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BIOLOGICAL STUDIES ON SOME SOUTH AFRICAN *CULICOIDES* SPECIES (DIPTERA: CERATOPOGONIDAE) AND THE MORPHOLOGY OF THEIR IMMATURE STAGES

By

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Blood-sucking midges of the genus Culicoides are found throughout the world, about 800 species having so far been described (Arnaud & Wirth, 1964). They are commonly known as "biting midges" in most parts of the world and as "sand-flies" in the central Americas and U.S.A. They are also called "punkies", "no-see-ums", "moose-flies", "gnats" and "jejenes" in other parts of the world (Wirth, 1952).

Their importance stems from the blood-sucking habit of the females. This has been the cause of extreme annoyance and irritation to man in many countries especially along the Atlantic seaboard of southern United States (Dove, Hall & Hull, 1932) and the Highlands of Scotland (Hill, 1947). In these instances retarded economic development and adverse effects on tourist trade have been attributed to these midges. Recently in Salvador, Brazil, widespread cases of a skin reaction called "dermatozoonosis" have been shown to be caused by the bites of C. paraensis (Goeldi) (Sherlock & Guitton, 1964, 1965).

Culicoides spp. have also been shown to be involved in the transmission of five species of filarial worms in man, horses and cattle; four species of protozoa in birds and monkeys; five virus diseases of sheep, horses, cattle and man (Kettle, 1965); and an allergic condition of horses known as Queensland Itch (Riek, 1954).

Except for the transmission of microfilariae, all the discoveries of diseases transmitted by Culicoides spp. were made during the last 22 years. Prior to 1944 Culicoides were mainly of taxonomic interest and studies on their biology were restricted to those species of nuisance value and to the transmitters of filarial diseases. Since Du Toit's discovery in 1944 that Culicoides spp. are the vectors of bluetongue virus in sheep and horsesickness virus, these midges have received increased world attention as potential disease transmitters, with a corresponding increase in studies on their taxonomy and biology.

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The identity of South Africa's Culicoides spp. was clarified by Fiedler (1951), and Caeiro described C. gulbenkiani in 1959, bringing the total to 22 species. Some of these species occur throughout Africa while one species viz. C. schultzei Enderlein is also found in India, Japan and recently in parts of Australia (A.L. Dyce, 1967 - personal communication).

To date little or no information exists on the biology and immature morphology of South African Culicoides spp. other than brief and scattered references from other parts of Africa, or incomplete pupal descriptions usually based on a single exuvia from which the identified adult had emerged. A thorough study of the many aspects making up the biology of a species as well as a complete morphological study of the immature stages of these species is needed, therefore. This is a very large task which cannot be accomplished in a few years. The following treatise will attempt to consolidate existing published and unpublished observations and will be supplemented with the writer's own studies. It is hoped that this study will contribute to a clearer understanding of the role played by these biting midges in the transmission of bluetongue and horsesickness viruses, and perhaps other diseases, the transmission of which has still to be explained satisfactorily.

Since the investigations cover two widely differing aspects, namely biology and morphology, they will be dealt with separately as Part I and Part II, respectively.

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PART I Biological Studies On Some South African *Culicoides* Species.

1. INTRODUCTION

The importance of biological studies on *Culicoides* species has recently been realized in most parts of the world especially those where *Culicoides* are of importance as vectors of disease organisms or where they are of nuisance value. In Africa, however, very few studies have been made on their biology. Carter, Ingram & Macfie (1920) were the first to make observations on the biology of African *Culicoides* species but until recently no other worthwhile attempts have been made.

In 1961, Nagaty and Morsy started taxonomic and biological studies on *Culicoides* in Egypt, but their observations have to date been limited (1961, 1962).

Since 1944 when Du Toit showed *Culicoides* spp. to be the vectors of bluetongue disease of sheep and African horsesickness, many observations have been made at Onderstepoort on the biology of these midges but these have never been published, and although seasonal variation in *Culicoides* numbers has been noticed yearly, it has until recently never been measured.

The following treatise on the biology of *Culicoides* species consists of a thorough study by the writer of five main aspects of *Culicoides* biology at Onderstepoort. These include studies on the life-cycle, breeding sites, time of flight, seasonal abundance and variation in abundance of various species throughout the year. Information gained from each of these aspects tends to supplement the others so that eventually an overall picture of the biology of *Culicoides* may be formed.

A list of *Culicoides* species found in South Africa appears below -

C. ravus De Meillon

C. engubandei De Meillon

C. schultzei Enderlein

C. nivosus De Meillon

C. cornutus De Meillon

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C. punctithorax Carter, Ingram and Macfie

C. nigripennis Carter, Ingram and Macfie

C. pycnostictus Ingram and Macfie

C. distinctipennis Austen

C. accraensis Carter, Ingram and Macfie

C. eriodendroni Carter, Ingram and Macfie

C. neavei Austen

C. onderstepoortensis Fiedler

C. bedfordi Ingram and Macfie

C. dutoiti De Meillon

C. babrius De Meillon

C. similis Carter, Ingram and Macfie

C. milnei Austen

C. pallidipennis Carter, Ingram and Macfie

C. hirtius De Meillon and Lavoipierre

C. magnus Colaço

C. gulbenkiani Caeiro

The species which received most attention were those which are the most abundant at Onderstepoort or which were reared in the laboratory viz. C. pallidipennis, C. pycnostictus, C. distinctipennis, C. nivosus, C. schultzei, C. milnei, C. magnus and C. bedfordi, although observations on some of the less abundant species have been included in studies on breeding sites.

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2. MATERIAL AND METHODS

(a) The Light Trap.

Most of the investigations depended upon a constant supply of wild-caught Culicoides midges. A suction-type light trap of similar design to the modified New Jersey light trap used by Du Toit (1944) was operated nightly over the last three years, 1963 to 1966 (Fig. 1). Insects were attracted to a 100 watt electric globe fixed beneath the 44 inch long cylindrical trap. A strong upward draught of air was created by a large extractor fan mounted in the top of this cylinder and all insects were caught in an organdie-covered cage interposed between the light and the fan. This cage had a wooden frame and was fitted on one side with glass or perspex through which the catch could be clearly seen and, using an aspirator, all Culicoides midges were removed via a sleeve over the inlet.

The trap was suspended three feet above the ground under a large "wag-n-bietjie" tree (Zizyphus sp.), the tree standing about 10 yards from a long open stable normally housing 35 mules and 11 horses at night. This position was found by Prof. R.M. du Toit (personal communication) to be the best site at Onderstepoort for trapping Culicoides. A similar trap situated only 50 yards from this position was found by the writer to catch only one third as many midges.

(b) Laboratory Studies on Life-cycle.

(1) Oviposition.

Live wild-caught Culicoides were fed on the shaven ear of a rabbit. The cage holding the midges was darkened except for a small area of light on the rabbit's ear. Since midges are attracted to light under these conditions, this served to concentrate their numbers in the vicinity of the ear. In this way approximately 50% would feed after two hours although many died, presumably due to desiccation and injury caused when large numbers are concentrated in a small area.

The living midges were then removed with an aspirator, anaesthetized with carbon dioxide (CO_2), and transferred to a sorting-chamber made of perspex and glass (Fig. 2). Here they could be kept anaesthetized for up to half an hour by a slow flow of CO_2 through the

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chamber. This chamber was designed to fit under a dissecting microscope so that the various species of Culicoides could be identified while alive. Engorged specimens of different species were then removed by aspirator to separate tubes for egg-laying.

These tubes were 2 x 1½ inches in size with a 3/4 inch layer of tamped-down moist cotton wool. Two layers of filter paper, exactly fitting these tubes, were placed on the cotton wool. This provided an even surface on which eggs could be laid and reduced the danger of wings becoming stuck to wet surfaces. The tubes were then held in a horizontal position and from one to twenty-five anaesthetized females of a single species were placed on the dry glass sides. This prevented their wings from adhering to the moist filter paper during their subsequent struggles on recovering from anaesthetization. At the same time the tubes were stoppered with cotton wool, and after recovery of the adults, about five minutes later, the tubes were placed erect and held in a room at 72°F for oviposition.

(2) Larvae and pupae.

Glass needle-boxes 3 1/4 inches in diameter by 2 1/4 inches deep with a loose glass top were used to rear the larvae of each species. Following the method of Jones (1960), sifted soil rich in humus and fresh bovine dung were thoroughly mixed in the ratio 2:1 to half-fill the container and was tamped down to provide a slope of about 45°. Tap water was added slowly until the medium was thoroughly wet and free water extended half way up the slope. In this way larvae could choose the correct amount of moisture to suit their requirements.

Eggs laid on the filter paper in the egg laying tubes were added to the larval medium by placing the filter paper discs with one edge submerged so that the paper remained very moist and thus assisted the newly emerged larvae in gaining the larval medium proper. It was seldom necessary to add more water as little evaporation took place. Sometimes an "oily" film would form on the surface of the water and this was removed with tissue or filter paper, immediately it was noticed.

Pupae were recovered from the medium by flooding which caused them to wriggle out of their positions in the medium and to float to the surface where they were

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removed by means of a suction tube. Many larvae were also recoverd in this way but for almost complete recovery of larvae it was necessary to make use of the method originally described by Ladell (1936) (cited by Kettle & Lawson, 1952). In this method the larval medium was first washed through 10 and 20 mesh sieves to remove very large particles and then through a 100 mesh sieve which retained all larvae and pupae. These were subsequently floated in a saturated solution of magnesium sulphate, removed to clean water using a suction tube, killed in hot water and preserved in 70 per cent ethyl alcohol for later morphological studies.

Observations on the life-cycle made while rearing the various Culicoides species, as well as certain related minor experiments, will be discussed later, but it must be emphasized that a thorough study of the life-cycle of the various Culicoides species was not the aim of this investigation.

(c) Breeding Sites.

To determine the situation of breeding sites, two methods were adopted. These consisted of the use of emergence cages placed over the suspected breeding area, and the removal of breeding medium to cages for emergence in the laboratory.

The emergence cage most commonly used was designed and used by Dr. C.G.H. Fiedler and Dr. R.M. du Toit in 1949 (unpublished data). It consisted of a wooden framework 14 x 9 x 9 inches covered with organdie. Two sheets of glass converged internally so that they formed a non-return route to the organdie-covered section of the cage. Midges were removed from this section through a sleeve at one end of the cage. The lower edge of the cage was fitted with a three inch metal "skirt" which could be pushed into the soil of the suspected breeding site. This anchored the cage and also prevented escape beneath the edges.

Experience with these cages has shown that they are not ideal as the glass non-return section is not altogether proof against return of the midges to the soil, and they are expensive and cumbersome. It is suggested therefore that future investigations on breeding sites should make use of the trap recently described by Davies (1966). Emergence cages placed in situ have the

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advantage of disturbing the breeding medium to the least possible extent but the disadvantage of having to be visited regularly. Furthermore, in winter few or no adults emerge so that it became essential to remove breeding medium to the controlled temperature of the laboratory. In the laboratory the medium was placed in a metal or glass container and covered with an organdie cage with perspex viewing window and sleeve for midge removal.

On occasions when a rapid check was required to determine whether a suspected breeding medium contained larvae, the method used by Bidlingmayer (1957) was adopted. This involved covering the surface of a sample of the medium with presifted washed sand to a depth of about one inch, adding water until the sand was just flooded, then leaving overnight for the larvae to make their way to the surface. The following morning the sand layer was quickly scooped out onto a sieve of a size sufficient to retain the sand but not larvae, and the larvae were washed out of the sand. The filtrate was then passed through a 100 mesh sieve and the contents of the sieve emptied into clean water. In this way the presence and approximate number of Culicoides larvae could be determined and the remainder of the sample could either be discarded or kept to determine the species present by adult emergence and identification.

(d) Hours of Activity.

Culicoides are seldom if ever seen during the day-time and appear to be active only at night as evidenced by large and regular light trap catches. To determine the period during the night when Culicoides are active, the cage in the light trap was replaced every two hours between 7 p.m. and 7 a.m. This was repeated on five nights in January, 1963. Each of the six two-hourly catches was counted in the laboratory the next morning.

On the nights analysed by this means, a thermohygrograph was operated a few feet from the light trap so that catches could be compared with temperature and relative humidity. Unfortunately it was not possible to record wind speed during these nights.

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(e) Seasonal Variation in Culicoides Abundance.

The Culicoides catches from the light-trap described above were recorded daily from 1963 to 1966. After removing the midges from the cage they were killed with ether vapour and unwanted insects were removed. If there were less than 1,000 Culicoides they were counted directly under a stereo dissecting microscope. If more than 1,000, the midges were weighed in grams to four decimal places and by comparison with the weight of a counted number of midges, normally 1,000, their numbers could be calculated. Early in the season (September to December) a high proportion of large species such as C. pycnostictus and C. distinctipennis are present, but as the season progresses and the percentage of small species (mostly C. pallidipennis) increases, the weight of 1,000 midges alters. This was taken into account by repeated checks of sample weights throughout the season.

Rainfall and temperature records for this period were obtained from the meteorological station at Onderstepoort in order to correlate seasonal variations in Culicoides abundance with weather conditions where possible.

From November, 1965 careful daily records were kept of catches from a similar trap at the farm "Kaalplaas" nearly four miles from Onderstepoort. In addition to being run as a farm, "Kaalplaas" is also a nature reserve of nearly 3,000 morgen of bush-covered hills and open veld. The trap was situated at a point where the light could be seen from the nearest hillock about 100 yards away and near some paddocks used on occasion for cattle or horses. These surroundings would appear to be more natural than those near the Onderstepoort light trap where buildings and camps housing large concentrations of fowls, horses, pigs, sheep and cattle are to be found. A comparison of catches from these two areas was most enlightening therefore.

(f) Seasonal Variation in Culicoides Species.

As previously mentioned there are approximately 22 Culicoides spp. present at Onderstepoort. These species are not all present at the same time of the year and although many species may be present throughout the summer season they are more numerous at certain times.

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To determine those times it was necessary to take regular samples of the light trap catches and to identify all the species present. A sample of between 500 and 1,000 specimens was preferred but if less than 500 then the entire catch was identified. The Onderstepoort catch was analysed at least twice a week between August, 1965 and May, 1966 (June and July having nil catches) and similar analyses were made of the "Kaalplaas" catches from November, 1965 onwards.

Identifications were made using the key drawn up by Fiedler (1951) and Caeiro's (1959) description of C. gulbenkiani.

3. DISCUSSION OF RESULTS

(a) Laboratory Studies on Life-cycle.

(1) Preoviposition period.

On numerous occasions when large numbers of eggs were required for attempts to start a colony, a thousand or more wild-caught midges, mostly C. pallidipennis, were fed on a rabbit's ear and provided with moist filter paper on which to lay eggs. At 72°F and about 45 per cent R.H. most eggs were laid three to four days after the blood-meal, while at 80°F eggs were laid after two days. This period was not determined for individual species.

(2) Eggs.

One batch of eggs is matured for each blood-meal taken (Kettle, 1962). By studying follicular relicts, Gluchova (1958) (cited by Kettle, 1962) has shown that up to four batches of eggs may be laid in nature by C. grisescens Edwards. The isolated specimens used in the present studies never laid more than one batch of eggs, however, since they either died immediately after egg laying or would not feed again.

Eggs of the various species varied in colour from light to very dark brown, but this colour difference was not consistent in every species. The eggs are normally laid in a double row resembling footprints. They are "sausage-shaped" being about 400 µ long and 50 µ wide.

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To determine the number of eggs each female is capable of laying in one batch, the eggs were dissected from females three days after engorging on blood, or the number of eggs oviposited by a female were counted and to these were added unlaid eggs removed by dissection in distilled water.

The number of eggs varied considerably between species but also between individuals of the same species as can be seen in Table 1.

Table 1. Number of eggs laid in a single batch by Culicoides spp.

Species	Number of females	Number of eggs	Average /female
C. pallidipennis	9	41-86	69
C. nivosus	9	126-264	162
C. distinctipennis	6	106-236	140
C. pycnostictus	3	91-126	110
C. milnei	1	93	93
C. schultzei	1	92	92
C. bedfordi	1	122	122
C. ravus	1	119	119
C. babrius	1	70	70
C. magnus	1	142	142
C. gulbenkiani	1	55	55

At a temperature of 70 to 75°F the incubation period for C. pallidipennis, C. distinctipennis, C. pycnostictus, C. nivosus and C. milnei was three days and four days for C. magnus. Most of these periods were determined for a batch of about 100 eggs laid by a single female except in the case of C. pallidipennis where thousands of eggs from hundreds of females were observed.

To test the effect of desiccation on Culicoides eggs a mixed population of wild-caught Culicoides were allowed to lay eggs on moist filter paper over a period of three days so that eggs of all ages from newly laid to those about to hatch were present. Initially very slow desiccation was attempted by slowly drying out a moist pad of cotton wool on which the filter paper with the eggs rested. This was done at 80°F and 85 per cent R.H. It was found, however, that eggs do not gradually collapse as the paper dries out, but remain turgid and

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normal until the paper is completely dry when they then collapse. Slow desiccation thus seems impossible as eggs apparently only require 100 per cent R.H. in order to remain turgid. Thereafter only the filter paper discs of eggs were dried at 85 per cent R.H. After drying for a few hours most of the eggs had collapsed. About one per cent were still turgid and these did not collapse on further drying. On immersion in water these turgid eggs hatched while those which collapsed never recovered. This suggests that in nature most eggs will survive desiccation while they are in a saturated atmosphere and should further drying take place a few eggs may still survive to give rise to a new generation when suitable conditions return.

It is appreciated that more detailed and exact studies such as those by Parker (1950) on the eggs of Scottish midges, are possible, but this was not the aim of the present study. Parker found that most species could not survive 48 hours desiccation and that the age of the egg also affected its ability to survive desiccation. Parker, however, made use of a calcium chloride desiccator which cannot be compared with the conditions of the above experiments.

Thousands of eggs laid over three days by wild-caught Culicoides spp., mostly C. pallidipennis, were immersed in water. Hatching started soon after immersion and continued until most of the eggs had hatched. Inundation of eggs in nature should therefore not adversely affect hatching.

Hundreds of similar eggs were kept on moist cotton wool in a closed petri-dish in the refrigerator at 44°F (6.5°C). Eggs were removed to room temperature at intervals and the numbers hatching noted. After seven days all eggs hatched, after 14 days only 43 per cent were still viable and after 37 days no eggs hatched. The exact time needed to prevent all eggs from hatching was not determined.

(3) Larvae.

The mixture of soil and bovine dung used as a breeding medium was not equally acceptable to all species of Culicoides. The four species, C. nivosus, C. distinctipennis, C. pycnostictus and C. bedfordi, appeared to find this medium suitable for development as many reached the pupal stage within 10 to 20 days of the eggs being placed on the medium.

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Only a few C. schultzei and C. milnei developed to the adult stage, while much difficulty was experienced in rearing C. magnus and C. pallidipennis to the pupal stage, only a few specimens being successful in reaching this stage.

Culicoides larvae are normally found in the surface layer of the medium where they "snake" in and out, or emerge and retreat into burrows as described by Linley (1966). All the species studied except C. bedfordi appeared to have this habit, and on studying the medium under a dissecting microscope with incident light, the larvae of C. distinctipennis remained exposed for from 10 to 20 seconds, appearing to bask in the heat of the light before reversing below the surface. Larvae of C. bedfordi on the other hand were never seen during their entire development and were only found on washing the medium through sieves and floating in $MgSO_4$ solution.

It is normally difficult to decide what the larvae are feeding on. Kettle (1962) reviewed the literature and suggested that those larvae with heavily built pharynges feed on algae, fungi and bacterial films, while those with light pharynges may feed on algae or detritus or be carnivorous. Thomsen (1937) states that Culicoides larvae are carnivorous and occasionally cannibalistic which agrees with her earlier statement that "the carnivorous larvae have a long narrow head, with the mouth-parts directed anteriorly, a weakly sclerotized labium, and a single-toothed mandible". During the present study fourth stage C. milnei larvae were twice seen devouring, tail first, live second instar larvae of the same species, and a number of alcohol preserved C. nivosus fourth stage larvae were found with half-eaten smaller larvae protruding from their mouths. Cannibalism does therefore definitely occur amongst the species studied.

To determine whether larvae can withstand periods of immersion, fourth instar larvae of all the species studied except C. schultzei were placed in petri-dishes of water and observed. Larvae of C. pycnostictus, C. nivosus and C. magnus survived immersion at 72°F for six days and longer, while those of C. distinctipennis, C. pallidipennis, C. milnei and C. bedfordi were still alive after 13 days and longer. Since these tests were not repeated the differences in the periods of survival

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could possibly be due to the physiological state of the larvae or some other factor. The main point illustrated by these tests, however, is that the fourth stage larvae and possibly other stages are capable of surviving long periods of immersion which are likely to occur in nature after periods of heavy rains. Kettle (1962) states that pupation does not occur if larvae are kept flooded. In the present study the odd larva of both C. pallidipennis and C. bedfordi pupated while submerged but died soon afterwards.

Media containing fourth instar larvae of C. nivosus, C. pycnostictus and C. milnei were placed in a refrigerator at 44°F (6.5°C) for from 10 to 14 days. On returning the media to room temperature development was completed in the normal time. No dead larvae were noticed and it would appear that the low temperature did not adversely affect the larvae but temporarily brought development to a complete standstill. Periods of refrigeration longer than 14 days were never attempted. This was found to be a very useful method of delaying development and can be compared with winter conditions in nature where over-wintering takes place in the larval stage. Overwintering appears to take the form of retarded development rather than a diapause since a sample of breeding medium brought into the laboratory at any time of the year will almost immediately give rise to adults.

At 70 to 75°F the period from egg-laying to the first adults averaged 20 days, with emergence extending from 11 to 66 days after oviposition. Larval periods for six species varied between seven and 25 days and the pupal period averaged four days. Males were normally the first to emerge. Under laboratory conditions therefore the minimum period for a generation to be completed is about 25 days (i.e. the period egg to egg). These times are very similar to those found by Jones (1964) for his colony of C. variipennis sonorensis Wirth & Jones. This life-cycle took about 24 days i.e. egg two, larva 15 (12 days and longer), pupa three, period prior to blood ingestion one, and preoviposition period three days.

(4) Pupae.

Pupation takes place in mud at the surface of the medium, the pupa manoeuvering itself into an upright position with the two respiratory horns protruding from

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the surface. On immersion most pupae wriggle free of the medium and float to the surface of the water where they hang from the horns. C. pallidipennis pupae, however, were the only ones which did not float to the surface, but after freeing themselves from the immersed substrate they lay loosely on its surface where they died within two days at room temperature. Immersed in water at 6.5°C they remained alive for more than six days but died within one day after removal to room temperature.

Dyce & Murray (1966) described three distinct patterns of pupal behaviour on immersion. Some species float to the surface and are unable to submerge. This is the case in all the South African species studied except C. pallidipennis. Other species repeatedly rise to the surface and then sink. In the third type of behaviour the pupae do not float but on immersion they actively burrow into the substratum. The behaviour of C. pallidipennis warrants it being placed in a fourth group since it does not float or burrow but lies loosely on the substratum until it drowns.

Cannon & Reye (1966) have found a similar type of behaviour in the Australian species C. brevitarsis Kieffer, but do not state whether immersion adversely affects this species. It is of interest to note that the larvae of this species were found in the moist lower regions of fairly dry cow pats. This type of pupal behaviour may therefore indicate a more terrestrial type of development.

(5) Adults.

Adults emerge from the pupal case by pushing forward the "operculum" and splitting the thorax dorsally for part of its length. Emergence can take place from floating pupae (except in C. pallidipennis) or from the surface of the larval medium. The adults are able to walk on water but wing surfaces must not touch the water surface as they appear to adhere to wet surfaces by surface tension. The body surface of the adults is rendered water repellent by a covering of fine hairs.

