AFROTROPICAL *CULICOIDES*: BIOSYSTEMATICS OF THE IMICOLA GROUP, SUBGENUS *AVARITIA* (DIPTERA: CERATOPOGONIDAE)

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dedicated to

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AFROTROPICAL *CULICOIDES*: BIOSYSTEMATICS OF THE IMICOLA GROUP, SUBGENUS *AVARITIA* (DIPTERA: CERATOPOGONIDAE)

2. A redescription of *C. (Avaritia) imicola* Kieffer, 1913 and *C. (A.) bolitinos* Meiswinkel, 1989, the latter reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa (Diptera: Ceratopogonidae)

3. Morphological comparison of *Culicoides (Avaritia) nudipalpis* Delfinado, 1961 and *C. (A.) imicola* Kieffer, 1913: reappraisal of their separate species status

4. *Culicoides (Avaritia) miombo* Meiswinkel, 1991, a widespread species closely allied to *C. (A.) imicola* Kieffer, 1913

5. *Culicoides (Avaritia) loxodontis* Meiswinkel, 1992, a member of the Imicola group associated with the African elephant (*Loxodonta africana*)

6. A redescription of *Culicoides (Avaritia) pseudopallidipennis* Clastrier, 1958 with description of its fruit-inhabiting sister species *C. (A.) tuttifrutti* sp. nov.

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

9. African horsesickness epidemiology: A two-year light-trap survey of the *Culicoides* of the southern Kruger National Park and adjoining livestock farms in the eastern Transvaal lowveld, South Africa

10. The 400 km^2 sandy dune field west of Port Elizabeth: the only *Culicoides imicola*-free zone in South Africa?

11. Subgenus *Avaritia* Fox, 1955: Redescription of the Orientalis and Imicola groups based on morphological separation of the adults (*Culicoides*; Diptera: Ceratopogonidae)

12. Subgenus *Avaritia* (*Culicoides*; Ceratopogonidae): key to nine world species of the Imicola group

SUMMARY

Bloodsucking flies of the genus *Culicoides* Latreille, 1809 came to world prominence in 1944 when Du Toit's research at Onderstepoort revealed that *C. imicola* Kieffer, 1913 transmitted the orbiviruses of bluetongue (BT) and African horsesickness (AHS); *C. imicola* is not only the most effective vector of these viruses in the Old World but it is also the most widespread species. To date, some 50 viruses have been isolated from species of the genus worldwide, and also certain filarial and protozoal parasites.

Culicoides are found almost anywhere on earth; there are ± 1 300 described species in 27 subgenera. One of these subgenera, *Avaritia* Fox, 1955 was erected for the distinctive Holarctic Obsoletus group. The taxonomy of *Avaritia* is complex as it comprises at least 10 species groups; 75 % of the 70 known world species are found in the Afrotropical and Oriental Regions. The Imicola group is the most important; its biosystematics form the subject of this thesis.

Fiedler (1951) recorded 22 species of *Culicoides* from South Africa. During this study, 112 species were collected; 23 belong to the subgenus *Avaritia*. While half are new to Science only the true congeners of *C. imicola*, constituting the Imicola group, are treated here. Due to confusion in the literature, the Imicola and Orientalis groups are redefined and distinguished, and nine Old World species are assigned to the firstnamed group (Chapter 11). The five presently recognized African species, i.e. *C. imicola*, *C. pseudopallidipennis* Clastrier, 1958, *C. bolitinos* Meiswinkel, 1989, *C. miombo* Meiswinkel, 1991 and *C. loxodontis* Meiswinkel, 1992, are redescribed (Chapters 2, 4–6), and two new species, *C. tuttifrutti* sp. nov. and *C. kwagga* sp. nov. described (Chapters 6, 7). The remaining two species, *C. brevitarsis* Kieffer, 1917 and *C. nudipalpis* Delfinado, 1961, occur outside Africa and are briefly compared with, and distinguished from, their respective sister species *C. bolitinos* (Chapter 2) and *C. imicola* (Chapter 3).

In Europe, the immature stages of three *Avaritia* species were first shown to inhabit animal dung (Kettle & Lawson 1952); later, in Australia, the abundant *C. brevitarsis* of the Imicola group was also reared from cattle dung. Coprophily in *Culicoides* occurs on all continents but is exclusive to some 10 species. These coprophiles are scattered amongst six of the 10 groups in *Avaritia*; four of them belong in the

Imicola group.

A bloodsucking insect developing in the dung of an animal is but a few wingbeats away from biting that animal; the transmission of viruses may form part of this association. Despite African diseases affecting domesticated livestock being harboured by indigenous game animals, no research has been done on *Culicoides* associated with wild herbivores in Africa. This thesis, to a modest extent, examines this association (Chapter 8). Emphasis is placed on unravelling the life-cycles of three species inhabiting the dung of the elephant, both species of rhinoceros, the buffalo, the blue wildebeest and the plains zebra (Chapters 2, 5 and 7); two of these have expanded their resource range and adapted to the dung of domesticates i.e. cattle and horses. This has obvious implications for disease transmission.

The study included a two-year survey of the *Culicoides* of the eastern Transvaal lowveld, the African horsesickness ‘hotspot’ of South Africa. Not only was the *Culicoides* fauna of a wilderness area (the Kruger National Park) compared with that found on adjoining farms, but the prevalence and abundance patterns of six species of the Imicola group were also investigated. This study reveals that Man can drive species to local extinction or cause a species to become widespread and superabundant (Chapter 9). In Chapter 10 the first *imicola*-free zone found in Africa is described. This has positive benefits as regards quarantining against AHS, and the holding of gymkhana events both local and international.

Collection methods involving light-trapping, truck-trapping and rearing from dung are briefly described. To improve observation and measurement of bodyparts, to obtain data of statistical value, and to facilitate greater accuracy during illustration, emphasis is placed on the study of long series of both sexes of a species, and on upgrading methods traditionally used in slide-mounting. Weaknesses in the descriptive format currently in use for world *Culicoides* are also addressed, and some new character states are employed (Chapter 1).

Finally, a key is provided for both sexes of the nine known species of the Imicola group (Chapter 12).

ABSTRACT

A biosystematic study of seven Afrotropical and two Oriental species of the Imicola species-group was undertaken; this group of *Culicoides* of the subgenus *Avaritia* includes *C. imicola* the most important vector of the viruses of bluetongue (BT) and African horsesickness (AHS) known in the Old World.

Five African species are redescribed i.e. *C. imicola, C. pseudopallidipennis, C. bolitinos, C. miombo* and *C. loxodontis*. Two new species are described, and the extralimital *C. brevitarsis* and *C. nudipalpis* are discussed where relevant. These nine species comprise the Imicola group, one of 10 groups constituting the subgenus worldwide. Due to confusion in the literature, the Imicola group is redefined and distinguished from the Orientalis group (also redefined); 21 species of approximately 70 world species of *Avaritia* are reassigned to either of the two groups. A key to all nine known species of the Imicola group is given; shortcomings in the taxonomy of the Orientalis group are discussed.

The adult morphology of both sexes of the nine Imicola group species was studied; this revealed deficiencies in the descriptive format currently used in taxonomic studies of world *Culicoides*. Accordingly, greater detail has been introduced into descriptions and includes the use of new character states. Methods for mounting *Culicoides* on glass slides are also improved to ensure more accurate observation and measurement of diagnostic taxonomic features; furthermore, the descriptions are based on long series of each sex. Illustrations were made from specimens mounted symmetrically, and no feature omitted from any bodypart illustrated.

Certain aspects of the life-cycle of most species were investigated but especially those of *C. bolitinos, C. loxodontis* and *C. kwagga*. The immatures of these develop exclusively in the dung of the elephant, the buffalo, the blue wildebeest, both species of rhinoceros and Burchell's zebra. Two of these species, *C. bolitinos* and *C. kwagga*, have broadened their resource range as they can invade and mature in the dung of cattle and horses. This has obvious implications for the transmission of viruses, especially where indigenous herbivores are run with domesticated livestock.

In an intensive two-year survey a comparison was made between the *Culicoides* fauna of a natural area, the Kruger National Park (KNP), and that found in areas changed by Man, namely livestock farms adjoining the KNP. This part of the eastern Transvaal lowveld is a main focus of AHS in South Africa. Light-trapping, rearing from dung, and pootering off live hosts, revealed that some species of the Imicola group are exclusively associated with certain herbivores; these include the elephant and the zebra which are suspected or proven reservoir hosts for AHS. The results thus throw further light on the epidemiology of this disease, and also show that Man plays a decisive role in determining the numbers, and distribution, of particular Imicola group species under certain conditions. In the case of *C. imicola*, the commonest and most widespread of all species, this range expansion, or establishment of foci, is due to man's maintenance of domesticated livestock in confined species, and especially where these are kept on irrigated pastures. However, the serendipitous discovery of a large *imicola*-free zone in South Africa indicates that edaphic conditions likely play an even more important role than Man and climate in determining the prevalence and abundance of *C. imicola*. This area is the sandy dune field west of Port Elizabeth and holds promise as a natural quarantine zone for the import and export of livestock.

OPSOMMING

’n Biosistematiese ondersoek van sewe Afrotropiese en twee Orientale spesies van die Imicola groep is gedoen; by hierdie groep *Culicoides* van die subgenus *Avaritia* word *C. imicola* wat as die mees belangrikste vektor van bloutong-(BT) en perdesiekte (AHS) virus in die Ou Wêreld beskou word, ingesluit.

Vyf Afrika spesies, nl. *C. imicola, C. pseudopallidipennis, C. bolitinos, C. miombo* en *C. loxodontis*, is herbeskryf. Twee nuwe spesies is beskryf asook die suid-oos Asiese spesies *C. brevitarsis* en *C. nudipalpis* word bespreek waar van toepassing. Hierdie nege spesies vorm die Imicola groep, een van die 10 groepe waaruit die subgenus *Avaritia* wêreldwyd bestaan. As gevolg van verwarring in die literatuur is die Imicola groep hergedefinieer en geskei van die Orientalis groep (ook hergedefinieer); 21 spesies van ongeveer 70 wêreld spesies van *Avaritia* is heringedeel in die twee groepe. ’n Sleutel vir al nege wêreld spesies van die Imicola groep, asook ’n verspreidingskaart vir elke spesie, word gegee. Tekortkominge in die taksonomie van die Orientalis groep word ook bespreek.

Die volwasse morfologie van beide geslagte van die nege Imicola groep spesies is bestudeer; dit het gebreke in die formaat wat tans vir die taksonomiese beskrywing van wêreld *Culicoides* gebruik word aan die lig gebring. Gevolglik is daar meer data in die beskrywings, wat nuwe karakterkenmerke insluit. Die metode van die montering van *Culicoides* op glasplaatjies is ook verbeter om meer akkurate ondersoek en meting van die diagnostiese kenmerke te verseker; verder, is beskrywings gebaseer op lang reekse van elke geslag. Illustrasies is gemaak van voorbeelde wat simmetries gemonteer is en geen kenmerk is uitgelaat van enige geïllustreerde liggaamsdeel.

Aspekte van die lewensiklus van die meeste van die spesies, veral *C. bolitinos, C. loxodontis* en *C. kwagga* is ondersoek. Die onvolwassenes van hierdie spesies ontwikkel slegs in die mis van olifante, buffels, wildebeeste, renosters en zebras. Twee van die spesies, *C. bolitinos* en *C. kwagga*, het hulle broeimediums van voorkeur vergroot en kan eiers lê en tot volwassenheid ontwikkel in die mis van beeste en perde. Dit het vanselfsprekende gevolge vir virusoordrag tussen inheemse herbivore en vee, veral in gebiede waar die twee groepe saamloop.

In ’n intensiewe twee-jaar studie, is ’n vergelyking gemaak tussen die *Culicoides* fauna soos aangetref in ’n ongerepte deel van Afrika, die Kruger Nasionale Park (KNP), en in gebiede aangrensend aan die KNP wat deur die mens in veeplase omskep is. Die deel van die Oos Transvaalse laeveld is bekend as ’n perdesiekte "hotspot" in Suid-Afrika. Ligvalvangste, uitbroei van *Culicoides* uit mis en versameling vanaf lewendige gashere het aangedui dat sekere spesies van die Imicola groep eksklusief met sekere herbivore geassosieer is; hierby ingesluit is die olifant en zebra wat onderskeidelik verdagte en bevestigde gashere van AHS is. Die resultate dra by tot die verklaring van die epidemiologie van hierdie siekte en wys ook dat die mens ’n beslissende rol speel in die vasstelling van die getalle sowel as die verspreiding van spesifieke spesies van die Imicola groep. In die geval van *C. imicola*, die mees algemeenste en wydverspreidste spesie, is die uitbreiding, of daarstelling van fokuspunte, te wyte aan die mens se instandhouding van vaste bloedbanke op besproeide weiding. Die toevallige ontdekking van ’n groot *imicola*-vry sone elders in Suid-Afrika dui daarop dat grondtipe moontlik ’n bepalende rol kan speel in die aanwesigheid en volopheid van *C. imicola*. Hierdie "skoon" area is die sandduine-veld wes van Port Elizabeth en lyk belowend as ’n natuurlike kwarantyn gebied vir die invoer en uitvoer van lewende hawe.

CHAPTER 1

1.1 INTRODUCTION AND PURPOSE OF STUDY

At this moment, throughout the tropics, a trillion tiny *Culicoides* adults are emerging from their pupal cases. Half of them will be females, and within 24 hours, after first copulating and storing the sperm for the remainder of their lives, 95 % of them will fly away in search of the blood of man, beast or bird. And so trillions of micrograms of blood are daily siphoned off a great variety of earth's creatures; these hosts will not only be irritated by the bites (hence such *Culicoides* names as *vexans, irritans, diabolicus, molestus, pungens, furens* and *damnosus*), but a small fraction will become intermediate host to one or more diseases. These include filarial worms, protozoan parasites and viruses. Most illnesses are relatively benign, but the viruses of bluetongue (BT) and African horsesickness (AHS) are killers.

In South Africa, the dreaded 'perreziekte' or 'pardenziekte' (AHS) was frequently referred to in the early records of the Dutch East India Company, having made its appearance soon after the first horses were imported from Europe and the East Indies (Theiler 1921). Losses occurred every year and clearly indicated that, in the Cape, the virus of horsesickness existed in some indigenous animals; today we can only surmise that one of them was the now extinct variety of Burchell's zebra, the kwagga. Of a number of severe epizootics which followed, the most virulent was in 1854/5 when 64,850 horses died, 40 % of the entire population of 160 784 horses in the Cape Colony (Henning 1956). Today, without the help of vaccines, less than 10 % of the horses in South Africa would survive the disease (Coetzer & Erasmus 1994).

In 1908 the Swiss veterinarian, Arnold Theiler, established the Onderstepoort Veterinary Institute (OVI) to campaign against a number of diseases that continued to devastate animal husbandry in South Africa. One that particularly intrigued Theiler was African horsesickness, and he was to remain interested in its epidemiology throughout his life. Although his work was seminal to producing the first efficacious vaccines against AHS, he did not live to see du Toit, in 1944, implicate *Culicoides* biting midges as the insects transmitting both AHS and BT. This happened after various attempts had failed to incriminate mosquitoes. Having now been shown capable of transmitting diseases of veterinary import, *Culicoides* became the focus of intensive research; to date some 50 arboviruses and various filarial and protozoal parasites have been isolated from the genus worldwide (Meiswinkel, Nevill & Venter 1994).

Although du Toit showed *Culicoides (Avaritia) imicola* to be a vector of African horsesickness virus (AHSV) in South Africa 50 years ago, there is still doubt as to which game animals, as asymptomatic carriers, harbour the disease, and which of the 112 species of *Culicoides* known to occur here, transmit it. Piecemeal serological investigations done in parts of Africa indicate a wide range of animal hosts for AHSV that includes zebras, donkeys, elephants, camels, goats, dogs, cattle and various carnivores (Lubroth 1991). A 90 % mortality rate has elevated AHS to being one of three African diseases whose introduction is most feared by the European community; since 1987 close on 2 000 horses have died during yearly epizootics in Spain and Portugal (Palmiter 1991; Mellor 1993). Despite quarantine measures and a lengthy sea voyage the disease is acknowledged to have arrived in a group of five zebras from Namibia, and reminds us sharply about our lack of knowledge of the epidemiology of AHS. While it is well established that the African *C. imicola* has penetrated Spain and Portugal up to latitude 41° N, and is considered the only vector of AHS there, the situation in Africa is far more complex. In this thesis it will be shown that at least seven species of the Imicola group of the subgenus *Avaritia* occur on this continent; five are redescribed and two are new. One of the new species is closely associated with zebras and so must be considered as a potential vector of AHS.

Five years after Theiler commenced his work at Onderstepoort, *C. imicola* was described from a single female collected at Tiwi on the southern Kenyan coast (Kieffer 1913). Its apt name derives from the Latin for 'lover of low-lying places'. This taxon, however, remained unrecognised as Kieffer was lax in preserving type specimens and seldom illustrated his *Culicoides* descriptions. In consequence, most of the African literature on *C. imicola* appears under the junior synonym *C. pallidipennis* described by Carter, Ingram & Macfie from the Gold Coast in 1920. This name was used in du Toit's original research on the transmission of BLU and AHS. Nearly 60 years after its description, the holotype of *C. imicola* was found in the holdings of the Museum national d'Histoire naturelle, Paris, and the species resurrected by Kremer (1972). Besides *C. pallidipennis*, a further three synonyms have since been established (Dyce & Wirth 1983), namely *C. iraqensis, C. minutus* and *C. pseudoturgidus*, all from the Near East and India. To date, *C. imicola* is known to occur as far east as Laos (Howarth 1985). Off the Asian mainland, in the Philippines and Indonesia, *C. imicola* is replaced by *C. nudipalpis* Delfinado, 1961. As noted by Wirth & Hubert (1989) '*Culicoides nudipalpis* is nearly identical with *C. imicola* ...'. The question as to whether they really are separate species will be examined in this thesis.

These developments in the taxonomic history of *C. imicola* reveal that its specific characters are still not properly understood nor is there clarity as to which other taxa worldwide are its closest relatives. Furthermore, there are problems with ranking above the species level; *C. imicola* has been variously assigned to either the Imicola or Orientalis groups, which have been regarded as part of the subgenus *Avaritia* or used in lieu of it (Wirth & Hubert 1989; Glick 1990). Others view the subgenus and group categories as interchangeable, or that their status be lowered to that of subgroups (Itoua & Cornet 1986). Regarding understanding of the subgenus *Avaritia* there is ambivalence in the literature, due perhaps to it being erected by Fox (1955) for the highly unusual (in the ♂ genitalia only) Holarctic Obsoletus group. The view defended in this thesis is:

(i) that *C. imicola* is one of a group of nine closely related species restricted to the Old World, seven of which occur in Africa;

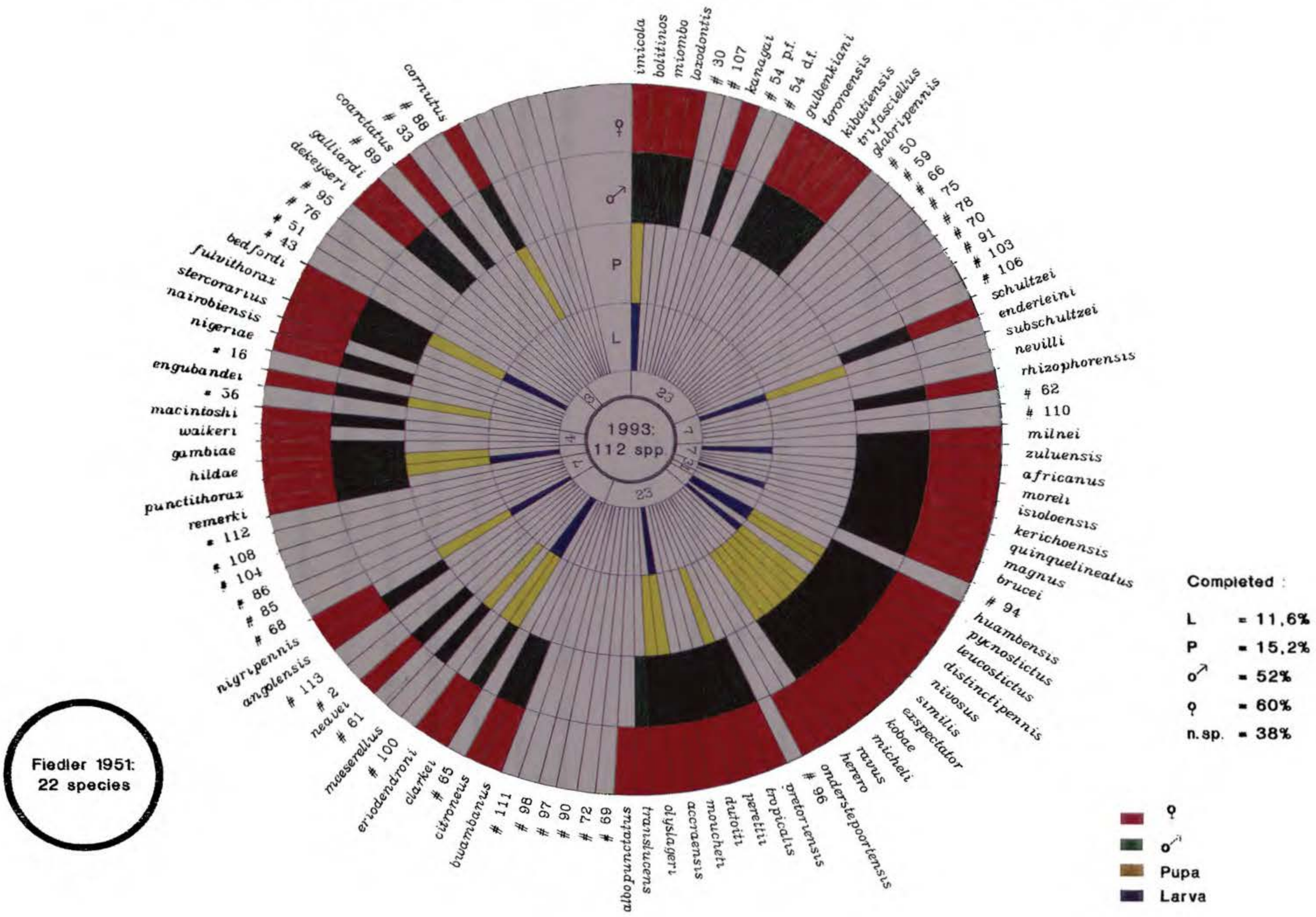
(ii) that the Imicola group, in the strict sense, is clearly separate from the Orientalis group, and that both groups are represented in Africa;

(iii) that these two groups, which are here redefined, are only two of at least 10 separate lineages that comprise the subgenus *Avaritia* worldwide; and

(iv) that subgroups are discernible within the Imicola group itself.

As a genus *Culicoides* comprises some 1 123 species distributed worldwide (Wirth & Dyce 1985); 136 have been recorded from the Afrotropical Region (Wirth, de Meillon & Haeselbarth 1980). Fiedler (1951) listed only 22 species from South Africa; it is known that at least 112 occur here. These are listed in Fig. 1 which also reveals by colour which species have been described, and whether both sexes, larva and pupa of each are known. The first 23 species belong to the subgenus *Avaritia*; more than half are

FIG. 1.1 South African *Culicoides*: state d'art of description 1994



new to science (denoted by numbers). Not all will be treated here, only those that are truly allied to *C. imicola* and constitute the Imicola group. This group will be defined, and all nine world taxa comprising it will be dealt with as follows:

(i) the five currently recognised Afrotropical species, *C. imicola*, *C. pseudopallidipennis, C. bolitinos, C. miombo* and *C. loxodontis* are redescribed;
(ii) two new African species are described;
(iii) the two extralimital species, *C. brevitarsis* and *C. nudipalpis,* are referred to in discussions and used in comparisons where relevant; and
(iv) a key to all nine species is given.

The introductory paragraphs show *C. imicola* to be an important vector of orbiviruses affecting livestock. One implication is that some of the species most closely related to it may also be involved in disease transmission, and for this basic reason it is necessary to describe them as accurately and thoroughly as possible.

As with species in general, members of any species group exhibit variations, either seasonally, altitudinally or nutritionally induced. These, when overlying genetical polymorphism, can cause puzzling overlaps of phenotypic change. To try, in such circumstances, to distinguish between intra- and interspecific variation can be difficult if the material has not been predetermined cytotaxonomically. This point has been reached in taxonomic studies on the genera *Anopheles* (Culicidae) and *Simulium* (Simuliidae); the discovery of cryptic species has become commonplace in well-studied groups in both the plant and animal kingdoms and suggests that they are widespread.

As the use of molecular taxonomy is almost non-existent in *Culicoides* studies, it is necessary to emphasize that ranking is simply a matter of scale that may only reflect the depth of a particular taxonomic investigation; the term ‘complex’ is therefore best reserved for well-studied species aggregates, and especially, those difficult to unravel morphologically. For this reason a ‘group’ designation is maintained here for the Imicola assemblage as the individual species are quite easily segregated on a number of character states, and in their bionomics. However, it needs to be conceded that there are species-pairs that form subgroups within the Imicola group for instance *kwagga/loxodontis* and *pseudopallidipennis/tuttifrutti*. At this point the implementation of molecular techniques is warranted.

In this study the morphological features examined on slide-mounted *Culicoides* adults are arranged on seven data sheets (Tables 1.1–1.7); except for the male of *C. brevitarsis* these were examined for a minimum of 20 adults per sex per species. Approximately 80 characters are scored; for half of these, namely those suitable for numerical analysis, sample size, average and range were obtained. All data were then included in the species descriptions to facilitate present and future comparisons between populations and species. Certain characters (length of palpal and antennal segments) were subjected to two-tailed T-tests, and bar diagrams were drawn.

In the process, weaknesses in the descriptive format currently in use for world *Culicoides* were exposed. Improvements are made, resulting in new character states being introduced to the literature to aid the identification and differentiation of species. To further maximize the retrieval of morphometric data it was found necessary to improve methods for mounting these tiny 1–3 mm-sized midges on glass slides; the methodology is given below.

As regards the biology of the known African species, only some aspects were investigated for certain species; special emphasis was placed on unravelling the ecology of four species as they are locally common in South Africa; the immatures of three of these live in the dung of African herbivores, notably the elephant, both rhinoceros species, the buffalo, the blue wildebeest and the plains zebra. Two of the coprophiles have expanded their resource range by moving into the dung of cattle and horses; one of the more obvious implications of this is that certain diseases endemic to African game animals can now be more easily transmitted to various breeds of introduced livestock. It needs to be assessed, however, whether these *Avaritia* species are indeed capable of vectoring the viruses of concern. This possibility, and ecological investigations on seasonal abundance, numerical prevalence and geographic distribution, are dealt with only cursorily as they either fall outside the ambit of a biosystematic study or are being investigated by colleagues at Onderstepoort.

1.2 MATERIALS AND METHODS

1.2.1 Collecting methods

These include light-trapping, truck-trapping and rearing from dung; they are mostly detailed under the relevant species in the various chapters.

a) Light-trapping

Light-trapping was done using a commercially available New Jersey-type downdraught trap equipped with an eight-watt U.V. tube. Usually only one light-trap per site was used; in the survey reported on in Chapter 9, one to six were operated at a site; these were spaced at 50 m intervals. Traps were always hung 1–2 m above ground. On farms they were placed as near to livestock as practicably possible, and operated from dusk to dawn.

Culicoides were always collected into a beaker containing 300 mℓ of water to which two tablespoons of Savlon antiseptic had been added. After retrieval, the catches were transported to the field laboratory, and either put in a refrigerator at 4 °C or washed immediately and transferred to 70 % ethyl-alcohol. Washing was done by pouring the catch into a very fine sieve, and gently rinsing it three or four times with a Savlon/water mixture. In this way, the midges were cleaned of extraneous moth-scales that tend to adhere to the wings, and become more difficult to remove once the insects have been stored in alcohol.

b) Truck-trapping

A trap based on the design of Dyce, Standfast & Kay (1972) was used. It was mounted on the roof of the vehicle; its opening was 0,5 m^2. Trapping using this method was done opportunistically at any time of the day. A ‘run’ could be 1,5–15 km in length and could last from five to 45 minutes. Insects were collected into a fine muslin bag; at the end of a run the bag would be removed, tied closed, and placed for 10 minutes in a bottle of potassium cyanide or ethyl acetate. The dead insects were then shaken out onto the centre page of a glossy magazine and funnelled into 70 % alcohol. The relevant data are given under material examined.

c) Rearing from dung

Methods used are detailed under the relevant species in Chapters 2, 5 and 7.

d) Subsampling of large light-trap collections

Catches made during the warm summer months are usually very large (1 500–500 000 *Culicoides*), and the labour involved to sort such catches exhaustively and identify them to species is prohibitive. Subsampling was done in the following way as described by Van Ark & Meiswinkel (1992):

(i) the *Culicoides* were suspended in a known volume of alcohol by lightly shaking the container;
(ii) a subsample of known volume was taken from the middle of the suspension using a pipette with an aperture of at least 2 mm, and all specimens were counted and identified;
(iii) subsampling was continued until approximately 500 specimens had been obtained;
(iv) the volume of the subsample was calculated in relation to the volume of the total catch and the subsample fraction for calculation of the estimated total catch determined.

1.2.2 Mounting *Culicoides* on glass slides

For accurate identification and description, *Culicoides* adults need to be mounted on glass slides and examined at 100–1000 x magnification. Methodologies vary with each researcher. In the main, adults are mounted with one wing, the abdomen and the head detached; the remainder of the thorax and legs, and one wing, are left in one piece and mounted laterally. All parts are placed under one large coverslip. This method is designed to speed up the mounting of larger series of midges, but is defective in that it leaves many delicate taxonomic features inadequately displayed or destroyed.

1.2.2.1 Disadvantages of current methods:

♀: a) Only one wing is presented under a thick layer of mountant; in many instances it is impossible to photograph these wings as they are not on a flat plane, and if the mountant is too thick, obscures nuances in the wing pattern which are extremely important for identification. The second wing can be equally useless as its base is invariably obscured by the thorax to which it is left attached.

b) The head may suffer in three ways:

  i) it may be squashed by excessive pressure during coverslipping and so parts are distorted or broken. For example it would give an incorrect P/H ratio where the length from the tip of the proboscis to the tormae is divided by the distance from the tormae to the interocular bristle on the anterior frons;

  ii) if not squashed, but lying in thick mountant, the distal segments of the palp either tend to droop downwards or curl upwards; this is not only a disadvantage for illustration but also introduces a severe error of parallax into measurements;

  iii) eyes often not cleared of pigment: this obscures observation as to whether they are joined or separated medially, and obscures the presence and nature of interfacetal pubescence.

c) If left on the head, the antennae suffer in four ways:

  i) they droop down from near the top of the head; this introduces error of parallax in length and width measurements made of each flagellar segment;

  ii) basal segments can be obscured by reddish-black pigmentation in eyes;

  iii) the preceding two problems obstruct the recording of sensilla lengths and their distribution on each flagellar segment; and

  iv) antennae that settle randomly in a thick mountant often assume an irregularly bent or spiralled position; this complicates illustration and measurement of individual or grouped segments, especially when obtaining the antennal ratio (AR).

d) If the thorax is mounted laterally and/or is squashed, it obscures:

  i) setation on scutum;

  ii) size and position of pale markings or vittae on scutum;

  iii) setation of scutellum.

e) An abdomen mounted laterally (if left attached to thorax) obscures:

  i) shape of sclerotization surrounding or reinforcing opening of gonopore; and

  ii) may result in one spermatheca being obscured by another, while the rudimentary third spermathecae and small ring on the common oviduct may be hidden entirely.

f) If abdomen is removed from thorax and mounted dorsally or ventrally:

  i) distal two or three segments may be telescoped which also obscures sclerotization surrounding gonopore, and complicates observation of spermathecae and ducts.

g) Legs left attached to the thorax may lie in a random, compressed jumble of 54 superimposed segments which:

  i) partially obscures banding patterns;

  ii) complicates measurements due to error of parallax; and

  iii) obscures setation (especially problematical in predatory genera of Ceratopogonidae).

♂ : Most of the disadvantages recorded above for females also apply to males, especially as regards the antennae. Others involve the genitalia:

a) If the abdomen is not fully stretched out and the genitalia are not properly positioned at its extremity but left retracted, the spiculate membrane of abdominal segment VIII can overlie that of segment IX; this not only obscures the spiculation of sternum IX (as to whether it is present or absent) but, in addition, the spiculation of sternum VIII will be incorrectly interpreted as belonging to that of sternum IX. The type of spiculation found on these two segments differs and should not be confused.

b) Genitalia may be twisted: i.e. if not mounted symmetrically and on a plane the detailed measurement, illustration and interpretation of various structures is seriously affected.

c) Genitalia not opened: distimeres folded inwards, so overlying other structures which are often membranous. Similarly the basimeres can be bent inwards, and so press against the aedeagus, parameres and tergum IX, hindering clear observation.

d) Genitalia squashed by excessive pressure during coverslipping; this distorts natural conformation and position of various structures, and results in illustrations that are misleading and not comparable to those generated from undistorted mounts.

1.2.2.2 **Improvements made to traditional methods:**

Midges collected by whatever method (light-trap, truck-trap or reared from collected pupae) are stored in 70–80 % ethyl-alcohol. When needed for slide mounting, preferably within six months of capture, a few individuals at a time are transferred to 96 % ethanol for approximately an hour. In the ethanol the wings are cut off and slide-mounted in a 50:50 phenol-balsam mixture. An effort is made, often laborious, to clean the wings of all extraneous hairs and scales, a problem with *Culicoides* captured in light-traps invaded by moths. The remainder of the insect is cleared in 10 % KOH at room temperature for 18–24 h, neutralized and dehydrated in 10 % acetic acid for 30 minutes, washed once through 96 % ethanol (lasting a few hours), and then finally stored in clove oil for a minimum of 12 h. From the clove oil dissection is done in a phenol-balsam mixture with electrically sharpened tungsten needles on the slide carrying the wings. The midge is eventually divided into six parts, all to be coverslipped separately:

a) Antennae are dissected from the head and are laid straight; the coeloconica should face dorsally, this checked during manipulation by quick scrutiny under a compound microscope at 100 x magnification. It is difficult to establish the exact numbers of coeloconica if these are mounted laterally, and because the width of the third flagellar segment is greater when measured across the face bearing the coeloconica.

b) The head is then dissected off the thorax and laid separately away from, but underneath, the wings. The cervical sclerites are left on the head to act as supportive 'struts' preventing the head from tilting backwards. The head is positioned so that the mouthparts lie flat on the surface of the slide. Every effort must be made to have the palps neither curled upwards nor drooping downwards.

c) With the remainder of the insect now displayed laterally on the slide, a cut is made longitudinally through the pleura to neatly sever the mesonotum from the abdomen and legs. This is done with regard to the halteres and scutellum remaining attached to the mesonotum. The thorax is then removed and positioned separately with scutum and its setation uppermost.

d) The abdomen is then dissected away from the legs, and positioned separately, ventral side up. If the distal two or three segments in the female are telescoped an effort must be made to extend them. This is not always easy to achieve because each individual, depending upon whether it is teneral or matured, reacts differently to the clearing process. Some contract and resist attempts at distension. Male genitalia are also mounted ventral side up, but are carefully manipulated to keep sternum IX separate from sternum VIII, and to ensure that the distimeres are not folded into the aedeagus and parameres; the basimeres are also separated fractionally to give an uninterrupted view of the aedeagus and parameres. During manipulation the results are checked at 100 x magnification under a compound microscope, to ensure that the genitalia are correctly positioned on a plane and that all extraneous bristles and scales are removed.

e) The legs are spread so that each lies flat and separate from the next to display banding patterns and facilitate accurate measurements of segment lengths, and the counting of bristles and sensillae.

1.2.2.3 Coverslipping

The slides are stored for at least three weeks for the various parts to become set in position. Setting ensures that the parts do not drift out of position when coverslipping is done. Each of the six bodyparts is coverslipped separately and differently, with square or rectangular coverslips cut to suit the size and shape of each part.

a) The wings are placed under a large coverslip with the minimal amount of Canada balsam. This is to ensure that the wings are on as flat a plane as possible, so that the wing is in focus throughout its length during photography.

b) A small coverslip (0,25 cm^2) with just enough balsam, is used for the antennae, to ensure that they are not squashed. Not only does squashing distort the shape of the segments but it also breaks or pulls sensilla off the segments. The chaetica are especially vulnerable if not already lost during capture or the clearing process.

c) The head is mounted under an equally small coverslip but in thicker balsam, also to ensure that it is not squashed or cracked; its exact form must be maintained to ensure accurate description, measurement and illustration.

d) The abdomen, especially that of the male, is coverslipped in a moderate amount of balsam to facilitate observation at 400 x magnification without the objective lens crushing the coverslip. The coverslip should not compress the genitalia as this distorts the shape of the various parts. For example, the aedeagus is fractionally concave; compression can disturb its position in relation to the articulating parameres and basimeres, and splays the legs of the aedeagus.

e) Legs are covered by moderately thin balsam and a fairly large coverslip.

f) The scutum is thickly mounted under a small coverslip the size of that used for the head, to avoid crushing it. Being a thick mount, the scutum can be examined at magnifications up to 100 x, which is sufficient for counting bristles, while the scutal pattern can be easily observed at 60 x magnification.

1.2.3 Species descriptions

A trend that pervades the world literature on *Culicoides* is that species descriptions are overly brief and that many new species are based on a single sex or represented by a single specimen. For example, 52 (31 %) of the 168 south-east Asian species dealt with by Wirth & Hubert (1989) are known from one sex only. This poor descriptive coverage of the genus reveals that we are not yet able to unravel or discover complexes of cryptic or sibling species. Such incomplete knowledge has some consequences:

(i) our knowledge of the bionomics of many species is confused; this has a ripple effect into studies on disease epidemiology, vector competence and geographic distribution;

(ii) insufficient numerical data mean we are unable to assess intra-specific variation; this in turn denies us access to character states necessary for accurate outgroup comparisons and for ranking above the species level. A further result is that —

(iii) no phylogenies can be generated; this robs us of insights into the history of the genus which go hand in hand with the development of hypotheses on speciation events, particular host adaptions, adult and immature habitats, and other aspects of niche occupation; phylogenies are also a useful aid in explaining biogeographic patterns both modern and historical.

As regards detailed species descriptions, it is instructive first to consider the methodology of mosquito systematists. Since the landmark studies of Belkin (1962) on Pacific Culicidae, it is deemed essential to describe the larva, pupa, male and female of a particular species from long series of pelts and adults reared from eggs laid by one or more wild-caught females. In this way, more than 1 000 characters are scored per species. Despite this comprehensive morphological approach, Zavortink (1990) still estimates that only 25–50 % of the extant mosquito species of the world have been discovered. As mosquitoes have been more intensively studied than *Culicoides* it is probable that fewer than 25 % of *Culicoides* species have been named. Despite an intensive search for diagnostic features in mosquitoes it is important to note that species complexes defying morphological separation exist, such as the *Anopheles gambiae* complex containing the principal vectors of human malaria in Africa. Here the identification of the six component species is dependent upon: (i) artificial cross-mating tests, (ii) chromosomal banding patterns, (iii) enzyme electrophoresis, (iv) cuticular hydrocarbons, and (v) the polymerase chain reaction amplification (PCR) of genomic sequences.

For the last 25 years the former three methods have been used widely on the Gambiae complex in Africa; consistent and comparable results attest to the 'reality' of these component species. The application of such molecular techniques has become known as the 'new systematics' and has, in specific cases, all but replaced the morphological approach where only the external attributes of a phenotype are examined. An important consequence of these developments is that the molecular methods have sharpened our vision of, and respect for, the subtle diversity of the animal world.

Compared to the use in mosquito systematics of molecular techniques coupled to an outstandingly detailed morphological system, the situation in *Culicoides* could be described as almost retrogressive. While the time is clearly ripe for implementing a Belkin-type method in the Cerotopogonidae, this thesis is restricted to upgrading the adult descriptions only. Larger series of *Culicoides* imagoes were examined in greater detail with emphasis on the genitalia and antennae of both sexes. The genitalia and antennae are described and illustrated in their entirety; the latter possess seven different types of sensilla which yield data suited to numerical analyses. Along with wing pattern, they provided the key characters for species identification. The ♂ genitalia proved more valuable than those of the ♀ but the inter-specific differences, have more to do with subtle differences in the shapes of various parts and their degree of sclerotisation, and so are not suited for numerical analysis. In the ♀, the number of long bristles found medially on the scutellum proved useful, and is another character hitherto not used in *Culicoides*. For comparative purposes, the description and illustrations of *C. imicola* by Wirth & Hubert (1989) are reproduced in Fig. 1.4. These reveal differences when compared with the expanded description of the same species on pp 5–23, Chapter 2. All morphometric data were recorded on 'character-state' sheets prior to analysis; these are detailed in Tables 1.1–1.7.

1.2.4 Taxonomic characters and ratios

Good accounts of the external morphology of *Culicoides* have been given by many workers (Campbell & Pelham-Clinton 1960; Khamala & Kettle 1971; Wirth & Hubert 1989). These are not repeated here except where improvements and changes were found necessary, and where new taxonomic characters aided the identification of species. In this study, the various characters scored and/or measured in both sexes are detailed in Tables 1.1–1.7.

a) **Antennal sensilla** (Fig. 1.2). Of the seven types of sensilla found on the female antenna, the coeloconica and chaetica are the most important for species identification. Most authors record only the distribution of the coeloconica on the flagellar segments, and ignore the chaetica entirely. In this study the precise number of coeloconica and chaetica are recorded for each flagellar segment as shown in Tables 1.1 and 1.2. The total and average number of sensilla are also recorded for both antennae of a specimen. Usually a minimum of 20 antennae are scored. The sensilla chaetica vary in length and thickness but being too thin to measure precisely, are only shown as longer or shorter in the illustrations.

b) **Flagellar lengths, total antennal length and antennal ratio (AR)**. In Table 1.3 the length of each flagellar segment for both antennae of a specimen is recorded. The average length of each segment is calculated at the bottom of the table. The total antennal length is obtained by adding the grouped measurements of III–X to those of XI–XV; these measurements include the intersegmental membrane. The antennal ratio is obtained from the combined length of segments XI–XV (measured as a single unit) divided by that of III–X (also measured as a single unit). The measuring of the individual antennal segments at 400 x magnification demands accuracy. For this reason the antennae are separated away from the head and mounted flat, but not squashed, and straight in a thin film of phenol-balsam. Measurements are thus derived from antennae in their natural shape and resting on a flat plane. Most researchers leave the antennae inposition near the top of the well-rounded head from where they droop downwards, and as

FIG. 1.2

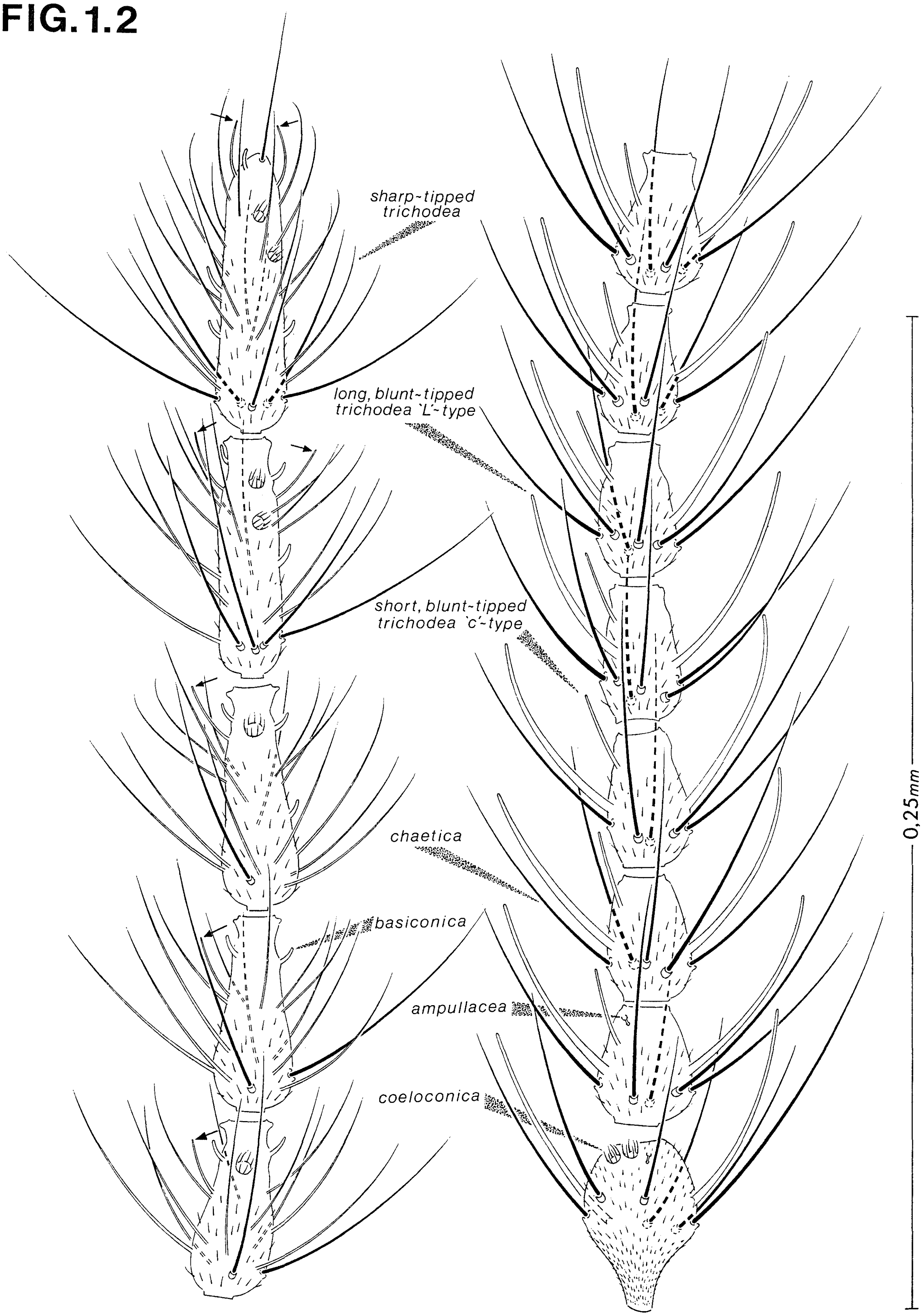


Fig. 1.2 *Culicoides*. Antenna, female: seven types of sensilla; arrows indicate short, blunt-tipped trichodea of the 'L'-type. Flagellar segments XI–XV on left, III–X on right

they settle randomly in the mountant, facing either inwards or outwards, they will assume an irregularly bent or spiralled position. Unless the head is completely squashed during coverslipping, measurements made from such antennae suffer from an error of parallax. This error is further compounded when it is considered that 26 individual segments may be measured per adult specimen over 10 or more specimens; the resultant antennal ratios derived from such material will contain an error factor difficult to predict or calculate. This is critical when one deals with a group of closely related species.

c) **Antennal trichodea ratio (AtR)** (Table 1.5; Fig. 2.1). A new character in which the length of one of the long blunt-tipped trichodea on female antennal segment VI (Fig. 2.1a) is divided by the length of segment VI (Fig. 2.1b). Only trichodea projecting laterally, and in focus from base to apex, are used to obtain the AtR. These blunt-tipped trichodea also differ in their width and are here classified as slender or inflated, but owing to their small size, this is only indicated in illustrations (Fig. 11.2a and 11.2b).

d) **Palp**. In Table 1.4 the measurement of the entire palp will be seen to differ from the totalled measurements of the individual segments. This is because segments I and II overlap diagonally where they articulate (Fig. 2.7). The number of sensilla chaetica on each of the five palpal segments, and the total, are also recorded in Table 1.4.

e) **Palpal ratio (PR)**. Obtained by dividing the length of palpal segment III by its greatest width.

f) **The proboscis/head ratio (P/H)**. Obtained by dividing the length of the proboscis (measured from the toothed tip of the labrum-epipharynx to the tormae) by the head height (measured from the tormae to the interocular seta). The measurements are derived only from specimens in which the head is not squashed. As noted by Boorman (1991) the P/H values given for *C. imicola* by Meiswinkel (1989) differed from those he (Boorman) had obtained from 123 specimens scored from North Africa and the Mediterranean. He concluded that the South African material might 'represent a distinct race of *C. imicola....*' The truth is simpler: Meiswinkel (1989, 1991 and 1992) erred in his method of deriving the P/H ratio. He measured from the tip of the fleshy labium, and as it always protrudes beyond the tip of the mouthparts, all the P/H ratios were too high. For *C. imicola, C. bolitinos, C. miombo* and *C. loxodontis* these have been corrected in Chapters 2–5. The revised P/H value for *C. imicola* ranges between 0,82–1,02 (n = 45) which compares well with that of 0,74–0,96 (n = 123) given by Boorman (1991).

g) **Wing length**. Measured from basal arculus to wing tip (Fig. 2.2).

h) **Wing breadth**. Measured at maximum breadth.

i) **Costal length**. Measured from basal arculus to tip of second radial cell.

j) **Costal ratio (CR)**. Obtained by dividing the costal length by the wing length.

k) **Tarsal ratio (TR)**. Obtained by dividing the length of the first segment of the hind tarsus by the length of the second segment.

l) **Scutum**. Mounted dorsal side up, bristles counted in both sexes (Fig. 2.22); new character.

m) **Scutellum**. Number of bristles counted in both sexes.

n) **Male antenna** (Table 1.7; Fig. 2.15). As in the female only straight antennae were used to obtain measurements. Flagellar segments III, XIII–XV were measured individually whereas segments IV–XII, being fused, were measured as a single unit. Counts were made of the number of sensilla coeloconica, chaetica and blunt-tipped trichodea (Table 1.7). The lengths of the latter two sensilla were also measured; in some species one of the trichodea can be underdeveloped on certain segments. This shortening is indicated as a fraction (Tables 6.4, 6.8

and 11.4 and Fig. 6.19).

o) **Male genitalia.** (i) basimere: its length (lb) and its width (wb) were measured as shown in Fig. 1.3.

(ii) distimere: its length (ld) was measured as shown in Fig. 1.3.

(iii) aedeagus: its length (la) and width (wa), and the height of the aedeagal arch (ha) were measured as shown in Fig. 1.3.

(iv) sternum nine: the range in number of spicules on the membrane was determined by counting only those spicules found in the excavated area (see Fig. 1.3).

1.2.5 Illustrations

Illustrations were executed on squared paper using a grid micrometer. Except for the wings and scutellum, these were done at 400 x magnification.

Only mounted specimens, aligned in balanced symmetry, lying on a flat plane, and not squashed during coverslipping, were used; if a specimen was slightly twisted or cramped it was discarded. While every effort was made to adhere strictly to this standard as it yields data and illustrations that are comparable between species, it was not always possible to do so for the antennae. For example, comparison of Figs. 2.15 and 2.16 will show ♂ or ♀ antennae to be either 'right-handed' or 'left-handed', i.e. the chaetica and blunt-tipped trichodea arise on opposite faces of the flagellar segments. This is not a morphological reality but merely an artefact of specimen choice, the priority being to find an antenna that was both straight and in possession of all its chaetica (these often lost during capture).

Antennae: As indicated above, only straight antennae mounted flat, but not squashed, were used for measurements and illustration. Once the entire series of antennae had been examined for a species, one antenna, approaching the mean, was chosen for illustration. All sensilla observed are illustrated, those occurring ventrally being drawn with broken lines.

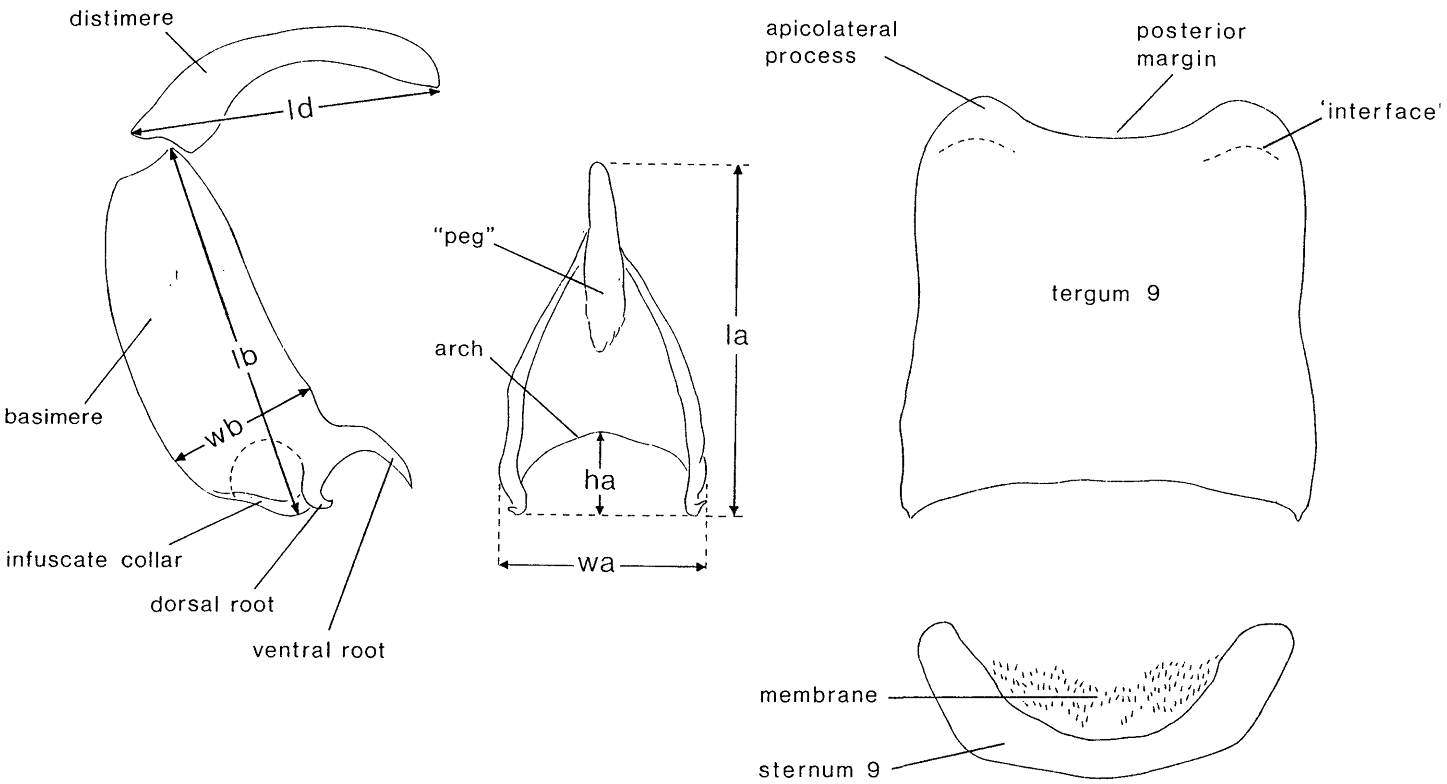


Fig.1.3

Fig 1.3 *Culicoides*. Genitalia, male: dissected; showing character states and measurements derived; ld (length distimere), lb (length basimere), wb (width basimere), la (length aedeagus), ha (height aedeagus), wa (width aedeagus)

Genitalia: Only ♂ genitalia mounted flat, and not compressed by excessive coverslipping were used for illustration. The specimen chosen had to have the anterior legs of the aedeagus and its posterior tip simultaneously in focus. The entire genitalia were illustrated from dorsal mounts, tergum IX from ventral mounts. Only one distimere is illustrated. The left basimere shows dorsal setation and spiculation, the right shows these on the ventral surface.

A ♀ specimen was chosen for genitalic illustration only if the spermathecae were not collapsed. The sclerotized plates on segment VIII that abut and reinforce the gonopore opening are described and illustrated; while they are a valuable group-specific character in *Avaritia*, they are of little use for separating species. Their exact conformation is often difficult to ascertain as the last three segments of the ♀ abdomen are often telescoped and need to be extended during mounting.

1.3 REFERENCES

BELKIN, J.H. 1962. The mosquitoes of the South Pacific (Diptera, Culicidae). Vols. 1 and 2. University of California Press, Berkeley and Los Angeles. xii + 608 pp. 412 Figs.

BOORMAN, J. 1991. A review of *Culicoides* subgenus *Avaritia* species (Insecta, Diptera, Ceratopogonidae) vectors of viruses of sheep, cattle and horses, with particular reference to *Culicoides imicola* in Europe and the Mediterranean region. *Report for The Overseas Development Administration*, The Natural History Museum, London. pp 1–54.

CAMPBELL, J.A. & PELHAM-CLINTON, B.A. 1960. A taxonomic review of the British species of "*Culicoides*" Latreille (Diptera, Ceratopogonidae). *Proceedings of the Royal Society of Edinburgh*, B, 67:181–302.

COETZER, J.A.W. & ERASMUS, B.J. 1994. African horsesickness. in *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press, 1:460–475.

DU TOIT, R.M. 1944. The transmission of bluetongue and horsesickness by *Culicoides*. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 19:7–16.

DYCE, A.L. & WIRTH, W.W. 1982. Reappraisal of some Indian *Culicoides* species in the subgenus *Avaritia* (Diptera: Ceratopogonidae). *International Journal of Entomology*, 25:221–225.

DYCE, A.L., STANDFAST, H.A. & KAY, B.H. 1972. Collection and preparation of biting midges (Fam. Ceratopogonidae) and other small Diptera for virus isolation. *Journal of the Australian Entomological Society*, 11:91–96.

FIEDLER, O.G.H. 1951. The South African biting midges of the genus *Culicoides* (Ceratopogonid: Dipt.). *Onderstepoort Journal of Veterinary Research*, 25:3–33.

FOX, I. 1955. New advances in the study of the genus *Culicoides* (in Spanish). *Boletin Laboratorio de la Clinica Luis Razetti*, 43–44:635–639.

GLICK, J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology*, 27:85–195.

HENNING, M.W. 1956. Animal diseases of South Africa. Third edition Pretoria: Central News Agency Ltd.

HOWARTH, F.G. 1985. Biosystematics of the *Culicoides* of Laos (Diptera: Ceratopogonidae). *International Journal of Entomology*, 27:1–96.

ITOUA, A. & CORNET, M. 1986. Les Ceratopogonidae (Diptera) du Mayombe Congolais. III. Revue taxonomique des espèces du genre *Culicoides* Latreille 1809. *Cahiers de l'office de la Recherche Scientifique et Technique Outre-Mer, Série Entomologie Medicale et Parasitologie*, 24:233–250.

KHAMALA, C.P.M. & KETTLE, D.S. 1971. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of East Africa. *Transactions of the Royal Entomological Society of London*, 123:1–95.

KIEFFER, J.J. 1913. Chironomidae et Cecidomyidae. Resultats scientifiques Voyage de Ch. Alluaud et R. Jeannel en Afrique orientale. (1911–1912 Diptera), 1:1–43.

KREMER, M. 1972. Redescription de *Culicoides imicola, C. alticola* et *C. tropicalis* Kieffer sur des exemplaires déterminer par l'auteur (Diptera, Ceratopogonidae). *Bulletin du Museum National d'Histoire Naturelle*, 58:645–655.

LUBROTH, J. 1991. The complete epidemiologic cycle of African horsesickness: our incomplete knowledge. *In*: WALTON, T.E. & OSBURN, B.I. (eds). *Bluetongue, African Horsesickness and Related Orbiviruses*. Proceedings of the Second International Symposium. CRC Press, Boca Raton, Florida, U.S.A.: 197–204.

MEISWINKEL, R. 1989. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:23–39.

MEISWINKEL, R. 1991. Afrotropical *Culicoides*: *C. (Avaritia) miombo* sp. nov., a widespread species closely allied to *C. (A.) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 58:155–170.

MEISWINKEL, R. 1992. Afrotropical *Culicoides*: *C. (Avaritia) loxodontis* sp. nov., a new member of the Imicola group (Diptera: Ceratopogonidae) associated with the African elephant in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 59:145–160.

MEISWINKEL, R., NEVILL, E.M. & VENTER, G.J. 1994. Vectors: *Culicoides* spp. in *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press, 1:68–89.

MELLOR, P.S. 1993. African horse sickness: transmission and epidemiology. *Veterinary Research*, 24:199–212.

PALMITER, C. 1991. Death stalks Iberia's horses. *Readers Digest*, 115–119.

THEILER, A. 1921. African horsesickness (pestis equorum). *Science Bulletin, No. 19*, Department of Agriculture, Union of South Africa.

VAN ARK, H. & MEISWINKEL, R. 1992. Subsampling of large light trap catches of *Culicoides* (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 58:183–189.

WIRTH, W.W., DE MEILLON, B. & HAESELBARTH, E. 1980. Family Ceratopogonidae in Catalogue of the Diptera of the Afrotropical Region, edited by R.W. Crosskey. British Museum (Natural History), London, pp. 150–174.

WIRTH, W.W. & DYCE, A.L. 1985. The current taxonomic status of the *Culicoides* vectors of bluetongue viruses. in *Bluetongue and related orbiviruses*, edited by T.L. Barber & M.M. Jochim. New York: Alan R. Liss, Inc. pp. 151–164.

WIRTH, W.W. & HUBERT, A.A. 1989. The *Culicoides* of south-east Asia. *Memoirs of the American Entomological Institute*, 44:1–508.

ZAVORTINK, T.J. 1990. Classical taxonomy of mosquitoes — a memorial to John N. Belkin. *Journal of the American Mosquito Control Association*, 6:593–599.

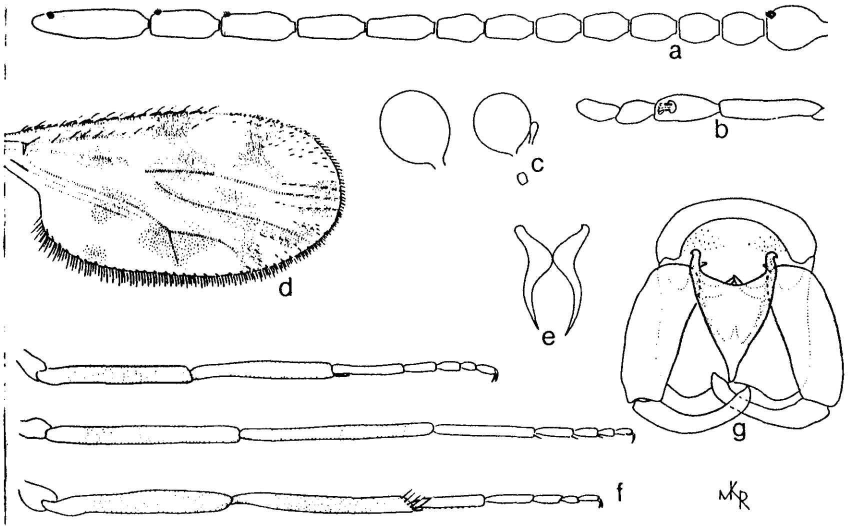


Fig. 119. *Culicoides imicola*: a. antenna; b. palpus; c. spermathecae; d. wing; e. parameres; f. legs; g. male genitalia, parameres omitted.

Figure 1.4: Sample of a contemporary illustration of *C. imicola*; sensu Wirth & Hubert 1989

Species: C. sp. # 30 1.1

pecimen umber:	Number of sensilla coeloconica on ♀ antennal flagellar segments: III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	Tota numb
Renosterkoppies 83	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	1	8
Renosterkoppies 89	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	1	8
Renosterkoppies 90	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	1	8
Renosterkoppies 91	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	4	—	—	—	—	—	—	—	1	1	1	1	1	9
Renosterkoppies 92	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	1	8
Renosterkoppies 93	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	2	9
Renosterkoppies 94	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	4	—	—	—	—	—	—	—	1	1	1	1	1	9
Renosterkoppies 95	3 (2 fused)	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	1	8
Renosterkoppies 96	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	1	8
Renosterkoppies 97	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	4	—	—	—	—	—	—	—	1	1	1	1	1	9
Renosterkoppies 98	4	—	—	—	—	—	—	—	1	1	1	1	1	9
	4	—	—	—	—	—	—	—	1	1	1	1	1	9
Renosterkoppies 100	3	—	—	—	—	—	—	—	1	1	1	1	1	8
	3	—	—	—	—	—	—	—	1	1	1	1	1	8
(n = 24) total	77	0	0	0	0	0	0	0	24	24	24	24	25	198
range	3-4	0	0	0	0	0	0	0	1	1	1	1	1-2	8-9
average	3,21	0	0	0	0	0	0	0	1,00	1,00	1,00	1,00	1,04	8,25
Grand Total: (n = 104)	348 3.35	0	0	0	0	0	0	4 0,04	101 0,97	104 1,00	104 1,00	[illegible] 1,00	106 1,02	871 8,38

Table 1.1: Female: Sample sheet of raw data collected on the number of sensilla coeloconica found on antennal flagellar segments III–XV in slide-mounted specimens.

1.2

Species: C. sp. # 30

Specimen number:	Number of sensilla chaetica on ♀ antennal flagellar segments:													Total number
	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	
Renosterkoppies	5	3	2	4	2	4	2	4	–	–	–	–	–	26
83	5	4	2	4	2	4	2	3	–	–	–	–	–	26
Renosterkoppies	5	4	2	4	2	4	2	3	–	–	–	–	–	26
89	5	4	2	4	2	4	2	3	–	–	–	–	–	26
Renosterkoppies	5	3	2	4	2	4	2	3	–	–	–	–	–	25
90	5	3	2	4	2	4	2	3	–	–	–	–	–	25
Renosterkoppies	4	3	2	4	2	4	2	3	–	–	–	–	–	24
91	4	3	2	4	2	4	2	3	–	–	–	–	–	24
Renosterkoppies	5	3	2	4	2	4	2	3	–	–	–	–	–	25
92	5	4	2	4	3	4	2	3	–	–	–	–	–	27
Renosterkoppies	5	4	2	4	2	3	2	3	–	–	–	–	–	25
93	5	3	2	4	2	4	2	3	–	–	–	–	–	25
Renosterkoppies	5	3	2	4	2	4	2	3	–	–	–	–	–	25
94	5	3	2	3	2	4	2	3	–	–	–	–	–	24
Renosterkoppies	5	3	2	3	2	4	2	3	–	–	–	–	–	24
95	5	3	2	4	2	4	2	3	–	–	–	–	–	25
Renosterkoppies	4	4	2	4	2	4	2	3	–	–	–	–	–	25
96	4	3	2	4	2	4	2	3	–	–	–	–	–	24
Renosterkoppies	5	3	2	4	2	5	2	3	–	–	–	–	–	26
97	5	3	2	3	2	4	2	3	–	–	–	–	–	24
Renosterkoppies	5	3	2	4	2	3	2	3	–	–	–	–	–	24
98	5	3	2	3	2	4	2	3	–	–	–	–	–	24
Renosterkoppies	5	3	2	4	2	4	2	3	–	–	–	–	–	25
100	5	3	2	3	2	4	2	3	–	–	–	–	–	24
(n = 24) total	116	78	48	91	49	95	48	73	0	0	0	0	0	598
range	4-5	3-4	2	3-4	2-3	3-5	2	3-4	0	0	0	0	0	24-27
average	4,83	3,25	2,00	3,79	2,04	3,96	2,00	3,04	0	0	0	0	0	24,92
Grand Total:	515	331	211	374	216	411	226	312	1	0	0	0	0	2598
(n = 105) $\bar{x}$:	4,90	3,15	2,01	3,56	2,06	3,91	2,15	2,97	0,01	0	0	0	0	24,74

Table 1.2: Female: Sample sheet of raw data collected on the number of sensilla chaetica found on antennal flagellar segments III–XV in slide-mounted specimens.

Species: C. sp. # 30

1.3

	lengths of ♀ antennal flagellar segments III – XV :																AR	
Specimen number:	III	IV	V	VI	VII	VIII	IX	X	Subtotal length III – X	XI	XII	XIII	XIV	XV	subtotal length XI – XV	Total length III – XV	XI – XV / III – X	
Renosterkoppies	15	10,5	10,5	11	11,5	11	11,25	12,25	–	17,5	18	18,75	18,5	27,75	–	200	20,4/19,6	1,04
83	15	10	10	11	11,25	10,75	11	12	–	17,5	17,25	18,5	18,75	28	–	198	20,2/19,4	1,04
Renosterkoppies	14	9,5	9,25	10,5	11	10,75	10,75	11,75		17	16	17	17,75	30,5	–	192,5	20/18,5	1,08
89	14	9,75	9,75	10,75	11	10,5	10,25	11,5	–	16,5	16	17,25	18	29,75	–	191,5	20/18,3	1,09
Renosterkoppies	14,25	9,75	10	10,25	11	11	10,75	11,5	–	16,5	17	17,75	17,25	27,5	–	192	19,5/18,9	1,03
90 *	14,75	9,75	9,25	10,25	11	11	10,75	11,5	–	17	17,25	17,5	16,5	28	–	192,5	19,7/18,8	1,05
Renosterkoppies	14	9,5	9,75	10,25	11	11,25	10,75	12	–	17,75	17	17,25	17,5	29,75	–	195,5	20,3/18,8	1,08
91	14,75	9,75	9.25	10,25	11	11,25	10,75	11,75	–	17,25	17	17,75	17	30	–	193,5	20/18,7	1,07
Renosterkoppies	15,5	10,75	10,5	11,25	11,5	11,75	11,5	12,75	–	17,5	18	18,5	19	29,5	–	205	20,8/20,2	1,03
92	15,75	11	10,75	11,75	12	11,5	11,75	12,75	–	18	17,25	18,75	18,75	29,75	–	206,5	20,9/20,4	1,02
Renosterkoppies	13	9,75	9	9,5	10,5	10	10,75	11,5	–	15,25	15	16	15,75	25,75	–	177	17,8/17,6	1,01
93	14	9	9	10,25	10,25	10,5	10,25	11,75	–	15,75	14,75	16,25	16.25	26,75	–	179	18,1/17,7	1,02
Renosterkoppies⊛	15	10,25	10	11	11,25	11,25	11	12,5	–	17,5	18,25	18,25	18,25	29,75	–	201,5	20,8/19,5	1,07
94	15	10,25	10,25	11	11	11,5	11	12,25	–	17,75	18,5	18,75	18,25	29,25	–	201,5	20,9/19,4	1,08
Renosterkoppies	15,25	10,75	10	10,75	11,75	11,75	11,75	13	–	18,75	18,25	18,25	19	28,75	–	204,5	20,9/20	1,05
95	15,25	10,25	9,75	11	11,25	12	12,25	13,5	–	18,5	18,25	18,75	18	27,75	.	202,5	20,5/20	1,03
Rnstrkpps	14,5	10,5	9,5	10,5	10,75	11	11	12	–	16,5	18	17,75	17,75	28	–	193,5	19,8/18,9	1,05
96	15	10	10	10,75	10,75	11	11	11,75	–	16,75	16,75	17,5	18,25	28	–	193,5	19,8/18,9	1,05
Rnstrkpps	15,75	10,25	10	11,25	11,25	11,5	11,5	12,5	–	17,75	18,75	18	18,25	30,5	–	204	20,8/20	1,04
97	16,25	10,5	10,25	11,25	11,5	11,5	11,5	12	–	18	18	18,5	18	30	–	206	20,9/[illegible]	1,03
Rnstrkpps	15,5	10,5	11	11,25	11,75	11,25	11,75	12,75	–	18,25	19	19	19,5	31	–	211,5	21,8/20,5	1,06
→ 98	15,75	11	10,5	11,5	11,75	11,75	11,5	13	–	17,5	18	19,75	19,25	32	–	210,5	21,7/20,4	1,06
Rnstrkpps	15	10	10	11	11,75	11,25	11,5	12,5	–	17,5	16,75	17,5	18,5	28	–	199,5	20/19,9	1,01
100	15	10	10,25	11	12	11,5	11	12	–	17,5	17	17,75	18	29,25	–	200	20,4/19,6	1,04
subtotal (n=24)	357,25	243,15	238,5	259,25	269,75	268,5	267,25	292,75	–	415,25	416,0	431,0	432,0	695,25		4751,5		25,1[illegible]
X̄	14,89	10,13	9,94	10,80	11,24	11,19	11,14	12,18	–	17,30	17,33	17,96	18,0	28,97		197,98		1,05
TOTAL	1566,75	1056,15	1036,5	1124,75	1176,75	1167,5	1166,25	1280,5	✓	1832,5	1828,25	1889,5	1910,5	3052		20723,5		109,76
(n=104)	15,06	10.16	9,97	10,81	11,31	11,23	11,21	12.31	–	17,62	17,58	18,17	18,37	29,35		199,26		1,06
μm	37,65	25,4	24,93	27,03	28,28	28,08	28,03	30,78	–	44,05	43,95	45,43	45,93	73,38		498,15		

Table 1.3: Female: Sample sheet of raw data collected on lengths of individual antennal flagellar segments III–XV; total antennal length and antennal ratio (AR) also recorded.

1.4

Species: C. tuttifrutti

	♀ palp :												PR :	
Specimen number :	length of palpal segments: I	II	III	IV	V	Total length	No. of sensilla chaetica/segment: I	II	III	IV	V	Total	length III / width III	
Rnstrkoppies 83	8	20	23	11,75	11	71,5	1	3	4	4	5	17	23/10,25	2,24
	8	21	23,5	11,25	10,75	69,5	1	3	4	4	5	17	23,5/11	2,14
Rnstrkoppies 89	9,5	18	22,5	11	10,5	66,5	1	4	5	3	5	18	22,5/11,25	2,00
	8,5	18	23,5	10,5	11	66	1	3	4	4	5	17	23,5/11,25	2,09
Rnstrkppies 90	9,5	19,5	21	11	10	67	1	3	4	5	5	18	21/10,25	2,05
	8,5	19	20,75	11,25	11	66	1	3	5	3	5	17	20,75/10,5	1,98
Rnstrkoppies 91	8	19,5	23	11,5	11,25	69	1	3	6	4	6	20	23/11	2,09
	←	—	bent	—	—	→	1	3	6	4	5	19	23,5/11,5	2,04
Rnstrkppies 92	10	20	24	13	10,25	72,25	1	3	8	5	5	22	24/11,75	2,04
	9,25	22	23	12	11,75	72,25	1	3	6	4	5	19	23/11	2,09
Rnshkppies 93	7	15	21,5	10	8,5	61	1	3	4	2	5	15	21,5/10	2,15
	←	—	missing	—	—	→	←	—	—	—	—	→	/	/
Rnshkppies 94	10	20,5	24,5	13	11	72,5	1	3	7	5	5	21	24,5/11	2,23
	10	22,5	24,75	12	10,25	72,75	1	4	7	4	5	21	24,75/12	2,06
Rnstrkppies 95	8	19,5	26	12	10	72	1	4	8	4	5	22	26/11	2,36
	8,5	20	25,5	13	10	73	1	3	4	4	5	17	25,5/11	2,32
Rnstrkppies 96	8	18	22	10,25	12	66,5	1	3	5	4	5	18	22/9,75	2,26
	8	18	22,5	10,5	12	67	1	4	6	4	5	20	22,5/9,75	2,31
Rnstrkppies 97	8,5	20	22,5	13	12,75	73	1	3	5	5	5	19	22,5/10,5	2,14
	8,5	20	22	12,75	12,75	72,5	1	3	4	5	5	18	22/10,5	2,10
Rnstrkppies 98	8,75	21	23,5	12,25	10	71	1	3	7	5	5	21	23,5/11	2,14
	10	20	24,5	11,75	11	71,25	1	3	5	3	5	17	24,5/11,25	2,18
Rnstrkppies 100	8,5	19,5	24,5	12	11	70	1	3	4	4	5	17	24,5/10,25	2,39
	8	18,5	22,75	11,75	11,75	67,75	1	3	4	5	5	18	22,75/10,25	2,22
(n = 22)	191	429,5	510,75	257,5	240,5	1530,25	23 (n = 23)	13				428	248,0	49,62 n = 23

Table 1.4: Female: Sample sheet of raw data collected on lengths of individual palpal segments I–V, and number of sensilla chaetica per segment; palpal ratio (PR) also recorded.

1.5

Species: C. tuttifrutti

Specimen no:	P/H	no. teeth on mandible	AtR	no. bristles medianally on scutellum	TR	no. spines hind tibia
Rnstrkoppies 83	14,3/14,5 = 0,99	15 + 15	18,5/11 = 1,68	1	13,3/8,7 = 1,53	5 + 5
Rnstrkppies 89	13,2/14,9 = 0,89	13 + 14	16,5/10,25 = 1,61	1	11,8/8,2 = 1,44	5 + 5
Rnstrkppies 90	13,1/14,4 = 0,91	14 + 15	16,5/10,5 = 1,57	1	12,2/7,5 = 1,63	5 + 5
Rnstrkppies 91	13,4/14,9 = 0,90	13 + 15	16,5/10,5 = 1,57	1	12,1/7,6 = 1,59	5 + 5
Rnstrkppies 92	14/14,6 = 0,96	13 + 14	15,5/11,75 = 1,32	1	13,8/8,6 = 1,60	5 + 5
Rnstrkppies 93	12,2/14,6 = 0,84	14 + 12	15,5/10 = 1,55	1	11,6/7,5 = 1,55	5 + 5
Rnstrkppies 94	14,3/16,6 = 0,86	16 + 14	17,5/11 = 1,59	1	13,4/8,5 - 1,58	5 + 5
Rnstrkppies 95	14,1/15 = 0,94	13 + 16	16,5/10,75 = 1,53	1	13,1/8,6 = 1,52	5 + 5
Rnstrkppies 96	13,4/14,3 = 0,94	15 + 14	17,25/10,25 = 1,68	1	13/8 = 1,63	5 + 5
Rnstrkppies 97	14,5/14,6 = 0,99	15	18/11 = 1,64	1	13,1/8,2 = 1,60	5 + 5
Rnstrkppies 98	14/15,3 = 0,92	14 + 14	19/11,25 - 1,69	1 *	13,1/8,5 - 1,54	5 + 5
Rnstrkppies 100	13,7/15,3 = 0,90	13 + 14	17/11 = 1,55	1	13/8,3 = 1,57	5 + 5
		325			18,78 n=12 1,565	

Table 1.5: Female: Sample sheet of raw data collected on the proboscis/head (P/H) ratio, number of teeth on mandible, antennal trichodea ratio (AtR), number of bristles medially on scutellum, tarsal ratio (TR) and number of spines on hind tibia.

1.6

Species :

Specimen number :	wing breadth	wing length	costa length	CR	Spermathecae		
					large	medium	rudi-mentary
Renosterkoppies 83	15,5 + 15,1	29,4 + 29,6	16,8 + 16,9	0,57 + 0,57	* 21 × 15,5	17,75 × 13,5	4 × 2
Rnstrkpps 89	14,2 + 14,4	28,3 + 28,2	16,4 + 16,6	0,58 + 0,59	-	-	-
Rnstrkpps 90	13,9 + 13,8	27,4 + 27,4	15,7 + 15,6	0,57 + 0,57	-	-	-
Rnstrkpps 91	14,2 + 14	27,6 + 27,6	15,5 + 15,5	0,56 + 0,56	-	-	-
Rnstrkpps 92	15,4 + 15,5	30,8 + 30,8	18,2 + 18	0,59 + 0,58	-	-	-
Rnstrkpps 93	14,2 + 13,9	26,6 + 26,5	14,8 + 14,8	0,56 + 0,56	20 × 15,75	15,5 × 13,5	3,5 × 2
Rnstrkpps 94	15 + 14,9	30,1 + 30,2	17,6 + 17,4	0,58 + 0,58	-	-	-
Rnstrkpps 95	15,2 + 15,1	29,8 + 30,1	16,8 + 17,2	0,56 + 0,57	-	-	-
Rnstrkpps 96	13,8 + 13,7	28 + 27,9	16,1 + 16	0,58 + 0,57	22 × 16	16,5 × 13,5	4,5 × 1,5
Rnstrkpps 97	14,6 + 14,6	30,2 + 30,2	17,5 + 17,3	0,58 + 0,57	21,75 × 16,25	—	5 × 1,5
Rnstrkpps 98	15,5 + 15,1	30,4 + 30,5	17,5 + 17,7	0,58 + 0,58	-	-	-
Rnstrkpps 100	14,8 + 14,7	29,4 + 29,1	16,9 + 16,9	0,57 + 0,58	-	-	-
n = 24	351,1 14,63	696,1 29,00	/	13,76 0,57 x̄	63,75 × 48 21,25 × 16	32 × 27 16 × 13,5	13 × 5 4,33 × 1,67
Grand total n = 58	1317,3 14,97	2610,6 29,67	/	50,51 0,57 n = 21-23 : x̄	441,5 × 342 21,02 × 16,29	347,25 × 280 16,54 × 13,33	131 × 45,25 5,70 × 2,00
range	12,9 – 17,0	25,5 – 33,5		0,55 - 0,60 um	52,55 × 40,73	41,35 × 33,33	14,25 × 5,00
mm	0,40 – 0,53	0,80 – 1,05	/				

Table 1.6: Female: Sample sheet of raw data collected on the wing length and breadth, costa length, costal ratio (CR), and size of spermathecae.

Specimen no: 1.7

Species & coll. data: C. sp. nr. pseudopallidipennis

lengths of segments, distribution of various sensillae on ♂ antennal flagellar segments III – XV:

Specimen no:		III	IV	V	VI	VII	VIII	IX	X	XI	XII	Subtotal length III – XII	XIII	XIV	XV	Subtotal length XI – XV	Total length	XIII–XV / III–XII	AR
White River 14 (Dodds)	lengths	29,5 29,5	← ←	— —	— —	— —	138 137,5	— —	— —	— —	→ →	167,5 167	37 37,5	30 28,5	36,5 36,5	103,5 102,5	271 269,5	103,5/167,5 102,5/167	0,62 0,61
	coeloconica	2 2	— —	— —	— —	— —	— —	— —	— —	— —	— —		1 1	1 1	2 2				
	chaetica	5 5	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	3 3	2 2	— —				
	trichodea	LL LL	LLc LLc	Lc L¼Lc	Lc L¼Lc	Lc Lc	Lc Lc	Lc Lc	c c	— —	— —								
White River 18 (Dodds)	lengths	27,5 27,5	← ←	— —	— —	— —	126 126,5	— —	— —	— —	→ →	153,5 154	35 36	28 27,5	34,5 33,5	97,5 97	251 251	97,5/153,5 97/154	0,64 0.63
	coeloconica	2 2	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	1 1	1 1	2 2				
	chaetica	5 5	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	3 3	2 2	0 0				
	trichodea	LL LL	Lc LLc	L¾Lc L½Lc	Lc Lc	Lc Lc	Lc Lc	Lc Lc	c c	— —	— —								
White River 19 (Dodds)	lengths	30 31,5	← ←	— —	— —	— —	138 138	— —	— —	— —	→ →	168 169,5	37 36,5	-29 29,5	37 37,5	103 103,5	271 273	103/168 103,5/169,5	0,61 0,61
	coeloconica	2 2	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	1 1	1 2	2 2				
	chaetica	5 5											3 3	2 2	0 0				
	trichodea	LL LL	LLc LLc	LLc L¾Lc	Lc L⅙Lc	Lc Lc	Lc Lc	Lc Lc	c c	— —	— —								

Table 1.7: Male: Sample sheet of raw data collected on the lengths of flagellar segments III–XV, and number of sensilla coeloconica, chaetica and trichodea; total antennal length and antennal ratio (AR) also recorded.

