

Comparative descriptions of the pupae of five species of the *Culicoides imicola* complex (Diptera, Ceratopogonidae) from South Africa

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ABSTRACT

NEVILL, HILDA, VENTER, G.J., MEISWINKEL, R. & NEVILL, E.M. 2007. Comparative descriptions of the pupae of five species of the *Culicoides imicola* complex (Diptera, Ceratopogonidae) from South Africa. *Onderstepoort Journal of Veterinary Research*, 74:97–114

The viruses causing the economically important livestock diseases of African horse sickness (AHS) and bluetongue (BT) are transmitted by biting midges of the genus *Culicoides* (Diptera, Ceratopogonidae). In the Old World the most important vectors of these diseases are *Culicoides imicola* Kieffer, 1913, *Culicoides brevitarsis* Kieffer, 1917 and *Culicoides bolitinos* Meiswinkel, 1989. All three of these vectors belong to the *Imicola* complex of the subgenus *Avaritia* Fox, 1955. This species complex now comprises 12 sibling species; ten occur in sub-Saharan Africa and are difficult to identify (based mostly on subtle variations in the wing patterns) and so additional methods of reliable identification are needed. The pupal exuviae of the five commonest sibling species (*C. imicola*, *C. bolitinos*, *Culicoides loxodontis* Meiswinkel, 1992, *Culicoides tuttifrutti* Meiswinkel, Cornet & Dyce, 2003 and *Culicoides* sp. # 107) harvested from a variety of large herbivore dung types and from decaying fruits, are described and illustrated in detail. It is shown that they can be differentiated clearly on a number of morphological characters and, furthermore, are separable into two distinct groups based (principally) on the shape of the respiratory organ. A key for identifying and differentiating these five pupae is provided. Also, the pupa of the Oriental-Australasian *C. brevitarsis* was compared with its allopatric sister taxon, *C. bolitinos*. Because they share a common larval habitat (cattle and buffalo dung) and are almost inseparable in the adult phenotype, the question of their possible synonymy is raised. However, their respective pupae could not be differentiated on gross morphology and so it is argued that this unresolved problem requires a molecular solution.

Keywords: Ceratopogonidae, *Culicoides bolitinos*, *Culicoides brevitarsis*, *Culicoides imicola* complex, *Culicoides loxodontis*, *Culicoides* sp. # 107, *Culicoides tuttifrutti*, Diptera, pupae, morphology, South Africa

INTRODUCTION

Culicoides biting midges are important vectors of a number of arboviruses causing disease in domesti-

cated livestock. The most economically significant diseases are bluetongue (BT) in sheep and African horse sickness (AHS) in equids (Mellor, Boorman & Baylis 2000). Bluetongue, in particular, is endemic to all areas of the tropics and subtropics where competent vector *Culicoides* occur. In southern Europe, specifically, BT has extended its range vastly since 1998 with outbreaks occurring up to latitude 44°30' N; this extension has been linked to the warming of our global climate (Purse, Mellor, Rogers, Samuel, Mertens & Baylis 2005). Although AHS remains essentially African, dramatic incursions have also occurred into Europe (Mellor, Boned, Hamblin & Graham

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Accepted for publication 12 January 2007—Editor

1990) and further afield into central India (Howell 1963).

Of the 75 viruses associated with *Culicoides* worldwide, 23 have been isolated from the Imicola complex of the subgenus *Avaritia* Fox, 1955 (Meiswinkel, Venter & Nevill 2004). By far the most important vector within this species complex is *Culicoides imicola*. It is also the most widespread, extending from the southernmost tip of Africa northwards into southern Europe and from there eastwards as far as India, Laos, Vietnam and southern China (Meiswinkel 1989; Meiswinkel *et al.* 2004). In South Africa—due to its exceptionally high abundance levels around nearly all breeds of livestock—*C. imicola* remains the most important vector of both bluetongue virus (BTV) and African horse sickness virus (AHSV). Despite its notoriety, little is known about the precise breeding habitat of *C. imicola*. Nevertheless, it has been retrieved consistently from moist, organically-enriched, clayey soils, which are either bare or covered by short grass only (Meiswinkel *et al.* 2004). The larva and pupa of *C. imicola* have been described (Nevill 1969) but under the earlier name, *Culicoides pallidipennis* Carter, Ingram & Macfie 1920.

In the last decade another species of the Imicola complex, i.e. *Culicoides bolitinos*, has emerged as an additional vector of BTV and AHSV in South Africa, especially in higher-lying areas where cooler conditions prevail (Meiswinkel & Paweska 2003). Its newly discovered role as a field vector is supported by laboratory oral susceptibility studies (Venter, Paweska, Van Dijk, Mellor & Tabachnick 1998; Venter, Graham & Hamblin 2000). The larval habitat of *C. bolitinos*, cattle and buffalo dung, differs markedly from that of *C. imicola* and likely accounts for dissimilarities in their respective abundances and geographic distribution patterns (Meiswinkel, Labuschagne, Baylis & Mellor 2004). Interestingly, the species considered most closely related phylogenetically to *C. bolitinos* is the Oriental-Australasian *Culicoides brevitarsis* which, in the last 200 years, has spread south and entered western, northern and eastern Australia. Like *C. bolitinos*, it is an important vector of BTV and, furthermore, breeds also exclusively in the fresh dung of wild and domesticated bovids. This, and the fact that these two taxa are virtually inseparable in the adult phenotype, raises the question of their possible synonymy.

Currently, the Imicola complex comprises 13 species, four of which remain to be described. A key to the adult stage of nine species has been provided (Meiswinkel 1995, 2003). The adult males and females are distinguishable on a limited number of

characters, most of which are not immediately accessible, making it difficult to identify them reliably. These key adult-distinguishing features are not due to intra-specific variation but do reflect genomic differences, as was confirmed using random amplified polymorphic DNA (RAPD) markers (Sebastiani, Meiswinkel, Gomulski, Guglielmino, Mellor, Malacrida & Gasperi 2001), and sequencing of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene (Linton, Mordue, Cruickshank, Meiswinkel, Mellor & Dallas 2002; Meiswinkel & Linton 2003). The accurate identification of adult *Culicoides* is based largely on subtle variations in the grey and white wing patterns. However, within subgenera, and especially within species complexes, basing identifications on wing pattern alone becomes increasingly more difficult, to the point of unreliability if not performed by a specialist (Meiswinkel 2003). Therefore, additional avenues for identifying sibling species of the Imicola complex are needed, not only to confirm adult identifications, but also to throw further light on the phyletic relationships amongst the various taxa.

Perhaps of greater significance is that studies on the immature stages help clarify the often unique biology of each taxon. Such studies—largely unpublished—have in the last two decades revealed the various taxa of the Imicola complex to be niche specialists exploiting a variety of ephemeral semi-moist habitats that include large herbivore dung, rotting fruits and other decaying vegetative materials (Meiswinkel 1989, 1992, 1995; Meiswinkel & Braack 1994; Meiswinkel & Linton 2003).

Our principal aim was to investigate whether the pupal stage possesses diagnostic taxonomic characters to reliably differentiate sibling species of the Imicola complex. To this end, live pupae of *C. bolitinos*, *Culicoides loxodontis*, *Culicoides tuttifrutti* and *Culicoides* sp. # 107 (= *Culicoides kwagga* Meiswinkel, unpublished 1995) were recovered from a variety of breeding media collected in the field and link-reared. As no fresh material of *C. imicola* was obtained, we redescribe the pupa using the original material of Nevill (1967; 1969) who obtained immature stages of *C. pallidipennis* (= *C. imicola*) by rearing them from eggs of blood-fed, field-collected females induced to oviposit under laboratory conditions.

Finally, pupal exuviae of the Oriental-Australasian *C. brevitarsis* were supplied by Alan L. Dyce of Sydney, Australia for comparison with those of its Afrotropical sister taxon *C. bolitinos* in the hope that the issue surrounding their possible synonymy could be resolved.

MATERIALS AND METHODS

Field collections

In 1973/74 Alan L. Dyce, during a 3-month sabbatical from the CSIRO, Australia, collected *Culicoides* pupae from a variety of habitats in the provinces of Gauteng, Mpumalanga, North West and Limpopo. He reared many of these to the adult stage, resulting in the description of the pupae of seven species of the Similis supergroup (Nevill & Dyce 1994). During the same period he also link-reared *C. tutti-frutti* and *C. bolitinos*. His early findings induced us to seek and link-rear further species of the Imicola complex, the results of which are reported here.

Except for that of *C. imicola*, the material of the remaining species was collected from a variety of larval habitats at a number of locations across South Africa, as indicated under each species description. Numerous pupae were retrieved from the dung of the African buffalo (*Syncerus caffer*), cattle (*Bos*), the white rhinoceros (*Ceratotherium simum*), the plains zebra (*Equus burchellii*) and the African elephant (*Loxodonta africana*). Other substrates sampled were the rotting fruits of the marula tree (*Sclerocarya birrea*) and the sausage tree (*Kigelia africana*). Once collected, the breeding material was placed in a 32 x 45 x 28 cm sealed, black plastic container. One side of the container was fitted with a white transparent funnel to which an unwaxed white cardboard cup was attached, its opening closed off with a fine gauze to prevent insects from escaping. The buildup of excess moisture was suppressed by lining the container with thick, absorbent cardboard and by evaporation through windows (screened with black gauze) in the lid. The container was then put in a well-ventilated and well-lit room at ambient temperature. Once the first emerging adult midges were seen to appear in the unwaxed cup, a portion of the medium was removed from the container and laboriously screened for live pupae. Small quantities of medium were mixed with water, the coarsest material removed, and sugar added and stirred until the pupae floated to the surface. The individual pupae retrieved were kept separately in stoppered vials to await eclosion. When the adult emerged it was kept alive for at least 24 h to promote hardening and darkening of the exoskeleton. Afterwards the vial was filled with 70% alcohol to preserve the insect and its exuvia.

