EXTERNAL MORPHOLOGY OF THE LARVAL, PUPAL AND ADULT STAGES OF *SIMULIUM* (*EUSIMULIUM*) *BAISASAE* DELFINADO (SIMULIIDAE, DIPTERA)

By

BENJAMIN L. CARIASO

Bachelor of Science

University of the Philippines

Diliman, Rizal

Philippines

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EXTERNAL MORPHOLOGY OF THE LARVAL, PUPAL AND ADULT STAGES OF *SIMULIUM* (*EUSIMULIUM*) *BAISASAE* DELFINADO (SIMULIIDAE, DIPTERA)

Thesis Approved:

D E Howell
Thesis Adviser

D E Bryan

Donald W. Twohy

Wendell Krull

G A Bieberdorf

Robert MacVicar
Dean of the Graduate School

PREFACE

This thesis manuscript on external morphology is an outgrowth of a previous preliminary observation on the biology and ecology of black flies which was carried out mainly at Molawin Creek, Philippines, from October 1958 to March 1959. The study was suggested by Dr. Bernard V. Travis who was then a Cornell University visiting professor. After the Creek was sprayed with DDT for the control of malarial mosquitoes the work on biology and ecology was discontinued.

The writer has collected a reasonable number of Philippine materials upon which the following work on morphology was based. Additional materials were sent by a friend who made collections from a tributary of the Molawin Creek.

Under the guidance of Dr. D. E. Howell, the thesis work was carried out at the Oklahoma State University, Stillwater, Oklahoma, extending from September, 1959, until July, 1960.

The writer is deeply indebted to his major adviser, Dr. D. E. Howell for his personal interest and for the many helpful suggestions made by him throughout the study and preparation of this manuscript. To Dr. W. H. Krull, Professor and Head of the Department of Veterinary Parasitology, sincere gratitude is due for his helpful suggestions. Many thanks are due to Dr. D. E. Bryan, Associate Professor of Entomology, Dr. W. A. Drew, Assistant Professor of Entomology, and Dr. D. W. Twohy, Assistant Professor of Zoology, for their helpful criticism of the manuscript.

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INTRODUCTION

While black flies are very important and have been studied extensively in many parts of the world, no biological and morphological studies on black flies have been published and possibly no attempt along these lines has been undertaken in the Philippines. As far as the writer knows there is only one worker engaged at the present time on the taxonomic study of Philippine simuliids. Many black flies are pestiferous, most are blood suckers and some are potential vectors of animal diseases. They are present in large numbers in the Philippines but their significance to man and animals is almost unknown. Apparently this important group of insects is not receiving the attention it merits.

These flies are called buffalo gnats because of a decidedly prominent hump on the dorsal part of the thorax. According to Riley (1880) about 6000 mules were killed in the Ouachita Valley in Louisiana as a result of the attack of great numbers of a "poisonous midge". Riley (1880) implicated black flies in this report. Forbes (1912) pointed out the possibility that these flies were carriers of pellagra. Lewis (1953) reported on the problem of human onchocerciasis pointing to Simulium damnosum Theobald as the sole vector. A similar human disease occurs in Guatemala according to Dalmat (1955).

This study is primarily concerned with the external morphology of the larval, pupal and adult stages of Simulium (Eusimulium) baisasae Delfinado. The feeding habits of this species are not known to the writer. A detailed comparative morphology of the adult male and female mouthparts may give a clue to their potential in inflicting injury to man and animals.

Larval stages are difficult to associate with the adults without studying the salient characters of the pupa, consequently detailed information concerning their external anatomy is imperative with regard to taxonomy. Rearing of the adults is necessary in order to identify the successively smaller larval instars for a specific species. According to Stone and Jamnback (1955) many species of adult simuliids are difficult to distinguish unless the larval and pupal stages are known.

REVIEW OF LITERATURE

Among the earliest workers with the larvae of Simuliidae in America was Osten Sacken (1870) who presented a short description of the larval mouthparts. McBride (1870) suggested the larval stage as being very destructive to young trout which, he stated, possibly get entangled with the silken threads of the larvae. Hagen (1881) though he did not study the early stages in detail, was able to point out the presence of filaments on the pupae. Owing to the importance of this group of insects many observers have produced valuable papers pertaining to the habits and morphology of the larvae among the more prominent of these are; Miall (1903), Johannsen (1903), Malloch (1914), Edwards (1920), Puri (1925) and Wu (1930).

Taxonomic studies on the larval and pupal stages have increased in number in recent years. Sommerman (1953), Freeman and de Meillon (1953), Dalmat (1955) and Stone and Jamnback (1955) have shown the relative significance to the taxonomy of the structural differences existing among the immature stages of simuliids. Chief characters usually used for separating species are the number of filaments of the pupal respiratory organs and their branching patterns, number of teeth on the submental plate of the larva as well as the shape and extent of the throat cleft. Other minor characters used in taxonomy are the shape and number of hooklets on the abdomen of the pupa and number of teeth on the mandibles and segments of antennae of the mature larva. Because these structures have not been carefully studied on Philippine black flies, it is the purpose of this study to work out in detail the morphology of the pupa and the larval instars to determine what differences exist in different species.

The mouthparts of some species of adult Simuliidae have been studied and described rather briefly by some authors, among whom are Blacklock (1926) and Smart (1935). Some recent papers on the subject are by Gibbins (1938) on *Simulium damnosum* Theobald, Krafchick (1942) on *Eusimulium lascivum* Twinn, Snodgrass (1944) on *Simulium venustum* Say and Nicholson (1945) on *Eusimulium dacotense* D. and S. Workers are not all in agreement as to the number of segments of the maxillary palpus of a simuliid adult. Tonnoir (1924), Snodgrass (1944) and Nicholson (1945) claim that there are five but Smart (1935) and Krafchick (1942) maintained that there are only four. These distinguished workers completely disagree with each other and it appears that their decisions are based on differences in interpretations. However, it could be due possibly to anatomical differences in species that were involved.

The vestiture of the thoraces of the male and female has a very important bearing on taxonomy. Its description is always given in a detailed manner, but the sclerites seem to have been neglected. Only a brief description of sclerites of the thorax have been found in the literature. Malloch (1914) discussed briefly the sclerites of the thorax of *Prosimulium pleurale* Malloch, Nicholson and Mickel (1950) of *Prosimulium hirtipes* (Fries) and Stone and Jamnback (1955) of *Prosimulium magnum* D. and S.

Some of the early taxonomists of the simuliid group used to a great extent the male and female terminalia. Edwards (1915) in his study of the Simuliidae of the British Isles pointed out the importance of the male genitalia. A year later, Pomeroy in his work on the North American Simuliidae emphasized the importance of the male genitalia. Many terminologies were later employed for the same structures found in the male genitalia which bewildered a new worker. Because of this chaotic situation in terminology Freeman (1950) proposed a terminology for the different structures of the male genitalia.

It appears that the use of the female genitalia is comparatively recent. Dyar and Shannon (1927) published with reference to taxonomy an account of their work illustrating the female genitalia of various species of North American simuliids.

MATERIALS AND METHODS

Collection and preservation of specimens. The various larval instars were collected from stones, leaves or twigs submerged in the running water of the Molawin Creek, Philippines. Fine forceps were used in removing the larvae from their place of attachment taking care not to injure the specimens. Vials containing either 95 percent ethyl alcohol or Bouin's fixative were used for killing the larvae.

Larvae and pupae were fixed in Bouin's for two hours, washed in 70 percent ethyl alcohol and preserved in vials with 70 percent ethyl alcohol. These specimens were prepared for use in the dissection of various body parts.

Pupae were also collected from the same habitat. Utmost care was employed in removing the pupae from stones, leaves and twigs. Sometimes it was necessary to loosen the cocoon from its place of attachment before it was removed by the forceps. Some of the pupae were killed in 95 percent ethyl alcohol, but many of those in advanced stages (dark brown to black) were placed in individual vials for rearing. Pupae attached to leaves were most favorable for rearing. A section of a leaf with a single pupa was placed inside a vial. Pupae were reared individually in these shell vials, 10 x 40 mm.

Each vial was supplied with a strip of absorbent paper about the diameter of the vial and placed lengthwise. It was kept moist by adding water and the vial was plugged with moistened cotton. The method of rearing the pupae was similar in principle to that employed by Nicholson and Mickel (1950) except that a strip of absorbent paper was used instead of cotton. Adults usually emerged in less than a week. They were kept for 15 or more hours to allow time for expansion of the wings and the hardening of the cuticula. The imago, with its cast skin and cocoon, was preserved in 95 percent ethyl alcohol and catalogued. Studying the pupal exuviae of the reared adults and then comparing their filaments with the developing pupal gill filaments of the fully grown larvae facilitated association of larvae and pupae in a species.

This method of collecting and rearing pupae has the distinct advantage of usually yielding both sexes. The two sexes in a particular species can easily be associated. Furthermore, the pupa as well as the cocoon are isolated for respective individuals and have indispensable characters of taxonomic value.

In this study it was desirable to separate the pupal filaments of the fully grown larva and for this purpose the procedure of Sommerman (1953) was followed. A pair of forceps was used to hold the larva and with an insect pin the integument was freed from the area of the developing pupal filaments. The pupal respiratory filaments were removed and characteristic number of branches and branching patterns determined.

Association of the fully grown larva with progressively smaller ones of the same species, collected previously at the same location, was based upon the submental plate and throat cleft and often upon the color patterns of the head capsule and the anal gills.

In this connection, identification based on the anal gills alone was quite difficult since a complete extrusion of the finger-like structures was necessary. This method made possible the determination of the species composition of weekly larval population samples over a six-month period. Sorting of the species was done after the larval characters had been established.

Because of the difficulties in taxonomy of larvae it was necessary to use some larval characters to identify certain categories of larval stages. Larvae in the first instar are small (0.67 to 1.81 mm.) with no apparent histoblasts and the egg burster on the head capsule is still present. Those of medium size (1.81 to 2.62 mm.) are in several intermediate instars and are provided with histoblasts not seen by the unaided eye but identifiable with the aid of the stereoscopic microscope. Near fully grown larvae (2.62 to 3.37 mm.) with slightly developed white irregularly shaped histoblasts which can be seen by the naked eye. Full grown larvae (3.17 to 4.75 mm.) are provided with dark histoblasts.

Mounting techniques. Two methods were employed. Some preserved specimens were cleared in five percent KOH and properly washed in a series of alcohols (50, 70, 85, 95 percent) and then placed in equal parts 95 percent ethyl alcohol and beechwood creosote for 20 or more minutes until clear. They were transferred to equal parts of beechwood creosote and balsam for 15 or more minutes and mounted on a slide in pure balsam. To obtain an even penetration of the liquid into the specimen, a minuten nadeln was used for perforating the body wall.

For convenience and speed of mounting larvae, pupae and adults, Hoyer's medium was used. Specimens preserved in 70 percent or 95 percent ethyl alcohol were passed through ethyl alcohol of decreasing concentrations and washed in distilled water. The specimens were pricked with a minuten nadeln and placed overnight in a five percent KOH. The specimens were then washed several times in distilled water and mounted directly in Hoyer's medium.

All structures that needed to be detached from the larvae, pupae and adults, were dissected on a glass slide in a drop of Hoyer's medium before mounting on the same slide.

Smaller structures, like the mouthparts, after having been dissected out of the head were left in the medium. Since the head capsule was not necessary in this mount it was removed. This procedure in mounting was followed for all small structures. Parts were oriented on the slide before putting the cover slip in place. Sharpened insect pins were used for manipulating the specimens. All dissections were done under the stereoscopic microscope.