CHAPTER 2

A redescription of *C. (Avaritia) imicola* Kieffer, 1913 and *C. (A.) bolitinos* Meiswinkel, 1989, the latter reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa (Diptera: Ceratopogonidae)

2.1 INTRODUCTION

In the Afrotropical Region, the single most important species of the *Culicoides* subgenus *Avaritia* is *C. imicola* as it is both a proven and suspected vector of certain viruses in livestock. The most noteworthy are bluetongue in sheep and African horsesickness (du Toit 1944). *Culicoides imicola,* mostly recorded under the old name of *C. pallidipennis* (Carter, Ingram & Macfie 1920) occurs both throughout and outside the African continent. Although Egypt, Morocco and Algeria remain the only north African countries from which *C. imicola* has been reported (Macfie 1943; Nagaty & Morsy 1959; Szadziewski 1984), it has also been found on the northern side of the Mediterranean in Spain and Portugal (Mellor, Jennings, Wilkinson & Boorman 1985), Cyprus and western Turkey (Jennings, Boorman & Ergün 1983), and on the Greek islands of Lesbos (Boorman & Wilkinson 1983) and Rhodes (Boorman 1986). It remains unrecorded from the central Mediterranean on mainland Italy and Greece (Mellor, Jennings & Boorman 1984), from the Balearic Islands, and the islands of Sardinia, Corsica, Sicily, Malta and Crete (Boorman, Jennings, Mellor & Wilkinson 1985). It has, however, been recorded from the Cape Verde islands (Boorman & van Harten 1992). Glick's 1990 record of *C. imicola* from France is likely an error; the northernmost record of *C. imicola* is from Cheires, Portugal at 41°17'N (Capela, Sousa, Pena & Caeiro 1993).

East of the Mediterranean, *C. imicola* occurs in Israel from which four bluetongue serotypes have been isolated to date (Braverman, Barzilai, Frish & Rubina 1985). It also ranges throughout western and southern Asia, being recorded from India under the names of *C. minutus* (Sen & Dasgupta 1959) and *C. pseudoturgidus* Dasgupta, 1962. These species were synonymized with *C. imicola* by Dyce & Wirth (1983).

It has also been recorded from Iraq under the name *iraqensis* (Khalaf 1957) and from Iran as *C. pallidipennis* (Navai & Mesghali 1968). Going still further beyond the Indian subregion, are the recent records of *C. imicola* from Laos (Howarth 1985), Sri Lanka, Thailand and Vietnam (Wirth & Hubert 1989).

Though known from Madagascar (De Meillon, 1961; Callot, Kremer & Brunhes 1968) and the smaller islands of Reunion (Clastrier 1959) and Mauritius (Boorman & Mellor 1992), some 2 500 km off the east coast of Africa, *C. imicola* still remains unrecorded from many mainland countries such as Malawi, Mozambique, Zambia, Botswana and Namibia. This is probably due to the paucity of collections, since in neighbouring countries such as Zimbabwe, Phelps, Blackburn & Searle (1982) showed *C. imicola* to comprise 61,7–96,8 % of biting midges caught near a paddock containing horses and cattle. Similarly, in South Africa, *C. imicola* can be said to be always present in certain situations and can comprise up to 94 % of collections made near mules, sheep and cattle (Nevill & Anderson 1972). Further afield, it has been reported also to be the commonest species in Kenya (Khamala 1971; Khamala & Kettle 1971; Davies & Walker 1974; Walker 1977) and in Nigeria (Dipeolu & Sellers 1977).

Though it can be said that these numbers reflect a veterinary bias which favours the collection of *C. imicola* on cattle farms, they do suggest that man is an important link who maintains and spreads this species. This is the opinion of Howarth (1985) who, in his treatment of the *Culicoides* of Laos, said that ‘many of the widespread species may have been spread indirectly through human activities. This is especially true for species closely associated with domestic animals, such as ... *C. imicola* and *C. brevitarsis*. From ancient times to the present, cattle drives have occurred between India and Thailand’. Similarly, in Australia the important immigrant *Avaritia* species *C. brevitarsis, C. brevipalpis* and *C. wadai* only established themselves and spread widely once cattle dung, the only suitable larval habitat for the immatures, became constantly available (Dyce 1982). Thus, the presence of *C. brevitarsis* in near pest-proportions in that country today is undoubtedly man-induced. In South Africa, a similar picture as regards the abundance of *C. imicola* is emerging, especially in heavily stocked areas, while the converse appears to be true in those places where man has interfered little with the environment. Though little or no quantitative work has been done in the wild areas of the Afrotropical Region, my recent studies in the Kruger National Park (KNP), South Africa, reveal that of 12 species of the subgenus

Avaritia found there, *C. imicola* remains uncommon and localized. This is not due to a lack of suitable hosts, but to the fact that the hosts can roam far and wide thus preventing a buildup of enormous foci of *C. imicola*. Equally important, the larval habitats in such natural areas are subject to the seasonal vagaries of both drought and flood, and so severely check the numbers of *C. imicola* (personal observations 1985/86). In the farmyard situation, irrigation is controlled and constant year round, and this, coupled to the maintenance of large sedentary populations of domesticated animals, exactly suits the needs of *C. imicola* and leads to an escalation in its populations. Studies in recent years have yielded a second member of the Imicola group, *C. bolitinos*, also associated with farming activities in southern Africa (Nevill, Venter, Edwardes, Pajor, Meiswinkel & Van Gas 1988). It is also one of the 12 *Avaritia* species found in the KNP, and is one of only two species which will breed in buffalo and cattle dung. This chapter will clarify its taxonomy and briefly indicate its possible veterinary importance.

As to the taxonomy of members of the subgenus *Avaritia*, there are 'unfortunately in each region difficult taxonomic problems in the accurate determination of some proven or potential vectors ... this includes the so-called *imicola* group ...' (Wirth & Dyce 1985). In the Afrotropical Region there is increasing evidence that *C. imicola* forms part of a complex of species within the Imicola group of the subgenus *Avaritia* with new species still to be described. To date five African species are described i.e. *C. imicola*, *C. pseudopallidipennis* Clastrier, 1958, *C. bolitinos* Meiswinkel, 1989, *C. miombo* Meiswinkel, 1991, and *C. loxodontis* Meiswinkel, 1992. Two new species are described in Chapters 6 and 7. A further two species of the Imicola group occur extralimitally in south-east Asia and Australia, i.e. *C. brevitarsis* Kieffer, 1917 and *C. nudipalpis* Delfinado, 1961.

It is for two reasons that the emphasis of this thesis will be on a numerical description of the various species dealt with. Firstly, it is forseeable that members of the Imicola group will soon be subjected to the modern techniques of enzyme and hydrocarbon cuticular analyses. Promising results have recently been obtained on certain European *Culicoides* by Waller, Belliard & Krêmer (1987) who examined 10 species including three members of the subgenus *Avaritia* to which *C. imicola* belongs. However, the value of such studies depends strongly upon species being as soundly described taxonomically as possible. For this to be achieved, a population needs to be sampled and significant numbers examined to assess the range of variation that exists within traditionally used characters and ratios, and to establish new parametres. This is best achieved from reared material. Such data are given additional depth by knowledge of the biology of the various species, as it is often the habits rather than the morphology that reveals the presence of sibling species. This is especially evident in vector complexes, such as *gambiae* in *Anopheles* and *damnosum* in *Simulium*. Indications are that the genus *Culicoides* will be no exception.

Finally, the presence of such cryptic species has obvious implications concerning the aetiology of disease transmission, and until an effort is made to define these species more accurately, both on the biological and taxonomic level, our isolations of viruses from species pools will remain bedevilled by generalities. Furthermore, it will invalidate conclusions about *Culicoides* dynamics in the important areas of distribution, feeding habits, transmission cycles and vector competence.

2.2 MATERIALS and METHODS

The redescription of *C. imicola* is based entirely on slide mounts of 136 of more than 6 000 adults reared under field conditions at Onderstepoort during April and May 1986, using the tent-trap emergence method described by Pajor (1987). The type series used for the description of *C. bolitinos* comprises 92 of 512 midges that were reared from half of a single African buffalo (*Syncerus caffer*) dung pat. The pat was collected off short grass adjoining a stand of *Phragmites* reeds on the lower banks of the dry Timbavati River near Roodewal in the west-central area of the Kruger National Park. These data are on occasion linked to and supplemented by further information gained from numerous specimens collected by light-trap or reared from cattle, buffalo and blue wildebeest (*Connochaetes taurinus*) dung in various localities of the Transvaal, Orange Free State, Cape and Natal as well as from Zimbabwe and Malawi. The dung collected by myself was always a single or half of a pat, placed in a cardboard box and then stored in a fine gauze net to await emergence of *Culicoides*, if present. The dung collected by E.M. Nevill, J.E. Randall, G.J. Venter and colleagues always comprised five to ten pats placed together in a large, black plastic box closed with a ventilated lid. Mounted over a hole made in one side of the box was a white exit cone onto which was fixed a 500 mℓ paper icecream cup. Emerging *Culicoides* attracted to the light make their way through the cone into the paper cup. These midges were removed daily to be identified, counted and sexed.

2.3 RESULTS

2.3.1 *Culicoides (Avaritia) imicola* Kieffer 1913

(Fig. 2.1–2.3, 2.5, 2.7, 2.9, 2.11, 2.13, 2.15, 2.17, 2.19; Table 2.1–2.7)

Culicoides imicola Kieffer 1913:11. Kenya.

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

Culicoides pallidipennis Carter, Ingram & Macfie; Fiedler 1951: 30. South Africa.

Culicoides iraqensis Khalaf 1957: 343. Iraq.

Culicoides pallidipennis Carter, Ingram & Macfie; Clastrier 1958: 194. Senegal.

Culicoides pallidipennis Carter, Ingram & Macfie; Nagaty & Morsy 1959: 71. Egypt.

Culicoides minutus Sen & Das Gupta 1959: 622: Dyce & Wirth 1983: 221. India.

Culicoides pallidipennis Carter, Ingram & Macfie; Caeiro 1961: 230. Angola.

Culicoides pseudoturgidus Das Gupta 1962: 538: India.

Culicoides pallidipennis Carter, Ingram & Macfie; Khamala & Kettle 1971: 41. Kenya, Uganda and Tanzania.

Culicoides imicola Kieffer; Krémer 1972: 648. Redescription of holotype.

Culicoides sigaensis Tokunaga; Ratanaworabhan 1975: 12 (misident.)

Culicoides imicola Kieffer; Boorman & Dipeolu 1979: 31. Nigeria.

Culicoides imicola Kieffer; Wirth & Hubert 1989: 272. Laos, Thailand, Vietnam.

Culicoides imicola Kieffer; Boorman 1989: 183. Bahrain, Oman, Saudi Arabia, U.A.E., Yemen.

Culicoides imicola Kieffer; Glick 1990: 120. Chad, Egypt, Ethiopia, Gambia, Israel, Kenya, Mauritius, Nigeria and Zimbabwe.

Female (Fig. 2.1–2.3, 2.5, 2.7, 2.9, 2.13; Table 2.1–2.7)

Head. Eyes (Fig. 2.3); bare, contiguous for a distance of between one and two facets. Antenna (Fig. 2.5, Table 2.1, 2.2, 2.3, 2.4, 2.5 and 2.7) slender, basal segments IV–X barrel-shaped, distal segments XI-

XIV faintly vasiform narrowing perceptibly subapically, XV nearly parallel-sided only narrowing apically; lengths of antennal segments III–XV: 37,4–24,4–24,2–25,9–27,1–26,9–27,3–29,8–41,3–43,4–44,1–44,3–70,8 μm (n = 25); total length of antenna: 435,0–494,5 mean 466,0 μm (n = 25); widths of antennal segments III–XV: 27,6–21,6–18,0–18,0–16,8–16,8–15,6–15,6–16,8–16,8–16,8–16,8–18,0 μm (n = 1); AR 0,95–1,10, mean 1,01 (n = 167); sensilla coeloconica present on segments III, XII–XV in 92,5 % of antennae examined (n = 172) (see Table 2.2 for deviations from the norm); sensilla chaetica distribution on segments III–XV was 5–3–2–3–2– 3–2–3–0–0–0–0–0 (n = 172) in 95 % of antennae examined (see Table 2.3 for deviations from the norm); sensilla trichodea distribution of the LLc type i.e. each of segments IV–X with two long and one short blunt-tipped trichodea, segment III with only two long blunt-tipped trichodea (n = 172); AtR 1,59–2,27, mean 1,86 (n = 173); segments XI–XV each with 9–23 sharp-tipped sensilla trichodea of varying lengths and thicknesses as also 1–9 short, blunt-tipped basiconica per segment; each antennal segment is uniformly clothed throughout with fine spiculae. The distributions of the sensilla coeloconica, chaetica and trichodea appear in Table 2.1. Palp (Fig. 2.7; Table 2.6): of a moderate length, slender, light brown throughout, lengths of palpal segments I–V: 18,96–54,24–46,85–30,64–26,97 (n = 160); total length 165,6–182,4 μm, mean 176,06 μm (n = 25); III moderately long and slender carrying only three to four rather short chaetica, with a small, round and shallow subapical pit with opening about half the width of segment in diameter, margin of pit smooth; PR 2,40–3,38, mean 2,86 (n = 172); P/H ratio 0,82–1,02, mean 0,90 (n = 45); mandible with 12–16 fine teeth (n = 42). Legs brown with fore and middle femora narrowly pale basally and subapically; hind femora brown, only narrowly pale basally; all tibiae brown with a distinct narrow subbasal pale band, only hind tarsi also narrowly pale apically; TR 1,52–1,76, mean 1,64 (n = 100); comb on apex of hind tibia with five spines, the first being the longest and only slightly longer than the second. Wing: (Fig. 2.2, 2.9): length 0,92–1,17 mm, mean 1,06 (n = 150); breadth 0,43–0,61 mm, mean 0,52 mm (n = 100); CR 0,54–0,59, mean 0,56 (n = 100); macrotrichia scanty, confined to distal third of wing in cells R_5, M_1, and M_2 only; microtrichia dense and coarse. Dark pattern of wing grey, pale areas white to yellowish well-defined but irregularly shaped; two radial cells, proximal half of first and distal half to two thirds of second cell pale. The more important species-specific wing pattern characters are: (i) pale costal spots 2 and 3, those that cover the r-m crossvein and distal half of second radial cell respectively, are rather broadly separated by a dark area below the radial cells, (ii) distal or fourth pale costal spot in cell R_5 broadly abuts wing margin and always has its proximal margin moderately to fairly strongly pointed, and (iii) entire posterior margin of vein M_2 dark, anterior margin is dark for proximal two thirds but distal third is more or less equally divided into a pale preapical section which touches and occasionally straddles vein M_2, followed by an equally broad dark distal section which always leaves the apex of vein M_2 broadly dark. Abdomen (Fig. 2.13): two moderately sclerotized slightly unequal spermathecae present, measuring 50 x 39 μm and 39 x 34 μm; both are round and entirely devoid of small hyaline punctations, with short narrow pigmented necks; rather small narrow rudimentary third spermatheca present measuring 18 x 8 μm; sclerotized ring on common spermathecal duct cylindrical, smooth and parallel-sided, a little longer than broad, less than half the length of the rudimentary spermatheca; sclerotization surrounding the oviduct as shown in Fig. 2.13. Thorax light brown in alcohol; scutum with 60–79, average 71 bristles (n = 10; Fig. 2.21) scutellum with one median bristle and one shorter bristle on each corner in 85/87 specimens, remaining two specimens differed in having two median bristles.

Male (Fig. 2.11, 2.15, 2.17, 2.19; Table 2.1 & 2.7)

Head. Eyes bare. Antenna (Fig. 2.15, Table 2.1): plume rather sparse, appressed, plume fibrillae light brown, almost completely encircle medianally each of segments IV–XII in a regular whorl; these segments with very few spiculae, distal segments XIII–XV densely and evenly clothed with spiculae; lengths of segments III–XV: 62,4–36,0–36,0–38,4–38,4–38,4–38,4–38,4–36,0–33,6–88,8–67,2–91,2 μm (n = 1); sensilla coeloconica distribution: 96 % with two on segment III, 98 % with 1 on XIII, 98 % with one on XIV and 96 % with two on XV (n = 50); 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 medianally (though very slender is one and a half times longer than segment) on XIII, two basally (both weak but of different lengths) on XIV, none basally on XV only one apically; sensilla trichodea distribution on segments III–XII: III with two long blunt-tipped trichodea, segments IV–VI with two long and one short blunt-tipped trichodea; segments VII–IX with one long and one short blunt-tipped trichodea, segment X with one short blunt-tipped trichodea only, segments XI and XII lacking trichodea (n = 25). The distributions of the sensilla coeloconica, chaetica and trichodea appear in Table 2.1. Wing: (Fig. 2.11). Abdomen. Genitalia (Fig. 2.17, 2.19): tergum 9 (Fig. 2.17) square, fractionally waisted medianally, finely spiculate throughout except for narrow strips of the anterior and posterior margins being bare, bearing 11–19 chaetica of different lengths, mean 15 (n = 45); apicolateral processes are replaced by thin, hyaline flanges these lacking spiculae but each carrying a single fine, rather short chaetica issuing from the interface that comprises the base of the flange and the adjoining spiculate fringe where the concave body of the tergum commences; the posterior margin of tergum which separates these flanges is gently concave and without median indentation or infuscation; two well-developed cerci (Fig. 2.19), each adorned with long spiculae and two long and two short setae apically, protrude well beyond posterior margin of tergum (Fig. 2.19); sternum 9 (Fig. 2.19) with moderately deep excavation, membrane within the excavated area can be sparsely to rather strongly spiculate the excavated area bearing from 8–145 spiculae, mean 47 (n = 50); basimere with dorsal and ventral spiculae and chaeticae as illustrated (Fig. 2.19), basimere 2,3–2,7, mean 2,5 x as long as broad (n = 20) with basal infuscate collar and well developed dorsal and ventral roots of the form typical of the subgenus *Avaritia*. Distimere (Fig. 2.19) 0,70–0,85, mean 0,77 x length of basimere (n = 20), rather stout, gently curved and broadly blunt-tipped; with basal half spiculate and carrying six bristles of varying lengths and thicknesses, extreme apex with about five very short, fine tactile sensilla as illustrated. Aedeagus (Fig. 2.19) shield-shaped, slender 1,35–1,75, mean 1,55 x longer than wide (n = 20) and 0,84–0,91, mean 0,88 x length of basimere (n = 20); basal margin concave lightly to moderately infuscate, distal margin or arch reaching to 0,21–0,30, mean 0,25 x length of aedeagus (n = 20); lateral margins of the aedeagus smooth and convex, darkly but narrowly infuscate and converging distad to end in a hyaline, round-tipped, parallel-sided terminal projection the base of which projects anteriorly into median area of aedeagus in the form of a raggedly infuscate "peg". Parameres (Fig. 2.19) separate, nearly touching medianally from where they diverge anteriad and posteriad at 45°, posterior halves are as two convex almost hyaline blades initially stout but tapering smoothly to sharp, simple, erect tips. Thorax with 51–61, average 57 bristles (n = 5; Fig. 2.22).

Slide material used in redescription

SOUTH AFRICA: 83 ♀♀ 53 ♂♂ Onderstepoort, Transvaal. April-May 1986, I.T.P. Pajor, reared from drainage furrow overgrown with kikuyu grass, using *in situ* tent-type emergence trap.

Additional slide material examined

Transvaal:

5 ♀♀ 27 ♂♂ Honeydew, northern Johannesburg, M. Wasserthal, blacklight, dates as

	follows: 3 ♀♀ 20 ♂♂ 8.II.1984; 1 ♂ 14.II.1984; 2 ♀♀ 6 ♂♂ 25.III.1984.
1 ♀ 1 ♂	Farm Krugerspan, Sentrum, north-western Transvaal, ♀ 21.IV.1987, ♂ 13. V. 1987, M. Ras, blacklight.
1 ♂	Farm Apel, Sekhukhuneland, central Transvaal, 6. III.1979, R. Meiswinkel & K. Newberry, white light on edge of sand river.
2 ♀♀ 1 ♂	Makonde, Vendaland, northern Transvaal, 23.IX.1980, R. Meiswinkel, blacklight on edge of vlei.
1 ♂	Eiland mineral baths, north-eastern Transvaal, 2.III.1984, R. Meiswinkel, blacklight on edge of vlei.
1 ♂	Bergpan saltworks, Soutpansberg district, northern Transvaal, 5.IX.1984, R. Meiswinkel, blacklight.
2 ♂♂	Tshipese mineral baths, northern Transvaal, 4.III.1984, R. Meiswinkel & J.E. Randall, blacklight on edge of vlei.
1 ♀	Mooketsi, northern Transvaal, 10.II.1980, R. Meiswinkel, blacklight.
10 ♀♀ 11 ♂♂	Farm Jaffray ± 15 km east of Tzaneen, northern Transvaal, 14.I.1984, R. Meiswinkel, blacklight in sheep pen.
1 ♀ 3 ♂♂	Skukuza, Kruger National Park, eastern Transvaal, 11.III.1984, R. Meiswinkel & L.E.O. Braack, blacklight on edge of Sabie river.

Natal:

2 ♀♀	Ngome Tea estate, northern Natal, 3.I.1981, R. Meiswinkel, blacklight.
4 ♀♀ 6 ♂♂	Mfomoti False Bay, northern Natal, 24.IX.1983, R. Bagnall, blacklight.
19 ♀♀ 18 ♂♂	Ndumu Game Reserve, northern Natal, 6.VI.1988, R. Meiswinkel, blacklight at main camp.

Cape:

8 ♀♀	Farm Welgevallen, Stellenbosch, 1 ♀ 7.IX.1983; 7 ♀♀ 20.VI.1984, A. Kriel, blacklight.
2 ♀♀ 5 ♂♂	Verlorenvlei near Redelinghuys, western Cape, 3.V.1987, G. v. Eeden, blacklight.

10 ♀♀ 2 ♂♂	Farm Veekos near Upington, northern Cape, 1 ♀ 1 ♂ 21.XI.1983; 1 ♂ 15.XII.1983; 4 ♀♀ 1.IX.1986; 2 ♀♀ 4.IX.1986; L. Jordaan, blacklight.
2 ♀♀	Augrabies National Park, northern Cape, 23.IX.1987, M. Edwardes, blacklight in main camp.
1 ♂	Grootfontein Agricultural College, Middelburg, Cape, 7.I.1984, J.C. van Straaten, blacklight.

Botswana:

10 ♀♀	Mamalakwe River near Maun, northern Botswana, 6.VI.1988, H.V. de V. Clarke, light-trap.

Swaziland:

31 ♀♀ 4 ♂♂	High Hope riding School, Manzini, 31.VIII.1988, G. v. Eeden, blacklight.

Malawi:

7 ♀♀ 1 ♂	Kawalazi estate ± 30 km east of Mzuzu, northern Malawi, 2 ♀♀ 23.X.1987; 1 ♂ 24.X.1987; 5 ♀♀ 14.II.1988, K. Verster, blacklight in *Brachystegia* woodland.
1 ♀ 1 ♂	Vizara Rubber Estate near Nkhata Bay, northern Malawi, 14-15.XI.1987, R. Meiswinkel, blacklight near cattle kraal.

Egypt:

11 ♀♀ 4 ♂♂	Aswan village, 15.VIII.1993, B.J.H. Barnard, blacklight at buffaloes, horses and goats.

Spain:

11 ♀♀	Andalucia Prov.; Sotogrande municipality, farm San Roque, VI.1991, J.A. Ferrer Romero, baffle light-trap at horses.

Iraq:

1 ♂	*C. iraqensis* (holotype), Baghdad, Iraq, 24.V.1954, K.T. Khalaf, light-trap.

Mauritius:

10 ♀♀ 1 ♂	S.O.D.I.A., Bambous, w. Mauritius, 30.X.1991, R. Meiswinkel, blacklight at ± 3 000 feedlot cattle.

Unmounted light-trap material examined

South Africa: Transvaal

371 ♀♀ 2 ♂♂	Skukuza buffalo boma K.N.P., 14.IV.1988, L.E.O. Braack & R. Meiswinkel, blacklight.
153 ♀♀ 14 ♂♂	Skukuza, K.N.P., 12.IV.1988, P.J. Meiswinkel, blacklight on banks of Sabie river.
33 ♀♀	Shingwidzi, K.N.P., 10.IV.1988, L.E.O. Braack & R. Meiswinkel, blacklight on banks of Shingwidzi river, 18h30-21h00.
16 ♀♀ 18 ♂♂	Manxeba Pan near Pafuri, K.N.P., 12.IV.1988, L.E.O. Braack & R. Meiswinkel, blacklight on edge of swamp plain 18h30-21h00.
51 ♀♀ 14 ♂♂	Tshalungwa springs north of Punda Maria, K.N.P., 7.XI.1985, L.E.O. Braack & R. Meiswinkel, blacklight 18h30-20h00.
131 ♀♀ 15 ♂♂	Bergpan saltworks, Soutpansberg district, northern Transvaal, 30.Xl.1984, R. Meiswinkel & G.J. Venter, blacklight at cattle kraal.

Namibia:

920 ♀♀	Farm Bergvlug ± 30 km east of Windhoek, central Namibia, XI-XII.1978, H.C. Biggs, light-trap.

Malawi:

348 ♀♀ 11 ♂♂	(202 nulliparous, 146 parous) Vizara Rubber Estate near Nkhata Bay, northern Malawi, 14-15.Xl.1987, R. Meiswinkel, blacklight near cattle kraal.

Unmounted host material examined

South Africa:

8 ♀♀	Skukuza, K.N.P., 13.VII.1985, L.E.O. Braack, aspirated off darted buffalo in boma.

2.3.2 ***Culicoides (Avaritia) bolitinos*** **Meiswinkel, 1989**

(Fig. 2.4, 2.6, 2.8, 2.10, 2.12, 2.14, 2.16, 2.18, 2.20; Table 2.1–2.8)

Culicoides bolitinos; Meiswinkel 1989: 30. South Africa.

Culicoides pallidipennis Carter, Ingram & Macfie; Nevill 1968: 61. South Africa (misident.).

Culicoides 1348; Braverman 1978: 167. Zimbabwe.

Culicoides sp. 49; Nevill, Venter, Edwardes, Pajor, Meiswinkel & Van Gas 1988: 103. South Africa.

Culicoides brevitarsis Kieffer; Boorman & Mellor 1992: Mauritius (misident).

Female (Fig. 2.4, 2.6, 2.8, 2.10, 2.14; Table 2.1-2.8)

Head. Eyes (Fig. 2.4); bare; contiguous for a distance of a little more than one facet. Antenna (Fig. 2.6, Table 2.1, 2.2, 2.3, 2.4, 2.5, 2.7 and 2.8); slender, basal segments IV–X barrel-shaped with V and VI only slightly longer than wide, segments XI–XIV faintly vasiform narrowing perceptibly subapically, XV nearly parallel-sided only narrowing apically; lengths of antennal segments III–XV (n = 25): 35,4–22,4– 22,5–24,0–25,3–25,1–25,8–28,1–39,2–39,7–39,8–39,7–66,8 μm; total length of antenna 392,4–480,0 μm mean 433,8 μm (n = 25); widths of antennal segments III–XV (n = 25): 26,6–20,1–17,5–16,8– 15,8–16,5-15,9–15,5–14,9–15,2–15,6–15,4–16,5 μm; AR 0,96–1,12 mean 1,04 (n = 54); sensilla coeloconica present on segments III, and one on each of segments XII–XV; one of 56 antennae examined had a coeloconica on XI (Table 2.2); sensilla chaetica distribution on segments III–XV: 5–3–2–3–2–3–2–3–0–0–0–0–0, with one of 59 antennae examined having one chaetica on XI (Table 2.3); sensilla trichodea distribution of the LLc type, i.e. each of segments IV–X with two long and one short blunt-tipped trichodea, segment III with only two long blunt-tipped trichodea; AtR 1,59–2,17 mean 1,85 (n = 56); segments XI–XV each with 13–33 sharp-tipped sensilla trichodea of varying lengths and thicknesses as also two to seven short blunt-tipped sensilla basiconica per segment (Fig. 2.6). The distributions of the sensilla coeloconica, chaetica and trichodea appear in Table 2.1. Palp (Fig. 8; Table 2.6): rather short, slender, pale brown throughout; lengths of palpal segments I–V: 18,3–44,5–38,7–23,4–24,5 μm (n = 25); total length 132,0–165,6 μm, mean 148,5 μ (n = 25); III rather short and slender almost barrel-shaped but not inflated, carrying three to four rather short chaetica, with a small round and shallow subapical pit of diameter about half the width of segment, margin of pit smooth in sideview; PR 1,86–2,72, mean 2,33 (n = 52); P/H ratio 0,62–0,89, mean 0,76 (n = 20), mandible with 12–14 fine teeth (n = 10); Legs: brown with all femora narrowly pale basally; fore-femora rather broadly pale subapically, middle femora narrowly pale subapically and hind femora entirely brown apically; all femoral-tibial knees dark; all tibiae brown with a distinct narrow subbasal pale band and with apices of fore-tibiae brown; apices of middle tibiae paling imperceptibly, those of hind tibiae broadly pale; tarsi pale brown; TR 1,54–1,75, mean 1,63 (n = 40); comb on hind tibia with five spines, the 1st being slightly longer and thicker than the remainder. Wing (Fig. 2.10): length 0,87–1,04 mm, mean 0,93 mm (n = 20); breadth 0,46–0,52 mm, mean 0,53 mm (n = 20); CR 0,53–0,60, mean 0,56 (n = 20); macrotrichia rather scanty confined to apical third of wing. Wing pattern very similar to that of *C. imicola,* main differences as follows: third dark costal spot in the centre of cell R_5 with its distal margin not indented medianally by a pointed extension of the fourth pale coastal spot in cell R_5 but is more or less straight and runs transversely, anteriad to posteriad, across cell R_5 and is often ragged rather than smooth; as a result the fourth pale costal spot adjoining the wing margin in R_5 has its proximal margin also straight, transverse and ragged rather than pointed as seen in *C. imicola.* It must be noted that this is subject to variation which includes those specimens which will have this proximal margin faintly convex thus the tendency for this fourth pale spot to have a muted form of the pointed extension always seen in *C. imicola.* The median third of the upper and lower margin of vein M_2 is broadly dark but gradually tapers and fades to leave the apex of vein M_2 pale or narrowly darkened. In darker variants the apex of vein M_2 can be fairly broadly darkened. In both pale and dark variants, however, vein M_2 never possesses the preapical pale excision that is always seen subapically on the upper margin in *C. imicola.* Abdomen (Fig. 2.14): Two round to gently ovoid rather darkly pigmented spermathecae, without hyaline punctations, each with rather short and narrow, smooth necks, subequal in size measuring 45 x 34 μm and 39 x 29 μm; rudimentary third spermatheca short and slender, slightly rugose with a small bulbous head this expanded in occasional specimens, measuring 12 x 7 μm; sclerotized ring on common spermathecal duct, cylindrical, smooth, and parallel-sided, a little longer than broad less than half the length of the rudimentary spermatheca; genital sclerotization surrounding oviduct as shown in Fig. 2.14. Thorax: light brown throughout when in alcohol; scutellum with one long median bristle and one shorter, thinner bristle on each corner (n = 20).

Male (Fig. 2.12, 2.16, 2.18, 22.0; Table 2.1 & 2.7)
Head. Eyes bare. Antenna (Fig. 2.16, Table 2.1): apparently inseparable from that of *C. imicola* having the same sensilla coeloconica, trichodea and chaetica distributions (n = 50; see under *C. imicola* and Table 2.1). Lengths of segments III–XV 55,2–31,2–31,2–31,2–31,2–31,2–31,2–28,8–28,8–28,8–72,0–62,4–76,8 µm (n = 1). Abdomen. Genitalia (Fig. 2.18, 2.20) very similar to *C. imicola.* Tergum 9 (Fig. 2.18) nearly square fractionally waisted medianally, not tapering distad, finely spiculate throughout except for narrow strips of the anterior and posterior margins, bears 11–16 chaetica, mean 13,6 (n = 20); apicolateral processes replaced on the lateral corners by broadly rounded relatively hyaline flanges that are devoid of spiculae but each carrying a fine, rather short chaetica issuing from the interface that comprises the base of the flange and the adjoining spiculate fringe where the concave body of the tergum commences; the posterior margin of tergum is gently concave and lacks a median indentation or infuscation; two well-developed cerci (Fig. 2.20) as seen in *C. imicola*. Sternum 9 (Fig. 2.20) with a moderately wide and deep excavation, membrane within the excavated area with 0–18 spiculae, mean 2,56 (n = 50), of these 40 % had no spiculae and only 2 % had more than 10 spiculae; basimere with dorsal and ventral spiculae these a little longer and more sparsely distributed than on tergum; basimere also possesses chaetica as illustrated (Fig. 2.20), basimere 2,5 x as long as broad with basal infuscate collar and well developed dorsal and ventral roots of the form typical for the subgenus *Avaritia.* Distimere as in *C. imicola.* Aedeagus also practically inseparable from that of *C. imicola,* in some specimens relatively more elongate; height of arch variable from being moderately low to fairly high; pigmented and shaped much as in *C. imicola,* 0,9 x as long as basimere; parameres 0,8 x the length of aedeagus, of the general shape and pigmentation as described for *C. imicola.*

Type material

South Africa: Holotype ♀ (slide Timbavati 8) Timbavati near Roodewal camp, west-central Kruger National Park, 17.XII.1985, R. Meiswinkel, reared from half of 1 buffalo dung pat collected off short grass on the margin of the dry Timbavati river.

50 ♀♀ 41 ♂♂	paratypes all from same dung pat. Slides from this type series have been deposited in the following Museums:
1 ♀ 1 ♂	paratype (slides Timbavati 24 and 15); British Museum (Natural History).
1 ♀ 1 ♂	paratype (slides Timbavati 21 and 14); United States National Museum, Washington.
1 ♀ 1 ♂	paratype (slides Timbavati 12 and 11); Australian National Insect Collection, Canberra.

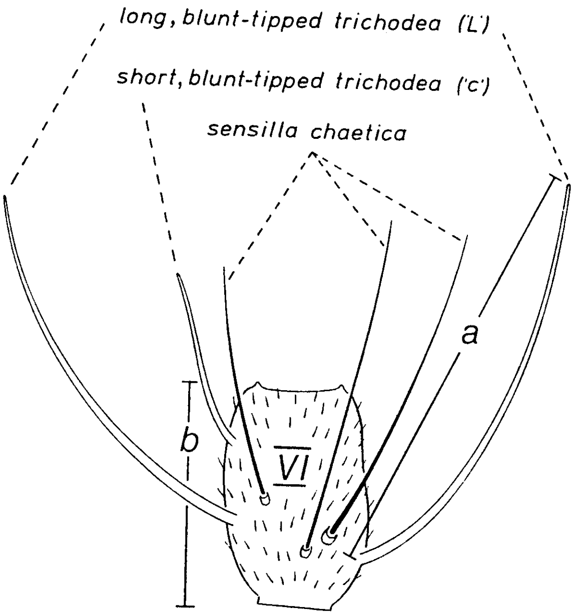
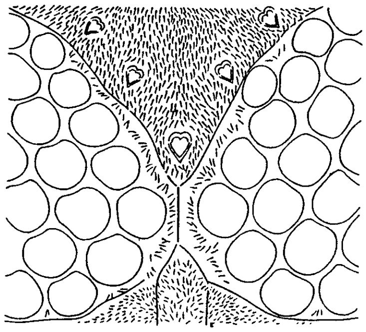
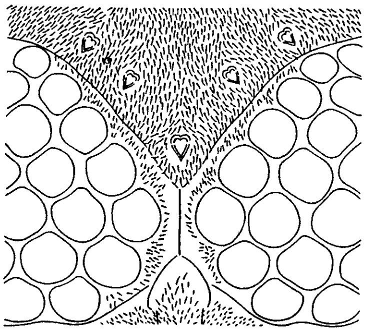


FIG. 2.1



2.3



2.4

FIG. 2.1 *C. (Avaritia) imicola.* Antenna, female, segment VI showing the measurement of a long blunt-tipped sensilla trichodea (a) divided by the length of the segment (b) to obtain the antennal trichodea ratio (AtR).

FIG. 2.3 *C. (Avaritia) imicola.* Eyes, female.

FIG. 2.4 *C. (Avaritia) bolitinos.* Eyes, female.

FIG. 2.2

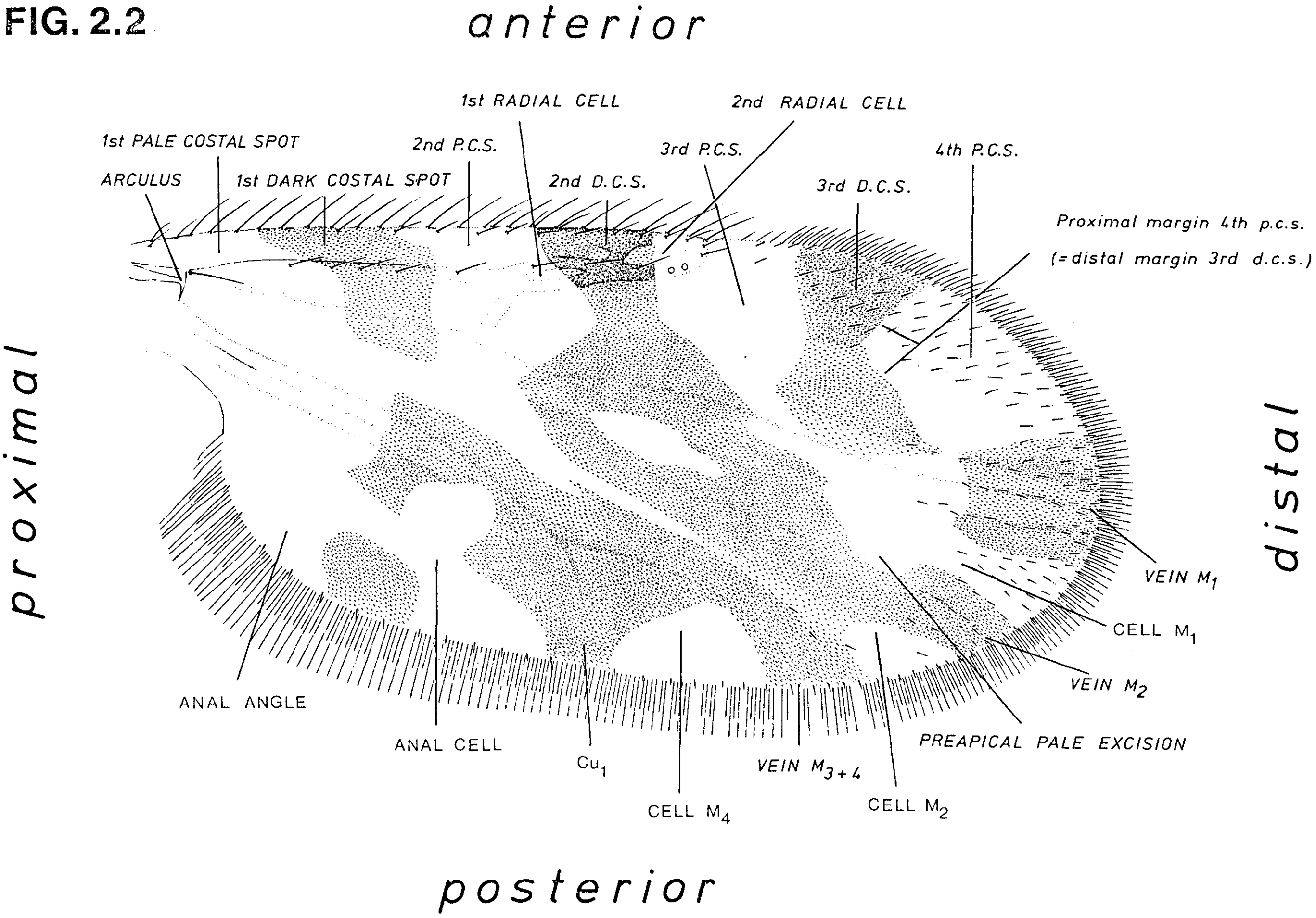


Fig. 2.2 *C. (Avaritia) imicola.* Wing, female: indicating important diagnostic characters (p.c.s. = pale costal spot; d.c.s. = dark costal spot).

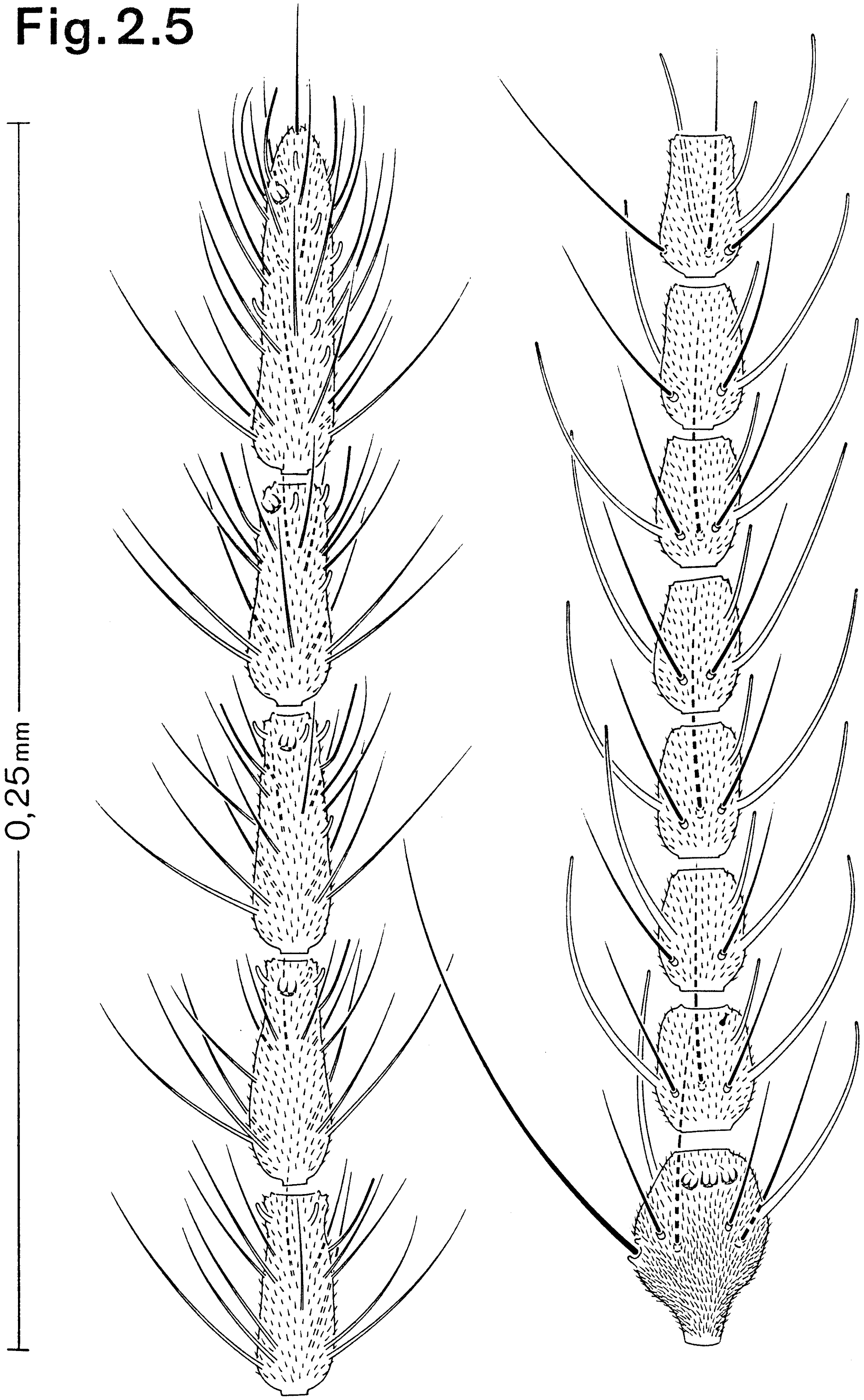


FIG. 2.5 *C. (Avaritia) imicola.* Antenna, female: segments XI–XV on left, segments III–X on right.

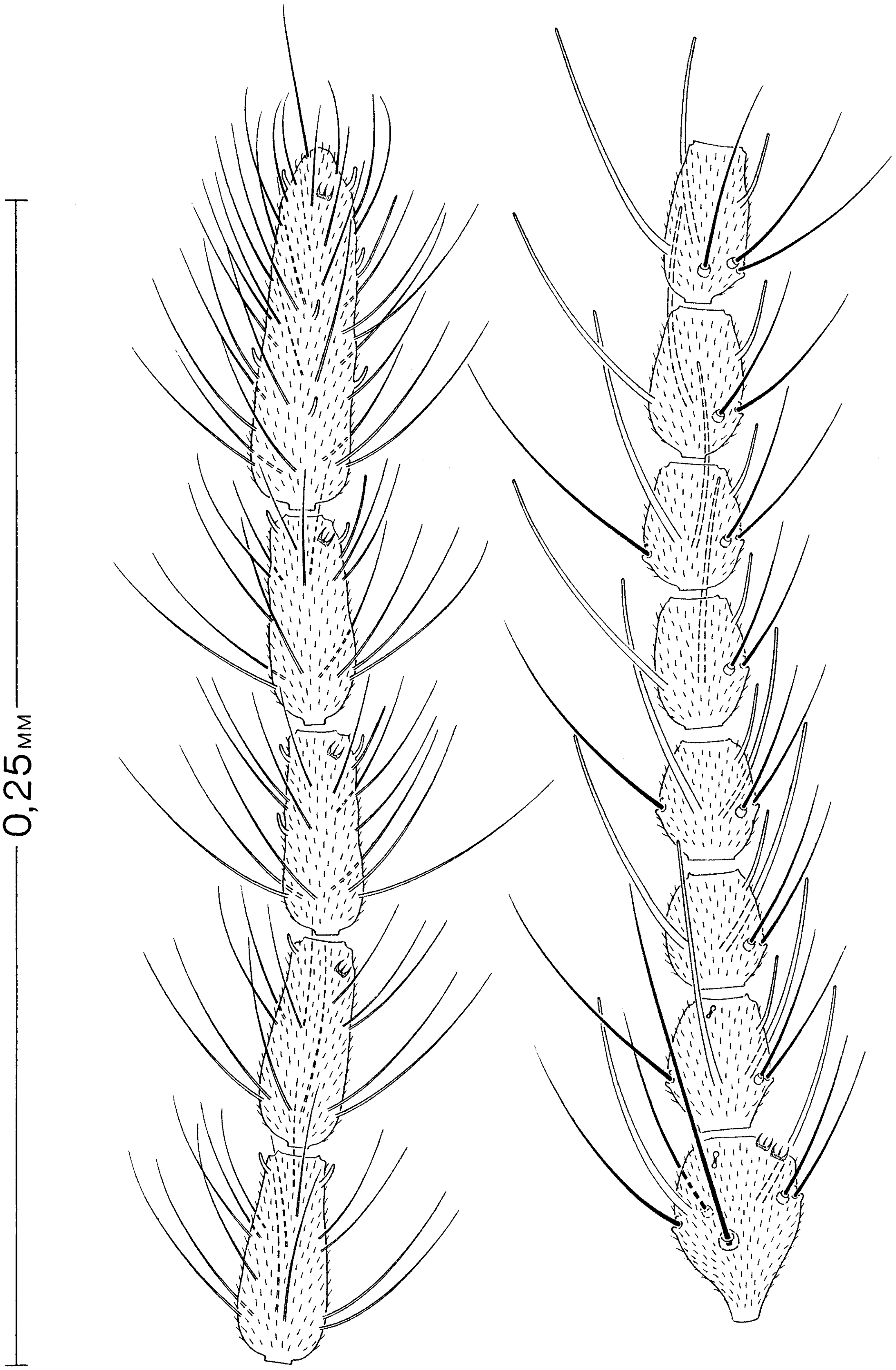


FIG. 2.6

Fig. 2.6 *C.* (*Avaritia*) *bolitinos*. Antenna, female: segments XI–XV on left, segments III–X on right

Fig. 2.7

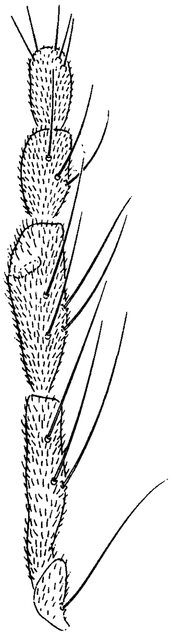
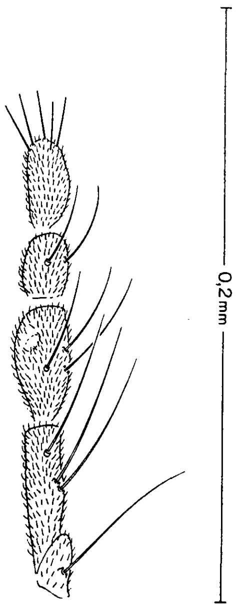


Fig. 2.8



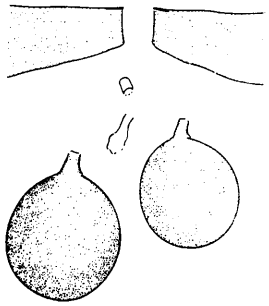


Fig. 2.13

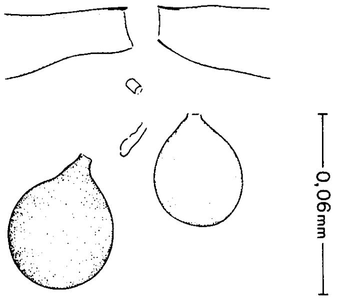


Fig. 2.14

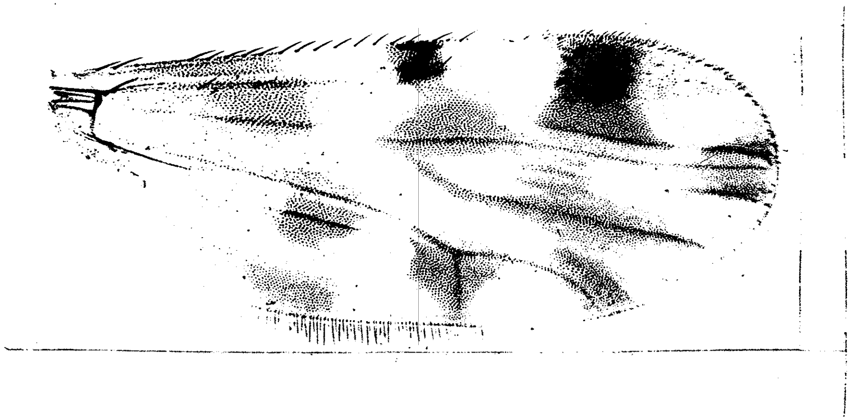
FIG. 2.7 *C. (Avaritia) imicola*. Palp, female.
FIG. 2.8 *C. (Avaritia) bolitinos*. Palp, female.

FIG. 2.13 *C. (Avaritia) imicola*. Genitalia, female: spermathecae and sclerotization surrounding gonopore.
FIG. 2.14 *C. (Avaritia) bolitinos*. Genitalia, female: spermathecae and sclerotization surrounding gonopore.

2.9 Female: *imicola*

2.10 Female: *bolitinos*

2.11 Male: *imicola*



2.12 Male: *bolitinos*

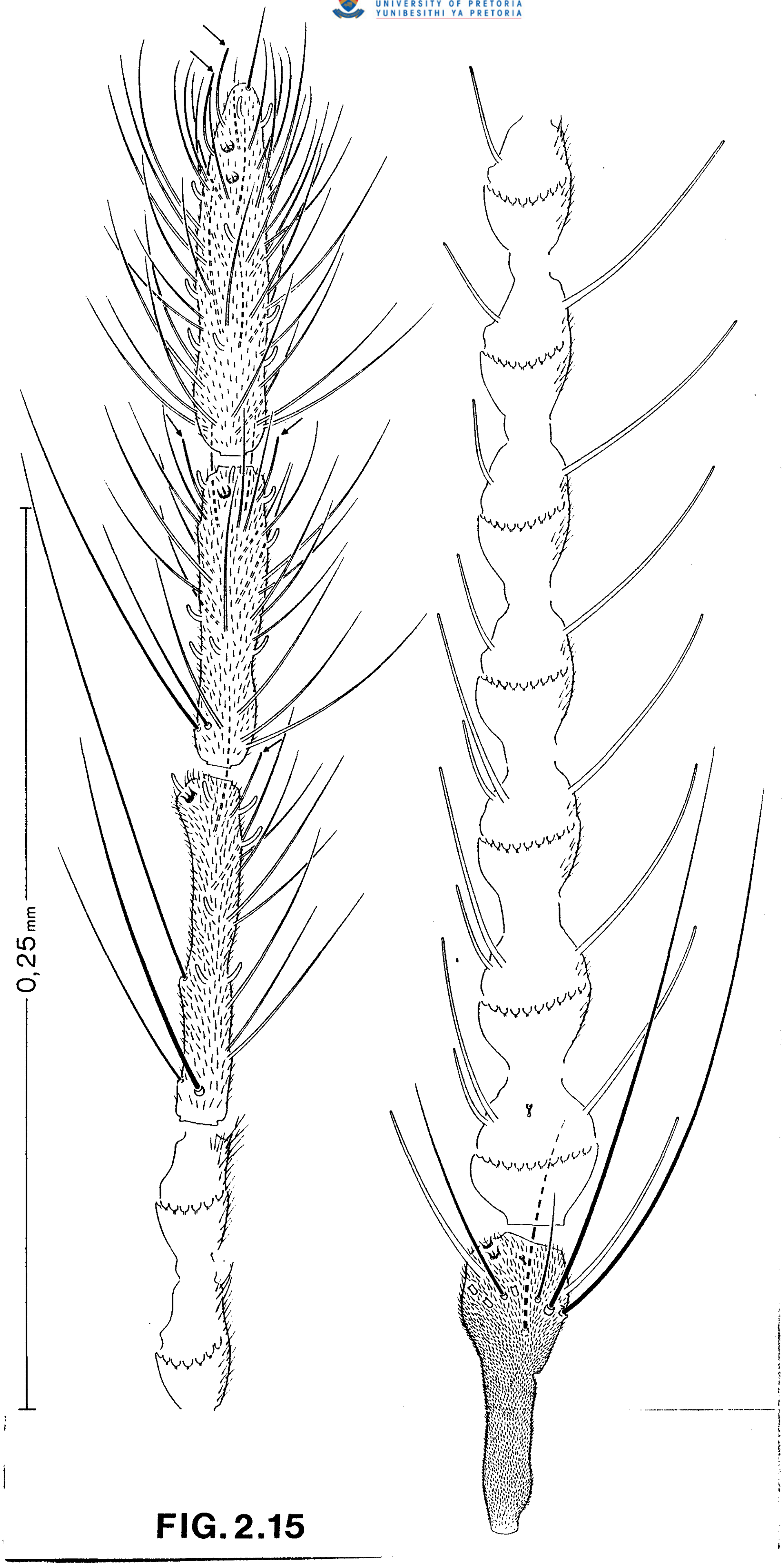


FIG. 2.15

FIG. 2.15 *C. (Avaritia) imicola.* Antenna male: segments XI–XV on left, segments III–X on right.

Fig. 2.16

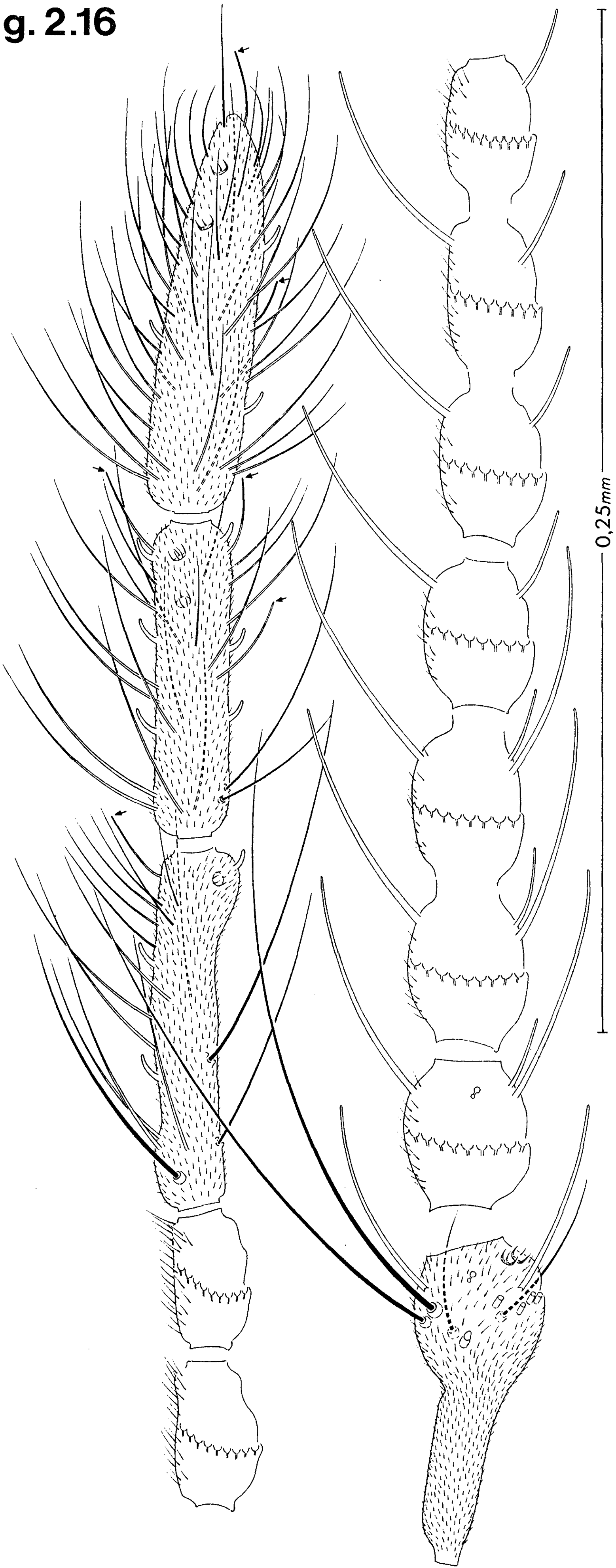


FIG. 2.16 *C. (Avaritia) bolitinos*. Antenna, male: segments XI–XV on left, segments III–X on right.

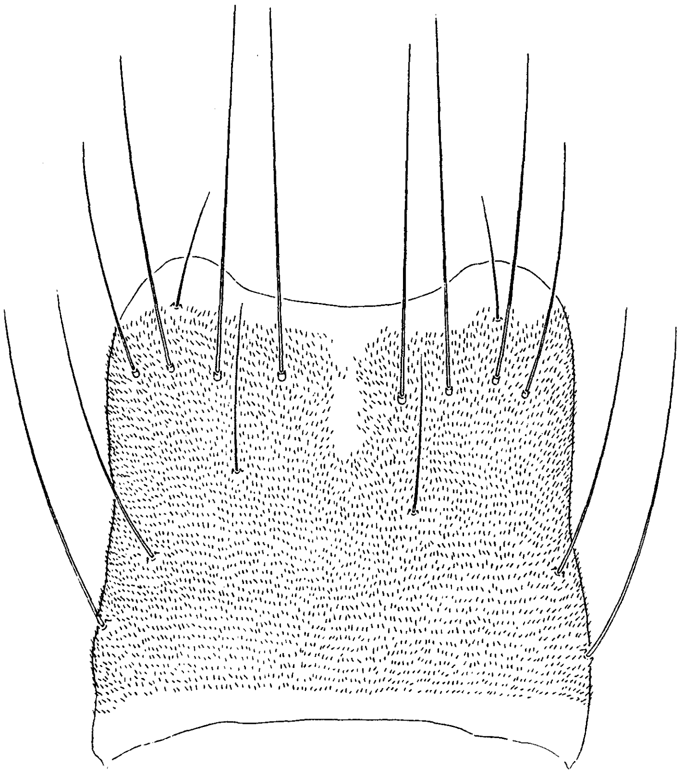


Fig. 2.17

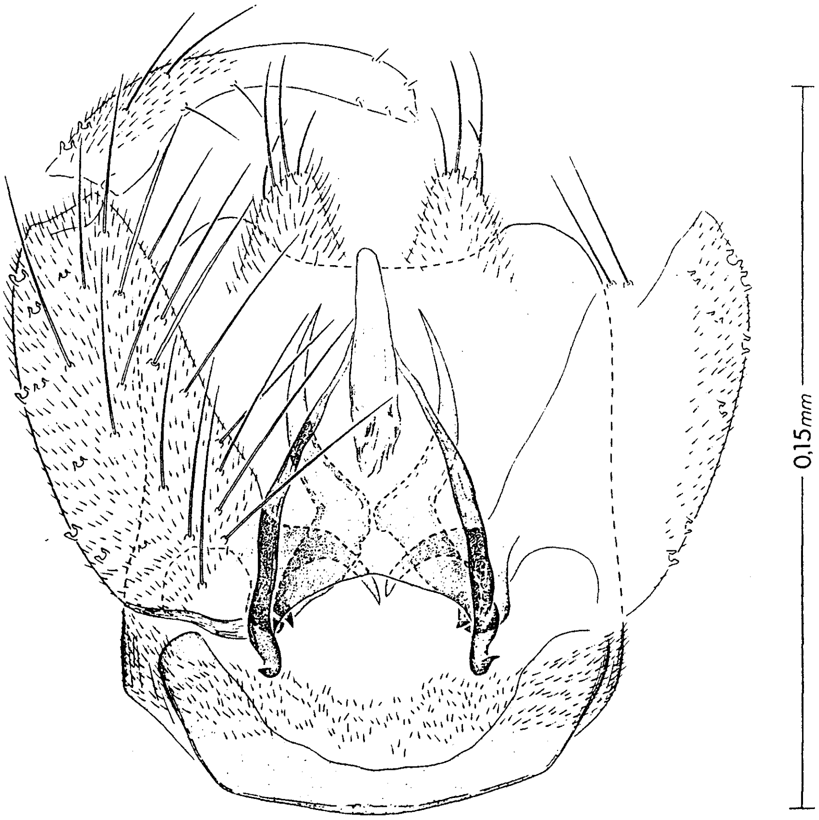


Fig. 2.19

FIG. 2.17 *C. (Avaritia) imicola.* Genitalia, male: tergum IX.
FIG. 2.19 *C. (Avaritia) bolitinos*. Genitalia, male.

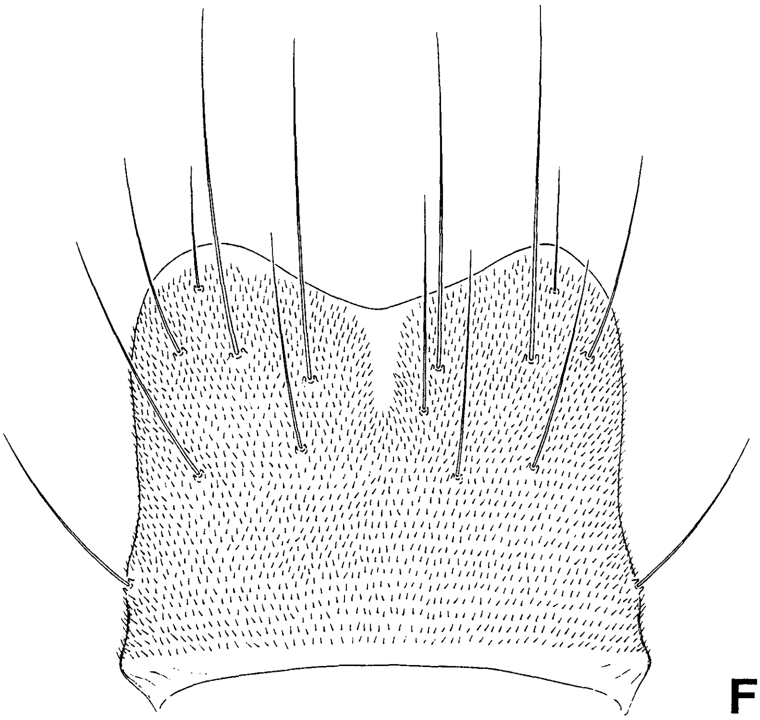


Fig. 2.18

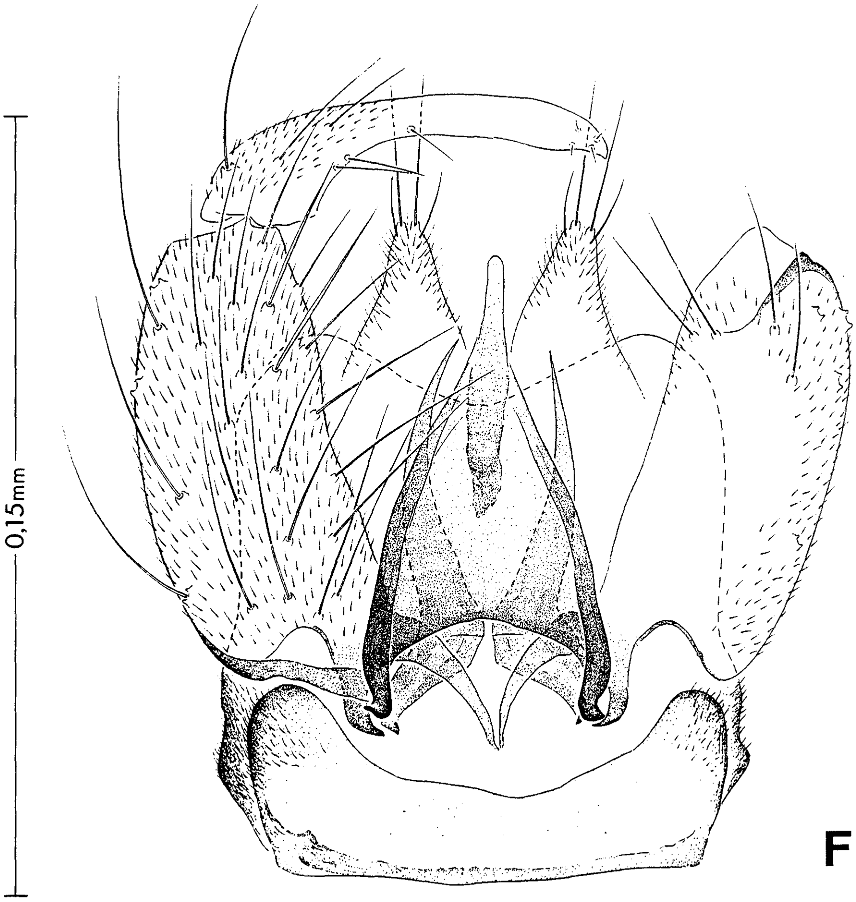


Fig. 2.20

Fig. 2.18 *C.* (*Avaritia*) *bolitinos*. Genitalia, male: tergum IX
Fig. 2.20 *C.* (*Avaritia*) *bolitinos*. Genitalia, male

Fig. 2.21

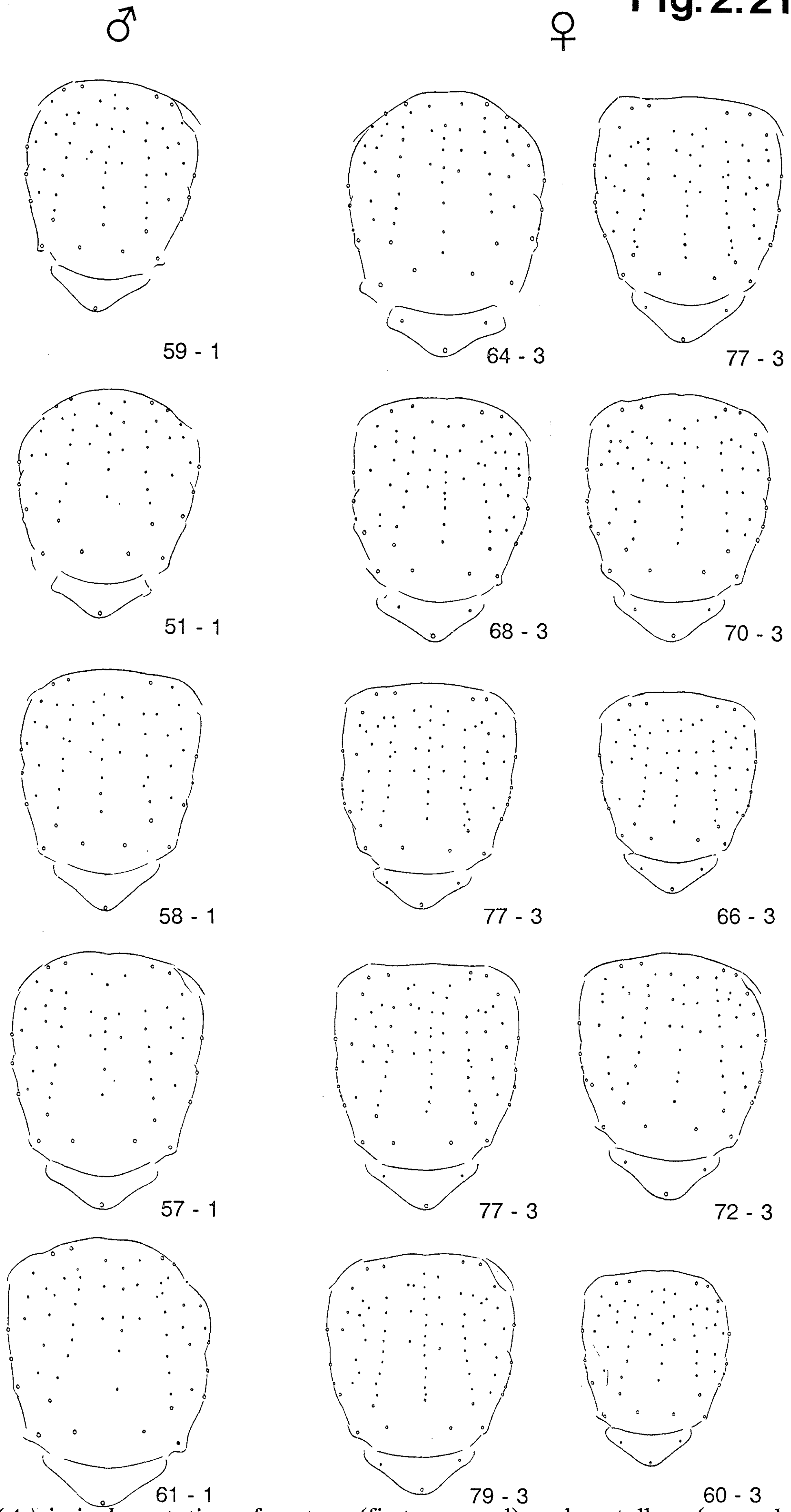


Fig. 2.21 *C. (A.) imicola*: setation of scutum (first numeral) and scutellum (second numeral); male (left column), female (two right columns)

1 ♀ 1 ♂	paratype (slides Timbavati 19 and 13); Museum National d'Histoire Naturelle, Paris.

Holotype ♀ and remaining paratype ♀♀ and ♂♂ in the Onderstepoort collection.

Other slide material examined

Transvaal:

28 ♀♀ 5 ♂♂	(collection No. 73.354), farm Ludwigs Lust, Hectorspruit, eastern Transvaal, 30.XI.1973, A.L. Dyce from a series of '55 larvae and pupae from cow pats floated out in sugar; pats 12-20 days old on bare ground near edge of gully with waterhole (obvious camp). Dung beetles had worked and were still active - those species that mine out underneath the pat but leave the outer crust intact; bottom of pat was firmly in contact with the soil'
2 ♂♂	Pafuri, northern K.N.P., 15.IV.1986, L.E.O. Braack and R. Meiswinkel, black light 14 m up *Acacia albida* in gallery forest lining Pafuri river.
2 ♀♀	Skukuza, K.N.P., 11.III.1984, L.E.O. Braack and R. Meiswinkel, blacklight on bank of Sabie river.
1 ♀ 3 ♂♂	Skukuza, K.N.P., 17.I.1985, R. Meiswinkel, blacklight along Sabie river.
5 ♀♀	Murangoni location, ± 15 km west of Thohoyandou, Vendaland, northern Transvaal, 30.I.1988, R. & P. Meiswinkel, truck-trap 18h42-19h01.
1 ♀ 2 ♂♂	Haenertzburg, northern Transvaal, 21.IV.1983, R. Meiswinkel, blacklight at dairy.
7 ♀♀	Ebenezer dam, northern Transvaal, 14.VI.1980, R. Meiswinkel, blacklight at dairy.
6 ♀♀ 18 ♂♂	Kransberg, Thabazimbi district, north-western Transvaal, IV.1985, I.T.P. Pajor, ex 3 cattle dung pats.
39 ♀♀ 12 ♂♂	Honeydew, near Johannesburg, M. Wasserthal, blacklight on smallholding, dates as follows: 1 ♂ 15.XII.1983; 1 ♂ 7.I.1984; 3 ♀♀ 1 ♂ 8.II.1984; 2 ♀♀ 3 ♂♂ 14.II.1984; 1 ♀ 8.IV.1984; 7 ♀♀ 14.IV.1984, 1 ♀ 25.X.1984; 10 ♀♀ 3 ♂♂ 28.X.1984; 16 ♀♀ 24.XII.1984; 1 ♂ 15.II.1985.
1 ♀	Onderstepoort, 22.VII.1986, G.J. Venter and I.T.P. Pajor, truck-trap near horses, 16h48-17h05.
9 ♀♀ 2 ♂♂	Onderstepoort, VI.1985, I.T.P. Pajor, ex cow pats.
9 ♀♀ 4 ♂♂	Farm Krugerspan, Sentrum, north-western Transvaal, 18.XI.1987, G.J. Venter, ex blue wildebeest dung.
4 ♀♀	Farm Krugerspan, Sentrum, north-western Transvaal, 15.IX.1987, G.J. Venter, ex blue wildebeest dung.
5 ♀♀ 7 ♂♂	Farm Krugerspan, Sentrum, north-western Transvaal, 14.IV.1987, G.J. Venter, ex blue wildebeest dung.

Natal:

3 ♀♀ 4 ♂♂	Ngome tea estate, northern Natal, I.1981, R. Meiswinkel, blacklight.
2 ♀♀	Umlalazi Coastal Nature Reserve, 128 km north of Durban, 20-23.VII.1988, E.M. & H. Nevill, blacklight at back of cabin.

Orange Free State:

1 ♀ 1 ♂	Golden Gate National Park, III.1985, L.E.O. Braack, blacklight.

Cape:

11 ♀♀ 1 ♂	Farm Welgevallen, Stellenbosch, 20.VI.1984, A. Kriel, blacklight.
1 ♀ 3 ♂	Jonkershoek near Stellenbosch, 26.XI.1986, M. Edwardes, blacklight.

Lesotho:

3 ♀♀	St. Mary's High School, Roma, western Lesotho, 10.II.1987, G. K. Sweatman, blacklight.

Zimbabwe:

2 ♀♀ 6 ♂♂	Rekomitjie Research Station, north-western Zimbabwe, 6.II.1988, R.J. Phelps, light-trap on stream bank.

Malawi:

1 ♀	Limphasa dambo near Nkhata Bay, northern Malawi, 26.X.1987, R. Meiswinkel, truck-trap 17h45.
18 ♀♀ 5 ♂♂	Vizara Rubber Estate near Nkhata Bay, northern Malawi, 14-15.XI.1987, R. Meiswinkel, blacklight near cattle kraal.

Kenya:

1 ♀	Naibor Keju (0° 59' N, 36° 48' E), 22.XII.1971, A.R. Walker, light-trap.

Nigeria:

1 ♀	Kankiya, II.1957, B. McMillan, at light.

Mauritius:

4 ♀♀	Farm Salazie, Nicoliere, n.w. Mauritius, 28.X.1991, R. Meiswinkel, blacklight at 100 sheep in open shed.
3 ♀♀ 2 ♂♂	S.O.D.I.A., Bambous, w. Mauritius, 30.X.1991, R. Meiswinkel, blacklight at ± 3 000 feedlot cattle.

Côte d'Ivoire:

3 ♂♂	Gofabo, ± 20 km n.e. of Yamoussoukro, 19.IX.1990, R. Meiswinkel, L.E.O. Braack and G.J. Venter, blacklight at 20 cattle.

1 ♀	Korhogo, n. Côte d'Ivoire, 26.IX.1990, R. Meiswinkel and G.J. Venter, blacklight at 15 cattle near ricefields.

Unmounted light-trap material examined

South Africa:

422 ♀♀ 4 ♂♂	Allerton Regional Veterinary laboratory, Pietermaritzburg, Natal, 15.V.1986, R. Parker, blacklight.
89 ♀♀ 6 ♂♂	(61 parous, 28 nulliparous) Skukuza buffalo boma, K.N.P., 14.IV.1988, R. Meiswinkel & L.E.O. Braack, blacklight.
6 ♀♀	Skukuza, K.N.P., 12.IV.1988, P.J. Meiswinkel, blacklight on bank of Sabie river.
60 ♀♀ 5 ♂	Masanje waterhole northern K.N.P., 6.XI.1985, R. Meiswinkel, black light 18h45-22h30.

Malawi:

70 ♀♀ 1 ♂	(40 nulliparous, 30 parous) Vizara Rubber Estate near Nkhata Bay, northern Malawi, 14-15.XI.1987, R. Meiswinkel, blacklight near cattle kraal.

Unmounted reared material examined

South Africa:

4 ♂♂	Nwaswitshaka, southern K.N.P., 5.II.1986, R. Meiswinkel, ex buffalo dung in middle of sand road.
62 ♀♀ 5 ♂♂	Masanje, northern K.N.P., 6.XI.1985. R. Meiswinkel & L.E.O. Braack, black light near waterhole (18h45-22h30).
20 ♀♀ 32 ♂♂	Masanje waterhole, northern K.N.P., 6.XI.1985, R. Meiswinkel, ex buffalo dung on bare soil.
16 ♀♀ 25 ♂♂	Tshalungwa springs, northern K.N.P., 8.XI.1985, R. Meiswinkel, ex buffalo dung on bare soil.
161 ♀♀ 250 ♂♂	Timbavati riverbed near Roodewal, central K.N.P., 17.XII.1985, R. Meiswinkel, ex half buffalo dung pat on short grass.
395 ♀♀ 261 ♂♂	Northern farm, Johannesburg, 16.II.1982 - 10.III.1982. J.E. Randall & E.M. Nevill, ex 20 cow pats on irrigated pasture of rye grass.
19 ♀♀ 17 ♂♂	Northern farm, Johannesburg, 1.III.1985, G.J. Venter, ex 8 cow pats on irrigated pasture of rye grass.
48 ♀♀ 41 ♂♂	Irene near Pretoria, 14.III.1985, G.J. Venter, ex 8 cow pats on short grass.
85 ♀♀ 89 ♂♂	Irene near Pretoria, 16.IV.1985, G.J. Venter, ex 8 cow pats on short grass.

18 ♀♀ 8 ♂♂	Kaalplaas, Onderstepoort, 22.XI.1985, G.J. Venter, ex 5 cow pats on bare soil under *Acacia* trees.
19 ♀♀ 13 ♂♂	Kaalplaas, Onderstepoort, 9.I.1986, G.J. Venter, ex 8 cow pats on bare soil under *Acacia* trees.
7 ♀♀ 17 ♂♂	Kondowe farm near Eiland, north-eastern Transvaal lowveld, 8.IX.1985, G.J. Venter, ex 8 cow pats on dry ground in mopane veld.
637 ♀♀ 336 ♂♂	Kaalplaas, Onderstepoort, 13.V.1986, G.J. Venter, ex 8 cow pats on short grass.
343 ♀♀ 634 ♂♂	Blinkwater near Sentrum, north-western Transvaal, 13.IV.1987, E.M. Nevill, G.J. Venter & J. van Gas, ex 8 cow pats on bare soil in mixed bushveld.
104 ♀♀ 77 ♂♂	Blinkwater near Sentrum, north-western Transvaal, 30.VI.1987, E.M. Nevill & G.J. Venter, ex 8 cow pats on bare soil in mixed bushveld.
121 ♀♀ 177 ♂♂	Witklip near Sentrum, north-western Transvaal, 12.VIII.1987, E.M. Nevill & G.J. Venter, ex 8 cow pats on bare soil in mixed bushveld.
89 ♀♀ 19 ♂♂	Hluhluwe Game Reserve, northern Natal, 2.VIII.1985, E.J. Wright, ex buffalo pats. 2. vm. 1985, E.J. Wright, ex buffalo pats.
6 ♀♀ 7 ♂♂	Hluhluwe Game Reserve, northern Natal, 2.I.1986, E.J. Wright, ex buffalo pats.
5 ♀♀ 15 ♂♂	Farm Krugerspan near Sentrum, north-western Transvaal, 14.IV.1987, G.J. Venter and E.M. Nevill, ex blue wildebeest pats on bare soil surrounding waterhole in mixed bushveld.
32 ♀♀ 101 ♂♂	Farm Krugerspan near Sentrum, north-western Transvaal, 18.XI.1987, G.J. Venter & E.M. Nevill, ex 15-20 blue wildebeest pats on bare soil surrounding waterhole in mixed bushveld.

Unmounted host material examined

8 ♀♀	Skukuza, K.N.P., 13.VII.1985, L.E.O.Braack, aspirated off darted buffalo in boma.

2.4 DISCUSSION

2.4.1 *Taxonomy*

Since Kieffer's description of *C. imicola* 82 years ago, an extensive literature has grown around this species, much of it under the old name of *C. pallidipennis* Carter, Ingram & Macfie. Nine of these many references can be positively said to refer to either the new species *C. bolitinos* or to as yet undescribed closely allied ones. The first reference to *C. bolitinos* is that by Nevill (1968) in his description of a significant new breeding site for *C. pallidipennis*. That this was not *C. imicola* was pointed out by Braverman (1978) in his study on the larval habitats of Zimbabwean *Culicoides.* Therein he states: 'M. Cornet (personal communication) has recently shown the species which Nevill obtained from cow dung was not *C. imicola* but another member of this group (C. 1348)'. This communication was noted and repeated by Howarth (1985). Two further sources which point to species other than *C. imicola,* are those of Dipeolu & Ogunrinade (1977) and Lubega & Khamala (1976). These have to do with larval habitats and will be re-evaluated under the biology of *C. bolitinos.* In Khamala & Kettle's review of the East African *Culicoides* (1971), *C. pallidipennis* was treated indifferently and these authors are in error when they state that the sensilla coeloconica distribution is 3,11–15 which is really that of *C. pseudopallidipennis. C. imicola* has a 3,12–15 distribution. In addition their synonymizing of *C. glabripennis* under *C. pallidipennis* further indicates that they misunderstood *C. imicola sensu stricto.* The wing picture given by Kitaoka, Kaneko & Shinonaga (1984) as that of *C. imicola* is clearly that of *C. miombo* which will be dealt with in Chapter 4. Finally, Boorman & Mellor (1992) recorded the Oriental/Australasian *C. brevitarsis* from Mauritius; examination of material shows that it was, in fact, *C. bolitinos*.