Preparation of specimens and descriptive format

The procedure for slide-mounting the adult and its associated pupal exuvia is described in Nevill &

Dyce (1994) who list also the full suite of pupal characters scored. The only change since is the dorsal illustration of the thorax for greater clarity. Character terminology, abbreviations used and positions of the various setae are as shown in the illustration of *C. imicola* (Fig. 1). Characters were studied from the following body regions: the prothoracic respiratory organ, the operculum, the head tubercles, the thorax, the fourth abdominal segment and the caudal segment. Furthermore, all setae on all body parts were scored.

RESULTS

Culicoides imicola Kieffer

(Fig. 1A–H; Tables 1–6)

Culicoides imicola Kieffer, 1913: 11. Kenya.

Culicoides pallidipennis Carter, Ingram & Macfie, 1920: 265. Ghana.

Culicoides pallidipennis Carter, Ingram & Macfie; Nevill 1969: 276. South Africa (larva and pupa).

Description

Mean total length 1.92 mm (1.82–2.02 mm; $n = 9$). Yellow-brown, cephalothorax can be darker and occasionally also the abdomen.

RESPIRATORY ORGAN (Fig. 1A; Tables 1, 2 and 6). Basal and distal one fifth a dark ochreous colour, medial portion paler. Organ moderately long, about 6x longer than wide, narrowest medially. Medial half to three fifths annulated. Scales absent. Lateral spiracles four (3–5) borne on slight prominences. Six to seven terminal spiracles, the most distal lateral spiracle sometimes indistinguishable from terminal ones. Pedicel short, 0.18x length of organ.

OPERCULUM (Fig. 1B; Tables 3 and 6). Yellow-brown. In both females ♀♀ and males ♂♂ the disc is wider than long. A single row of long setaceous spines along anterolateral margins of disc, these joined by a narrow, transverse band of spines anteriorly. Rows of nodules radiate in semi-circles from lateral margins towards midline of disc. Large protuberance posteromedianly on disc.

HEAD TUBERCLES AND SETAE (Fig. 1B, D and F; Tables 5 and 6). Anteromarginal (am) tubercle with sharply pointed spur; seta very long, stout; basal sensillum present (Fig. 1B). Ventromedian (vm) sensillae and setae absent. Two fine ventrolateral (vl) setae of subequal length and of almost equal

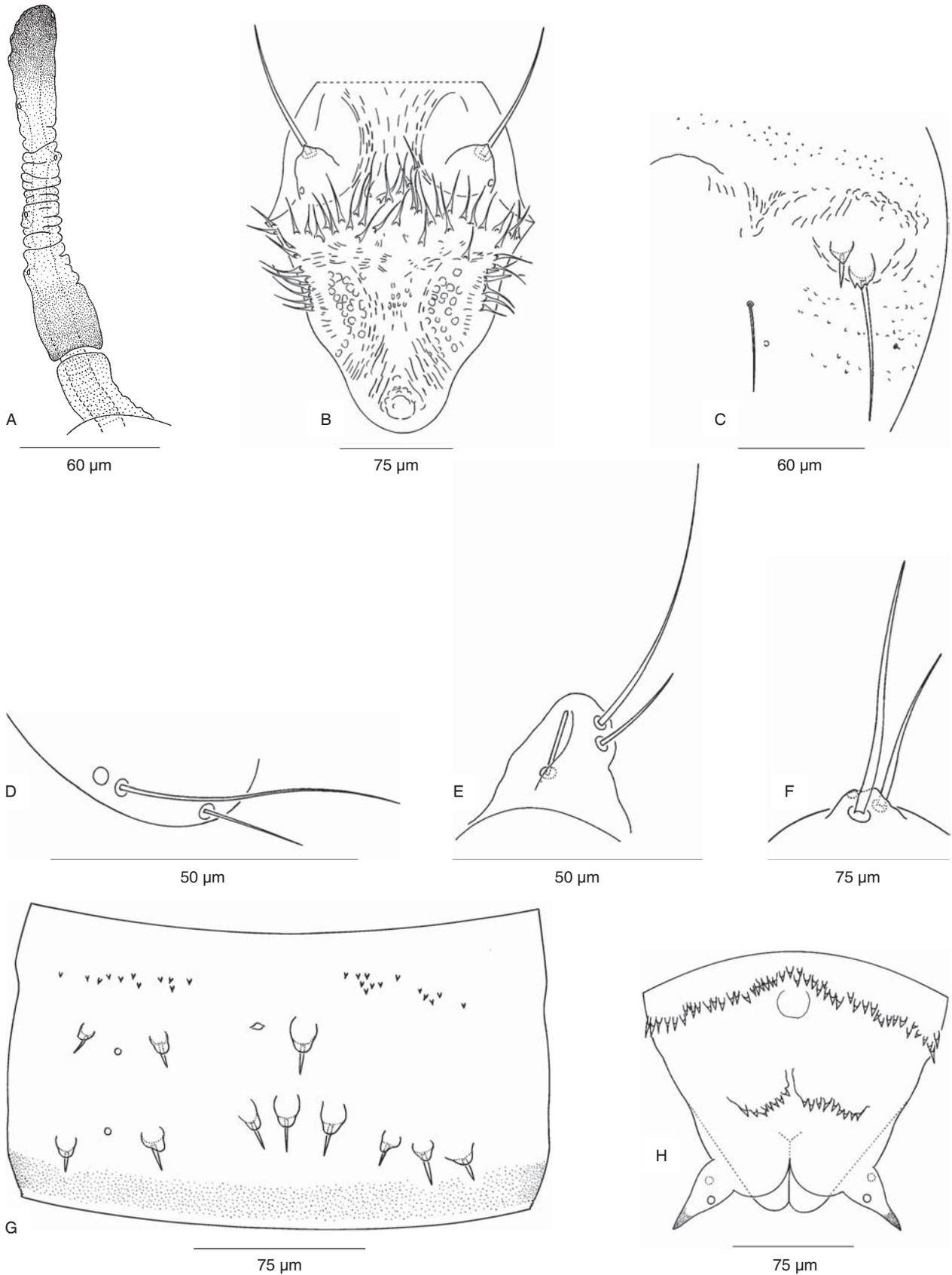


FIG. 1 The pupa of *Culicoides imicola*: (A) prothoracic respiratory organ; (B) ♀ operculum; (C) thorax; (D) ventrolateral setae (vl); (E) dorsolateral tubercle (dl); (F) anterodorsal tubercle (ad); (G) fourth abdominal segment; (H) caudal segment

stoutness; a third sensillum present (Fig. 1D). Antero-dorsal (ad) tubercle without sharply pointed spurs; seta i very long, stout; seta ii shorter, moderately stout; a third sensillum present (Fig. 1F).

THORAX (Fig. 1C and E; Tables 5 and 6). Dorsal (d) tubercles small; d i and d ii rather closely approximated each with 1–2 sharply pointed spurs; d i moderately stout, shorter than d ii; d ii long, slightly stouter than d i; d iii minute; d iv not on a tubercle, seta long and fine; d v sensillum present; small pits around and between tubercles (Fig. 1C). Dorsolateral (dl) tubercle with three setae: the one terminally is long and fine; the one laterally is short, but finer than the terminal seta, while the one basally is difficult to discern (Fig. 1E).

ABDOMEN (Fig. 1G; Tables 5 and 6). All tubercles rather small with apices rounded; dorsal postero-marginal (dpm) tubercles ii, iii and v absent. Base of dpm i rounded, seta short, moderately stout, sharply pointed; base of dpm iv rounded, seta slightly longer and stouter than dpm i and sharply pointed; sensillum present between dpm i and iv. Bases of lateral posteromarginal (lpm) tubercles i–iii oval, setae short, moderately stout, sharply pointed; lpm ii seta slightly longer and finer than lpm i and iii setae. Base of ventral posteromarginal (vpm) tubercle i rounded, seta short, moderately stout, sharply pointed; vpm ii similar to vpm i, seta slightly longer and stouter than vpm i and iii; base of vpm iii broader, seta short, moderately stout, sharply pointed. Base of dorsal anterosubmarginal (dasm) tubercle i rounded, seta short, moderately stout, sharply pointed; dasm ii similar to dasm i, seta slightly stouter; sensillum present between dasm i and ii. Lateral anterosubmarginal (lasm) tubercle similar to lpm i and iii. Anterolateral spiracle (sp) present. Anterior band of spinules interrupted dorsally, laterally and ventrally; posterior margin of segment granular; integument without spinules.

CAUDAL SEGMENT (Fig. 1H; Tables 4 and 6). Anterior band of large, triangular spines, not interrupted dorsally. Dorsomedian row of 23–28 spines interrupted medianly; remainder of integument smooth. Posterolateral processes short with rather sharp tips; two sensillae present on each process; spinules absent; apical quarter lightly pigmented.

