CHAPTER 6

Afrotropical *Culicoides*: a redescription of *C. (Avaritia) pseudopallidipennis* Clastrier, 1958 with the description of its fruit-inhabiting sister species *C. (A.) tuttifrutti* sp. nov. (Diptera: Ceratopogonidae)

6.1 INTRODUCTION

Culicoides pseudopallidipennis was described by Clastrier in 1958 from four females collected in the Niokolo-Koba National Park and in Dakar, Senegal. In the ensuing years it went virtually unnoticed, seldom being referred to in the literature, and only redescribed once (Glick, 1990).

From Angola, Caeiro (1961) reported specimens of *C. imicola* that perfectly matched Clastrier's description of *C. pseudopallidipennis*. However, he felt, firstly, that they represented individual variation normal to *C. imicola*, and thus did not warrant species status, and secondly, that the final acceptance of *C. pseudopallidipennis* depended upon a detailed study of more females and the discovery of the still unknown male. Cornet (1969) noted that *C. pseudopallidipennis* was rare in Senegal, having seen only two females from Niokolo-Koba, while Boorman & Dipeolu (1979) listed it as one of the known West African species not encountered during their taxonomic survey of the *Culicoides* of Nigeria. Wirth & Hubert (1989) assigned *C. pseudopallidipennis* to the Orientalis species group of the subgenus *Avaritia*, whereas Meiswinkel (1991, 1992) placed it in the Imicola group. In 1990 Glick recorded it for the first time from Kenya (four ♀♀) and Zimbabwe (one ♀), and described what appeared to be the hitherto unknown male from Ol Doinyo Sabachi, Kenya. He noted that 'the immature stages, larval habitat ... and feeding habits of the adults' of *C. pseudopallidipennis* remained unknown. In short, its rarity in collections has meant that understanding of the taxonomic status of *C. pseudopallidipennis* s.s. and knowledge of its biology have developed little over the past 35 years.

During the abnormally rainy summer of 1973/74, an *imicola*-like species was reared by Alan Dyce (unpublished data) from rotting fruits of the sausage tree (*Kigelia africana*; Bignoniaceae) in the eastern

Transvaal lowveld, South Africa. At the time, in personal correspondence, Dr. Michel Cornet of ORSTOM identified it as a new species allied to *C. pseudopallidipennis*. Independently, I commenced a study of South African *Culicoides* in the late 70's and collected low numbers of the same species in light-traps in the north-eastern Transvaal and northern Natal, referring to it as C. sp. #30.

Only in 1990, after a good series of males and females of *C. pseudopallidipennis* s.s. had been caught near Yamoussoukro, central Côte d'Ivoire, did it become obvious that two, albeit closely related, species existed, the West African material having paler wings and legs and a significantly higher antennal ratio in the female. Three of Clastrier's original type series of four specimens were then obtained on loan; though they are in a relatively poor state, the holotype has the pale legs and wing, and high antennal ratio, typical of the central Côte d'Ivoire material on which the redescription of *C. pseudopallidipennis* is now based. The description of *C. pseudopallidipennis* and *C. tuttifrutti* sp. nov. are preceded by brief notes on the condition and identity of the holotype (a) and two paratypes (b, c) of *C. pseudopallidipennis*. In Clastrier's original description of *C. pseudopallidipennis* four ♀♀ were recorded from Senegal. He informs us that he was unable to trace the third female from Niokolo-Koba. These notes are followed by brief observations on the condition and status of two males (d and e) reared from a banana stump in The Gambia and reported upon by Clastrier & Wirth (1961).

Although larvae and pupae of *C. tuttifrutti* sp. nov. retrieved from rotting *Sclerocarya* fruits by ALD in 1993 constitute part of the type series, they will be described elsewhere (Hilda Nevill, personal communication, OVI, 1994).

6.2 MATERIALS AND METHODS

Specimens were collected in blacklight traps of the type described in Meiswinkel (1989) or reared from the fallen fruits of the maroela (*Sclerocarya*) and the sausage tree (*Kigelia*). Fruits were either brought in from the field and stored in a dark container to await emergence of midges or were immediately soaked and washed in water, pupae and larvae being brought to the surface using sugar flotation. All slide material has been mounted in Canada balsam after being cleared in 10 % KOH, neutralized in 10 % acetic acid, washed once in 96 % ethanol, stored in clove oil and finally dissected in a 50:50

phenol/balsam mixture.

6.2.1 Statistical analyses

As *C. pseudopallidipennis* and *C. tuttifrutti* are considered to be sister species within the taxonomically complex Imicola group of six known species in the Region, statistical analyses are done on the numerical data generated from the lengths of the antennal flagellar segments.

Comparison of the mean antennal flagellar lengths of segments III–XV were made between three separate populations: *C. pseudopallidipennis* (central Côte d'Ivoire), *C. tuttifrutti* sp. nov (South Africa) and *C. tuttifrutti* sp. nov. (northern Côte d'Ivoire); 21 randomly chosen specimens (raw measurements) per population were compared by means of an analysis of variance for a completely random design. For some measurements, heterogeneity of variances occurred at $P > 0{,}001$ and consequently the non-parametric Kruskal-Wallis analysis was used. Means for the latter test are given in brackets (Table 6.9b). The Bonferroni method was used for multiple comparisons of means for the parametric tests and the Dunn method for non-parametric tests.

6.3 RESULTS

6.3.1 Brief notes on holotype and paratypes of *C. (A.) pseudopallidipennis*

a. Holotype ♀; label data: *C. pseudopallidipennis* n.sp. ♀. Type. 1535A. No other data. 'Type' is written in pencil near the top of the label and ½-encircled with red crayon; a red 'T' has been written over the pencilled word. Dr. Clastrier (in litt., 1991) informs me that batch no. 1535 was collected at ultraviolet light by E. Abonnenc at Badi, Niokolo-Koba National Park, July 1953. Holotype mounted under two coverslips in a resin; in fair condition but mountant deteriorating, with numerous small spots of brown flocculent developing; wings and one set of legs separate from remainder of insect; coverslip under which head with one attached antenna, scutum, three legs and abdomen are found is cracked through the middle; two large air-bubbles are forming. Margins of bodyparts are also drying out, creating a blackish refractive halo rendering precise observation of shapes and enumeration of setae and sensillae difficult. Furthermore, all bodyparts are squashed or distorted by shrinkage making measurements imprecise. The values and ratios given in the following brief redescription must therefore be accepted with caution: Head squashed, eyes bare, cibarium unarmed; P/H ± 0,91; length entire palp 185 μm; length palp III 57,5 μm, width palp III 30 μm, PR 1,92; palp III is obviously inflated but is also squashed; mandible with 14 fine teeth; one antenna missing; other attached to head and in rather poor condition: most segments either squashed or semi-collapsed; left margin of most basal segments blackened by air-bubbles thus obscuring sensillae; basal segments short and roundish, AR ± 1,15; blunt-tipped sensilla trichodea on basal segments inflated and rather short, AtR 1,5; sensilla coeloconica III, XI–XV; exact number of sensilla chaetica impossible to establish as alveoli of missing chaetica may be present or absent but obscured by blackening of lateral margins of basal segments. Scutum facing dorsally, squashed, with 80 bristles; scutellum attached to abdomen with one central and two lateral bristles; abdomen mounted laterally, squashed; two neckless spermathecae collapsed/squashed; three legs attached to abdomen appear entirely pale but absence of banding pattern may be due to bleaching; three legs mounted with wings under second coverslip are brownish throughout due to ageing mountant which again obscures true banding pattern; one wing (Fig. 6.5) in good condition, predominantly pale: second and third pale costal spot (p.c.s.) broadly fused under radial cells leaving dark spot covering radial cells isolated on anterior wing margin; anterior margin of distal pale spot in R_5 not strongly pointed; apex M_2 mostly pale.

b. Paratype ♀; label data: *C. pseudopallidipennis* n.sp. ♀. 1426 F. No other data. Dr. Clastrier (in litt., 1991) advises me that this specimen comes from the same collection as the holotype ♀ and should be re-numbered 1535 F. I have done so in red crayon, and merely crossed through, but not deleted, the black-inked '1426 F'. Specimen in similar, if not poorer, condition as the holotype. Legs attached to abdomen are not as bleached as in holotype and display reduced brown areas leaving the legs predominantly pale. Examination of character states reveals it be representative of the same species as the holotype.

c. Paratype ♀; label data: *C. pseudopallidipennis* n. sp. ♀. 1519 A. No other data. Dr. Clastrier (in litt., 1991) states that batch no. 1519 came from Dakar, coming to light in the evening, collected by E. Abonnenc, October 1957, i.e. four years after the three Niokolo-Koba females had been captured. This specimen is in a similarly poor state, but eight features persuade me that it is a species different from the Niokolo-Koba pair; these character states typify *C. tuttifrutti* sp. nov. and are:

- wings darker: second and third p.c.s. rather broadly separated under radial cells
- anterior margin distal pale spot in cell R_5 more definitely pointed
- both margins of M_2 moderately darkened
- dark spot adjoining wing margin medially in anal cell is narrowly fused with dark spot covering medio-cubital fork
- AR ± 1,08
- scutum has at least 90 bristles but likely more as parts of the squashed and fragmented scutum appear to be missing
- basal antennal segments barrel-shaped and not rounded
- legs with more extensive dark areas

As regards the poor condition of the type series of *C. pseudopallidipennis*, Dr. Clastrier has communicated (in litt., 1991) that he had in earlier years mounted in various media. These included a resin that Grenier and Taufflieb had recommended but which had, over time, catastrophic effects: all bodyparts became deformed to an irreversible degree. He therefore advises that one must rely on his published drawings, and less on the slide preparations. An example of the negative effects of shrinkage and compression can be seen in the differing PR measurements. Clastrier's illustration and text on *C. pseudopallidipennis* gives the PR as 2,20 and 2,35 respectively while ratios obtained from the holotype and a paratype (1426 F) are 1,92 and 1,83 respectively. These values fall below the range recorded for *C. pseudopallidipennis* (n = 46) from Côte d'Ivoire, but compression of the palps would artificially expand their width and so lower the PR value.

d,e Two ♂♂; label data: BR.W.AFRICA, Gambia, Keneba, W. Kiang Dist., 15 Nov 1959. D.H. Murphy Ga 316 Manduar, W. Kiang, banana stump. *Culicoides pallidipennis* (C.I. & M.). A 'U' in black ink is found on both labels. Excepting for the wings, both specimens are poorly mounted; in the genitalia the basimeres have collapsed and the membrane of sternum IX is obscured; the antennae are collapsed and are either missing or obscured by the head; the scutum is crushed. Nevertheless, these two males possess six features typical of *C. pseudopallidipennis*:

- legs very pale with little sign of banding; femoral-tibial knees obviously darkened;
- wings pale: vein M_2 without preapical excision, apex pale;
- anterior margin of distal pale spot in R_5 not markedly pointed;
- genitalia: though basimere collapsed is ± 2,9 × as long as wide;
- palps: though badly collapsed appear to be inflated;
- segment VI of one antenna could be examined only with difficulty: second long blunt-tipped trichodea present but clearly underdeveloped.

6.3.2 *Culicoides (Avaritia) pseudopallidipennis* Clastrier 1958.

Culicoides pallidipennis Carter, Ingram & Macfie: Clastrier & Wirth, 1961: 307 (misident.)

(Fig. 6.1–6.9; Table 6.1–6.4)

Female (Fig. 6.1–6.6, 6.20; Table 6.1–6.3)

Head. Eyes (Fig. 6.1); bare, contiguous over a distance equal to the diameters of 1,1–1,9 (mean 1,5) adjacent facets (n = 15). Antenna (Fig. 6.2, 6.20; Table 6.1–6.3) slender basal segments IV–VI almost round, VII–X barrel-shaped, distal segments XI and XII slightly flask-shaped narrowing subapically, XIII and XIV more or less parallel-sided, XV more or less parallel-sided only narrowing apically; mean lengths of antennal segments III–XV: 36,8–22,2–22,0–24,2–25,3–25,6–25,8–28,2–45,8–46,3–47,4–46,8–74,8 μm (n = 46); total length of antenna: 453,8–530,0 μm, mean 488,0 μm (n = 46); widths of antennal segments III–XV: 30,0–24,4–21,3–21,3–19,4–20,0–18,8–18,8–16,9–17,5–17,5–17,5–17,5 μm (n = 1); AR 1,13–1,24, mean 1,19 (n = 45); sensilla coeloconica present on segments III, XI–XV in all antennae examined (n = 48); antenna with a total of eight to nine coeloconica, mean 8,1 (n = 48) see Table 6.1 for number and frequency of coeloconica present per segment; mean sensilla chaetica distribution on segments III–XV: 4,98–3,04–2,00–3,83–2,00–3,83–2,00–2,98–0–0–0–0–0 (n = 48), antenna with a total of 23–26 chaetica, mean 24,67 (n = 48) see Table 2 for number and frequency of chaetica present per segment; blunt-tipped sensilla trichodea distribution of the LLc type, i.e. each of segments IV–X with two long and one short sensilla trichodea, segment III with only two long sensilla trichodea (n = 48); the long trichodea are of a moderate length and strongly swollen (Fig. 6.2); each of segments XI–XV with one or two short and thin blunt-tipped trichodea, these indicated by an arrow in Fig. 6.2. AtR 1,60–1,98 mean 1,74 (n = 23); segments XI–XIV each with 14–17 sharp-tipped sensilla trichodea of varying lengths and thicknesses distributed in a basal and subapical whorl; XV with approximately twice as many trichodea, these distributed almost throughout segment (Fig. 6.2); similarly the short blunt-tipped basiconica number from three to nine on each of the distal flagellar segments XI–XV (Fig. 6.2); segments III and IV with a single sensilla ampullacea; all antennal segments uniformly clothed throughout with fine spiculae (Fig. 6.2). The range and mean number of sensilla coeloconica, chaetica and blunt-tipped trichodea are given in Table 6.3. Palp (Fig. 6.3): moderately long, light brown, third segment swollen, mean length of segments I–V: 24,13–51,55–56,88–28,55–26,63 μm (n = 46) total length of palp 157,5–195,6, mean 175,3 μm (n = 46); segment I with one moderately long chaetica (n = 46), II with three to four slightly shorter chaetica, mean 3,07 (93 % with three chaetica; n = 46); III rather long and swollen bearing three to six chaetica, mean 4,15 (26 % with three; 41 % with four and 24 % with five chaetica; n = 46), width 22,5–26,25, mean 23,95 μm (n = 46), with a moderately large, round but rather shallow subapical pit with diameter of opening 0,39–0,63, mean 0,49 (n = 28) the width of segment, margin of pit smooth and well defined; IV with two to five short chaetica, mean 3,09 (76 % with three chaetica; n = 46); V with no chaetica on median portion of segment but bears four to six mean 5,0 (n = 46) short erect bristles apically; palp with a total of 14–19 chaetica, mean 16,3 (n = 46); PR 2,03–2,65, mean 2,38 (n = 46); P/H ratio 0,83–0,99, mean 0,91 (n = 17); mandible with 12–16 fine teeth, mean 14,1 (n = 45).

Thorax. Legs: all femora predominantly pale with median ⅓ brown; tibiae also predominantly pale, possessing a narrow subbasal pale band followed by a moderately narrow brown band which indistinctly fades into a pale apical ⅓–½; TR 1,50–1,64, mean 1,58 (n = 23); comb on apex of hind tibia with five or six spines, mean 5,07 (n = 45), the first being the longest and only slightly longer than the second. Wing (Fig. 6.6): length 0,83–0,97 mm, mean 0,89 mm (n = 45), breadth 0,40–0,47 mm, mean 0,43 mm (n = 45), CR 0,57–0,61, mean 0,59 (n = 45); macrotrichia scanty, confined to distal ⅓ of cell R_5 with fewer at the apex of cell M_1 and almost none in M_2; microtrichia dense and coarse. Dark areas of wing grey, pale areas white to yellowish; pale areas of wing extensive, fairly well defined but irregularly shaped; two short radial cells equal in length, proximal ½ of first and distal ⅔ of second pale. Due to the increase in size of pale areas, and reduction of the dark areas, the wing of *C. pseudopallidipennis* is paler than that of *C. imicola, C. bolitinos* and *C. miombo*, but comes closest to that of *C. loxodontis*. Important but variable species-specific wing-pattern characters of *C. pseudopallidipennis* are:

(i) in darker specimens the second and third pale costal spots are narrowly separated under the radial cells, but usually these spots are narrowly to broadly fused under the radial cells; in the palest specimens this fusion leaves the dark area straddling the radial cells clearly isolated from the dark area that straddles vein M_1 proximally;

(ii) proximal margin of the distal pale spot in cell R_5 more rounded than pointed; this spot always abuts wing margin broadly;

(iii) vein M_2 variable: can have both margins darkened and gradually tapering to leave the apex of M_2 narrowly dark, i.e. no preapical excision present; in paler specimens, on both margins, a broad, poorly defined excision leaves the entire apical ⅓ of M_2 pale with only the extreme apex of the vein indistinctly darkened;

(iv) dark spot found medially in anal cell and abutting posterior wing margin, is small and clearly isolated from the dark area covering the Cu_1 and $M_{3\ +\ 4}$ fork; only rarely, and in darker specimens, are these two dark areas narrowly fused;

(v) anal angle pale.

Scutum entirely brown in alcohol, bearing 76–109 bristles of varying lengths mean 89 (n = 17); scutellum entirely brown, bearing one to two long median bristles (one in 96 % of specimens; n = 23) and one shorter bristle in each corner. Haltere knobs pale. Abdomen (Fig. 6.4): two moderately sclerotized, slightly unequal spermathecae present, measuring 50,0–64,4 × 40,0–45,0 μm, mean 57,2 × 42,4 μm (n = 14) and 45,0–52,5 × 35,0–40,0 μm, mean 49,1 × 37,2 μm (n = 15), both devoid of small hyaline punctations, with moderately long, narrow, rudimentary third spermathecae present measuring 10,0–22,5 × 3,8–15,6 μm, mean 15,9 × 6,5 μm (n = 13), small sclerotized ring on common spermathecal duct cylindrical, smooth and parallel-sided, a little longer than broad, and about half the length of the rudimentary spermatheca; sclerotization surrounding the oviduct as shown in Fig. 6.4.

Male

Head. Eyes bare. Antenna (Fig. 6.19, Table 6.3, 6.4): plume moderately sparse, fibrillae light brown, almost completely encircling medially each of segments IV–XII in a regular whorl; these segments with very few spiculae which are on one face only; distal segments XIII–XV densely and evenly clothed with spiculae; mean lengths of segment III: 71,3, IV–XII: 322,9, XIII–XV: 89,6–70,2–87,5 μm; total mean length 641,5 μm (n = 34); sensilla coeloconica distribution: segment III with two (n = 34); XIII with one to two, mean 1,09 (92 % with one coeloconica; n = 34); XIV with one to two, mean 1,03 (97 % with one coeloconica; n = 34); XV with one to two, mean 1,97 (97 % with two coeloconica; n = 34); antenna with a total of five to seven coeloconica, mean 6,12 (n = 34); sensilla chaetica distribution: five of varying lengths and thicknesses on III; two basally (first long and robust, second shorter and weaker) and one medially (being very slender and 1,5 × longer than segment) on XIII; two basally (both slender but of different lengths, the one a little shorter than segment, the other fractionally longer than segment) on XIV; none basally on XV, only one apically (n = 34); blunt-tipped sensilla trichodea distribution on segment III–XII variable especially on segments V and VI (Table 6.4; Fig. 6.19): III with two long trichodea, IV with two long and one short trichodea (exceptionally one of the long trichodea may be shorter and thinner), V with two long and one short trichodea (exceptionally one of the long trichodea absent while in 35 % of the specimens it will be shorter and thinner), VI with two long and one short trichodea (in 15 % of specimens one of the long trichodea absent while in 68 % of specimens it will be shorter and thinner), VII–IX with one long and one short trichodea (only in one antenna was an additional long but under-developed trichodea found on VII), X with one short trichodea only while XI and XII lack trichodea (n = 34). The range and mean number of sensilla coeloconica, chaetica and trichodea appear in Table 6.3; the marked variability in blunt-tipped trichodea distributions is detailed in Table 6.4.

Thorax: Scutum with 68–78 bristles mean 72 (n = 8); scutellum with one median bristle only (n = 19). Wing: (Fig. 6.7). Genitalia (Fig. 6.8, 6.9): tergum nine (Fig. 6.8) square, slightly waisted medially, finely spiculate throughout except for narrow strips of the anterior and posterior margins being bare, bearing 15–21 chaetica of different lengths, mean 17,7 (n = 19); apicolateral processes replaced by broadly rounded but thinly membranous hyaline flanges lacking spiculae, but each carrying a single fine, rather short sensilla chaetica inserted at the base of each of the flanges where the main spiculate body of the tergum commences; posterior margin of tergum which separates flanges is gently to moderately concave, medially sparsely striated but not infuscate (Fig. 6.8); two well-developed cerci (Fig. 6.9), each adorned with long spiculae and two long and two short chaetica; cerci protrude well beyond posterior margin of tergum; sternum nine (Fig. 6.9) with a rather deep and wide excavation, membrane within the excavated area with 0–20 spiculae, mean 6,8 (n = 19), of these 10 % had no spiculae; basimere with dorsal and ventral spiculae and chaetica as illustrated (Fig. 6.9), basimere 2,7–3,0 mean 2,9 × as long as broad (n = 10) with basal infuscate collar and well-developed dorsal and ventral roots of the form typical of the subgenus *Avaritia*. Distimere (Fig. 6.9) 0,70–0,75, mean 0,72 × length of basimere (n = 10), rather stout, gently curved and broadly blunt-tipped; with bristles and spiculae as illustrated. Aedeagus (Fig. 6.9) shield-shaped, slender 1,7 × longer than wide and 0,85 × length of basimere (n = 10); basal margin concave lightly to moderately infuscate, distal margin of arch reaching to 0,24–0,32, mean 0,27 × length of aedeagus (n = 10); lateral margins of the aedeagus smooth and gently convex, darkly but narrowly infuscate and converging distad to end in a hyaline, moderately long, parallel-sided terminal projection whose apex is smoothly rounded (Fig. 6.9); base of this terminal projection inserts anteriorly into median area of aedeagus in the form of a raggedly infuscate ‘peg’ (Fig. 6.9). Parameres (Fig. 6.9) separate, nearly touching medially from where they diverge anteriad and posteriad at approximately 45 °; posterior halves as two convex almost hyaline blades, basally stout but tapering smoothly to sharp, simple, erect tips.

Slide material examined

SENEGAL:

One ♀ (holotype)	*Culicoides pseudopallidipennis* n. sp., slide 1535A; no further data on label except a pencilled notation ‘Type’ half-encircled in red crayon.
Two ♀♀ (paratype)	*Culicoides pseudopallidipennis* n. sp., slides 1519 A and 1426 F; no further data on labels as to possible status, locality or dates. See notes as to identification under ‘Materials and Methods’.

IVORY COAST:

24 ♀♀, 19 ♂♂	slides labelled and numbered to sex, the collection data as follows:
♀♀	Slides Ivory Coast 333–335, 337, 339, 341, 343, 345, 348, 349, 351, 353, 356, 370–373, 426, 428, 430, 432, 434, 436, 438; Gofabo, ± 20 km n.e. of Yamoussoukro; blacklight at 20 cattle in kraal; 19.IX.1990, R. Meiswinkel, L.E.O. Braack & G.J. Venter.
♂♂	Slides Ivory Coast 336, 338, 340, 342, 344, 346, 352, 354, 355, 422–425, 427, 429, 431, 435, 437; same collection data as for females.

THE GAMBIA:

Two ♂♂ BR.W.AFRICA, Gambia, Keneba, W. Kiang Dist., 15 Nov. 1959. D.H. Murphy Ga 316 Manduar, W. Kiang, banana stump.

6.3.3 *Culicoides (Avaritia) tuttifrutti* sp. nov. (Fig. 6.10–6.20; Table 6.5–6.7)

Culicoides pseudopallidipennis Clastrier: Clastrier 1958:197. Senegal, Dakar; paratype ♀ 1426 F (misident.)

Culicoides pseudopallidipennis Clastrier: Glick 1990:127. Kenya, Zimbabwe (misident.)

Female (Fig. 6.10–6.13, 6.15, 6.16, 6.20: Table 6.5–6.7)

Head. Eyes (Fig. 6.10); bare, contiguous over a distance equal to the diameters of 1,2–1,9, mean 1,66 adjacent facets (n = 20). Antenna (Fig. 6.11, 6.20; Table 6.5–6.7) slender basal segments IV and V almost round, VI-IX barrel-shaped, X slightly flask-shaped, distal segments XI–XIV flask-shaped, XV more or less parallel-sided only narrowing apically; mean lengths of antennal segments III–XV: 37,65–25,40–24,93–27,03–28,28–28,08–28,03–30,78–44,05–43,95–45,43–45,93–73, 38 µm (n = 104); total length of antenna: 405,0–551,25, mean 498,15 µm (n = 104); widths of antennal segments III–XV: 31,25–25,00–22,50–21,88–19,38–20,00–19,38–17,50–16,25–16,88–16,88–16,88–17,50 µm(n = 1);AR 0,98–1,13, mean 1,06 (n = 104); sensilla coeloconica present on segments III, X–XV in 4 %, III, XI–XV in 93 %, and III, XII–XV in 3% of all antennae examined (n = 104); antenna with a total of seven to ten coeloconica, mean 8,38 (n = 104) see Table 6.5 for number and frequency of coeloconica present per segment; an unusual feature is that on 15 antennae 18 pairs of coeloconica on segment III were fused; these were broader than a normal coeloconica and were counted as two. Mean sensilla chaetica distribution on segments III–XV: 4,90–3,15–2,01–3,56–2,06–3,91–2,15–2,97–0,01–0–0–0–0 (n = 105); antenna with a total of 23–28 chaetica, mean 24,74 (n = 105) see Table 6.6 for number and frequency of chaetica present per segment; blunt-tipped sensilla trichodea distribution of the LLc type, i.e. each of segments IV–X with two long and one short sensilla trichodea, segment III with only two long trichodea (n = 105); the long trichodea are of moderate length and strongly swollen (Fig. 6.11); each of segments XI–XV with one or two short and thin blunt-tipped trichodea, these indicated by an arrow in Fig. 6.11; AtR 1,24–1,86, mean 1,59 (n = 55); segments XI–XIV each with 18–20 sharp-tipped sensilla trichodea of varying lengths and thicknesses distributed in a basal and subapical whorl; XV with approximately twice as many trichodea, these distributed almost throughout segment (Fig. 6.11); segments XI–XV each with three to ten short blunt-tipped basiconica (Fig. 6.11); segments III and IV each with a single sensilla ampullacea; all antennal segments uniformly clothed throughout with fine spiculae (Fig. 6.11). The range and mean number of sensilla coeloconica, chaetica and blunt-tipped trichodea are given in Table 6.7. Palp (Fig. 6.12): moderately long, light brown, third segment swollen; mean lengths of segments I–V: 21,32–48,95–56,62–28,66–27,92 μm (n = 95), total length of palp 140,0–196,25, mean 172,43 μm (n = 95); segment I with one moderately long chaetica (n = 102), II with three to five slightly shorter chaetica, mean 3,20 (81 % with three chaetica; n = 102); III rather long and swollen bearing three to eight chaetica, mean 4,75 (14 % with three; 34 % with four; 26 % with five chaetica and 16 % with six chaetica; n = 102), width 21,88–31,25 mean 26,37 μm (n = 101), with a moderately large, round but rather shallow subapical pit with diameter of opening 0,36–0,62, mean 0,49 (n = 20) the width of segment, margin of pit smooth and well defined; IV with two to six short chaetica, mean 3,75 (32 % with three chaetica, 38 % with four chaetica and 16 % with five chaetica; n = 102); V with no chaetica on median portion of segment but bears four to six, mean 5,0 (n = 102) short erect bristles apically; total chaetica on palp 14–22, mean 17,7 (n = 102); PR 1,80–2,57, mean 2,15 (n = 101); P/H ratio 0,82–0,99, mean 0,90 (n = 25); mandible with 12–16 fine teeth, mean 13,7 (n = 100).

Thorax. Legs: all femora predominantly brown, narrowly pale basally and subapically, this subapical pale area inconspicuous on hind femora; fore and middle tibiae also predominantly brown with well defined narrow subbasal pale band only; hind tibia with subbasal pale band followed by a moderately broad brown band occupying median half of tibia but fading imperceptibly into a moderately broad pale apical third; TR 1,44–1,70, mean 1,57 (n = 52); comb on apex of hind tibia with four to six spines, mean five (n = 103) the first being the longest and only slightly longer than the second. Wing (Fig. 6.13): length 0,80–1,05 mm, mean 0,93 mm (n = 88), breadth 0,40–0,53 mm, mean 0,47 mm (n = 88), CR 0,55–0,60, mean 0,57 (n = 88); macrotrichia scanty confined to distal ⅓ of cell R_5 with fewer at the apex of cell M_1 and almost none in M_2; microtrichia dense and coarse. Dark areas of wing grey, pale areas white to yellowish; pale areas of wing extensive, fairly well defined but irregularly shaped; two short radial cells equal in length, proximal half of first and distal half of second pale. While the wing of *C. tuttifrutti* is predominantly pale it shows some variability especially the patterning along vein M_2 and in the anal cell. The wing comes closest to that of *C. pseudopallidipennis* and *C. loxodontis*. The slender third palpal segment of the latter species readily aids separation of specimens in alcohol. However, *C.*

pseudopallidipennis and *C. tuttifrutti* both have this segment swollen. Indeed, if these two species were to be found sympatrically it would be difficult to separate them on wing pattern alone. Some small differences to be noted are:

(i) in *C. tuttifrutti* the second and third pale costal spots are mostly narrowly separated under the radial cells, but never fuse to leave the dark area straddling the radial cells isolated from the proximal dark area straddling vein M_1 as is often seen in *C. pseudopallidipennis*;

(ii) proximal margin of the distal pale spot in cell R_5 always moderately to strongly pointed, not rounded or slightly pointed as in *C. pseudopallidipennis*; this spot always abuts wing margin broadly;

(iii) vein M_2 very variable: preapical excision present from being broad and well defined, to very broad, almost losing definition when nearly obliterating dark apex of vein M_2 by broadly straddling the vein;

(iv) dark spot found medianally in anal cell and abutting posterior wing margin can be either small and isolated or larger and fusing with the dark area covering the Cu_1 and M_{3+4} fork. Anal angle always pale.

Scutum (Fig. 6.15) entirely brown in alcohol, bearing 82–147 bristles of varying lengths mean 112 (n = 12); scutellum entirely brown, bearing one to two long median bristles (one in 95 %; n = 52) and one shorter bristle in each corner. Haltere knobs pale. Abdomen (Fig. 6.16): two moderately sclerotized, slightly unequal spermathecae present, measuring 47,5–59,4 × 37,5–43,8 μm, mean 52,6 × 40,7 μm and 37,5–46,3 × 30,0–36,3 μm, mean 41,7 × 33,2 μm (n = 21), both devoid of small hyaline punctations; moderately long, narrow rudimentary third spermathecae present measuring 12,5–19,4 × 3,1–6,9 μm, mean 14,8 × 5,1 μm (n = 24), small sclerotised ring on common spermathecal duct cylindrical, smooth and parallel-sided, a little longer than broad, and about half the length of the rudimentary spermatheca; sclerotisation surrounding the oviduct as shown in Fig. 6.16.