Ten character states separate *C. imicola* and *C. bolitinos.* These are summarized in Table 2.7. Five are discussed in detail below. The first two, when used in combination, are the most reliable for separating the females of the two species under the dissecting microscope. Of the remaining eight character states, seven require slide mounted material.

a. Female. The shape of the proximal margin of the distal pale spot in cell R_5: In *C. imicola* it is distinctly pointed medially (Fig. 2.2, 2.9) whereas in *C. bolitinos* this margin is usually straight and runs transversely, anteriad to posteriad, across cell R_5 (Fig. 2.10). Furthermore, in *C. bolitinos* this margin is often ragged, not always smooth. However, it must be noted that this character shows variation in both species: in *C. imicola* the pointed proximal margin can be blunted whereas in *C. bolitinos* there can be a hint of a point appearing medially, especially in the male wing. This can lead to error in identification but can be avoided when this character is considered in combination with the next (2).

b. Female. Vein M_2: In *C. imicola* the distal third of the posterior margin of this vein is dark. However, the distal third of the anterior margin differs in that it is more or less equally divided into a pale preapical section followed by an equally broad dark distal section. This always leaves the apex of vein M_2 broadly dark. This juxtaposition of a pale and a dark area on the anterior distal third of vein M_2 is the single most diagnostic character when the identification of *C. imicola* is based on wing pattern alone. This feature was named by Howarth (1985) as a 'pale preapical excision' and is adopted here. Importantly it appears in both Carter, Ingram & Macfie's original wing illustration of *C. pallidipennis* and in Kremer's redescription of the holotype of *C. imicola.* In *C. bolitinos* the median third of both the posterior and anterior margins of vein M_2 are broadly and entirely dark but taper and fade simultaneously leaving the apex of vein M_2 pale. Only in specimens showing a darkened wing pattern, which can be quite common, will the apex of vein M_2 be narrowly to fairly broadly darkened on one or both margins. In either pale or dark variants, however, *C. bolitinos* never possesses the pale preapical excision so diagnostic for *C. imicola.*

c. Female. Palps: A t-test (two tailed) was performed on the palpal measurements data to establish if there were any significant differences in segmental lengths between *C. imicola* and *C. bolitinos.* It was found that segments II–V in *C. imicola* were significantly longer than those of *C. bolitinos* (Table 2.6). The result is that *C. imicola* has a longer palp overall than *C. bolitinos*. In consequence, the shorter palp and proboscis of *C. bolitinos* means that this species has a lower proboscis/head ratio (P/H) than *C. imicola* (Table 2.7).

d. Female. Antennae: Two t-tests were performed: (1) comparison of the lengths of female antennal segments III–XV between *C. imicola* and *C. bolitinos*, and (2) a comparison between the two species of the ratios of these segments where the length of each segment is divided by the width. In the first test each antennal segment of *C. imicola* was found to be significantly longer than its counterpart *C. bolitinos* (Table 2.4). However, most showed a moderate discriminatory ability with the highest t-values seen only from segments IV, XII–XIV (Table 2.4). The second test showed the ratios to be even less discriminatory with four segments showing no significance, but as in the first test segments IV, XII–XIV were again the most discriminatory (Table 2.5). These antennal ratio findings are of interest as they give a result that highlights one of the key characters used by Howarth (1985) to separate the two species *C. imicola* and *C. brevitarsis* collected in Laos. He stated that the ratio for antennal segments VI–IX in *C. brevitarsis* was 1,4 as opposed to 1,7 seen in *C. imicola.* The ratios found on segments VI–IX in South African material of *C. imicola* (n = 25) agreed quite well with those of Howarth, but were of no real value in separating this species from C. *bolitinos* (n = 25) whose ratios overlapped considerably with those of *C. imicola* (Table 2.5). However, a higher discriminatory value is to be found between segments XII–XIV (Table 2.5), and though there is still a fair amount of overlap between the two species, it is more useful than the ratios of segments VI–IX. As the Afrotropical *C. bolitinos* appears to be both the taxonomic and ecological equivalent of the Asian, Australian, eastern Palearctic *C. brevitarsis,* it is important that these two species can be separated from their congener, *C. imicola,* by the use of ratios derived from different groups of female antennal segments. This is good evidence that *C. bolitinos* and *C. brevitarsis* are not conspecific and, furthermore, illustrates that these measured differences are species-specific and not induced by the shared environmental factor of having cow and buffalo dung as a larval habitat.

e. Female. Antennae: To further test for differences between *C. brevitarsis* and *C. bolitinos* the antennal flagellar lengths of two populations of the former (one each from northern Australia and northern Thailand) were compared against those of one population of the latter (type series, South Africa). Table 2.8 shows few significant differences between the two *C. brevitarsis* populations despite originating from opposite ends, and at differing altitudes, of their large geographic range. However, the lengths of all flagellar segments of *C. brevitarsis* were significantly shorter than those of *C. bolitinos*. While it can be argued that the *C. bolitinos* series simply consisted of larger specimens, it is notable that the F–value differences of basal segments III–X are much higher than those of distal segments XI–XV. Therefore, proportionately smaller specimens of *C. bolitinos* would still differ from *C. brevitarsis* in the lengths of the basal segments.

f. Male. The males of *C. imicola* and *C. bolitinos* differ most significantly in that the membrane of sternum 8 in *C. imicola* (Fig. 2.19) is sparsely to rather heavily spiculate (8–145 spiculae, mean 47; n = 50) whereas in *C. bolitinos* (Fig. 2.20) this membrane has 0–18 spiculae, mean 2,56 (n = 50). More subtle differences involve the shape of the aedeagus and the precise shape of the parameres and the hyaline, apicolateral flanges of tergum 9 (Fig. 2.17, 2.18). The appreciation of these differences requires near perfectly mounted series and even so remain difficult to quantify.

2.4.2 ***Larval habitat***

Extensive rearing from a variety of substrates over the past four years in South Africa has shown that *C. bolitinos* has the dung of the African buffalo and that of various races of domesticated *Bos* as its primary larval habitat. *C. bolitinos,* on a few occasions, has also been reared in low numbers from the dung of the blue wildebeest. During the rainy season the dung pats of this animal resemble small cow or buffalo pats and will then yield low numbers of *C. bolitinos.* In the dry season, however, wildebeest dung is more pellet-like and thus lacks the necessary moisture to sustain *Culicoides* immatures (personal observations; G.J. Venter, Veterinary Research Institute, Onderstepoort, unpublished data). At best, the *C. bolitinos*/wildebeest association is a short one, confined to the rainy season, and it will be considered no further.

As described by Nevill (1968) the larval habitat of *C. bolitinos* (= *C. pallidipennis* in that study) is 'dry pats with a very hard crust, a dry sponge-like centre, and a moist lower layer about an inch thick in direct contact with damp soil underneath.' In South Africa *C. bolitinos* has never been reared from dung dropped in streams or waterlogged areas where it disintegrates and enriches the surrounding medium of mud and water organically. This, in part, satisfies the requirements of *C. imicola*, though this species occurs in highest numbers where there is also grass cover, preferably short (Mellor & Pitzolis 1979; I.T.P. Pajor, unpublished data 1987).

Fresh cattle and buffalo dung will produce the first adult *C. bolitinos* after 8–10 days and can continue to yield for a further 20 days (personal observations; G.J. Venter, unpublished data). This is strongly dependent on the drying out of the dung which hastens or triggers pupation. Larval life is prolonged in dung which is brought in from the field and stored in containers in the laboratory, these pats being protected from such important extrinsic factors as the heating and drying rays of the sun and the working of dung beetles. An important intrinsic factor that almost totally inhibits either oviposition by adults or larval development is the acidification and subsequent fermentation of dung from high-energy supplement-fed cattle that are rarely or never put out to pasture (personal observations; A.L. Dyce, 48 Queens rd, Asquith, N.S.W. 2077, Australia, personal communication 1987). The factors that govern oviposition and increased or depressed emergence merit further investigation.

On irrigated rye-grass pastures, an average of 200 adult *C. bolitinos* will emerge from a single cow pat (J.E. Randall, E.M. Nevill, unpublished data). In the wild or natural state, the highest number of *C. bolitinos* yet reared was 512 adults from half of a very large *Syncerus caffer* pat collected on short, dry grass in the dry bed of the Timbavati river, Kruger National Park.

In light of the above data, four studies on the larval habitats of Afrotropical *Culicoides* merit reappraisal. These are the papers on Kenyan *Culicoides* by Lubega & Khamala (1976) and Walker (1977), that on Nigerian *Culicoides* by Dipeolu & Ogunrinade (1977) and finally, the study on various Zimbabwean species (Braverman 1978). Lubega & Khamala (1976) found the immatures of *C. imicola* 'in mud from edges of puddles, pools, lakes rivers and streams, exposed or covered by growing vegetation, usually frequented by livestock for drinking water'. They also reared it from 'drying cowdung pats in open grassland'. Similarly, Walker (1977), in his study on the seasonal fluctuations of Kenyan *Culicoides*, reared three specimens of *C. pallidipennis* from 61 cow pats collected from four sampling sites. In Nigeria, Dipeolu & Ogunrinade (1977), conducting their studies at the research farm of the University of Ibadan, said that *C. imicola,* comprising 95,4 % of the total *Culicoides* reared, was dominant 'in an open dairy paddock containing mostly white Fulani cattle. The paddock was littered with cattle dung pats over which emergence traps were placed'. In these three studies the rearing of *Culicoides imicola* from cow pats matches the larval habitat of *C. bolitinos* in South Africa. Though this niche may in those countries be filled by a species other than *C. bolitinos*, it will quite likely not be *C. imicola.* In this regard it is relevant to note that the wing photograph published by Kitaoka, Kaneko & Shinonaga (1983) of a Nigerian specimen of *C. imicola* is in fact that of *C. miombo* which has two prominent yellow, admedian vittae on the scutum (personal observations; S. Kitaoka, Mitaka, Tokyo 181, Japan, personal communication 1986) these being absent in both *C. imicola* and *C. bolitinos*. Such taxonomic uncertainty renders the results from the above larval habitat studies imprecise.

The fourth study, that by Braverman on the larval habitats of Zimbabwean *Culicoides*, has produced results only in partial agreement with South African studies. From '221 cow pats incubated in the laboratory which yielded 698 *Culicoides,* only 44 *C. imicola* were reared and they were confirmed by Dr M. Cornet to be true *C. imicola'.* Furthermore '*C. zuluensis, C. schultzei* grp. and *C. onderstepoortensis* were present together with *C. gulbenkiani* which was abundant (comprising 92 % of those reared) mainly in cow dung situated over damp soil' (Braverman 1978). In South Africa the only species to be reared along with *C. bolitinos* from cow dung pats is *C. gulbenkiani.* However, *C. gulbenkiani* is often the only species reared from cow pats as it has larval habitat requirements subtlely different from that of *C. bolitinos* (Nevill *et al.* 1988). *Culicoides zuluensis, C. schultzei* grp. *C. onderstepoortensis* and most importantly, *C. imicola,* have never in South Africa been reared from dung pats as was reportedly found in Zimbabwe by Braverman (1978). He did, however, qualify these findings by adding that this was 'most probably because in several instances the cow dung was collected with the soil underneath which contained odd individuals of these species'. In larval habitat studies, it is essential to record in detail whether the dung was collected from a stream margin along with mud and vegetation or whether it was taken in well irrigated, long-grassed pasture with some of the soil underneath. These two situations will, in the main, produce *Culicoides* species different to those found in discrete pats dropped on hard, bare or shortly grassed ground. A combination of these sites would explain the heterogeneity of the species reared by Braverman (1978).

2.5 CONCLUSION

The present study has shown that *C. bolitinos,* like *C. imicola,* is widespread in the Afrotropical Region, and that it occurs throughout the Republic of South Africa. The larval habitat preference of *C. bolitinos* can lead to it being either rare or common but localized in its distribution. Of importance is that *C. bolitinos* is now recognized as a species clearly separate from *C. imicola*, with which it has been confused in the past.

The intimate association with game animals such as the African buffalo and the blue wildebeest and with cattle, strongly suggests *C. bolitinos* as a vector of cattle viruses. For this reason, and also because it appears to be both the morphological and ecological equivalent of the Oriental-Australasian-eastern Palaearctic *C. brevitarsis, C. bolitinos* is deserving of detailed investigations into its biology about which too little is known.

2.6 REFERENCES

BOORMAN, J. 1986. Presence of bluetongue virus vectors on Rhodes. *Veterinary Record,* 118:21.

BOORMAN, J. 1989. *Culicoides* (Diptera: Ceratopogonidae) of the Arabian peninsula with notes on their medical and veterinary importance. *Fauna of Saudi Arabia*, 10:160–224.

BOORMAN, J. & DIPEOLU O.O. 1979. A taxonomic study of adult Nigerian *Culicoides* Latreille (Diptera: Ceratopogonidae) species. *Occasional Publications of the Entomological Society of Nigeria,* 22:1–121.

BOORMAN, J. & MELLOR, P.S. 1992. *Culicoides* vectors of bluetongue and African horse sickness viruses in Mauritius. *Medical and Veterinary Entomology*, 6:306.

BOORMAN, J. & VAN HARTEN, A. 1992. Vectors of African horse sickness in the Cape Verde Islands. *The Veterinary Record*, 131:56.

BOORMAN, J.P.T. & WILKINSON, P.J. 1983. Potential vectors of bluetongue in Lesbos, Greece. *The Veterinary Record,* 113:395–396.

BOORMAN, J., JENNINGS, M., MELLOR P. & WILKINSON, P. 1985. Further data on the distribution of biting midges in southern Europe and the Mediterranean area with special reference to *Culicoides* imicola. Bluetongue and related Orbiviruses. Alan R. Liss, New York.

BRAVERMAN, Y. 1978. Characteristics of *Culicoides* (Diptera: Ceratopogonidae) breeding places near Salisbury, Rhodesia. *Ecological Entomology,* 3:163–170.

BRAVERMAN, Y., BARZILAI, E., FRISH, K. & RUBINA, M. 1985. Bluetongue virus isolation from pools of *Culicoides* spp. in Israel during the years 1981 to 1983. Bluetongue and related Orbiviruses. Alan R. Liss. New York.

CAEIRO, V.M.P. 1961. Contribuicao para o estudo das espécies Angolanas do género *Culicoides* Latreille, 1809. *Estudos, Ensaios e Documentos Junta de Investigacoes do Ultramar,* 86:5–359.

CALLOT, J., KREMER, M. & BRUNHES, J. 1968. Etude de *Styloconops spinosifrons* et de *Culicoides* entomophages (Diptères Cératopogonides) dont certains sont nouveaux pour la faune de Madagascar. *Cahiers de l'Office de la Recherche Scientifique et Technique OutreMer*, 6:103–112.

CAPELA, R., SOUSA, C., PENA, I. & CAEIRO, V. 1993. Preliminary note on the distribution and ecology of *Culicoides imicola* in Portugal. *Medical and Veterinary Entomology*, 7:23–26.

CARTER, H.F., INGRAM, A. & MACFIE, J.W.S. 1920. Observations on the ceratopogonine midges of the Gold Coast with descriptions of new species. Part I & part II. *Annals of Tropical Medicine and Parasitology*, 14:187–274.

CLASTRIER, J. 1958. Notes sur les Cératopogonides. IV. Cératopgonides d'Afrique occidentale francaise. *Archives de l'Institut Pasteur d'Algérie,* 36:192–258.

CLASTRIER, J. 1959. Notes sur les Cératopogonides. VIII. Cératopogonides de l'ile de la Réunion. *Archives de l'Institut Pasteur d'Algérie,* 37:412–446.

DAS GUPTA, S.K. 1962. Some *Culicoides* of Calcutta and the neighbouring areas. *Science and Culture*, 28:537–539.

DAVIES, F.G. & WALKER A.R. 1974. The distribution in Kenya of bluetongue virus and antibody, and the *Culicoides* vector. *Journal of Hygiene, Cambridge,* 72:265–272.

DE MEILLON, B. 1961. The Madagascan Ceratopogonidae. *Revista de Entomologia de Mocambique*, 4:37–64.

DIPEOLU O.O. & OGUNRINADE, A.F. 1977. Studies on *Culicoides* species of Nigeria. VII. The biology of some Nigerian Culicoides species. *Zeitschrifl für Parasitenkunde,* 51:289–298.

DIPEOLU, O.O. & SELLERS, R.C. 1977. Studies on the *Culicoides* species of Nigeria. I. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of Ibadan with notes on the other ceratopogonids collected by light-trap. *Nigerian Journal of science*, 11:33–65.

DU TOIT, R.M. 1944. The transmission of bluetongue and horsesickness by *Culicoides*. *Onderstepoort Journal of Veterinary Research*, 19:7–16.

DYCE, A.L. 1982. Distribution of *Culicoides (Avaritia) spp.* (Diptera: Ceratopogonidae) west of the Pacific Ocean. *In*: ST. GEORGE, T.D. & RAY, B.H. (ed.). *Arbovirus Research in Australia.* Proceedings Third Symposium, 35–43.

DYCE, A.L. & WIRTH, W.W. 1983. Reappraisal of some Indian *Culicoides* species in the subgenus *Avaritia* (Diptera: Ceratopogonidae). *International Journal of Entomology*, 25:221–225.

FIEDLER, O.G.H. 1951. The South African biting midges of the genus *Culicoides* (Ceratopogonid: Dipt.). *Onderstepoort Journal of Veterinary Research*, 25:3–33.

GLICK, J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology*, 27:85–195.

HOWARTH, F.G. 1985. Biosystematics of the *Culicoides* of Laos (Diptera: Ceratopogonidae). *International Journal of Entomology,* 27:1–96.

JENNINGS, M., BOORMAN, J.P.T. & ERGUN H. 1983. *Culicoides* from western Turkey in relation to bluetongue disease of sheep and cattle. *Revue de'Elevage et de Médicine Veterinaire des Pays Tropicaux,* 36:67–70.

KHALAF, K.T. 1957. Heleids from Iraq, with description of new species (Diptera: Heleidae). *Bulletin, Société entomologique d'Egypte,* XLI:335–350.

KHAMALA, C.P.M., 1971. Ecological distribution of East African *Culicoides* Latreille (Dipt., Ceratopogonidae) as shown by light-traps. *Bulletin Entomological Research,* 60:549–557.

KHAMALA, C.P.M. & KETTLE, D.S. 1971. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of East Africa. *Transactions of the Royal Entomological Society of London*, 123:1–95.

KIEFFER, J.J. 1913. Chironomidae et Cecidomyidae. Resultats scientifiques Voyage de Ch. Alluaud et R. Jeannel en Afrique orientale. (1911-1912 Diptera), 1:1–43.

KITAOKA, S., KANEKO, K. & SHINONAGA, S. 1984. Survey of blood sucking species of *Culicoides* in Ife, Nigeria. *Journal of the Aichi Medical University Association*, 12:454–458.

KREMER, M. 1972. Redescription de *Culicoides imicola, C. alticola* et *C. tropicalis* Kieffer sur des exemplaires déterminer par l'auteur (Diptera, Ceratopogonidae). *Bulletin du Museum National d'Histoire Naturelle,* 58:645–655.

LUBEGA, R. & KHAMALA, C.P.M. 1976. Larval habitats of common *Culicoides* Latreille (Diptera, Ceratopogonidae) in Kenya. *Bulletin Entomological Research*, 66:421–425.

MACFIE, J.W.S. 1943. Ceratopogonidae (Diptera) from Egypt. *Proceedings of the Royal Entomological Society of London*, 12:145–159.

MEISWINKEL, R. 1989. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:23–39.

MELLOR, P.S. & PITZOLIS, G. 1979. Observations on breeding sites and light-trap collections of *Culicoides* during an outbreak of bluetongue in Cyprus. *Bulletin Entomological Research,* 69:229–234.

MELLOR, P.S., JENNINGS, M. & BOORMAN, J.P.T. 1984. *Culicoides* from Greece in relation to the spread of bluetongue virus. *Revue d'Elevage et de Médicine Veterinaire des Pays Tropicaux,* 37:286–289.

MELLOR, P.S., JENNINGS, D.M., WILKINSON, P.J. & BOORMAN, J.P.T. 1985. *Culicoides imicola:* a bluetongue virus vector in Spain and Portugal. *Veterinary Record,* 116:589–590.

NAGATY, H.F. & MORSY, T.A. 1959. *Culicoides pallidipennis*, C.I.&M. (Diptera: Ceratopogonidae) an Egyptian biting midge. *Journal of the Egyptian Public Health Association*, 34:71–75.

NAVAI, S. & MESGHALI, A. 1968. Ceratopogonidae (Diptera) of Iran. II. More records of *Culicoides* Latreille, 1809. *Journal of Natural History*, 2241–246.

NEVILL, E.M. 1968. A significant new breeding site of *Culicoides pallidipennis* Carter, Ingram and Macfie (Diptera: Ceratopogonidae). *Journal of the South African Veterinary Medical Association,* 39(3):61.

NEVILL, E.M. & ANDERSON, D. 1972. Host preferences of *Culicoides* midges (Diptera: Ceratopogonidae) in South Africa as determined by precipitin tests and light-trap catches. *Onderstepoort Journal of Veterinary Research,* 39:147–152.

NEVILL, E.M., VENTER, G.J., EDWARDES, M., PAJOR, I.T.P., MEISWINKEL, R. & VAN GAS, J.H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the Western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 55:101–106.

OZAWA, Y. 1985. Bluetongue and related Orbiviruses: overview of the world situation. Bluetongue and related Orbiviruses. Alan R. Liss, New York.

PAJOR, I.T.P. 1987. A collapsible, semi-automatic, tent-type, emergence trap, suitable for sampling *Culicoides* (Diptera: Ceratopogonidae) from a wide range of habitats. *Onderstepoort Journal of Veterinary Research*, 5499–101.

PHELPS, R.J., BLACKBURN, N.K. & SEARLE, L. 1982. Culicoides (Diptera: Ceratopogonidae) catches and virus isolations from them in the Mukwadzi Valley, Zimbabwe. *Journal of the Entomological Society of Southern Africa*, 45:195–200.

RATANAWORABHAN, N. 1975. An illustrated key to the female *Culicoides* of Chiangmai Valley, Thailand. U.S. Army Med. Comp. SEATO, Bangkok, 49 pp.

SEN. P. & DAS GUPTA, S.K. 1959. Studies on Indian *Culicoides* (Ceratopogonidae: Diptera). *Annals of the Entomological Society of America*, 52:617–630.

SZADZIEWSKI, R. 1984. Ceratopogonidae (Diptera) from Algeria. VI. *Culicoides* Latr. *Polskie Pismo Entomologiezne,* 54:163–182.

WALKER, A.R. 1977. Seasonal fluctations of *Culicoides* species (Diptera: Ceratopogonidae) in Kenya. *Bulletin Entomological Research*, 67:217–233.

WALLER, J., BELLIARD, J. & KREMER, M. 1987. First results of the application of isozyme analysis to study of *Culicoides* (Diptera: Ceratopogonidae). *Journal of the American Mosquito Control Association*, 3:360–368.

WIRTH, W.W. & DYCE, A.L. 1985. The current taxonomic status of the *Culicoides* vectors of bluetongue virus. Bluetongue and related Orbiviruses. Alan R. Liss, New York.

WIRTH, W.W. & HUBERT, A.A. 1989. The *Culicoides* of south-east Asia. *Memoirs of the American Entomological Institute*, 44:1–508.

TABLE 2.1 Lengths (μm) of segments and mean distributions of sensillae on the male and female antennae of C. *(Avaritia) imicola* and C. *(A.) bolitinos*

	Antennal segments												
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
C.imicola													
Female:													
Sens. coeloconica	3	0	0	0	0	0	0	0	0	1	1	1	1
Sens. chaetica	5	3	2	3	2	3	2	3	0	0	0	0	0
Sens. trichodea	LL	LLc	LLc	LLc	LLc	LLc	LLc	LLc	-	-	-	-	-
Lengths of segments	37,4	24,4	24,2	25,9	27,1	26,9	27,3	29,8	41,3	43,4	44,1	44,3	70,8
Male:													
Sens. coeloconica	2	0	0	0	0	0	0	0	0	0	1	1	2
Sens. chaetica	5	0	0	0	0	0	0	0	0	0	3	2	0
Sens. trichodea	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	-	-	-
Lengths of segments	62,4	36,0	36,0	38,4	38,4	38,4	38,4	38,4	36,0	33,6	88,8	67,2	91,2
C. bolitinos													
Female:													
Sens. coeloconica	3	0	0	0	0	0	0	0	0	1	1	1	1
Sens. chaetica	5	3	2	3	2	3	2	3	0	0	0	0	0
Sens. trichodea	LL	LLc	LLc	LLc	LLc	LLc	LLc	LLc	-	-	-	-	-
Lengths of segments	35,4	22,4	22,5	24,0	25,3	25,1	25,8	28,1	39,2	39,7	39,8	39,7	66,8
Male:													
Sens. coeloconica	2	0	0										
Sens. chaetica	5	0	0										
Sens. trichodea	LL	LLc	LLc	0	0	0	0	0	0	0	1	1	2
Lengths of segments	55,2	31,2	31,2	0	0	0	0	0	0	0	3	2	0
				LLc	Lc	Lc	Lc	c	-	-	-	-	-
				31,2	31,2	31,2	31,2	28,8	28,8	28,8	72,0	62,4	76,8

TABLE 2.2 Number and frequency of coeloconica present on each of female antennal segments III-XV of *C. (Avaritia) imicola* and *C. (A.) bolitinos*

No. coeloconica per segment *C. imicola*	**Antennal segments**							
	III	**IV-IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	-	172	171	165	8	-	-	-
1	-	-	1	7	164	172	172	170
2	1	-	-	-	-	-	-	2
3	169	-	-	-	-	-	-	-
4	2	-	-	-	-	-	-	-
No. antennae examined	**172**	**172**	**172**	**172**	**172**	**172**	**172**	**172**
C. bolitinos								
0	-	56	56	55	-	-	-	-
1	-	-	-	1	56	56	56	55
2	-	-	-	-	-	-	-	1
3	56	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
No. antennae examined	**56**	**56**	**56**	**56**	**56**	**56**	**56**	**56**

TABLE 2.3 Number and frequency of chaetica present on each of female antennal segments III-XV of *C. (Avaritia) imicola* and *C. (A.) bolitinos*

No. of chaetica per segment *C. imicola*	**Antennal segments**												
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	-	-	-	-	-	-	-	-	168	172	171	172	172
1	-	-	1	-	-	-	1	-	1	-	1	-	-
2	-	-	160	-	168	-	167	5	-	-	-	-	-
3	-	152	11	161	4	155	4	157	-	-	-	-	-
4	3	17	-	11	-	16	-	10	-	-	-	-	-
5	154	3	-	-	-	1	-	-	-	-	-	-	-
6	114	-	-	-	-	-	-	-	-	-	-	-	-
7	1	-	-	-	-	-	-	-	-	-	-	-	-
No. of antennae examined	**172**	**172**	**172**	**172**	**172**	**172**	**172**	**172**	**172**	**172**	**172**	**172**	**172**
C. bolitinos													
0	-	-	-	-	-	-	-	-	58	59	59	59	59
1	-	-	1	-	-	-	-	-	1	-	-	-	-
2	-	1	58	-	59	-	58	1	-	-	-	-	-
3	-	56	-	59	-	59	1	58	-	-	-	-	-
4	2	1	-	-	-	-	-	-	-	-	-	-	-
5	54	1	-	-	-	-	-	-	-	-	-	-	-
6	3	-	-	-	-	-	-	-	-	-	-	-	-
No. of antennae examined	**59**	**59**	**59**	**59**	**59**	**59**	**59**	**59**	**59**	**59**	**59**	**59**	**59**

TABLE 2.4 Comparison of lengths (μm) of female antennal segments III-XV in *C. imicola* (n=25) and *C. bolitinos* (n=25), and calculated t-values of significance.

Antennal segments	***C. imicola***		***C. bolitinos***		**t-value**
	Range	**Mean**	**Range**	**Mean**	
III	36,0 - 40,2	37,4	32,4 - 38,4	35,4	4,6888
IV	24,0 - 26,4	24,4	20,4 - 24,6	22,4	6,2352
V	21,6 - 25,8	24,2	20,4 - 26,4	22,5	4,7140
VI	24,0 - 27,6	25,9	21,0 - 27,0	24,0	4,3643
VII	24,0 - 28,8	27,1	21,6 - 27,6	25,3	4,0819
VIII	24,0 - 28,8	26,9	21,6 - 25,8	25,1	3,9692
IX	24,0 - 28,8	27,3	24,0 - 28,8	25,8	3,4904
X	26,4 - 33,6	29,8	25,2 - 30,0	28,1	3,8624
XI	36,0 - 45,6	41,3	36,0 - 45,6	39,2	3,1263
XII	39,6 - 48,0	43,4	36,0 - 44,4	39,7	5,4674
XIII	39,6 - 49,2	44,1	36,0 - 44,4	39,8	5,9936
XIV	39,6 - 49,2	44,3	34,8 - 44,4	39,7	6,0013
XV	63,6 - 78,0	70,8	57,6 - 81,6	66,8	2,6019
Total lengths	**435,0 - 494,4**	**466,0**	**391,8 - 480,0**	**433,8**	**5,2481**

TABLE 2.5 Comparison of ratios of female antennal segments III-XV in *C. imicola* (n=25) and *C. bolitinos* (n=25), and calculated t-values of significance (n.s. = not significant)

Antennal segment	***C. imicola***		***C. bolitinos***		**t-value**
	Range	**Mean**	**Range**	**Mean**	
III	1,20 - 1,51	1,37	1,25 - 1,50	1,36	0,4954 (n.s.)
IV	1,00 - 1,29	1,18	1,03 - 1,25	1,12	3,3549
V	1,24 - 1,45	1,34	1,17 - 1,48	1,28	2,9962
VI	1,33 - 1,61	1,50	1,29 - 1,56	1,43	2,8473
VII	1,46 - 1,85	1,66	1,38 - 1,77	1,60	2,2460
VIII	1,46 - 1,85	1,64	1,33 - 1,70	1,52	3,6955
IX	1,43 - 1,92	1,72	1,48 - 1,83	1,67	3,0541
X	1,63 - 2,16	1,88	1,62 - 1,92	1,81	1,9752 (n.s.)
XII	2,31 - 3,08	2,71	2,31 - 2,92	2,63	1,6532 (n.s.)
XII	2,44 - 3,08	2,78	2,44 - 2,79	2,60	4,4999
XIII	2,54 - 3,15	2,82	2,22 - 2,85	2,54	4,2294
XIV	2,36 - 3,08	2,76	2,07 - 2,88	2,50	4,4039
XV	3,53 - 4,69	4,11	3,69 - 4,62	4,04	0,8226 (n.s.)

TABLE 2.6 Comparison of lengths (μm) of female palpal segments I-V in *C. imicola* (n=25) and *C. bolitinos* (n=25), and calculated t-values of significance (n.s. = not significant)

Palpal segment	***C. imicola***		***C. bolitinos***		**t-value**
	Range	**Mean**	**Range**	**Mean**	
I	15,6 - 24,0	19,0	13,2 - 21,6	18,3	1,0961 (n.s.)
II	50,4 - 57,6	54,2	39,6 - 49,2	44,5	14,0000
III	40,8 - 51,6	46,8	33,6 - 43,2	38,7	10,2607
IV	25,2 - 32,4	30,6	20,4 - 24,0	23,4	10,4543
V	24,0 - 30,0	27,0	18,0 - 28,8	24,5	3,4126
Total length	**165,6 - 182,4**	**176,1**	**132,0 - 165,6**	**148,5**	**14,0530**

TABLE 2.7 Ten characters used to separate *C. imicola* from *C. bolitinos*

C. imicola	*C. bolitinos*
Taxonomic	
♀ – proximal margin of distal pale spot in cell R5 pointed	– this margin almost straight, transverse and ragged, or gently rounded
– apex of vein M_2 broadly dark this preceded by a broad, pale preapical excision	– apex of vein M_2 pale or occasionally darkened on upper and lower margin; no pale preapical excision
– palp longer: 165,6–182,4 µm mean 176,1 µm (n = 25)	– palp shorter: 132,0–165,6 µm mean 148,5 µm (n = 25)
– 2nd palpal segment longer, 50,5–57,6 µm, mean 54,2 (n = 25)	– 2nd palpal segment shorter, 39,6–49,2 µm, mean 44,5 (n = 25)
– 3rd palpal segment longer, 40,8–51,6 µm, mean 46,8 µm (n = 25)	– 3rd palpal segment shorter, 33,6–43,2 µm, mean 38,7 µm (n = 25)
– PR 2,40–3,38 mean 2,86 (n = 172)	– PR 1,86–2,72 mean 2,33 (n = 52)
– P/H ratio 0,82–1,02, mean 0,90 (n = 45)	– P/H ratio 0,62–0,89 mean 0,76 (n = 20)
– antennal segments IV, XII–XIV longer (see Table 2.4)	– antennal segments IV, XII–XIV shorter (see Table 2.4)
♂ – membrane of sternum 9 with 8–145 spiculae, mean 47 (n = 50)	– membrane with 0–18 spiculae, mean 2,56 (n = 50), 40 % have the membrane bare
Biological	
Larval habitat is the humus-rich soil of permanently moist, grassed margins of streams, furrows and vleis, especially where grass is kept short by grazing animals	Larval habitat exclusively the dung of large herbivores such as the African buffalo, blue wildebeest and cattle

TABLE 2.8 Comparison of length of ♀ antennal segments using Bonferrioni's method of multiple comparison of means: *C. brevitarsis* Thailand (n = 26) vs *C. brevitarsis* Australia (n = 27) vs *C. bolitinos* South Africa (n = 25); means underlined are not significantly different at 5 %

Antennal segment	Australia	Thailand	South Africa	F–value
III	12,833	13,212	14,740	61,392***
IV	7,769	8,192	9,340	78,311***
V	7,491	8,077	9,380	93,536***
VI	8,593	8,798	10,000	47,634***
VII	8,963	9,173	10,540	54,294***
VIII	9,157	9,269	10,470	38,190***
IX	9,028	9,183	10,740	63,497***
X	10,056	9,875	11,730	69,889***
XI	14,917	14,846	16,330	19,398***
XII	15,194	15,279	16,560	16,979***
XIII	15,528	15,625	16,530	7,169**
XIV	15,620	15,269	16,540	13,623***
XV	25,407	24,625	27,840	15,888***

CHAPTER 3

Morphological comparison of *Culicoides (Avaritia) nudipalpis* Delfinado, 1961 and *C. (A.) imicola* Kieffer, 1913: reappraisal of their separate species status.

3.1 INTRODUCTION

Culicoides nudipalpis was described by Delfinado (1961) from four females collected on the island of Luzon, Philippines. The holotype has apparently been lost (Dyce 1979); Wirth & Hubert (1989) gave further notes on types and synonymy, and described the male for the first time from Sumbawa, Indonesia. To date, *C. nudipalpis* has been recorded only from various islands comprising the Philippines and Indonesia i.e. from Luzon in the north-west to Timor in the south-east. These fall east of Huxley's modification of the Wallace line (Rosen 1988). Except for the record of the type series taken in a carabao-baited trap, nothing is known about the biology of *C. nudipalpis*. Judging from its sparse treatment in the literature, it is either rare and/or seasonal in its presence or is being misidentified as one of the other commoner Oriental/Australasian species of the subgenus *Avaritia*. These would include *C. orientalis* Macfie, 1932, *C. brevitarsis* Kieffer, 1917, *C. flavipunctatus* Kitaoka, 1975 and *C. wadai* Kitaoka, 1980. *Culicoides imicola*, however, is very abundant and widespread, being found through most of Africa into the Mediterranean, and from thence across the Near and Middle East as far as Laos. In distribution *C. imicola* and *C. nudipalpis* are not contiguous as neither has been recorded from peninsular Malaysia, Sumatra, Java, Borneo and the Celebes.

Regarding their status as two species, it is notable that almost the entire redescription of *C. nudipalpis* by Wirth & Hubert (1989) word-for-word duplicates that given by them for *C. imicola*. Not surprisingly, they conclude that *C. nudipalpis* 'is nearly identical with *C. imicola* ... and very similar to *C. brevitarsis* ...'. Furthermore, comparison of Fig. 284 and 287 in Wirth & Hubert (1989) show the wings of *C. imicola* and *C. nudipalpis* to be remarkably similar if not inseparable. The single feature used by these authors to distinguish females of *C. nudipalpis* is 'the virtual absence of the palpal pit; the third palpal segment of *C. nudipalpis* has a round pitlike area on the surface, but it lacks depth'. For *C. imicola* the third segment is described as 'without definite sensory pit but with sensilla grouped in a circular area on surface of segment'. While the wording may differ, the meaning is similar. The same character is used to separate the males. Comparison of their descriptions suggests two further differences between *C. imicola* and *C. nudipalpis*: palpal ratio (PR) 2,3 vs 0,68 and proboscis/head ratio (P/H) 0,88 vs 0,68 respectively. The large difference between the palpal ratios is clearly a lapsus as measurement of Fig. 122b in Wirth & Hubert (1989) gives a PR of 2,25. However, they did not refer to the significant difference in P/H ratios.

This synopsis begs the question as to whether these are indeed two good species. Doubt as to their separate status deepens when one considers that two Indian species, *C. minutus* Sen & Das Gupta, 1959 and *C. pseudoturgidus* Das Gupta, 1962, have already been found to be synonyms of *C. imicola* (Dyce & Wirth 1983). If *C. nudipalpis* is shown to be yet another synonym, it would mean a further extension in the already large geographic range of *C. imicola*, from the southern tip of Africa to the northern doorstep of Australia. This possibility of synonymy is important as *C. imicola* is probably the most important vector of the viruses of bluetongue (BT) and African horsesickness (AHS) known in the Old World.

These questions, and the recent acquisition of five females and two males of *C. nudipalpis*, prompted its present comparison with *C. imicola*. As the sample size of *C. nudipalpis* is too small to attempt a full redescription of *C. nudipalpis*, only key characters are compared.

3.2 MATERIALS AND METHODS

Initial examination of fresh unmounted *C. nudipalpis* females in alcohol indicated the species to be smaller than *C. imicola*, especially as regards relative length of the proboscis. The taxonomic value of this apparent difference was further evaluated in slide-mounted specimens, along with eight other character states. The results are summarized (Table 3.1) and depicted graphically (Fig. 3.1). The graph compares proboscis length against wing-length; unconverted raw data were used. For tabulation the data were converted to μm.

To negate seasonal effects and geographic clines in the *C. imicola* data, 121 individuals representing eight populations from various ecological zones of Africa were randomly chosen, these augmented by three extralimital populations (Spain, Israel and Thailand). They are listed in Table 3.2. Data on males were taken from Meiswinkel (1989). *Culicoides nudipalpis* was represented by four females and two males from Mindoro Island, Philippines, and one female from the island of Timor (Table 3.1). Too few specimens of *C. nudipalpis* were available for a more detailed comparison.

3.2.1 **Statistical analyses**

Only five females of *C. nudipalpis* were available for analysis. To make fair comparisons, and to have equal representativeness, data on *C. imicola* in Table 3.2 were obtained from five randomly chosen specimens from each of nine populations. These 45 females form part of the 121 used to generate Fig. 3.1. Comparison between populations was done by means of an analysis of variance for a completely random design. Population means were compared by the Bonferroni method.

3.3 **RESULTS**

Nine character states were examined; the taxonomic value of each is assessed separately:

a. **Wing length**: Fig. 3.1 compares proboscis length against wing length. While separation of *C. imicola* (n = 121; 11 populations) from *C. nudipalpis* (n = 5; 1 population) is clear Bonferroni's analysis of variance revealed wing length to be an unstable character. Although considerable overlap occurred between the populations tested, *C. nudipalpis* did have the shortest wings (Table 3.1). Future scrutiny of a longer series may well prove *C. nudipalpis* to be smaller than *C. imicola*. As regards *C. imicola*, it is notable that little to no overlap exists in wing length between three South African populations, i.e. Ndumu, Upington and Stellenbosch. This result could be interpreted as representing three species, but as these same populations exhibit consistency in proboscis length, and all other

Fig. 3.1

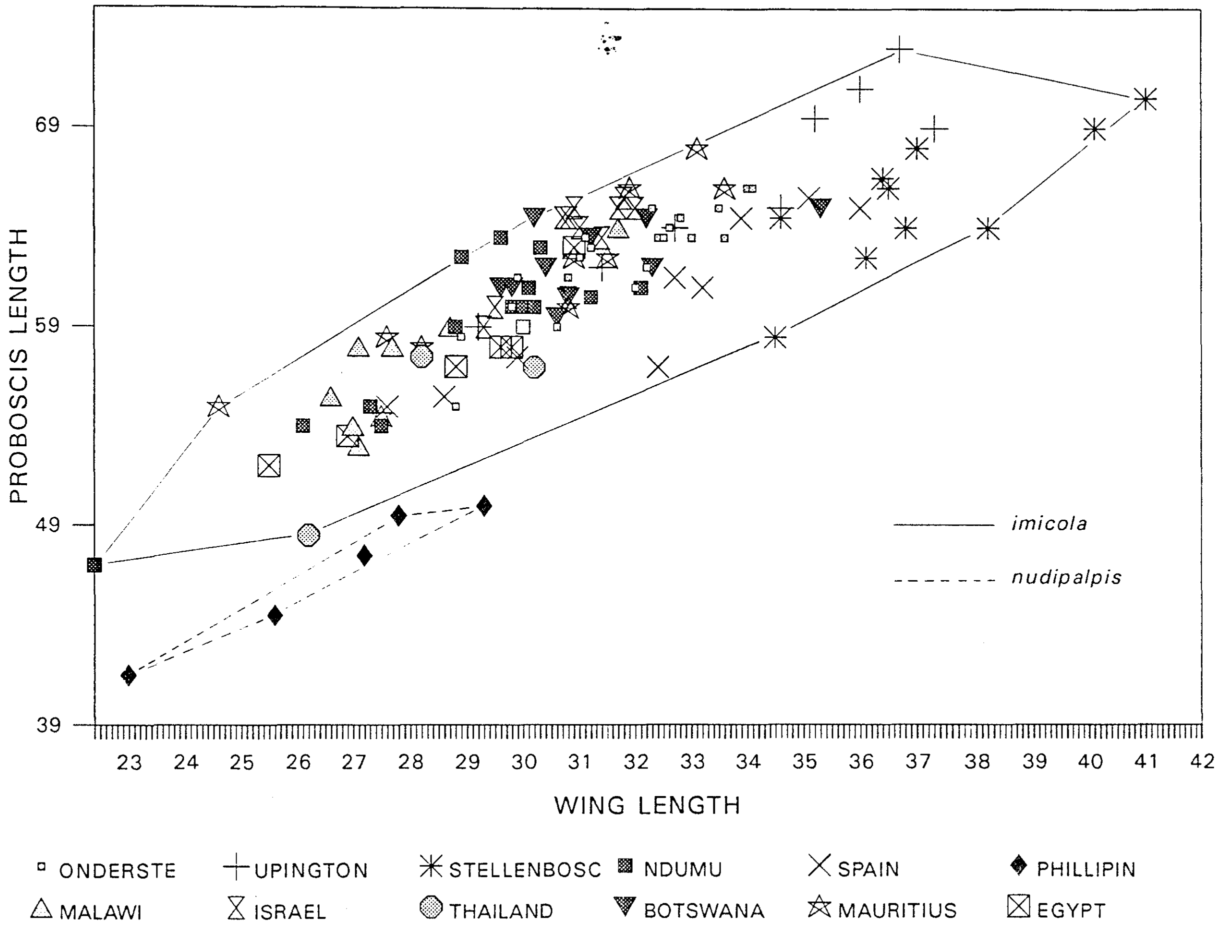


Fig. 3.1 Scatter diagram of proboscis length against wing length: separation of 11 populations of *C. imicola* (n = 121) from one of *C. nudipalpis* (n = 5).

characters, I do not hesitate to classify them as one species. This supports the earlier inference that wing length is unreliable, being more variable under environmental influences, whether nutritional or climatic. Hensley & Atchley (1977), in a study of natural and laboratory populations of the N. American *C. variipennis*, also found that there are 'highly significant differences among temperature groups in continuous body measurements, e.g., wing length and width ...'

b. **Proboscis length**: Table 3.1 and Fig. 3.1 show the proboscis of *C. nudipalpis* to be significantly shorter than that of *C. imicola*. Its usefulness as a distinguishing feature is graphically illustrated in Fig. 3.1: 24 % of the randomly chosen *C. imicola* specimens (representing ten of the 12 populations) have a wing length overlapping that of *C. nudipalpis*, but only 7 % (two specimens) share a similar proboscis length. One of the two *C. imicola* outliers is from northern Thailand and falls so near the *C. nudipalpis* data set in Fig. 3.1 that it could just as easily be identified as *C. nudipalpis*. While its intermediate position is suggestive of a geographic cline, it and the remaining two specimens from Thailand, have the antennal ratio, better defined palpal pit and coeloconica distribution typical of *C. imicola*. Such outliers alert us to the danger of basing identifications and descriptions on single specimens.

c. **P/H ratio**: A short proboscis results in a low proboscis/head (P/H) ratio. The data in Table 3.1 and 3.2 show that the difference in P/H between *C. nudipalpis* (0,66–0,73) and *C. imicola* (0,82–1,02) is highly significant. Indeed I consider this to be the most reliable way of separating these two species. These data support those provided by Wirth & Hubert (1989) for *C. nudipalpis*, and by Boorman (1991) for *C. imicola*.

d. **Antennal length**: Despite its small size the total antennal length of *C. nudipalpis* appears to be inseparable from that of *C. imicola* (Table 3.1). More data are needed to confirm this.

e. **Antennal ratio (AR)**: *Culicoides nudipalpis* has a significantly higher AR than *C. imicola* (n = 167; Onderstepoort population). Fig. 3.1 shows 24 Onderstepoort specimens to fall in the centre of all *C. imicola* populations scored, and thus to be reliably representative of the species.

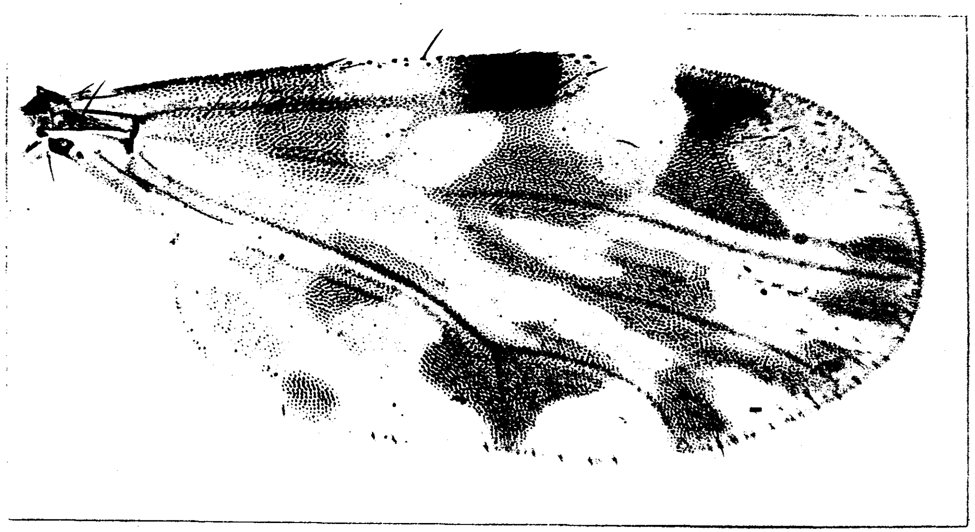
A higher antennal ratio indicates that the combined length of the distal five flagellar segments is longer than that of the basal eight segments. In *C. imicola,* a smaller difference exists between these two measurements resulting in a lower AR. The AR data provided by Delfinado (1961) and Wirth & Hubert (1989) differ from those presented here (Table 3.1). This may be due to mixed series or differing methods of mensuration.

f. **Sensilla coeloconica distribution**: Four of the nine *C. nudipalpis* female antennae examined have a coeloconica distribution of 3, 11–15; the remaining five (56 %) have 3, 12–15. In *C. imicola* only seven (4 %) of 172 antennae examined had a coeloconica on segment 11 (Onderstepoort population). The difference between the two species is significant but unreliable for identification, as more than half of the individuals examined of both species shared the coeloconica distribution of 3, 12–15.

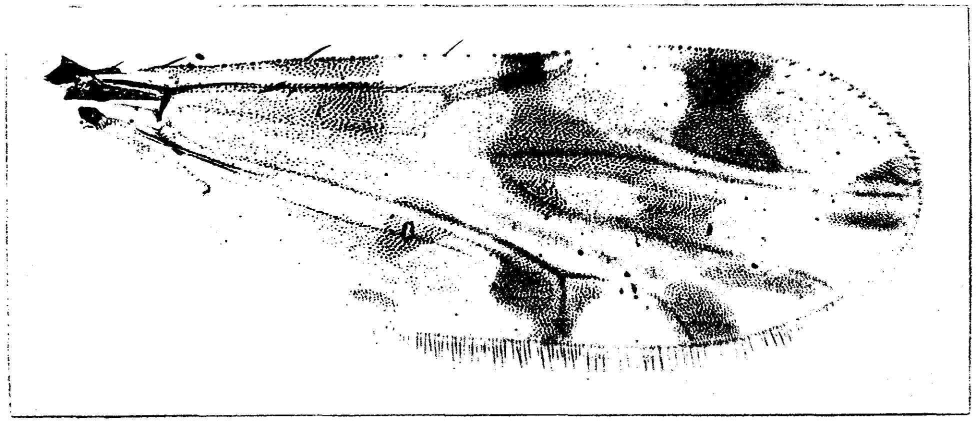
g. **Third palpal segment**: In their key to the south-east Asian *Culicoides*, Wirth & Hubert 1989) separate *C. nudipalpis* and *C. imicola* in that the former has 'third palpal segment with indistinct pit, the margins not definite'; the latter has the 'segment with distinct pit with well-defined sunken margins'. While this differentiation is more positive than the vague textual one made by Wirth & Hubert (1989) the key does pinpoint what appears to be an important difference between *C. nudipalpis* and *C. imicola.* Although few specimens of *C. nudipalpis* were examined, the palpal pit conformed with both the Delfinado (1961) and Wirth & Hubert (1989) descriptions. In most of the *C. imicola* material examined the palpal pit was better defined and deeper. However, the palpal pit may be a subjective character as its small size and variable angle of display can make accurate examination difficult. Furthermore, some specimens of *C. imicola* do have a shallow pit with poorly defined margins. To ensure accurate identification, the palpal pit is best used in combination with the P/H ratio.

h. **Wing pattern:** Comparison of Fig. 284 (*C. imicola*) with Fig. 287 (*C. nudipalpis*) in Wirth & Hubert (1989) shows the wing pattern of these two species to be virutally indistinguishable. This is confirmed by comparing two wing photographs of *C. imicola* (Thailand: Fig. 3.2 and 3.3) and two of *C. nudipalpis* (Timor: Fig. 3.4; West Irian: Fig. 3.5). The wing pattern feature

3.2



3.3



3.4

3.5

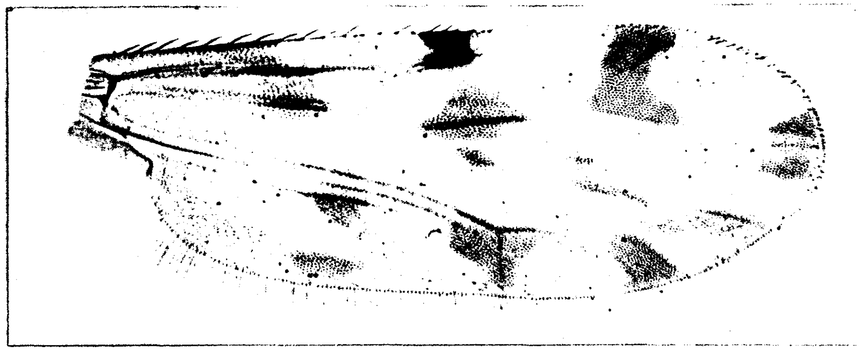


Fig. 3.2 *C. imicola*: Wing, female; Thailand (A.L. Dyce collection).

Fig. 3.3 *C. imicola*. Wing, male; Thailand (A.L. Dyce collection).

Fig. 3.4 *C. nudipalpis*. Wing, female; Timor (A.L. Dyce collection).

Fig. 3.5 *C. nudipalpis*. Wing, male; West Irian (A.L. Dyce collection).

that most reliably separates *C. imicola* from its known congeners *C. brevitarsis, C. pseudopallidipennis, C. bolitinos, C. miombo*, and *C. loxodontis*, is the preapical pale excision on the anterior margin of vein M_1 which leaves the apex of the vein broadly and squarely darkened. The same feature exists in *C. nudipalpis*, even though the preapical excision is inclined to straddle vein M_1 more deeply and broadly, almost separating the dark spot at the apex of the vein from the dark area straddling the same vein medially. However, this is a nicety; there is no doubt that the wings of *C. nudipalpis* and *C. imicola* are more similar to one another than to any other known member of the Imicola group worldwide. This strong similarity, if not representative of one species, at least confirms the two species to be closely related.

i. **Male genitalia**: The two specimens of *C. nudipalpis* had 84 and 112 spicules on the sternital membrane, so are moderately densely spiculate. This is approximately double the mean of 47 found in 50 specimens of *C. imicola* (Meiswinkel 1989). However, the range in *C. imicola* is considerable: 8–145 spicules. This comfortably embraces the number found in *C. nudipalpis*. Otherwise the genitalia of *C. nudipalpis* are very similar to, if not inseparable from, those of *C. imicola.*

3.4 CONCLUSION

Culicoides imicola and *C. nudipalpis* are good species, most easily distinguished in proboscis length and P/H ratio. Characters of secondary significance are body size, antennal length, antennal ratio (AR), sensilla coeloconica distribution, and definition of sensillar pit on the third palpal segment. Current data suggest that *C. imicola* and *C. nudipalpis* occur allopatrically; they are found west and east of the Wallace line respectively. This allopatry is another useful aid to identification. The two species are virtually inseparable in wing pattern and male genitalia. Longer series of both sexes of *C. nudipalpis* need to be examined to more fully quantify species differences.

Taxonomically there seems little doubt that *C. imicola* and *C. nudipalpis* are true sister species; they form a subgroup within the Imicola group of the subgenus *Avaritia*. The Imicola group comprises seven currently known species; five are found in Africa i.e. *C. imicola, C. pseudopallidipennis, C. bolitinos,* *C. miombo* and *C. loxodontis*, the remaining two (*C. nudipalpis* and *C. brevitarsis*) being in the Oriental/Australasian Region. The presently known allopatric distribution pattern of *C. imicola* and *C. nudipalpis*, west and east of the Wallace line respectively, suggests ancient vicariance involving the splitting of an ancestral species into two populations with subsequent speciation. It may be argued that *C. nudipalpis* is a subspecies of *C. imicola*; only studies involving cross-mating and/or enzyme electrophoresis would clarify whether they are indeed genetically isolated, as this morphological study suggests.

In Africa, *C. imicola* predominates in the medium rainfall (300–750 mm/annum) savannas and savanna-woodland areas. It declines rapidly to virtually disappear in the higher-rainfall forested tropics and frost-prone highland regions (Itoua & Cornet 1986; Meiswinkel 1991; Venter & Sweatman 1989; Venter & Meiswinkel 1994). Under optimal conditions, *C. imicola* can become superabundant, especially in situations where man husbands various kinds of livestock and maintains them on sunlit, short-grassed, irrigated pastures. In such locales, *C. imicola* can comprise 96 % of very large *Culicoides* collections made (Nevill 1971). Virtually nothing is known about the host preferences of *C. nudipalpis*, besides the fact that the type series was collected in a carabao-baited trap. In a recent study of *Culicoides* associated with poultry on the island of Luzon, Philippines, not one specimen of *C. nudipalpis* was recorded among >10 000 specimens of 17 species collected (Abella, Manuel, Cariaso & Kamiya 1994). This further suggests that *C. nudipalpis* is strictly mammalophilic.

Where known, the immature stages of *Avaritia* species worldwide thrive in semi-moist habitats such as animal dung, rotting fruits, humus, leaf-litter and fungi. The pupae do not float at the water surface, and are thus prone to drowning in any situation that becomes waterlogged (e.g. ricefields). These realities may help explain why *C. imicola*, and its sister-species *C. nudipalpis*, do not occur in abundance in the higher rainfall tropics of Africa and south-east Asia despite the presence of animal hosts. In this regard it is relevant to note that in a survey of *Culicoides* associated with cattle in Indonesia, the most specimens of *C. nudipalpis* were collected at Kupang on the island of Timor; although this locale experiences a relatively high rainfall (1 250 mm/annum) this is offset by a long eight-month dry season (Sukarsih, Daniels, Sendow & Soleha 1994).

Culicoides imicola is recognized to be one of the most important vectors of bluetongue and African horsesickness known worldwide. It remains to be established whether its congener, *C. nudipalpis*, is equally efficient at vectoring these orbiviruses. It is clear that all aspects of the bionomics of *C. nudipalpis* need further research; these should include studies into its distribution and prevalence, and the factors restricting its abundance.

3.5 REFERENCES

ABELLA, J.A., MANUEL, M., CARIASO, B. & KAMIYA, M. 1994. Abundance and prevalence of *Culicoides* (Diptera: Ceratopogonidae) on some Philippine chicken farms. *Journal of Medical Entomology*, 31:45–48.

BOORMAN, J. 1991. A review of *Culicoides* subgenus *Avaritia* species (Insecta, Diptera, Ceratopogonidae), vectors of viruses of sheep, cattle and horses, with particular reference to *Culicoides imicola* in Europe and the Mediterranean region. Report prepared for The Overseas Development Administration, pp 1–54.

DELFINADO, M.D. 1961. The Philippine biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae). *Fieldiana: Zoology*, 33:627–675.

DYCE, A.L. 1979. *Culicoides radicitus* Delfinado; a synonym of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae). *Journal of the Australian Entomological Society*, 18:52.

DYCE, A.L. & WIRTH, W.W. 1983. Reappraisal of some Indian *Culicoides* species in the subgenus *Avaritia* (Diptera: Ceratopogonidae). *International Journal of Entomology*, 25:221–225.

HENSLEIGH, D.A. & ATCHLEY, W.R. 1977. Morphometric variability in natural and laboratory populations of *Culicoides variipennis* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 14:379—386.

ITOUA, A. & CORNET, M. 1986. Les Ceratopogonidae (Diptera) du Mayombe Congolais. II. Revue taxonomique des espèces du genre *Culicoides* Latreille 1809. *Cahiers de l'office de la Recherche Scientifique et Technique Outre-Mer, Série Entomologie Medicale et Parasitologie*, 24:233–250.

MEISWINKEL, R. 1989. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:23–39.

MEISWINKEL, R. 1991. Afrotropical *Culicoides*: *C. (Avaritia) miombo* sp. nov., a widespread species closely allied to *C. (A.) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 58:155–170.

NEVILL, E.M. 1971. Cattle and *Culicoides* biting midges as possible overwintering hosts of bluetongue virus. *Onderstepoort Journal of Veterinary Research*, 38:65–72.

ROSEN, B.R. 1988. From fossils to earth history: Applied historical biogeography. Chapter 14, pp. 437–481. *in*: MYERS, A.A. & GILLER, P.S. (eds). *Analytical Biogeography: An integrated approach to the study of animal and plant distributions*. Chapman & Hall, London.

SUKARSIH, DANIELS, P.W., SENDOW, I. & SOLEHA, E. 1994. Longitudinal studies of *Culicoides* associated with livestock in Indonesia. *Arbovirus Research in Australia. Proceedings 6th Symposium*, 203–209.

VENTER, G.J. & MEISWINKEL, R. 1994. The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. *Onderstepoort Journal of Veterinary Research*, 61:327–340.

VENTER, G.J. & SWEATMAN, G.K. 1989. Seasonal abundance and parity of *Culicoides* biting midges associated with livestock at Roma, Lesotho (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 56:173–177.

WIRTH, W.W. & HUBERT, A.A. 1989. The *Culicoides* of southeast Asia (Diptera: Certopogonidae). *Memoirs of the American Entomological Institute*, 44:1–508.

Table 3.1 Six character states separating *C. nudipalpis* and *C. imicola*; data according to Delfinado (1961), Wirth & Hubert (1989), and this study

Character	***C. nudipalpis***	**X**	**n**	***C. imicola***	**X**	**n**
Wing length	0,66–0,91	0,82	5	0,92–1,17	1,06	150
Definado	0,77–1,02	0,85	9	-	-	-
Wirth & Hubert	0,80	?	?	0,79–0,86	0,82	5
Proboscis length	103,8–125,0	116,5	5	117,5–177,5	156,8	45
Delfinado	-	-	-	-	-	-
Wirth & Hubert	-	-	-	-	-	-
Proboscis/head ratio (P/H)	0,66–0,73	0,70	5	0,82–1,02	0,90	45
Delfinado	-	-	-	-	-	-
Wirth & Hubert	0,68	-	-	0,88	-	-
Antennal length	428,8–502,5	477,5	5	435,0–494,5	466,0	25
Delfinado	-	-	-	-	-	-
Wirth & Hubert	-	-	-	-	-	-
Antennal ratio (AR)	1,06–1,12	1,07	5	0,95–1,10	1,01	167
Delfinado	1,09–1,18	1,14	4	-	-	-
Wirth & Hubert	1,20	-	-	1,17–1,19	1,18	2
Sensilla coeloconica	3,11–15 (44 %)	-	9	3,11–15 (4 %)	-	172
	3,12–15 (56 %)	-	9	3,12–15 (96 %)	-	172
Delfinado	3,11–15	-	-	-	-	-
Wirth & Hubert	3,11–15 (?)	_	_	3,11–15 (?)	-	-
	3,12–15 (?)	-	-	3,12–15 (?)	-	-

TABLE 3.2 Comparison of means (Bonferroni) of nine populations of *C. imicola* with one of *C. nudipalpis*; means connected by a continuous vertical line are not significantly different at 5 %

Species and population		Proboscis length (μm)		P/H ratio		Wing length (raw data)	
C. nudipalpis	(n = 5):						
	(n = 4)	Philippines	46,600	Philippines	0,700	Philippines	26,580
	(n = 1)	Timor					
C. imicola	(n = 45):						
		Malawi	57,600	Israel	0,858	Malawi	28,320
		Ndumu	57,900	Ndumu	0,880	Ndumu	28,640
		Spain	61,700	Mauritius	0,884	Mauritius	30,360
		Mauritius	61,900	Stellenbosch	0,896	Botswana	30,740
		Botswana	62,600	Upington	0,900	Israel	31,080
		Israel	63,600	Spain	0,904	Onderstepoort	32,420
		Onderstepoort	64,000	Onderstepoort	0,908	Spain	32,740
		Stellenbosch	66,800	Malawi	0,908	Upington	35,180
		Upington	68,200	Botswana	0,976	Stellenbosch	36,920
F-value			13,048		17,590		8,492

CHAPTER 4

Culicoides (Avaritia) miombo Meiswinkel, 1991, a widespread species closely allied to *C. (A.) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae)

4.1 INTRODUCTION

During a study monitoring bluetongue virus (BTV) activity in a Nigerian dairy herd, Herniman, Boorman & Taylor (1983) collected >70 000 *Culicoides* over a 12 month period. They found that only two species, *imicola* and *schultzei*, occurred throughout the wet and dry seasons, and these were thus deemed the most likely to be involved in bluetongue transmission. However, they noted that each species 'may be placed with a few close relatives, distinguishable only with difficulty, into species groups. It was impractical to differentiate species on a project of this scale and accordingly these insects were enumerated in terms of species groups ...; for convenience these species groups are referred to ... as *imicola*, *milnei* or *schultzei*.' Elsewhere they note that the species closely related to *C. imicola* are *C. brosseti*, C. *grahamii* and *C. kibatiensis*, and that they occurred in proportions of 'approximately 2:1:1:0,5', and that these proportions remained much the same throughout the period of their study.

The four last-mentioned species all belong in the subgenus *Avaritia*, which is commonly, but mistakenly, equated in the literature with the Imicola group (Itoua & Cornet 1986; Glick 1990). In fact, these four species belong in four separate species groups; these groupings are recognized by Itoua & Cornet (1986) but are ranked by them as subgroups only. In my assessment, approximately 10 groups represent the 70 species constituting the subgenus *Avaritia* worldwide. Their biologies are diverse, and within each group each species possesses a unique life-cycle. As conceded by Herniman *et al.* (1983), 'the relative importance of individual members of the *imicola* and other groups is unknown but it is clearly possible that differences in feeding habit or vector competence might differentiate between the species.'

In this chapter it will be shown that the species referred to by Herniman *et al.* (1983) as *C. brosseti* is in fact the recently described *C. miombo* Meiswinkel, 1992. Conversion of the Nigerian data show it can achieve some measure of abundance, namely that it represented approximately 6–8 % of all *Culicoides* captured. Although few data are available, those presented in this chapter indicate that *C. miombo* is tropical in its distribution, and in wetter climates can be more common than *C. imicola*. Herniman *et al.* (1983) also state that '*imicola*... showed a clear preference for cattle blood ...' and further reinforced their conclusion that it (or rather the group) is the principal vector of BTV in Nigeria.

As *C. miombo* forms a significant part of this group, and is taxonomically very closely related to *C. imicola* s.s., it may also be playing a role in virus transmission. It follows that the lumping of species with diverse life strategies into groups can detrimentally affect interpretations as regards the epidemiology of a particular disease. The situation can be improved, in part, by refining the taxonomy of Afrotropical *Culicoides*. This, in turn, should promote more detailed investigations into their larval habitats, prevalence, geographic distribution and vector competence. Only then can each species be more reliably linked to, or disassociated from, the presence of a specific orbiviral disease.

The redescription of *C. miombo* is introduced with a brief description of the floristic composition and geographical extent of wetter Zambezian miombo woodland south of the equator, and of drier Guineo-Congolian rainforest and Sudanian woodland north of the equator. This is done to indicate the basic climatic preferences of *C. miombo* and, to initiate the exploration and definition of the ecozone preferences of various *Culicoides* species in Africa. It is the sympatric occurrence of various biota that will produce the patterns needed to elucidate the biogeography of this genus, a subject which has been almost wholly neglected in the region.

4.2 RESULTS

Africa, including Madagascar, is divided into 20 major phytochoria, which are further subdivided into 80 major vegetation units (White 1983). *Culicoides miombo* is for the present recorded from or very near to 14 of these units which fall into six of the major phytochoria; these 14 units form part of forest, forest transitions, woodlands and woodland transition zones. These phytocoria and vegetation zones are listed in Table 4.1 and then briefly discussed in the text to indicate the broad climatic preferences of *C. miombo*. This is followed by a more detailed description of topography, vegetation and climate of the type locality of the new species in Malawi.

4.2.1 Phytochoria with which *C. miombo* is associated

The type locality of *C. miombo* in Malawi is dominated by *Brachystegia* woodland (Leguminosae: Caesalpinioideae), and falls into the mapping unit 25 of White (1983) classified as 'wetter Zambezian miombo woodland'. Wetter miombo, however, is only one of 20 vegetation types comprising the Zambezian regional centre of endemism. For a detailed account see White (1983).

As to climate, almost the entire Zambezian region falls within the tropical summer rainfall zone. There is a single rainy season, chiefly from November to April with rainfall being between 500–1400 mm/annum. Precipitation in general decreases from north to south, but there are pronounced regional variations. Mean and annual temperatures range from 18–24 °C. There are three seasons: 1. Wet season November–April, where rain falls mostly as thunder-showers and heavy showers, with a fair amount of sunshine. 2. Cool season May–August, where day temperatures are moderately high with continuous sunshine; night temperatures are low and ground frosts may occur in sheltered valleys. 3. Hot season September–November, where temperatures and atmospheric humidity progressively increase until the oppressive feeling in the air is relieved by the advent of rains.

On the African mainland south of the equator, *C. miombo* has been positively identified from five other localities i.e. Rekomitjie (16°08'S, 29°24'E), north-western Zimbabwe; Maun (20°01'S, 23°25'E), northern Botswana; Ndumu (26°55'S, 32°15'E), northern Natal, South Africa; Skukuza (24°58'S, 31°36E) north-eastern Transvaal, and Tiwi (4°14S, 39°34'E) south-eastern Kenya. The records of *C. brosseti* from Dundo (9°46'S, 14°42'E), north-western Angola by Kremer (1972) and the species labelled as A–1 and A–10 by Kitaoka & Zulu (1990) from Chilanga (15°54'S, 28°35'E), Zambia in all probability refer to *C. miombo* (Fig. 4.1).

North of the equator *C. miombo* has been recorded as *C. imicola* from Ife, Nigeria (Kitaoka, Kaneko & Shinonaga 1984), and as *C. brosseti* Vattier & Adam, 1966 from Ibadan and Vom, Nigeria (Boorman & Dipeolu 1979). The record of *C. brosseti* from Simousso, Burkina Faso (Cornet 1969) quite likely also refers to *C. miombo*. It is now recorded for the first time from five localities in the Ivory Coast, i.e. Parhadi (8°27'N, 3°29'W), Ganse (8°35'N, 3°54'W), Korhogo (9°27'N, 5°39'W), Yamoussoukro (6°49'N, 5°15'W) and Biankouma (7°45'N, 7°18'W).

There are important parallels between the Sudanian phytochorion north of the equator and the Zambezian one south of the equator. Their climates are broadly similar, especially with regard to rainfall. In the Sudanian, however, temperatures are appreciably higher (24–28 °C) and nearly constant year-round, while frost is unknown; the dry season is more severe. Floristically, Sudanian *Isoberlinia* woodland (units 27, 30 and 32) can be regarded as an impoverished variant of miombo woodland.

Off the African mainland, the single record of *C. brosseti* (Kremer & Brunhes 1972) from Manakara, Madagascar, is quite likely a misidentification of *C. miombo*. Manakara (22°06'S, 48°00'E) is on the eastern coast, an area that falls into White's major phytochoria XIX (the East Malagasy regional centre of endemism) and in vegetation unit 11b, a mosaic of lowland rainforest and secondary grassland.

Table 4.1 lists all these localities and briefly indicates the vegetation unit, altitude, mean annual temperature, rainfall and incidence of frost at these localities in western and southern Africa. Fig. 4.1 and 4.2 map the localities and the phytochoria they occupy. Localities accompanied by a question mark are those from which I have not seen specimens of *C. miombo*.

4.2.2 **Type locality (Fig. 4.3)**

Description of the lakeshore plains of Malawi

Malawi is a small landlocked country that lies partially in the southernmost extension of the great African Rift Valley. Where the rift runs through Malawi it is 40–90 km wide, but much of it is under Lake Malawi (570 km long x 80 km wide). Broadly speaking, Malawi comprises five major landscape types (Fig. 4.3a).

The type series of *C. miombo* consists of 142 specimens (74 ♀♀ 68 ♂♂) collected at Kawalazi (11°37'S, 34°06'E) and Limphasa (11°38'S, 34°13'E) in north-eastern Malawi (Fig 4.3b). These locales are 12 km apart and lie 600–700 m a.s.l. between the towns of higher-lying Mzuzu (1 235 m; 11°27'S, 34°01'E) and lower-lying Nkhata Bay (481 m; 11°36'S, 34°18'E). This hot lowland area with a mean maximum temperature in November of 35 °C, lies in the Rift Valley trough, known as the lakeshore plains; the trough runs along most of the central length of Malawi. The plains, in which isolated hills

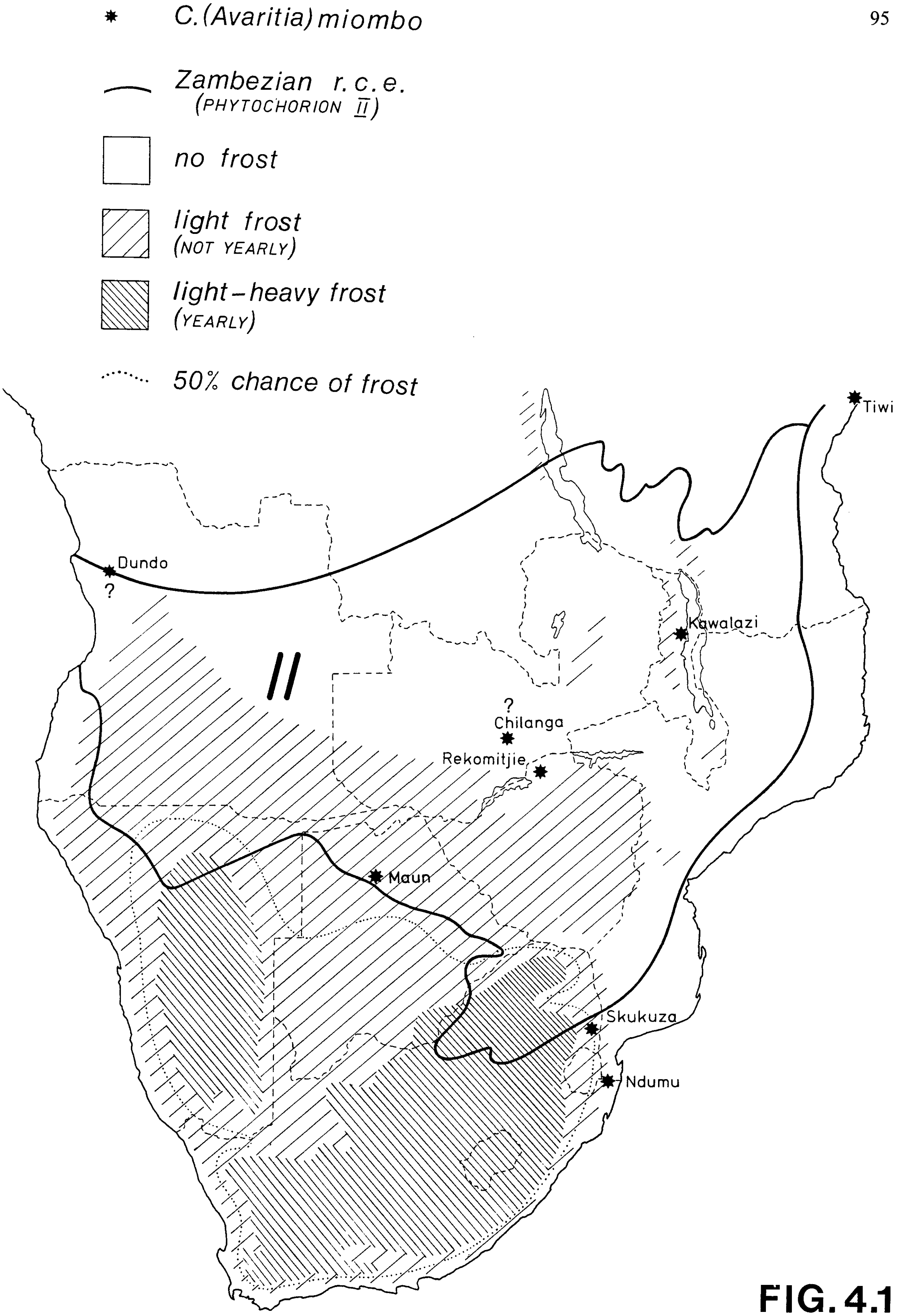


FIG. 4.1

Fig. 4.1 Map of southern Africa detailing the extent of the Zambezian regional centre of endemism (r.c.e.) (phytochorion II), the prevalence of frost, and the localities where *C. (Avaritia) miombo* has been collected.

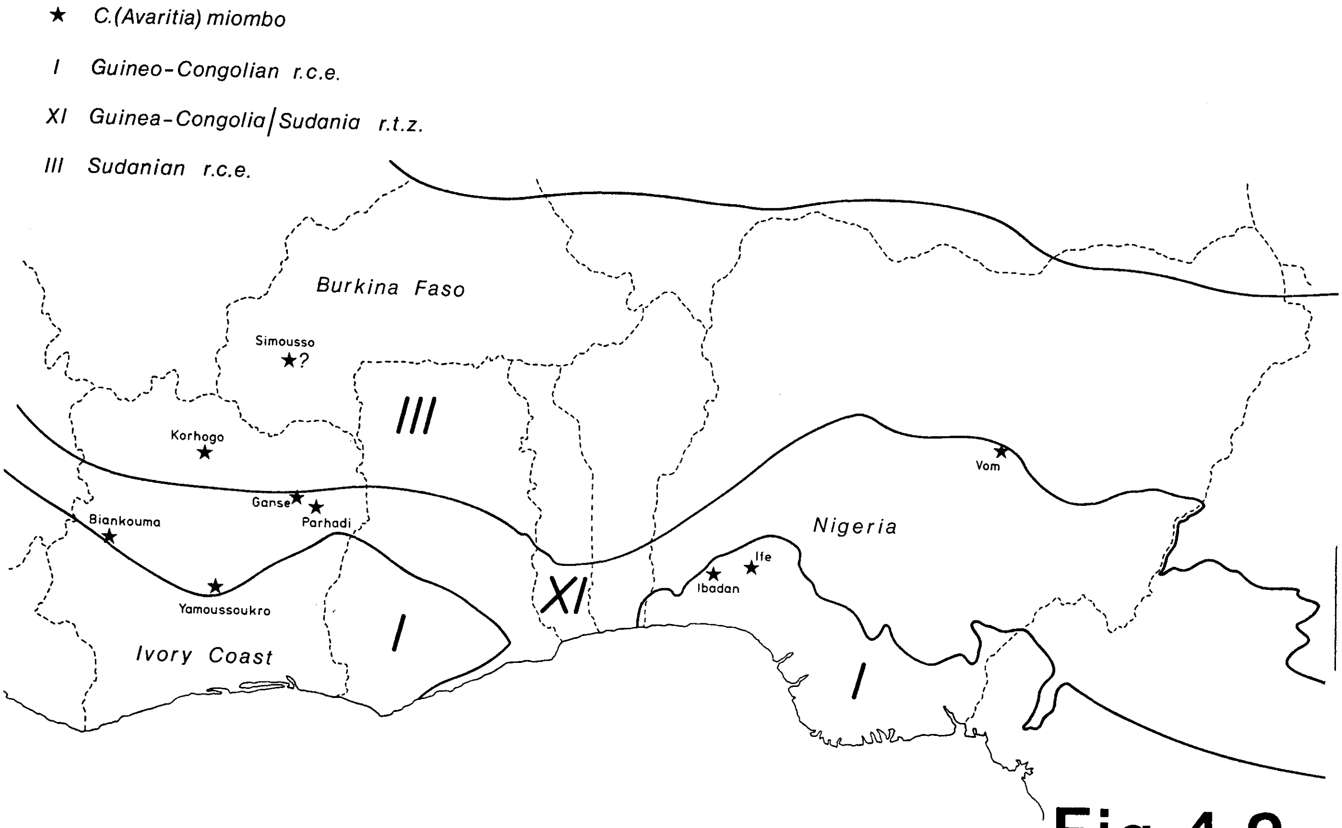


Fig.4.2

Fig. 4.2 Map of western Africa detailing the extent of the Guineo-Congolian and the Sudanian regional centres of endemism (phytocorion I and III); the Guinea-Congolia/Sudanian regional transition zone (r.t.z.) (phytochorion XI), and the localities where *C. (Avaritia) miombo* has been collected.

frequently arise, vary in extent but may be as wide as 25 km. In these wider sections, such as between Kawalazi and Nkhata Bay, the natural vegetation is lowland woodland with large and small swampy areas fed by numerous streams and rivers; these waters originate in the jumble of hills and ridges that make up the western escarpment. The fertile red alluvial soils of the plains have been developed for large-scale agricultural projects, as well as subsistence agriculture. The escarpment, which forms the western border of the lakeshore plains, consists of a series of low terraces which represents old shorelines of the lake; most of these slopes are covered in *Brachystegia* woodland and are generally not highly populated. Rainfall and temperature data for Kawalazi are not available, but during the years 1963–1977 higher-lying Mzuzu had a mean annual rainfall of 1 218 mm, a mean minimum temperature of 11,7 °C, and a mean maximum of 17,8 °C. For the years 1955–1977, lower-lying Nkhata Bay had corresponding figures of 1 695 mm, 18,8 °C and 27,8 °C. The data for Kawalazi would be most similar to that of Nkhata Bay, except for rainfall averaging nearer 1 400 mm per annum (Piet Verster, Kawalazi Estates, personal communication 1990).

At Kawalazi the *Brachystegia* woodlands (miombo) are not in a pristine state, but are under some pressure from man. Much of the area between Mzuzu and Nkhata Bay is cultivated, with large tracts planted to *Hevea* rubber (Vizara estate), tea and coffee (Chombe and Kawalazi estates) and eucalyptus. Besides such corporate investments, Malawians also clear smaller areas for fruit, cassava and grain crops. Because their protein intake comprises largely fish, not many cattle or other domesticated breeds are kept in this area. Between the cultivated sectors, however, fairly sizeable tracts of *Brachystegia* woodland still exist. At Kawalazi the light-trap was operated on the western slope of the Mphezi hills (Fig. 4.3b), in largely undisturbed *Brachystegia* woodlands with a moderate amount of foliage between the sparse field layer and the lower canopy at 5–8 m. A shaded, moist, and well-vegetated depression was located 100 m away from the light-trap and was fed by a small, constantly-running stream. No domesticated stock occurred in the immediate vicinity; game consisted of a small troop of baboons, smaller mammals and birds. Kawalazi was randomly monitored by light-trap over most months starting October 1987 through to April 1989; this revealed *C. miombo* to be entirely absent during the drier months of October and November. It was only after rains had commenced that *C. miombo* appeared but always in low numbers, for example in April 1989 only 54 specimens (0,9 %) of a total of 6 043 *Culicoides* collected were of this species.

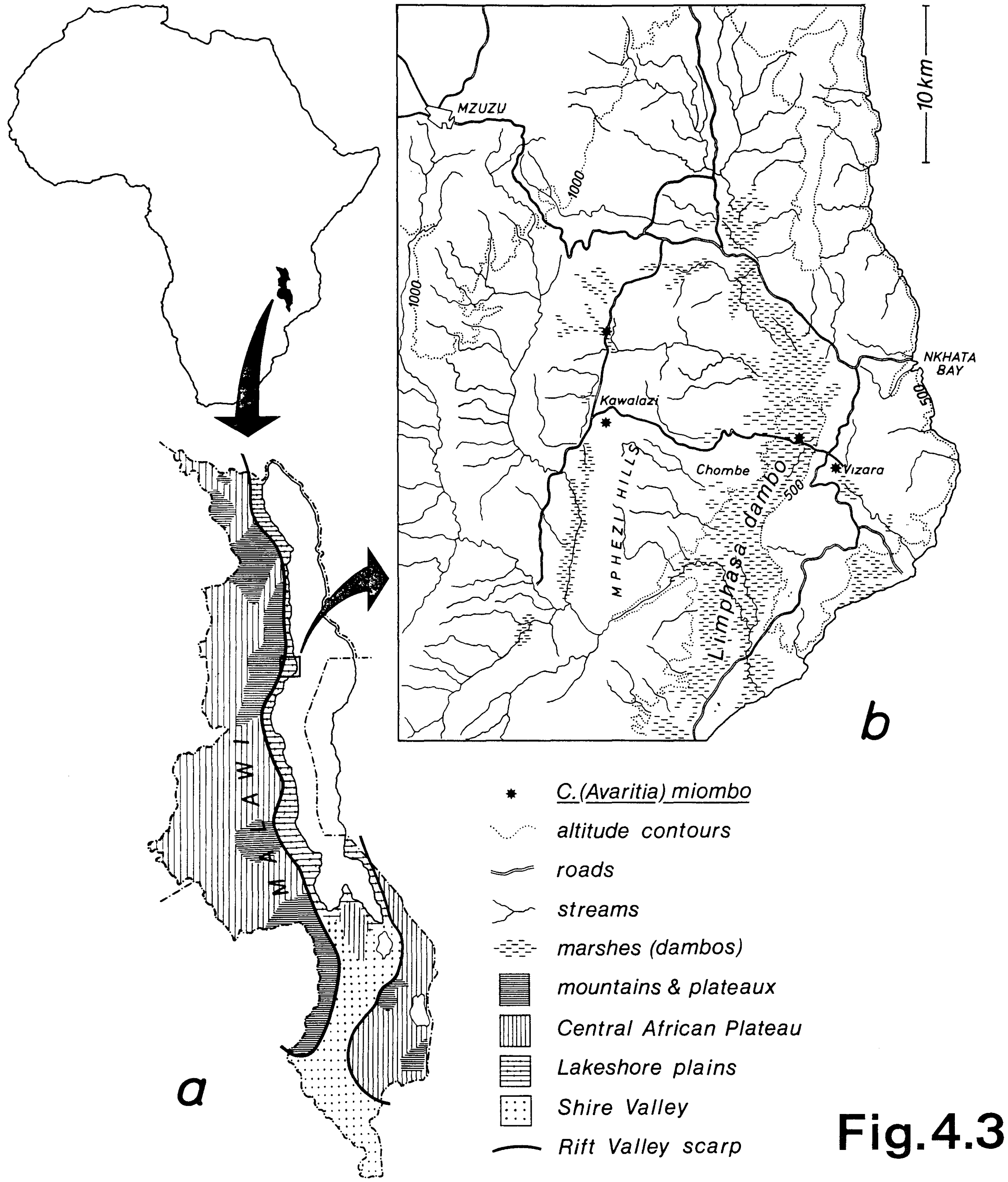


Fig.4.3

Fig. 4.3 (a) Map of Malawi showing five major landscape types, and (b) in greater detail, the topography of the type locality of *C. (Avaritia) miombo*.