Material examined

GAUTENG PROVINCE. 7 ♀♀ 2 ♂♂, ARC-OVI (25°39' S; 28°09' E), Pretoria, 1963.xi.05–1963.xii.20; 2 ♀♀ 6 ♂♂, same data, 1966.vii.26, E.M. Nevill, laboratory-reared from eggs oviposited by wild-caught females.

***Culicoides bolitinos* Meiswinkel**

(Fig. 2A–H; Tables 1–6)

Culicoides bolitinos Meiswinkel, 1989: 23. South Africa.

Description

Mean total length 1.83 mm (1.68–2.01 mm; $n = 21$). Abdomen light yellow, cephalothorax a darker yellow.

RESPIRATORY ORGAN (Fig. 2A; Tables 1, 2 and 6). Basal two fifths darkest, distal fifth darker than medial two fifths. Organ moderately long, about 6x longer than wide, basal third widest, narrowest medially. Medial third annulated. Scales absent. Lateral spiracles four (3–5), the medial spiracles on slight, unpigmented prominences. Four (3–5) terminal spiracles, the most distal lateral spiracle sometimes indistinguishable from terminal spiracles. Pedicel short, 0.2x length of organ.

OPERCULUM (Fig. 2B; Tables 3 and 6). Dark yellow. In both ♀♀ and ♂♂ the disc is wider than long. Long setaceous spines on anterior half of disc and sometimes between am tubercles. Nodules posteriorly on disc and on large posteromedian protuberance.

HEAD TUBERCLES AND SETAE (Fig. 2B, D and F; Tables 5 and 6). Am tubercle with blunt to sharply pointed spur; seta very long, stout; basal sensillum present (Fig. 2B). Vm sensillum and setae absent. Two fine vl setae of subequal length and width; a third sensillum present (Fig. 2D). Ad tubercle with small, bluntly pointed spur; seta i very long, moderately stout; seta ii slightly shorter and finer; a third sensillum present (Fig. 2F).

THORAX (Fig. 2C and E; Tables 5 and 6). Dorsal tubercles small; d i and d ii rather closely approximated each with 3–5 spurs; d i seta short, moderately stout, sharply pointed; d ii seta long, stouter than d i, tip rounded; d iii seta minute; d iv not on a tubercle, seta moderately long and fine; d v sensillum present. Small pits around and between dorsal tubercles (Fig. 2C). Dorsolateral tubercle with three setae: the one terminally is moderately long and fine; the one laterally is shorter and fine and the one basally (in a fold) is short, fine and of equal width throughout (Fig. 2E).

ABDOMEN (Fig. 2G; Tables 5 and 6). All tubercles very small and apices mostly with a single, sharply pointed spur; dpm ii, iii and v absent; dpm i minute; base of dpm iv rounded, tubercle mostly with single, sharply pointed spur, seta short, fine, sharply pointed; sensillum present between dpm i and dpm iv.

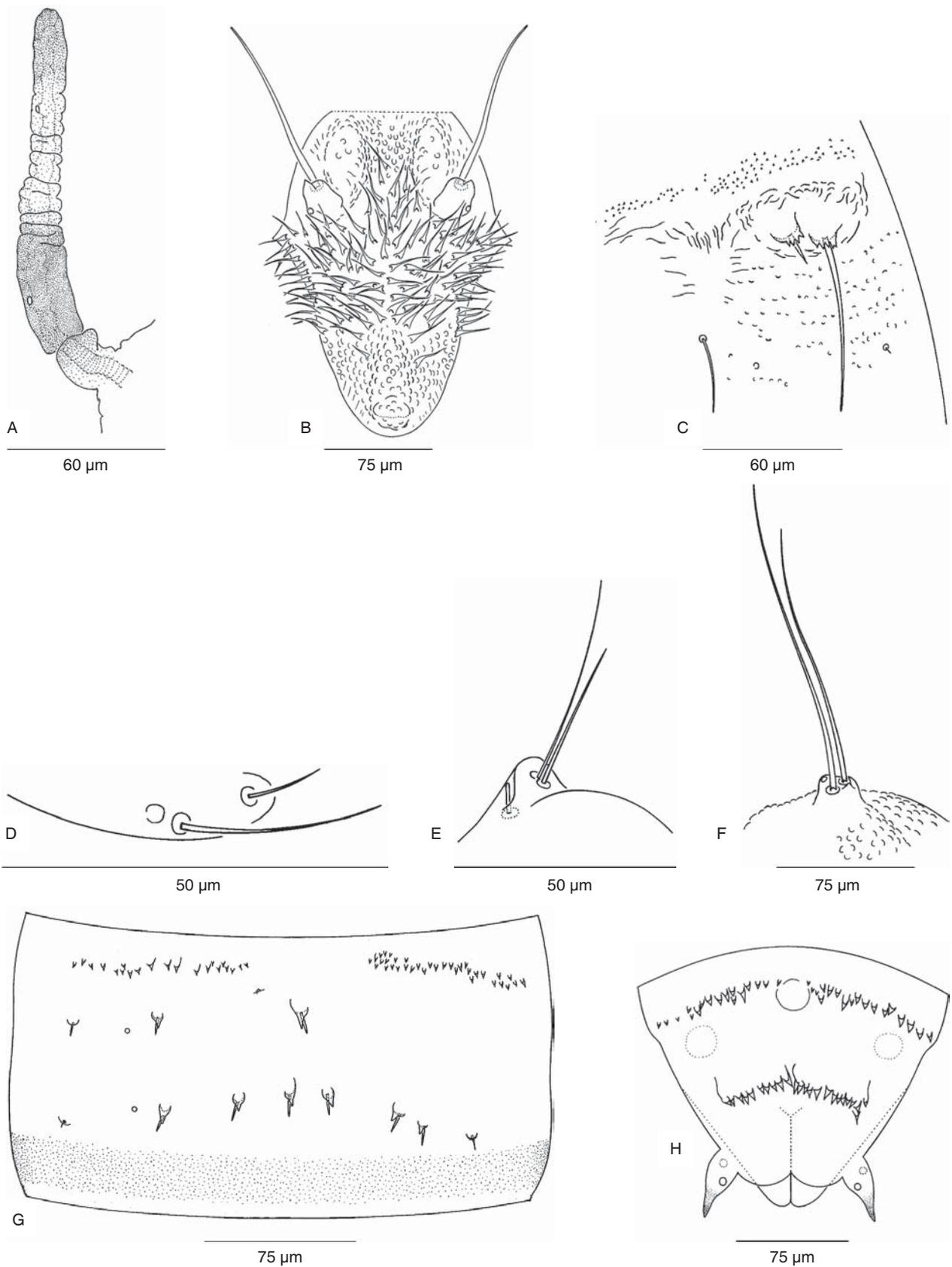


FIG. 2 The pupa of *Culicoides bolitinos*: (A) prothoracic respiratory organ; (B) ♂ operculum; (C) thorax; (D) ventrolateral setae (vl); (E) dorsolateral tubercle (dl); (F) anterodorsal tubercle (ad); (G) fourth abdominal segment; (H) caudal segment

Bases of lpm i–iii oval, each tubercle with a large, sharply pointed spur, seta short, moderately stout, sharply pointed. Base of vpm i rounded, tubercle bearing small, sharply pointed spur, seta short, moderately stout, sharply pointed; base of vpm ii rounded, tubercle bearing blunt or sharply pointed spur, seta short, moderately stout, sharply pointed; base of vpm iii broad, bluntly pointed spur, seta slightly shorter than vpm i and ii, moderately stout, sharply pointed. Base of dasm i rounded, spur absent, seta short, fine, sharply pointed; dasm ii similar to dpm iv; sensillum present between dasm i and dasm ii. Lasm similar to lpm i–iii. Anterolateral spiracle present. Anterior band of spinules interrupted dorsally, laterally and ventrally; nodules sometimes discernible posterior to dpm, lpm and vpm tubercles; posterior margin of segment granular; integument without spinules.

CAUDAL SEGMENT (Fig. 2H; Tables 4 and 6). Anterior band of large and small triangular spines interrupted dorsally. Dorsomedian row of 15–39 spines, interrupted medianly; remainder of integument smooth. Posterolateral processes short with rather sharp tips; two sensillae on each process; spinules absent; apical quarter lightly pigmented.

Material examined

MPUMALANGA PROVINCE. 5 ♀♀ 5 ♂♂, Farm “Lodwicks Lust” (25°26' S; 31°41' E), district Hectorspruit, 1993.iv.18, A.L. Dyce, ex cattle dung.

KWAZULU-NATAL PROVINCE. 10 ♀♀ 10 ♂♂, Maphumulo, Hluhluwe Game Reserve (28°07' S; 32°05' E), 1992.viii.10, H. Nevill, E.M. Nevill and K. Kappmeier-Green, ex buffalo dung.

Additional material examined

MPUMALANGA PROVINCE. 27 ♀♀ 4 ♂♂, Farm “Lodwicks Lust” (25°26' S; 31°41' E), district Hectorspruit, 1973.xi.30, A.L. Dyce, ex cattle dung.

GAUTENG PROVINCE. 1 ♂, ARC-OVI, Pretoria (25°39' S; 28°09' E), 1973.xii.12, A.L. Dyce, ex cattle dung.