The cephalic fan was separated easily from the larva by pulling at the base or stalk with fine pointed forceps. The fan was expanded by simply holding the base, dorsal side up, with the forceps and applying pressure on the elbowed joint with a dissecting needle so as to spread the filaments. If it was done on a slide in a drop of water a cover slip was placed on top and the preparation examined under the compound microscope. Some of the filaments usually stuck together because of minute lateral processes which made counting of the filaments difficult. A slight pressure applied on the cover slip with a rotary motion usually separated the filaments. Some adults and larvae were stained in acid fuchsin. Powdered acid fuchsin was added to 95 percent ethyl alcohol until a deep red color was produced. When properly stained the cells in the anal gills of the larva were easily discerned. For most of the work on morphology the staining method was abandoned since it was not necessary.

Illustrations and Measurements. Illustrations were made in various ways. Some of the structures were drawn under the camera lucida but as worked progressed a microprojector with three objectives was used to project the image of the specimen on a sheet of semi-transparent drawing paper. The basic outline of the specimen was traced on this paper and further details were drawn freehand using a compound microscope for verification and measurement. Drawings were finally transferred to a drawing paper and inked. All specimens and their corresponding structures were measured with a calibrated ocular micrometer disc from specimens preserved in 70 percent ethyl alcohol.

OBSERVATIONS

Description of the Female

The body length is 1.9 mm. (plate I, fig. 1).

Head.

The head is laterally ovate, reniform in frontal view, tending to surround the bases of the antennae, dichoptic, and light brown in color. The fronto-ocular triangles (Fr.O.) (plate III, fig. 2) are present above the bases of the antennae. The frons (Fr.) is rather broad, flaring dorsally. Below the frons the clypeus (Clp.) is not prominently convex and bears fine, recumbent, shiny, golden yellow hairs. The vestiture of the frons and vertex bears conspicuous golden yellow hairs. The cuticles of the clypeus, frons and vertex are glistening. The antennae (Ant.) (plate III, fig. 3) are eleven jointed, slightly tapering with the first to the third segment light yellow-orange. The remaining segments are light gray. The second, third, and terminal segments are twice as long as any of the other segments. The last segment is conical.

The maxillary palpi are generally light brown but the third segments are darker in color. The occiput (plate V, fig. 8) and postgenae (Pge.) are provided with long, brown hairs but these are not as prominent as in the male.

Mouthparts of the female.

Labrum-epipharynx. The wall of the labrum-epipharynx (plate II, fig. 1) is a delicate semi-transparent membrane strengthened by three rod-like heavily sclerotized processes, i.e., the labrum. There are two lateral processes (L.P.) and one median (M.P.) which is dorsal to the other two. These three processes form a three-sided pyramid with the basal part attached to the ventral part of the head. The median process (M.P.) is expanded distally and armed with two sets of three highly chitinized curved terminal teeth (T.T.). Many circular spots known as sensory pegs (S.p.) extend from the base of the labrum-epipharynx to its apex. The epipharynx is the area between the two lateral rods of the labrum.

Mandibles. The thickened base of the mandible (plate II, fig. 3) articulates with a lateral process of the clypeus. From near the base of the mandible on the posterior side, is a sclerotized rod projecting along free of the outer edge which joins the middle part of the mandible lateral to the depression (D.). The outer margin of the apex has approximately seven terminally directed teeth. The faint longitudianl striations which can be seen for a short distance on the mandible tip originate from points between the marginal teeth.

Hypopharynx (Hyp.) (plate II, fig. 2). This is a trough-like rigid structure. It bears teeth-like projecting structures around the distal edge. Around this edge are short posteriorly directed, stiff hairs. The salivary duct (S.D.) extends from the distal to the proximal ends of the midventral part of the hypopharynx. The lateral parts of the hypopharynx are conspicuously sclerotized and undoubtedly strengthened the entire structure.

Cibarial pump. The cibarial pump (Cb.) (plate II, fig. 2) is a vertical, trough-shaped structure with two circular spots on the lateral areas. On the disto-lateral edge of the cibarial pump are articulated two rod-like structures, the interlora of the lacinia (Il.La.) (plate II, fig. 2.). Attached to the proximal part of the cibarial pump are the sclerotized cornuae (Cc.).

Pharyngeal pump. The pharyngeal pump (Ph.) (plate II, fig. 2) is vertically attached to the proximal end of the cibarial pump. It lies in the midline of the head extending to the level of the foramen magnum.

Maxillae. The galea (Ga.) (plate II, fig. 8) is lanceolate and flattened. It does not exceed the length of the first two segments of the palpus taken together. The distal fourth of the galea is flattened and bears about twenty-two pointed, recurved teeth around the apical margin. These teeth occur on the posterior face of the galea. The anterior face is smooth. The maxillary palps are four-segmented with a sensory organ (S.O.) on the second segment.

Labium. The labium (plate II, fig. 9) is composed of a basal postmentum (Pmt.) (plate V, fig. 8) the theca (The.) (plate II, fig. 9) and the labella (1 lb, 2 lb) which are concave and form a deep gutter anteriorly concealing the other mouth parts when at rest. At the base of and between the labella is the membranous ligula (Li.) with tree-like branches.

Thorax.

The mesoscutum (Sct.) is dark brown with fine adpressed golden yellow hairs. On the cuticle of the mesoscutum can be seen three faint longitudinal stripes extending to its posterior third. The posterior margin of the mesoscutellum (Scl.) is fringed with long, erect, brown hairs. The pleural tufts (P.t.) are golden yellow.

Legs.

Leg I; length, 1.94 mm. (plate I, fig. 3). The coxa, femur, and tibia are light gray with dark brown tarsal segments. The basitarsus (Bt.) and third tarsal segments (T2) are each provided with two outwardly directed long hairs on their distal margins. Measurement of the basitarsus shows that it is almost as long as the rest of the tarsal segments taken together.

Leg II; length, 1.59mm. (plate I, fig. 4). The coxa, trochanter and femur are light gray. The distal third of the basitarsus and tibia is dark brown. The inner margin of the basitarsus is provided with nine to ten short spines. This segment is slightly longer than other tarsal segments.

Leg III; length, 1.89 mm. (plate I, fig. 5). The coxa is dark gray, trochanter light gray, and the femur is moderately dark gray. The basal one third of the tibia is light gray but becomes gradually darker toward the distal fourth. The distal third of the tibia (plate I, fig. 5) bulges outward. On the dorsal margin of the tibia are five to six long hairs. On plate 1, fig. 6 can be seen the hind basitarsus produced posteriorly as a flattened lobe. This is the calcipala (Cal.) which is well developed and covers the pedisulcus (Ped.), a dorsal notch on the second hind tarsal segment. The pedisculcus is widely notched on its basal third. Claws (Cl.) (plate I, fig. 5) on all the legs are simple. The distal ends of all tibiae are each provided with two massive spines.

Wings.

Wings; (plate III, fig. 4) length, 1.9 mm.; width, 0.9 mm. The costa (C) is clothed with spiniform and hair-like macrotrichiae. A single row of hair-like macrotrichiae extends along the proximal third of the subcosta (Sc), the remainder being bare. The proximal one-sixth of the radial sector (Rs) is bare but the rest of its length is provided with a single row of hair-like macrotrichiae. The entire length of the radius (R_1) bears a single row of hair-like macrotrichiae. The medial veins (M_1, M_2) have a common base for a short distance. Posteriorly the submedian fork (S.f.) appears as a fold, extending to but barely touching the inner wing margin. The cubitus (Cu_2) is sinuous. The anal vein (An_1) curves slightly forward and ends just short of joining Cu_2 distally. The hairs on the stem vein (St.v.) are light yellow. The pedicels of the halteres (H.) are light brown with an opaque, pale yellow knob.

Abdomen.

The basal scale (Abs.) (plate V, fig. 9), an expansion of the first abdominal segment, is fringed with silvery, extremely short hairs which do not reach the basal half of the second abdominal segment. All segments are generally grayish-brown. Brown hairs are evenly distributed throughout but scarce on segments two and three. Conspicuous dark, semicircular patches are found on the mid-dorsal tergites. The sclerotized area of the eighth sternite has a crescent-shaped dark brown patch midventrally.

Female genitalia (plate III, fig. 5,7).

Dorsally, the cercus (Cer.) is dome-shaped and almost twice as high as long. It is covered with a few long, strong hairs directed posteriorly except on the dorsal portion where the hairs are more nearly erect. Many minute hairs cover the entire surface of the cercus. Ventrally, the mesal margin of the cercus is slightly concave and covered with many adpressed short hairs and is twice as long as broad.

The anal lobe (A.l.), which is ventral to the cercus, is provided with a similar investiture. When seen from the lateral view it is almost oval and twice as large as the cercus, but ventrally it is more than twice as large. It is seen from plate III, fig. 7 that the genital fork (G.f.) is slender and slightly bent sinistrally. The arms of this structure are spread almost at a ninety-degree angle. The two arms are nearly as long as the rod but broader and each carries ventrally a highly sclerotized tooth-like projection. The two arms are continuous with the inner surface of the cuticle. Laterally, the genital fork is bent downwards, the two arms curved upwards.

The spermatheca (Spt.) is a dark brown, chitinous capsule. It is oval in shape with the spermathecal duct (Spt.d.) attached to its terminal pole.

Description of the Male

The body length is 2.1 mm. (plate I, fig. 2).

Head.

The head is triangular in lateral view with the wider part on the dorsal side. The compound eyes (C.E.) (plate III, fig. 1) are holoptic with the dorsal facets about twice as large as those in the female. The dividing line between the dorsal and ventral facets is abrupt without showing any sign of gradual transition from in front and from the sides. In preserved specimens the large faceted portion of the eyes is orange-yellow turning dark red in pinned specimens. The fronto-ocular triangles are absent. The frons (Fr.) above the bases of the antennae (Ant.) is depressed. Below the frons is the strongly convex, glistening clypeus (Clp.). The clypeus and frons are clothed with fine recumbent golden-yellow hairs. Each of the antennae are eleven-jointed, with the third segment twice as long as wide. Segments one to six are light brown in color becoming gradually darker towards the last conical segment.

On the posterior area of the head is the slightly flattened occiput (Oc.) (plate V, fig. 8) with conspicuous dark brown hairs projecting upwards from its dorsal margin. The four-segmented maxillary palpi (plate II, fig. 7) are brownish throughout their entire length. A detailed discussion of this is given on the section dealing with the morphology of the mouth parts.

Mouthparts of the male.

Labrum-epipharynx. The labrum-epipharynx (plate II, fig. 4) is similar to the female except that the sets of teeth on the female labrum-epipharynx are absent on its apex.

Mandibles. The mandibles (plate II, fig. 6) are also blade-like structures but less sclerotized than those of the female. Distally each is almost a thin membrane. The widest part is at the level of the clear area (Cl.a.) at the center. This clear area is not homologous with the depression which occurs on the mandible of the female. At the base can be seen two circular spots.

Hypopharynx. The hypopharynx (Hyp.) (plate II, fig. 5) is trough-like and articulates with the distal end of the cibarial pump. In the female, the distal extremity of the hypopharynx is somewhat flattened and broader than in the male. The apex of the hypopharynx tapers steadily and bears a fringe of long setae.

Cibarial pump. The cibarial pump (Cb.) (plate II, fig. 5) is the same as in the female but the cornuae are less prominent.

Pharyngeal pump. (Ph.) (plate II, fig. 5). This structure is the same as in the female.

Maxillae. (plate II, fig. 7). The palps are similar to the female but the galeae (Ga.) are thin and weakly sclerotized bearing hairs at their extremities.

Labium. The structures that make up the labium are essentially the same as those in the female.

Thorax.

Laterally, the mesoscutum (plate I, fig. 2) is seen to be strongly arched and dark brown but with adpressed yellowish hairs. The mesoscutellum is dark brown and posteriorly fringed with erect brown hairs. The pleural tufts are golden yellow.

Legs.

Leg I; length, 1.97 mm. In appearance, the first, second and third pairs of legs are very similar to those of the female except for the coloration. The coxa and trochanter and basal one-third of the femur and tibia are clothed with light brown hairs. The rest of the segments including the tarsi are dark brown. The two prominent hairs on the antero-distal tip of the basitarsus are approximately as long as the second tarsal segment. The third tarsal segment also has two long hairs on its antero-distal tip which are about as long as this segment.