Male (Fig. 6.14, 6.15, 6.17–6.19; Table 6.7, 6.8)

Virtually inseparable from *C. pseudopallidipennis*. Tables 6.3, 6.4, 6.7 and 6.8 list differences in:

1. degree of atrophication of the long blunt-tipped sensilla trichodea on flagellar segments IV–VI (Fig. 6.19); as mentioned elsewhere, the fluctuation in size of this sensilla has not been measured meticulously, and until fully assessed through larger series and various populations, cannot at this stage be considered reliable for species separation;

2. differences in the cumulative total length of flagellar segments IV–XII are significant but here cognisance must be taken of the size of a specimen examined;

3. the degree of sclerotisation of the anterior margin of the aedeagus: as shown in Fig. 6.9 (*C. pseudopallidipennis*) and Fig. 6.18 (*C. tuttifrutti*), it can differ between the species but also varies intraspecifically;

4. bristles on the scutum: *C. pseudopallidipennis* has 68–78, mean 72 (n = 8) fewer than the 88–110, mean 101 (n = 8) recorded for *C. tuttifrutti*. Though not investigated, the size of an individual may correlate positively with the number of bristles on the scutum. If this is so, it may lead to overlap in bristle numbers, and so complicate accurate identification.

Slide material examined

Type series:

SOUTH AFRICA: Transvaal

Holotype ♀ (White River 58), farm Sunrise 2 km s. of White River, eastern Transvaal (23°53’S, 28°53’E), blacklight at horse-stables, 16.I.1993, R. Meiswinkel

Paratypes: 21 ♀♀ (slides White River 39, 41, 43, 45, 52–57, 59, 61–65, 67, 69, 71–73), farm Sunrise 2 km s. of White River, eastern Transvaal, blacklight at horse-stables, 16.I.1993, R. Meiswinkel.

Ten ♂♂ (slides White River 42, 44, 46–50, 66, 68, 70), farm Sunrise 2 km s. of White River, eastern Transvaal, blacklight at horse-stables, 16.I.1993, R. Meiswinkel.

Three ♀♀ (slides White River 9–11), farm Kumula 3 km s.w. of White River, eastern Transvaal, blacklight at homestead, 16.IV.1992, D. & J. Dodds.

One ♂ (slide White River 14), farm Kumula 3 km s.w. of White River, eastern Transvaal, blacklight at homestead, 16.IV.1992, D. & J. Dodds.

Six ♀♀ (slides White River 27–32), farm Kumula 3 km s.w. of White River, eastern Transvaal, blacklight at horse-stables, 11.III.1992, R. Meiswinkel. Two ♂♂ (slides White River 18, 26), farm Kumula 3 km s.w. of White River, eastern Transvaal, blacklight at horse-stables, 22.III.1992, R. Meiswinkel.

Twelve ♀♀ (slides Skukuza 352–363), Skukuza horse-stables and animal bomas, southern K.N.P., blacklight, 16.XI.1992, R. Meiswinkel.

Ten ♂♂ (slides Skukuza 370–379), Nwaswitshaka research camp, Skukuza, southern K.N.P., 5.III.1993, A.L. Dyce, ex maroela fruits (*Sclerocarya caffra*), + pupal exuviae.

Ten ♀♀ (slides Skukuza 380–389), Nwaswitshaka research camp, Skukuza, southern K.N.P., 5.III.1993, A.L. Dyce, ex maroela fruits (*Sclerocarya caffra*), + pupal exuviae.

Four ♂♂ (slides Renosterkoppies 82, 88, 99), Renosterkoppies hills ± 20 km south of Skukuza, southern K.N.P., blacklight, 18.XI.1992, R. Meiswinkel.

Twelve ♀♀ (slides Renosterkoppies 83, 89–98, 100), Renosterkoppies hills ± 20 km south of Skukuza, southern K.N.P., blacklight, 18.XI.1992, R. Meiswinkel.

One ♀ (slide Heuningneskrans 151), Heuningneskrans ± 10 km north of Onderstepoort, 2.II.1983, blacklight, R. Meiswinkel (wing Fig. 6.13b).

Three ♀♀ (slides Mooketsi 2, 5, 7), Zurmussen farm, Mooketsi, north-eastern Transvaal, 10.II.1980, blacklight near homestead, R. Meiswinkel.

One ♂ (slide Mooketsi 6), Zurmussen farm, Mooketsi, north-eastern Transvaal, 10.II.1980, blacklight near homestead, R. Meiswinkel (wing Fig. 6.14).

Three ♀♀ (slides 74–153–8,–9, –10: A.L. Dyce collection), Hectorspruit, eastern Transvaal, 31.I.1974, A.L. Dyce, ex sausage fruits (*Kigelia africana*); slide 74–153–8 + pupal exuvia.

One ♂ (slide Skukuza 12), on banks of Sabie River, Skukuza, southern K.N.P., 11.III.1984, blacklight, R. Meiswinkel & L.E.O. Braack.

One ♀ (slide Skukuza 145), on banks of Sabie River, Skukuza, southern K.N.P., 17.I.1985, blacklight, R. Meiswinkel & L.E.O. Braack.

Fig. 6.2

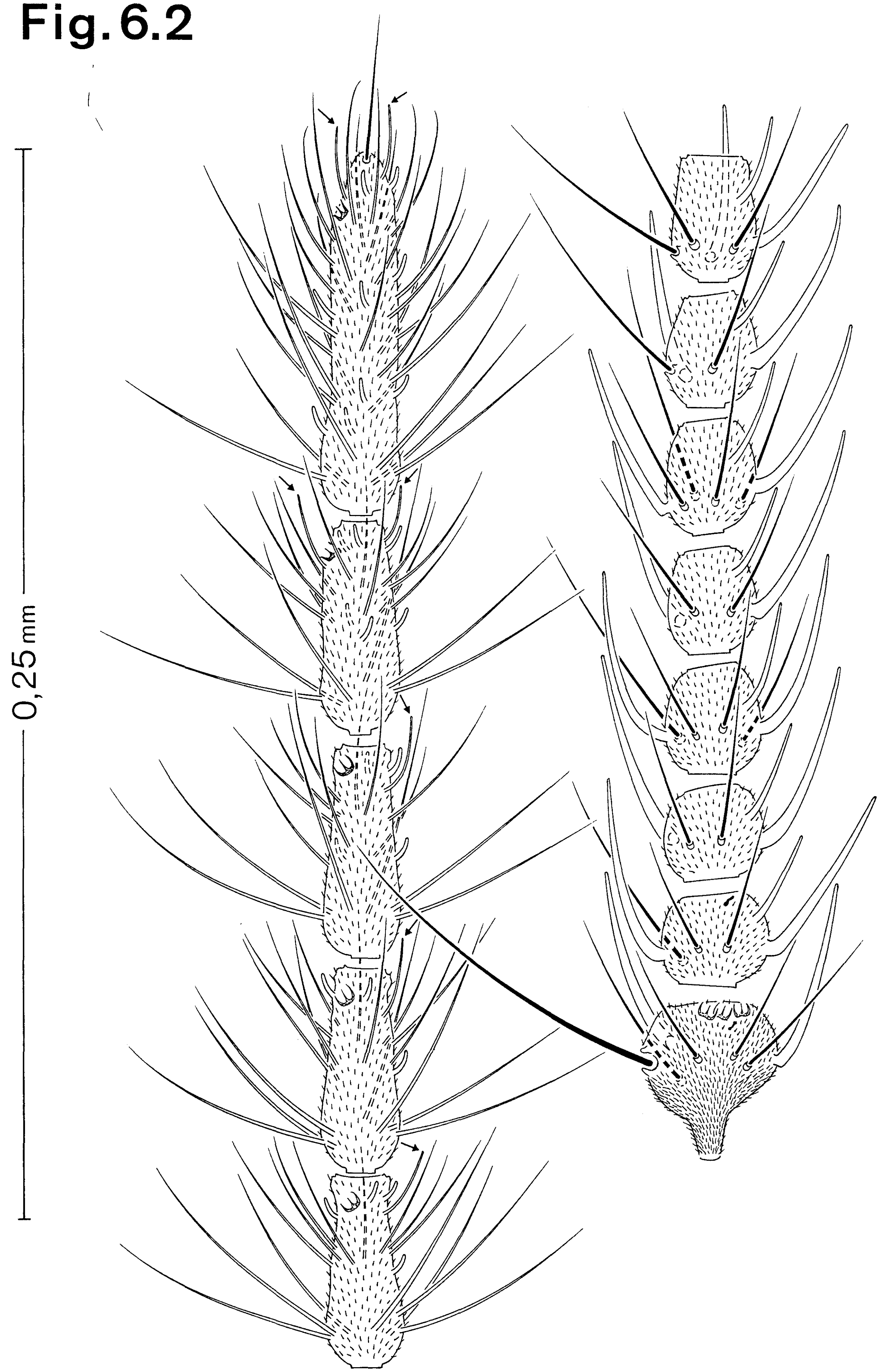


Fig. 6.2 *C. (Avaritia) pseudopallidipennis*. Antenna, female: segments XI–XV on left, segments III–X on right (Ivory Coast 343)

Fig. 6.12

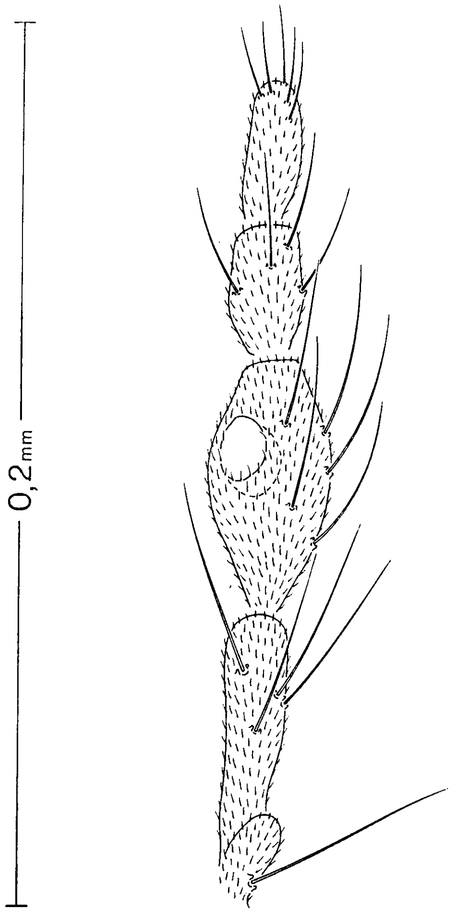
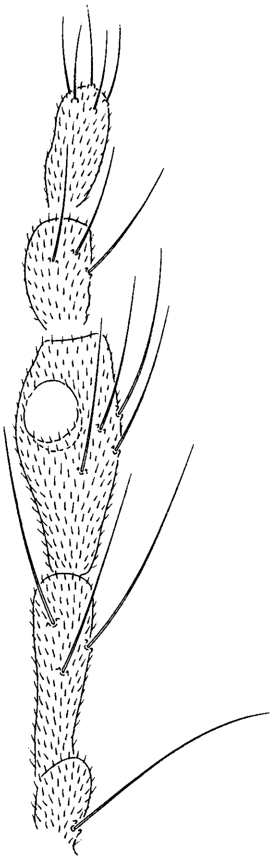


Fig. 6.3



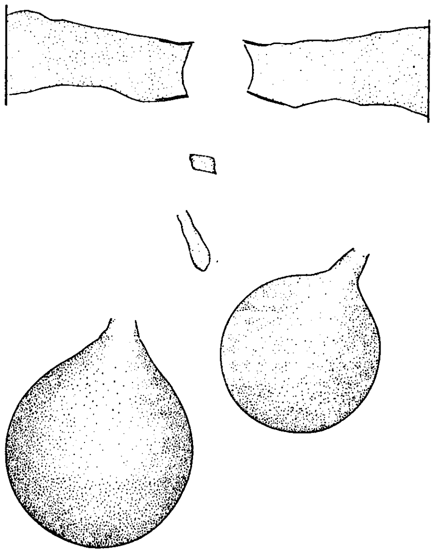


Fig. 6.16

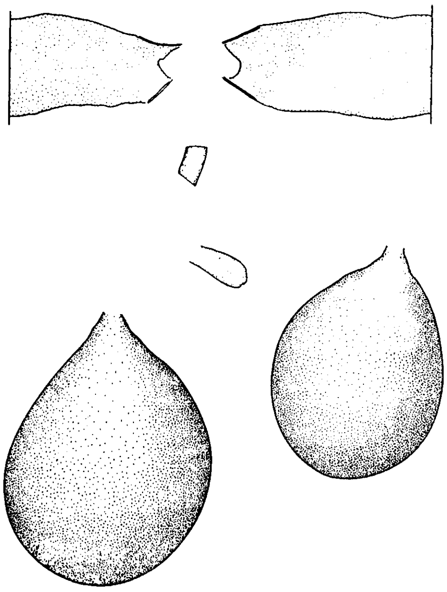


Fig. 6.4

Fig. 6.3 *C. (Avaritia) pseudopallidipennis*. Palp, female (Ivory Coast 348)
Fig. 6.4 *C. (Avaritia) pseudopallidipennis*. Genitalia, female (Ivory Coast 436)
Fig. 6.12 *C. (Avaritia) tuttifrutti* sp. nov. Palp, holotype female (White River 58)
Fig. 6.16 *C. (Avaritia) tuttifrutti* sp. nov. Genitalia, holotype female (White River 58)

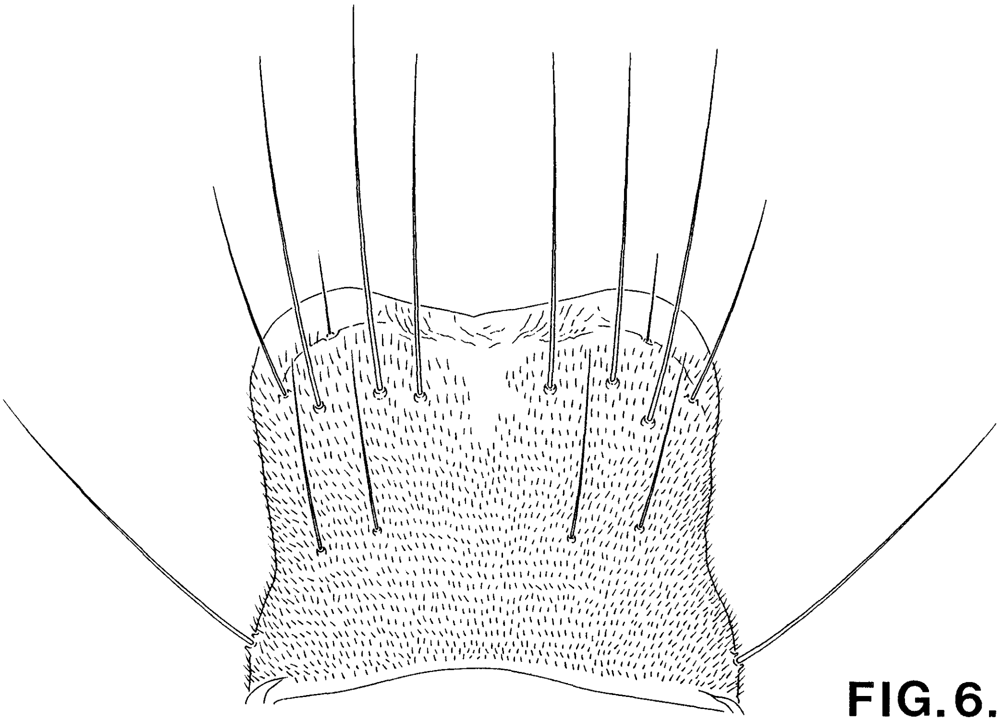


FIG.6.8

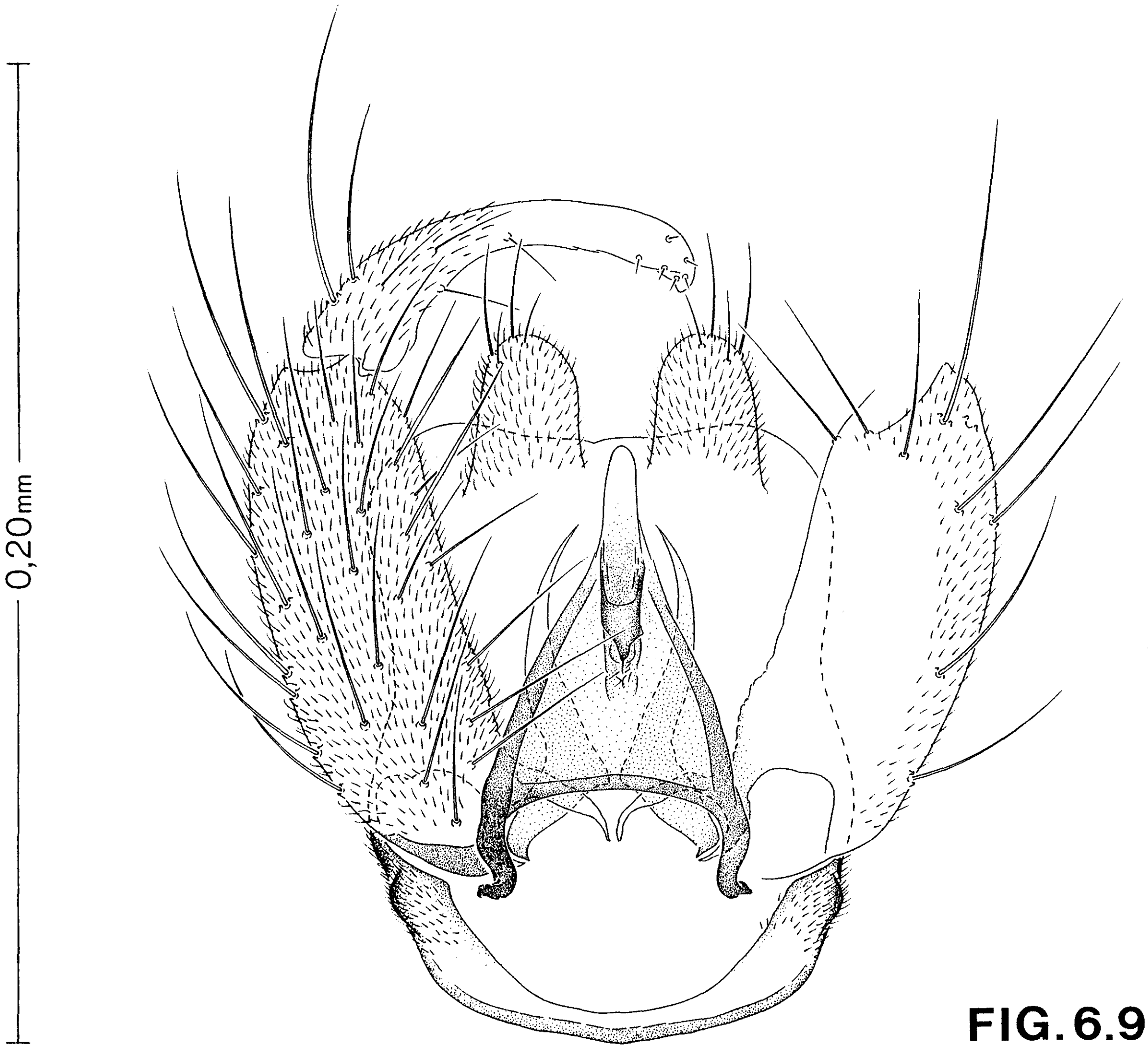


FIG.6.9

Fig. 6.8 *C. (Avaritia) pseudopallidipennis*. Genitalia, male: tergum 9 (Ivory Coast 342)

Fig. 6.9 *C. (Avaritia) pseudopallidipennis*. Genitalia, male (Ivory Coast 342)

Fig. 6.11

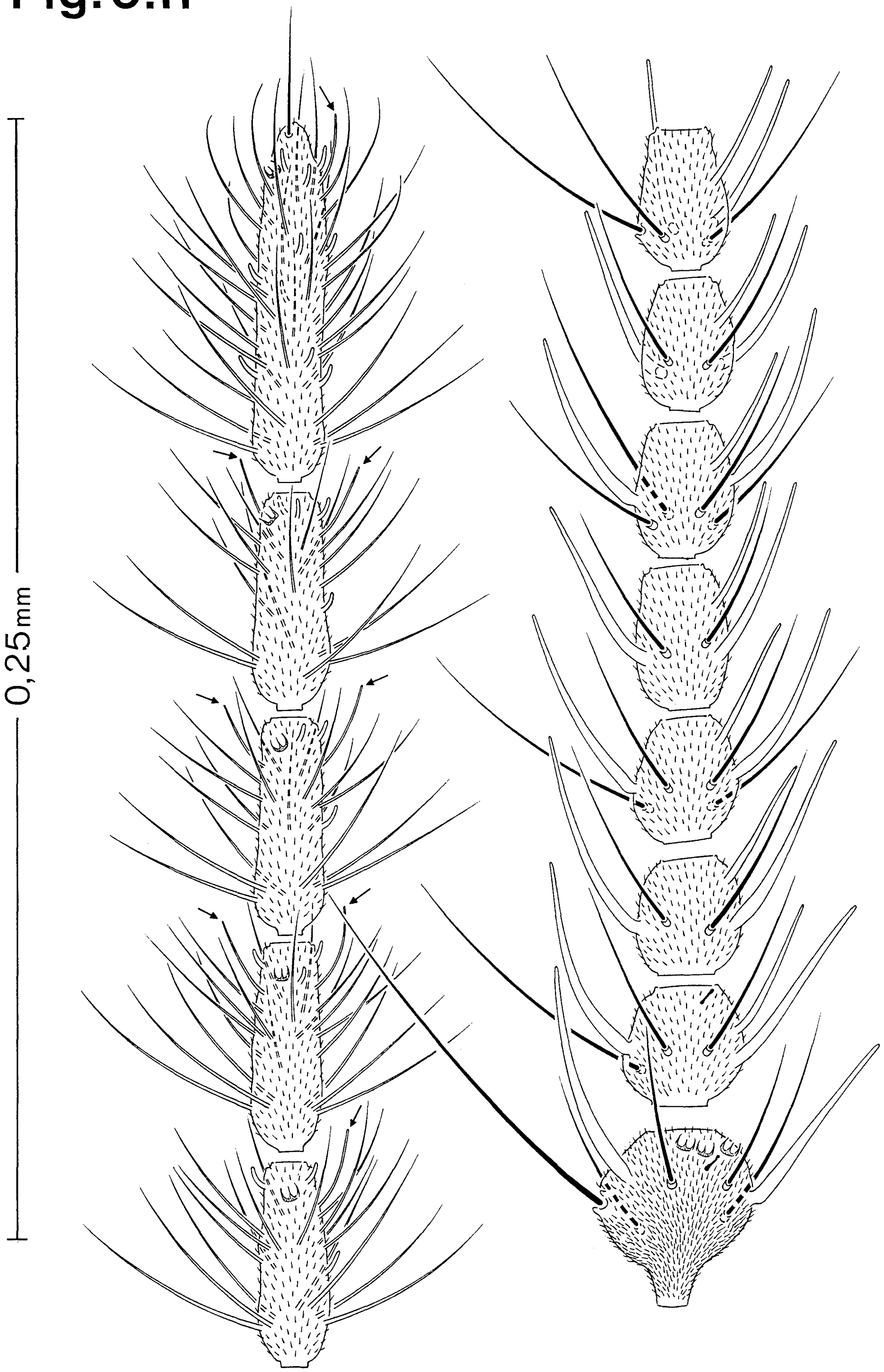


Fig. 6.11 *C. (Avaritia) tuttifrutti* sp. nov. Antenna, female: segments XI-XV on left, segments III-X on right (White River 63)

Fig. 6.13 a

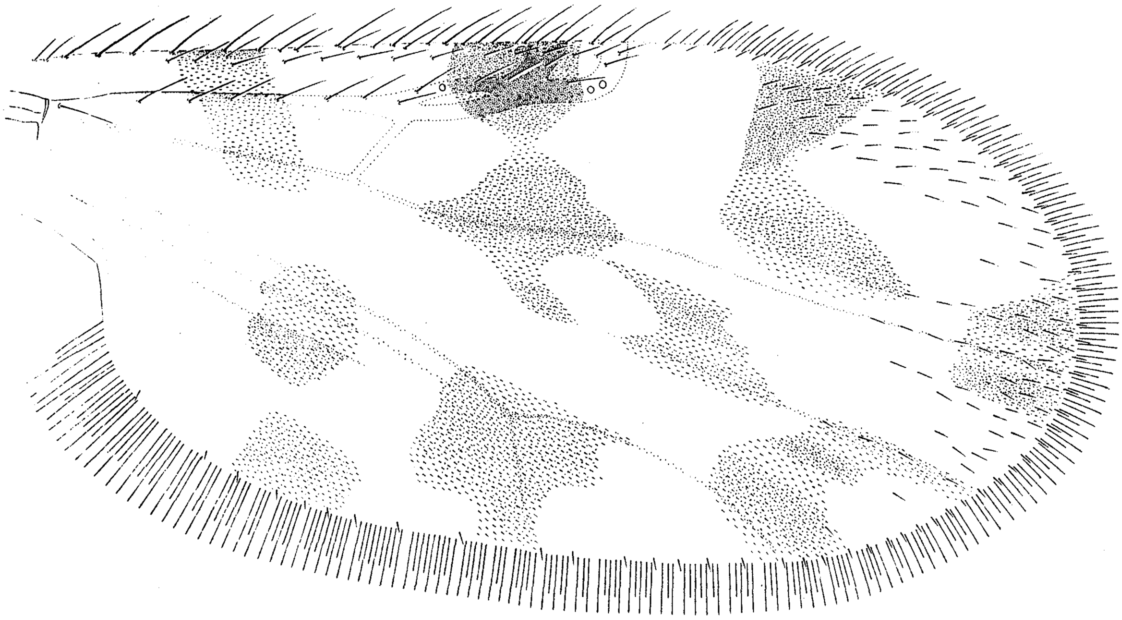


Fig. 6.13a *Culicoides (Avaritia) tuttifrutti* sp. nov. Wing, female.

6.13 b

6.14

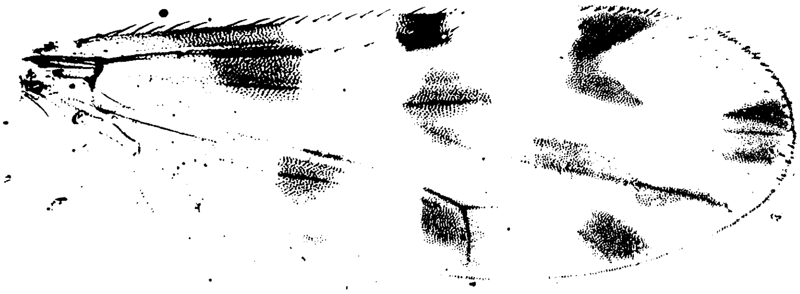


Fig. 6.13b *Culicoides (Avaritia) tuttifrutti* sp. nov. Wing, female (paratype Heuningeskrans 151).

