

by Lloyd E. Rozeboom

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GORGAS MEMORIAL LABORATORY

1. Introduction

Although mosquitoes have annoyed man and other animals throughout the ages, in former years only a few people cared enough about these insects to study them, and these individuals, being entomologists, were abnormal anyway. But in 1877 Sir Patrick Manson showed that filaria worms developed within the bodies of mosquitoes; in 1898 Sir Ronald Ross demonstrated that malaria was transmitted by these insects, and in 1900 Walter Reed and his co-workers proved that the devastating epidemics of yellow fever in tropical cities were caused by Aedes aegypti, which was able to carry the virus of the disease from the sick to the healthy. Nevertheless, immediately after these startling discoveries, the majority of those interested in tropical diseases, thought "Well, what of it?" Mosquitoes were going to keep on biting people whether they carried diseases or not. Fortunately, several far-sighted men thought differently. The practically-minded Ross immediately realized that malaria could be controlled by attacking the mosquitoes, and in 1901 he performed an experiment in Sierre Leone to show that mosquito control was possible. In the same year William C. Gorgas abolished yellow fever from Havana. In 1902 Sir Malcolm Watson began his mosquito reduction campaigns in the Federated Malay States. The most striking demonstration of the practicability of anti-mosquito campaigns was the work of Gorgas in Panama, which made this region of the tropics safe for gringos and made possible the building of the Panama Canal.

These results impressed upon the minds of the students of tropical medicine the importance of mosquitoes, and it was soon realized that very little was known about the insects. When it was found that only certain kinds of mosquitoes transmitted tropical diseases, and that it was necessary to know where and how

they lived before satisfactory control measures could be applied, intensive research on their systematics and bionomics was carried out by both entomologists and medical men throughout the world. The first monograph on this family of insects was published by F. V. Theobald, of the British Museum, during the years 1901-1910. In the New World, L. O. Howard, H. G. Dyar and F. Knab were some of our pioneer students, and in 1912 they published a monograph called "The Mosquitoes of North and Central America and the West Indies." Dyar improved upon this monograph when, in 1928, he published "The Mosquitoes of the Americas." The last monograph on the Culicidae was published in 1932 by F. W. Edwards, of the British Museum, and it is his classification that we shall follow. We are indebted to Dr. D. P. Curry for straightening out the systematics of the Anopheles mosquitoes in Panama.

## II. The Position of Mosquitoes in the Animal Kingdom.

The Animal Kingdom is divided, by most biologists, into twelve main groups called Phyla. The phyla are composed of Classes, the classes of Orders, the orders of Families, the families of Genera, the genera of species. However, there are often subdivisions of these groups; this is especially true in the mosquitoes.

Taking Culex pipiens as an example, in order to place it properly in the Animal Kingdom, we would proceed as follows:

Animal Kingdom

Phylum Arthropoda

Class Insecta

Order Diptera

Family Culicidae

Subfamily Culicinae

Tribe Culicini

Genus Culex

Subgenus Culex

Species pibiens

III. The Classification of the Family Culicidae.

As F. W. Edwards is recognized as the greatest authority on mosquitoes at present, we shall follow his classification.

The family Culicidae is divided into three subfamilies, but we need consider only one of them, the subfamily Culicinae. These are the true mosquitoes; the wings have a particular kind of venation, which we shall study later, the veins of the wings have scales attached to them, and the mouth-parts are long and used for biting. These three characters distinguish the mosquitoes from all other insects.