Leg II; length, 1.64 mm. The coxa and trochanter and basal parts of the femur and tibia are covered with light brown hairs. The rest of the segments including the tarsus are dark brown.

Leg III; length, 1.92 mm. The coxa is clothed with dark brown hairs.

Its outer surface is provided with a longitudinal row of longer hairs extending throughout its length. The trochanter is generally covered with light gray hairs and the femur with dark brown. The basal one-third of the tibia bears light gray hairs and the rest is dark brown. The basal two-thirds of the basitarsus is light gray and the remainder is dark brown. Claws and spines on the distal ends of all the tibiae are similar to those of the female.

Wings.

Wings; length, 1.7, width, 0.8 mm. The venation and hairs on the veins are essentially the same as those of the female except the subcosta which is bare throughout its entire length. The hairs on the stem vein are brown. The pedicels of the halteres are light brown, the knobs opaque pale yellow.

Abdomen.

The abdomen is generally dark brown with light gray areas on the ventral half of segments two, three and four. The basal scale is fringed with fine long brown hairs extending laterally to the base of the third abdominal segment. Dorsally a fringe of hairs extends to about the anterior fourth of the second abdominal segment.

Male genitalia (plate III, fig. 6).

The external genitalia are better studied and understood when separated from the posterior part of the abdomen. A large basal area, the basistyle (Bs.), is seen as semi-quadrate and longer than it is wide. Its apical half is sparsely covered with short setae. Dorsally, the base is cleft for a short distance. The ventral surface of the two basistyles is slightly flattened. Attached to the basistyles are the dististyles (Ds.) which are produced as semitube-like structures with their inner margins concave and apices curving toward each other. Both dististyles bear a conspicuous subterminal, tooth-like projection. Along the two lateral margins are four to five long setae. The dististyles are about four times as long as wide.

Between the basistyles is a plate-like sclerotized structure, the ventral plate (V.p.). When seen in ventral view it is almost twice as wide as long, the basal process being expanded. The hairs on the body of the ventral plate are short and adpressed and densely setose on the triangular keel.

On the postero-lateral margins of the ventral plate are the parameres (P.), which are both bent mesally almost at right angles toward each other. Hooks (P.h.), along their extremities, are arranged in a linear fashion but are difficult to count due to their arrangement one on top of the other. Three large hooks on the ventral margin are more than twice as long as the smaller hooks.

Description of the Fully Grown Larva

The body is eleven-segmented, 5.5 mm. in length, cylindrical in shape and slightly constricted in the middle (plage IV, fig. 5).

Head (plate IV, fig. 5,6).

The head capsule is light yellow and pigment spots are absent. Ventrally the epicranial plates are separated on the posterior half by the throat cleft (Th.c.). The cleft is deep, gradually tapering anteriorly with the sides almost indistinguishable. The medio-lateral sides of the cleft are widely set apart and rounded. The fronto-clypeal plate

(F.p.) makes up the dorso-median portion of the head. It is separated from the epicranial plate by a well defined suture. The posterior rim of the epicranial plates are strongly sclerotized, but even more so on the postero-ventral extremities where the throat pits (Th.p.) are located.

The submentum (Sm.) is armed with pointed and highly sclerotized teeth. There are nine teeth in all, one larger median tooth and four lateral pairs. The median tooth is twice as long as any of the four pairs of the lateral teeth. The margin of the tips of the four pairs of teeth are situated approximately on a straight anterior line. The sides of the submentum are serrate with the tips directed anteriorly. Though slightly variable in number there were in a large majority of specimens examined, four pairs of long setae near the lateral margins of the submentum. These setae are arranged in an antero-posterior direction.

Cephalic fans (plate IV, fig. 9). The filaments (F.) of the cephalic fan are scythe-shaped, flattened dorso-ventrally, and widened in the basal one-fifth of their length. Distal to the widened base are regularly interspersed hairs along the entire length of the filaments. The filaments vary from 34 to 45 in number. On the ventral area of the stalk, immediately below the basal articulation of the primary fan are shorter filaments. These shorter filaments vary from 26 to 30 in number. These are collectively termed the secondary fan (S.f.). The distal half of these filaments extend somewhat beyond the basal attachment of the primary fan.

Antennae (Ant.) (plate V, fig. 7). The antennae are located on the sides of the head dorsad to the base of the cephalic fan. They are cylindrical and consist of four segments. Segment one is approximately as long as segment two. The third segment is a little shorter than either of the preceding and the fourth or distal segment is very short and pointed.

Mandibles (plate IV, fig. 7). On the apical margins of each mandible are three sclerotized terminal teeth (Tt.). When viewed dorsally, one of the terminal teeth is partially covered by the other two. Along the inner margin, proximal to these teeth, is a row of three small, light colored teeth. Immediately following these are 10 to 11 tiny bristle-like teeth which decrease in size towards the base of the mandible. At the base of this row is a flattened tooth-like process (T.p.) followed by a smaller one. On the dorsal side, along the medio-distal half, is a row of long setae which partially cover 7 to 8 larger setae. Distal to the base of this row of setae, on the outer margin, are two prominent setae. On the inner border, at the level of a transverse cleft, is another row of setae. Below these are 7 stout, long setae, the apices of which are produced into short projections. Dorsally and ventrally at the bases of the mandibles can be found a varying number of short sensory hairs. The characteristic forms and structures of the groups of setae are shown in plate IV, fig. 7.

Maxillae (plate V, fig. 1). Groups of conspicuous, long setae are found on the dorsal and ventral surfaces. The apical one-fifth bears a cluster of long setae. A finger-like projection can be seen on the inner margin at the level of the base of this cluster of setae. On the inner and outer margins are two separate groups of shorter setae which can be seen both from the dorsal and ventral sides. A clear area between these two groups of setae is set with small protruberances. Arising near the base of the palp is another group of setae. Many sensory setae occur on the ventral side of the maxillae showing no definite pattern. The cylindrical one-segmented palp (Mx.p.) is stout, slightly truncated distally and bears 7 to 8 sensory papillae. A few sensory setae are scattered along its entire length.

Hypopharynx and prementum (plate V, fig. 4). The prementum (Pm.) is a double plate and together with the hypopharynx forms the floor of the mouth cavity. Along the anterior border of the prementum are long brush-like setae which tend to hide the palp-like (P.) structures lying medio-dorsally. There are two pairs of posterior processes; first, the internal processes (Ip.) which penetrate the mentum; and second, the freely ascending processes (Ap.).

Labrum and epipharynx (plate V, fig. 2). The semi-circular labrum is covered with long setae dorsally and extends beyond the mouth opening. An anteriorly directed group of hooklets (Gh.) are sparsely covered with long setae along the median line. Ventrally there are only a few short setae. The labrum is continued posteriorly forming the epipharynx which serves as the dorsal wall of the mouth. There are three groups of short setae, one median and two lateral.

Thorax (plate IV, fig. 5).

A dark conspicuous, semi-ovate, pigmented structure is found on each side of the light gray thorax. These are the developing pupal respiratory organs (P.o.). The single median thoracic proleg is attached to the ventral surface of the thorax. It is an elongate, truncate process the extremity of which is provided with tiny curved hooks by which the larva fixes itself when progressing from place to place. There are 22 to 28 radiating rows of hooks in the anterior crochet ring. The short distal joint is capable of retraction into the much larger basal joint. Behind this crown of hooks are chitinous lateral plates (Lp.), one on each side. The anterior border is prolonged obliquely and is armed with pointed processes in groups of two to three.

Abdomen (plate IV, fig. 5).

The abdomen is light gray in color, eight-segmented, constricted on the second and third segments and posteriorly enlarged. On the eighth segment lies the median transverse slit-like anus, through which the retractable anal gill (A.g.) protrudes. The anal gill is formed with three finger-like processes. Posterior to the anal gill are the anterior branches of the anal cross-piece (A.p.) (plate IV, fig. 8). This is an x-shaped dark sclerotized sclerite with the anterior arms terminating near the region of the anus and the posterior arms extending along the anterior border of the posterior crochet ring (P.c.). The length of the posterior arms is less than twice the length of the anterior arms. The posterior crochet ring lies caudal to the anal cross-piece. Its plane is almost at right angles to the longitudinal axis of the body. The crochet ring is composed of 81 to 94 rows of flattened hooks. The numbers of hooks diminish on the medio-dorsal and medio-ventral rows and are distributed as follows:

Row	Number of Hooks
1	4
2	6
3	7
4	9
5	11

The remainder have 12 to 16 hooks per row.

The first stage larva has the same shape as that of the fully grown larva except that an egg burster (Eb.) (plate V, fig. 5) is still present dorsally near the center of the head capsule. It is a small sclerotized tooth directed dorso-anteriorly. Under low magnification (10x), it appears as a dark spot in the middle of the back of the head. About 4 or 5 sensory hairs are found posterior to the egg burster which are not visible in the second stage larva. The head is comparatively large in relation to its body size. Each antenna appears to be three-segmented with two small projections on the antero-distal end of the first segment. The first segment is almost as long as the second but stouter at the base. The last segment which terminates in a pointed tip is five times as short as the first and second segments. Each of the primary cephalic fans has 17 to 28 filaments. These increase in number to about 36 in the next stage. In the posterior crochet ring, there are about nine hooks in each row with the exception of the first seven or eight rows on both sides of the medio-dorsal and medio-ventral rows. Although the mandibles are small and appear to be less sclerotized, the first stage larva possesses the same structures found in the fully grown larva. Their medio-lateral margins tend to be slightly angular. All other external features of the first stage larva are similar to those of the fully grown larva. This also holds true for the medium-sized and near fully grown larva. However, the number of rows of hooks and number of filaments in the primary cephalic fans differ as seen in table I.

Table I. Body lengths, number of primary cephalic fan filaments, and rows of hooks on the posterior crochet ring for four larval stages of Simulium baisasae Delfinado; College, Laguna, Philippines, 1958-1959.

	Length of larva (mm)	Number of filaments in primary fan	Number of rows of hooks on posterior crochet ring
First instar	0.7 - 1.8	17 - 28	60 - 70
Medium sized	1.8 - 2.6	27 - 36	63 - 76
Near fully grown	2.6 - 3.4	35 - 40	72 - 89
Fully grown	3.2 - 4.8	36 - 41	86 - 94

Description of the Pupa

The pupa (plate IV, figs. 1,3) measures 2.8 mm. in length. One of the most striking morphological features of this stage is the large respiratory organ (2.2 mm.) with its six filaments. The head of the pupa is tucked beneath the large thorax. Posterior to the head are the developing legs. On the lateral aspects are the wing pads in their early development forming large plate-like structures.

The smooth, strongly convex pronotum and mesonotum appear to be fused. The metanotum is composed of small triangular plates. From the thoracic pleura the dorsal petioles of the pupal respiratory organs (plate IV, fig. 1,2) arise almost vertically upwards for a short distance and the ventral petioles arise obliquely ventrally in a vertical plane, with each petiole dividing into two filaments. A single median petiole arises directly anteriorly and gives rise to two filaments. The sets of six respiratory filaments are each attached to a common stalk on the antero-lateral sides of the thorax. The cuticle forming the tube of the filaments is very thin and varies from 1.16 to 3.06 microns in width increasing toward the base.

When viewed dorsally (D) (plate IV, fig. 3), the abdomen shows nine distinct segments with characteristic hooklets and spines. Ventrally (V), the two anterior segments are concealed from view by the developing leg sheaths and plate-like wing pads. Hooklets (R.h.) on the ventral part of the abdomen are shown in plate IV, fig. 3. Dorsally, the third and fourth abdominal segments are provided with four pairs of short, stout hooklets. Along the anterior margins of the 6th, 7th, 8th, and 9th segments there is one irregular transverse row of posteriorly directed triangular spines (T.s.), the number of which vary. Near the dorso-posterior end of the ninth abdominal segment there are a pair of short blunt hooks (A.h.). Ventrally, the fifth abdominal segment is provided with four retrorse, bifurcate hooklets. Also, the sixth abdominal segment is provided with four, distinct, retrorse hooklets (R.h.) with the two lateral hooklets simple and tapering, and the two median hooklets bifurcate. Four retrorse hooklets are also found on the seventh abdominal segment. All are bifurcate except one on the sinistral side which is simple. The terminal hooks on the tip of the abdomen are short and blunt.

