

# Afrotropical *Culicoides*: Description and comparison of the pupae of seven species of the Similis supergroup (Diptera: Ceratopogonidae)

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## ABSTRACT

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The pupae of *Culicoides exspectator* Clastrier, *C. kobae* Cornet & Chateau, *C. micheli* Cornet & Chateau, *C. olysageri* Kremer & Nevill, *C. ravus* De Meillon and *C. (Synhelea) tropicalis* Kieffer are described and illustrated for the first time, and the pupa of *C. similis* Carter, Ingram & Macfie is redescribed. All seven species belong to the Similis supergroup; the immature stages of six are found in ground waters while those of *C. olysageri* occur in tree holes. A concise key is presented for differentiating the seven species. Short notes on their larval habitats are given. Reference is made to *C. accraensis* Carter, Ingram & Macfie, also found in tree holes.

Available data suggest that pupal morphology can be used to differentiate closely related species; group characters also exist for those species within the Similis supergroup which can be used to separate ground-water species from those utilizing tree holes. Indications are that the pupae are of limited help in resolving the systematic subdivision of the Similis supergroup.

## INTRODUCTION

Of 136 *Culicoides* species recorded from the Afrotropical Region (Wirth, De Meillon & Haeselbarth 1980), the pupal stage of only 19 have been described or redescribed. They were from:

- The Gold Coast (= Ghana) (Carter, Ingram & Macfie 1920): *C. accraensis*, *C. clarkei*, *C. eriodendroni*, *C. inornatipennis*, *C. nigripennis*, *C. punctithorax*

- and *C. schultzei* Enderlein, 1908 (probably *C. sp. #4* of Glick 1990, which = *C. sp. #3* of R. Meiswinkel, Onderstepoort Veterinary Institute [OVI])
- South Africa (De Meillon 1936): *C. alexis* and *C. meeserellus*
- South Africa (De Meillon 1937): *C. cornutus*, *C. engubandei* and *C. nivosus*
- South Africa (Nevill 1969): *C. bedfordi* Ingram & Macfie, 1923, *C. distinctipennis* Austen, 1912 (later identified as *C. leucostictus* Kieffer, 1911), *C. milnei* Austen, 1909 (later identified as *C. zuluensis* De Meillon, 1936), *C. nivosus* De Meillon, 1937, *C. pallidipennis* Carter, Ingram & Macfie, 1920 (= *C. imicola* Kieffer, 1913), *C. pycnostictus* Ingram & Macfie, 1925 and *C. schultzei* Enderlein, 1908 (probably *C. sp. #4* of Glick 1990, which = *C. sp. #3* of Meiswinkel)

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- South Africa (Cornet & Nevill 1979): *C. hildae*
- Laos (Howarth 1985): *C. similis* Carter, Ingram & Macfie, 1920

Based solely on adult morphology, Cornet & Chateau (1971) placed 16 species of Afrotropical *Culicoides* within the Similis group. Meiswinkel & Dyce (1989) reassessed this group and while they, in part, accepted the definition of Cornet & Chateau (1971), they concluded that the broad Similis group is distributed worldwide and constitutes a number of species groups with complex affinities. For the Afrotropical Region, Meiswinkel & Dyce (1989) listed 28 species which belong to the Similis fraternity and these, except for a few outliers, can be split into three main entities—the Similis, Accraensis and Tropicalis groups. For the latter group, they resurrected the subgenus *Synhelea* Kieffer to include ten species that share two apomorphies in the male and female adult stages. The broad Similis group of species worldwide is now referred to as the Similis supergroup (R. Meiswinkel, OVI, personal communication 1992).

This paper presents original descriptions of pupae of seven Afrotropical *Culicoides* species belonging to the Similis supergroup, which includes a redescription of the pupa of *C. similis*, based on African specimens. Meiswinkel and Dyce (1989) have placed *Culicoides expectator* Clastrier, 1959, *C. kobae* Cornet & Chateau, 1971, *C. micheli* Cornet & Chateau, 1971 and *C. ravus* De Meillon, 1936 in the Similis group, and *C. tropicalis* Kieffer, 1913 in the subgenus *Synhelea*. The immatures of these six species all inhabit the margins of ground-water bodies. The remaining species, *C. olysageri* Kremer & Nevill, 1972, belongs to the Accraensis group and uses tree holes for its larval habitat. Brief comparisons are drawn between the character states of the above species and those described by Carter *et al.* (1920) for *C. accraensis*, which also inhabits tree holes.

Finally, the question whether pupal character states are useful for the differentiation of closely related species and as morphological indicators of group and intergroup relationships, is briefly discussed.

## MATERIALS AND METHODS

One hundred and one slide-mounted pupae were studied; the majority of specimens (78) used for the descriptions in this paper were from link-reared adult and pupal collections made by one of us (A.L.D.) in the western and northern Transvaal and the northern Cape during an extensive study trip in 1973/1974. Additional pupae (23) were collected during 1988–1993 at Umlalazi Coastal Nature Reserve on the Zululand coast (H.N.), in the Transvaal Lowveld (A.L.D.) and near the Wonderboom, Pretoria (A.L.D. & H.N.) Comparisons with *C. accraensis* are based on descriptions and illustrations by Carter *et al.*

1920. Slide-mounted specimens were not available, therefore it was not possible to compare all character states in detail.

## Preparation of specimens

Each adult midge and its matching pupal case was processed and mounted as follows: the wings were removed in 96% ethanol and mounted in a drop of Canada Balsam/phenol/ethanol mixture on a glass slide and left to dry. The remainder of the adult and its pupal case were placed in a 10% KOH solution overnight, at room temperature, to clear. The solution was drawn off and the KOH remaining in the specimens neutralized with a few drops of glacial acetic acid for 10–15 min. The specimens were held in 96% ethanol overnight to dehydrate sufficiently and then removed to clove oil for 1–3 d.

The adults and pupal cases were dissected and each body section placed in a small drop of Canada Balsam. The body sections of the pupa were positioned as follows:

- Left prothoracic respiratory horn (PRH) (Fig. 1)—lateral view for easier measuring and illustrating
- Operculum (O) (Fig. 1 and 2)—dorsal view, immediately cover-slipped to prevent curling
- Caudal segment (Fig. 1)—dorsal view to note spinules and to measure the length of the posterolateral processes (PP)
- Remaining cephalothorax and abdomen (Fig. 1)—lateral view, specifically to note presence and size of apicolateral processes (spines) on lateral posteromarginal (lpm) tubercles of fourth abdominal segment (Fig. 3). In this position the dorsomedian seta is obscured and therefore omitted from the descriptions and illustrations

One to three weeks later, when the body parts had dried in position, the specimens were cover-slipped.

## Terminology, measurements and illustrations

(Fig. 1, 2 and 3)

The terminology used, as well as techniques for measuring and illustrating, follows a number of authors: Carter *et al.* (1920), Lawson (1951), Jones (1961), Nevill (1969), Kettle & Elson (1976; 1978), Cornet & Nevill (1979) and Howarth (1985) (Fig. 1, 2 and 3).

Measurements were made under 312x magnification with an ocular micrometer and converted to  $\mu\text{m}$ . To compare measurements in this study and to be able to relate these measurements to other species in future studies, it was necessary to examine and measure characters of the pupae of a number of species (23), ranging from the smaller species of the subgenus *Avaritia* to the larger species of the subgenus *Monoculicoides*. For the sake of convenience, the

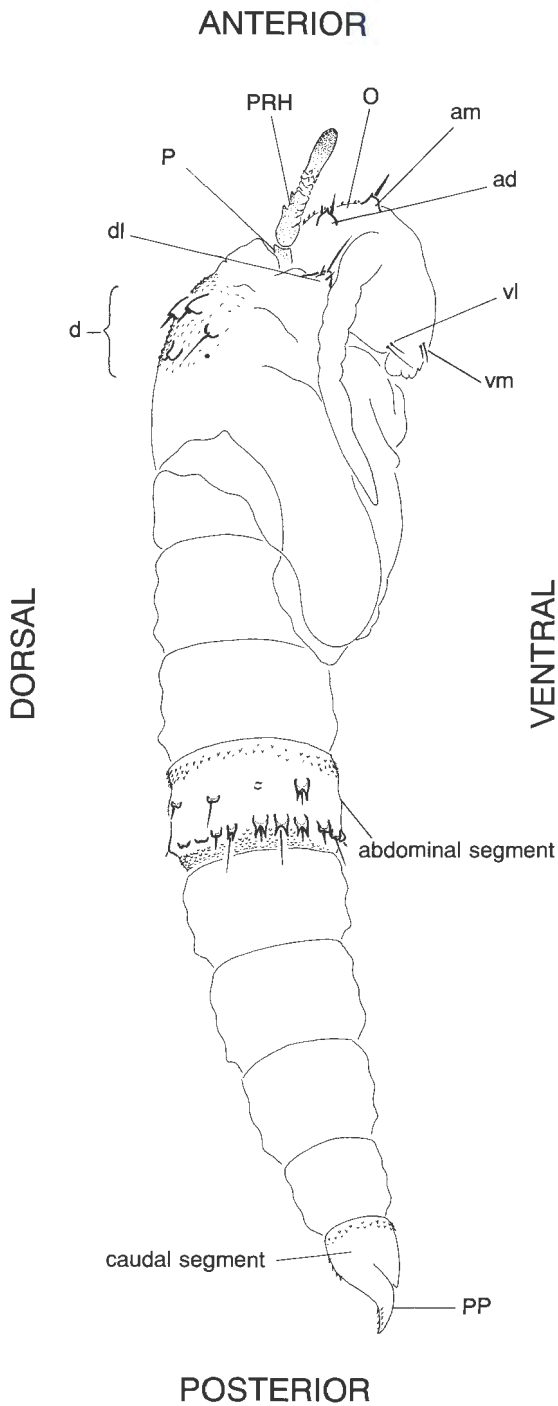


FIG. 1 Lateral view of *Culicoides similis* pupa indicating the structures studied

- Head:** (O) operculum with (am) anteromarginal tubercle; (ad) anterodorsal tubercle; (vl) ventrolateral setae; (vm) ventromedian setae
- Thorax:** (PRH) prothoracic respiratory horn; (P) pedicel; (dl) dorsolateral tubercle; (d) dorsal tubercles
- Abdomen:** (PP) posterolateral processes

following categories for differentiation were then adopted: very short – short – moderate – long – very long for the respiratory horn, pedicel and posterolateral processes; minute – short – moderate – long – very long for the setal lengths and thin – moderate – stout – very stout for the setal widths. Pupal length was measured in lateral view (Fig. 1), from the most anterior point on the cephalothorax to the tip of the posterolateral processes—only the sclerotized areas of the abdominal segment were included in the measurement. Use was made of two ratios—one derived from measurements of the horn and pedicel (Fig. 1) and one from the disc of the operculum (Fig. 2):

$$\text{Pedicel/horn ratio (P/H)} = \frac{\text{Length of pedicel}}{\text{Length of horn}}$$

$$\text{Operculum width/operculum length ratio (OW/OL)} = \frac{\text{Distance between lateral angles}}{\text{Distance from lateral angles to posterior margin}}$$

Measurements and ratios of the operculum were calculated separately for males and females (Table 3). No other differentiation was made between the sexes.

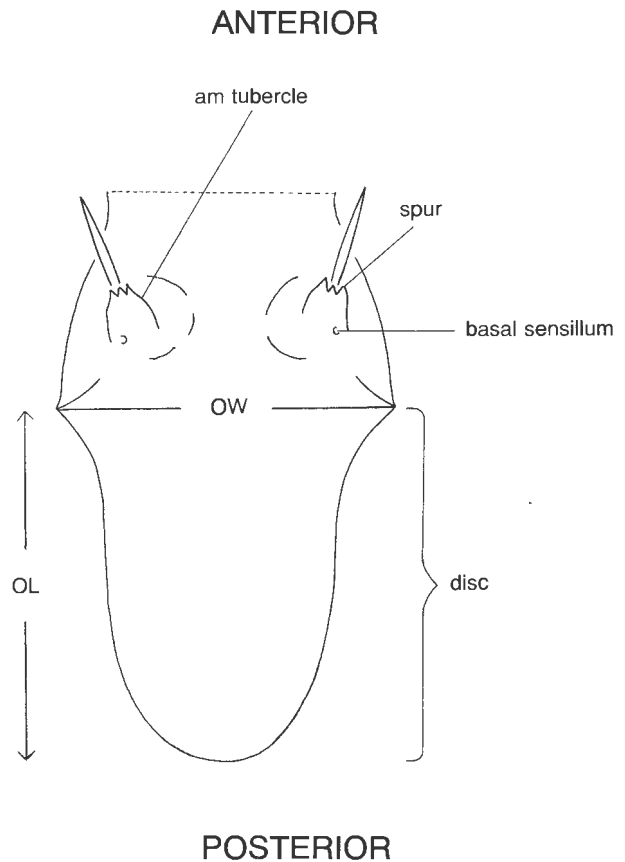


FIG. 2 Dorsal view of operculum indicating measurements taken: (OL) operculum length; (OW) operculum width

The abdominal tubercles were numbered from the middorsal line (Fig. 3). There was great variation in the angle of divergence of the posterolateral processes, probably due to compression when cover-slipped. These measurements were therefore omitted.