Twelve km to the east of Kawalazi, near Vizara, is found the extremely large Limphasa dambo (Fig. 4.3b) or marsh that is some 30 km long and 0,5–3 km wide. It is thickly vegetated with 1 m high grasses and herbage, is saturated underfoot, and fed by quietly flowing, meandering streams. Occasionally very large palms and associated bush trace an eccentric line through the dambo. Elsewhere, small parts of the dambo are planted to rice. A number of small scattered villages have arisen on the low banks of the dambo; here some cattle are kept and spend their days grazing the drier parts of the marsh.

The collecting of *Culicoides* at Limphasa was done on two occasions, once only during each of the hot, dry months of October and November, 1987, by truck-trapping on a slightly elevated road that transected the dambo. On these two occasions a total of four females and more than 100 males of *C. miombo* was caught at dusk. The presence of so many males could have been the result of a mating swarm being captured which in turn suggests that the larval habitat of *C. miombo* was in close proximity.

One other site was sampled once by light-trap in November 1987, this was the cattle-kraal at the Vizara rubber estate 4 km east of Limphasa. Here only two ♀♀ of *C. miombo* were caught amongst 359 *C. imicola* and 71 *C. bolitinos*.

4.2.3 ***Culicoides (Avaritia) miombo*** **(Fig. 4.4–4.14; Table 4.2–4.7)**

Culicoides brosseti Vattier & Adam: Boorman & Dipeolu 1979: 17. Nigeria (misident.).
Culicoides imicola Kieffer: Kitaoka, Kaneko & Shinonaga 1984: 458. Nigeria (misident.).

Female (Fig. 4.4–4.8, 4.13, 4.14; Table 4.2–4.7)

Head. Eyes (Fig. 4.4); seemingly bare but mostly sparsely to moderately hairy, with hairs absent from a transverse band formed by the median three to eight rows of facets; eyes contiguous for a distance of between one and two facets. Antenna (Fig. 4.5, 4.13; Table 4.2–4.5) slender, basal segments IV–IX barrel-shaped, distal segments X–XIV faintly vasiform narrowing perceptibly subapically, XV nearly parallel-sided only narrowing apically; mean lengths of antennal segments III–XV: 36,8–23,3–23,6–25,7–28,1–27,3–28,2–32,7–44,6–46,0–48,4–49,5–83,3 μm (n = 68); total length of antenna: 470,0–537,5 mean 505,2 μm (n = 36); widths of antennal segments III–XV:

28,1–20,0–17,5–16,3–15,0–15,6–15,6–15,0–14,4–14,4–14,4–13,7–15,0 μm (n = 1); AR 1,09–1,25, mean 1,15 (n = 66); sensilla coeloconica present on segments III, XII–XV in 97 % of antennae examined (n = 70), see Table 4.3 for deviations from the norm; sensilla chaetica distribution on segments III–XV was 5–3–2–3–2–3–2–3–0 (n = 70) in 97,5 % of antennae examined, see Table 4.4 for deviations from the norm; sensilla trichodea distribution of the LLc type i.e. each of segments IV–X with two long and one short blunt-tipped trichodea, segment III with only two long blunt-tipped trichodea (n = 70); AtR 1,27–1,76, mean 1,54 (n = 68); segments XI–XIV each with 8–12 sharp-tipped sensilla trichodea of varying lengths and thicknesses distributed in a basal and subapical whorl; XV with two times as many trichodea these distributed in a basal, median and subapical whorl (Fig. 4.5); similarly the short blunt-tipped basiconica range from 2–7 on each of the distal flagellar segments XI–XV (Fig. 4.5); all antennal segments uniformly clothed throughout with fine spiculae.

The distributions of sensilla coeloconica, chaetica and trichodea are given in Table 4.2. Palp (Fig. 4.6, 4.14; Table 4.6): of a moderate length, slender, light brown throughout, mean lengths of palpal segments I–V: 19,85–49,77–45,90–25,60–26,8 μm (n = 70); total length 147,5–177,5 μm, mean 162,6 μm (n = 70); palpal segment I with only one rather long chaetica (n = 70), II with two to four rather short chaetica mean 3,06 (91 % with three chaetica; n = 70); III moderately long and slender carrying only one to four rather short chaetica, mean 2,87 (75 % with three chaetica; n = 70), with a small, round and shallow subapical pit with opening about half the width of segment in diameter, margin of pit smooth but well-defined, IV with one to four chaetica, mean 2,44 (44 % with three chaetica; n = 70), V with no chaetica on median portion of segment but does always bear five short erect bristles apically; these bristles appear to be different in form and function to those chaetica found on the basal segments and are thus dealt with separately; PR 2,44–3,00, mean 2,80 (n = 70); P/H ratio 0,87–0,96, mean 0,91 (n = 20); mandible with 13–18 fine teeth, mean 15 (n = 64).

Thorax. Legs: brown with all femora narrowly pale basally and with fore and middle femora indistinctly pale apically. All tibiae with a narrow well-defined basal pale band; remainder of fore and middle tibiae brown while apices of hind tibiae are pale, TR 1,51–1,84, mean 1,66 (n = 70); comb on apex of hind tibia with five spines, the first being the longest and only slightly longer than the second (n = 70). Wing (Fig. 4.8): length 0,83–1,01 mm, mean 0,94 mm (n = 66); breadth 0,41–0,51 mm, mean 0,46 (n = 66),

Fig. 4.4

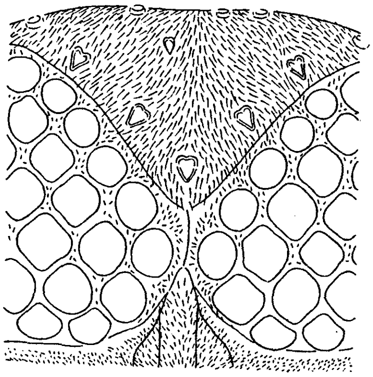


Fig. 4.4 *Culicoides (Avaritia) miombo*. Eyes, female (paratype Malawi 825).

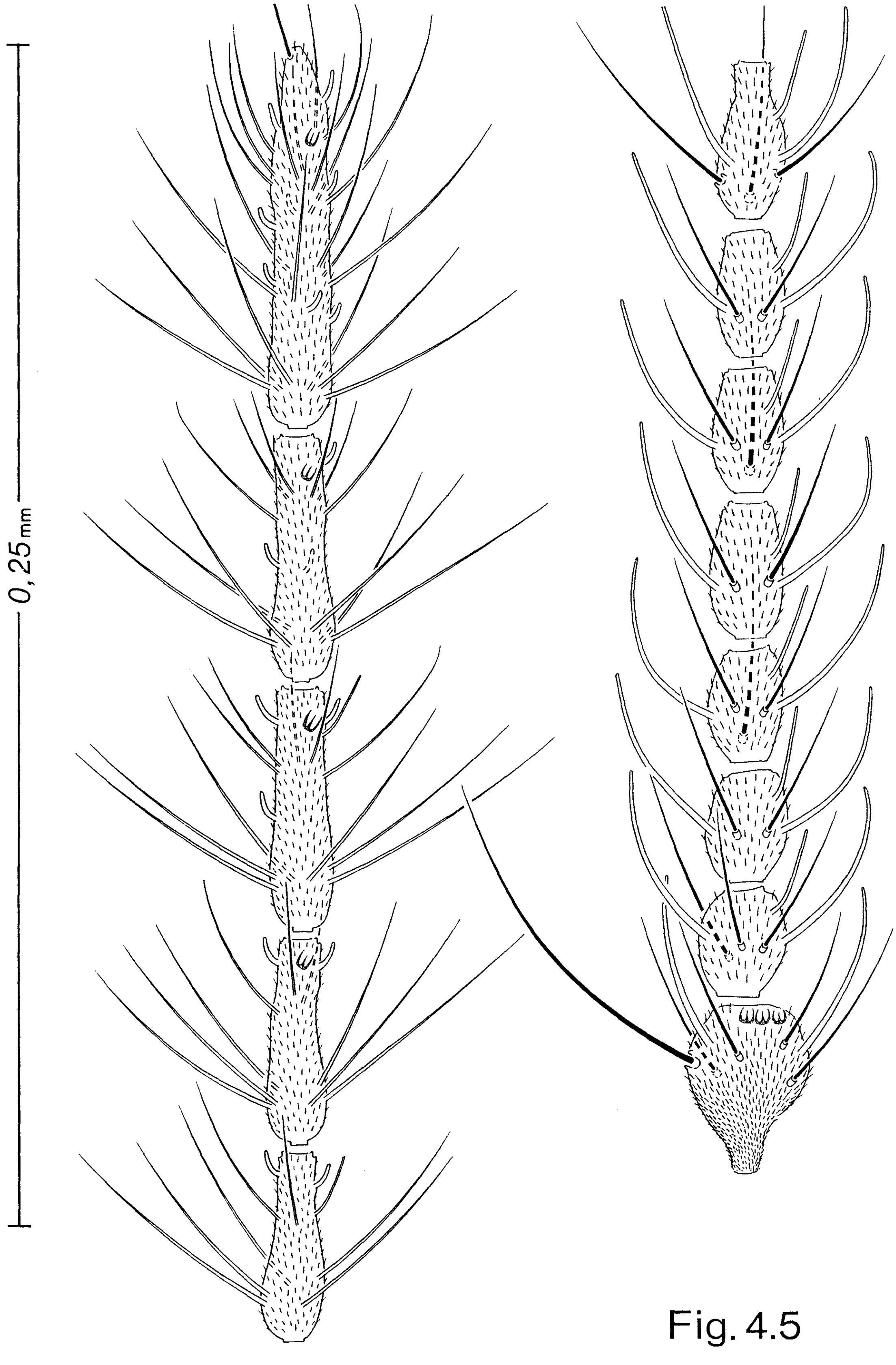
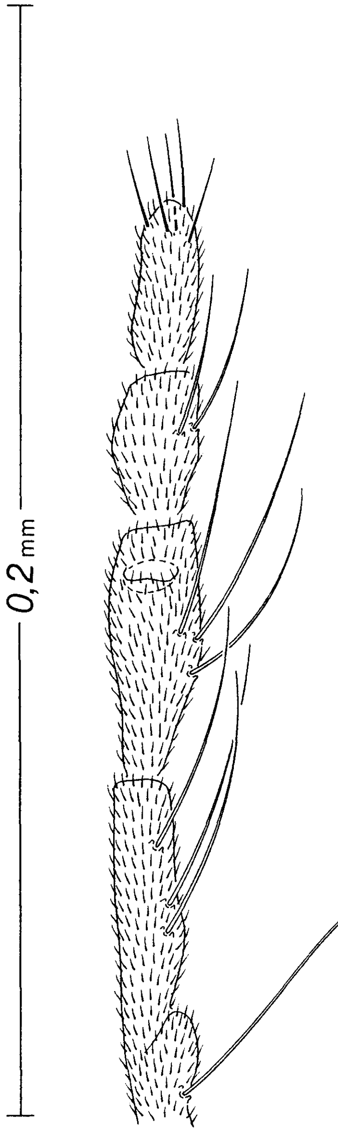


Fig. 4.5

Fig. 4.5 *Culicoides (Avaritia) miombo*. Antenna, female: segments XI–XV on left, segments III–X on right (paratype Malawi 822).



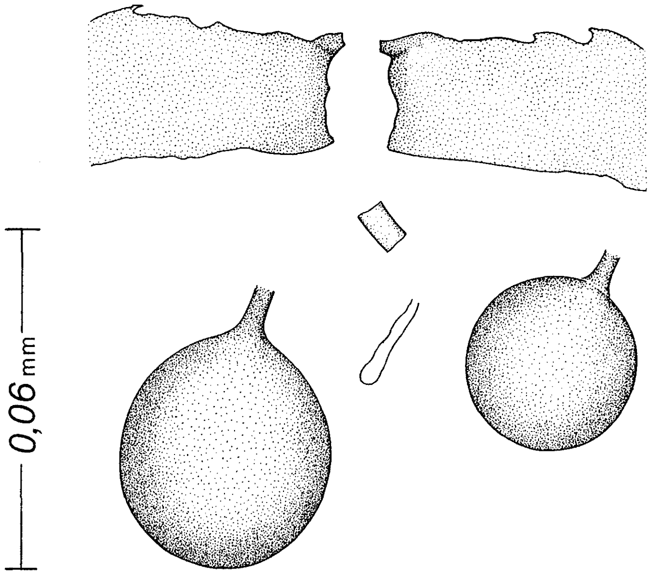


FIG. 4.7

FIG. 4.6

Fig. 4.6 *Culicoides (Avaritia) miombo*. Palp, female (paratype Malawi 813).

Fig. 4.7 *Culicoides (Avaritia) miombo*. Genitalia, female: spermathecae and sclerotization surrounding gonopore (paratype Malawi 809).

Fig.4.8

Fig.4.9

Fig. 4.8 *Culicoides (Avaritia) miombo*. Wing, female (holotype Malawi 820).

Fig. 4.9 *Culicoides (Avaritia) miombo*. Wing, male (holotype Malawi 951).

CR 0,56–0,60, mean 0,58 (n = 66); macrotrichia scanty, confined to distal third of wing in cells R_5, M_1 and M_2 only; microtrichia dense and coarse.

Dark pattern of wing greyish-brown, pale areas faint yellowish, fairly well defined but irregularly shaped; two radial cells, proximal half of first and distal third of second cell, pale. The wing of *C. miombo* is clearly darker than that of its congeners *C. imicola, C. pseudopallidipennis* and *C. bolitinos* as a result of the general reduction in the size of the pale areas. The most distinctive feature of the wing pattern is the elongate dark smudge which arises at the proximal base of the anal cell, cuts diagonally across the cell to terminate at the posterior wing margin well proximad of the apex of vein Cu_1. It is important to note that this smudge is divided into two by a fairly broad pale interruption subapically. Only in rare instances will these 2 dark areas be indistinctly and narrowly fused; only the smaller distal dark area is found in *C. imicola, C. bolitinos* and *C. pseudopallidipennis*. Other important species-specific wing pattern characters are: (i) pale costal spot two that straddles the r–m crossvein is reduced in size, slightly waisted medially, but expands to fairly broadly abut the anterior wing margin; (ii) distal or fourth pale costal spot in cell R_5 abuts wing margin, but in many specimens this spot is both reduced in size and isolated by a narrow dark strip that runs along the antero-distal wing margin; in rare dark forms this distal pale spot in cell R_5 almost disappears; (iii) proximal margin of this 4th pale costal spot rounded rather than sharply pointed; (iv) median third of anterior margin of vein M_1 never entirely pale but brownish; (v) vein M_2 has both margins very broadly darkened for most of its length, does not have a preapical excision on the anterior margin only but instead can be fairly abruptly tapered on both margins near its apex; however, this tapering still leaves both margins at the apex of vein M_2 dark, while in some darker specimens the tapering may again flare into a more broadly brown apex. Scutum brown in alcohol but strikingly adorned with two fairly large, yellow, very narrowly separated admedian vittae, scutellum narrowly brown medially, lateral margins broadly yellow; bearing one median bristle and one shorter bristle on each corner in 36/37 specimens, remaining specimen different in having two median bristles. Haltere knobs distinctly brown.

Abdomen (Fig. 4.7): Two moderately sclerotized, slightly unequal spermathecae present, measuring 40–50 x 35–41 μm and 34–44 x 29–35 μm (n = 20); both round and devoid of small hyaline punctations, with moderately long, narrow, pigmented necks; rather small, narrow, rudimentary third

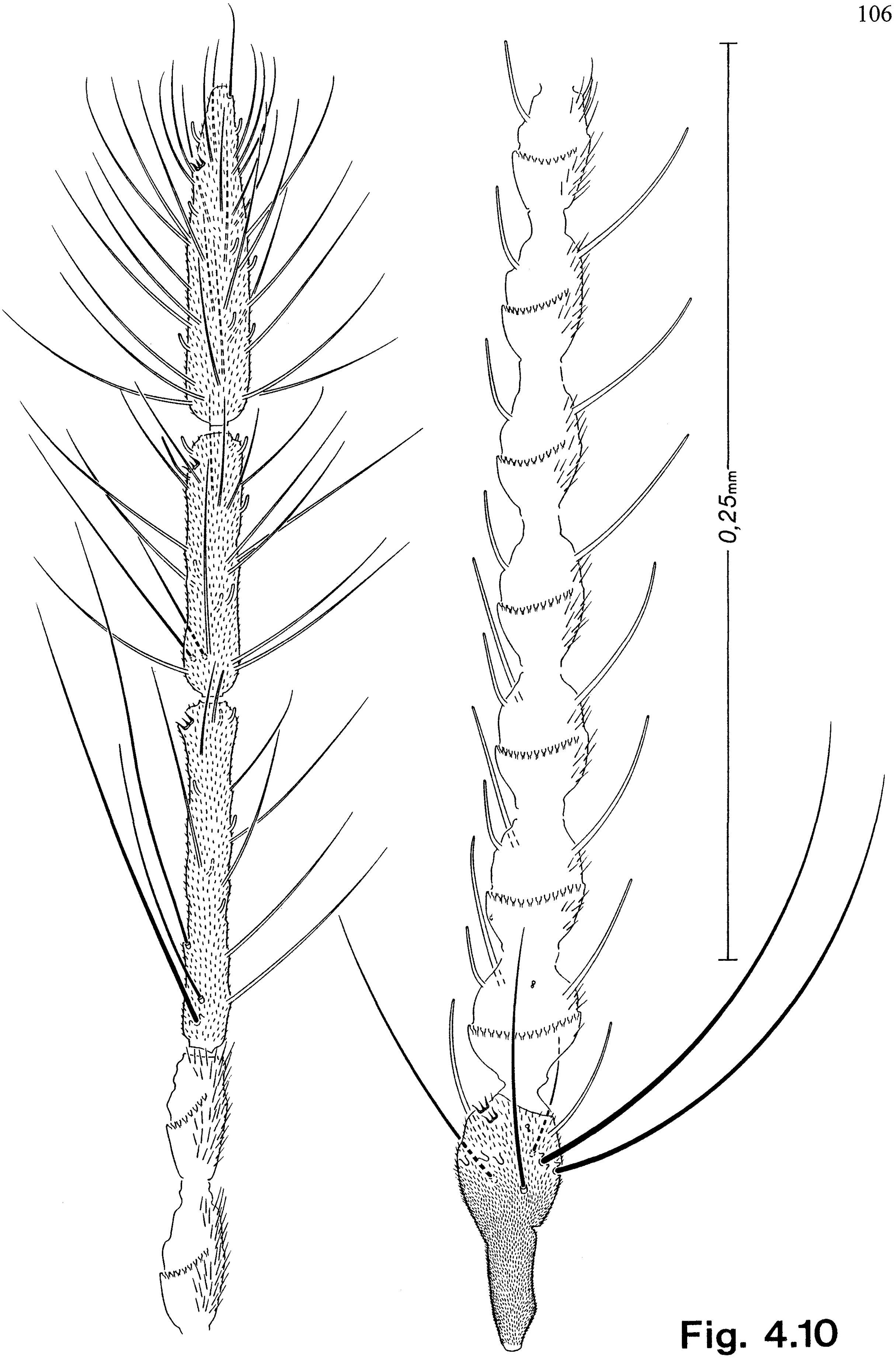


Fig. 4.10

Fig. 4.10 *Culicoides (Avaritia) miombo*. Antenna, male: segments XI-XV on left, segments III-X on right (paratype Malawi 834).

spermatheca present measuring 14–21 x 4 μm; 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 gonopore as shown in Fig. 4.7.

Male (Fig. 4.9–4.12; Table 4.2, 4.7)

Head. Eyes sparsely hairy between most facets. Antenna (Fig. 4.10, Table 4.2): 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, distal segments XIII–XV densely and evenly clothed with spiculae; lengths of segments III–XV: 71,3–36,3–37,5–37,5–37,5–37,5–37,5–37,6–37,5–36,3–96,3,75,0–92,5 μm (n = 1); sensilla coeloconica distribution: 100 % with two on segment III, 100 % with one on XIII, 100 % with one on XIV and 92 % with two and 8 % with one on XV (n = 25); 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 x longer than segment) on XIII, 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 = 25); sensilla trichodea distribution on segment III–XII: III with two long blunt-tipped trichodea, segments IV–VI with two long and one short blunt-tipped trichodea, segments VII–IX with one long and one short blunt-tipped trichodea, segment X with one short blunt-tipped trichodea only, segments XI and XII lacking trichodea (n = 25). The only deviation from the norm noted was an additional long blunt-tipped trichodea in two antennae on segment VII; this extra trichodea was shorter than normal and obviously aberrant. The distributions of the sensilla coeloconica, chaetica and trichodea are identical to those found in *C. imicola* and *C. bolitinos* and appear in Table 4.2.

Wing: (Fig. 4.9). Genitalia (Fig. 4.11, 4.12), tergum nine (Fig. 4.11) almost square, fractionally waisted transversely at midlength, finely spiculate throughout except for narrow strips of the anterior and posterior margins being bare, bearing 14–17 chaetica of different lengths, mean 15 (n = 25); apicolateral processes replaced by thin, hyaline flanges lacking tiny spiculae but each carrying a single fine, rather short straight chaetica issuing from the interface that comprises the base of the flange and the adjoining spiculate fringe where the concave body of the tergum commences, posterior margin of tergum which separates these flanges gently concave, lacking marked indentation or infuscation; two well-developed

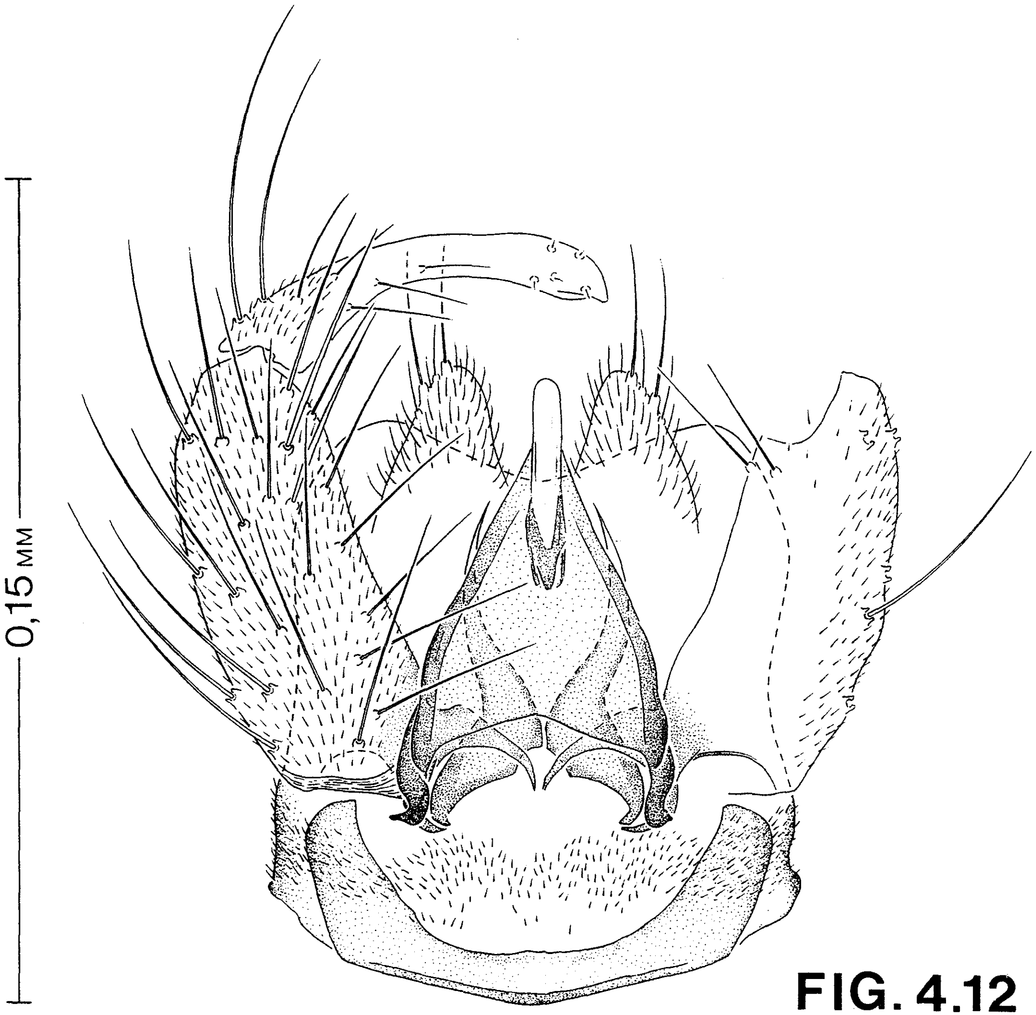


FIG. 4.12

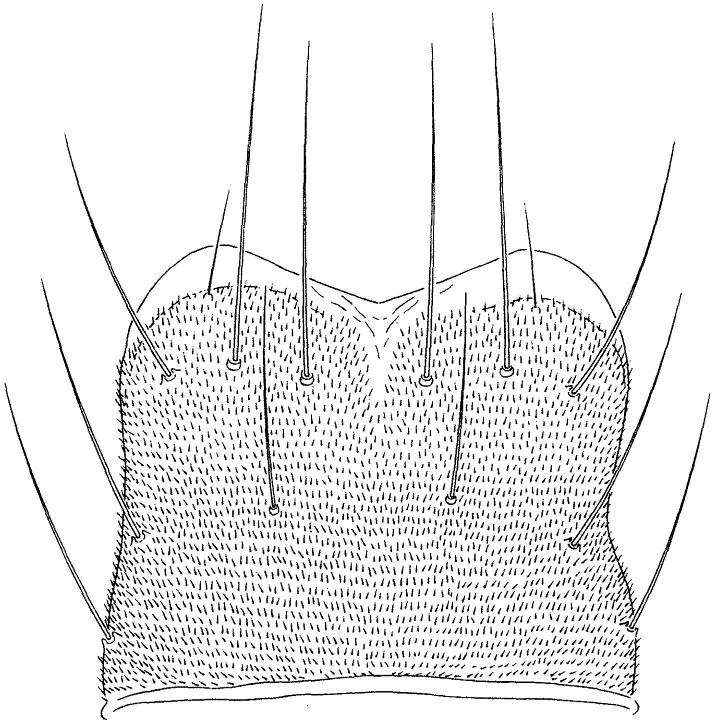


FIG. 4.11

Fig. 4.11 *Culicoides (Avaritia) miombo*. Genitalia, male: tergum IX (paratype Malawi 161).

Fig. 4.12 *Culicoides (Avaritia) miombo*. Genitalia, male (paratype Malawi 161).

FIG. 4.13

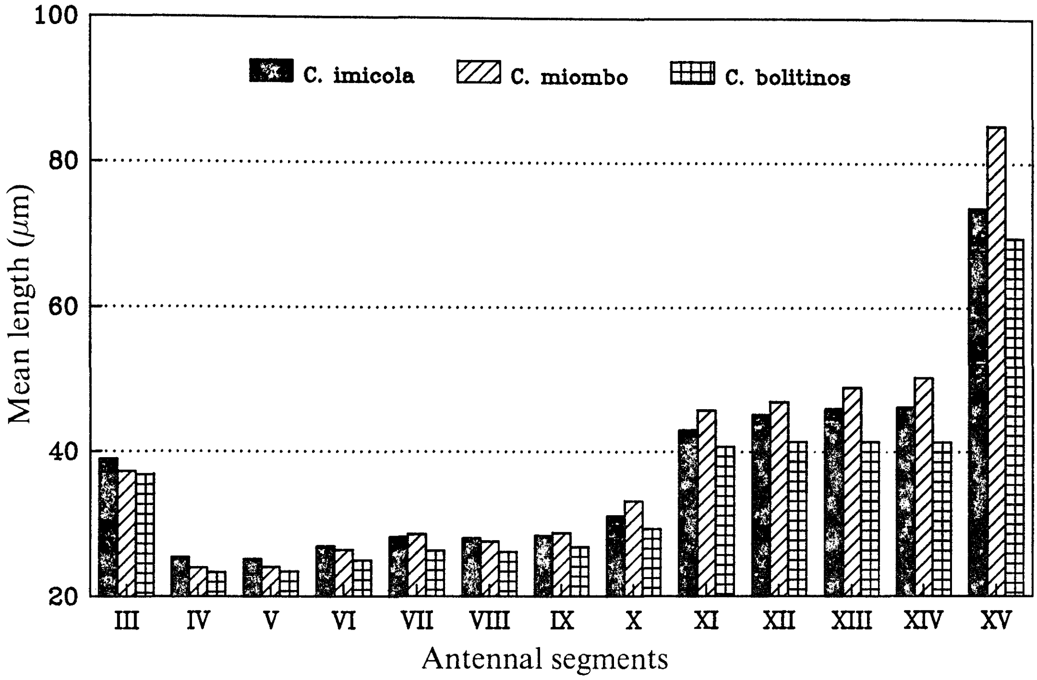


FIG. 4.14

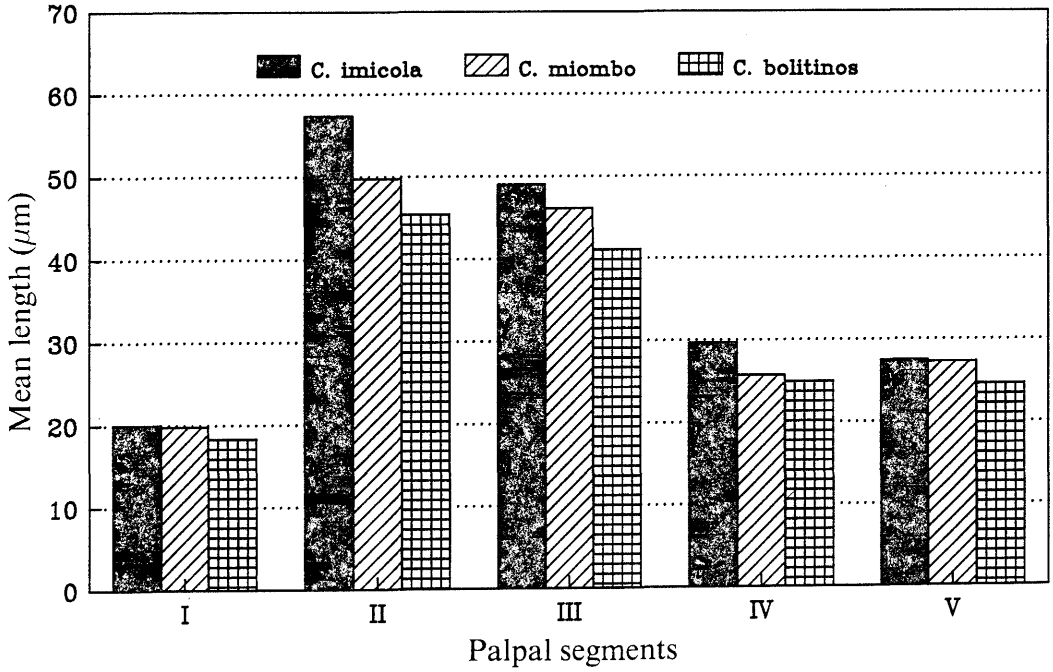


Fig. 4.13 Comparison of mean lengths (μm) of each of female antennal segments III-XV of *C. (Avaritia) imicola, C. (A.) miombo* and *C. (A.) bolitinos.*

Fig. 4.14 Comparison of mean lengths (μm) of each of female palpal segments I-V of *C. (Avaritia) imicola, C. (A.) miombo* and *C. (A.) bolitinos.*

cerci (Fig. 4.12), each bearing with long spiculae and two long and two short chaetica apically; cerci protruding well beyond posterior margin of tergum; sternum nine (Fig. 4.12) with a rather deep excavation, membrane within the excavated area always densely spiculate, bearing 80–280 spiculae, mean 170 (n = 16), basimere with dorsal and ventral spiculae and chaetica as illustrated (Fig. 4.12), basimere 2,6 x as long as broad with basal infuscate collar and well-developed dorsal and ventral roots of the form typical for the subgenus *Avaritia.* Distimere 0,8 x length of basimere, rather stout, gently curved and broadly blunt-tipped; basal half spiculate carrying six to seven bristles of varying lengths and thicknesses, extreme apex with about five very short fine tactile sensilla. Aedeagus (Fig. 4.12) subtriangular, slender, almost equal in length to the basimere; basal arch concave, only fractionally infuscate on lateral margins, distal margin of 4arch reaching to nearly 0,3 x length of aedeagus; lateral margins of aedeagus smooth and gently convex, darkly but narrowly infuscate and converging distad to end in a hyaline, round-tipped, parallel-sided terminal projection whose base projects anteriorly into median area of aedeagus in the form of a raggedly infuscate peg. Parameres (Fig. 4.12) separate, nearly touching medially from where they diverge anteriad and posteriad at 45 °, posterior halves as two convex almost hyaline blades initially stout but tapering smoothly to sharp, simple, erect tips.

Etymology. In southern Africa the widespread *Brachystegia* woodland is locally known as ‘miombo’. Its distribution correlates strongly with that of the new species. All biota in these areas are sensitive to temperate conditions.

Type material

MALAWI: Holotype ♀ (Slide Malawi 820), Kawalazi (11°37’S, 34°06’E) 20 km south-east of Mzuzu, northern Malawi, IV.1989, P. & K. Verster, blacklight at homestead in *Brachystegia* woodland.

73 ♀♀ 68 ♂♂	paratypes, slides labelled and numbered to sex, the collection data as follows:
4 ♀♀	(slides Malawi 153–155, 166), Limphasa dambo, 8 km south-west of Nkhata Bay, 15.XI.1987, R. Meiswinkel truck-trap dusk.
10 ♂♂	(slides Malawi 156–165), Limphasa dambo, 8 km south-west of Nkhata Bay, 15.XI.1987, R. Meiswinkel, truck-trap, dusk.
5 ♀♀	(slides Malawi 208–212), Kawalazi, 20 km south-east of Mzuzu, northern Malawi,

	14.II.1988, P. & K. Verster, blacklight at homestead in *Brachystegia* woodland.
47 ♂♂	(slides Malawi 243–254, 267–270, 917–947), Limphasa dambo, 8 km south-west of Nkhata Bay, 26.X.1987, R. Meiswinkel, truck-trap, dusk 17:00–18:00.
1 ♂	(slide Malawi 60), Kawalaz, 20 km south-east of Mzuzu, northern Malawi, 14.XI.1987, R. Meiswinkel, truck-trap in marshy and wooded area five km north of woodland light-trapping site, dusk.
14 ♀♀	(slides Malawi 854–856, 902, 904–907, 909–914), Kawalazi, 20 km south-east of Mzuzu, northern Malawi, II.1989, P. & K. Verster, blacklight at homestead in *Brachystegia* woodland.
2 ♂♂	(slides Malawi 857, 915), Kawalazi, 20 km south-east of Mzuzu, northern Malawi, II.1989, P. & K. Verster, blacklight at homestead in *Brachystegia* woodland.
6 ♀♀	(slides Malawi 837–842), Kawalazi, 20 km south-east of Mzuzu, northern Malawi, III.1989, P. & K. Verster, blacklight at homestead in *Brachystega* woodland.
1 ♂	(slide Malawi 858), Kawalazi, 20 km south-east of Mzuzu, northern Malawi, III.1989. P. & K. Verster, blacklight at homestead in *Brachystegia* woodland.
44 ♀♀	(slides Malawi 801–819, 821–833, 843–853, 953), Kawalazi, 20 km south-east of Mzuzu, northern Malawi, IV.1989, P. & K. Verster, blacklight at homestead in *Brachystegia* woodland.
7 ♂♂	(slides Malawi 834–836, 950–952, 976), Kawalazi, 20 km south-east of Mzuzu, northern Malawi, IV.1989, P. & K. Verster, blacklight at homestead in *Brachystegia* woodland.

Paratype slides from this type series will be deposited in the following Museums:

1 ♂ 1 ♀;	The Natural History Museum, London.
1 ♂ 1 ♀;	United States National Museum, Washington, D.C.
1 ♂ 1 ♀;	Museum National d'Histoire naturelle, Paris.
1 ♀ 1 ♂;	Australian National Insect Collection, Canberra.

Holotype ♀ and remaining paratype ♀♀ and ♂♂ in the Onderstepoort collection.

Other slide material examined but not forming part of type series:

ZIMBABWE:

20 ♀♀ 10 ♂♂	(slides Zimbabwe 113–142), Rekomitjie Research Station (16°08'S, 29°24'E), north-western Zimbabwe, 16.III.1988, R.J. Phelps, light-trap, warthog pen.

SOUTH AFRICA:

1 ♀	(slide Ndumu 11), Ndumu Game Reserve (26°55'S, 32°15'E), northern KwaZulu, Natal, 6.VI.1988, R. & P. Meiswinkel, blacklight in camp.
1 ♂	(slide Renosterkoppies 85), 20 km s of Skukuza, Kruger National Park, 18.XI.1992, R. Meiswinkel, blacklight in veld.

BOTSWANA:

5 ♀♀	(slides Botswana 1–5), Mamalakwe river near Maun (20°01'S, 23°25'E), northern Botswana, 6.VI.1988, H.V. de V. Clarke, light trap.

IVORY COAST:

5 ♀♀ 8 ♂♂	(slides Ivory Coast 1–13), Parhadi (8°27'N, 3°29'W), north-eastern Ivory Coast, 25.IX.1990. R. Meiswinkel & J.C. Koffauth, blacklight at 15 cattle on edge of village in disturbed forest.

NIGERIA:

1 ♀ 1 ♂	Vom (9°45'N, 8°46'E), north-eastern Nigeria, 31.VIII.1975, W. Taylor, at light.

Unmounted material examined

IVORY COAST:

125 ♀♀ 1 ♂	Yamoussoukro (6°49'N, 5°16'W), central Ivory Coast, 13.IX.1990, R. Meiswinkel & L.E.O. Braack, blacklight at cattle kraal.
24 ♀♀	Ganse (8°35'N, 3°54'W), north-eastern Ivory Coast, 24.IX.1990, R. Meiswinkel & G.J. Venter, blacklight at sheep and goats in village.
1 ♀	Biankouma (7°45'N, 7°18'W), western Ivory Coast, 28.IX.1990, R. Meiswinkel & J.C. Koffauth, blacklight at four cattle in dense forest.

15 ♀♀ Parhadi (8°27'N, 3°29'W), north-eastern Ivory Coast, 25.IX.1990, R. Meiswinkel & J.C. Koffauth, blacklight at 15 cattle on edge of village in disturbed forest.

23 ♀♀ Korhogo (9°27'N, 5°39'W), northern Ivory Coast 26.IX.1990, R. Meiswinkel & J.C. Koffauth, blacklight at 40 cattle in cultivated area adjoining ricefields.

KENYA:

19♀♀ 3♂♂ Tiwi, 20 km south of Mombasa, 12.VIII.1994, R. & P. Meiswinkel, blacklight at 33 goats.

SOUTH AFRICA:

4 ♀♀ Kruger National Park, 10 km east of Lower Sabie, 28.VIII.1991, R. Meiswinkel, blacklight 500 m from the heavily reeded and treed confluence of the Mlondozi and Sabie rivers.

1 ♀ Kruger National park, 10 km east of Lower Sabie, 28.VIII.1991, R. Meiswinkel, blacklight on the banks of the Mlondozi river 1 km north of where it joins the Sabie river.

1 ♀ Kruger National Park, 10 km east of Lower Sabie, 17.IX.1991, R. Meiswinkel, blacklight on banks of Mlondozi river.

1 ♀ Kruger National Park, Skukuza, 19.II.1992, R. Meiswinkel, blacklight in research camp on margins of reeded bed of Nwaswitshaka river.

1 ♀ Kruger National park, Skukuza, 16.XI.1992, R. Meiswinkel, blacklight at buffalo boma.

1 ♀ Kruger National Park, 8 km east of Lower Sabie, 9.XII.1992, R. Meiswinkel & C. Kiss, blacklight in open veld 2 km north of Sabie river.

1 ♀ Kruger National Park, Skukuza, 13.I.1993, R. Meiswinkel, blacklight at buffalo boma.

3 ♀♀ Kruger National Park, Skukuza, 23.III.1993, R. Meiswinkel, blacklight at empty elephant and rhinoceros bomas 1 km south of Sabie river.

1 ♀ Kruger National Park, Manzimahle, 19.V.1993, R. Meiswinkel, blacklight at water-filled clay dam 20 km north-east of Skukuza.

2 ♀♀ Kruger National Park, Mutlumuvi, 20.V.1993, R. Meiswinkel, blacklight in dense thicket at confluence of Mutlumuvi and Sand rivers 12 km n.e. of Skukuza.

1 ♀ Kruger National Park, Skukuza, 22.VI.1993, R. Meiswinkel, blacklight at horse stables.

1 ♂ Kruger National Park, ± 3 km east of Skukuza, 4.III.1993, H.A. Standfast & A.L. Dyce, 5-watt

light-trap on edge of dense forests fringing Sabie river, 01:18–02:18; collection no. KNP 93.141/H.

4.3 DISCUSSION

4.3.1 Taxonomy

Culicoides (Avaritia) miombo appears to have been included six times previously in studies on Afrotropical *Culicoides.* Boorman & Dipeolu (1979) identified a Nigerian series as belonging to *C. (A.) brosseti* Vattier & Adam 'on account of the very prominent and extensive yellow markings on the anterior half of the mesonotum'. They did, however, note that their specimens differed in having a higher palpal ratio and lower costal ratio than were described for *C. brosseti* by the original authors Vattier & Adam (1966). I have examined a slide-mounted male and female of the Boorman & Dipeolu (1979) series collected at Vom and consider them to be *C. miombo.* Their photograph of the female wing of *C. brosseti* is inseparable from the one figured above; especially characteristic is the dark streak at the extreme base of the anal cell. Boorman & Dipeolu erroneously described the male genitalia as having the 'tergite without lateral processes, posterior margin almost straight' and the membrane of sternum nine as 'bare'. *Culicoides miombo* in fact has a very densely spiculate membrane and moderately developed apicolateral flanges, exactly as in the Vom male before me. *Culicoides brosseti* appears to be the only truly cavernicolous species of *Culicoides* currently known from the Afrotropical Region. However, *C. brosseti* is not a member of the Imicola group, but, as pointed out by Itoua & Cornet (1986), belongs to the Trifasciellus group which contains a third species, *C. dubitatus* Kremer, Rebholtz-Hirtzel & Delecolle, 1976. The Trifasciellus group differs from the Imicola group in a number of morphological features, involving the male genitalia and the distribution pattern of the long and short blunt-tipped sensilla trichodea on the antennae of both sexes. *C. brosseti,* like *C. miombo,* possesses prominent yellow admedian vittae on the anterior half of the mesonotum, and this is likely the reason why *C. miombo* has been mistakenly identified as *C. brosseti* for the last 20 years.

The second reference to *C. miombo is* one made by Kitaoka, Kaneko & Shinonaga (1984). Their wing photograph of a specimen of *C. imicola* from Ife, Nigeria is clearly that of *C. miombo,* the dark streak at the base of the anal cell once again being highly diagnostic.

The third reference is that of Cornet (1969) who recorded a single female from Simousso, Burkina Faso. It is likely that he was dealing with *C. miombo* and not *C. brosseti.*

The fourth reference is that of Kremer (1972) who recorded 'trés nombreux... femelles et mâles' of *C. brosseti* from Dundo, Angola. Though no taxonomic data were given, it is likely that he too had *C. miombo* before him.

The fifth reference is the record of 16 ♀♀ and 1 ♂ *C. brosseti* from Manakara, Madagascar (Kremer & Brunhes 1972). As in the Angolan series, no taxonomic data were given, but as the two studies were published by Kremer in the same year the material examined may have been conspecific.

The final reference (Kitaoka & Zulu 1990) deals with two unnamed species of *Culicoides* from Chilanga, Zambia, provisionally labelled as C. A–1 and C. A–10. These authors note that they are 'members of the *brosseti* subgroup of the subgenus *Avaritia* . . . which have two yellowish vittae on the thorax', and that together they comprised only 4 % of nearly 10 000 *Culicoides* collected around a guinea-pig run. It is certain that these represent *C. miombo* and that their rarer C. A–10 is simply an abnormally dark variant.

I have not seen the material detailed above in the last four references; their identification is thus tentative, and for this reason a question mark is placed next to these records in Fig. 4.1 and 4.2.

4.3.2 **Differential diagnosis**

Fifteen character states that easily separate the two species *C. imicola* and *C. miombo* are summarized in Table 4.7. To differentiate *C. miombo* not only from *C. imicola* but also from *C. bolitinos,* statistical analyses were conducted on the antennal and palpal measurements of the three species. The mean measurement of each antennal and palpal segment, taken from 25 specimens of each species, was first tabulated and then tested for significant differences (Table 4.5, 4.6). It was found that *C. bolitinos* and *C. miombo* showed no difference in measurements between antennal segment III–V and palpal segment IV, while in turn a comparison of *C. imicola* and *C. miombo* showed antennal segments VI–IX and palpal segments I and V to be inseparable. It must be noted that heterogeneous variances occurred in the measurements of antennal segments IV and V but these were accepted as homogeneous mainly because the small size of these segments may have affected the accuracy of their measurements in all three species. Table 4.5 and Fig. 4.13 show that as a trio the three species are most easily separated by significant differences in the relative lengths of antennal segments X–XV. The same separation can be obtained using palpal segments II and III (Table 4.6, Fig. 4.14). Of the 15 character states that separate *C. miombo* and *C. imicola,* six are discussed in detail below. Three characters are very reliable for separating *C. miombo* and *C. imicola* under the dissecting microscope: The former has (a) prominent, pale yellow, admedian vittae on the scutum, (b) haltere knobs brown, and (c) the prominent dark smudge at the base of the anal cell of the wing. These and five other character states are discussed in further detail below; where data are available these two species are also differentiated from *C. bolitinos* and *C. pseudopallidipennis.*

1. Female. Wing: The wing of *C. miombo* is distinctly darker than that of *C. imicola, C. pseudopallidipennis* or *C. bolitinos.* The following wing characters more clearly define *C. miombo* as a good species:

    a. The second pale costal spot is round where it straddles the r–m crossvein, is rather strongly waisted medially only to expand and broadly abut the anterior wing margin. This spot is more quadrate and not waisted medially in the remaining three species of the Afrotropical Imicola group.

    b. The shape of the distal pale spot in the apex of cell R_5: its proximal margin is weakly pointed to rounded; this spot is quite often, especially in darker specimens, indistinctly separated or isolated from the wing apex by a narrow dark strip that runs along the antero-distal wing margin; in still darker specimens the pale spot may almost vanish. In *C. imicola, C. pseudopallidipennis* and *C. bolitinos*, this pale spot always broadly abuts the wing margin and is never isolated from the wing margin.

    c. The median third of the anterior margin of vein M_1 is more brown than pale; predominantly to entirely pale in *C. imicola, C. pseudopallidipennis* and *C. bolitinos.*

    d. Vein M_2 is broadly darkened for most of its length and does not have the very obvious preapical excision seen on the anterior margin in *C. imicola.* In *C. miombo* vein M_2

is near its apex moderately to fairly abruptly tapered on both margins; however, this tapering still leaves both margins of vein M_2 at the apex narrowly dark and never entirely pale as in *C. bolitinos*. Furthermore, in the majority of specimens of *C. miombo,* this preapical tapering once again expands leaving the extreme apex of vein M_2 more broadly dark.

e. Extreme base of anal cell with a prominent, long, dark smudge; in *C. imicola, C. pseudopallidipennis* and *C. bolitinos* this area is entirely pale.

2. Female. Eyes: The extent of hairiness of the eyes in *C. miombo* is a deceptive character state that shows considerable variation. Firstly, the areas between the facets are at most only sparsely adorned with short hairs. These hairs will not be seen in specimens whose eyes are poorly cleared of dark pigment. Secondly, the median transverse band of three to eight rows of facets is usually devoid of hairs with only the more lateral, and thus obscured, interfacetal areas weakly haired. The eyes of *C. miombo* therefore need to be carefully examined in material which is properly prepared. *Culicoides miombo* appears to be the only member of the Imicola group worldwide that possesses hairy eyes.

3. Female. Palps: The mean length of the entire palp in *C. miombo* (mean 169,8 μm; n = 25) is almost exactly intermediate between that of *C. imicola* (mean 183,6 μm, n = 25) and *C. bolitinos* (mean 154,6 μm, n = 25) as shown in Fig. 4.14. This is because the three species are quite easily separable on the mean lengths of palpal segments II and III.

   In *C. pseudopallidipennis* the mean length of the palp is 175,3 μm, i.e. very similar to *C. imicola* and *C. miombo*. However, *C. pseudopallidipennis* differs in that it has a swollen third palpal segment.

4. Female. Antennae: As to the length of the entire antenna the converse is true; that of *C. miombo* is the longest (mean 506,0 μm; n = 25); with *C. imicola* intermediate (mean 485,5 μm; n = 25) and that of *C. bolitinos* again shortest (mean 451,9 μm; n = 25). As shown in Table 5 and Fig. 4.13 the three species are easily separable on the mean lengths of antennal segments X–XV.

Those segments with statistically non-significant differences in length are underlined in Table 4.5.

5. Female. Antennae: The antennal trichodea ratio (AtR) gives an equally interesting result in that, despite having the longest antennae of the three species, *C. miombo* carries the shortest long blunt-tipped trichodea on antennal segment VI. These trichodea are longer but more or less equal in size in *C. imicola* and *C. bolitinos.* As illustrated by Clastrier (1958), these trichodea are rather short and unusually swollen in *C. pseudopallidipennis.*

6. Male. The males of the three species differ most significantly in the extent of spiculation on the membrane of sternum nine of the genitalia. In *C. miombo* it is moderately to densely spiculate (80–280 spiculae, mean 170; n = 16), in *C. imicola* it is sparsely to moderately spiculate (8–145 spiculae, mean 47; n = 50) whereas in *C. bolitinos* it is normally bare, occasionally carrying a few spiculae (0–18 spiculae, mean 2,56; n = 50); this membrane is also bare in *C. pseudopallidipennis.* There are subtle differences between the former three species in the shape of the posterior margin of tergum nine, but these are difficult to quantify.

4.3.3 Larval habitat

Unknown; suspected to be in well-vegetated marshy areas. The dung of cattle or any other large herbivore is thought not to be the larval habitat of *C. miombo* as none of nearly 400 specimens examined had the phoretic deutronymph stage of *Myianoetus* mites attached to its abdomen, an association that is commonly found amongst the adults of those *Avaritia* species whose immatures develop exclusively in animal dung.

4.3.4 Vector status

Unknown; however, one needs to re-examine the findings of Lee (1979) who assayed 270 000 *Culicoides* caught during the years 1967–1970 at the dairy herd of the University of Ibadan, Nigeria. Seventy isolates of 16 arboviruses belonging to seven groups were made either from single *Culicoides* species pools or from multiple species pools that comprised at least 14 species. These quite likely included *C. miombo* sp. nov., as Lee reported *C. imicola* to be one of the four most abundant species taken during the study. The likelihood that *C. miombo* formed part of Lee's collection is supported by the statement made by Boorman & Dipeolu (1979) that *C. brosseti* (misidentification for *C. miombo)* was taken 'in large numbers with *C. imicola'* at Ibadan.

4.3.5 Distribution

In discussing biota that are strictly tropical in their occurrence (a subject admirably explored for certain sections of the southern African fauna by Stuckenberg 1969) it is worthwhile to recall the words of Nix (1983) '. . . the available evidence indicates that the 20 °C isotherm encloses virtually all occurrences of tropical savanna on all continents' where 'annual mean rainfall ranges between 1 000 and 1 500 mm, annual mean air temperature exceeds 24 °C and mean minimum temperature of the coldest month is between 13–18 °C. However, significant occurrences of tropical savannas are also found between the 8 °C and 13 °C isotherm for the coldest month and the absence of freezing temperatures', while the 8 °C isotherm 'coincides very approximately with the 50 % probability of a freezing temperature occurring in any one year'. Importantly, Nix also notes that frost is a very '... meaningful boundary condition for living organisms'.

This definition fits *C. miombo*, as in Table 4.1 it can be seen that it is largely restricted to those parts of Africa below 1 000 m in altitude, where frost is absent or rare, and where the rainfall is relatively high (700–1 500 mm/annum), especially if this rainfall is distributed through all or most months of the year. It thus seems reasonable to predict that these ecological preferences will preclude *C. miombo* from ever becoming established in the more arid or cooler, more temperate areas of southern Africa, and likely explain why it occurs in such low numbers in Ndumu, South Africa; Maun, Botswana; Rekomitjie, Zimbabwe and Kawalazi, Malawi. All these localities are marginally embraced by the 8 °C minimum isotherm and thus experience frost occasionally (Fig. 4.1) and furthermore will have four to six months of the year hot and dry. For example at Kawalazi, Malawi, during the hot dry months of October and November, *C. miombo* was rarely collected and then only near marshy areas. It was only found more widely once rains had commenced but even so remained uncommon, representing only 0,9 % of 6 043 *Culicoides* collected in the rainy month of April, 1989 (van Ark & Meiswinkel 1992).

In light of *C. miombo*'s apparent need for fairly high temperatures and a high average rainfall, it is pertinent to look north of the equator and repeat Boorman and Dipeolu's 1979 observation that *C. brosseti* (= *C. miombo*) occurred in large numbers in Nigeria. Here the minimum temperature of the coldest month is in the region of 18–22 °C, considerably higher than the 8 °C found in areas sampled for *C. miombo* in southern Africa. Similarly, in the Ivory Coast *C. miombo* was always present in collections made in the northern half of the country (R. Meiswinkel, unpublished data 1990), where at least some rain falls in each month of the year and frost is absent. Only once, however, was *C. miombo* the most abundant species in the Ivory Coast, comprising 26,2 % of a subsample of 500 *Culicoides* caught at zebu cattle on the outskirts of Yamoussoukro. In the same collection *C. imicola* only constituted 0,8 % of the catch (R. Meiswinkel, unpublished data 1990). It is important to note that not a single specimen of *C. miombo* was found amongst ± 10 000 *Culicoides* collected at four sites in the wetter southern half of the Ivory Coast. Although there is a paucity of data, this suggests that *C. miombo* is virtually absent from the very high rainfall forested regions of equatorial Africa. When this is linked to the fact that no specimens of *C. miombo* have been collected in 30 years in the semi-arid and warm-temperate regions of South Africa, the implication is strong that *C. miombo* evolved in the tropical woodlands immediately adjacent to the wetter rainforest block of equatorial Africa.

4.4 CONCLUSIONS

Culicoides miombo is a new member of the Imicola group; it is widespread and can be common in the tropical woodlands of Africa. Because of its preference for high temperatures, good rainfall and frost-free regimes, it is unlikely that *C. miombo* will ever become established in the more arid or temperate parts of southern Africa. It has until now been confused taxonomically with *C. brosseti* and *C. imicola*, and as a result little is known about its host preferences, seasonal abundance and prevalence. Its larval habitat also remains undiscovered. Finally, *C. miombo* deserves mention as a potential vector of cattle viruses.

4.5 REFERENCES

BOORMAN, J. & DIPEOLU, O.O. 1979. A taxonomic study of adult Nigerian *Culicoides* Latreille (Diptera: Ceratopogonidae) species. *Occasional Publications of the Entomological Society of Nigeria,* 22:1–121.

BOORMAN, J. & MELLOR, P.S. 1992. *Culicoides* vectors of bluetongue and African horse sickness viruses in Mauritius. *Medical and Veterinary entomology*, 6:306.

CARTER J. 1987. Malawi: wildlife, parks and reserves. Macmillan, London.

CLASTRIER, J. 1958. Notes sur les Cératopogonidés. IV. Cératopogonidés d'Afrique occidentale francaise. *Archives de l'Institut Pasteur d 'Algérie,* 36:192–258.

CLASTRIER, J. 1959. Notes sur les Cérotopogonidés. VIII. Cératopogonidés de I'Ile de la Réunion. *Archives de l'Institut Pasteur d'Algérie*, 37:412–446.

CORNET, M. 1969. Les *Culicoides* (Diptera: Ceratopogonidae) de l'ouest africain (1ère note). *Cahiers de l'office de la Recherche Scientifique et Technique Outre-Mer, Série Entomologie Medicale et Parasitologie*, 15:171–176.

DU TOIT, R.M. 1944. The transmission of bluetongue and horsesickness by *Culicoides. Onderstepoort Journal of Veterinary Research,* 19:7–16.

DYCE, A.L. 1982. Distribution of *Culicoides (Avaritia)* spp. (Diptera: Ceratopogonidae) west of the Pacific Ocean. *In*: ST. GEORGE, T.D. & KAY, B.H. (eds). *Arbovirus Research in Australia.* Proceeding Third Symposium, 35–43.

DYCE, A.L., STANDFAST, H.A. & KAY, B.H. 1972. Collection and preparation of biting midges (Fam. Ceratopogonidae) and other small Diptera for virus isolation. *Journal of the Australian Entomological Society,* 11:91–96.

GLICK J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology,* 27:85–195.

HOWARTH, F.G. 1985. Biosystematics of the *Culicoides* of Laos (Diptera: Ceratopogonidae). *International Journal of Entomology*, 27:1–96.

ITOUA, A. & CORNET, M. 1986. Les Ceratopogonidae (Diptera) du Mayombe Congolais. III. Revue taxonomique des espéces du genre *Culicoides* Latreille 1809. *Cahiers de l'office de la Recherche Scientifique et Technique Outre-Mer, Série Entomologie Medicale et Parasitologie,* 24:233–250.

KITAOKA, S. 1985. Japanese *Culicoides* (Diptera: Ceratopo-gonidae) and keys for the species. I. *Bulletin of the National Institute for Animal Health*, 87:73–89.

KITAOKA, S., KANEKO, K. & SHINONAGA, S. 1984. Survey of blood-sucking species of *Culicoides* in Ife, Nigeria. *Journal of the Achi Medical University Association,* 12:454–458.

KITAOKA, S. & ZULU, F.P. 1990. Species composition of *Culicoides* (Diptera: Ceratopogonidae) found at Chilanga near Lusaka, Zambia. *Bulletin of the Niigata Sangyo University*, 4:197–206.

KREMER, M. 1972. *Culicoides* (Diptera: Ceratopogonidae) de la region éthiopienne et particulièrement d'Angola (IIe Note). (Espèces nouvelles, redescription et chorologie). *Das Publicaçoes Culturais da Companhia de Diamantes de Angola,* Lisboa, 84:81–107.

KREMER, M. & BRUNHES. J. 1972. Description de *Culicoides bisolis* (Diptère: Cératopogonide) de Madagascar. *Cahiers de l'office de la Recherche Scientifique et Technique Outre-Mer, Serie Entomologie Medicale et Parasitologie,* 10:287–290.

KREMER, M., REBHOLTZ-HIRTZEL. C. & DELECOLLE, J.C. 1976. Description d'une espèce nouvelle: *C. dubitatus* n. sp. (Diptera: Ceratopogonidae) de la région éthiopienne. *Cahiers de l'office de la Recherche scientifique et Technique Outre-Mer, Serie Entomologie Médicale et Parasitologie,* 13:233–236.

LEE, V.H. 1979. Isolation of viruses from field populations of *Culicoides* (Diptera: Ceratopogonidae) in Nigeria. *Journal of Medical Entomology*, 16:76–79.

MEISWINKEL, R. 1987. Afrotropical *Culicoides:* A redescription of *C. (Avaritia) kanagai* Khamala & Kettle, 1971, reared from elephant dung in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 54:585–590.

MEISWINKEL, R. 1989a. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research,* 56:23–39.

MEISWINKEL, R. 1989b. Afrotropical *Culicoides: C. (Avaritia) spinifer* Khamala & Kettle, 1971, a name based on an artefact (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research,* 56:287–288.

MULLER, M.J., STANDFAST, H.A., ST. GEORGE, T.D. & CYBINSKI, D.H. 1982. *Culicoides brevitarsis* (Diptera: Ceratopogonidae) as a vector of arboviruses in Australia. *Arbovirus Research in Australia.* Proceedings Third Symposium, 43–49.

MURRAY, M.D. 1987. Local dispersal of the biting midge *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) in south-eastern Australia. *Australian Journal of Zoology,* 35:559–573.

MURRAY, M.D. & NIX, H.A. 1987. Southern limits of distribution and abundance of the biting-midge *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) in south-eastern Australia: An application of the GROWEST model. *Australian Journal of Zoology,* 35:575–585.

NIX, H.A. 1983. Climate of tropical savannas. Chapter 3, pp. 37-62. *In*: BOURLIèRE, F. (ed.). Ecosystems of the world. 13. Tropical Savannas. Elsevier Scientific Publishing Company, Amsterdam.

ROSEN, B.R. 1988. From fossils to earth history: Applied historical biogeography. Chapter 14, pp. 437–481. *In:* MYERS, A.A. & GILLER, P.S. (eds). Analytical Biogeography: An integrated approach to the study of animal and plant distributions. Chapman & Hall, London.

ST. GEORGE, T.D. & MULLER, M.J. 1984. The isolation of a bluetongue virus from *Culicoides brevitarsis*. *Australian Veterinary Journal*, 61:95.

STUCKENBERG, B.R. 1969. Effective temperature as an ecological factor in southern Africa. *Zoologica Africana*, 4:145–197.

VAN ARK, H. & MEISWINKEL, R. 1992. Subsampling of large light trap catches of *Culicoides* (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 59:183–189.

VATTIER, G., & ADAM, J.P. 1966. Capture de Ceratopogonidae (Diptera) dans des grottes de la Republique Gabonaise. *Biologia Gabon,* 2:295–309.

WHITE, F. 1983. The vegetation of Africa. A descriptive memoir to accompany the Unesco/AeTFAT/UNSO vegetation map of Africa and Maps. Paris. UNESCO.

WIRTH, W.W. & DYCE, A.L. 1985. The current taxonomic status of the *Culicoides* vectors of bluetongue virus. Bluetongue and related Orbiviruses. Alan R. Liss, New York.

WIRTH, W.W. & HUBERT, A.A. 1989. The *Culicoides* of south-east Asia (Diptera: Ceratopogonidae). *Memoirs of the American Entomological Institute*, 44:1–508.

TABLE 4.1 List of 18 localities from which *C. (Avaritia) miombo* has been recorded in Africa and Madagascar with details of associated major phytochorion, vegetation unit, altitude, mean annual temperature, rainfall and incidence of frost

Locality	Co-ordinates	Altitude (a.s.l.)	Major phytochorion (after White, 1983)	Vegetation unit	Mean annual temperature	Rainfall (mm per annum)	Frost/ no frost
Ibadan **NIGERIA**	7°17'N, 3°30'E	< 500m	Transition between Guineo-Congolian regional centre of endemism (I) and the Sudanian regional centre of endemism (III)	2	26° C	1200	no frost
Ife, NIGERIA	7°28'N, 4°34'E	< 500m		2 11a,12			
Vom, NIGERIA	9°45'N, 8°46'E	1220 m	(III)	27, 29a, 32	24–28° C	1400	no frost
Simousso, BURKINA FASO	11°01'N, 4°03'W	< 500m	(III)	27, 29a,	24–28° C	1400	no frost
Parhadi, IVORY COAST	8°27'N, 3°29'W	300–400 m	(III)	11a	25,5° C	1100	no frost
Ganse, IVORY COAST	8°35'N, 3°54'W	100–200 m	(III)	11a	25,5° C	1100	no frost
Korhogo, IVORY COAST	9°27'N, 5°39'W	300–400 m	(III)	27	26° C	1300–1400	no frost
Yamoussoukro, IVORY COAST	6°49'N, 5°16'W	200–300 m	Guinea-Congolia/Sudania regional transition zone XI	2, 11a	25,5° C	1100–1200	no frost
Biankouma, IVORY COAST	7°45'N, 7°18'W	500–700 m	(XI)	11a	24,5° C	1400–1500	no frost

Dundo, ANGOLA	9°46'S, 14°42'E	< 500 m	Guineao-Congolia/Zambezian regional transition zone (X)	29c	21° C	700–900	no frost
Kawalazi & Limphasa, MALAWI	11°37'S, 34°06'E	600–700 m	Zambezian regional centre of endemism (II)	25	18–24° C	1400	frost localized to above 1200m altitude and in depressions
Rekomitjie, ZIMBABWE	16°08'S, 29°24'E	500–1000 m	(II)	26, 28	18–24° C	1400	frost localized to above 1200m altitude and in depressions
Maun, BOTSWANA	20°01'S 23°25'E	500–1000 m	(II)	28, 35a	18–24° C	1400	frost localized to above 1200m altitude and in depressions
Ndumu, SOUTH AFRICA	26°55'S, 32°15'E	0–100 m	Tongoland-Pondoland regional mosaic (XV) of undifferentiated woodland in transition to bushland: 20 % of woody plants are Zambezian linking species	29e, 16c	22° C	600–1000	no frost
Manakara, MADAGASCAR	22°06'S, 48°00'E	0–100 m	East Malagasy regional centre of endemism (XIX) of lowland rain forest and secondary grassland	11b	23–25° C	2000–3000	no frost
Masai-Mara G.R., KENYA	1°30'S, 35°05'E	1700-1900 m	The Somalia-Masai regional centre of endernism (IV)	45	25-30° C	500-1000	no frost
Tiwi, KENYA	4°14'S, 39°34'E	0-200 m	The Zanzibar-Inhambane regional mosaic (XIII)	16a	26° C	800-1200	no frost
Skukuza, SOUTH AFRICA	24°58'S, 31°36'E	230 m	Tongoland-Pondoland regional mosaic (XV)	29e	22° C	500	frost very rare

TABLE 4.2 Mean lengths (μm) of segments and mean distribution of sensillae on the female and male antennae of *C. (A.) miombo*

C. miombo	**Antennal segments**												
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
Female:													
Sens. coeloconica	3	0	0	0	0	0	0	0	0	1	1	1	1
Sens. chaetica	5	3	2	3	2	3	2	3	0	0	0	0	0
Sens. trichodea (blunt-tipped	LL	LLc	LLc	LLc	LLc	LLc	LLc	LLc	–	–	–	–	–
Lengths of segments	36,8	23,3	23,6	25,7	28,1	27,3	28,2	32,7	44,6	46,0	48,4	49,5	83,3
Male:													
Sens. coeloconica	2	0	0	0	0	0	0	0	0	0	1	1	2
Sens. chaetica	5	0	0	0	0	0	0	0	0	0	3	2	0
Sens. trichodea (blunt-tipped)	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	–	–	–	–	–
Lengths of segments	71,3	36,3	37,5	37,5	37,5	37,5	37,5	37,6	37,5	36,3	96,3	75,0	92,5

TABLE 4.3 Number and frequency of coeloconica present on each of female antennal segments III–XV of *C. (Avaritia) miombo*

No. of coeloconica per segment of *C. miombo*	**Antennal segments**							
	III	**IV-IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	–	70	70	69	–	–	1	1
1	–	–	–	1	70	70	68	69
2	–	–	–	–	–	–	1	–
3	69	–	–	–	–	–	–	–
4	1	–	–	–	–	–	–	–
No. of antennae examined	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**

TABLE 4.4 Number and frequency of chaetica on each of female antennal segments III–XV of *C. (Avaritia) miombo*

No. of chaetica per segment *C. miombo*	**Antennal segments**												
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	–	–	–	–	–	–	–	–	68	70	70	70	70
1	–	–	1	–	1	–	–	–	2	–	–	–	–
2	–	–	69	–	69	4	70	3	–	–	–	–	–
3	–	69	–	69	–	64	–	66	–	–	–	–	–
4	–	1	–	1	–	2	–	1	–	–	–	–	–
5	70	–	–	–	–	–	–	–	–	–	–	–	–
No. of antennae examined	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**

TABLE 4.5 Comparison of mean lengths (μm) of female antennal segments III–XV of 3 *Culicoides* species*

Antennal segment	**Species**			**F. value**
	C. imicola **μm**	***C. miombo*** **μm**	***C. bolitinos*** **μm**	
III	39,00	37,30	36,85	11,171
IV	25,45	24,00	23,35	20,261
V	25,20	24,08	23,45	12,500
VI	26,95	26,45	25,00	11,641
VII	28,23	28,63	26,35	14,755
VIII	28,08	27,63	26,18	9,656
IX	28,43	28,80	26,85	13,085
X	31,10	33,20	29,33	32,047
XI	43,00	45,75	40,75	26,595
XII	45,20	46,95	41,40	40,739
XIII	45,98	48,95	41,43	58,127
XIV	46,20	50,30	41,35	56,764
XV	73,78	85,25	69,60	63,220
Total	485,48	506,0 μ	451,85	40,346

All F-values are significant at 5 %; means underlined are not significantly different at 5 %; n = 25 for each species

TABLE 4.6 Comparison of mean lengths (mm) of female palpal segments I–V of 3 *Culicoides* species*

Palpal segment	**Species**			**F-Value**
	C. imicola	***C. miombo***	***C. bolitinos***	
I	20,05	19,85	18,35	6,333
II	57,40	49,80	45,45	96,885
III	49,05	46,15	41,10	45,363
IV	29,65	25,70	24,90	54,368
V	27,45	27,25	24,55	14,207
Total	**183,6**	**169,75**	**154,55**	**118,044**

* All F-values are significant at 5 %; means underlined are not significantly different at 5 %; n = 25 for each species

TABLE 4.7 Fourteen morphological character states used to separate *C. imicola* from *C. miombo*

C. imicola		***C. miombo***	
♀	thorax entirely brown in alcohol	-	thorax with two prominent yellow admedian vittae
-	scutellum entirely brown	-	scutellum narrowly brown medially and broadly yellow laterally
-	base of anal cell entirely pale	-	base of anal cell with a prominent brown streak
-	anterior margin of second pale costal spot straddling the r–m crossvein straight	-	anterior margin strongly indented medially
-	proximal margin of distal pale spot in cell R_5 pointed	-	this margin weakly pointed to rounded
-	median third of anterior margin of vein M_1 pale	-	this area brownish
-	apex of vein M_2 broadly dark on both margins this preceded by a broad, pale and rather deep preapical excision on the anterior margin only; this excision usually touches and sometimes straddles M_2	-	apex of vein M_2 narrowly dark on both margins, this preceded by only a small, pale and indistinctly shallow preapical excision on both the anterior and posterior margins; these excisions do not touch or straddle vein M_2
-	haltere knobs pale	-	haltere knobs brown
-	palp slightly longer: 165,6–182,4 μm mean 176,1 μm (n = 25)	-	palp slightly shorter: 147,5–177,5 μm mean 162,6 μm (n = 70)
-	antennal segments X–XV shorter (see Table 4.4)	-	these segments longer (see Table 4.4)
-	AR 0,95–1,10 mean 1,01 (n = 167)	-	AR 1,09–1,25 mean 1,15 (n = 66)
-	AtR 1,59–2,27 mean 1,86 (n = 173)	-	AtR 1,27–1,76 mean 1,54 (n = 68)
-	eyes bare	-	eyes sparsely to moderately hairy
♂	membrane of sternum nine with eight to 145 spiculae, mean 47 (n = 50)	-	membrane with 80–280 spiculae, mean 170 (n = 16)

CHAPTER 5

Culicoides (Avaritia) loxodontis Meiswinkel, 1992, a member of the Imicola group (Diptera: Ceratopogonidae) associated with the African elephant in the Kruger National Park, South Africa

5.1 INTRODUCTION

The first species of Afrotropical *Culicoides* found to inhabit large herbivore dung was reported by Nevill (1968) to be *C. pallidipennis* Carter, Ingram & Macfie, 1920 (= *C. imicola*) which he had reared from cattle dung in the Transvaal, South Africa. This record was, however, shown by Meiswinkel (1989) to refer not to *C. imicola* but to the previously unknown species *C. bolitinos,* a species that has since been repeatedly reared from cattle dung as well as from the dung of the African buffalo *(Syncerus caffer)* and the blue wildebeest *(Connochaetes taurinus)*. Today *C. bolitinos* is recognised as the species most closely related to the Oriental-Australasian-eastern Palaearctic *C. brevitarsis* Kieffer, the immatures of which also develop exclusively in the dung of Bovidae such as the water buffalo *(Bubalus bubalis)* and cattle (Dyce 1982).

Subsequent to the record by Nevill (1968), Mr. Alan Dyce of Sydney, Australia, during a four-month visit to Onderstepoort in 1973/74, also reared *C. bolitinos* from the dung of the African buffalo and cattle in South Africa. In addition, he made the discovery that three other *Culicoides* species of the subgenus *Avaritia* lived in the dung of the African elephant and the plains zebra *(Equus burchellii).* The *Culicoides* species reared were identified as *C. gulbenkiani* Caeiro, 1959, C. sp. # 54 and C. sp. # 50. None of these is a member of the Imicola group. These findings were made in the Kruger National Park and were reported upon in a short communication (Dyce & Marshall 1989).

In 1985, following upon these leads, I continued investigations on the dung-inhabiting *Culicoides* fauna of the large herbivores of the Kruger National Park, and since then have reared six species of the subgenus *Avaritia* from elephant dung alone. One of these, *C. (A.) kanagai* Khamala & Kettle, 1971, has been reported upon (Meiswinkel 1987). A further three, *C. tororoensis* Khamala & Kettle, 1971, C. sp. # 54 and C. sp. # 50, in large measure support the findings of Dyce & Marshall (1989), the only discrepancies being that their record of *C. gulbenkiani* is most likely a misidentification of *C. tororoensis* and that C. sp. # 54 consists of two closely related species difficult to separate in the adult stage. As regards the taxonomic status of *C. gulbenkiani* and *C. tororoensis*, I disagree with Glick (1990) who stated the latter to be a synonym of the former. These are two valid but closely similar species that need reappraisal both biologically and taxonomically.

The sixth species reared from elephant dung is *C. (A.) loxodontis* which is redescribed below. Unlike the others known from elephant dung, it is a member of the Imicola group, which in turn is one of at least six groups that constitute the subgenus *Avaritia* in the Afrotropical Region. In Africa five species of the Imicola group have thus far been described: *C. imicola, C. pseudopallidipennis, C. bolitinos, C. miombo* and *C. loxodontis*. Only *C. bolitinos* and *C. loxodontis* have their immatures developing in animal dung.

As in previous chapters, some emphasis is placed on a numerical description of *C. loxodontis* so as to differentiate it more clearly from three of its taxonomic congeners *C. imicola, C. bolitinos* and *C. miombo*. While *C. pseudopallidipennis* will be redescribed in Chapter 6, two character states easily separate it from *C. loxodontis* and these will be discussed.

5.2 MATERIALS AND METHODS

The type material of *C. loxodontis* was collected in the Kruger National Park, either by light-trap or reared from elephant dung. The dung was randomly collected, and placed in a cardboard box, and then stored in a fine gauze net to await emergence of *Culicoides*, if present. The dung belonged to two age categories. Either it was (i) a discrete round bolus or boli only a few hours old, or (ii) was a one to two day-old subsample of one to five boli that had been flattened by

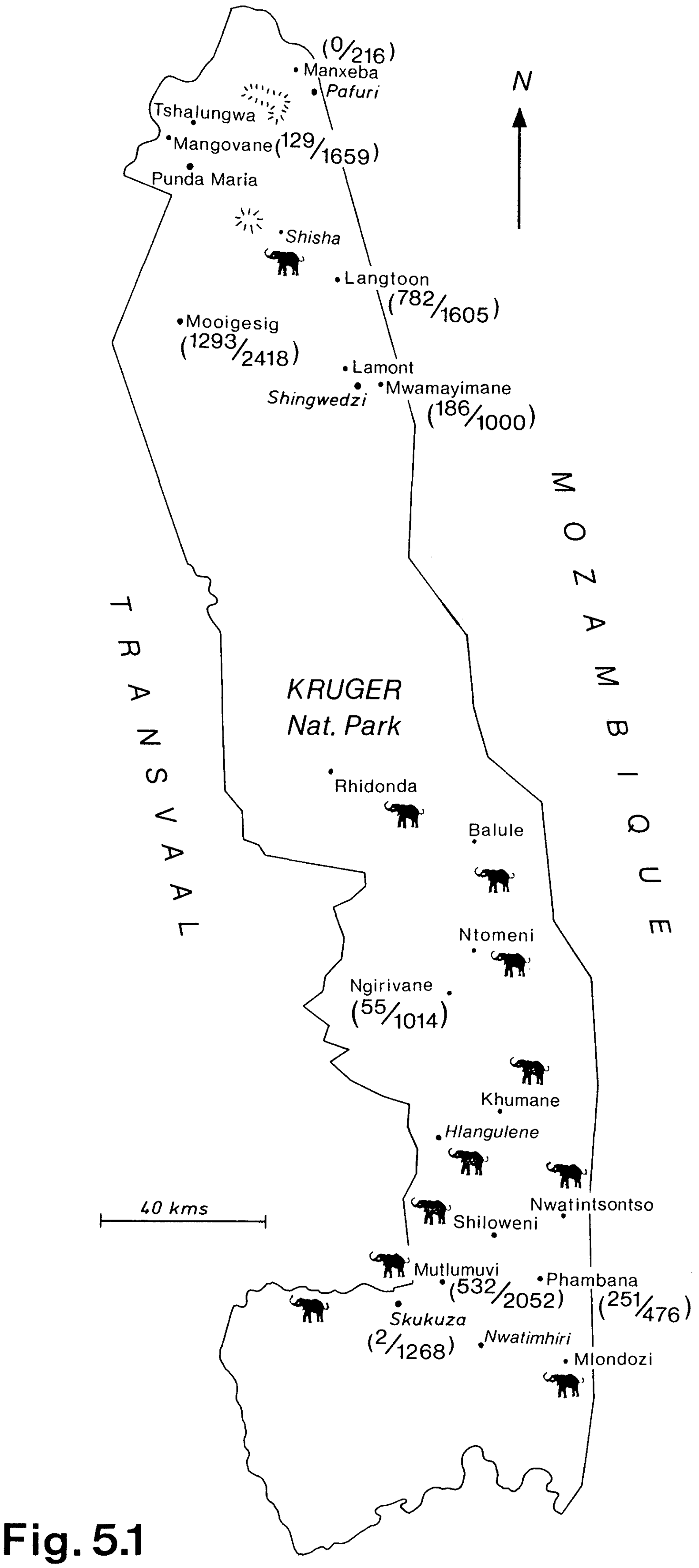


Fig. 5.1 A map of the Kruger National Park indicating localities where *C. (Avaritia) loxodontis* was either reared from elephant dung (elephant symbols) or collected in light-traps; in parentheses the number of *C. (A.) loxodontis* captured is given as a fraction of the total number of *Culicoides* midges collected.

the workings of various dung-beetle (Scarabaeoidea) species into a large (± 1 m diameter) 30–50 mm high amorphous mass. All dung was collected off hard substrates, mostly tarred or sand roads, or from similarly bare unvegetated patches away from roads. None of the samples included soil, water or vegetation that was not evacuated by the elephant itself. Of the more than 200 specimens used for the description of *C. loxodontis*, only one female, 24 males and one associated pupal exuvia reared from elephant dung, are included in the type series. Under field conditions it proved difficult to keep such tiny insects alive for more than 48 hours in order to have specimens sufficiently mature for mounting in Canada balsam.

5.3 RESULTS

5.3.1 ***Culicoides (Avaritia) loxodontis*** (Fig. 5.2–5.15; Table 5.1–5.6)

Female (Fig. 5.2-5.7, 5.13–5.15; Table 5.1–5.6)

Head. Eyes (Fig. 5.2); bare, contiguous over a distance equal approximately to the diameters of between one and two adjacent facets. Antenna (Fig. 5.3, 5.14; Table 5.1–5.4) slender, basal segments IV–X barrel-shaped distal segments XI–XIV more or less parallel-sided only narrowing fractionally medially, XV parallel-sided only narrowing apically; mean lengths of antennal segments III–XV: 39,6–25,2–24,5–26,6–26,3–25,8–26,2–28,5–38,6–42,1–43,9–42,6–63,5 μm (n = 80); total length of antenna: 428,8–516,3 μm, mean 453,1 μm (n = 80); widths of antennal segments III–XV: 28,8–22,5–20,0–19,4–18,8–18,8–17,5–17,5–16,3–16,9–16,3–16,9–18,1 (n = 1); AR 0,92–1,08, mean 0,99 (n = 80); sensilla coeloconica present on segments III, XII–XV in 92,5 % of antennae examined (n = 81), see Table 5.2 for deviations from the norm; antenna with a total of six to eight coeloconica, mean 6,93 (n = 81); mean sensilla chaetica distribution on segments III–XV is 5,00–3,52–2,15–4,04–2,06–4,00–2,04–3,02–0,05–0–0–0–0 (n = 81), see Table 5.3 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 = 81); AtR 1,35–1,67 mean 1,47 (n = 42); segments XI–XIV each with 13–17 sharp-tipped sensilla trichodea of varying lengths and thicknesses distributed in a basal and subapical whorl, XV with approximately

Fig. 5.2

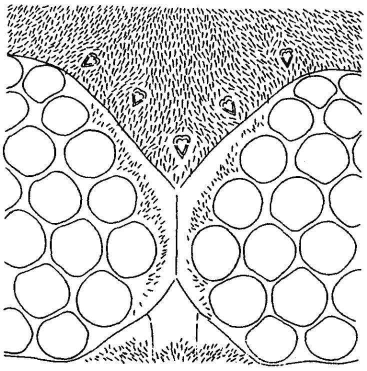


Fig. 5.2 *Culicoides (Avaritia) loxodontis*. Eyes, female (paratype Pafuri 6).