The cocoon (plate IV, fig. 4) is 3.6 mm. long, light brown in color and has a closely woven parchment-like texture. Its general configuration has been described as the "wall-pocket" type because it narrows posteriorly towards its flattened bottom. The anterior margin is thickened and is provided with a medio-dorsal projection which is often broken but the base of the projection may be visible.

DISCUSSION

Comparative Morphology of the Male and Female Mouthparts

Relationships of the various mouthparts were determined by Jobling (1927) who showed the close resemblance in arrangement of _Culicoides_ sp. mouthparts with those of _Simulium_ _ornatum_ Mg. Most of the workers since then have confined their investigations to the female mouthparts. In this study the terminology adopted is mainly that of Nicholson (1945); however, that of Jobling (1927) has been used to some extent.

Hocking and Pickering (1954) in their study of the bionomics of _Simulium_ _vittatum_ Zetterstedt observed that most of the females were heavily engorged with nectar although they are known to be blood feeders. The feeding habits of both sexes of _Simulium_ (_Eusimulium_) _baisasae_ are unknown to the writer at the present time although it is probable that the female is a blood feeder.

Head.

The head of the simuliids is hypognathous in position, that is, the mouthparts are directed downward. The compound eyes occupy almost the entire lateral areas of the head. The frons, though almost obliterated between the antennae, is considered by Krafchick (1942) and Gibbins (1938) to be fused with the clypeus, but according to Snodgrass (1944) it is separated from the clypeus (Clp.) (plate III, fig. 2). Posteriorly, the head is made up of the occiput (Oc.) (plate V, fig. 8), postgenae (Pge.)

and postmentum (Pmt.). These sclerites surround the foramen magnum (For.). When seen from the front, the clypeus is a shield-like, convex sclerite below the bases of the antennae. The antennae are attached to the head capsule between the ventral margin of the vertex and frons. As seen from in front or behind the head of the female is almost spherical.

Labrum-epipharynx (plate II, fig. 1,4). Studies made by Jobling (1927) show that the labrum-epipharynx is a product of the fusion of those parts. It is suspended by a membrane from the head capsule. The sets of teeth on the median process (M.P.) of the labrum-epipharynx of the female are absent in the male. Gibbins (1938) strongly believed that the teeth serve as devices to anchor the labrum-ephipharynx in the punctured wound while in the act of feeding. On the distal margin near the outer extremities of the sets of teeth are fine setae.

There are four circular light spots. These are the sensory pegs (S.p.) of Nicholson (1945) on the broadened area where the three rods of the labrum are fused together. These sensory pegs are variable in position extending from the base of the labrum-epipharynx to the tip. Nicholson (1945), who made a careful study of these sensory pegs in Eusimulium dacotense D. and S. and Simulium venustum Say stated that there is a minute spicule at the center. The writer corroborates this view. In the male, the four circular spots are also present, but, in addition, there are two larger spots.

Mandibles. The mandibles (plate II, fig. 3) of the female are paired and each is a blade-like, heavily sclerotized structure. The bridge-like sclerotized strip of the mandible is called the "abductor of the mandible" by Gibbins (1938) in Simulium damnosum Theobald. Nicholson (1945) describing S. venustum Say proposed the term pseudotendon (P.M.) since "abductor of the mandible" connotes that the sclerotized structure is a muscle.

In the center of the anterior view of each mandible is a small elevated area which appears as a depression (D.) on the posterior side. This projection on the anterior side is best seen when the mandible is examined from the lateral view. In Culicoides pulicaris L., Jobling (1927) described this projection on the posterior side. As reported by Gibbins (1938), the mandibles of Simulium damnosum Theobald function in the same manner as a pair of scissors and that they lie right over left with the projections serving as the pivot. A close perusal of the figure shown by Gibbins (1938) shows them in the reverse position. However, Snodgrass (1944) states that the "mandibles overlap each, the left over the right", a view with which the writer entirely agrees. Since the apical margin of the mandible in the female is serrate, it is probable that it could be used to cut or puncture the skin. In the male the pointed tips of the mandibles are almost membranous and devoid of teeth and would appear to be very inefficient cutting or puncturing structures.

Cibarial pump (Cb.) (plate II, fig. 2,5). This structure has two circular spots on its medio-lateral areas. Nicholson (1945) reported that in Eusimulium dacotense D. and S. and Simulium venustum Say, each of these circular spots or sensory pegs contain two minute, dark-pigmented setae which he presumed to be sensory. These setae are on the inner surface making it possible for them to come in contact with food materials. Emery (1913) in his work on Simulium vittatum Zetterstedt, called these setae gustatory sensillae. To see these sensillae a dark phase contrast microscope should be used.

Maxillae (plate II, fig. 7,8). The maxillae are paired organs attached to the head at the antero-lateral areas of the postmentum (Pmt.) (plate V, fig. 8). Each consists of basal stipes (St.), galea (Ga.) and palps (Seg. 1,2,3,4). Krafchick (1942) considers the cardo and stipes to be fused. The shape of the galeae and number of recurved teeth vary according to species according to a report of the U. S. Army, 406th Medical Laboratory (1955).

Studies on the musculature in Culicoides pulicaris L. by Jobling (1927) reveal that the galeae move by protraction and retraction. Although the females of Simulium baisasae have never been observed to bite, the writer strongly believes that their type of mandibles and galeae are well adapted for inflicting wounds. In the male the mandibles and galeae are weakly sclerotized. The galeae taper acutely and are provided only with fine setae along their apical margins. Because of their structure they apparently could not be used effectively in biting.

The maxillary palps of both sexes are evidently four-segmented as a result of the fusion of the first two segments. Although the intersegmental suture is distinct, the first two segments are immovably joined. However, Tonnoir (1924), Snodgrass (1944) and Nicholson (1945) claim that there are five segments. On the other hand, two proponents, Smart (1935) and Krafchick (1942) maintained that there are four segments since the first two are partially fused. The dilated second segment bears internally a sensory organ (S.O.). A histological section of this organ was demonstrated by Nicholson (1945) to contain spatulate sensillae set in pits with short pointed setae between them. Jobling (1927) in his work on Culicoides pulicaris L. described these sensillae to be connected with the palpal nerve. The last segment of the palpus is narrow, but more than twice as long as any of the other segments. All the segments carry long setae.

Labium (plate II, fig. 9). According to Jobling (1927) the thick-walled theca (Thc.) is a result of the fusion of the palpigers. Two lobe-like structures form the labella (1 Lb., 2Lb.) at the distal extremity. Numerous long setae and minute spines cover the labella. There are a few on the theca. The free labella according to Jobling (1927) represent the modified labial palps. The ligula (Li.) has been described by Snodgrass (1944) as representing the glossae and paraglossae.

The structures of the male and female labium are essentially the same.

Thorax of the adult stage (plate V, fig. 9, 10, 11).

The thoraces of both sexes are peculiarly formed. The most striking feature about the mesothorax is the well developed scutum (Sct.) which is arched dorsally (more so in the male) and the much reduced prescutum (Psc.) which assumes a triangular outline at least in lateral view. From the lateral view the mesothorax has a large membranous field (M) in the pleura which divides the upper region of the episternum (Eps.) into anterior and posterior regions. A transverse suture has markedly separated the episternal plates of this segment.

Another remarkable feature is the presence of the pleural tuft (P.t.) of hairs. This upward but obliquely projecting group of hairs can be found on the mesoepimeron (Epm_2), in front of the posterior spiracle (Sp.2).

The triangularly-shaped sclerite above coxa 2 (Cx_2) is the divided mesosternum (S_2) of Malloch (1914). Along the posterior border and above the base of coxa 3(Cx_3) is a much reduced sclerite, the metaepimeron (Epm_3). The metaepisternum (Eps_3) is rather large and bears on its anterior margin, the posterior spiracle.

The prothoracic sternal region contains a longitudinally divided anterior presternal sclerite (Prt.), followed by a more or less triangular furcasternum (Fs). The large mesothoracic episterna (Eps_2) meet ventrally, thereby separating the connection of the furcasternum with the mesosternum. A closer view of the mesosternum shows that it is convex and longitudinally divided by a midventral suture (S.g.). The metasternum (Met.) is narrower and also divided by a midventral suture.

The general plan of the sclerites on the mesonotum and metanotum is relatively simple. In the mesonotum, the prescutum (Psc.) is broader than long. It is concealed in the dorsal view by the enlarged mesoscutum (Sct.). The slightly convex mesoscutellum (Scl.) is produced medio-posteriorly. On each of the latero-anterior margins of the mesoscutellum are depressed areas of the mesoscutum which are similar to the parascutellum described by Crampton (1919) in <u>Tipula</u> <u>spp</u>. Posterior to the mesoscutellum is a more convex, semi-circular sclerite, the mesothoracic postnotum (Pn_2). This sclerite is followed by a narrow metathoracic postnotum (Pn_3) which is constricted on the median line. The postphragma (Pph.), which is irregularly shaped appears to be slightly sclerotized.

Cervical sclerites (plage V, fig. 9, 11). Crampton (1925) who worked on the homologies of the thoracic sclerites of non-tipuloid nematocerous Diptera noted that the shape of the lateral cervicals (Lcs.) in the Ceratopogonidae closely resemble the Simuliidae. The lateral cervical sclerites are elongate, very slender and are produced ventromesally to meet the base of the ventral cervicals (Vcs.). These two sclerites are embedded in the membrane of the neck region. The lateral cervicals articulate with the two mesal protrusions along the dorsal third of the foramen magnum (For.) and prothoracic episterna (Eps_1). The slender ventral cervicals extend mesally meeting at the midventral region anterior to the presternum. These cervical sclerites act as supporting structures of the pendant head.

Studies were made by Martin (1916) on the origin of these cervical sclerites and their homologies. According to him these sclerites "may be either detached areas of the head or of the prothorax or of both". On the basis of homology Crampton (1917) also demonstrated that the cervical sclerites are distinct intersegmental plates between the labial segment and the prothoracic segment. These intersegmental plates, according to him, are homologous with the intersegmental plates of the mesothorax and metathorax.

Wings (plate III, fig. 4).

The anterior wing veins (C, Sc, R_1, Rs) are markedly thicker than the posterior veins (M_1, M_2, Cu_1, Cu_2, An_1, An_2). The radius and the simple, unbranched radial sector (Rs) run into the anterior margin to join the costa (C) and subcosta (Sc) which terminate before the wing apex. The medial veins (M_1, M_2) have a common short stalk with M_1 terminating approximately at the wing apex. A remarkable forked vein-like crease, the submedian fork (S.f.), is found between M_2 and Cu_1.

The basal one-fourth of the subcosta bears a row of five to six sensillae and two are found at the base of the radial sector. Along the axillary region are short, fine, delicate long hairs which can be seen on plate III, fig. 4.

Halteres (plate V, fig. 9, 10). These are knobbed organs immediately behind the base of the first pair of wings. The basal area is enlarged and slightly thickened. The stalk is short and straight terminating in a knob which is slightly cleft on its outward aspect. Pringle (1948) who studied the gyroscopic mechanism of the halteres of Diptera found that the end-knobs of the halteres are specialized structures for equilibrium control.

Abdomen of the adult stage.