The respiratory horns, thoraces, fourth abdominal segments, ventromedian (vm), ventrolateral (vl), anterodorsal (ad) and dorsolateral (dl) tubercles and setae (Fig. 1) were illustrated under 625x magnification (because of its large size the fourth abdominal segment of *C. olysageri* was illustrated under 390x magnification); the opercula and caudal segments under 500x magnification. Illustrations were made using a tracing device. Evaluation of horn pigmentation, especially the paler areas, was made under phase contrast as well as brightfield illumination. The slide material examined, from which the seven species were described, is deposited in the OVI collection.

**RESULTS**

**Key to pupae of seven Afrotropical *Culicoides* species belonging to the *Similis* supergroup**

1. Apicolateral spines on abdominal tubercles absent (Fig. 8h) . . . . . *ravus*  
Apicolateral spines on abdominal tubercles present . . . . . 2
2. Scales present on most of respiratory horn (Fig. 7a) . . . . . *olysageri*

3. Scales absent from most of or entire respiratory horn . . . . . 3
3. A few scales present distad of folds on respiratory horn (Fig. 9a) . . . . . *similis*  
Scales absent from respiratory horn . . . . . 4
4. Respiratory horn with apical fan-like process and one lateral spiracle (Fig. 6a) . . . . . *miceli*  
Respiratory horn without apical process and possessing more than one lateral spiracle . . . . . 5
5. Narrow transverse band of opercular spines anteriorly on disc (Fig. 4b) . . . . . *expectator*  
Transverse band of spines absent . . . . . 6
6. Large, well-defined spines on lateral margins of disc (Fig. 10b) . . . . . *tropicalis*  
Large, round structures, with an occasional very small spine, on lateral margins of disc (Fig. 5b) . . . . . *kobae*

***Culicoides expectator* Clastrier**

(Fig. 4a-i; Tables 1-6)

*Culicoides expectator* Clastrier, 1959:177. Senegal

*Description*

Mean total length 1,69 mm (1,62-1,77 mm; n = 6). Light brown; darker brown on thorax, anteriorly and on dorsal half of abdomen.

RESPIRATORY HORN (Fig. 4a; Tables 1, 2 and 6). Basal half and distal third uniformly brown, distinct pale area medially. Horn short; comma-shaped, very nar-

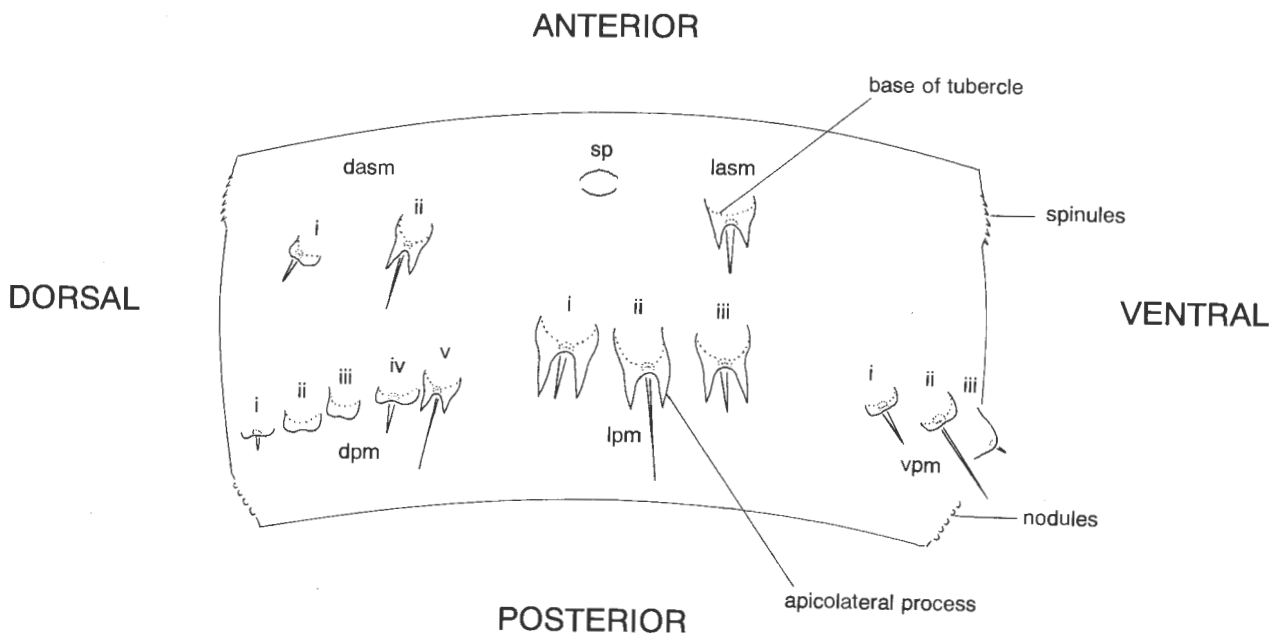


FIG. 3 Lateral view of fourth abdominal segment of *Culicoides* pupa showing position of tubercles: (dasm) dorsal anterosubmarginal tubercle; (dpm) dorsal posteromarginal tubercle; (lasm) lateral anterosubmarginal tubercle; (lpm) lateral posteromarginal tubercle; (vpm) ventral posteromarginal tubercle; (sp) anterolateral spiracle

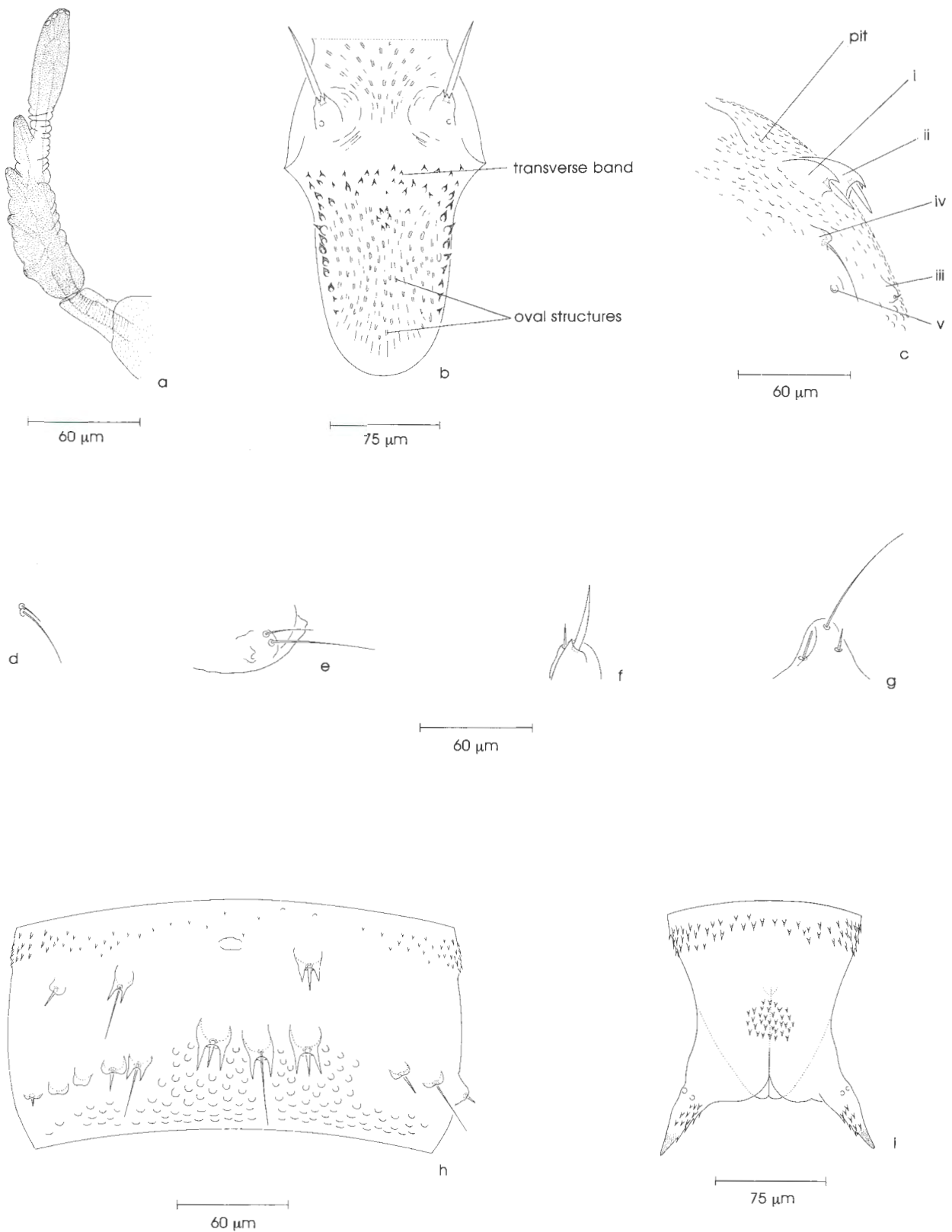


FIG. 4 Pupa of *Culicoides expectator*: (a) prothoracic respiratory horn; (b) ♀ operculum; (c) thorax; (d) ventromedian setae (vm); (e) ventrolateral setae (vl); (f) anterodorsal tubercle (ad); (g) dorsolateral tubercle (dl); (h) fourth abdominal segment; (i) caudal segment

row beyond distal lateral spiracle, widens slightly to narrow towards apex which bears four terminal spiracles. Slight folds present on basal third, folds become more prominent medially; annular beyond distal spiracle. Scales absent. On basal three-fifths of horn 4–5 (4) lateral spiracles, basal spiracle and sometimes second and third spiracle on very slight prominences, distal lateral spiracle on large, pigmented prominence. Pedicel short, 0,185 length of horn.

**OPERCULUM** (Fig. 4b; Tables 3 and 6). Brown. In ♀♀ and ♂♂, disc longer than wide. A single row of small, rather delicate, dark brown spines on lateral margins of disc, tips occasionally extend beyond margin; rows of spines joined anteriorly by a thin, transverse band of smaller spines; a small group of spines occasionally present posteromedially of transverse band; distinct semi-circular rows of oval structures radiate from lateral margins to unite medially; oval structures present between and anterior of am tubercles.

**HEAD TUBERCLES AND SETAE** (Fig. 4b, d–f; Tables 4 and 6). Am tubercle with 2–3 sharp spurs, seta moderate to long, stout, sharp; basal sensillum present (Fig. 4b). Two thin vm setae of subequal length and width (Fig. 4d). Two thin vl setae of subequal length and width, on prominence; a sensillum on slight prominence sometimes discernible dorsad of vl setae (Fig. 4e). Ad tubercle with 1–2 sharp spurs; seta i moderate length, stout, sharp and seta ii short, thin and sharp, c. one third the length of seta i (Fig. 4f).

**THORAX** (Fig. 4c and g; Tables 4 and 6). Dorsal tubercles small; d i and d ii closely approximated, both with two sharp spurs, setae short, moderate width, sharp; d iii seta minute; d iv seta moderate length, thin; d v sensillum on very slight prominence. Pits present between and surrounding tubercles; a few nodules lateromedial of d ii (Fig. 4c). Dl tubercle with three setae: one terminal, moderate length, thin; one lateral, short, thin, sharp and one basal, short, in fold, difficult to discern (Fig. 4g).