The life span of the adult in the field is unknown but newly emerged adults of the species studied survive from two to three weeks in the laboratory if provided with 10 per cent honey water and a relative humidity above 50 per cent. Laboratory reared adults would not

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feed on rabbits' ears or mate, however, so that it has been impossible to establish a colony. To date only Megahed (1956), Jones (1964) and Hair & Turner (1966) have managed to maintain Culicoides colonies. Megahed maintained a strain of C. nubeculosus (Meigen) for six years before it weakened and died out, while Jones has maintained a strain of C. variipennis sonorensis since 1957. The colony of C. guttipennis (Coquillet) established by Hair and Turner is only one year old. Establishment of a colony apparently depends upon the selection of a suitable strain from the offspring of many thousands of wild-caught midges.

(6) Conclusions.

These laboratory observations on the life-cycle of some of our Culicoides species may provide some information as to the ecology of these species.

The most important observation is the fact that all larvae are good swimmers and that only C. pallidipennis pupae do not float and soon drown. This means that the inundation by heavy rains of a breeding site common to a few species will affect them differently. In the larval stage they will all survive and will be able to swim to drier parts. If in the pupal stage, however, C. pallidipennis will drown while the pupae of the other species will float and give rise to adults. These adults will then be in a position to mate and lay eggs in the new breeding grounds created by the heavy rains. Inundated C. pallidipennis larvae will be forced to wait for the breeding site to dry out somewhat before further development, pupation and adult emergence can take place. This probably explains the low catches of C. pallidipennis during periods of good rain and their increase in numbers during drier periods which follow. The correlation between Culicoides catches and rainfall will be discussed later.

Another important conclusion which can be made from the study of the life-cycle is that a number of generations can be completed during the summer. Theoretically, according to developmental periods in the laboratory, this should amount to about one generation a month.

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(b) Breeding Sites

Culicoides breed in a variety of situations ranging from rot-holes in trees to manure and manure-polluted mud at the edge of pools. Kettle (1962) reviewed larval habitats for Culicoides in the world and concluded that "the larvae are neither genuinely aquatic nor terrestrial, but occupy the ecotone between, occurring in very wet soil". Very little is known of the breeding sites of Culicoides in the Ethiopian region. Carter et al. (1920) found Culicoides breeding in the following situations :-

C. accraensis - rot-holes in flamboyant tree, Cynometra sp., Eriodendron sp. and other trees.
C. inornatipennis Carter, Ingram and Macfie - rot-holes in Eriodendron sp. and in the stumps of banana plants.
C. clarkei Carter, Ingram and Macfie - rot-holes in Cynometra sp. and Eriodendron sp.
C. similis - bottom of waterlogged canoes.
C. schultzei - bottom of waterlogged canoes, in mud around puddles at a "stand-pipe" or tap and at washing place in the backwaters of a river.
C. eriodendroni - rot-holes in stump of Eriodendron sp. and mango tree.
C. punctithorax - as for C. eriodendroni
C. confusus Carter, Ingram and Macfie - rot-hole in Eriodendron sp.
C. nigripennis - rot-hole in mango tree.

De Meillon (1936, 1937) found C. meeserellus (syn. C. pycnostictus) in a rot-hole in a papaw tree; C. nivosus, C. pycnostictus, C. cornutus and C. schultzei in the mud around rain water pools; and C. engubandei in a rock pool. Fiedler (1951) found C. engubandei and C. onderstepoortensis in the mud at the edge of pools.

Since the early 1940's when Du Toit showed Culicoides to be the transmitters of bluetongue and horsesickness, searches have been conducted at Onderstepoort to discover the breeding places of these midges especially C. pallidipennis, which accounts for up to 90% of the light trap catches in summer. A thorough search was undertaken by Dr. R.M. du Toit and Dr. O.G.H. Fiedler in 1949-1950 (unpublished data). They started by taking samples of suspected breeding areas and recording emergence in the laboratory. Between 16th March and 10th October, 1949 they collected

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36 samples of mud from the edge of a small water course (Newtown spruit) leading into Bon Accord dam near Onderstepoort, from around the dam itself and from numerous other water furrows. Their best results are summarized in Table 2.

Table 2. Summary of Culicoides emerging from mud samples from Newtown spruit.

Species	Date						
	16.3.49	18.3.49	7.6.49	7.6.49	11.7.49	11.7.49	Total
C. pycnostictus	43	7	43	431	137	273	934
C. cornutus	33	-	3	8	28	51	123
C. nivosus	56	-	4	29	20	9	118
C. schultzei	6	2	6	25	19	10	68
C. distincti-pennis	-	-	-	20	19	-	39
C. ravus	15	2	-	18	-	2	37
C. pallidi-pennis	-	3	-	-	-	-	3
C. similis	-	2	-	-	-	-	2
Total	153	16	56	531	223	345	1,324

Table 2 shows C. pycnostictus to be predominant with C. pallidipennis and C. similis very rare. The Table also shows that larvae are present in July; further evidence that Culicoides overwinter in the larval stage.

During the first three months of 1950 Du Toit and Fiedler used emergence traps. They showed that small numbers of C. pycnostictus were breeding in moist ground covered by short grass on the eastern side of Bon Accord dam when 23 adults were caught in 23 trap nights. On 27th January, 1950 one trap caught 33 C. pallidipennis over 8 nights from a swampy kikuyu grass-covered area at a leaking cement dam at "Kaalplaas". Thereafter 9 to 12 traps were used per night on a large kikuyu grass-covered area at Onderstepoort, part of the area being under water. Over 13 nights from 24th February to 20th March very good catches of C. pallidipennis were made, the catch being as follows :-

C. pallidipennis	661
C. nivosus	182
C. distinctipennis	58
C. pycnostictus	33
C. schultzei	24

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It is important to note that only the traps placed over swampy kikuyu grass-covered areas yielded C. pallidipennis.

The writer repeatedly tested similar situations between 1963 and 1965 but was never able to trap more than the odd adult of this species. These odd specimens were also recovered from very moist areas covered with kikuyu grass and from the banks of the Apies River. The other species mentioned in Table 2 were also found except C. cornutus which has never again been seen although so abundant in 1949. Every conceivable breeding site was investigated ranging from various types and ages of manure, algae in water troughs, compost, stagnant bogs, edges of rivers, lucerne fields, etc. Those samples which yielded fair numbers of adults are given in Table 3. Adults emerged from these samples over a period of from 13 to 22 days at ± 75°F.

It is a mystery why C. pallidipennis which is so abundant in the light trap (Table 7) cannot be found breeding in large numbers in nature. Jones (1961) has suggested that "A preponderance of one species in light traps is often a result of low-density breeding over an extensive area". This may well be the case with C. pallidipennis. Evidence was forwarded earlier that C. pallidipennis may develop in much drier habitats than commonly expected for Culicoides midges. A thorough examination of such sites may eventually reveal the breeding place of C. pallidipennis.

(c) Hours of Activity.

Kettle (1962) reviewed observations throughout the world on the time of flight of Culicoides species and concluded : "Most Culicoides are crepuscular, showing great activity at dawn and dusk. Most crepuscular species, except C. grahamii Austen, continue to be active throughout the night, although they are less abundant after midnight, and they also bite during the day on calm, dull days".

Reuben (1963) studying C. impunctatus Goetghebuer in Scotland used a suction trap and showed these midges to be active throughout the day but most active at night. Kitaoka & Morii (1964a) compared meteorological conditions with light trap catches of six Japanese Culicoides species. The different species varied in their time of flight, some being more abundant just after sunset and near sunrise, while others were present

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Table 3. Summary of Culicoides emerging from mud samples from Onderstepoort area.

Date	Suspected breeding site	Culicoides species							
		nivosus	pycnostictus	distinctipennis	schultzei	pallidipennis	milnei	neavei	similis
16.7.63	Stagnant mud at bog near transport stable	6	83						
22.7.63	Stagnant mud in kikuyu field	20	9						
29.1.65	Mud from edge of slime-covered pool in dung-polluted paddock	680	21	2	1				
25.3.65	Dung-polluted mud at leaking water trough	1	44	3	1				
1.7.65	Mud from water-level of Apies river	13	51	11		3			
1.7.65	Mud from swampy kikuyu-covered area	4	7	21		2	2	1	
1.7.65	Mud from swampy veld-grass covered area near river	74	200	45	1	3	2	12	11
Total		798	415	82	3	8	4	13	11

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throughout the night. Changes in temperature, humidity, cloudiness and rain did not affect catches directly but strong wind and low temperatures appeared to reduce activity.

In South Africa Culicoides are seldom noticed biting man or animals during the day-time and Du Toit (1944) only noticed their presence at Onderstepoort on operating a suction-type light trap for the first time. The time of flight, however, was unknown. The results of an analysis of two-hourly catches during five nights in January, 1963 at Onderstepoort, are given in Table 4.

Table 4. Comparison of 2-hourly light trap catches with temperature and humidity.

2-hourly periods	Date					
	5.1.63	11.1.63	17.1.63	22.1.63	25.1.63	Mean
	Number of Culicoides					
7 - 9 p.m.	605	281	747	138	108	378.8
9 - 11	3,873	183	7,819	3,509	43	3,085.4
11 - 1 a.m.	2,677	271	3,864	4,391	318	2,304.2
1 - 3	7,693	5,930	1,739	5,566	1,745	4,534.6
3 - 5	1,658	6,533	706	4,337	2,272	3,101.2
5 - 7	9	34	3	37	25	21.6
	Mean Temperature (^{o}C)					
7 - 9 p.m.	22.8	24.7	28.6	30.0	24.7	26.1
9 - 11	18.9	21.4	26.7	27.5	21.9	23.3
11 - 1 a.m.	15.8	19.4	25.0	26.1	18.9	21.1
1 - 3	13.6	18.6	23.6	25.8	18.9	20.1
3 - 5	15.6	17.5	21.7	25.0	18.9	19.7
5 - 7	17.8	17.5	20.6	24.4	18.9	19.8
	Mean Relative Humidity (per cent)					
7 - 9 p.m.	41.0	57.5	57.5	60.0	73.5	57.9
9 - 11	53.5	70.0	70.5	76.5	89.5	72.0
11 - 1 a.m.	65.0	77.0	79.0	80.5	97.0	79.7
1 - 3	75.0	78.5	82.5	77.0	96.0	81.8
3 - 5	82.0	82.5	87.5	78.5	89.0	83.9
5 - 7	79.5	80.5	85.5	79.5	81.5	81.3

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In January the sun sets at about 7.00 p.m. and rises at about 5.25 a.m. This means that at least one hour of complete darkness will remain in the period 7 to 9 p.m. but that it will already be light by 5 a.m. Catches made during the first period (7 to 9 p.m.) may still be compared with later catches, but those between 5 and 7 a.m. must be ignored and are possibly due to Culicoides in the vicinity of the trap being accidentally sucked in by the strong updraught.

The results show that Culicoides (mostly C. pallidipennis) had no single peak hour of activity but that this varied nightly between 9 p.m. and 5 a.m. The peak period was not correlated with temperature or humidity but appeared to be influenced by some factor not measured such as wind, heavy rain, etc. It may be noted that temperatures during the period of the study ranged from 13.6°C to 30.0°C (56.5°F to 86°F) and that Culicoides were plentiful at temperatures throughout this range. Relative humidity ranged from 41.0 per cent to 97.0 per cent and many midges were caught while the humidity was 53.5 per cent (5.1.63). Although catches were always poor between 7 and 9 p.m. this could not be attributed to low humidity alone as it was nearly always above the 53.5 per cent figure mentioned earlier. However, when low humidity (50 to 60 per cent) and high temperatures (above 22°C) occurred simultaneously, low catches were always the result. These conditions were found during the 7 to 9 p.m. catch period.

(d) Seasonal Variation in Culicoides Abundance.

Kettle (1962) has shown that in temperate climates there may be only one or two generations in a year while in the tropics adult Culicoides can be present constantly. In temperate climates a peak in adult numbers probably represents the completion of a generation while Kettle found that seasonal fluctuations in the tropics could be related to tides or rainfall depending of course on the species concerned.

The climate at Onderstepoort borders on sub-tropical except that frosts may occur between May and August. The average catch per night on which the light trap was operated is recorded in Table 5 for each half-month for the period June, 1963 to May, 1966. A summary of temperatures and rainfall over the same period is given in Table 6. These records are expressed in graph form in Figure 3.

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Table 5. Seasonal variation in light trap catches of Culicoides (expressed as catches per trap night).

Month		Onderstepoort						Kaalplaas	
		Nights	1963-1964	Nights	1964-1965	Nights	1965-1966	Nights	1965-1966
June	1*	9	0	0	-	11	0	-	-
	2	10	7	7	0	11	0	-	-
July	1	10	17	9	0	10	0	-	-
	2	12	8	12	0	11	0	-	-
Aug.	1	11	17	10	1	10	0	-	-
	2	11	121	11	22	12	76	-	-
Sept.	1	7	777	10	659	10	706	-	-
	2	10	968	11	519	11	1,400	-	-
Oct.	1	9	1,391	11	1,265	9	342	-	-
	2	10	2,719	8	144	10	1,214	-	-
Nov.	1	9	6,297	8	1,812	11	1,510	-	-
	2	10	1,620	8	1,899	9	2,117	5	424
Dec.	1	7	6,882	8	1,528	5	3,526	5	266
	2	7	3,642	8	1,085	10	3,769	4	111
Jan.	1	9	3,054	9	2,780	8	6,186	6	171
	2	12	3,637	10	30,063	8	1,912	5	197
Feb.	1	7	11,262	10	10,706	9	3,772	9	390
	2	7	15,089	9	13,762	8	9,680	9	2,154
March	1	9	16,176	11	17,796	10	12,290	9	443
	2	8	23,299	11	12,025	11	43,156	11	1,320
April	1	0	-	10	3,527	9	9,050	6	945
	2	8	1,920	9	982	9	8,476	10	426
May	1	7	2,050	9	2,654	10	5,733	10	123
	2	7	571	9	409	10	138	9	56

* Refers to first and second halves of month.

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Table 6. Weather records for Onderstepoort.
June, 1963 - May, 1966.

Month		Mean Temperatures (°C)						Rainfall (mm.)		
		Maximum			Minimum			1963-1964	1964-1965	1965-1966
		1963-1964	1964-1965	1965-1966	1963-1964	1964-1965	1965-1966			
June	1*	19.8	21.9	18.4	0.7	1.0	1.8	34.0	0	0
	2	18.4	15.5	19.0	2.5	-3.7	0.8	19.0	0	0
July	1	17.1	18.4	19.7	2.0	-2.1	0.4	10.5	0	0
	2	20.8	20.6	20.5	0.5	-1.8	2.8	0	0	0
Aug.	1	21.4	21.0	23.7	0.6	0.8	4.4	0	0	0
	2	25.1	24.8	25.3	2.4	5.7	7.5	0	0	1.5
Sept.	1	27.4	25.9	26.9	6.8	7.7	5.7	0	13.0	0
	2	29.6	29.7	26.4	8.8	10.7	9.6	0	0	2.5
Oct.	1	30.1	28.6	24.3	11.5	14.0	8.0	20.7	11.7	12.0
	2	27.2	24.3	30.5	12.9	14.2	10.2	27.6	137.5	1.2
Nov.	1	28.6	30.0	27.3	14.4	15.7	14.3	82.6	28.5	60.2
	2	27.2	27.8	29.0	16.5	14.0	15.3	29.5	14.0	58.2
Dec.	1	29.0	27.6	30.9	15.1	16.8	15.5	44.3	59.0	20.0
	2	32.2	26.1	32.4	13.6	14.9	16.2	0.7	106.0	40.7
Jan.	1	29.7	28.4	34.7	15.3	16.7	17.8	103.0	62.5	10.0
	2	28.8	30.3	30.2	15.7	17.5	17.9	98.5	14.0	129.3
Feb.	1	30.4	31.4	26.4	15.9	17.6	17.4	36.5	55.0	86.7
	2	33.4	32.1	29.3	16.6	17.6	15.6	4.5	19.0	21.0
March	1	32.0	30.6	28.2	16.3	13.6	13.2	22.5	16.0	37.1
	2	30.7	28.7	30.4	14.8	15.5	13.4	2.5	16.5	0
April	1	30.3	26.0	26.1	12.5	15.0	11.9	10.7	110.7	31.5
	2	24.0	22.0	23.4	7.1	10.0	7.7	0.9	7.0	0.4
May	1	22.7	23.6	25.0	4.2	7.9	8.0	4.0	0	13.5
	2	24.5	22.4	23.6	3.7	3.8	3.0	0	0	0
Total rainfall								552.0	670.4	525.8

* Refers to first and second halves of month.

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From Table 5 and Figure 3 it can be seen that Culicoides adults were completely absent or were present only on an isolated night from the beginning of June to the middle of August. Thereafter catches increased steadily and by the end of September they reached about 1,000 per night. Between the end of September and January or February, catches fluctuate between 1,000 and 3,800 with an occasional rapid increase into the 6,000's as in November and December, 1963 and in January, 1966. During January or February a very sharp increase in numbers takes place and this high population is maintained for about 2½ months until the end of March or April. In 1964 and 1966 this rise in numbers took the form of a threefold increase in catch which continued to peaks of 23,299 and 43,156 Culicoides per night, respectively, four to six weeks later. In 1965, however, a peak of 30,063 was reached immediately with the first sudden increase in catches but catches of from 10,000 to 17,000 were maintained for two months thereafter. Catches gradually decrease in April and May and by the beginning of June Culicoides are usually completely absent.

It would be useful to know the reason for these seasonal fluctuations in light trap catches of Culicoides as this would lead to a clearer understanding of the ecology of these midges. Williams (1961) has studied the effect of weather conditions on the activity and abundance of various insects taken chiefly in light traps. He started this work in 1933 and by the use of partial and multiple regressions has been able to correlate catches with various climatic factors especially temperature and rainfall. He has found it possible also to predict future catches by studying temperature and rainfall up to three months before the time. This type of statistical correlation, however, requires many years of unbroken records and all climatic factors such as rainfall, temperatures, wind, cloudiness, etc. should be taken into account. In his very comprehensive 1961 paper he noted that "With only 3 years data for three seasons the analysis of the data from the suction trap was so unreliable as not to warrant publication. Even the 4 years available for the light trap was lower than desirable". Bursell (1964) examining Williams' work from the physiological point of view questioned the soundness of correlating catches with various climatic factors, if the exact relationships between the

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biology of the insects concerned and these factors are unknown. He points out certain anomalies which he says "give one the uncomfortable impression of being in the realm of pure mathematics". He does eventually admit, however, that "Even if no casual relationship underlies the observed correlations they might still prove valuable in enabling predictions to be made of the level of population density in future seasons".

From the foregoing discussion it is clear that there would be little point in trying to correlate statistically Onderstepoort light trap catches with temperature and rainfall, especially since there are records for only three years. These include nil catches for nearly three months a year, and no wind records exist. However, a careful visual examination of the records does show certain correlations which are repeated each year.

In 1964 and 1965 no Culicoides were taken between 1st June and 15th August and this can be assumed to be due to low temperatures. However, in 1963 the same temperatures were experienced but the odd Culicoides adult was taken from 16th June onwards. The winter of 1963, however, differed from the two following years in that it had 63.5 mm. of rain while no rain fell during this period in 1964 and 1965. Winter rainfall may perhaps explain the presence of adults in winter.

Once the maximum temperature increases above about 24°C (after 16th August) Culicoides appear regularly, increasing in numbers with rising temperatures until about 1,000 are taken per night by the beginning of October. Thereafter temperatures are consistently high (minimum temperature above 10°C) until the first half of April when they start dropping slowly until winter temperatures are reached. During the period of consistently high temperatures the catch fluctuates between 1,000 and 3,800 for from 3½ to four months. The sudden increase in numbers in the second half of January or in February cannot therefore be correlated with a sudden change in temperature.

There is evidence that this sharp increase can be directly correlated with the first heavy rains of the summer, the increase occurring exactly three months after more than 80 mm. of rain had fallen within less than a month. The size of the increase can also be correlated with the amount of rain falling and the time

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taken for more than 80 mm. to be recorded. Thus 32.6 mm. in the first half of November, 1963 led to a threefold increase in catch (11,262) in the first half of February, 1964. Again 137.5 mm. in the second half of October, 1964 led to an elevenfold increase (30,063) in the second half of January, 1965, and rain which started at the beginning of November, 1965 but only exceeded 80 mm. during the second half of November led to a threefold increase in catch (9,680) in the second half of February, 1966. In all cases, however, after the initial sharp increase the catch remained high for about eight weeks, either dropping a little where the initial increase was very high (elevenfold) as in 1964-1965, or increasing considerably where the initial increase was only threefold as in the other two seasons.

As can be seen from Table 6 and Figure 3, there is often a six month period of drought between 16th April and 15th October. The first heavy rains of the summer serve to break this drought and probably re-establish the summer breeding sites of Culicoides. Subsequent rains may then maintain these sites in a suitable state for Culicoides development.

From the second half of April onwards the decline in catches can be correlated with lower temperatures and the almost complete cessation of rain until the cold dry conditions of winter are reached and larval development ceases almost completely.

The fact that there is a delay of three months between the first heavy rains which probably initiate breeding in new breeding sites, and the emergence of the resulting adults, suggests that about three months are required to complete a generation in summer (Fig. 3). This period does not agree with the one month suggested earlier after a study of the life-cycle in the laboratory. A possible and very plausible explanation as to why a generation may take three months in the field has been advanced by Mr. D.W. du Plooy*. He suggests that although heavy rains in early summer may create new breeding sites and that many eggs will be laid here, the development of the larvae will be interrupted periodically thereafter since the surface layers of these sites will dry out during the dry periods between the thunderstorms which account for most

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of the summer rainfall. The larvae will then migrate to lower moist layers where they can survive, but will have to wait until rain again provides suitable moist surface layers in which to continue their development. In this way a generation which may be completed in one month under the optimum conditions of the laboratory could easily be extended to three months in the field.

An experiment was conducted in the laboratory to test if desiccation followed by moistening of the larval medium could delay larval development. Two six inch diameter glass dishes three inches deep, were used. Thousands of eggs were added to the larval medium in each dish. The dishes were left open in a small warm room at 80°F. The one dish acted as control and was kept moist, while the medium in the second dish was allowed to dry out. Before the medium was completely dry on the surface, adults started to emerge. They continued to emerge from the 11th to the 18th day after which the surface of the medium was very dry and no further emergence took place. The medium was then allowed to dry out still further for four days until the medium formed a clod which could be removed from the dish and broken into pieces which were dry, except for a slight amount of moisture in the centre.

Water was added to this dry medium and no pupae were seen to loosen themselves from the substrate or to float to the surface. After 13 days during which the medium was kept moist, adults began to emerge. This period was similar to the original period for development to the adult stage noticed earlier in the experiment. From this experiment it may be concluded that Culicoides development may be delayed on desiccation of the breeding medium and that development will be resumed on the medium receiving sufficient moisture. Whether this delay takes place in the egg or the larval stage, or perhaps both stages, has still to be proved.

Alternatively it has been suggested by Prof. R.M. du Toit (personal communication) that a generation may well be completed in one month but that the summer breeding sites will only start to become re-established after sufficient rain has fallen during the early part of the season to restore the moisture level in the soil after the winter drought. He argues that the early summer rains of October or November are not sufficient to restore the parched summer breeding

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sites to the necessary moisture level for larval survival.

Evidence for this argument is the fact that the sharp increase in catches may also be correlated with very heavy rains one month earlier (Fig. 3). For example the catch of 11,262 in the first half of February, 1964 followed a rainfall of 103.0 mm. in the first half of January. Similarly in 1965 a catch of 30,063 in the second half of January followed a rainfall of 106.0 mm. in the second half of December, 1964 and in 1966 a catch of 9,680 in the second half of February followed a rainfall of 129.3 mm. in the second half of January. Thus heavy rains in the middle of summer following on the earlier lighter rains could perhaps be the explanation for the sudden increase in catches one month later. The fact that a generation takes about one month in the laboratory also lends weight to this observation.

Another explanation for the sharp increase in catches could be the following: The heavy rains one month before the increased catch each year, bring about a drop in the catch during the period that the rains are falling, i.e. there is a direct adverse effect of rainfall upon catch (Fig. 3). Larvae in permanent breeding sites may, under these conditions delay development to the pupal stage until drier conditions are available. Simultaneously normal egg laying and early larval development probably continues. To this may be added those larvae which have been lingering in fairly dry sites and for which heavy rains have now made it possible to complete their development. Thus a combination of factors may bring about a mass emergence once the breeding sites have dried out slightly.