The subfamily Culicinae contains three tribes: the Culicini, Megarhini, and the Anophelini. The tribe Megarhinini is a small one; these mosquitoes are not numerous. They are very large in size, and the larvae are usually found in tree holes, and feed on other mosquito larvae. It is necessary, for our purposes, to differentiate only between the two main genera of the tribes Culicini and Anophelini: the genus Culex and the genus Anopheles, and the characters for doing so are summarized in the following table:

<u>Adults</u>	<u>Anopheles</u>	<u>Culex</u>
1. Resting attitude	at angle to surface	parallel to surface
2. Wings	spotted	not spotted
3. Palpi		
female	long, slender	very short
male	long, clubbed	long, slender
4. Scutellum	evenly rounded	trilobed

<u>Eggs</u>	<u>Anopheles</u>	<u>Culex</u>
1. Laid	singly	in rafts
2. Shape	boat-shaped	elongate, oval, or conical
3. Lateral air-chambers	present	not present

### Larvae

1. Position at surface	horizontal	oblique or vertical
2. Air tube	absent	present
3. Shape of head	ovoid	rounded or squarish
4. Palmate hairs	usually present	never present

### Pupae

1. Position in lateral view	bent forward	more upright
2. Outline in dorsal view	elliptical	ovoid
3. Breathing trumpets	short, scoop-shaped split nearly to base	slender, tubular, not split to base
4. Paddles at tip of abdomen	with a small hair on blade in addition to terminal hair	with terminal hair only

There are some exceptions to these rules: Some Anopheles have wings that are entirely dark, Chagasia has a trilobed acutellum, the Megarhinini have a rounded scutellum, and there are other exceptions that need not be considered here because they are not found in the mosquitoes with which we shall deal.

In Panama we have 15 different species of Anopheles, and according to Edwards they are classified as follows:

Family Culicidae

Subfamily Culicinae

Tribe Anophelini

Genus Chagasia

Chagasia bathanus Dyar

Genus Anopheles.

Subgenus Stethomyia

Anopheles kompi Edwards

Subgenus Anophelos

Anopheles group

Anopheles eiseni Coquillett

( (Patagiamyia group) Edwards does not recognize the Patagiamyia group; he places these mosquitoes in the Anopheles group.)

Anopheles pseudopunctipennis Theobald

Arribalzagia group

Anopheles punctimacula Dyar & Knab

Anopheles apicimacula D. & K.

A. neomaculipalpus Curry

Subgenus Nyssorhynchus

Nyssorhynchus group

Tarsimaculatus series

A. albimanus Wiedemann

A. bachmanni Petrocchi

A. strodei Root

A. oswaldoi Peryassu

A. tarsimaculatus Goeldi

Argyritarsis series

A. argyritarsis Rob.-Des.

A. albitarsis Lynch-Arribalzaga

A. cruzii D. & K. (A. bellator D. & K.)

IV Identification of the Adult Anophelines of Panama.

A. Structure of the Adult

Before we can identify mosquitoes, it will be necessary to review briefly the structure of these insects. The body is divided into three regions: the head, Thorax, and abdomen. The large compound eyes cover most of the surface of the head. The piercing mouth-parts are composed of several slender stylets; they are sheathed in a gutter-shaped structure called the labium. Just above the mouth-parts is a pair of palpi, consisting of three to five joints, depending upon the species and sex of the mosquito. On the anterior, upper side of the head, between the eyes, are the sensory antennae, composed of fifteen joints, each joint bearing a whorl of hairs. In the males the antennae are very bushy.

Mosquitoes, like all other insects, have an exoskeleton; that is, the hard structures supporting the muscles are on the outside of the body instead of being internal, such as bones of higher animals. The exoskeleton consists of a number of chitinous plates, called sclerites. A sclerite on the dorsal surface of the body is called a tergita; one on the side of the body, pleura; and one on the ventral side, sternum.

The dorsal surface of the thorax of the mosquitoes is covered by a single large rounded plate, the mesonotum. Immediately behind the mesonotum is a narrow transverse strip of chitin which bears the name scutellum. In Anopheles, this scutellum is evenly rounded, while in Culicine mosquitoes it has three lobes.

To the thorax are attached three pairs of legs, and a pair of wings. The legs are made up of five joints: the nearest to the body is the coxa; then comes the trochanter. Both of these are small, and of no importance in classification.

Then comes a long joint, the femur, and other long joint, the tibia. Last of all is the tarsus, which is subdivided into five joints. The markings on these tarsal joints are very important in identifying mosquitoes.