**ABDOMEN** (Fig. 4h; Tables 4 and 6). Dpm i base broad, seta minute; dpm ii base more rounded than i, seta absent; dpm iii smaller than ii, base rounded, sometimes with sharp apicolateral spines, seta absent; dpm iv base rounded, sometimes with two sharp apicolateral spines; seta short, moderate width, sharp; dpm v same as iv but smaller, with two sharp apicolateral spines, seta longer than iv, thin. Lpm tubercles i–iii extended, oval bases, two long, sharp apicolateral spines; lpm i and iii each with seta short, moderate width, sharp; lpm ii seta longer than i and iii, thin. Vpm i small, base rounded, apices rounded, seta short, stout, sharp; vpm ii base wider than i, apices rounded, seta longer than i, thin; vpm iii base broad, seta minute. Dasm i same as vpm i, seta very

short, thin, sharp; dasm ii same as dpm v. Lasm slightly smaller than lpm iii, seta short, moderate width, sharp. Anterolateral spiracle present. Anterior band of spinules laterally reduced to a few small spinules; a few nodules present anterolaterally, posterior to lpm tubercles and on posterior margin of segment, interrupted dorsally and ventrally.

**CAUDAL SEGMENT** (Fig. 4i; Tables 5 and 6). Anterior band of spinules narrowed to interrupted dorsally; dorsomedian patch of 17–30 spinules. Posterolateral processes of moderate length; only inner aspects of processes with spinules; apical quarter to half pigmented; tips bluntly rounded.

#### Material examined

TRANSVAAL. 6 ♀♀ 1 ♂, Farm "Lodwicks Lust" (25° 26'S; 31° 41'E), district Hectorspruit, eastern Transvaal, 1973.xi.29, A.L. Dyce, ground-water margin from wheel tracks crossing drainage stream from sugar-cane irrigation.

2 ♀♀, Jam Tin Creek (25° 31'S; 31° 35'E), Nelspruit–Komatipoort road, eastern Transvaal, 1973.xii.02, A.L. Dyce, margin of clear, running stream.

1 ♀, Sterkrivier (23° 55'S; 28° 24'E), 30 km south-west of Potgietersrust, northern Transvaal, 1974.i.03, A.L. Dyce, ground-water body—seepage from grassed irrigation drain next to road, water milky, soil red, gritty, gravelly loam, marginal vegetation open to sunlight.

#### Associated species

*C. expectator* was collected in association with *C. ravus* (2x), *C. similis* (2x), *C. sp.* #1-10 (Meiswinkel) (2x), *C. bedfordi* (1x), *C. micheli* (1x) and *C. nivosus* (1x).

#### *Culicoides kobae* Cornet & Chateau

(Fig. 5a–i; Tables 1–6)

*Culicoides kobae* Cornet & Chateau, 1971:148. Senegal

#### Description

Mean total length 1,50 mm (1,43–1,60 mm; n = 6). Light brown; darker brown on thorax and anteriorly.

**RESPIRATORY HORN** (Fig. 5a; Tables 1, 2 and 6). Light brown, distal quarter to third darker. Horn short; slightly wider basally and distally, bearing 5–7 (6) terminal spiracles. Annular folds cover median three-fifths of horn. Scales absent. Two lateral spiracles on basal half of horn, the distal spiracle always on a medium-sized, unpigmented prominence. Pedicel short, 0,166 length of horn.

**OPERCULUM** (Fig. 5b; Tables 3 and 6). Rather dark brown. In ♀♀ and ♂ disc longer than wide. One or

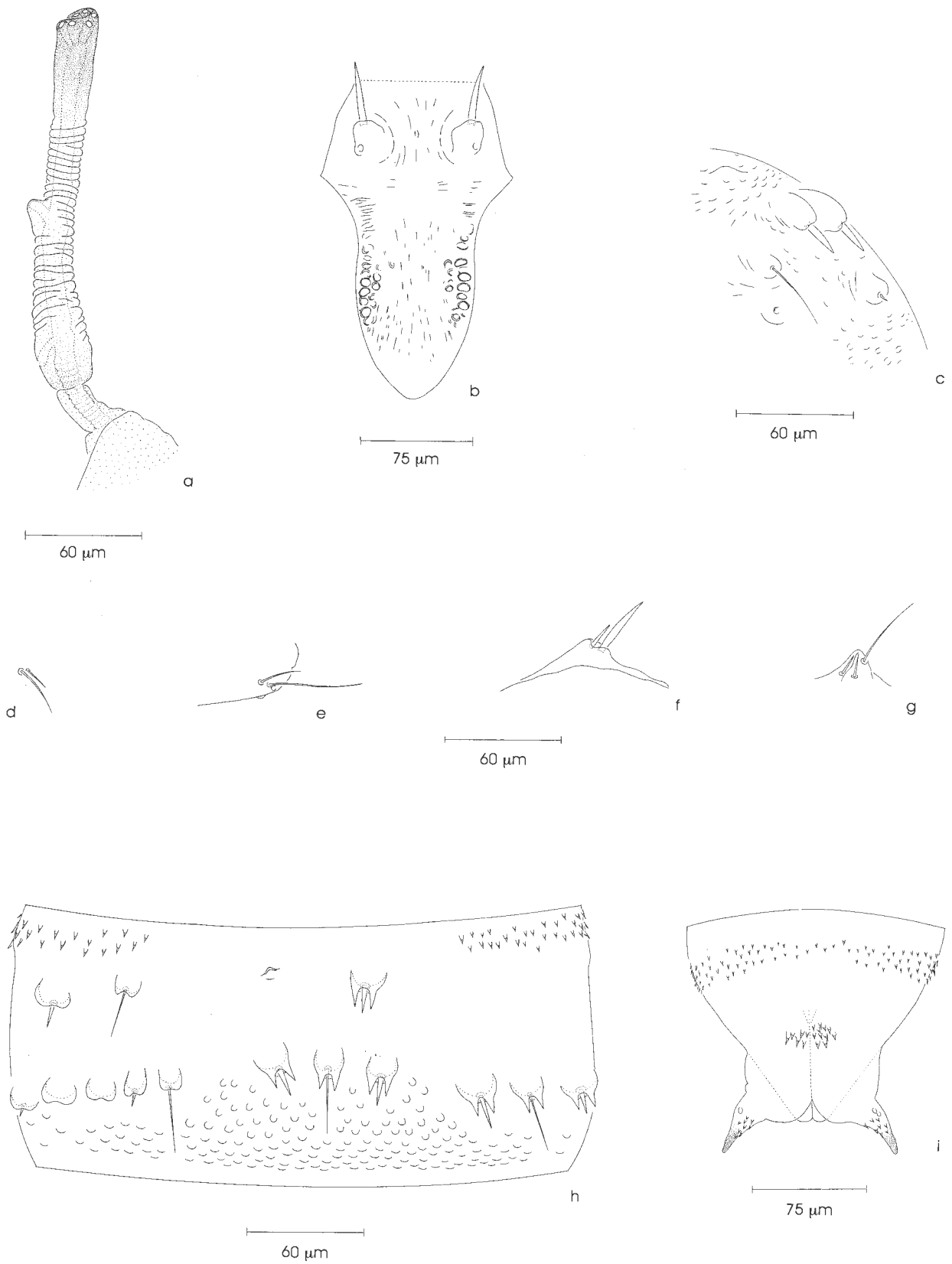


FIG. 5. Pupa of *Culicoides kobae*: (a) prothoracic respiratory horn; (b) ♀ operculum; (c) thorax; (d) ventromedian setae; (e) ventrolateral setae; (f) anterodorsal tubercle; (g) dorsolateral tubercle; (h) fourth abdominal segment; (i) caudal segment

more rows of large, round structures on each lateral margin of disc, occasionally a very small, sharp spine present on structure. Oval structures present on remainder of disc; a few oval structures occasionally between am tubercles.

**HEAD TUBERCLES AND SETAE** (Fig. 5b, d–f; Tables 4 and 6). Am tubercle with blunt spurs; seta moderate length, stout, sharp; basal sensillum present (Fig. 5b). Two thin vm setae of subequal length and width (Fig. 5d). Two thin vl setae of subequal length and width on slight prominence; a sensillum present (Fig. 5e). Ad tubercle without spurs; seta i sharp, of moderate length and width and seta ii short, thin, *c.* one third the length of seta i (Fig. 5f).

**THORAX** (Fig. 5c and g; Tables 4 and 6). Dorsal tubercles small; d ii larger than d i, closely associated, spurs blunt, setae short, moderate width, sharp; d iii seta minute; d iv seta moderate length, thin; d v sensillum on very slight prominence. Pits present anterior to tubercles, a few pits sometimes between, around and posteriad of tubercles (Fig. 5c). DI tubercle with three setae: one terminal, moderate length, thin; one lateral, short, sharp, thin and one basal, in fold, short, difficult to discern (Fig. 5g).

**ABDOMEN** (Fig. 5h; Tables 4 and 6). Dpm i base broad, seta minute; dpm ii and dpm iii bases more rounded, setae absent; dpm iv base more elongate, apices rounded, seta short, thin, sharp; dpm v same as dpm iv, seta moderate length, thinner. Lpm i–iii extended, apicolateral spines long, sharp; lpm setae i and iii sharp, longer, stouter than dpm iv, lpm ii seta longer than lpm i and lpm iii, thinner. Vpm i–iii with sharp apicolateral spines; vpm i base rounded, seta same as lpm i and lpm iii, thinner; vpm ii base more elongate, seta longer, thinner than vpm i; vpm iii base broader, seta slightly shorter, stouter than vpm i. Dasm i base rounded, seta longer than dpm iv, same width; dasm ii smaller than dasm i, seta moderate length, thin. Lasm same as lpm i and lpm iii. Anterolateral spiracle present. Anterior band of spinules interrupted laterally; nodules present posteriad of lpm tubercles and on posterior margin of segment, interrupted dorsally and ventrally.

**CAUDAL SEGMENT** (Fig. 5i; Tables 5 and 6). Anterior band of spinules interrupted dorsally; dorsomedian patch of 17–28 spinules. Posterolateral processes rather short; inner and dorsal aspects of processes with spinules; apical third pigmented; tips bluntly rounded.

#### *Material examined*

TRANSVAAL. 6 ♀♀ 1 ♂, Pafuri, Kruger National Park (24°59'S; 31° 32'E), 1993.ii.17, A.L. Dyce & L.E.O. Braack, inlet to side creek of the Levuvhu River, red silt over sand.

#### *Associated species*

*C. kobae* was collected in association with *C. tropicalis* (1x), *C. ravus* (1x), *C. sp.* #3 (Meiswinkel) (1x) and *C. sp.* #110 (Meiswinkel) (1x).

#### ***Culicoides micheli* Cornet & Chateau** (Fig. 6a–i; Tables 1–6)

*Culicoides micheli* Cornet & Chateau, 1971:164.  
Senegal

#### *Description*

Mean total length 1,64 mm (1,60–1,68 mm; n = 5). Light brown; darker on thorax and anteriorly.

**RESPIRATORY HORN** (Fig. 6a; Tables 1, 2 and 6). Basal four-fifths uniformly lightly pigmented, distal fifth darker. Horn short; narrowest medially, wider distally; fan-like process present on apex of horn, bearing 5–7 (6) small terminal spiracles. Annulations medially. Scales absent. One lateral spiracle on very small, sharp-tipped prominence on proximal third of horn. Pedicel short, 0,185 length of horn.

**OPERCULUM** (Fig. 6b; Tables 3 and 6). Brown. In ♀♀ and ♂♂ disc longer than wide. A single row of short, sharp spines on lateral margin of disc, tips do not extend beyond margin, joined anteriorly by a sparse, transverse band of spines and spinules; a double row of spines radiates from each lateral row in a semi-circle towards midline of disc; a few spines and oval structures medially on disc, oval structures anteriad of am tubercles.

**HEAD TUBERCLES AND SETAE** (Fig. 6b, d–f; Tables 4 and 6). Am tubercle with sharp spur, seta long, stout, sharp; basal sensillum (Fig. 6b). Two thin vm setae of subequal length and width (Fig. 6d). Two thin vl setae of subequal length and width and a sensillum present (Fig. 6e). Ad tubercle with 1–2 blunt to sharp spurs; seta i short, moderately stout, sharp seta and seta ii shorter, thin, *c.* one third the length of seta i (Fig. 6f).