The value of the above theories, however, can only be judged after accurate studies have been made of the life-cycle of Culicoides in the field. This will not be easy since C. pallidipennis is the main species concerned and its breeding sites have so far proved extremely difficult to locate.

As can be seen in Table 5, light trap catches at "Kaalplaas" were only a fraction of the Onderstepoort catches. This could be due to the fact that the "Kaalplaas" light trap was not situated near high concentrations of animals as in the case of the Onderstepoort

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trap. It has already been mentioned that high catches in the Onderstepoort trap may be correlated with its close proximity to an open stable of 46 horses and mules while a similar trap only 50 yards from this position caught an average of three times fewer Culicoides during the same season. In fact it has recently been observed by the writer that Sunday night catches are consistently smaller than those made during the week, since the mules and horses are not stabled over the weekend.

Another reason for the low catches at "Kaalplaas" could be that the trap is further from breeding sites or that prevailing winds, or some local factor do not favour this trap. However, it was of interest to note that sharp increases in catches took place at the same time as in the Onderstepoort trap and extended over much the same period. "Kaalplaas" catches did differ in that catches for the first half of March, 1966 were low and the largest number of Culicoides were taken in the second half of February and not in the second half of March as would be expected from the Onderstepoort catches.

(e) Seasonal Variation in Culicoides Species.

Maximum numbers of the different Culicoides species are not necessarily found at the same time of the year. Nishijima & Ono (1963) showed this very clearly in their study on the seasonal prevalence of seven Japanese species; three species showing one peak of abundance while the other four species had two peaks. One is tempted to conclude that the number of peaks represent the number of generations, but this is probably only seldom so. Kettle (1950) showed C. impunctatus to have a bimodal seasonal distribution and Reuben (1963) substantiated this some years later with an even more detailed study. Kettle concluded that the bimodal distribution could not be accounted for by climatic factors alone, and that it was unlikely that this species was bivoltine. Results of similar studies at Onderstepoort and the farm "Kaalplaas" are recorded in Table 7 and in graph form in Figure 4. In discussing these results it is most necessary to bear in mind some of the points discussed earlier.

When studying the prevalence of the six most abundant species present at Onderstepoort (Fig. 4, solid lines) it can be clearly seen that C. pallidipennis and C. schultzei have only one peak of abundance. This is in late summer starting in January with the highest

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Table 7. Seasonal variation in Culicoides species at Onderstepoort and "Kaalplaas". August, 1965 - May, 1966.

(expressed as average catch per trap night).

Month	Trap*	No. of nights	Species						Per cent C. pal.
			C. pal.	C. pyc.	C. dist.	C. niv.	C. sch.	C. mil.	
Aug.	O.P.	6	133.7	6.0	6.2	4.0	0.2	0.3	88.9
	K.P.	-	-	-	-	-	-	-	-
Sept.	O.P.	11	852.5	25.3	12.9	4.5	1.1	1.5	95.0
	K.P.	-	-	-	-	-	-	-	-
Oct.	O.P.	7	957.2	43.0	11.9	19.3	18.4	0.9	91.1
	K.P.	-	-	-	-	-	-	-	-
Nov.	O.P.	11	1,088.3	54.9	48.1	39.9	6.7	3.7	87.7
	K.P.	7	117.9	103.7	52.1	48.7	0.3	0	36.5
Dec.	O.P.	6	1,191.0	102.3	331.1	201.1	14.7	0.8	64.7
	K.P.	5	47.4	36.6	29.2	8.2	0.2	0	39.0
Jan.	O.P.	8	3,740.9	47.3	83.5	54.0	86.8	0.8	93.2
	K.P.	8	83.6	13.0	16.1	12.3	3.6	0	65.0
Feb.	O.P.	9	5,557.1	175.8	257.8	99.3	376.2	5.0	85.9
	K.P.	14	770.7	43.0	70.3	27.0	41.5	0	80.9
March	O.P.	9	32,213.0	29.4	380.7	11.1	401.5	21.1	97.4
	K.P.	13	576.7	28.5	59.2	7.7	20.3	0	83.3
April	O.P.	7	9,971.1	107.9	195.7	23.4	109.0	15.3	95.7
	K.P.	5	1,011.0	211.2	236.4	17.0	26.2	0	67.3
May	O.P.	5	4,267.6	46.6	40.4	12.2	9.0	14.0	97.2
	K.P.	5	34.8	34.6	26.2	2.6	0.8	0	35.2
Per cent Females	O.P.		99.2	88.9	88.0	91.2	92.8	99.8	
	K.P.		97.3	75.5	65.0	73.9	82.1	-	

* O.P. = Onderstepoort.
K.P. = "Kaalplaas".

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catches in March. C. milnei followed a similar pattern but catches were too small to warrant further study. The remaining three species, viz. C. pycnostictus, C. distinctipennis and C. nivosus all showed an early peak in December and one or two additional peaks between February and April.

In the previous section various theories were advanced to explain the sudden increase in Culicoides numbers in January or February. These theories must also apply to C. pallidipennis since as can be seen from the last column of Table 7, C. pallidipennis may account for up to 97.4 per cent of the light trap catch at Onderstepoort. These theories may thus explain its single peak of abundance in late summer and perhaps that of C. schultzei as well.

The fact that the other three species show an earlier peak of abundance in December as well as later peaks suggests that they require different conditions for breeding, these conditions apparently being present throughout most of the summer. This has in fact already been shown earlier when discussing breeding sites, where any muddy area high in organic matter produced all these species. These breeding sites do not depend on rainfall for their establishment or maintenance but rainfall may possibly adversely affect their suitability as breeding sites, or govern in some way the abundance of these species.

The "Kaalplaas" light trap was situated about four miles from the trap at Onderstepoort. The peaks of abundance at "Kaalplaas" although not always coinciding with those at Onderstepoort nevertheless occurred at similar times. The catches from November, 1965 when the trap was put into operation are shown in Figure 4 by the broken lines. C. pallidipennis showed two peaks at the end of February and April, but since the catch for March was very high this curve can be compared with the single peaked curve of the Onderstepoort catch. The curve for C. schultzei showed a similar tendency. The other three species had an early peak of abundance in November and further peaks between February and April.

A comparison of catches from the two traps is very interesting and shows many differences which if they can be explained will lead to a clearer understanding of the

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ecology of the various species. From Figure 4 it can be seen that in the species other than C. pallidipennis and C. schultzei, the numbers taken at "Kaalplaas" compare reasonably with those taken at Onderstepoort so that it is possible to plot them on the same scale of the graph. The numbers of C. pallidipennis taken at "Kaalplaas", however, had to be multiplied 10 times so that they could be compared on the same graph as the Onderstepoort catch of this species. The enormous numbers of C. pallidipennis in the Onderstepoort trap therefore accounts for the very noticeable differences in the size of the catches from the two traps. This species may be particularly abundant due to a number of reasons. The first is that the Onderstepoort trap may be situated near very large breeding sites of C. pallidipennis while the "Kaalplaas" trap may be almost out of the flight range of midges from these sites, or the "Kaalplaas" trap may only catch C. pallidipennis from a few small local breeding sites. Secondly the area around the Onderstepoort trap is well illuminated at night thus providing the initial attraction to this area. "Kaalplaas" on the other hand is almost completely in darkness. Thirdly high concentrations of stock are kept in stables and paddocks at Onderstepoort compared with the odd few animals at "Kaalplaas" where most are in the veld. Finally the exact position of the Onderstepoort trap 10 yards from an open stable housing 46 mules and horses may be particularly attractive to C. pallidipennis. Here it is interesting to refer to a study made by Kitaoka & Morii (1964b) on the composition of light trap catches in poultry houses and cow sheds. They found C. arakawae (Matsumura) to account for 88.9 per cent of the Culicoides species taken in poultry houses but this species made up only 17.5 per cent of the catch from cow sheds. The predominant species from cow sheds was never taken in poultry houses. Thus in the case of the Onderstepoort trap considerable bias may exist in favour of C. pallidipennis. The writer has in fact recently observed a marked reduction in the number of C. pallidipennis in the Sunday night catch and this can be correlated with the absence of mules from the stable over the weekend.

The last column of Table 7 summarises the percentage C. pallidipennis in the two traps throughout the summer season. At "Kaalplaas" it was only between January and April that C. pallidipennis accounted for more than

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40 per cent of the catch, the maximum being 83.3 per cent in March. At Onderstepoort, however, C. pallidipennis accounted for more than 85 per cent of the catch in all months except December when large catches of the other species reduced this figure to 64.7 per cent.

Kettle (1962) when reviewing the use of light traps for sampling Culicoides populations noted that the percentage males is often low indicating that males disperse less widely or that they are shorter lived. If the former theory is correct then catches with a high percentage males should indicate that the trap is situated close to the breeding sites and vice versa. The last two lines of Table 7 show the percentage females of each species taken in the two traps. More than 99 per cent of C. pallidipennis and C. milnei caught in the Onderstepoort trap consisted of females, indicating perhaps that their breeding sites were some distance away. Catches of the other four species were made up of from 88 to 92.8 per cent females, which although high could indicate that some breeding sites for these species would be found nearby. Breeding sites of these species have in fact been found in the vicinity of the Onderstepoort trap. At "Kaalplaas" C. pallidipennis catches again consisted chiefly of females (97.3 per cent) suggesting again that breeding sites are fairly distant. The other four species, however, had from 65 to 82.1 per cent females so that breeding sites of these species are probably not very far from the trap site.

4. SUMMARY AND CONCLUSIONS

Five aspects of the biology of Culicoides midges in the Onderstepoort area were studied. They were the life-cycle in the laboratory, breeding sites, hours of activity, seasonal variation in abundance and its correlation with weather, and seasonal variation in abundance of five species. Most of these studies depended upon the use of suction-type light traps.

Laboratory studies on the life-cycles of Culicoides species consisted of observations made while rearing eight species for later studies on the morphology of the immature stages (Part II). The following species were reared :-

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C. pallidipennis, C. pycnostictus, C. distinctipennis, C. nivosus, C. schultzei, C. milnei, C. magnus and C. bedfordi.

The life-cycles of these species are similar, the average period from egg to egg being 25 days. Theoretically therefore, a generation can be completed in 25 days at 70 to 75°F. The number of eggs per batch varied from 69 to 162 between species. Desiccation below 100 per cent R.H. killed most eggs but a few survived which suggests that short dry spells may be survived in the egg stage. Eggs hatched while submerged and larvae survived immersion for more than six days indicating that inundation would apparently not adversely affect these stages. Pupae of all species except C. pallidipennis, floated to the surface on being immersed in water with no adverse effects upon adult emergence. Since C. pallidipennis pupae drown, it can be expected that in inundated areas larval development to the pupal stage will be delayed until such areas have dried out somewhat. Eggs stored at 6.5°C all hatched after seven days storage. Thereafter viability decreased rapidly with longer storage. Overwintering is thus unlikely to take place in this stage. Larvae were unaffected by this temperature for 14 days, longer periods not being tested. Adults were reared from larvae collected in the field in the middle of winter. Overwintering can therefore take place in the larval stage. A mixture of soil and bovine manure was suitable for rearing C. pycnostictus, C. distinctipennis, C. nivosus and C. bedfordi, but poor for the other species. Culicoides larvae are probably carnivorous and C. milnei and C. nivosus were seen to be cannibalistic. Larvae normally feed in the surface layers except C. bedfordi which was never seen during its entire larval stage. Attempts at establishing a laboratory colony of Culicoides were unsuccessful since laboratory-reared adults would not mate or take a blood-meal.

Dr. R.M. du Toit and Dr. C.G.H. Fiedler in 1949 - 1950, found C. pallidipennis breeding in three sites, all of which were moist kikuyu grass-covered areas. The main breeding site of this species has yet to be determined but can be expected to be comparatively dry (terrestrial) since C. pallidipennis pupae drown.

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Other species were found breeding in manure-polluted mud around leaking water troughs, in marshy areas and in mud along the margins of pools and streams.

A study of Culicoides activity showed them to be abundant at any time between 9 p.m. and 5 a.m. depending on prevailing weather conditions. The period of poor catches between 7 p.m. and 9 p.m. could be correlated with a combination of high temperature and relatively low humidity.

A study of the seasonal variation in abundance of Culicoides midges was conducted over a period of three years. Attempts were made to correlate seasonal abundance of midges with temperature and rainfall. It was concluded that temperature only played a minor role. Low temperatures in winter delayed larval development so that few or no adults were caught in June, July and the first half of August. Rainfall apparently determines the size of the catch between September and May. Each year a sudden threefold to elevenfold increase in catch was recorded in January or February. Three possible explanations are tendered. The first correlates the increase with heavy rains early in summer, three months before the increase, the delay in development being due to periods of partial desiccation of the breeding areas. The second explanation correlates the sharp increase with heavy summer rains one month earlier. This agrees with the laboratory observation that a generation may be completed in one month. The third explanation is a combination of the first two but takes into account the observation that catches always drop during the period of heavy summer rainfall, one month prior to the sharp increase. Inundation of breeding areas caused by these rains, may delay development to the pupal stage and result in a build-up of Culicoides larvae and the subsequent sharp increase in catches one month later.

A study of the seasonal variation in five Culicoides species showed C. pallidipennis and C. schultzei to have one peak of abundance occurring between February and April. The remaining species showed an early peak in November or December with one or more later peaks between February and April. Since C. pallidipennis may constitute up to 97.4 per cent of the Onderstepoort

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catch, the same theories forwarded earlier can be used to explain its abundance at certain times of the year. The other species are apparently not so dependent upon rainfall. A light trap at "Kaalplaas" showed similar tendencies in catches but C. pallidipennis accounted for less than 40 per cent of the catch before January. One explanation for the high percentage C. pallidipennis taken in the Onderstepoort trap, was its proximity to an open mule stable which served to concentrate this species in this area.

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PART II The Morphology Of The Immature Stages Of Some South African *Culicoides* Species.

1. INTRODUCTION

The immature stages of insects have always been relatively unknown except where they are responsible for direct annoyance or damage to man and his environment. *Culicoides* adults were relatively unknown until their importance as transmitters of various disease organisms was recognized. Their immature stages have on the whole been ignored. Only with the change in emphasis from the taxonomy of the adult transmitter, to a study of its biology and ecology, has attention been focussed on the larval and pupal stages. Recognition of these stages is essential when studying the biology of the species.

Hill (1947) made a thorough survey of the literature dealing with the immature stages of *Culicoides* species and noted only two references dealing with tropical species and five on European species. In 1952 Kettle and Lawson made a thorough study of the early stages of British biting midges, and in the same year Wirth reviewed existing knowledge on the midges of California. The position in Africa, however, is that apart from the pioneering work of Carter, Ingram and Macfie in the Gold Coast in 1920, and that of De Meillon (1936, 1937) in South Africa, no careful systematic work has been done on the immature stages of *Culicoides* in the Ethiopian region.

Carter *et al.* (1920) gave good general descriptions of the pupae of seven *Culicoides* species, viz. *C. inornatipennis*, *C. punctithorax*, *C. clarkei*, *C. schultzei*, *C. accraensis*, *C. eriodendroni* and *C. nigripennis*, and described the larvae of the last four of these species. They were the first workers to describe the morphology of *Culicoides* larvae and pupae, and drew attention to the number and shape of teeth on the posterior margin of what they called the "hypo-pharyngeal-sclerites". This character is today recognized as being of considerable value in larval identification. Of the species mentioned above, only *C. clarkei* and *C. inornatipennis* have not been recorded from South Africa. The only other work on the immature stages of South African *Culicoides* species is that of De Meillon (1936). He described the pupae of

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C. meeserellus and C. alexis. Fiedler (1951) has shown that these are synonyms for C. pycnostictus so that De Meillon's two pupal descriptions refer to the same species. These were described from single specimens and differ considerably, especially in the features of the prothoracic horn and caudal segment. In 1937 De Meillon described C. cornutus, C. engubandei and C. nivosus pupae, but the descriptions and drawings are inadequate to permit a thorough comparison.

It is the purpose of the following investigation to make a thorough morphological study of fourth instar larvae and of pupae, paying particular attention to certain morphological characters which other workers have found to be of value, but at the same time to investigate possible new characters, and to construct a tentative key for the identification of the species studied. This work will hopefully form a basis for future studies and the eventual construction of a key for the identification of the immature stages of all Culicoides species in South Africa.

For these studies the writer was able to rear fourth instar larvae of C. pallidipennis, C. pycnostictus, C. distinctipennis, C. nivosus, C. schultzei, C. milnei, C. magnus and C. bedfordi; and pupae of all these species except C. magnus.

2. MATERIAL AND METHODS

(a) Source of Material for Study.

Nearly all the material studied were the laboratory-reared progeny of a number of females identified prior to egg laying. This procedure has been fully described in Part I since this method also allowed a close study of the life-cycle of the species concerned.

In order to obtain fourth instar larvae and pupae, the rearing media were closely observed and when the first adults emerged all larvae and pupae were collected by the magnesium sulphate flotation method described in Part I. These were then killed in hot water and preserved for study in 70% ethyl alcohol.

Another means of obtaining material is by field collection of larvae and pupae. Fairly accurate larval identification is possible if a proportion of the larvae

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are removed and preserved and the remainder allowed to develop to adults. If the adults are of one species then it is fairly safe to conclude that this is the identity of the larvae. This method was not used to obtain larvae for the present study as it was common for more than one species to emerge from a single sample.

Pupae collected in the field, however, can be safely used in these studies. Each pupa collected was placed on moist tissue paper in a small stoppered bottle. The emerging adult was then identified and preserved together with its pupal exuvia.

(b) Preparation and Study of Material.

The preparation of material and the method of study depends upon the character(s) to be studied and especially the stage of development, since pupae can withstand more drastic treatment than the soft bodied larvae. It is as well to deal with larvae and pupae separately.

(1) Larvae.

The more prominent larval features such as head colour, eye-spots, pigmentation, etc. could be clearly seen by viewing larvae in alcohol under a dissecting microscope, with incident light, a white background and x 50 magnification.

Measurements of preserved larvae were made under a compound microscope using transmitted light, with the larvae temporarily mounted in water under a glass coverslip. About 20 larvae per species were measured and where duplicate material was available this was repeated as a check. After measurement the larvae were returned to storage in 70% alcohol. Measurements were made using an eye-piece micrometer which was standardized with a stage micrometer to give readings in microns.

A method of preparation for permanent larval mounts which was suitable for viewing most structures including the setae of the head and body, thoracic pigmentation, eye-spots, etc. was to allow alcohol-preserved larvae to remain at room temperature in Berlese's fluid[1] for at least one night before mounting in the same fluid. An

[1]Formula for Berlese's fluid -
60 grams Gum arabic
40 grams Glycerine
400 grams Chloral hydrate
100 grams Distilled water

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alternate method was to leave the larvae overnight in lacto-chloro-phenol[2] before mounting in Berlese's fluid. The latter method did, however, tend to make the eye-spots fainter.

To study the pharyngeal skeleton in detail it was necessary to open the head capsule of treated larvae, and using minuten pins to dissect out the mandibles, hypopharynx and epipharynx. Treatment prior to dissection consisted of gently boiling the larvae for about five minutes in a 10 per cent aqueous solution of caustic potash (KOH) or leaving them for some weeks in lactic acid. The KOH method is that used by Kettle & Lawson (1952) who also dissected out the pharyngeal skeleton. Linley and Kettle (1964) studied the pharyngeal skeleton *in situ* after prolonged treatment of the larvae in warm (60°C) lactic acid. The writer found, however, that only the larger combs could be clearly seen *in situ*, the smaller combs and hypopharynx being obscured. The components of the pharyngeal skeleton were mounted in Berlese's fluid and studied under an oil immersion lens.

(2) Pupae.

In the mounting of intact pupae or pupal exuviae it is essential that most of the body contents be removed and that setae, especially on the cephalo-thorax, be made clearly visible by suitable treatment prior to mounting. Linley & Kettle (1964) recommended clearing specimens in warm lactic acid for several hours. This method was found to be effective but better results were obtained by keeping pupae in warm (± 52°C) lacto-chloro-phenol for at least one night before mounting in Berlese's fluid.

In pupal studies certain characters must be viewed dorsally and some laterally. For this reason it was found useful to mount half the pupae intact in a lateral position while the remaining half were dissected and mounted under separate coverslips on one slide as follows :- The operculum was dissected off and mounted for dorsal view under one coverslip; the last two or three abdominal segments were mounted dorsal side up

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[2]Formula for lacto-chloro-phenol -
Chloral hydrate - 2 parts by volume
Lactic acid - 1 part by volume
Phenol - 1 part by volume

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under another coverslip; and the remainder of the pupa was mounted in the same position under a third coverslip.

All drawings were made using a drawing tube attached to a compound microscope.

3. DISCUSSION OF RESULTS

(a) Fourth Instar Larvae.

(1) Recognition.

Apart from an increase in size with each instar, other larval characters such as pigmentation and the development of the pharyngeal skeleton reach their final form in the fourth instar. It is in this stage that these characters can be seen at their best and is an important reason for the study of this stage.

It is, however, not easy to differentiate between the last two instars of a mixed sample of unknown species. The first two instars are seldom collected because of their very small size and may be easily differentiated from fourth instar larvae on head length alone. For example first instar larvae of the largest species studied, viz. C. milnei have a head length of between 70 and 80 μ while the head of the smallest species C. pallidipennis is between 59 and 66 μ long. Second instar C. milnei have a head length of less than 120 μ, which cannot be confused with fourth instar C. pallidipennis which have an average head length of more than 130 μ (Table 9).

Where head length is considerably longer than that of the third instar of the largest species, viz. C. milnei ($\pm$ 160 μ) it is probably safe to assume that the specimens are in the fourth instar. Where head lengths are shorter than this, however, as in the fourth instars of C. pallidipennis, C. bedfordi and C. schultzei, further ways of recognizing fourth instar larvae are necessary (Table 9).

Perhaps the most reliable method is to try and detect signs of pupation. "The first signs of this are the hollowing out of the mesothoracic and metathoracic paired lateral bodies as they are pushed in by the growing imaginal buds. Much later the typical pupal structures, such as the respiratory horns and the caudal spines, become visible but by this time the larva is so distorted

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as to make identification very difficult". (Kettle & Lawson, 1952).

In heavily pigmented species the paired lateral bodies of the meso and metathorax cannot be seen easily but the same "hollowing out" effect is obtained due to the layer of pigment cells being shouldered up by the imaginal buds to form a clearly defined indented lateral margin to the pigmented areas (Fig. 6) (Lawson, 1951).

Where a single species is present it is easy to recognize the various instars on the basis of Dyar's law that head measurements increase by a certain fixed ratio for a species at each successive moult. Since first instar head lengths for the eight species studied vary between 59 and 80 μ it is simple to calculate which instars are present. Kettle and Lawson (1952) give a full explanation and examples of the procedure to adopt in their study of British biting midges.

Larval length varies greatly between species but must also depend on conditions in the larval medium. From Table 9, however, it is probably safe to conclude that larvae shorter than 3.0 mm. are not in the fourth instar, and that larvae longer than 3.0 mm. are probably either in the third or fourth instar.

(2) General Features.

Features which may be used for the direct identification of some species or to sort the larvae into groups for later more detailed study are : the colour of the head, the shape of the eye-spots, the presence or absence of pigmentation on the thorax and the distribution of this pigmentation. The general features of the eight species studied are summarised in Table 8 and shown in Figures 5 to 12. The following conclusions can be drawn from Table 8 :

Head colour.

C. pallidipennis, C. magnus and C. milnei all have brown or amber-coloured heads, the heads of the other species being dark cream or straw-coloured.

Eye-spots.

C. pallidipennis generally shows a pair of almost circular spots compared with the comma-shaped eye-spots of the other seven species.

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Table 8. General features of fourth instar larvae of 8 Culicoides species.