The wings of mosquitoes are supported by a number of veins, and the arrangement of these veins is very characteristic of this family. The entire margin of the wing is surrounded by the costal vein, although when we speak of the costa, we usually mean the upper, or anterior edge of the wing. Just beneath the costal vein is a short vein, called the subcostal vein. Then there is a long vein, extending to the tip of the wing; this is vein #1. The next long vein, #2, arises from near the base of #1; it also extends to the tip of the wing, and is split into two branches apically. Vein #3 is short, single, and arises about at the middle of ven #2. Vein #4 is long, extending all the way from the base of the wing to the margin, and is also split apically into two branches. Vein #5 extends from the base of the wing to the margin, and is branched near its middle. The last vein, #6, is single. All of these veins have scales attached to them.

The abdomen has 10 segments, but the last two are modified into anal and genital structures, so that only eight can easily be seen. Each segment has a dorsal plate, the tergite, and a ventral plate, the sternite. Groups of scales may be present on the sternites or tergites, which sometimes aid in identification.

### B. Characters Used for Identification.

#### 1. Palpi

The joints of the palpi often have spots or rings of white scales, the size of which and their location are sometimes of value in recognizing species.

#### 2. Legs

White bands or spots are often found on the joints of the legs. In the

subgenus *Stethomyia* and the *Anopheles* group, no white markings are present on the legs, although *A. eiseni* has a broad white band at the tip of the tibia. *Chagasia* has broad white bands on the tarsi. The legs of the *Arribalzagia* mosquitoes are beautifully mottled with yellow-white spots, while the members of the *Nyssorhynchus* group are called the "white hind-footed" mosquitoes because part of the second, and all of the third, fourth, and fifth tarsal joints are entirely white, except for a black ring that is sometimes present on the fifth hind tarsal joint. Those having this ring belong to the *tarsimaculatus* series; those without it belong to the *argyritarsis* series. Sometimes there are white rings on the tarsi of the middle and front legs as well. White rings on the middle tarsi help to distinguish *A. albitarsis* from *A. argyritarsis*, as the latter does not have such rings on this location. *A. cruzii* has broad white markings on the tarsi of the front and middle legs as well as on the hind tarsi.

### 3. Wings.

The more primitive Anophelines have wings with scales entirely dark. *Anopheles kompi* has no light scales on the wings; this is also true of *A. eiseni*, except for a group of yellowish-white scales at the tip of the wing, and for a few light scales on the first vein about one-third of the distance from the base of the wing. *Chagasia bathanus* has some light scales on the wings, but they are mixed with the black ones, and do not form definite light spots.

In the rest of the mosquitoes we wish to study, we find that the white scales have become grouped together, so that there are black and white spots on the wings. The second, fourth and sixth veins of *A. cruzii* are entirely dark-scaled, although there are white spots on the rest of the wing, particularly along the costal border, where four evenly-spaced white spots are usually found, involving both the costa and the first vein, in addition to a very small spot near the base of the wing.