**THORAX** (Fig. 6c and g; Tables 4 and 6). Dorsal tubercles small; d i and d ii rather closely approximated, both with two sharp spurs, setae short, moderate width; d iii seta minute; d iv seta longer than d i and d ii, d v sensillum on slight prominence. Pits present between and surrounding tubercles; a few nodules posteriorly (Fig. 6c). DI tubercle with three setae: one terminal, moderate length, thin; one lateral, short, thin and one basal, in fold, difficult to discern (Fig. 6g).

**ABDOMEN** (Fig. 6h; Tables 4 and 6). All tubercles with long, sharp apicolateral spines except dpm i and vpm iii. Dpm i base broad, seta minute; dpm ii and



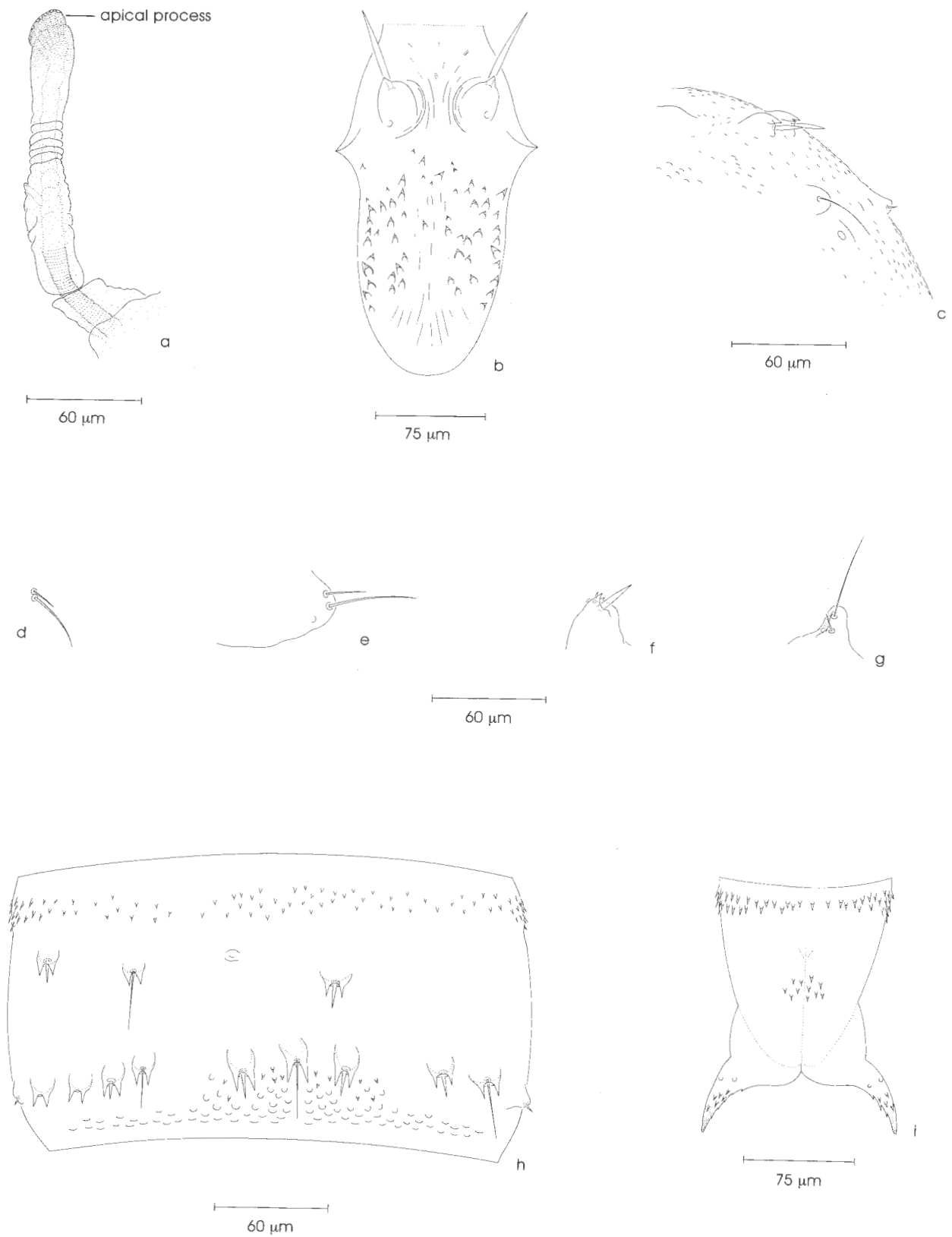


FIG. 6 Pupa of *Culicoides micheli*: (a) prothoracic respiratory horn; (b) ♂ operculum; (c) thorax; (d) ventromedian setae; (e) ventrolateral setae; (f) anterodorsal tubercle; (g) dorsolateral tubercle; (h) fourth abdominal segment; (i) caudal segment

iii base rounded, setae absent; dpm iv base rounded, seta short, thin, sharp, dpm v base rounded, seta longer than iv, thinner. Lpm i–iii extended, bases oval; lpm i and iii setae short, moderate width, sharp; lpm ii seta longer than i and iii, thin. Vpm i base rounded, seta short, thin; vpm ii base rounded, seta longer than i, thinner; vpm iii base broader, seta minute. Dasm i base rounded, seta short, thin, sharp; dasm ii base rounded, seta longer than i, thinner. Lasm seta short, moderate width, sharp. Anterolateral spiracle present. Anterior band of spinules not interrupted laterally. Spinules and nodules present posteriad of lpm tubercles; nodules on posterior margin of segment, interrupted dorsally and ventrally.

**CAUDAL SEGMENT** (Fig. 6j; Tables 5 and 6). Anterior band of spinules narrowed dorsally; dorsomedian patch of 9–20 spinules. Posterolateral processes of moderate length; inner aspects of processes with spinules; apical quarter pigmented; tips bluntly rounded.

#### *Material examined*

TRANSVAAL. 5 ♀♀ 6 ♂♂, Jam Tin Creek (25°31'S; 31°35'E), Nelspruit-Komatipoort road, eastern Transvaal, 1973.xii.02, A.L. Dyce, margin of clear, running stream.

#### *Associated species*

*C. micheli* was collected in association with *C. bedfordi* (1x), *C. ravus* (1x) and *C. sp.* #110 (Meiswinkel) (1x).

#### ***Culicoides olysageri* Kremer & Nevill** (Fig. 7a–i; Tables 1–6)

*Culicoides olysageri* Kremer & Nevill, 1972:467.  
South Africa

#### *Description*

Mean total length, 1.86 mm (1.76–2.10 mm; n = 8). Uniformly rather dark yellow-brown.

**RESPIRATORY HORN** (Fig. 7a; Tables 1, 2 and 6). Rather dark yellow-brown throughout, except for pale medial quarter. Horn short; widest basally, narrowest medially, widens again towards apex to bear 6–9 (8) terminal spiracles. Folds medially. Sharp scales present between basal seventh and distal quarter of horn. On basal two-thirds of horn 2–4 (3) lateral spiracles, basal spiracle on small pigmented prominence, larger prominences distally. Pedicel short, 0.115 length of horn.

**OPERCULUM** (Fig. 7b; Tables 3 and 6). Rather dark yellow-brown. In ♀♀ and ♂♂, disc slightly wider than long. A single row of rather small, broad-based, sharp spines on lateral margins of disc, some tips

extend slightly over margin; medial V-shaped band of spines; patch of smaller spines in centre of V; lateral rows and V-shape joined anteriorly by a thin, transverse band of smaller spines; oval structures discernible on disc, between and anterior of am tubercles.

**HEAD TUBERCLES AND SETAE** (Fig. 7b, d–f; Tables 4 and 6). Am tubercle with small, blunt to sharp spur, seta very long, moderate width; basal sensillum (Fig. 7b). Two vm setae of subequal length and width (Fig. 7d). Two vl setae of subequal length and width; a sensillum present on same, rather large, prominence (Fig. 7e). Ad tubercle large, with a rather blunt spur; seta i long, moderately wide, sharp and seta ii short, thin, sharp, c. one ninth the length of seta i (Fig. 7f).

**THORAX** (Fig. 7c and g; Tables 4 and 6). Dorsal tubercles large, d i and d ii very closely approximated, both with two small, blunt to sharp spurs, setae short, stout, sharp; d iii large, seta minute; d iv seta long, thin; d v sensillum on large prominence. Pits present between tubercles; surface of thorax surrounding tubercles densely covered with rather large nodules, a few nodules between tubercles (Fig. 7c). Dl tubercle with three setae: one terminal, very long, thin, sharp; one lateral, short, thin, sharp and one basal, in fold, difficult to discern (Fig. 7g).

**ABDOMEN** (Fig. 7h; Tables 4 and 6). Dpm i base broad, apices rounded, minute seta; dpm ii and iii bases more rounded than dpm i, setae absent, dpm iv same as iii, seta short, thin, sharp; dpm v smaller than iv, apices rounded, seta moderate length, thinner than iv. Lpm i–iii more extended, bases oval, each tubercle with two sharp apicolateral spines; setae i and iii short, moderate width, sharp; seta ii long, thinner than i and iii. Vpm i small, base rounded, apices rounded, seta short, thin, sharp; vpm ii same as i, larger, seta long, thinner than i; vpm iii base broad, apices rounded, seta short, thin. Dasm i and ii small, bases rounded, apices rounded, dasm i seta short, thin, sharp; dasm ii seta long, thinner than i. Lasm same as lpm i and iii, seta short, moderate width, sharp. Anterolateral spiracle present. Anterior band of fine spinules slightly interrupted laterally, continues half-way down segment dorsally between dasm i tubercles; distinct mosaic pattern covers whole integument except for triangle of five bare circles dorsally and two ventrally. Nodules present posteriad of lpm tubercles and on posterior margin of segment, uninterrupted.

**CAUDAL SEGMENT** (Fig. 7i; Tables 5 and 6). Anterior band of spinules interrupted dorsally by a round, bare area; spinules continue posteriorly to cover most of segment dorsally, except for an inverted V-shaped bare area posteromedially. Posterolateral processes long; inner, ventral and dorsal aspects of

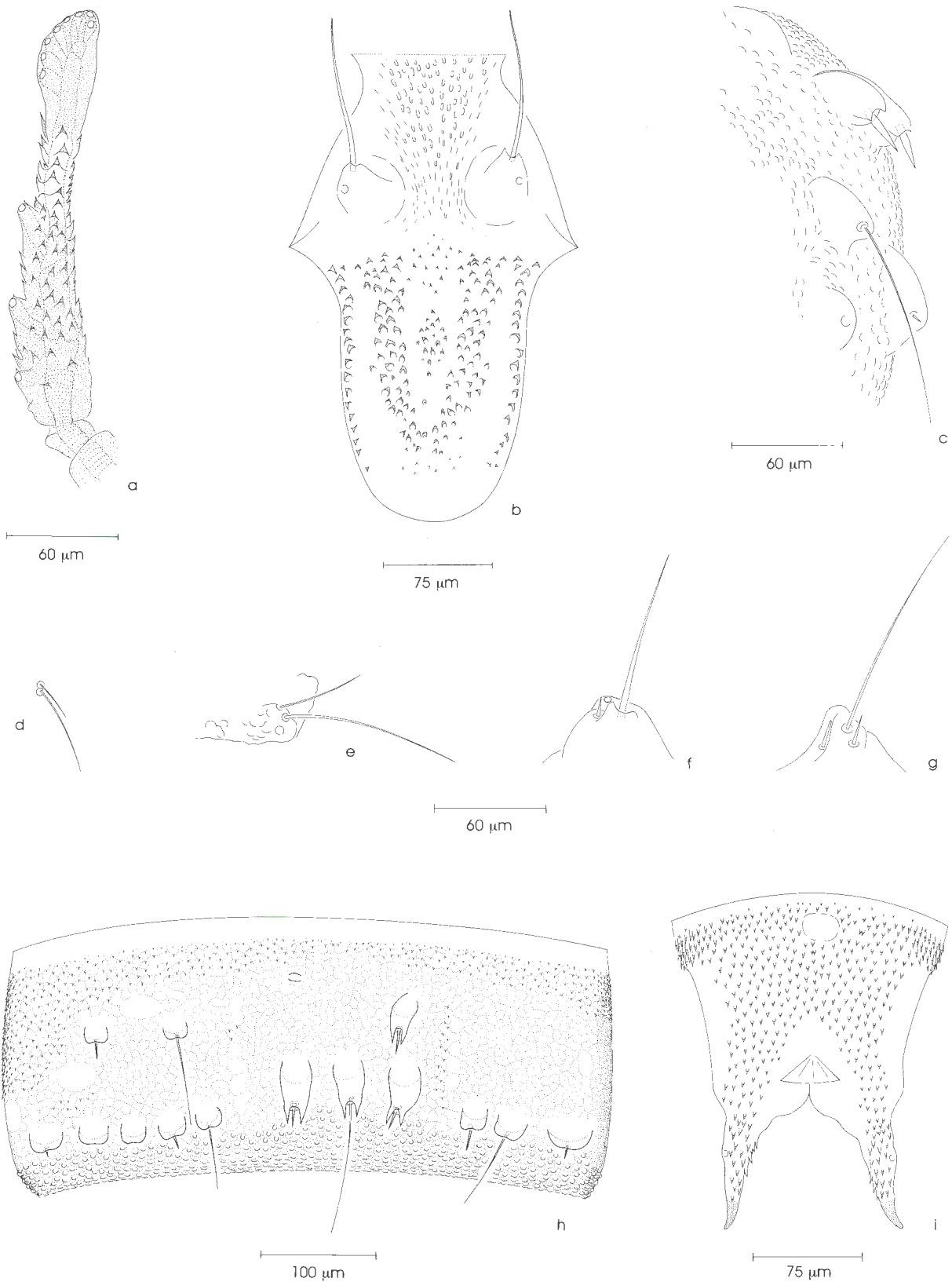


FIG. 7 Pupa of *Culicoides olysageri*: (a) prothoracic respiratory horn; (b) ♂ operculum; (c) thorax; (d) ventromedian setae; (e) ventrolateral setae; (f) anterodorsal tubercle; (g) dorsolateral tubercle; (h) fourth abdominal segment; (i) caudal segment

processes covered with spinules; apical quarter pigmented; tips bluntly rounded.