Species	Head	Eye-spots	Neck	Prothorax	Mesothorax	Metathorax
C. distinctipennis (Fig. 5)	Dark cream	Comma-shaped	Unpigmented except for odd spot.	Brown pigment fills segment. Open spots in pigment sometimes present.	Pigment similar to prothorax. Narrows posteriorly. Two dark lateral bodies sometimes present.	Pigmentation reduced. Two dark lateral bodies sometimes present.
C. pycnostictus (Fig. 6)	Dark cream	Comma-shaped	Unpigmented	As above	As above	As above
C. nivosus (Fig. 7)	Dark cream	Comma-shaped	Irregular central pigment usually present.	As above	As above	As above
C. schultzei (Fig. 8)	Dark cream	Comma-shaped	Brown irregular pigment for most of width.	As above	As above	As above
C. milnei* (Fig. 9)	Brown	Comma-shaped	Sometimes pigmented.	Brown localized narrow pigment strips anteriorly and laterally.	Pigmentation similar to prothorax.	Lateral bands of pigment only.
C. magnus (Fig. 10)	Brown	Comma-shaped	Unpigmented	Unpigmented	Unpigmented	Unpigmented
C. bedfordi (Fig. 11)	Dark cream	Comma-shaped	Unpigmented	Unpigmented	Unpigmented	Unpigmented
C. pallidipennis (Fig. 12)	Brown	Circular	Unpigmented	Unpigmented	Unpigmented	Unpigmented

* In C. milnei isolated black spots may occur superficially on the thorax.

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Pigmentation.

When present, pigmentation is most pronounced in the prothorax and usually diminishes in the meso and metathoraces. The small segment between the head and prothorax forms the so-called "neck" which may also be pigmented (Fig. 10).

The pigment layer is very superficial and is usually restricted to the dorsal and dorso-lateral regions although it is sometimes found as a narrow ventral band in the posterior region of the metathorax. Pigment appears to consist of very small particles giving a diffuse effect, but particles are often formed into circular groups giving a more mottled appearance. Open circular unpigmented areas are common in certain species especially C. pycnostictus and C. nivosus.

From Table 8 and Figures 5 to 12, it can be seen that the eight species may be arranged into three groups according to the type of pigmentation. The first group includes those species which have a diffuse type of pigmentation which fills the prothorax and diminishes posteriorly in the meso and metathorax, viz. C. distinctipennis, C. pycnostictus, C. nivosus and C. schultzei. The second group is made up of one species, C. milnei, which has narrow bands of very well defined areas of pigmentation; and the third group includes the three unpigmented species C. magnus, C. bedfordi and C. pallidipennis.

(3) Size.

The following measurements were made of fourth instar larvae of the eight species studied :- head length, head breadth, width at oral ring, distance from anterior margin of labrum to eye-spot, distance between eyes, and larval length. An indication of the shape of the head is given by the ratio head length as to head breadth and called the head ratio. Head length was measured from the anterior margin of the labrum along the mid-line to the posterior margin of the post-occipital ridge. Breadth was measured at the widest part of the head, oral ring width was taken from a straight line connecting the hind borders of the sub-genal band, and the length of the larvae did not include anal papillae (see Fig. 13). Only five C. magnus were measured due to a shortage of material. Means and standard deviations (S.D.) from the means were calculated and the results are given in Table 9.

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Table 9. Mean measurements and standard deviation from mean (S.D.) of fourth instar Culicoides larvae (in microns).

Species	Date Reared	Number Measured	Parameter	Head Length	Head Breadth	Head ratio	Oral ring	Labrum to eye	Distance between eyes	Larval length
C. pallidipennis	5.11.63	20	Mean ± S.D.	136.1 ± 3.6	102.4 ± 3.8	1.33 ± 0.05	68.5 ± 1.9	74.2 ± 7.0	54.7 ± 6.1	3398 ± 187
	12. 2.65	20	M. ± S.D.	134.7 ± 2.1	95.9 ± 2.5	1.40 ± 0.04	61.3 ± 1.6	70.7 ± 3.6	50.6 ± 3.6	3048 ± 100
C. bedfordi	15. 4.65	20	M. ± S.D.	145.3 ± 3.1	96.2 ± 3.5	1.51 ± 0.06	60.8 ± 2.0	86.4 ± 7.2	57.5 ± 3.9	3346 ± 235
C. schultzei	26.11.63	20	M. ± S.D.	154.9 ± 8.4	116.4 ± 8.7	1.33 ± 0.07	75.8 ± 5.5	84.7 ± 5.8	57.6 ± 5.6	3716 ± 245
C. distinctipennis	11.11.63	20	M. ± S.D.	170.0 ± 3.2	115.6 ± 3.0	1.47 ± 0.03	78.1 ± 2.0	102.6 ± 6.4	61.6 ± 4.6	3676 ± 139
	26. 3.65	20	M. ± S.D.	164.0 ± 4.6	114.5 ± 4.4	1.43 ± 0.06	74.5 ± 2.4	95.8 ± 7.2	63.4 ± 4.0	3510 ± 128
C. nivosus	31.10.63	20	M. ± S.D.	177.4 ± 5.3	129.0 ± 5.3	1.38 ± 0.04	89.2 ± 2.7	112.3 ± 9.0	71.5 ± 8.5	4455 ± 183
	8. 4.64	20	M. ± S.D.	179.6 ± 7.3	131.8 ± 9.2	1.36 ± 0.07	86.0 ± 4.2	99.4 ± 5.2	73.6 ± 6.4	3810 ± 448
C. pycnostictus	25. 3.64	20	M. ± S.D.	183.2 ± 5.4	132.6 ± 5.9	1.38 ± 0.07	87.0 ± 3.8	99.5 ± 4.3	74.3 ± 4.6	3717 ± 174
	9. 3.65	20	M. ± S.D.	180.4 ± 5.6	127.9 ± 4.5	1.41 ± 0.02	82.9 ± 3.3	96.4 ± 4.4	72.2 ± 4.6	3432 ± 211
C. magnus	9. 3.65	5	M. ± S.D.	203.5 ± 16.1	133.9 ± 4.4	1.52 ± 0.14	85.4 ± 3.8	105.2 ± 10.6	74.6 ± 2.4	3778 ± 317
C. milnei	8.11.63	20	M. ± S.D.	223.2 ± 3.8	146.7 ± 5.3	1.52 ± 0.07	102.4 ± 3.4	122.3 ± 4.7	91.8 ± 5.5	4141 ± 168
	16. 2.65	13	M. ± S.D.	224.5 ± 5.9	150.2 ± 3.2	1.49 ± 0.04	100.2 ± 2.2	121.4 ± 4.0	93.1 ± 6.6	4093 ± 372

45. / ...

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The measurements of the distance between labrum and eye, and between eyes were included for their possible taxonomic value, but they add nothing to the information given by the other measurements suggested by Kettle & Lawson (1952). There appears to be little or no correlation between the length or breadth of the head and the larval length, e.g. in the duplicate sample of C. nivosus, the head measurements were slightly larger than in the first sample whereas larval length was very much shorter. The larval lengths therefore can only serve as rough indicators of the species or instar, and are probably dependent on larval age within the instar and on conditions in the larval medium. For the species studied it may be sufficient to say that larval length varied from approximately 3.0 mm. for the smallest species to about 4.1 mm. for the largest.

Head length, head breadth and the width of the oral ring are very useful indicators of the identity of a species where this cannot be determined by easier means. Most of the eight species studied are fairly easy to identify under x 50 magnification using the characters in Table 8 such as the presence or absence of pigmentation, etc., or by a careful study of the dorsal comb of the epipharynx (see later). C. distinctipennis and C. pycnostictus cannot, however, be distinguished on these characters and resort to differences in head measurements may have to be made.

(4) Detailed Study of the Heads.

Under oil immersion the pharyngeal skeleton and mandibles could be clearly discerned, but no attempt was made to trace and study the antennae, labrum, maxillae and labium, since as pointed out by Linley & Kettle (1964), "a large amount of careful work on properly prepared specimens would be necessary to appreciate them and at the best they could only be employed as confirmatory characters on a few larvae of doubtful identity, and would not be applicable to large numbers".

Where possible the mandibles, epipharynges, and hypopharynges of six larvae of each species were drawn under oil immersion, and the clearest and most representative of these drawings appear in Figures 15 to 32. Dorsal and lateral diagramatic views of a larval head showing all the structures to be discussed, appear in Figures 13 and 14.

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(a) Mandibles.

Each Culicoides larva possesses two mandibles which articulate antero-dorsally, and move almost parallel to each other in a vertical plane. As pointed out by Kettle and Lawson (1952), mandibles are difficult to describe and compare as "a slight alteration in their position makes a considerable change in their appearance". The mandibles appearing in Figures 15 to 22 therefore give only a general indication of their structure, shape and size. The presence of a seta or setal socket postero-laterally, and the possession of a single tooth, appears to be characteristic for the species studied. The lengths of the mandibles are given in Table 10.

Table 10. Length of mandibles of fourth instar Culicoides larvae.

Species	Number measured	Range (μ)
C. pallidipennis	6	30.7 - 34.2
C. bedfordi	5	34.8 - 38.9
C. schultzei	3	40.6 - 41.3
C. distinctipennis	5	44.1 - 49.3
C. nivosus	6	50.5 - 57.5
C. pycnostictus	8	51.0 - 55.1
C. magnus	2	47.6 - 54.0
C. milnei	9	51.6 - 55.6

Mean measurements in Table 9 of head length, head breadth, and width of the oral ring, are noticeably greater in C. milnei than in the other species. In Table 10, however, the length of the mandibles of C. milnei does not differ significantly from those of C. nivosus, C. pycnostictus and C. magnus. Mandible length in relation to the other head measurements just mentioned, is thus another character which could possibly be used in the identification of species.

(b) The Pharynges.

The pharynges or pharyngeal skeleton consist of two main parts, the hypopharynx and the epipharynx. Together these form the walls of the pre-oral cavity (Lawson, 1951).

(i) Hypopharynx.

This is made up of a concave or trough-shaped membrane which forms the floor of the pre-oral cavity and which is suspended from two sclerotized lateral arms.

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The anterior region is of a membranous nature and is prolonged anteriorly into what appears to be a tube. Lawson (1951) has shown that in C. nubeculosus the salivary duct opens in this anterior section of the hypopharynx.

The hypopharynges of seven of the eight species studied appear to be very similar in structure and a single figure (Fig. 23) is representative of these seven species. C. schultzei, however, differs markedly in that the trough of the hypopharynx is dark and fibrous in nature, and not as delicate as that of the other species (Fig. 24). In all species the posterior edge of the trough is prolonged into fine membranous teeth and the tip of the so-called "anterior hypopharynx" is flanked by four or five very small teeth or "denticles".

The fact that the hypopharynges are not all alike, suggests that they may provide useful characters in larval identification.

(ii) Epipharynx.

The epipharynx makes up the other half of the pharynges and forms the dorsal wall to the pre-oral cavity (Lawson, 1951). It consists of a number of plate-like sclerites closely associated with one another to form the epipharynx proper, and this is suspended in the trough of the hypopharynx by two heavily sclerotized lateral arms (Figs. 13 and 14). These arms are of minor importance from the taxonomic viewpoint since their appearance varies considerably depending upon the angle in which they are mounted (Kettle & Lawson, 1952). The arms may possess small sclerites or membranous teeth which may serve for muscle attachments.

Kettle & Lawson (1952) describe two types of epipharynx. In the first type or group there are basically four sclerites present. The posterior sclerite is divided into a pair of roughly triangular sclerites, the posterior edges of which are toothed. The posterior edges of the other sclerites may be smooth or have rounded projections or definite teeth. The epipharynx in this group is heavily sclerotized and pigmented (C. nubeculosus group). The epipharynx in the second group is more lightly pigmented and sclerotized, and here all sclerites are toothed and are referred to as "combs". The dorsal or posterior comb is the most prominent and is divided into two roughly triangular sclerites, the teeth of which can usually be clearly made out under oil

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immersion. This is regarded as the first comb of the series so that as one proceeds ventrally the others have been named "second", "third", and "fourth" combs (Kettle & Lawson, 1952). The fourth comb is sometimes called the ventral comb. These combs may not all be present and often even when present they are very difficult to see due to their fine structure or because they may be obscured by some overlying structure.

The eight species studied appear to fall into the second group since most have three or more combs with well-defined teeth. In C. pallidipennis, however, only two combs can be seen. They are the dorsal comb and what must be the ventral comb (fourth comb) since it is well developed and there is space enough between it and the dorsal comb for the two missing combs. The Figures 25 to 32 represent ventral views of dissected and in situ epipharynges. They may be called diagramatic since for clarity it has been necessary to draw each comb distinct from the next, whereas in reality the combs are superimposed.

The dorsal comb is the clearest structure of the epipharynx and provides some very useful characters for taxonomic purposes. The number of teeth on each half of this comb can be fairly clearly distinguished even in an intact treated specimen, and as can be seen from Table 11, there exists a significant difference between the species studied.

C. schultzei is the most striking example, having only three large teeth on each half of the dorsal comb. These combs in turn are flanked laterally by a single large tooth or projection which appears to be part of the lateral arms. The remaining species may be grouped roughly into those with less than 14 and those with 14 or more teeth on each half of the dorsal comb. The latter includes two species, viz. C. pallidipennis and C. magnus.

Careful dissection and mounting is necessary for a study of the other three combs. Except in C. distinctipennis, C. nivosus and C. pycnostictus, it was almost impossible to determine accurately the number of teeth in these combs. No trace of a second or third comb could be found in C. pallidipennis. These combs in C. nivosus appear to have more teeth than those of its close relatives C. distinctipennis and C. pycnostictus, which are almost identical, otherwise they are probably of little or no diagnostic value.

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Table 11. Details of epipharyngeal combs of fourth instar Culicoides larvae.

Species	Number of teeth per comb				Width of half dorsal comb (μ)	No. of specimens
	Dorsal*	Second	Third	Fourth		
C. pallidipennis	(14)-19	-	-	(6)-9	12.8 - 17.4	9
C. bedfordi	(11-12)	( )	(14)	(14)	11.6 - 13.3	5
C. schultzei	3	( )	( )	(12-20)	10.5 - 11.1	5
C. distinctipennis	8-9	5-6	8	16-19	13.9 - 16.2	6
C. nivosus	10-12	8-10	11-12	(20)	15.7 - 16.8	10
C. pycnostictus	9-10	5-7	8-11	16-(19)	14.5 - 17.4	8
C. magnus	15-(18)	( )	( )	( )	19.2 - 21.5	5
C. milnei	10-(13)	(4)	(7)	(8)-12	20.3 - 23.2	11

*Refers to number of teeth on each half of dorsal comb.

( ) Number of teeth uncertain.

The size of the teeth of the dorsal comb may serve as a diagnostic character. In most species teeth are almost equal in size. In C. milnei, however, the first two to four teeth on the mesal side of each half of the dorsal comb are considerably larger than the others (Fig. 31).

The shape of each half of the dorsal comb varies between species. In three species, viz. C. pallidipennis, C. milnei and C. magnus, the two halves of the dorsal comb when adjacent to each other give the dorsal comb an almost perfect semicircular serrated outline (Figs. 25, 31 and 32). However, in C. distinctipennis, C. pycnostictus, C. nivosus and C. bedfordi, each half of the dorsal comb has the shape of a hand so that when adjacent, the outline of the comb has a distinct indentation in the centre (Figs. 26, 27, 28 and 30).

Kettle & Lawson (1952) attached some value to the width of the dorsal combs as a means of distinguishing between otherwise morphologically similar species. For this reason the widths of each half of the dorsal combs (measured at the widest section) of the eight species studied, are included in Table 11. The width across

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the complete comb was not used, as in many cases the two halves of the comb overlapped or became widely separated on mounting.

The value of the width of the dorsal comb as a diagnostic character is probably limited. In the eight species studied, once the various species have been separated on such characters as number of teeth on the combs, size of teeth, and shape of combs, then width of dorsal comb can be used as an additional character to differentiate between those species which fall in the same groups. For example, C. pallidipennis, C. magnus and C. milnei have combs of a similar shape but C. pallidipennis may be easily recognized since the dorsal comb is much narrower (Table 11). Similarly C. bedfordi has a narrower dorsal comb than C. distinctipennis, C. nivosus and C. pycnostictus, although their combs have the same basic shape.

In summing up, the following characters of the epipharynx are of possible taxonomic value :- The number of teeth on the dorsal comb, the size of these teeth, the shape of the dorsal comb, the width of the dorsal comb and the number of teeth on the second and third combs.

(5) Structure and Chaetotaxy of the Head.

The head of Culicoides larvae is made up of three sclerites. They are the fronto-clypeus, a lateral/ventral sclerite, and the post-occipital ridge or collar. These are separated from one another by the frontal (ecdysial) and post-occipital sutures, respectively (Kettle & Lawson, 1952) (Figs. 13, 14 and 39). Neither these authors nor Linley & Kettle (1964) attached much importance to the sutures or sclerites as taxonomic characters since they noted very little variation between species.

The eight South African species studied conformed to a greater or lesser extent with the overseas species referred to above. However, it would be unwise to ignore the possible taxonomic value of at least one of these characters, viz. the post-occipital ridge. As can be seen from Figures 33 to 48, variations between species in type and degree of sclerotization of this collar do exist, e.g. C. pallidipennis consistently shows a narrow ventral anterior projection of the median section of the post-occipital ridge, which makes it readily distinguishable from the other species. This can even be seen at x 50 magnification (Fig. 46).

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The position, size, and number of setae on the head were studied and appear in Figures 33 to 48. No attempt has been made to label them or to go into a detailed description since the differences which were observed between species were trivial. Linley & Kettle (1964) sum up the position with regard to chaetotaxy as follows :- "The value of the chaetotaxy of the head as a source of useful characters for specific identification is very limited. The arrangement of the setae and pits on the head seems to be constant within the genus, and only small size and positional differences occur from species to species".

However, while studying the chaetotaxy of the larval body, measurements were made of the length of head setae, and setae on the prothorax and anal segment. It was very difficult to discern the tips of most of the setae even under oil immersion. Exact measurements could not always be made therefore, but since repeated measurements of a number of specimens gave about the same lengths, and since only large differences in lengths are of interest, these approximate measurements are given in Table 12 and Table 13. It was also seldom possible to measure a particular seta since some were strongly curved or obscured so that only the longest of the straight and clearly visible setae were measured, be they ventral or dorsal. One seta per specimen was measured.

As far as the chaetotaxy of the head is concerned these measurements suggest that it may be useful to compare the length of the longest head setae with head length. The results of this comparison for the eight species studied appear in Table 12.

It is clear from Table 12 that two species, viz. C. bedfordi and C. magnus differ markedly from the others in having very long head setae in relation to head length. This ratio may therefore have some use in the identification of certain species.

(6) Chaetotaxy of the Body.

Culicoides larvae possess a fixed pattern of setae on each body segment. The arrangement has been described by Lawson (1951) for C. nubeculosus and the setae each identified with a letter. Linley & Kettle (1964) adhered to this terminology when studying C. furens Poey and C. hoffmani Fox and concluded that :- "The chaetotaxy of the larval body is probably fairly constant throughout the genus, ...".

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Table 12. Comparison of length of head and longest head setae of fourth instar Culicoides larvae.

Species*	Mean head length (μ)	Mean seta length (μ)	Ratio of length head : seta
C. pallidipennis	135	35	3.9
C. bedfordi	145	53	2.7
C. schultzei	155	37	4.2
C. distincti-pennis	167	42	4.0
C. nivosus	179	42	4.3
C. pycnostictus	182	47	3.9
C. magnus	204	75	2.7
C. milnei	224	62	3.6

*2 - 5 specimens measured for each species.

In the present study a similar examination was made of each species but the only differences noted between species were in the size and position of the setae. In many instances setae were at first thought to be absent only to be found after a careful search under oil immersion.

Setae appeared to differ greatly in size depending on the species and the location of the setae. It can generally be said that the setae of the anal segment are the longest, followed by those of the prothorax and remaining body segments. The mean lengths of the longest setae on the head, prothorax, and anal segment are recorded in Table 13 together with the ratios of the length of the head setae to prothoracic and anal setae.

From the last two columns in Table 13 it can be seen that the various setae within a species differ little in length, seven of the eight species having ratios very close to 1.0. In C. pallidipennis, however, setae differ markedly in length since the setae of the head are 2.1 times as long as those of the prothorax and 1.4 times longer than those of the anal segment. This character would therefore seem to be of very definite value in separating C. pallidipennis from other fourth instar larvae.

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Table 13. Length of longest setae of fourth instar Culicoides larvae (in microns).

Species	Number measured	Head	Pro-thorax	Anal segment	Ratio setae Head : Prothorax	Ratio setae Head : Anal segment
C. pallidi-pennis	5	35	17	25	2.1	1.4
C. bedfordi	5	53	53	63	1.0	0.8
C. schultzei	5	37	37	46	1.0	0.8
C. distincti-pennis	2	42	49	53	0.9	0.8
C. nivosus	5	42	39	56	1.1	0.8
C. pycnostictus	5	47	48	54	1.0	0.9
C. magnus	2	75	86	71	0.9	1.1
C. milnei	5	62	66	72	0.9	0.9

(7) Anal Papillae.

Linley & Kettle (1964) found the variations in the form of the anal papillae to be of value in larval identification. In the present study, however, difficulty was experienced in obtaining specimens with suitably extruded papillae or with papillae which could be clearly seen through the body wall. No attempt was therefore made to compare anal papillae of the various species.

(8) Key to Fourth Instar Larvae.

1. Thorax pigmented dorsally. 2
   Thorax unpigmented dorsally. 6

2.(1) Pigmentation diffuse, almost filling the segments. 3
   Pigmentation restricted to lateral and anterior bands (Fig. 9).

..... C. milnei

3.(2) Pigment absent from the "neck" or only an odd spot present. 4
   Pigment present on the "neck". 5

4.(3) Head ± 160-173 µ long and ± 110-118 µ wide (Fig. 5).

... C. distinctipennis

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Head ± 175-189 μ long and
± 123-139 μ wide (Fig. 6).

..... C. pycnostictus

5.(3) Three teeth present on each half of dorsal comb of epipharynx, trough of hypopharynx dark and fibrous (Figs. 8, 29 and 24).

..... C. schultzei

More than eight teeth present on each half of dorsal comb of epipharynx, trough of hypopharynx pale and delicate (Figs. 7, 28 and 23).

..... C. nivosus

6.(1) Eye-spots comma-shaped, ratio head length : length of head setae ± 2.7 7

Eye-spots circular, ratio head length : length of head setae > 3.6, post-occipital ridge possesses a ventral median anterior projection (Figs. 12 and 42).

..... C. pallidipennis

7.(6) Head brown, more than 14 teeth on each half of dorsal comb of epipharynx (Figs. 10 and 32).

..... C. magnus

Head dark cream or straw coloured, more than 8 but less than 14 teeth on each half of dorsal comb of epipharynx (Figs. 11 and 30).

..... C. bedfordi

(b) Pupae.

Pupae are more easily recognized than larvae since they have a large number of sclerotized structures which may be used in identification. Pupae are also comparatively easy to collect and the exuviae show all the characters of the intact pupa.

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Dorsal and lateral views of Culicoides pupae are given in Figures 49 and 50. The more important characters are labelled and these will be discussed below. Unfortunately no pupae of C. magnus could be reared so only seven species were studied. Additional material of C. pycnostictus and C. distinctipennis was collected in the field and was compared with the laboratory-reared material.

(1) Colour.

This character has been used by some workers, e.g. Linley & Kettle (1964) to differentiate between species, but in the present study specimens of the seven species all had a light brown abdomen and a darker cephalo-thorax.

(2) Length.

Twenty specimens of each species were measured in alcohol in a petri-dish and their average lengths from the anterior margin of the cephalo-thorax to the tip of the caudal processes, determined. Some specimens appeared to have stretched in the alcohol while some had a shrunken appearance. Measurement of the more normal appearing specimens showed C. nivosus, C. pycnostictus, and C. milnei to be large species with an average length of from 2.2 to 2.4 mm., while C. distinctipennis, C. pallidipennis, C. schultzei, and C. bedfordi were noticeably shorter, averaging 1.8 to 1.9 mm. However, in field collected specimens C. distinctipennis was only slightly smaller than C. pycnostictus.

(3) Prothoracic Respiratory Horn.