Fig. 6.14 *C. (Avaritia) tuttifrutti* sp. nov. Wing, male (Mooketsi 6)

FIG. 6.15

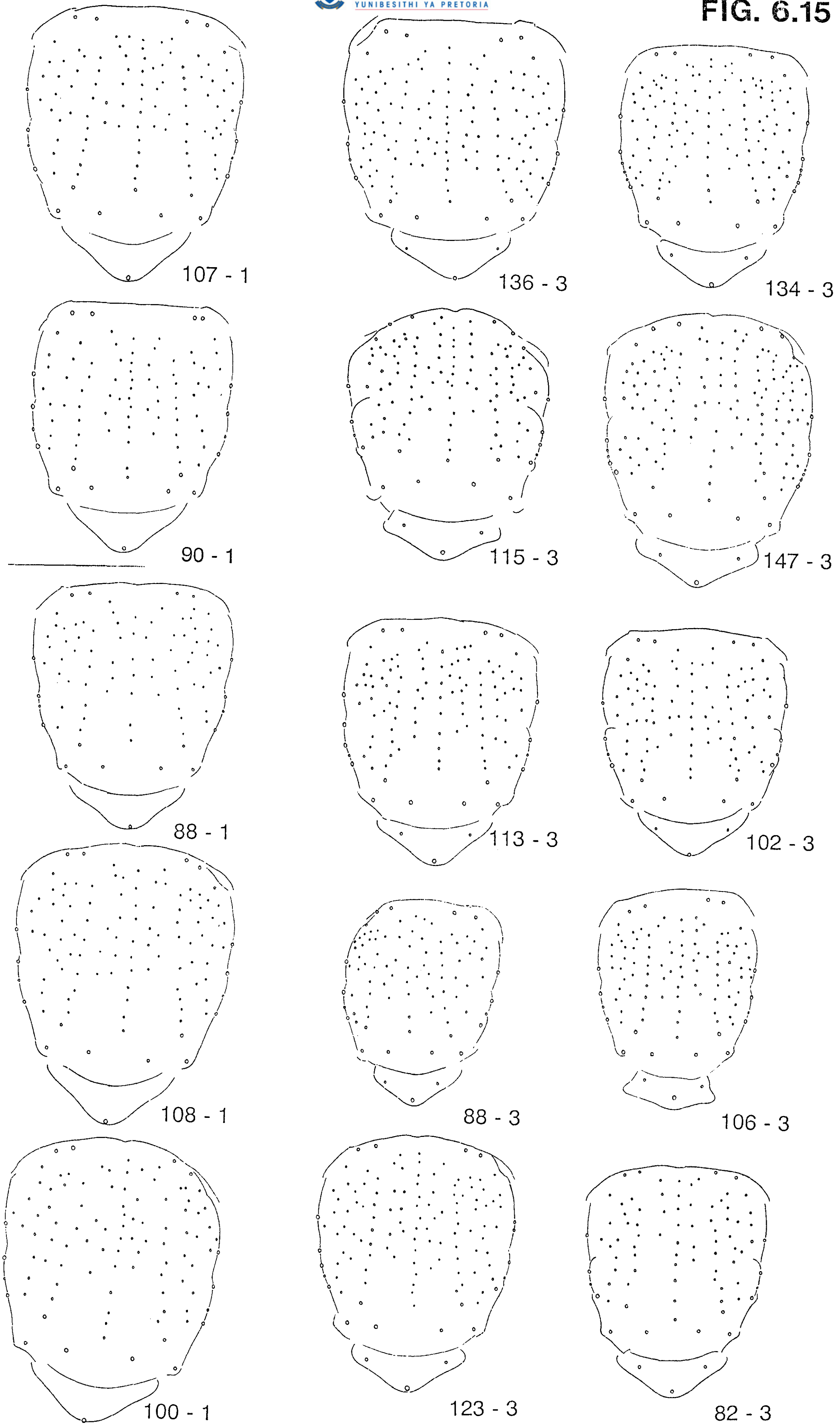


Fig. 6.15 *C. (A.) tuttifrutti* sp. nov.: setation of scutum (first numeral) and scutellum (second numeral); male (left column), female (two right columns)

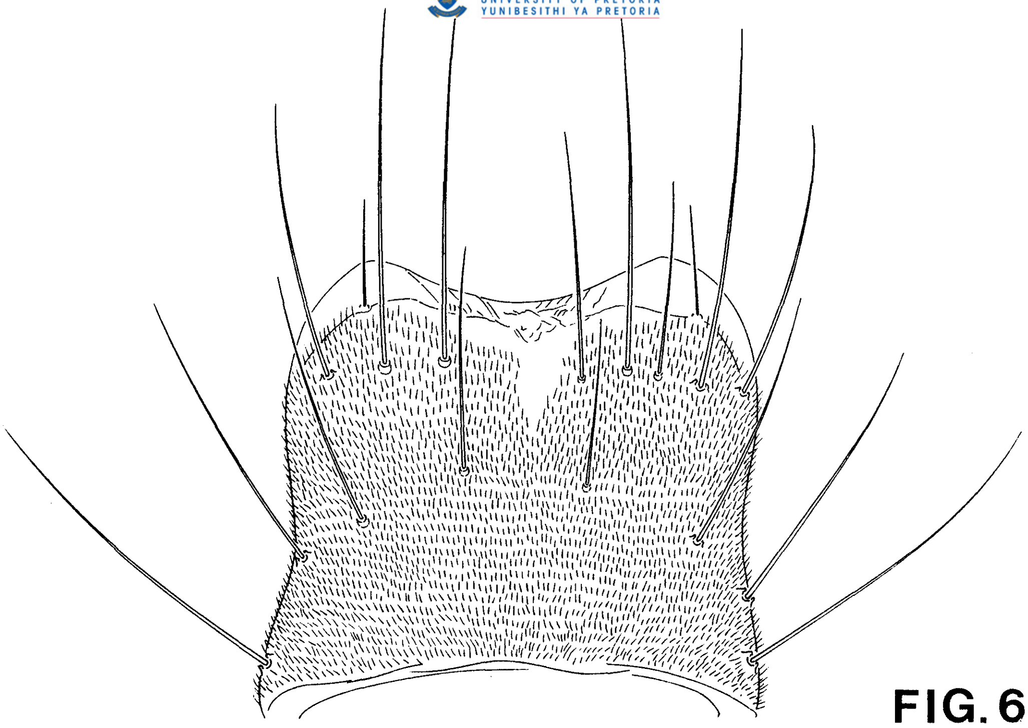


FIG. 6.17

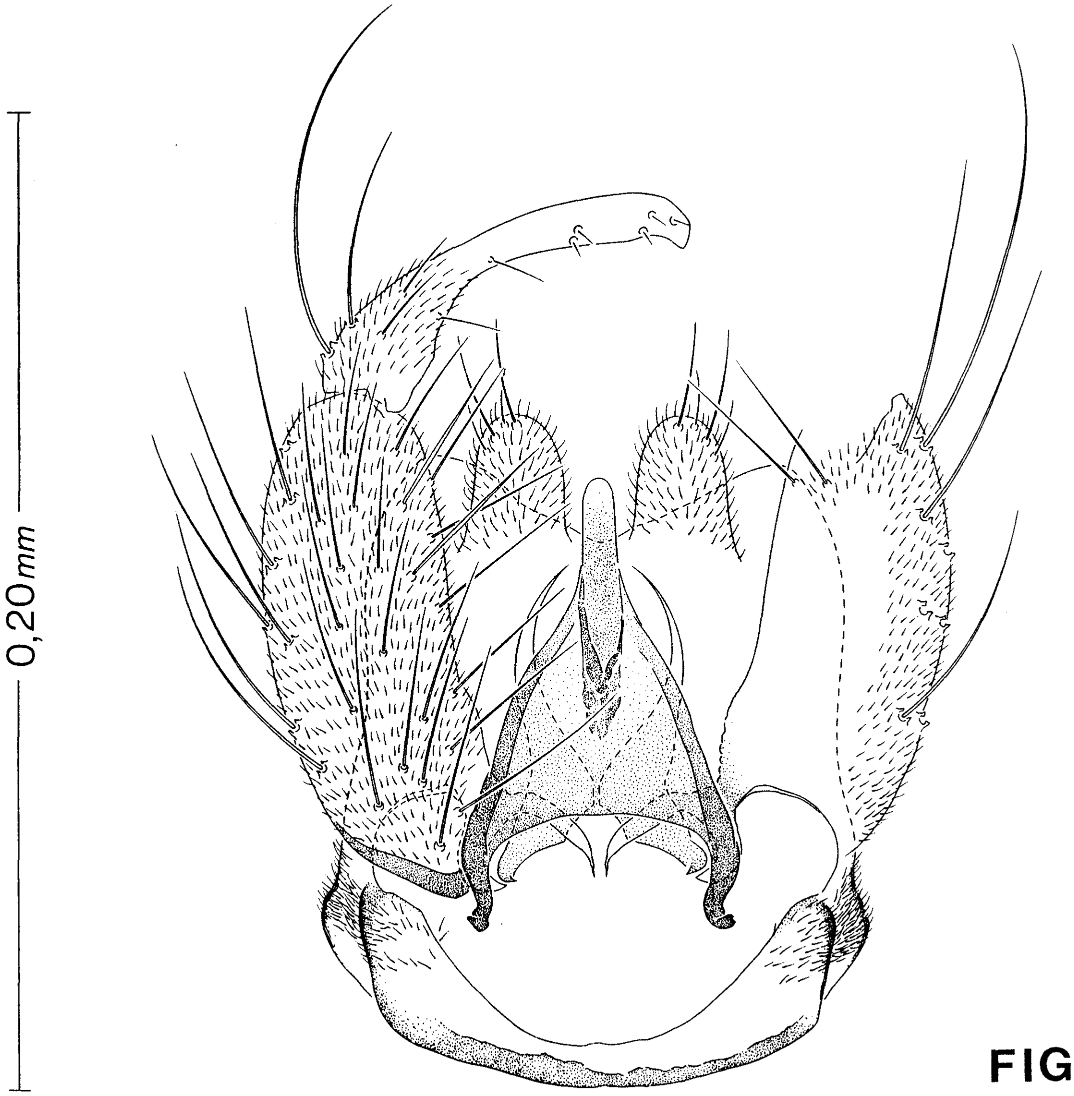


FIG. 6.18

Fig. 6.17 *C. (Avaritia) tuttifrutti* sp. nov. Genitalia, male: tergum 9 (White River 42)
Fig. 6.18 *C. (Avaritia) tuttifrutti* sp. nov. Genitalia, male (White River 46)

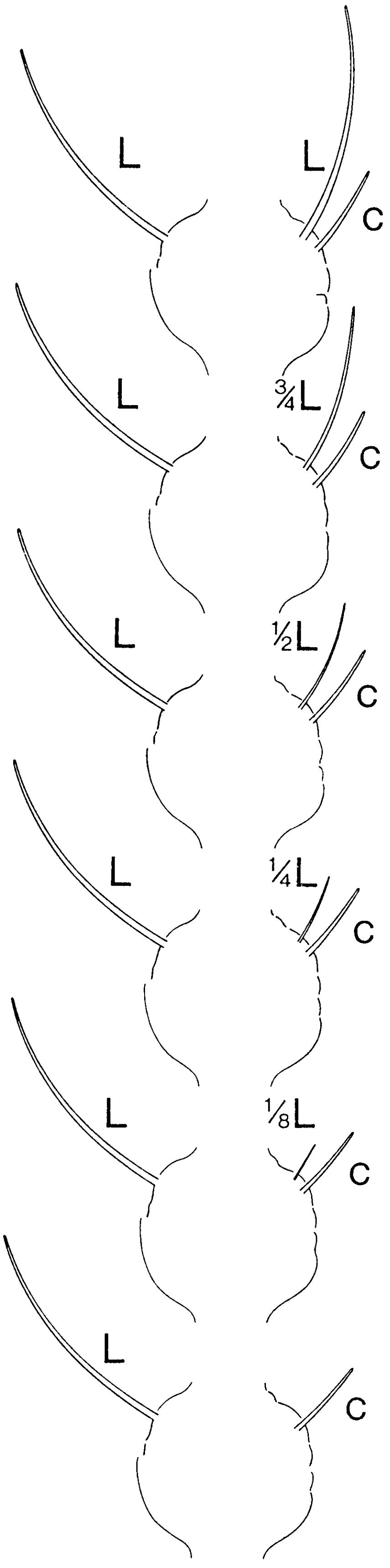


FIG. 6.19

Fig. 6.19 *C. (Avaritia) tuttifrutti* sp. nov. and *C. (A.) pseudopallidipennis*: schematic presentation of variability in the loss of or partial to full development of one of the long (L) blunt-tipped sensilla trichodea on male flagellar segment VI. Similar but less manifest reductions occur also on segment V. C = short blunt-tipped sensilla trichodea.

Fig. 6.20

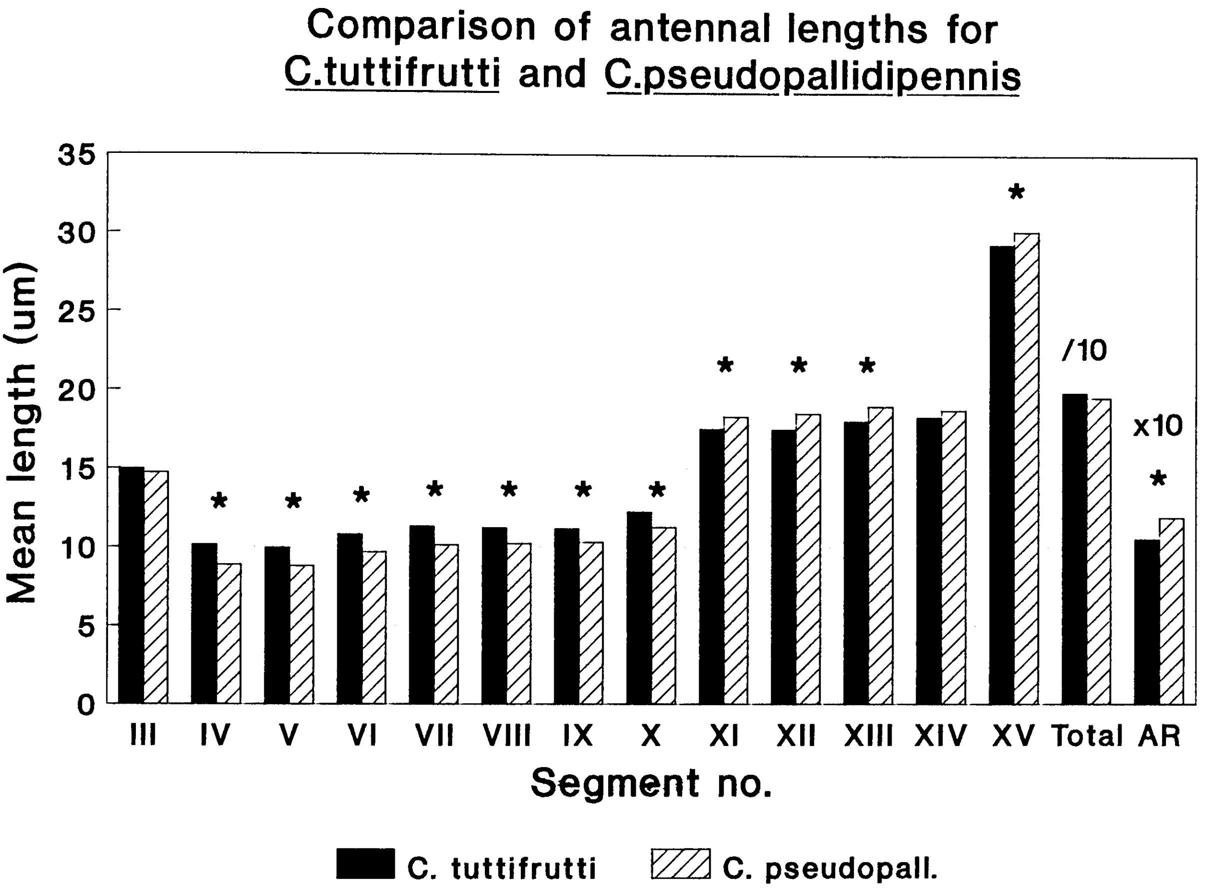


Fig. 6.20 Comparison of mean lengths (μm) of female antennal flagellar segments III–XV of *C. (Avaritia) tuttifrutti* sp. nov. and *C. (A.) pseudopallidipennis*. * = significantly different.

One ♀ (slide Skukuza 294), on banks of Sabie River, Skukuza, southern K.N.P., 5.II.1986, blacklight, R. Meiswinkel & L.E.O. Braack.

One ♀ (slide Bergpan 107), Bergpan saltworks, 60 km west of Louis Trichardt, northern Transvaal, 30.XI.1984, blacklight in forested foothills of Soutpansberg, R. Meiswinkel & G.J. Venter.

One ♀ (slide Sentrum 120), farm Krugerspan 35 km north of Thabazimbi, western Transvaal, blacklight in bush on gamefarm, 21.IV.1987, R. Meiswinkel.

Four ♀♀ (slide Sentrum 132, 133, 137, 138), farm Krugerspan 35 km north of Thabazimbi, western Transvaal, blacklight in bush on gamefarm, 13.V.1987, R. Meiswinkel.

Two ♀♀ (slide Sentrum 123, 127), farm Krugerspan 35 km north of Thabazimbi, western Transvaal, blacklight in bush on gamefarm, 28.I.1987, R. Meiswinkel.

One ♀ (slide Louis Trichardt 2), blacklight, 14.X.1983, J. van Gas.

One ♂ (slide Louis Trichardt 1), blacklight, 14.X.1983, J. van Gas.

Two ♀♀ (slides Tzaneen 12, 13), Pusela farm on eastern edge of Tzaneen, north-eastern Transvaal, 28.I.1980, blacklight, R. Meiswinkel.

Two ♂♂ (slides Tzaneen 27, 29), Pusela farm on eastern edge of Tzaneen, north-eastern Transvaal, 7.II.1980, blacklight, R. Meiswinkel.

Two ♀♀ (slides Ofcolaco 31, 35), Carpe Diem farm, Ofcolaco, north-eastern Transvaal, 13.XI.1979, blacklight, H. Cross.

One ♂ (slide Ofcolaco 34), Carpe Diem farm, Ofcolaco, north-eastern Transvaal, 13.XI.1979, blacklight, H. Cross.

Three ♂♂ (slides Letsitele 3–5), ± 20 km east of Tzaneen, north-eastern Transvaal, I.1980, blacklight, T. Ikin.

One ♂ (slide Gravelotte 1), J.C.I. mines, ± 45 km east of Tzaneen, north-eastern Transvaal, 18.I.1980, blacklight, T. Ikin.

Natal

Two ♀♀ (slides 3575, 3576: M. Cornet collection), Pietermaritzburg, XII.1970, light-trap, E.M. Nevill.

Three ♀♀ (slides Ndumu 41–43), Ndumu Game Reserve, northern Natal, Hlabeni thickets, truck-trap 17:27–18:00, 1.III.1989, R. Meiswinkel & G.J. Venter.

One ♂ (slide Ndumu 93), Ndumu Game Reserve, northern Natal, Hlabeni thickets, truck-trap

18:00–19:00, 28.II.1989, R. Meiswinkel & G.J. Venter.

Two ♀♀ (slides Ndumu 106, 107), Ndumu Game Reserve, northern Natal, Manzibomvu, truck-trap 18:05–19:05, 1.III.1989, R. Meiswinkel & G.J. Venter.

Three ♂♂ (slides Ndumu 103–105), Ndumu Game Reserve, northern Natal, Manzibomvu, truck-trap 18:00–19:00, 28.II.1989, R. Meiswinkel & G.J. Venter.

One ♀ (slide Ndumu 217), Ndumu Game Reserve, northern Natal, Manzibomvu, light-trap, 28.II.1989, R. Meiswinkel & G.J. Venter.

Three ♀♀ (slides Ngome 89, 97, 125), Ngome Tea Estates, northern Natal, blacklight indigenous forest, 8.I.1981, R. Meiswinkel.

One ♂ (slide Ngome 190), Ngome Tea Estates, northern Natal, blacklight indigenous forest, 1.III.1982, R. Meiswinkel.

One ♀ (slide Ngome 261), Ngome Tea Estates, northern Natal, blacklight at vlei with mature *Syzygium cordatum* trees, 30.XII.1982, R. Meiswinkel.

One ♀ (slide Ngome 284), Ngome Tea Estates, northern Natal, blacklight at vlei with mature *Syzygium cordatum* trees, 23.XII.1982, R. Meiswinkel.

One ♂ (slide Ngome 372), Ngome Tea Estates, northern Natal, blacklight at vlei with mature *Syzygium cordatum* trees, 10.I.1983, R. Meiswinkel.

Four ♀♀ (slides Ngome 408, 413, 420, 421), Ngome Tea Estates, northern Natal, blacklight at vlei with mature *Syzygium cordatum* trees, 4.I.1983, R. Meiswinkel.

One ♂ (slide Ngome 658), Ngome Tea Estates, northern Natal, blacklight at pumphouse on edge of indigenous forest, 24.XII.1986, R. Meiswinkel.

One ♀ (slide Ngome 696), Ngome Tea Estates, northern Natal, blacklight at pumphouse on edge of indigenous forest, 24.XII.1986, R. Meiswinkel.

Two ♀♀ (slides St. Lucia lake 6, 7), Hells Gate, Zululand, blacklight indigenous bush, 7.IX.1993, G.J. Venter.

Cape

12 ♀♀ (slides Port Elizabeth 34–45), Barclay Bridge, blacklight at cattle along bushed margins of Sundays river, 29.IV.1995, R. Meiswinkel & G.J. Venter.

Material not forming part of type series:

ZIMBABWE

Four ♀♀ (slides Zimbabwe 157–160), Rekomitjie, n.w. Zimbabwe, light-trap at thicket site, 9.I.1988, R.J. Phelps.

MALAWI

Three ♀♀ (slides Malawi 203–205), Kawalazi coffee estates 25 km east of Mzuzu, northern Malawi, blacklight at homestead in *Brachystegia* woodlands, 14.II.1988, K. Verster.

One ♂ (slide Malawi 220), Mzuzu, northern Malawi, blacklight, III.1988, K. Verster.

IVORY COAST

11 ♀♀ (slides Ivory Coast 602–613), Ganse village on southern edge of Comoe National Park, n.e. Ivory Coast, blacklight at small group of sheep and goats, 23.IX.1990, R. Meiswinkel & G.J. Venter.

6.4 DISCUSSION

6.4.1 Differential diagnosis

During 15 years (1978–1993) of collecting in South Africa, a largish *Avaritia* species similar to, but distinct from, *C. imicola* was infrequently captured. It usually comprised <10 individuals/light-trap collection, was patchy in its distribution, and appeared to be seasonal. Slide-mounted material was labelled as C. sp. # 30 nr. *pseudopallidipennis* (= *C. tuttifrutti* sp. nov.). The female had the distinguishing features of a slightly paler wing, an inflated third palpal segment, swollen blunt-tipped sensilla trichodea on basal flagellar segments III–X, and coeloconica on segments III, XI–XV. In these characters the specimens all matched Clastrier's original description of *C. pseudopallidipennis* from Senegal.

In 1990, smallish specimens of an unrecognizable *Avaritia* were collected by me 20 km north-east of Yamoussoukro, Côte d'Ivoire. Under the dissecting microscope, the distinguishing features were the unusually pale wings and legs; due to the proximal margin of the distal pale wing spot in cell R_5 being gently rounded, the specimens were provisionally dubbed as a 'pale *bolitinos*-like species with swollen palps'. These proved to be *C. pseudopallidipennis* s.str. *Culicoides tuttifrutti* differs in having darker legs, and the proximal margin of the distal pale spot in R_5 is more clearly defined and pointed, much as seen in *C. imicola.*

These initial observations indicated that the West African *C. pseudopallidipennis* was not conspecific with the southern African *C. tuttifrutti.* The search for distinguishing morphological features proved rather exacting, as the females of both species show considerable variation and overlap in their wing patterns, and in the sensilla chaetica and coeloconica distributions found on the antennae. The males of both species also display an unusual polymorphism in the fluctuating size and presence of one of the long blunt-tipped sensilla trichodea found on basal flagellar segments V and VI (Fig. 6.19). This plasticity appears to be genetically fixed, but it is difficult to explain why it is restricted to these two members of the Imicola group only. It is tempting to interpret this heterogeneity as thermally or nutritionally induced, linked to the ephemerality of rotting fruit as a larval habitat. The fermentation of different-sized fruits of various kinds, at differing rates in sunny or shady situations, is likely to exert pressure on the immatures and may result in a variable adult phenotype. However, there is no evidence of any gross morphological variations such as those reported by Horsfall (1974) in the adults of several species of mosquitoes reared under different temperatures.

While Bonferroni's multiple means method revealed significant differences between the individual flagellar lengths of three light-trap populations (White River, Renosterkoppies, Skukuza) of *C. tuttifrutti* captured within a 100 km radius in the eastern Transvaal lowveld, the antennal ratios (AR) were remarkably stable. This latter fact convinced me that one is dealing with a size cline rather than a mixture of cryptic species, and that the high degree of variability in the male and female antennal setation, though unusual for the Imicola group, is intraspecific. All the southern African populations of *C. tuttifrutti* were therefore lumped and used as a single data set for statistical comparisons with *C. tuttifrutti* from the northern Côte d'Ivoire and *C. pseudopallidipennis* from central Côte d'Ivoire. The results are presented below and summarized in Tables 6.9a and 6.9b and Fig. 6.20. Each of the few species-specific character states is discussed separately; also discussed are those states that showed slighter trends i.e. giving only partial separation of *C. pseudopallidipennis* and *C. tuttifrutti*. The features that distinguish the former species from *C. imicola* are given in Table 6.11.

a. Female. Wing: Although the wing of *C. pseudopallidipennis* is paler than that of *C. tuttifrutti*, the range in intraspecific variation is too considerable for only single wing-pattern character to be chosen as reliably diagnostic. In fact, the longer series of adults of *C. tuttifrutti* reared from rotting fruits and taken in single light-trap collections have shown this species, in wing pattern, to be the most variable of all Imicola group species known. While the Ivorean material of *C. pseudopallidipennis* studied was paler and more homogeneous in wing patterning it is not known if this is a single-population artefact. Therefore, while wing characters distinguishing the two species are listed in Table 6.10, they reveal some overlap and should be used with caution, and only in combination with other characters especially the antennal ratio (AR). Despite the difficulty in quantifying these wing-pattern differences, it is relatively easy to see that one of Clastrier's Senegalese paratypes is conspecific with *C. tuttifrutti* from northern Côte d'Ivoire and southern Africa.

b. Both species are unique for the Imicola group in possessing a swollen third palpal segment. While the mean length of palp III does not differ significantly between the two species, 56,62 μm for *C. tuttifrutti* (n = 101) and 56,88 μm for *C. pseudopallidipennis* (n = 46), there is a significant difference in their mean widths: 26,37 vs 23,95 μm respectively. This accounts for the significant difference in their palpal ratios (PR): 2,15 vs 2,38.

c. Female. Antennae: Differences in antennal flagellar lengths and antennal ratio (AR) most reliably separate *C. pseudopallidipennis* from *C. tuttifrutti*. As can be seen from Table 6.9a and Fig. 6.20, *C. tuttifrutti* has segments IV–X significantly longer, while segments XI–XIII, XV are significantly shorter than those found in *C. pseudopallidipennis*. While these result in highly significant differences in the AR of the two species, they counter-balance one another to yield no significant differences in their respective total antennal lengths (Table 6.9a). These inverted differences, i.e. shorter-longer in *C. pseudopallidipennis* vs longer-shorter in *C. tuttifrutti*, appear to be genetically fixed and not environmentally induced. As mentioned above, comparison of a cline of smaller to larger specimens of *C. tuttifrutti* showed concomitant increases in flagellar lengths resulting in a stable AR being maintained. There is thus no evidence of intra-specific size inversions or group reversals as found interspecifically between the basal and distal flagellar segments of *C. pseudopallidipennis* and *C. tuttifrutti*.

d. Female. Antennae: While both species are also unique for the Imicola group in having the long blunt-tipped sensilla trichodea on basal flagellar segments III–X unusually inflated, they are not separable on mean length; the significantly lower mean antennal trichodea ratio (AtR) of 1,59 for *tuttifrutti* vs 1,74 for *C. pseudopallidipennis* is due to antennal segment VI being shorter in the latter species.

e. Female. Antennae: As shown in Table 6.1, 96 % of the 48 antennae of *C. pseudopallidipennis* examined had only three sensilla coeloconica on segment III, and, furthermore, none of these was fused to form a larger 'double' pit. In *C. tuttifrutti*, however, 32 % of 104 antennae had four or five coeloconica on III (Table 6.5), and in 15 % of these two of the coeloconica were fused to form a larger 'double' pit.

f. Female. Antennae: 87 % of the *C. pseudopallidipennis* specimens had four sensilla chaetica basally on segment VI; in *C. tuttifrutti* only 57 % had four chaetica, the remainder had three. These inversions in coeloconica and chaetica numbers, though less marked, reinforce differences discussed above under 3. *Culicoides loxodontis* is the only other species of the Imicola group known to have four chaetica on flagellar segment VI.

g. Female. Scutum: *Culicoides tuttifrutti* has 82–147, mean 112 bristles on the scutum (n = 12) significantly more than found in *C. pseudopallidipennis* with 76–109, mean 89 (n = 17). Fewer bristles occur in *C. imicola* (57–79, mean 67; n = 17) similar to the number found in *C. bolitinos, C. loxodontis* and *C. miombo* (R.M., unpublished data 1993).

h. Male. Antennae: As members of the Imicola group, both *C. tuttifrutti* and *C. pseudopallidipennis* are unusual in having one of the long blunt-tipped trichodea on flagellar segments V and/or VI either fully developed, underdeveloped (i.e. shorter and thinner) or absent (Fig. 6.19; Table 6.4, 6.8). An underdeveloped trichodea can range from being almost fully developed (¾L) to almost non-existent (⅛L). Their exact classification was not done by laborious measurement, but by visually comparing them with their fully developed (L) counterpart on the same segment. Care was taken not to confuse this undeveloped trichodea with the short blunt-tipped trichodea (c) found accompanying the long trichodeas on segments IV–X. Only one population of *C. tuttifrutti* (White River; n = 30) was compared with the Ivorean population of *C. pseudopallidipennis* (n = 34). While both species showed underdevelopment of these long trichodea it was more evident in *C. tuttifrutti*; the trichodea distributions of both species are detailed in Tables 6.4 and 6.8. As in the female, only partial separation is possible: 18 % of *C. pseudopallidipennis* males have two fully developed long trichodea on VI as opposed to 0 % in *C. tuttifrutti*.

i. Male. Genitalia: Apparently inseparable.

6.4.2 **Taxonomy**

Culicoides (Avaritia) tuttifrutti appears to have been dealt with once only in previous studies on Afrotropical biting midges. Judging from the antennal ratio data, the females of *C. pseudopallidipennis* described by Glick (1990) from Kenya and Zimbabwe appear to be *C. tuttifrutti*, while the hitherto unknown male he described belongs to *C. loxodontis*. This male was one of three from Ol Doinyo Sabachi, Kenya, donated by Dr. Michel Cornet of ORSTOM to the Smithsonian Natural History Museum, Washington. I have seen a second male of this series; it undoubtedly belongs to *C. loxodontis* as it has the tip of the aedeagus minutely papillate. This was not illustrated by Glick (1990). Its presence in Kenya extends the recorded range of *C. loxodontis* which was previously known only from the Kruger National Park, South Africa.

In their large treatise on the 168 species of south-east Asian *Culicoides*, Wirth and Hubert (1989) split the subgenus *Avaritia* into the smaller Actoni and larger Orientalis groups. They placed *C. pseudopallidipennis* in the latter. Their Orientalis group, however, comprises at least six species groups, and is a complex clearly distinct from the Imicola group (Chapter 11). In his treatment of 55 species of

Kenyan *Culicoides*, Glick (1990) correctly placed *C. pseudopallidipennis* in the Imicola group, but by default as he employs the group in the broad sense, as a replacement for *Avaritia*. Accordingly his Imicola group of seven species is also an agglomerate of some five species groups, and it mirrors the definition presented by Khamala and Kettle (1971) for their Pallidipennis group.

Boorman (1991) assembled these and four other subgenus and species-group definitions, and noted that 'there may be justification for suggesting another group within *Avaritia* not previously recognized: that of the Montanus group'. It comprises the Palaearctic *C. montanus* and the Afrotropical *C. pseudopallidipennis*, united in 'that the female antennae bear a series of very characteristic stout sensilla trichodea on the basal segments III–X'. However, Boorman does note that *C. montanus* 'is very like *obsoletus* in both sexes'. This justifies its inclusion in the Palaearctic/NearcticObsoletus group of species characterizedby highly unusual male genitalia. The Obsoletus group appears to constitute a monophyletic lineage within the subgenus *Avaritia*, but does not include *C. pseudopallidipennis*. While the swollen trichodea on the basal flagellar segments are unique, it seems that they arose independently within the Obsoletus and Imicola groups. The swollen trichodea may be used by ovipositing females in search of fermenting fruits, but this remains conjectural as the larval habitats of *C. montanus* remains unknown while the one rearing of *C. pseudopallidipennis* from a banana stump likely does not reflect its true preference. It is notable that *C. montanus* also has a swollen third palpal segment similar to that seen in *C. pseudopallidipennis* and *C. tuttifrutti*, and may point to a second parallelism: ornithophily.

In the final analysis, both *C. pseudopallidipennis* and *C. tuttifrutti* are regarded as true members of the Imicola group within the subgenus *Avaritia*. However, their close resemblance indicates recent speciation (perhaps the result of habitat vicariance), and they form a distinct subgroup within the Imicola group. The Montanus group is artificial, or paraphyletic.

6.4.3 **Distribution**

Culicoides pseudopallidipennis: only the West African records of Clastrier (1958), Clastrier & Wirth (1961), Cornet (1969) and the present material from Gofabo, central Côte d'Ivoire, are considered to be of this species. They fall within the high-rainfall equatorial belt and suggest that *C. pseudopallidipennis* is restricted to this tropical zone.

Culicoides tuttifrutti: the Kenyan and Zimbabwean material identified by Glick (1990) as *C. pseudopallidipennis* are misidentifications of *C. tuttifrutti*. It is now also recorded from South Africa, Malawi and northern Côte d'Ivoire (Comoe National Park). All these records are from the lower rainfall subtropical and tropical woodlands that adjoin and encircle the equatorial forest block.

In South Africa *C. tuttifrutti* has been found throughout the northern and eastern Transvaal, northern Natal and the southern Cape coast. This distribution correlates very positively with frost-free areas but only partially overlaps the known occurrence of *Sclerocarya* (maroela) and *Kigelia* (sausage tree) whose fruits are inhabited by the immatures of *C. tuttifrutti*. In the Port Elizabeth area *C. tuttifrutti* is moderately common and widespread, and clearly indicates it to breed in other, unknown, fruits.