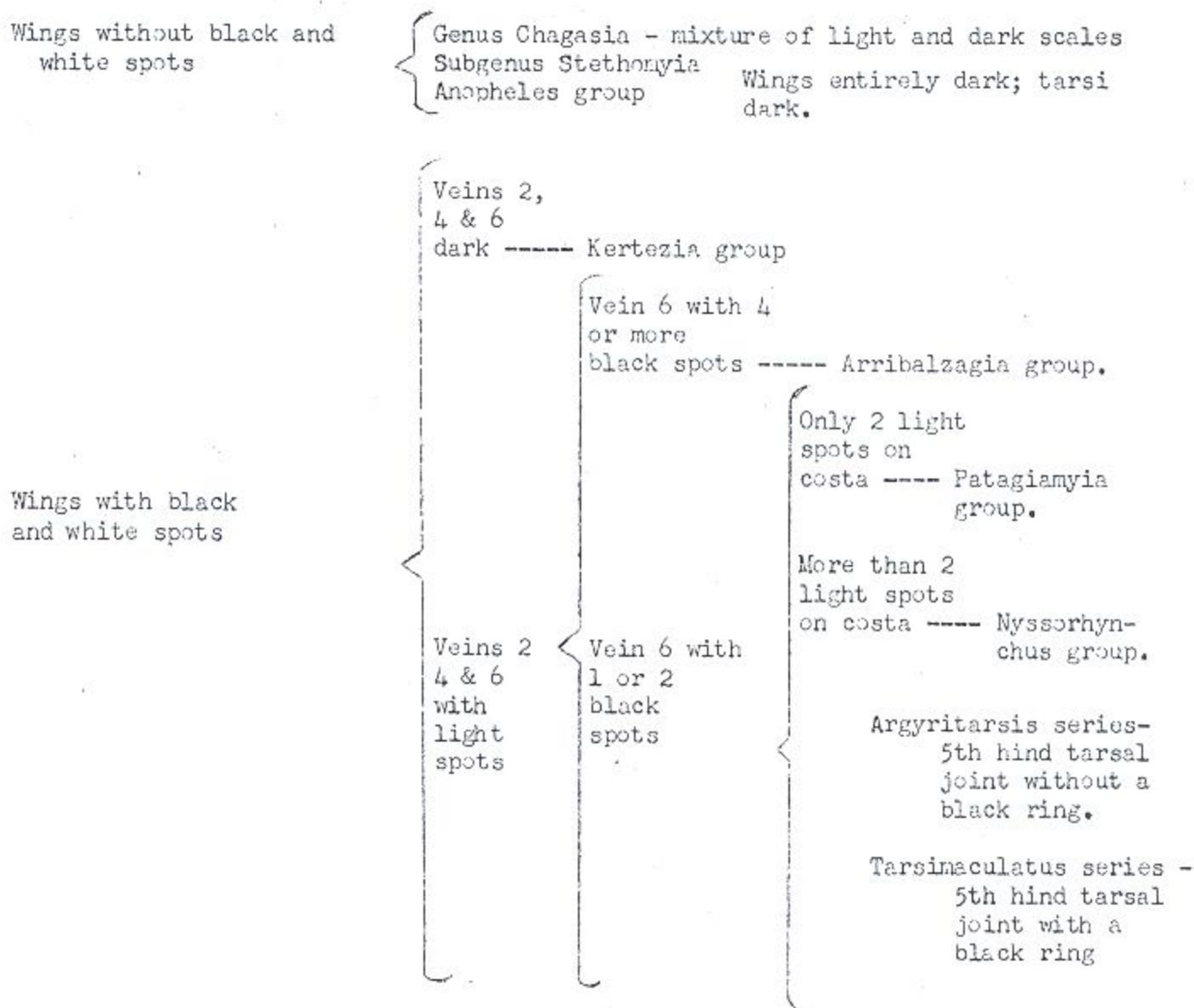


This now leaves the Patagiamyia and the Arribalzagia groups of the subgenus Anopheles, and the Nyssorhynchus group of the subgenus Nyssorhynchus. In the Patagiamyia group there are just two light spots on the costal border. A.pseudopunctipennis is the only member of this group in Panama. The Arribalzagia group and the Nyssorhynchus group both have more than two light spots along the costa; these two groups are separated from one another easily. In the Arribalzagia mosquitoes there are many small white spots on the wings, while in the Nyssorhynchus group, although the light areas are larger, they are fewer in number. The easiest character for differentiation is the sixth vein; in the Nyssorhynchus group it is mainly white, with a black spot near each end, while in the Arribalzagia group it has at least four small black spots. The species of Arribalzagia and Nyssorhynchus mosquitoes are very difficult to separate on the basis of the characters of the females, as the size and location of the wing and leg markings are very variable. One can seldom be absolutely certain of one's identification if it is based solely on the females. In the Nyssorhynchus group, we find, on the costal border, near the base of the wing, three white spots. The first is at the very base of the wing, and it has been named spot B<sub>1</sub>. Then there is a black area, followed by spot B<sub>2</sub>, and spot B<sub>3</sub> is found a little further apically. The size of these spots varies greatly, but we find that in some species they may consistently be larger than in others. Thus A.albimanus is separated from A.bachmanni by spot B<sub>2</sub>, which is usually larger than the black spot just basad of it in the former species, while in A.bachmanni it is smaller than the black spot basad of it. In A.albitarsis the light areas at the base of the costa are large, and spots B<sub>1</sub> and B<sub>2</sub> may even run together, eliminating the black spot between them.