#### Material examined

TRANSSVAAL. 3 ♀♀, Farm "Sterkstroom" (22°59'S; 30°05'E), northern Transvaal, 1973.xii.27, A.L. Dyce, large open crotches in *Albizia* tree near house.

1 ♂, Farm "Varsvlei", (24°40'S; 27°30'E), Thabazimbi, north-western Transvaal, 1973.xii.10, E.M. Nevill, moist frass from *Cerambycid* borer hole exit in *Aca-cia karroo*.

2 ♂♂, Farm "Mooiplaas", (23°32'S; 29°48'E), Lydenburg, eastern Transvaal, 1973.xi.27, A.L. Dyce, open leafy cavity on stump, free water present, but not obvious, beetle frass.

8 ♀♀, Bekker Agricultural High School (25°59'S; 27°33'E), Magaliesburg, western Transvaal, 1973.xi.13, A.L. Dyce, open rot cavity in tree, adjacent to chicken house.

1 ♂, Onderstepoort Veterinary Institute (25°39'S; 28°09'E), Pretoria, 1973.xii.13, A.L. Dyce, rot pocket in tree adjacent to poultry run.

1 ♀, Onderstepoort Veterinary Institute (25°39'S; 28°09'E), 1973.xii.08, A.L. Dyce, rot hole holding water, in *Acer negundo*.

4 ♀♀, Annlin (25°39'S; 28°09'E), Pretoria, 1988.x.01, A.L. Dyce & H. Nevill, open tree fork with water, in *Celtis africana*.

#### Associated species

In all seven samples *C. olysageri* was the only species present.

#### *Culicoides ravus* De Meillon (Fig. 8a–i; Tables 1–6)

*Culicoides ravus* De Meillon, 1936:151. South Africa

#### Description

Mean total length 1,73 mm (1,65–1,79 mm; n = 10). Light brown; darker brown on thorax, anteriorly and on dorsal half of abdomen.

RESPIRATORY HORN (Fig. 8a; Tables 1, 2 and 6). Basal third of horn brown, distal third darker, pale area medially. Horn short to medium length; basal quarter widest, narrowest distad of basal three-fifths, widens towards apex to bear 4–8 (5) terminal spiracles. Folds basally, which become deeper and annular beyond distal lateral spiracle. Scales absent. On basal three-fifths of horn 2–4 (3) lateral spiracles, the basal spiracle usually not elevated, the other spiracles on small, rather pointed, pigmented prominences. Pedicel short to moderate length, 0,172 length of horn.

OPERCULUM (Fig 8b; Tables 3 and 6). Brown. In ♀♀ and ♂♂, disc longer than wide. A single row of short, stout spines on lateral margins of disc, tips extend beyond margin; rows of spines joined anteriorly by a transverse band of spines; two rows of spines radiate in erratic semi-circles from lateral margins towards midline of disc; oval structures discernible on disc, between and anterior of am tubercles.

HEAD TUBERCLES AND SETAE (Fig. 8b, d–f; Tables 4 and 6). Am tubercle with 0–3 sharp spurs, seta moderate length, sharp, stout; basal sensillum (Fig. 8b). Two thin vm setae subequal length and width (Fig. 8d). Two thin vl setae subequal length, almost equal width; two sensilla present: one on large prominence anterior of setae and one posterior of setae, smaller, not elevated. A few nodules and spinules dorsad of setae (Fig. 8e). Ad tubercle with 1–2 rather sharp spurs; seta i moderate length, stout, sharp and seta ii short, thin, c. one third the length of seta i (Fig. 8f).

THORAX (Fig. 8c and g; Tables 4 and 6). Dorsal tubercles small; d i and d ii closely approximated, both with two sharp spurs, setae short, moderate width, sharp; d iii seta minute; d iv seta longer than d i and d ii, thin; d v sensillum on slight prominence. Pits present between and surrounding tubercles; nodules posteriorly (Fig. 8c). Dl tubercle with three setae: one terminal, moderate length, thin; one lateral, short, thin, sharp and one basal, in fold, short, of equal width throughout (Fig. 8g).

ABDOMEN (Fig. 8h; Tables 4 and 5). All tubercles with apices rounded. Dpm i base broad, seta minute, dpm ii and iii bases more rounded, setae absent, dpm iv seta short, thin; dpm v small, seta longer than iv, thinner. Lpm i–iii more extended, bases oval; lpm i and iii setae short, medium width; lpm ii seta longer than i and iii, thin. Vpm i base rounded, seta short, thin; vpm ii base broader, seta longer, thinner than i; vpm iii base broad, seta minute. Dasm i seta short, thin; dasm ii seta longer, thinner than i. Lasm smaller than lpm i–iii, seta short, medium width. Anterolateral spiracle present. Anterior band of spinules narrowed laterally; nodules posterior to lpm tubercles and on posterior margin of segment, interrupted dorsally and ventrally.

CAUDAL SEGMENT (Fig. 8i; Tables 5 and 6). Anterior band of spinules interrupted dorsally by a round, bare area; dorsomedian patch of 10–32 spinules. Posterolateral processes of moderate length; only inner aspects of processes with spinules; apical quarter to third pigmented; tips bluntly rounded.

#### Material examined

TRANSSVAAL. 1 ♀ 1 ♂, Sterkrivier (23°55'S; 28°24'E), 30 km south-west of Potgietersrust, northern Trans-

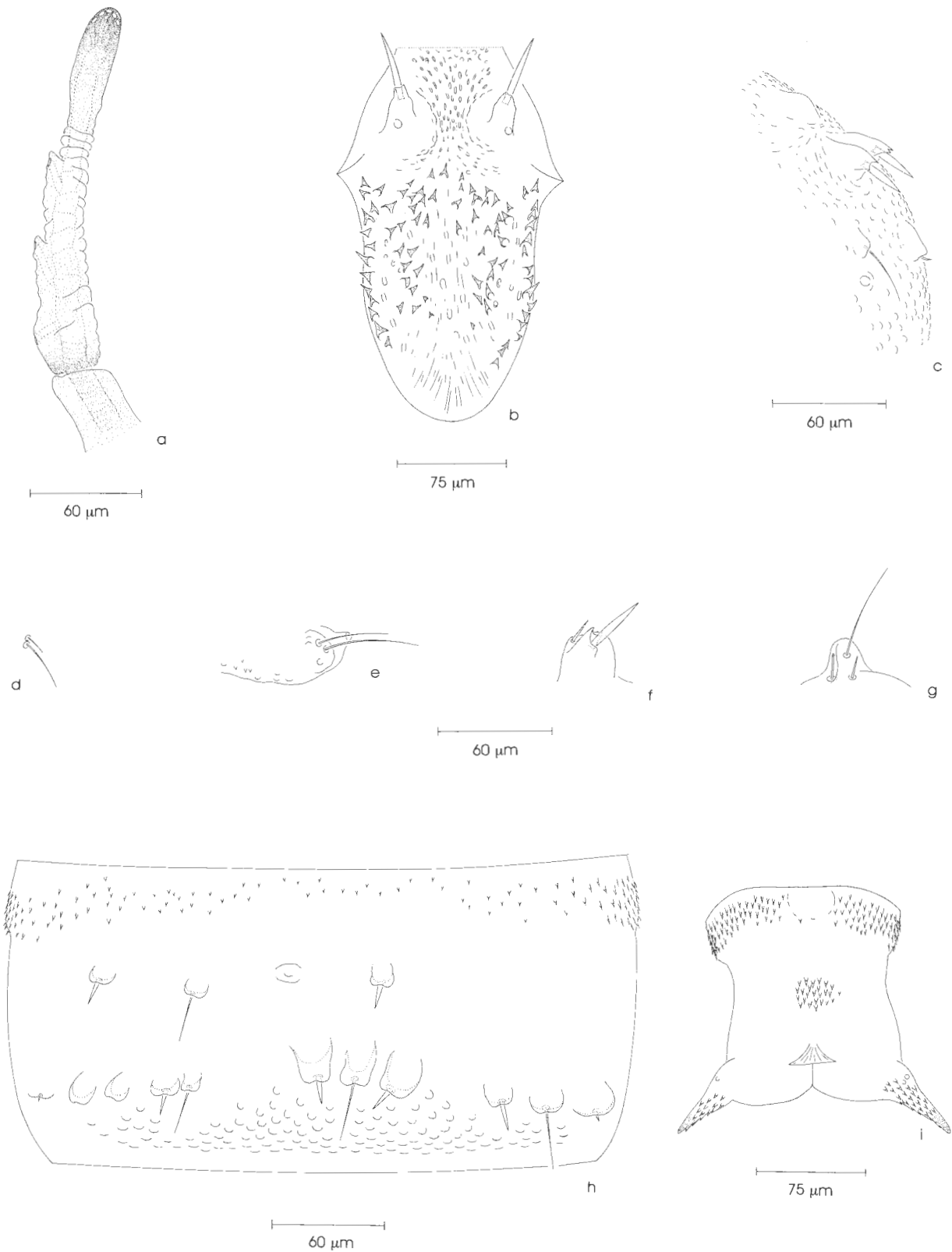


FIG. 8 Pupa of *Culicoides ravus*: (a) prothoracic respiratory horn; (b) ♂ operculum; (c) thorax; (d) ventromedian setae; (e) ventrolateral setae; (f) anterodorsal tubercle; (g) dorsolateral tubercle; (h) fourth abdominal segment; (i) caudal segment

vaal, 1974.i.03, A.L. Dyce, ground-water body—seepage from grassed irrigation drain next to road, water milky, soil red, gritty, gravelly loam, marginal vegetation open to sunlight.

10 ♀♀ 7 ♂♂, Farm "Zoutpan" (25°24'S; 28°05'E), north-west of Pretoria, 1974.i.10, A.L. Dyce, vegetation-covered, sandy storm-water gully. Scattered silty depressions with recently rain-filled hoof marks, grey silt.

2 ♀♀ 3 ♂♂, Farm "Zoutpan" (25°24'S; 28°05'E), north-west of Pretoria, 1974.i.10, A.L. Dyce, margins of vegetated, brackish spring on outer perimeter of crater salt lake.

1 ♂, Onderstepoort Veterinary Institute (25°39'S; 28°09'E), Pretoria, 1973.xii.16, A.L. Dyce, moist soil from bottoms of drying pool holes in bogged drain overflow.

1 ♀ 2 ♂♂, Dendron (23°23'S; 29°20'E), northern Transvaal, 1974.i.01, A.L. Dyce, margins of polluted, trampled ground water near drinking troughs, muddy.

1 ♂, Jam Tin Creek (25°31'S; 31°35'E), Nelspruit-Komatipoort road, eastern Transvaal, 1973.xii.02, A.L. Dyce, margin of clear, running stream.

