Onderstepoort J. vet. Res. (1969), 36 (2), 265—284
Printed in the Republic of South Africa by T. W. Hayne Ltd. (H. & G.), Johannesburg, for the Government Printer, Pretoria.

THE MORPHOLOGY OF THE IMMATURE STAGES OF SOME SOUTH AFRICAN *CULICOIDES* SPECIES (DIPTERA: CERATOPOGONIDAE)

E. M. NEVILL, Veterinary Research Institute, Onderstepoort

ABSTRACT

NEVILL, E. M. The morphology of the immature stages of some South African *Culicoides* species (Diptera: Ceratopogonidae). *Onderstepoort J. vet. Res.*, 36 (2), 265-284.

The morphology of the fourth larval stage of eight South African *Culicoides* species and of the pupae of seven species was studied. The value of existing taxonomic characters was tested and several useful new characters were found. Keys were constructed for the identification of both these stages. It is hoped that these studies will form a basis for future taxonomic work on the remaining South African *Culicoides* species.

CONTENTS

Received for publication on 4 August 1969.- Editor

Introduction

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 the U.S.A.

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 the southern United States (Dove, Hall & Hull, 1932) and in the Highlands of Scotland (Hill, 1947). In these areas 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 by Sherlock & Guitton (1964, 1965) to be caused by the bites of *Culicoides paraensis* (Goeldi, 1905).

Culicoides spp. are known to transmit five species of filarial worms in man, horses and cattle; three or perhaps four species of protozoa in birds and monkeys; bluetongue disease in sheep and possibly four other virus diseases of horses, cattle and man (Kettle, 1965). An allergic condition of horses known as Queensland Itch has been attributed by Riek (1954) to the bites of *Culicoides robertsi* Lee & Reye, 1953.

Except for the transmission of microfilariae, all the discoveries concerning diseases transmitted by *Culicoides* spp. have been made during the last 23 years. Prior to 1944 *Culicoides* were mainly of taxonomic interest and studies on their biology were restricted to 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 possibly of horse-sickness virus, these midges have received increased world attention as potential disease transmitters, with a corresponding increase in studies on their taxonomy and biology.

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 and one, *C. schultzei* Enderlein, 1908, has also been found in India, Japan and, recently, in parts of Australia (A. L. Dyce, McMaster Laboratory, Sydney, personal communication, 1967).

The change in emphasis from studies on the taxonomy of the adult transmitters to investigations on their biology and ecology has drawn attention to the necessity of differentiating the larval and pupal stages of various species of *Culicoides*.

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 & Lawson studied the early stages of British biting midges, and in the same year Wirth reviewed existing knowledge on the midges of California. In the Ethiopian region of Africa, however, apart from the pioneering work of Carter, Ingram & 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*.

Carter *et al.* (1920) described the adults, pupae and larvae of *C. accraensis*, *C. eriodendroni* and *C. nigripennis;* the adults and pupae of *C. inornatipennis*, *C. punctithorax* and *C. clarkei;* and the pupa and larva of *C. schultzei*. 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 "hypopharyngeal-sclerites". This character is today recognized as being of considerable value in larval identification. Of the species mentioned above, *C. clarkei* alone has not been recorded from South Africa, while *C. inornatipennis* has only been recorded here recently (Nevill, unpublished observations).

De Meillon (1936) described the adults and pupae of *C. meeserellus* and *C. alexis*, which Fiedler (1951) regarded as synonyms of *C. pycnostictus* Ingram & Macfie, 1925. However, despite the fact that in these circumstances De Meillon's two pupal descriptions, each based on a single specimen, should refer to one species they do 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* adults and pupae, but his descriptions and drawings of the pupae are inadequate for a thorough comparison between these and other species.

In the investigation described below a thorough morphological study was made of laboratory-reared fourth instar larvae of the following species of South African *Culicoides:*

C. pallidipennis	Carter, Ingram & Macfie, 1920
C. pycnostictus	Ingram & Macfie, 1925
C. distinctipennis	Austen, 1912
C. nivosus	De Meillon, 1937
C. schultzei	Enderlein, 1908
C. milnei	Austen, 1909
C. magnus	Colaço, 1946
C. bedfordi	Ingram & Macfie, 1923

Laboratory-reared pupae of all these species except *C. magnus* were also studied. Particular attention was paid to certain characters which other workers have found to be of value taxonomically, possible new characters were investigated and a tentative key was constructed for the identification of the species studied.

Material and Methods

(*a*) *Source of material for study*

Wild *Culicoides* midges were taken in a suction-type light trap of similar design to the modified New Jersey trap used by Du Toit (1944) [Fig. 1 (*a*)]. They were fed on the shaven ear of a rabbit in a cage 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 them in the vicinity of the ear. In this way approximately 50 per cent would feed within 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 per-

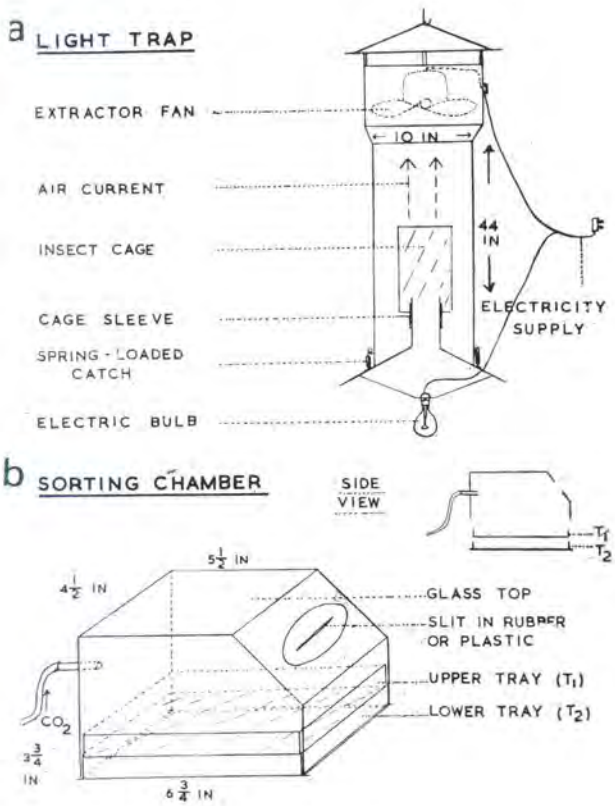

FIG. 1(*a*).—Suction-light trap
FIG. 1(*b*).—*Culicoides* sorting chamber

spex and glass [Fig. 1 (*b*)]. Here they could be kept anaesthetized for up to half an hour by a slow flow of CO_2 through the chamber, which was designed to fit under a dissecting microscope so that the various species of *Culicoides* could be identified while alive. Engorged, identified specimens were then removed with an aspirator to separate tubes for egglaying.

These tubes were 5.0 × 3.8 cm in size with a 2.0 cm layer of tamped-down moist cotton wool at the bottom. Two layers of filter paper, exactly fitting the tubes, were placed on the cotton wool. This provided an even surface on which the eggs could be laid and reduced the danger of the females' wings becoming stuck to wet surfaces. Initially the tubes were held in a horizontal position and from 1 to 25 anaesthetized females of a single species were placed on the dry glass sides. This prevented their wings adhering to the moist filter paper during their struggles whilst recovering from anaesthetization. The tubes were stoppered with cotton wool. About 5 min after the adults had recovered the tubes were placed upright in a room at 22°C for oviposition to take place.

Glass needle-boxes 8.3 cm in diameter by 5.7 cm in depth with a loose glass top were used to rear the larvae. Following the method of Jones (1957), the container was half-filled with a mixture of two parts of sifted soil rich in humus and one part of fresh bovine dung, tamped down to form 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 amount of moisture they required. The filter paper discs on which the eggs had been laid were placed on this larval medium with one edge submerged; thus the paper remained very moist and the newly-emerged larvae could move more easily from it into the medium. It was seldom necessary to add more water as little evaporation took place. Sometimes an oily-looking film formed 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 it: this caused them to wriggle out of the medium and float to the surface of the water, from which they were removed by means of a pipette. Many larvae were also recovered in this way but for almost complete recovery of larvae the method originally described by Ladell (cited by Kettle & Lawson, 1952) was used. In this method the larval medium is first washed through 10 and 20 mesh sieves to remove very large particles and then through a 100 mesh sieve which retains all the larvae and pupae. These are subsequently floated in a saturated solution of magnesium sulphate, removed to clean water using a pipette, killed in hot water and preserved in 70 per cent ethyl alcohol for morphological studies.