LIMPOPO PROVINCE. 1 ♀, Waterpoort (22°54' S; 29°37' E), 1974.i.01, A.L. Dyce, ex cattle dung.

***Culicoides tuttifrutti* Meiswinkel, Cornet & Dyce** (Fig. 3A–H; Tables 1–6)

Culicoides tuttifrutti Meiswinkel, Cornet & Dyce, 2003: 47. South Africa.

Description

Mean total length 1.95 mm (1.69–2.15 mm; $n = 12$). A light yellow-brown colour throughout.

RESPIRATORY ORGAN (Fig. 3A; Tables 1, 2 and 6). Basal and distal quarter ochreous, paler medially. Organ moderately long, about 6x longer than wide, widest basally and distally. Annulations present on medial half of organ. Scales absent. Bears 4–7 (5) terminal spiracles. Proximal six sevenths bears 5–8 (6) lateral spiracles, the medial ones on prominences. Pedicel short, 0,18x length of organ.

OPERCULUM (Fig. 3B; Tables 3 and 6). Dark yellow. In both ♀♀ and ♂♂ the disc is wider than long. Long setaceous spines densely cover anterior half of disc, extending between am tubercles. Nodules occur anterior to the am tubercles and on the large postero-medial protuberance of the disc.

HEAD TUBERCLES AND SETAE (Fig. 3B, D and F; Tables 5 and 6). Am tubercle occasionally with one sharply pointed spur, seta very long, stout; basal sensillum present (Fig. 3B). The vm sensillum and setae absent. Two fine vl setae of almost equal length and stoutness; a third sensillum present (Fig. 3D). Ad tubercle sometimes with 1–2 sharply pointed spurs; seta i and ii very long, stout; seta ii slightly shorter; a third sensillum present (Fig. 3F).

THORAX (Fig. 3C and E; Tables 5 and 6). Dorsal tubercles rather small; d i and d ii rather closely approximated each with 3–5 spurs; d i short, moderately stout, sharply pointed; d ii long, moderately stout; d iii minute; d iv moderately long and fine; d v sensillum borne on slight prominence. Pits around and between tubercles (Fig. 3C). The dl tubercle bears three setae: the one terminally is long and fine; the one laterally is short and fine while the one basally is short, fine and of equal stoutness throughout (Fig. 3E).

ABDOMEN (Fig. 3G; Tables 5 and 6). All tubercles rather small and mostly with a sharply pointed spur; dpm ii, iii and v absent. Base of dpm i broad, occasionally with sharply pointed spur, seta minute; base of dpm iv rounded with sharply pointed spur, seta short, moderately stout, sharply pointed; sensillum present between dpm i and iv. Bases of lpm i and iii oval, each tubercle with a large, sharply pointed spur and a short, moderately stout, sharply pointed seta; lpm ii similar to lpm i and iii but with longer, fine seta. Base of vpm i rounded, with sharply pointed spur, seta short, moderately stout, sharply pointed; vpm ii similar to vpm i but with longer, fine seta; base of vpm iii broader, occasionally with a sharply pointed spur, seta shorter, finer than vpm i, sharply pointed. Base of dasm i rounded, occasionally with sharply pointed spur, seta short, moderately stout, sharply pointed; dasm ii larger, base rounded, spur sharply

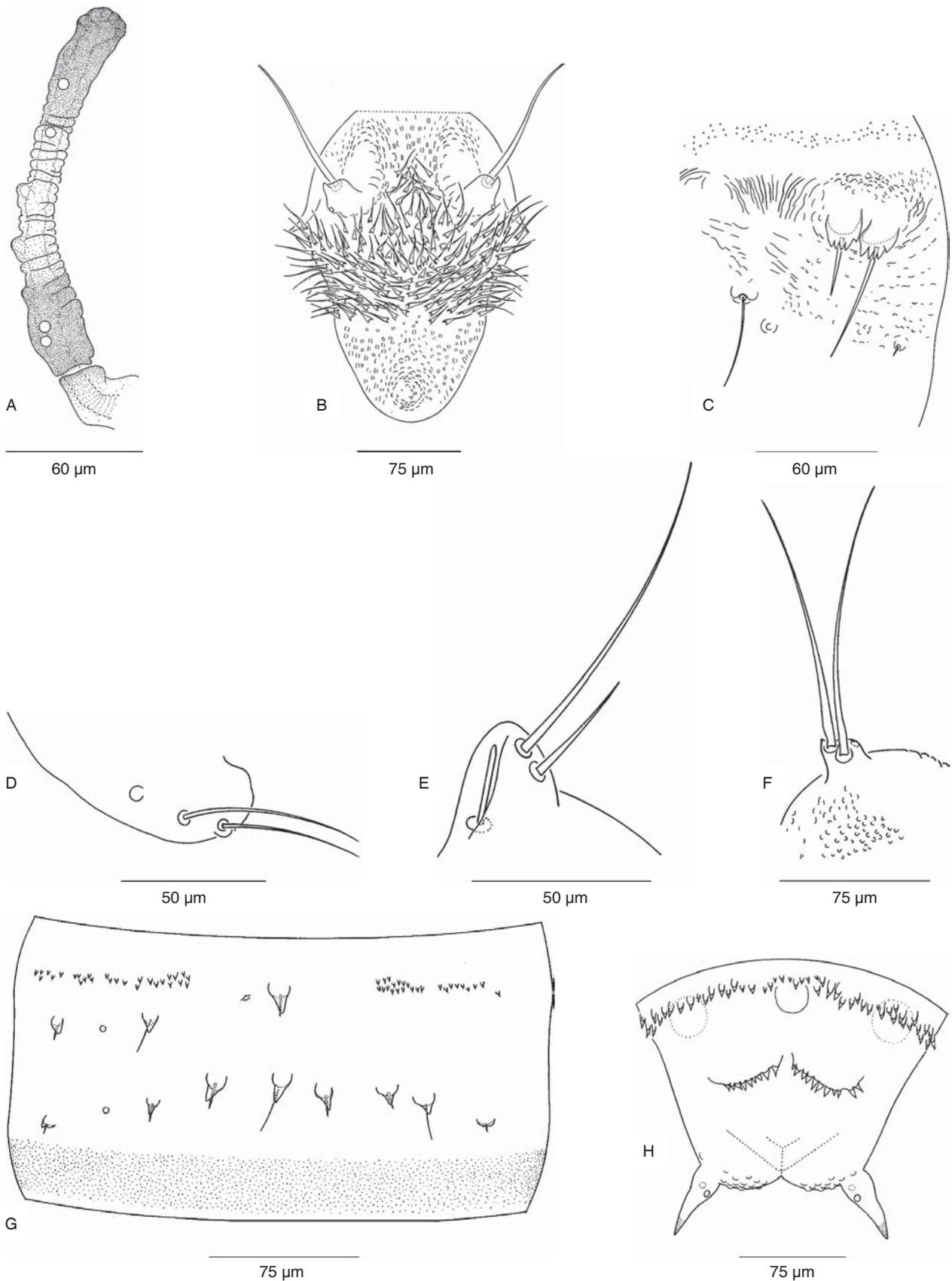


FIG. 3 The pupa of *Culicoides tuttifrutti*: (A) prothoracic respiratory horn; (B) ♀ operculum; (C) thorax; (D) ventrolateral setae (vl); (E) dorsolateral tubercle (dl); (F) anterodorsal tubercle (ad); (G) fourth abdominal segment; (H) caudal segment

pointed, seta longer than dasm i and fine; sensillum present between dasm i and ii. Lasm similar to lpm i and iii. Anterolateral spiracle present. Anterior band of spinules interrupted dorsally, laterally and ventrally, integument faintly pitted; integument without spinules; posterior margin of segment granular.

CAUDAL SEGMENT (Fig. 3H; Tables 5 and 6). Anterior band of large, triangular spines occasionally interrupted dorsally. Dorsomedian row of 20–53 spines interrupted medianly; integument faintly pitted, and bearing nodules. Posterolateral processes short with rather sharp tips; two sensillae on each process; spinules absent; apical quarter lightly pigmented.

Material examined

MPUMALANGA PROVINCE. 4 ♂♂, Farm “Lodwicks Lust” (25°26' S; 31°41' E), district Hectorspruit, 1973.xi.29, A.L. Dyce, ex decaying sausage tree fruit (*Kigelia africana*).

7 ♀♀ 5 ♂♂, Farm “Lodwicks Lust” (25°26' S; 31°41' E), district Hectorspruit, 1974.i.31, A.L. Dyce, ex decaying sausage tree fruit (*Kigelia africana*).

10 ♀♀ 1 ♂, Nwaswitshaka research camp, Skukuza (24°59' S; 31°35' E), Kruger National Park (KNP), 1993.iii.05, A.L. Dyce and L.E.O. Braack, ex decaying marulas (*Sclerocarya birrea*)

3 ♀♀, Skukuza (24°59' S; 31°35' E), KNP, 1993. iv.19, A.L. Dyce, ex decaying sausage tree fruit (*Kigelia africana*).

***Culicoides loxodontis* Meiswinkel**

(Fig. 4A–H; Tables 1–6)

Culicoides loxodontis Meiswinkel, 1992: 145. South Africa.

Description

Mean total length 1.71 mm (1.44–1.88 mm; $n = 9$). Yellow throughout, cephalothorax slightly darker than abdomen. Integument of cephalothorax with nodules, that of abdomen with spinules.