This organ and its functions have been adequately described by Lawson (1951), and Kettle & Lawson (1952) have made much use of its characters in describing British biting midges. Its size and prominence make it a very easy structure to study. It possesses a number of characters of taxonomic value. These include the degree of pigmentation as well as the region pigmented, the number of lateral and terminal spiracular papillae (Fig. 51), and the presence of external folds and pointed or dentate scales.

Table 14 and Figures 51 to 57 summarise and illustrate this information for the seven species studied.

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Table 14. Summary of the most important characters of the prothoracic horns of Culicoides pupae.

Species	Number of specimens studied	Pigmentation	Number of spiracular papillae		Presence of scales, folds, etc.
			Lateral	Terminal	
C. nivosus	11	Distal ¼ of horn dark brown.	2-3	4-7(6)*	Dentate scales and folds in central region.
C. pycnostictus	9	Distal ¼ of horn slightly darker than remainder.	4-5	3-6(4)	Dentate scales and folds in central region.
" Field specimens	9	"	3-5(4)	4-7(5)	"
C. distinctipennis	10	Distal ¼ of horn slightly darker than remainder.	2-4(3)	2-5(3,4)	Dentate scales and folds in central region.
" Field specimens	8	"	2-4(3)	3-5(4)	"
C. pallidipennis	6	Proximal ¼ and distal ¼ of horn darker than central region.	4-6(4)	3-6(6)	Annulated rings or folds in central region. Scales absent.
C. schultzei	6	Distal ¼ of horn darker than remainder.	2-4(3)	4-7(6)	Annulated rings or folds in central region. Lateral tubercles pronounced. Scales absent.
C. bedfordi	7	Entire horn dark brown.	3-5(4)	3-6(4,5)	A few small dentate scales on proximal 2/3 of horn. Slight folds may be present on proximal ⅓.
C. milnei	6	Entire horn dark brown.	0	7-11 (10)	Small dentate scales scattered over proximal 2/3 of horn.

* Number in brackets indicates most common number of papillae found.

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It can be seen that three types of pigmentation of the prothoracic horn are found, viz. complete pigmentation, tip pigmented, or base and tip pigmented. This provides a very easy method for identification, at least into groups.

The arrangement and number of tracheal openings or "papillae" is another useful character. C. milnei for example is the only species having no lateral papillae. This character was used by Kettle & Lawson (1952) to distinguish the C. pulicaris group from the other two main groups of British Culicoides species.

The number of papillae, however, is of limited value as a diagnostic character, since it is seldom possible to distinguish all the terminal papillae, and the number of papillae differ not only between individuals of the same species, but also between the horns of a single specimen.

The presence or absence of small pointed or dentate scales and of transverse folds is another useful character for distinguishing groups of species. C. nivosus, C. pycnostictus, and C. distinctipennis are the only species which possess scales as well as folds and thus form one group. A second group possesses annulated rings or folds only and includes C. pallidipennis and C. schultzei. The third group includes those species having small dentate scales but no rings or folds, viz. C. milnei and C. bedfordi which have already been grouped together on the basis of horn colour.

It was at first thought that the length of the horn would be a useful character to distinguish between very similar species such as C. pycnostictus and C. distinctipennis, as measurements of laboratory-reared specimens showed considerable differences in horn lengths (Table 15). However, measurement of the horns of specimens of these species collected in the field showed them to be of equal length, so that horn length apparently depends on conditions in the larval medium. Unfortunately no field specimens of the other species were collected so that this comparison between laboratory-reared and field-collected specimens could not be extended. The examples above should, however, be sufficient warning not to depend on a comparison of measurements for species differentiation.

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Table 15. Average lengths of prothoracic horns and antero-marginal setae of Culicoides pupae.

Species*	Prothoracic Horn (μ)	Antero-marginal seta (μ)
Lab.-reared		
C. nivosus	267	54
C. pycnostictus	239	62
C. distinctipennis	161	54
C. milnei	160	71
C. pallidipennis	160	94
C. schultzei	227	30
C. bedfordi	139	51
Field-collected		
C. pycnostictus	196	54
C. distinctipennis	205	54

*9 - 21 specimens measured for each species.

(4) Operculum.

"The dorsum of the head is occupied largely by the "operculum", which is bounded by the arms of the ecdysial suture and therefore corresponds to the fronto-clypeus. The arms of the suture do not converge anteriorly, so that when the adult emerges, the operculum, a convenient name to retain, is simply reflected forward, as on a hinge, and returns more or less to its original position after the adult has emerged. The anterior border of the operculum bears two large tubercles, which each carry a single, large, articulated seta. These are the antero-marginal tubercles". (Lawson, 1951).

A large area of the operculum may also be covered with spines of sizes varying from minute projections of the integument (spinules), to long seta-like structures.

Kettle & Lawson (1952) although recognizing variations between opercula of different groups, found differences between species within a group to be non-significant, and were therefore reluctant to include characters of the opercula in their key to the identification of British biting midges. The fact that an unobstructed view required the operculum to be dissected off, also detracted from its value as a taxonomic character.

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The writer is, however, inclined to agree with Linley & Kettle (1964) that, "when specific differences are being sought between a relatively small number of species that are not necessarily closely related, the operculum forms a useful character".

A short description of the opercula of each of the seven species studied, follows below. The antero-marginal (a-m) tubercles and setae will be discussed separately in the section dealing with head tubercles and setae.

C. nivosus (Fig. 58).

Spines ranging from medium to short, sturdy, densely cover entire area posterior of a-m tubercles but absent from posterior quarter and between a-m tubercles.

C. pycnostictus (Fig. 60).

Spines short, sturdy, decreasing in size to minute rounded projections (spinules). Spines cover most of the area posterior to a-m tubercles but are absent from posterior quarter. Spinules are present anterior to a-m tubercles and sometimes between these tubercles.

Field collected specimens similar.

C. distinctipennis (Fig. 59).

Spines of same size and distribution as C. pycnostictus except that spinules are absent anterior to a-m tubercles.

Field collected specimens similar.

C. pallidipennis (Fig. 62).

Spines very long and flexible of about equal size. Seldom more than 50 along lateral margins and posterior to a-m tubercles. Sometimes between a-m tubercles but never anterior to them. Central area and posterior third of operculum bare. Spinules absent.

C. schultzei (Fig. 63).

Operculum almost rectangular in shape, not narrowing gradually posteriorly as in the other species. Spines short, sturdy, scattered over area well posterior to a-m tubercles especially along lateral margins. Absent from posterior sixth. Spinules abundant, interspersed between the short spines, and present between and anterior to the a-m tubercles.

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C. bedfordi (Fig. 61).

Spines short, sturdy, scattered over area well posterior to a-m tubercles especially along lateral margins. Absent from most of posterior quarter, from small central region and between a-m tubercles. Spinules absent.

C. milnei (Fig. 64).

Spines very short. Sparsely scattered along lateral margins and in median band posterior to a-m tubercles. Spinules present between a-m tubercles. Posterior third of operculum bare.

From the above descriptions and figures it is possible to distinguish between the seven species studied. It may be noted that the first three species and C. bedfordi are very similar and can only be separated on minor differences. For example C. pycnostictus differs from C. distinctipennis in having spinules present anterior to the antero-marginal tubercles. The other three species differ more markedly and are very easy to recognize by reference to Figures 62 to 64.

(5) Head Tubercles and Setae.

The pupal head possesses two prominent dorsal tubercles, the antero-marginal and the antero-dorsal tubercles, which bear one and two setae, respectively. Ventrally there are two groups of two setae each, viz. the ventro-lateral and ventro-median tubercles. The positions of these tubercles and setae are shown in Figure 50, while detailed drawings for each species are given in Figures 65 to 71. The nomenclature used in naming the tubercles and setae of the head and thorax is that proposed by Carter et al. (1920).

The antero-marginal tubercles are situated on the operculum (Figs. 58 to 64), and may bear a short stout seta as in C. schultzei, a medium length seta in most species, or a long thin seta as in C. pallidipennis. Generally a distinct setal socket is evident at the base of this tubercle. Average lengths of these setae are shown in Table 15, where it can be seen that laboratory-reared and field-collected C. pycnostictus differ slightly in setal length although C. distinctipennis measurements are identical. It would therefore be unsafe to place reliance on exact measurements for differentiation of species.

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The antero-dorsal tubercles bear two setae subequal in length. These are very long in C. pallidipennis and C. milnei, the longest setae being more than twice as long as the corresponding setae of the other five species. The shorter setae in the two aforementioned species are always more than half the length of the longer setae, while in the other five species the shorter setae are usually half or less than half the length of the longer setae.

Two thin setae subequal in length are present in all the species in the ventro-lateral position.

Similar setae are present in the ventro-median position in all the species except C. pallidipennis where these setae cannot be discerned.

The length of the setae of the antero-marginal and antero-dorsal tubercles can therefore be used to separate C. pallidipennis and C. milnei from the other species.

(6) Thoracic Tubercles and Setae.

In the anterior region of the prothorax there are two groups of small setae of very limited importance (Figs. 49 and 51). The most prominent group is situated on the dorso-lateral tubercle, laterad of the base of the prothoracic respiratory horns. Two or three small setae may be present but are not easily seen.

On the inner side of the horn base is a slight swelling normally bearing a single small seta and called the dorso-median tubercle by Lawson (1951). In all the species studied only a single seta could be seen, and this only with difficulty. Both these groups are clearly illustrated in Figures 51 to 57.

On the dorsal hump of the thorax are a group of tubercles and setae called the "dorsals" (Figs. 49 and 50 (dors.)). There are normally five setae or setal sockets and these are numbered and illustrated in Figures 72 to 78. The first two setae are prominent and arise from pronounced tubercles. The first seta is short and sturdy and similar in all the species. The second seta, however, is very long in C. pallidipennis and C. milnei but short and stout in the other five species. In this respect they agree with the antero-marginal and antero-dorsal setae discussed earlier. The third seta is very short in all species except C. pallidipennis, where it can hardly be made out. The fourth seta is long and slender in all species, and the

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fifth seta is represented by an empty socket in all species.

A markedly longer second dorsal seta can therefore be used as an additional character to separate C. pallidipennis and C. milnei from the other species.

(7) Metathorax.

The posterior margin of the scutellum is prolonged posteriorly in the mid-dorsal line and projects into an indentation in the anterior margin of the metathorax. Kettle & Lawson (1952) showed that this indentation divides the metathorax completely in almost all the British Culicoides species but has a different appearance in other genera of the same family. They used this character in their pupal key to separate Culicoides from other genera.

In the seven South African Culicoides species studied, all have the metathorax divided dorsally by the scutellum and no great differences exist between species. This character is illustrated in Figure 49, which is representative of the seven species studied.

(8) Fourth Abdominal Segment.

The abdomen is composed of nine segments. The ninth or caudal segment differs markedly from the others so that it will be considered separately. Segments one to eight may possess outgrowths of the integument called tubercles, which may or may not bear a seta. Lawson (1951) has shown segments one, two and eight to differ considerably in the number of tubercles and setae, while segments three to seven have a fairly constant arrangement. Carter et al. (1920) named five groups of tubercles according to their position on the segment. This terminology has been adhered to by all workers to the present day. These groups of tubercles are :-

dorsal antero-submarginal tubercles (d.a.s.m.)
dorsal postero-marginal tubercles (d.p.m.)
lateral antero-submarginal tubercles (l.a.s.m.)
lateral postero-marginal tubercles (l.p.m.)
ventral tubercles (vn.)

Their positions are shown in Figure 82. Lawson (1951) took the fourth abdominal segment as being representative of all the segments, and his lead has been followed by Kettle & Lawson (1952) and Linley & Kettle (1964). For the purpose of comparison therefore, the fourth segment of the seven species under study has been studied in

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detail and lateral views are given in Figures 79 to 85.

Small spinules are present on the anterior part of the fourth segment of all species. They may be very numerous and completely encircle the segment as in C. milnei (Fig. 79) or may be absent laterally and reduced to a few odd patches as in C. pallidipennis (Fig. 85).

A spiracle can sometimes be made out as a circular depression situated antero-laterally (Fig. 82), but is not visible in all the specimens.

The shape and number of tubercles, the presence or absence of setae on the tubercles, and the form and length of the setae are important taxonomic characters.

In most species the tubercles are rounded or may project slightly to form a blunt point or shoulder as in the l.p.m. tubercles of C. milnei (Fig. 79). In C. bedfordi, however, the l.p.m. and l.a.s.m. tubercles are prolonged into two spines with a seta arising from the centre of the fork formed by these spines (Fig. 83). Kettle & Lawson (1952) used this character to distinguish the C. obsoletus group from the two other major groups of British Culicoides species.

In six of the seven species studied, the number of tubercles in the positions named above is constant. However, C. pallidipennis differs radically in that the d.p.m. tubercles are reduced from the normal five to two (Fig. 85). This character is probably of considerable taxonomic value.

A single seta is present on each tubercle except in the d.p.m. tubercles where the first three tubercles are usually naked. Sometimes a small spine is present on the first of these tubercles.

The setae on the various tubercles differ in shape and length. These differences appear to be common to most species, e.g. the second l.p.m. tubercle always possesses a longer seta than tubercles one and three. This rule is not adhered to by C. pallidipennis, however, where all setae are of almost equal length (Fig. 85).

The posterior margin of each segment has a ring of pale closely packed nodules forming a wide border which is common to nearly all species. C. milnei, however, differs in that the posterior margin has a mosaic or mottled appearance (Fig. 86). Kettle & Lawson (1952) noted this "crazy paving" effect in some British Culicoides species.

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(9) Caudal Segment.

When viewed dorsally under high power (x 620), the caudal segment shows a number of characters which can be of great value in identification. Kettle & Lawson (1952) in their study of 28 British Culicoides spp. used only the colour of the tips of the caudal spines in their key, and included the angle of the spines to the long axis in only some of their descriptions. Linley & Kettle (1964), however, found the features of the caudal segment to be quite useful taxonomically especially since "they are readily seen under low magnification without much preliminary work". In the present study the angle of the caudal spines was found to be of little or no value compared with other more obvious characters. A dorsal view of the caudal segment of the seven species studied is given in Figures 86 to 92.

A broad ring of small pointed scales or spinules encircles the anterior end of the caudal segment of all the species. C. pallidipennis differs slightly from the other six species in that the scales are restricted to one or two rows (Fig. 89).

Small pointed scales are also found dorsally confined to a patch or to rows in the centre of the caudal segment in five of the species but are absent in C. nivosus and C. schultzei (Figs. 88 and 87). In C. milnei scattered scales connect this central patch with the anterior rows described earlier (Fig. 86). In C. pallidipennis large scales are arranged across the centre of the caudal segment to form a single or double row (Fig. 89).

The tips of the caudal spines are darkly pigmented or sclerotized in all seven species, and one or two sensillary sockets are present at the base of each spine.

The dorsal surface of the caudal spines of six species is covered to a greater or lesser extent with small scales, but these are absent in C. pallidipennis.

In the eighth segment anterior scales are present as in the caudal segment, but these are never very large and the rows of scales may be broken in places. In C. milnei scattered scales extend backwards on the dorsal side almost as far as the bases of the posterior tubercles. This segment is otherwise very similar to the fourth abdominal segment.

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(10) Key to Pupae.

1. Tip of prothoracic horn darker than rest of horn (Figs. 51 to 55). 2

Entire prothoracic horn dark brown (Figs. 56 and 57). 5

2.(1) Small dentate scales and transverse folds present on prothoracic horn (Figs. 51 to 53). 3

Annulated rings or transverse folds present on prothoracic horn. Scales absent (Figs. 54 and 55). 6

3.(2) Very small dentate scales absent from central dorsal area of caudal segment (Fig. 88).

..... C. nivosus

Very small dentate scales present on central dorsal area of caudal segment (Figs. 90 and 91). 4

4.(3) Spinules on operculum present anterior to antero-marginal tubercles (Fig. 60).

..... C. pycnostictus

Spinules on operculum never anterior to antero-marginal tubercles (Fig. 59).

..... C. distinctipennis

5.(1) Lateral papillae on prothoracic horn absent (Fig. 56).

..... C. milnei

Lateral papillae on prothoracic horn present (Fig. 57).

..... C. bedfordi

6.(2) Small dentate scales present on central dorsal area of caudal segment (Fig. 89).

..... C. pallidipennis

Small dentate scales absent from central dorsal area of caudal segment (Fig. 87).

..... C. schultzei

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4. CONCLUSIONS

Within the limited number of Culicoides species studied it has been possible to find sufficient characters in both larvae and pupae to be able to identify each species after suitable preparation and mounting. Whether the same characters will enable the identification of further species remains to be seen, but some combination of the many characters studied should make this possible.

Certain characters which have been used by other workers were found to be of little or no value in identification, e.g. head ratio and anal papillae in larvae, and pupal colour and angle of caudal spines. A few new characters were found to be useful as confirmatory characters. These included a comparison of lengths of the head and setae on the head and body. In one instance the post-occipital ridge was found to be very distinctive as well as the shape of the larval eye-spots. In pupae, comparisons of setal lengths on head and thorax, and the presence or absence of small dentate scales on the central area of the caudal segment and on the caudal spines, were useful new characters.

Although features such as larval pigmentation, head colour, pupal horns, etc. are fairly easy to see in an unprepared specimen, they can only be used to place the specimen into a group. Accurate identification will depend upon a careful study of mounted specimens under high power, and for larval setae and epipharyngeal studies, oil immersion is necessary.

5. SUMMARY

Almost no work has been done on the morphology of the larvae and pupae of Culicoides midges in Africa. This study on the fourth instar larvae of eight Culicoides species and of the pupae of seven species is intended as a foundation for further studies. A search has therefore been made among the many morphological characters for those of possible taxonomic value.

The fourth instar is the larval instar which lends itself most to morphological and taxonomic study. Characters studied under low magnification included head colour, shape of eye-spots, the presence or absence of

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pigmentation on the neck and thoracic segments, larval length, head length, head breadth, width of oral ring, and others. Many characters could only be seen under high magnification which involved careful preparation and mounting on slides. Characters found to be of possible taxonomic value were the epipharyngeal skeleton (made up of a number of toothed combs and a hypopharynx), the mandibles, and the ratio of the length of head setae to head length. This was extended to the remainder of the body where the lengths of setae on the anal segment and prothorax were compared with those of the head. A tentative key to the eight species studied is included.

Pupae were somewhat more rewarding to study since once suitably mounted they provided many clear characters which could possibly be used in identification. These included the prothoracic respiratory horns, the operculum, tubercles and setae on the head, thorax, and abdomen, and characters of the caudal segment. It was thus easy to draw up a key for the identification of the seven pupae studied.

GENERAL SUMMARY

This study is divided into two parts. Part I deals with the biology of some Culicoides species found in the Onderstepoort area and includes investigations on the life-cycle in the laboratory, breeding sites, hours of activity, seasonal abundance, and variation in species abundance. In most of these studies extensive use was made of a suction-type light trap.

For morphological studies the larvae of C. pallidipennis, C. pycnostictus, C. distinctipennis, C. nivosus, C. schultzei, C. milnei, C. bedfordi, and C. magnus were reared in the laboratory, as were the pupae of all these species except C. magnus. Laboratory observations on the life-cycles of these species showed :-

(1) The average period from egg to egg for all species is 25 days at ± 72°F.
(2) Eggs hatch while submerged and larvae survive immersion for more than six days.

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(3) Pupae of ~~seven~~ six species float on water and adult emergence is unaffected. Pupae of C. pallidipennis drown.

(4) Low temperatures adversely affect egg viability but have no effect on larvae. Culicoides overwinter in the larval stage.

(5) Four of the eight species reared flourished on a bovine manure/soil medium. C. pallidipennis did not.

Field observations and light trap catches revealed :-

(1) Many species breed in manure-polluted mud and in marshy areas.

(2) The main breeding site of C. pallidipennis is still unknown. In 1950 C. pallidipennis was found breeding in moist kikuyu grass-covered areas.

(3) Culicoides are most abundant between 9 p.m. and 5 a.m. when humidity is high and temperatures comparatively low.

(4) Culicoides are seldom caught in June, July, or early August due to low winter temperatures. As temperatures rise so the catch increases.

(5) Each year in January or February there is a sudden threefold to elevenfold increase in catch, these numbers being maintained until the winter drop.

(6) Three theories relating this sudden increase to rainfall have been tendered.

(7) C. pallidipennis and C. schultzei have one peak of abundance occurring between February and April.

(8) C. nivosus, C. pycnostictus, and C. distinctipennis have an early peak in November or December and one or more later peaks between February and April.

(9) C. pallidipennis may constitute up to 97.4 per cent of the Onderstepoort catch. Theories relating catch to rainfall thus apply to C. pallidipennis as well.

Part II deals with the morphology of fourth instar larvae and pupae of Culicoides. Under x50 magnification larvae can be identified or sorted into groups on the basis of head colour, shape of eye-spots, presence or absence of pigmentation, and its distribution. Under high power, characters such as number and size of teeth on the dorsal comb of the epipharynx, shape and width of comb, head measurements, ratio of setal lengths, and structure of the hypopharynx can be used.

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After suitable mounting on a slide, pupae can be identified using characters of the prothoracic respiratory horns, opercula, caudal segments, and the tubercles and setae on the head, thorax, and abdomen.

Keys for the identification of fourth instar larvae and pupae are presented.

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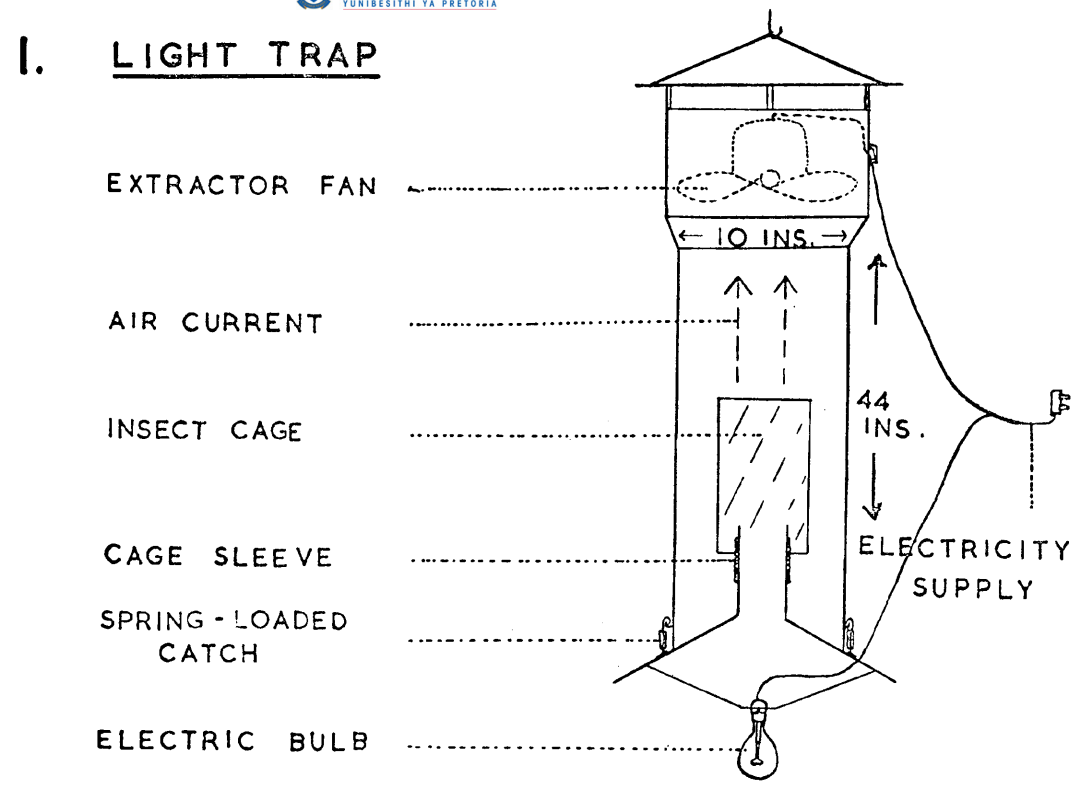

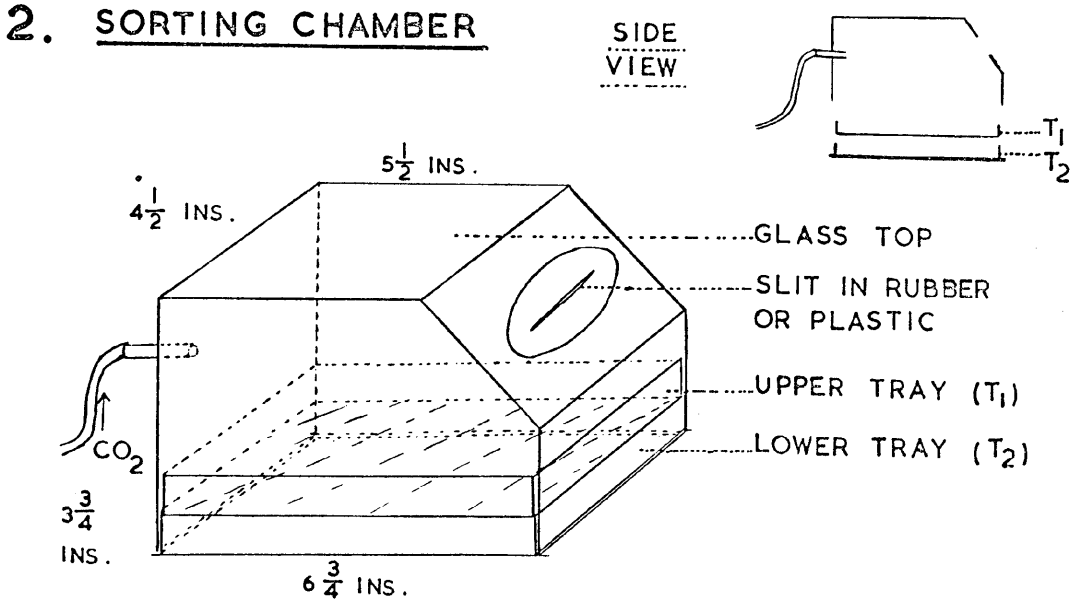

Fig. 1 - Suction-light trap.