Pupae collected in the field were also used in these studies. Each pupa 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 depended upon the character(s) to be examined and especially the stage of development, since pupae can withstand more drastic treatment than the soft bodied larvae. Larvae and pupae were dealt with 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 × 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 the measurements were repeated. After measurement the larvae were returned to 70 per cent alcohol. Measurements were made using an eye-piece micrometer which was standardized with a stage micrometer to give readings in microns.

A method of preparing 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* for at least one night before mounting them in the same fluid. An alternative method was to leave the larvae overnight in lacto-chlorophenol** before mounting them in Berlese's fluid. The latter method, however, tended 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, dissect out the mandibles, hypopharynx and epipharynx. Treatment prior to dissection consisted of gently boiling the larvae for about 5 min in a 10 per cent aqueous solution of caustic potash (KOH) or leaving them for some weeks in lactic acid. The KOH method was used by Kettle & Lawson (1952), who also dissected out the pharyngeal skeleton. Linley & Kettle (1964) studied the pharyngeal skeleton *in situ* after prolonged treatment of the larvae in warm (60°C) lactic acid. It was 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:* When mounting intact pupae or pupal exuviae it is essential to remove most of the body contents and to make setae, especially on the cephalo-thorax, clearly visible by suitable treatment prior to mounting. Linley & Kettle (1964) recommended clearing specimens in warm lactic acid for several hours. Although this method was effective even better results were obtained by keeping pupae in warm (± 52°C) lacto-chloro-phenol for at least one night before mounting them in Berlese's fluid.

In pupal studies certain characters must be viewed laterally and some dorsally. Half of the available pupae were therefore mounted intact, lying on their sides. Each of the others was dissected into three pieces, — the operculum, the last two or three abdominal segments and the remainder of the pupa. The pieces from each specimen were then mounted, dorsal side up, on a slide under three separate coverslips.

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

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 and can be seen best in the fourth instar.

It is not, however, easy to differentiate between the last two instars in a mixed sample of unknown species. The first two instars are seldom collected because they are very small; they can 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 70 to 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, and thus cannot be confused with fourth instar *C. pallidipennis*, which has an average head length of more than 130 μ (Table 2).

Where the head length is much greater than that of the third instar of the largest species, viz. *C. milnei* (± 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 methods of recognizing fourth instar larvae are necessary (Table 2).

Perhaps the most reliable method is to try and detect signs of pupation. According to Kettle & Lawson (1952): "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 as to make identification very difficult."

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. 2(b)] (Lawson, 1951).

Where a single species is present it is easy to recognize the various instars on the basis of Dyar's law, which states 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 & 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 and also depends on conditions in the larval medium. As shown in Table 2, 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 either for the direct identification of some species or to sort the larvae into groups for more detailed study later 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 1 and shown in Fig. 2.

(*) Formula for Berlese's fluid —
60 g Gum arabic
40 g Glycerine
400 g Chloral hydrate
100 g Distilled water

(**) Formula for lacto-chloro-phenol —
Chloral hydrate — 2 parts by volume
Lactic acid — 1 part by volume
Phenol — 1 part by volume

TABLE 1.— *General features of fourth instar larvae of eight* Culicoides *species*

Species	Head	Eye-spots	Neck	Prothorax	Mesothorax	Metathorax
C. distinctipennis [Fig. 2(a)]	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. 2(b)]	Dark cream	Comma-shaped	Unpigmented	As above	As above	As above
C. nivosus [Fig. 2(c)]	Dark cream	Comma-shaped	Irregular central pigment usually present	As above	As above	As above
C. schultzei [Fig. 2(d)]	Dark cream	Comma-shaped	Brown irregular pigment for most of width	As above	As above	As above
*C. milnei** [Fig. 2(e)]	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. 2(f)]	Brown	Comma-shaped	Unpigmented	Unpigmented	Unpigmented	Unpigmented
C. bedfordi [Fig. 2(g)]	Dark cream	Comma-shaped	Unpigmented	Unpigmented	Unpigmented	Unpigmented
C. pallidipennis [Fig. 2(h)]	Brown	Circular	Unpigmented	Unpigmented	Unpigmented	Unpigmented

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

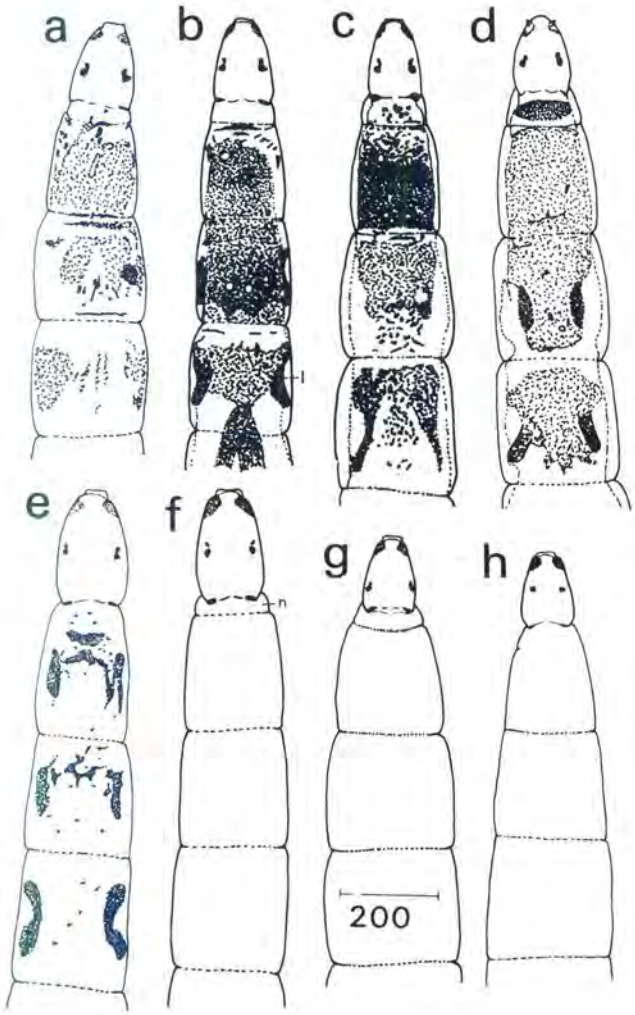

FIG. 2.—Dorsal view of fourth instar *Culicoides* larvae
(*a*) *C. distinctipennis* (*b*) *C. pycnostictus*
(*c*) *C. nivosus* (*d*) *C. schultzei*
(*e*) *C. milnei* (*f*) *C. magnus*
(*g*) *C. bedfordi* (*h*) *C. pallidipennis*
l, "lateral body"; n, "neck"

The following conclusions can be drawn from Table 1:

Head colour: *C. pallidipennis*, *C. magnus* and *C. milnei* all have brown or amber-coloured heads, while the heads of the other species are dark cream or straw-coloured.

Eye-spots: *C. pallidipennis* [Fig. 6(b)] generally has a pair of almost circular spots compared with the comma-shaped eye-spots of the other seven species [Fig. 6(d)].

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 which forms the so-called "neck" may also be pigmented [Fig. 2(f)].

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 which may give a diffuse effect, or may be formed into circular groups, giving a more mottled appearance. Open circular unpigmented areas are common in certain species, especially *C. pycnostictus* and *C. nivosus* [Fig. 2 (b and c)].

From Table 1 and Fig. 2, it can be seen that the eight species can be arranged into three groups according to the type of pigmentation. The first group includes those species with 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 of the head length to head breadth, which is termed 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, and its breadth was measured at the widest part. Oral ring width was taken on a straight line connecting the hind borders of the subgenal band (see Fig. 3). The length of the larvae did not include the anal papillae. Only five *C. magnus* were measured owing to a shortage of material. Means and standard deviations (S. D.) from the means were calculated and the results are given in Table 2.