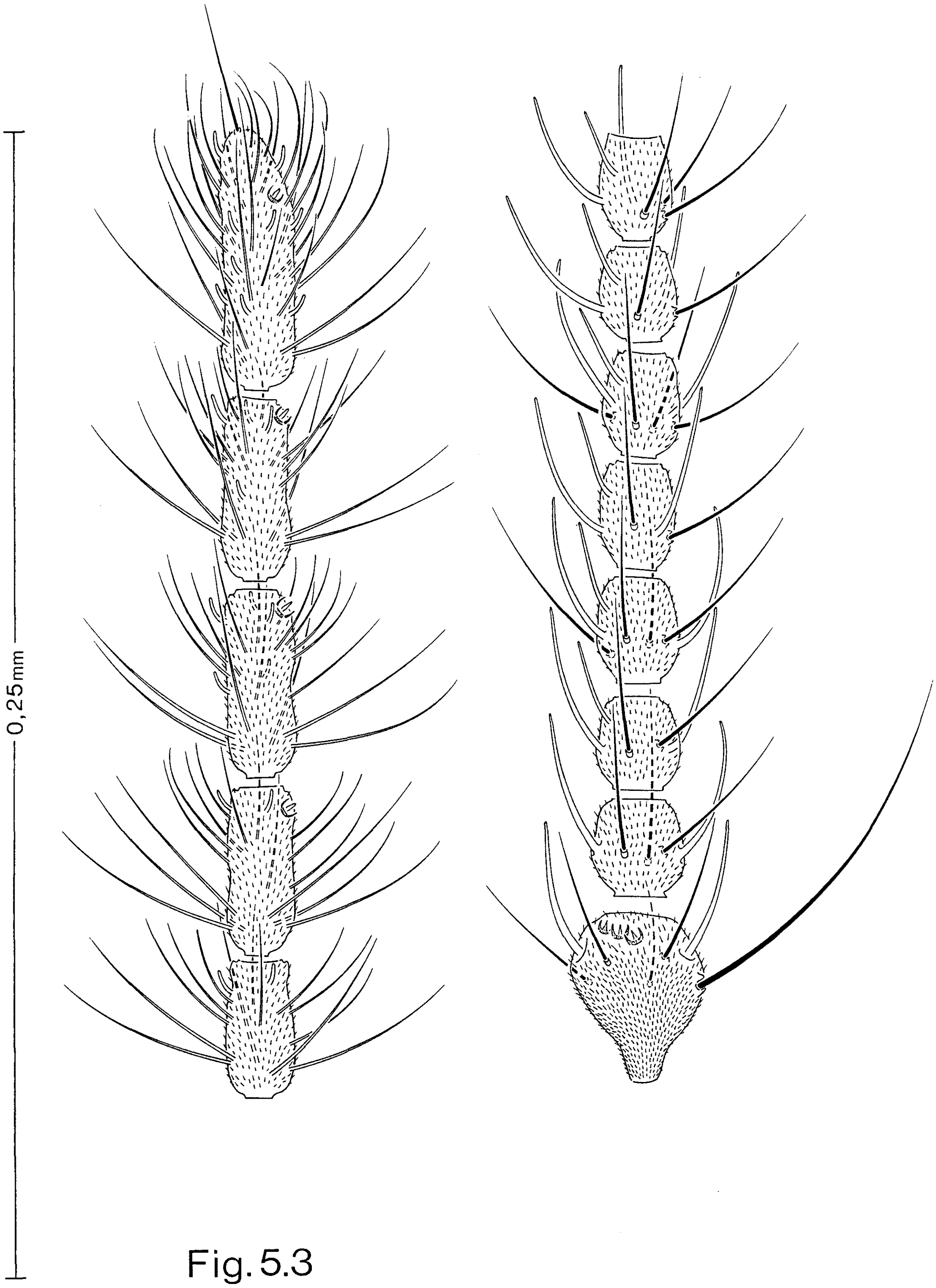


Fig. 5.3

Fig. 5.3 *Culicodes (Avaritia) loxodontis*. Antenna, female: segments XI–XV on left, segments III–X on right (paratype Shingwedzi 268).

twice as many trichodea, these distributed almost throughout segment (Fig. 5.3); similarly the short blunt-tipped basiconica range from two to nine on each of the distal flagellar segments XI–XV (Fig. 5.3); all antennal segments uniformly clothed throughout with fine spiculae (Fig. 5.3). The range and mean number of sensilla coeloconica, chaetica and blunt-tipped trichodea are given in Table 5.1. Palp (Fig. 5.4, 5.15; Table 5.5); of a moderate length, slender, light brown throughout mean length of palpal segments I–V; 22,65–54,43–44,95–25,75–25,73 μm (n = 63); total length 147,5–175,0 μm, mean 163,3 μm (n = 63); palpal segment I with one to two rather long chaetica, mean 1,01 (n = 77), II with two to four rather short chaetica, mean 3,00 (97 % with three chaetica; n = 77); III of a moderate length, slightly swollen and carrying three to five rather short chaetica, mean 3,07 (93 % with three chaetica, n = 70), with a small, round and shallow subapical pit with diameter of opening 35–50 % the width of segment, margin of pit smooth but well defined; IV with one to four short chaetica, mean 2,66 (45 % with three chaetica; n = 77); V with no chaetica on median portion of segment but always bears five short erect bristles apically; PR 2,07–2,92, mean 2,53 (n = 80); P/H ratio 0,80–0,89, mean 0,84 (n = 20); mandible with 12–16 teeth, mean 13,2 (n = 75).

Thorax. Legs: brown, with all femora narrowly pale basally, and with fore and middle femora indistinctly pale apically. All tibiae with a narrow, well-defined subbasal pale band; remainder of fore and middle tibiae brown while apices of hind tibiae are indistinctly paler; TR 1,44–1,75, mean 1,68 (n = 40); comb on apex of hind tibia with five spines, the first being the longest and only slightly longer than the second (n = 81). Wing: (Fig. 5.7, 5.13), length 0,77–0,96 mm, mean 0,88 mm (n = 80), breadth 0,38–0,48 mm, mean 0,44 mm (n = 80), CR 0,55–0,59, mean 0,57 (n = 80); 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 greyish-brown, pale areas whitish with microtrichia, and macrotrichia 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 to two-thirds of second cell pale. As a result of the general increase in the size of the pale areas and concomitant reduction of the dark areas, the wing of *C. loxodontis* is paler than that of *C. imicola, C. bolitinos* and *C. miombo,* but comes closest to that of *C. pseudopallidipennis.* The most distinctive feature of the wing pattern in the majority of specimens is for the proximal margin of the distal pale

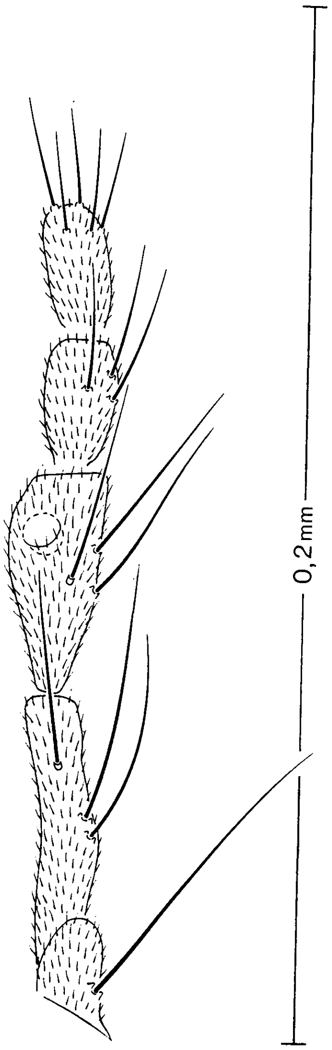


Fig. 5.4

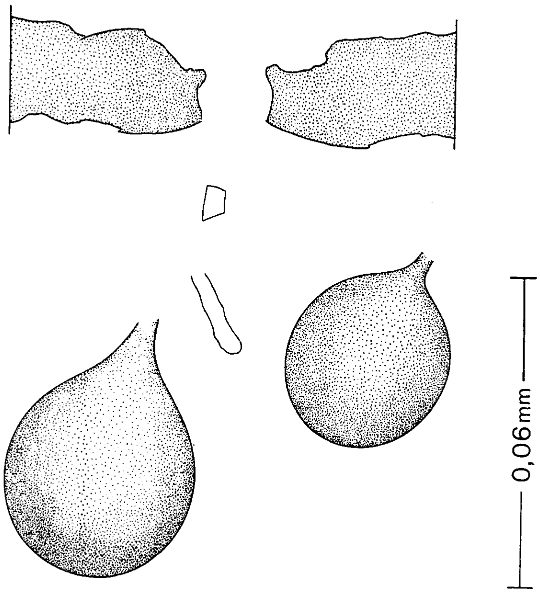


Fig. 5.5

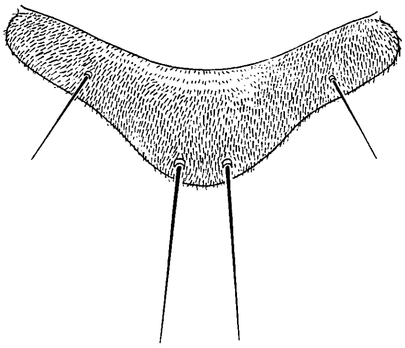


Fig. 5.6

Fig. 5.4 *Culicoides (Avaritia) loxodontis*. Palp, female (paratype Shingwedzi 218).

Fig. 5.5 *Culicoides (Avaritia) loxodontis*. Genitalia, female: spermathecae and sclerotization surrounding gonopore (paratype Shingwedzi 37).

Fig. 5.6 *Culicoides (Avaritia) loxodontis*. Scutellum, female (paratype Shingwedzi 232).

spot in cell R_5 to be more strongly pointed than is found in its taxonomic congeners; occasionally this pointed margin may even fuse narrowly with the distal margin of the third pale costal spot that covers the tip of the second radial cell (Fig. 5.13). Other important specific wing pattern characters are:

(i) The distal margin of the second pale costal spot that straddles the r–m crossvein is expanded medially and tends almost to fuse, under the radial cells, with the third pale costal spot whose proximal margin is expanded medially;

(ii) posterior margin of vein M_2 entirely darkened but anterior margin broadly excised to leave the apex of vein M_2 pale or only narrowly and indistinctly darkened on both margins; this preapical excision is broader than, but not nearly as clearly defined as, that seen in *C. imicola*;

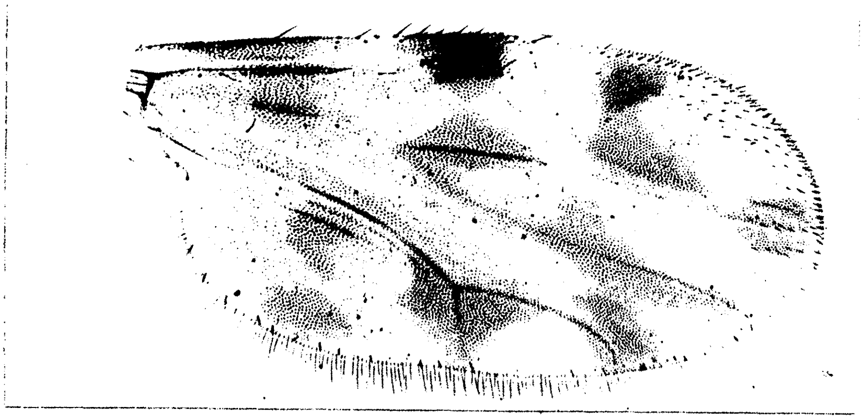
(iii) proximal base of anal angle pale.

Scutum entirely brown in alcohol; scutellum (Fig. 5.5) entirely brown, bearing two long median bristles and one shorter bristle on each corner (n = 41). Haltere knobs pale. Abdomen (Fig. 5.6): two moderately sclerotized slightly unequal spermathecae present, measuring 47,5–56,3 x 35,0– 42,5 μm, mean 52,8 x 41,0 μm and 42,5–43,8 x 30,0–35,0 μm, mean 40,3 x 32,3 μm (n = 20), both round and devoid of small hyaline punctations, with moderately long narrow rudimentary third spermatheca present measuring 11,3–22,5 x 3,1–8,8 μm mean 16,8 x 4,3 μm (n = 27), 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. 5.6.

Male (Fig. 5.8–5.12; Table 5.1).

Head. Eyes bare. Antenna (Fig. 5.9; Table 5.1): 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: 72,6, IV–XII: 338,8, XIII–XV: 75,2–63,1–85,8; total mean

5.7



5.8

Fig. 5.7 *Culicoides (Avaritia) loxodontis*. Wing, female (paratype Pafuri 7).

Fig. 5.8 *Culicoides (Avaritia) loxodontis*. Wing, male (paratype Pafuri 8).

length 635,4 μm (n = 24); sensilla coeloconica distribution: segment III with two (n = 26); XIII with nought to one, mean 0,96 (96 % with one coeloconica, n = 26), XIV with one to two, mean 1,77 (87 % with two coeloconica, n = 26), XV with two coeloconica (n = 26); antenna with a total of five to seven coeloconica, mean 6,65 (n = 26); sensilla chaetica distribution: five of varying lengths and thicknesses on III; two (sometimes three) basally (first long and robust, second shorter and weaker) and one medially (being very slender and 1,5 times longer than segment) on XIII (the deviations from the norm were an additional long and robust sensilla chaetica basally on segment XIII in five antennae, while one antenna had the long medially-occurring sensilla chaetica absent); 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 = 26); blunt-tipped sensilla trichodea distribution on segment III–XII: III with two long sensilla trichodea, 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 = 26), the only deviation from the norm noted was an additional long blunt-tipped sensilla trichodea in one antenna on segment V, and a short blunt-tipped trichodea in one antenna on segment XI; these additional trichodea were shorter than normal and obviously aberrant. The distributions of the sensilla coeloconica, chaetica and trichodea are identical to those of *C. imicola, C. bolitinos* and *C. miombo,* and appear in Table 5.1. Those of *C. pseudopallidipennis* differ to some degee and are detailed in Chapter 6 (Table 6.3). Thorax: Scutellum with two median bristles and no lateral bristles (n = 47). Wing: (Fig. 5.8). Genitalia: (Fig. 5.10–5.12) tergum nine (Fig. 5.10, 5.11) square, slightly waisted medially, finely spiculate throughout except for a narrow strip of the anterior and a broad strip of the posterior margins being bare, bearing 12–23 chaetica of different lengths, mean 16,7 (n = 63); apicolateral processes replaced by thin, hyaline flanges lacking spiculae but each carrying a single fine, rather short sensilla chaetica; posterior margin of tergum which separates these flanges most often straight as in Fig. 5.11, to moderately but very rarely deeply concave as in Fig. 5.10; median area not infuscate but bearing a number of striations (Fig. 5.10); two well-developed cerci (Fig. 5.11), each adorned with long spiculae and two long and two short chaetica; cerci protruding well beyond posterior margin of tergum; sternum nine (Fig. 5.11) with a rather deep and wide excavated area with nought to three spiculae, mean 0,21 (n = 80), of these 87,5 % had no spiculae; basimere with dorsal and

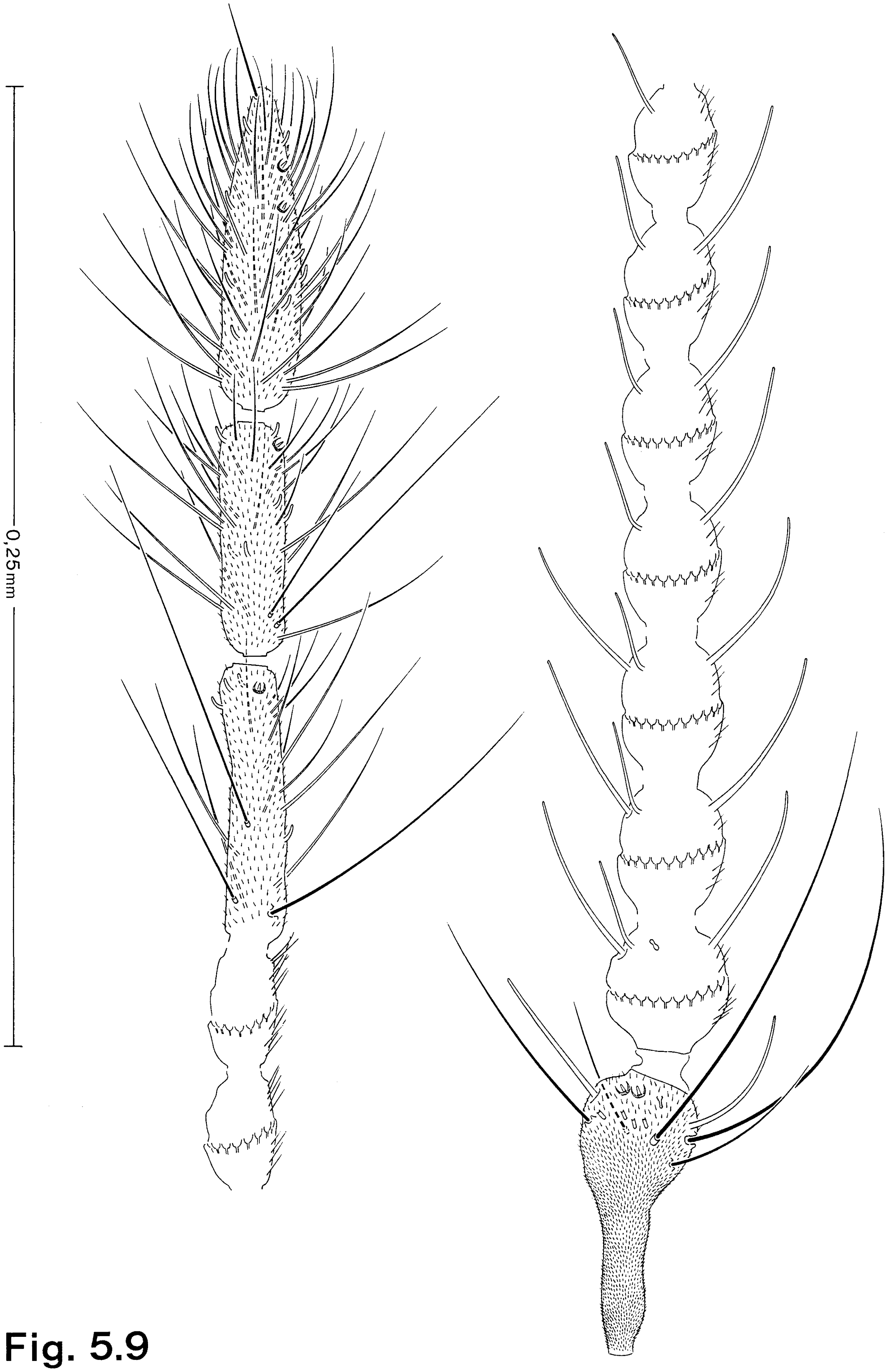


Fig. 5.9

Fig. 5.9 *Culicoides (Avaritia) loxodontis*. Antenna, male: segments XI–XV on left, segments III–X on right (paratype Shingwedzi 283).

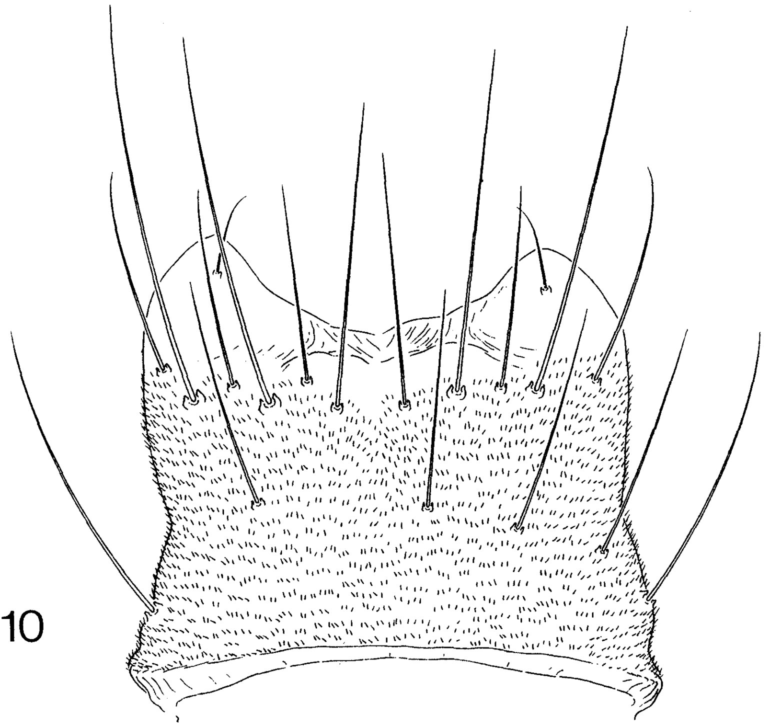


Fig. 5.10

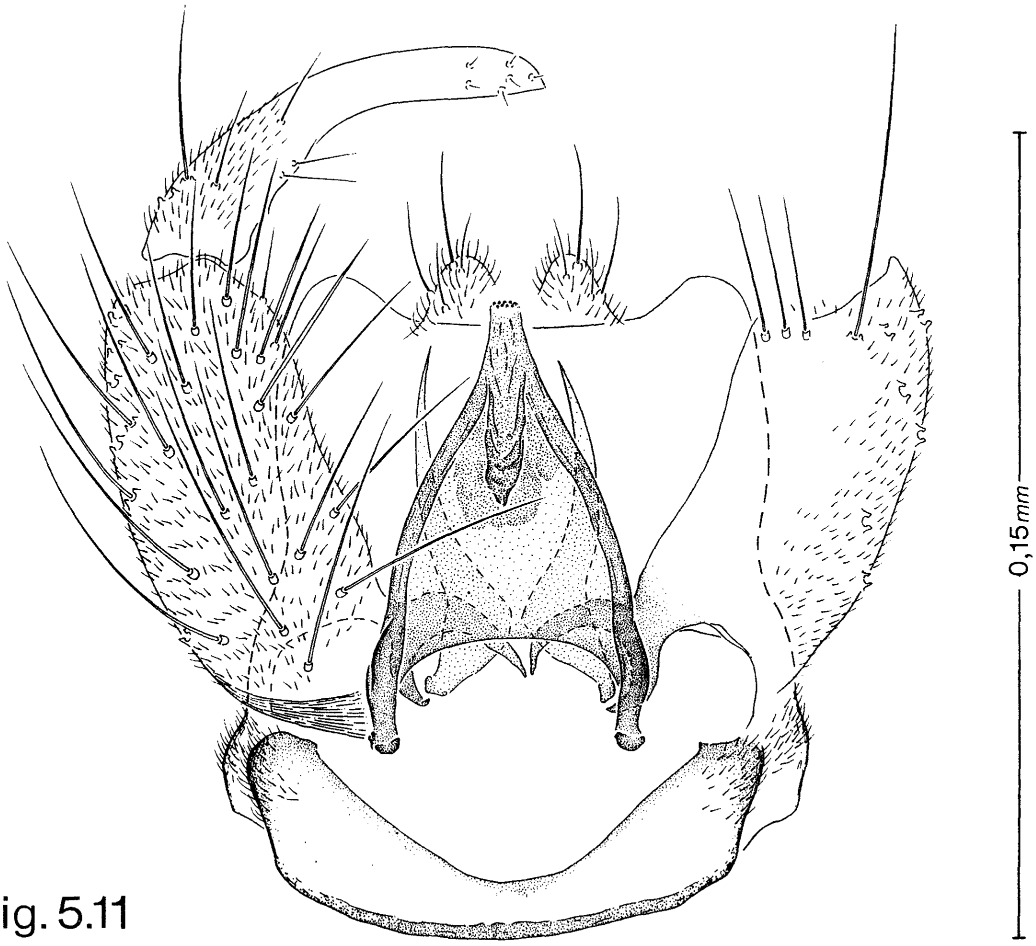


Fig. 5.11

Fig. 5.10 *Culicoides (Avaritia) loxodontis*. Genitalia, male: tergum IX (paratype Hlangulene 16).

Fig. 5.11 *Culicoides (Avaritia) loxodontis*. Genitalia, male (paratype Shingwedzi 117).

ventral spiculae and chaetica as illustrated (Fig. 5.11), basimere 2,4 times as long as broad with basal infuscate collar and well-developed dorsal and ventral roots of the form typical of the subgenus *Avaritia.* Distimere (Fig. 5.11) 0,88 x length of basimere (n = 7), rather stout, gently curved and broadly blunt-tipped, basal half spiculate, carrying six to seven bristles of varying lengths and thicknesses, extreme apex with about six very short fine sensillae. Aedeagus (Fig. 5.11, 5.12) shield-shaped, slender, 1,63–1,86, mean 1,71 x longer than wide (n = 25) and 0,9 x length of basimere (n = 7); basal margin convex, only narrowly and lightly infuscate towards lateral margins, distal margin of arch reaching to 0,18–0,26 x length of aedeagus (n = 7); lateral margins of the aedeagus smooth and gently convex, darkly but narrowly infuscate and converging distad to end in a hyaline, rather short parallel-sided terminal projection whose extreme apex is not smooth but serrated with five to eight tiny sclerotized papillae (Fig. 5.11, 5.12); base of this terminal projection inserts anteriorly into median area of aedeagus in the form of a raggedly infuscate 'peg' (Fig. 5.11). Parameres (Fig. 5.11) 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 distribution pattern of the new species correlates with that of the African elephant *Loxodonta africana* in the KNP; in addition, its immatures appear to develop exclusively in elephant dung, further evidence of a close association.

Type material

SOUTH AFRICA: Holotype ♀ (slide Shingwedzi 224), Lamont windmill (23°06'S, 31°24'E) on banks of Mphongolo river six km north-west of Shingwedzi rest camp, northern Kruger National Park, 20.11.1990, R. Meiswinkel and L.E.O. Braack, blacklight 18:00–22:00.

60 ♀♀ 178 ♂♂	paratypes, slides labelled and numbered to sex, the collection as follows:
6 ♀♀	(slides Shingwedzi 37, 45, 48, 54, 64, 68), Shingwedzi rest camp, northern KNP, 1.IX.1979, B. de Meillon and R. Meiswinkel, blacklight.
3 ♂♂	(slides Shingwedzi 43, 57, 66), (collection data as above).

6 ♀♀	(slides Shingwedzi 82–84, 86, 90, 113), Shingwedzi rest camp, northern KNP, 19.IV.1983, R. Meiswinkel, blacklight.
2 ♂♂	(slides Shingwedzi 100, 117), (collection data as above).
1 ♀	(slide Shingwedzi 78), Shingwedzi rest camp, northern KNP, 7.IX.1984, R.Meiswinkel, blacklight.
40 ♀♀	(slides Shingwedzi 167–171, 173–175, 210–223, 225–233, 261–269), Lamont windmill on banks of Mphongolo river, six km n.w. of Shingwedzi rest camp, northern KNP, 20.II.1990, R. Meiswinkel & L.E.O. Braack, blacklight 18:00–22:00.
24 ♂♂	(slides Shingwedzi 160–165, 177, 234–250), (collection data as above).
94 ♂♂	(slides Shingwedzi 276–295, 373–466), Mooigesig dam area, 45 km w. of Shingwedzi rest camp, northern KNP, 25.IV.1990, R. Meiswinkel, L.E.O. Braack & D. de Klerk, blacklight in *Colophospermum mopane*/*Acacia nigrescens* savanna, 17:00–22:00.
28 ♂♂	(slides Shingwedzi 301–328), Langtoon dam area, 30 km n. of Shingwedzi rest camp, northern KNP, 24.IV.1990, R. Meiswinkel, L.E.O. Braack & D. de Klerk, blacklight in *Colophospermum mopane* shrub-veld, 17:00–22:00.
1 ♀	(slide Pafuri 7), Pafuri anthrax camp on banks of Luvuvhu river, northern KNP, 15.IV.1986, R. Meiswinkel & L.E.O. Braack, blacklight 13 m up *Acacia albida* tree, 18:00–23:00.
1 ♂	(slide Pafuri 8), (collection data as above).
1 ♀	(slide Skukuza 18), Skukuza rest camp, southern KNP, 11.III.1984, R. Meiswinkel & L.E.O. Braack, blacklight on banks of Sabie river.
1 ♂	(slide Skukuza 122), Skukuza rest camp, southern KNP, 15.I.1985, R. Meiswinkel & L.E.O. Braack, blacklight on banks of Sabie river.
1 ♀	(slide Hlangulene 10), 50 km n. of Skukuza rest camp, southern KNP 26.II.1986, R. & P. Meiswinkel, reared from elephant dung.
23 ♂♂	(slides Hlangulene 1–9, 11–16, 19, 22, 25–30), (collection data as above).

1 ♀	(slide Tshalungwa 13), 18 km n. of Punda Maria rest camp n.w. KNP, 10.IV.1986, R. Meiswinkel & L.E.O. Braack, blacklight 18:30–21:00.
1 ♂	(slide Tshalungwa 14), (collection data as above).
3 ♀♀	(slides Phambana 1–3), 28 km e. of Skukuza rest camp, southern KNP, 8.V.1990, D. de Klerk, blacklight 17:00–22:00.
1 ♂	+ pupal exuvia (slide Skukuza 351), ex elephant dung on sand road 20 km w. of Skukuza rest camp, southern KNP, 14.V.1991, H. Nevill.

Unmounted light-trap material examined

248 ♀♀ 3 ♂♂	Phambana, 28 km e. of Skukuza rest camp, southern KNP, 8.V.1990, D. de Klerk, blacklight 17:00–22:00.
74 ♀♀ 55 ♂	Mangovane spring, 10 km n. of Punda Maria rest camp, northern KNP, 3.III.1991, R. Meiswinkel, L.E.O. Braack, Alan L. Dyce & M. Swanepoel, blacklight 18:30–20:15.
157 ♀♀ 29 ♂♂	Mwamayimane windmill, six km e. of Shingwedzi rest camp, northern KNP, 20.II.1990, R. Meiswinkel & L.E.O. Braack, blacklight 17:30–24:00.
1161 ♀♀ 39 ♂♂	Mooigesig dam area, 45 km w. of Shingwedzi rest camp, northern KNP, 25.IV.1990, R. Meiswinkel, L.E.O. Braack & D. de Klerk blacklight in *Colophospermum mopane/Acacia nigrescens* savanna, 17:00–22:00.
728 ♀♀	Langtoon dam area, 30 km n. of Shingwedzi rest camp, northern KNP, 24.IV.1990, R. Meiswinkel, L.E.O. Braack & D. de Klerk, blacklight in *Colophospermum mopane* shrubveld, 17:00–22:00.
50 ♀♀ 5 ♂♂	Ngirivane windmill, 12 km w. of Satara rest camp, central KNP, 18.IX.1991, R. Meiswinkel, H. van Ark & M. Swanepoel, blacklight 18:00–24:00.
14 ♀♀ 1 ♂	At confluence of Mutlumuvi and Sand rivers, 12 km n.e. of Skukuza rest camp, southern KNP, 19.IX.1991, R. Meiswinkel, H. van Ark & M. Swanepoel, blacklight in dry thickets, 18:00–24:00.
454 ♀♀ 78 ♂♂	At confluence of Mutlumuvi and Sand rivers, 12 km n.e. of Skukuza rest camp, southern KNP, 22.I.1992, R. Meiswinkel, blacklight in dry thickets, 18:45–24:00

Unmounted reared material examined

42 ♀♀ 68 ♂♂	Rhidonda windmill, 23 km w. of Letaba rest camp, central KNP, 16.XI.1979, R. Meiswinkel.
30 ♀♀ 45 ♂♂	Shilolweni dam, 38 km n.e. of Skukuza, southern KNP, 22.I.1991, L.E.O. Braack.
163 ♀♀ 323 ♂♂	Khumane dam, 67 km n.e. of Skukuza, southern KNP 22.I.1991, L.E.O. Braack.
57 ♀♀ 87 ♂♂	Nwatintsontso, 50 km n.e. Skukuza, southern KNP, 22.I.1991, L.E.O. Braack.
30 ♀♀ 18 ♂♂	Mlondozi, six km e. of Lower-Sabie rest camp, south-eastern KNP, emerged 16–17.IX.1991 from dung evacuated ± 45 year-old elephant bull 29.VIII.1991, R. Meiswinkel.
14 ♀♀ 26 ♂♂	At confluence of Mutlumuvi and Sand rivers, 12 km n.e. of Skukuza rest camp, southern KNP, emerged 30.IX–4.X.1991 from dung evacuated by ± 12 year-old male 19.IX.1991, R. Meiswinkel.
1 ♀ 9 ♂♂	12 km e. of Skukuza rest camp, southern KNP, emerged 25–26.X.1991 from dung evacuated by ± 35 year-old elephant bull 16.X.1991, R. Meiswinkel, G. J. Venter & E.M. Nevill.
56 ♀♀ 77 ♂♂	13 km s. of Balule rest camp, central KNP, emerged 6.II.1992, from dung evacuated 29.I.1992, R. Meiswinkel.
82 ♀♀ 230 ♂♂	Ntomeni windmill, 15 km n. of Satara rest camp, central KNP, emerged 6.II.1992 from dung evacuated by large elephant bull 29.I.1992, R. Meiswinkel.

5.4 DISCUSSION

5.4.1 *Taxonomy*

Culicoides (Avaritia) loxodontis does not appear to have been dealt with previously in studies on Afrotropical biting midges. Since its discovery in 1979, I have referred to it as C. sp. # 71 in unpublished reports and correspondence.

5.4.2 *Differential diagnosis*

The 12 character states that separate *C. imicola* and *C. loxodontis* are summarized in Table 5.6 and are discussed in further detail below. Where data are available, these two species are also differentiated from *C. bolitinos, C. miombo* and *C. pseudopallidipennis.*

Three character states reliably separate *C. loxodontis* from *C. imicola* under the dissecting microscope. The former has (a) a generally paler wing, with the proximal margin of the distal pale spot in cell R_5 strongly pointed, (b) has the preapical pale excision on the anterior margin of vein M_2 more extensive and ill-defined, leaving the apex of M_2 pale or only slightly and indistinctly darkened, and (c) has two median bristles on the scutellum in both sexes; these bristles are, however, difficult to detect under the dissecting microscope, especially if one or both have been knocked off during capture. The fact that *C. loxodontis* appears to be exclusively associated with the African elephant means that it will only be captured in the presence or territory of these animals, whereas *C. imicola* will also be widespread where man husbands livestock. Finally, because the immature stages of *C. loxodontis* develop exclusively in the dung of elephants, the adults, during eclosion, become infested with phoretic *Myianoetus* mites. Counts made of the number of mites carried by the females of two subpopulations of *C. loxodontis* captured in light-traps at Mooigesig and Langtoon (Fig. 5.1) showed that at the former site 186 of 697 (26,7 %) carried from one to 13 mites/midge, while at the latter site 84 of 728 (11,5 %) had from one to nine mites. Recently emerged nulliparous females have the highest infestation rate (22 % at Langtoon, 46 % at Mooigesig) (R. Meiswinkel & L.E.O. Braack, unpublished observations 1990). The presence of these mites on *C. loxodontis is* therefore a useful aid to identification as *C. imicola,* a species whose immatures do not live in dung, is entirely free of them.

Based on wing pattern, it is rather difficult to separate *C. loxodontis* from *C. pseudopallidipennis* as both species have predominantly pale wings. However, *C. pseudopallidipennis* has a swollen third palpal segment which can quite easily be seen under the dissecting microscope, thus differentiating it from the slender third segment seen in *C. imicola, C. bolitinos, C. miombo* and *C. loxodontis.*

In slide-mounted specimens, the following 11 character states facilitate the identification and separation of *C. loxodontis* from its four taxonomic congeners quite easily:

1. Female. Wing: The wing of *C. loxodontis* is distinctly paler than that of *C. imicola, C. bolitinos* or *C. miombo*, but closely resembles that of *C. pseudopallidipennis*. The next four wing characters more clearly define *C. loxodontis* as a good species and are labelled in Fig. 5.13.

   (a) The shape of the distal pale spot in the apex of cell R_5: its proximal margin usually strongly pointed (Fig. 5.13). In some specimens this point narrowly fuses with the distal margin of the third pale costal spot covering the tip of the second radial cell. In *C. imicola, C. bolitinos, C. miombo* and *C. pseudopallidipennis*, the proximal margin is moderately pointed to nearly straight.

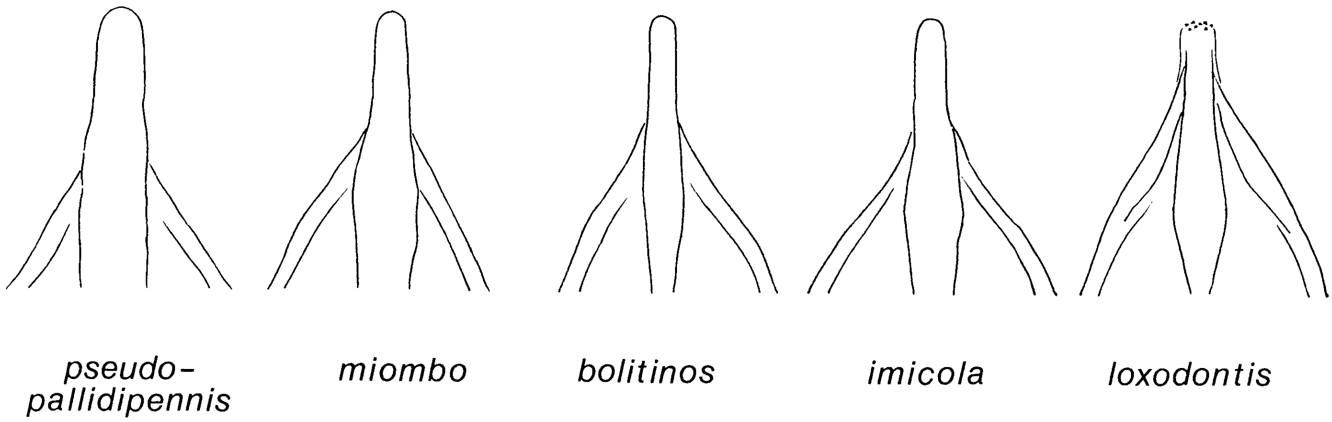
   (b) In *C. loxodontis* and *C. pseudopallidipennis* the second and third pale costal spots nearly fuse under the radial cells (Fig. 5.7, 5.13). In the remaining species these pale spots are well separated from one another by a dark area.

   (c) In *C. loxodontis* vein M_2 has an extensive and ill-defined pale excision on its anterior margin. This leaves the apex of the vein either pale or only narrowly and indistinctly darkened (Fig. 5.7, 5.13). This is again very similar to the configuration seen in *C. pseudopallidipennis* and in part also *C. bolitinos* and *C. miombo. C. imicola is* distinctive in that it has a clearly defined preapical excision that leaves the apex of vein M_2 broadly and obviously darkened.

   (d) As in *C.imicola, C.bolitinos* and *C.pseudopallidipennis*, the extreme base of the anal cell of *C. loxodontis* is pale (Fig. 5.13); in *C. miombo* there is a highly diagnostic, prominent, long, dark smudge.

2. Female. Palps: A low P/H ratio (mean 0,84; n = 20) indicates that *C. loxodontis* has a short proboscis which in turn indicates short palps. As can be seen in Table 5.5 and Fig. 5.15, *C. loxodontis* comes close to *C. bolitinos* in total palpal length while *C. miombo* and *C. imicola* have longer palps. Glick (1990) describes the proboscis of *C. pseudopallidipennis* as ‘long’. Clastrier (1958) gives the measurements of segments II–V of *C. pseudopallidipennis* which indicates that the palps are about as long as those of *C. imicola.* Furthermore, *C. pseudopallidipennis* has a swollen third palpal segment as opposed to the slender one found

Fig. 5.12



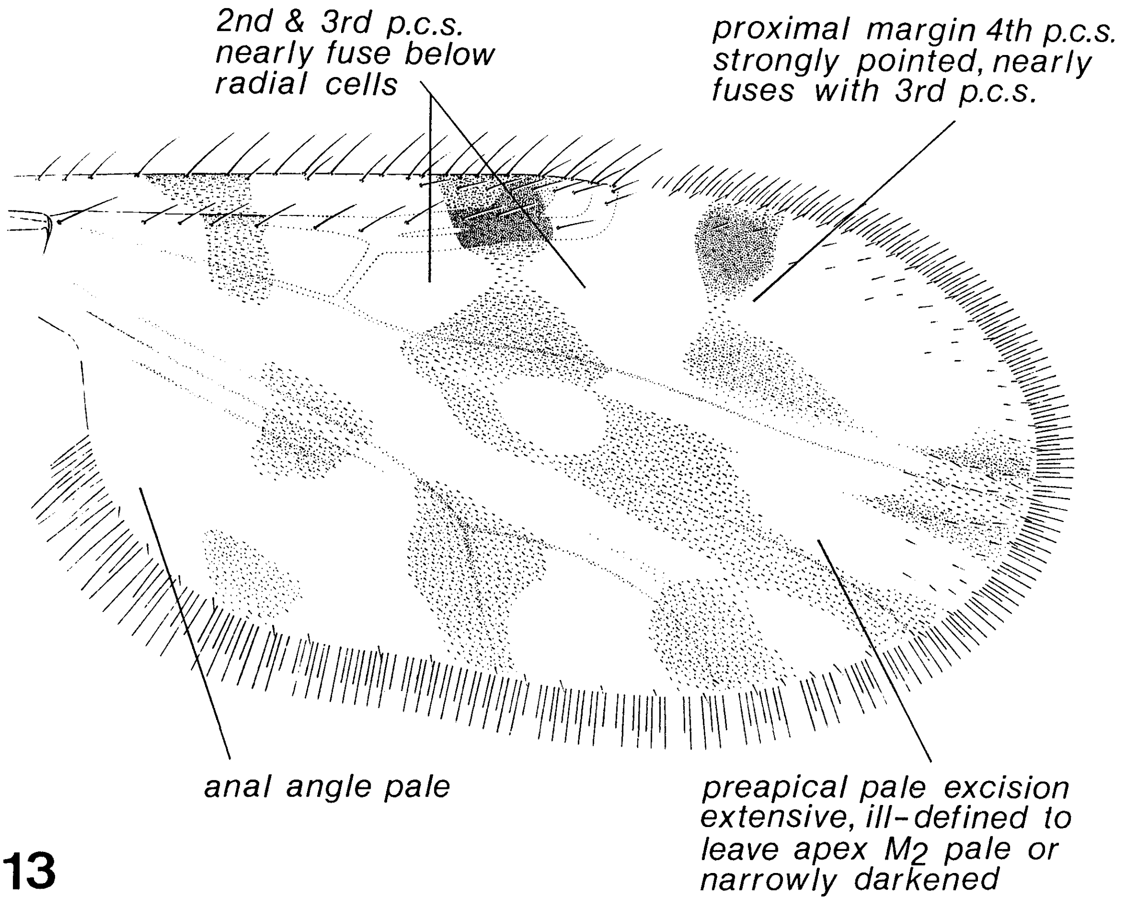


Fig. 5.13

Fig. 5.12 Genitalia male: comparison of the apices of the aedeagi of five species of the Imicola group.

Fig. 5.13 *Culicoides (Avaritia) loxodontis.* Wing, female: diagnostic features.

Fig. 5.14

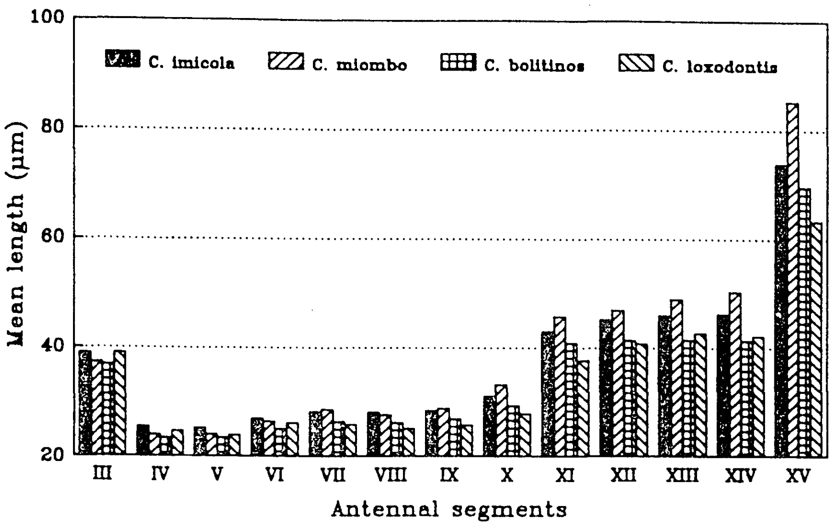


Fig. 5.15

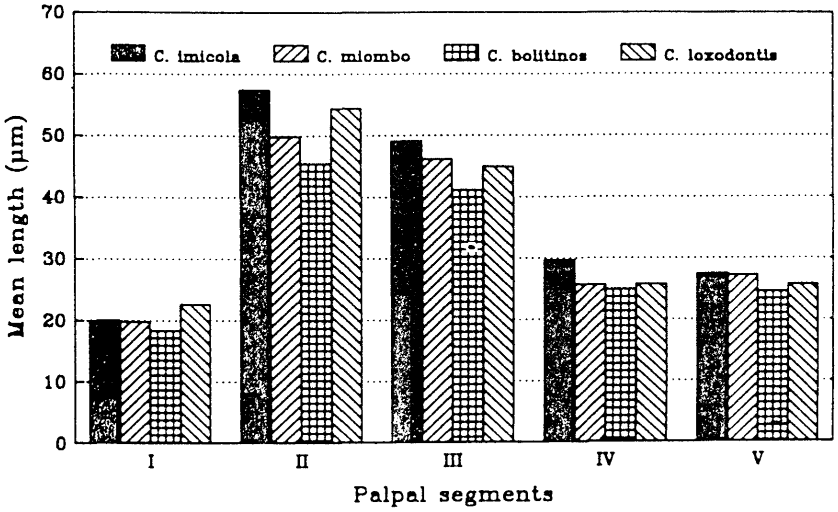


Fig. 5.14 Comparison of mean lengths (μm) of each of the female antennal segments III-XV of *C. (Avaritia) imicola, C. (A.) miombo, C. (A.) bolitinos* and *C. (A.) loxodontis*.

Fig. 5.15 Comparison of mean lengths (μm) of each of female segments I-V of *C. (Avaritia) imicola, C. (A.) miombo, C. (A.) bolitinos* and *C. (A.) loxodontis*.

in its four taxonomic congeners.

3. Female. Antennae: As can be seen in Table 5.4 and Fig. 5.14, *C. loxodontis* has the longest antennal segment III but has the shortest segments VII–XII and XV. Once again it comes closest to *C. bolitinos* in total antennal length. The four species *C. loxodontis, C. imicola, C. bolitinos* and *C. miombo*, are all separable on the mean lengths of antennal segments X, Xl and XV the last-named being the most significant (Table 5.4). The data provided for *C. pseudopallidipennis* by Clastrier (1958) come near that for *C. loxodontis*, except that segment III of the former is given as only two-thirds the length of the four species listed in Table 5.4.

4. Female. Antennae: *C. loxodontis* and *C. miombo* both have the long blunt-tipped sensilla trichodea on antennal segment VI rather short, and as a result share a low antennal trichodea ratio (AtR): mean 1,47 in the former vs 1,54 in the latter, while *C. bolitinos* (mean 1,85) and *C. imicola* (mean 1,86) have longer trichodea and share a higher AtR ratio. As illustrated by Clastrier (1958), these trichodea are rather short and unusually swollen in *C. pseudopallidipennis*, and its AtR ratio will in all likelihood cluster with that of *C. loxodontis* and *C. miombo*. In the latter two species, however, the blunt-tipped trichodea are not swollen.

5. Female. Antennae: One of the most striking and reliable taxonomic differences between *C. loxodontis* (n = 81) and its three congeners *C. imicola* (n = 172), *C. bolitinos* (n = 59) and *C. miombo* (n = 70), is that in *C. loxodontis* antennal segments VI and VIII always bear four sensilla chaetica basally (Fig. 5.3 and Table 5.3), whereas in the other three species three chaetica are the norm. The number of chaetica in *C. pseudopallidipennis* are variable as shown in Chapter 6 (Table 6.3).

6. Female. Scutellum: *C. loxodontis* (n = 41) always carries two long bristles medially (Fig. 5.5); *C. imicola* (n = 87), *C. bolitinos* (n = 20), and *C. miombo* (n = 37) each have only one bristle medially. Clastrier (1958) recorded one bristle medially for *C. pseudopallidipennis.* This consistent presence of two median bristles on the scutellum I have found to be the easiest and most reliable way of identifying a slide-mounted specimen of *C. loxodontis*. Even if one or

both bristles have been lost during capture, their insertion sockets or alveoli remain visible.

7. Male. The extent of spiculation on the membrane of sternum nine of the genitalia once again groups *C. loxodontis* with *C. bolitinos*, and *C. imicola* with *C. miombo*; in the former pair the membrane is mostly bare or occasionally adorned with a few spicules, whereas in the latter pair the membrane is seldom sparsely but mostly moderately to densely spiculate. This membrane bare in *C. pseudopallidipennis.*

8. Male. The most diagnostic feature of the genitalia of *C. loxodontis* is that the extreme apex of the aedeagus is not smoothly rounded and hyaline as in its four taxonomic congeners, but is distinctly roughened or serrated by five to eight tiny, darkly pigmented papillae (Fig. 5.11, 5.12). The apex of the aedeagus of *C. loxodontis* is also shorter than in its four congeners which all have it a little longer and largely inseparable in shape (Fig. 5.12).

5.4.3 ***Larval habitat***

Culicoides loxodontis has on a number of occasions been reared from the dung of the African elephant in the Kruger National Park, these records represented by the symbol of an elephant in Fig. 5.1. Despite my persistent efforts during the past seven years, it has never been reared from the dung of either the white or black rhinoceros, zebra, wildebeest, buffalo, cattle or horse, either within or outside the Kruger National Park.

As mentioned earlier, six species of the subgenus *Avaritia* have been found to inhabit the dung of *Loxodonta*, the commonest of these being two species provisionally labelled C. sp. # 54 pale form and C. sp. # 54 dark form (R. Meiswinkel & H. Nevill, unpublished observations 1985–1991). *Culicoides loxodontis* is third in abundance; up to 500 specimens have been reared from 2,5 kg of wet elephant dung. In the height of summer, the first individuals of *C. loxodontis* will emerge after eight to ten days from dung collected when only a few hours old.

The recently discovered pupa of *C. loxodontis* is quite remarkable and is included in the type series. It will be described elsewhere (H. Nevill, personal communication 1991).

5.4.4 *Distribution*

Culicoides (A.) loxodontis is known only from the Kruger National Park, South Africa, and a single record from Ol Doinyo Sabachi, central Kenya. Fig. 5.1 shows the holotype locality (Lamont windmill, Shingwedzi), and paratype locales (Pafuri, Tshalungwa, Mangovane spring, Langtoon dam, Mooigesig dam, Hlangulene, Phambana and Skukuza) from which the new species has been commonly collected, either in light-traps or reared from elephant dung. As noted earlier, only one ♀ and 23 ♂♂ reared from elephant dung collected at Hlangulene, and one ♂ and its associated pupal exuvia retrieved from elephant dung found 20 km west of Skukuza, form part of the type series of *C. loxodontis*. Rearings made from dung collected at Khumane dam, Shiloweni dam, Nwatintsontso, Rhidonda and Shisha were too teneral to be slide-mounted in Canada balsam.

On occasion, *C. loxodontis* has been found to be the dominant species in certain areas of the KNP and can comprise up to 52 % of 500–2500 *Culicoides* collected in light-traps operated during the first four to six hours following nightfall. The light-trap data for nine locales are indicated by the numbers in parentheses in Fig. 5.1; rather than providing percentages, the actual numbers of *C. loxodontis* out of the total number of *Culicoides* captured are given. At four of these sites *C. loxodontis* was the dominant *Culicoides* of 16–24 species collected (R. Meiswinkel & L.E.O. Braack, unpublished data 1987–1990).

Conversely, *C. loxodontis* can be either absent or rare in light-trap catches, as shown by the nil result for Manxeba Pan and the presence of only two specimens out of more than 1 000 *Culicoides* collected on the banks of the Sabie river in Skukuza. Manxeba Pan falls into that far northern part of the KNP that is visited only by lone elephant bulls and small bachelor herds at certain times of the year. These are culled during the winter months to protect the rare vegetation. The virtual absence of elephants is paralleled by the absence of *C. loxodontis* in light-trap collections. A similar situation prevails in the immediate surrounds of Skukuza, where the presence of larger groups of elephants is sporadic as this is an area generally used only by bachelor bulls in the dry season. Just 5–15 km north, south and east of Skukuza, however, large herds of elephants are to be found at certain times of the year, but are too far distant for their attendant dung-inhabiting *Culicoides* to be captured in a light-trap operated in Skukuza. At one such site, i.e. Mutlumuvi 12 km north-east of Skukuza, *C. loxodontis* is one of the most abundant *Culicoides* species present, especially during the hotter, wetter months.

These statistics imply strongly that *C. loxodontis* will only be sampled in abundance in areas directly frequented by large numbers of elephant. This appears to be borne out by its dominance at Mooigesig, Langtoon, Mwamayimane, Mutlumuvi and Phambana, all locales that were sampled on the same day that large groups of elephants were sighted, and known to carry large elephant herds year after year (van Wyk & Fairall 1969).

Although more precise comparative data need to be assembled, it seems that the ebb and flow in the distribution and abundance of *C. loxodontis* reflects strongly the dynamics of elephant herd as regards their daily food and water requirements, clan ranges, reproductive strategies and seasonal movements. Furthermore, it is likely that the breeding herds, which make up approximately 85 % of the entire elephant population of ± 7 500 individuals in the KNP (A. Hall-Martin & I. Whyte, unpublished data 1973–1990), are most pivotal to the spread and abundance of *C. loxodontis*, as these herds, each numbering from 50–300 individuals, would provide large amounts of dung for the immature stages of *C. loxodontis* and would likely also provide bloodmeals for the adult females. To conclude: not only can *C. loxodontis* be abundant, but it is also found widely in areas inhabited by elephants irrespective of vegetation or landscape type.

Since I first became aware in 1979 of its status as a good species, subsequent collections made randomly in the animal husbandry areas of South Africa have failed to yield a single specimen of *C. loxodontis*. It has also not been found on nine game parks, reserves or hunting lodges sampled in the provinces of Transvaal and Natal, all of which harboured dung-producing herbivores such as the white rhinoceros *(Ceratotherium simum)*, the black rhinoceros *(Diceros bicornis)*, buffalo, zebra and wildebeest. A number of dung-inhabiting *Avaritia* species have been collected at these sites but no specimen of *C. loxodontis*. In South Africa there are eight locales besides the Kruger National Park and adjoining game reserves that carry small populations of mostly introduced elephants. None of these reserves has been sampled for *C. loxodontis*.

The failure, during 12 years of collecting, to find *C. loxodontis* outside the KNP, in areas where elephants do not occur, is further reason for now asserting that the distribution of *C. loxodontis* is tightly linked to that of the African elephant. As regards this association and the resultant distribution of *C. loxodontis*, there are some deeper issues that still need to be researched. Elephants, in being both grazers and browsers, 'are catholic in their habitat requirements' (Smithers 1983) and thus widespread in Africa where man allows them to be. Although it seems safe to assume that *C. loxodontis* will be similarly widespread in the savanna woodland areas of eastern and southern Africa, it is premature to conclude that *C. loxodontis*, despite its dependence on elephant dung, will be as tolerant as the elephant of changes in vegetation and climate in the rest of Africa. In South Africa it will be difficult to assess whether *C. loxodontis* has any ecozone preferences, as its present pattern of occurrence is, in great measure, artificial, the elephant having been eliminated from most of its former range. Therefore, if any vegetational or climatic constraints are exerted on the distribution of *C. loxodontis*, their unravelling will depend on collections still to be made throughout Africa, especially in areas such as hot and dry semi-deserts (Namibia), cooler higher-lying plateaux (eastern Zaire; western Uganda) and the wetter, more humid tropical forests (Zaire; Gabon; Cameroun).

As regards the historical distribution of elephants in South Africa, it is interesting to note that at the end of the 15th century elephants were reported as far south as Mossel Bay on the Cape coast (Smithers 1983). For the next four centuries, their extermination was ferociously pursued to the point where Stevenson-Hamilton (1903) reported that elephants were locally extinct in the Transvaal, even in the Sabi Game Reserve (now the KNP) proclaimed five years previously. However, two years later, the first 10 elephants were recorded in this reserve, having entered from Mozambique (Stevenson-Hamilton 1905). Their numbers slowly increased to stand at 131 in 1931 (Van Wyk & Fairall 1969); today the Kruger National Park is home to ± 7 500 elephant, the maximum number the vegetation in this 20 000 km^2 reserve can support without being degraded.

It remains a matter of speculation whether *Culicoides loxodontis* had enjoyed a similarly widespread distribution in South Africa. Its dependence on elephant dung as a larval habitat does imply that this biting midge species must have receded, along with the elephant, from some areas of its range, as there is still no evidence that *C. loxodontis* will switch resources and utilize the dung of any other herbivore. I therefore believe that the dominance of *C. loxodontis* in parts of the KNP today is directly the result of the spectacular rebound in elephant numbers in the same area over the last 90 years. The abrupt absence of *C. loxodontis* outside the western Park boundaries seems to bear this out, as no concomitant changes in rainfall or vegetation could account for this marked break in distribution.

If it is true that *C. loxodontis* re-entered the Transvaal with elephants from Mozambique, it is likely that it occurs throughout the remainder of southern and eastern Africa and may eventually be found westwards north and south of the equator in the savanna woodland zones encircling the equatorial forest block.

5.5 **CONCLUSION**

Culicoides loxodontis is the fifth member of the Imicola group of the subgenus *Avaritia* to be described from the Afrotropical Region. It appears to be exclusively associated with the African elephant as the distribution of *C. loxodontis* parallels that of the elephant in the KNP, and it has been reared only from elephant dung.

Taxonomically, *C. loxodontis* can be confused with *C. imicola* and, especially, *C. pseudopallidipennis*. The separation of these two species from *C. loxodontis* depends primarily upon the examination of slide-mounted material, as all three species may occur in ‘elephant country’. Judging from light-trap catches made at a number of sites in the Kruger National Park, *C. loxodontis* can be quite abundant. Because of its abundance and its close association with elephants, *C. loxodontis* deserves attention as a potential vector of orbiviruses to an animal that has been suggested as being a possible reservoir host for African horsesickness (Davies & Otieno 1977).

5.6 REFERENCES

CLASTRIER, J. 1958. Notes sure les Ceratopogonides. IV. Ceratopogonides d'Afrique Occidentale Française. *Archives de l'Institut Pasteur d'Algerie,* 36:192–258.

DAVIES, F.G. & OTIENO, S. 1977. Elephants and zebras as possible reservoir hosts for African horsesickness virus. *The Veterinary Record,* 100:291–292.

DYCE, A.L. 1982. Distribution of *Culicoides (Avaritia) spp.* (Diptera: Ceratopogonidae) west of the Pacific Ocean. *In*: ST. GEORGE, T.D. & KAY B.H. (eds). *Arbovirus Research* in Australia. Proceedings Third Symposium, 35–43.

DYCE, A.L. & MARSHALL, B.D. 1989. An early record of *Culicoides* species (Diptera: Ceratopogonidae) developing in the dung of game animals in southern Africa. *Onderstepoort Journal of Veterinary Research,* 56:85–86.

GLICK, J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology,* 27:85–195.

MEISWINKEL, R. 1987. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) kanagai* Khamala & Kettle, 1971, reared from elephant dung in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research,* 54:585–590.

MEISWINKEL, R. 1989. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research,* 56:23–39.

NEVILL, E.M. 1968. A significant new breeding site of *Culicoides pallidipennis* Carter, Ingram & Macfie (Diptera: Ceratopogonidae). *Journal of the South African Medical Association,* 39:61.

SMITHERS, R.H.N. 1983. The mammals of the southern African subregion. The University of Pretoria, Pretoria, Republic of South Africa.

STEVENSON-HAMILTON, J. 1903. Transvaal Administration Report for 1903. Pretoria: Government Printer.

STEVENSON-HAMILTON, J. 1905. Transvaal Administration Report for 1905. Pretoria: Government Printer.

VAN WYK, P. & FAIRALL, N. 1969. The influence of the African elephant on the vegetation of the Kruger National Park. *Koedoe*, 12:57–89.

TABLE 5.1: Mean lengths (µm) of segments, range in number, mean number and distribution of sensillae on the female and male antennae of *C. (A.) loxodontis*

		Antennal segments													Total and mean total
		III	**IV**	**V**	**VI**	**VII**	**VIII**	**VIX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**	
Female: (n = 80–81)															
Sens. coeloconica:	range/segment	2–3	0	0	0	0	0	0	0	0–1	0–1	1	1	0–1	6–8
	mean no./segment	2,91	0	0	0	0	0	0	0	0,05	0,98	1,00	1,00	0,98	6,93
Sens. chaetica:	range/segment	4–7	3–5	2–3	4–5	2–3	3–5	1–3	2–4	0–1	0	0	0	0	24–29
	mean no./segment	5,00	3,25	2,15	4,04	2,06	4,00	2,04	3,02	0,05	0	0	0	0	25,89
Sens. trichodea (blunt-tipped)		LL	LLc	LLc	LLc	LLc	LLc	LLc	LLc	–	–	–	–	–	
Mean lengths of segments		39,6	25,2	24,5	26,6	26,3	25,8	26,2	28,5	38,6	42,1	43,9	42,6	63,5	453,1
Male: (n = 24–26)															
Sens. coeloconica:	range/segment	2	0	0	0	0	0	0	0	0	0	0–1	1–2	1–2	5–7
	mean no./segment	2	0	0	0	0	0	0	0	0	0	0,96	1,92	1,92	6,65
Sens. chaetica:	range/segment	5	0	0	0	0	0	0	0	0	0	2–4	2	0	9–11
	mean no./segment	5	0	0	0	0	0	0	0	0	0	3,42	2,00	0	10,15
Sens. trichodea (blunt-tipped)		LL	LLc	LLc	LLc	Lc	Lc	Lc	c	0	0				
Mean lengths of segments		72,6	<-- 338,8 -->									75,2	63,1	85,8	635,5

TABLE 5.2: Number and frequency of coeloconica present on each of female antennal segments III–XV of *C. (Avaritia) loxodontis*

No. of coeloconica per segment of *C. loxodontis*	**Antennal segments**							
	III	**IV–IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	–	81	81	77	1	–	–	1
1	–	-	–	4	80	81	81	80
2	7	–	–	–	–	–	–	–
3	74	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–
No. of antennae examined	81	81	81	81	81	81	81	81

TABLE 5.3: Number and frequency of chaetica present on each of female antennal segments III–XV of *C. (Avaritia) loxodontis*

No. of chaetica per segment *C. loxodontis*	**Antennal segments**												
	III	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
0	–	–	–	–	–	–	–	–	77	81	81	81	81
1	–	–	–	–	–	–	1	–	4	–	–	–	–
2	–	–	69	–	76	–	76	1	–	–	–	–	–
3	–	42	12	–	5	2	4	77	–	–	–	–	–
4	2	36	–	78	–	76	–	3	–	–	–	–	–
5	78	3	–	3	–	3	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–	–	–	–	–
7	1	–	–	–	–	–	–	–	–	–	–	–	–
No. of antennae examined	81	81	81	81	81	81	81	81	81	81	81	81	81

TABLE 5.4: Comparison of mean lengths (μm) of female antennal segments III–XV of four *Culicoides* species of the Imicola group (n = 25)

Antennal segment	Species and mean lengths				F-value
III	*bolitinos*	*miombo*	*imicola*	*loxodontis*	
	36,85	37,30	39,00	39,05	10,245
IV	*bolitinos*	*miombo*	*loxodontis*	*imicola*	x^2 = 26,464
	23,35	24,00	24,65	25,45	
V	*bolitinos*	*loxodontis*	*miombo*	*imicola*	x^2 = 19,705
	23,45	23,93	24,08	25,20	
VI	*bolitinos*	*loxodontis*	*miombo*	*imicola*	7,878
	25,0	26,15	26,45	26,95	
VII	*loxodontis*	*bolitinos*	*imicola*	*miombo*	19,329
	25,85	26,35	28,23	28,63	
VIII	*loxodontis*	*bolitinos*	*miombo*	*imicola*	19,755
	25,15	26,18	27,63	28,08	
IX	*loxodontis*	*bolitinos*	*imicola*	*miombo*	20,800
	25,75	26,85	28,43	28,80	
X	*loxodontis*	*bolitinos*	*imicola*	*miombo*	49,212
	27,85	29,33	31,10	33,20	
XI	*loxodontis*	*bolitinos*	*imicola*	*miombo*	48,404
	37,65	40,83	43,00	45,75	
XII	*loxodontis*	*bolitinos*	*imicola*	*miombo*	41,128
	40,80	41,10	45,20	46,95	
XIII	*bolitinos*	*loxodontis*	*imicola*	*miombo*	44,926
	41,43	42,70	45,98	48,95	
XIV	*bolitinos*	*loxodontis*	*imicola*	*miombo*	51,949
	41,35	42,15	46,20	50,30	
XV	*loxodontis*	*bolitinos*	*imicola*	*miombo*	85,413
	63,30	69,60	73,78	85,25	
TOTAL	*bolitinos*	*loxodontis*	*imicola*	*miombo*	
	451,85	445,00	485,48	506,00	42,611

TABLE 5.5: Comparison of mean lengths (raw data) of female palpal segments I–V of four *Culicoides* species of the Imicola group (n = 25)

Antennal segment	**Species and mean lengths**				**F-value**
I	*bolitinos* 7,34	*miombo* 7,94	*imicola* 8,02	*loxodontis* 8,68	11,985
II	*bolitinos* 18,18	*miombo* 19,92	*loxodontis* 20,94	*imicola* 22,96	64,934
III	*bolitinos* 16,44	*loxodontis* 17,88	*miombo* 18,46	*imicola* 19,62	31,806
IV	*bolitinos* 9,96	*loxodontis* 10,24	*miombo* 10,28	*imicola* 11,86	37,672
V	*bolitinos* 9,82	*loxodontis* 10,08	*miombo* 10,90	*imicola* 10,98	12,810
TOTAL	*bolitinos* 61,82	*loxodontis* 63,94	*miombo* 67,90	*imicola* 73,44	88,541

TABLE 5.6: Twelve morphological and biological character states used to separate *C. imicola* from *C. loxodontis*

	C. imicola		***C. loxodontis***
Morphological			
♀	scutellum with one median bristle (n = 87)	-	scutellum with two median bristles (n = 41)
-	wing generally darker with proximal margin of distal pale spot in cell R_5 moderately pointed; this point never fuses with the third pale costal spot covering radial cells	-	wing paler with this margin moderately or strongly pointed; this point occasionally fuses narrowly with the third pale costal spot
-	apex of vein M_2 broadly dark on both margins, this preceded by a well-defined excision	-	apex either pale or only narrowly dark on both margins; this is a result of the pale excision almost entirely eliminating anterior dark areas at apex of M_2
-	palp longer; 165,6–182,4, mean 176,1 μm (n = 25)	-	palp shorter; 147,5–175,0, mean 163,3 (μm) (n = 63)
-	proboscis longer; P/H ratio 0,82–1,02, mean 0,90 (n = 45)	-	proboscis shorter; P/H ratio 0,80–0,89, mean 0,84 (n = 20)
-	antennal segments IV, V, VII–XV significantly longer (Table 5.4)	-	these segments shorter (Table 5.4)
-	AtR 1,59–2,27, mean 1,86 (n = 173)	-	AtR 1,35–1,67, mean 1,47 (n = 42)
-	antennal segments VI and VIII with three sensilla chaetica basally in 90-94 % of specimens (n = 172)	-	these segments with four or five chaetica in 98 % of specimens (n = 81)
♂	membrane of sternum nine with eight to 145 spiculae, mean 47 (n = 50)	-	membrane with nought to three spiculae, mean 0,21 (n = 80)
-	extreme apex of aedeagus hyaline, round-tipped, smooth	-	apex not smooth but serrated by five to eight tiny sclerotised papillae
-	scutellum with one median bristle (n = 38)	-	scutellum with two median bristles (n = 41)
Biological			
-	larval habitat in short-grassed, moist pastures and margins of organically enriched drainage furrows and swamps	-	immature stages found exclusively in the dung of the African elephant

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

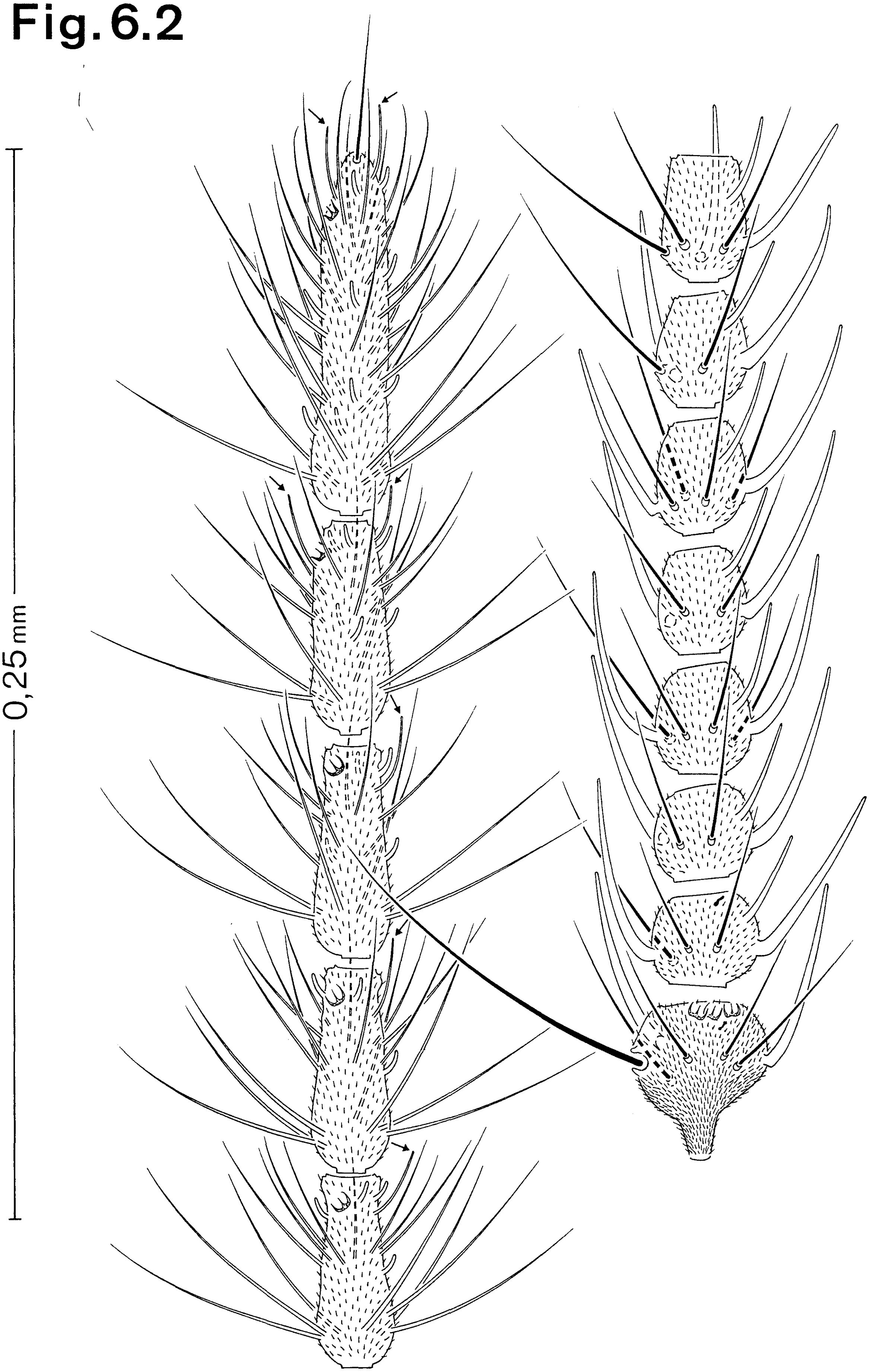


Fig. 6.2 *C. (Avaritia) pseudopallidipennis*. Antenna, female: segments XI-XV on left, segments III-X on right (Ivory Coast 343)

Fig. 6.12

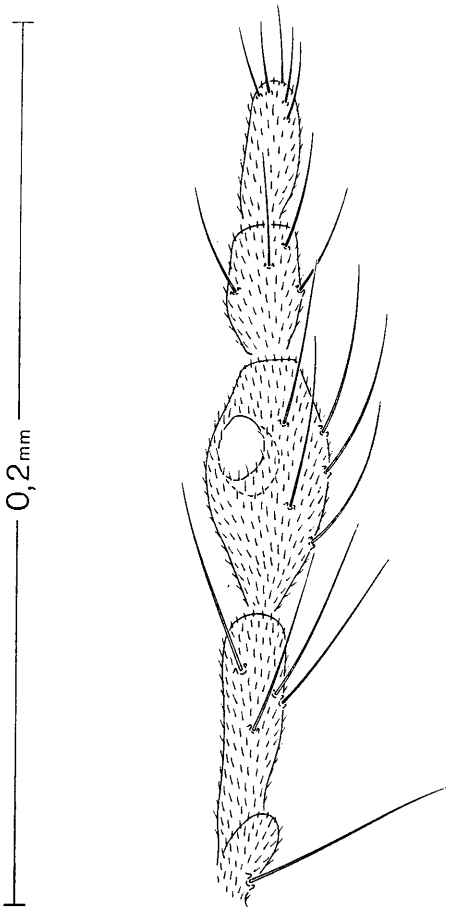
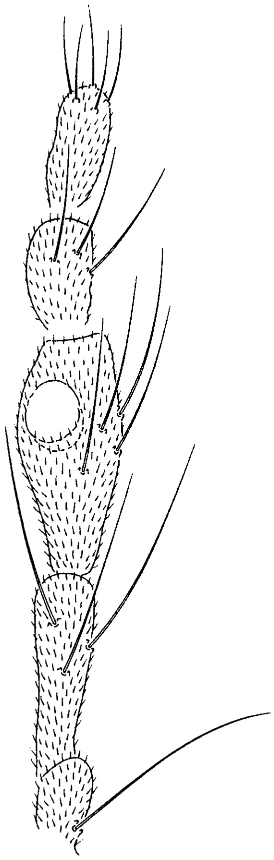


Fig. 6.3



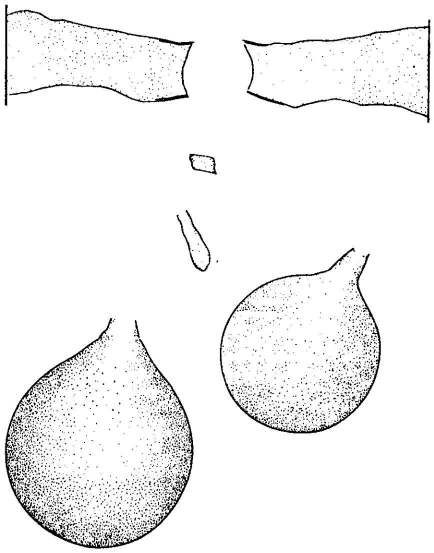


Fig. 6.16

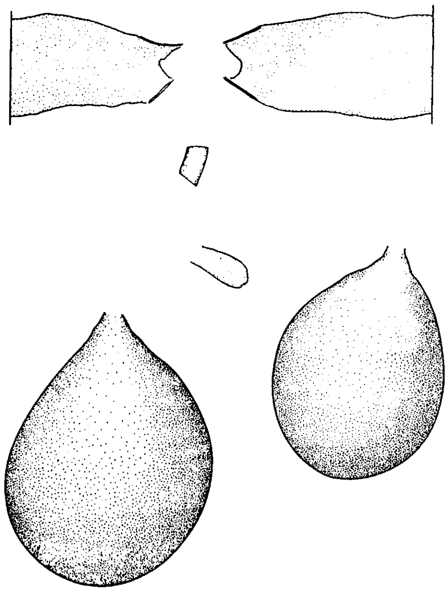


Fig. 6.4

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)

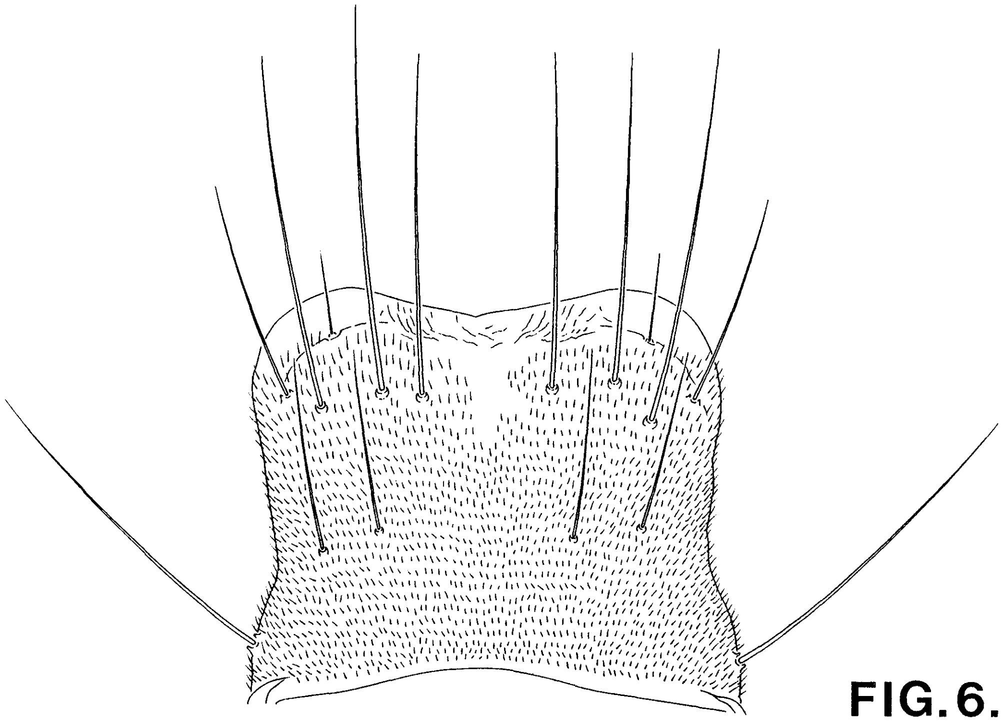


FIG.6.8

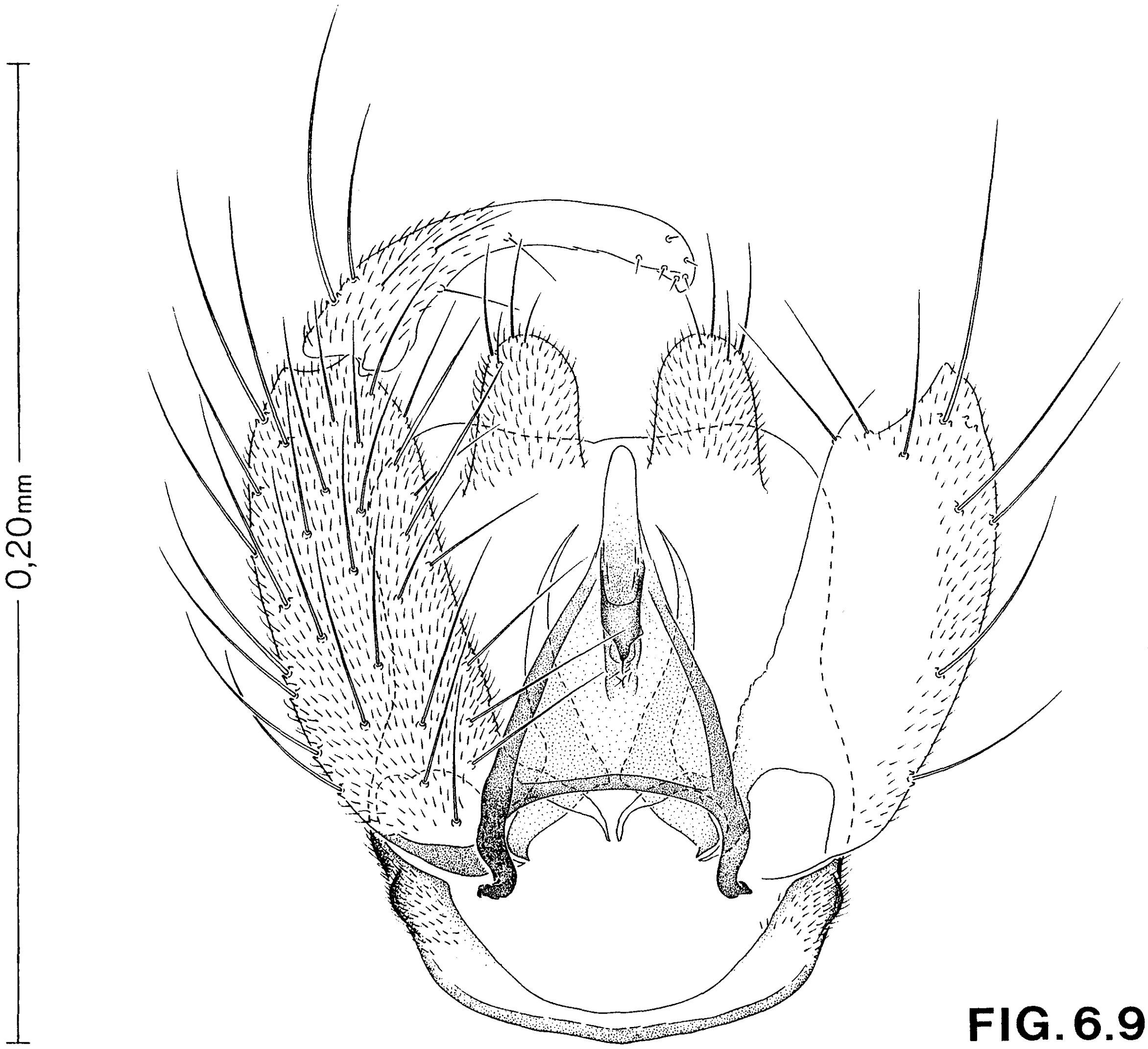


FIG.6.9

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

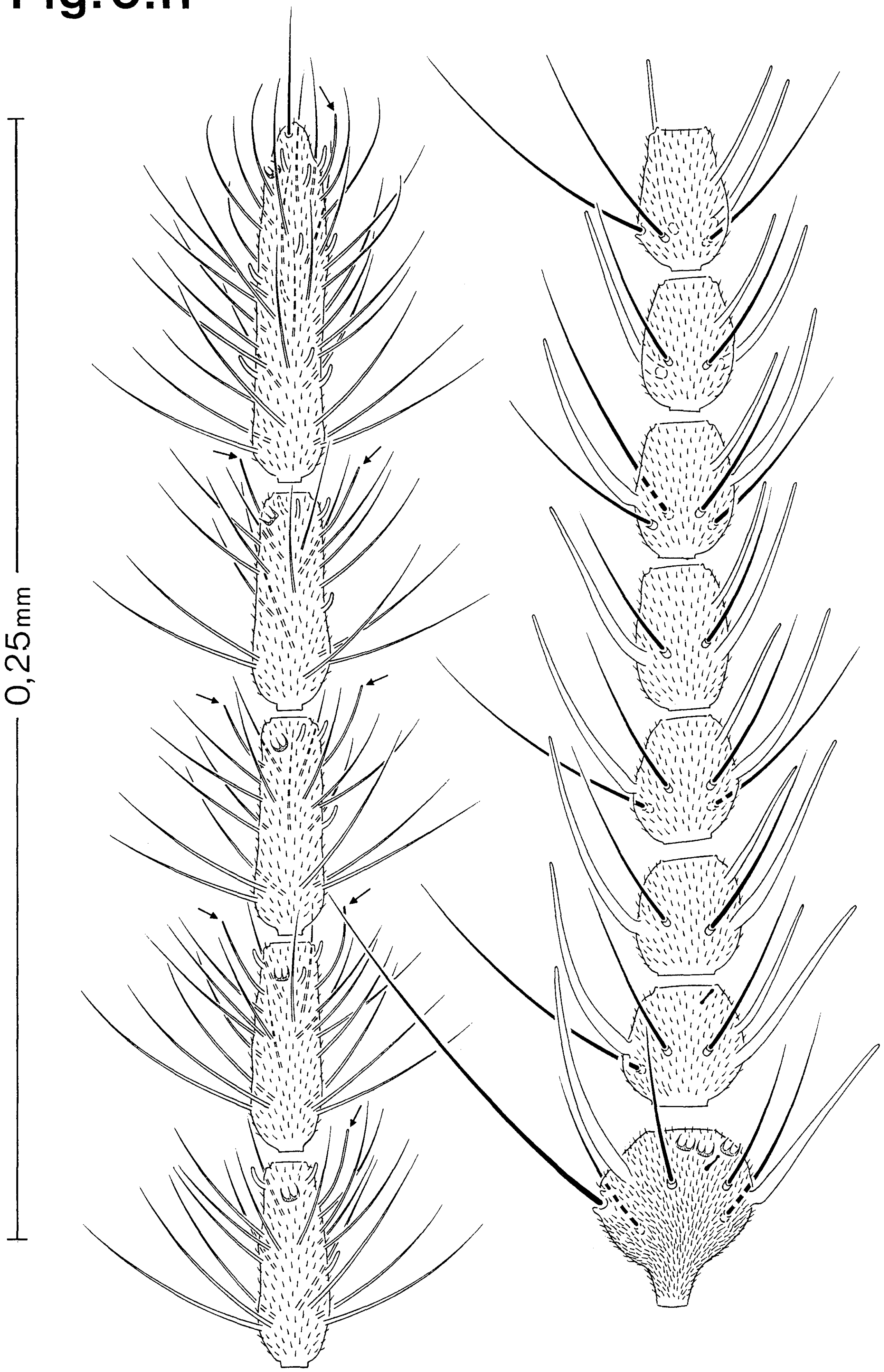


Fig. 6.11 *C. (Avaritia) tuttifrutti* sp. nov. Antenna, female: segments XI-XV on left, segments III-X on right (White River 63)

Fig. 6.13 a

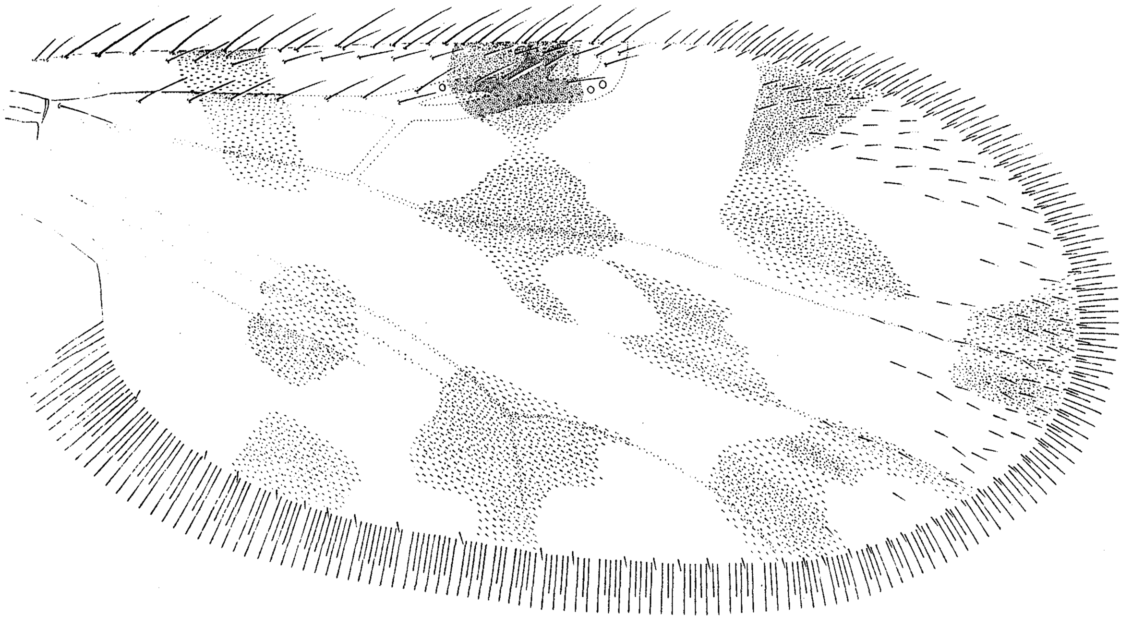


Fig. 6.13a *Culicoides (Avaritia) tuttifrutti* sp. nov. Wing, female.