The distribution records of both species are extremely sparse and thus the interpretation that *C. pseudopallidipennis* is restricted to the higher-rainfall equatorial regions, and *C. tuttifrutti* to the encircling lower-rainfall woodlands, may be modified in future. I suggest it is coincidental that the West African records of the two species fall on the transition between equatorial forest and tropical woodlands. The intergradation of these two phytochoria is complex in this region (White 1983), and might explain why *C. pseudopallidipennis* and *C. tuttifrutti* are here found in close proximity. However, it would be premature to thus assume that these two species occur sympatrically. It is clear that the immatures of *C. tuttifrutti* are resource specific, and this is the major factor limiting its abundance and distribution. In all probability this applies to *C. pseudopallidipennis* too, but, until more is known about its larval habitat, and that of *C. tuttifrutti* more completely known, our understanding of precisely which factors determine the distribution of these two species remains preliminary and partly inferential.

As remarked by Kingdon (1990) Africa has experienced numerous oscillations between wet and dry climatic phases which 'have pushed animals and plants back and forth across the forest-savanna frontier'. One consequence is that in various taxonomic groups 'closely related forest and non-forest sibling species' have developed. It is notable that immigration from the savannas into forests is common; indeed there is a predominance of 'derived' species in the forest fauna. This may have been accompanied by a change in larval habitat, as both *Kigelia* and *Sclerocarya* are also retricted to the savannas and subtropical woodlands of Africa.

6.4.4 Abundance and prevalence

Prior to the discovery that certain indigenous fruits are its preferred larval habitat, the rarity and patchy occurrence of *C. tuttifrutti* was thought to be an artefact of discontinuous sampling.

Monthly sampling during a recent two-year survey conducted at 65 sites in the eastern Transvaal lowveld confirmed *C. tuttifrutti* to be uncommon but widespread. It was also quite prevalent, being found in 77 (43 %) of 180 light-trap collections sorted (Table 6.12), but it was never the dominant *Culicoides* species at any site at any given time. The 30 000 km^2 study area embraced the Kruger National Park (43 sampling sites) and adjoining farms (22 sites). On farms, *C. tuttifrutti* was four times more abundant and twice as prevalent as in the KNP. While extensive areas of natural bush still occur on and between farms, much of this bush has been replaced by cultivated orchards of bananas, mangoes, avocadoes, pawpaws and litchis. Clastrier & Wirth's Gambian record of *C. pseudopallidipennis* (misidentified as *imicola*) from a rotting banana stump suggests that its sister species *C. tuttifrutti* could also be more catholic in its choice of larval habitat and may switch from wild to domestic fruits. If so, this may explain its significantly higher prevalence in the farming arena, and its persistence throughout the year. However, it must be noted that both *Sclerocarya* and *Kigelia* are protected by law, and that farmers often establish their homes, dairies or stables in the shade of magnificent specimens of these trees. This would markedly skew the presence of *C. tuttifrutti* in light-traps operated nearby.

In the KNP, where no exotic fruit trees are cultivated, *C. tuttifrutti* can also be found throughout the year. Although there is no evidence of this as yet, *C. tuttifrutti* may inhabit the fallen fruits of some of the ten or more *Ficus* (Moraceae) species known to be widespread in the KNP. Not only do they overlap in their fruiting seasons but they can bear more than once per annum. Alternatively, *C. tuttifrutti* may rely on the long persistence of *Kigelia* fruits; these can be extremely large (1 m × 18 cm), thick-skinned and heavy. The fibrous pulp decomposes slowly, a process that can last through four seasons. Whatever the reality, it is evident that the fruiting season of *Sclerocarya*, during any two months between January and June, is too short for it to be the sole host that sustains *C. tuttifrutti* year-round.

6.5 CONCLUSION

Culicoides pseudopallidipennis and *C. tuttifrutti* are sister species that form a subgroup within the Imicola group of the subgenus *Avaritia*. The immature stages of *C. tuttifrutti* develop in the fallen fruits of certain indigenous trees; it is likely that those of *C. pseudopallidipennis* do the same. Further research may show that the immatures of both species also develop in decaying cultivated fruits and other types of rotting vegetation. The specificity and limited availability of this type of larval habitat is likely the major cause for their rarity and patchy distribution. Low abundances and suspected ornithophily imply that *C. pseudopallidipennis* and *C. tuttifrutti* are unlikely to be involved in the transmission of orbiviruses to domesticated stock such as cattle, horses and sheep.

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TABLE 6.1: Number and frequency of coeloconica present on each of female antennal flagellar segments III–XV of *C. (Avaritia) pseudopallidipennis*

No. of coeloconica/segment	**Flagellar segments**						
	III	**IV–X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	-	48	-	-	-	-	-
1	-	-	46	48	48	48	47
2	-	-	2	-	-	-	1
3	46	-	-	-	-	-	-
4	2	-	-	-	-	-	-
No. of antennae examined	48	48	48	48	48	48	48

TABLE 6.2: Number and frequency of chaetica present on each of female antennal flagellar segments III–XV of *C. (Avaritia) pseudopallidipennis*

No. of chaetica/segment	**Flagellar segments**												
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	-	-	-	-	-	-	-	-	48	48	48	48	48
1	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	48	-	48	-	48	1	-	-	-	-	-
3	-	46	-	8	-	9	-	47	-	-	-	-	-
4	1	2	-	40	-	38	-	-	-	-	-	-	-
5	47	-	-	-	-	1	-	-	-	-	-	-	-
No. of antennae examined	48	48	48	48	48	48	48	48	48	48	48	48	48

TABLE 6.3: Mean lengths (μm) of flagellar segments, range in number, mean number and distribution of sensillae on the female and male antennae of *C. (A.) pseudopallidipennis* Clastrier

	Flagellar segments													**Total and mean total**
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**	
Female: (n = 46–48)														
Sens. coeloconica: range/segment	3–4	0	0	0	0	0	0	0	1–2	1	1	1	1–2	8–9
mean no./segment	3,04	0	0	0	0	0	0	0	1,04	1,0	1,0	1,0	1,02	8,1
Sens. chaetica: range/segment	4–5	3–4	2	3–4	2	3–5	2	2–3	0	0	0	0	0	23-26
mean no./segment	4,98	3,04	2,00	3,83	2,0	3,83	2,00	2,98	0	0	0	0	0	24,67
Sens. trichodea (blunt-tipped)	LL	LLc	LLc	LLc	LLc	LLc	LLc	LLc	-	-	-	-	-	
Mean lengths of segments	36,8	22,2	22,0	24,2	25,3	25,6	25,8	28,2	45,8	46,3	47,4	46,8	74,8	488,0
Male: (n = 34)														
Sens. coeloconica: range/segment	2	0–1	0	0	0	0	0	0	0	0	1-2	1-2	1-2	5-7
mean no./segment	2	0,03	0	0	0	0	0	0	0	0	1,09	1,03	1,97	6,12
Sens. chaetica: range/segment	5	0	0	0	0	0	0	0	0	0	3	2	0	10
mean no./segment	5	0	0	0	0	0	0	0	0	0	3	2	0	10
Sens. trichodea (blunt-tipped)	LL	L0,97Lc	L0,82Lc	L0,42Lc	L0,01Lc	Lc	Lc	c	0	0	-	-	-	-
Mean lengths of segments	71,3	<------------------------------ 322,9 ------------------------------>									89,6	70,2	87,5	641,5

TABLE 6.4: Distribution and variation in size of the long (L) and short (c) blunt-tipped sensilla trichodea on male antennal flagellar segments III–XII of *C. (Avaritia) pseudopallidipennis* (n = 34)

Slide no.	**Flagellar segments**									
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
Ivory Coast **336**	L L L L	L L c L L c	**L L c** **L L c**	L L c L L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast 338	L L L L	L L c L L c	L L c L L c	L¼L c L½L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **340**	L L L L	L L c L L c	L½L c L L c	L¼L c L½L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **342**	L L L L	L L c L L c	L½L c L L c	L c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **344**	L L L L	L L c L L c	L½L c L½L c	L⅛L c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **352**	L L L L	L L c L L c	L L c L L c	L L c L L c	L½L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **354**	L L L L	L L c L L c	L L c L L c	L⅛L c L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **355**	L L L L	L L c L L c	L L c L L c	L¾L c L L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **422**	L L L L	L L c L L c	L¾L c L L c	L¾L c L¾L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **423**	L L L L	L½L c L½L c	L¾L c L½L c	L¾L c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **424**	L L L L	L L c L L c	L L c L¾L c	L⅛L c L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **425**	L L L L	L L c L L c	L½L c L c	L⅛L c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **427**	L L L L	L L c L L c	L½L c L L c	L⅛L c L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **429**	L L L L	L L c L L c	L L c L L c	L¾L c L½L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **431**	L L L L	L L c L L c	L¾L c L L c	L¾L c L L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **435**	L L L L	L L c L L c	L L c L L c	L c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
Ivory Coast **437**	L L L L	L L c L L c	L L c L½L c	L⅛L c L½L c	L c L c	L c L c	L c L c	c c	- -	- -
mean no./seg.	**L L**	**L0,97L c**	**L0,82L c**	**L0,42L c**	**L0,01L c**	**L c**	**L c**	**c**	**-**	

TABLE 6.5: Number and frequency of coeloconica present on each of female antennal flagellar segments III–XV of *C. (Avaritia) tuttifrutti* sp. nov.

No. of coeloconica/segment	**Flagellar segments**							
	III	**IV–IX**	**X**	**XI**	**XII**	**XII**	**XIV**	**XV**
0	-	104	99	3	-	-	-	-
1	-	-	5	101	104	104	104	102
2	-	-	-	-	-	-	-	2
3	71	-	-	-	-	-	-	-
4	30	-	-	-	-	-	-	-
5	3	-	-	-	-	-	-	-
No. of antennae examined	104	104	104	104	104	104	104	104

TABLE 6.6: Number and frequency of chaetica present on each of female antennal flagellar segments III–XV of *C. (Avaritia) tuttifrutti* sp. nov.

No. of chaetica/segment	**Flagellar segments**												
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	-	-	-	-	-	-	-	-	104	105	105	105	105
1	-	-	-	-	-	-	-	-	1	-	-	-	-
2	-	-	104	-	99	-	89	4	-	-	-	-	-
3	1	90	1	46	6	20	16	100	-	-	-	-	-
4	10	14	-	59	-	75	-	1	-	-	-	-	-
5	92	1	-	-	-	9	-	-	-	-	-	-	-
6	2	-	-	-	-	1	-	-	-	-	-	-	-
No. of antennae examined	105	105	105	105	105	105	105	105	105	105	105	105	105

TABLE 6.7: Mean lengths (μm) of flagellar segments, range in number, mean number and distribution of sensillae on the female and male antennae of *C. (A.) tuttifrutti* sp. nov.

	Flagellar segments													**Total and mean total**
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**	
Female: (n = 104)														
Sens. coeloconica: range/segment	3–4	0	0	0	0	0	0	0–1	1	1	1	1	1–2	8–9
mean no./segment	3,35	0	0	0	0	0	0	0,04	0,97	1,00	1,00	1,00	1,02	8,38
Sens. chaetica: range/segment	4–5	3–4	2	3–4	2–3	3–5	2	3–4	0	0	0	0	0	24–27
mean no./segment	4,83	3,25	2,00	3,79	2,04	3,96	2,00	3,04	0	0	0	0	0	24,92
Sens. trichodea (blunt-tipped)	LL	LLc	LLc	LLc	LLc	LLc	LLc	LLc	-	-	-	-	-	-
Mean lengths of segments	37,7	25,4	24,9	27,0	28,3	28,1	28,0	30,8	44,1	44,0	45,4	45,9	73,4	498,2
Male: (n = 28–30)														
Sens. coeloconica: range/segment	2	0	0	0	0	0	0	0	0	0	1	1–2	2	6–7
mean no./segment	2	0	0	0	0	0	0	0	0	0	1	1,50	2,00	6,50
Sens. chaetica: range/segment	4–6	0	0	0	0	0	0	0	0	0	3	2	0–1	9–12
mean no./segment	4,96	0	0	0	0	0	0	0	0	0	3	2	0,07	10,04
Sens. trichodea (blunt-tipped)	LL	L0,95Lc	L0,37Lc	L0,2Lc	Lc	Lc	Lc	c	0	0	-	-	-	-
Mean lengths of segments	73,7	<-- 331,2 -->									90,0	72,9	92,3	661,1

TABLE 6.8: Distribution and variation in size of the long (L) and short (c) blunt-tipped sensilla trichodea on male antennal flagellar segments III–XII of *C. (Avaritia) tuttifrutti* (n = 30)

slide no.	**Flagellar segments**									
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
White River 18	L L L L	L c L L c	L½L c L½L c	L c L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 19	L L L L	L L c L L c	L L c L¾L c	L L c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 20	L L L L	L L c L L c	L L c L L c	L⅛L c L¾L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 21	L L L L	L L c L L c	L L c L L c	L‹ c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 22	L L L L	L L c L L c	L L c L¾L c	L¼L c L⅛L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 23	L L L L	L L c L L c	L¾L c L L c	L c L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 24	L L L L	L L c L L c	L¾L c L½L c	L¾L c L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 25	L L½L L L	L L c L L c	L L c L L c	L¾L c L¾L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 26	L L L L	L L c L L c	L¾L c L L c	L⅛L⅛c L½L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 42	L L L L	L L c L L c	L c L c	L c L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 44	L L L L	L L c L L c	L¼L c L½L c	L c L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 46	L L L L	L L c L L c	L¤L c L¾L c	L¼L c L L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 47	L L L L	L L c L½L c	L½L c L c	L c L c	L c L c	L c L c	L c L c	c c	- -	- -
White River 48	L L L L	L L c L L c	L½L c L⅛L c	L c L¼L c	L c L c	L c L c	L c L c	c ¼L c	- -	- -
White River 50	L L L L	L L c L L c	L c L⅛L c	L c L c	L c L c	L c L c	L ½c L c	c c	- -	- -
X	L L	L 0,95 L c	L 0,37 L c	L 0,2 L c	L c	L c	L c	c	-	-

TABLE 6.9a Comparison of mean lengths (raw data) of female antennal segments III–XV between three South African populations (White River, Renosterkoppies, Skukuza) of *C. tuttifrutti* sp. nov. and one Ivorean population of *C. pseudopallidipennis*

Antennal segment	**Species and mean lengths**		**t-values**	
	C. tuttifrutti **(n = 77)**	***C. pseudopallidipennis*** **(n = 46)**		
III	14,961	14,728	1, 845	NS
IV	10,133	8,870	13,000	
V	9,932	8,793	12,531	
VI	10,792	9,685	11,065	
VII	11,302	10,136	11,143	
VIII	11,214	10,223	8,163	
IX	11,156	10,315	7,160	
X	12,237	11,272	7,689	
XI	17,510	18,299	-3,709	
XII	17,481	18,505	-4,795	
XIII	18,000	18,962	-4,807	
XIV	18,263	18,723	-1,773	NS
XV	29,249	30,098	-2,116	
Total length	198,156	195,200	1,528	NS
AR	1,053	1,190	-25,108	

TABLE 6.9b: Comparison of mean lengths (raw data) of female antennal segments III–XV between *C. pseudopallidipennis* (Gofabo, central Côte d'Ivoire), *C. tuttifrutti* sp. nov. (South Africa) and *C. tuttifrutti* (Ganse, northern Côte d'Ivoire); n = 21 for each series. Means followed by the same letter are not significantly (NS) different at P = 0,05

Antennal segment	**Species and mean lengths**			**F–values (Bonferroni)**	**Kruskal-Wallis X^2 (Dunn)**
	C. pseudopallidipennis	***C. tuttifrutti* (South Africa)**	***C. tuttifrutti* northern Côte d'Ivoire**		
III	(14,726)^{a}	(14,833)^{ab}	(15,155)^{b}		6,931*
IV	(8,952)^{a}	(10,107)^{b}	(10,071)^{b}		33,005***
V	8,952^{a}	9,893^{b}	9,679^{b}	18,367***	
VI	(9,809)^{a}	(10,750)^{b}	(10,309)^{b}		28,440***
VII	10,238^{a}	11,155^{b}	10,929^{b}	22,295***	
VIII	10,190^{a}	11,143^{b}	10,762^{c}	23,646***	
IX	10,333^{a}	11,107^{b}	10,821^{b}	13,897***	
X	11,226^{a}	12,131^{b}	11,619^{a}	13,638***	
XI	18,310^{a}	17,274^{b}	17,571^{b}	5,790**	
XII	18,619^{a}	17,345^{b}	17,845^{b}	7,639**	
XIII	(18,976)^{a}	(17,905)^{b}	(18,345)^{a}		12,687**
XIV	18,560^{a}	17,917^{a}	18,048^{a}	1,949 NS	
XV	30,214^{a}	28,857^{b}	27,679^{b}	10,937***	
Total length	(195,524)^{a}	(197,214)^{a}	(192,619)^{a}		2,375 NS
AR	1,195^{a}	1,049^{b}	1,073^{b}	95,398***	

TABLE 6.10: Morphological and geographical separation of *C. pseudopallidipennis* and *C. tuttifrutti* sp. nov.

C. pseudopallidipennis	*C. tuttifrutti*
Morphological	
♀ wing paler, with proximal margin of distal pale spot in cell R_5 moderately to gently rounded to overtly pointed; this spot narrowly separated from third pale costal spot partially covering second radial cell	- wing slightly darker with pale areas more clearly defined; proximal margin of distal pale spot in R_5 more clearly pointed and moderately broadly separated from third pale costal spot
- second and third pale costal spots usually narrowly to broadly fused under radial cells	- these spots usually narrowly to moderately separated, occasionally narrowly fused
- in paler specimens dark area straddling radial cells on anterior wing margin is markedly reduced and is clearly isolated from the dark spot that straddles vein M_1 proximally	- these dark spots almost never separated; if so the one covering the radial cells is not markedly reduced
- vein M_2 variable: can have both margins darkened which gradually taper to leave the apex of M_2 narrowly dark i.e. no preapical excision present; in paler specimens a poorly defined excision broadly straddles the vein to leave almost the entire apical third of M_2 pale with just the extreme apex of the vein indistinctly darkened	- vein M_2 variable: preapical excision present from being broad and well-defined (rather *imicola*-like) to very broad thereby losing definition i.e. nearly obliterates dark apex of vein M_2 by broadly straddling the vein
- femora and tibiae predominantly pale	- predominantly brown
- antennal segments IV–X significantly shorter (Table 6.9)	- significantly longer (Table 6.9)
- antennal segments XI–XIII, XV significantly longer (Table 6.9)	- significantly shorter (Table 6.9)
- AR 1,13–1,24, mean 1,19 (n = 45)	- AR 0,98–1,13, mean 1,06 (n = 104)
- 96 % with three coeloconica on flagellar segment III, mean 3,04 (n = 48); no coeloconica fused	- 68 % with three coeloconica, 32 % with four or five coeloconica, mean 3,35 (n = 104); 15 % with two coeloconica fused to form a larger 'double' pit
- PR 2,03–2,65 mean 2,38 (n = 46) i.e. palp III not as inflated	- PR 1,80–2,57, mean 2,15 (n = 101) i.e. palp III slightly more inflated
- scutum with 76–109, mean 89 bristles (n = 17)	- scutum with 82–147, mean 112 bristles (n = 12)
Geographical	
- restricted to higher-rainfall equatorial regions	- restricted to lower-rainfall, frost-free treed savannas and subtropical escarpments

TABLE 6.11: Morphological and biological character states separating *C. imicola* from *C. pseudopallidipennis*

C. imicola	*C. pseudopallidipennis*
Morphological	
♀ wing darker; second and third pale costal spots broadly separated under radial cells	- wing markedly paler; second and third pale costal spots seldom separated under radial cells; usually these spots narrowly to broadly fused
- apex of vein M_2 broadly dark on both margins, this preceded by a well-defined, not too broad preapical excision which very seldom straddles vein M_2	- vein M_2 variable: can have both margins darkened which gradually taper to leave the apex of M_2 narrowly dark, no preapical excision; in paler specimens M_2 with a broad, poorly defined excision on both margins to leave entire apical $^1/_3$ of vein pale or rarely with extreme apex indistinctly darkened
- antennal segments III–X significantly longer	- these segments shorter
- coeloconica on segments III, XII–XV	- coeloconica on III, XI–XV
- long blunt-tipped sensilla trichodea on basal antennal flagellar segments moderately long and slender	- these trichodea slightly shorter and obviously inflated
- in 94 % of specimens antennal segments VI and VIII with 3 sensilla chaetica basally (n = 172)	- in 94 % of specimens at least one of segments VI and VIII with four sensilla chaetica; 69 % with four chaetica on both segments (n = 48)
- third palpal segment moderately long and slender with a rather small sensory pit	- this segment equal in length but inflated and bearing a larger sensory pit
- legs darker, bands more clearly defined	- legs paler, bands narrow and poorly defined
- AR 0,95–1,10, mean 1,01 (n = 167)	- AR 1,13–1,24, mean 1,19 (n = 45)
- scutum with 57–79 bristles, mean 67 (n = 17)	- 76–109, mean 89 (n = 17)
♂ membrane sternum 9 with 8–145 spiculae, mean 47 (n = 50)	- membrane with 0–20 spiculae, mean 6,8 (n = 19)
- basimere 2,3–2,7 mean 2,5 x as long as broad	- 2,7–3,0 mean 2,9 × as long as broad (n = 10)
- both long blunt-tipped sensilla trichodea on antennal flagellar segments V and VI fully developed	- in most antennae one of these trichodea either stunted or missing, especially on VI
- scutum with 51–63 bristles, mean 57 (n = 11)	- 68–78, mean 72 (n = 8)
Biological	
- Larval habitat is the permanently moist grassed margins of streams, furrows and vleis especially where grass is kept short by grazing animals	- two males once "reared from banana stump;" immatures probably inhabit various fallen fruits and certain forms of decomposing vegetation

TABLE 6.12: Numbers and % abundances of 5 species of the Imicola group subgenus *Avaritia* captured in light-traps in the Kruger National Park and on adjoining farms; August 1991 – May 1993

	KNP	FARMS
No. of sites sampled	43	22
No. of collections made	700	125
No. of collections analysed	120	60
Total *Culicoides* identified	217 146	380 760
No. *Culicoides* spp./trap	3–30; x: 16,1	1–26; x: 14,2
% subgenus *Avaritia*	52,6	74,2
No. *Avaritia* spp.	10	11
% *imicola*	19,58	72,48
% *bolitinos*	10,87	0,83
% *loxodontis*	5,81	0,002
% *miombo*	0,006	0
% *tuttifrutti*	0,26	0,62
No. *tuttifrutti*	557	2 352
Frequency in traps (*tuttifrutti*)	38/120 (31,7 %)	39/60 (65 %)

CHAPTER 7

Afrotropical *Culicoides* (Diptera: Ceratopogonidae): *C. (Avaritia) kwagga* sp. nov., a potential vector of African horsesickness virus (AHSV) reared from the dung of the plains zebra, the white rhinoceros and the horse in South Africa

7.1 INTRODUCTION

On 20 June 1987 a small group of six zebras (*Equus burchellii*), shipped from Namibia, was released into the 100 hectare El Rincón safari park 60 km south-west of Madrid, Spain. Five weeks later, horses in the safari park began to die, and in early September, when the death toll stood at 29, the cause was identified as African horsesickness. The epizootic spread into the southern half of Spain and Portugal, and persisted for well over three years; nearly 2 000 horses died (Palmiter 1991; Rodrigues, Hooghuis & Castano 1992). Current data indicate the virus being spread principally by one species of biting midge, *Culicoides imicola* (Mellor, Boned, Hamblin & Graham 1990), a widespread African species that has become established in some Mediterranean countries and is known to occur as far east as Laos.

Research done in South Africa has further substantiated the link between zebras and African horsesickness (Barnard 1993). Over a 10-month period (August 1991 — May 1992), sera were collected every six to 12 weeks from 123 free-living zebras in the Satara area of the central Kruger National Park (KNP), and their changing antibody titres to AHSV determined. Four noteworthy facts emerged:

(i) by the end of their first year close to 100 % of young zebra foals, after losing their colostral immunity at five to six months of age, had seroconverted to all nine known serotypes of AHSV;

(ii) the highest incidence of natural infection did not occur during the summer to mid-autumn months (December–May) as is the norm in other parts of South Africa, but during the drier winter and spring months of July–October;

(iii) the high and rapid rates of seroconversion occurred during the severest drought experienced in the KNP this century; this drought may have caused the

(iv) severely depressed populations of *C. imicola*, and so suggests that other species of *Culicoides* were involved in the transmission of AHS amongst zebra in the KNP.

The sero-study on the epidemiology of AHS in the African veld was done in tandem with a survey of *Culicoides* found associated with elephant, black and white rhinoceros, buffalo and plains zebra, as some of these are suspected reservoir hosts for AHSV. *Culicoides* were collected with light-traps, pootered off culled hosts, or reared from their dung. (The association between five *Culicoides* species of the subgenus *Avaritia* and elephants has been reported on in Chapters 5 and 8.) Simultaneously, stock farms (mostly cattle) and horse stables adjoining the western and southern borders of the KNP were monitored with light-traps; this part of the eastern Transvaal lowveld is known as the African horsesickness 'hotspot' of South Africa.

Monthly collections were made over two years (August 1991 — August 1993); a total of 75 species of *Culicoides* was collected at 65 sites (43 in the KNP, 22 on farms). Sixteen (21 %) of the species are of the subgenus *Avaritia*, and include *C. imicola* the primary vector of AHS. Half the *Avaritia* species collected in the lowveld are new to science, and include C. sp. # 107 which is here described as *Culicoides kwagga* sp. nov. The fact that this new species is closely associated with the plains zebra, is also caught on farms only around horses, uses the dung of these two equids for development of its immatures, is most prevalent in the late winter — early spring months exactly when zebra seroconvert to all serotypes of AHS, and is very closely related to *C. imicola* taxonomically, greatly increases its potential for being a natural vector of African horsesickness. It is most closely related to *C. (A.) loxodontis* which is associated with the African elephant, and is described in Chapter 5.

7.2 RESULTS

7.2.1 ***Culicoides (Avaritia) kwagga* sp. nov.** (Fig. 7.1–7.6; Table 7.1–7.9)

FEMALE (Fig. 7.1, 7.2; Table 7.1–7.3, 7.5)

Head. Eyes bare, contiguous over a distance equal approximately to the diameters between one and two adjacent facets. Antenna (Fig. 7.1, Table 7.1–7.3, 7.7) slender, basal segments IV–IX barrel-shaped, X more flask-shaped, distal segments XI–XIV more or less parallel-sided, narrowing fractionally subapically, XV parallel-sided only except apically narrowed to a broadly rounded apex; mean lengths of antennal segments III–XV: 39,2–25,1–24,9–26,3–26,9–26,8–27,1–28,8–42,1– 44,6–47,4–46,0–71,2 μm (n = 31); total length of antenna: 427,5–531,5 μm, mean 495,5 μm (n = 31); widths of antennal segments III–XV: 29,4–23,1–20,6–20,0–18,8–18,8–18,8–17,5–16,3– 16,3–16,3–16,3–18,8 (n = 1); AR 0,96–1,12, mean 1,06 (n = 31); sensilla coeloconica present on segments III, XII–XV in 93,8 % of antennae examined (n = 32), see Table 7.2 for deviations from the norm; antennae with a total of six to eight coeloconica, mean 7,00 (n = 32); mean sensilla chaetica distribution on segments III–XV is 5,06–3,03–2,03–3,13–2,06–3,03–1,97–3,00–0–0–0– 0–0 (n = 32), see Table 7.3 for deviations from the norm; blunt-tipped sensilla trichodea distribution of the LLc type, i.e. each of segments IV–X with two long and one short sensilla trichodea, segment III with only two long sensilla trichodea (n = 32); AtR 1,41–1,65 mean 1,54 (n = 12); segments XI–XIV each with 16–19 sharp-tipped sensilla trichodea of varying lengths and thicknesses distributed in a basal and subapical whorl, XV with approximately twice as many trichodea, these distributed almost throughout segment (Fig. 7.1); similarly the short blunt-tipped basiconica range from three to seven on each of the distal flagellar segments XI–XV (Fig. 7.1); similarly the short blunt-tipped basiconica range from three to seven on each of the distal flagellar segments XI–XV (Fig. 7.1); all antennal segments uniformly clothed throughout with fine spiculae (Fig. 7.1). The range and mean number of sensilla coeloconica, chaetica and blunt-tipped trichodea are given in Table 7.1. Palp: of a moderate length, slender, light-brown throughout, mean length of palpal segments I–V: 24,32–54,43–50,52–31,98–29,38 μm (n = 24); total length 165,0–192,5 μm, mean 179,6 μm (n = 24); palpal segment I with one rather long chaetica (n = 24), II with three to four rather short chaetica, mean 3,17 (83 % with three chaetica; n = 24); III of a moderate length, slightly swollen and bearing two to five rather short chaetica, mean 3,42 (58 % with three chaetica, 29 % with four, n = 24), with a well-defined but small, round and shallow subapical pit with diameter of opening 0,44–0,56, mean 0,48 (n = 29) the width of segment, margin of pit smooth but well defined; IV with two to five short chaetica, mean 3,58 (42 % with three chaetica, 33 % with four; n = 24); V with no chaetica on median portion of segment, but always bears five short erect bristles apically; PR 2,22–2,93, mean 2,60 (n = 24); P/H ratio 0,85–1,02, mean 0,93 (n = 20); mandible with 12–15 teeth, mean 13,6 (n = 24).

Fig.7.1

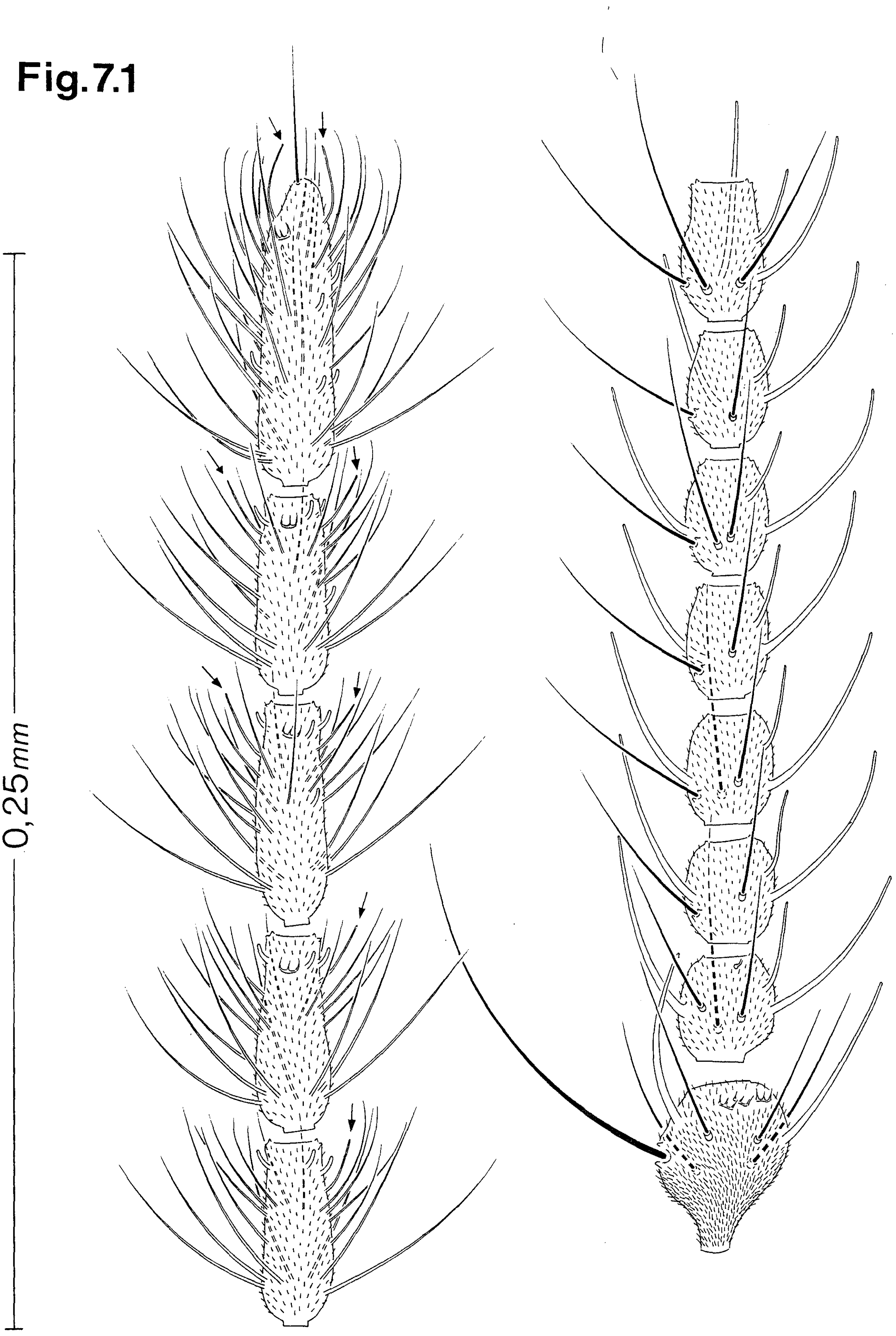

Fig. 7.1 *Culicoides (Avaritia) kwagga* sp. nov. Antenna, female: segments XI–XV on left, segments III–X on right (slide Pilanesberg).