The measurements of the distance between the labrum and the eye, and between the eyes, were included for their possible taxonomic value, though they appear to 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 they were in the first sample although the larvae were very much shorter. The larval lengths can therefore only serve as rough indicators of the species or instar, and are probably dependent both on larval age within the instar and on conditions in the larval medium. For the species studied, 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 when this cannot be determined by any easier means. Most of the eight species studied are fairly easy to identify under × 50 magnification using the characters in Table 1, such as the presence or absence of pigmentation, etc., or by a careful study of the dorsal comb of the epipharynx [see (4) (b) (*ii*) page 273.] *C. distinctipennis* and *C. pycnostictus* cannot, however, be distinguished on these characters and differences in their head measurements may have to be used.

TABLE 2.— *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	Mean ± 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

(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, using the oil immersion lens, and the clearest and most representative of these drawings are presented in Fig. 4 and 5. Dorsal and lateral diagrammatic views of a larval head showing all the structures to be discussed, are given in Fig. 3 (a and b).

(a) Mandibles: Each *Culicoides* larva possesses two mandibles which articulate antero-dorsally, and move almost parallel to each other in the vertical plane. As pointed out by Kettle & 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 shown in Fig. 4 (a to h) 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 3.

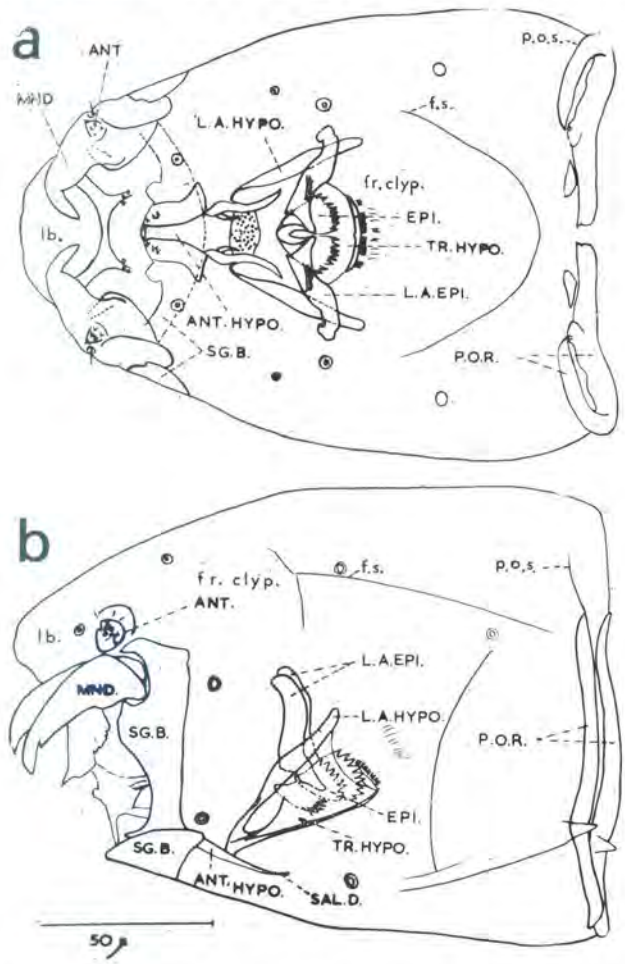

FIG. 3.—Diagrammatic views of heads of fourth instar *Culicoides* larvae
(*a*) Dorsal view
(*b*) Lateral view
ant: antenna; ant. hypo: anterior hypopharynx; epi: epipharynx; f.s: frontal suture; fr. clyp: fronto-clypeus; l. a. epi: lateral arms of epipharynx; l. a. hypo: lateral arms of hypopharynx; 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

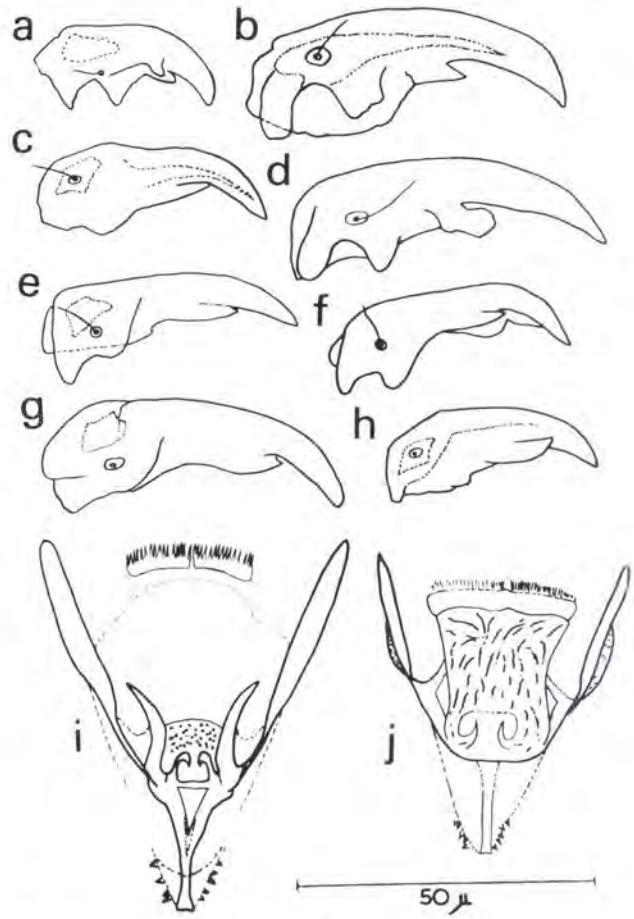

FIG. 4 (*a* to *h*).—Lateral view of mandibles of fourth instar *Culicoides* larvae

(*a*) *C. pallidipennis*	(*b*) *C. milnei*
(*c*) *C. distinctipennis*	(*d*) *C. magnus*
(*e*) *C. pycnostictus*	(*f*) *C. schultzei*
(*g*) *C. nivosus*	(*h*) *C. bedfordi*

FIG. 4(*i*).—Hypopharynx representative of all species other than *C. schultzei*

FIG. 4(*j*).—Hypopharynx of *C. schultzei*

Although the mean measurements of head length, head breadth and width of the oral ring are noticeably greater in *C. milnei* than in the other species (Table 2), the length of its mandibles does not differ significantly from those of *C. nivosus*, *C. pycnostictus* and *C. magnus* (Table 3). Mandible length in relation to the other head measurements is thus another character which could possibly be used in the identification of species.

(b) 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).

TABLE 3.—*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

(*i*) Hypopharynx: This consists of a concave or trough-shaped membrane which forms the floor of the pre-oral cavity and is suspended from two sclerotized lateral arms. It is prolonged anteriorly into a tubular structure, whose tip is flanked by four or five very small teeth or denticles. Lawson (1951) has shown that in *C. nubeculosus* the salivary duct opens in this anterior section of the hypopharynx. The posterior edge of the trough is prolonged into fine membranous teeth.

The hypopharynges of seven of the eight species studied appear to be very similar in structure and may be represented by a single figure [Fig. 4(i)]. *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. 4(j)].

(*ii*) Epipharynx: The epipharynx forms the dorsal wall of 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, which is suspended in the trough of the hypopharynx by two heavily sclerotized lateral arms [Fig. 3 (a and b)]. These arms are of little importance from the taxonomic viewpoint since their appearance varies considerably depending upon the position in which they are mounted (Kettle & Lawson, 1952). The arms may possess small sclerites or membranous teeth which possibly serve as muscle attachments.