6.13 b

6.14

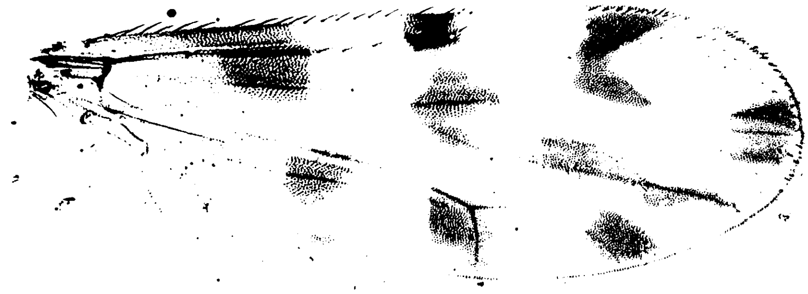


Fig. 6.13b *Culicoides (Avaritia) tuttifrutti* sp. nov. Wing, female (paratype Heuningeskrans 151).

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

FIG. 6.15

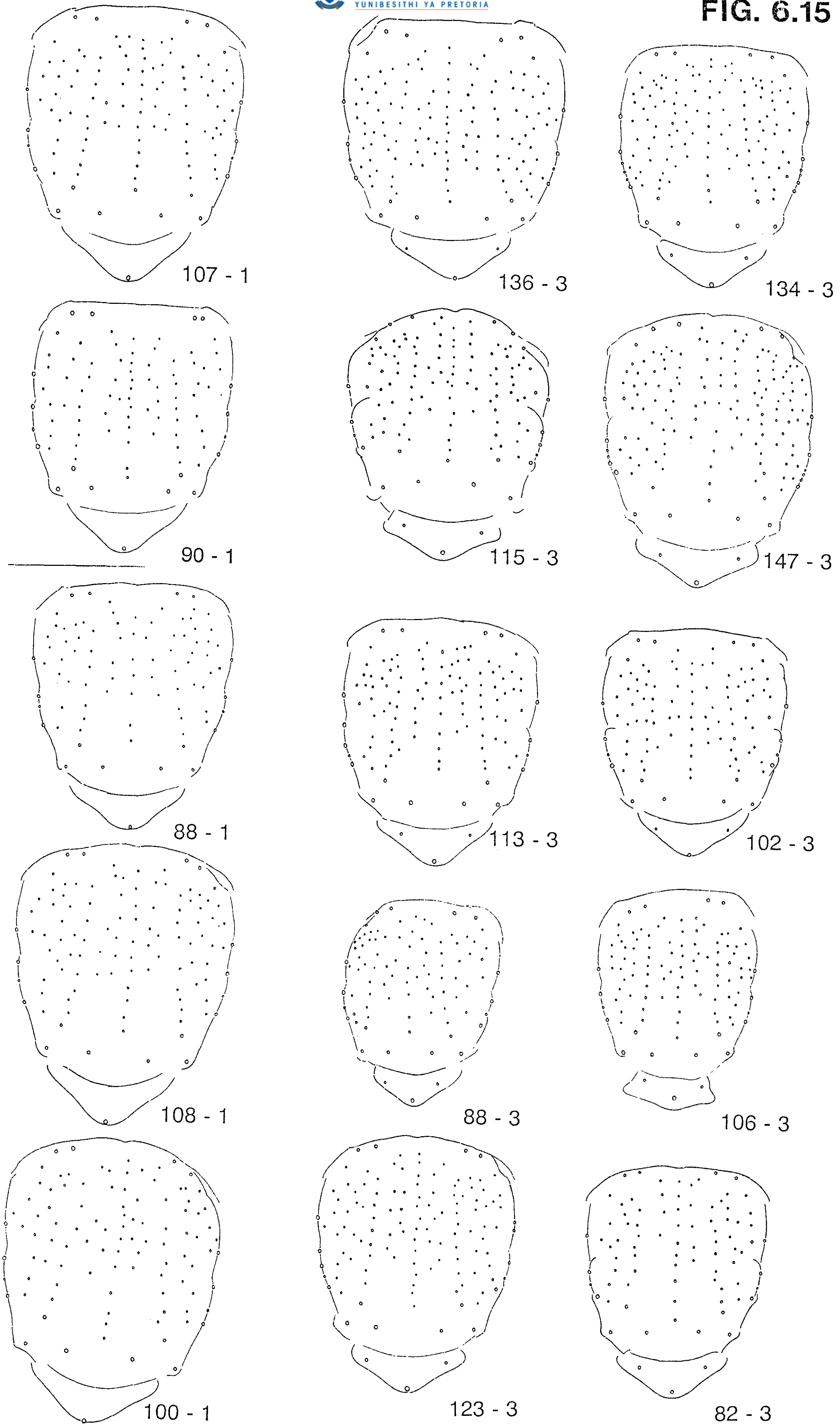


Fig. 6.15 *C. (A.) tuttifrutti* sp. nov.: setation of scutum (first numeral) and scutellum (second numeral); male (left column), female (two right columns)

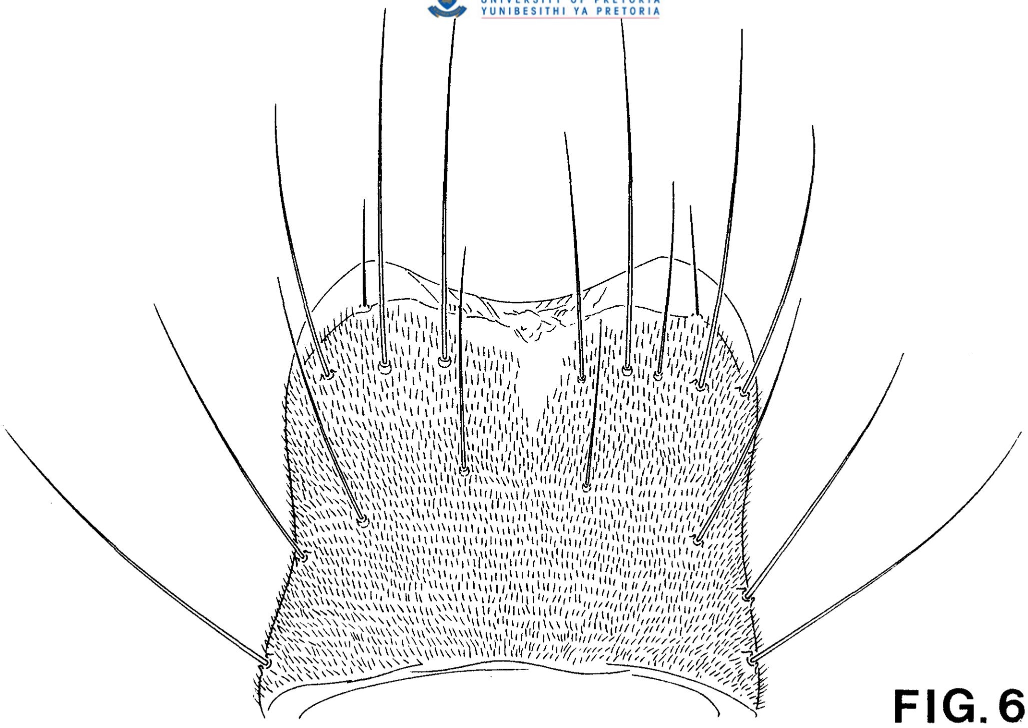


FIG. 6.17

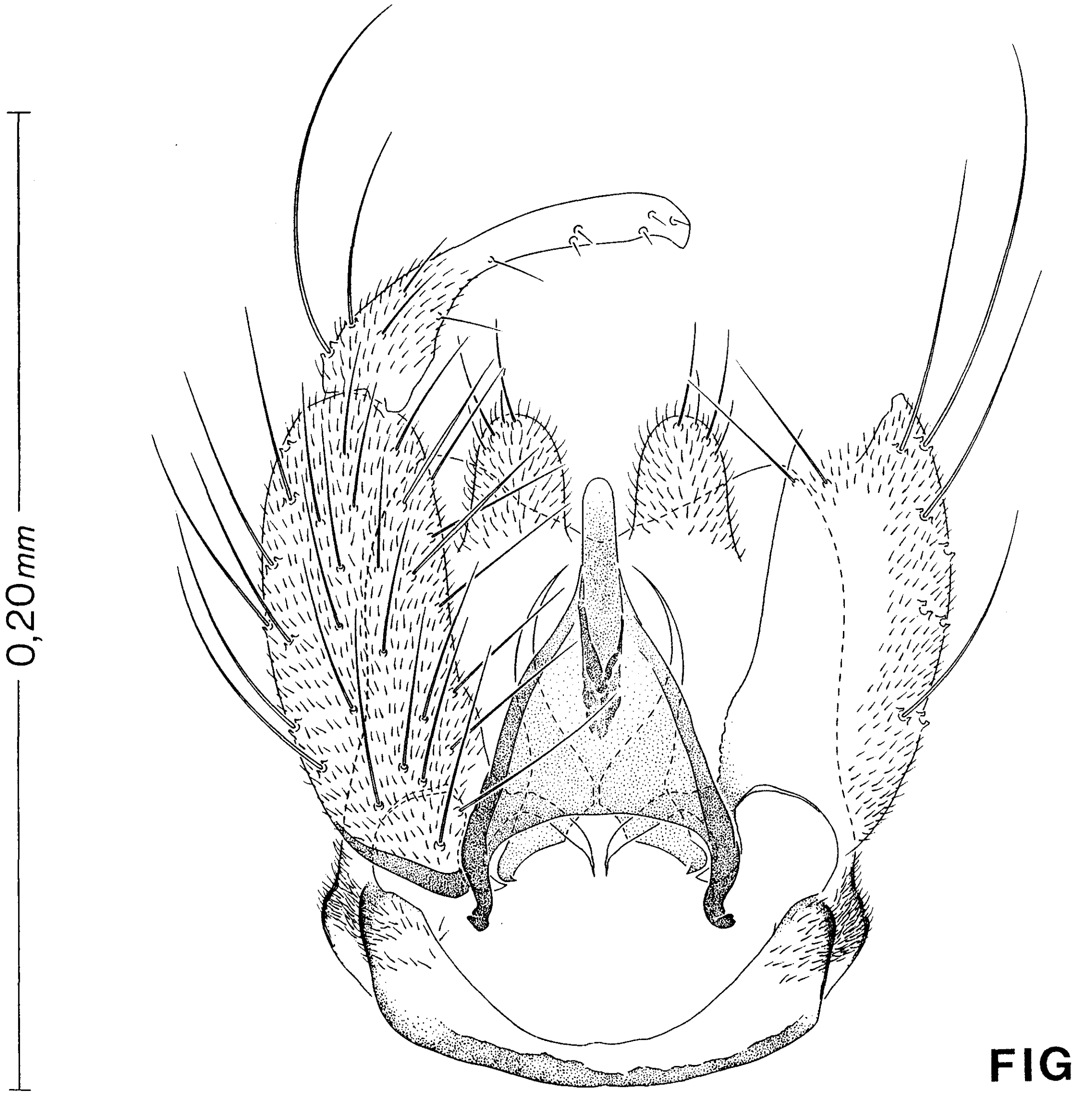


FIG. 6.18

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)

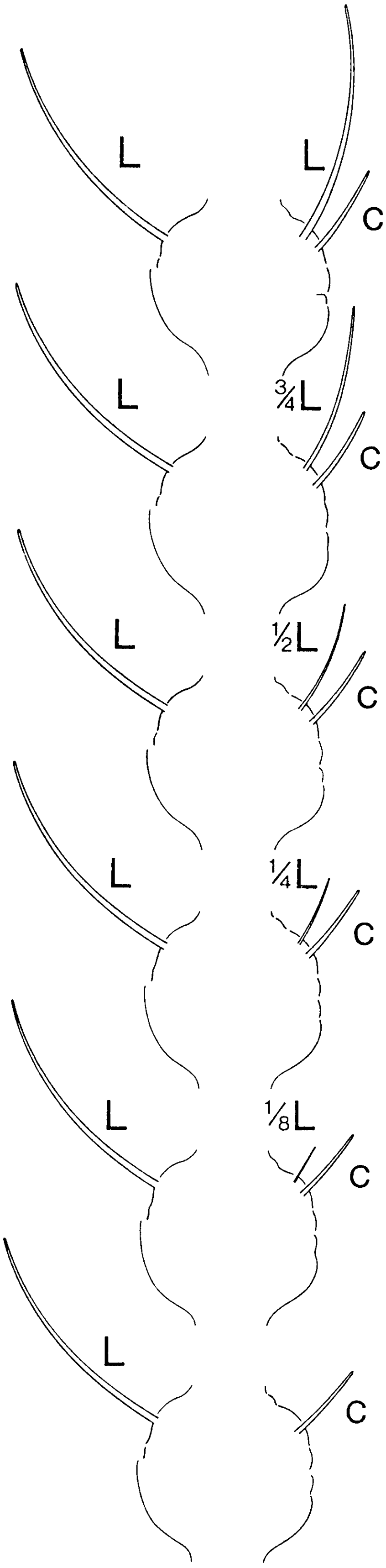


FIG. 6.19

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

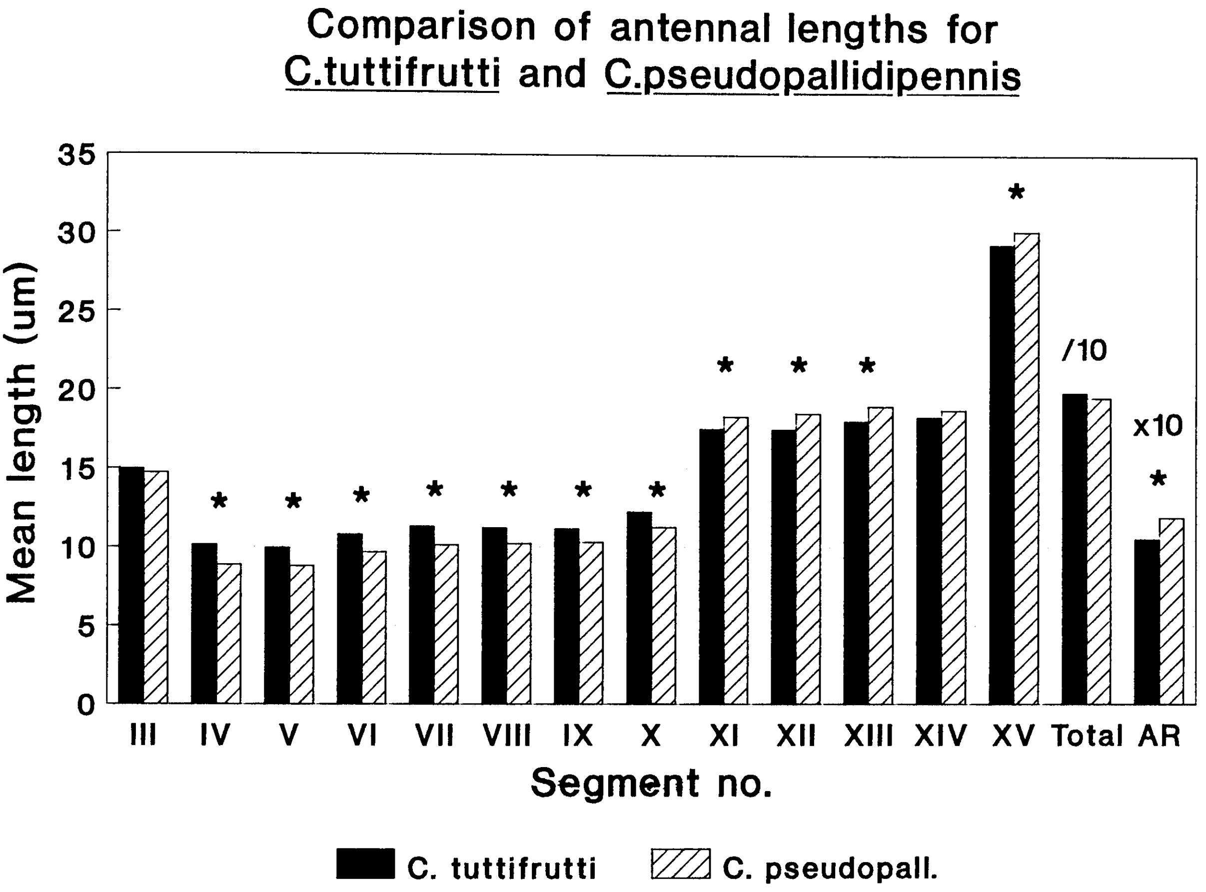


Fig. 6.20 Comparison of mean lengths (μm) of female antennal flagellar segments III–XV of *C. (Avaritia) tuttifrutti* sp. nov. and *C. (A.) pseudopallidipennis*. * = significantly different.

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.

6.6 REFERENCES

BOORMAN, J. 1991. A review of *Culicoides* subgenus *Avaritia* species (Insecta, Diptera, Ceratopogonidae), vectors of viruses of sheep, cattle and horses, with particular reference to *Culicoides imicola* in Europe and the Mediterranean region. Report prepared for The Overseas Development Administration, pp 1–54.

BOORMAN, J. & DIPEOLU, O.O. 1979. A taxonomic study of adult Nigerian *Culicoides* Latreille (Diptera: Ceratopogonidae) species. *Occasional Publications of the Entomological Society of Nigeria*, 22:1–121.

CAEIRO, V.M.P. 1961. Contribuicao para o estudo das espécies Angolanas do género *Culicoides* Latreille, 1809. *Estudos, Ensaios e Documentos junta de Investigacoes do Ultramar*, 86:5–359.

CLASTRIER, J. 1958. Notes sur les Cératopogonidés. IV. Cératopogonidés d'Afrique Occidentale Française. *Archives de l'Institut Pasteur d'Algerie*, 26:192–258.

CLASTRIER, J. & WIRTH, W.W. 1961. Notes sur les Cératopogonidés. XIV. Cératopogonidés de la Région éthiopienne (2). *Archives de l'Institut Pasteur d'Algerie*, 39:302–337.

CORNET, M. 1969. Les *Culicoides* (Diptera, Ceratopogonidae) de l'Quest Africain (1ére note). *Cahiers de l'Office de la Recherche Scientifique et Technique Outre-Mer, Série Entomologie Médicale et Parasitologie*, 15:171–176.

GLICK, J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology*, 27:85–195.

HORSFALL, W.R. 1974. Heteromorphic development of Aedine mosquitoes reared at abnormal temperatures. *Annales Zoologici Fennici*, 11:224-236.

MEISWINKEL, R. 1989. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:23–39.

MEISWINKEL, R. 1991. Afrotropical *Culicoides*: *C. (Avaritia) miombo* sp. nov. a widespread species closely allied to *C. (A.) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 58:155–170.

MEISWINKEL, R. 1992. Afrotropical *Culicoides*: *C. (Avaritia) loxodontis* sp. nov. a member of the Imicola group (Diptera: Ceratopogonidae) associated with the African elephant in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 59:145–160.

KHAMALA, C.P.M. & KETTLE, D.S. 1971. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of East Africa. *Transactions of the Royal Entomological Society of London*, 123:1–95.

KINGDON, J. 1990. Island Africa: The evolution of Africa's rare animals and plants. William Collins Sons & Co. Ltd.

WHITE, F. 1983. The vegetation of Africa. A descriptive memoir to accompany the Unesco/AETFAT/UNSO vegetation map of Africa, and Maps. Paris: Unesco.

WIRTH, W.W. & HUBERT, A.A. 1989. The *Culicoides* of southeast Asia (Diptera: Ceratopogonidae). *Memoirs of the American Entomological Institute*, 44:1–508.

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

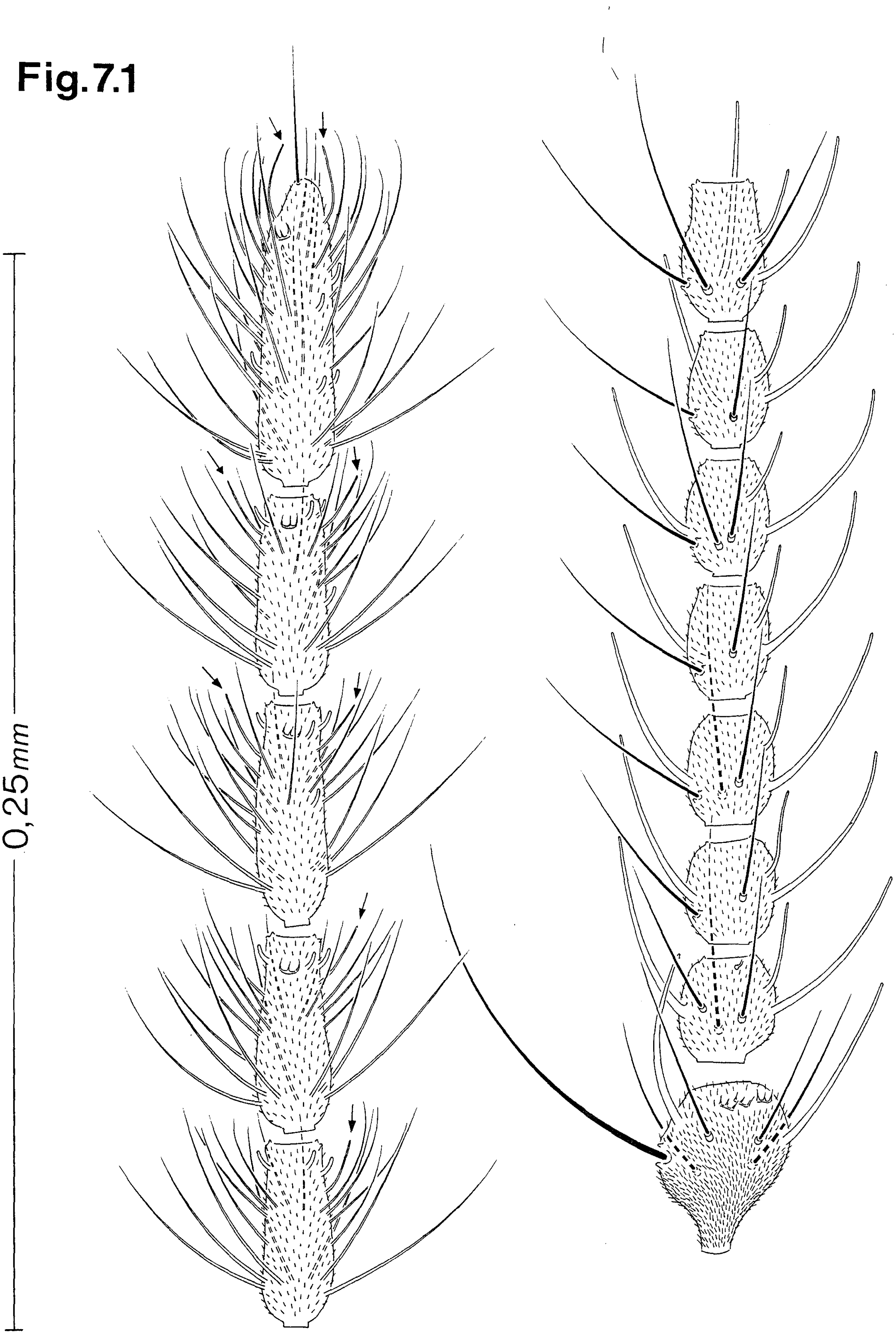


Fig. 7.1 *Culicoides (Avaritia) kwagga* sp. nov. Antenna, female: segments XI–XV on left, segments III–X on right (slide Pilanesberg).

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

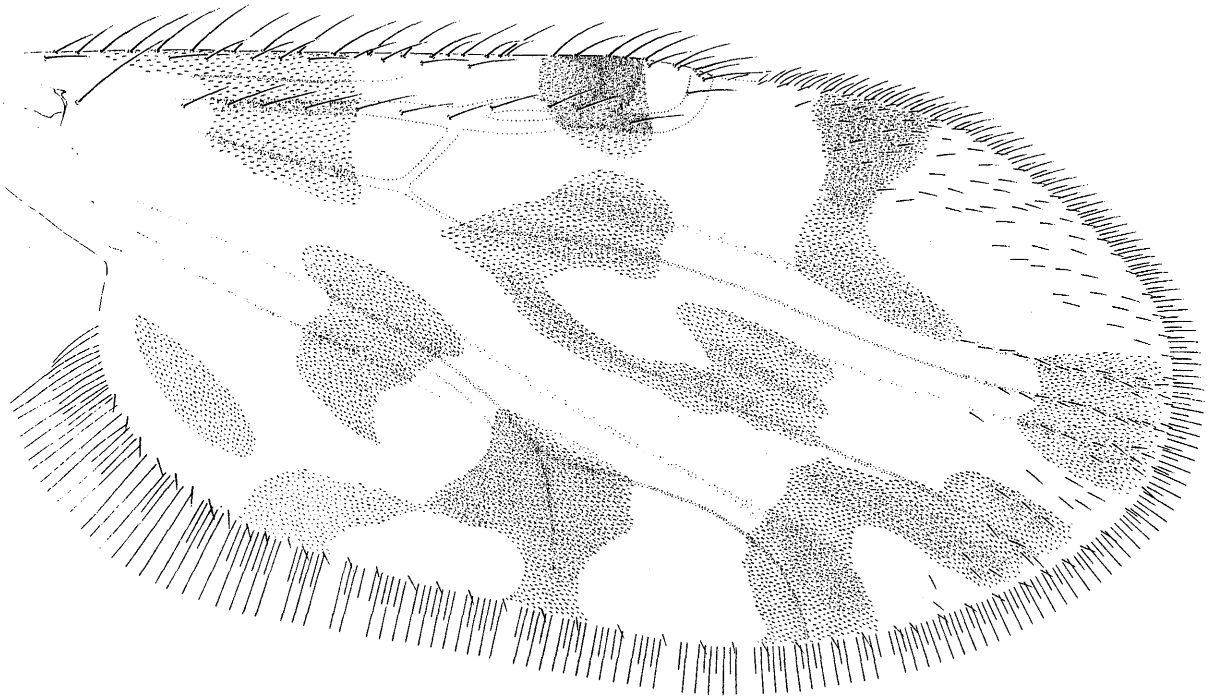


Fig. 7.2 *Culicoides (Avaritia) kwagga* sp. nov. Wing, female (slide Pilanesberg 5).

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.

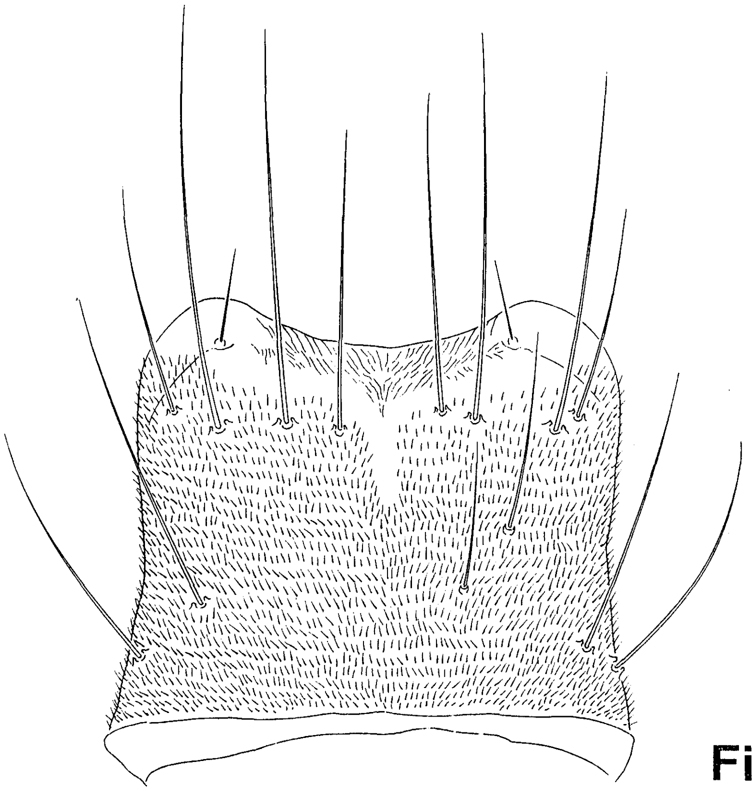


Fig. 7.3

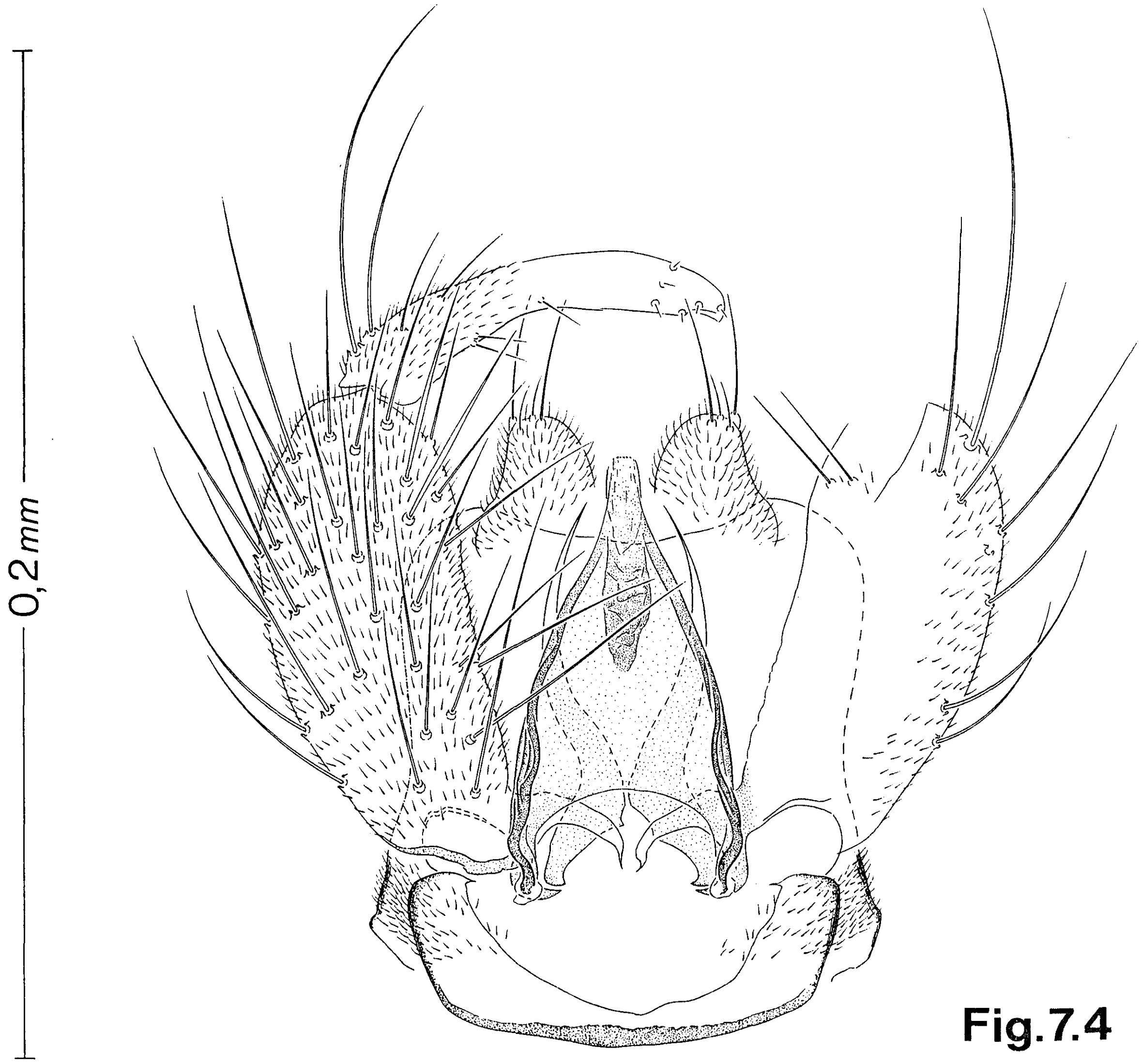


Fig. 7.4

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,

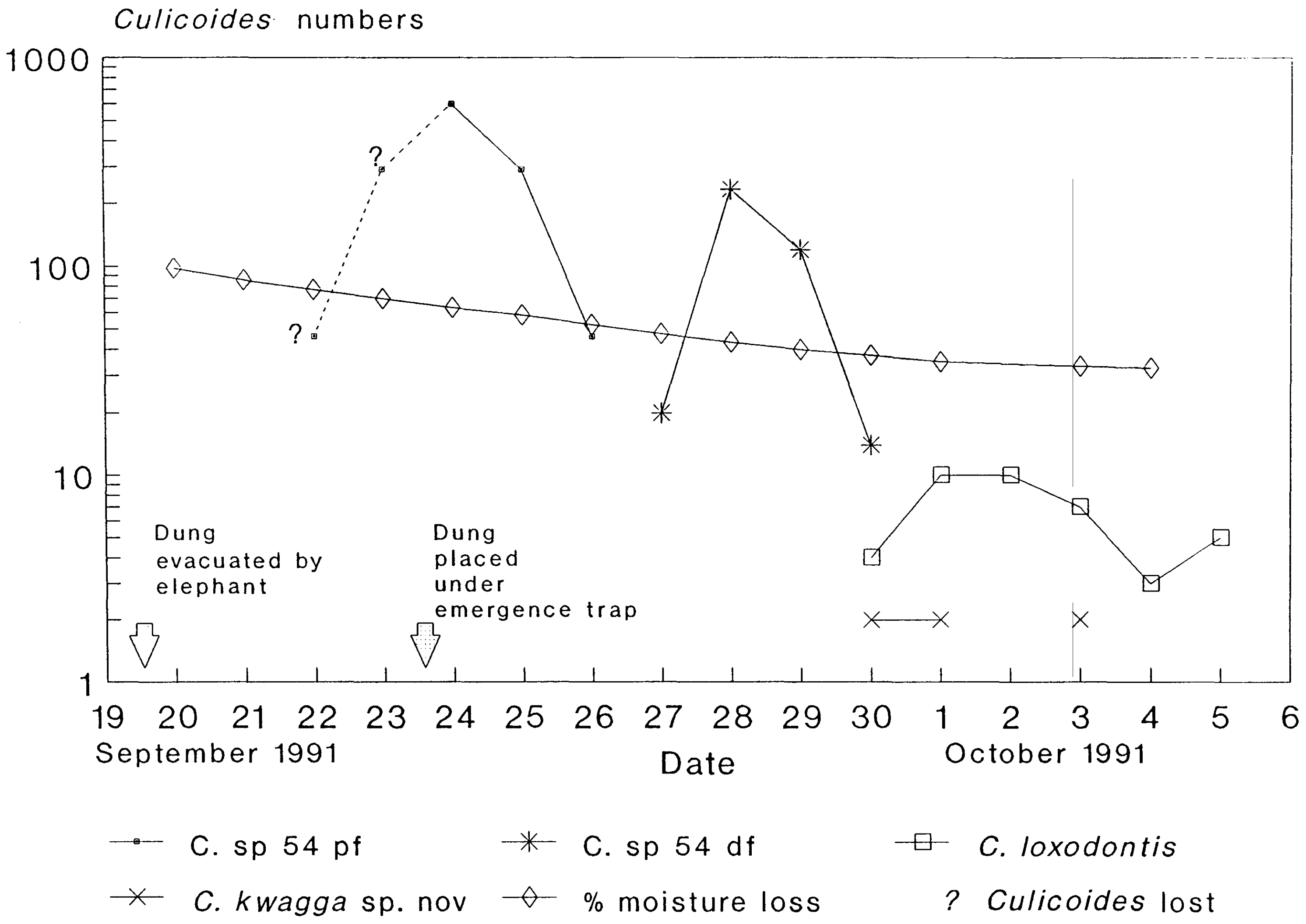


FIG. 7.5 Numbers of four *Culicoides* spp. reared from 7,825 kg of elephant dung collected on 19/9/1991 at Mutlumuvi 12 km n. of Skukuza (southern KNP); dung a few hours old. Collection no. KNP 91.26.

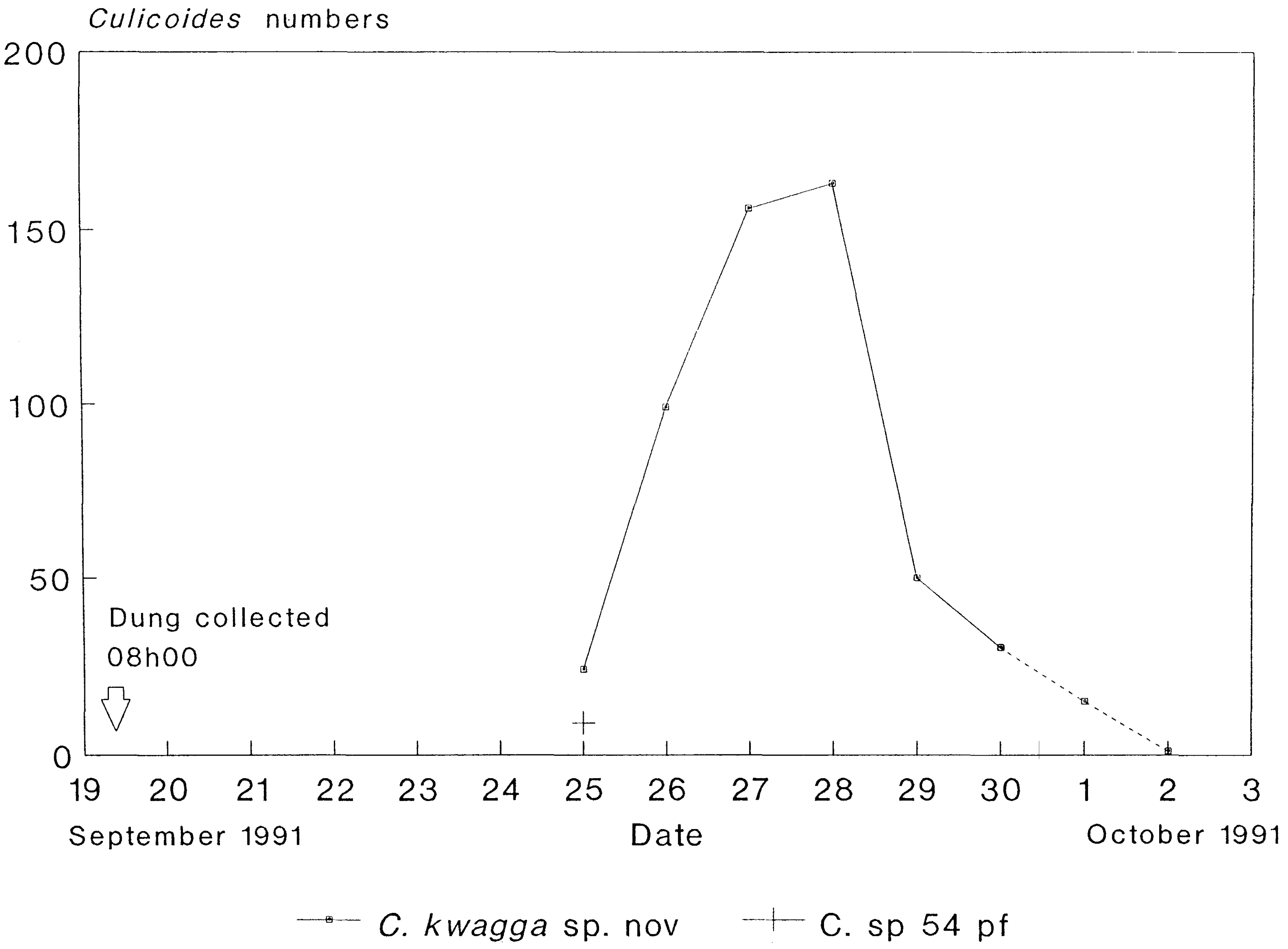


FIG. 7.6 Numbers of two *Culicoides* spp. reared from 4,5 kg of zebra dung collected on 19/9/1991 at Ngirivane 12 km w. of Satara (central KNP); dung a few hours to 1,5 days old. Collection no. KNP 91.27.

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

BARNARD, B.J.H. 1993. Circulation of African horsesickness virus in zebra *(Equus burchelli)* in the Kruger National Park, South Africa, as measured by the prevalence of type specific antibodies. *Onderstepoort Journal of Veterinary Research,* 60:111–117.

BARNARD, B.J.H. 1994. Epidemiology of African horse sickness: zebra as virus reservoir. In working document of: OIE scientific conference on the control of Foot and Mouth Disease, African Horse Sickness and Contagious Bovine Pleuropneumonia. Gaborone (Botswana), 20–23 April 1994:57–58.

EDINGTON, A. 1895. Horse sickness — Oedema mycosis. History. In: Report of the Director of the Colonial Bacteriological Institute for the year 1894. Department of Agriculture. Cape of Good Hope. Cape Town: W.A. Richards & Sons, Government Printers.

LUBROTH, J. 1991. The complete epidemiologic cycle of African horsesickness: our incomplete knowledge. *In*: WALTON, T.E. & OSBURN, B.I. (eds). *Bluetongue, African Horsesickness and Related Orbiviruses*. Proceedings of the Second International Symposium. CRC Press, Boca Raton, Florida, U.S.A.: 197–204.

MEISWINKEL, R. 1992. Afrotropical *Culicoides*: *C. (Avaritia) loxodontis* sp. nov., a new species of the Imicola group closely associated with the African elephant in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 59: 145–160.

MELLOR, P.S., BONED, J., HAMBLIN, C. & GRAHAM, S. 1990. Isolations of African horse sickness virus from vector insects made during the 1988 epizootic in Spain. *Epidemiology and Infection*, 105:447–454.

PALMITER, C. 1991. Death stalks Iberia's horses. *Readers Digest*, 115–119.

RODRIGUEZ, M., HOOGHUIS, H. & CASTANO, Ma. 1992. African horse sickness in Spain. *Veterinary Microbiology*, 33:129–142.

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

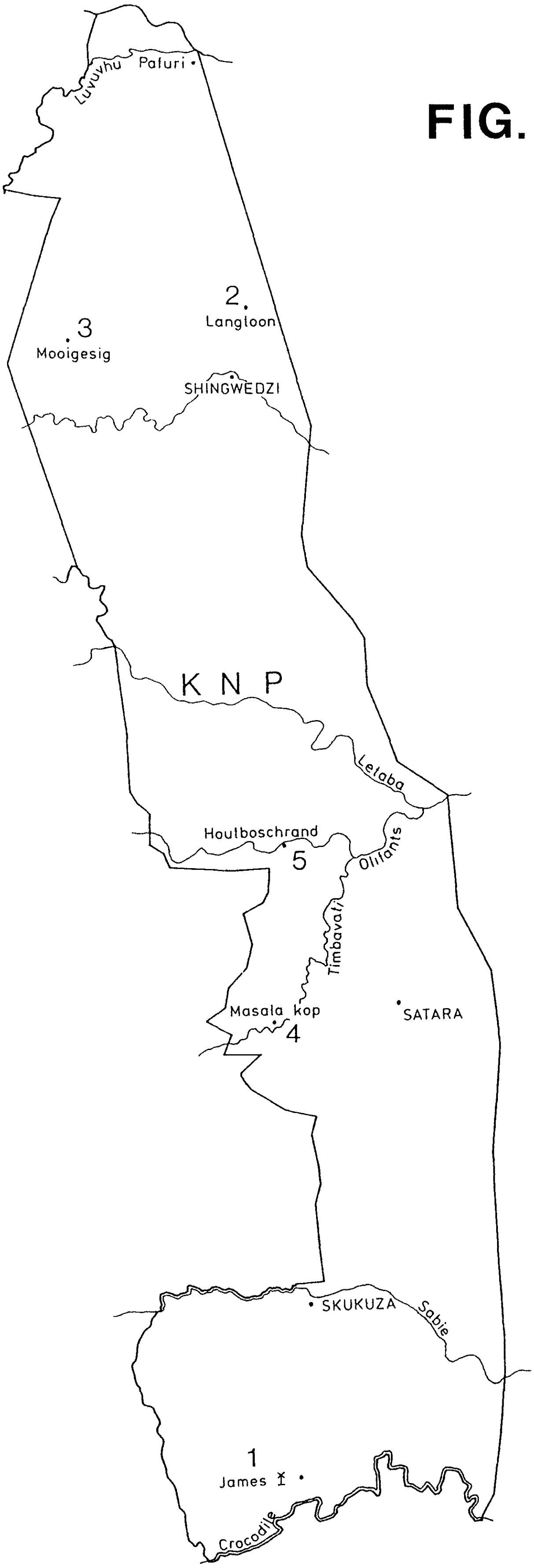


Fig. 8.1 Map of the Kruger National Park detailing elephant culling sites 1–5, major rivers and camps.

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.

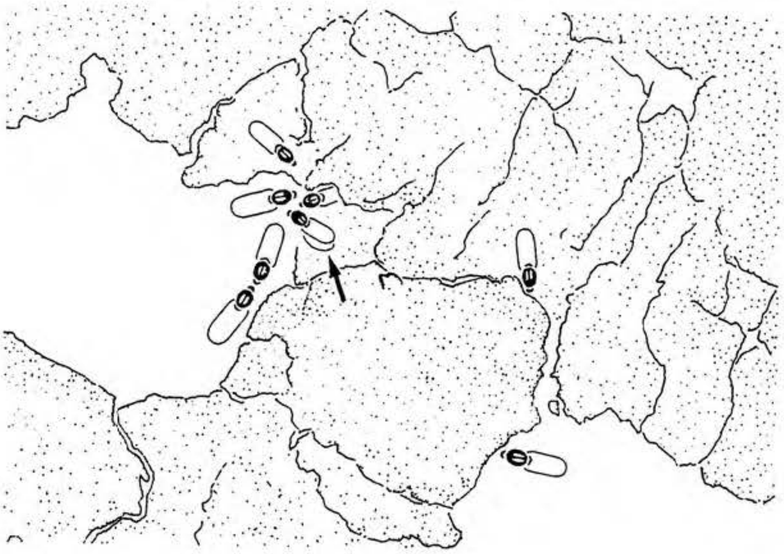
The presence of only one gravid female *Culicoides* behind elephant ears suggests strongly that sated midges leave the host to digest the blood and develop their ovaries in a suitable micro-habitat which quite likely is in the nearby vegetation. Besides fanning themselves with their ears constantly during 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.

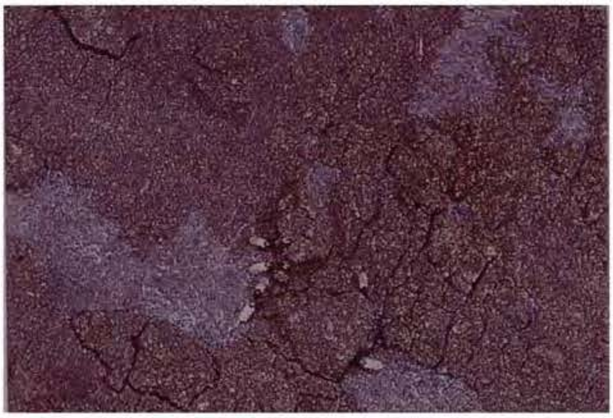
Although four elephants were examined at Langtoon, only one nulliparous specimen of C. sp. # 54 (p.f.) was found (not tabulated). This finding must be interpreted with caution. It is quite possible that not all individuals in a herd of elephants have attendant *Culicoides*, but it must also be noted 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.

8.5 REFERENCES

ACOCKS, J.P.H. 1975. Veld types of South Africa with accompanying veld type map. *Memoirs of the Botanical Survey of South Africa*, No. 40:128.

BANNISTER, A. & SKINNER, J. 1987. *South African Animals in the Wild.* 2nd edition. Central News Agency, Johannesburg, 2001.

BARNARD, B.J.H. 1993. Circulation of African horsesickness virus in zebra *(Equus burchelli)* in the Kruger National Park, South Africa, as measured by the prevalence of type specific antibodies. *Onderstepoort Journal of Veterinary Research,* 60:111-117.

BINEPAL, V.S., WARIRU, B.N., DAVIES, F.G., SOI, R. & OLUBAYO, R. 1992. An attempt to define the host range for African horsesickness virus (Orbivirus, Reoviridae) in East Africa, by a serological survey in some Equidae, Camelidae, Loxodontidae and Carnivore (sic). *Veterinary Microbiology*, 31:19-23.

BRAVERMAN, Y. 1992. Host detection, hourly activity, and the preferred biting sites of *Culicoides imicola* (Diptera: Ceratopogonidae) on a calf in Israel. *In*: WALTON, T.E. & OSBURN, B.I. (eds.). *Bluetongue, African Horse Sickness, and Related Orbiviruses.* Proceedings of the Second International Symposium. CRC Press, Boca Raton, Florida, U.S.A.: 327-332.

BRAVERMAN, Y. & PHELPS, R.J. 1981. Species composition and bloodmeal identification in samples of *Culicoides* (Diptera: Ceratopogonidae) collected near Salisbury, Zimbabwe in 1976-77. *Journal of the Entomological Society of Southern Africa,* 44:315-323.

CLASTRIER, J. 1958. Notes sur les Cératopogonidés IV. — Cératopogonidés d'Afrique occidentale française. *Archives de l'Institut Pasteur d'Algerie*, 36:192–258.

COE, M.J. 1972. Defaecation by African elephants (*Loxodonta africana africana)* Blumenbach. *East African Wildlife Journal*, 10:165-174.

DAVIES, F.G. & LUND, L.J. 1974. The application of flourescent antibody techniques to the virus of African horsesickness. *Research in Veterinary Science*, 17:128-130.

DAVIES, F.G. & WALKER, A.R. 1974a. The distribution in Kenya of bluetongue virus and antibody and the *Culicoides* vector. *Journal of Hygiene*, Cambridge, 72:265-272.

DAVIES, F.G. & WALKER, A.R. 1974b. The isolation of ephemeral fever virus from cattle and *Culicoides* midges in Kenya. *The Veterinary Record*, 95:63-64.

DAVIES, F.G. & JESSETT, D.M. 1985. A study of the host range and distribution of antibody to Akabane virus (genus bunyavirus, family Bunyaviridae) in Kenya. *Journal of Hygiene*, Cambridge, 95:191-196.

DAVIES, F.G. & OTIENO, S. 1977. Elephants and zebras as possible reservoir hosts for African horsesickness virus. *The Veterinary Record*, 100:291-292.

DAVIES, F.G., SHAW, T. & OCHIENG, P. 1975. Observations on the epidemiology of ephemeral fever in Kenya. *Journal of Hygiene*, Cambridge, 75:231-235.

DE MEILLON, B. & HARDY, F. 1953. New records and species of biting insects from the Ethiopian region. IV. *Journal of the Entomological Society of Southern Africa*, 16:17-35.

DIPEOLU, O.O. 1976. Studies on *Culicoides* species of Nigeria. II. Species collected around wild animals at Ibadan. *Veterinary Parasitology*, 1:257-263.

DOUGALL, H.W. & SHELDRICK, D.L.W. 1964. The chemical composition of a day's diet of an elephant. *East African Wildlife Journal*, 2:51-59.

DOWNES, J.A. 1950. Habits and life-cycle of *Culicoides nubeculosus* Mg. *Nature,* 166:510-511.

DU TOIT, R.M. 1962. The role played by bovines in the transmission of bluetongue in sheep. *Journal of the South African Veterinary Medical Association,* 33:483-490.

DYCE, A.L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Journal of the Australian Entomological Society*, 8:11-15.

DYCE, A.L. & MARSHALL, B.D. 1989. An early record of *Culicoides* species (Diptera: Ceratopogonidae) developing in the dung of game animals in southern Africa. *Onderstepoort Journal of Veterinary Research*, 56:85-86.

ERASMUS, B.J., YOUNG, E., PIETERSE, L.M. & BOSHOFF, S.T. 1976. The susceptibility of zebra and elephants to African horsesickness virus. *Proceedings of the 4th International Conference on Equine Infectious Diseases,* Lyon: 409-413.

FIEDLER, O.G.H. 1951. The South African biting midges of the genus *Culicoides* (Ceratopogonid.: Dipt.). *Onderstepoort Journal of Veterinary Research,* 25:3-33.

GERTENBACH, W.P.D. 1983. Landscapes of the Kruger National Park. *Koedoe*, 26:9-121.

GLICK, J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology*, 27:85-195.

HAMBLIN, C., ANDERSON, E.C., JAGO, M., MLENGEYA, T. & HIRJI, K. 1990. Antibodies to some pathogenic agents in free-living wild species in Tanzania. *Epidemiology and Infection*, 105:585-594.

HARESNAPE, J.M., TAYLOR, W.P. & LUNGU, S.A.M. 1988. The epidemiology of bluetongue in Malawi. *Epidemiology and Infection*, 100:493-499.

HARWOOD, R.F. & JAMES, M.T. 1979. *Entomology in Human and Animal Health.* 7th edition. Washington State University, Pullman.

HESS, W.R. 1988. Chapter 14. African horsesickness. *In*: MONATH, T.P., (ed.). The arboviruses: epidemiology and ecology. Vol II. CRC Press, Inc., Boca Raton, Florida, USA: 1-18.

KANGWAGYE, T.N. 1973. Diurnal and nocturnal biting activity of flies (Diptera) in western Uganda. *Bulletin Entomological Research*, 63:17-29.

KANGWAGYE, T.N. 1974. The seasonal incidence of biting flies (Diptera) in Rwenzori National Park and Kigezi Game Reserve, Uganda. *Bulletin Entomological Research,* 63:535-549.

KHAMALA, C.P.M. 1968. Survey of *Culicoides* (Diptera: Ceratopogonidae) of East Africa: their taxonomy and distribution. PH.D. thesis, University of East Africa, Nairobi.

KHAMALA, C.P.M. & KETTLE, D.S. 1971. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of East Africa. *Transactions of the Royal Entomological Society of London,* 123:1-95.

LUBROTH, J. 1991. The complete epidemiologic cycle of African horsesickness: our incomplete knowledge. *In*: WALTON, T.E. & OSBURN, B.I. (eds). *Bluetongue, African Horsesickness and Related Orbiviruses*. Proceedings of the Second International Symposium. CRC Press, Boca Raton, Florida, U.S.A.: 197-204.

MEISWINKEL, R. 1987. Afrotropical *Culicoides*: a redescription of *C. (Avaritia) kanagai* Khamala & Kettle, 1971, reared from elephant dung in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research,* 54:585-590.

MIRCHAMSY, H. & HAZRATI, M. 1973. A review of aetiology and pathogeny of African horsesickness. *Archives Institut Razi,* 25:23-46.

NEITZ, W.O. 1933. The blesbuck (*Damaliscus albifrons*) as a carrier of heartwater and blue tongue. *Journal of the South African Veterinary Medical Association*, 4:24-26.

NEVILL, E.M. 1971. Cattle and *Culicoides* biting midges as possible overwintering hosts of bluetongue virus. *Onderstepoort Journal of Veterinary Research,* 38:65-72.

NEVILL, E.M. & ANDERSON, DORA 1972. Host preferences of *Culicoides* midges (Diptera: Ceratopogonidae) in South Africa as determined by precipitin tests and light trap catches. *Onderstepoort Journal of Veterinary Research,* 39:147-152.

NEVILL, E.M., VENTER, G.J., EDWARDES, M., PAJOR, I.T.P., MEISWINKEL, R. & VAN GAS, J.H. 1988. *Culicoides* associated with livestock in the Stellenbosch area of the western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research,* 55:101-106.

REUBEN, RACHEL 1963. A comparison of trap catches of *Culicoides impunctatus* Goetghebuer (Diptera: Ceratopogonidae) with meteorological data. *Proceedings of the Royal Entomological Society of London* (A), 38:181-193.

SIMPSON, V.R. 1978. Serological evidence of bluetongue in game animals in Botswana. *Tropical Animal Health Production,* 10:55-60.

SMITHERS, R.H.N. 1983. *The Mammals of the Southern African Subregion.* University of Pretoria.

WALKER, A.R. & BOREHAM, P.F.L. 1976. Blood feeding of *Culicoides* (Diptera: Ceratopogonidae) in Kenya in relation to the epidemiology of bluetongue and ephemeral fever. *Bulletin of Entomological Research,* 66:181-188.

WALKER, A.R. & DAVIES, F.G. 1971. A preliminary survey of the epidemiology of bluetongue in Kenya. *Journal of Hygiene*, Cambridge, 69:47-60.

WHITE, F. 1983. *The Vegetation of Africa.* A descriptive memoir to accompany the Unesco/AETFAT/UNSO vegetation map of Africa, and maps. Paris: Unesco.

WHYTE, I. 1993. The rhino and elephant foundation helps fund elephant research in the Kruger National Park. *The Rhino and Elephant Journal,* 8:34-37.

YOUNG, E. 1972. The elephant's temperature-control mechanisms. *Custos,* 1:32-37.

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

		James W/mill	Mooigesig Dam	Mooigesig Dam	Mooigesig Dam	Houtboschrand	Masala Kop. 28.iv.1992	Totals
Date:		22.iv.1988	25.iv.1990	25.iv.1990	25.iv.1990	14.iv.1992	14:55-15:35	Totals
Time:		16:00-16:40	15:30-15:45	15:00-15:20	15:00-15:15	14:30-14:55	3	
No. of elephants:		4	1	1	1	4	Behind ears	14
Position:		Behind ears	Base of ear	Behind ears	Behind ears	Behind ears		
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:	Langtoon Dam	Langtoon Dam	Langtoon Dam	Mooigesig Dam	Masala Koppies	Totals
Date:	24.iv.1990	24.iv.1990	24.iv.1990	25.iv.1990	28.iv.1992	
Time:	16:10–16:35	16:00–16:25	16:40–16:50	16:15–16:30	15:40–16:01	
No. of elephants:	2	1	1	1	1	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**	**FEMALES**				**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

CHAPTER 9

African horsesickness epidemiology: A two-year light-trap survey of the *Culicoides* of the southern Kruger National Park and adjoining livestock farms in the eastern Transvaal lowveld, South Africa

9.1 INTRODUCTION

Fifty years ago, du Toit (1944) incriminated *Culicoides imicola* as the vector of the orbiviruses of bluetongue (BT) and African horsesickness (AHS). Only since the late 1960's, however, have various workers begun to report on the prevalence, abundance, geographic distribution and host preferences of *C. imicola*; less attention has been paid to its taxonomy and competence to vector viruses.

Some 40 studies (Table 9.1) reveal *C. imicola* to be one of the most widespread species of the genus worldwide. It occurs throughout Africa, into countries on both sides of the Mediterranean and eastwards as far as Laos. It can also be phenomenally abundant — Nevill (1971) found that *C. imicola* could comprise 97 % of up to 124 000 *Culicoides* captured nightly at Onderstepoort. In a three-year light-trap survey, Venter (1991) showed that *C. imicola* was also the most prevalent and dominant *Culicoides* species in South Africa. It was present at 33 of 34 sites sampled countrywide, and comprised 71,4 % of > 3 000 000 *Culicoides* captured.

There seems little doubt that *C. imicola* is also catholic in its choice of hosts, as it can be found in high numbers near all classes of domesticated livestock, i.e. cattle, sheep, horses, pigs and poultry (Table 9.1). Further perusal of these tabulated data, however, reveals that *C. imicola* can also be poorly represented, for example as low as 1,4 % of catches in high-altitude frost-prone Lesotho (Venter & Sweatman 1989), 0,8 % in the higher-rainfall forested central Côte d'Ivoire (Meiswinkel 1991) and 2,0 % in the dry savannas of the northern Kruger National Park (KNP), South Africa (see Chapter 8). It appears, therefore, that factors other than host preference cause the wide amplitude in *C. imicola* abundances; these will not only be climatic, as suggested by Mellor (1992), but soil-type may also play an equally important role, as will be indicated in Chapter 10.

All but two of the 40 studies summarized in Table 9.1 deal with *Culicoides* found near exotic breeds of livestock imported into Africa. It needs to be investigated whether the veterinary bias of these studies correlates positively with the high recorded abundance of *C. imicola*. As mooted by Howarth (1985), species closely associated with domestic animals 'such as *C. imicola* ... may have been spread indirectly through human activities.' In Chapter 2 it was similarly noted that 'in the farmyard situation ... the maintenance of sedentary populations of domesticated animals on irrigated pastures ... exactly suits the needs of *C. imicola* and leads to an escalation in its populations.' However, these views remain somewhat anecdotal and need substantiation.

Culicoides imicola has penetrated Spain and Portugal up to latitude 41°30'N (Mellor & Rawlings 1994). It was the major vector involved in the African horsesickness epizootic that spread through the southern parts of Iberia, and led to the death of some 2 000 horses between 1987 and 1990. This outbreak had its origin in a small group of zebras imported from Namibia and released 60 km southwest of Madrid. Recent work done in the KNP, South Africa, has confirmed that zebra do play a role in the epidemiology of AHS (Barnard 1993). Interestingly, his findings showed that while zebra foals seroconverted during every month of the year, there was a peak period in winter when *Culicoides* populations, especially those of *C. imicola*, were at their lowest. Not only do these observations lead one to surmise that the kwagga (*Equus quagga*) was a source of AHS resulting in the severe epizootics that decimated the horse populations of the Cape since the late 17th century (Henning 1956; Barnard 1994), but also that other species of *Culicoides* may be involved in its transmission.

Today the eastern Transvaal lowveld, which embraces the KNP, is recognized as South Africa's AHS 'hotspot' (B.J. Erasmus, personal communication 1992). It is generally believed that the disease spreads southwards from here, supposedly due to winds carrying infective *Culicoides* (Bosman 1994).

This two-year study was initiated to test this belief and the suspicion that man is a cause of the localised superabundance of *C. imicola*. The primary method adopted was to compare the numbers and prevalence of *C. imicola* in a largely undisturbed piece of Africa (KNP) with those found in areas altered by man (livestock farms adjoining the KNP). Aspects studied were the following:

a) which species of the Imicola group, other than *C. imicola*, occur widely in the area;

b) differences in species composition and population sizes between the two areas;

c) change in *Culicoides* numbers and species composition across an altitudinal transect;

d) to establish indirectly whether *Culicoides* are windborne namely whether there are noticeable distribution patterns, and if so, whether there are occasional, but obvious, intrusions from the KNP onto farms or *vice versa*;

e) consider whether species other than *C. imicola* have the potential to vector African horsesickness.

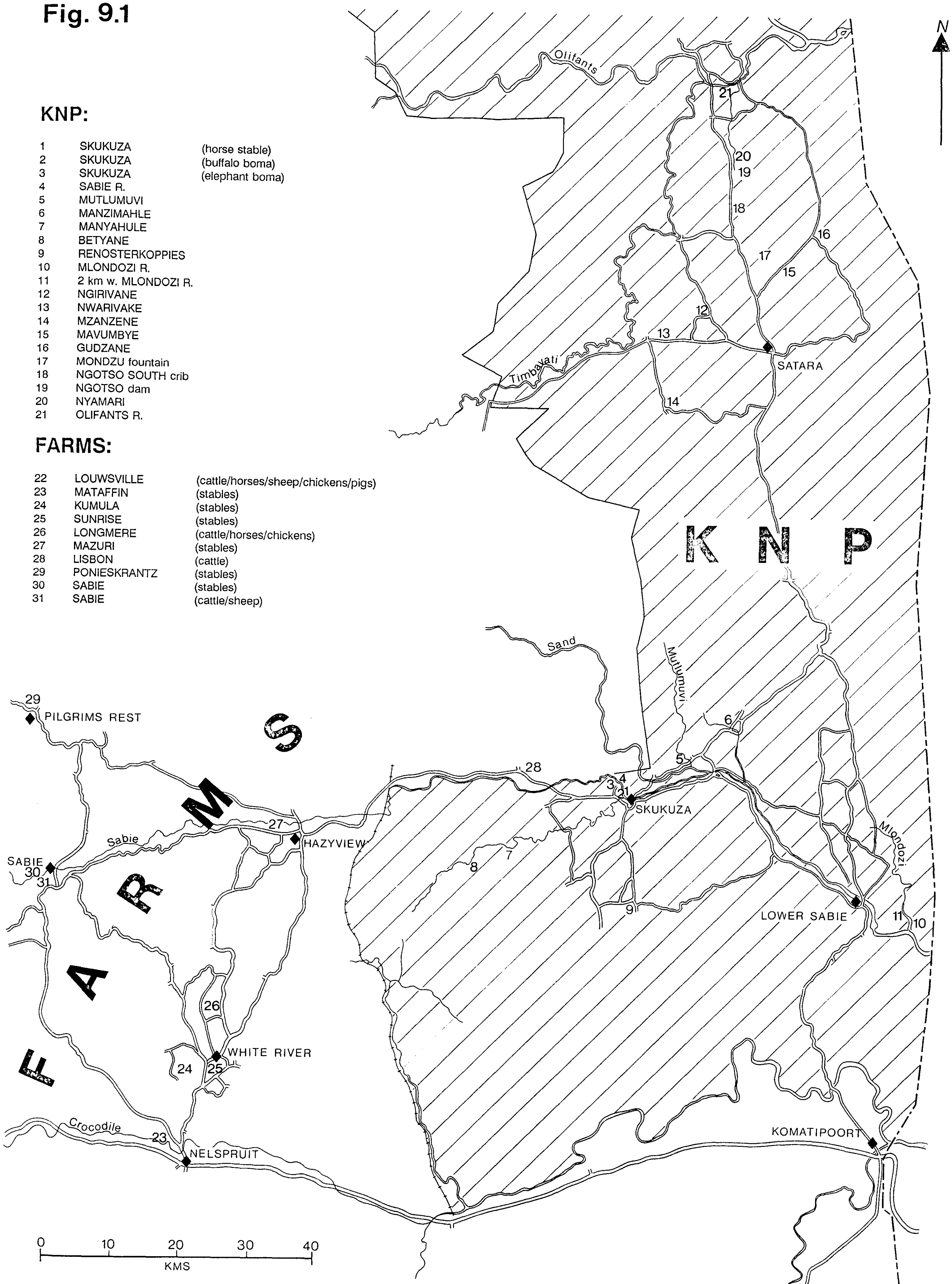
9.2 MATERIALS AND METHODS

9.2.1 Light-trap collections

KNP: Blacklight suction traps were operated monthly from VIII.1991-VIII.1993 in the southern half of the KNP (Fig. 9.1); in two years a total of 43 sites was sampled, but only 12 were monitored continuously for a year or more. A total of 695 light-trap collections were made in the triangle including Skukuza — Lower Sabie — Satara. Sampling was done at one to three sites/night, and the number of light-traps operated varied from one to nine/night. Trapping was done over a period of five to 12 days each month, and, depending on the weather, five to 65 collections (average 28) were made monthly. At most sites, three light-traps were operated simultaneously and were hung in trees and bushes at one to two metre height and 50 m apart.

FARMS: During the same period collections were made at horse stables, and at cattle and sheep farms adjoining the southern and western boundaries of the KNP; the majority were sited in the Nelspruit — White River — Hazyview — Sabie — Pilgrims Rest area (Fig. 9.1). A total of 183 collections were made on 22 farms, and only one, occasionally two, traps were operated/night; seven farms were monitored for a year or more. Traps were hung on the eaves of stables or on fences of pastures carrying cattle and/or sheep.

Fig. 9.1



KNP:

1	SKUKUZA	(horse stable)
2	SKUKUZA	(buffalo boma)
3	SKUKUZA	(elephant boma)
4	SABIE R.	
5	MUTLUMUVI	
6	MANZIMAHLE	
7	MANYAHULE	
8	BETYANE	
9	RENOSTERKOPPIES	
10	MLONDOZI R.	
11	2 km w. MLONDOZI R.	
12	NGIRIVANE	
13	NWARIVAKE	
14	MZANZENE	
15	MAVUMBYE	
16	GUDZANE	
17	MONDZU fountain	
18	NGOTSO SOUTH crib	
19	NGOTSO dam	
20	NYAMARI	
21	OLIFANTS R.	

FARMS:

22	LOUWSVILLE	(cattle/horses/sheep/chickens/pigs)
23	MATAFFIN	(stables)
24	KUMULA	(stables)
25	SUNRISE	(stables)
26	LONGMERE	(cattle/horses/chickens)
27	MAZURI	(stables)
28	LISBON	(cattle)
29	PONIESKRANTZ	(stables)
30	SABIE	(stables)
31	SABIE	(cattle/sheep)

Fig. 9.1 *Culicoides* collecting sites in the Kruger National Park (KNP) and on adjoining livestock farms (FARMS); blacklight traps VIII.1991–VIII.1993.

9.2.2 Classification of sites

KNP: a) In Table 9.2 the collecting sites are classified as 'veld' and 'bomas/stables'. The former category (627 collections) is considered natural, with little interference by Man as regards the daily movements and range of various game species. The latter category (68 collections) is unnatural in that animals are held year-round in the Skukuza bomas; game constantly held there were 15 buffaloes, 30 impalas, and a few kudu. Occasionally other species, including zebra, were brought into the bomas, but for short periods only. Alongside the bomas are 11 horses kept in well-constructed stables; by day they are released into an adjoining paddock, 1 ha in size and sandy. These bomas/stables were sampled monthly for two years. The elephant and rhino bomas, situated 2 km west, have animals only for three to four months during winter and early spring; these were sampled monthly for one year.

b) In Table 9.3 the veld collections are divided into 'wet veld' and 'dry veld'. The former denotes collecting sites along rivers, near dams and lakes, and other watering points, mostly man-made. The 'dry veld' includes sites in open grassed or bushed veld some 400–5 000 m from any water.

c) In Table 9.4 and Fig. 9.2 the 22 farms are arranged along an altitudinal transect commencing from the lower flatlands ('lowveld') and ascending the eastern escarpment of the northern Drakensberg; they are classified as 'lower scarp' and 'middle/upper scarp'.

d) In Table 9.3 and 9.4 the two scarp classes are combined as *Culicoides* numbers and species composition did not differ significantly.

9.2.3 Subsampling and classification of species

a) The 175 (25 %) KNP, and 94 (51 %) FARMS collections studied were subsampled using the method of Van Ark & Meiswinkel (1992); in the majority of collections 500–700 specimens/catch were identified, age-graded and sexed. During the winter, catch sizes regularly fell below the 500 threshold, and so all specimens were studied.

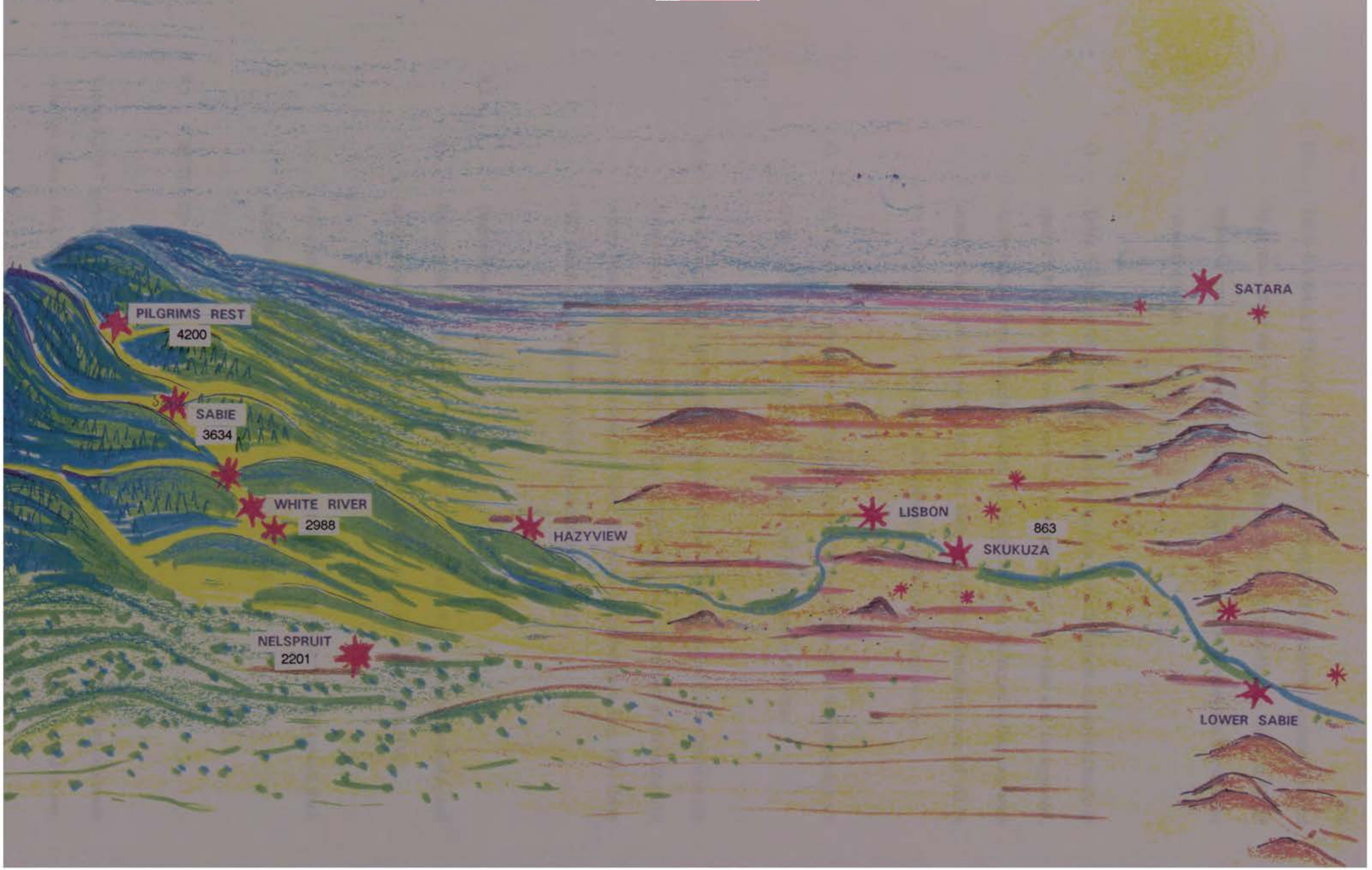


Fig. 9.2 Panoramic view of the Transvaal lowveld commencing with escarpment (FARMS) on the left and the low-lying KNP to the right. Not all light-trap sites shown; altitudes given in feet.

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b) Table 9.4 lists all 75 *Culicoides* species collected, and their relative numbers (denoted by a star-rating on a scale of 0–5). Each species is classified as either a 'KNP endemic' or a 'scarp endemic', while species common to both zones are listed in the 'shared' column.

c) Table 9.3 shows the dominant *Culicoides* species in the 20 largest collections made in each of five zones: 'dry veld', 'wet veld', 'bomas/stable', 'lowveld' and 'escarpment'. At the head of the columns are shown the months in which the largest collections were made, and range in catch size. This table summarises identifications of 68 % (> 3 000 000) of ± 4 500 000 *Culicoides* collected in the entire survey.

d) In Table 9.5 the abundance and prevalence data of six species of the Imicola group are given, along with those of five other species of the subgenus *Avaritia.*

e) In Tables 9.8–9.11 a breakdown is given of the 35 species of *Culicoides* found in the Satara area (sites 12–21; Fig. 9.1); the 200 light-trap collections were made in the area where Barnard (1993) collected sera from 123 zebra darted between Aug. '91—May '92, which gave evidence of rapid seroconversion to AHS.

9.2.4 Discussion of species

a) Each of the six species of the Imicola group in Table 9.5 is briefly discussed, and explanations given for their differential presence in the KNP and on FARMS.

b) The ecology of each of the 13 dominant species shown in Table 9.3 is discussed; their known larval habitat preferences are given in Table 9.7.

9.3 RESULTS

Tables 9.2–9.7 give a breakdown of the 4 416 272 *Culicoides* taken in 878 light-trap collections made over two years at 65 sites in the KNP (43) and on FARMS (22). Selected aspects are discussed below, with emphasis on *C. imicola* and 10 other species of the subgenus *Avaritia*. Tables 9.8–9.11 represent a subset of the findings made in the Satara area; these are appraised separately as they were generated in 'zebra country' during a period when seroconversions to AHS had been demonstrated amongst zebras (Barnard 1993).

a) *Culicoides* (all species) were 3,4 times more abundant on FARMS than in the KNP. On FARMS a remarkable 94,1 % were trapped in the flatter, hotter lowveld areas. In the lower to middle/upper escarpment there was a marked decline in *Culicoides* numbers (Table 9.2), even though the traps were set at similarly large groups of animals and were operated on the same nights.

b) On FARMS *C. imicola* comprised 79,7 % (± 1,5 million) of all *Culicoides* caught, and was present in 92 of 94 catches analysed; 96 % of the *C. imicola* specimens were caught in the hot lowveld (Table 9.2). The sharp decline of numbers of this species as one ascends the escarpment correlates strongly with the decline in recorded AHS cases, and is most probably due to good water run-off on an undulating topography which would prevent a build-up of large saturated breeding areas. Higher up the escarpment *C. imicola* remained the dominant species, but a combination of sloping topography, lower temperatures and a more widely spread livestock biomass, further suppressed its numbers.

c) The single largest light-trap collection made over two years was 533 984 *Culicoides* (von Landsberg, farm Mazuri, 11 horses; Hazyview 8.II.1993); subsampling showed *C. imicola* to comprise 90,41 % i.e. 482 807 individuals. The second largest catch made was 293 497 (Lisbon Estates, 99 cattle; 6 km west of Paul Kruger gate, 20.XI.1992) 73,2 % of which were *C. imicola*. The third largest catch of 190 456 was made at the Skukuza bomas (15 buffaloes; 8.XII.1992); again *C. imicola* was dominant (50,1 %). These three collections make up nearly 25 % of all *Culicoides* collected throughout the survey, and suggests that confined livestock lead to massive *Culicoides* foci, invariably dominated by *C. imicola* (Table 9.3). While catch sizes at Hazyview horses and Skukuza buffaloes responded positively to increases in temperature and rainfall in summer, and declined with the onset of cold in winter, the catches crashed at Lisbon Estates cattle in December 1992; two light-trap collections yielded 8 000 and 12 000 *Culicoides* respectively, a 30-fold decrease from the previous month. No evidence is available but this was most likely due to implementation of the summer acaricide programme for cattle which may impact heavily on *Culicoides*; their numbers increased gradually over the next five months to a total of 33 363 in May 1993. Nineteen of the 20 largest FARMS collections were made at horse-stables. Two factors may explain this:

- horses are sensitive to dips, pour-ons and similar treatments, and are thus not treated with acaricides;
- the kikuyu pastures in horse paddocks are usually cropped short; dairy cattle are mostly kept on lush kikuyu 15–30 cm deep. Such long grass does not seem to favour *C. imicola* as much as the cropped grass. Whether it is repetitive acariciding or a shaded larval habitat that inhibits *C. imicola* on dairy farms remains to be investigated.

d) In the KNP *C. imicola* was far less abundant, comprising 30 % of the ± 1 million *Culicoides* analysed of > 2 000 000 captured (Table 9.2). Significantly, 93 % of the *imicola* specimens were found at the 'bomas/stable' in and around Skukuza. Only 6,6 % of the *Culicoides* trapped in the KNP veld were *C. imicola*; there it was absent in 8 % of the light-trap collections made, while < 10 individuals were found in a further 40 %. Despite this, *C. imicola* remained one of the most prevalent species in the KNP veld as it was found in 127 of 139 collections analysed (Table 9.5). In Table 9.2 the 'between site ratio' shows *C. imicola* is 273 times more abundant on 'lowveld' FARMS than in the KNP veld, despite the two locales being at about the same altitude and having a similar climate. Such high numbers of *C. imicola* on FARMS, and its dominance at the Skukuza bomas/stable, strongly suggest that man's maintenance of readily available feeding resources (whether these be cattle, horses, buffaloes or elephants) leads to a perpetuation of large populations of *C. imicola*. Despite a large biomass of game animals in the KNP veld, the reasons are at least two-fold as to why *C. imicola* is poorly represented there:

- breeding sites are limited in a predominantly dry, low-rainfall landscape; and
- the movement patterns of game animals, in being somewhat random, would further

inhibit the development of *Culicoides* foci.

e) Reference to Table 9.3 in which the KNP veld has been divided into 'dry veld' and 'wet veld', raises the question: why is *C. imicola* not dominant in the flat 'wet veld' where high numbers of *Culicoides* occur? If one eliminates the random prevalence of game animals as the direct cause (as it would also apply in the dry veld and should affect other species of *Culicoides*), it seems more likely that the unsuitability of the available larval habitats is of greater import. Some reasons are:

- the most important reason, perhaps, is that the pupae of all *Avaritia* species, in being unable to float, drown in flowing waters i.e. those of rivers and streams
- where quiet pockets of water do exist in rivers the type of micro-organisms available may not suit *Avaritia* species; this could be exacerbated by the good drainage of coarse sands;
- while other pockets of organically-rich silted sands might be a suitable feeding resource of the larvae, these sites tend to become thickly vegetated and shaded; this may not suit *C. imicola* which seems to prefer shortly vegetated, sunlit larval habitats. Given that the immatures of *C. imicola* are dependent on moisture (like all species of *Culicoides*), it seems ironic that it does better in the 'dry veld'. Although not shown in Table 9.3, *C. imicola* only becomes dominant in the 'dry veld' after the rains begin to fall between November and April. This suggests that *C. imicola* invades the margins of rain pools that form in the veld (which effectively transform the 'dry veld' into 'wet veld'). These pools would be standing, strongly sunlit, and marginally vegetated. Furthermore, pools would form in areas with impeded drainage, so the margins would remain moist for longer periods, and would benefit the immatures of *C. imicola*. Finally, pools on clay-based soils would seem to be richer in the nutrients sustaining the food organisms appropriate for *C. imicola*, but this remains to be demonstrated. On FARMS, the

irrigation of pastures in low-lying areas would produce conditions analogous to such wet veld pools; the constant maintenance of livestock in their immediate vicinity would provide blood for the midges, and could result in constant maintenance of large populations in cyclical patterns.

f) As the maintenance of livestock in confined spaces leads to the development of large *Culicoides* foci why then are the catches made at the bomas/stables not larger than those made in the 'wet veld'? It is an oversimplification to classify the KNP as an untouched piece of African wilderness. Since the early 1950's, the trustees of the National Parks Board have pursued a policy of providing water for game during droughts; these are not infrequent in this low rainfall savanna. In the southern half of the KNP at least 100 water boreholes are windmill-driven. At each site there is either a reservoir and/or trough, the former for elephants, the latter for all other types of game. At some places small earthen dams have also been constructed into which excess water is piped. These constituted most of the 'wet veld' trapping sites; three other sites were on the banks of two of the six large sand rivers that transverse the KNP from west to east. Their margins can be either tree-lined or densely reeded; during even the driest month of this century (October 1992) they continued to flow at very low levels and rates.