The abdomen consists of nine visible segments. The first segment which can be easily confused as part of the thorax is expanded at the sides in the form of lamellae which are termed the basal scales (Abs.) (plate V, fig. 9) by most workers in this group. Malloch (1914) called this the "basal segment". It extends only on the dorsal and lateral parts of the abdomen. The lateral parts of the basal scale appear to be convex and their entire posterior margins are fringed with long hairs. This fringe of brown hairs is present in both sexes but much longer in the male, sometimes extending past the base of the third abdominal segment. Edwards (1915) was of the opinion that this basal scale represented the first abdominal tergite a peculiar characteristic of Simuiidae. This assumption is substantiated by Gibbins (1935) who found a small spiracle on both sides of the median area of the basal scale indicating that it is associated with the first abdominal segment.

In the male, sternites 2, 3, and 4 are light gray. With the exception of sternite two, the rest of the sternites have each a conspicuous semi-circular brown patch on their medio-ventral areas. These patches are provided with straight brown hairs directed ventrally. Longitudinal ridges, or fold-like wrinkles, extend throughout the entire length of the abdominal sternites.

Dorsally, the female has brown semi-circular patches on all tergites. These patches are absent in the male. The spiracles are closed only on the second abdominal segment. Although the tenth abdominal segment cannot be seen externally, it can be demonstrated in cleared specimens.

Male genitalia (plate III, fig. 6). The terminology of the male genitalia is confusing owing to the fact that many investigators have given their own terms to the structures. For this reason the proposed terminology of Freeman (1950) on the male genitalia has been used except for the terms coxite and style. The terms basistyle (Bs.) and dististyle (Ds.) of Stone (1955) appear to be more commonly used.

It has been shown by Gibbins (1935) that the ninth abdominal segment is entire; that is, it encircles the dorsal, lateral, and ventral surfaces of the abdomen. The mid-dorsal tergite is broadest and narrows gradually towards the mid-ventral line. Beneath this broad dorsal tergite lies the cloaca and the much reduced tenth abdominal segment. According to Freeman (1950) the ventral plate (V.p.) is possibly supportive and sensory in function. Posterior to the ventral plate are the two basistyles (Bs.). Ventrally, the basistyles are seen to be slightly flattened and with a cleft base. The tube-like dististyles (Ds.) are attached to the basistyles. A subterminal tooth-like structure is attached to each of the mesal margins of the dististyles. The bent parameres (P.) are provided with three large hooks and many smaller hooks which are difficult to count.

Female genitalia (plate III, fig. 5, 7). Considering the importance of the genital fork (G.f.) and spermatheca (Spt.) to taxonomy an attempt was made to dissect them out of the abdominal cavity. According to Smart (1935) the genital fork is a cuticular ingrowth at the external opening of the vagina. The arms of the genital fork are almost twice as broad as its rod. The spermathecal duct (Spt.) is terminally attached to the oval, dark brown spermatheca (Spt.). It was noted by the 406th Medical Group (1955) that the color and shape of the spermatheca and the attachment of the spermathecal duct is a supplementary index in separating some species of Prosimulium Roub., Cnephia End. and Simulium Latr. Under the high power objective (430x) spicule-like structures can be seen throughout the length of the spermathecal duct. The dome-shaped cercus (Cer.) is covered with a few long hairs. Ventrally it is twice longer than broad and laterally it is twice as high as long. The anal lobe (A.l.) appears twice as large as the cercus when seen laterally and more than twice as large when viewed ventrally.

External Morphology of the Larvae

Preserved specimens in 70 percent ethyl alcohol are light gray with the dorsal part slightly darker than the ventral part. Since the ventral area is lighter in color, the thoracic ganglia, silk glands and alimentary tract are visible when viewed with a stereoscopic microscope. However, some of the larvae have a dirty brown rust colored pattern scattered on the sides of the abdomen.

A fully grown larva is about 5.5 mm. long. The body is cylindrical in shape and slightly constricted in the middle. The posterior half of the abdomen is much larger than the anterior half, hence club-shaped. Johannsen (1903) counted 12 poorly defined segments excluding the head but a closer examination of the larvae of Simulium baisasae shows that there are only eleven segments. The spiracles in these larvae are closed. The conspicuously dark-colored pupal respiratory organ (P.o.) (plate IV, fig. 5) is visible in this instar.

Head.

The highly chitinous head capsule (plate IV, fig. 6) is cylindrical with its antero-dorsal surface projecting and sloping downward above the labrum. Laterally on each side of the center of the head capsule there are two closely set, black pigmented eye-spots (Ey.s.) (plate IV, fig. 5). Emery (1913) suspected that these were eyes or light receptor organs. Dorsally and ventrally the head capsule is devoid of pigment spots. On the postero-ventral margins are the throat pits (Th.p.). Sommerman (1953) in her work on black fly larvae identification, used the throat pits to mark the posterior limits of the throat cleft (Th.c.).

Cephalic fans (plate IV, fig. 9). These are peculiarly shaped organs situated in front of the antennae and lateral to the anterior end of the head. A short but stout stalk bears along its distal border a row of filaments (plate V, fig. 6) which when expanded is semicircular and fan-like in shape. As reported by Emery (1913), Puri (1925) and Wu (1930), the cephalic fans are used for the collection of food. Observations of the larvae in their habitat revealed that these fans open and close frequently. These observations were made possible by the use of a magnifying lens. In preserved specimens a closer examination of the cephalic fans showed that when they are closed the tips of their filaments come just to the mouth opening.

Antennae (Ant.) (plate V, fig. 7). The antennae of a fully grown larva are each composed of four segments. As reported by Johannsen (1903) and Malloch (1914) the fully grown larva has only three antennal segments. However, Puri (1925) and Edwards (1920) reported that there are four segments. The second segment bears at its distal extremity two short projections which are the sensory processes (S.p.). Puri (1925) believed that these sensory processes were supplied by a nerve arising from the antennal ganglion located at the base of the antennae.

Mandibles (plate IV, fig. 7). These are paired, highly sclerotized structures located below the cephalic fans. The mandibles move horizontally which can be demonstrated by moving them with a dissecting needle. These are attached to thickened areas of the epicranial plates. Each mandible is rounded on the lateral margin and almost rectilinear on the inner margin.

Maxillae (plate V, fig. 1). It appears that the maxillae are slightly sclerotized but strengthened by thickenings in certain areas. The maxillae are made up of a stout, fleshy basal piece and a single segmented palp (Mx.p.). Osten Sacken (1870) claimed that the palpus is two-jointed.

Hypopharynx and prementum (plate V, fig. 4). These are double plate structures forming the floor of the mouth cavity. They are weakly sclerotized, but transverse sclerotized structures and thickenings, on the lateral margins, along the ventral side of the prementum provide an excellent support for this combined structure.

Thorax.

On the lateral areas of the thorax (plate IV, fig. 5), are dark pigmented structures, the developing respiratory organs (P.o.). When dissected, the pupal respiratory organs are seen to be spirally coiled. They are used in associating the larva with the proper pupa. Other organs seen on the sides of the thorax were noted by Puri (1925) who called them imaginal discs of the future legs (L_1, L_2, L_3), wings (W.) and halteres (H.). The size, shape and relative position of these organs are illustrated in plate IV, fig. 5.

Abdomen.

The abdomen (plate IV, fig. 5,8), is eight-segmented with segments six and seven much arched dorsally but flattened ventrally. Anterior to the anus is the simple anal gill. This gill is a rectal evagination and can be dissected easily with an insect pin by making a transverse incision through the integument above the region of the anus. Removing the fecal material in the previously incised portion of the gut will expose the three hollow, finger-like processes of the anal gill. When retracted, this gill lies flat on the rectum, but when fully extended it curves upward and toward the head. The gill walls can be stained with aceto-carmine showing the large mononucleate cells.

Koch (1938) who conducted an experiment on Chironomus sp. and Culex sp. larvae concluded that the most important function of the anal gill was the uptake of chloride ions. As reported by Headlea (1906) the anal gill of Simulium pictipes Hagen serves both as a respiratory device due to the presence of blood and tracheae in this gill. It has been found by Puri (1925) that this gill is supplied with paired erector and retractor muscles. Osten Sacken (1870) regarded the anal gill as a tracheal gill, while Johannsen (1903) considered it to be a blood gill (devoid of trachea or provided with only a few small branches). Experiments conducted by Thorpe (1933) on Chironomus sp. and Simulium sp. revealed that the anal gill is not the site of active oxygen uptake. On the other hand, Miall (1903) pointed out the possibility of cutaneous respiration because the cuticle is richly supplied with a fine network of tracheoles. Wigglesworth (1933) reported that the thin-walled anal gill of culicid and some nematocerous larvae is of little significance in respiration but that gills are permeable to water and salts. Furthermore, water is continuously taken up as a component of the blood and eliminated by the Malpighian tubules.

The arms of the anal cross-piece (A.p.) are highly sclerotized. Stone and Jamnback (1955) used the characteristic shape of the anal cross-piece in separating Simulium decorum Walker, Twinnia tibblesi Stone and Prosimulium hirtipes (Fries).

The rows of hooks on the posterior crochet ring (P.c.) are arranged radially leaving a hollowed-out, sucker-like center. No doubt the function of these hooks is for attachment to objects in the substratum and possibly the sucker-like disc aids also in obtaining a firm attachment. According to Tonnoir (1924), however, the sucker-like disc cannot function as a sucker since there are no muscles connected to it. Nevertheless, Puri (1925) observed strong muscles attached to the center of the disc and also a salivary (sic) secretion which filled up the hollowed area of the disc. Along the ventro-lateral margins and anterior to the posterior crochet ring are two ventral tubercles (Vt.). The first instar larva has the same schematic plan of body parts as that of the fully grown larva except that the number of hooks per row and the number of radiating rows in the posterior crochet ring are reduced.

An attempt was made to correlate the four major categories of the larval developmental stages with body length, number of filaments of the primary cephalic fan, and the number of rows of hooks on the posterior crochet ring. These data were based on fifty individuals representing each developmental stage, that is first instar, medium, near fully grown, and fully grown.

It is doubtful if newly hatched larvae are included with the first instar category as employed by the writer. In some of the larval forms collected the egg burster was still present which indicates with certainty that they belonged to the first instar. Many larvae without the conspicuous egg burster were classified in the first instar because the histoblasts on the lateral areas of the thoraces could not be seen under the binocular microscope. For this reason there was a wide range of variation in length of the first instar category as presented in table I. In spite of variations in the number of filaments and rows of hooks in the four different larval groups, it is interesting to note that larval length is accompanied by an increase in the number of filaments and rows of hooks.

External Morphology of the Pupa

The entire length of the pupal respiratory organs is almost as long as the pupa (plate IV, fig. 1). The six long slender filaments of each respiratory organ (plate IV, fig. 2) and the mode of branching, which is a diagnostic character, is constant for all the pupae taken from the Molawin Creek. All the filaments are transversely sclerotized and the outer surface is roughened with microscopic protuberances. The cocoon (plate IV, fig. 4) is closely woven and with a thickened anterior margin. It is of a "wall-pocket" type with a medio-dorsal projection.

Ecological Notes of the Larval and Pupal Stages of *Simulium baisasae* Delfinado

Molawin Creek, from which the material was taken, is located at College, Laguna, Philippines. The creek is permanent with rocks, stones and other organic debris forming the substratum. The water is clear and cool, the source of which arises on Mt. Makiling. Two local branches of the creek served as study areas for observations and weekly collections of samples of the larvae and pupae. Random samples from populations were obtained at each weekly visit for a six-month period extending from October 1958 to March 1959.

The larvae and pupae were collected from trailing vegetation, submerged roots, floating leaves, twigs, rocks and stones in the creek. All three simuliid species, namely *Simulium* (*Eusimulium*) spp., *Simulium* (Simulium) *baltazare* Delfinado and *Simulium* (*Eusimulium*) *baisasae* Delfinado were often collected in association with each other, although generally, each species was found in habitats segregated from each other. It appears that each species has its own ecological requirements but further data are necessary.