Kettle & Lawson (1952) describe two types of epipharynx. In the first type (*C. nubeculosus* group) the epipharynx is heavily sclerotized and pigmented and consists basically of four sclerites. The posterior sclerite is subdivided into a pair of roughly triangular sclerites, whose rear edges are toothed. The rear edges of the other sclerites may be smooth or may have rounded projections or even definite teeth. In the second type (which includes the remaining British *Culicoides* spp.) the epipharynx is more lightly pigmented and sclerotized. All the sclerites are toothed and are referred to as "combs". The dorsal or posterior comb, which is the most prominent, is subdivided into two roughly triangular sclerites whose teeth can normally be clearly distinguished under oil immersion. This is regarded as the first comb of the series and, as one proceeds ventrally, the others have in turn been named the 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 even when present are often very difficult to see owing 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: the dorsal comb and what is thought to be the ventral (fourth) comb. The latter is well developed and there is enough space between it and the dorsal comb for the two missing combs. Ventral views of epipharynges dissected and *in situ* are shown in Fig. 5 (a to h). They are diagrammatic in that for clarity each comb has been shown 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 taxonomic characters. 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 4, significant differences exist between the species studied.

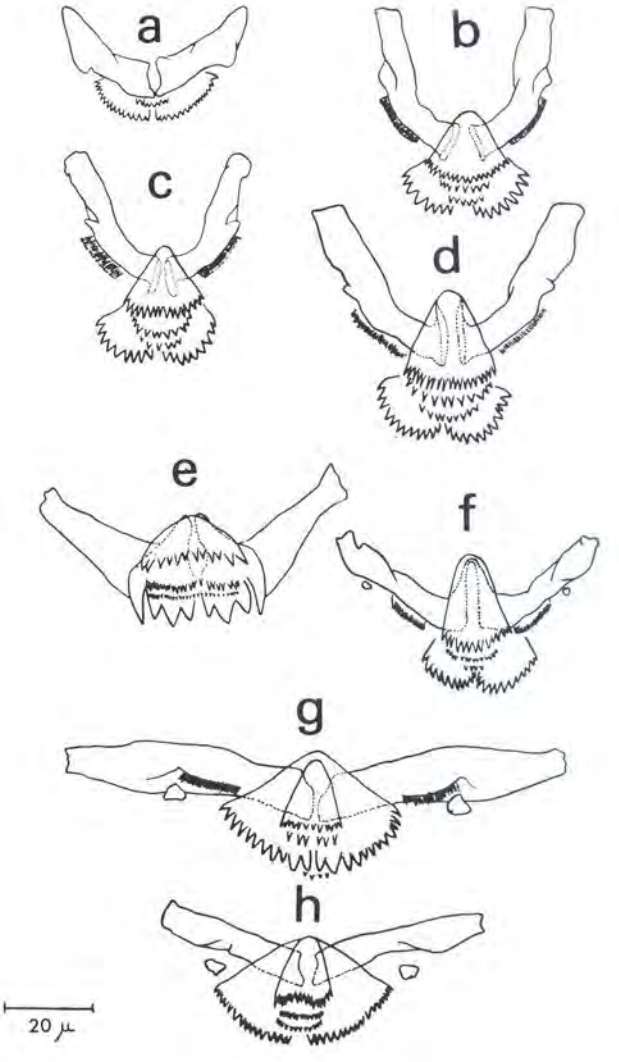

FIG. 5.—Ventral view of epipharynges of fourth instar *Culicoides* larvae

(*a*) *C. pallidipennis* (*b*) *C. distinctipennis*
(*c*) *C. pycnostictus* (*d*) *C. nivosus*
(*e*) *C. schultzei* (*f*) *C. bedfordi*
(*g*) *C. milnei* (*h*) *C. magnus*

TABLE 4. — *Details of epipharyngeal combs of fourth instar* Culicoides *larvae*

Species	No. of specimens	Number of teeth per comb				Width of half dorsal comb (μ)
		Dorsal*	Second	Third	Fourth	
C. pallidipennis	9	(14)—19	—	—	(6)—9	12.8—17.4
C. bedfordi	5	(11—12)	( )	(14)	(14)	11.6—13.3
C. schultzei	5	3	( )	( )	(12—20)	10.5—11.1
C. distinctipennis	6	8—9	5—6	8	16—19	13.9—16.2
C. nivosus	10	10—12	8—10	11—12	(20)	15.7—16.8
C. pycnostictus	8	9—10	5—7	8—11	16—(19)	14.5—17.4
C. magnus	5	15—(18)	( )	( )	( )	19.2—21.5
C. milnei	11	10—(13)	(4)	(7)	(8)—12	20.3—23·2

* Refers to number of teeth on each half of dorsal comb
( ) Number of teeth uncertain

C. schultzei is the most striking species, having only three large teeth on each half of the dorsal comb. These combs in turn are flanked 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 teeth on each half of the dorsal comb (*C. bedfordi*, *C. distinctipennis*, *C. nivosus*, *C. pycnostictus* and *C. milnei*) and those with more than 14 teeth (*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 on these combs. No trace of a second or third comb could be found in *C. pallidipennis*. In *C. nivosus* these combs appear to have more teeth than they do in its close relatives *C. distinctipennis* and *C. pycnostictus*, which are almost identical.

The size of the teeth of the dorsal comb may be diagnostic. In most species they 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 much larger than the others [Fig. 5(g)].

The shape of each half of the dorsal comb varies between species. In three, *C. pallidipennis*, *C. milnei* and *C. magnus*, the two halves of the dorsal comb when adjacent to each other form an almost perfect semicircular serrated outline [Fig. 5 (a, g and h)]. However, in *C. distinctipennis*, *C. pycnostictus*, *C. nivosus* and *C. bedfordi*, each half of the dorsal comb is hand-shaped so that, when they are adjacent, its outline has a distinct indentation in the centre [Fig. 5 (b, c, d and f)].

Kettle & Lawson (1952) attached some value to the width of the dorsal combs as a means of distinguishing between morphologically similar species. The maximum widths of each half of the dorsal combs of the eight species studied are therefore included in Table 4. The width across the complete comb was not used, as in many cases the two halves of the comb either overlapped or became widely separated on mounting.

The value of this feature as a diagnostic character is probably limited. In the eight species studied, once the various species have been separated on such characters as the number of teeth on the combs, the size of teeth and the shape of combs, then the width of the dorsal combs 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 similarly shaped combs but that of *C. pallidipennis* is much narrower than the others (Table 4). *C. bedfordi* also has a narrower dorsal comb than *C. distinctipennis*, *C. nivosus* and *C. pycnostictus*, although their combs have the same basic shape.

To sum up, the following characters of the epipharynx are of possible taxonomic value:— the number and size of the teeth on the dorsal comb, the shape and width of this comb, and the number of teeth on the second and third combs.

(5) *Structure and chaetotaxy of the head:* The head of a *Culicoides* larva is made up of three sclerites: 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) [Fig. 3 (a and b) and 6 (d)]. 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 heads of the eight South African species studied conformed more or less with those of the overseas species referred to above. However, it would be unwise to ignore the possible taxonomic value of at least one of these characters, the post-occipital ridge. As can be seen in Fig. 6 variations between species in the type and degree of sclerotization of this collar do exist, e.g. *C. pallidipennis* [Fig. 6(a)] consistently shows a narrow ventral anterior projection of the median section of the post-occipital ridge while *C. nivosus* [Fig. 6(c)], which is representative of the other seven species, lacks this projection.

The position, size, and number of setae on the head were studied and are shown in Fig. 6 (a to d). No attempt has been made to label or describe them in detail since the differences which were observed between species were trivial. Linley & Kettle (1964) sum up the position 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".