Thorax. Legs: brown with all femora narrowly pale basally, fore femora narrowly and indistinctly pale apically. All tibiae with a narrow, well-defined, subbasal pale band; remainder of all tibiae brown; TR 1,56–1,76, mean 1,64 (n = 12); comb on apex of hind tibia with five or six spines, mean 5,06 (n = 34), the first being the longest and only slightly longer than the second. Wing: (Fig. 7.2), length 0,93–1,07, mean 1,01 (n = 26), breadth 0,47–0,54, mean 0,51 (n = 26), CR 0,54–0,58, mean 0,56 (n = 28); macrotrichia scanty, confined to distal third of cell R_5, with very few at the apex of cells M_1 and M_2 only; microtrichia dense and coarse. Dark areas of wing pattern grey, pale areas white with macrotrichia yellowish in pale areas and brown in grey areas; pale areas of wing extensive, well-defined but irregularly shaped; two short radial cells equal in length, proximal half of first and distal half to two-thirds of second pale. The wing of *C. kwagga* is paler than that of *C. imicola, C. bolitinos* and *C. miombo*, coming closest to those of *C. loxodontis* and *C. tuttifrutti*. Distinctive and specific wing pattern characters serve to separate *C. kwagga* from its six known taxonomic congeners. These are:

(i) extreme apex of vein M_2 narrowly but distinctly pale in most specimens; this leaves the preapical dark spot clearly isolated from wing margin;

(ii) preapical excision on vein M_2 well-defined, much as seen in *C. imicola*; excision either broadly touches the vein or straddles it shallowly to deeply;

(iii) anal angle with a brown smudge similar to that found in *C. miombo* but usually smaller.

Scutum entirely brown in alcohol; scutellum entirely brown, bearing one long median bristle (one of 50 examined had two bristles) and one shorter bristle in each corner (n = 50). Haltere knobs pale. Abdomen: two moderately sclerotized slightly unequal spermathecae present, measuring 48,1–52,2 x 36,9–40,6 μm, mean 50,9 x 39,8 μm and 36,9–40,0 x 30,0–31,9 μm, mean 38,7 x 31,3 μm (n = 7), both round to slightly ovoid and lacking hyaline punctations, with moderately long, narrow, rudimentary third spermatheca present measuring 11,3–20,0 x 3,1–5,0 μm, mean 15,4 x 4,2 (n = 7), small sclerotized ring on common spermathecal duct cylindrical, smooth and parallel-sided, a little longer than broad, and almost half the length of the rudimentary spermatheca; sclerotization surrounding gonopore much as seen in other species of the Imicola group.

Fig. 7.2

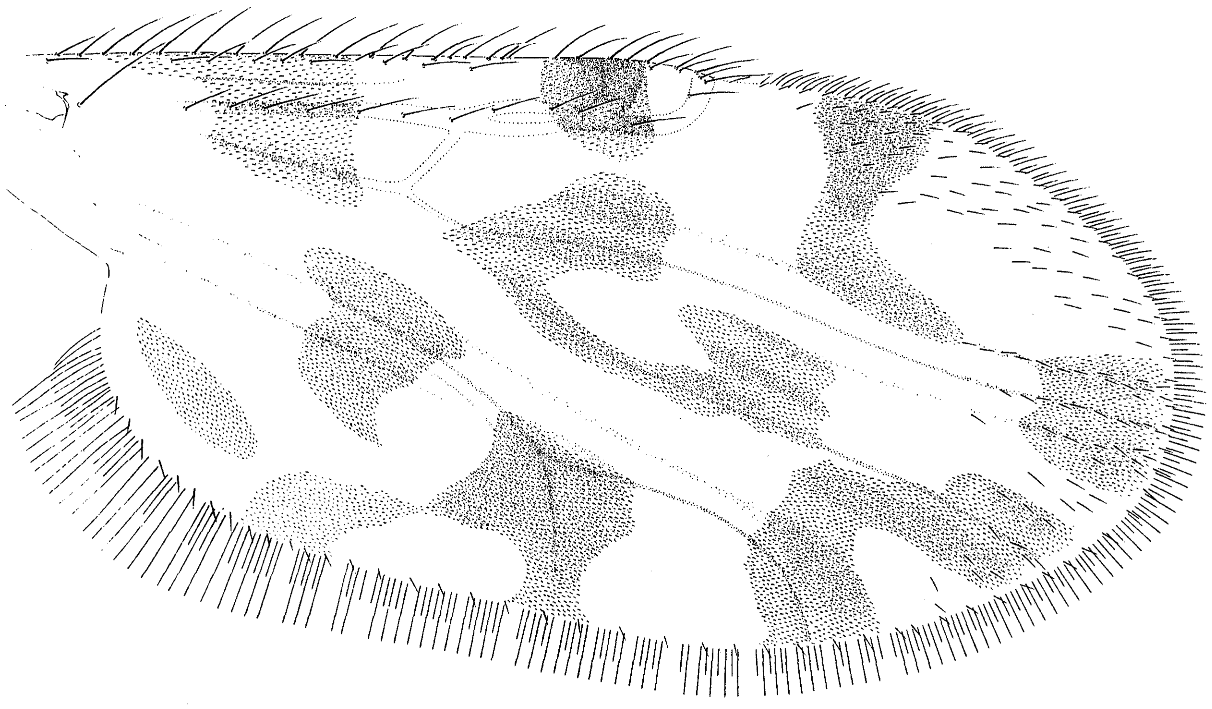

Fig. 7.2 *Culicoides (Avaritia) kwagga* sp. nov. Wing, female (slide Pilanesberg 5).

Male: (Fig. 7.3, 7.4; Table 7.1, 7.4–7.6)

Head. Eyes bare. Antenna (Table 7.1, 7.4): plume rather sparse, fibrillae light brown, almost completely encircling medially each of segments IV–XII in a regular whorl; these segments with very few spiculae which are on one face only, distal segments XIII–XV densely and evenly clothed with spiculae; mean lengths of segment III: 74,3, IV–XII: 366,2, XIII–XV: 92,7–75,0–103,1; total mean length 711,3 (n = 38); sensilla coeloconica distribution: segment III with one to two, mean 1,97 (n = 38); XIII with nought to one, mean 0,97 (n = 38); XIV with one (n = 38); XV with one to two, mean 1,97 (n = 38); antenna with a total of five to six coeloconica, mean 5,92 (n = 38); sensilla chaetica distribution: five of varying lengths and thicknesses on III; two (rarely three) basally (first long and robust, second shorter and weaker) and one medially (being very slender and 1,2 times longer than segment) on XIII (the deviations from the norm were an extra long and robust sensilla chaetica basally in one antenna); two basally (both very slender but of different lengths, the one a little shorter than segment, the other fractionally longer than segment) on XIV; none basally on XV, only one apically (n = 38); blunt-tipped sensilla trichodea distribution on segment III–XII: III with two long, segments IV–VI each with two long and one short sensilla trichodea, segments VII–IX with one long and one short sensilla trichodea, segment X with one short sensilla trichodea only, segments XI and XII lacking sensilla trichodea (n = 36); deviations from the norm are rare and are shown in Table 7.4. The distributions of the sensilla coeloconica, chaetica and trichodea are identical to those of *C. imicola, C. bolitinos, C. miombo* and *C. loxodontis*, and appear in Table 7.1. Thorax: Scutellum with one median bristle and no lateral bristles (n = 25).

Genitalia (Fig. 7.3, 7.4) tergum nine (Fig. 7.3) square, slightly waisted medially, finely spiculate throughout except for a narrow strip of the anterior and a broader strip of the posterior margins being bare, bearing 13–21 chaetica of different lengths, mean 15,7 (n = 50); apicolateral processes replaced by thin, hyaline flanges lacking spiculae but each carrying a single fine, rather short sensilla chaetica; posterior margin of tergum which widely separates these flanges gently concave (Fig. 7.3); median area not infuscate but bearing a number of striations (Fig. 7.3); two well-developed cerci (Fig. 7.4), each adorned with long spiculae and two long and two short chaetica; cerci protruding well beyond posterior margin of tergum; sternum nine (Fig. 7.4) with a rather deep and wide excavated area with nought to 64 spiculae, mean 5,4 (n = 63), of these 25 % had no spiculae; basimere with dorsal and ventral spiculae and chaetica as illustrated (Fig. 7.4), basimere 2,44–2,81, mean 2,66 times as long as broad (n = 15) with basal infuscate collar and well-developed dorsal and ventral roots of the form typical of the subgenus *Avaritia*. Distimere (Fig. 7.4) 0,76–0,82, mean 0,78 x length of basimere (n = 7), rather stout, gently curved and broadly blunt-tipped, basal half spiculate, carrying seven bristles of varying lengths and thicknesses, extreme apex with about six very short fine sensillae. Aedeagus (Fig. 7.4) shield-shaped, slender, 1,71–2,03, mean 1,87 x longer than wide (n = 16) and 0,82–0,90, mean 0,86 x length of basimere (n = 15); basal margin convex, only narrowly and very lightly infuscate towards lateral margins, distal margin of arch reaching to 0,19–0,28, mean 0,23 x length of aedeagus (n = 16); lateral margins of aedeagus moderately smooth to quite sinuous as in Fig. 7.4, gently convex, darkly but narrowly infuscate and converging distad to end in a hyaline, rather short parallel-sided terminal projection; extreme apex is not smooth but appears sclerotized and slightly irregular due either to minute papillae or fractional recurve or folding (Fig. 7.4); base of terminal projection inserts anteriorly into median area of aedeagus in the form of a raggedly infuscate 'peg' (Fig. 7.4). Parameres (Fig. 7.4) separate, nearly touching medially from where they diverge anteriad and posteriad at approximately 45°, posterior halves as two convex almost hyaline blades, basally stout but tapering smoothly to sharp, simple, erect tips.

Etymology. The association between the new species and equines is strong; 'quagga', or the Afrikaans derivative 'kwagga', is apparently the Hottentot name for the zebra, and mimics its distinctive barking alarm call 'kwa-ha-ha'. The now extinct *Equus quagga* Boddaert, 1785 earlier occurred in most of the Cape Province, and is likely the species the Afrikaner peoples and their horses first encountered during their colonization of southern Africa three centuries ago. As suggested by Barnard (1994), the kwagga may have been the reservoir host for African horsesickness that ignited a number of epizootics, the most virulent being in 1854/5 when 64 850 horses died, nearly 40 % of the entire Cape horse population (Edington 1895).

Slide-mounted material examined

1 ♀ (slide Loskop 1), Loskop nature reserve, central Transvaal, 5.X.1985, D. de Klerk, ex white rhinoceros dung.

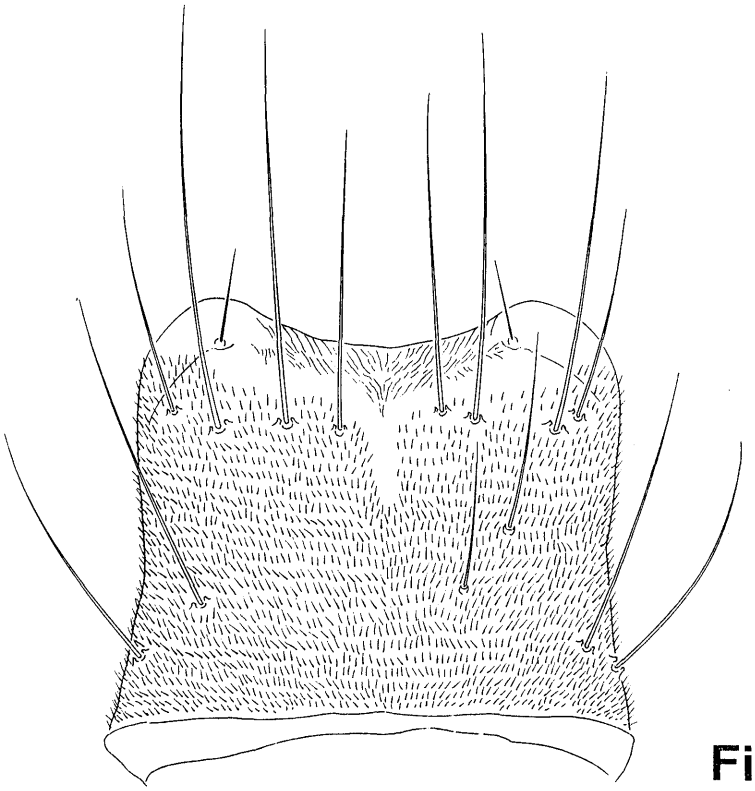

Fig. 7.3

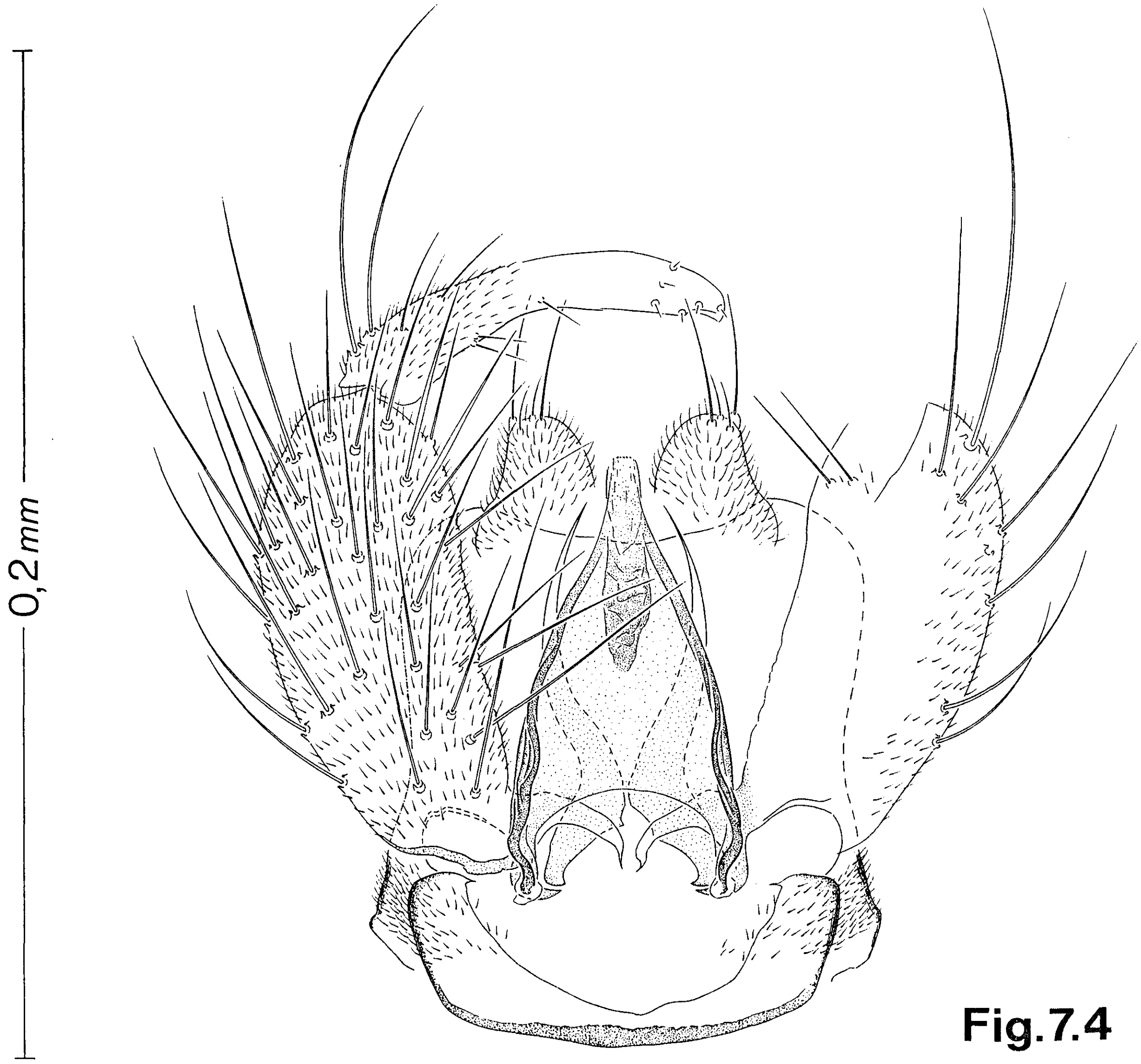

Fig. 7.4

Fig. 7.3 *Culicoides (Avaritia) kwagga* sp. nov. Genitalia, male: tergum IX (slide Pilanesberg 39).
Fig. 7.4 *Culicoides (Avaritia) kwagga* sp. nov. Genitalia, male (slide Pilanesberg 39).

1 ♂ (slide Loskop 2), Loskop nature reserve, central Transvaal, 5.X.1985, D. de Klerk, ex white rhinoceros dung.

1 ♀ (slide Onderstepoort 259), Kaalplaas horse stable, Onderstepoort, south-central Transvaal, emerged 21.IV.1986 from horse dung evacuated 13.IV.1986, G.J. Venter and R. Meiswinkel.

2 ♂♂ (slides Onderstepoort 260, 261), Kaalplaas horse stable, Onderstepoort, south-central Transvaal, emerged 21.IV.1986 from horse dung evacuated 13.IV.1986, G.J. Venter and R. Meiswinkel.

1 ♂ (slide Sentrum 51), Krugerspan game farm, Sentrum, north-western Transvaal, 20.I.1987, E.M. Nevill, M. Edwardes, G.J. Venter and R. Meiswinkel, blacklight.

2 ♂♂ (slides Sentrum 58, 62), Krugerspan game farm, Sentrum, north-western Transvaal, 16.VI.1987, E.M. Nevill, M. Edwardes, G.J. Venter and R. Meiswinkel, blacklight.

1 ♂ (slide Sentrum 84), Krugerspan game farm, Sentrum, north-western Transvaal, 30.VI.1987, E.M. Nevill, M. Edwardes, G.J. Venter and R. Meiswinkel, blacklight.

3 ♀♀ (slides Sentrum 60, 63, 66), Krugerspan game farm, Sentrum, north-western Transvaal, 16.VI.1987, E.M. Nevill, M. Edwardes, G.J. Venter and R. Meiswinkel, blacklight.

5 ♀♀ (slides Sentrum 73, 76, 78, 80, 88), Krugerspan game farm, Sentrum, north-western Transvaal, 30.VI.1987, E.M. Nevill, M. Edwardes, G.J. Venter and R. Meiswinkel, blacklight.

6 ♀♀ (slides Sentrum 188, 191–195), Krugerspan game farm, Sentrum, north-western Transvaal, ex zebra dung 3.V.1987, G.J. Venter and R. Meiswinkel.

4 ♂♂ (slides Sentrum 186, 187, 189, 190), Krugerspan game farm, Sentrum, north-western Transvaal, ex zebra dung 3.V.1987, G.J. Venter and R. Meiswinkel.

7 ♂♂ (slides Ndumu 144, 145, 147, 167, 202, 204, 210), Manzibomvu, Ndumu game reserve, northern Natal, 1.III.1989, R. Meiswinkel and G.J. Venter, truck-trap 18:05–19:05.

8 ♀♀ (slides Ndumu 154, 156, 182, 183, 187, 215, 219, 220), Manzibomvu, Ndumu game reserve, northern Natal, 1.III.1989, R. Meiswinkel and G.J. Venter, truck-trap 18:05–19:05.

2 ♂♂ (slides Ndumu 244, 257), Manzibomvu, Ndumu game reserve, northern Natal, 28.II.1989, R. Meiswinkel and G.J. Venter, blacklight.

2 ♀♀ (slides Ndumu 240, 265), Manzibomvu, Ndumu game reserve, northern Natal, 28.II.1989, R. Meiswinkel and G.J. Venter, blacklight.

22 ♀♀ (slides Pilanesberg 1–22), Pilanesberg nature reserve, 50 km north of Rustenburg, western Transvaal, D. de Klerk, ex white rhinoceros dung collected 12.XII.1989.

27 ♂♂ (slides Pilanesberg 23–49), Pilanesberg nature reserve, 50 km north of Rustenburg, western Transvaal, D. de Klerk, ex white rhinoceros dung collected 12.XII.1989.

8 ♀♀ (slides Pilanesberg 53–57, 76–78), Pilanesberg nature reserve, 50 km north of Rustenburg, western Transvaal, P.J. Meiswinkel, ex white rhinoceros dung collected I.1990.

21 ♂♂ (slides Pilanesberg 50–52, 58–75), Pilanesberg nature reserve, 50 km north of Rustenburg, western Transvaal, P.J. Meiswinkel, ex white rhinoceros dung collected I.1990.

18 ♀♀ (slides Manzimahle 1–7, 9–19), Manzimahle crib, ± 20 km north of Skukuza, south-central Kruger National Park, eastern Transvaal, R. Meiswinkel, blacklight 19.VIII.1992; Onderstepoort collection no. KNP 92.195b.

1 ♂ (slide Manzimahle 8), Manzimahle crib, ± 20 km north of Skukuza, south-central Kruger National Park, eastern Transvaal, R. Meiswinkel, blacklight 19.VIII.1992; Onderstepoort collection no. KNP 92.195b.

8 ♂ (slides Manzimahle 24–27, 37–39, 43), Manzimahle crib, ± 20 km north of Skukuza, south-central Kruger National Park, eastern Transvaal, R. Meiswinkel, blacklight 24.IX.1992; Onderstepoort collection no. KNP 92.221b.

14 ♂♂ (slides Manzimahle 20–23, 28–36, 45, 46), Manzimahle crib, ± 20 km north of Skukuza, south-central Kruger National Park, eastern Transvaal, R. Meiswinkel, blacklight 24.IX.1992; Onderstepoort collection no. KNP 92.221b.

Unmounted material examined

3 ♀♀ 3 ♂♂ Zoutpan horse stable, 40 km north of Onderstepoort, south-central Transvaal, J. Matthee, blacklight, 24.III.1989; Onderstepoort collection no. 89.49.

3 ♀♀ 2 ♂♂ Zoutpan horse stable, 40 km north of Onderstepoort, south-central Transvaal, J. Matthee, blacklight, 3.XI.1989; Onderstepoort collection no. 89.66.

44 ♀♀ Glentig farm, Alma district, Waterberg, western Transvaal, blacklight in vicinity of zebras and wildebeest, 17:00–24:00, 14.III.1990, R. Meiswinkel, G.J. Venter and M. Swanepoel.

11 ♀♀ Borakalalo nature reserve, central Transvaal, blacklight in vicinity of zebras and white rhinos, 19:00–22:00, 4.V.1991, R. Meiswinkel.

3 ♀♀	Dikhololo nature reserve, 40 km west of Onderstepoort, south-central Transvaal, M. Edwardes, blacklight in the vicinity of zebras, 4.V.1991.
13 ♀♀	Lugmag dam, south-western Kruger National Park, R. Meiswinkel, H. van Ark and M. Swanepoel, four blacklight traps, 12.VIII.1991; collection no. KNP 91.1.
12 ♀♀	Ngotso-south crib, 23 km north of Satara, central Kruger National Park, R. Meiswinkel, H. van Ark and M. Swanepoel, four blacklight traps, 13.VIII.1991; collection no. KNP 91.2.
12 ♀♀ 24 ♂♂	500 m from confluence of Mlondozi and Sabie rivers, 10 km east of Lower Sabie camp, southeastern Kruger National Park, R. Meiswinkel, blacklight, 28.VIII.1991; collection no. KNP 91.4a.
106♀♀ 14♂♂	banks of Mlondozi river, 1 km north of collection no. KNP 91.4a site; four blacklight traps, 28.VIII.1991; collection no. KNP 91.5a, b, c, d.
28 ♀♀ 13 ♂♂	2 km west of collection no. KNP 91.5 site; three blacklight traps, 29.VIII.1991; collection no. KNP 91.6a, b, c.
31 ♀♀ 1 ♂♂	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 30.VIII.1991; collection no. KNP 91.9.
2 ♀♀	Nwaswitshaka research camp, 300 m west of Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 31.VIII.1991; collection no. KNP 91.10.
6 ♀♀ 1 ♂♂	banks of Mlondozi river, 10 km east of Lower Sabie camp, south-eastern KNP, blacklight, R. Meiswinkel, 17.IX.1991; collection no. KNP 91.14b.
1 ♀	2 km west of collection no. KNP 91.14b site; R. Meiswinkel, three blacklight traps, 17.IX.1991; collection no. KNP 91.15a, b, c.
26 ♀♀ 5 ♂♂	Ngirivane crib and reservoir, 12 km west of Satara, central KNP, R. Meiswinkel, two blacklight traps, 18.IX.1991; collection no. KNP 91.16a, b.
6 ♀♀	Mutlumuvi, 13 km north-east of Skukuza, southern KNP, three blacklight traps in thicket near confluence of Mutlumuvi and Sand rivers, R. Meiswinkel, 19.IX.1991; collection no. KNP 91.18a, b, c.
121 ♀♀ 1 ♂♂	Manzimahle crib, 20 km north-east of Skukuza, southern KNP, three blacklight traps, R. Meiswinkel, 20.IX.1991; collection no. KNP 91.19a, b, c.

1 ♀	Renosterkoppies dam, 13 km south of Skukuza, southern KNP, blacklight, R. Meiswinkel, 20.IX.1991; collection no. KNP 91.20a.
5 ♀♀	Manzimahle crib, 20 km north-east of Skukuza, southern KNP, blacklight, R. Meiswinkel, 14.X.1991; collection no. KNP 91.30a.
3 ♀♀	Mutlumuvi, 13 km north-east of Skukuza, southern KNP, blacklight, R. Meiswinkel, 14.X.1991; collection no. KNP 91.31b.
48 ♀♀	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 14.X.1991; collection no. KNP 91.32.
75 ♀♀	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 17.X.1991; collection no. KNP 91.38.
2 ♀♀ 2 ♂♂	Muntshe crib, 20 km north of Lower Sabie camp, eastern KNP, blacklight, R. Meiswinkel, 18.X.1991; collection no. KNP 91.40b.
3 ♂♂	Manzimahle crib, 20 km north-east of Skukuza, southern KNP, blacklight, R. Meiswinkel, 24.XI.1991; collection no. KNP 91.51a.
40 ♀♀	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 26.XI.1991; collection no. KNP 91.57.
26 ♀♀	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 14.VII.1992; collection no. KNP 92.156.
14 ♀♀ 3 ♂♂	Manzimahle crib, 20 km north-east of Skukuza, southern KNP, three blacklight traps, R. Meiswinkel, 14.VII.1992; collection no. KNP 92.157a, b, c.
1 ♀	Skukuza elephant bomas, blacklight, R. Meiswinkel, 14.VII.1992; collection no. KNP 92.159.
13 ♀♀ 2 ♂♂	Manzimahle crib, 20 km north-east of Skukuza, southern KNP, four blacklight traps, R. Meiswinkel, 19.VII.1992; collection no. KNP 92.175a, b, c, e.
17 ♀	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 18.VIII.1992; collection no. KNP 92.191.
1 ♀	Mutlumuvi, 13 km north-east of Skukuza, southern KNP, blacklight, R. Meiswinkel, 19.VIII.1992; collection no. KNP 92.194b.
43 ♀♀ 2 ♂♂	Manzimahle crib, 20 km north-east of Skukuza, southern KNP, three blacklight traps, R. Meiswinkel, 19.VIII.1992; collection no. KNP 92.195a, b, c.

36 ♀♀ 8 ♂♂	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 23.XI.1992; collection no. KNP 92.216.
54 ♀♀ 38 ♂♂	Manzimahle crib, 20 km north-east of Skukuza, southern KNP, blacklight, R. Meiswinkel, 24.IX.1992; collection no. KNP 92.221a.
1 ♀	Ngotso-south crib, 23 km north of Satara, central Kruger National Park, R. Meiswinkel, 14.X.1992; collection no. KNP 92.230c.
95 ♀ 143 ♂♂	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 19.X.1992; collection no. KNP 92.240.
4 ♀♀ 19 ♂♂	Mutlumuvi, 13 km north-east of Skukuza, southern KNP, blacklight, R. Meiswinkel, 19.X.1992; collection no. KNP 92.241c.
29 ♀♀ 44 ♂♂	Manzimahle crib, 20 km north-east of Skukuza, three blacklight traps, R. Meiswinkel, 19.X.1992; collection no. KNP 92.242a, b, c.
232 ♀♀ 7 ♂♂	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 16.XI.1992; collection no. KNP 92.250.
151 ♀♀	Skukuza buffalo bomas, blacklight, R. Meiswinkel, 16.XI.1992; collection no. KNP 92.251.
15 ♀♀ 2 ♂♂	Renosterkoppies, 13 km south of Skukuza, southern KNP, two blacklight traps, R. Meiswinkel, 18.XI.1992; collection no. KNP 92.254a, b.
4 ♀♀	Manzimahle crib, 20 km north-east of Skukuza, blacklight, R. Meiswinkel, 20.XI.1992; collection no. KNP 92.260d.
351 ♀♀	Skukuza horse stables/bomas, blacklight, R. Meiswinkel, 7.XII.1992; collection no. KNP 92.266.
1 ♀ 1 ♂	Louwsville farm, 20 km south of Komatipoort, south-eastern Transvaal, blacklight at livestock including horses, R. Meiswinkel, H. van Ark and M. Swanepoel, 19.X.1991.
7 ♀♀	Sunrise farm, 2 km south-east of White River, eastern Transvaal, blacklight at horse stables, R. Meiswinkel, 26.I.1992.
2 ♀♀	Hoheisen research station, Orpen Gate, west-central KNP, blacklight, V. Bodenstein, 23.VII.1992.
2 ♀♀	Sunrise farm, 2 km south-east of White River, eastern Transvaal, blacklight at horse stables, R. Meiswinkel, 27.VIII.1992.