CAPE PROVINCE. 1 ♀, 300 m from "The Eye", Kuruman (27°28'S; 23° 26'E), northern Cape, 1974.i.21, A.L. Dyce, margin of running stream, algal growth, sandy silt.

#### *Associated species*

*C. ravus* was collected in association with *C. leucostictus* (4x), *C. pycnostictus* (4x), *C. similis* (4x), *C. nivosus* (3x), *C. sp. #110* (Meiswinkel) (3x), *C. tropicalis* (2x), *C. cornutus* (1x), *C. bedfordi* (1x), *C. exspectator* (1x) and *C. micheli* (1x).

#### ***Culicoides similis* Carter, Ingram & Macfie**

(Fig. 1 and 9a–i; Tables 1–6)

*Culicoides similis* Carter, Ingram & Macfie, 1920: 255. Ghana

*Culicoides similis* Carter, Ingram & Macfie; Howarth 1985:85. Laos (pupa)

#### *Description*

Mean total length 1.61 mm (1.49–1.80 mm; n = 7). Light brown; darker brown on thorax, anteriorly and on dorsal half of abdomen.

RESPIRATORY HORN (Fig. 1 and 9a; Tables 1, 2 and 6). Basal third to half of horn light brown, distal third darker, palest area medially. Horn short; widest basally, narrowest medially, widens towards apex to bear 4–8 (7) terminal spiracles. A few slight folds basally becoming closer, more numerous, deeper

and almost annular medially, distad of lateral spiracles. A few sharp scales evolve from most distad folds. On basal half of horn 2–4 (3) lateral spiracles on rather large, pigmented prominences; basal spiracle usually not elevated. Pedicel short to moderate length, 0.213 length of horn.

OPERCULUM (Fig. 1 and 9b; Tables 3 and 6). Light brown. In ♀♀ and ♂♂, disc longer than wide. A single row of short, rather stout, sharp, dark brown spines on lateral margins of disc, tips not extending beyond margin; rows joined anteriorly by a thin, transverse band of spines and spinules; rows of a few spines and oval structures radiate in erratic semi-circles from lateral margins towards midline of disc; oval structures occasionally discernible between and anteriorly of am tubercles.

HEAD TUBERCLES AND SETAE (Fig. 1, 9b, d–f; Tables 4 and 6). Am tubercle with 2–3 sharp spurs, seta moderate length, stout, sharp; basal sensillum (Fig. 1, 9b). Two thin vm setae of subequal length and width (Fig. 9d). Two thin vl setae on slight prominence, of subequal length, almost equal width; a sensillum present posterior to vl setae (Fig. 9e). Ad tubercle with 1–3 sharp spurs; seta i moderate length, stout, sharp and seta ii short, moderately thin, sharp, c. one half to one third the length of seta i (Fig. 9f).

THORAX (Fig. 1, 9c and g; Tables 4 and 6). Dorsal tubercles small; d i and d ii closely approximated, both with two sharp spurs, setae short, stout, sharp; d iii seta minute; d iv seta medium length, thin; d v sensillum on very slight prominence. Pits surrounding and between tubercles; nodules present posteriorly (Fig. 1, 9c). D1 tubercle with three setae: one terminal, moderate length, thin; one lateral, short, thin, sharp and one basal short, in fold, difficult to discern (Fig. 9g).

ABDOMEN (Fig. 1 and 9h; Tables 4 and 6). Dpm i base broad, seta minute; dpm ii and iii base more rounded than i, apices rounded, seta absent; dpm iv same as iii, seta short, moderate width, sharp; dpm v smaller than iv, sometimes with sharp apicolateral spurs, seta longer than iv, thin. Lpm i–iii bases slightly elongated, each with two sharp apicolateral spines; lpm i and iii seta longer, stouter than dpm iv, sharp; lpm ii seta longer than lpm i and iii, thin. Vpm i and ii same as dpm iv and v, respectively, vpm i seta longer than dpm iv; vpm ii larger than vpm i, seta longer, thinner; vpm iii base broader, seta minute. Dasm i and ii same as dpm iv and v respectively, dasm ii sometimes with small, sharp apicolateral spines. Lasm same as lpm i and iii, tubercle smaller, seta slightly shorter. Anterolateral spiracle present. Anterior band of spinules not interrupted, sometimes reduced laterally; a few nodules antero-

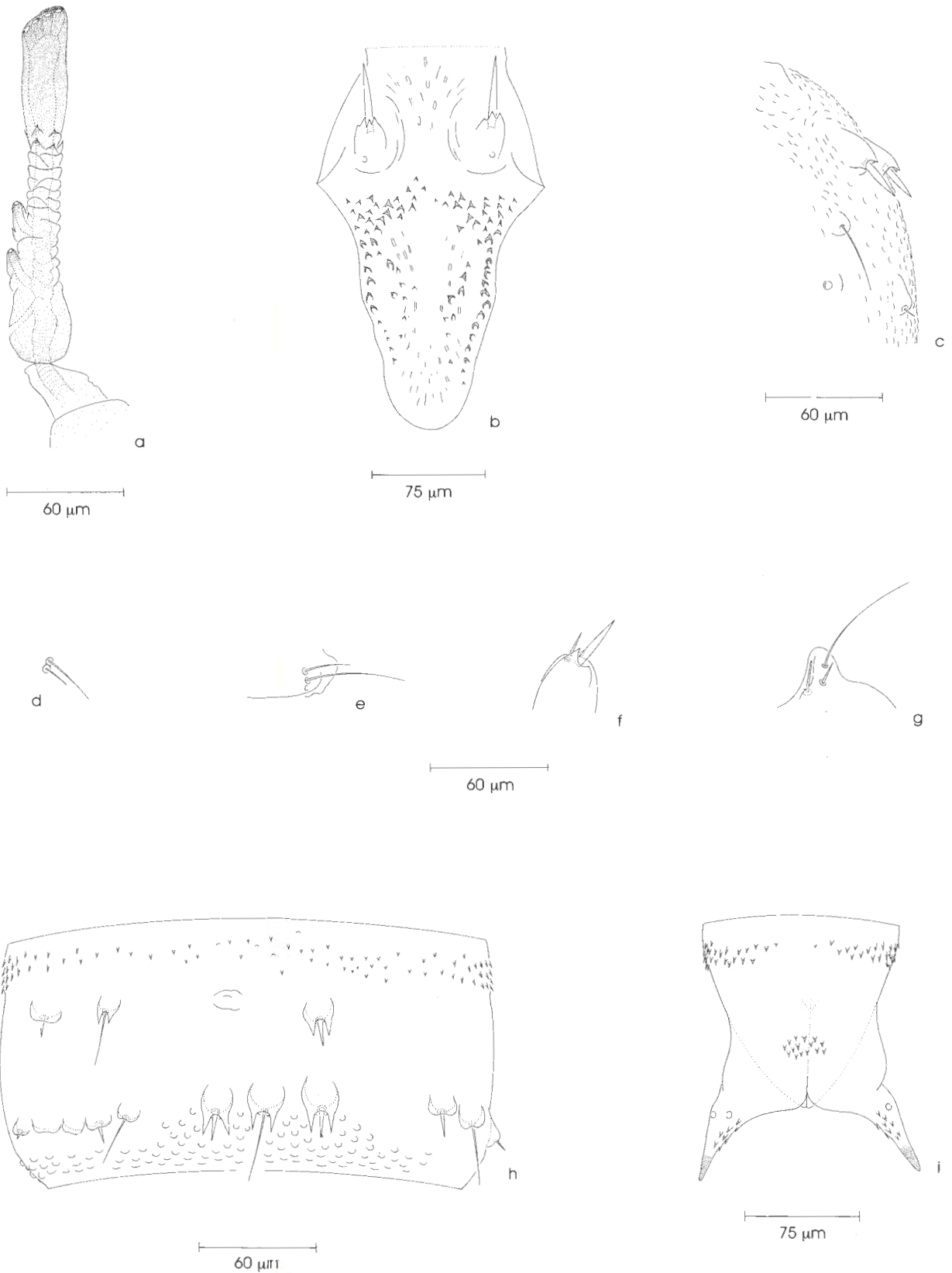


FIG. 9 Pupa of *Culicoides similis*: (a) pröthoracic respiratory horn; (b) ♂ operculum; (c) thorax; (d) ventromedian setae; (e) ventrolateral setae; (f) anterodorsal tubercle; (g) dorsolateral tubercle; (h) fourth abdominal segment; (i) caudal segment

laterally, nodules posteriad of lpm tubercles and on margin of segment, interrupted ventrally.

CAUDAL SEGMENT (Fig. 1 and 9j; Tables 5 and 6). Anterior band of spinules interrupted dorsally; dorso-medial patch of nine to 20 spinules. Posterolateral processes moderate length; only inner aspects of processes with spinules; apical quarter pigmented; tips bluntly rounded.

#### Material examined

TRANSVAAL. 1 ♀, Jam Tin Creek (25°31'S; 31°35'E), Nelspruit-Komatipoort road, eastern Transvaal, 1973.xii.02, A.L. Dyce, margin of clear, running stream.

3 ♀♀, Sterkrivier (23°55'S; 28°24'E), 30 km southwest of Potgietersrust, northern Transvaal, 1974.i.03, A.L. Dyce, ground-water body—seepage from grassed irrigation drain next to road, water milky, soil red, gritty, gravelly loam, marginal vegetation open to sunlight.

1 ♀ 1 ♂, Farm "Zoutpan" (25°24'S; 28°05'E), north-west of Pretoria, 1974.i.10, A.L. Dyce, vegetation-covered, sandy storm-water gully—scattered silty depressions with recently rain-filled hoof marks, grey silt.

NATAL. 1 ♂, Umlalazi Coastal Nature Reserve (28°58'S; 31°46'E), Mtunzini, Zululand, 1988.vii.21, H. & E.M. Nevill, margins of fast-flowing clear stream, full sunlight, soil sandy, light.

3 ♀♀ 1 ♂, Umlalazi Coastal Nature Reserve (28°58'S; 31°46'E), Mtunzini, Zululand, 1990.ix.19, H. & E.M. Nevill, margins of clear-water bodies in river-bed, semi-shaded by reeds and sedges, soil fine, dark.

CAPE PROVINCE. 3 ♂♂, 300 m from "The Eye", Kuruman (27°28'S; 23°26'E), northern Cape, 1974.i.21, A.L. Dyce, margin of running stream, algal growth, sandy silt.

#### Associated species

*C. similis* was collected in association with *C. ravus* (3x), *C. leucostictus* (2x), *C. expectator* (2x), *C. tropicalis* (2x), *C. bedfordi* (1x), *C. micheli* (1x), *C. nivosus* (1x), *C. pycnostictus* (2x), *C. sp. #110* (Meiswinkel) (1x) and *C. sp. #69* (Meiswinkel) (1x).

#### *Culicoides (Synhelea) tropicalis* Kieffer (Fig. 10a–i; Tables 1–6)

*Culicoides tropicalis* Kieffer, 1913:10. Kenya

*Culicoides (Synhelea) tropicalis* Kieffer; Meiswinkel & Dyce 1989:147. South Africa

#### Description

Mean total length 1.59 (1.51–1.66 mm; n = 3). Light brown; darker brown on thorax, anteriorly and on dorsal half of abdomen.

RESPIRATORY HORN (Fig. 10a; Tables 1, 2 and 6). Basal third of horn paler brown than distal third, medial area palest. Horn short; widest basally, narrowest distad of basal half, widens to bear 5–7 (6) terminal spiracles. Annular folds cover median two-thirds of horn, becoming deeper and more ring-like distally. Scales absent. Three lateral spiracles usually on basal half of horn, the basal spiracle slightly elevated, the other spiracles on rather large prominences. Pedicel short, 0.176 length of horn.

OPERCULUM (Fig. 10b; Tables 3 and 6). Rather dark brown. In ♀♀ and ♂♂ disc longer than wide. A single row of short, rather stout, sharp, dark brown spines on each lateral margin of disc, tips do not extend beyond margin; sometimes a small group of spines posterolaterad of am tubercles, not joined transversely. Oval structures radiate from lateral margins in semi-circles towards midline of disc; oval structures between am tubercles extend towards anterior margin.

