative comb scales of *Orthopodomyia alba* Baker. Top row. Comb scales from anterior rows. Bottom row. Comb scales from posterior rows. Drawings not made to scale.

Fig. 10. Comb scale from anterior row, second instar.
Fig. 11. Comb scale from anterior row, third instar.
Fig. 12. Comb scale from anterior row, fourth instar.
Fig. 13. Comb scale from posterior row, second instar.
Fig. 14. Comb scale from posterior row, third instar.
Fig. 15. Comb scale from posterior row, fourth instar.