1 ♀ 1 ♂	Reniers farm, 2 km east of White River, eastern Transvaal, blacklight at horse stables, R. Meiswinkel, 26.VIII.1992.
11 ♀♀	Sunrise farm, 2 km south-east of White River, eastern Transvaal, blacklight at horse stables, R. Meiswinkel, 28.IX.1992.
4 ♀♀	Reniers farm, 2 km east of White River, eastern Transvaal, blacklight at horse stables, R. Meiswinkel, 28.IX.1992.
2 ♀♀	Mataffin farm, 4 km west of Nelspruit, eastern Transvaal, blacklight at horse stables, R. Meiswinkel, 28.IX.1992.
13 ♀♀	Mazuri farm, 3 km west of Hazyview, eastern Transvaal, blacklight at horse stables, R. Meiswinkel, 22.IX.1992.
4 ♀♀	Kumula farm, 3 km south-west of White River, eastern Transvaal, blacklight at horse stables, R. Meiswinkel and J. Dodds, 24.IX.1992.
69 ♀♀	Mazuri farm, 3 km west of Hazyview, eastern Transvaal, blacklight at horse stables, R. Meiswinkel and W. von Landsberg, 23.X.1992.
46 ♀♀	Mazuri farm, 3 km west of Hazyview, eastern Transvaal, blacklight at horse stables, R. Meiswinkel and W. von Landsberg, 23.XI.1992.
67 ♀♀ 22 ♂♂	Sunrise farm, Rottcher riding school, 2 km south-east of White River, eastern Transvaal, blacklight at first horse stables (11 horses), R. Meiswinkel, 15.XI.1992.
11 ♀♀	Sunrise farm, Rottcher riding school, 2 km south-east of White River, eastern Transvaal, blacklight at second horse stables (3 horses), R. Meiswinkel, 15.XI.1992.

7.3 DISCUSSION

7.3.1 *Differential diagnosis*

The nine character states that separate *C. imicola* and *C. kwagga* are summarized in Table 7.5, and are discussed in further detail below. In Table 7.6 nine character states distinguish *C. kwagga* from its sister species *C. loxodontis*.

Two states most reliably separate *C. kwagga* from all other members of the Imicola group when viewed under the dissecting microscope. While *C. kwagga* has a generally paler wing, it is unusual in having

(a) the dark spot on the apex of vein M_2 usually block-like in shape and indistinctly to clearly isolated from the wing margin by a narrow pale band, and (b) has a variable-sized, but obvious, dark smudge in the anal angle of wing. This diagnostic smudge is also found in *C. miombo* but this species is easily distinguished from *C. kwagga* in having a darker wing, dark halteres and two yellow admedian vittae on the scutum.

In slide-mounted specimens, the following six character states facilitate the separation of *C. kwagga* from its six Afrotropical congeners:

1. Female. Wing: The wing of *C. kwagga* is paler than that of *C. imicola, C. bolitinos* and *C. miombo*, but more closely resembles that of *C. loxodontis, C. tuttifrutti* and *C. pseudopallidipennis*. The following two wing characters more clearly define *C. kwagga* as a good species (Fig. 7.2):

   (a) the dark area at the apex of vein M_2 is indistinctly to clearly isolated from the wing margin; furthermore, the preapical pale excision may so deeply straddle vein M_2 that it isolates this dark area and leaves it block-like in shape

   (b) anal angle with an elongate smudge which may become smaller or be divided in two. This spot is also found in *C. miombo* but in this darker species it is larger and more clearly defined, and never divided.

2. Female. Antennae: In Table 7.6 the mean length of each antennal segment of each Afrotropical species of the Imicola group is given a comparative size ranking of one to seven; these mean lengths are also totalled for basal segments III–X against that of the distal segments XI–XV. While this method is not as sensitive as the multiple comparison of means test, it does show that all species differ from each other (even if the totals do not quite reflect this). The antenna of *C. kwagga* comes closest to that of *C. imicola* and *C. tuttifrutti*, but differs in having segments VII–X significantly shorter.

3. Female. Antennae: The seven Afrotropical species, i.e. *imicola, pseudopallidipennis, bolitinos,*

miombo, loxodontis, tuttifrutti and *kwagga*, also differ to some extent from one another in the number of sensilla chaetica and coeloconica, and in the length and degree of inflation of the blunt-tipped sensilla trichodea. These features are utilized in the key (Chapter 12).

4. Male. Genitalia: In *C. imicola* and *C. miombo* respectively the membrane of sternum nine is moderately to densely spiculate; rarely are less than 10 spicules seen in *C. imicola*. In *C. kwagga* the membrane is usually bare, and if not, rarely with more than 10 spicules. While *C. kwagga* is easily separated from these two species, it shares a bare sternital membrane with *C. pseudopallidipennis, C. bolitinos, C. loxodontis* and *C. tuttifrutti*.

5. Male. Genitalia: In *C. imicola, C. pseudopallidipennis, C. bolitinos, C. miombo* and *C. tuttifrutti*, the extreme apex of the aedeagus is moderately long and smoothly rounded; in *C. kwagga* and *C. loxodontis* the apex is not only shorter but its posterior margin is flattened and slightly roughened or darkened by a few tiny sclerotized papillae. The aedeagus of *C. kwagga* thus resembles most closely that of *C. loxodontis*, but differs in that it is more elongate or slender, being 1,71–2,03 times longer than wide; in *C. loxodontis* it ranges between 1,63–1,86. Furthermore, in most specimens, the sclerotized lateral margins of the aedeagus of *C. kwagga* are more sinuous than in *C. loxodontis*.

7.3.2 ***Taxonomy***

A small series of findings led to the stepwise realization that *C. kwagga* is a good species; a brief account of these reveals its discovery, and its separation from the closely-related *C. loxodontis*.

The first specimens of *Culicoides (Avaritia) kwagga* were reared in 1985 from white rhinoceros (*Ceratotherium simum*) dung collected in the Loskop Nature Reserve, central Transvaal. Only a single male and female emerged; these were slide-mounted and provisionally, but erroneously, labelled as C. sp. # 71, which was described as *C. loxodontis* by Meiswinkel (1992). In 1986, a few more specimens of *C. kwagga* were reared from horse dung at Onderstepoort, but were also wrongly identified as C. sp. # 71. The following year, larger numbers were reared from zebra dung, and caught in light-traps, on the game farm Krugerspan, western Transvaal. While the wings were pale, the consistent presence of only one median scutellar bristle and a slender third palpal segment indicated it to be a species different from the pale-winged *C. loxodontis* (two median bristles) and *C. tuttifrutti* (inflated palp); the males lacked spicules on the sternital membrane and so also differed from *C. imicola* which has a spiculate membrane, but, like *C. kwagga*, has a slender third palpal segment and one median bristle on the scutellum. In December 1989 and January 1990, fairly large numbers of *C. kwagga* were reared from white rhinoceros dung collected in the Pilanesberg National Park, western Transvaal; again all had only one median bristle on the scutellum.

It had become evident that *C. kwagga* was potentially widespread, and, beyond doubt, was associated with animal dung. It remained a puzzle as to why this species (still labelled in collections as C. sp. # 71) should consistently have one median bristle on the scutellum in the western half of the Transvaal, whereas in the east (the Kruger National Park) it always had two bristles. This prompted re-examination, and additional mounting, of large series of *C. loxodontis* from the northern KNP, but all consistently had two bristles medially on the scutellum. In the interim, more specimens of *C. kwagga* became available from other parts of the Transvaal, and from the Ndumu Game Reserve, northern Natal. The latter all had only one bristle medially on the scutellum, and so revealed the artificiality of the apparent east/west cline in bristle number.

At this point a second pattern emerged: all sites yielding *C. kwagga* did not have elephants but had either the white rhinoceros, zebras or horses. However, its apparent absence in the KNP still needed explanation as all these game species, and horses, occur there.

In 1991, two-pronged investigations were initiated into the epidemiology of African horsesickness in the KNP. The first aim was to conduct a sero-survey of zebras to establish if they harboured the virus (Barnard 1993), and the second was to establish which species of *Culicoides* were associated with elephants and zebras; both are suspected reservoir hosts for AHSV (Lubroth 1991). A major part of the survey was conducted in the Satara area of the central KNP, known to have the densest zebra populations, and not previously explored for *Culicoides*.

It was fortuitous that the study commenced in August [1991], as on the second night of the two-year survey *C. kwagga* was found for the first time in the KNP in four light-traps operated at Ngotso-south water trough (Chapter 9, Fig. 9.1). Copious amounts of zebra dung covered the area surrounding the trough, which is situated in a waterless, rocky grassland sparsely dotted with stunted *Acacia nigrescens* trees. Despite a cool breeze and temperatures dropping to 5 °C, 12 specimens of *C. kwagga* were collected and made it the dominant species in a very small catch of 28 *Culicoides*. Remarkably, this proved to be only one of two times that *C. kwagga* dominated light-trap collections during the entire two-year survey. The next month some 500 specimens of *C. kwagga* were reared from zebra dung collected at Ngirivane reservoir and trough. This further reinforced the growing realization that *C. kwagga* was associated with zebras, and explained why no *C. kwagga* had hitherto been collected in the KNP: all previous *Culicoides* research had been conducted in 'elephant country'.

Subsequent to this, two sites were chosen to further explore the association between *C. kwagga* and zebras. The first site was Mutlumuvi 12 km north-east of Skukuza, in an area traversed almost daily in winter by elephant family groups en route from the dry hinterland to water in the Sabie river (Chapter 9, Fig. 9.1). Because the area is densely thicketed, zebras are absent. Eight km north of Mutlumuvi lies Manzimahle which has a cement trough filled by a windmill (Chapter 9, Fig. 9.1). Nearby, a man-made earthen dam, 0,5 km^2 in size, becomes filled during the rainy season but by mid-winter has dried out. As the winter, commencing June/July, gets progressively drier, zebras trickle in from far and wide to get water at the Manzimahle trough; the build-up in zebra numbers is accompanied by the deposition of dung. Throughout the year Manzimahle is, however, irregularly visited by lone bulls or small family groups of elephants. Both Mutlumuvi and Manzimahle were sampled monthly for two years; usually three blacklight traps were operated at each site on the same night.

During this period, 371 *C. kwagga* were captured at Manzimahle in 38 light-trap collections analysed, i.e. 54 % of all *C. kwagga* specimens collected in the KNP veld throughout the two-year survey; at Mutlumuvi only 31 specimens (4,5 %) were found in 24 collections analysed. This suggests that the elephant, despite dropping enormous amounts of dung, plays little or no role in sustaining *C. kwagga* locally, and reinforced the earlier conclusion as to why *C. kwagga* had previously not been found in 'elephant country'. Indeed this species was only once reared from 7,825 kg of fresh elephant dung collected at Mutlumuvi in September 1991; a total of six specimens emerged amongst 1 249 *Culicoides* of three other species, i.e. two adults on each of days 11, 12 and 14 post-evacuation (Fig. 7.5). As mentioned above, 534 specimens of *C. kwagga* were reared from five pooled heaps of zebra dung that weighed 4,5 kg and varied from being fresh to 1,5 d old. The first individuals emerged on day eight, with peak emergence between days nine to 11 (Fig. 7.6).

In Fig. 7.5 it can be seen that in elephant dung each *Culicoides* species has a specific time of emergence; this pattern has been demonstrated repeatedly in multiple rearings made from elephant dung over the years. However, the simultaneous emergence of the two species, *C. loxodontis* and *C. kwagga*, breaks the pattern of temporal succession displayed by all other species, and suggests that *C. loxodontis* and *C. kwagga* do not normally occupy the same dung type. Indeed, this is the only occasion that *C. kwagga* emerged from over 100 samples of elephant dung. More research is required to establish whether *C. kwagga* and *C. loxodontis* are as resource specific as their names suggest. The fact that the former has more than once been reared from *Ceratotherium* dung may suggest otherwise. However, it must be borne in mind that the white rhino, like the zebra, is a grazer and so its dung is more finely textured. This may suit the needs of *C. kwagga*, and where the zebra and white rhino occur together, it will utilize both dung types. In this regard it is notable that *C. loxodontis* has on two occasions been reared from the dung of the black rhinoceros (*Diceros bicornis*) which is exclusively a browser; the dung is thus coarser, and in the textural sense approximates that of the elephant. Although the elephant is both a grazer and a browser, and so might on occasion be able to sustain the immatures of both *C. kwagga* and *C. loxodontis*, too little is known of the precise ecology of the immature stages within dung for one to speculate further on this possibility. The pupal stage of both species has been collected; gross morphological differences attest to their separate species status.

7.3.3 *Seasonal prevalence*

Of a total of 695 light-trap collections made in the KNP between August 1991 and August 1993, 627 were made in the veld and 68 at the animal bomas/horse stables, Skukuza (Chapter 9); 139 and 36 of these were analysed respectively; all specimens were identified, age-graded and sexed. During the same period, 183 collections were made on adjoining livestock farms and at horse stables; 94 were analysed (Chapter 9). The precise numbers of *C. kwagga* collected monthly are shown in Table 7.8. A most unusual feature of its phenology is that *C. kwagga* disappears from light-trap collections in the summer,

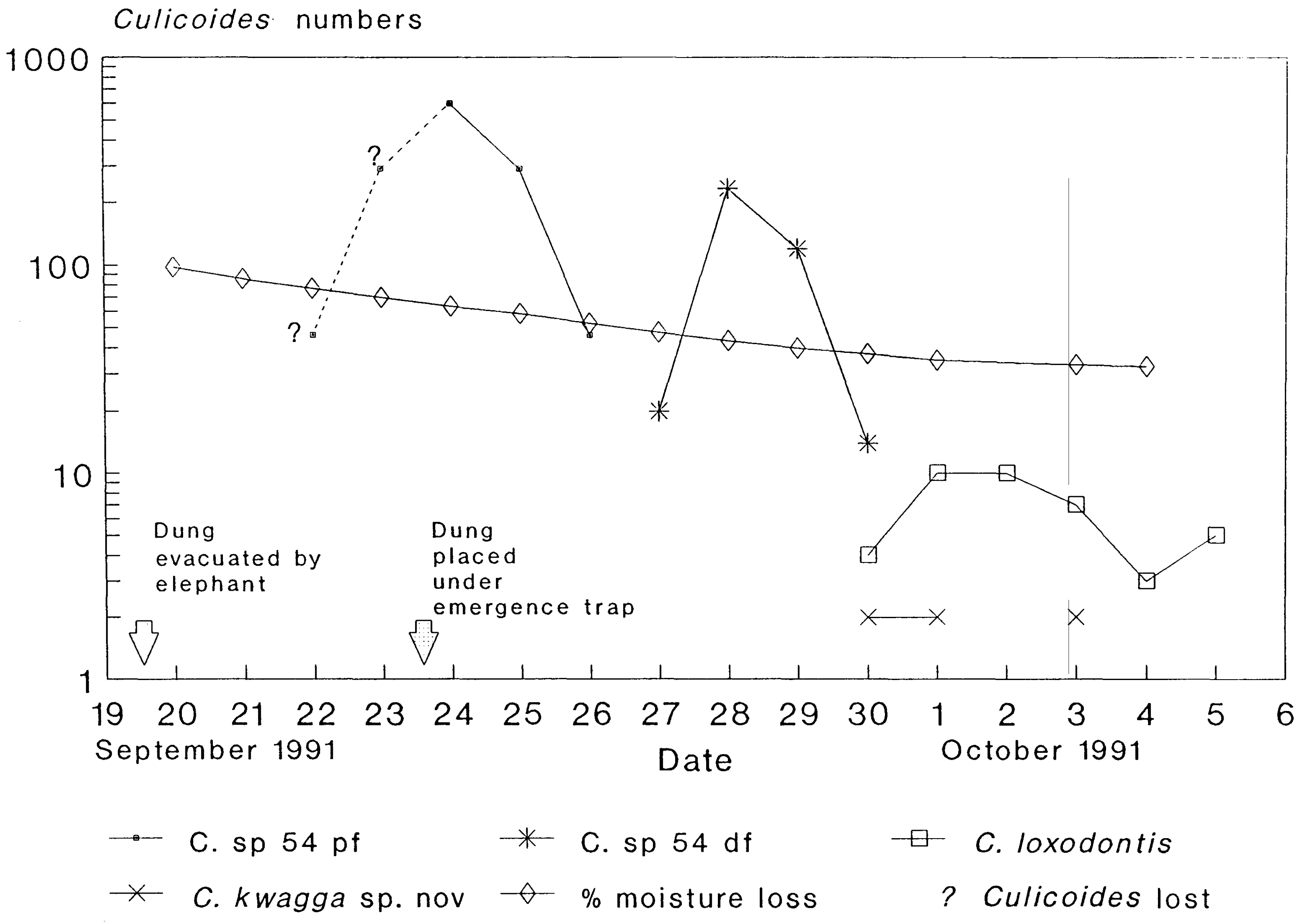

FIG. 7.5 Numbers of four *Culicoides* spp. reared from 7,825 kg of elephant dung collected on 19/9/1991 at Mutlumuvi 12 km n. of Skukuza (southern KNP); dung a few hours old. Collection no. KNP 91.26.

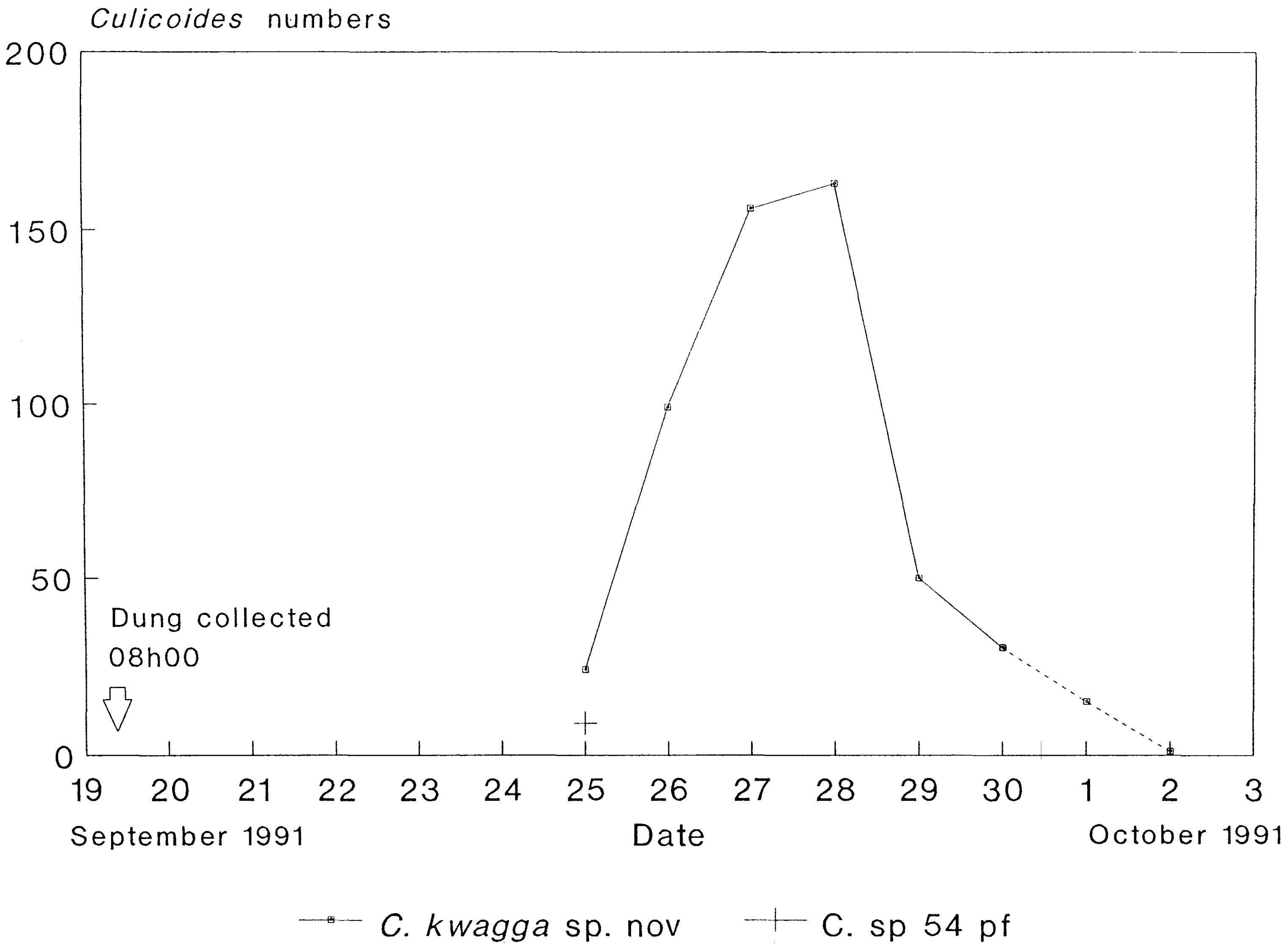

FIG. 7.6 Numbers of two *Culicoides* spp. reared from 4,5 kg of zebra dung collected on 19/9/1991 at Ngirivane 12 km w. of Satara (central KNP); dung a few hours to 1,5 days old. Collection no. KNP 91.27.

autumn and early winter months of January to June. Individuals have been collected sporadically during these months elsewhere in South Africa, and indicate that *C. kwagga* can persist through the height of summer but is less prevalent.

In the KNP, its increased prevalence during the late winter/early spring months can be correlated directly with the temporary gathering of large groups of zebra around waterholes, such as happens at Manzimahle. This leads to a build-up of dung which results in an escalation in *C. kwagga* numbers. If dung is a prerequisite for its persistence, why then does *C. kwagga* also disappear at the Skukuza stables where horses are maintained year-round? In April 1992, a simple experiment was performed at Ngotso-south crib in the KNP, in which three lots of fresh zebra dung were weighed at intervals over three days and their percentage weight-loss calculated (Table 7.9). The dung was one hour old when first collected and weighed. Each lot was gently moved onto a square of brown paper (to minimize disturbance during subsequent weighing), and separately placed under small, sparse thorny bushes, so as to be virtually unshaded as originally found. At the end of the first day 25–38 % wet-weight loss was registered. Dung lot A had termites well established under the paper square, and while no termites were active in the dung they may have taken moisture at some stage to account for lot A's significantly higher weight loss. Nevertheless, within 48 h lots B and C showed a 60–63 % weight loss, and were almost desiccated. This shows clearly that at the height of summer zebra dung dries out within three days; this would halt development of *Culicoides* immatures. As shown in Fig. 7.6, the first adults of *C. kwagga* only emerge after spending seven or eight days in the dung as immatures. This lengthy development period would thus only be achievable in the cooler winter months, or in larger dung piles that retain higher moisture levels for longer periods. In this regard, it is not uncommon to find large heaps of zebra dung, the result of multiple defaecations by territorial stallions, when zebras mass together. Another factor that would impact negatively on *Culicoides* immatures in dung is that after the first rains have fallen (October/November), dung beetles become active and for the following three to five months utilize and remove dung swiftly. These pressures on the invertebrate fauna resident in dung likely explains why *C. kwagga* never becomes abundant in winter, and disappears in summer. After the first rains, zebras abandon sites like Manzimahle and disperse into the veld where water is then widely available; *C. kwagga* populations crash in consequence, and the species disappears entirely from light-trap collections, only to reappear the following winter when zebras once again congregate.

How and where *C. kwagga* persists in the KNP during the summer months is unknown. The collection data (material examined) reveals that *C. kwagga* can be found during the summer months but only outside the KNP. Here, however, it only achieves some measure of abundance when white rhinoceroses are present, possibly because they produce larger quantities of dung daily. As yet, no continuous sampling has been done in such locales to unravel more precisely the seasonal prevalence of *C. kwagga*. Nor has it been established how large its populations become in more artificial circumstances where herbivores are confined to small areas, and are unable to disperse in response to seasonal changes in weather and forage.

7.3.4 *Abundance*

A two-year light-trap survey in the eastern Transvaal lowveld (Chapter 9) showed *C. kwagga* to be uncommon to rare. In the KNP, it represented a mere 0,36 % of 2,275 million *Culicoides* collected, while at horse stables adjoining the KNP it was even rarer (0,01 %). As mentioned above, its dependence on certain dung-types means that *C. kwagga* can never become abundant. The largest number of *C. kwagga* yet collected in a single light-trap is 351 at the Skukuza horse-stables in December 1992, this being 0,65 % of 53 592 *Culicoides* captured (52 % *C. imicola*). This implies that horses maintain *C. kwagga*, but this remains to be proven as the Skukuza stables not only adjoin the bomas (in which eight zebras had been held in the preceding three months) but also are in a habitat traversed by various game species (but never zebra).

As shown in Chapter 8, a number of *Avaritia* species are active in daylight; *C. kwagga* may also be diurnally active, and if so, this would lower its percentage prevalence in light-traps.

7.3.5 *Distribution*

While current data are fragmentary, *C. kwagga* appears to be widespread. It has been collected across the breadth of the central Transvaal, and once in northern Natal. However, it is probably localized due to its dependence on white rhino, zebra and horse dung. A 1990 survey of 10 cattle and sheep farms and horse stables in the Alma area of the Waterberg, central Transvaal, yielded no specimens of *C. kwagga*. It was recorded on a single game farm carrying various antelope species, wildebeest and zebras, and situated centrally in the Alma survey area. While this suggests *C. kwagga* does not disperse widely locally, it does show that *C. kwagga* becomes established where the appropriate hosts or dung-types occur.

7.4 CONCLUSION

Culicoides kwagga is a new species of the Imicola group of the subgenus *Avaritia*, and is most closely related to *C. loxodontis*; this suggests a fairly recent split in evolutionary terms. The apparent dependence of *C. kwagga* on moderately finely textured dung-types means it is only found in association with the white rhinoceros, zebras and horses; current evidence indicates that the coarsely-textured dung of the elephant and the black rhinoceros utilized by *C. loxodontis*, are unsuited to the survival of *C. kwagga*.

During hotter periods, zebra dung dries out too quickly to sustain the immatures of *C. kwagga*; this may be the principal explanation for its apparent seasonality in appearing in mid-winter and disappearing in early summer. Individuals have nevertheless been collected sporadically throughout the year, and show that *C. kwagga* will persist given the right conditions. While it is clear that *C. kwagga* has the potential to become widespread, its dependence on a specific resource causes it to be very localized in its occurrence.

It is notable that the appearance of *C. kwagga* coincides precisely with the time of peak sero-conversion of young zebra foals in the KNP to African horsesickness virus. While this may be coincidental, the gathering of large groups of zebras around waterholes in the late winter/early spring does lead to an increase in numbers of *C. kwagga*; the stage thus appears set for the transmission of a virus. Although the six-month break in the prevalence of *C. kwagga* suggests other species of *Culicoides* are involved in the transmission of AHS (a virus that requires year-round cycling), it remains a possibility that adults of *C. kwagga* accompany zebras on their treks, and somehow persist elsewhere in the veld in summer. Whatever the reality, it is probable that *C. kwagga* feeds on zebras, and if so, would make it a prime suspect in the transmission of AHS. Even though *C. kwagga* is uncommon to rare, its competence to transmit AHS merits investigation for two reasons: firstly, it is associated with equines, and, secondly, is very closely related to *C. imicola* which is the most effective vector of AHS known in the Old World.

7.5 REFERENCES

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TABLE 7.1: Mean lengths (μm) of segments, range in number, mean number, and distribution of sensillae on the female and male antennae of *C. (A.) kwagga* sp. nov.

	Antennal segments													Total and mean total
	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	
Female: (n = 32) Sens. coeloconica: range/segment	3	0	0	0	0	0	0	0	0-1	0-1	1	1	1	6-8
mean no./segment	3,00	0	0	0	0	0	0	0	0,03	0,97	1,00	1,00	1,00	7,00
Sens. chaetica: range/segment	5-6	3-4	2-3	3-4	2-3	2-5	1-2	3	0	0	0	0	0	22-27
mean no./segment	5,06	3,03	2,03	3,13	2,06	3,03	1,97	3,00	0	0	0	0	0	23,31
Sens. trichodea (blunt-tipped)	LL	LLc	LLc	LLc	LLc	LLc	LLc	LLc	-	-	-	-	-	
Mean lengths of segments	39,2	25,1	24,9	26,3	26,9	26,8	27,1	29,8	42,1	44,6	47,4	46,0	71,2	495,5
Male: (n = 36-38) Sens. coeloconica: range/segment	1-2	0	0	0	0	0	0	0	0	0	0-1	1	1-2	5-6
mean no./segment	1,97	0	0	0	0	0	0	0	0	0	0,97	1	1,97	5,92
Sens. chaetica: range/segment	5	0	0	0	0	0	0	0	0	0	3-4	2	0	10-11
mean no./segment	5	0	0	0	0	0	0	0	0	0	3,03	2,00	0	10,02
Sens. trichodea (blunt-tipped)	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	0	0				
Mean lengths of segments	74,3	366,2									92,7	75,0	103,1	711,3

TABLE 7.2: Number and frequency of coeloconica present on each of female antennal segments III-XV of *C. (Avaritia) kwagga* sp. nov.

No. of coeloconica per segment of *C. kwagga*	Antennal segments							
	III	IV-IX	X	XI	XII	XIII	XIV	XV
0	-	32	32	31	1	-	-	-
1	-	-	-	1	31	32	32	32
2	-	-	-	-	-	-	-	-
3	32	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
No. of antennae examined	32	32	32	32	32	32	32	32

TABLE 7.3 Number and frequency of chaetica present on each of female antennal segments III-XV of *C. (Avaritia) kwagga* sp. nov.

No. of chaetica per segment of *C. kwagga*	Antennal segments												
	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
0	-	-	-	-	-	-	-	-	32	32	32	32	32
1	-	-	-	-	-	-	1	-	-	-	-	-	-
2	-	-	31	-	30	2	31	-	-	-	-	-	-
3	-	31	1	28	2	28	-	32	-	-	-	-	-
4	-	1	-	4	-	1	-	-	-	-	-	-	-
5	30	-	-	-	-	1	-	-	-	-	-	-	-
6	2	-	-	-	-	-	-	-	-	-	-	-	-
No. of antennae examined	32	32	32	32	32	32	32	32	32	32	32	32	32

TABLE 7.4: Distribution and variation in size of the long (L) and short (c) blunt-tipped sensilla trichodea on male antennal flagellar segments III-XII of *C. (Avaritia) kwagga* sp. nov. (n = 38)

Slide no.	Flagellar segments									
	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Pilanesberg 23	LL LL	LLc LLc	LLc LLc	LLc LLc	L½Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 24	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 25	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	L½Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 26	LL LL	LLc ½LLLc	LLc LLc	LLc LLc	Lc L¾Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 27	LL LL	LLc LLc	LLc LLc	LLc LLc	L¼Lc L¼Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 28	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 29	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 30	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc L½Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 31	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc L¾Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 32	LL LL	LLc LLc	LLc LLc	LLc ½LLLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 33	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 34	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -

Slide no.	Flagellar segments									
	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Pilanesberg 35	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- ½c	- -
Pilanesberg 36	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- ½c	- -
Pilanesberg 37	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 38	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 39	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Pilanesberg 40	LL LL	LLc LLc	LLc LLc	LLc LLc	Lc Lc	Lc Lc	Lc Lc	c c	- -	- -
Mean no./seg.	LL	LLc	LLc	LLc	L0,1Lc	Lc	Lc	c	-	-

TABLE 7.5: Nine morphological and biological character states used to separate *C. imicola* from *C. kwagga* sp. nov.