In this preliminary study, it has been observed that larvae congregate more in places where the current is the strongest. Experiments conducted by Wu (1930) showed that larvae of *Simulium venustum* Say were still well established in a water current rate of about one foot per second. Hocking and Pickering (1954) suggested that the attachment of larvae is determined by a mechanical factor, that of "water velocity gradient conditioned by visual stimuli". The developing larvae are usually found in parts of the creek that are shallow, ranging from about 0.12 inch from the water surface to as deep as six inches. Larval attachment to the substrate is accomplished by means of a posterior crochet ring (P.c.) (plate IV, fig. 5). These crochets or hooks enable the larvae to retain their position in strong water currents. They prefer a clean substratum for attachment rather than those that are covered with growing algae or gelatinous, slimy deposits. The larvae are motionless and fixed in their location except when disturbed by some aquatic animals, like snails, in which case the larvae quickly move downstream attached to their silken threads. They can crawl back on the threads to their original place by using the mouthparts and crochet-armed thoracic proleg. Their movement can best be observed when placed in a petri dish filled with water, where they move about in a manner very much similar to a geometrid caterpillar.

The last instar larva spins a silken cocoon on the substratum in which pupation takes place. The three larval species have their own characteristic pattern and form of spinning cocoons, which greatly aids in species determination. The pupa breathes by means of its gill filaments, thus the water current helps in supplying the necessary dissolved oxygen not only to the larvae but to the pupae as well. The pupal filaments not protected by the cocoon extend out on the downstream side.

When there was a drop in the water level, there was no evidence that larvae were stranded on their places of attachment. They either moved downward by their characteristic looping movement or released themselves while attached to their silken threads and reattached in a much lower water level. Simulium baisasae larvae, collected from Molawin Creek, when placed in a petri dish filled with creek water, died in about 6 to 8 hours. This was possibly due to the lack of dissolved oxygen or the inability to take in food.

Examination of the gastral contents of the larvae showed that they feed on organic matter, diatoms and to a lesser extent small bits of crustaceans. Riley (1886) observed minute crustaceans in the digestive tract of simuliid larvae. Miall (1912), Emery (1913), Wu (1930) also found diatoms and particles of plant and animal tissues. According to Sommerman, Sailer, and Esselbaugh (1955) diatoms are natural enemies of black fly larvae.

Pupation of the species under consideration takes place in the same habitat as the larvae. The cocoon is spun by the mature larva on objects below the water surface. A general external lateral view of the mature papa is shown in plate IV, fig. 1. One can see the prominence of the convex pronotum and mesonotum which appear to be fused. When the imago is ready to emerge, the pupal cuticle splits longitudinally on the mid-dorsal surface of the thorax. The developing pupa lies within the cocoon with the head filaments and anterior half of the thorax exposed. Its respiratory filaments which are thin, cylindrical and flexible, freely float with the current. Pupae when first formed are of a yellowish brown color which later turns darker as the imago develops. When in their habitat, the pupa rest securely within this cocoon, whose pointed tip is directed up-stream while the open mouth is turned down-stream. Minute particles of debris are incorporated within the silken framework of the cocoon thereby adding strength to the silken material. A thinly woven floor is present only on the posterior two-thirds of the cocoon. The developing pupa is prevented from being dislodged by the current by anchoring its hooklets and spines to the dorsal and ventral walls of the cocoon. The shape and structure of the cocoon are fairly constant in Simulium baisasae and are of taxonomic value.

SUMMARY AND CONCLUSIONS

Larval, pupal and adult stages of Simulium (Eusimulium) baisasae were used in this study of external morphology. Larval and pupal samples were collected weekly from Molawin Creek, Laguna, Philippines for a six-month period extending from October 1958 to March 1959. Adult flies were reared from pupae placed in individual shell vials. Pupae taken from leaves below the water surface proved most satisfactory for rearing.

Adult and larval association was determined by comparing the respiratory filaments of the pupal exuvium of a reared adult with the pupal gill filaments of a full grown larva. The smaller larval instars were associated with the mature larvae using the characteristic features of their submental plates and the throat clefts as an index to classification. Most often the color patterns of the head capsule and the simple anal gill confirmed the association of the larval instars.

It has been found in this study that the six filaments in each pupal respiratory organ are constant in number. The branching pattern of the filaments is apparently satisfactory for separation of the pupa of Simulium baisasae from those of the other two species occurring in the same habitat. Also, the characteristic shape and structure of the cocoon aid in the separation of the pupa from the other two species.

As a result of this study the evidence shows that the mouthparts of male S. baisasae appear to be inefficient for biting, whereas, the female has mouthparts which have effective cutting or puncturing structures.

Sexual dimorphism in the adult stage is apparent. The male can be separated from the female by the shape of its head, in being holoptic, by the strongly convex thorax, by the characteristics of the mouthparts and general color pattern of the body.

The larval and pupal stages collected at a depth of 0.12 to 6 inches were found to spend their life stages in swift water currents but it would be desirable to investigate the current velocity that can be tolerated by these immature forms. The fragmentary information on these aspects serves to emphasize the need for detailed, long range investigation of other ecological factors.

The larvae attach themselves to stones, rocks, twigs, trailing vegetations and other similar objects below the water surface by means of their posterior crochet rings. Pupation takes place in the same habitat of the larvae.

This study indicates that the first instar larvae have the least number of filaments in the cephalic fan and the smallest number of rows of hooks in the posterior crochet ring, while the fully grown forms have the greatest number. Successive molts are accompanied by an increase in the number of fan filaments and number of rows of hooks in the posterior crochet ring. It is suggested that further studies on the different larval instars be made in relation to changes in number and shape of other external body structures.

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APPENDIX

PLATE I

Fig. 1. Lateral view of adult female. (22x)

Fig. 2. Lateral view of adult male. (20x)

Fig. 3. Prothoracic leg of female. (64x)

Fig. 4. Mesothoracic leg of female. (72x)

Fig. 5. Metathoracic leg of female, inner portion. (68x)

Fig. 6. Outer portion of basitarsus and second tarsal joint with hairs removed. (123x)

Bt. --------------Basitarsus

Cal. ------------Calcipala

Cl. --------------Claw

Cx. --------------Coxa

Emp. ------------Empodium

Fm. --------------Femur

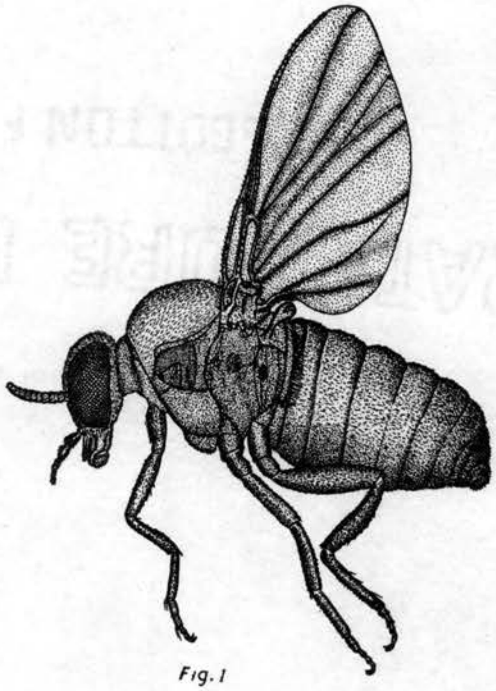
Ped. ------------Pedisulcus

T_1, T_2, T_3, T_4 ------Tarsal segments

Tb. --------------Tibia

Tr. --------------Trochanter

PLATE I



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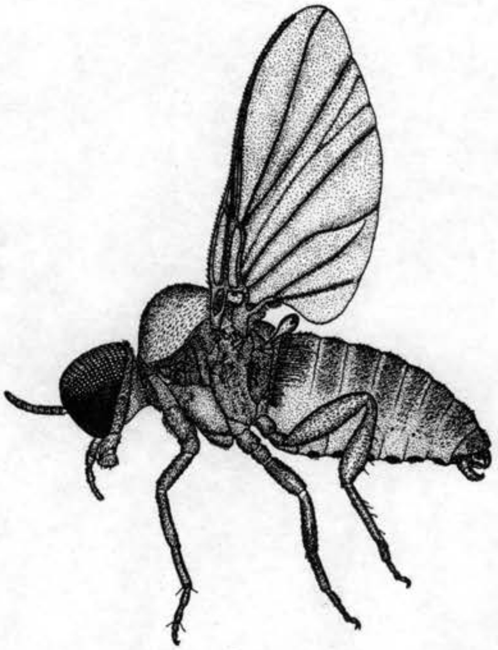

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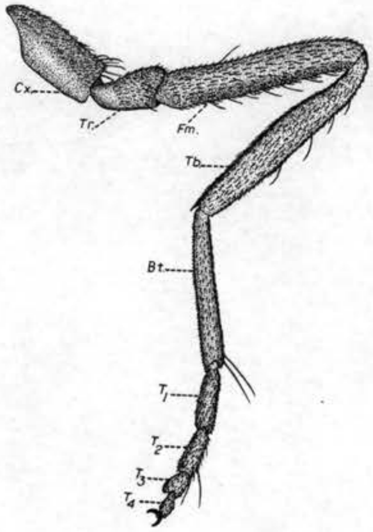

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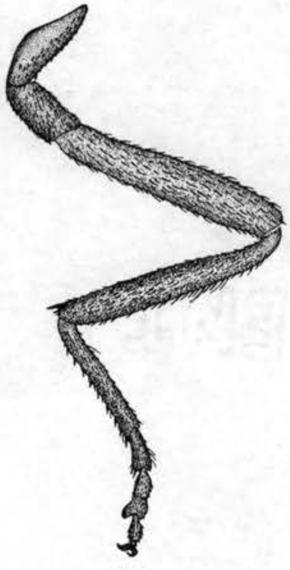

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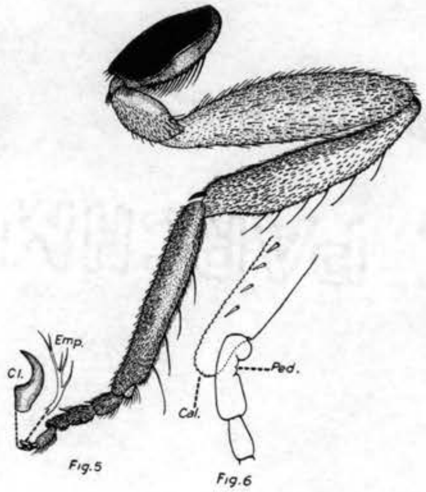


Fig. 5 Fig. 6

PLATE II

Fig. 1. Posterior view of labrum-epipharynx of female. (305x)

Fig. 2. Posterior view of hypopharynx, cibarial pump and pharyngeal pump of female. (117x)

Fig. 3. Posterior view of right mandible of female. (390x)

Fig. 4. Posterior view of labrum-epipharynx of male. (315x)

Fig. 5. Posterior view of hypopharynx, cibarial pump and pharyngeal pump of male. (143x)

Fig. 6. Posterior view of right mandible of male. (33x)

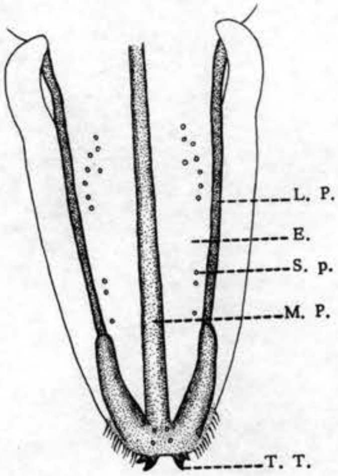
Fig. 7. Anterior view of right maxilla of male. (166x)

Fig. 8. Posterior view of right maxilla of female. (140x)

Fig. 9. Posterior view of labium of female. (188x)