In the Arribalzagia mosquitoes, there may be three large groups of black scales on the anterior margin of the wing, or only two. A.neomaculipalpus has

only two such spots, while A. apicimacula and A. punctimacula both have three. The last two can be separated from one another by the color of the light scales on the wings; in A. apicimacula they are pure white, while in A. punctimacula they have a yellowish color.

The characters used for separating the groups are perhaps better shown in the following table:



C. Key to the Anopheline mosquitoes of Panama, based on the characters of the adults.

1. Wings entirely dark-scaled, or with a mixture of light and dark scales; never with definite white and black spots caused by groups of scales of these colors ..... 2  
 Wings with black and white spots ..... 3
2. Wings with a mixture of light and dark scales; tarsi with conspicuous white bands ----- Chagasia bathanus.  
 Wings entirely dark, except for a yellowish-white spot at the tip of the wing; and a very small light spot on the first vein about one-third the distance from the base of the wing. Tip of hind tibia with a broad white band ..... Anopheles eiseni.  
 Wings dark; legs dark ..... A.kompi.
3. Veins 2, 4, and 6 entirely dark scaled. Broad white markings on tarsi of all the legs ..... A.cruzi.  
 Veins 2, 4, and 6 with white and black spots ..... 4
4. Costa with a white spot at the tip of the subcostal vein, and another at the tip of the first vein; vein 6 mostly dark, with a long white spot at the base, and a very small one at the tip ..... A.pseudopunctipennis.  
 Costa with more than two white spots ..... 5
5. Vein 6 mostly white, with two black spots ..... 6  
 Vein 6 with four or more black spots ..... 12
6. Fifth hind tarsal joint entirely white ..... 7  
 Fifth hind tarsal joint with a black ring ..... 8
7. Second hind tarsal joint one-half or more black; first abdominal sternite with a stripe of white scales on each side of the median line.  
 White rings on the tarsi of the middle pair of legs .... A.albitarsis  
 Second hind tarsal joint less than half black; first abdominal sternite without the double stripe of white scales. No definite white rings on the mid-tarsi ..... A.argyritarsis.
8. Second hind tarsal joint about one-half black ..... 9  
 Second hind tarsal joint less than one-half black ..... 10
9. Spot B<sub>2</sub> of wing longer than black spot basad of it; tip of lower branch of 2nd vein white scaled ..... A.albimanus.  
 Spot B<sub>2</sub> shorter than black spot basad of it; tip of lower branch of vein 2 black ..... A.bachmanni.
10. Second hind tarsal joint with only a very little bit of black at its base ..... A.oswaldoi.  
 Second hind tarsal joint about one-fourth to one third black ..... 11

11. Light colored wing-scales usually yellowish ..... A. tarsimaculatus.  
 Light colored wing-scales white ..... A. strodei.
12. Wings with two large black spots on costa, and one at apex of the wing; light-colored scales white ..... A. neomaculipalpus.  
 Wings with three large black spots on costa, and with either another black spot at apex of wing, or with a diffuse group of spots at the apex ..... 13
13. Wing-scales all pure white; a black spot at apex of wing ..... A. apicimacula.  
 Wing-scales on lower part of wing yellowish in color; diffuse group of spots at apex of wing ..... A. punctimacula