RESPIRATORY ORGAN (Fig. 4A; Tables 1, 2 and 6). Of an even yellow colour throughout. Organ very short, not more than 2x longer than wide, almost triangular in shape. Lacking both annulations and scales. Bears five (2–6) terminal spiracles; lateral spiracles absent. Pedicel very short, 0.27x length of organ.

OPERCULUM (Fig. 4B; Tables 3 and 6). Yellow. In both ♀♀ and ♂♂ the disc is wider than long. Setaeous spines absent. Protuberances present laterally and posteromedianly. Nodules cover most of the operculum and the am tubercles.

HEAD TUBERCLES AND SETAE (Fig. 4B, D and F; Tables 5 and 6). Am tubercle without spurs, borne on very large, distinct protuberance; seta very long, stout; basal sensillum present (Fig. 4B). Vm sensillae and setae absent; vl setae of subequal length and of almost equal stoutness; a third sensillum present (Fig. 4D). Ad tubercle without spurs; seta i and ii very long and stout, but with seta i longer and slightly stouter than ii; a third sensillum present (Fig. 4F).

THORAX (Fig. 4C and E; Tables 5 and 6). Dorsal tubercles vary in size; d i small bearing 3–4 small spurs, seta short, moderately stout, sharply pointed; d ii larger than d i, also bearing four spurs, seta very long, stouter than d i which is not closely approximated to d ii; d iii minute; d iv tubercle small, seta short, fine; d v sensillum borne on a slight prominence. Nodules densely adorn entire integument (Fig. 4C). The dl tubercle bearing three setae: the one terminally is moderately long and fine, the one laterally is shorter, while the one basally is very short and fine (Fig. 4E).

ABDOMEN (Fig. 4G; Tables 5 and 6). All tubercles large and covered with nodules, apices rounded; dpm ii, iii and v are absent. Bases of dpm i and iv rounded, setae very short and moderately stout, sharply pointed, seta i slightly finer than seta iv; elevated sensillum between dpm i and dpm iv. Bases of lpm tubercles i–iii are oval, setae very short and moderately stout, sharply pointed. The bases of vpm i and ii rounded; base of vpm iii broader, setae very short and moderately stout, sharply pointed. Dasm i and dasm ii similar to dpm i and dpm ii; elevated sensillum between tubercles. Lasm similar to lpm i–iii. Anterolateral spiracle present. Anterior band of spinules interrupted dorsally, laterally and ventrally; integument covered with spinules; posterior margin of segment granular.

CAUDAL SEGMENT (Fig. 4H; Tables 4 and 6). Anterior band of spines usually not interrupted dorsally. Dorsomedian row of 16–46 spines interrupted medianly; small spines adorn the anterodorsal two-thirds and ventral third of segment; remainder of the segment covered with nodules. Posterolateral processes of moderate length with rather blunt tips; two sensillae on each process; inner and dorsal aspect of processes sparsely adorned with spinules; apical quarter to one third lightly pigmented.

Material examined

MPUMALANGA PROVINCE. 10 ♂♂, north of Skukuza rest camp (24°59' S; 31°35' E), KNP, 1991.v.13, H. Nevill, H. Heyne and B. du Toit, ex elephant dung.

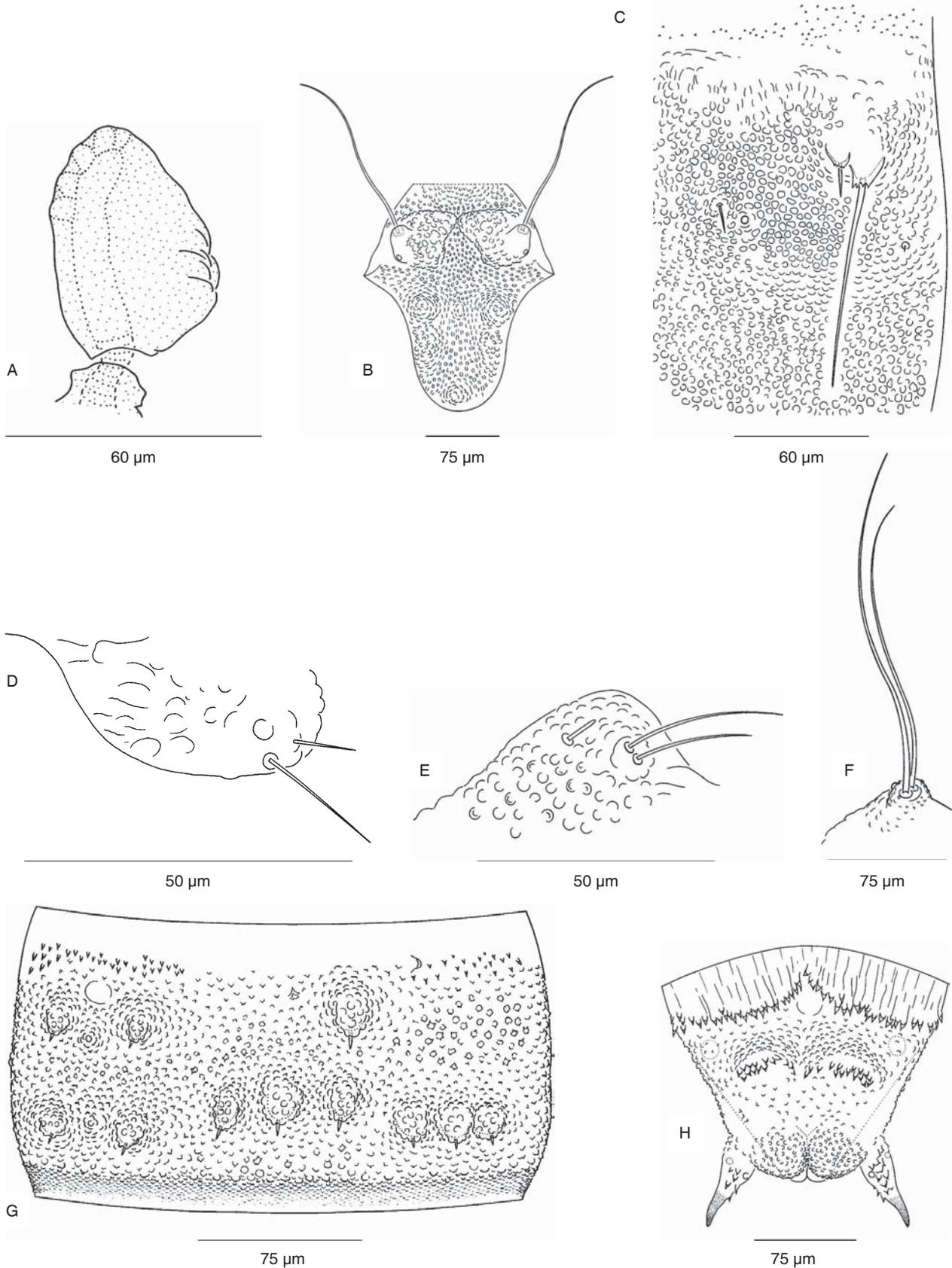


FIG. 4 The pupa of *Culicoides loxodontis*: (A) prothoracic respiratory organ; (B) ♂ operculum; (C) thorax; (D) ventrolateral setae (vl); (E) dorsolateral tubercle (dl); (F) anterodorsal tubercle (ad); (G) fourth abdominal segment; (H) caudal segment

2 ♂♂, west of Skukuza rest camp (24°59' S; 31°35' E), KNP, 1991.v.14, H. Nevill and H. Heyne, ex elephant dung.

1 ♂, west of Skukuza rest camp (24°59' S; 31°35' E), KNP, 1991.v.14, H. Nevill, K. Newberry and I. McKay, ex elephant dung.

LIMPOPO PROVINCE. 6 ♂♂, south of Letaba rest camp (23°52' S; 31°35' E), KNP, 1993.ii.03, A.L. Dyce, ex elephant dung.

KWAZULU-NATAL PROVINCE. 1 ♀, Hluhluwe Game Reserve (28°07' S; 32°05' E), 1992.ix.10, H. Nevill, E.M. Nevill and K. Kappmeier-Green, ex elephant dung.

***Culicoides* sp. # 107**

(Fig. 5A–H; Tables 1–6)

Culicoides kwagga (Meiswinkel, unpublished data 1995: 217. South Africa).

Description

Mean total length 1.96 mm (1.79–2.12 mm; $n = 16$). Dark yellow throughout, cephalothorax slightly darker than abdomen.

RESPIRATORY ORGAN (Fig. 5A; Tables 1, 2 and 6). Uniformly yellow throughout. Organ very short, not more than 2x longer than wide, almost oval in shape. Annulations and scales absent. Bears 3–5 (4) terminal spiracles, lateral spiracles absent. Pedicel very short, 0.34x length of organ.

OPERCULUM (Fig. 5B; Tables 3 and 6). Yellow. In both ♀♀ and ♂♂ the disc is wider than long. Setaceous spines absent. Protuberances present laterally and posteromedianly. Most of the operculum densely covered with nodules.

HEAD TUBERCLES AND SETAE (Fig. 5B, D and F; Tables 5 and 6). Am tubercle on slight protuberance, without spurs; seta very long, stout, basal sensillum present (Fig. 5B). The vm sensillae and setae are absent; vl setae of subequal length and stoutness; sensillum present (Fig. 5D). Ad tubercle without spurs; seta i very long, stout; seta ii about half the length, moderately stout, a third sensillum present (Fig. 5F).