Cb. ---------------- Cibarial pump
Cl. a. ---------------- Clear area
Co. ---------------- Cornua
D. ---------------- Depression
E. ---------------- Epipharynx
Ga. ---------------- Galea
Hyp. ---------------- Hypopharynx
Il.La. ---------------- Interlora of lacinia
1 Lb. ---------------- First segment of labellum
2 Lb. ---------------- Second segment of labellum
Li. ---------------- Ligula
L.P. ---------------- Lateral process of labrum
M.P. ---------------- Median process of labrum
Ph. ---------------- Pharyngeal pump
P.M. ---------------- Pseudotendon of mandible
S.D. ---------------- Salivary duct
Seg. 1,2,3,4 ---------------- Segments of maxillary palpus
S.O. ---------------- Sensory organ
S.p. ---------------- Sensory peg
St. ---------------- Stipes
Thc. ---------------- Theca
T.T. ---------------- Terminal teeth

PLATE II



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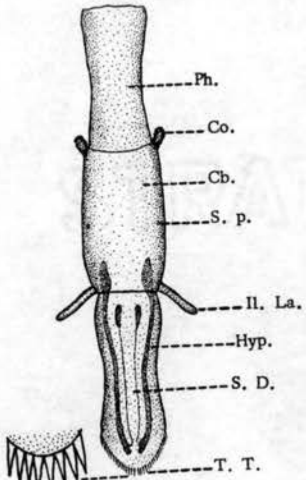

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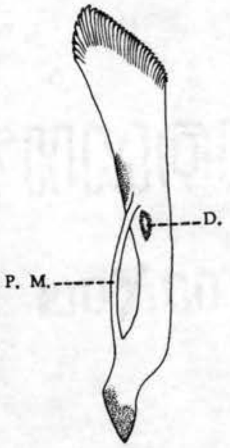

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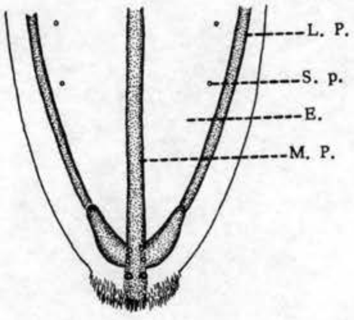

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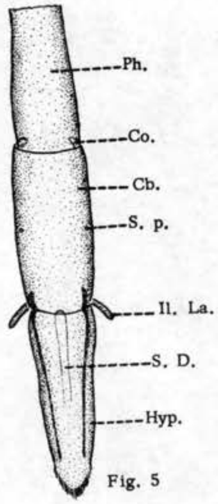

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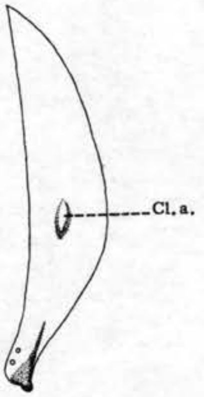

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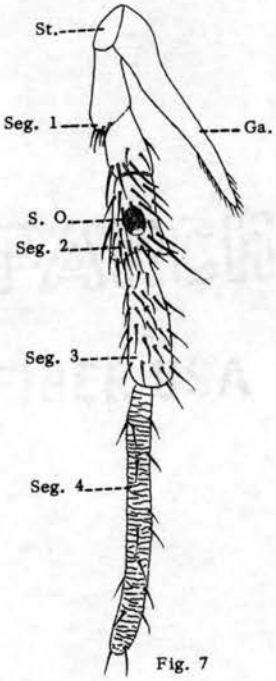

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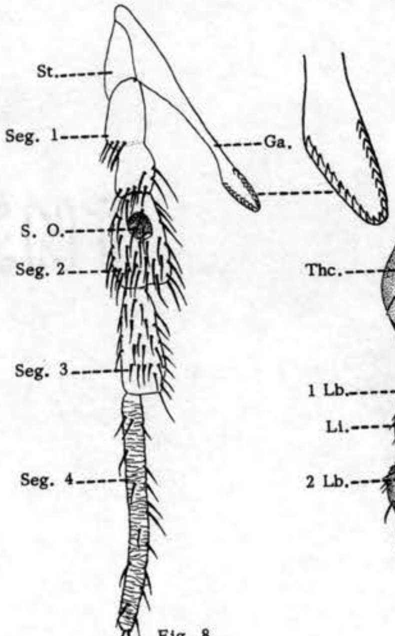


Fig. 8

Fig. 9

PLATE III

Fig. 1. Head of male, anterior view. (52x)

Fig. 2. Head of female, anterior view. (44x)

Fig. 3. Antenna of female. (200x)

Fig. 4. Right wing of female and detail of costa. (44x)

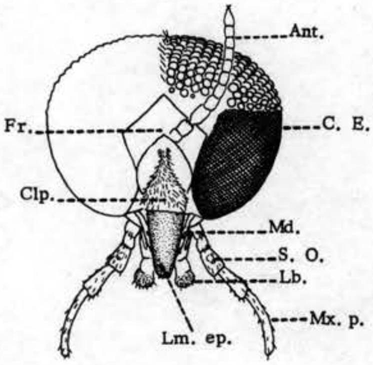
Fig. 5. Female genitalia, laterial view. (136x)

Fig. 6. Male genitalia, ventral view. Basistyle and dististyle, dorsal view. (310x). Paramere (470x). Ventral plate (575x).

Fig. 7. Female genitalia, ventral view. Anal lobe and cercus (180x). Genital fork (195x). Spermatheca (166x).

A.l.	Anal lobe
An_1, An_2	Anal veins
Ant.	Antenna
Bs.	Basistyle
C.	Costa
C.E.	Compound Eye
Cer.	Cercus
Clp.	Clypeus
Cu_1, Cu_2	Cubitus
Ds.	Dististyle
Fr.	Frons
Fr.o.	Fronto-ocular triangle
Ga.	Galea
G.f.	Genital fork
Lb.	Labium
Lm.ep.	Labrum-epipharynx
M_1, M_2	Media
Md.	Mandible
Mx.p.	Maxillary palpus
Ov.	Ovipositor
P.	Paramere
P.a.	Parameral arm
P.h.	Parameral hooks
R_1	Radius
Rs.	Radial sector
Sc.	Subcosta
S.f.	Submedian fork
S.O.	Sensory organ
Spt.	Spermatheca
Spt.d.	Spermatheca1 duct
V.	Vertex
St.v.	Stem vein
V.p.	Ventral plate

PLATE III



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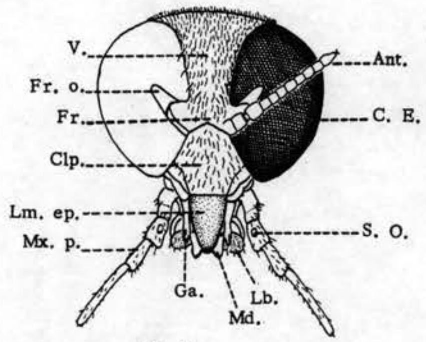

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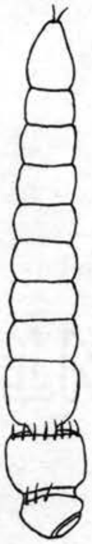

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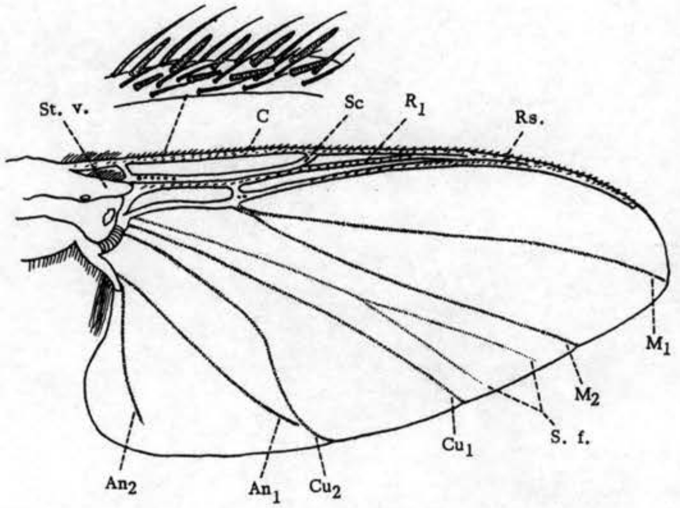

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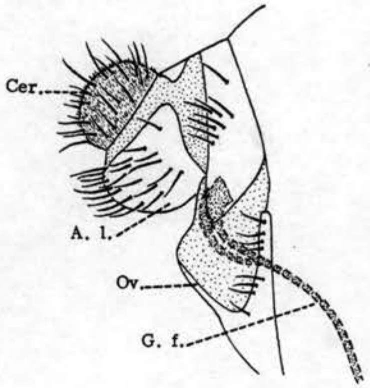

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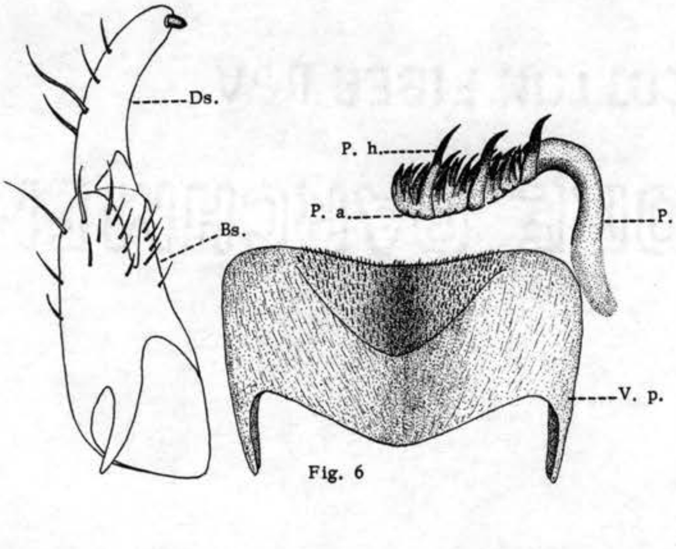


Fig. 6

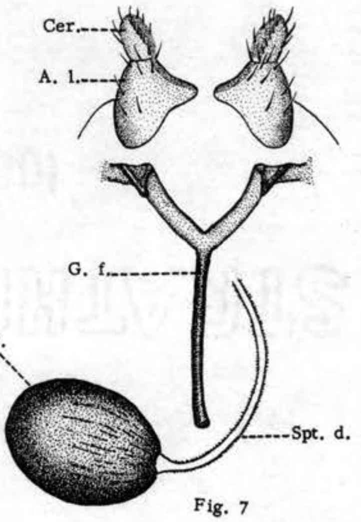


Fig. 7

PLATE IV

Fig. 1. Pupa, lateral view. (16x)

Fig. 2. Pupal respiratory organ and detail of filament. (73x)

Fig. 3. Dorsal (D) and ventral (V) longitudinal halves of pupa. (21x)

Fig. 4. Cocoon, lateral view. (14x)

Fig. 5. Lateral view of fully grown larva. (14x)

Fig. 6. Head capsule showing submentum and throat cleft. (100x)

Fig. 7. Dorsal view of the right mandible. (140x)

Fig. 8. Dorsal view of tip of abdomen. (67x)

Fig. 9. Ventral view of the cephalic fan when extended. (41x)

A.g.	Anal gills
A.h.	Apical hook
A.p.	Anal cross piece
Ant.	Antenna
C.f.	Cephalic fan
Ey.s.	Eye spot
F.	Filament
F.p.	Fronto-clypeal plate
H.	Imaginal disc of halter
L_1, L_2, L_3	Imaginal discs of the first, second and third legs.
L.t.	Lateral teeth
Md.	Mandible
M.p.	Medio-dorsal projection
M.t.	Median tooth
Mx.	Maxilla
P.c.	Posterior crochet ring
P.f.	Primary cephalic fan
Pg.	Proleg
P.o.	Developing pupal respiratory organ
R.h.	Retrorse hooklet
S.	Stalk of cephalic fan
S.f.	Secondary cephalic fan
Sm.	Submentum
Th.c.	Throat cleft
Th.p.	Throat pit
T.p.	Tooth-like process
T.s.	Triangular spine
T.t.	Terminal teeth
V.t.	Ventral tubercle
W.	Imaginal disc of wing