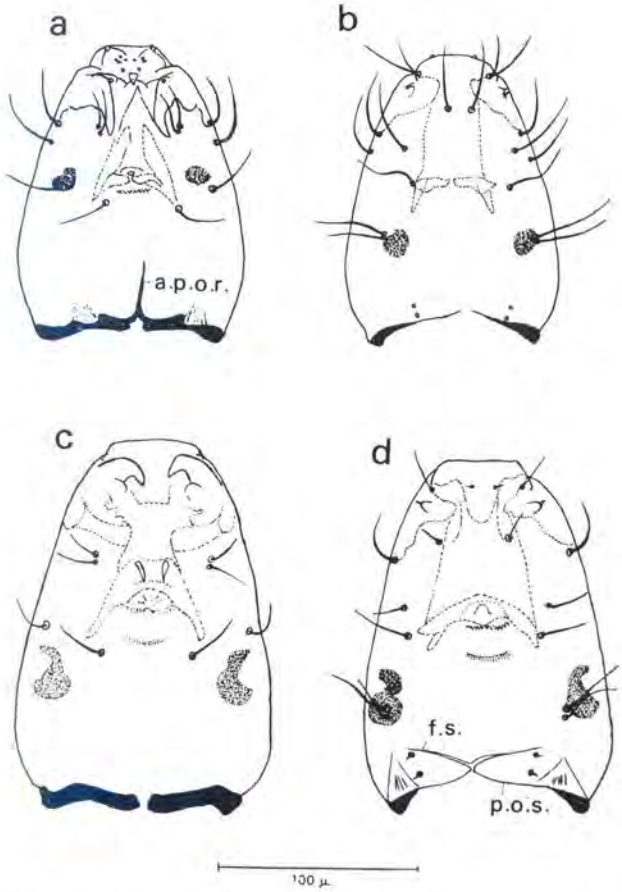

FIG. 6.—Heads of fourth instar *Culicoides* larvae
(*a*) *C. pallidipennis*, ventral view
(*b*) *C. pallidipennis*, dorsal view
(*c*) *C. nivosus*, ventral view
(*d*) *C. nivosus*, dorsal view
a.p.o.r: anterior projection of post-occipital ridge; f.s: frontal suture; p.o.s: post-occipital suture

However, while studying the chaetotaxy of the larval body, measurements were made of the length of the head setae and the setae on the prothorax and anal segment. It was very difficult to see the tips of most of them, even under oil immersion, so exact measurements could not always be made, but as repeated measurements of a number of specimens gave about the same lengths, and as only large differences in lengths are of interest, these approximate measurements are given in Tables 5 and 6. It was seldom possible to measure a particular seta, since some were strongly curved or they were obscured, so only the longest of the straight, clearly visible setae, dorsal or ventral were measured. One seta per specimen was measured.

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 are given in Table 5.

It is clear from Table 5 that two species, viz. *C. bedfordi* and *C. magnus*, differ markedly from the others in having very long head setae in relation to their head length. This ratio may therefore be of some use in the identification of certain species.

TABLE 5.—*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. distinctipennis	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

(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 each seta identified with a letter. Linley & Kettle (1964), who adhered to this terminology when studying *C. furens* (Poey, 1853) and *C. hoffmani* Fox, 1946 concluded that:— "The chaetotaxy of the larval body is probably fairly constant throughout the genus, . .".

In this 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 and were only found after a careful search under oil immersion.

Setae appeared to differ greatly in size, depending on their location and on the species. In general those on the anal segment are the longest, followed by those on the prothorax and the remaining body segments. The mean lengths of the longest setae on the head, prothorax, and anal segment are recorded in Table 6 together with the ratios of the length of the head setae to prothoracic and anal setae.

From the measurements recorded in the last two columns in Table 6 it can be seen that the various setae usually differ little in length within a species, seven of the eight species having ratios very close to 1.0. In *C. pallidipennis*, however, they do differ markedly, the setae of the head being 2.1 times as long as those of the prothorax and 1.4 times longer than those of the anal segment. This character therefore seems to be of definite value in separating *C. pallidipennis* from other fourth instar larvae.

(7) *Anal papillae:* Linley & Kettle (1964) found that the variations in the form of the anal papillae were of value in larval identification. In the present study, however, difficulty was experienced in obtaining specimens with suitable 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

TABLE 6. — *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. pallidipennis	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. distinctipennis	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

Pigmentation restricted to lateral and anterior bands [Fig. 2(e)]
.. *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. 2(a)]
.. *C. distinctipennis*
Head 175-189 μ long and 123-139 μ wide [Fig. 2(b)]
.. *C. pycnostictus*

5. (3) Three teeth present on each half of dorsal comb of epipharynx, trough of hypopharynx dark and fibrous [Fig. 2(d), 4(j) and 5(e)]
.. *C. schultzei*
More than eight teeth present on each half of dorsal comb of epipharynx, trough of hypopharynx pale and delicate [Fig. 2(c), 4(i) and 5(d)]
.. *C. nivosus*

6. (1) Eye-spots comma-shaped, ratio head length: length of head setae $\pm$ 2.7 7
Eye-spots circular, ratio head length: length of head setae > 3.6, post-occipital ridge possesses a ventral median anterior projection [Fig. 2(h) and 6(a)]
.. *C. pallidipennis*

7. (6) Head brown, more than 14 teeth on each half of dorsal comb of epipharynx [Fig. 2(f) and 5(h)]
.. *C. magnus*
Head dark cream or straw coloured, more than 8 but less than 14 teeth on each half of dorsal comb of epipharynx [Fig. 2(g) and 5(f)]
.. *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. They are also comparatively easy to collect and the exuviae show all the characters of the intact pupa.

Dorsal and lateral views of *Culicoides* pupae are given in Fig. 7. The more important characters are labelled and are 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

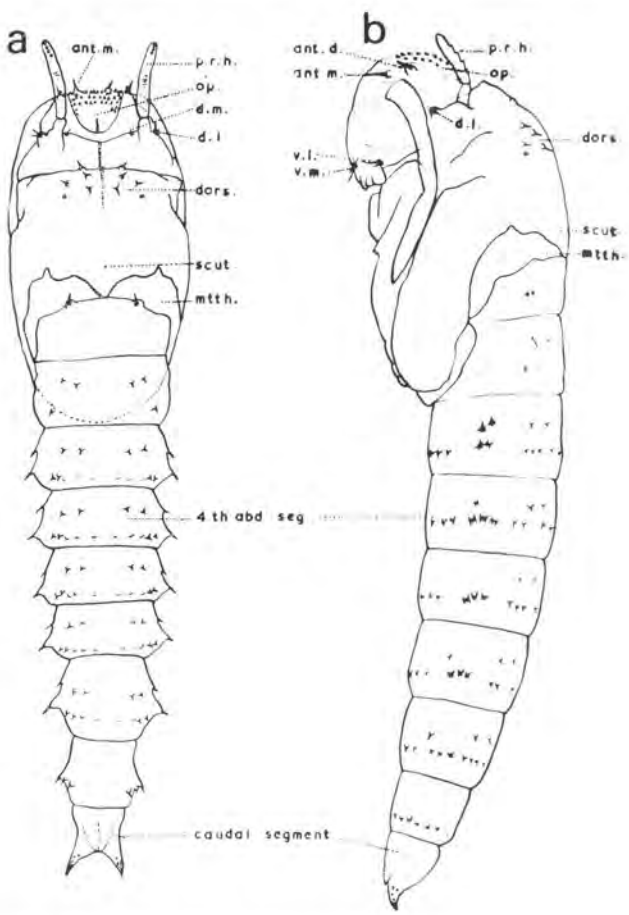

FIG. 7(*a*).—Dorsal view of *Culicoides* pupa

FIG. 7(*b*).—Lateral view of *Culicoides* pupa
ant. d: antero-dorsal tubercle; ant. m: antero-marginal tubercle; d.l: dorso-lateral tubercle; d.m: dorso-median 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

of all seven species 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, taken from the anterior margin of the cephalo-thorax to the tip of the caudal processes, determined. Some specimens seemed to have stretched in the alcohol while some had a shrunken appearance. Measurements of the more normal looking 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. Field collected specimens of *C. distinctipennis* were, however, 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, including the degree of pigmentation as well as the region