THORAX (Fig. 5C and E; Tables 5 and 6). Dorsal tubercles vary in size; di small, without spurs, seta short, moderately stout, sharply pointed; d ii larger, without spurs, seta very long, slightly stouter than d i; d i and d ii not closely approximated; d iii minute; d iv tubercle small, seta long, moderately stout; d v sensillum on slight prominence. Nodules adorn entire integument (Fig. 5C). The dl tubercle bears

three setae: the one terminally is short and fine; the one laterally is shorter and fine, while the one basally is very short and fine (Fig. 5E).

ABDOMEN (Fig. 5G; Tables 5 and 6). All tubercles rather large and with rounded apices; dpm ii, iii and v absent. Base of dpm i wide, seta i very short, slightly finer than seta iv, sharply pointed; base of dpm iv round, seta very short, moderately stout, sharply pointed; elevated sensillum present between dpm i and dpm iv. Bases of lpm i–iii oval, setae very short, moderately stout, sharply pointed. Base of vpm i small, rounded, seta very short, moderately stout, sharply pointed; base of vpm ii larger, seta very short, moderately stout, sharply pointed; base of vpm iii broad, seta very short, finer than vpm i and ii, sharply pointed. Bases of dasm i and ii small and rounded, setae very short, moderately stout, sharply pointed; sensillum present between tubercles. Lasm similar to lpm i–iii. Anterolateral spiracle present. Anterior band of spinules interrupted dorsally, laterally and ventrally; integument densely adorned with nodules; posterior margin of segment granular.

CAUDAL SEGMENT (Fig. 5H; Tables 4 and 6). Anterior band of large, triangular spines, occasionally interrupted dorsally. Dorsomedian row of 20–65 spines interrupted medianly; integument densely adorned with nodules. Posterolateral processes moderately long with blunt tips; two sensillae on each process; inner and dorsal aspects of processes with spinules; apical quarter to one third lightly pigmented.

Material examined

NORTH WEST PROVINCE. 3 ♂♂, Pilanesberg Nature Reserve (25°13' S; 27°06' E), 1989.xii.28, H. Nevill and D.G. de Klerk, ex white rhinoceros dung.

2 ♀♀ 4 ♂♂, Dikhololo Nature Reserve near Brits (25°37' S; 27°45' E), 1992.ix.15, H. Nevill and M. Edwards, ex zebra dung.

1 ♀ 7 ♂♂, same data, 1993.ix.19.

2 ♂♂, Emaweni Nature Reserve, Melkrivier (23°59' S; 28°23' E), 1990.vi.11, H. Nevill and E.M. Nevill, ex white rhinoceros dung.

MPUMALANGA PROVINCE. 1 ♀, Ngirivane (24°22' S; 31°41' E), KNP, 1991.ix.18, H. Nevill and R. Meiswinkel, ex zebra dung.

1 ♀ 2 ♂♂, south of Skukuza rest camp (24°59' S; 31°35' E), KNP, 1991.ix.20, H. Nevill, K. Newberry and K. Kappmeier-Green, ex white rhinoceros dung.

KWAZULU-NATAL PROVINCE. 5 ♀♀, Umfolozi Game Reserve (28°50' S; 31°35' E), 1990.ix.20, H. Nevill and E.M. Nevill, ex zebra dung.

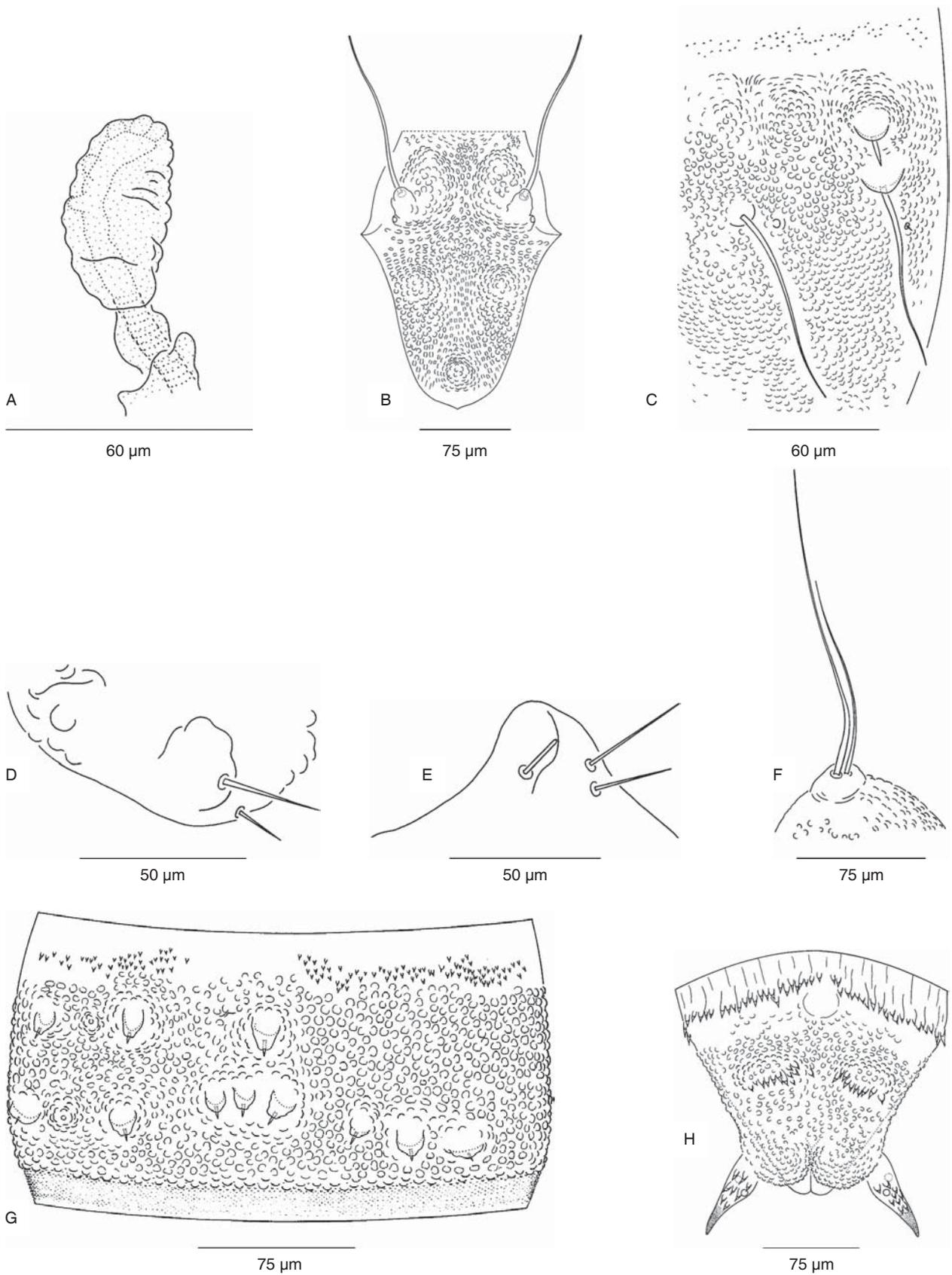


FIG. 5 The pupa of *Culicoides* sp. # 107: (A) prothoracic respiratory organ; (B) ♀ operculum; (C) thorax; (D) ventrolateral setae (vl); (E) dorsolateral tubercle (dl); (F) anterodorsal tubercle (ad); (G) fourth abdominal segment; (H) caudal segment

TABLE 1 Respiratory organ, pedicel lengths (μm) and P/H ratios of five species of *Culicoides* of the *Imicola* complex

Species	Horn (H)		Pedicel (P)		Ratio (P/H)	
	Range	Mean (n)	Range	Mean (n)	Range	Mean (n)
<i>C. imicola</i>	147.5–160.5	154.2 (15)	25.0–34.5	28.8 (15)	0.16–0.21	0.19 (14)
<i>C. bolitinos</i>	116.0–149.0	131.6 (36)	23.0–31.0	26.1 (41)	0.17–0.24	0.20 (36)
<i>C. tuttifrutti</i>	140.0–195.5	175.5 (42)	25.0–40.0	31.0 (40)	0.14–0.25	0.18 (39)
<i>C. loxodontis</i>	45.0–65.0	51.4 (33)	10.0–17.5	13.7 (19)	0.18–0.39	0.27 (18)
<i>C. sp. # 107</i>	35.0–54.5	44.3 (38)	10.0–20.5	15.4 (29)	0.28–0.46	0.35 (29)

TABLE 2 The number and greatest frequency of lateral and terminal spiracles on the respiratory organs of the pupae of five species of *Culicoides* of the *Imicola* complex

Species	Lateral spiracles		Terminal spiracles	
	Range	Highest frequency (n)	Range	Highest frequency (n)
<i>C. imicola</i>	3–5	4 (12)	6–7	7 (16)
<i>C. bolitinos</i>	3–5	4 (36)	3–5	4 (44)
<i>C. tuttifrutti</i>	5–8	6 (49)	4–7	5 (50)
<i>C. loxodontis</i>	0	0 (27)	2–6	5 (27)
<i>C. sp. # 107</i>	0	0 (36)	3–5	4 (36)