HEAD TUBERCLES AND SETAE (Fig. 10b, d–f; Tables 4 and 6). Am tubercle with very blunt to sharp spur; seta moderate length, stout, sharp; basal sensillum (Fig. 10b). Two thin vm setae of subequal length and width (Fig. 10d). Two thin vl setae on slight prominence of subequal length and width; a sensillum present (Fig. 10e). Ad tubercle with 1–2 sharp spurs; seta i moderate length, stout, sharp and seta ii short, thin, sharp, c. one-third the length of seta i (Fig. 10f).

THORAX (Fig. 10c and g; Tables 4 and 6). Dorsal tubercles small; d i and d ii closely approximated, sometimes with two sharp spurs, short and moderate width, sharp setae; d iii seta minute; d iv seta moderate length, thin; d v sensillum on very slight prominence. Pits surrounding and between tubercles; a few nodules posteriorly (Fig. 10c). Dl tubercle with three setae; one terminal, moderate length, thin; one lateral, short, sharp, thin and one basal, short in fold, difficult to discern (Fig. 10g).

ABDOMEN (Fig. 10h; Tables 4 and 6). Dpm i base broad, seta minute; dpm ii base more rounded, seta absent; dpm iii smaller, seta absent; dpm iv larger than iii, apices rounded, seta short, thin, sharp; dpm v smaller than iv, seta longer, thinner. Lpm i–iii extended, bases oval, apicolateral spines long, sharp; lpm setae i and iii sharp, slightly longer, stouter than dpm iv; lpm ii seta longer than lpm i and lpm iii, thinner. Vpm i same as dpm iv, sometimes with small sharp spines; vpm ii larger than i, base broader, apices rounded, seta longer than i, thinner; vpm iii base broader, apices rounded, seta minute. Dasm i base rounded, seta short, thin, sharp; dasm ii same as dpm v. Lasm same as lpm i and iii, seta shorter, thinner. Anterolateral spiracle present.



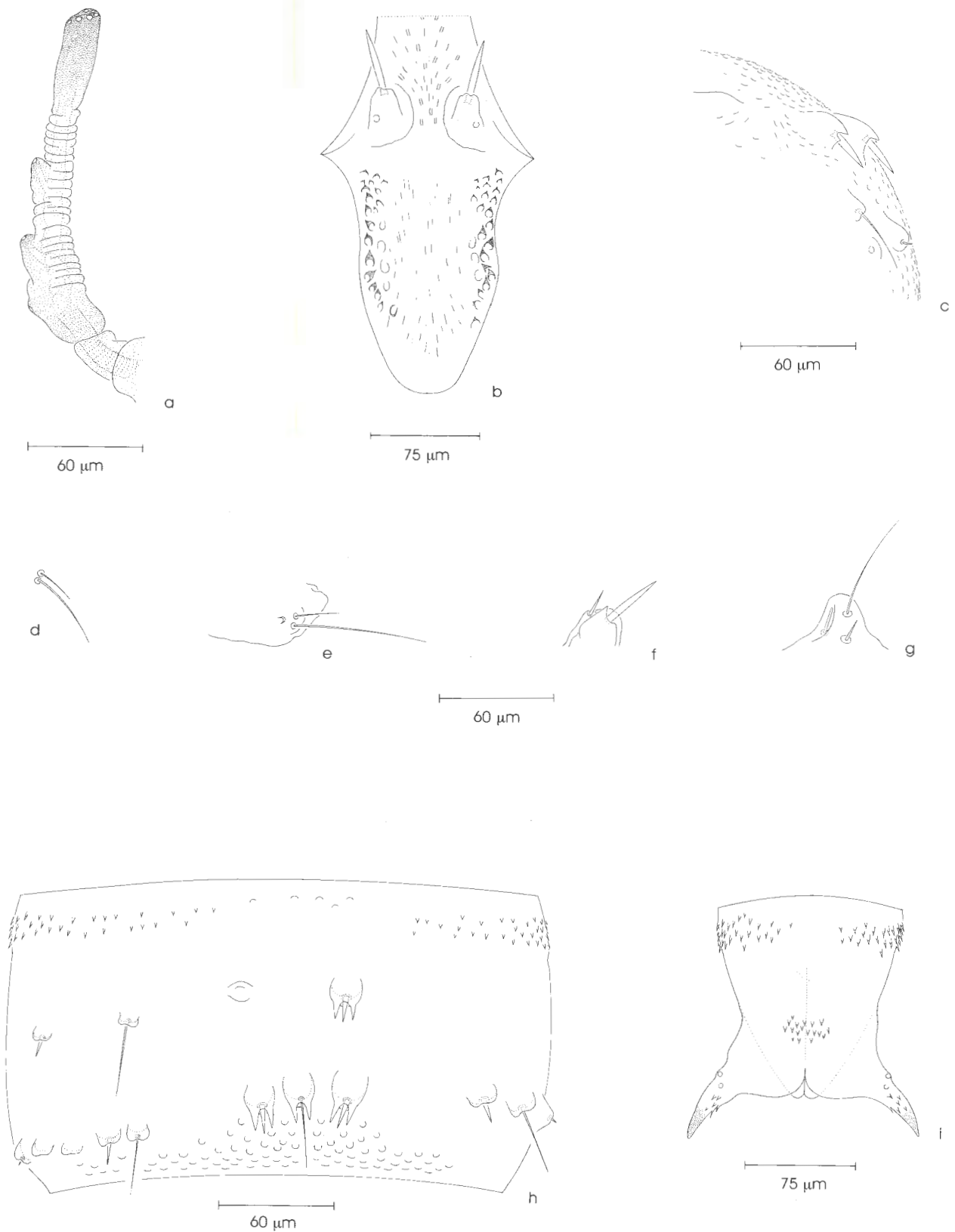


FIG. 10 Pupa of *Culicoides tropicalis*: (a) prothoracic respiratory horn; (b) ♂ operculum; (c) thorax; (d) ventromedian setae; (e) ventrolateral setae; (f) anterodorsal tubercle; (g) dorsolateral tubercle; (h) fourth abdominal segment; (i) caudal segment

Anterior band of spinules interrupted laterally; a few nodules anterolaterally, nodules posteriad of 1pm tubercles and on posterior margin of segment, interrupted dorsally and ventrally.

CAUDAL SEGMENT (Fig. 10i; Tables 5 and 6). Anterior band of spinules interrupted dorsally; dorsomedian patch of 13–26 spinules. Posterolateral processes of moderate length; inner aspects of processes with spinules; apical quarter to third pigmented; tips bluntly rounded.

#### Material examined

TRANSVAAL. 3 ♀♀ 1 ♂, east of Skukuza, Kruger National Park (24°59'S; 31°32'E) 1991.iii.09, A.L. Dyce, R. Meiswinkel & M. Swanepoel, moist seepage areas away from margins of pool, no running water visible, green algal bloom, full sunlight, no vegetation, sandy.

1 ♀ 1 ♂, Farm "Zoutpan" (25°24'S; 28°05'E), north-west of Pretoria, 1974.i.10, A.L. Dyce, vegetation-covered, sandy storm-water gully. Scattered silty depressions with recently rain-filled hoof marks, grey silt.

CAPE PROVINCE. 1 ♀, 300 m from "The Eye", Kuruman (27°28'S; 23° 26'E), northern Cape, 1974.i.21, A.L. Dyce, margin of running stream, algal growth, sandy silt.

#### Additional material examined

TRANSVAAL. 1 ♀ 2 ♂♂, Pafuri, Kruger National Park (24°59'S; 31°32'E), 1993.ii.17, A.L. Dyce & L.E.O. Braack, inlet to side creek of the Levuvhu River, red silt over sand.

#### Associated species

*C. tropicalis* was collected in association with *C. leucostictus* (2x), *C. pycnostictus* (2x), *C. similis* (2x), *C. sp. #110* (Meiswinkel) (3x), *C. nivosus* (1x), *C. kobae* (1x), *C. ravus* (1x) and *C. sp. #9* (Meiswinkel) (1x).

## DISCUSSION

### Differential diagnosis

A summary of the character states differentiating the seven species belonging to three groups of the *Similis* supergroup, is given in Table 6 and discussed below. Differences and similarities between the seven species and *C. accraensis* are also noted.

*C. olysageri* (Fig. 7), together with *C. accraensis* (Carter *et al.* 1920: Fig. 9a and b), stands distinctly apart from the six ground-water species:

- Abundant scales on the respiratory horn (Fig. 7a).

TABLE 1 Respiratory horn and pedicel lengths (µm) and P/H ratios of the pupae of seven *Culicoides* species

Species	Horn			Pedicel			P/H		
	Range	Mean	n	Range	Mean	n	Range	Mean	n
<i>C. exspectator</i>	145,0–160,0	151,0	12	25,0–30,0	27,8	10	0,17–0,20	0,19	8
<i>C. kobae</i>	161,0–180,5	170,2	14	25,5–32,5	28,1	14	0,15–0,20	0,17	14
<i>C. micheli</i>	125,0–152,0	137,2	17	20,0–30,0	25,3	10	0,16–0,22	0,19	10
<i>C. olysageri</i>	161,5–222,5	197,4	26	16,0–26,0	22,7	18	0,10–0,14	0,12	15
<i>C. ravus</i>	153,7–220,0	183,9	30	25,0–40,0	30,4	20	0,14–0,22	0,17	17
<i>C. similis</i>	127,5–192,5	163,9	23	25,0–40,0	32,6	12	0,20–0,23	0,21	9
<i>C. tropicalis</i>	150,0–195,0	172,8	13	25,0–37,5	30,8	7	0,15–0,20	0,18	7

TABLE 2 The number and highest frequency of lateral and terminal spiracles on the respiratory horns of the pupae of seven *Culicoides* species

Species	Lateral spiracles			Terminal spiracles		
	Range	Highest freq.	n	Range	Highest freq.	n
<i>C. exspectator</i>	4–5	4	11	4	4	10
<i>C. kobae</i>	2	2	14	5–7	6	14
<i>C. micheli</i>	1	1	13	5–7	6	10
<i>C. olysageri</i>	2–4	3	29	6–9	8	26
<i>C. ravus</i>	2–4	3	24	4–8	5	17
<i>C. similis</i>	2–4	3	23	4–8	7	14
<i>C. tropicalis</i>	3	3	12	5–7	6	12

TABLE 3 Measurements ( $\mu\text{m}$ ) of the opercula discs ( $\text{♀}$  and  $\text{♂}$ ) of the pupae of seven *Culicoides* species

Species and gender	n	Length (OL)		Width (OW)		Ratio (OW/OL)	
		Range	Mean	Range	Mean	Range	Mean
<i>C. exspectator</i> ♀	6	134,5–148,5	142,3	125,0–142,5	132,2	0,88–0,96	0,93
	♂	1	150,0	127,5	127,5	0,85	0,85
<i>C. kobae</i> ♀	6	138,0–145,5	141,8	109,5–120,0	116,5	0,78–0,87	0,82
	♂	1	154,5	115,5	115,5	0,75	0,75
<i>C. micheli</i> ♀	1	150,0	150,0	135,5	135,5	0,90	0,90
	♂	4	137,5–154,0	143,1	115,0–122,5	119,9	0,80–0,86
<i>C. olysageri</i> ♀	13	151,0–187,0	168,2	165,5–201,0	182,3	0,99–1,15	1,08
	♂	3	153,5–163,5	157,3	161,0–172,5	166,5	1,04–1,08
<i>C. ravus</i> ♀	8	140,0–166,5	156,6	135,0–165,0	143,1	0,87–0,97	0,92
	♂	9	150,0–186,0	163,3	132,5–152,0	141,0	0,81–0,92
<i>C. similis</i> ♀	7	135,0–155,0	145,1	120,0–140,5	130,6	0,81–0,96	0,90
	♂	5	140,0–161,0	150,9	120,0–149,0	128,9	0,78–0,93
<i>C. tropicalis</i> ♀	4	140,0–150,0	146,8	130,0–140,0	137,0	0,92–0,95	0,93
	♂	2	148,0–150,0	149,0	117,5–127,5	122,5	0,78–0,86

TABLE 4 Lengths ( $\mu\text{m}$ ) of some cephalothoracic and abdominal setae of the pupae of seven *Culicoides* species