C. imicola		*C. kwagga* sp. nov.	
Morphological			
♀	apical ¼-1/3 of vein M_2 broadly darkened along both margins; dark area broadly abuts wing margin	-	apex of vein M_2 less broadly darkened, and dark area is indistinctly to clearly isolated from wing margin by a narrow pale band
-	anal angle pale	-	anal angle with a variably-sized dark smudge which can be separate from or fused to dark area found medially in anal cell and abutting posterior wing margin
-	antennal segments VII–X, XV longer	-	these segments shorter
-	antennal trichodea ratio (AtR) 1,59-2,27 mean 1,86 (n = 173)	-	AtR 1,41–1,65 mean 1,54 (n = 12)
♂	membrane of sternum nine with eight to 145 spiculae, mean 47 (n = 50)	-	membrane with nought to 64 spiculae, mean 5,4 (n = 63)
-	extreme apex of aedeagus moderately long, hyaline, round-tipped and smooth	-	apex shorter, not smooth or hyaline but slightly roughened or darkened by a few tiny chitinized papillae or fold
-	aedeagus more squat 1,35–1,75, mean 1,55 x longer than wide (n = 20)	-	aedeagus more slender 1,71–2,03, mean 1,87 x longer than wide (n = 16)
-	sclerotized lateral margins of aedeagus smooth and of uniform width	-	these margins may be sinuous and of variable width
Biological			
-	larval habitat in short-grassed, moist kikuyu pastures and margins of organically enriched drainage furrows and swamps	-	immature stages abundantly found in the dung of the plains zebra and the white rhinoceros, and more rarely in that of the horse and the African elephant

TABLE 7.6: Nine morphological and biological character states used to separate *C. kwagga* sp. nov. from *C. loxodontis*

C. kwagga		*C. loxodontis*	
Morphological			
♀	dark spot at apex of vein M_2 clearly defined, blocklike	-	this dark spot poorly defined almost absent
-	anal angle with elongate dark smudge which may be divided in two	-	anal angle pale
-	third and fourth palpal segments longer	-	these segments shorter
-	antennal segments VII–XV longer; mean AR higher (1,06)	-	these segments shorter; mean AR lower (0,99)
-	antennal segments VI and VIII with three sensilla chaetica only	-	these segments always bear four chaetica
-	scutellum with one median bristle	-	with two median bristles
♂	aedeagus more elongate 1,71–2,03, mean 1,87 times longer than wide (n = 16)	-	aedeagus more squat 1,63–1,86, mean 1,71 times longer than wide (n = 25)
-	scutellum with one median bristle	-	with two median bristles
Biological			
	immature stages most abundant in the dung of the zebra and the white rhino; seldom found in the dung of the horse and the African elephant		almost exclusively found in elephant dung; has also been reared from black rhino dung

TABLE 7.7: Mean lengths of female flagellar segments III–XV ranked from one to seven for all Afrotropical species of the Imicola group

Species	Antennal segment													
	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	total III-X total XI-XV
imicola	5	7	7	6	5	6	6	6	4	5	4	5	5	48/23
bolitinos	2	2	2	2	3	3	3	3	2	2	1	1	2	20/8
loxodontis	6	4	3	3	2	1	1	1	1	1	2	2	1	21/7
miombo	3	3	4	5	7	5	7	7	6	7	7	7	7	41/34
pseudopallidipennis	1	1	1	1	1	2	2	2	7	6	5	6	6	11/30
tuttifrutti	4	6	6	7	6	7	5	5	5	3	3	3	4	46/18
kwagga	7	5	5	4	4	4	4	4	3	4	6	4	3	37/20

TABLE 7.9: Percentage moisture loss of one-hour-old zebra dung as determined in the field over four days; Ngotso-south crib 23 km n. of Satara, central Kruger National Park

Date	Time	Zebra dung lots					
		A		B		C	
		weight (kg)	% moisture loss	weight (kg)	% moisture loss	weight (kg)	% moiture loss
24.IV.1992	07:27	1,135	-	1,450	-	1,000	-
24.IV.1992	18:22	0,700	38,3	1,010	30,4	0,750	25,0
26.IV.1992	19:05	destroyed by aardvark?		0,580	60,0	0,375	62,5
28.IV.1992	06:37	-		0,505	65,2	0,375	62,5

TABLE 7.8 Monthly numbers and seasonal prevalence of *C. kwagga* sp. nov. collected over two years (VIII.1991–VIII.1993) in light-traps operated at various sites in the southern Kruger National Park (KNP) and at horse stables and livestock farms (FARMS) adjoining the KNP. In KNP column circled numbers = catches made at Skukuza horse stables/bomas; uncircled = KNP veld

Month and year	**KNP**			**FARMS**		
	Total catches made	Total catches analysed	No. of *C. kwagga*	Total catches made	Total catches analysed	No. of *C. kwagga*
VIII.1991	21	14	13-12-36-17-22-40-41-27-9-5-2-㉜	0	0	
IX.1991	21	13	7-1-9-26-5-1-2-93-27-1	1	1	2
X.1991	24	8	5-3-㊽-(75)-4	1	1	
XI.1991	19	3	3-㊵	1	1	
XII.1991	2	0		0	0	
I.1992	17	4		7	5	7
II.1992	27	7		3	2	
III.1992	33	9		15	11	
IV.1992	67	17		13	9	
V.1992	47	8		7	3	
VI.1992	62	8		9	5	
VII.1992	61	13	㉖-9-8-①-1-6-6-2	8	6	2
VIII.1992	42	9	⑰-1-3-21-21	9	3	2-2
IX.1992	16	4	㊹-92	16	19	11-4-2-13-4
X.1992	57	12	1-(238)-23-8-45-20	5	4	69
XI.1992	27	6	(239)-(151)-7-10-4	16	8	46-89-11
XII.1992	21	8	(351)	5	3	
I.1993	23	5		9	4	
II.1993	18	3		7	5	
III.1993	9	5		12	4	
IV.1993	23	7		9	6	
V.1993	31	8		10	3	
VI.1993	13	3		11	1	
VII.1993	10	1		10	1	
VIII.1993	8	0		7	0	

CHAPTER 8

African horsesickness epidemiology: five species of *Culicoides* (Diptera : Ceratopogonidae) collected live behind the ears and at the dung of the African elephant in the Kruger National Park, South Africa

8.1 INTRODUCTION

In the Afrotropical Region, certain arboviruses are biologically transmitted by bloodsucking *Culicoides* midges, the most important being those of bluetongue in sheep, African horsesickness, and, to a lesser extent, Bovine ephemeral fever and Akabane. Since these viruses appear to be endemic to Africa, wild ungulates must act as reservoir and amplifying hosts. Many game animals at least circulate the viruses, as indicated by antibody surveys (Walker & Davies 1971; Davies & Walker 1974 a,b; Davies, Shaw & Ochieng 1975; Davies & Otieno 1977; Simpson 1978; Davies & Jessett 1985; Hamblin, Anderson, Jago, Mlengeya & Hirji 1990; Binepal, Wariru, Davies, Soi & Olubayo 1992; Barnard 1993), and by transmission and infectivity trials such as those conducted on blesbuck (*Damaliscus albifrons*) by Neitz (1933), and on zebra and elephants by Erasmus, Young, Pieterse & Boshoff (1976). In many parts of the continent these natural hosts have been eradicated, and in the case of bluetongue the reservoir role has now been taken over by cattle (du Toit 1962; Nevill 1971). As regards African horsesickness virus, limited data gathered in the last 20 years indicate that the African elephant may be a reservoir. As summed up by Lubroth (1991) 'experimental inoculations ... did not equivocally exonerate the elephant having a role in AHS epizootiology but did underline the need for further field and experimental studies'. Further elucidation of the epidemiology of AHS can best be achieved against a backdrop of knowledge gleaned from a natural situation such as occurs in the Kruger National Park (KNP), South Africa, where large numbers of Africa's major savanna herbivores still abound.

Since 1979, piecemeal studies, mainly by light-trapping, have been done on the *Culicoides* biting midges of the KNP. In 1983, seven females of the undescribed *Culicoides* (*Avaritia*) species #54 pale form (p.f.) were found behind the ears of an elephant culled in the Pafuri area, northern KNP, but the significance of this finding was not fully appreciated at the time. Two years later, a six-month study was initiated to rear *Culicoides* species from the dung of elephants and other large herbivores in the KNP. This project was largely inspired by unpublished data assembled by Mr. Alan Dyce of Sydney, Australia, during a four-month fellowship to South Africa in 1973/74, and later presented in a short communication (Dyce & Marshall 1989). The rearing of *C. kanagai* and four other species of the subgenus *Avaritia* from elephant dung led Meiswinkel (1987) to contend that an intimate relationship seemed to exist between these biting midge species and *Loxodonta.* He had found that *C. kanagai*, and a few other dung-inhabiting *Culicoides* species, were always caught in higher numbers in the immediate vicinity of elephants, and that the near absence of these midge species throughout the rest of South Africa, in parallelling the disappearance of elephants from the same locales, further supported the idea of mutualism. More conclusive data were needed.

Since then, information has been gathered that supports the view that a close relationship exists between five dung-inhabiting species of *Culicoides* of the subgenus *Avaritia*, and the African elephant. Especially revealing were observations made in 1988, 1990 and 1992, when 14 elephant carcasses were examined for *Culicoides* in the southern, northern and central areas of the KNP. These data are presented in detail. To reveal more clearly the relationship that exists between the elephant and these biting midge species, I also draw on unpublished notes made on their larval habitat, i.e. elephant dung, and their geographic distribution and abundance gained during more than ten years of light-trapping. This research has culminated in what I believe is a fairly complete understanding of the life-cycle of these *Avaritia* species, and appears to be the best evidence yet gathered on the wild ungulate hosts preferentially fed on by any species of Afrotropical *Culicoides*.

While only one of the five species examined in this chapter is a member of the Imicola group, i.e. *C. loxodontis*, all will be discussed. To omit four of the species would not only weaken elucidation of the overall pattern of association between *Culicoides* midges and the African elephant, but would also prevent one from indicating subtle differences to be found in the poorly understood bionomics of each species.

8.2 MATERIALS AND METHODS

8.2.1 Culling sites, vegetation and prevailing weather.

According to White (1983), the Kruger National Park falls into two major phytochoria: II (Zambezian regional centre of endemism) and XV (Tongaland — Pondoland regional mosaic). The northern three-quarters of the KNP falls into phytochorion II, comprising vegetation units 28 and 29d, the former described as *Colophospermum mopane* woodland and scrub woodland, the latter as South Zambezian undifferentiated woodland. The southern quarter falls into phytocorion XV, vegetation unit 29e, known as a transition from the above mentioned 29d to Tongaland-Pondoland bushland. Colloquially these are referred to respectively as lowveld, arid lowveld and mopani veld (Acocks 1975). Gertenbach (1983) introduced detail into the above categories by zoning the KNP into 35 landscapes, each defined as an area 'with a specific geomorphology, climate, soil and vegetation pattern together with an associated fauna'.

Culling of elephants during the present study occurred at five sites in four of these landscapes (Fig. 8.1). These sites are briefly described to pinpoint areas of activity, rather than to imply that the vegetational and climatic patterns solely dictate the distribution of the elephants and their associated *Culicoides* species. A brief description of each site, weather conditions and temperature at the time of culling are given.

Culling site 1

James Windmill, s. KNP; landscape five: mixed *Combretum spp./Terminalia sericea* woodland; covers 8 % of the KNP; topography undulating with dense bush savanna vegetation on the uplands, open tree savanna in the bottomlands and with dense riparian vegetation on the banks of rivers and streams; altitude 350–500 m a.s.l.; climate more temperate than elsewhere in the KNP with sporadic frost in the bottomlands; rainfall 550–600 mm/annum. Elephants are commonly found in this landscape. Time and date of culling: 16:00; 22.IV.1988. Weather: windless; clear, blue autumn sky; temperature ± 28 °C. No light-trap collection made.

FIG. 8.1

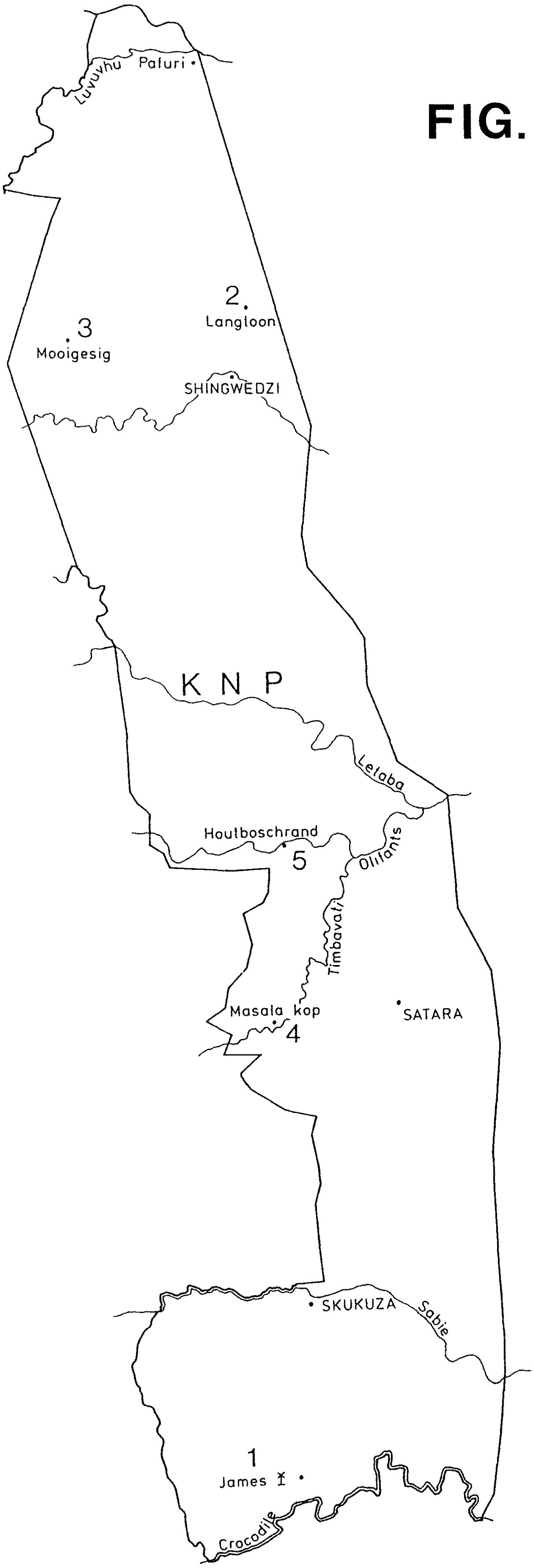


Fig. 8.1 Map of the Kruger National Park detailing elephant culling sites 1–5, major rivers and camps.

Culling site 2

Langtoon dam area, n. KNP; landscape 23: *Colophospermum mopane* shrubveld; is the largest and most homogeneous landscape, covering 10 % of the KNP; consists of flat to concave plains with a number of drainage-channels often ending in marshes; woody vegetation is dominated by multi-stemmed mopane shrubs one to two m in height; altitude 300–400 m a.s.l.; summers very hot; rainfall 450–500 mm/annum. Zebra and buffalo are found in large numbers here; elephant bulls are common, while breeding herds occasionally move through to other landscapes. Time and date of culling: 15:15; 24.IV.1990. Weather: windless, clear blue sky; temperature 31 °C. Light-trap operated from 17:00–22:00.

Culling site 3

Mooigesig dam area, n. KNP; landscape 12; *Colophospermum mopane/Acacia nigrescens* savanna; covers 5,5 % of the KNP; topography relatively flat; four rivers drain this landscape, and three hot springs and a number of pans occur; vegetation is an open tree savanna with occasional low shrubs; grass cover dense; altitude 400–460 m a.s.l.; temperatures a little milder than in landscape 23, frost seldom occurs; rainfall 500–600 mm/annum. This landscape is a preferred habitat for a variety of game, with large numbers of zebra, buffalo and elephant present. Time and date of culling: 14:50; 25.IV.1990. Weather; windless, blue sky with much scattered cloud but sun shone throughout collection period until 16:45; temperature 28 °C. Light-trap operated from 17:00–22:00.

Culling site 4

Masala koppie, 30 km w. of Satara, central KNP; landscape five: see under culling site 1. Time and date of culling: 14:45; 28.IV.1992. Weather: blue sky with scattered cloud, periodic slight breeze; temperature 31 °C. No light-trap collection made as strong winds from the south began blowing at 17:30.

Culling site 5

Houtboschrand, n.w. of Timbavati, central KNP; landscape 6: *Combretum species/Colophospermum mopane* woodland of the Timbavati area; covers 2,4 % of the KNP; topography undulating and drained by a number of tributaries of the Timbavati River; vegetation on uplands is open bush-savanna with a moderate shrub layer and dense grass cover; altitude 300–500 m a.s.l.; hot summer and cool winters, frost exceptional; rainfall 450–550 mm/annum. Habitat suitable for sable antelope, elephant, buffalo, kudu and impala whith zebra in small groups of about six individuals. Time and date of culling: 14:30; 14.IV.1992. Weather: windless; clear blue sky; temperature 32 °C. No light-trap collection made.

8.2.2 Elephant culling.

As part of the annual culling programme to restrict the number of elephants to ± 7500 in the 20,000 km^2 KNP, small family groups of eight to 20 animals are randomly tracked down, rounded up by helicopter, and destroyed. This is done each year during the autumn and winter months. Within minutes after being drug-darted from the air the elephants are bled (throats severed) and disembowelled. Over the next one to two hours, various data pertaining to their age, size and sex are collected, and the elephants are then loaded onto flatbed trucks to be taken to the abattoirs at Skukuza. During the short period the elephants lay in the field, 14 elephants were examined as thorougly as possible for *Culicoides* midges.

8.2.3 Collection of *Culicoides*.

1. **Behind elephant ears**. Live *Culicoides* were collected off ears using a suction pooter comprising a 50 cm rubber tube connected to a 30 cm glass tube, the joint interrupted by a fine gauze net prevent *Culicoides* from being swallowed during collecting. After five to ten midges had been collected, they were blown into a small bottle of 70 % ethyl alcohol. As elephants usually are on their side, only half the anatomy and one ear were examined; only at Mooigesig could both ears of one elephant be examined as it had died on its haunches. Owing to brevity of collecting time, no effort was made to establish exactly which part of the ear carries the majority of *Culicoides*.

2. **At intestinal dung**. Soon after culling, all elephants are disembowelled. In six animals a slit was made in the large intestine near the anus to expose the fully digested, bronze-coloured browse. These ruptures were monitored for ten to 25 minutes, and as many as possible of the *Culicoides* seen attracted to the dung were collected with a suction pooter. In two animals a slit was also made higher up in the small intestine to reveal a large amorphous mass of partly digested, pale green

browse in the early stages of fermentation. This incipient dung was monitored for ten minutes on each occasion.

3. **Light-trapping**. Immediately after the culling operation had been completed and all personnel had departed, light-traps were operated at the Langtoon and Mooigesig culling sites. One commercially available New Jersey-type down-draught trap equipped with an eight watt UV light, was run at each site between 17:00–22:00. A small generator was used to provide 220 v electricity.

8.2.4 Age-grading

All of the *Culicoides* (4 705) collected behind ears, at dung and in light-traps were identified and sexed. All females were graded as either nulliparous, empty parous, gravid or freshly bloodfed, using the method of Dyce (1969).

8.2.5 Taxonomy

More than 500 males and females of the five *Avaritia* species collected were slide mounted in Canada balsam; these provide the data on which the discussion of their taxonomy is based.

Finally, where a *Culicoides* species is referred to as having been reared from the dung of elephants and other herbivores, most of the information is drawn from unpublished data accumulated during the last seven years in the KNP, and from miscellaneous findings made elsewhere in South Africa.

8.3 RESULTS AND DISCUSSION

8.3.1 What is known about the wild host preferences of Afrotropical *Culicoides?*

Very little is known about the preferred wild hosts of *Culicoides* in Africa south of the Sahara. It must be appreciated that the collection of an unbiased sample of such small (1 mm-sized) primarily nocturnal midges off Africa's wild animals is difficult to achieve.

The host data that do exist reflect a strong veterinary bias in that they nearly all originate from domesticated animals (Walker & Davies 1971; Nevill & Anderson 1972; Walker & Boreham 1976; Braverman & Phelps 1981; Nevill, Venter, Edwardes, Pajor, Meiswinkel & van Gas 1988). Besides these studies there are, however, nine reports which in one way or another deal with *Culicoides* and Africa's endemic mammals; these either contain limited data resulting from opportunistic collecting, or their data are biased as the host animals were either tamed or kept in man-made environments away from their natural habitats.

In their introduction to a taxonomic study of 61 East-African *Culicoides* species, Khamala & Kettle (1971) refer to specimens received from Mr. T.N. Kangwagye that were collected off tame African buffaloes (*Syncerus caffer*). This note is best ignored as the authors do not name the *Culicoides* species involved, make no further textual reference to these host records, and, furthermore, appear to have misquoted the data assembled by Kangwagye (1973, 1974). The latter author, in a study conducted at Katookye on the River Ntungwe, western Uganda, collected biting Diptera off 'four tethered, brown, Ankole longhorn bullocks (*Bos taurus*)' and from 'two juvenile buffaloes (*Syncerus caffer caffer*)'. His results reveal that 109 specimens of four species of *Culicoides* (*C. fulvithorax, C. grahamii, C. pallidipennis* and *C. schultzei*) were found only on the *Bos taurus* bullocks, and that the two *Syncerus* buffaloes examined for biting flies were only sampled during the day and yielded no *Culicoides* whatsoever. Elsewhere this information was corroborated by Khamala (Ph.D. thesis, Univ. of East Africa, 1968) who states that 'Mr. T.N. Kangwagye . . . sent specimens of *Culicoides* collected off bullocks . . . along the River Ntugu'. The data on *Culicoides* presented in Kangwagye's second study (1974) are inconclusive as he lists under Ceratopogonidae the same four species he collected off bullocks at Katookye (Kangwagye 1973), but he gives no clue as to where, how or off which host these specimens were caught. It appears, therefore, that these data were merely transcribed from his 1973 study.

In another study on Kenyan biting midges, Walker & Boreham (1976) identified the bloodmeal source of 682 *Culicoides* belonging to 21 species collected with light-traps. Eighteen of these species, comprising 390 specimens, had fed on bovids i.e. 'any member of the Bovidae family except sheep and goats but includes cattle and wild Bovidae'. Although their collections were made at sites where 'there was a wide and plentiful variety of other hosts including humans, wild and domestic birds, rodents, dogs, wild Bovidae, viverrids, rabbits and reptiles' Walker & Boreham add that 'there was a deliberately strong bias towards sheep and cattle as potential hosts; the traps were usually at or near their pens and other livestock were ignored'. These results are therefore inconclusive as regards the wild host preferences of African *Culicoides*.

In a study purporting to deal with wild host-associated *Culicoides* in Nigeria, Dipeolu (1976) collected 33 biting midge species around two sitatungas (*Tragelaphus spekei*), two crowned duiker (*Cephalophus rufilatus*), two Maxwell's duiker (*Philantomba maxwellii*), six kob (*Kobus kob*), two red-bellied monkeys (*Cercopithecus erythrogaster*), four cherry-crowned mangabey (*Cercocebus tergautus*) and 58-60 giant rats (*Cricetomys gambianus* or *emini*) in the zoological gardens of the University of Ibadan. The animals were held in three enclosures, two of which were vegetated. At best, such situations must be considered man-made, and, whether stocked with exotics or endemics displaced from their natural habitat, would probably attract only a specific range of *Culicoides*, especially those species which do not succumb to, or alternatively, respond positively to, man's manipulation or development of the surrounding environment.

A similar, but less severe, bias is to be found in Chapter 2 where eight females each of *C. (Avaritia) bolitinos* and *C. (A.) imicola* were captured live off a darted specimen of an African buffalo kept in man-made bomas at Skukuza in the KNP. This finding is categorised as only partly artificial as the bamboo-fenced bomas are small in area (± 1 000 sq.m.), open above, and are situated in undisturbed bushveld thickets where much game abounds, including buffalo. Furthermore, there is little doubt that *C. bolitinos* has a close association with buffalo, as it has been reared from buffalo dung on numerous occasions (Chapter 2; Dyce & Marshall 1989), and also from the dung of the blue wildebeest (*Connochaetes taurinus*) and domesticated cattle. A two-year unpublished light-trapping survey (Chapter 9) has shown that the strongly mammalophilic *C. imicola* is one of four dominant species at the bomas; in the KNP, this is the site with the largest known *C. imicola* population. This herd of ten to 15 buffaloes has been held in these bomas for some eight years; the maintenance of this confined feeding resource is probably responsible for the *C. imicola* focus.

The last reference to *Culicoides* and wild hosts, and the one most pertinent to this study, concerns the following important note made by de Meillon & Hardy (1953) on *C. (Avaritia) grahamii*: '... several females, Entebbe, Uganda, 1949, from under an elephant's ear where they were firmly attached. Forwarded ... for identification by Dr. O. Fiedler of Onderstepoort. Some of these females had blood in their intestines and there seems little doubt that they were feeding on the elephant'. Unfortunately there is no record as to how these *Culicoides* were collected. The identity of these midges will be discussed elsewhere.

8.3.2 **Live *Culicoides* found behind the ears of elephants.** (Fig. 8.2, 8.3 & 8.4; Table 8.1)

After the first collections of *Culicoides* were made from behind elephant ears in the northern KNP in 1983, the entire anatomy of another 30 elephants, either culled or darted at a variety of sites in the KNP, was examined in ensuing years. If *Culicoides* were present, they occurred only behind the ears. Here the skin is at its thinnest and smoothest, and, being liberally supplied with blood vessels, would seem to be the area most suitable for biting midges to obtain their bloodmeals. As described by Bannister & Skinner (1987), the elephant's bulk makes heat loss difficult, especially as it has no sweat glands. Cooling is achieved through the enormous ears. Weighing 20 kg apiece, but comprising some 20 % of the elephant's surface area, the ears provide the major avenue for heat loss. Blood passes through them at a rate of five to 12 litres/minute, and this is responsible for as much as three-quarters of the heat loss needed to maintain a normal body temperature. Although *Culicoides* were found on almost any part of the back of the ear, there appeared to be a preponderance of midges on the upper half of the ear where they often congregated in groups of eight or more (Fig. 8.3 & 8.4). It is interesting to note that all elephants, young and old, have the characteristic ear 'turnover' where the upper margin of the ear limply folds back (Fig. 8.2). Under this fold the skin will be almost permanently shaded, and this may further suit the needs of bloodfeeding *Culicoides*. On one of the Mooigesig elephants, a large number of midges were also collected at the base of the ear. Although it is noted by Young (1972) that elephants' ears 'exude a large quantity of moisture' and that elephants captured on hot days are 'usually noticeably wet behind the ears', none of the ears examined by us was damp or moist, and the 2–3 mm thick layer of clay that caked variable parts of the ear was always dry (Fig. 8.4). Moisture loss from the ear surface may be reduced during the cooler autumn months, the period when culling is done in the KNP.

Table 8.1 gives the numbers and age-status of a total of 511 *Culicoides* of five species found live behind the ears of 14 elephants culled at the four sites of James Windmill, Mooigesig, Houtboschrand and Masala. Of these, 204 individuals (39,9 %) were nulliparous, 293 (57,3 %) empty parous, 13 (2,5 %) freshly bloodfed while only one female (0,2 %) was gravid. The age composition of the *Culicoides* found behind the ears clearly indicates that the *Culicoides* are there to feed; as in all biting Nematocera (Diptera), it is the empty parous and nulliparous individuals that are hungry. This interpretation agrees with that made by Downes (1950) and Reuben (1963) concerning the Palaearctic species *C. nubeculosus* and *C. impunctatus* respectively; and more recently, it is supported by Braverman (1992) who found that of 522 *C. imicola* captured on a calf in Israel, 65,5 % were nulliparous and 34,5 % parous; of these, 11 % were freshly bloodfed.

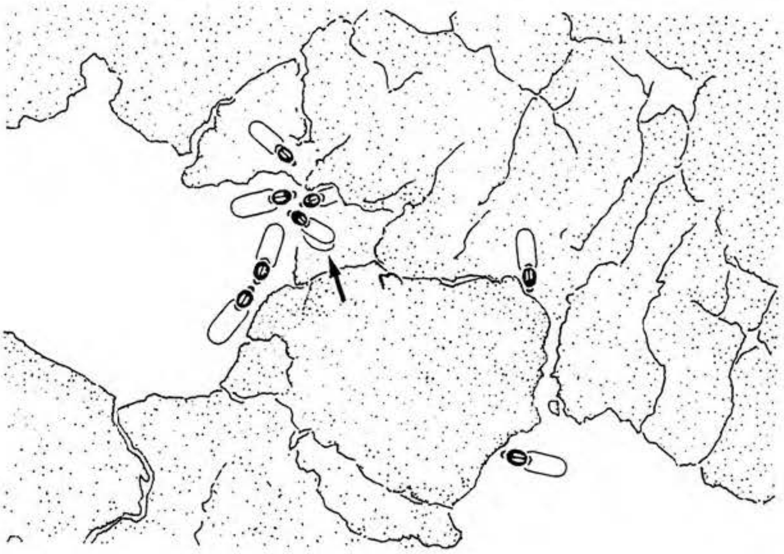
The presence of only one gravid female *Culicoides* behind elephant ears suggests strongly that sated midges leave the host to digest the blood and develop their ovaries in a suitable micro-habitat which quite likely is in the nearby vegetation. Besides fanning themselves with their ears constantly during the hottest hours of the day, elephants are also ‘partial to wallowing in mud or dusting themselves to protect the skin from the hot sun or to rid themselves of parasites’ (Smithers 1983). It seems realistic to suggest that these activities of pachyderms must be hazardous for tiny biting midges, and as ovarian development in *Culicoides* in hot climes takes two to three days, it seems unlikely that the midges would hold on behind the ears of an active host while maturing their ovaries. I also discount the possibility that, having taken blood, *Culicoides* will migrate to other parts of the elephants’ anatomy, such as near the anus, to develop their ovaries while awaiting the evacuation of dung. As stated before, the entire anatomy of 30 elephants was examined for various biting arthropods over a number of years; *Culicoides* have been seen only behind the ears. Finally, the capture of large numbers of gravid females in light-traps further indicates that *Culicoides* mature their ovaries away from the host. There is thus no evidence that gravid females of the various *Culicoides* species remain on the host as apparently is the case with the adult hornfly *Haematobia irritans* (Muscidae: Stomoxyinae) which ‘remains on the host day and night, the females leaving only briefly to deposit their eggs’ (Harwood & James 1979).

Of some interest is the fact that 13 freshly bloodfed *Culicoides* were captured off ears in daylight. In the photograph (Fig. 8.4), taken in full sunshine at 15:00 at the Mooigesig dam site, it can be seen that one of the *Culicoides* is still feeding and is almost fully engorged. This individual is arrowed in Fig. 8.3. This gainsays the general belief that all *Culicoides* are exclusively nocturnal in their activity patterns, especially during that of bloodsucking.