TABLE 3 Measurements (μm) of the opercular discs (♀♀ and ♂♂) and spine lengths (μm) of the pupae of five species of *Culicoides* of the *Imicola* complex

Species, gender	n	Operculum length (OL)		Operculum width (OW)		Ratio (OW/OL)		Spine length	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
<i>C. imicola</i> ♀+♂	1	154.0	154.0	177.5	177.5	1.15	1.15	30.5–32.5	31.4
	3	140.0–157.5	147.2	155.5–160.0	158.7	1.02–1.11	1.08		
<i>C. bolitinos</i> ♀+♂	11	130.5–143.0	136.5	150.0–169.5	163.4	1.15–1.27	1.20	25.0–35.5	30.2
	17	127.5–145.5	138.1	154.5–170.5	162.2	1.09–1.26	1.18		
<i>C. tuttifrutti</i> ♀+♂	4	140.0–141.0	140.3	170.0–190.0	182.5	1.21–1.35	1.30	28.0–50.0	37.1
	18	132.5–165.0	151.1	170.0–201.0	182.6	1.09–1.30	1.20		
<i>C. loxodontis</i> ♀+♂	1	138.5	138.5	186.0	186.0	1.34	1.34	Absent	
	14	132.5–162.5	148.4	170.0–198.5	184.0	1.09–1.36	1.24		
<i>C. sp. # 107</i> ♀+♂	9	125.0–156.0	144.0	161.0–180.0	172.2	1.10–1.36	1.20	Absent	
	16	142.5–176.0	153.3	157.5–175.0	167.5	1.01–1.21	1.09		

TABLE 4 Lengths (μm) of posterolateral processes and number of dorsal spinules on the caudal segment of the pupae of five species of *Culicoides* of the *Imicola* complex

Species	Posterolateral processes (length)			Number of dorsal spinules		
	Range	Mean	n	Range	Mean	n
<i>C. imicola</i>	48.5–54.0	50.5	6	23–28	25.0	3
<i>C. bolitinos</i>	34.0–42.5	35.6	52	15–39	26.3	27
<i>C. tuttifrutti</i>	35.0–57.5	43.9	37	20–53	37.7	14
<i>C. loxodontis</i>	45.0–63.0	54.2	37	16–46	31.9	18
<i>C. sp. # 107</i>	56.0–81.0	68.8	53	20–65	40.3	24

TABLE 5 Lengths (μm) of specific cephalothoracic and abdominal setae of the pupae of five species of *Culicoides* of the *Imicola* complex

Species	<i>C. imicola</i>		<i>C. bolitos</i>		<i>C. tuttifrutti</i>		<i>C. loxodontis</i>		<i>C. sp. # 107</i>	
	Range	Mean (n)	Range	Mean (n)	Range	Mean (n)	Range	Mean (n)	Range	Mean (n)
Setae										
am	90.5–103.5	97.1 (5)	92.5–127.5	110.2 (56)	89.5–120.0	107.6 (32)	130.5–187.5	170.6 (24)	107.5–155.5	139.1 (27)
ad i	97.5–119.5	109.2 (7)	135.0–184.0	165.7 (37)	113.0–150.0	133.6 (19)	175.0–203.5	191.7 (20)	157.5–185.5	174.3 (19)
ad ii	66.0–91.0	73.9 (8)	100.0–142.5	133.6 (36)	109.0–145.0	125.7 (19)	141.0–175.5	155.5 (19)	79.5–115.0	92.0 (16)
D i	15.0–21.0	18.4 (7)	11.5–25.0	16.8 (35)	17.5–30.0	25.5 (34)	10.0–24.5	15.8 (24)	10.0–26.0	17.0 (35)
D ii	46.0–69.0	59.1 (8)	36.0–73.0	56.5 (35)	45.0–67.5	55.2 (36)	85.5–135.0	108.0 (23)	80.0–130.0	105.2 (24)
D iv	49.0–58.0	53.4 (8)	18.0–31.0	26.4 (27)	35.0–57.5	45.3 (35)	9.0–15.0	11.1 (21)	60.0–107.0	81.0 (31)
Dpm iv	10.0–19.5	16.3 (8)	7.0–11.0	9.1 (24)	6.5–10.5	8.2 (11)	5.5–7.5	6.0 (14)	5.5–8.0	6.3 (11)
dasm i	11.5–16.0	14.5 (8)	6.0–10.0	8.8 (25)	6.3–11.0	8.9 (25)	5.5–7.0	6.0 (14)	5.5–9.0	6.9 (16)
dasm ii	15.0–19.5	16.3 (8)	7.0–10.5	8.9 (26)	10.0–40.0	25.9 (22)	5.0–7.5	6.1 (15)	5.0–9.5	6.6 (17)
Lpm i	15.0–20.0	16.9 (8)	9.0–13.0	10.7 (26)	8.8–17.5	11.3 (16)	5.0–8.0	6.3 (16)	5.5–10.0	7.5 (15)
Lpm ii	18.0–26.0	22.0 (8)	8.5–14.0	10.7 (26)	17.5–40.0	27.6 (17)	5.5–8.0	6.3 (16)	5.5–9.0	7.2 (15)
Lpm iii	16.0–21.0	18.6 (8)	7.5–11.0	10.1 (27)	7.5–15.0	10.7 (16)	5.0–8.0	6.1 (16)	5.5–9.5	7.1 (15)
Lasm	15.0–20.0	16.9 (8)	9.0–14.0	11.2 (27)	7.5–14.5	10.2 (18)	5.0–8.5	6.5 (15)	6.5–10.0	8.0 (15)
Vpm i	12.5–15.5	14.6 (7)	7.5–10.0	9.3 (26)	7.5–10.5	8.9 (15)	5.0–7.5	5.6 (15)	5.0–9.0	6.7 (15)
Vpm ii	15.0–21.0	17.8 (8)	8.0–11.0	9.5 (25)	8.8–30.0	20.0 (16)	5.0–6.0	5.6 (15)	5.5–8.5	6.2 (14)
Vpm iii	13.5–16.0	14.9 (7)	6.0–11.0	8.6 (26)	5.0–10.5	6.6 (14)	5.0–7.5	5.7 (15)	4.5–6.0	5.2 (15)

TABLE 6 Morphological and ecological similarities and differences amongst five species of *Culicoides* of the *Imicola* complex

Species	<i>C. imicola</i>	<i>C. bolitinos</i>	<i>C. tuttifrutti</i>	<i>C. loxodontis</i>	<i>C. sp. #.107</i>
<i>Morphological</i>					
RESPIRATORY ORGAN					
Length (µm)	147.5–160.5	116.0–149.0	140.0–195.5	45.0–65.0	35.0–54.5
Pigmentation	Basal, distal fifths dark ochreous, paler medially	Basal two-fifths darkest	Basal, distal quarters ochreous, paler medially	Uniformly yellow	Uniformly yellow
Annulations	Medial half to three-fifths	Medial third	Medial half	Absent	Absent
Scales	Absent	Absent	Absent	Absent	Absent
Lateral spiracles	3–5	3–5	5–8	Absent	Absent
Terminal spiracles	6–7	3–5	4–7	2–6	3–5
OPERCULUM					
Disc width/length ratio	Wider than long	Wider than long	Wider than long	Wider than long	Wider than long
Spines	Long, setaceous, along anterolateral margins, and with narrow transverse band	Long, setaceous, on anterior half, occasionally between tubercles	Long, setaceous on anterior half and between tubercles	Absent	Absent
CEPHALOTHORAX					
Length of am seta (µm)	90.5–103.5	92.5–127.5	89.5–120.0	130.5–187.5	107.5–155.5
Length of d ii seta (µm)	46.0–69.0	36.0–73.0	45.0–67.5	85.5–135.0	80.0–130.0
Length of d iv seta (µm)	49.0–58.0	18.0–31.0	35.0–57.5	9.0–15.0	60.0–107.0
ABDOMEN					
Tubercle size	Moderately small	Very small	Moderately small	Large, covered with nodules	Moderately large
Apices/spur	Rounded apices	Single, sharp spur	Single, sharp spur	Small, rounded apices	Rounded apices
Setal length, width	lpm i–iii, vpm i–iii equal length and width	lpm i–iii, vpm i–iii equal length and width	lpm ii, vpm ii longer, thinner than lpm I, iii, vpm I, iii	lpm i–iii, vpm i–iii equal length and width	lpm i–iii, vpm i–iii equal length and width
Integument	Smooth	Smooth	Smooth	Adorned with spinules	Adorned with nodules
<i>Ecological</i>					
Larval habitats	Organically enriched grassy margins of drainage furrows, irrigated pastures	Cattle, buffalo and blue wildebeest dung	Decaying fruits of indigenous flora	Elephant dung	White rhinoceros, zebra and horse dung

DISCUSSION

Differential diagnosis

Based on pupal morphology the five species of the *Imicola* complex can be separated into two groups:

- The first group (*C. imicola*, *C. bolitinos*, *C. brevitarsis* and *C. tuttifrutti*) is defined by the possession of a long respiratory organ, by the presence of long setaceous spines on the operculum and by the abdominal integument being almost bare of spinules or nodules.
- The second group (*C. loxodontis* and *C. sp. # 107*) is defined by the respiratory organ being markedly shortened and bulbous, by the absence of long setaceous spines on the operculum and by the abdominal integument being adorned densely with either nodules or spinules.