V. Identification of the Larval Anophelines of Panama.

A. Structure of Larvae.

Like the adults, the body of the larva is divided into three regions: head, thorax, and abdomen. The head is an oblong structure; laterally are the elongated, dark-colored eyes, in front of the eyes are the stout, cylindrical antennae, with a small antennal hair at about the middle, and on the dorsal surface of the head is a transverse row of long, forward-pointing hairs, the frontal hairs. In A. cruzii these hairs are single and unbranched, but in the rest of the Panama Anophelines they have lateral branches. Behind the frontal hairs, and between the eyes, are two pairs of small hairs, the inner and outer occipital hairs. At the base of the antenna is a subantennal hair. On the lower, anterior side of the head is the mouth opening, surrounded by maxillae, mandibles, and most conspicuous of all, the mouth brushes, with which food-laden water is swept into the mouth. Posteriorly, the mouth opening is bordered by a heavily-pigmented, triangular labial or mental plate. On the front margin of the head is a small, squarish plate, the clypeus, which bears four very important clypeal hairs. These hairs may be evenly spaced from one another, or the inner hairs may be very close together. They may be single, but often they are divided into a number of small branchlets.

The thorax is a short, bulging structure, composed of three segments fused together. Ventrally there are some large, feathered pleural hairs, which, unfortunately, can not be used for classification of American mosquitoes. There are many other hairs on the thorax, but the only ones of importance are two groups of hairs immediately behind the head, the anterior submedian thoracic hairs, which, in the subgenus Nyssorhynchus, are very important. Each group is composed of three hairs, lying on either side of the median line. Those lying nearest to the median line are the inner hairs of the anterior submedian thoracic group, and their structure is very variable. They may be small, with a few fine lateral branchlets, they may be long and feathered, resembling the middle hairs of this group (A. albimanus), or they may resemble a palmate hair, having short, broad leaflets arising from a common base. The middle hairs are long and feathered, and show no differences between species, and the outer hairs are very short and single, and are also similar in all the species.

The abdomen is composed of nine segments, the last two being modified by respiratory and anal structures. On the dorsal surface of the segments, on either side of the median line, is a conspicuous, circular, palmate hair. Each hair is composed of a number of radiating leaflets, all arising from a common base. Sometimes, as in the case of the Nyssorhynchus larvae, these hairs may be present on the first seven segments, but in others these hairs may be missing on the first segment (A. eiseni), or missing on the first two segments (Arribalzagia), while Chagasia bathanus has palmate hairs only on the 3rd, 4th and 5th abdominal segments. In the Nyssorhynchus larvae the edges of the leaves of the palmate hairs are smooth, while in the Anopheles and Arribalzagia larvae they are notched along the edges. In Chagasia bathanus they are widely expanded apically, and from the center of the apex arises a single long hair.

On the first three segments of the abdomen are found long, feathered abdominal hairs. These hairs are present on the first three segments of all the Panama Anophelines, with the exception of Chagasia bathanus, which has them only on the first two segments. On the fourth, fifth, and sometimes the sixth abdominal segments are the long lateral abdominal hairs, which vary in their structure, so that they are very useful in classification. They are absent in Chagasia bathanus. In the subgenus Nyssorhynchus the long lateral hairs are single and unbranched, and are present on segment 6 as well as on segments 4 and 5. In the Arribalzagia group they are single and unbranched also, with the exception of A. apicimacula, but are absent on segment 6. In the Anopheles group they are double, triple, or even multiple, and in A. pseudopunctipennis they have many short lateral branches.