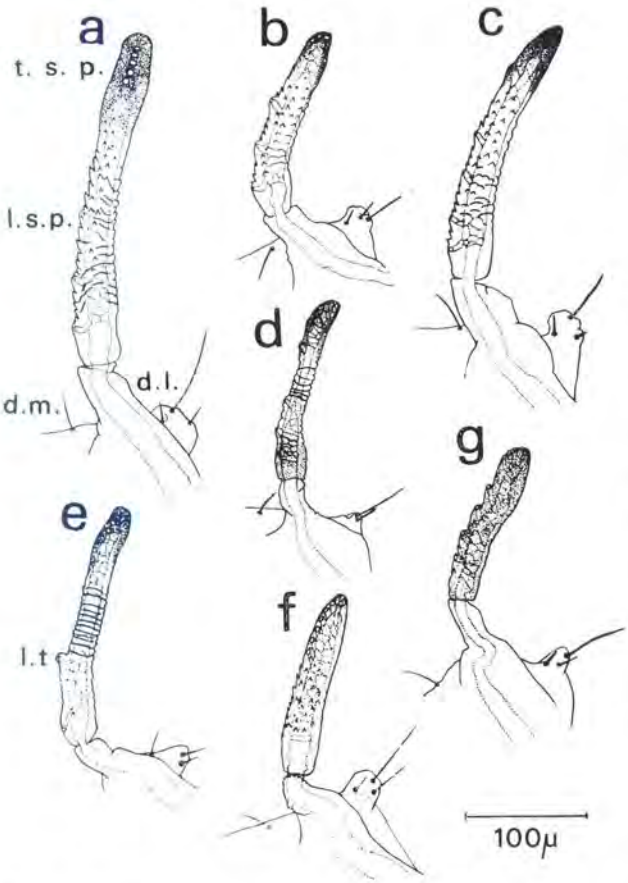

FIG. 8.—Prothoracic respiratory horns of *Culicoides* pupae
(*a*) *C. nivosus* (*b*) *C. distinctipennis*
(*c*) *C. pycnostictus* (*d*) *C. pallidipennis*
(*e*) *C. schultzei* (*f*) *C. milnei*
(*g*) *C. bedfordi*
d.l: dorso-lateral tubercle; d.m: dorso-median tubercle; l.s.p: lateral spiracular papillae; l.t: lateral tubercle; t.s.p: terminal spiracular papillae

pigmented, the number of lateral and terminal spiracular papillae [Fig. 8(a)], and the presence of external folds and pointed or dentate scales.

Table 7 and Fig. 8 summarize and illustrate these characters for the seven species studied.

Three types of pigmentation of the prothoracic horn are found, viz. complete pigmentation, tip pigmentation, or base and tip pigmentation. This provides a very easy method for separation of the species, 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 lacking lateral papillae. This character was used by Kettle & Lawson (1952) to distinguised the *C. pulicaris* group from the other two main groups of British *Culicoides* species.

The number of papillae, however, is of limited diagnostic value. 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 useful for distinguishing groups of species. One group, *C. nivosus*, *C. pycnostictus*, and *C. distinctipennis* possesses scales as well as folds. A second group including *C. pallidipennis* and *C. schultzei* possesses annulated rings or folds only. The third group includes those species having small dentate scales but no rings or folds, viz. *C. milnei* and *C. bedfordi*. These two species 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 for distinguishing between very similar species, such as *C. pycnostictus* and *C. distinctipennis*, as measurements of laboratory-reared specimens showed considerable differences in horn lengths (Table 8). However, measurements of the horns of field-collected specimens of these species showed them to be of equal length, so horn length apparently depends on conditions in the larval medium. Unfortunately no field specimens of the other species were collected so this comparison between laboratory-reared and field-collected specimens could not be extended.

The examples above should, however, act as a warning against dependence on comparative measurements for species differentiation.

(4) *Operculum:* Lawson (1951) states: "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 frontoclypeus. 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".

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

TABLE 7.— *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 [Fig. 8(a)]	11	Distal $\frac{1}{4}$ of horn dark brown	2—3	4—7(6)*	Dentate scales and folds in central region
C. pycnostictus [Fig. 8(c)]	9	Distal $\frac{1}{4}$ of horn slightly darker than re-remainder	4—5	3—6(4)	Dentate scales and folds in central region
C. pycnostictus Field specimens	9	,,	3—5(4)	4—7(5)	,,
C. distinctipennis [Fig. 8(b)]	10	Distal $\frac{1}{4}$ of horn slightly darker than remainder	2—4(3)	2—5(3,4)	Dentate scales and folds in central region
C. distinctipennis Field specimens	8	,,	2—4(3)	3—5(4)	,,
C. pallidipennis [Fig. 8(d)]	6	Proximal $\frac{1}{4}$ and distal $\frac{1}{4}$ of horn darker than central region	4—6(4)	3—6(6)	Annulated rings or folds in central region. Scales absent
C. schultzei [Fig. 8(e)]	6	Distal $\frac{1}{4}$ 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 [Fig. 8(g)]	7	Entire horn dark brown	3—5(4)	3—6(4,5)	A few small dentate scales on proximal $\frac{2}{3}$ of horn. Slight folds may be present on proximal $\frac{1}{3}$
C. milnei [Fig. 8(f)]	6	Entire horn dark brown	0	7—11(10)	Small dentate scales scattered over proximal $\frac{2}{3}$ of horn

*Number in brackets indicates most common number of papillae found

TABLE 8.—*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

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

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 operculum of each of the seven species studied, is given below. The antero-marginal (a-m) tubercles and setae will be discussed separately in the section dealing with head tubercles and setae respectively.

C. nivosus [Fig. 9(a)]

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

C. pycnostictus [Fig. 9(c)]

Spines short: sturdy; decrease 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. 9(b)]

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

Field collected specimens similar.

C. pallidipennis [Fig. 9(e)]

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

C. schultzei [Fig. 9(f)]

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, also between and anterior to the a-m tubercles.

C. bedfordi [Fig. 9(d)]

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. 9(g)]

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 can be seen that the first three species and *C. bedfordi* are very similar and are only separable 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 easily recognizable by reference to Fig. 9 (e to g).

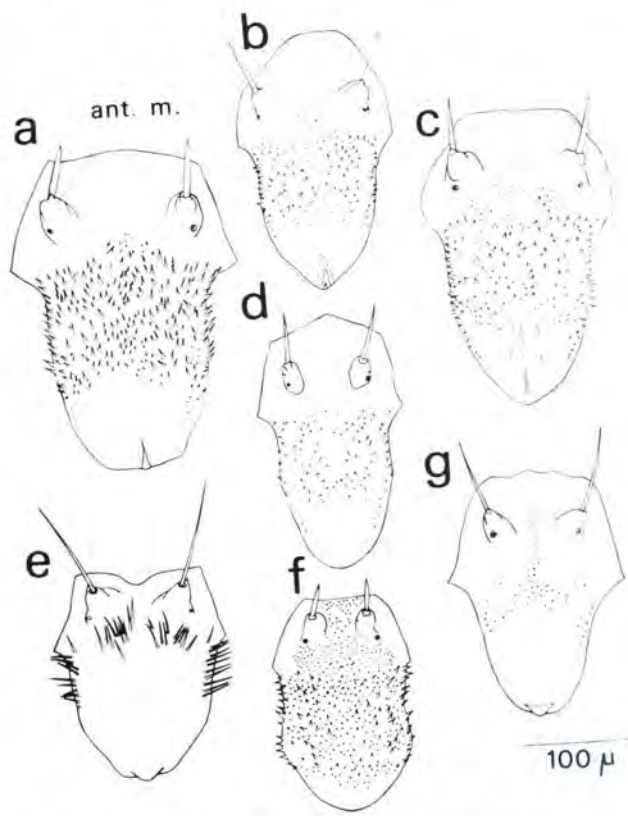

FIG. 9.—Dorsal view of opercula of *Culicoides* pupae
(*a*) *C. nivosus* (*b*) *C. distinctipennis*
(*c*) *C. pycnostictus* (*d*) *C. bedfordi*
(*e*) *C. pallidipennis* (*f*) *C. schultzei*
(*g*) *C. milnei*
ant. m: antero-marginal tubercle

(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 tubercles, viz. the ventro-lateral and ventro-median tubercles. The positions of these tubercles and their setae are shown in Fig. 7(b) and detailed drawings for each species are given in Fig. 10. The nomenclature used in naming the tubercles and setae of the head and thorax is that proposed by Carter *et al.* (1920).