Fig. 2 - *Culicoides* sorting chamber.

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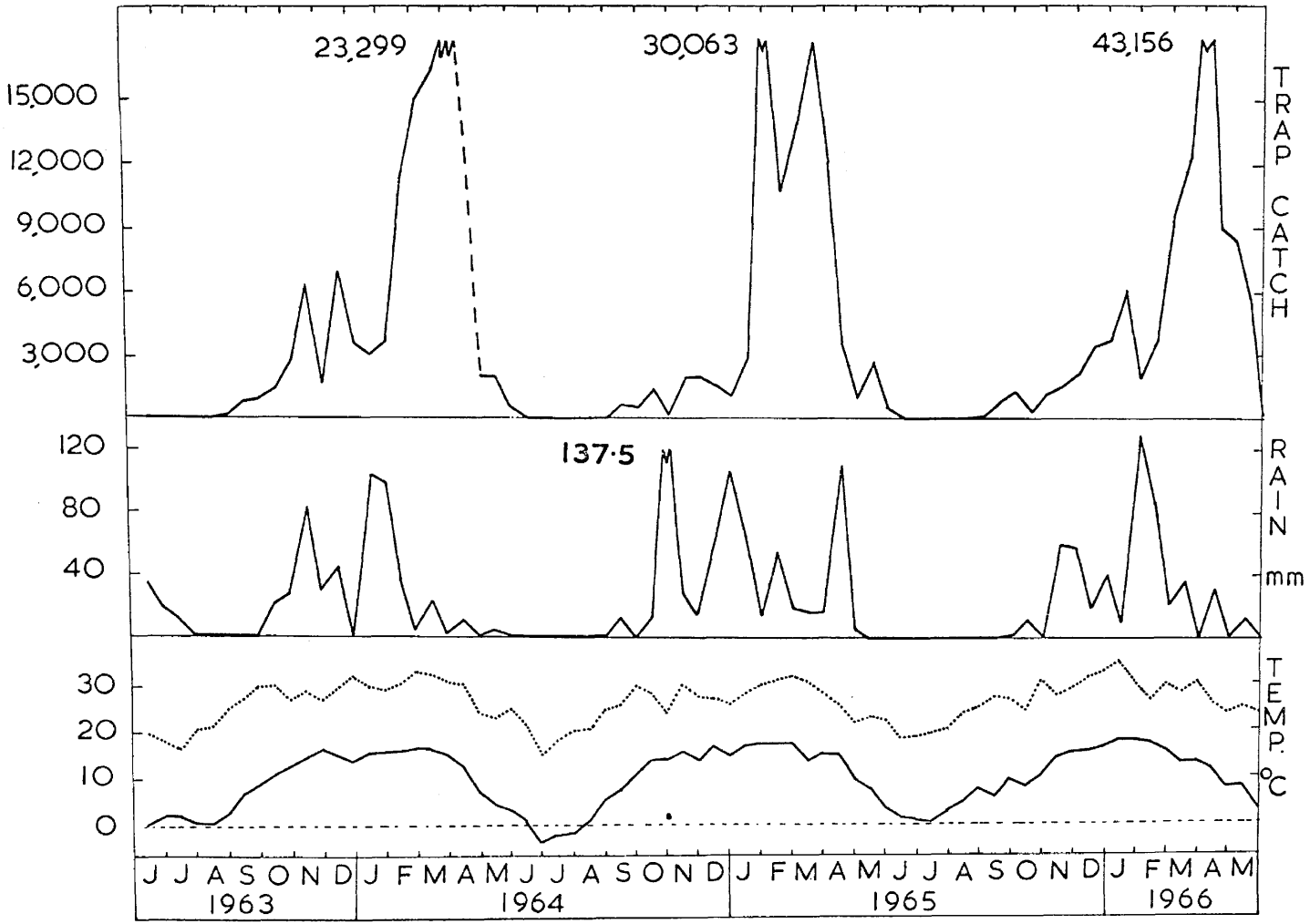

Fig. 3 -

Graphical presentation of Culicoides light trap catches, rainfall, and maximum and minimum temperatures, at Onderstepoort for the period 1st June, 1963 to 31st May, 1966.

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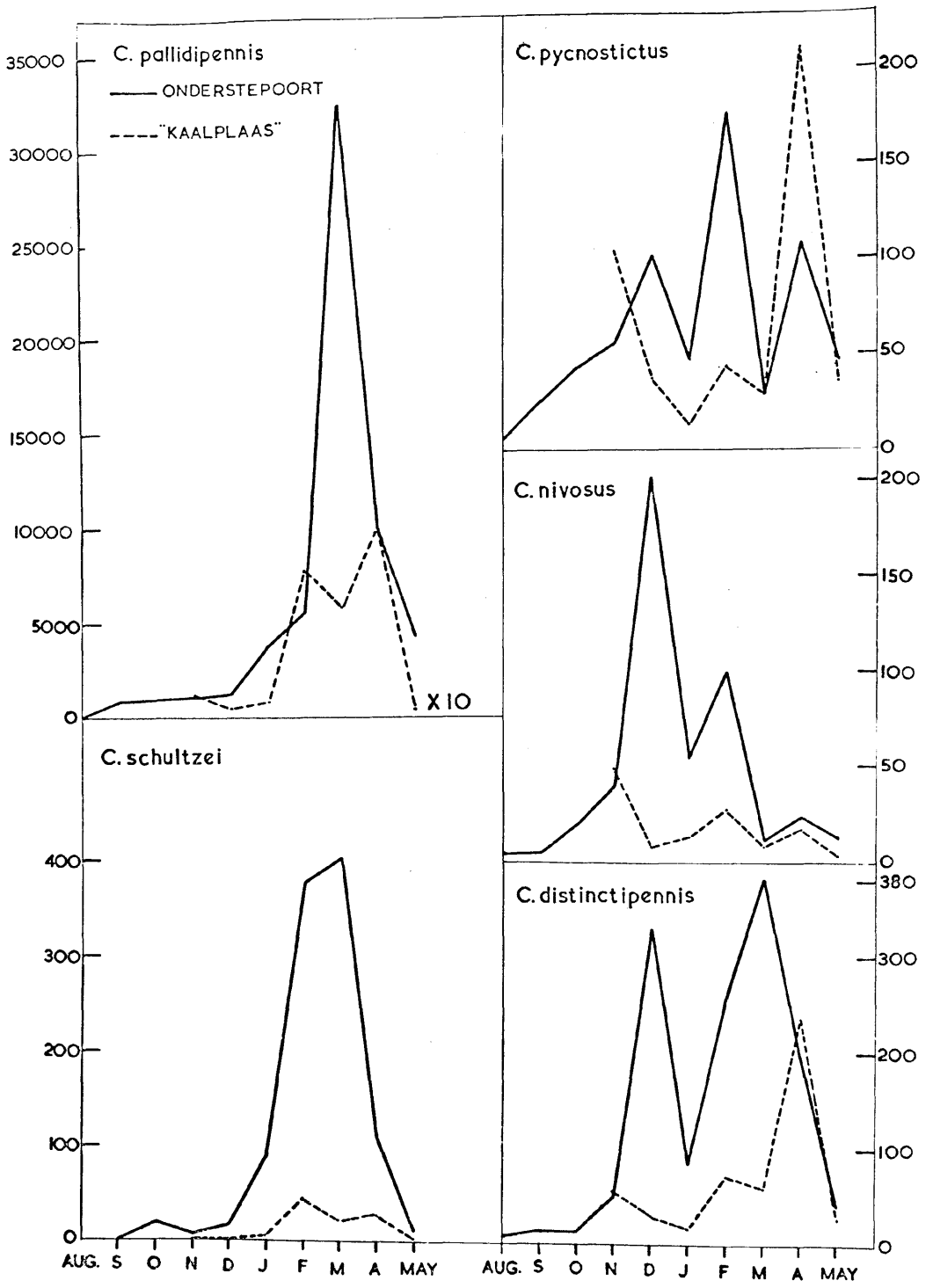

Fig. 4 - Graphical presentation of the seasonal abundance of five Culicoides species taken in light traps at two localities during 1965 - 1966.

Solid line represents "Onderstepoort" trap.

Broken line represents "Kaalplaas" trap.

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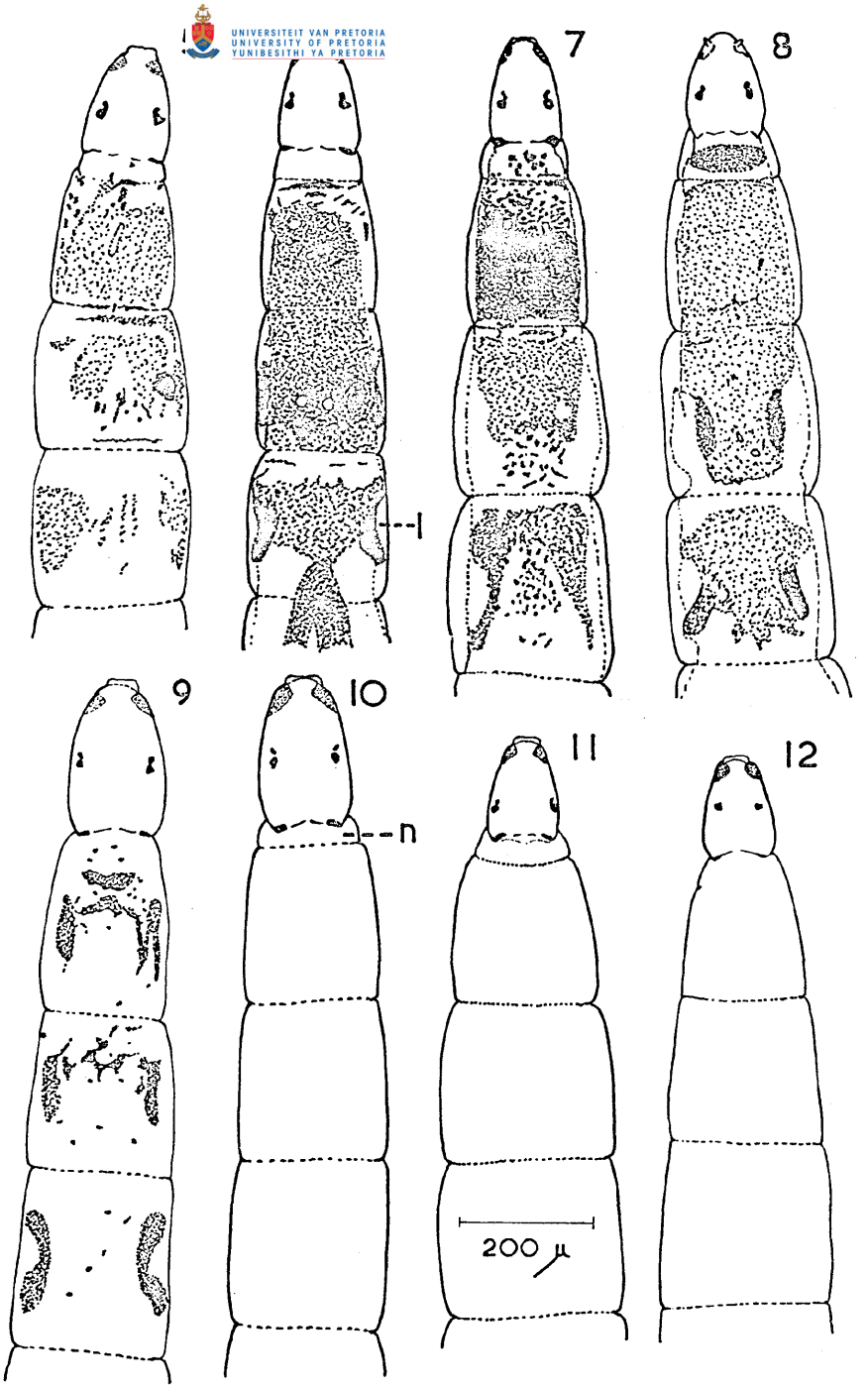

Dorsal view of fourth instar Culicoides larvae.

Fig. 5 - C. distinctipennis.
Fig. 6 - C. pycnostictus.
Fig. 7 - C. nivosus.
Fig. 8 - C. schultzei.
Fig. 9 - C. milnei.
Fig. 10 - C. magnus.
Fig. 11 - C. bedfordi.
Fig. 12 - C. pallidipennis.

l, "lateral body"; n, "neck".

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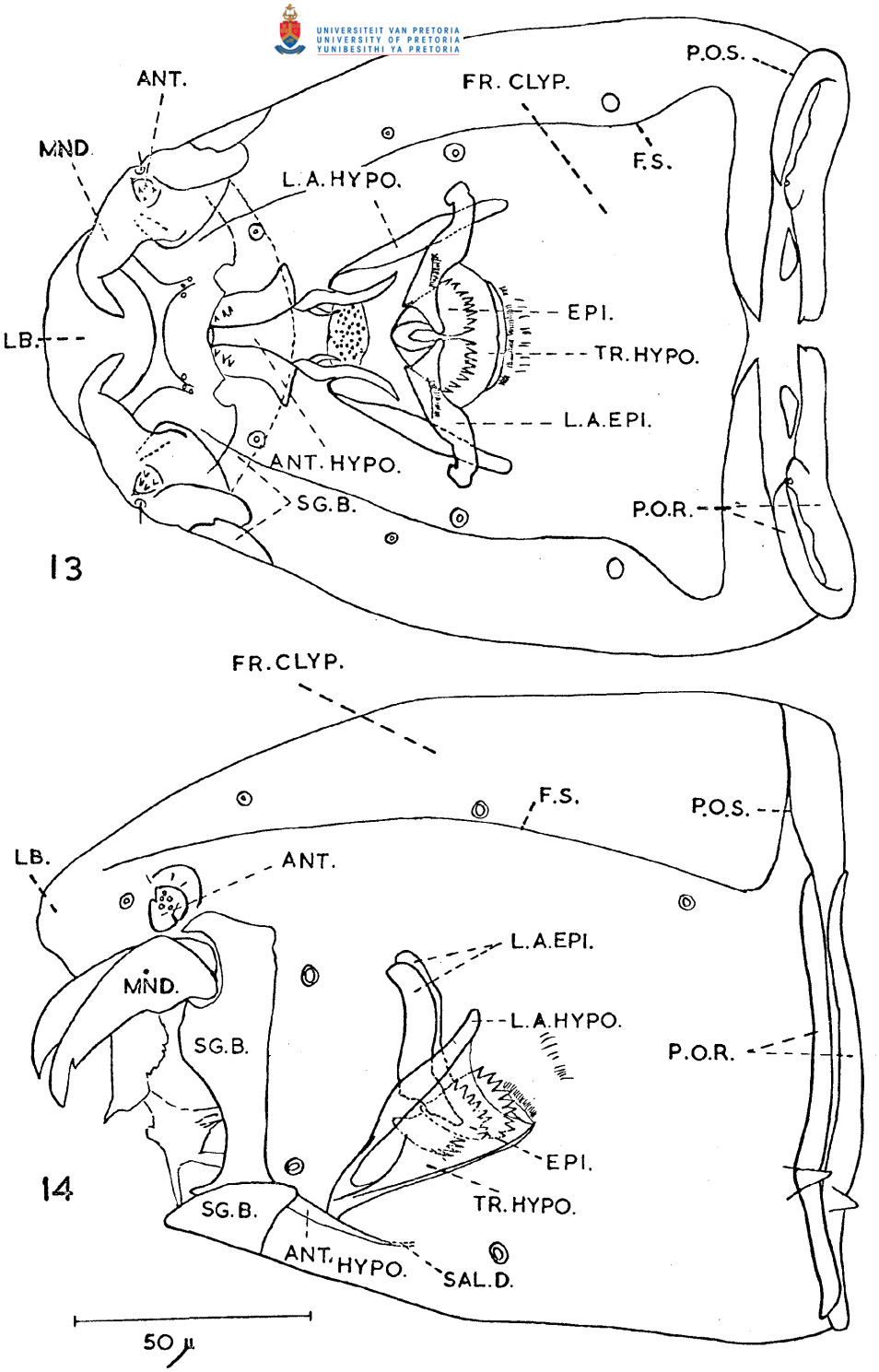

Diagramatic views of heads of fourth instar Culicoides larvae.

Fig. 13 - Dorsal view.
Fig. 14 - Lateral view.

ant, antenna; ant. hypo, anterior hypopharynx; epi, epipharynx; f.s, frontal suture; fr. clyp, fronto-clypeus; l.a.epi, lateral arms of epipharynx; l.a.hypo, lateral arms of hypopharynx; lb, labrum; mnd, mandibles; p.o.r, post occipital ridge; p.o.s, post occipital suture; sal.d, salivary duct; sg.b, subgenal band; tr.hypo, trough of hypopharynx.

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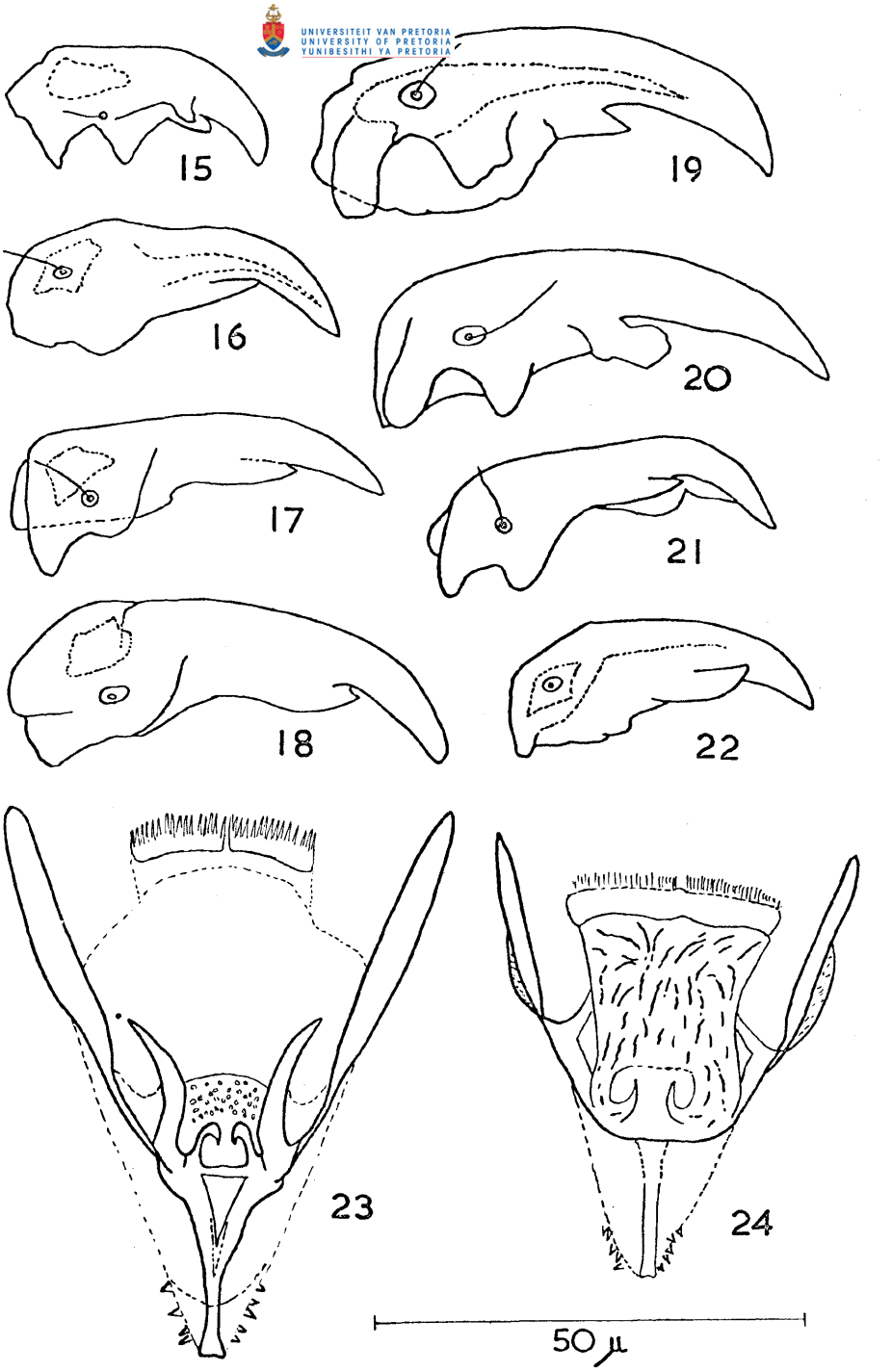

Figs. 15 to 22 - Lateral view of mandibles of fourth instar Culicoides larvae.

Fig. 15 - C. pallidipennis.
Fig. 16 - C. distinctipennis.
Fig. 17 - C. pycnostictus.
Fig. 18 - C. nivosus.
Fig. 19 - C. milnei.
Fig. 20 - C. magnus.
Fig. 21 - C. schultzei.
Fig. 22 - C. bedfordi.

Figs. 23 to 24 - Hypopharynges of fourth instar Culicoides larvae.

Fig. 23 - Hypopharynx representative of all species except C. schultzei.

Fig. 24 - Hypopharynx of C. schultzei.