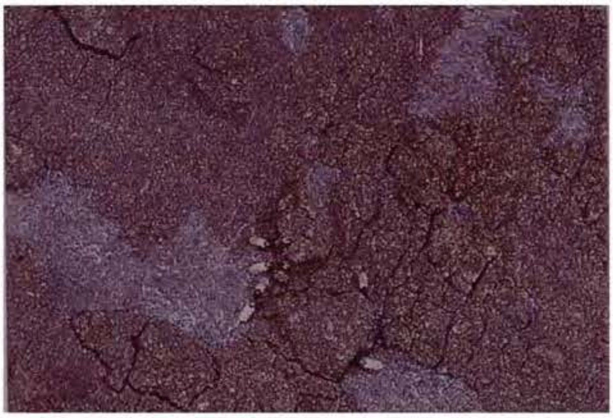
Although four elephants were examined at Langtoon, only one nulliparous specimen of C. sp. # 54 (p.f.) was found (not tabulated). This finding must be interpreted with caution. It is quite possible that not all individuals in a herd of elephants have attendant *Culicoides*, but it must also be noted that culling is a deeply traumatic experience for these animals as they are herded into a tight group by a helicopter mere metres above them. In addition, the vortex created by the helicopter blades raises clouds of dust and even lifts the ears of some elephants. Under these circumstances it is remarkable that any *Culicoides* remain attached behind the ears, as to do so they must undergo a frenzied period of herd panic that may last from one to five minutes. However, some of my observations left me in no doubt as to the tenacity of these tiny insects. For example, at the Mooigesig culling site, where 328 *Culicoides* were collected off three elephants, it was extremely difficult to suck the midges up with a pooter. Even if they were touched with the tip of the pooter and sucked at simultaneously, they would not release their foothold, but would either remain motionless or would walk away to creep in and hide between the cracks in the mud which thinly layers large areas of the ears (Fig. 8.4). Only midges which were seen to alight during collecting periods were easily disturbed and would fly away. In fact, these *Culicoides* seemed aware of any movements I made, and were difficult to capture.

From Table 8.1 it can be seen that *C. kanagai* and C. sp # 54 p.f. made up 96,3 % of all midges found behind elephant ears. It is difficult to explain why *C. loxodontis, C. tororoensis* and C. sp. # 50 were so rare. Judging from the high numbers caught in light-traps, *C. loxodontis* may be more nocturnal in its feeding habits, but the capture of similarly large numbers of C. sp. # 54 p.f. in light-traps, a species also found commonly behind elephant ears by day, indicates that factors other than nocturnalism/ diurnalism may be at play. *Culicoides kanagai* presents yet another problem with interpretation; while it was a co-dominant species behind elephant ears, it was rare in the light trap collections (2,28 %). What seems evident is that

Pen sketch of Fig. 8.4: arrowed *Culicoides* sp. # 54 p.f. freshly bloodfed. **Fig. 8.3**



8.2 Upper margin of an elephant's ear showing venation, flecks of dried mud, and shaded area beneath characteristic "turn-over"



8.4 Mooigesig culling site: eight *Culicoides* females behind elephant's ear, with grey areas of smooth skin between thin layer of cracked mud; though not clearly visible, one of the central group of six midges is freshly bloodfed (see Fig. 3); obvious wing pattern indicates all except one to be *C*. sp. #54 p.f.; patternless species *C. kanagai*

8.5 Mooigesig culling site: slit intestines of elephant exposing partly digested, pale green browse (left) and mature, fully digested, bronze-coloured browse (right); gravid *Culicoides* females only attracted to the latter for ovipositing

C. loxodontis, C. kanagai and C. sp. #54 p.f., in being common near elephants, feed on them, and in this broad sense they can be said to occupy the same niche. The disparities in their prevalence and abundance patterns implies that each species differs from the next in certain unknown details of its life-cycle. Finally, as many other game species occupy the same territory as elephants, the possibility exists that these may also provide bloodmeals, but I strongly doubt that any of them will play a more important role in the life-cycle of these *Avaritia* species than the elephant does.

8.3.3 **Live *Culicoides* attracted to the intestinal dung of disembowelled elephants** (Fig. 8.5; Table 8.2).

At the Langtoon, Mooigesig and Masala culling sites, the large intestine of six elephants was slit near the anus to leave the fully digested bronze-coloured dung exposed (Fig. 8.5). These ruptures were monitored for ten to 25 minutes, and as many as possible of the *Culicoides* seen attracted to the dung were collected with a suction pooter.

While monitoring these ruptured entrails, *Culicoides* could be seen approaching upwind on an almost imperceptible breeze (exact windspeed unknown but certainly <1 m/sec). They would then fly around actively and soon alight on the neighbouring whitish intestine or on the dung itself. Most midges would swiftly enter the crevasses in the dung, presumably to oviposit. Using a suction pooter, about 30 female *Culicoides* could be collected in 20 minutes. They were not easy to capture, as most of the females either entered the dung quickly, or gripped firmly on to the wet areas. Others had alighted on blood on the ruptured intestine, where they struggled to free themselves. A fourth category consisted of those walking actively on the sun-dried parts of the intestines, but these were easily disturbed and thus difficult to approach and collect by pooter. During 15–25 minutes of collecting, a slight decrease occurred in the number of *Culicoides* visiting the dung, but there never was a total absence. It appears therefore that dung remains attractive to ovipositing *Culicoides* for at least an hour.

At the Mooigesig and Masala sites, a slit was also made higher up in the small intestine of two elephants to reveal a large amorphous mass of partly digested pale green browse in the early stages of fermentation

(Fig. 8.5). This incipient dung was monitor for ten minutes but no *Culicoides* was seen. This dung probably lacks the necessary attractant volatiles that emanate from the darker and more mature khaki-coloured dung that is found lower down the intestine near the anus, where it is compressed into boli ready for evacuation.

Elephant dung, after being dropped to the ground, is invaded within minutes by tens to thousands of arthropods, principally larger Diptera and Coleoptera. Their presence, especially during the height of summer, makes close observation difficult as they get into the eyes, nose and throat of the observer. From present observations, made during the autumn months when insect activity is on the decline in the KNP, it now seems evident that *Culicoides* midges are amongst the first insects to arrive at dung, and therefore may also be amongst the first to leave. Early oviposition may be part of an evolutionary adaptation enabling the now empty parous females to locate an elephant before it, or the herd, has moved 100 metres or more. In this way the cycle of feeding behind the ear can begin again. The quick arrival of gravid *Culicoides* to dung also implies that they must have been in close attendance near elephants. As the search has thus far failed to reveal gravid female midges on any part of the anatomy of elephants, it is suggested that they most likely reside in a suitable microhabitat in the vegetation, and will only be stimulated into activity by elephants passing in close proximity, which they will then follow, awaiting the evacuation of dung.

Table 8.2 gives the number and age status of 171 *Culicoides* of three species caught live at dung. Of these, three individuals (1,8 %) were nulliparous, 25 (14,6 %) were empty parous, 142 (83,0 %) were gravid, while one male (0,6 %) was captured. The results are interesting in that they reveal the age structure of this part of the population of *Culicoides* collected to be diametrically opposed to that found at the same time behind the ears of elephants. These two samples are compared in Table 8.3. As the clear majority of females at dung were gravid (83,0 %), it seems superfluous to say that they were there to lay eggs. However, it may be argued that these midges were visiting dung to obtain moisture in an environment that is very dry at this time of the year. But if this is indeed part of the survival strategy of *Culicoides*, one would expect nulliparous females, and also males, to take part. Perhaps the most cogent evidence against the argument that these midge adults are solely at dung to imbibe moisture is that during studies done in the KNP from 1985–1992, all the presently tabled *Avaritia* species were reared from elephant dung, proving beyond doubt that this is the preferred larval habitat of their immature stages. The capture of 25 empty parous females (14,6 %) at dung were probably individuals that had just completed ovipositing.

Using the data contained in Tables 8.1–8.3, the adult ecology of these five *Culicoides* species is broadly sketched:

i) after feeding behind the ears of elephants the females —

ii) vacate this host to develop their ovaries in a suitable microhabitat in the nearby vegetation;

iii) once fully gravid, two to three days later, they may disperse in search of elephants; alternatively, they may only be stimulated into flight and egg-laying activity once elephants pass nearby;

iv) an elephant, or a herd, is then followed and eggs laid immediately dung is evacuated;

v) egg-laying is complete within a few minutes and the now empty parous female *Culicoides* leaves the dung to find an elephant which may not have progressed more than 100 metres or so, and

vi) by feeding once again behind the ear, is able to repeat this cycle.

The question remains as to how nulliparous female *Culicoides,* newly mated and freshly emerged from dung, locate their hosts. Though further research is needed, it is surmised that host location by nullipars is fairly random, and that dispersion in search of hosts may take place towards dusk when local conditions, such as relative humidity, are more congenial. Alternatively, as suggested above for gravid females, they may also rest in vegetation and await the passing of elephants. Such strategies may be considered far too disadvantageous for such tiny insects giving them little chance of tracking down elephants in large tracts. Elephants, however, are 'gregarious and live in family groups' while a number of groups 'may coalesce to form herds' (Smithers 1983). In the KNP, the *ca.* 7 500 elephants comprise at least 22 clans, each clan having a distinct home range or territory (A. Hall-Martin, unpublished data 1984; Whyte 1993). Depending upon the whereabouts and availability of watering points, elephants will crisscross their clan range in an almost predictable pattern. As stated by Smithers (1983), elephants create 'well worn paths which are regularly used over many years and are a feature of "elephant country"'. It is certain that their dung will be randomly dropped along these routes, at feeding points, and at watering holes. The existence of clan ranges thus favours a buildup of coprophilic *Culicoides* species, and also favours contact between newly emerged midges and elephants.

Similarly, if freshly engorged *Culicoides* abandon a host after taking a bloodmeal, to develop their ovaries in the surrounding vegetation, it seems likely that a good proportion of them, when fully mature and ready to lay eggs, will quite easily locate an elephant two or three days later and follow it until dung is evacuated. Grown elephants eat, on average, between 170–300 kg of green food per day (Guy 1975, cited in Smithers 1983), approximately half of which will be dropped as dung daily. Dougall & Sheldrick (1964), in Kenya, observed a ten year-old elephant male, weighing a little over 1800 kg, to evacuate 75 kg of dung in 12 hours (diurnal). Coe (1972), also in Kenya, observed a ten year-old captive elephant to evacuate over four days and three nights an average of 100,36 kg every 24 hours. He found that the time between each defaecation for four age-groups of elephants, ranged from 1,41–1,91 hours, and these revealed 'no significant difference between the grouped rates for day and night, suggesting that digestive activity and perhaps feeding do not change appreciably'. Such a consistent rate of defaecation means that within each elephant clan range dung will be plentiful, and, furthermore, will be rather evenly distributed along all regularly used paths. It is along these routes that a large percentage of blood-engorged *Culicoides* would have originally abandoned their hosts, and thus would be in the near vicinity two or three days later when elephants revisited the area. My observations, recorded above, as to how quickly gravid *Culicoides* come to the dung of freshly disembowelled elephants, seems to bear out this supposition. Furthermore, my failure to find behind elephant ears either gravid females or individuals with partly digested, blackened blood in their abdomens, seems to further reinforce the notion that they do not stay on the elephant to develop their ovaries.

8.3.4 Geographic distribution of dung-inhabiting *Culicoides* species

Unpublished data reveal that two of the commonest species in the KNP, *C. loxodontis* and C. sp. # 54 p.f., are very resource specific as regards their larval habitat as they will rarely, if ever, oviposit in dung other than that of the elephant. As reported in Chapter 5, *C. loxodontis* is exclusively found in 'elephant country' where it is often the dominant *Culicoides* amongst 20 or more species recorded in light-trap catches. Outside the range of larger elephant groups, as in lone bull territory, *C. loxodontis* declines markedly in numbers and disappears entirely from areas where elephants do not occur even if those areas are only a few kilometres distant. Because of this strong dependence on elephant dung, *C. loxodontis* has never been found in those parts of South Africa where no elephants occur. There is mounting evidence that most *Culicoides* species relying on the dung of a number of indigenous herbivores have undergone a parallel extinction in the farming arena, where man, in the process of establishing his farms, has exterminated Africa's game animals. This explains why *C. kanagai, C. tororoensis, C. loxodontis,* C. sp. # 50 and C. sp. # 54 p.f., which are dependant on elephants, are either new records or only recently discovered taxa in South Africa, as no research had previously been done on *Culicoides* in game parks.

From the foregoing, it seems clear that the geographic distribution of coprophilic *Culicoides* is unequivocally dependent on the whereabouts and availability of herbivore dung, and that this association is the primary factor determining the geographic distribution of the five *Avaritia* species concerned. Climate likely plays a secondary role, but here more evidence is needed.

8.3.5 **Light-trapping** (Table 8.4, 8.5)

At two of the five culling sites light-trap collections were made from 17:00–22:00; each specimen was sexed, and the females age-graded (Tables 8.4 & 8.5).

Based on their known larval habitat preferences, the 21 species captured fall either into the dung-utilizing or groundwater-inhabiting, or aquatic, sectors of the genus *Culicoides.* While it is premature to speculate on what has been collected in ten hours on two consecutive nights at two sites 35 km apart, the significant disparity in abundance between the 'dung' and 'groundwater' groups of *Culicoides* in dry open bushveld warrants brief comment:

1. The light-trap catches were dominated by the same five *Avaritia* species found live behind the ears, and at the dung, of culled elephants; these constituted 89,8 % and 97,9 % of the catches at Mooigesig and at Langtoon respectively. Similarly 96 % of the 250 males found in the traps also

belonged to these species; this shows that in the preceding weeks elephants had been resident in, or passing through, the area and had dropped fair amounts of dung.

2. Of the remaining 16 species caught in light-traps, 15 (6,24 %) rely solely on groundwaters for their larval habitat, and include *C. imicola* (1,96 %). Their low prevalence is likely the result of few breeding sites in predominantly dry bushveld, their rarity perhaps exacerbated by a feeding preference for other hosts. Two aspects of the biology of the 'groundwater' species restrict their ability to establish viable populations in new locales once they have dispersed. The first is the utter dependence of the immature stages on moist habitats such as are found on the edges of rivers, dams and marshes. Secondly, in these situations, the adults would in all likelihood obtain their required bloodmeals as there is an assured and varied biomass of game that visits daily because of their reliance on water. For this reason it is unlikely that 'groundwater' *Culicoides* disperse widely from their riverine habitats. However, if they should, it is probable that the mortality rate amongst gravid females would be high because of the distances involved to locate suitably moist oviposition sites in large expanses of dry bushveld. There is no evidence that 'groundwater' *Culicoides* species will utilize animal dung as an alternative larval habitat.

My unpublished observations reveal it is untrue that the relative scarcity of this 'groundwater' group in the present collections is solely due to their populations being low during the autumn months of the year, or that they are not attracted to light-traps. Much unpublished light-trap data collected over the years reveals that most of these species can be abundant at any time of the year but mostly along the larger sand rivers such as the Luvuvhu, Shingwedzi, Letaba, Olifants and Sabie (Fig. 8.1), and that their numbers decline as one collects further away from water into the drier bushveld.

As regards the coprophilic *Avaritia* spp. found in the light-trap collections, it is sufficient to note that *C. loxodontis* and C. sp. # 54 p.f. are amongst the most abundant species of *Culicoides* found in the KNP, and that *C. kanagai, C. tororoensis* and C. sp. # 50 are always caught in light-traps, or reared from dung, in low numbers. In fact, the highest numbers of *C. kanagai* yet collected in the KNP are the 200 females recorded from behind elephant ears at Mooigesig.

As already indicated elsewhere, explanations for the differences observed in the prevalence and abundance patterns of these five elephant-dung-inhabiting *Avaritia* species require further investigations into their bionomics. Light-traps are biased in the data they provide, and, for obvious reasons, would help little to establish whether a particular species is more diurnal than nocturnal in its habits, nor can they clarify why some species persist at such low population levels. Species may also be rare because they tolerate poorly the seasonally dry conditions that persist in the KNP, and if there are indeed preferences in terms of climate it needs to be recognised that the KNP may be too small and homogeneous to enable us to probe such trends. In addition, the severe depletion in numbers of the African elephant and the fragmentation of its distribution range into ever smaller enclaves throughout the continent would further complicate such investigations. Taking the discussion in another direction, one may also ask: in the process of maintaining a constant number of 7 500 elephants on 2 000 000 hectares, and in preventing them from moving further afield, is Man not creating unnaturally high populations of some elephant-associated *Culicoides*? There are much unpublished data indicating that when Man maintains large numbers of domestic stock in confined spaces, and irrigates pastures and builds dams, he works against those natural laws that use animal migration and drought cycles to suppress escalating parasite populations.

8.3.6 **Taxonomy**

Taxonomic understanding of the subgenus *Avaritia* is far from complete; more than half the African species still need to be described, and the six species groups defined. For this reason, each species will only be briefly discussed below.

1. *Culicoides kanagai* Khamala & Kettle, 1971, has on a few occasions been reared from elephant dung. It is rarely caught in light-traps, and in fact the largest numbers taken are those herein reported from behind elephant ears. *Culicoides kanagai* was originally described from two females from Kenya; later both sexes of *C. kanagai* were described and brief notes given on its distribution and biology in the KNP (Meiswinkel 1987). There is still some doubt as to its taxonomic status as

it is very similar to *C. dasyops* Clastrier, 1958, described on a single male from the Niokolo-Koba National Park, Senegal.

2. *Culicoides tororoensis* Khamala & Kettle, 1971, is another species rarely caught in light-traps; it has, on a few occasions only, been reared in low numbers from elephant, zebra and white rhinoceros dung (R. Meiswinkel & H. Nevill, unpublished data 1985-1992). Glick (1990) placed *C. tororoensis* in synonymy with *C. gulbenkiani* Caeiro, 1959, but our unpublished data indicate that these are two valid but closely related species that need to be re-examined both biologically and taxonomically. Dyce & Marshall (1989) recorded *C. gulbenkiani* as reared from elephant dung in the KNP but probably had *C. tororoensis* before them.

3. *Culicoides loxodontis* (Chapter 5) is a member of the Imicola group that can be abundant, and also appears to be exclusively associated with the African elephant. Certain aspects of its biology and geographic distribution were touched upon during its recent description.

   Because *C. loxodontis* is morphologically very similar to *C. imicola*, these two species can be easily confused. While they may occur sympatrically, major differences in their larval habitat, and most likely host preferences, has meant that these two species have never been found to be co-dominant at any site in South Africa.

4. C. sp. # 54 sensu lato (s.l.) is common and widespread in the KNP. It consists of two species very similar morphologically. These have been provisionally categorised as C. sp. # 54 pale form (p.f.) and C. sp. # 54 dark form (d.f.). Morphologically, they differ in that the latter is generally smaller and has a darker wing pattern. They also differ in aspects of their biology (R.M., unpublished data 1985–92) and pupal morphology (H. Nevill, unpublished data 1991). C. sp. # 54 p.f. appears also to be exclusively associated with the elephant, and has been reared in large numbers from its dung. The dark form, however, has been reared from a variety of dung types including elephant, zebra, white and black rhinoceros (R. Meiswinkel, L.E.O. Braack & H. Nevill, unpublished data

1985–1992). Dyce & Marshall (1989) reared C. sp. # 54 (s.l.) from elephant and zebra dung, and while their series from elephant dung might have included both forms, that from zebra would likely have been only the dark form. Taxonomically C. sp. # 54 (s.l.) is closely related to *C. kanagai*, the major difference being that the former have strongly patterned wings whereas the latter possesses two pale spots along the costal margin only.

5. The fifth species found behind elephant ears, C. sp. # 50, is a small member of the Grahamii group. It is uncommon in light-trap collections and has on only a few occasions been reared from elephant dung in the K.N.P. In this regard it is important to evaluate the note made by de Meillon & Hardy (1953) that they had seen 'several females of *C. grahamii* from under an elephant's ear' from Entebbe, Uganda. They were '... forwarded ... for identification to Dr. O. Fiedler of Onderstepoort'. This note prompted a search through the original manuscript of Fiedler's 1951 revision of 22 South African *Culicoides* species. A page of drawings was found, labelled: '*Culicoides* species from ear of elephant, Uganda.' The sketches show two views of the female palp, one each of spermathecae, apex of hind tibia, and the recorded measurements and outline of the distal four segments of the antenna. Although these resemble C. sp. # 50 (to be expected as they are both members of the Grahamii group), Fiedler's measurements of the antennal segments do not match those of C. sp. # 50. I am aware of other undescribed species belonging to the problematical Grahamii group, and it is possible that one of these may be associated with elephants further north in equatorial Africa. It is likely that the species recorded by de Meillon & Hardy was not *C. grahamii sensu stricto*, as this species is known to be strongly anthropophilic in Central and West Africa. It is also true that females of the Grahamii group are particularly difficult to identify as there are only minute specific differences in the wing pattern. Ultimately, accurate identification of species of this group will depend strongly on detailed examination of the various sensillae found on the antennae of both sexes, and the male genitalia.

8.4 CONCLUSIONS

Studies in the Kruger National Park, South Africa show that five species of *Culicoides* of the subgenus *Avaritia* require the blood and dung of elephants to complete their life cycles, and that they will also feed in daylight. One consequence is that the midges will be found wherever elephants occur, and are thereby enabled to penetrate even the driest tracts of savanna where they can comprise up to 90 % of all *Culicoides* captured in light-traps.

It is reasonable to conclude that the geographic distribution of all coprophilic *Avaritia* spp. in the Afrotropical Region will be found to be congruent with the distribution pattern of those animals that produce copious quantities of dung; these would include the elephant, rhinoceroses, buffaloes, cattle, zebras, horses and wildebeests. This association between midge and mammal not only gives the *Culicoides* a unique dispersal capability, but also casts suspicion on them as potential vectors of disease. As regards the elephant, certain studies indicate it to be a possible reservoir host for African horsesickness virus (Mirchamsy & Hazrati 1973; Davies & Lund 1974; Erasmus et al. 1976; Davies & Otieno 1977; Binepal et al. 1992).

This host-mediated dispersal of *Culicoides* invites us to examine the statement by Hess (1988) that 'it is unlikely that the host animals of *C. imicola* are involved in their dispersal over any more than relatively short distances'. This may be so in the farming arena where confined domesticated animals facilitate the build-up of large foci of *C. imicola*; while the need for any long-distance flights may be obviated in such situations, the converse appears to be true for species like *C. kanagai, C. tororoensis, C. loxodontis,* C. sp. # 54 p.f. and C. sp. # 50, which have to be in the same locale as their roaming hosts. Unfortunately, too little is known about the dynamics of this association to establish what distances are involved, what the midge mortality rates are, and what influence seasonal factors and climatic fluctuations have on their populations. In addition, the confinement of herds in National Parks will also neutralize the negative impact that animal migrations and long-term droughts may have on dung-inhabiting *Culicoides*, and may, instead, lead to an increase in their numbers. Whatever the realities, it remains true that coprophilic midges are widespread and can be both abundant and dominant in areas where 'groundwater' *Culicoides*, including *C. imicola,* are virtually absent.

This association between a midge and a host also partly undermines the hypothesis that in each season infective *Culicoides* need to be transported on the wind to cause disease outbreaks in distant places, as was suggested for bluetongue in Malawi (Haresnape, Taylor & Lungu 1988). Hosts with attendant *Culicoides* could ensure that a cycle of orbivirus infection is maintained year-round, regardless of movement patterns of the hosts.

Most of the seven species of the subgenus *Avaritia* treated here are poorly understood taxonomically, and so will be confused with other apparently better known African species e.g. *C. loxodontis* as *C. imicola, C. tororoensis* as *C. gulbenkiani,* and C. sp. # 50 as *C. grahamii.* While the taxonomic differences between each species pair at first glance appear negligible, there are major differences in their bionomics which have interesting and important implications as regards the epidemiology of certain viral and filarial pathogens.

If elephants do play a role in the epizootiology of African horsesickness, as has been mooted by several studies, it now seems probable that other *Avaritia* species besides *C. imicola* would be involved in the transmission of this virus.

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Table 8.1 *Culicoides* females found on the ears of elephants culled at four sites in the Kruger National Park, 1988-1992

Site: Date: Time: No. of elephants: Position:	James W/mill 22.iv.1988 16:00-16:40 4 Behind ears	Mooigesig Dam 25.iv.1990 15:30-15:45 1 Base of ear	Mooigesig Dam 25.iv.1990 15:00-15:20 1 Behind ears	Mooigesig Dam 25.iv.1990 15:00-15:15 1 Behind ears	Houtboschrand 14.iv.1992 14:30-14:55 4 Behind ears	Masala Kop. 28.iv.1992 14:55-15:35 3 Behind ears	Totals 14
Species: ***C. kanagai***							
nulliparous	15	87	11	3	0	0	116
parous	25	61	23	12	0	1	122
gravid	0	0	0	0	0	0	0
bloodfed	0	2	1	0	0	0	3
Total	40 (33,3 %)	150 (67,0 %)	35 (59,3 %)	15 (33,3 %)	0	1 (2,2 %)	241 (47,2 %)
Species: ***C. sp # 54 pf***							
nulliparous	29	17	4	6	9	14	79
parous	47	46	18	18	4	31	164
gravid	0	0	0	1	0	0	1
bloodfed	0	3	0	2	2	0	7
Total	76 (63,3 %)	66 (29,5 %)	22 (37,3 %)	27 (60,0 %)	15 (88,2 %)	45 (97,8 %)	251 (49,1 %)
Species: ***C. tororoensis***							
nulliparous	0	1	0	0	0	0	1
parous	3	0	0	0	0	0	3
gravid	0	0	0	0	0	0	0
bloodfed	0	0	0	0	0	0	0
Total	3 (2,5 %)	1 (0,4 %)	0	0	0	0	4 (0,8 %)
Species: ***C. sp # 50***							
nulliparous	0	6	0	1	0	0	7
parous	1	1	0	1	0	0	3
gravid	0	0	0	0	0	0	0
bloodfed	0	0	1	0	0	0	1
Total	1 (0,8 %)	7 (3,%)	1 (1,7 %)	2 (4,4 %)	0	0	11 (2,1 %)
Species: ***C. loxodontis***							
nulliparous	0	0	0	0	1	0	1
parous	0	0	0	0	1	0	1
gravid	0	0	0	0	0	0	0
bloodfed	0	0	1	1	0	0	2
Total	0	0	1 (1,7 %)	1 (2,2 %)	2 (11,8 %)	0	4 (0,8 %)
Grand Total	120	224	59	45	17	46	511

Table 8.2 *Culicoides* females attracted to the intestinal dung of six elephants disembowelled at three sites in the Kruger National Park, 1990–1992

Site: Date: Time: No. of elephants:	Langtoon Dam 24.iv.1990 16:10–16:35 2	Langtoon Dam 24.iv.1990 16:00–16:25 1	Langtoon Dam 24.iv.1990 16:40–16:50 1	Mooigesig Dam 25.iv.1990 16:15–16:30 1	Masala Koppies 28.iv.1992 15:40–16:01 1	Totals 6
Species: ***C. kanagai***						
nulliparous	0	0	0	1	0	1
parous	0	1	0	1	0	2
gravid	1	1	0	0	0	2
bloodfed	0	0	0	0	0	0
Total	1 (3,2 %)	2 (3,7 %) + 1 male	0	2 (4,2 %)	0	5 (2,9 %)
Species: ***C. sp. # 54 pf***						
nulliparous	0	0	2	0	0	2
parous	2	9	6	5	2	24
gravid	26	42	5	41	22	136
bloodfed	0	0	0	0	0	0
Total	28 (90,3 %)	51 (94,4 %)	13 (100,0 %)	46 (95,8 %)	24 (100,0 %)	162 (95,3 %)
Species: ***C. loxodontis***						
nulliparous	0	0	0	0	0	0
parous	0	0	0	0	0	0
gravid	2	1	0	0	0	3
bloodfed	0	0	0	0	0	0
Total	2 (6,4 %)	1 (1,9 %)	0	0	0	3 (1,8 %)
Grand Total	31	54	13	48	24	170

Table 8.3 Comparison of gonotrophic status of *Culicoides* (all species) from behind the ears and attracted to the intestinal dung of elephants in the Kruger National Park, 1988–1992

Gonotrophic status of *Culicoides*	**Behind ears of elephants**	**Attracted to intestinal dung of elephants**
nulliparous	204 (39,9 %)	3 (1,8 %)
parous	293 (57,3 %)	26 (14,6 %)
gravid	1 (0,2 %)	141 (83,0 %)
bloodfed	13 (2,5 %)	0
males	0	1 (0,6 %)
Total	511	171

Table 8.4 Mooigesig dam elephant culling site, KNP; analysis of species and age-grading of *Culicoides* caught in light-trap, 25.iv.1990.

***Culicoides* species**	**F E M A L E S**				**MALES**	**TOTAL**	**%**
	nullipar	**parous**	**b/f**	**gravid**			
C. loxodontis *	383	369	5	404	132	1293	53,47
C. sp. # 54 p.f. *	101	94	5	535	11	746	30,85
C. imicola	1	0	0	63	0	64	2,65
C kanagai *	11	8	0	33	6	58	2,40
C. tororoensis *	33	4	0	3	16	56	3,32
C kobae	2	0	1	39	0	42	1,74
C. ravus	3	0	2	27	3	35	1,45
C. tropicalis	1	0	3	19	0	23	0,95
C. sp. # 50 *	4	6	0	6	2	18	0,74
C. bolitinos	2	7	0	8	0	17	0,70
C. leucostictus	1	0	1	9	3	14	0,58
C. similis	2	0	0	6	3	11	0,45
C. nigeriae	6	0	0	2	2	10	0,41
C. sp. # 3 (Schultzei grp.)	1	0	0	8	0	9	0,37
C. walkeri	0	0	1	6	1	8	0,33
C. pycnostictus	1	0	0	3	0	4	0,16
C. dutoiti	1	0	0	2	0	3	0,12
C. schultzei s.s.	0	0	0	2	0	2	0,08
C. micheli	0	1	0	1	0	2	0,08
C. sp. # 35 (Schultzei grp.)	0	0	0	2	0	2	0,08
C. nivosus	0	0	0	1	0	1	0,04
						= 1 248	

* *Culicoides* species caught behind elephant ears and reared from their dung

Table 8.5 Langtoon dam elephant culling site, KNP; analysis of species and age-grading of *Culicoides* caught in light-trap, 24.iv.1990

***Culicoides* species**	**FEMALES**				**MALES**	**TOTAL**	**%**
	nullipar	**parous**	**b/f**	**gravid**			
C. loxodontis *	206	199	1	322	27	755	47,04
C. sp. # 54 p.f. *	391	63	1	247	40	742	46,23
C. kanagai *	9	4	0	20	2	35	2,18
C. tororoensis *	6	9	0	13	1	29	1,81
C. imicola	1	1	0	13	0	15	0,93
C. bolitinos	0	5	0	7	0	12	0,75
C. sp. # 50 *	5	6	0	0	0	11	0,68
C. ravus	1	0	0	1	0	2	0,12
C. pycnostictus	1	0	0	1	0	2	0,12
C. kobae	0	0	0	1	0	1	0,06
C. leucostictus	0	0	0	0	1	1	0,06
						=1 605	

* *Culicoides* species caught behind elephant ears and reared from their dung