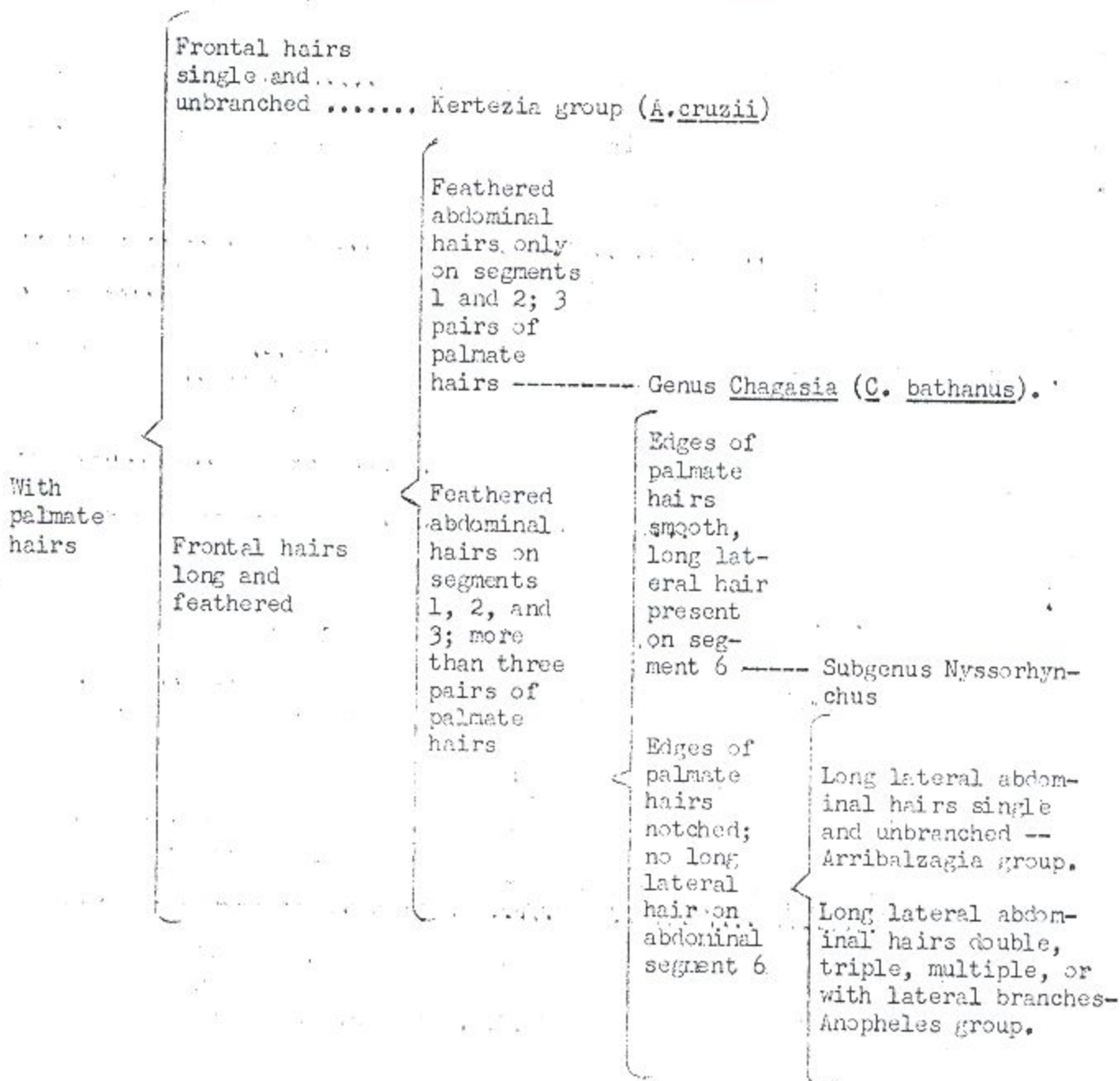
The spiracles of Anopheline larvae are not situated at the tip of a long air-tube, as in the case of the Culicine mosquitoes, but are found on the dorsal surface of the eighth abdominal segment, surrounded by four small flaps; a small anterior flap, two small lateral ones, and a large, scoop-shaped posterior one.

On the posterior, lateral side of the eighth segment is a row of short spines called the pecten. The teeth of the pecten of A. cruzii are all of the same length. but in the other Anophelines in Panama there is a mixture of long and short ones. There may be a regular alternation of long and short teeth, or there may be several short teeth together between the long teeth.

The ninth, or anal segment bears posteriorly four transparent anal gills. Above the gills is a pair of long feathered hairs, and, on either side of the feathered hairs, long, curved grappling hairs, which the larva uses to anchor itself to bits of floating debris in the water. Below the gills, on the median line, is the ventral brush, composed of a longitudinal row of long, branched hairs.