Given the presence of rivers, occasional springs and numerous artificial waterpoints, it would be accurate to say that the maintenance of large populations of game in the KNP is extensively assisted through management practices. In the broad sense, this could be termed farming. Thus the choice to use such animal congregation points for the monitoring of *Culicoides* was biased, and probably explains the similarity in *Culicoides* catch sizes between KNP 'bomas/stable' and 'wet veld'. Five natural or 'dry veld' sites were sampled but only two were monitored regularly. These were sited 400–5 000 m from water. Table 9.3 shows the 'dry veld' catches to be significantly smaller than those made in the 'wet veld'; this is apparently due to a lack of breeding sites and the random or transient presence of animals. However, a closer examination of the *Culicoides* found in 'dry veld' catches provides interesting data as regards dispersal. The Nwarivake collections (Table 9.6) were not only large for the 'dry veld' category but were also unusual in that > 80 % of the *Culicoides* of 12 species captured belonged to one species only:

C. bolitinos. On this occasion the two light-traps had been set opportunistically in the path of a herd of 60 buffalo sighted at dusk. The data in Table 9.6 suggest:

- *C. bolitinos*, known to breed primarily in buffalo dung, must have been following the herd of buffalo. The high percentage of gravid females implies that they were seeking dung in which to lay eggs, while the high number of nulliparous and parous females indicates they were in search of a bloodmeal. The virtual absence of males indicates that no breeding in dung occurred locally which in turn means the buffalo were not confined to this small area, nor did they traverse it daily.

- If these data have been correctly interpreted, we can surmise that *C. bolitinos* is closely associated with, and dependent upon, buffaloes, and will accompany herds (for some distance at least) on their daily treks. Table 9.5 shows *C. bolitinos* is present in 80 % of 627 collections made in the KNP veld, but averaged only 110 specimens/trap. At the bomas/stable it was 10 times more abundant, averaging 1 040/trap; the largest catch of 14 087 was made at the Skukuza buffalo boma. Only on three occasions in two years were more than 1 000 specimens of *C. bolitinos* taken in veld light-traps. Two of these were at Nwarivake and reinforce the notion that *Culicoides* do follow game animals (see Chapter 8). If such mobile foci exist, then the *Culicoides* species composition of light-trap collections could change daily, perhaps even hourly, as different game animals criss-cross the same habitat. This would be especially true for coprophilic *Culicoides* species of the subgenus *Avaritia*; in this way they would disperse widely. Such dispersal would not be passive but host-mediated.

g) A large total of 75 species of *Culicoides* was collected along the east-west altitudinal gradient spanning 132 km from Lower Sabie (KNP) to Pilgrims Rest (FARMS) (Fig. 9.2; Table 9.4). Species turnover, or beta diversity, between the 'lowveld' and escarpment habitats was marked. Table 9.4 categorizes the species as 'KNP endemics', 'shared' and 'escarpment endemics', and gives each species a star rating as to its relative abundance. The 'shared' column has asterisks to the left and right to denote a dual prevalence. While species diversity is to be expected in a transect community that is not homogeneous either floristically or climatically, comparisons are futher complicated by the fact that the eastern third (KNP) comprises largely undisturbed habitat home to indigenous game animals, while the western two-thirds (FARMS) have been greatly affected by man through 'deterministic extinction'; the flora has been replaced with exotic fruit monocultures or afforested, and all the larger game herbivores replaced by domesticates including cattle, horses and sheep. Nevertheless, it can still be seen clearly that the *Culicoides* fauna comprises a temperate (escarpment) and savanna (lowveld) sector, with a small tropical element on the eastern fringes. Even where the former two overlap and species are 'shared', there remain marked differences in their relative abundances.

h) In attempting to unravel the epidemiology of African horsesickness, it is pertinent to examine the 12–15 most abundant species in the 'shared' column. More than half of these (*loxodontis*, C. sp. # 110, *tropicalis*, *kobae*, C. sp. # 54 p.f., C. sp. # 54 d.f. and *C. kwagga*) are rare or uncommon in the farming arena, and so might play no role in AHS transmission there. This leaves four species for further consideration: *C. imicola*, *C. bolitinos*, C. sp. # 3 (Schultzei grp.) and *C. exspectator*. *Culicoides bolitinos* can perhaps be eliminated because of its dependence on cattle and buffaloes, C. sp. # 3 too as it appears only briefly in large numbers during the hottest months (Jan./Feb.), while *C. exspectator* may be ornithophilic. *Culicoides imicola* remains as the prime candidate for AHS transmission in the farming community; its overwhelming dominance on farms is illustrated by the shaded blocks in Table 9.3. At the Mataffin stables, Nelspruit, where as many as seven horses died daily in the 1973/74 horsesickness epizootic, *C. imicola* made up > 80 % of consistently large *Culicoides* catches made; this strongly suggests it to have been responsible for virus transmission in those years.

i) While *C. imicola* was superabundant and dominant on farms, 18 of the 52 species collected in the KNP were dominant in at least one of the 175 light-trap catches analysed; the 60 largest catches of 695 made in the KNP were dominated by 12 species (Table 9.3). They belonged to the Similis group (*C. tropicalis*, *C. kobae* and *C. micheli*), the Schultzei group (*C. schultzei*, C. sp. # 3 and C. sp. # 110), the subgenus *Avaritia* (*C. imicola, C. bolitinos, C. loxodontis*, C. sp. # 54 p.f. and C. sp. # 54 d.f.) and the subgenus *Meijerehelea* (*C. nivosus*). Where known, their larval habitat preferences are listed in Table 9.7; although it is an overly brief summary (mainly due to our incomplete knowledge), it does show that larval preferences greatly influence their abundance and geographic distribution. For instance, members of the Similis and Schultzei groups can become very abundant along the margins of broad-bottomed sand rivers, but their populations will crash when these rivers flood; outside the KNP, these species will always be poorly represented on the escarpment slopes where rivers are still narrow, fast-flowing and colder, and lack sunlit sandy margins. Similarly, a handful of *Avaritia* species dependent on elephant dung become locally extinct (as on FARMS) when elephants are exterminated.

j) *Culicoides bolitinos* (Imicola group): this species was the second most abundant and prevalent member of the Imicola group. It is known to inhabit the dung of buffaloes and wildebeest and has switched, with great success, to cattle dung. It probably feeds predominantly on these animals and so is unlikely to be a vector of African horsesickness. In the eastern Orange Free State (OFS), *C. bolitinos* comprises > 50 % of large numbers of 32 species of *Culicoides* found near livestock; the area is free of AHS.

k) *Culicoides loxodontis* (Imicola group) and C. sp. # 54 p.f. (group unknown): as regards elephants (suspected reservoir hosts for AHS), seven species of *Culicoides* have been found to breed in their dung; these do not include *C. imicola* (see Chapter 8). Two of these, *C. loxodontis* and C. sp. # 54 p.f., can become dominant in the KNP veld, and are very prevalent (Table 9.5, 9.7). It is becoming increasingly obvious that they are exclusively associated with elephants, being dependent on them for blood and dung. Except for sporadic individuals, they are not found on farms. While a few serology surveys elsewhere in Africa have indicated *Loxodonta* to be a reservoir host for AHSV, recent studies on the sera of KNP elephants suggest otherwise (B.J.H. Barnard and R. Bengis, personal communication 1995). This implies that elephant-associated *Culicoides* may not vector the virus, an observation supported by the fact that AHSV occurs widely outside the range of these two *Culicoides* species and elephants in South Africa.

l) *Culicoides kwagga* (Imicola group) and C. sp. # 54 d.f. (group unknown): these two species have greater contact with zebras (a proven reservoir host for AHS), and, importantly, also occur around horses on FARMS but in very low numbers. Of the two, *C. kwagga* appears to be almost exclusively associated with zebras; up to 500 adults have been reared from 4,5 kg of zebra dung. At Manzimahle, 20 km north-east of Skukuza, a congregation point for zebras during the drier months, *C. kwagga* manifested itself during the months of June–November in two successive seasons. On occasion it can be the dominant species in light-traps, but it never becomes particularly abundant; the largest number collected in a single light-trap was 357. It is interesting to note that *C. kwagga* appears on adjoining FARMS (at horse stables only) during the same winter/spring months, and as in the KNP, also disappears during the summer months. However, it is rarer on FARMS than in the KNP (Table 9.5); this may be due to larger groups of zebras providing a larger mass of dung for *C. kwagga* to breed in. It is important to note that Barnard (1993) found that young zebra foals between the ages of six to 12 months seroconverted to all nine serotypes of AHS between the months June–December in the Satara area. This correlates perfectly with the upswing of both *C. kwagga* and C. sp. # 54 d.f. While these two species must be considered potential AHS vectors, especially the former as it is closely related to *C. imicola*, a number of other *Culicoides* species can be present during the same period (see 9.4 below). As noted in Chapter 7, the disapperance of *C. kwagga* from light-trap collections in the hotter summer months is due to zebra dung drying out too quickly (two to three days) for immatures requiring seven to 10 days to complete their cycle. How *C. kwagga* maintains itself in the KNP during the summer months remains unknown. Elsewhere in the Transvaal, however, it has been reared in large numbers from white rhino dung but only from complete boli. Numerous rhino middens sampled in the KNP have proved negative for *C. kwagga*, presumably because the dung is spread out thinly by kicking and so dries out quickly. Very rarely are whole boli of white rhino dung seen in the KNP. The white rhino is rare in the Satara area and seems unlikely to be involved in the rapid cycling of AHS locally.

m) C. sp. # 54 d.f. (group unknown): This species has the same seasonal prevalence pattern as *C. kwagga*, but it is difficult to pinpoint its precise relationship with zebra as it breeds in higher numbers in elephant dung, whereas *C. kwagga* does not. This implies that elephants, in criss-crossing zebra habitats, and visiting the same waterholes, may be introducing and maintaining

C. sp. # 54 d.f. locally. However, it is also known that elephants are not the sole hosts of C. sp. # 54 d.f., as it is also found at horse-stables outside the KNP, and in game reserves throughout the Transvaal where only zebra and/or white rhino are kept. Why C. sp. # 54 d.f. disappears during the hotter summer months, both in the KNP and in the cooler escarpment areas, remains to be explained. In Table 9.5, 99 % of the FARMS specimens recorded under C. sp. # 54 s.l. are C. sp. # 54 d.f.; it is only slightly more prevalent and abundant than *C. kwagga*.

n) *Culicoides miombo* (Imicola group): An extremely rare species in the KNP (0,005 %) and absent on FARMS. The data support the contention that *C. miombo* is more tropical in its distribution, only becoming abundant in high-rainfall, frost-free areas. The most individuals (63) found in a single light-trap collection were captured at the Skukuza buffalo bomas; this suggests that *C. miombo* is mammalophilic.

o) Do winds disperse *Culicoides* over a wide area? It seems not, judging from the high level of beta diversity in species between zones in the KNP and FARMS (Table 9.4). It is also notable that not a single specimen of one of the commonest species in the KNP, *C. nigeriae*, was found on FARMS in two years. However, some dispersal is evident as individual specimens of the elephant-associated C. sp. # 54 p.f. and *C. loxodontis* were collected on FARMS, but only on those closest to the KNP. Table 9.4 reveals that even species shared between the KNP and the escarpment were seldom found to be equally abundant. Although it must be acknowledged that monthly sampling, with a few widely distributed light-traps, is unlikely to trap low numbers of wind-dispersed individuals, the large disparities in species composition clearly show that *Culicoides* are not dispersed and commingled by some agent, such as wind, in the wider eastern Transvaal lowveld. If *C. imicola* is the main vector of AHS in South Africa, the present data adequately show that it is maintained in great abundance on FARMS. If a virus were to be introduced onto FARMS, epizootics would develop rapidly (in the absence of vaccination), as *C. imicola* is already in place.

9.4 *CULICOIDES* IN THE SATARA AREA

During the 22-month period, 200 light-trap collections were made at 17 sites in the Satara area (Table 9.8; 10 sites, no's 12–21, are shown in Fig. 9.1); the data form a subset of the entire KNP survey, but are assessed separately to help discuss the prevalence and abundance of those *Culicoides* species found in the zone where Barnard (1993) showed zebras to seroconvert rapidly to African horsesickness. Ninety per cent of the catches were obtained between February — November 1992. A total of 590 722 *Culicoides* was collected; of these, 233 621 (40 %), and representing 47 collections, were identified, age-graded and sexed. The exact number of each species, and prevalence ranking, are given in Table 9.9. The commoner species, and the uncommon *C. imicola*, are discussed below as regards their potential to transmit African horsesickness.

a) *Culicoides nivosus, C. schultzei* and C. sp. # 110 (Schultzei grp.): These were the first-, second- and fourth-most abundant species, and made up 56 % of all *Culicoides* collected. Only three sites produced 94–97 % of the specimens; these were the Mondzu and Nyamari springs, and Mavumbye (a spring-fed, clay-bottomed lake). The development of such large, but localized, foci is due to the preference of these species for sunlit, muddy habitats, especially those of mature lakes undergoing changes in pH values and salinity. While nothing is known of the ability of these three species to transmit viruses in Africa, their lower prevalence (both spatial and temporal), and larval habitat specificity, would seem to weaken their potential to vector AHSV as rapidly and widely as has been documented in the Satara zebras (Barnard 1993).

b) *Culicoides kobae, C. similis, C. ravus* and *C. tropicalis* (20 %): These species are restricted to the warmer parts of South Africa, and are abundantly found along flat-bottomed rivers with sandy beds where they breed in the moist, sunlit margins. Such rivers, both large and small, drain the Satara area and explains the high abundances and widespread occurrences of these four species. Nothing is known as regards their feeding habits. However, it is notable that they all possess a swollen third palpal segment; this indicates ornithophily, but it remains to be proven. If true, this would limit their role in the transmission of AHS as there is no evidence that this virus persists in birds.

c) *Culicoides imicola* (0,94 %): This remains the only species proven to transmit African horsesickness in the continent. The low numbers in the Satara area at first glance suggest it not responsible for the rapid seroconversion rates seen in local zebra. However, *C. imicola* not only turned out to be the fifth-most prevalent species, but was also found in all habitats sampled, and in the presence of a variety of game animals throughout the year. In Table 9.10 the 'total *imicola*: total *Culicoides*' ratio reveals that *C. imicola* assumes some measure of dominance in the driest habitats where zebra occur, e.g. Ngotso south. It was also significantly more numerous in the company of the Nwarivake buffaloes, despite this 60-strong herd being 'in transit', and 5 km from the nearest potential breeding sites of *C. imicola*. This species was found to be dominant only at Bangu, known to be a congregation point for large numbers of zebras (and other game) in the drier months. During the summer, grass-margined rainpools form around Bangu, and, depending on their size, persist into the winter months. These provide ideal breeding sites for *C. imicola*. At certain times similar pools occurred at Gudzane, Ngirivane, Mondzu and Nyamari where the highest numbers of *C. imicola* were found. Although 1992 was the driest year on record in the KNP this century, and most of these pools had dried out, *C. imicola* still maintained a broad prevalence. Although its numbers were low, it was still found in the late winter/early spring months (August to October) when Barnard (1993) showed zebras to be seroconverting to AHS. While it is unknown how large a population of *C. imicola* is needed to transmit the virus, its high vector capacity implies it may have been plentiful enough to cycle virus amongst zebras. Furthermore, the data amply demonstrate that *C. imicola* can persist under the driest conditions, and that such small core populations would fairly rapidly spread and grow with the onset of rains. Limited breeding areas in a predominantly sandy terrain would preclude *C. imicola* from ever achieving abundance in the Satara area.

9.5 CONCLUSIONS

Marked differences and interesting similarities characterize the *Culicoides* faunas of the KNP and adjoining FARMS, and would affect the underlying dynamics of the epidemiology of African horsesickness in the area. In the farming community AHS is generally considered to be a 'late summer — early autumn' disease, whereas in the KNP Barnard (1993) found that zebras seroconverted rapidly to all nine serotypes of AHS in the late winter — early spring. This may simply be a coincidence, as winter is the period in the KNP when five- to six-month old foals lose their colostral immunity and so become fully susceptible. Whether transmission at this time is effected by a number of *Culicoides* species, and so accounting for rapid seroconversion, or is due to the increased prevalence of a single species such as *C. kwagga* (which is closely associated with zebras), remains unknown. The poor overlap in *Culicoides* species composition, seasonal prevalence and abundances in the KNP and on FARMS, must contribute further to the difference in incidence of AHS. There are also major disparities in the kind of hosts available (game vs. domesticated livestock), their AHS reservoir status, and in the timing of birth and recruitment rates of foals. All are essential elements for a virus such as AHS that needs to cycle continously. Finally, vaccination of horses against AHS further obscures the true epidemiology of this disease in the African context.

The overwhelming dominance of *C. imicola* on FARMS implies that it is the sole vector of AHS there. Although *C. imicola* is quite prevalent in the KNP veld, the average number of specimens/light-trap is half that found on FARMS in the middle/upper escarpment where AHS is rare to absent. At first glance this suggests that other species of *Culicoides* are involved in AHS transmission in the KNP veld, but the far higher prevalence of reservoir hosts, and the absence of vaccination, likely render this inference erroneous. In the final analysis, we simply do not know what population levels are required for *C. imicola* to effect transmission. However, the low numbers of this species in the driest KNP veld, where zebras persist with the help of artificial water supplies, and show rapid seroconversion rates against all nine serotypes of AHS, do suggest that other species of *Culicoides* may be involved in its transmission.

Eighteen of at least 52 species of *Culicoides* found in the KNP were, at one or other time, the dominant species in a light-trap collection. This is attributable to seasonal changes in the quality or availability of niches occupied by *Culicoides* species, and to the perpetual movement of game animals into and out of areas. For instance, members of the Similis and Schultzei groups can become abundant along the margins of broad-bottomed sand rivers, but their populations will crash when these rivers flood; the same species will not be found on FARMS as the character of a river changes as one ascends the escarpment; they become colder, and flow fast through rocky gorges that largely lack sunlit, sandy margins. Similarly, a handful of *Avaritia* species that breed rapidly and abundantly in elephant dung (constituting 6 % of all KNP *Culicoides*) are extinct on FARMS where elephants have been exterminated. Indeed, if light-trap data are depicted graphically, the seasonal and abundance patterns of one species seldom correlates with that of the next. Sensible comparison of data from various sites is further impeded when it is realized that a number of *Culicoides* species are found only where a particular animal resides. The daily and seasonal movements of these hosts fundamentally influence *Culicoides* density at a particular site in the KNP, and can even affect species prevalence and composition within the hour. The hosts referred to are the elephant, rhino, zebra, buffalo and wildebeest, and in the KNP veld, their dung is the larval habitat of 13 % of all specimens of *Culicoides* specimens captured. There are seven species of the subgenus *Avaritia* known to also feed exclusively on these herbivores, some of which are implicated as, or proven to be, reservoir hosts for African horsesickness. This close relationship between insect and animal may include the transmission of viruses, and for this reason the capacity of these *Avaritia* species to vector AHS merits investigation. The epidemiology of AHS in the KNP will be better understood only once virus transmission is unequivocally linked to one or more species of *Culicoides*, and the reservoir status of all suspected herbivores clarified.

9.6 AHS EPIDEMIOLOGY IN SOUTH AFRICA

It was hoped that this investigation would throw further light on the epidemiology of African horsesickness, and perhaps provide a better understanding as to why the eastern Transvaal lowveld is the AHS ‘hotspot’ of South Africa. Perhaps it is pertinent to re-examine the comments made by Theiler (1930) who was particularly intrigued by the disease. His knowledge was built on 40 years’ experience and, in the epidemiological sense, is the most accurate available. Although it was developed at a time when vaccines did not mask the natural course and spread of outbreaks in South Africa, the indiscriminate hunting of game may have had considerable impact on the geographic distribution of AHS due to elimination of reservoir hosts. Theiler concluded: ‘Horse sickness is only found in certain localities and under certain orographic, telluric and climatic conditions. During the late summer months, after the rains, it occurs regularly with more or less severity in enzootic form in the low-lying coast lands, valleys and swamps. The appearance of the disease and the severity of the outbreak are governed by the amount and distribution of the rainfall. When the rains appear late, horse sickness also appears late. Enzootics usually terminate with the first frosts. Continuous rains do not favour the spread of the disease, but heavy rains alternating with hot spells, or followed by drought, furnish optimal conditions for its dissemination. In South Africa, March and April constitute the usual horse-sickness season for the middle and high veld, whereas in low-lying lands it may occur during May and June, and in years with very late rains may be found throughout the winter.' As regards the comments surrounding the occurrence of AHS in 'low-lying lands', it is useful also to quote the remarks of Stevenson-Hamilton (1905), the first warden of the Sabi Reserve (later to grow and become the Kruger National Park): 'Horse-sickness is another obstacle ... It is no exaggeration to say that ... a horse brought down from the high country will, as a rule, not survive for a longer period than a month, while from November until the following May a fortnight is as long as he can be depended upon.' Elsewhere, Stevenson-Hamilton (1937) notes that horse-sickness is present 'almost everywhere at altitudes below 4 000 feet in the wet season ...' In the following discussion an attempt will be made to correlate current findings with these past experiences, and so pinpoint the species of *Culicoides* most likely to be involved in AHS transmission.

As regards the eastern Transvaal lowveld, the above quotes reveal that AHS can occur throughout the year and is twice as prevalent during the rainy summer season, and that the onset of cold reduces, but does not necessarily eliminate, incidence. This study adequately shows that many *Culicoides* species, including *C. imicola*, will be caught in light-traps throughout the winter. During winter a significant nulliparous rate amongst females proves that breeding is continuous; for this to happen, adults must be actively seeking and obtaining blood, and laying eggs. With reference to the seasonal transmission of AHS, Barnard (1993) showed that young zebra foals in the KNP seroconvert very rapidly to all nine serotypes of AHS, and, surprisingly, that seroconversion peaked in winter after an unexpected dip in the summer months (December — February). How can this be explained? These months mark the start of more regular rains, and soon the larger rivers are in flood or flowing wider than normal. This depresses *Culicoides* populations, especially the sand river species, as rushing waters scour out their larval habitats. Furthermore, these rivers would take some time to settle (if repeated rains fell in the escarpment) and so would prevent *Culicoides* from establishing significant new foci. With special reference to *C. imicola*, this study reveals that it seldom, if ever, becomes dominant along the margins of larger rivers (see 'wet veld' Table 9.3). Instead it does better in the 'dry veld' (Table 9.3), but only after the summer rains have commenced. This is ascribed to the creation of rainpools in the open veld, principally where clayey depressions occur. While this remains to be more conclusively proven, it is probable that such pools are rapidly colonized by *C. imicola*, and if visited by game, foci of this species would soon develop.

Therefore while rivers are flowing and rainpools are 'raw' (December — March), the growth in *Culicoides* populations would be inhibited; the subsequent climb in AHS seroconversions in the later months correlates positively with the stabilization of larval habitats and the increase in *C. imicola* numbers. By mid- to late-winter, many of the pools will have vanished and then game animals, especially zebras, will begin to concentrate at more permanent watering points, some natural, some man-made. These late-season gatherings of herbivores would lead to the build-up of *Culicoides* at the same sites. The stage would now be set for the transmission of African horsesickness.

Let us ignore *C. imicola* and consider whether AHS may be transmitted by other species of *Avaritia*, as suggested in previous chapters. Those meriting examination first are the coprophilic species that can become locally dominant where animals gather, and can also be among the most prevalent species in the KNP veld. Their complete reliance on the blood and dung of larger herbivores indicates them as potential vectors of viruses. Furthermore, their ability to use dung as a larval habitat serves to isolate them from the vagaries of droughts and floods, and means that they will always be found in the vicinity of the appropriate herbivore. Another consequence of coprophily is that *Avaritia* population levels are more stable, and always proportionate to the amount of dung dropped locally. However, the fact that this stability in numbers is not mirrored by an equally consistent seroconversion rate suggests that these *Avaritia* species are indeed not involved in AHS transmission. This conclusion may be erroneous, as the mid-summer dip in seroconversions noted by Barnard (1993) amongst zebras may simply be the result of a more prevalent colostral immunity. The majority (75 %) of foals in the KNP are born in November/December, and so would be largely refractory to infection during the following 5–6 months.

Another aspect of the life-cycle of coprophilic *Avaritia* species that would seem to interfere with their ability to disseminate AHSV, is that they are very host-specific. For example the elephant-associated species will never be found in the dung of buffaloes and, with rare exceptions, are also not found in the dung of the zebra, and *vice versa*. It thus seems reasonable to assume that these *Avaritia* species will also not feed regularly on alternative hosts. Subsequent to European colonization, hunting led to a swift decline in numbers of the big-dung-five (elephant, rhino, buffalo, wildebeest and zebra) in South Africa. This study provides ample evidence that dung-dependent *Culicoides* disappear when their hosts become locally extinct. Despite this, AHS has remained as widespread in South Africa as two centuries ago, and

Could any species other than *C. imicola* be involved in the transmission of AHS? As noted in Chapter 7 the new species, *C. kwagga*, was found to be closely associated with zebras, and because of this, and its close taxonomic relationship to *C. imicola*, it must be considered a potential vector of African horsesickness. A remarkable feature of its occurrence is that *C. kwagga* is found only in 'zebra country' in the KNP, and secondly, appears only in winter (June/July), disappearing in November/December. This interrupted prevalence is directly the result of its dependence on zebra dung; in the summer this dries out within three days, too soon for the larvae of *C. kwagga* which require moisture for a minimum of six days. What happens to this species during the hotter summer months remains unknown. While there is no evidence yet that *C. kwagga* will at this time utilize coarser elephant dung, it does seem to thrive in the finer-textured dung of the white rhinoceros. As regards its potential to transmit AHS, light-trap collections reveal that numbers of *C. kwagga* in the KNP do climb between July to November, the months in which Barnard (1993) noted a peak in AHS seroconversions amongst zebras. However, once again, this could merely be coincident with the simultaneous loss of colostral immunity in zebra foals, and, furthermore, would not eliminate *C. imicola* as being also involved, or solely responsible, for AHS transmission. At least the later summer (February — May) preponderance of AHS in most parts of South Africa, which coincides with the increased prevalence of *C. imicola*, would be achieved in the absence of *C. kwagga*. It needs to be stressed, though, that *C. kwagga*, on the cooler highveld, can be collected during the summer months, and, remarkably, has been found in every isolated pocket sampled where zebras and/or the white rhino are kept. Importantly, it has on one occasion been reared from horse dung at Onderstepoort; its appearance in the winter/spring months in the eastern Transvaal lowveld, at horse stables only, further confirms its close association with equines. Even if *C. kwagga* is ever shown to be a competent vector of AHS, it is highly unlikely that it be involved in the swift spread of epizootics; its presence is too limited and patchy for it ever to rival *C. imicola*. It seems that elephant-associated *Avaritia* species are even less likely to be involved in the epidemiology of African horsesickness; it is inescapable that the distribution of AHS in South Africa is far wider than that of the elephant, and its associated biting midges.

As regards the local distribution of AHS, it was remarked by Edington (1893) that 'certain conditions of locality seem, most markedly, to favour the production of the disease; thus it prevails mostly or more generally in low-lying districts. In connection with this statement, however, it must be noted that *the* *absolute altitude* of the district has not so much to do with its production, as its *comparative altitude* with regard to the district immediately around. Thus it may occur in the kloofs and vleys of a district which may stand at an altitude of 2 000 feet above the seas level.' It is notable how accurately this 100-year-old observation reflects the situation as regards *C. imicola* in the escarpment areas of the eastern Transvaal lowveld today. Although the great majority of specimens (94 %) were taken in the hotter, flatter, lower-lying areas, *C. imicola* still remained the dominant species (Table 9.2), but in far lower numbers, in the foothills and escarpment, where moist depressions rarely form due to efficient water runoff on a sharply sloping topography. Pilgrims Rest, a small community at 4 200 feet, and completely encircled by mountains, lies in a small, flat valley where two streams converge. At this marshy corner is sited the local Ponieskrantz stable (60 horses), fronted by an acre of kikuyu pasture; here *C. imicola* was the dominant species throughout summer, and in February 1993 (peak season) comprised 86 % of 3 300 *Culicoides* collected. A high nulliparous rate of 38 %, and numerous males (3%), attest that *C. imicola* is breeding locally. It seems clear that only the virus of AHS needs to be introduced into the Pilgrims Rest valley for an outbreak to occur in the absence of vaccination. The introduction in 1987 of just five infective zebra into central Spain, and the resultant deaths of some 2 000 horses over the next three years, illustrates how effectively *C. imicola* can cycle the virus in any locale. Before it was known that AHS was caused by a virus, and that it was transmitted by blood-sucking insects, these patterns of introduced infection were already being noted in the previous century. Edington (1895) observed: 'It also made its appearance in areas which had formerly been supposed to be safe, areas in which ... persons were in the habit of sending their horses ... during the season, as being there out of reach of it. One such area is known as the Bontebok flats, and I have been informed that several horses were sent there, of which one died of the sickness very soon after its arrival, and some weeks later the disease made its appearance among others. I am of opinion that this is to be accounted for by the fact of one horse dying there as a result of infection acquired elsewhere, and thus infecting the area.'

From the foregoing it is patently clear that of 112 species of *Culicoides* found in South Africa, *C. imicola* still remains the only one able to fulfil all requirements needed for the transmission of African horsesickness. Indeed, it seems quite fortuitous that this species, the most competent at vectoring AHS, is also the one that thrives best under man's hand: its catholic choice of hosts, and its ability to breed in areas saturated by consistent irrigation, means it can become superabundant in the presence of a variety of domesticated livestock. Furthermore, peak populations of *C. imicola* occur between February to May, the period when most cases of horsesickness are recorded in South Africa. While it is true that *C. imicola* is also the most widespread species, and so further mirroring the widespread occurrence of the disease, three factors appear seriously to limit its prevalence in the subregion:

1. Low mean temperatures in the central highlands restrict *C. imicola* to low populations; these areas are also free of AHS.
2. A sloping topography with good water-runoff also inhibits *C. imicola*; moist depressions are found intermittently in these escarpment areas, and can harbour *C. imicola*. Here the occurrence of AHS is similarly sporadic.
3. Limited data reveal that well-drained, sandy soils are remarkably free of *C. imicola*; the larvae cannot survive the lack of moisture and nutrients. While parts of the Cape Peninsula are considered to be horsesickness-free, these are likely to be only the sand flats and narrow coastal strips. Along the peninsular foothills and further inland, a change in soil type benefits *C. imicola* and large populations will then be found. This coincides remarkably well with severe outbreaks of AHS recorded this century in Ceres, Porterville, Clanwilliam, Wellington, Robertson, Montagu and Worcester. In support of soil type being influential, it is to be noted that the poorly drained black cotton soils of Onderstepoort support large populations of *C. imicola*.

Further north in equatorial Africa, there is some evidence that *C. imicola* declines in the higher- rainfall forested regions, dense shade and waterlogging of habitats are unfavourable conditions. It thus seems reasonable to postulate that *C. imicola* is restricted to the moderate rainfall (300–700 mm) savannas that surround the equatorial block. Its distribution is thus centered amongst the greatest concentration and diversity of herbivores found in Africa.

9.7 REFERENCES

BARNARD, B.J.H. 1993. Circulation of African horsesickness virus in zebra *(Equus burchelli)* in the Kruger National Park, South Africa, as measured by the prevalence of type specific antibodies. *Onderstepoort Journal of Veterinary Research,* 60:111-117.

BARNARD, B.J.H. 1994. Epidemiology of African horse sickness: zebra as virus reservoirs. In working document of: OIE scientific conference on the control of Foot and Mouth Disease, African Horse Sickness and Contagious Bovine Pleuropneumonia. Gaborone (Botswana), 20–23 April 1994: 43, 44.

BIRLEY, M.H. & BRAVERMAN, Y. 1987. Diversity and association among *Culicoides* (Diptera: Certopogonidae) fauna in Israel. *Israel Journal of Entomology*, 21:39–50.

BOORMAN, J. 1974. *Culicoides* (Diptera, Ceratopogonidae) from Cyprus. *Cahiers de l'Office de la Recherche Scientifique et Technique Outre-Mer. Série Entomologie Médicale et Parasitologie*, 12:7–13.

BOORMAN, J.P.T. & MELLOR, P.S. 1982. Notes on *Culicoides* (Diptera, Ceratopogonidae) from the Sudan in relation to the epidemiology of bluetongue virus disease. *Revue d'Elevage et de Médecine Veterinaire des Pays Tropicaux*, 35:173–178.

BOORMAN, J.P.T. & WILKINSON, P.J. 1983. Potential vectors of bluetongue in Lesbos, Greece. *Veterinary Record*, 113:395-396.

BOSMAN, P. 1994. African horse sickness: surveillance systems and regionalisation. In working document of: OIE scientific conference on the control of Foot and Mouth Disease, African Horse Sickness and Contagious Bovine Pleuropneumonia. Gaborone (Botswana), 20–23 April 1994: 40.

BRAVERMAN, Y. & GALUN, R. 1973. The medical and veterinary importance of the genus *Culicoides* (Diptera, Ceratopogonidae). *Refuah Veterinarith*, 30:62–68.

BRAVERMAN, Y. & PHELPS, R.J. 1981. Species composition and bloodmeal identification in samples of *Culicoides* (Diptera: Ceratopogonidae) collected near Salisbury, Zimbabwe in 1976-77. *Journal of the Entomological Society of Southern Africa,* 44:315-323.

BURGU, I., URMAN, H.K., AKCA, Y., YONGUC, A., MELLOR, P.S. & HAMBLIN, C. 1992. Serologic survey and vector surveillance for bluetongue in southern Turkey, in *Bluetongue, African Horse sickness and related orbiviruses*, edited by T.E. Walton & B.I. Osburn. *Proceedings of the Second International Symposium*. Boca Raton, Florida, USA: CRC Press: 168–174.

CAPELA, R., SOUSA, C., PENA, I. & CAEIRO, V. 1993. Preliminary note on the distribution and ecology of *Culicoides imicola* in Portugal. *Medical and Veterinary Entomology*, 7:23–26.

DAVIES, F.G. & WALKER, A.R. 1974. The distribution in Kenya of bluetongue virus and antibody and the *Culicoides* vector. *Journal of Hygiene*, Cambridge, 72:265-272.

DIPEOLU, O.O. 1976a. Studies on *Culicoides* species of Nigeria. II. Species collected around wild animals at Ibadan. *Veterinary Parasitology*, 1:257-263.

DIPEOLU, O.O. 1976b. Species distribution and abundance of *Culicoides* Latreille (Diptera: Ceratopogonidae) in Nigeria. *Bulletin of Entomological Research*, 66:685–693.

DIPEOLU, O.O. 1977. Potential vectors of bluetongue in Nigeria. *Bulletin of Animal Health and Production in Africa*, 25:17–23.

DIPEOLU, O.O. 1978. Studies on *Culicoides* species of Nigeria. III. Physiological conditions of species caught with light traps. *Nigerian Journal of Entomology*, 3:35–44.

DIPEOLU, O.O., DUROJAIYE, O.A. & SELLERS, K.C. 1974. Preliminary observations on the distribution of *Culicoides* species (Diptera:Ceratopogonidae) on the teaching and research farm of the University of Ibadan. *Nigerian Journal of Entomology*, 1:35–42.

DIPEOLU, O.O. & OGUNRINADE, A.F. 1976. Species of *Culicoides* breeding on rocks and riverbanks in Nigeria. *Ecological Entomology*, 1:267–274.

DIPEOLU, O.O. & SELLERS, K.C. 1977. Studies on the *Culicoides* species of Nigeria. I. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of Ibadan with notes on the other Ceratopogonids collected by light traps. *Nigerian Journal of Science*, 11:33–65.

DU TOIT, P.J. 1924. Horsesickness in 1923. *Journal of the Department of Agriculture, Union of South Africa*, 8:370–382.

DU TOIT, R.M. 1944. The transmission of bluetongue and horsesickness by *Culicoides. Onderstepoort Journal of Veterinary Science and Animal Industry*, 19:7–16.

EDINGTON, A. 1893. Report on the Colonial Bacteriological Institute for the year 1892. Cape of Good Hope. Department of Lands, Mines and Agriculture. Cape Town: W.A. Richards & Sons, Government Printers. pp 1–40 + 4 photographs of Bacteriological Institution, Grahamstown.

EDINGTON, A. 1895. Report of the Director of the Colonial Bacteriological Institute for the year 1894. Cape of Good Hope. Department of Agriculture. Cape Town: W.A. Richards & Sons, Government Printers. pp 1–70.

HENNING, M.W. 1956. African horsesickness, Perdesiekte, Pestis Equorum. In: Animal Diseases in South Africa. Being an account of the infectious diseases of domestic animals. Third edition. Central News Agency Ltd., South Africa. pp 785–808.

HERNIMAN, K.A.J., BOORMAN, J.P.T. & TAYLOR, W.P. 1983. Bluetongue virus in a Nigerian dairy cattle herd. I. Serological studies and correlation of virus activity to vector population. *Journal of Hygiene, Cambridge*, 90:177–193.

HOWARTH, F.G. 1985. Biosystematics of the *Culicoides* of Laos (Diptera: Ceratopogonidae). *International Journal of Entomology*, 27:1–96.

JENNINGS, M., BOORMAN, J.P.T. & ERGÜN, H. 1983. *Culicoides* from western Turkey in relation to bluetongue disease of sheep and cattle. *Revue d'Elevage et de Médecine Veterinaire des Pays Tropicaux*, 36:67–70.

JUPP, P.G., McINTOSH, B.M. & NEVILL, E.M. 1980. A survey of the mosquito and *Culicoides* faunas at two localities in the Karoo region of South Africa with some observations on bionomics. *Onderstepoort Journal of Veterinary Research*, 47:1–6.

KHAMALA, C.P.M. 1971. Ecological distribution of East African *Culicoides* (Dipt., Ceratopogonidae) as shown by light traps. *Bulletin of Entomological Research*, 60:549–557.

KITAOKA, S. & ZULU, F.P. 1990. Species composition of *Culicoides* (Diptera: Ceratopogonidae) found at Chilanga near Lusaka, Zambia. *Bulletin of the Niigata Sangyo University*, 4:197–206.

MEISWINKEL, R. 1991. Afrotropical *Culicoides*: *C. (Avaritia) miombo* sp. nov., a widespread species closely allied to *C. (A.) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 58:155–170.

MEISWINKEL, R. & BRAACK, L.E.O. 1994. 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. *Onderstepoort Journal of Veterinary Research*, 61:155–170.

MELLOR, P.S. 1992. *Culicoides* as potential orbivirus vectors in Europe, in *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E. Walton & B.I. Osburn. *Proceedings of the Second International Symposium*. Boca Raton, Florida, USA: CRC Press: 278–283.

MELLOR, P.S., JENNINGS, D.M., WILKINSON, P.J. & BOORMAN, J.P.T. 1985. *Culicoides imicola*: a bluetongue virus vector in Spain and Portugal. *Veterinary Record*, 116:589–590.

MELLOR, P.S. & PITZOLIS, G. 1979. Observations on breeding sites and light-trap collections of *Culicoides* during an outbreak of bluetongue in Cyprus. *Bulletin of Entomological Research*, 69:229–234.

MELLOR, P.S. & RAWLINGS, P. 1994. African horse sickness and the overwintering of *Culicoides* in Iberia. In working document of: OIE scientific conference on the control of Foot and Mouth Disease, African Horse Sickness and Contagious Bovine Pleuropneumonia. Gaborone (Botswana), 20–23 April: 55, 56.

MOHAMMED, M.E.H. & MELLOR, P.S. 1990. Further studies on bluetongue and bluetongue-related orbiviruses in the Sudan. *Epidemiology and Infection*, 105:619–632.

NEVILL, E.M. 1971. Cattle and *Culicoides* biting midges as possible overwintering hosts of bluetongue virus. *Onderstepoort Journal of Veterinary Research,* 38:65-72.

NEVILL, E.M. & ANDERSON, DORA 1972. Host preferences of *Culicoides* midges (Diptera: Ceratopogonidae) in South Africa as determined by precipitin tests and light trap catches. *Onderstepoort Journal of Veterinary Research,* 39:147-152.

NEVILL, E.M., VENTER, G.J. & EDWARDES, M. 1992. Potential *Culicoides* vectors of livestock orbiviruses in South Africa, in *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E. Walton & B.I. Osburn. *Proceedings of the Second International Symposium*. Boca Raton, Florida, USA: CRC Press: 306-313.

NEVILL, E.M., VENTER, G.J., EDWARDES, M., PAJOR, I.T.P., MEISWINKEL, R. & VAN GAS, J.H. 1988. *Culicoides* associated with livestock in the Stellenbosch area of the western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research,* 55:101-106.

PATON, T. 1863. The "Horse Sickness" of the Cape of Good Hope. *The Veterinarian*, 36–9:480–494.

PHELPS, R.J., BLACKBURN, N.K. & SEARLE, L. 1982. *Culicoides* (Diptera: Ceratopogonidae) catches and virus isolations from them in the Mukwadzi Valley, Zimbabwe. *Journal of the Entomological Society of Southern Africa*, 45:195–200.

RANDALL, J.E. 1982. The distribution and ecology of *Culicoides* species (Diptera: Ceratopogonidae) at three sites in Natal. B.Sc. Hons. submitted to the University of Natal, Pietermaritzburg.

STEVENSON-HAMILTON, J. 1905. Chapter VI. Game preservation in the Transvaal. Addresses and Papers read at the joint meeting of the British and South African Associations for the Advancement of Science, 1:350–368.

STEVENSON-HAMILTON, J. 1937. South African Eden. From Sabi Game Reserve to Kruger National Park. Collins, St. James's Place, London.

TAYLOR, W.P., AL BUSAIDY, S.M. & MELLOR, P.S. 1991. Bluetongue in the Sultanate of Oman, a preliminary epidemiological study. *Epidemiology and Infection*, 107:87–97.

THEILER, A. 1930. Chapter XXXI. African horse sickness in A system of Bacteriology in relation to Medicine. His Majesty's Stationery Office, London, 7:362–375.

VAN ARK, H. & MEISWINKEL, R. 1992. Subsampling of large light-trap catches of *Culicoides* (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 59:183–189.

VENTER, G.J. 1991. 'n Studie van die verspreiding en seisoenale voorkoms van *Culicoides* spp. (Diptera: Ceratopogonidae) en gekoppelde virussiektes in die R.S.A. M.Sc. Universiteit van die Oranje-Vrystaat.

VENTER, G.J. & SWEATMAN, G.K. 1989. Seasonal abundance and parity of *Culicoides* biting midges associated with livestock at Roma, Lesotho (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 56:173–177.

WALKER, A.R. 1977. Seasonal fluctuations of *Culicoides* species (Diptera: Ceratopogonidae) in Kenya. *Bulletin of Entomological Research*, 67:217–233.

WALKER, A.R. & DAVIES, F.G. 1971. A preliminary survey of the epidemiology of bluetongue in Kenya. *Journal of Hygiene*, Cambridge, 69:47-60.

Table 9.1 Percentage prevalence of *C. imicola* around vertebrate hosts (domestic and wild) in Africa, the Mediterranean and the Middle East according to authors

Authors	Country	Site	Hosts present (secondary hosts in brackets)	*C. imicola* % prevalence
Khamala 1971	Kenya, Tanzania, Uganda	Countrywide	Not listed	11 %
Nevill 1971	South Africa	Onderstepoort		97,4 %
Walker & Davies 1971	Kenya	Various	Sheep, cattle	0–30 %
Nevill & Anderson 1972	South Africa	Onderstepoort	Cattle Mules & horses Sheep Poultry	97,8 % 97,1 % 94,2 % 47,1 %
Braverman & Galun 1973	Israel	Bet Dagan	Sheep	60–100 %
Dipeolu, Durojaiye & Sellers 1974	Nigeria	Ibadan	Cattle (sheep & goats) Pigs	18 % 7,4 % (8,7 %)
Davies & Walker 1974	Kenya			1 %
Boorman 1974	s.e. Cyprus	Various	Sheep	Dominant
Dipeolu 1976a	Nigeria	Ibadan	'wild' game	36,9 %
Dipeolu 1976b	Nigeria	Various	Poultry, cattle; sm. ruminants	17,8 %
Dipeolu & Ogunrinade 1976	Nigeria	Eruwa	?	12–13,6 %
Dipeolu 1977	Nigeria	Countrywide	Various livestock	37,6 %
Dipeolu & Sellers 1977	Nigeria	Countrywide?	Various	38 %
Walker 1977	Kenya	Ruiru Kiboko Makindu	Cattle Sheep, goats, cattle Sheep, goats	18,1 % 12,9 % 37,4 %
Dipeolu 1978	Nigeria	Ibadan Univ.	Cattle (sheep, goats)	36,3% (44,8%)
Mellor & Pitzolis 1979	Cyprus	Various	Sheep	< 10 %
Jupp, McIntosh & Nevill 1980	South Africa	Bethulie; s. OFS	Sheep	< 1 %
Braverman & Phelps 1981	Zimbabwe	Harare area	Poultry (horses, pigs, cows nearby)	53,7 %
Phelps, Blackburn & Searle 1982	Zimbabwe	Harare	Horses, cattle	61,7 – 96,8 %
Randall 1982	South Africa	Pietermaritzburg; Natal	Cattle Sheep	53,9 – 80,8 % 57,7 – 67,8 %
Boorman & Mellor 1982	Sudan	Soba Nishishiba 7km s. Khartoum	Cattle, horse pens Cattle ?	12,0 % 18,0 % 2,1 %
Jennings, Boorman & Ergün 1983	Western Turkey	Various	Cattle, sheep	7,1 %
Herniman, Boorman & Taylor 1983	Nigeria	Vom	Cattle	35,7 %
Boorman & Wilkinson 1983	Greece	Island of Lesbos	Sheep?	24 %
Mellor, Jennings, Wilkinson & Boorman 1985	Spain/Portugal	Lisboa area	Sheep, cattle	9,0 %
Birley & Braverman 1987	Israel	Various	Sheep Cow Horse Turkey Zoo Mixed: pigs, donkey, horse	33 – 97 % 5 – 55 % 25 – 60 % 84 % 71 % 90 %

Authors	Country	Site	Hosts present (secondary hosts in brackets)	*C. imicola* % prevalence
Nevill *et al.* 1988	South Africa	Stellenbosch; w. Cape	Cattle (11,2%) Sheep (41,2%) Horse (29,4%) Goats (13,1%) Pigs (13,6%) Poultry (16,7%)	19,6 %
Venter & Sweatman 1989	Lesotho	Roma	Cattle, sheep, chickens, pigs	< 2 %
Kitaoka & Zulu 1990	Zambia	Lusaka	Guinea-pigs, cattle, chickens, rabbitry	34,8 %
Mohammed & Mellor 1990	Sudan	Khartoum (Shambat)	Cattle	2,0 %
Taylor, Al Busaidy & Mellor 1991	Oman	Rumais	Cattle	27,4 %
		Salalah	Cattle	12,4 %
		Salalah	Horses	5,0 %
		Seeb	Horses	35,3 %
Meiswinkel 1991	Côte d'Ivoire	Yamoussoukro	Zebu cattle	0,8 %
Venter 1991	South Africa	Countrywide	Various	71 %
Van Ark & Meiswinkel 1992	South Africa	Skukuza, KNP	400m from game bomas and horse stables	13,8 %
Van Ark & Meiswinkel 1992	Malawi	Kawalazi, e. of Mzuzu	Small game; birds	14,3 %
Burgu, Urman, Akca, Yonguc, Mellor & Hamblin 1992	Turkey	Countrywide	Various domesticates	10 %
Nevill, Venter & Edwardes 1992	South Africa	Countrywide	Various domesticates	
			E. Cape	> 10 %
			Central Cape	< 2 %
			N. Cape	> 50 %
			N.W. Cape	2 – 10 %
			Natal	> 10 %
			Transvaal	> 50 %
			Onderstepoort	> 50 %
Capela, Sousa, Pena & Caeiro 1993	Portugal	17 localities widespread	Various Pigs	57,4 %
			Horses	29,6 %
			Goats	5,8 %
			Cattle	3,8 %
			Sheep	3,4 %
Meiswinkel & Braack 1994	South Africa	n. KNP	Elephants & other game	2,0 %

Table 9.2 Total *Culicoides* captured in 878 light-trap collections made between VIII.1991 – VIII.1993 at 65 sites in the KNP (2 zones) and on FARMS (3 zones), with detailed breakdown of the prevalence and abundance of *C. imicola*

	KNP		**FARMS**			**TOTAL**
Zone	Bomas/stables	Veld	Lowveld	Lower escarpment	Middle/upper escarpment	
No. catches made	68	627	68	74	41	878
Total *Culicoides*	2 274 770		2 141 502			4 416 272
Subtotal *Culicoides* % No. *Culicoides*/trap between site ratio	742 135 32,6 10 914 30	1 532 635 67,4 2 444 7	2 014 885 94,1 29 631 81	110 573 5,2 1 508 4	15 044 0,7 367 1	
no. catches analysed total *Culicoides* analysed % analysed	36 602 205 81,1	139 465 924 30,4	36 1 845 453 91,3	37 98 252 88,1	21 9 523 63,3	269 (31%) 3 021 357 68
total *C. imicola* % no. imicola/trap between site ratio	297 329 49,4 8 259 56	21 166 4,5 152 1	1 494 160 89,1 41 504 273	55 695 56,7 1 505 10	7 001 735 333 2	1 875 351
no. traps *imicola* absent no. traps *imicola* < 10 range in no. *imicola*	0 0 91 – 96 429	12 (8%) 58 (40%) 0 – 7 714	0 0 69 – 482 807	0 2 (5%) 3 – 16 066	2 (9%) 9 (43%) 0 – 2 845	

Table 9.3 The dominant *Culicoides* species in the 20 largest light-trap collections made in each of 5 zones (3 in the KNP, 2 on FARMS). *C. imicola* (shaded) is clearly dominant where sedentary animals are kept year-round either on pastures or in bomas. In the natural veld *C. imicola* has to compete with 12 other species for dominance; roman numerals indicate months of dominance. C. sp. # 3 and C. sp. # 110 are both undescribed species of the Schultzei group

KNP			FARMS	
I–V, VIII, IX, XI, XII 587 – 4 981 **dry veld**	I–V, VII–X, XII 13 884 – 157 760 **wet veld**	I–VI, X–XII 8 294–190 456 **bomas/stables**	I–VI, X–XII 24 208–533 984 **lowveld**	I–V, X, XI 1 522–17 072 **escarpment**
bolitinos	*micheli*	*imicola*	*imicola*	*imicola*
imicola	*micheli*	*imicola*	*imicola*	C. sp. # 3
imicola	*nivosus*	*imicola*	*imicola*	*imicola*
tropicalis	*micheli*	*imicola*	*imicola*	*zuluensis*
bolitinos	*schultzei*	C. sp. # 3	*imicola*	*imicola*
loxodontis	*kobae*	C. sp. # 3	*imicola*	*imicola*
imicola	*kobae*	*imicola*	*imicola*	*imicola*
loxodontis	*schultzei*	*imicola*	*imicola*	*imicola*
tropicalis	*kobae*	C. sp. # 3	*imicola*	*imicola*
kobae	*nivosus*	C. sp. # 110	*imicola*	*bolitinos*
loxodontis	*micheli*	C. sp. # 3	*imicola*	C. sp. # 3
kobae	*kobae*	C. sp. # 54 p.f.	*imicola*	*imicola*
C. sp. # 54 d.f.	C. sp. # 54 p.f.	*imicola*	*imicola*	*imicola*
imicola	C. sp. # 54 p.f.	*imicola*	C. sp. # 110	*imicola*
imicola	*micheli*	*imicola*	*imicola*	*imicola*
schultzei	*schultzei*	*imicola*	*imicola*	*imicola*
loxodontis	*imicola*	*bolitinos*	*imicola*	*imicola*
imicola	*bolitinos*	*imicola*	*imicola*	*imicola*
imicola	C. sp. # 54 d.f.	*imicola*	*imicola*	*imicola*
C. sp. # 3	*kobae*	*imicola*	*imicola*	*imicola*

Table 9.4 Relative abundance and distribution of 75 *Culicoides* species in the eastern Transvaal lowveld

KNP 'endemics'		Shared			Escarpment 'endemics'	
***	*nigeriae*	***	*imicola*	*****	*onderstepoortensis*	***
**	*kanagai*	***	*bolitinos*	**	*neavei*	**
**	*pretoriensis*	***	sp. # 3	**	*engubandei*	**
*	*miombo*	***	sp. # 54 p.f.	*	*zuluensis*	**
*	*dutoiti*	***	sp. # 54 d.f.	*	*ovalis*	**
*	*meeserellus*	***	*kobae*	*	*gulbenkiani*	**
*	*moreli*	***	*tropicalis*	*	*brucei*	**
*	*remerki*	**	*exspectator*	**	*angolensis*	*
*	sp. # 61	***	sp. # 110		sp. # 35	*
*	*accraensis* sp. # A	***	*schultzei*		sp. # 48	*
*	*accraensis* sp. # B	***	*loxodontis*		*kibatiensis*	*
*	*nigripennis* sp. # A	**	*micheli*	*	sp. # 75	*
*	*nigripennis* sp. # B	**	*nivosus*	*	*magnus*	*
	moucheti	**	*kwagga*	*	*stercorarius*	
	trifasciellus	**	sp. # 50	*	*brucei* p.f.	
	distinctipennis		*leucostictus*	**	sp. # 69	
	sp. # 59	*	*tuttifrutti*	**	*dekeyseri*	
		*	*pycnostictus*	**	*huambensis*	
		**	*nigripennis*	*	*milnei*	
			sdp. # 9	**	*citroneus*	
		*	*ravus*	*	*kerichoensis*	
		*	*similis*	*	*olyslageri*	
		*	*bedfordi*	*	*gambiae*?	
		*	*coarctatus*	*		
		*	*accraensis*	*		
		*	*tororoensis*			
		*	sp. # 56			
		*	*eriodendroni*			
		*	*perettii*			
		*	*punctithorax*			
			sp. # 16	*		
		*	*africanus*			
		*	*walkeri*			
		*	*glabripennis*			
		*	*quinquelineatus*			

superabundant *****; v. common ****; common ***; uncommon **; rare *; extremely rare

Table 9.5 Subgenus *Avaritia*: comparative abundances and prevalence of six species of the Imicola group (and other species of the subgenus) in the KNP and on adjoining FARMS of the eastern Transvaal lowveld; VIII.1991–VIII.1993

	KNP				**FARMS**	
	bomas/stables	prevalence in traps	veld	prevalence in traps		prevalence in traps
no. catches made	68		627		183	
total *Culicoides*	**2 274 770**				**2 141 502**	
no. catches analysed	36		139		94	
subtotal *Culicoides* (%)	742 135 (32,6)		1 532 635 (67,4)		2 141 502 (100)	
total analysed (%)	602 205 (81,1)		465 924 (30,4)		1 953 228 (91,2)	
total *C. imicola* (%)	297 329 (49,4)	36/36	21 166 (4,5)	127/139	1 556 856 (79,7)	92/94
total *C. bolitinos* (%)	37 442 (6,2)	33/36	16 292 (3,3)	112/139	17 565 (0,9)	79/94
total *C. loxodontis* (%)	4 511 (0,75)	28/36	19 801 (4,25)	119/139	468 (0,02)	10/94
total *C. tuttifrutti* (%)	1 372 (0,23)	25/36	214 (0,05)	30/139	6 628 (0,34)	61/94
total *C. kwagga* (%)	1 262 (0,21)	12/36	699 (0,15)	44/139	254 (0,01)	14/94
total *C. miombo* (%)	98 (0,02)	4/36	27 (0,006)	8/139	0 (–)	–
total Imicola group (%)	342 373 (56,9)		57 191 (12,3)		1 581 771 (81,0)	
no. dung-breeders (%) (Imicola group)	43 574 (12,7)		35 784 (62,6)		18 287 (1,16)	
no. C. sp. # 54 s.l. (%)	14 955 (2,48)	19/36	25 967 (5,57)	130/139	894 (0,05)	30/94
no. C. sp. # 50 (%)	1 244 (0,21)	18/36	437 (0,09)	41/139	185 (0,009)	2/94
no *C. kanagai* (%)	251 (0,04)	7/36	342 (0,07)	43/139	0	
no *C. tororoensis* (%)	111 (0,02)	1/36	77 (0,02)	8/139	2	1/94
no. *C. trifasciellus* (%)	2 (0,0003)	1/36	0 (0)	0	0	
no. dung-breeders (other groups)	16 561		26 823			
total dung-breeders	**122 742 (11,5%)**				**19 366 (0,99)**	
	60 135 (9,99%)		62 607 (13,4%)			
total subg. *Avaritia*	358 936 (59,60%)		84 014 (18,0%)		1 582 850 (81,0%)	

Table 9.6 Nwarivake grasslands 20 km w. Satara, central KNP; analysis of species and age-grading of *Culicoides* caught in light-trap set near herd of 60 buffalo, 24.V.1993. Collection no. KNP 93.67a

Culicoides species	FEMALES				MALES	TOTAL	%
	nullipar	parous	b/f	gravid			
C. bolitinos	123	242	3*	94	17	479	84,78
C. loxodontis	6	5	-	5	-	16	2,83
C. imicola	8	2	-	4	-	14	2,48
C. sp. # 54 p.f.	2	3	-	7	-	12	2,12
C. tropicalis	1	1	-	9	1	12	2,12
C. leucostictus	1	-	-	7	1	9	1,59
C. kanagai	1	2	-	1	4	8	1,42
C. ravus	3	3	-	1	-	7	1,24
C. exspectator	3	-	-	-	-	3	0,53
C. kobae	2	-	-	1	-	3	0,53
C. accraensis grp.	-	-	-	1	-	1	0,18
C. similis	-	1	-	-	-	1	0,18
						= 565**	

* one of the bloodfeds fresh, remaining two dark red (± 10 h old)

** 11,34 % of entire catch of 4 981 *Culicoides* analysed; subgenus *Avaritia* = 5 species = 93,64 %; dung-breeders = 4 species = 91,16 %

Table 9.7 The known larval habitat preferences of the commonest *Culicoides* species in the eastern Transvaal lowveld

Subgenus or species group	Species	Larval habitat	Extent of availability of larval habitat
subg. *Avaritia* (Imicola group)	*C. imicola*	organically enriched, sunlit, shortly vegetated areas on saturated clays	widespread on flat terrain; localized pockets of irrigated man-made pasture
	C. bolitinos	buffalo/wildebeest/cattle dung	widespread but localized to presence of these animals
	C. loxodontis	elephant dung	only found in elephant country
subg. *Avaritia* (group unknown)	C. sp. # 54 p.f.	elephant dung	only found in elephant country
subg. *Remmia*	*C. schultzei*	restricted to saline waters; seepages and pools in calcareous rock; tolerant of high temps. and high pH	more widespread and abundant in dry western regionsof S.A.; occasionally abundant but very localized in the east
	C. sp. # 3	sunlit, bare, sandy clays	widespread
	C. sp. # 110	?	widespread in arid western regions of S.A.; occasionally abundant but localized in east
Similis group	*C. tropicalis*	sunlit margins of broad-bottomed sandrivers	restricted to sand rivers and their tributaries
	C. kobae	"	"
	C. micheli	?	appears to be more prevalent along forested margins of sand rivers
Group unknown	*C. nigeriae*	?	"
subg. *Meijerehelea*	*C. nivosus*	sunlit, unvegetated putrefying sites in clay soils	locally abundant; restricted to contaminated habitats, 'dying' lakes, etc.

Table 9.8 Number of *Culicoides* and light-trap collections (in brackets) made at 17 sites in the Satara area, central KNP (August 1991 — May 1993).

	VIII 91	IX 91	X 91	XI 91	XII 91	I 92	II 92	III 92	IV 92	V 92	VI 92	VII 92	VIII 92	IX 92	X 92	XI 92	XII 92	I 93	II 93	III 93	IV 93	V 93	Total
Ngotso-south	123 (3)						103 (3)	6178 (3)	10784 (21)	5554 (3)	936 (9)	1712 (3)	1268 (3)		442 (6)	1929 (3)					17 (3)	383 (4)	(64)
Ngirivane		2319 (3)					27220 (1)	214 (2)	19695 (3)	1692 (3)	2308 (3)	2830 (3)	976 (4)		2624 (3)								(25)
Ngotso dam									52064 (3)	2038 (3)	384 (3)	1821 (3)	6735 (2)		525 (1)	186 (3)							(18)
Mzanzene							37356 (2)	210 (2)	15923 (3)	555 (3)	598 (1)	511 (2)	599 (2)		1074 (2)								(17)
Gudzane							5124 (1)	11972 (3)	27016 (3)	491 (3)	206 (3)	438 (1)			2197 (3)								(17)
Nyamari									4393 (3)		6923 (2)	13395 (1)	38680 (2)		8550 (2)	9599 (2)					408 (2)		(14)
Ntomeni															8051 (12)							434 (3)	(15)
Olifants R										1260 (1)	11251 (1)	26459 (1)			3485 (1)	6750 (1)					19271 (1)	2924 (1)	(7)
Mondzu															160699 (3)								(3)
Mavumbye															32195 (2)								(2)
Nwarivake																						6799 (2)	(2)
Bangu																						281 (2)	(2)
Kambana											4 (2)												(2)
Sweni											1169 (3)												(3)
Rockvale												206 (1)											(1)
2 km s. of Ngotso south								320 (2)															(2)
Nsemani											137 (2)												(2)
Total collections	3	3	-	-	-	-	7	12	36	16	29	15	13	-	35	9	-	-	-	-	3	8	196
Total *Culicoides*							69803	18894	129875	11590	23916	47372	48258		219842	18464					19696	10821	
Average/trap	43	773	-	-	-	-	9972	1575	3608	724	825	3158	3712		6182	2052					6565	1353	

Table 9.9 Number of *Culicoides* collected and number analysed in 200 light-trap collections; Satara area, central KNP (August 1991 — May 1993).

	No. light traps	No. analysed	Total *Culicoides*	Total analysed
Ngotso south (trough)	65	29	29 329	11 561
Ngotso dam	18	2	63 753	17 290
Mzanzene	17	0	40 042	-
Gudzane	17	1	31 521	1 530
Ntomeni	15	1	8 485	814
Nyamari fountain	14	1	81 948	25 480
Olifants R.	7	2	71 400	22 756
Mondzu fountain	3	1	160 699	99 720
Sweni	3	0	1 169	-
Nwarivake	2	2	6 799	6 799
Mavumbye	2	1	32 195	26 486
Bangu	2	1	281	213
Nsemani dam	2	0	137	-
Kambana	2	0	4	-
2 km s. Ngotso south	2	2	320	320
Rockvale	1	0	206	-
Ngirivane	28	6	62 434	2 564
	200	48	590 722	231 540
		24% subsampled		39,2% analysed

Table 9.10 Total number, percentage and prevalence ranking of 35 species of *Culicoides* collected in the Satara area, central KNP (August 1991 — May 1993).

Species	(larval habitat)	Total	%	Prevalence (rank)
nivosus	(mud lakes)	65 881	28,24	28/47 (9)
schultzei	(mud lakes)	43 386	18,59	21/47 (12)
kobae	(rivers)	31 285	13,41	38/47 (3)
C. sp. # 110 (Schultzei grp)	(mud lakes)	21 265	9,11	18/47 (14)
C. sp. # 54 p.f.	(elephant dung)	13 285	5,69	40/47 (1)
loxodontis	(elephant dung)	12 099	5,19	40/47 (2)
bolitinos	(buffalo dung)	11 960	5,13	25/47 (10)
similis	(rivers)	9 556	4,10	33/47 (6)
leucostictus		5 135	2,20	32/47 (7)
C. sp. # 3 (Schultzei grp)		4 841	2,07	14/47 (17)
ravus	(rivers)	3 968	1,70	37/47 (4)
tropicalis	(rivers)	3 424	1,47	30/47 (8)
imicola	(grass-margined rain pools)	2 198	0,94	34/47 (5)
bedfordi		1 096	0,47	8/47
nigeriae	(rivers)	756	0,32	11/47 (20)
pycnostictus		752	0,32	25/47 (11)
walkeri		394	0,17	5/47
C. sp. # 54 d.f.	(elephant & zebra dung)	390	0,17	17/47 (15)
exspectator		355	0,15	15/47 (16)
pretoriensis	(rock pools)	348	0,15	9/47
micheli		198	0,08	9/47
C. sp. # 30	(rotting fruits)	179		20/47 (13)
kanagai	(elephant dung)	147		12/47 (19)
neavei		131		2/47
dutoiti		99		7/47
C. sp. # 61		62		14/47 (18)
C. sp. # 107	(zebra dung)	44	0,02	4/47
coarctatus		31		2/47
remerki	(tree holes)	17		8/47
C. sp. # 56		16		5/47
eriodendroni	(tree holes)	13		3/47
Accraensis grp.	(tree holes)	10		2/47
C. sp. # 50	(elephant dung)	6		3/47
africanus		1		1/47
punctithorax	(tree holes)	1		1/47
		233 330		
		= 39,5% of total captured		

Table 9.11 Numbers of, localities, and months in which *C. imicola* was collected in the Satara area, central KNP (August 1991 — May 1993).

Collection No.	Date	Locality	Total *imicola*	Total *Culicoides*	*imicola*: *Culicoides* ratio	Rank	Months dominant
KNP 91.2	13.VIII.1991	Ngotso south trough	-	28	-		
KNP 91.16 a	18.IX.1991	Ngirivane	7	697	1:100	18	
KNP 91.16 b	"	"	15	317	1:21	4	September
KNP 92.33	26.II.1992	"	163	27 220	1:67		
KNP 92.34 abc	27.II.1992	Ngotso south	-	103	-		
KNP 92.49 a	25.III.1992	2 km s. Ngotso south	1	259	1:259		
KNP 92.49 b	"	"	2	56	1:28	6	March
KNP 92.50 a	"	Ngotso south	4	3 132	1:783		
KNP 92.50 b	"	"	5	555	1:111		
KNP 92.70 a	20.IV.1992	"	5	529	1:106	20	
KNP 92.70 c	"	"	18	2 390	1:133		
KNP 92.73 a	21.IV.1992	"	-	96	-		
KNP 92.73 b	"	"	1	245	1:245		
KNP 92.76 a	23.IV.1992	Ngotso dam	160	14 000	1:88	17	
KNP 92.79 a	24.IV.1992	Ngotso south	-	53	-		
KNP 92.79 b	"	"	-	52	-		
KNP 92.79 c	"	"	-	72	-		
KNP 92.80 B	26.IV.1992	"	6	712	1:119		
KNP 92.81 c	27.IV.1992	"	6	1 030	1:172		
KNP 92.109 b	26.V.1992	"	31	2 563	1:83	16	
KNP 92.113 c	28.V.1992	Ngirivane	8	842	1:105	19	
KNP 92.144 a	16.VI.1992	Ngotso south	3	179	1:60	13	
KNP 92.144 b	"	"	2	92	1:46	10	June
KNP 92.144 c	"	"	2	141	1:70	14	
KNP 92.176 a	20.VII.1992	"	3	392	1;131		
KNP 92.206 a	24.VIII.1992	"	7	199	1:28	5	August

Collection No.	Date	Locality	Total *imicola*	Total *Culicoides*	*imicola*: *Culicoides* ratio	Rank	Months dominant
KNP 92.207 a	"	Ngotso dam	20	3 290	1:165		
KNP 92.208 b	"	Nyamari fountain	479	25 480	1:53	11	August
KNP 92.209 b	27.VIII.1992	Ngirivane	-	115	-		
KNP 92.225 abc	13.X.1993	Ngotso south	7	81	1:12	3	October
KNP 92.227	14.X.1992	Olifants R.	90	3 485	1:39	7	October
KNP 92.230 a	"	Ngotso south	-	158	-		
KNP 92.230 c	"	"	-	116	-		
KNP 92.231 a	15.X.1992	Gudzane	213	1 530	1:7	2	October
KNP 92.232 b	"	Mavumbye	85	26 486	1:312		
KNP 92.235 a	16.X.1992	Ngirivane	4	566	1:142		
KNP 92.236 a	18.X.1992	Ntomeni	10	814	1:81	15	
KNP 92.237 a	17.X.1992	Mondzu fountain	151	88 720	1:588		
KNP 92.261 a	23.X.1992	Ngotso south	12	543	1:45	9	November
KNP 93.50	26.IV.1993	Olifants R.	364	19 271	1:53	12	
KNP 93.67 a	24.V.1993	Nwarivake	124	4 981	1:40	8	May
KNP 93.67 b	"	"	88	1 818	1:21	4	May
KNP 93.66 a	23.V.1993	Bangu	103	213	1:2	1	May
			2 198	233 621			
			0,94%				

CHAPTER 10

The 400 km^2 sandy dune field west of Port Elizabeth: The only *Culicoides imicola*-free zone in South Africa?

10.1 INTRODUCTION

Culicoides midges (Diptera, Ceratopogonidae) are among the most plentiful biting insects; up to 500 000 can be caught at a horse stable on a warm summer night.

In South Africa, *C. imicola* is without doubt the commonest and most prevalent of all *Culicoides* species. In a countrywide study it was found on 33 of 34 farms surveyed and comprised 71 % of > 3 000 000 midges captured (Venter 1991). A second study (see Chapter 9) done in the eastern Transvaal lowveld, the African horsesickness 'hotspot' of South Africa, confirmed these data; its superabundance appears to be largely artificial as the maintenance of cattle, horses, sheep, and other livestock on irrigated pastures exactly suits the needs of this midge. Under more natural conditions, where drought, floods and the constant movement of animals are the norm, large populations of *C. imicola* are unable to develop.

The occurrence of *C. imicola* throughout the farming community of South Africa means that animals always run the risk of contracting any one of the following viruses: bluetongue (BT), African horsesickness (AHS), three-day stiffsickness (BEF), equine encephalosis (EE), and Akabane (AKA); *C. imicola* has a proven involvement in the transmission of some of these diseases (du Toit 1944). South Africa thus stands classified as an enzootic zone, with the result that free trade in live animals, semen and embryos is disallowed, and participation in overseas equestrian events is complicated as horses have to undergo lengthy quarantine periods.

The re-acceptance of South Africa into the international arena has meant that current embargoes on animal exports needed reappraisal and modification. In the case of African horsesickness, the European community has developed the concept of regionalization; low-risk zones, known to be relatively free of disease, are selected for quarantine purposes. The Cape Peninsula to some extent fulfils the requirements, and so is under consideration as a possible horsesickness-free zone (Bosman, Brückner & Faul 1995). However, it must subscribe to a restrictive protocol. Not only does this protocol rely on the integrity of involved persons/owners/farmers/officials, but the controls also include a ban on vaccination, strict monitoring of animals into and out of the zone, constant surveillance of insect populations, obligatory notification of mortalities, and mandatory post-mortems (Bosman 1994). This protocol is, however, necessary as *Culicoides* surveys in the s.w. Cape have shown that not only is *C. imicola* present at all sites but at > 20 % of these it is also the dominant species (Nevill *et al.* 1988; Nevill *et al.* 1994), and probably explains the rare intrusion of AHS into the area. It is thus clear that in the presence of *C. imicola* animals are at risk in this subregion; in the absence of vaccination, only a virus needs to be introduced to result in losses.

Most aspects of the complex protocol outlined above could be eliminated if an *imicola*-free zone were to be found in South Africa. Such a zone would not only have obvious benefits as regards the quarantining of animals, but their safety also would be assured at a far lower cost in both man-hours and money.

New data, indicating that an *imicola*-free area does exist in South Africa, are presented here. Not only is the area well-suited for horses and other livestock, but it is also accessible and possesses an excellent infrastructure that includes a harbour and an airport. This area is the sandy dune field immediately west of Port Elizabeth. Possible reasons for the absence of *C. imicola* are advanced and the potential quarantine area shown on a general and a detailed map. A four-part research plan, designed to resolve remaining questions, is presented.

10.2 MATERIALS AND METHODS

Culicoides were collected with a single U-V light-trap site set on most nights between 21–31.XII.1993. Eight plots or farms were sampled — the majority were situated in the Sardinia Bay area west of Port Elizabeth; four of the sites had only horses, two only cattle, and two had a mixture of both (Table 10.1). The sites (Fig. 10.2) and weather prevailing on the day and night of trapping are respectively indicated at the top and bottom of Table 10.1. Each specimen of 4 749 *Culicoides* captured was identified and sexed (Table 10.1).

A brief resumé of the climate and geomorphology of the Port Elizabeth area is necessary to allow for a more sensible evaluation of the collected data (adapted from Marker 1988).

10.2.1 Climate:

- Frost-free; the average monthly minimum temperature ranges between 7,3–16,9 °C; maximum averages between 19,5–25,5 °C.
- Rainfall year-round; classified as a winter maximum rainfall area; precipitation ranges from a low of 33,4 mm (February) to a high of 65,1 mm (June). Mean annual rainfall (1960–1980): 613,7 mm. On average rain falls every 3,5 days.
- Winds: dominated by westerlies and south-westerlies; hot winds from the north are rare and of short duration; on average, only one in five days is calm. Known as one of the windiest parts of southern Africa.

10.2.2 Geomorphology:

- Most of the coastal belt is rural, and intensive agriculture is scarce, the soils being poor. The dominant coastal landform is of dunes and shelving arcuate sandy beaches separated by low rocky promontories fronting a stepped coastal plain. The underlying geology of this coastal belt is complex as can be seen in Fig. 10.1. The most recent events occurred during the Pleistocene when multiple northern hemisphere glaciations caused repeated drops in sea level. This created a straight coast from Cape St. Francis to East London and resulted in the bays becoming dry land. Calcareous sands were then blown onshore from the exposed sea floor and deposited against any uneven ground. The resultant dunes are of various ages; the oldest are the inland aeolianite solified dunes and the youngest are the low fore-dunes on the present beaches. The scale of these events can be appreciated when one examines the extent of the yellow areas (sand) in Fig. 10.1; Fig. 10.2 details the exact extent of these dune fields west of Port Elizabeth, an area of ± 400 km^2 in which collections of *Culicoides* were made. To the north and north-west of Port Elizabeth are found the Alexandria dune fields, the largest in southern Africa.

10.2.3 Vegetation: - The Sardinia Bay area is characterizedby abundant dune thicket, and stretches of coastal fynbos; coastal forest is occasionally present; coastal grasslands and acacia savanna are absent. Extensive areas have been planted to kikuyu pasture.

10.3 RESULTS AND DISCUSSION

Three facts emerge from the light-trap data given in Table 10.1:

a) In South Africa the concurrence of a number of factors causes large populations of *Culicoides* midges to develop. These factors occur in the Port Elizabeth area and are:

- a frost-free regime
- good rainfall
- extensive irrigation of kikuyu pastures, and
- the presence of large groups of animals providing blood for biting *Culicoides*.

Although these preconditions are to be found in the Port Elizabeth dune field, *Culicoides* numbers, and species diversity, are low. The most immediate reasons appear to be:

- windy circumstances: only one in five days is calm. Windspeeds of more than 4 m/sec are known to suppress flight in *Culicoides*, and
- sandy soils, being poor in organic matter, and having excellent drainage, would inhibit the development of *Culicoides* larvae.

b) The great majority of *Culicoides* caught (92,54 %) in the Port Elizabeth area belong to only two species, which are known to inhabit the dung of cattle elsewhere in South Africa. These are *C. bolitinos* and *C. gulbenkiani*; neither of these has yet been found to transmit viruses. This is only one of three sites known in the country where dung-loving *Culicoides* overwhelmingly dominate other species. Not only is dung largely independent of the soil type on which it is deposited, but it carries its own suite of micro-organisms on which *Culicoides* larvae, of a few species only, will feed. It seems improbable that the currently recorded catches will increase markedly if sampling were to be done at any other time of the year, as these data clearly show that the dominant *Culicoides* in the dune fields are coprophilic; their population levels are thus determined by the amount of dung available. This, in turn, is governed by cattle density. It is unknown what role horse-dung plays in the maintenance of local *Culicoides*.

c) Twelve of the remaining 13 species (7,42 %) of *Culicoides* captured are 'groundwater' breeders; their larvae and pupae develop on the edges of water bodies, either large or small. These would include lakes, streams, drainage furrows, overflows from leaking cement dams, and similar places. The low prevalence of groundwater *Culicoides* suggests a lack of these larval habitats, but this is certainly not the case; it is more likely the result of the sandy substrate not remaining water-logged for sufficiently long periods (10–20 days). Rapidly drying soils would adversely affect moisture-loving *Culicoides* larvae, and if the soils are also poor in organic matter, would further exacerbate conditions for a species such as *C. imicola* so dependent upon prey organisms which thrive in organically enriched, semi-moist situations. As moisture is essential for *Culicoides* larvae, aridity would depress overall species abundance and diversity. The total number of groundwater *Culicoides* (355 individuals), and the number of species (12), is the lowest recorded for any group of sites yet sampled over 15 years in South Africa; even the cold eastern Orange Free State has three times this number of groundwater species (Venter & Meiswinkel 1994). This reinforces the notion that it is not climate but organically poor, single origin, young soils that are responsible for the short *Culicoides* species list in the Port Elizabeth area. Wind would affect *Culicoides* numbers too, but not overall species diversity.

10.4 CONCLUSIONS

a) The total absence of *C. imicola* in a particular area surveyed is unprecedented in South Africa. Only once previously in 15 years of sampling throughout the Republic has *C. imicola* been found to be absent at a site. This was at Rhodes — a small high-altitude enclave in the southern Drakensberg — but it was sampled for one night only (Venter 1991). Its absence in the Port Elizabeth area is all the more suprising when we consider that elsewhere in South Africa *C. imicola* is by far the commonest *Culicoides* found in association with cattle, horses or sheep (Venter 1991). For instance, in the eastern Transvaal lowveld where African horsesickness is most prevalent, up to 500 000 *C. imicola* females can be caught on a single summer night near horses (R. Meiswinkel, personal observation 1992).

b) The absence of *C. imicola* in the Port Elizabeth area is a cause for celebration, as it is still the only species proven to be an efficient vector of the viruses of bluetongue and African horsesickness (du Toit 1944; Venter, Hill, Pajor & Nevill 1991). The sandy dune field area would thus serve as an excellent natural quarantine station, possessing not only an excellent infrastructure including a harbour and an airport. Furthermore, if South Africa were in future to host the Olympic Games, the Port Elizabeth area would seem to be the ideal venue for all gymkhana events; in the absence of vaccination, valuable horses would not be exposed to *C. imicola* and thus African horsesickness. In addition, recent efforts to export breeding stock of goats and sheep to Australia and America necessitated huge enclosures that could only be insect-proofed with difficulty and at great cost. A vector- or disease-free area in South Africa would be invaluable for such ventures.

c) *Culicoides bolitinos* was by far the most common species collected in the Port Elizabeth area. Its role in the transmission of AHSV is unknown, but it is thought not to be a vector since *C. bolitinos* also dominates in the eastern Orange Free State where African horsesickness is absent (Venter & Meiswinkel 1994). Nevertheless, it remains essential that this species be tested in the laboratory for its ability to multiply AHSV and other arboviruses.

d) As discussed, the total absence of *C. imicola* appears to be the result of nutrient-poor, well-drained sandy soils being unable to sustain the immature stages. This suggests that many groundwater *Culicoides* persist in slow-drying clay-based soils that not only retain moisture but are generally richer in nutrients and micro-organisms. Some evidence does exist that *C. imicola* predominates in regions with clayey soils: (i) it is the most abundant and prevalent species in the greater Onderstepoort area, much of which is situated on dense black cottonsoils (Edwardes, personal communication 1989); and (ii), of 1 568 *Culicoides* of nine species reared by Nevill *et al.* (1988) in the Stellenbosch area, 18 (1,1 %) were *C. imicola*; while the total is very low, half were reared from the only one of five sites sampled that had the soil 'clay-like and medium moist'. At the remaining four sites no *C. imicola* or few individuals were reared from situations that were either 'well-drained', 'dry and sandy' or 'with fine particle size (silt)'. It remains to be proved that the highly variable prevalence of *C. imicola* in light-traps in the 540 km^2 area surveyed by Nevill *et al.* (1988) correlates positively with latitudinal changes in soil types.

10.5 RECOMMENDATIONS

a) Three two-week light-trap surveys should be carried out: one each in spring, summer and autumn. During each visit, five north-south transects, each 50–100 km in length, and bisecting various ecozones and/or soil types, should be surveyed. Ten to 20 farms should be sampled along each transect, and seven to 10 farms sampled per night. Random samples should also be done between transects where any sizeable groups of livestock are encountered, so as to improve the chances of finding *C. imicola* amongst any *Culicoides* foci. Each of the two-weekly surveys should yield 150–200 light-trap collections; this large number is needed to give adequate coverage of a large area, to reduce the effects of poor weather which will significantly depress the size of four of every five catches made, and to more accurately define the geographic limits of *C. imicola*. Collections should be made near all kinds of livestock.

b) After the first transect survey has been completed, two farms should be chosen at each of three well-separated points along one transect, and the traps run weekly by farmers. This year-round sampling in three zones where *C imicola* is abundantly present, sporadically present, and absent, will help establish:

(i) whether *C. imicola* is truly absent throughout the year in a 'clean' area, namely the sandy dune field;

(ii) when, for how long, and in what numbers, *C. imicola* intrudes into the sporadic zone;

(iii) just how abundant *C. imicola* is on those farms where it occurs permanently, to compare with abundance data collected elsewhere in South Africa; and finally,

(iv) compare in greater detail, overall *Culicoides* abundances and species composition on different soil types.

c) On 35 farms, blood should be drawn from six animals and screened for antibodies to BT, AKA, BEF and AHS. Not only will this reveal the geographic distribution of these viruses but, if present, will also indicate:

(i) Farm-specific and recent circulation of viruses. On 'positive' farms the dominant *Culicoides* should be artificially infected with the relevant virus/es, and attempts made to re-isolate the virus/es in the laboratory seven to 10 days later.

(ii) Whether higher levels of virus transmission are occurring on one or other soil type. If a distinct pattern emerges, it will give an indication as to which *Culicoides* species are involved, and what numbers are needed to effect transmission.

10.6 REFERENCES

BOSMAN, P.P. 1994. African horse sickness: surveillance systems and regionalisation. In working document of: OIE scientific conference on the control of Foot and Mouth Disease, African Horse Sickness and Contagious Bovine Pleuropneumonia. Gaborone (Botswana), 20–23 April 1994:54.

BOSMAN, P., BRÜCKNER, G.K. & FAUL, A. 1995. African horse sickness surveillance systems and regionalisation/zoning: the case of South Africa. *Revue Scientifique et Technique de l'Office International des Épizooties*, 14:645-653.

DU TOIT, R.M. 1944. The transmission of blue-tongue and horse sickness by *Culicoides*. *Onderstepoort Journal of Veterinary Science and animal Industry*, 19:7–16.