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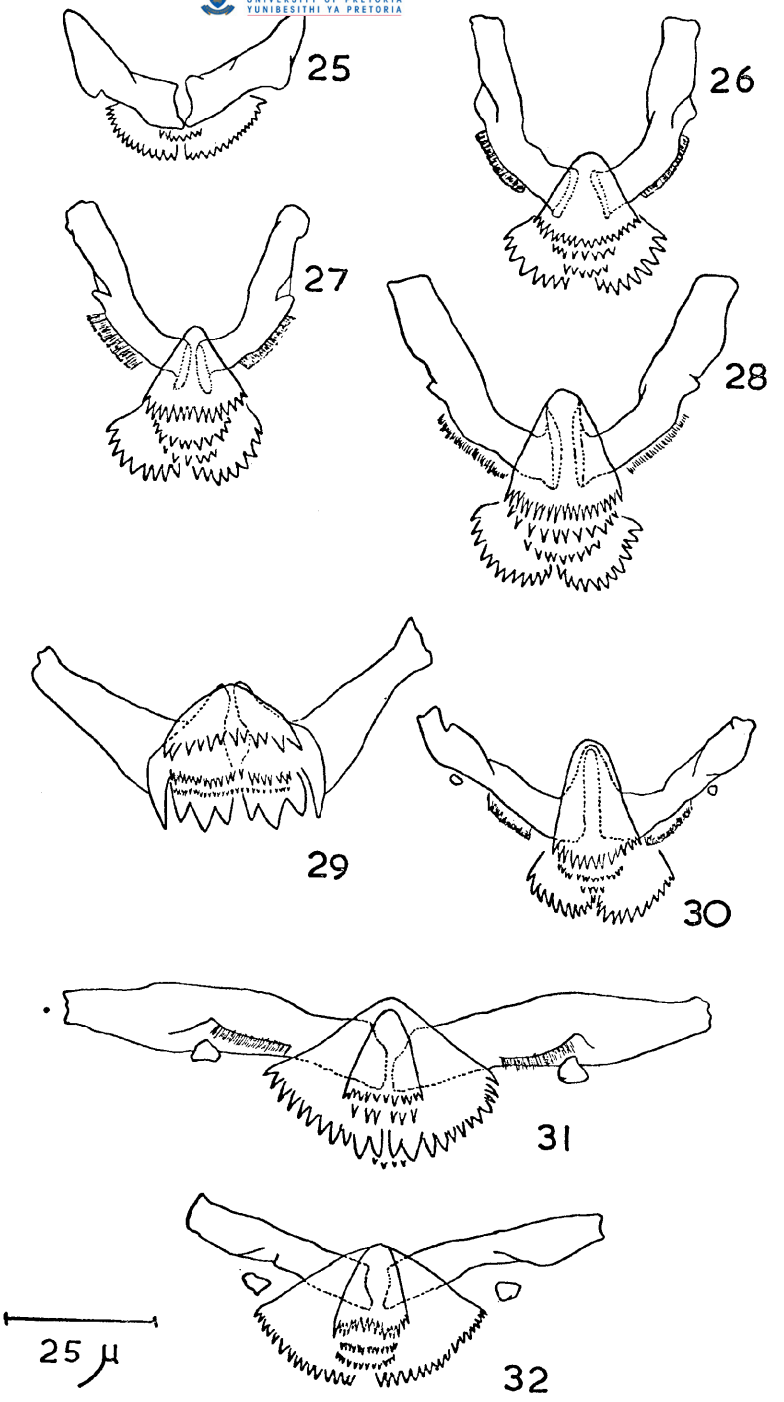

Ventral view of epipharynges of fourth instar *Culicoides* larvae.

Fig. 25 - *C. pallidipennis*.
Fig. 26 - *C. distinctipennis*.
Fig. 27 - *C. pycnostictus*.
Fig. 28 - *C. nivosus*.
Fig. 29 - *C. schultzei*.
Fig. 30 - *C. bedfordi*.
Fig. 31 - *C. milnei*.
Fig. 32 - *C. magnus*.

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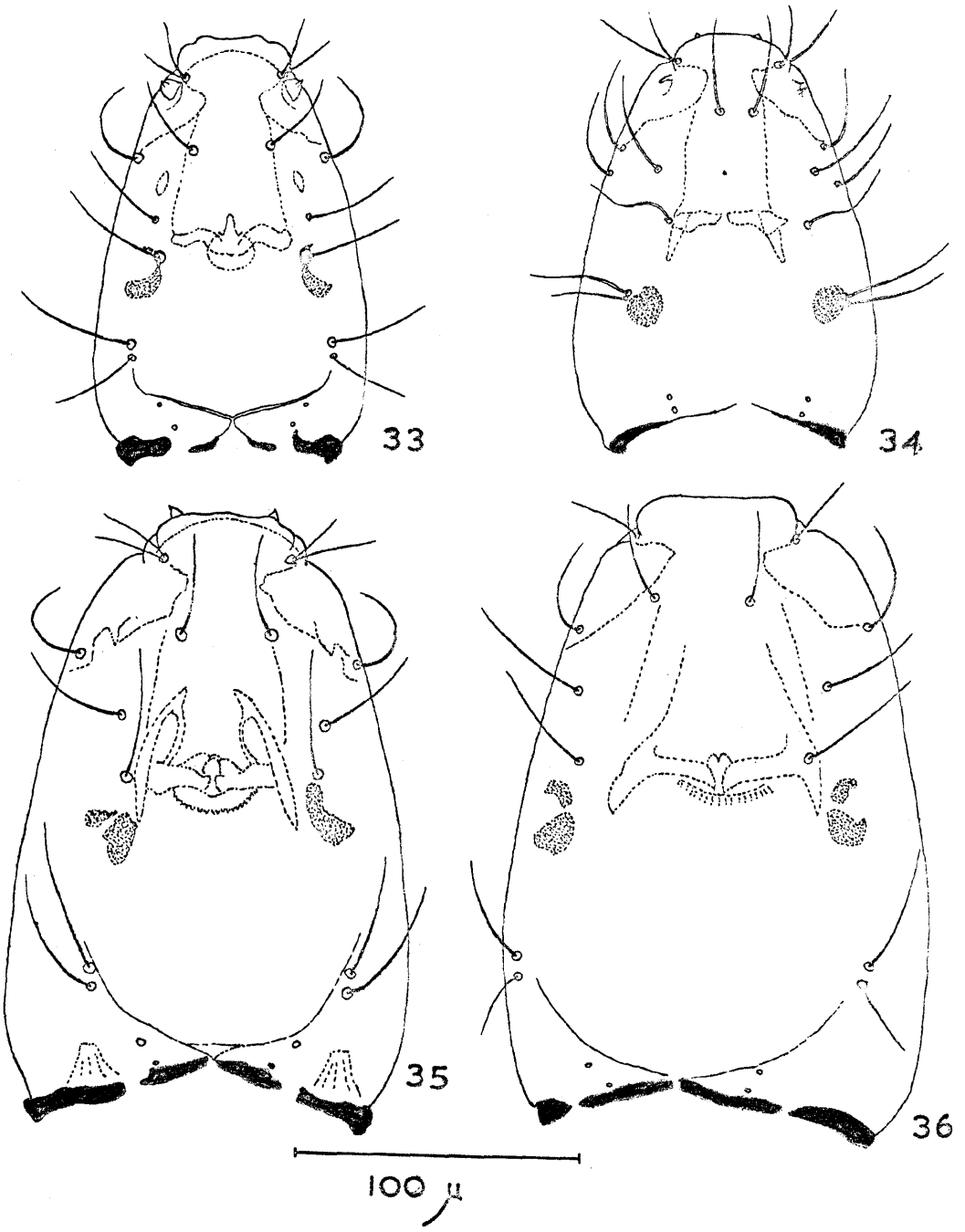

Dorsal view of heads of fourth instar Culicoides larvae.

Fig. 33 - C. bedfordi. Fig. 34 - C. pallidipennis

Fig. 35 - C. magnus. Fig. 36 - C. milnei.

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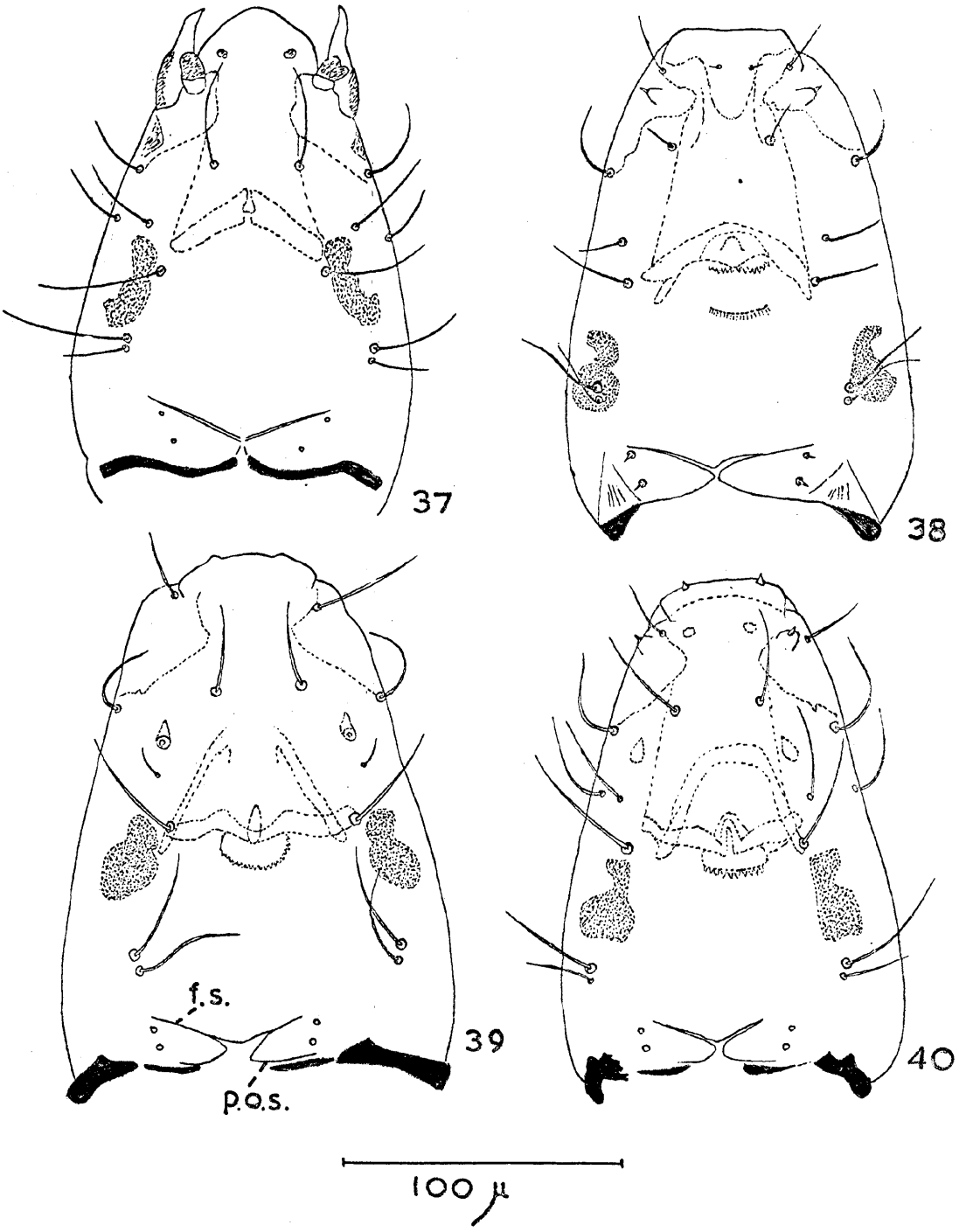

Dorsal view of heads of fourth instar Culicoides larvae.

Fig. 37 - C. schultzei. Fig. 38 - C. nivosus.

Fig. 39 - C. pycnostictus. Fig. 40 - C. distinctipennis.

f.s, frontal suture; p.o.s, post occipital suture.

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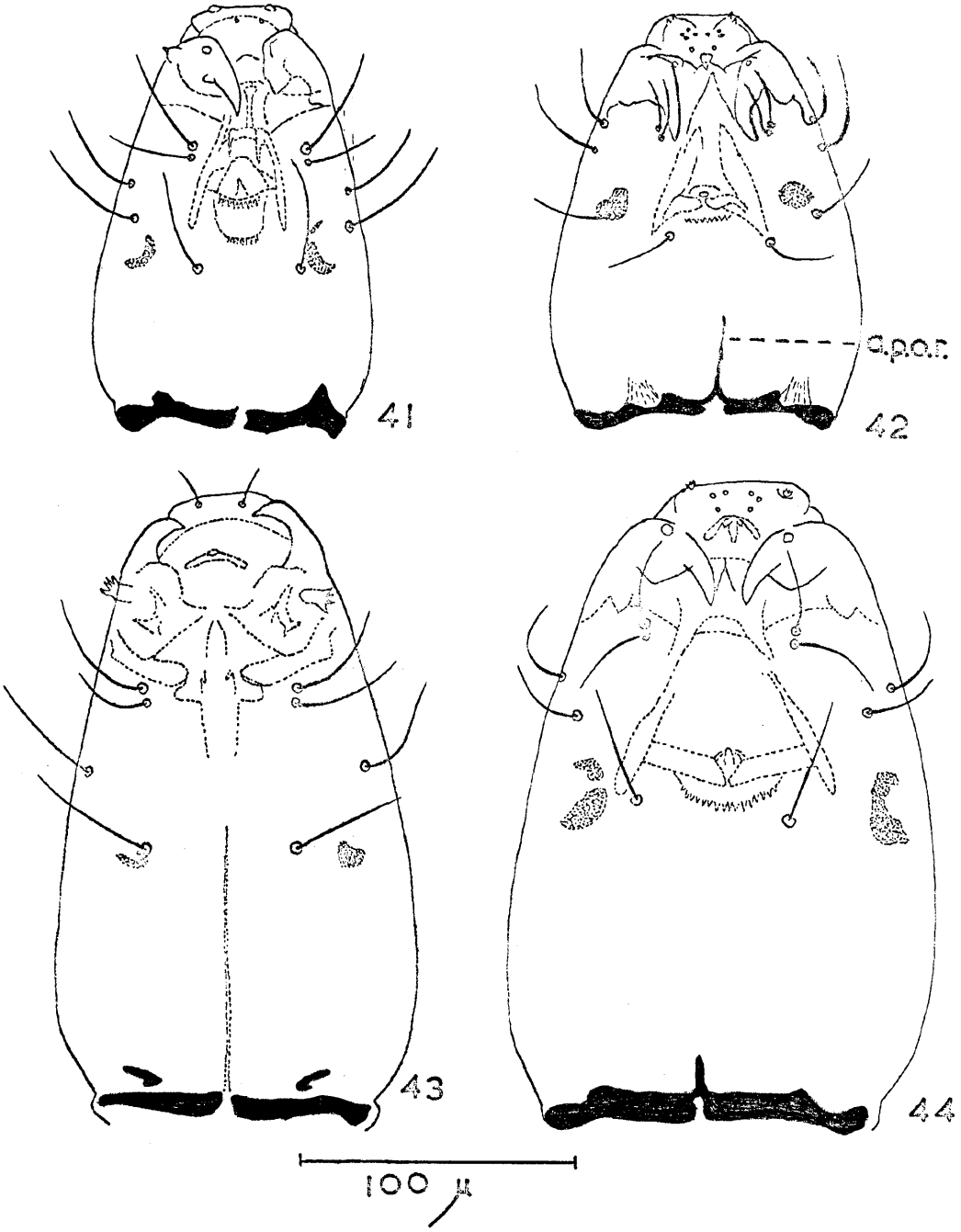

Ventral view of heads of fourth instar Culicoides larvae.

Fig. 41 - C. bedfordi. Fig. 42 - C. pallidipennis.

Fig. 43 - C. magnus. Fig. 44 - C. milnei.

a.p.o.r, anterior projection of post occipital ridge.

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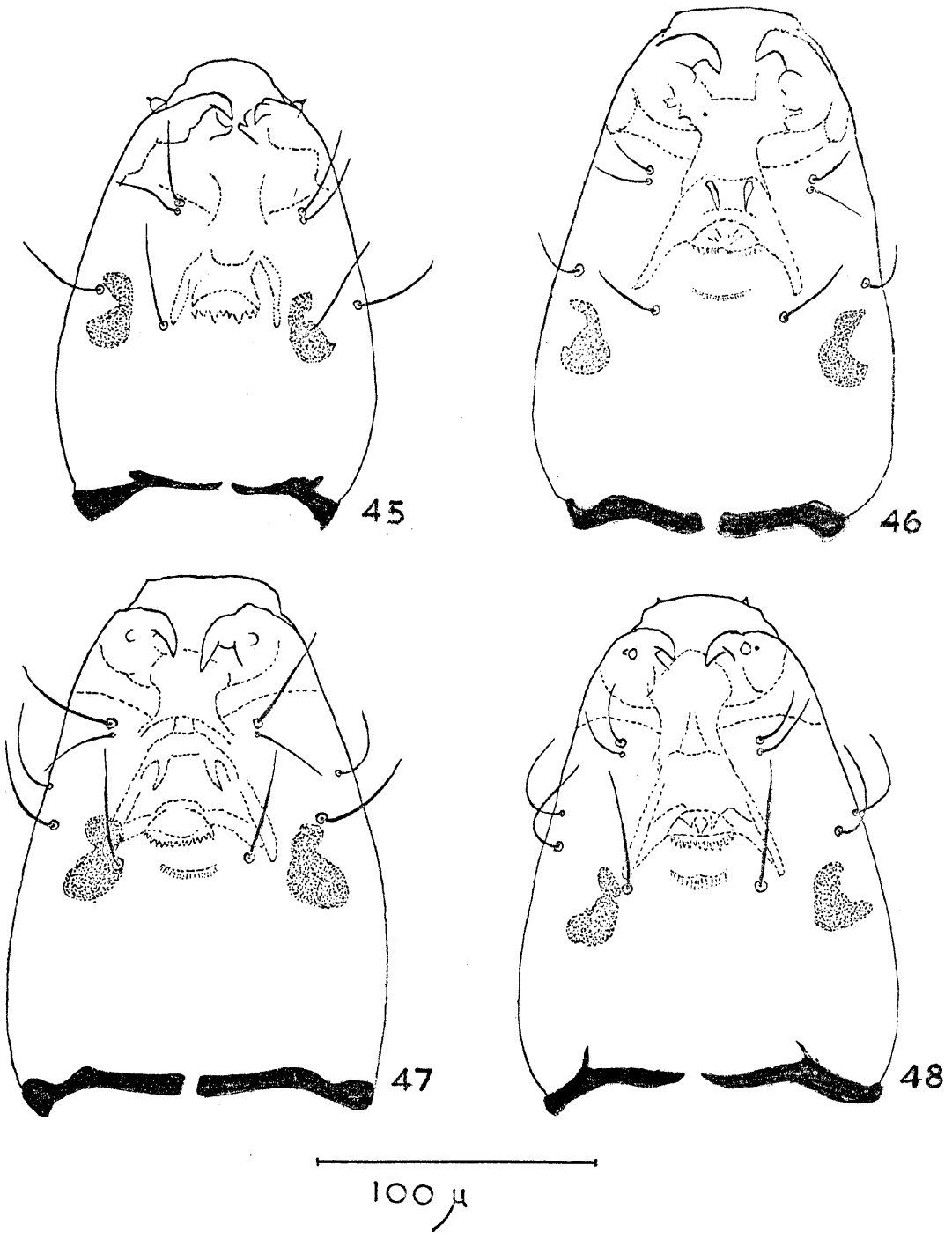

Ventral view of heads of fourth instar Culicoides larvae.

Fig. 45 - C. schultzei. Fig. 46 - C. nivosus.

Fig. 47 - C. pycnostictus. Fig. 48 - C. distinctipennis.

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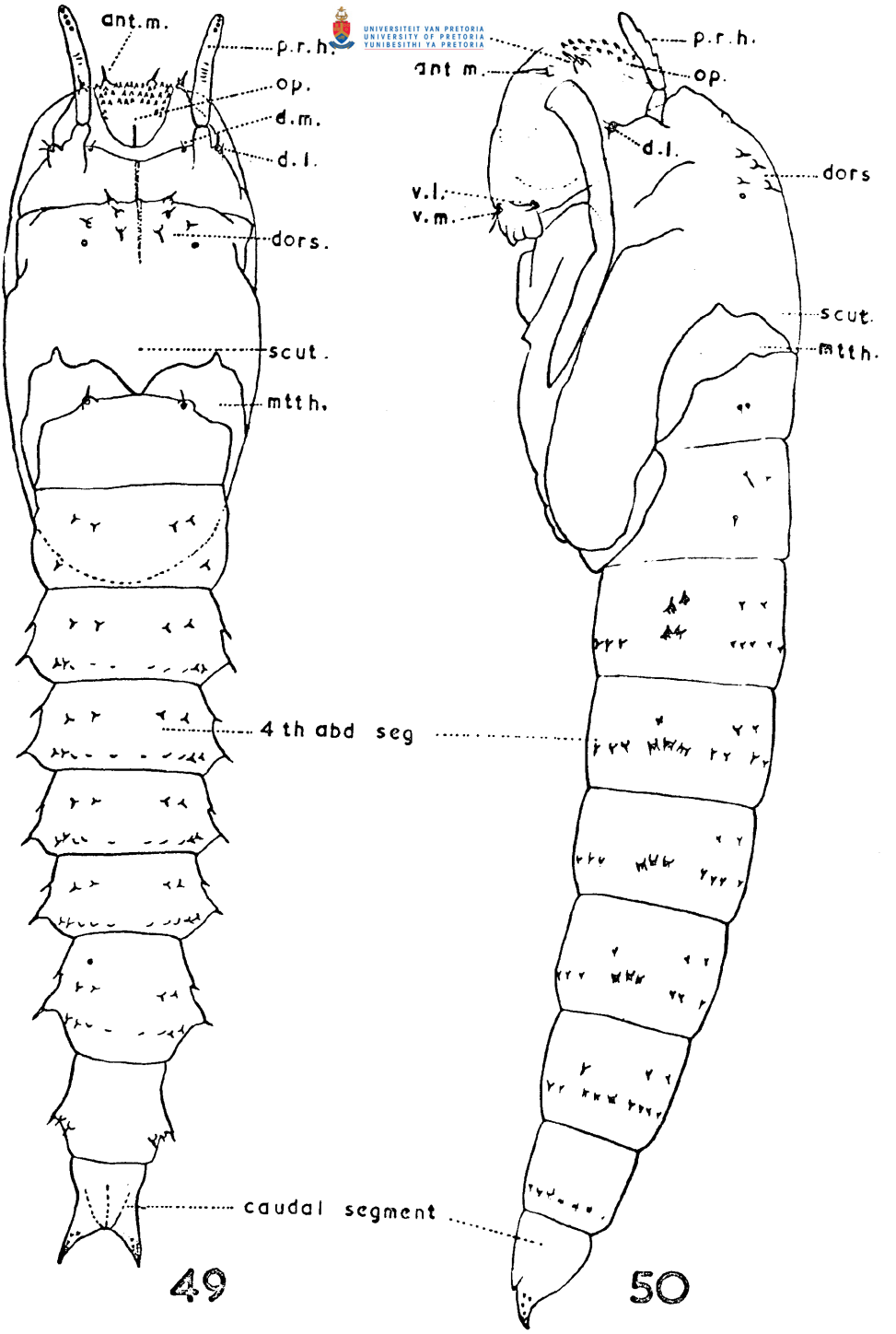

Fig. 49 - Dorsal view of Culicoides pupa.

Fig. 50 - Lateral view of Culicoides pupa.

ant.d, antero-dorsal tubercle; ant.m, antero-marginal tubercle; d.l, dorso-lateral tubercle; dors, dorsal tubercles ("dorsals"); 4th abd. seg, fourth abdominal segment; mtth, metathorax; op, operculum; p.r.h, prothoracic respiratory horn; scut, scutellum; v.l, ventro-lateral tubercle; v.m, ventro-median tubercle.