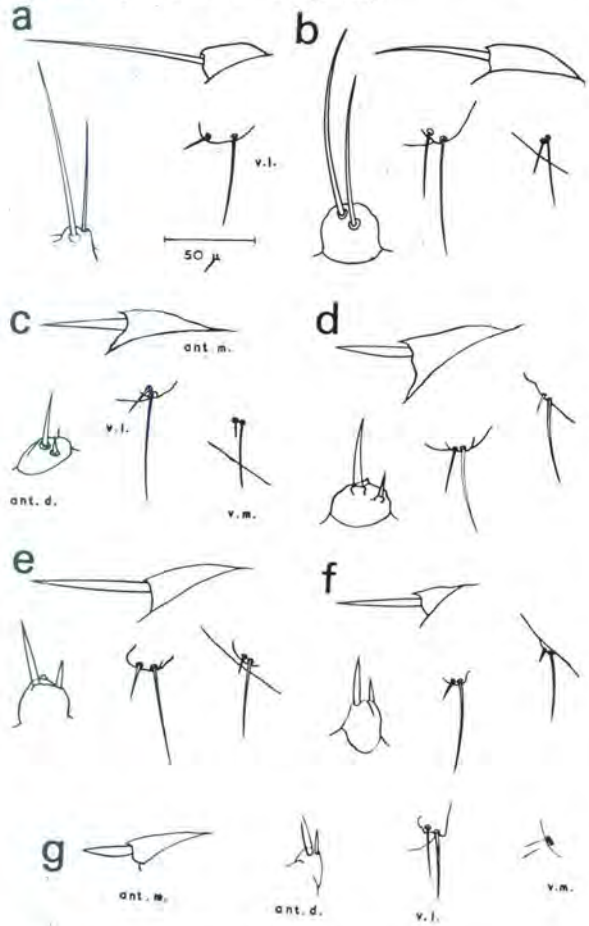

FIG. 10.—Head tubercles of *Culicoides* pupae
(*a*) *C. pallidipennis* (*b*) *C. milnei*
(*c*) *C. distinctipennis* (*d*) *C. nivosus*
(*e*) *C. pycnostictus* (*f*) *C. bedfordi*
(*g*) *C. schultzei*
Letters as in Fig. 7

The antero-marginal tubercles are situated on the operculum (Fig. 9) and may bear a short stout seta as in *C. schultzei;* a medium length seta, as 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 8 from which it can be seen that laboratory-reared and field-collected *C. pycnostictus* differ slightly in setal length although *C. distinctipennis* measurements are identical. Exact measurements are therefore unreliable for the differentiation of species.

The antero-dorsal tubercles bear two subequal setae. These are very long in *C. pallidipennis* and *C. milnei,* the longer 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 of the length of the longer setae. These setae can therefore be used to separate *C. pallidipennis* and *C. milnei* from the other species.

Two thin subequal setae are present ventro-laterally in all the species.

Similar setae are present in the ventro-median position in all the species except *C. pallidipennis,* in which they cannot be seen.

(6) *Thoracic tubercles and setae:* On the anterior region of the prothorax there are two groups of small setae but they are of little importance [Fig. 7(a) and 8(a)]. 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 they are not easily seen.

On the inner side of the horn base is a slight swelling, normally bearing a single small seta, called the dorso-median tubercle by Lawson (1951). In all the species studied only one seta could be seen, and this only with difficulty. Both these groups are clearly illustrated in Fig. 8.

On the dorsal hump of the thorax there is a group of tubercles and setae called the "dorsals" [Fig. 7 (a and b) (dors.)]. There are normally five setae or setal sockets, which are numbered and illustrated in Fig. 11. The first two setae are prominent and arise from pronounced tubercles. One is short and

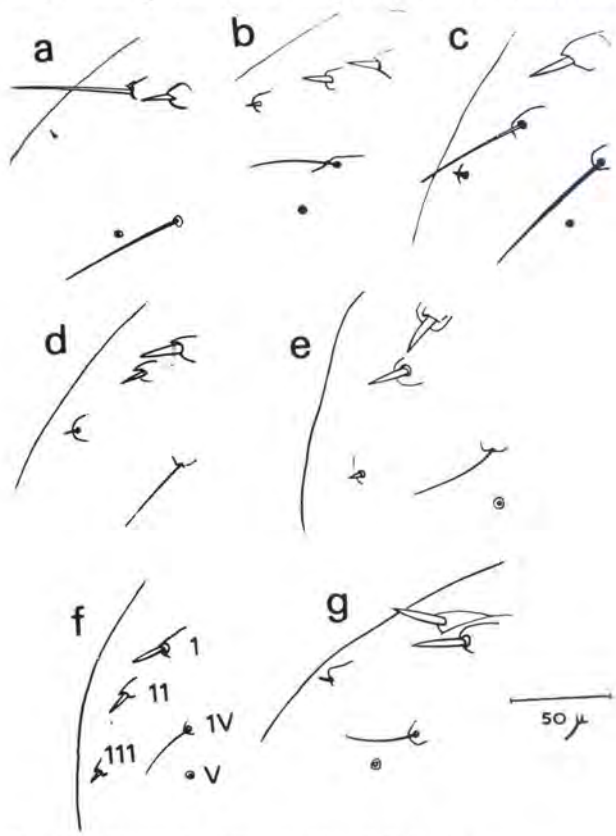

FIG. 11.—Dorsal thoracic tubercles of *Culicoides* pupae
(*a*) *C. pallidipennis* (*b*) *C. nivosus*
(*c*) *C. milnei* (*d*) *C. distinctipennis*
(*e*) *C. pycnostictus* (*f*) *C. bedfordi*
(*g*) *C. schultzei*

sturdy and similar in all the species. The other, however, is very long in *C. pallidipennis* and *C. milnei* but short and stout in the other five species. In this respect they resemble the antero-marginal and antero-dorsal setae discussed earlier. The third seta is very short in all the species except *C. pallidipennis*, where it can hardly be distinguished. The fourth seta is long and slender, while the fifth is represented by an empty socket, in all the 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 fits 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 differs in other genera belonging to the same family. They used this character in their pupal key to separate *Culicoides* from other genera.

In all seven of the South African *Culicoides* species studied the metathorax is divided dorsally by the scutellum [Fig. 7(a)]. No great differences exist between the species.

(8) *Fourth abdominal segment:* The abdomen is composed of nine segments of which the ninth or caudal segment differs markedly from the others and 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 that segments one, two and eight 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 and their terminology has been used by all workers up 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 Fig. 12(d). Lawson (1951) took the fourth abdominal segment as being representative of all the segments, as have Kettle & Lawson (1952) and Linley & Kettle (1964). For comparative purposes, therefore, the fourth segment of the seven species described here has been studied in detail and lateral views are given in Fig. 12.

Small spinules are present on the anterior part of the fourth segment of all species. In *C. milnei* [Fig. 12(a)] they are numerous and completely encircle the segment but in the other species they are interrupted laterally to a greater or lesser extent. They are most reduced in *C. pallidipennis* [Fig. 12(g)], where only a few isolated spinules remain.

A spiracle can sometimes be made out as a circular depression situated antero-laterally [Fig. 12(d)], but is not visible in all the specimens.

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

In most species the tubercles are rounded, or they may project slightly to form a blunt point or shoulder as in the l.p.m. tubercles of *C. milnei* [Fig. 12(a)]. In *C. bedfordi*, however, the l.p.m. and l.a.s.m. tubercles are prolonged into two spines, forming a fork from whose centre a seta arises [Fig. 12(e)]. Kettle & Lawson (1952) used this character to distinguish the *C. nubeculosus* group from the two other major groups of British *Culicoides* species.

In six of the seven species studied, the number of tubercles in the above positions is constant. However, *C. pallidipennis* differs radically in that the d.p.m. tubercles are reduced from the normal five to two [Fig. 12(g)]. This character may be of considerable taxonomic value.

A single seta is present on each tubercle except the d.p.m. tubercles of which the first three are usually naked, though a small spine is sometimes present on the first.