Species		<i>C. exspectator</i>	<i>C. kobae</i>	<i>C. micheli</i>	<i>C. olysageri</i>	<i>C. ravus</i>	<i>C. similis</i>	<i>C. tropicalis</i>
Anteromarginal seta	Range	40,0–60,0	34,5–41,0	50,0–57,5	85,5–109,5	30,0–57,5	32,5–49,0	37,0–48,0
	Mean (n)	<b>50,8</b> (12)	<b>37,5</b> (10)	<b>53,0</b> (10)	<b>98,4</b> (13)	<b>41,4</b> (27)	<b>43,4</b> (16)	<b>42,2</b> (11)
Anterodorsal seta i	Range	37,5	30,0–40,0	20,0–22,5	80,0–105,0	23,7–43,8	27,0–31,5	30,0–35,0
	Mean (n)	<b>37,5</b> (2)	<b>33,4</b> (10)	<b>21,3</b> (7)	<b>89,1</b> (13)	<b>30,3</b> (14)	<b>28,6</b> (7)	<b>33,3</b> (4)
Dorsal seta iv	Range	41,0–47,5	32,5–56,0	30,0–40,0	70,5–100,0	27,0–35,0	30,0–40,0	35,0–40,0
	Mean (n)	<b>44,5</b> (6)	<b>46,6</b> (13)	<b>34,2</b> (3)	<b>89,0</b> (11)	<b>31,5</b> (10)	<b>33,4</b> (6)	<b>38,0</b> (3)
Dpm v	Range	20,0–27,5	22,5–35,0	11,0–15,0	32,5–49,0	20,0–25,0	16,0–25,5	30,0–30,5
	Mean (n)	<b>25,0</b> (4)	<b>27,7</b> (7)	<b>13,7</b> (3)	<b>42,4</b> (9)	<b>23,0</b> (6)	<b>21,8</b> (6)	<b>30,4</b> (4)
Dasm ii	Range	25,0–30,0	25,0–35,0	25,0–30,0	45,0–75,5	21,0–30,0	21,5–30,0	30,0–37,5
	Mean (n)	<b>26,3</b> (6)	<b>28,6</b> (7)	<b>27,5</b> (2)	<b>61,0</b> (13)	<b>24,6</b> (10)	<b>27,1</b> (10)	<b>33,0</b> (5)
Lpm ii	Range	30,0–32,5	21,0–33,0	20,0–30,0	62,5–105,5	20,0–32,5	25,0–35,0	36,0–46,0
	Mean (n)	<b>30,5</b> (6)	<b>28,3</b> (7)	<b>25,0</b> (4)	<b>77,7</b> (7)	<b>25,8</b> (9)	<b>30,2</b> (10)	<b>40,8</b> (4)
Vpm ii	Range	25,0–27,5	23,0–31,0	20,0–25,0	40,0–85,5	25,0–32,5	30,0–35,0	30,0–39,0
	Mean (n)	<b>25,8</b> (6)	<b>26,5</b> (7)	<b>22,5</b> (2)	<b>57,7</b> (15)	<b>27,9</b> (6)	<b>33,5</b> (6)	<b>34,3</b> (6)
Vpm iii	Range	6,0	10,0–15,0	5,0–5,5	6,0–11,0	4,9–5,1	6,0–10,0	3,8–6,0
	Mean (n)	<b>6,0</b> (4)	<b>11,4</b> (7)	<b>5,3</b> (2)	<b>8,1</b> (15)	<b>5,0</b> (5)	<b>7,0</b> (12)	<b>4,7</b> (7)

TABLE 5 Lengths ( $\mu\text{m}$ ) of posterolateral processes and number of dorsal spinules on the caudal segment of the pupae of seven *Culicoides* species

Species	Posterolateral processes (length)			Number of dorsal spinules		
	Range	Mean	n	Range	Mean	n
<i>C. exspectator</i>	50,0–65,0	52,8	9	17–30	23,6	10
<i>C. kobae</i>	31,5–45,5	40,6	14	17–28	22,7	7
<i>C. micheli</i>	42,5–55,0	49,1	7	9–20	13,4	5
<i>C. olysageri</i>	60,0–84,5	70,7	27	Many	Many	14
<i>C. ravus</i>	35,0–70,0	54,1	30	10–32	18,5	18
<i>C. similis</i>	45,0–67,5	54,9	14	9–20	11,6	14
<i>C. tropicalis</i>	45,0–65,0	54,6	12	13–26	20,1	7

TABLE 6 Summary of character states which differentiate the pupae of seven *Culicoides* species belonging to the Similis, Accraensis and Tropicalis (S, A and T) groups of the Similis supergroup; major differences indicated by bold type (Fig. 4–10; Tables 2–5)

Character states	<i>C. expectator</i> (S)	<i>C. kobae</i> (S)	<i>C. micheli</i> (S)	<i>C. oylslageri</i> (A)	<i>C. ravus</i> (S)	<i>C. similis</i> (S)	<i>C. tropicalis</i> (T)
<i>Respiratory horn</i>							
Pigmentation	Basally and distally uniformly pigmented, distinctly pale medially	Uniformly pigmented, darker distally	Uniformly pale throughout, apex darker	Basally, distally, uniformly pigmented, palest medially	Paler base, darkest distally, palest medially	Paler base, darkest distally, slightly paler medially	Paler base, darkest distally, slightly paler medially
Annulations	Restricted	<b>Extensive</b>	Restricted	Restricted	Restricted	Restricted, fold-like	<b>Extensive</b>
Scales	Absent	Absent	Absent	<b>Abundant</b>	Absent	<b>A few distad of folds</b>	Absent
Apical fan-like process	Absent	Absent	<b>Present</b>	Absent	Absent	Absent	Absent
Terminal spiracles	4	5–7	5–7	6–9	4–8	4–8	5–7
Lateral spiracles	4–5	2	1	2–4	2–4	2–4	3
Lateral spiracular prominences (excluding basal spiracle)	1–2 very small proximally, large distally	Rather large	Very small	Rather large throughout	Small throughout	Rather large throughout	Rather large throughout
<i>Operculum</i>							
Width/length ratio	Longer than wide	Longer than wide	Longer than wide	<b>Wider than long</b>	Longer than wide	Longer than wide	Longer than wide
Spines on disc	Transverse band present anteriorly; small clutch medially	<b>Transverse band absent; large round structures on lateral margins only, no spines medially</b>	Transverse band present anteriorly; a few rows medially	Transverse band present anteriorly; <b>distinct V-shaped pattern medially</b>	Transverse band present anteriorly; a few spines medially	Transverse band present anteriorly; a few spines medially	<b>Transverse band absent; large spines on lateral margins only, no spines medially</b>
<i>Cephalothorax</i>							
Combined range of am, ad i & d iv setal lengths (µm)	37,5–60,0	30,0–56,0	20,0–57,5	<b>70,5–109,5</b>	23,7–57,5	27,0–49,0	30,0–48,0
<i>Abdomen</i>							
Apicolateral spines	Present on lpm, i–iii, lasm, sometimes dpm iii–v	Present on lpm i–iii, lasm, vpm i–iii	Present on all tubercles except vpm iii, dpm i	Present on lpm i–iii, lasm	<b>Absent</b>	Present on lpm i–iii, lasm, sometimes dpm v, dasm ii	Present on lpm i–iii, lasm, vpm i
Combined range of dpm v, dasm ii, lpm ii & vpm ii setal lengths (µm)	20,0–32,5	21,0–35,0	11,0–30,0	<b>32,5–105,5</b>	20,0–32,5	16,0–35,0	30,0–46,0
Mosaic pattern on integument	Absent	Absent	Absent	<b>Present</b>	Absent	Absent	Absent
Presence of spinules on abdominal integument	Anterior band, laterally reduced	Anterior band interrupted laterally	Anterior band, not interrupted laterally; <b>posteriad of lpm tubercles</b>	Anterior band narrowed laterally, <b>extended between dasms dorsally</b>	Anterior band, narrowed laterally	Anterior band, not interrupted	Anterior band, interrupted laterally
<i>Caudal segment</i>							
Mean no. of dorsal spinules	23,6	22,7	13,4	<b>&gt; 50% of segment covered dorsally</b>	18,5	11,6	20,1
Mean lengths of posterolateral processes	52,8	40,6	49,1	<b>70,7</b>	54,1	54,9	54,6

- a well-defined V-shaped pattern of spines on operculum of *C. olysageri* (Fig. 7b)
- Operculum wider than long (OW/OL > 1) in *C. olysageri* (Fig. 7b; Table 3)
- Very long am, d iv and ad i setae (Fig. 7b, c and f; Table 4); *C. accraensis* appears to have two d iv setae
- Very long dpm v, lpm ii, vpm ii and dasm ii setae (Fig. 7h; Table 4)
- The integument of the abdomen of *C. olysageri* is "mosaic"-patterned (Fig. 7h)
- Spinules present dorsally between dasm i tubercles of *C. olysageri* (Fig. 7h); abdominal integument of *C. accraensis* appears to be completely covered in spinules
- Spinules cover more than 50% of caudal segment dorsally in *C. olysageri* (Fig. 7i; Table 5) and apparently completely in *C. accraensis*
- Long posterolateral processes (Fig. 7i; Table 5)

Each of the six species inhabiting ground waters, can be differentiated from one another by one or more distinct character states. *C. ravus* is easily distinguished by the complete absence of apicolateral spines from the abdominal tubercles (Fig. 8h), a feature apparently shared with *C. accraensis*; *C. micheli* is distinguished by the presence of apicolateral spines on all abdominal tubercles except dpm i and vpm iii, spinules posteriad of lpm tubercles (Fig. 6h), a unique process protruding from the apex of the horn and the presence of only one lateral spiracle (Fig. 6a). *Culicoides exspectator*, *C. kobae*, *C. similis* and *C. tropicalis* can be separated by features of the horn, operculum and abdomen; the other morphological differences between these latter four species are slight. The main differences between these four species are:

#### *C. exspectator* (Fig. 4a)

- Comma-shaped horn unicolorous proximally and distally, with a prominently pale medial area
- Lateral spiracular prominences on horn become progressively larger towards apex
- Only four terminal spiracles observed in each of ten horns examined

#### *C. similis* (Fig. 9a)

- A few scales distad to the medial fold-like annulations on horn immediately distinguishes this species

#### *C. tropicalis* (Fig. 10a and b)

- Extensive annulations on horn, three lateral spiracles in all 18 specimens examined

- Anterior transverse band of opercular spines absent from disc, large spines present on lateral margins only

#### *C. kobae* (Fig. 5a, b and h)

- Extensive annulations on horn, two lateral spiracles in all 14 specimens examined
- Anterior transverse band of opercular spines absent from disc; large round, structures, occasionally with a very small spine, present on lateral margins
- Apicolateral spines present on all lateral and ventral tubercles on abdominal segment in all seven specimens examined

The only two features shared by all seven species, including *C. accraensis*, are annulations or fold-like rings on the horn, resulting in the horn being narrowed medially (Fig. 4a–10a), and the close approximation of d i and d ii tubercles, each with a short seta ranging in width from moderate to stout.

No differences could be seen between the *C. similis* pupae from Africa and those from Laos (Howarth 1985).

## CONCLUSION

*Culicoides olysageri*—belonging to the *Accraensis* group of the *Similis* supergroup—has certain morphological character states which strongly resemble not only *C. accraensis* and other African *Culicoides* species which utilize tree holes for their larval habitat (H. Nevill, unpublished data 1992), but also resemble the tree-hole-frequenting *Culicoides* species from Australia (Kettle & Elson 1975; Kettle, Elson & Dyce 1976; Kettle & Elson 1980) and North America (Linley & Kettle 1964; Linley 1965; Jamnback 1965; Linley 1970; Lamberson, Pappas & Pappas 1992). Many of these species are not even distantly related taxonomically, but their common larval habitat appears to induce convergence or homogeneity in features.

On the other hand, the six ground-water species can easily be distinguished from *C. olysageri*, as well as from one another. However, no clear-cut differences were found to warrant separation of *C. tropicalis* of the subgenus *Synhelea*, from the remaining five species belonging to the *Similis* group. On the contrary, *C. kobae* shares more character states with *C. tropicalis* than with either *C. exspectator*, *C. micheli*, *C. ravus* or *C. similis*. This suggests that pupal morphology is of limited help towards the systematic subdivision of the *Similis* supergroup, but can be used to distinguish those species in the supergroup which utilize tree holes from those inhabiting ground-water margins. However, more species need to be studied before definite conclusions can be drawn regarding the value of *Culicoides* pupal morphology as an indicator of group or subgeneric relationships.

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