The following table summarizes the characters used for separating the main divisions of the Anophelinae. There are some exceptions to the characters listed below, so that it is difficult to separate Anopheline larvae according to groups. In Panama, the only exception is A. apicimacula, which, instead of having single lateral abdominal hairs, as do the other two local Arribalzagia mosquitoes, these hairs are double.

Without palmate hairs ----- Subgenus Stethomyia (A. kompi)



B. Key to the Species of Anopheline Larvae of Panama.

1. Without palmate hairs ..... A.kompi. 2  
 With palmate hairs .....
2. Long feathered abdominal hairs present only on segments 1 and 2; no long lateral hairs; palmate hairs on abdominal segments 3, 4, and 5; leaves of palmate hairs expanded apically ..... Chagasia bathanus.  
 Long feathered abdominal hairs present on segments 1, 2, and 3; long lateral hairs present; more than three pairs of palmate hairs ..... 3
3. Frontal hairs of head long and feathered ..... 4  
 Frontal hairs of head shorter, with few or no lateral branches .....  
A.cruzi
4. Long lateral abdominal hairs of segments 4 and 5 single and unbranched (Arribalzagia and Nyssorhynchus) ..... 5  
 Long lateral abdominal hairs of segments 4 and 5 double, triple, multiple, or with lateral branches (Anopheles and A.apicimacula) ..... 13
5. No palmate hairs on abdominal segments 1 and 2; edges of leaves of palmate hairs notched; long lateral hairs absent from abdominal segment 6 (Arribalzagia) ..... 6  
 Palmate hairs present on abdominal segments 1 and 2, edges smooth; long lateral hair present on abdominal segment 6 (Nyssorhynchus) ..... 7
6. Outer clypeal hairs branched into about 12 branchlets ... A.punctimacula.  
 Outer clypeal hairs forked at middle or slightly branched ....  
A.neomaculipalpus.
7. Inner clypeal hairs very close together ..... 8  
 Inner clypeal hairs as far apart from one another as they are from the outer clypeal hairs ..... 9
8. Inner hairs of anterior submedian thoracic group resembling a palmate hair, i. e., with about 15 flat, leaf-like branches all arising at about the same level ..... A.strodei.  
 Inner hairs of anterior submedian thoracic group smaller, with seven or eight hair-like branches arising along a short main stem .....  
A.argyritarsis.
9. Inner hair of anterior submedian thoracic group resembling the middle hair, with many hair-like branches arising from a short, thick main stem, usually over half as long as the middle hairs .....  
A.albimanus.  
 Inner hair of anterior submedian thoracic group resembling a palmate hair ..... 10
10. Inner hairs of anterior submedian thoracic group large, with broad, heavily pigmented leaf-like branches, the inner hairs of each side close together ..... A.albitarsis.



- Inner hairs of anterior submedian thoracic group smaller; the branches finer, and not heavily pigmented; the inner hairs of each side further apart ..... 11
11. Inner hairs of anterior submedian thoracic group with 13 to 18 fine, almost hair-like leaflets ..... A. bachmanni.  
 Inner hairs of anterior submedian thoracic group with about eleven broad leaflets ..... 12
12. Clypeal hairs less heavily branched. Fresh water breeder .....  
A. oswaldoi  
 Clypeal hairs more heavily branched. Brackish water breeder ...  
A. tarsimaculatus.
13. Long lateral hairs of abdominal segments 4 and 5 double ..... 14  
 Long lateral hairs of abdominal segments 4 and 5 with a single main stem and a number of well-developed lateral branches ...  
A. pseudopunctipennis.
14. Palmate hairs present on second abdominal segment; alteration on long and short teeth in pecten not regular .....  
A. eiseni.  
 Palmate hairs not present on abdominal segment 2; teeth of pecten mostly alternately long and short ..... A. apicimacula.

Information used in this paper is based on publications of F. W. Edwards, H. G. Dyar, F. M. Root, D. P. Curry, R. C. Shannon, and on the writer's personal notes.

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