The setae on the various tubercles differ in shape and length and the differences appear to be common to most species. For example the second l.p.m. tubercle always possesses a longer seta than tubercles one and three, except in *C. pallidipennis*, in which all the setae are almost equal in length [Fig. 12(g)].

The posterior margin of each segment has a ring of pale, closely packed nodules forming a wide border seen in nearly all the species. *C. milnei*, however, differs in that the posterior margin has a mosaic or mottled appearance [Fig. 13(a)]. Kettle & Lawson (1952) noted this "crazy paving" effect in some British *Culicoides* species.

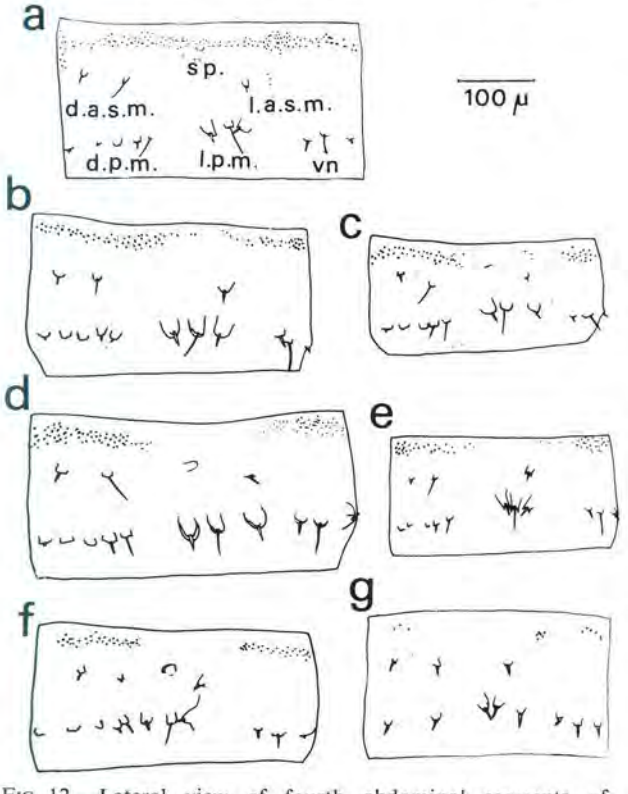

FIG. 12.—Lateral view of fourth abdominal segments of *Culicoides* pupae
(*a*) *C. milnei* (*b*) *C. nivosus*
(*c*) *C. distinctipennis* (*d*) *C. pycnostictus*
(*e*) *C. bedfordi* (*f*) *C. schultzei*
(*g*) *C. pallidipennis*
d.a.s.m: dorsal antero-submarginal tubercles; d.p.m: dorsal postero-marginal tubercles; l.a.s.m: lateral antero-submarginal tubercles; l.p.m: lateral postero-marginal tubercles; sp: spiracle; vn: ventral tubercles

(9) *Caudal segment:* When viewed dorsally under high power (× 620), the caudal segment shows a number of valuable morphological characters. 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 quite useful taxonomically, especially as "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 Fig. 13.

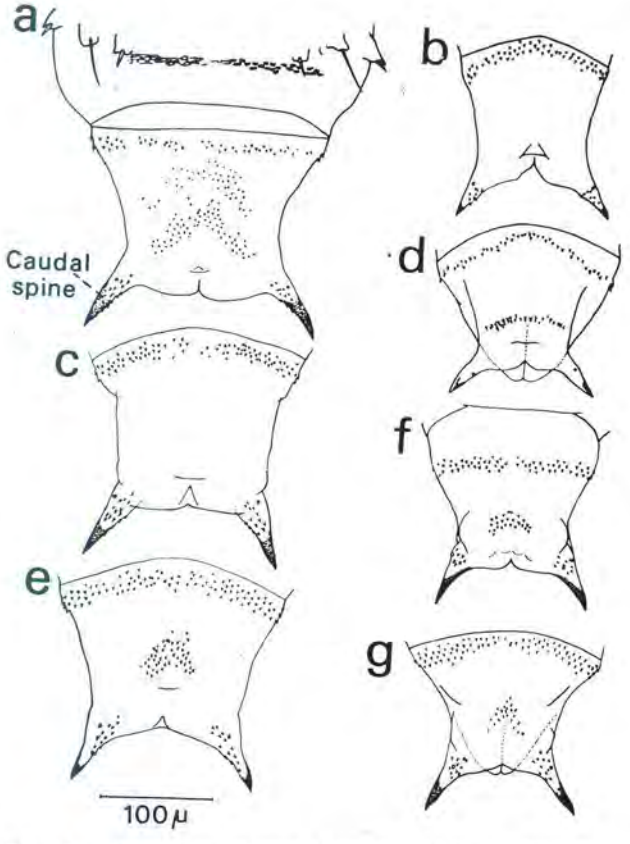

FIG. 13.—Dorsal view of caudal segments of *Culicoides* pupae
(*a*) *C. milnei* ♀ (*b*) *C. schultzei* ♀
(*c*) *C. nivosus* ♀ (*d*) *C. pallidipennis* ♂
(*e*) *C. pycnostictus* ♀ (*f*) *C. distinctipennis* ♀
(*g*) *C. bedfordi* ♂

A broad ring of small pointed scales or spinules encircles the anterior end of the caudal segment of all the species except *C. pallidipennis*, which differs slightly in that the ring is formed of one or two rows of scales only [Fig. 13(d)].

Small pointed scales are also found dorsally. They are 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* [Fig. 13 (c and b)]. In *C. milnei* scattered scales connect this central patch with the anterior rows described earlier [Fig. 13(a)]. In *C. pallidipennis* large scales are arranged across the centre of the caudal segment to form a single or double row [Fig. 13(d)].

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

(10) *Key to pupae:*

1. Tip of prothoracic horn darker than rest of horn [Fig. 8 (a to e)] 2
   Entire prothoracic horn dark brown [Fig. 8 (f and g)]. 5
2. (1) Small dentate scales and transverse folds present on prothoracic horn [Fig. 8 (a to c)] 3
   Annulated rings or transverse folds present on prothoracic horn. Scales absent [Fig. 8 (d and e)] 6
3. (2) Very small dentate scales absent from central dorsal area of caudal segment [Fig. 13 (c)]
   .. *C. nivosus*
   Very small dentate scales present on central dorsal area of caudal segment [Fig. 13 (e and f)] 4
4. (3) Spinules on operculum present anterior to antero-marginal tubercles [Fig. 9 (c)]
   .. *C. pycnostictus*
   Spinules on operculum never anterior to antero-marginal tubercles [Fig. 9 (b)]
   .. *C. distinctipennis*
5. (1) Lateral papillae on prothoracic horn absent [Fig. 8 (f)]
   .. *C. milnei*
   Lateral papillae on prothoracic horn present [Fig. 8 (g)]
   .. *C. bedfordi*
6. (2) Small dentate scales present on central dorsal area of caudal segment [Fig. 13 (d)]
   .. *C. pallidipennis*
   Small dentate scales absent from central dorsal area of caudal segment [Fig. 13 (b)]
   .. *C. schultzei*

CONCLUSIONS

Within the limited number of *Culicoides* species studied it has been possible to find sufficient characters in both the larvae and pupae for the identification of each species after suitable preparation and mounting. Whether the same characters will enable further species to be identified remains to be seen, though 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 of South African spp., e.g. the head ratio and anal papillae in the larvae, and pupal colour and the angle of the caudal spines. A few new characters were found to be useful as confirmatory characters. In the larvae 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 the 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, setal and epipharyngeal studies, oil immersion is necessary.

Summary

Little 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 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.

Acknowledgements

The writer wishes to thank Prof. J. J. Matthee, Prof. R. M. du Toit, Dr. Gertrud Theiler, Miss Jane Walker and Mr. K. P. N. Kleynhans for their helpful criticism of the manuscript. For technical help many thanks are due to Messrs. D. W. du Plooy, R. B. Weber, M. C. de Lange and Miss K. Roos. The writer is also indebted to the photographic section at Onderstepoort, especially Mr. A. M. du Bruyn, for photographs of drawings.

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