These, and other, differences are exploited in the key to the five species given below.

Key to the pupae of five Afrotropical species of the *Imicola* complex

1. Respiratory organ moderately long (116.0–195.5 µm), about six times as long as wide and bearing 3–8 spiracles laterally; operculum adorned with long setaceous spines (25.0–50.0 µm); abdominal integument smooth; anteromarginal setae long (89.5–127.5 µm); dorsal ii setae moderately long (36.0–73.0 µm) 2
 Respiratory organ very short (35.0–65.0 µm), not more than twice as long as wide and lateral spiracles absent; operculum without setaceous spines; abdominal integument bearing distinctive nodules or spinules; anteromarginal setae very long (107.5–187.5 µm); dorsal ii setae very long (80.0–135.0 µm) 4
2. Single row of long setaceous spines along anterolateral margins of opercular disc, joined anteriorly by narrow transverse band of spines (Fig. 1B); spur on abdominal tubercles rounded (Fig. 1G) ***C. imicola***
 Dense mass of long setaceous spines on anterior half of opercular disc and between anteromarginal tubercles (Fig. 2B, 4B); spur on abdominal tubercles sharp (Fig. 2G, 4G) 3
3. Respiratory organ moderately long (116.0–149.0 µm) (Fig. 2A); organ bearing 3–5 (4) lateral and terminal spiracles (Fig. 2A); lateral posteromarginal i–iii setae, ventral posteromarginal i–iii setae almost of the same length and width (Fig. 2G) ***C. bolitinos***
 Respiratory organ longer (140.0–195.5 µm) (Fig. 3A); organ bearing 5–8 (6) lateral spiracles and 4–7 (5) terminal spiracles (Fig. 3A); lateral posteromarginal ii setae, ventral posteromarginal ii setae longer and finer than lateral posteromarginal i and iii setae and ventral posteromarginal i and iii setae, respectively (Fig. 3G) .. ***C. tuttifrutti***
4. Anteromarginal tubercles borne on large conical protuberances which almost touch medianly (Fig. 4B); abdominal integument with spinules (Fig. 4G); dorsal iv setae

short, fine (9.0–15.0 µm) (Fig. 4C); anterodorsal ii setae very long, stout (141.0–175.5 µm) (Fig. 4F) .. ***C. loxodontis***

Anteromarginal tubercles borne on slight protuberances (Fig. 5B); abdominal integument with nodules (Fig. 5G); dorsal iv setae long and moderately stout (60.0–107.0 µm) (Fig. 5C); anterodorsal ii setae long and moderately stout (79.5–115.0 µm) (Fig. 5F) ***C. sp. # 107***

This study has shown the pupae to possess highly diagnostic characters that can be used to differentiate species reliably within the *Imicola* complex. Indeed, in some taxa, the differences were marked enough to be visible even at low magnifications. For example, the extremely short respiratory organs of *C. loxodontis* and *C. sp. # 107* are unique not only to the *Imicola* complex but also to the genus *Culicoides*. Also unique are the large paired protuberances, which almost meet medianly on the operculum of *C. loxodontis* and, like the bulbous respiratory organs, can be viewed easily under a stereoscopic microscope.

The division of the five species into two groups—based principally on whether the respiratory organ is bulbous or elongate—to some extent reflects relationships based on the adult imago of both sexes. In this scheme, the majority of the 12 species form distinct species pairs: *C. tuttifrutti*–*Culicoides pseudopallidipennis*, *C. bolitinos*–*C. brevitarsis*, *C. imicola*–*Culicoides nudipalpis* and *C. loxodontis*–*C. sp. # 107*. The relationship between the latter pair is more tenuous, whilst *Culicoides miombo* Meiswinkel, 1991, though showing some affinity with *C. imicola*, stands more isolated. However, these groupings, based on shared similarities in the phenotype, are less convincingly mirrored in a phylogeny based on partial nucleotide sequences of the COI gene (Meiswinkel & Linton 2003) in which *C. tuttifrutti*, along with *C. pseudopallidipennis*, fell separate from all the remaining species (*C. imicola*, *C. bolitinos*, *C. loxodontis* and *C. sp. # 107*). This phylogeny, however, omitted the two taxa *C. brevitarsis* and *C. nudipalpis*, and created significant gaps that, when filled, might significantly alter the topology of the COI tree. Clearly, more studies, both cladistic and molecular, and utilising all constituent taxa, are required to more fully resolve the phylogeny of the *Imicola* complex across its Old World range and to reveal whether the sharing of a bulbous respiratory organ is indeed a homoplasy due to convergence induced in response to certain external pressures rather than the result of direct inheritance from a common ancestor.

As noted above, the Afrotropical *C. bolitinos* is considered to be the sister species of the Oriental–Australasian *C. brevitarsis* and because they are

virtually inseparable in the adult phenotype the question of their possible synonymy has been raised. This possibility is supported by the fact that they are ecological equivalents, both taxa breeding solely in the dung of cattle (*Bos*) and buffalo (*Syncerus* and *Bubalus*). In an attempt to resolve this issue of possible synonymy we compared their respective pupal exuviae in detail but no differences were found. Thus, for the present, it is perhaps best to accept their current status as two good species that arose from a common ancestor, and because the ancestral stocks have continued to exploit the dung of bovids, no species-specific morphological adaptations have arisen consequent to their geographic isolation. It is also assumed that *C. bolitinos* and *C. brevitarsis* have become reproductively isolated under sustained allopatry. However, all these suppositions would benefit from further scrutiny at the molecular level. In regard to intraspecific variation, none was observed to occur amongst the pupae of three Afrotropical species reared from different biotopes. The pupae and biotopes compared were *C. bolitinos* from buffalo and cattle dung, *C. tuttifrutti* from the fruits of the sausage and marula trees, and *C. sp. # 107* from white rhinoceros and zebra dung.

The pupae of most *Culicoides* spp. are aquatic and have the ability to float at the water's surface where the respiratory organs, attached to the meniscus, obtain oxygen. Interestingly, where known, the pupae of species of the subgenus *Avaritia* differ fundamentally from those of all other species of *Culicoides* in that, once submerged, the pupae are unable to rise to the surface and so will eventually drown (Jamnback & Wirth 1963; Nevill 1967). It is therefore not surprising that the larval habitats of species of the subgenus *Avaritia* are restricted to substrates that, though moist, are not waterlogged. These substrates include organically-enriched damp soil (*C. imicola*), elephant dung (*C. loxodontis*), white rhinoceros, zebra and horse dung (*C. sp. # 107*), buffalo, cattle and wildebeest dung (*C. bolitinos*), decaying fruits (*C. tuttifrutti*) and other rotting vegetative materials such as fallen banana plant stems (*C. pseudo-pallidipennis*).

Four species of the *Imicola* complex breed exclusively in the dung of herbivores indigenous to Africa and south-east Asia. This co-dependency ensures the spread of coprophilic *Culicoides* into areas newly colonised by their preferred vertebrate host. For example, *C. loxodontis* is found only in the near vicinity of elephants (Meiswinkel 1992; Meiswinkel & Braack 1994) but because it seems unable to switch to another breeding resource—such as cattle dung—

it has not been able to disperse into the livestock farming community. The opposite is true for *C. bolitinos*. Its successful switch from buffalo to cattle dung has enabled it to penetrate into all areas where cattle occur. Coincidentally, it has emerged that *C. bolitinos* is also an important vector of both BTV and AHSV which, along with *C. imicola*, ensures the almost constant circulation of orbivirus diseases amongst husbanded livestock throughout much of South Africa. This ability of vector *Culicoides* spp. to switch from one vertebrate host to another, should stimulate investigations into the possible involvement of *C. sp. # 107* in the transmission of AHS. There is little doubt that *C. sp. # 107* is closely associated with the plains zebra, which is known to asymptotically circulate all nine serotypes of AHSV (Barnard 1993). It is also known that *C. sp. # 107* will appear around horse stables in the warmer regions of South Africa but mostly during the winter season (Meiswinkel 1995).

CONCLUSION

This study brings to 26 the total number of pupae described for Afrotropical *Culicoides*, which represents a mere 17% of the 157 species known. This statistic illustrates how slowly studies on the breeding habitats of biting midges are progressing. The preferred tool for sampling *Culicoides* in the field in Africa is the light trap. However, its usefulness is limited to providing only single-point measurements of relative midge abundance and geographic distribution. This gives little insight into the ecologies of the many species that exist in nature. To elucidate these ecologies, it is necessary to go a step further, namely to extract larvae and pupae from an infinite variety of moist and semi-moist substrates. Only by pinpointing the precise breeding habitat of a species can the edaphic factors determining its occurrence in nature be identified. This information is vital for refining models predicting vector distribution patterns, which are essential if areas at risk to *Culicoides*-borne orbiviral diseases are to be more accurately delineated.

ACKNOWLEDGEMENTS

We wish to sincerely thank Alan L. Dyce (Australian order of Merit), Honorary Research Fellow, CSIRO, Canberra, Australia for providing the pupae and associated link-reared adults of *C. tuttifrutti* and other species of South African *Culicoides* and for pupae of *C. brevitarsis* from Australia. We would also like to thank our various colleagues who assisted with

the field collection of zebra, white rhinoceros and elephant dung. All the research was funded by the ARC-Onderstepoort Veterinary Institute.

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