PLATE IV

Fig. 1

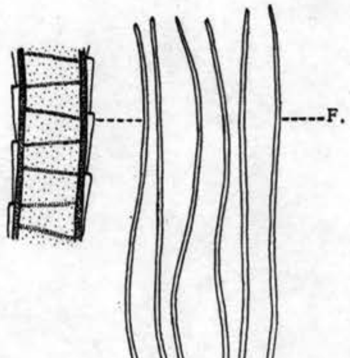


Fig. 2

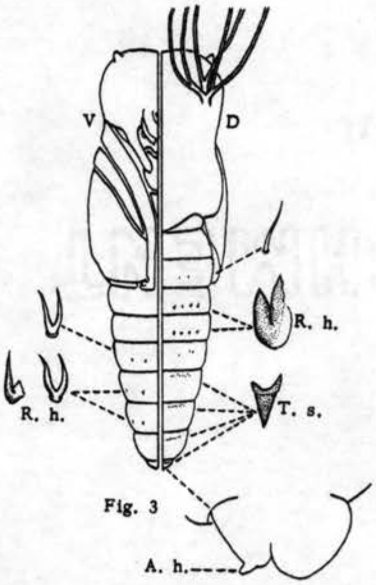


Fig. 3

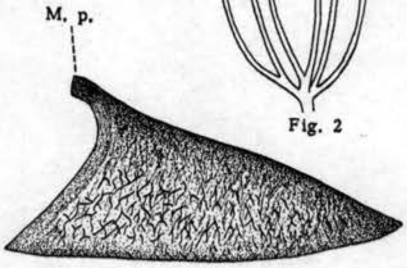
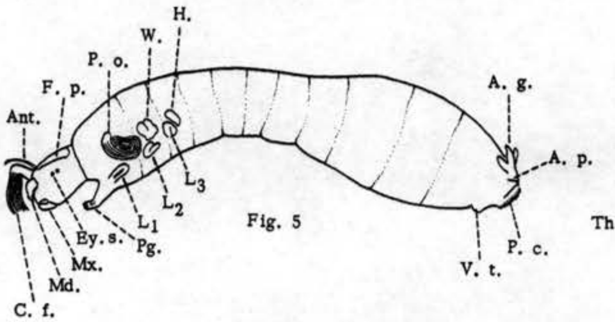


Fig. 4



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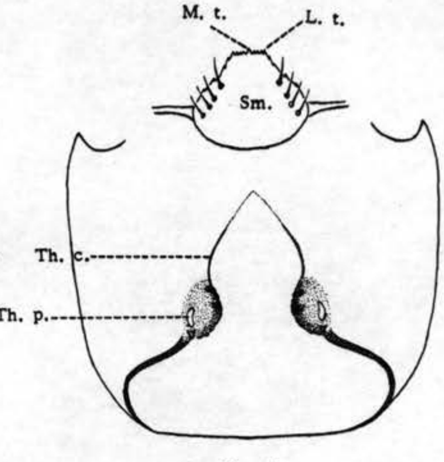


Fig. 6

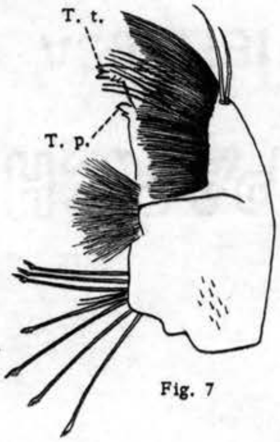
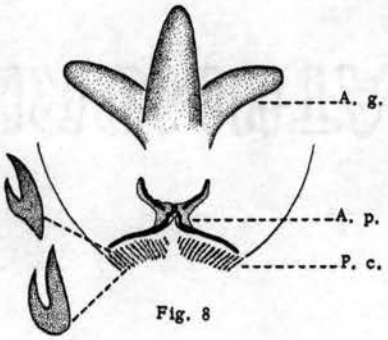


Fig. 7



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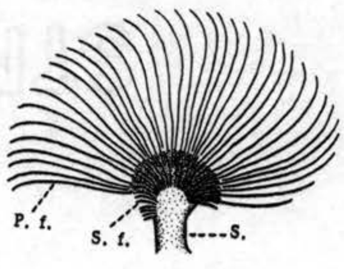


Fig. 9

PLATE V

Fig. 1. Dorsal view of the left maxilla. (380x)
Fig. 2. Dorsal view of the labrum. (146x)
Fig. 3. Lateral view of the thoracic proleg. (160x)
Fig. 4. Dorsal view of the hypopharynx and the prementum. (182x)
Fig. 5. Portion of head capsule of a first stage larva. (130x)
Fig. 6. A filament from the primary cephalic fan. (94x)
Fig. 7. Antenna of a full grown larva. (60x)
Fig. 8. Head of a female, posterior view. (44x)
Fig. 9. Lateral view of thorax and first abdominal scale of female. (43x)
Fig. 10. Dorsal view of thorax of female. (33x)
Fig. 11. Ventral view of thorax of female. (55x)

Abs. ———————— Abdominal basal scale
Ant. ———————— Antenna
A.p. ———————— Ascending process of prementum
Cx_1, Cx_2, Cx_3 ———— Coxae
Eb. ———————— Egg burster
Epm_1, Epm_2, Epm_3 ——— Pro-, meso-, metathoracic epimera.
Eps_1, Eps_2, Eps_3 ——— Pro-, meso-, metathoracic episterna.
Ey.s. ———————— Eye spot
For. ———————— Foramen magnum
Fs. ———————— Furcasternum
Gh. ———————— Group of hooklets
H. ———————— Halter
Ip. ———————— Internal process of the prementum
Lbl. ———————— Labellum
Lcs. ———————— Lateral cervical sclerite
Li. ———————— Ligula
Lp. ———————— Lateral plate on proleg
M. ———————— Pleural membrane
Md. ———————— Mandible
Met. ———————— Metasternum
Mx. ———————— Maxilla
Mx.p. ———————— Maxillary palpus
N. ———————— Pronotum
Oc. ———————— Occiput
P. ———————— Palp of prementum
Pm. ———————— Prementum
Pmt. ———————— Postmentum
Pn_2, Pn_3 ———————— Meso-, metathoracic postnota
Pph. ———————— Postphragma
Prt. ———————— Presternum
Ps_2, Ps_3 ———————— Meso-, metathoracic sutures
Psc. ———————— Prescutum
P.t. ———————— Pleural tuft
Pt.p. ———————— Posterior tentorial pit

S_2 —— Divided mesosternum
Scl. - Scutellum
Sct. - Scutum
Sg. —— Median sternal groove
S.h. —— Sensory hair
S.p. —— Sensory process
Sp.$_1$, Sp.$_2$ —— Anterior, posterior spiracles
St. —— Stipes
Thc. - Theca
Vcs —— Ventral cervical sclerite
Wp. —— Pleural wing process

PLATE V

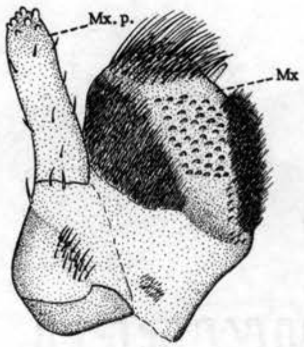


Fig. 1

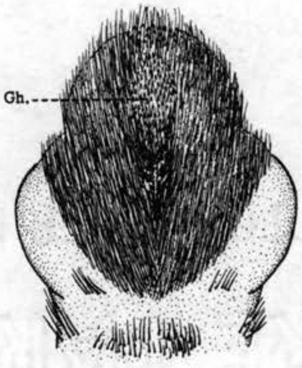


Fig. 2

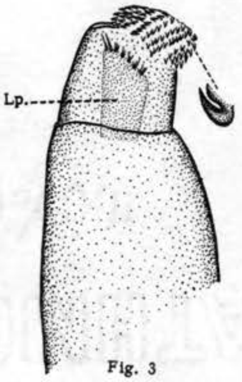


Fig. 3

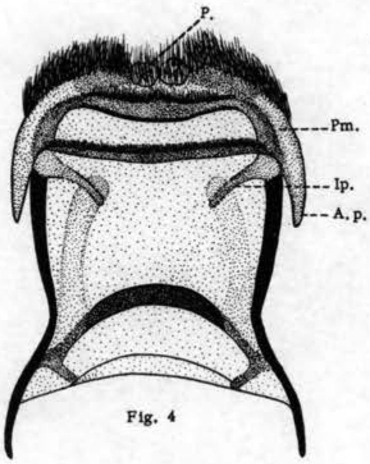


Fig. 4

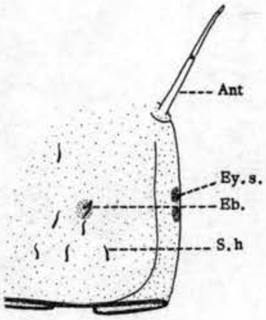


Fig. 5

Fig. 6

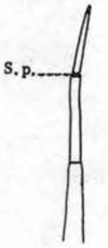


Fig. 7

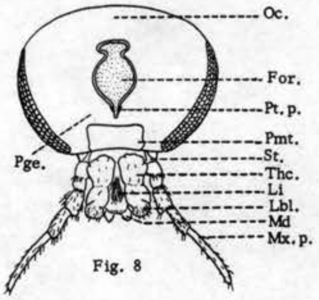


Fig. 8

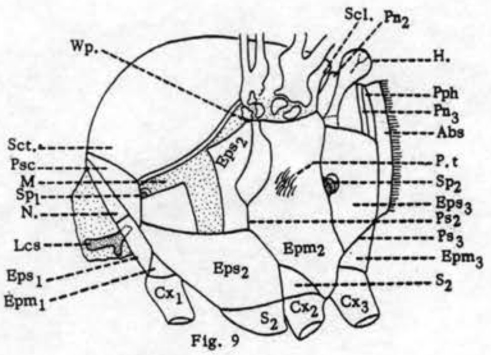
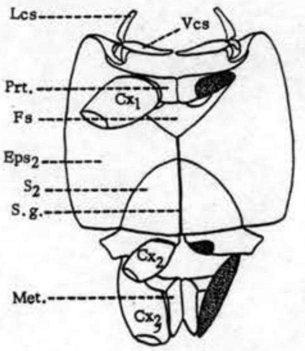


Fig. 9



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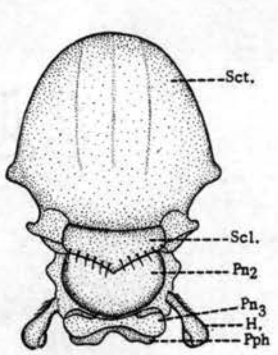

Fig. 10

VITA

Benjamin L. Cariaso

Candidate for the Degree of

Master of Science

Thesis: EXTERNAL MORPHOLOGY OF THE LARVAL, PUPAL AND ADULT STAGES OF SIMULIUM (EUSIMULIUM) BAISASAE DELFINADO (SIMULIIDAE, DIPTERA)

Major Field: Entomology

Biographical:

Personal Data: Born in Manila, Philippines, December 1, 1927, the son of Urbano B. and Anastacia L. Cariaso.

Education: Graduated from High School, Far Eastern University, Manila, Philippines, in 1949; received the Bachelor of Science degree from the University of the Philippines, with a major in Zoology, in May, 1954; completed requirements for the Master of Science degree in August, 1960.

Experience: Student Research Assistant, Department of Zoology, University of the Philippines, 1953. Research Assistant in Zoology, Department of Entomology, College of Agriculture, University of the Philippines, 1954-1956. Assistant instructor in Entomology, College of Agriculture, University of the Philippines, 1956-1959.

Organizations: Zoological Society, University of the Philippines; Entomological Society, University of the Philippines; Los Baños Biological Club; Sanborn Entomology Club.