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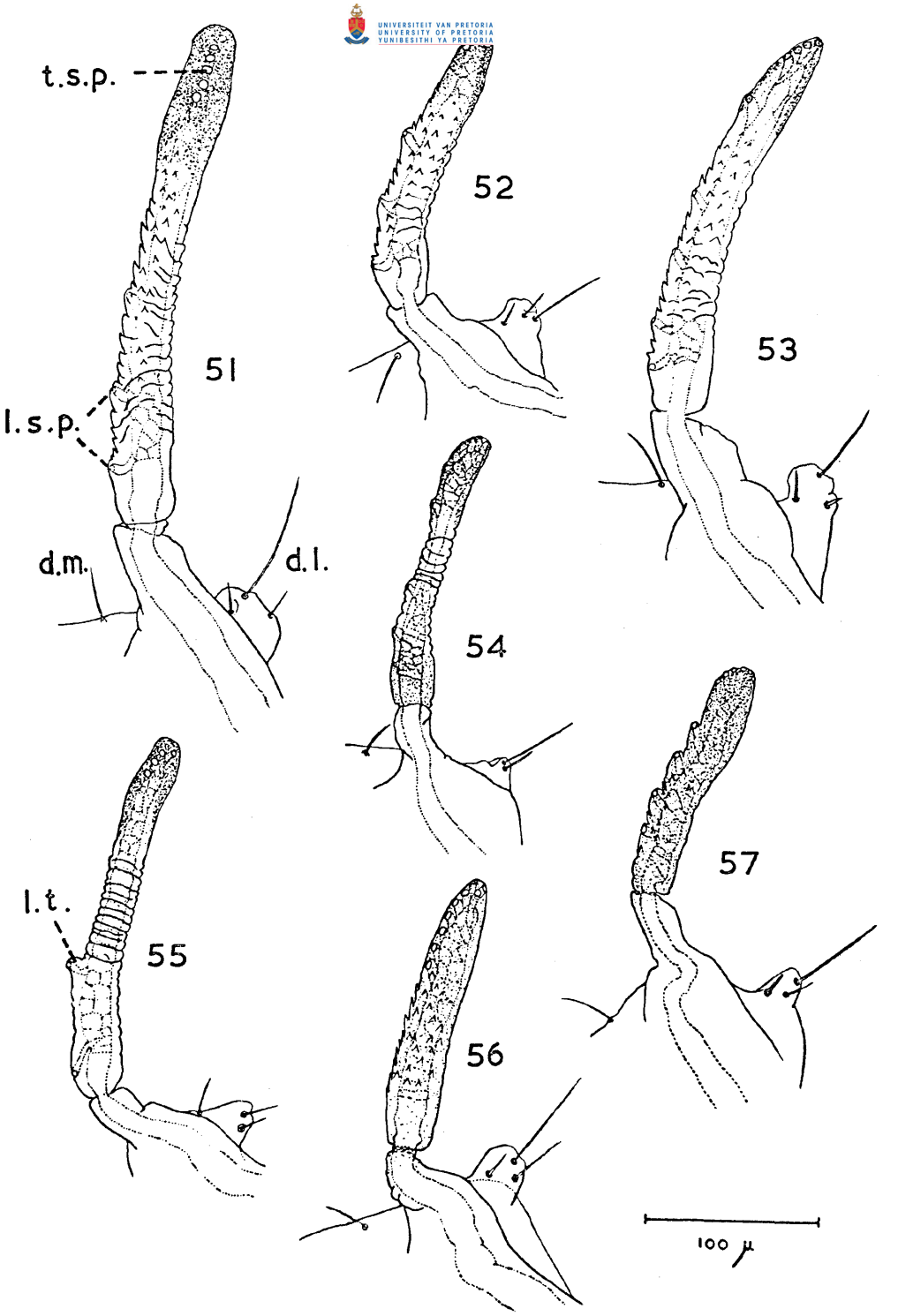

Prothoracic respiratory horns of Culicoides pupae.

Fig. 51 - C. nivosus.
Fig. 52 - C. distinctipennis.
Fig. 53 - C. pycnostictus.
Fig. 54 - C. pallidipennis.
Fig. 55 - C. schultzei.
Fig. 56 - C. milnei.
Fig. 57 - C. bedfordi.

d.l, dorso-lateral tubercle; d.m, dorso-median tubercle; l.s.p, lateral spiracular papillae; l.t, lateral tubercle; t.s.p, terminal spiracular papillae.

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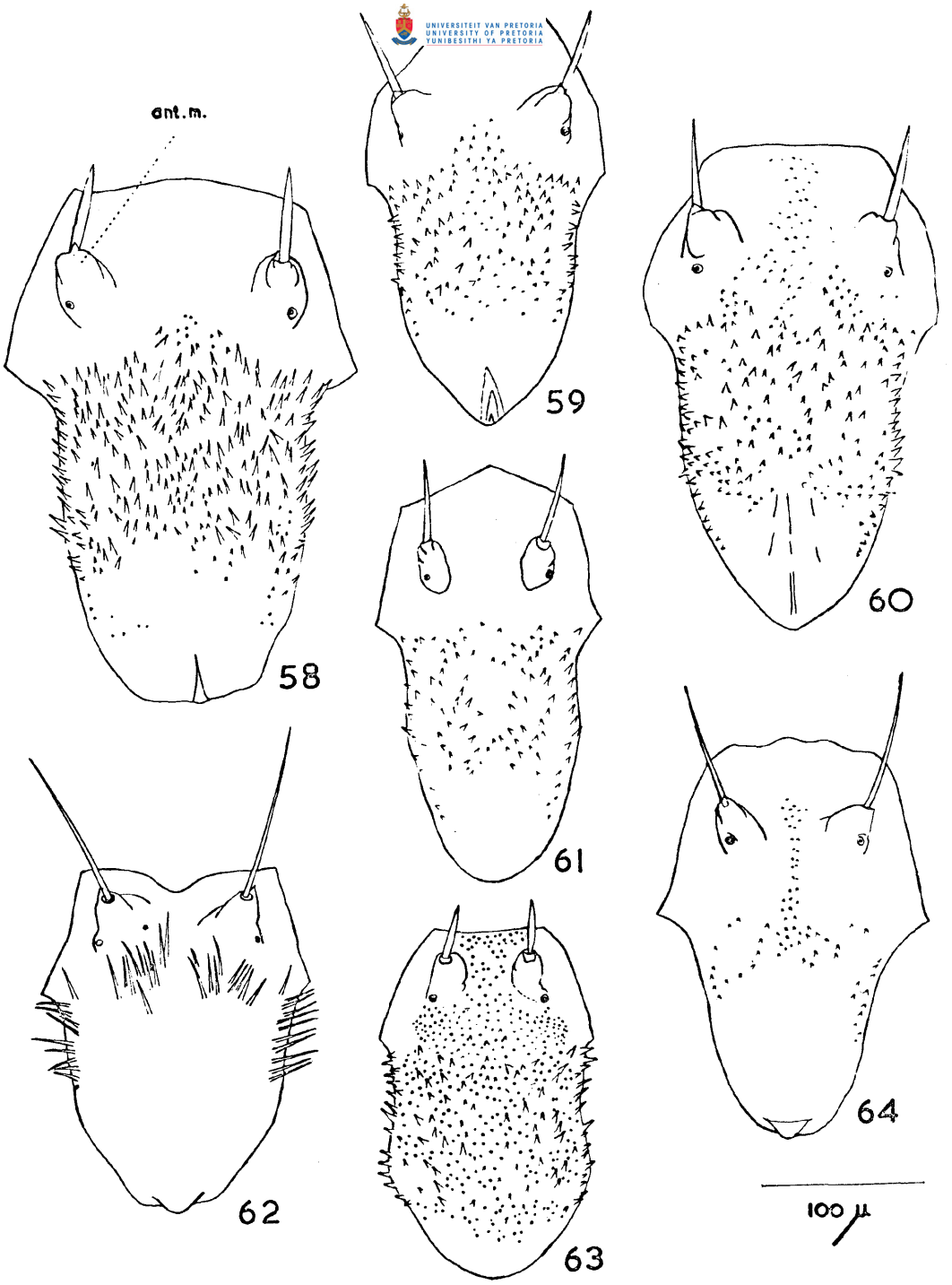

Dorsal view of opercula of Culicoides pupae.

Fig. 58 - C. nivosus.
Fig. 59 - C. distinctipennis.
Fig. 60 - C. pycnostictus.
Fig. 61 - C. bedfordi.
Fig. 62 - C. pallidipennis.
Fig. 63 - C. schultzei.
Fig. 64 - C. milnei.

ant.m, antero-marginal tubercle.

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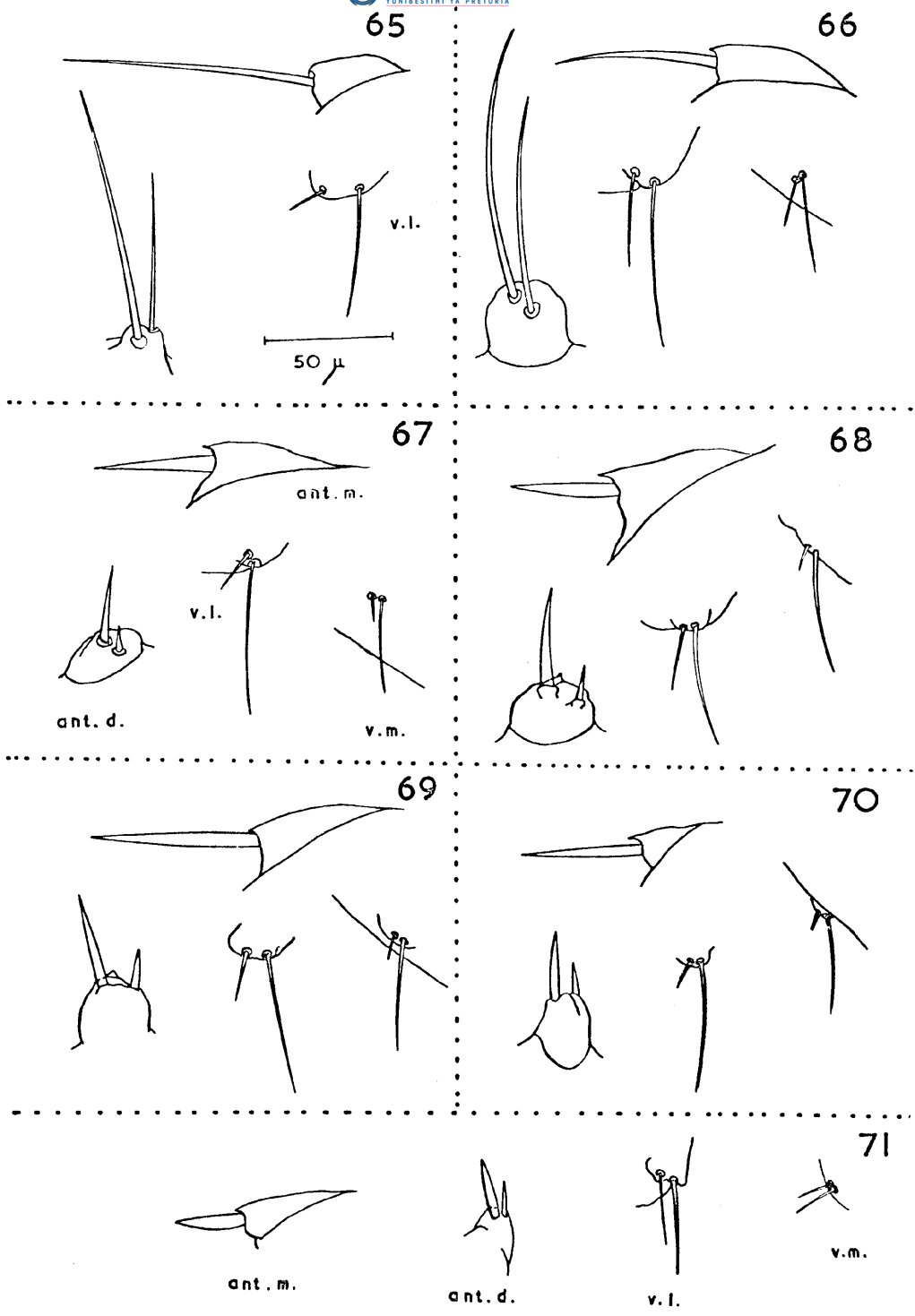

Head tubercles of Culicoides pupae.

Fig. 65 - C. pallidipennis.
Fig. 66 - C. milnei.
Fig. 67 - C. distinctipennis.
Fig. 68 - C. nivosus.
Fig. 69 - C. pycnostictus.
Fig. 70 - C. bedfordi.
Fig. 71 - C. schultzei.

Lettering as in Figs. 49 and 50.

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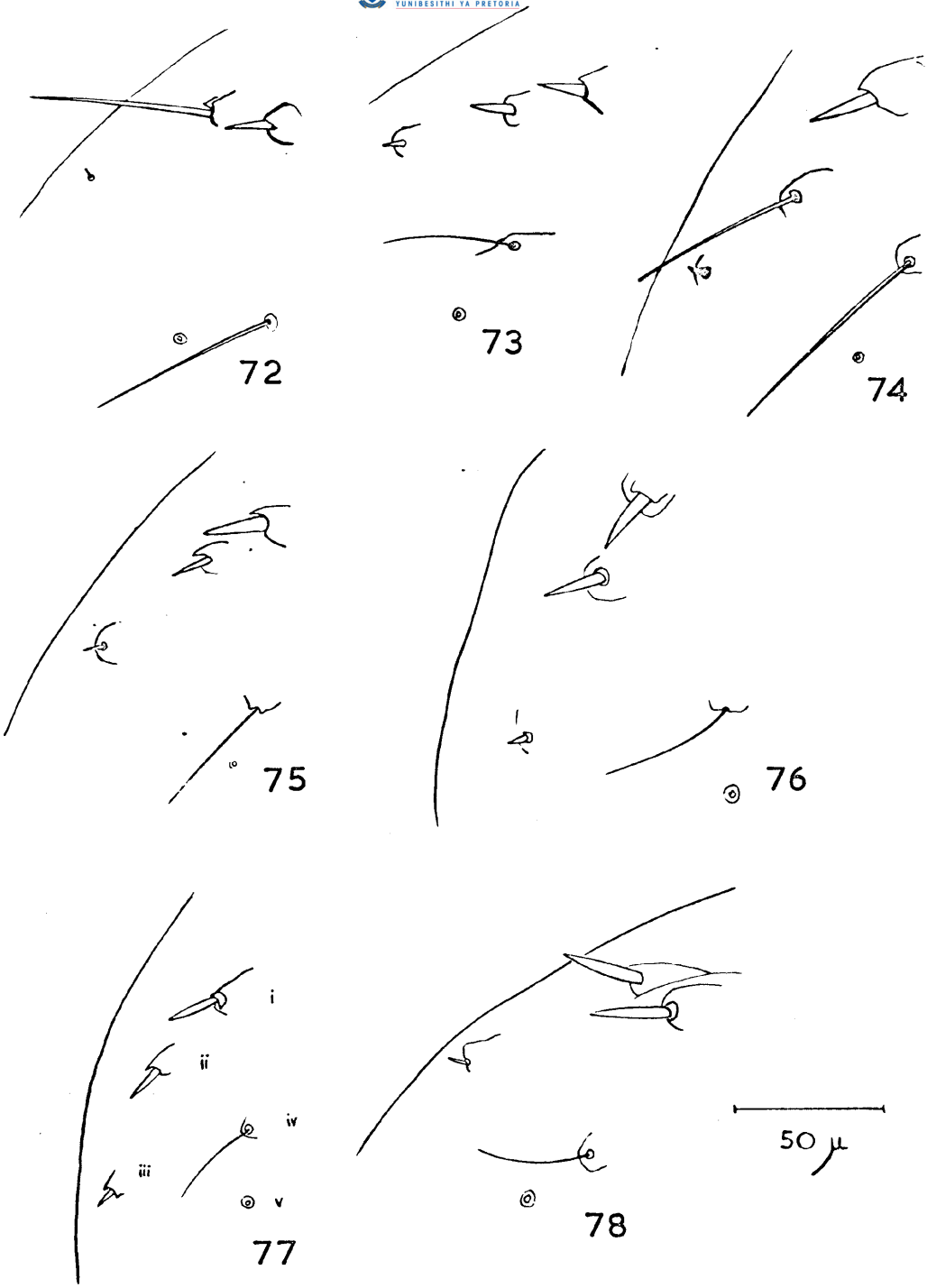

Thoracic dorsal tubercles of Culicoides pupae.

Fig. 72 - C. pallidipennis.
Fig. 73 - C. nivosus.
Fig. 74 - C. milnei.
Fig. 75 - C. distinctipennis.
Fig. 76 - C. pycnostictus.
Fig. 77 - C. bedfordi.
Fig. 78 - C. schultzei.

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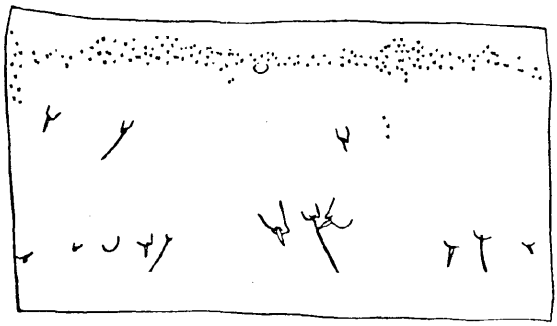

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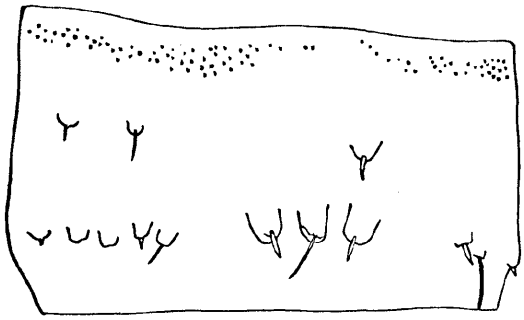

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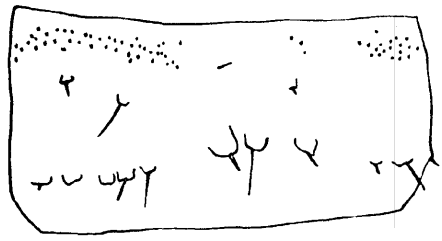

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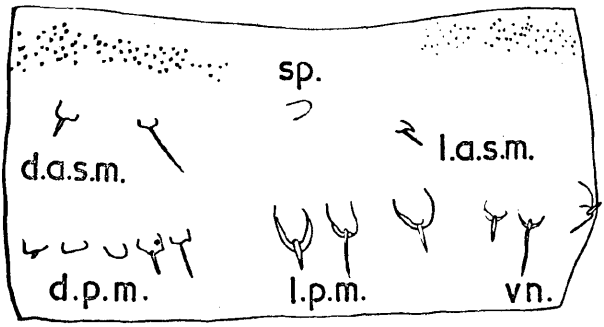

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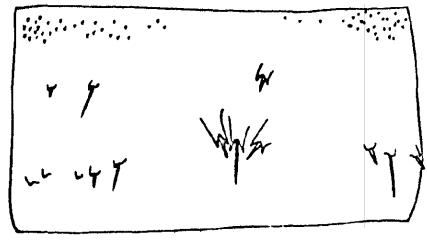

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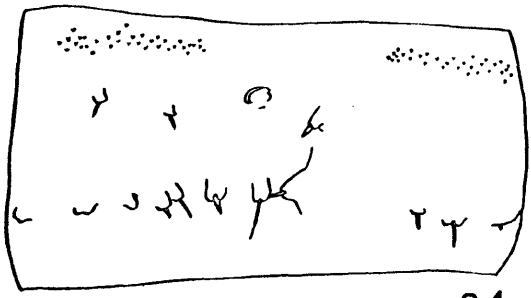

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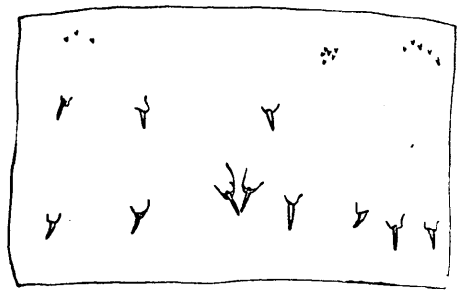

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Lateral view of fourth abdominal segments of *Culicoides* pupae.

Fig. 79 - *C. milnei*.
Fig. 80 - *C. nivosus*.
Fig. 81 - *C. distinctipennis*.
Fig. 82 - *C. pycnostictus*.
Fig. 83 - *C. bedfordi*.
Fig. 84 - *C. schultzei*.
Fig. 85 - *C. pallidipennis*.

d.a.s.m, dorsal antero-submarginal tubercles;
d.p.m, dorsal postero-marginal tubercles;
l.a.s.m, lateral antero-submarginal tubercles;
l.p.m, lateral postero-marginal tubercles;
sp, spiracle; vn, ventral tubercles.

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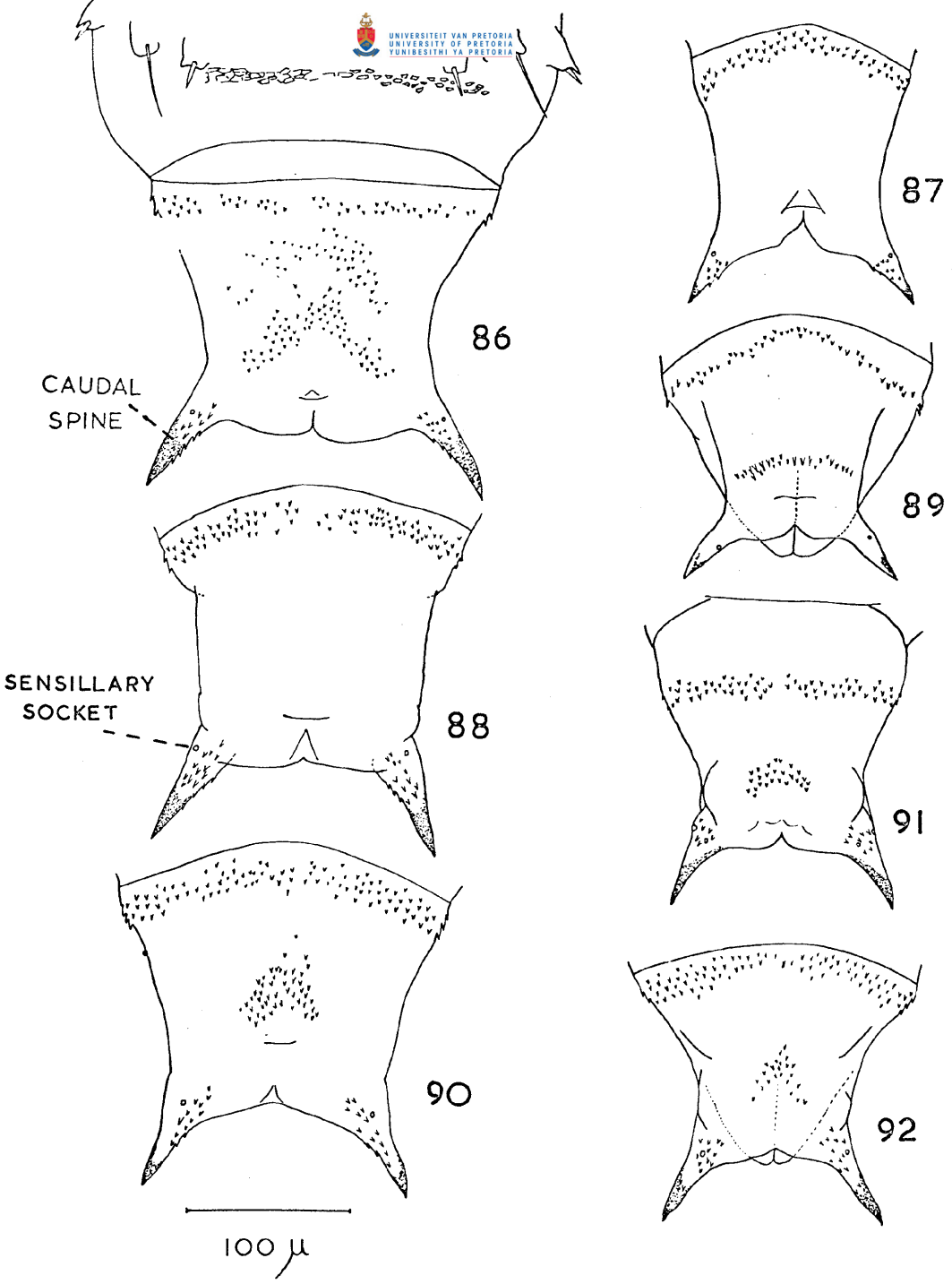

Dorsal view of caudal segments of Culicoides pupae.

Fig. 86 - C. milnei ♀.
Fig. 87 - C. schultzei ♀.
Fig. 88 - C. nivosus ♀.
Fig. 89 - C. pallidipennis ♂.
Fig. 90 - C. pycnostictus ♀.
Fig. 91 - C. distinctipennis
Fig. 92 - C. bedfordi ♂.

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