MARKER, M.E. 1988. Geology and geomorphology. In: LUBKE, R.A., GESS, F.W. & BRUTON, M.N. (eds.). A field guide to the eastern Cape Coast. The Grahamstown Centre of the Wildlife Society of Southern Africa; pp. 1–520.

NEVILL, E.M., VENTER, G.J., EDWARDES, M., PAJOR, I.T.P., MEISWINKEL, R. & VAN GAS, J.H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 55:101–106

NEVILL, E.M., VENTER, G.J., LE ROUX, V. & BARNARD, B.J.H. 1994. The vector potential for African horse sickness of *Culicoides* species on Robben island and nearby s.w. Cape mainland. In working document of: OIE scientific conference on the control of Foot and Mouth Disease, African Horse Sickness and Contagious Bovine Pleuropneumonia. Gaborone (Botswana), 20–23 April 1994:52, 53.

VENTER, G.J. 1991. 'n Studie van die verspreiding en seisoenale voorkoms van *Culicoides* spp. (Diptera: Ceratopogonidae) en gekoppelde virussiektes in die R.S.A. M.Sc, Universiteit van die Oranje-Vrystaat.

VENTER, G.J., HILL, Elaine, PAJOR, I.T.P. & NEVILL, E.M. 1991. The use of a membrane feeding technique to determine the infection rate of *Culicoides imicola* (Diptera, Ceratopogonidae) for 2 bluetongue virus serotypes in South Africa. *Onderstepoort Journal of Veterinary Research*, 58:5–9.

VENTER, G.J. & MEISWINKEL, R. 1994. The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in the colder, high-lying area of the eastern Orange Free State, South Africa, and its implications for the transmission of arboviruses. *Onderstepoort Journal of Veterinary Research*, 61:327–340.

TABLE 10.1: Numbers and species of *Culicoides* midges collected at eight horse stables and dairy farms west of Port Elizabeth (21.XII. — 31.XII.1993)

Site No.	1	2	3	4	5	6	7	8			
Site:	Hustlers' six horses, Sardinia Bay, P.E. 21.XII.1993	El Paso, two cattle, horses. Sardinia Bay 22.XII.1993	G & P Scallen; Farm Broadlands, Sardinia Bay, nine horses 23.XII.1993	Farm Avonlea, Sardinia Bay, P.E. — David Emslie 24 horses 26.XII.1993	B & N Bartlett, Under Milkwoods, Sardinia Bay. 30 horses & cattle 28.XII.1993	Yellow Sea dairy, P.E. 160 Jersey herd, blacklight 29.XII.1993	Fairview Racecourse, Green bushes, P.E. 360 horses 30.XII.1993	Bushy Park Dairy ± 400 cattle, Sardinia Bay 31.XII.1993	Male	Total (Male + female)	%
C. bolitinos	2 916	1	416	218	635	95	1	16	56	4 354	91,72
C. pycnostictus	2	1	2	4	86	16	3	16	16	146	3,08
C. nivosus	1		1	5	20	6		9	1	43	0,91
C. leucostictus					9				1	10	0,21
C. zuluensis						3			18	21	0,44
C. gulbenkiani	1					24			14	39	0,82
C. magnus	1			1		61	19	1	13	96	2,02
C. neavei	1					12	1		8	22	0,46
C. angolensis						1				1	0,02
C. onderstepoortensis				2	1	1			1	5	0,11
C. sp. # 69						1				1	0,02
C. bedfordi	2							3	2	7	0,15
C. similis				1						1	0,02
C. tropicalis	1									1	0,02
C. accraensis group		1		1						2	0,04
	2 925	3	419	232	751	220	24	45	130	4 749	
Weather conditions:	Warm/calm	Very windy	Coolish/calm	Cool/misty/calm	Coolish/calm	Intermittently windy	Windy	Windy			

Entire catches analysed: subgenus *Avaritia* = two species = 92,54 % = dung-breeders
C. imicola: 0 %

Identified by: R. Meiswinkel and M.E. Botha

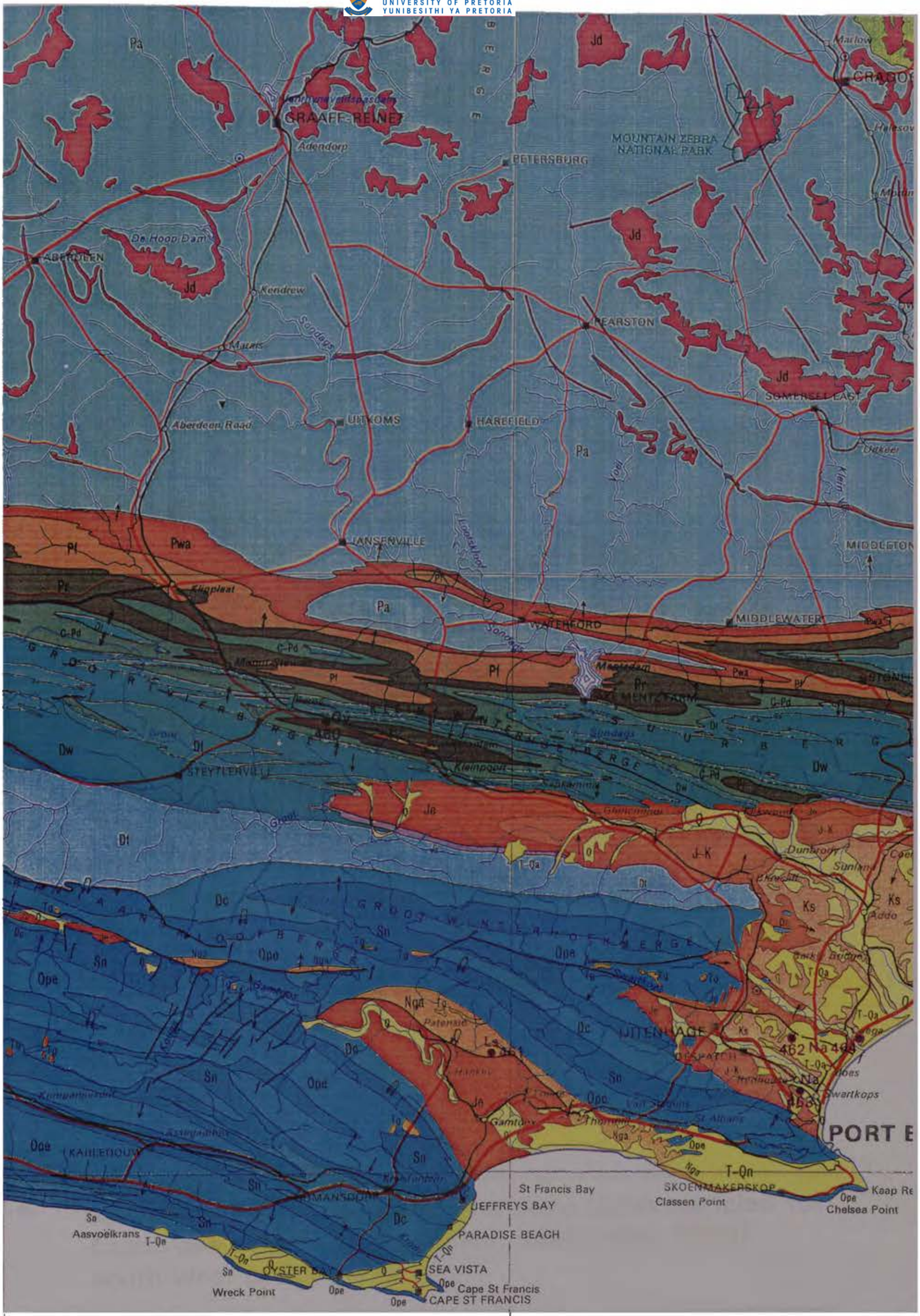


Fig. 10.1 Southern Cape coast: extent of ancient, nutrient-poor sea sands (yellow) blown onland during past eons.

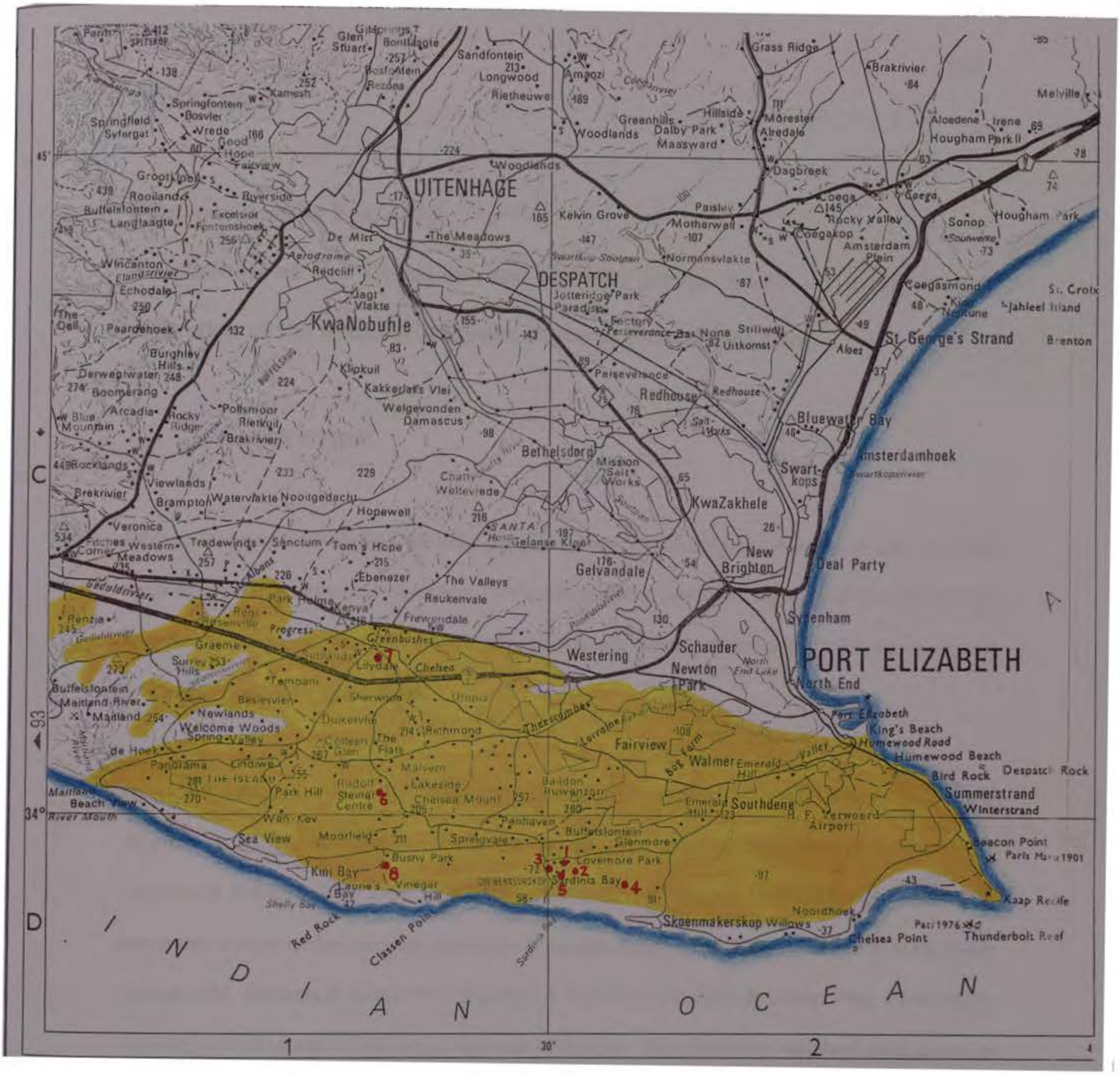


FIG. 10.2 Location of 8 horse stables and dairy farms sampled for *Culicoides* midges on the sandy coastal strip (▭) south-west of Port Elizabeth

CHAPTER 11

Subgenus *Avaritia* Fox, 1955:

Redefinition of the Orientalis and Imicola groups based on morphological separation of the adults (*Culicoides*; Diptera: Ceratopogonidae)

11.1 INTRODUCTION

Of the 109 names available for *Culicoides* species belonging to the subgenus *Avaritia*, 63 are considered valid and 46 to be synonyms (Boorman 1991). Allowing for omissions, subsequent descriptions and resurrection from synonymy, the number of valid *Avaritia* species stands nearer to 70, approximately 5 % of the *Culicoides* world fauna.

Fox (1955) created *Avaritia* and chose the Holarctic species *C. obsoletus* (Meigen) as subgenotype. While *Avaritia* was adopted by researchers worldwide, its usage was rather random (Wirth & Blanton 1959; Campbell & Pelham-Clinton 1960; Vargas 1960; Delfinado 1961; Atchley 1967; Blanton & Wirth 1979) and in time a more refined tendency developed to use species-groups instead (Jamnback & Wirth 1963; Kremer 1965; Khamala & Kettle 1971; Boorman & Dipeolu 1979; Itoua & Cornet 1986; Glick 1990); however, it resulted also in the more limited use of *Avaritia*. This dichotomy may have been due to an emergent realization that no *Avaritia* species in other regional faunas has the complex, almost flamboyantly aberrant, genitalia that characterize *C. obsoletus* and seven other species of the Obsoletus group. Vargas (1962, 1973) also sent mixed signals when he incorrectly excluded taxa from *Avaritia*, while assigning other, clearly unrelated, ones to it.

Although the pendulum has recently swung back to using *Avaritia*, this seems to be more a case of nomenclatorial legalism (Wada & Kitaoka 1977; Dyce 1980, 1983; Dyce & Wirth 1983; Howarth 1985; Kitaoka 1985; Wirth, Dyce & Peterson 1985; Glukhova 1989; Mirzaeva 1989; Boorman 1989; Kitaoka & Zulu 1990). While there is some merit in rejecting the informal species-group category, its *ad hoc* replacement with *Avaritia* cannot be seen as progressive as this only means that new species are now simply being gathered under a 'named' umbrella, one that is growing ever larger and more disparate. Furthermore, by 'securely' placing a species within a formal subgenus we may neglect to explore the relationships that undoubtedly exist between its constituent taxa.

In the last eight years, however, a small group of workers has begun to recognize that *Avaritia* is a polyphyletic assemblage, and that it can be split (Itoua & Cornet 1986; Meiswinkel 1987, 1989, 1991; Wirth, Dyce & Spinelli 1988; Wirth & Hubert 1989; Boorman 1991). To date seven species groups have been proposed. These are the Actoni, Andicola, Imicola (= Pallidipennis), Montanus, Obsoletus, Orientalis and Pusillus groups (Wirth, Dyce & Spinelli 1988; Wirth & Hubert 1989; Glick 1990; Boorman 1991). While all except the Montanus group of Boorman (1991) appear to be valid, they are either not delineated (Andicola and Pusillus groups), have been established in the female only (Actoni and Orientalis groups), or are too broadly defined (Imicola and Orientalis groups). As a result their usage is confused, especially as regards the Orientalis and Imicola groups.

While some authors have begun to establish species groups within *Avaritia*, others do not recognize the subgenus; they prefer to use the group caterogy in lieu of *Avaritia* (Itoua & Cornet 1986; Glick 1990). This complicates levels of ranking, however, as discrete lineages subsequently discovered must be accommodated by further subdivision of the said group, a reality that led Itoua & Cornet (1986) to present, but not define, the Afrotropical Grahamii, Trifasciellus and Imicola subgroups of the Imicola group. Not only do subgroups at this level hamper further fragmentation involving species-pairs, but also the discordance in hierarchy obscures the fact that their first two subgroups are, in part, synonymous with the south-east Asian Actoni and Orientalis groups of Wirth & Hubert (1989).

Before a proper cladistic analysis is done to clarify the various levels of relatedness that appear to exist between the taxa comprising each group, I suggest that the three subgroups of Itoua & Cornet (1986) be recognized as groups. This would bring studies on the Afrotropical *Avaritia* fauna in line with those done in other Regions; the Orientalis group is here used instead of the Trifasciellus group as the nominal species of the former was described earlier. The Orientalis and Imicola groups are here understood to clearly belong within the subgenus *Avaritia*, and are not equal to or meant to replace it.

Although there may be problems with hierarchial ranking, it is more important to note that the undisputed presence of closely related species in two or more regional faunas is tacit acknowledgement that discrete lineages exist within *Avaritia*, and that current species-group definitions are failing to expose them clearly enough. As mooted in Chapter 4, I believe that some 10 species groups consitute *Avaritia* worldwide. In this chapter two of these, the Orientalis and Imicola groups, are redefined and separated. Not only have they been used by authors as a catchall for a miscellany of species, but they also contain the most important vectors of bluetongue, African horsesickness and Akabane viruses currently known to occur in the Old World. This analysis has consequences and recommendations that include:

(i) adjustments being made to current species group lists;

(ii) new and seldom-used characters are presented that should be employed in future species descriptions and group definitions;

(iii) in future studies, larger series of both sexes should be scored to better understand inter- and intra-specific variation;

(iv) the accurate observation of small but important character states also requires that *Avaritia* specimens be more carefully prepared during slide-mounting;

(v) a combination of (ii) — (iv) has left the systematics of the south-east Asian Orientalis group in some disarray, and is discussed.

11.2 MATERIALS AND METHODS

Nearly 600 slide-mounted males and females of nine species of the Imicola group were examined, and provided a better understanding of the taxa than can be claimed for the Orientalis group. Fifty males and females of the latter group, collected in Thailand and the Philippines, were studied. As the males provide more group-specific data, they were intensively studied and are used to spearhead arguments that the systematic study of the Orientalis group in south-east Asia is in some disarray. Data on the male antennal trichodea distributions of the three known Afrotropical species of the Trifasciellus subgroup (synonymous with the Orientalis group) were taken from Itoua & Cornet (1986), and supplemented by examination of 20 specimens of *C. trifasciellus* in the Onderstepoort collection.

The 11 Orientalis group species are lettered sp. #A — sp. #K; only four have been identified with confidence, namely *jacobsoni* (sp. #A), *dumdumi* (sp. #B), *pungens* (sp. #C) and *fulvus* (sp. #D). It is probable that sp. #E is *maculatus* and sp. #F *orientalis*. Each putative species was identified using either keys, illustrations or descriptions found in the works cited.

Illustrations were made at 400 x magnification from slide-mounted material in which the genitalia were clearly and symmetrically displayed. Each one of numerous spicules on the ninth sternal membrane has been illustrated as observed, to faithfully reproduce their size, position and density of distribution accurately. In the same figures the aedeagus and sternum have been fractionally displaced anteriad of the tergum so that the sclerotised median cleft of the tergum can be clearly depicted. Excepting for the short bristle found at the base of the apicolateral processes of most species, the spiculation and setation of the basimere and tergum nine have been omitted.

The long and short blunt-tipped sensilla trichodea on male and female flagellar segments III–XII are labelled L and c respectively, following the nomenclature of Itoua & Cornet (1986). Sensilla that are atrophied or under-developed are shown as a ¾L, ½L or ¼L in Tables 11.4 and 11.5; Table 11.2 is less detailed, giving only presence/absence data. Derivation of the wing costal ratio (CR), antennal ratio (AR), and antennal trichodea ratio (AtR) are given in the text where appropriate.

11.3 RESULTS AND DISCUSSION

Despite problems with specific identifications, it is clear that the Orientalis and Imicola groups represent two lineages within *Avaritia*, and can be quite easily separated on the 18 character states listed in Table 11.1; the nine that appear in bold type are considered the more important. Each of the characters is discussed below, and comments are made on its reliability and variability both within and between species. In the female two are compared in Figs. 11.1a–11.1d, in the male seven are shown in Figs. 11.3a–h, while the eighth, the antennal blunt-tipped trichodea distribution on flagellar segments III–XII, is presented in Table 11.2. The data on the Orientalis group in Table 11.2 (including the three African species of the Trifasciellus group) summarize what is given in greater detail in Table 11.4, and is provided to demonstrate that species of the Orientalis group mostly have more trichodea than those of the Imicola group. In Table 11.3, all Old World species are assigned to their respective groups; synonyms are included following Wirth & Hubert (1989) and Boorman (1991). The south-east Asian species *C. hui* is included under the Orientalis group; reservations as to its true position within *Avaritia* are submitted and discussed. Eleven of the 19 species included by Wirth & Hubert (1989) in the Orientalis group are omitted; they belong either in the Imicola group (i.e. *C. brevitarsis, C. imicola, C. nudipalpis* and *C. pseudopallidipennis*), or to as yet undefined groups (i.e. *C. boophagus, C. brevipalpis, C. dikhros, C. fragmentum, C. pastus, C. suzukii* and *C. wadai*).

The broader group definitions are followed by a more detailed appraisal of slide-mounted specimens of the Orientalis group species, with special emphasis on the male; detailed data on their antennal trichodea distributions are given in Table 11.4. In Table 11.5 other male character states are listed that correlate with species-specific differences indicated in Table 11.4, and support the contention that at least 11 species (12 if *C. hui* is included) of the Orientalis group exist in south-east Asia. The more obvious differences, such as the shape of posterior margin of tergum nine and spiculation of sternal membrane, are illustrated (Figs. 11.4a–k). The data are used to explore incongruities in the literature on the south-east Asian Orientalis group, and help to explain why it is difficult to identify individual specimens held in collections. As regards females of the Orientalis group, Table 11.6 summarizes data on the antennal ratio (AR) as given by various authors, and is compared with data on the Imicola group. While there is a paucity of data, the great variability in recorded values reinforces the notion that the Orientalis group embraces more species than currently recognized.

11.3.1 **Character states defining the Orientalis and Imicola groups** (Table 11.1)

♀: 1. **Abdomen: dorsal pigmented tergites**

As shown in Fig. 11.1a, species of the Orientalis group always have large, rectangular tergites dorsally on abdominal segments II–VII whereas in the Imicola group they are reduced on segments III–V being smaller and appear almost round at low magnifications (Fig. 11.1c). This feature is the most reliable for assigning a specimen to its respective group when viewed under a dissecting microscope. Unfortunately, the tergites are only clearly visible in nulliparous females as the burgundy pigmentation laid down in the abdomens of older parous and gravid females tends to obscure them. Similarly, during slide-mounting, these tergites fade in material treated in KOH, and are further obscured in females whose abdomens are mounted ventral side up for better examination of the spermathecae and sclerotized plates surrounding the gonopore. While the shape of these tergites remains unknown for other species groups, it is probable that some will share the conformations described here.

2. Abdomen: sclerotized plates surrounding gonopore

As illustrated (Fig. 11.1b, 11.1d), the precise form of these plates differs between the two groups. In the Imicola group they are simpler i.e. lack the forefinger and thumb-like projections that partially embrace the gonopore opening, and seen in all Orientalis group species. Their precise delineation depends strongly on specimens being carefully prepared, especially cleared of pigment and the abdomen stretched as the distal segments can be telescoped. To my knowledge the shape of these plates has only been used once before as a taxonomic character in the genus *Culicoides* (Wirth & Hubert 1989), and its usefulness for defining other species-groups still needs to be ascertained.

3. Legs: hind tibial comb

The first, and longest, of five spines comprising the hind tibial comb is equally long in both species groups. While it appears to be more darkly pigmented and more robust in the Orientalis group, it must be noted that degree of pigmentation can be affected by the clearing process during slide-mounting. Also, due to its relatively small size, this spine is difficult to measure accurately and thereby establish the degree of difference that may exist between the two groups.

4. Wing: Costal ratio (CR)

This is obtained by dividing the length of the costa (measured from the arculus to the tip of the second radial cell) by the total wing length (also measured from the arculus). As shown in Table 11.1, this value is higher in the Orientalis group, i.e. the costa in each species of this group is fractionally longer than that found in members of the Imicola group.

5. Antenna: Antennal ratio (AR)

As indicated in Table 11.1, the antennae of species of the Orientalis group are more slender than those of the Imicola group; this may create the impression, falsely, that they are also longer. However, there is little exact data on total antennal length in the literature as they are either omitted by authors (Kitaoka

FIG.11.1

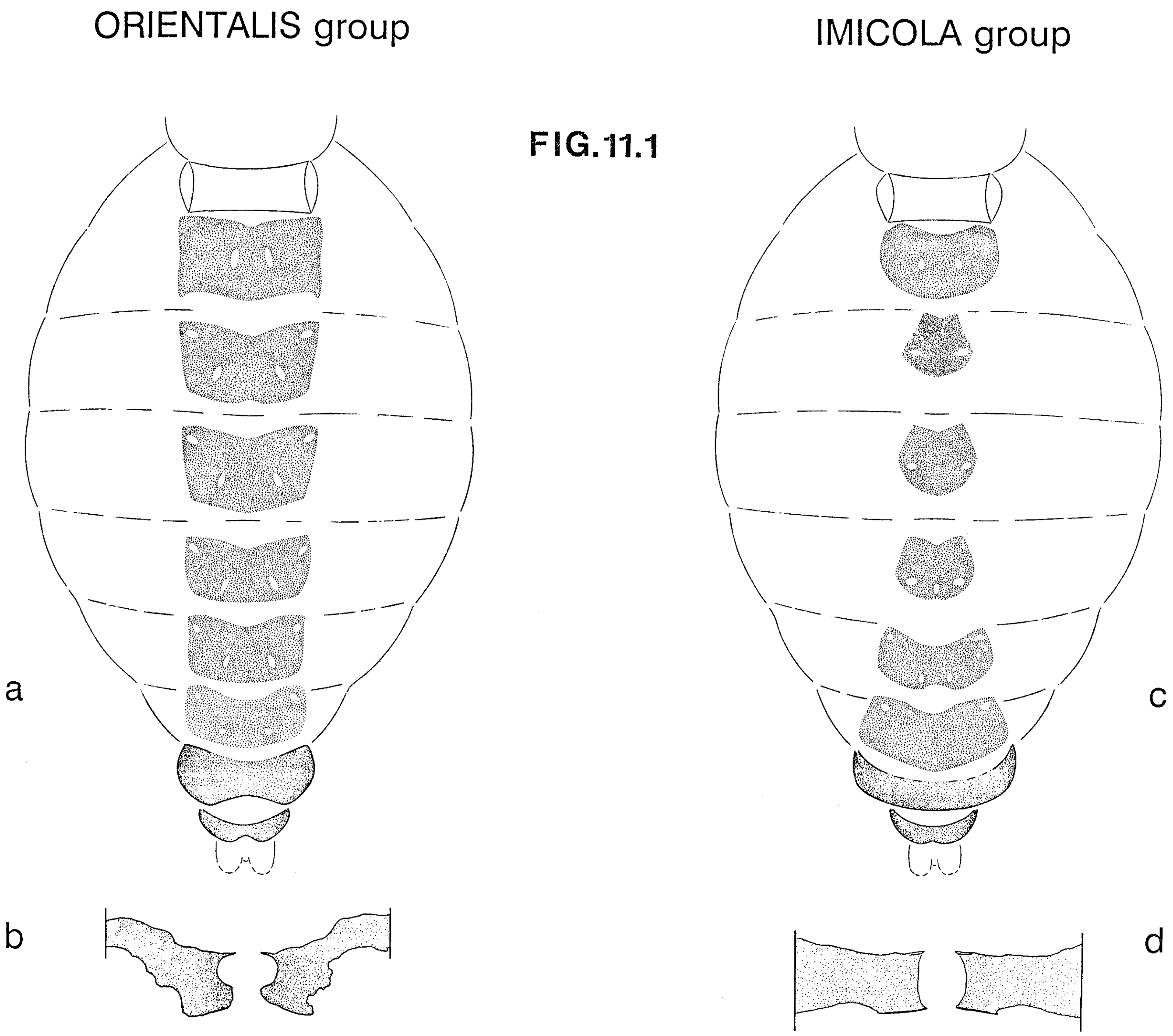


Fig. 11.1a Female abdomen (Orientalis group): dorsal pigmented plates

Fig. 11.1b Female abdomen (Orientalis group): sclerotized plates surrounding gonopore

Fig 11.1c Female abdomen (Imicola group): dorsal pigmented plates

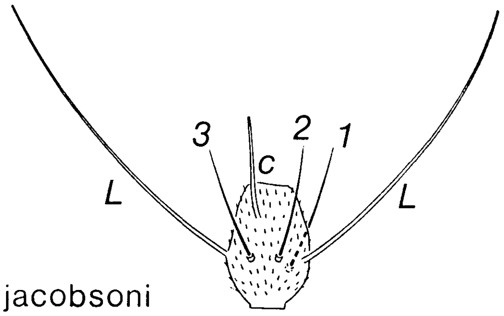
Fig. 11.1d Female abdomen (Imicola group): sclerotized plates surrounding gonopore

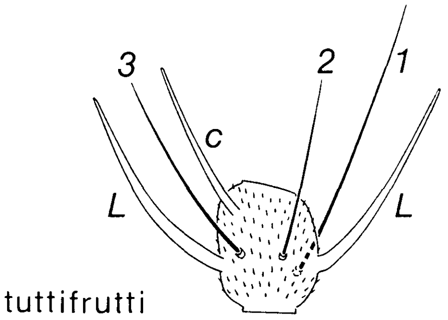
1985), or flagellar segment lengths are only given as proportions (Wirth & Hubert 1989). To overcome the range in variation that measurements of small to large specimens of a species would give, the antennal ratio (AR) has been widely adopted by researchers working in *Culicoides*; the AR is obtained by dividing the sum of the lengths of the five apical segments (XI–XV) by the sum of the basal eight segments (III–X). As remarked upon by Campbell & Pelham-Clinton (1960), the AR must be based on precise measurements if it is to be of any value. In Table 11.6, the AR data on 17 species of the Imicola and Orientalis groups are presented according to 12 sets of authors. If perused carefully, it reveals discrepancies between authors describing the same species. For example, Wirth & Hubert (1989) give an antennal ratio for eight Orientalis group species (43 specimens) that ranges between 1,07–1,47; for five of these species, Kitaoka (1985) gives a narrower range of 1,04–1,26 (number of specimens unknown), which is comparable to the 1,09–1,28 generated in this study for seven species (10 specimens).

The Imicola group AR data generated here from 562 specimens (nine species; Table 11.6) show a lower group-ratio, ranging between 0,92–1,25. This does, however, overlap considerably with ratios published on the Orientalis group. While it can be argued that far too few specimens of Orientalis group species were examined by the cited workers, it must be pointed out that the range in their values should thus have been smaller, and not larger, as shown in Table 11.6. For example, Wirth & Hubert (1989) derived from 10 specimens of *C. pungens* an AR that ranged between 1,27–1,47. In this study, 167 specimens of *C. imicola* gave a more conservative range of 0,95–1,10. Indeed, fairly large samples of the remaining Imicola group species (Table 11.6) revealed a consistent deviation between upper and lower values. This consistency is entirely absent in the Orientalis group data, and either indicates mixed species or technical problems. As discussed under Materials and Methods (Chapter 1) only antennae removed from the head, mounted straight and flat, were used to obtain the Imicola group measurements presented in this study. Antennae left on a rounded head and/or lying curled, and at varying angles in thick mountant, would contribute to the type of inconsistent data that currently exist for the Orientalis group.

Despite apparent shortcomings in the data available for the Orientalis group, it seems justified to conclude that its AR is higher than that found in the Imicola group. However, considerable overlap nullifies its value as a group character. These two groups can be separated on other features of the

FIG. 11.2





		C. jacobsoni		*C. tuttifrutti*
length segments 3-10	:	213		209
length segments 11-15	:	194		224
total antennal length	:	407		433
antennal ratio (AR)	:	0,91	*	1,07
length segment VI	:	20,5		23
width segment VI	:	14,5		18
no. chaetica segment VI	:	3		3 or 4
no. trichodea segment VI	:	LLc		LLc
length short trichodea (c)	:	19	*	29
length long trichodea (L)	:	61	*	38
length chaetica 1	:	22,5	*	50
antennal trichodea ratio (AtR)	:	2,98	*	1,65

Fig. 11.2a Female antenna (Orientalis group): Various sensilla on flagellar segment VI of *C. jacobsoni*

Fig. 11.2b Female antenna (Imicola group): various sensilla on flagellar segment VI of *C. tuttifrutti* antenna as will be shown for the male. Figs. 11.2a and 11.2b are of the sixth antennal segment of *C. jacobsoni* (Orientalis group) and *C. tuttifrutti* (Imicola group) respectively. Accompanying the illustrations are various measurements and ratios; those values showing significant differences are indicated with an asterisk. These reveal that, in spite of the two species having antennae of similar length and the same sensilla coeloconica distributions, they can be easily separated on the antennal ratio (AR) and the respective lengths and thicknesses of the long trichodea (L) and chaetica (1, 2 and 3). The large difference in long trichodea length explains the discrepancy in antennal trichodea ratios (AtR), where the length of L is divided by the length of segment VI; in *C. jacobsoni*, these trichodea are also very slender. In the same species, the flagellar segment is also narrower than that of *C. tuttifrutti* and so makes for an antenna that is more slender than, but nearly equal in length to, that of the more robust *C. tuttifrutti*.

6. **Antenna: sensilla coeloconica**

As shown in Table 11.1, all species of the Orientalis group have sensilla coeloconica distributed on flagellar segments III, XI–XV. Seven of the nine species of the Imicola group differ in having a III, XII–XV distribution; two, *C. pseudopallidipennis* and *C. tuttifrutti*, have coeloconica on III, XI–XV, while a third, *C. nudipalpis*, has this distribution in approximately 50 % of specimens. It is fair to say that more than 80 % of the 70 species of the subgenus *Avaritia* found worldwide will share one of these two distribution patterns, irrespective of species group, and therefore they have limited value as a group character.

♂: 7. **Genitalia: aedeagus**

In the Orientalis group, the infuscated 'peg', a prolongation of the distal process that projects into the aedeagus, narrows anteriorly to end in a sharp to blunt point (Figs. 11.3b, 11.4a–k), and is rather smoothly pigmented. In the Imicola group, the anterior end of the peg differs in that it usually expands in a more irregular manner (Figs. 11.3f, 2.20, 2.21, 4.12, 5.12, 6.9, 6.18) and, is not smoothly pigmented, but granular.

8. **Genitalia: aedeagus**

In the Orientalis group, the infuscated peg is connected to the lateral converging sclerotised arms of the

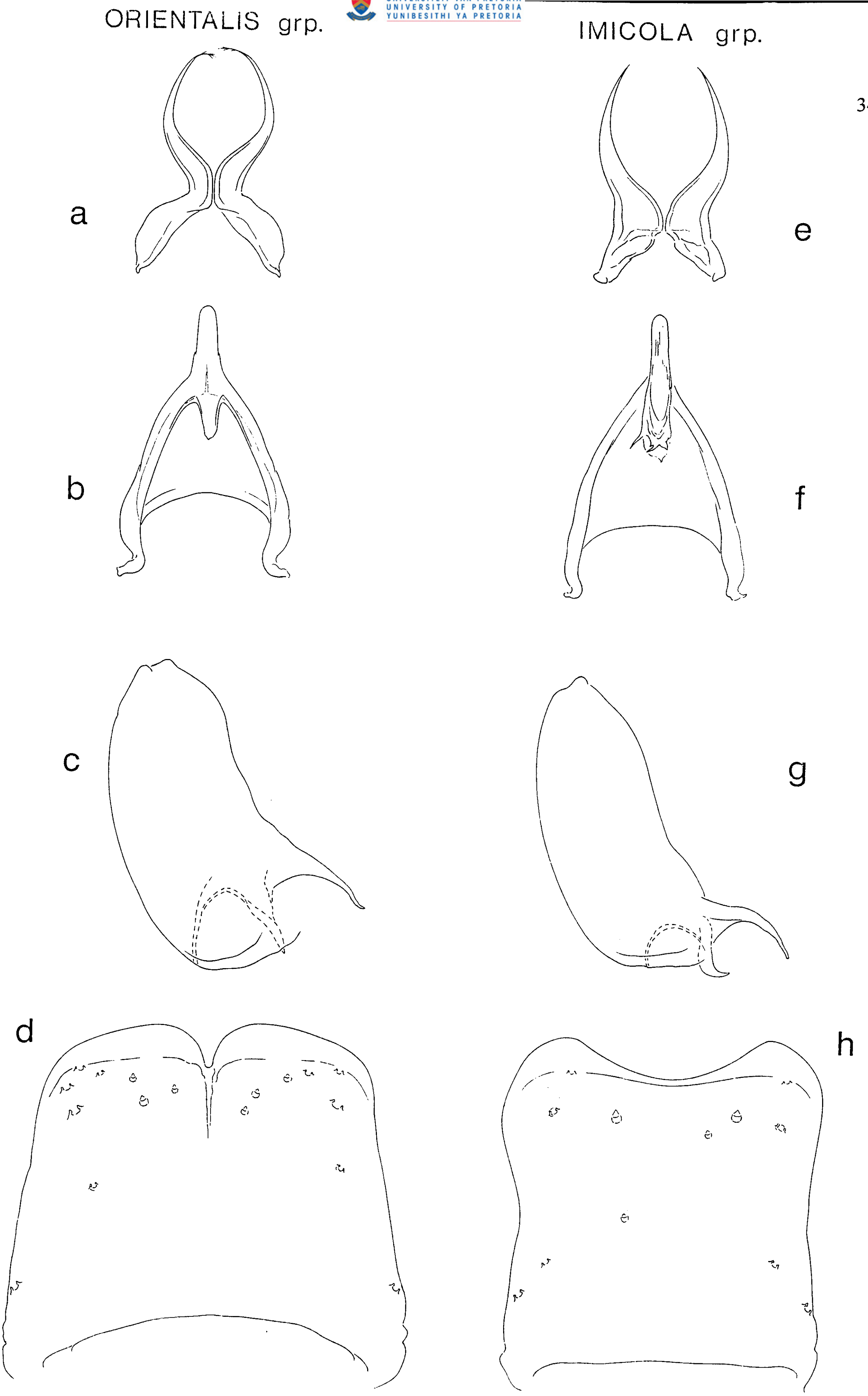


FIG. 11.3

Fig. 11.3a–d Male genitalia (Orientalis group: sp. #F); a: parameres; b: aedeagus; c: basimere; d: tergum nine

Fig. 11.3e–h Male genitalia (Imicola group: *C. imicola*); e: parameres; f: aedeagus; g: basimere; h: tergum nine aedeagus by a lightly pigmented membranous arch (Fig. 11.4a–k). This arch is better developed in some species (sp. #D, #E, #H, and #K) than in others; it is absent in all species of the Imicola group.

9. **Genitalia: parameres**

Comparison of the parameres in Fig. 11.3a and e indicates more than one difference to exist between the two species groups. These are the angle of projection, the degree of development, the shape of the anterior feet, and the configuration of the posterior tips. In the Orientalis group, the tips of the parameres are always sinuous, most often being limp and recurved, and can be bare (seldom) or inconspicuously to obviously filamentous or feathered (Fig. 11.3a; Table 11.5). In some species this tip has been described as bare but its precise observation depends on high magnification (800–1000 x) and clearly prepared material. For example Wirth & Hubert (1989) described the parameres of *C. flavipunctatus* and *C. orientalis* as 'tapering distally to a simple filamentous tip', but illustrated them as feathered. This could be the result of mixed series, or conflicting observations made by the taxonomist and co-operating illustrator. In the Imicola group, all species, without exception, have the tip of the parameres erect, sharp and simple (Fig. 11.3e).

10. **Genitalia: tergum nine**

Figs. 11.3d, 11.4a–k show that in the Orientalis group, the apicolateral processes are broad, subtlely triangular in shape, and, importantly, the apex of the process is found sublaterally. Furthermore, these processes are always narrowly and fairly abruptly separated medially by a deep and clearly pigmented subtriangular excision (Figs. 11.3d, 11.4a–k). In the Imicola group, the apicolateral processes are not as broadly developed, and arise on the posterolateral corners of the tergum. The processes are thus broadly separated by a gently concave posterior margin that is not infuscated but, at most, may be striated medially (Figs. 4.11, 5.11, 6.8, 6.17). While the configuration of the apicolateral processes described as characterizing the Imicola group may be found outside the group, that described for the Orientalis group does not seem to occur in other species of *Avaritia*.

11. **Genitalia: basimere**

Fig. 11.3c shows the basal half of the basimere of most species of the Orientalis group to be broader than the apical half, the ventral root arises at a shallower angle (45 °– 75 °), and the dorsal root is

Fig.11.4

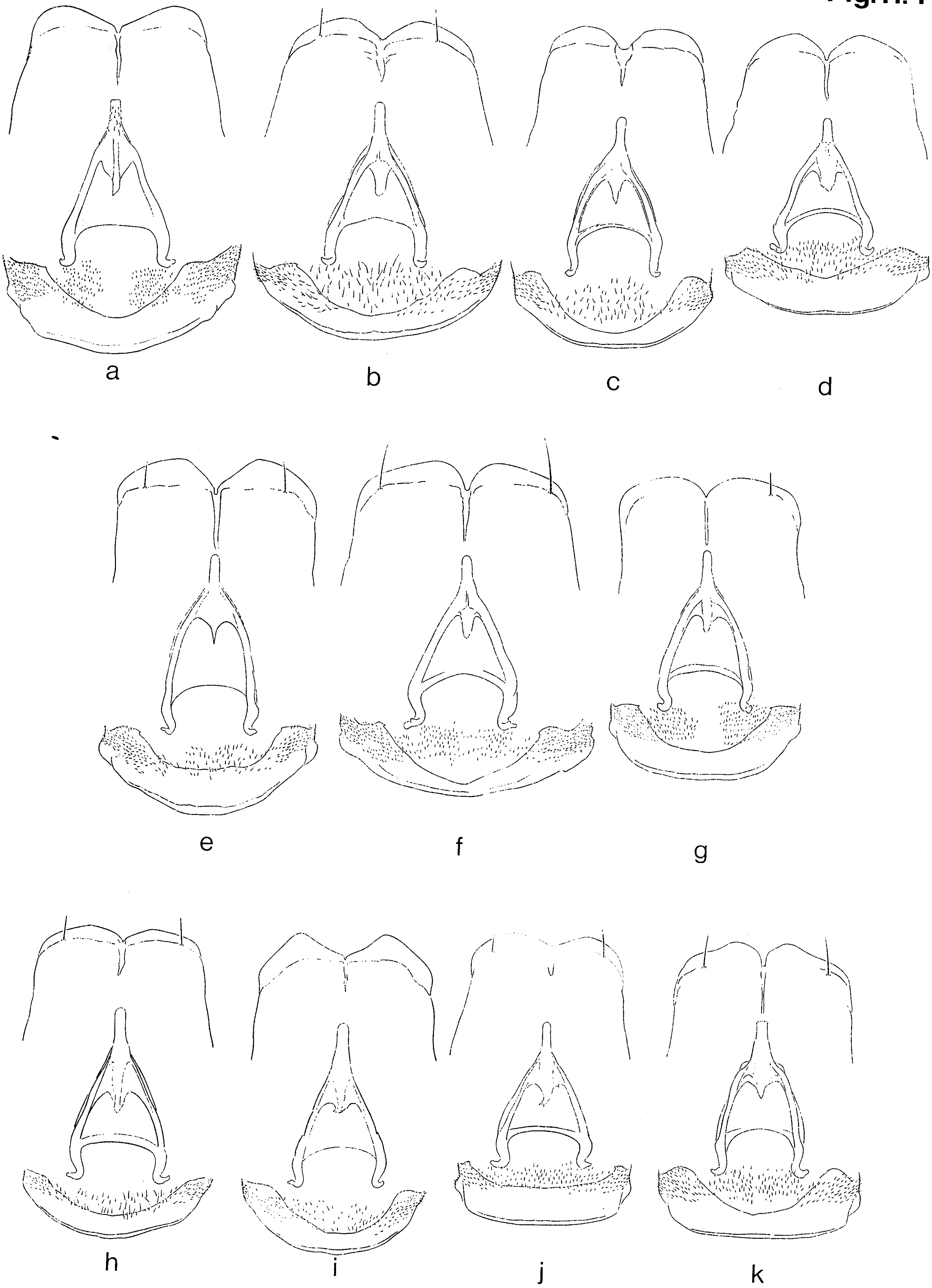


Fig. 11.4a–k Male genitalia (Orientalis group) showing posterior half of tergum nine, aedeagus and spiculation on membrane of sternum nine of 11 putative species.

almost straight. In the Imicola group (Fig. 11.3g), the basimere is nearly parallel-sided for its entire length, and the dorsal and ventral roots are distinctly bowed almost like a curled forefinger and thumb. Furthermore, the ventral root almost arises at right-angles (75 °–90 °) from the basimere. In all species of the Imicola group, the shorter dorsal root is rather broad and always curls around the base of the paramere.

12. **Genitalia: tergum nine**

In the Orientalis group, the tergum is trapezoidal in shape, gradually narrowing posteriorly (Fig. 11.3d, 11.4a–k); in the Imicola group, it is almost square, as wide anteriorly as posteriorly, and noticeably waisted medially (Fig. 11.3h). This character is best observed in specimens where the genitalia have not been squashed by coverslipping. In compressed specimens the anterior half of the tergum, in being three-dimensional where it is fused to the narrow strip that forms sternum nine, tends to splay outwards i.e. becomes trapezoidal, and so gives a false impression of its true shape.

13. **Genitalia: sternum nine**

In the Orientalis group, the membrane of sternum nine is never bare but always spiculate. In Figs. 11.4a–k it can be seen that this spiculation is fairly diagnostic for each species, being either sparse to moderately abundant (spp. #C, #E, #H and #I), or abundant to very abundant (spp. #A, #D, #G and #J), short (spp. #A, #F and #G), moderately long (spp. #C, #D, #E, #I and #K) to long or very long (spp. #H and #B), or distributed evenly (most species) or in two discrete patches (spp. #A and #G). In the Imicola group, two-thirds of the species i.e. *bolitinos, brevitarsis, loxodontis, pseudopallidipennis*, *tuttifrutti* and *kwagga*, have the membrane bare or with a few spicules found near the margin of the excavation (Figs. 2.20, 5.12, 6.9, 6.18). In the remaining species, *imicola, miombo* and *nudipalpis*, the spiculation ranges from being moderate to dense, but the spicules do not vary in size, being all short (Figs. 2.21, 4.12).

Although spicules on the sternital membrane can be present or absent in a group, and thus of limited value when assigning species, the degree, size and precise distribution of these spicules is indifferently treated in taxonomic studies. For example Wirth & Hubert (1989) describe the sternal membranes of *jacobsoni* and *maculatus* as spiculate but illustrate them as bare, while Kitaoka (1975), in his original description of *flavipunctatus*, stated the membrane to be bare, whereas his 1985 illustration of the same species shows it to be spiculate. In some instances the position of the spicules appear to be group- rather than species-specific. I am aware of *Avaritia* species not having spicules on the membrane but spread across the sclerotised anterior part of the sternum. As they do not lie exposed on the membrane in the excavated area, they may not be referred to, or noticed, by systematists. This rather important issue of spiculation is further complicated when the eighth abdominal segment is allowed to lie folded over sternum nine during preparation of slide-mounts. The dense spiculation of sternum eight thus overlies and obscures that which may be present on nine; while the spiculation is of a different type on these preceding segments, it is difficult to distinguish them as the respective membranes tend to subside onto a single plane during coverslipping.

14. **Antenna: Long (L) and short (c) blunt-tipped sensilla trichodea**

Comparison of the blunt-tipped trichodea distributions found in the Orientalis and Imicola species group (Tables 11.2 and 11.3) reveals that:

a) Despite some variation, the 14 Orientalis group species have significantly more trichodea on flagellar segments III–XII. These number 17–31/antenna as compared to the 16–18 found in the Imicola group (nine species). This is mainly due to most Orientalis group species having an additional long trichodea on X, and usually an additional short one on XI and XII.

b) While the trichodea distributions in the Imicola group are remarkably stable and similar, there exists a greater degree of interspecific variation in the Orientalis group. The trichodea distribution of *C. jacobsoni* is unusual, but this species must be regarded as an outlier as it is also the only one bearing retrorse spinules along the tip of the aedeagus (Fig. 11.4a). However, *C. jacobsoni* bears a number of Orientalis group distinctions that include the shape of the basimere, configuration of apicolateral processes, and ‘arched’ peg of the aedeagus. The same comments apply to *C. fulvus*, which also has a reduced number of antennal trichodea. This diversity in the Orientalis group implies an active and long evolutionary history and suggests that subgroups remain to be discovered.

c) In Table 11.2 the large number of trichodea encased in brackets suggests that a higher degree of intraspecific variation occurs within the Orientalis than in the Imicola group, but the more detailed data given in Table 11.4 indicates that this is not so. For example four specimens of sp. #F consistently had one of the L trichodea underdeveloped (exactly as recorded for *C. pseudopallidipennis* and *C. tuttifrutti* of the Imicola group). It can be seen that a similar pattern of reduction marks the antennae of sp. #G. Indeed, the two species (#F and #G) are very difficult to separate, differing only in the shape of the apicolateral processes and in that the former possesses a seta at the base of each process (Fig. 11.4f, g). Species #G is illustrated as having the spicules on the membrane in two distinct patches (Fig. 11.4g), whereas in #F they are shown as narrowly fused (Fig. 11.4f). However, they occur in two patches in a second specimen (Thailand 227; Table 11.5). Either one of those two species could be *C. orientalis* s.s.; it appears to be sp. #F if we compare the genitalia (Fig. 11.3a–d) to those given in its redescription by Dyce (1980). Along with sp. #E (Fig. 11.4e), which strongly resembles *C. maculatus* as illustrated by Kitaoka (1985) and Wirth & Hubert (1989), these two species indicate the presence of a subgroup. It is notable that the aforementioned *C. pseudopallidipennis* and *C. tuttifrutti* also have atrophied trichodea, and also form a distinct subgroup within the Imicola group. Various other characters justify the latter pair of species being split off as a subgroup. This aberration in the development of antennal trichodea has been consistently found in various populations of *C. tuttifrutti*, and has not been linked to cryptic speciation. Its unexpected existence underscores the necessity of examining long series of adults to obtain data that more accurately define a species.

11.3.2 Anomalies in the literature on the Orientalis group

As noted earlier, the systematics of this group in south-east Asia is in partial disarray. Incongruities in the published literature create doubt as to the exact identity of specimens, and this is exacerbated by descriptions being too brief, illustrations too stylized, and too few specimens being examined. Some of these difficulties are discussed below; notes are also given on the annectent taxonomic position of *C. hui*.

1. a) The key to species of *Culicoides* of south-east Asia, commencing on p. 57 in Wirth

& Hubert (1989), contains an error that leads the user astray: couplet 108 (p. 68) gives two choices, these leading to couplets 109 and 110; the latter should be numbered 111. Similarly, in couplet 109 the numbered choice should be '110' and not '111' as given.

b) In couplet 113 it is stated that 'sensilla chaetica (verticils) on segments XI–XV usually reduced' or 'well developed'. *Avaritia* species never have sensilla chaetica on these segments, only on segments III–X. Should read: 'sharp-tipped sensilla trichodea ...'

2. *C. fulvus* Sen & Das Gupta, 1959

Wirth & Hubert (1989) state:

a) 'ninth sternum with broad, shallow caudomedian excavation'. Fig. 117g (p. 268) shows it to be narrow if compared with all other Orientalis group species.

b) 'aedeagus 1,8 x as long as basal breadth ...' Measurement of Fig. 117g shows it to be 1,35.

c) 'aedeagus ... basal arch low, a fifth of total length ...' Fig. 117g shows it to be closer to a third.

d) parameres described (and illustrated) as 'tapering distally to a slender tip bearing minute fringing hairs'. In the discussion it is said that 'the long distal portion tapers to a fine tip.'

e) 'basistyle with ventral root long and slender ...' Fig. 117g shows ventral root entirely absent.

Similar discrepancies between the text and illustrations were noted above for other species of the Orientalis group described by Wirth & Hubert (1989). As observed, this could be the result of conflicting observations made by the taxonomist and the co-operating illustrator, or could be due to mixed series. It is pertinent to comment that Wirth & Hubert's material of many of the 168 species covered in their treatise of south-east Asian *Culicoides* came from a broad geographic area. While the localities are recorded, we are given hardly any information as to which specimens were described, or which were illustrated or photographed. It is thus impossible to link described material with a precise locality, or to establish whether the males were captured with the females or came from widely separated locales.

3. *C. flavipunctatus* Kitaoka, 1975 and *C. fulvus* Sen & Das Gupta, 1959

There appears to be some confusion as to the status of these two species. Wirth & Hubert (1989) separate them on central wing markings and shape of the apicolateral processes on tergum nine. They do not refer to, or use, the characters employed by Howarth (1985) who distinguishes them on colouration of the halteres and thorax, the intensity of darkness of the first costal wing spot, and the shape of the spermathecae. Unfortunately, Howarth did not redescribe these species but did note that *C. fulvus* 'is confusingly similar to' *C. flavipunctatus*, and 'except for the differently shaped spermathecae ... could be considered to represent pale specimens of *C. fulvus*. He goes on to say '... they occur together ... from widely scattered localities in Laos'. Wirth & Hubert (1989) echo the conclusions of Howarth in noting 'that the wing markings of *C. flavipunctatus* intergrade gradually into those of *C. fulvus* ... the two forms occur together in Indonesia.' Doubt is heightened when one refers to Kitaoka's original description of *C. flavipunctatus*. Judging from his wing photographs, the male and female are correctly associated; the ninth sternal membrane is described and illustrated as bare, but in a later publication it is figured as spiculate (Kitaoka 1985). If one accepts that Kitaoka was originally mistaken (as no members of the Orientalis group have bare membranes), one can then compare his 1985 illustration with that given by Wirth & Hubert (1989). Two differences become apparent: (i) the apicolateral processes on tergum nine appear to differ in shape, and (ii) the aedeagus illustrated by Wirth & Hubert (1989) has rather pronounced shoulders (but not described). The genitalia figured by Wirth & Hubert (1989) strongly resemble those illustrated here for C. sp. #K (Thailand 228); both have the unusual wavy shape of the posterior margin of tergum nine, shoulders on the aedeagus and same shape of basimere and distimere. Indeed, the posterior of tergum nine of C. sp. #K is very similar to that illustrated by Tokunaga (1951) in his original description of *C. suborientalis*. The females collected in southern Thailand in association with the C. sp. #K male nearly key out to *C. flavipunctatus* in Wirth & Hubert (1989), but fail as they do not have the dark spot at the base of wing vein M_2 isolated from, but have them broadly joined to, the dark area over base of vein M_1. In Kitaoka (1975, 1985), this spot also appears to be isolated but is too indistinctly figured, and not described. It is relevant to note that the narrow membranous area between the base of vein M_1 and the subbasal part of M_2 is concave or sunken; when photographed, this area is usually out of focus and can create the impression of being fractionally paler.

As noted earlier, Howarth (1985) separated *C. flavipunctatus* from *C. fulvus* on spermathecal size being 'about 1,4 x as long as wide' in the former and 'about 1,3 x ...' in the latter. The measurements for both spermathecae of *C. flavipunctatus* given by Kitaoka (1975) and Wirth & Hubert (1989) range between 1,24–1,31, thus matching those given for *C. fulvus* by Howarth (1985). The latter species was originally described from four males (Sen & Das Gupta 1959), and the female later described by Das Gupta (1962). Dyce & Wirth (1983) examined this material, noting that the 'unusual asymmetrical enlargments on the terminal sector of both parameres' illustrated by Sen & Das Gupta were in fact 'droplets of an oily residue.' While Dyce & Wirth (1983) recognized *C. fulvus* as a valid species, they did not redescribe it, and it is unknown whether Wirth & Hubert (1989) used part of the type series housed in the USNM in their later redescription. Furthermore, they do not refer to Howarth's distinguishing features, but instead compare *C. fulvus* with *C. brevitarsis*, a more simple exercise when one considers that they belong to two species groups.

As regards the biology of *C. flavipunctatus*, Howarth (1985) reared two males from 'old decomposed elephant feces collected ... in a shaded damp woodland ... Laos.' In the Afrotropical Region, five *Avaritia* species have been reared from elephant dung (see Chapter 8). These species are resource specific, and furthermore only utilize dung for oviposition when it is absolutely fresh. Howarth does not estimate the age of the elephant dung from which he reared *C. flavipunctatus*; we only know the two males emerged 12 days post-harvest. It is therefore unknown whether *C. flavipunctatus* utilizes fresh dung only, or will lay its eggs in any type of damp decomposing plant material. The distinction is important: is *C. flavipunctatus* primarily associated with elephants and their dung (as has been shown for five African species), or is it not? If it is, then one may go on to ask: are all mainland south-east Asian records conspecific with Kitaoka's original type series of *C. flavipunctatus* which was collected on the Nansei island where no elephants occur? It is notable that two other species of the Orientalis group have been reared: *C. dumdumi* from rotting seaweed, and *C. jacobsoni* from decaying fruits and mushrooms, ginger flowers and banana stems. These data suggest that *C. flavipunctatus* could utilize a range of moist vegetative materials for development of the immatures. The five species reared from elephant dung in Africa belong to four species groups within the subgenus *Avaritia*, but not to the Orientalis group. However, occasional specimens of a species resembling *C. trifasciellus* of this group have been collected in South Africa, and found to carry *Myianoetus* mites, a sure sign that the adults had emerged from dung. Unfortunately, this species is rare; like *C. flavipunctatus*, its precise association with dung also remains to be discovered.

This resumé indicates a clear need for *C. flavipunctatus* and *C. fulvus* to be redescribed and carefully compared. As conceded by Wirth & Hubert (1989), '... more data need to be gathered and checked' as '... it is possible that differences exist in proboscis length and antennal ratio.' It is also possible that more than two closely related species exist.

4. *C. pastus* Kitaoka, 1980

Examination of Wirth & Hubert's 1989 redescription of the female reveals that they have taken the numerical data directly from Kitaoka's original description of *C. pastus*, but introduced minor changes and made omissions, in the description of the palpus, legs and spermathecae. For the male they have added the note that the ninth tergum has a 'median cleft present, caudal margin with broad low rounded lobes.' This is reflected in the illustrated genitalia and is as typically seen in the Orientalis group. However, their tergum is distinctly different from that shown by Kitaoka (1980, 1985) who figures the apicolateral processes widely separated, much as those found in the Imicola group. There is also confusion as to whether the ninth sternal membrane of *C. pastus* is spiculate or not. Wirth & Hubert (1989) describe it as bare but illustrate it as spiculate; Kitaoka (1980, 1985) consistently illustrates and describes it as bare.

It seems clear that Wirth & Hubert did not have the male of *C. pastus* before them; I follow the original author, as his male and female are correctly associated judging from the wing photographs. However, his description of *C. pastus* is too superficial for it to be assigned to the Imicola group. Indeed, *C. pastus* probably belongs in an as yet undefined group, as it has large spermathecae with irregular margins and the apices of the parameres limp; the apices have been described both as feathered (Kitaoka 1980) and simple (Kitaoka 1985).

5. *C. hui* Wirth & Hubert, 1961

As noted elsewhere, I have been reluctant to include this species in the Orientalis group (Table 11.3) for the following reasons:

♀: a) Wing pattern unusual, but more like that found in species of the Imicola group. Pale spot in the base of cell M_1 does not align with those found at the apex of the second radial cell, medially in cell M_2 and M_{3+4}. It thus creates the overt 'zigzag' typifying species of the Imicola group.

b) Costal ratio (CR) of 0,61–0,64 is at the bottom end of the Orientalis group range, and at the top of the Imicola group range.

c) Pigmented terga on dorsum of abdomen appear to be large and rectangular as in the Orientalis group.

d) Pigmented sclerites surrounding the gonopore are simple, much as seen in the Imicola group.

e) First spine of hind tibial comb not markedly pigmented or robust like that seen in Imicola group.

f) Basal antennal segments IV and V shortish (Imicola group), VI–X slightly longer and barrel-shaped (Imicola/Orientalis group).

g) Antennal ratio (AR) ranges between 1,00–1,07, average 1,04, in two specimens available (1,12 according to Wirth & Hubert 1989); overlaps the Imicola group range of 0,92–1,25, rather than that of 1,04–1,47 recorded for the Orientalis group (Table 11.6).

h) Sensilla coeloconica III, XI–XV like that of the Orientalis group but not excluding Imicola group.

i) Blunt-tipped sensilla trichodea on basal flagellar segments IV–X moderately long and robust (Imicola group).

♂ j) Ninth tergum: illustrated as subtlely trapezoidal by Kitaoka (1985) and Wirth & Hubert (1989), like that of the Orientalis group.

k) Ninth tergum: posterior margin ‘broadly rounded caudad’ but illustrated as straight (Wirth & Hubert 1989); in Kitaoka (1985) the margin is also shown as almost straight, and though it appears to be fractionally indented medially, it is obscured by parameres and tip of aedeagus (Imicola group).

l) Ninth tergum: according to Wirth & Hubert (1989) posterior margin ‘with only slight trace of caudomedian indentation’ (Imicola group).

m) Parameres: in Wirth & Hubert (1989) described ‘as tapering and curving ventrally to ... slender filament; due to poor preparation presence or absence of minute hairs at tip not determined.’ Their illustration (Fig. 118e), however, shows the parameres as erect (Imicola group) but with hairs at tip (Orientalis group). Kitaoka (1985) illustrates the tips of the parameres as recurved (Orientalis group) but bare (either group).

n) Parameres: in both Kitaoka (1985) and Wirth & Hubert (1989) the anterior feet of the parameres do not diverge widely and are also shaped much as seen in the Imicola group.

o) Sternum nine: both sets of authors describe and/or illustrate the membrane as bare (Imicola group).

p) Basimere: both sets of authors illustrate the ventral roots as not arising at right angles (Orientalis group).

q) Aedeagus: both sets of authors illustrate the ‘peg’ as not being joined by an arch to lateral converging margins of aedeagus (Imicola group).

This examination suggests that *C. hui* falls within the Imicola group, but half the characters scored are too poorly described in the literature for more definite conclusions to be drawn. Furthermore, certain characters are likely to be shared by other, as yet undefined species groups of *Avaritia*. For the interim, *C. hui* is retained in the Orientalis group.

11.4 CONCLUSIONS

The Orientalis and Imicola groups of the subgenus *Avaritia* are redefined. Both contain species widely distributed in, but restricted to, the Old World. The former group is more tropical in its distribution, with most species found in the Oriental Region; it includes the three Afrotropical species comprising the Trifasciellus subgroup of Itoua & Cornet (1986). Only eight of the 19 species assigned by Wirth & Hubert (1989) to the Orientalis group are retained; four are moved to the Imicola group, while the remaining seven belong to unknown species groups. Nine species compose the Imicola group; seven are African and are found in abundance outside the equatorial forest block in the lower rainfall subtropical woodlands and savanna grasslands. The Orientalis and Imicola group definitions *sensu* Wirth & Hubert (1989) and Glick (1990) respectively, are here considered too broad. Only three characters in the Wirth & Hubert (1989) diagnosis indicate that they were dealing with species in the Orientalis group. These are (i) high antennal ratio (AR), (ii) sensilla coeloconica on III, XI–XV, and (iii) costa moderately long. These and remaining characters occur widely outside the two groups under review. Not one of the characters listed by Glick (1990) defines the Imicola group; his diagnosis is only appropriate for the subgenus *Avaritia*.

Under the dissecting microscope, adult females of the two groups are easily separated on the abdominal tergal pattern. However, its use is largely limited to nulliparous females, and furthermore, it is unknown whether these tergal patterns are also found in other species groups. Although the wing patterns offer some group-specific clues, they are too interspecifically variable to be used reliably. Three features deserve mention: (i) as a general rule, the pale spot found at the tip of the second radial cell in all species of the Orientalis group combines with those found at the base of cell M_1, and medially in cells M_2 and M_4, to form a broadly pale and straight line that bisects the wing longitudinally. In the Imicola group, the small, but prominent, pale spot in the base of cell M_1 is positioned more proximally and thus a subtle zigzag of pale areas bisects the wing. (ii) In the Orientalis group, the short, slanting vein that forms the proximal boundary of the second radial cell is always thickened where it meets the costa. This thickening does not occur in the Imicola group. (iii) Most species of the Orientalis group have the anal angle dark; in the Imicola group seven of the nine species have it pale. It seems probable that each of these characters will also find expression in other species groups of *Avaritia*, and so lose their diagnostic value. As for slide-mounted specimens, the two groups can be easily separated on nine character states and, to a lesser degree, on another nine. When these are assessed in combination they will ensure correct assignation of a species. While both sexes are not essential for grouping, the male does offer more distinguishing features. It is likely that some of the parameters given here also occur outside the Orientalis and Imicola groups.

Evidence is presented that the systematics of the Orientalis group is in disarray. A preliminary study indicates that at least 11 species of this group exist in south-east Asia, three more than the number currently recognized. The admission by Wirth & Hubert (1989) that 'due to poor preparation' of slide-mounted specimens certain characters were 'not determined' to a great extent sums up problems surrounding the systematics of world *Culicoides*. For example, the males of 28 % of the 168 south-east Asian species treated by Wirth & Hubert (1989) remain unknown. The use of insufficient numbers of specimens of both sexes, and the lack of attention to detail as regards their preparation, description and illustration, are hampering progress in a genus, and specifically the subgenus *Avaritia*, that can only be described as very challenging taxonomically.

Finally, the descriptive format used worldwide on the genus is considered too superficial and stylized. New and seldom-used characters are presented here, and discussed. Workers are urged to adopt them if our understanding of *Avaritia* is to grow and attain precision. Correct identifications are essential for studies on the phylogeny, biology and biogeography of each species; in turn, these form the foundation for more detailed studies on exactly which species are most competent at vectoring viral diseases such as bluetongue and African horsesickness.

11.5 REFERENCES

ATCHLEY, W.R. 1967. The *Culicoides* of New Mexico (Diptera: Ceratopogonidae). *The University of Kansas Science Bulletin*, 46:937–1022.

BLANTON, F.S. & WIRTH, W.W. 1979. The sand flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae). *Arthropods of Florida*, 10:1–204.

BOORMAN, J. 1989. *Culicoides* (Diptera: Ceratopogonidae) of the Arabian Peninsula with notes on their medical and veterinary importance. *Fauna of Saudi Arabia*, 10:160–224.

BOORMAN, J. 1991. A review of *Culicoides* subgenus *Avaritia* species (Insecta, Diptera, Ceratopogonidae) vectors of viruses of sheep, cattle and horses, with particular reference to *Culicoides imicola* in Europe and the Mediterranean region. Report for The Overseas Development Administration, The Natural History Museum, London pp 1–54.

BOORMAN, J. & DIPEOLU, O.O. 1979. A taxonomic study of adult Nigerian *Culicoides* Latreille (Diptera: Ceratopogonidae) species. *Occasional Publications of the entomological Society of Nigeria*, 22:1–121.

CAMPBELL, J.A. & PELHAM-CLINTON, B.A. 1960. A taxonomic review of the British species of "*Culicoides*" Latreille (Diptera, Ceratopogonidae). *Proceedings of the Royal Society of Edinburgh*, B, 67:181–302.

DAS GUPTA, S.K. 1962. Some *Culicoides* of Calcutta and the neighbouring areas. *Science and Culture*, 28:537–539.

DELFINADO, M.D. 1961. The Philippine biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae). *Fieldiana, Zoology*, 33:629–675.

DYCE, A.L. 1980. Redescription of *Culicoides orientalis* (Diptera: Ceratopogonidae). *Pacific Insects*, 21:286–292.

DYCE, A.L. 1983. Reviews of published records of *Culicoides* species in subgenus *Avaritia* (Diptera: Ceratopogonidae) from the New Guinea subregion. *International Journal of Entomology*, 25:276–280.

DYCE, A.L. & WIRTH, W.W. 1983. Reappraisal of some Indian *Culicoides* species in the subgenus *Avaritia* (Diptera: Ceratopogonidae). *International Journal of Entomology*, 25:221–225.

FOX, I. 1955. New advances in the study of the genus *Culicoides* (in Spanish). *Boletin Laboratorio de la Clinica Luis Razetti*, 43–44:635–639.

GLICK, J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology*, 27:85–195.

GLUKHOVA, V.M. 1989. Bloodsucking midges of the genera *Culicoides* and *Forcipomyia* (Ceratopogonidae). *Fauna SSSR*, 3:1–406. (In Russian).

HOWARTH, F.G. 1985. Biodsystematics of the *Culicoides* of Laos (Diptera: Ceratopogonidae). *International Journal of Entomology*, 27:1–96.

ITOUA, A. & CORNET, M. 1986. Les Ceratopogonidae (Diptera) du Mayombe Congolais. III. Revue taxonomique des espèces du genre *Culicoides* Latreille 1809. *Cahiers de l'office de la Recherche Scientifique et Technique Outre-Mer, Série Entomologie Medicale et Parasitologie*, 24:233–250.

JAMNBACK, H. & WIRTH, W.W. 1963. The species of *Culicoides* related to *obsoletus* in eastern North America. *Annals of the Entomological Society of America*, 56:185–198.

KHAMALA, C.P.M. & KETTLE, D.S. 1971. The *Culicoides* Latreille (Diptera: Ceratopogonidae) of East Africa. *Transactions of the Royal Entomological Society of London*, 123:1–95.

KITAOKA, S. 1975. Five new species of *Culicoides* (Diptera: Ceratopogonidae) of the Nansei Islands. *National Institute of Animal Health Quarterly*, 15:192–200.

KITAOKA, S. 1980. Six new species and males of some species of Japanese *Culicoides* (Diptera: Ceratopogonidae). *National Institute of Animal Health Quarterly*, 20:11–22.

KITAOKA, S. 1985. Japanese *Culicoides* (Diptera: Ceratopogonidae) and keys for the species. I. *Bulletin of the National Institute of Animal Health*, 87:73–89.

KITAOKA, S. & ZULU, F.P. 1990. Species composition of *Culicoides* (Diptera: Ceratopogonidae) found at Chilanga near Lusaka, Zambia. *Bulletin of the Niigata Sangyo University*, 4:197–206.

KREMER, M. 1965. Contribution a l'etude du genre *Culicoides* Latreille particulierement en France. *Encyclopedic Entomologique*, Serie A, 39:3–299.

LEE, T.S. 1978. Diptera. Biting midges. Chinese Economic Entomology, 13:1–124. Acad. Sinica Zool. Edit. Committee. Science Publishers, Peking (In Chinese).

McDONALD, J.L. & LU, L.C. 1972. Female *Culicoides* of Taiwan with descriptions of new species (Diptera, Ceratopogonidae). *Journal of Medical Entomology*, 9:396–417.

MEISWINKEL, R. 1987. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) kanagai* Khamala & Kettle, 1971, reared from elephant dung in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 54:585–590.

MEISWINKEL, R. 1989. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:23–39.

MEISWINKEL, R. 1991. Afrotropical *Culicoides*: *C. (Avaritia) miombo* sp. nov., a widespread species closely allied to *C. (A.) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 58:155–170.

MIRSAEVA, A.G. 1989. Bloodsucking midges (Diptera, Ceratopogonidae) of Siberia and the Far East. Sibirskoe Otdelenie Biologicheskii Institut. Novosibirsk Nauka. 230 pp (In Russian).

SEN, P. & DAS GUPTA, S.K. 1959. Studies on Indian *Culicoides* (Ceratopogonidae: Diptera). *Annals of the Entomological Society of America*, 52:617–630.

TOKUNAGA, M. 1951. Some Javanese Biting midges (Ceratopogonidae, Diptera). *Scientific reports of the Saikyo University*, 1:101–110.

TOKUNAGA, M. 1959. New Guinea biting midges (Diptera: Ceratopogonidae). *Pacific Insects*, 1:177–313.

TOKUNAGA, M. 1962. Biting midges of the genus *Culicoides* from New Guinea (Diptera: Ceratopogonidae). *Pacific Insects*, 4:457–516.

VARGAS, L. 1960. The subgenera of *Culicoides* of the Americas (Diptera: Ceratopogonidae). *Revista de Biologia Tropical*, 8:35–47.

VARGAS, L. 1962. Los subgeneros de *Culicoides* de la U.R.S.S. (Diptera: Heleidae). *Medicina Revista Mexicana*, 42:45–47.

VARGAS, L. 1973. Los subgeneros de *Culicoides* (Diptera: Ceratopogonidae). *Revista de Investigación en Salud Pública*, 32:116–129.

WADA, Y. & KITAOKA, S. 1977. Preliminary arrangement of Japanese *Culicoides* into groups (Diptera: Ceratopogonidae). *Tropical Medicine*, 19:169–176.

WIRTH, W.W. & BLANTON, F.S. 1959. Biting midges of the genus *Culicoides* from Panama (Diptera: Heleidae). *Proceedings of the United States National Museum*, 109:237–482.

WIRTH, W.W., DYCE, A.L. & PETERSON, B.V. 1985. An atlas of wing photographs, with a summary of the numerical characters of the Nearctic species of *Culicoides* (Diptera: Ceratopogonidae). *Contributions of the American Entomological Institute*, 22:1–46.

WIRTH, W.W., DYCE, A.L. & SPINELLI, G.R. 1988. An atlas of wing photographs, with a summary of the numerical characters of the Neotropical species of *Culicoides* (Diptera: Ceratopogonidae). *Contributions of the American Entomological Institute*, 25:1–72.

WIRTH, W.W. & HUBERT, A.A. 1989. The *Culicoides* of South-East Asia (Diptera: Ceratopogonidae). *Memoirs of the American Entomological Institute*, 44:1–508.

Table 11.1 Subgenus *Avaritia*: 18 male and female character states defining and separating the Orientalis and Imicola groups (most important features in **bold** type)

Character state	**Orientalis group**	**Imicola group**
♀: abdomen	dorsal pigmented plates on segments II–VII more or less equal in size and shape i.e. large and rectangular (Fig. 11.1a)	pigmented plates vary in size, those on segments III–V significantly smaller and roundish (Fig. 11.1c)
genitalia	sclerotized plates partly surrounding gonopore with finger-like projections both anteriad and posteriad of opening (Fig. 11.1b)	sclerotized plates simpler, lacking pronounced finger-like projections, and thus not partly embracing gonopore opening (Fig. 11.1d)
legs	first spine of hind tibial comb moderately darkly pigmented, and longer and more robust than second spine	first spine less robust and not as darkly pigmented
wing	costal ratio (CR) higher ranging between 0,56–0,67, average 0,63 (eight species)	CR lower; 0,54–0,61, average 0,57 (nine species)
antenna	sensilla coeloconica distributed on flagellar segments III, XI–XV	sensilla coeloconica III, XII–XV except for *C. pseudopallidipennis* and *C. tuttifrutti* (III, XI–XV)
antenna	antennal ratio (AR) higher: 1,07–1,47; all segments more slender with basal ones elongate barrel- or flask-shaped (Fig. 11.2a)	AR lower: 0,92–1,25; all segments stouter with basal ones most nearly spherical to barrel-shaped (Fig. 11.2b
antenna	blunt-tipped trichodea on segments III–X moderately long to long and always slender (Fig. 11.2a)	trichodea vary in length from short to moderately long and may be slender to rather swollen (Fig. 11.2b)
♂: paramere	apex sinuous usually limp and recurved with tip inconspicuously to obviously feathered (Fig. 11.3a)	apex always erect with tip sharp and simple (Fig. 11.3e)
aedeagus	anterior end of infuscate 'peg' somewhat V-shaped and smoothly pigmented (Figs. 11.3b, 11.4a–k)	infuscate 'peg' irregularly globular and pigmentation granular (Fig. 11.3f)
aedeagus	infuscate 'peg' joined to lateral converging margins of aedeagus by a roundly arched, window-like pigmented area (Figs. 11.3b, 11.4a–k)	this arched connection absent (Fig. 11.3f)
tergum nine	apicolateral processes broad and overtly triangular in shape with apex of process usually positioned mesally along posterior margin of tergum (Figs. 11.3d, 11.4a–k)	apicolateral processes narrower and arising on postero-lateral corners of tergum (Fig. 11.3h)
tergum nine	apicolateral processes narrowly separated by a clearly infuscate median cleft or ravine (Figs. 11.3d, 11.4a–k)	processes broadly separated by a gently concave posterior margin that is not infuscated (but may be striated) medially (Fig. 11.3h)
tergum nine	trapezoidal in shape gradually narrowing posteriorly (Fig. 11.3d)	square in shape noticeably waisted medially (Fig. 11.3h)

Character state	**Orientalis group**	**Imicola group**
basimere	basal half broader than apical half (Fig. 11.3c)	more parallel-sided with basal half fractionally broader (Fig. 11.3g)
basimere	ventral root arising at 45° and moderately bowed; dorsal root straight and not 'cupping' base of paramere (Fig. 11.3c)	ventral root most nearly arising at right-angles and noticeably bowed; dorsal root broader and curls around base of paramere (Fig. 11.3g)
sternum nine	membrane adorned with long or short spiculae, never bare (Fig. 11.4a–k)	membrane bare in most species, or adorned with short spicules only
antenna	all species (excepting *jacobsoni*) with one long trichodea on segment X and most with a short trichodea on XI and XII	all species without these long and short trichodea on segments X–XII
antenna	bearing a total of 17–31 long and short blunt-tipped trichodea on flagellar segments III–XII (Table 11.2)	with a lower total of 16–18 trichodea (Table 11.2)

Table 11.2 Subgenus *Avaritia*: presence/absencedistribution of long (L) and short (c) blunt-tipped trichodea on male flagellar segments III–XII of Old World species of the Orientalis and Imicola groups

Species group	Species	Flagellar segments										Total trichodea
		III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Orientalis:	*jacobsoni* (#A)	LL	LLc	LLc	LLc	(L)Lc	c	Lc	c	-	-	17-18
Southeast Asia	*dumdumi* (#B)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	-	20
	pungens (#C)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	c	21
	fulvus (#D)	LL	LLc	(L)Lc	(L)Lc	Lc	Lc	Lc	Lc	(c)	(c)	18-20
	maculatus? (#E)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	c	21
	orientalis? (#F)	LL	LLc	LLc	LLc	LLc	(L)Lc	(L)Lc	Lc	c	(c)	21-24
	? (#G)	LL	LLc	LLc	LLc	LLc	(L)Lc	(L)Lc	Lc	c	c	22-24
	? (#H)	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	c	24
	? (#I)	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	c	24
	? (#J)	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	c	24
	flavipunctatus? (#K)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	-	20
Africa	*brosseti*	LL	LLc	LLc	(L)Lc	Lc	Lc	Lc	Lc	c	c	20-21
	trifasciellus	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	(c)	23-24
	dubitatus	LLc	LLc	LLc(c)	LLc	LLcc	LLc	LLcc	Lc(c)	c(c)	(cc)	26-31
Imicola:	*brevitarsis*	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
Southeast Asia	*nudipalpis*	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
Africa	*bolitinos*	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	imicola	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	loxodontis	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	miombo	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	pseudopallidipennis	LL	LLc	(L)Lc	(L)Lc	Lc	Lc	Lc	c	-	-	16-18
	tuttifrutti	LL	LLc	(L)Lc	(L)Lc	Lc	Lc	Lc	c	-	-	16-18
	kwagga	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18

Table 11.3 Subgenus *Avaritia*: revised listing (alphabetical) of 21 *Culicoides* species constituting the Old World Orientalis and Imicola groups including author, original date and country of description, and synonyms (valid species in **bold** type, synonyms indented)

Orientalis group	Imicola group
Oriental/Palaearctic Regions:	Oriental/Palaearctic/Australasian Regions:
dumdumi Sen & Das Gupta, 1959: India	***brevitarsis*** Kieffer, 1917: Australia
flavipunctatus Kitaoka, 1975: Nansei Islands	*radicitus* Delfinado, 1961: Philippines
fulvus Sen & Das Gupta, 1959: India	*robertsi* Lee & Reye, 1953: Australia
hui Wirth & Hubert, 1961: Taiwan	*superfulvus* Das Gupta, 1962: India
jacobsoni Macfie, 1934: Sumatra	***nudipalpis*** Delfinado, 1961: Philippines
buckleyi Macfie, 1937: Malaysia	***imicola*** Kieffer, 1913: Kenya
kitaokai Tokunaga, 1955: Japan	*iraqensis* Khalaf, 1957: Iraq
unisetiferus Tokunaga, 1959: Papua New Guinea	*minutus* Sen & Das Gupta, 1959: India
maculatus (Shiraki), 1913: Taiwan	*pseudoturgidus* Das Gupta, 1962: India
kii Tokunaga, 1937: Japan	
kyotoensis Tokunaga, 1937: Japan	
sigaensis Tokunaga, 1937: Japan	
suborientalis Tokunaga, 1951: Java	
tainana Kieffer, 1916: Taiwan	
orientalis Macfie, 1932: Malaysia, India, Java (syntype series)	
nayabazari Das Gupta, 1963: India	
pungens de Meijere, 1909: Sumatra	
obscurus Tokunaga & Murachi, 1959: Caroline Islands	
Afrotropical Region:	Afrotropical Region:
brosseti Vattier & Adam, 1966: Gabon	***bolitinos*** Meiswinkel, 1989: South Africa
dubitatus Kremer, Rebholtz-Hirtzel & Delecolle, 1976: Angola	***imicola*** Kieffer, 1913: Kenya
trifasciellus Goetghebuer, 1935: Zaire	*pallidipennis* Carter, Ingram & Macfie, 1920: Ghana
	loxodontis Meiswinkel, 1992: South Africa
	miombo Meiswinkel, 1991: South Africa
	pseudopallidipennis Clastrier, 1958: Senegal
	tuttifrutti sp. nov
	kwagga sp. nov.

TABLE 11.4 Subgenus *Avaritia*: variation in size and presence of long (L) and short (C) blunt-tipped trichodea on male flagellar segments III-XII of south-east Asian Orientalis group species; unidentified specimens grouped under alphabet symbol

Specimen no.	**Species**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
Thailand 66	*jacobsoni* (# A)	L L	L L c	L L c	L L c	L c	c	L c	c	–	–
Thailand 145	"	L L L L	L L c L c	L L c L c	L L c L c	L L c L c	c c	L c L c	c c	– –	– –
Thailand 585	*jacobsoni* (# A)	L L L L	L½L c L¾L c	L½L c L¾L c	L½L c L¾L c	L c L½L c	c c	L c L c	c c	– –	– –
Philippines 25 Philippines 24	*dumdumi* (# B) *dumdumi* (# B)	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	L c L c	c c	c –
Thailand 27	*pungens* (# C)	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	c
Thailand 633	*fulvus* (# D)	L L L L	L L c L L c	L L c L c	L½L c L¼L c	L c L c	L c L c	L c L c	L c L c	– c	– c
Thailand 712	"	L L L L	L L c L L c	L L c L L c	L½L c L¼L c	L c L c	L c L c	L c L c	L c L c	c –	– –
Thailand 1032	"	L L L L	L L c L L c	L L c L¾L c	L c L c	L c L c	L c L c	L c L c	L c L c	– –	– –
Thailand 1054	"	L L L L	L L c L L c	L L c L L c	L c L½L c	L c L c	L c L c	L c L c	L c L c	– –	– –
Thailand 736	sp.# E (*maculatus*?)	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	c
Thailand 1058	"	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	c
Philippines 15	sp.# F (*orientalis* ?)	L L L L	L½L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L½L c L c	L c L c	L c L c	c c	– –
Thailand 55	"	L L L L	L¾L c L¾L c	L½L c L½L c	L½L c L½L c	L½L c L½L c	L½L c L½L c	L c L¼L c	L c L c	c c	c c
Thailand 227	"	L L L L	L¾L c L½L c	L½L c L½L c	L½L c L½L c	L½L c L½L c	L c L½L c	L c L c	L c L c	c c	c c

Thailand 676	"	L L L L	L¾L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L½L c L½L c	L½L c L c	L c L c	c c	c c
Thailand 353	sp.# G	L L L L	L L c L L c	L L c L L c	L L c L¾L c	L L c L¼L c	L c L¼L c	L c L¼L c	L c L c	c c	c c
Thailand 354	"	L L L L	L L c L L c	L L c L L c	L L c L¾L c	L¾L c L¼L c	L½L c L½L c	L c L c	L c L c	c c	c –
Thailand 355	"	L L L L	L½L c L¾L c	L½L c L¾L c	L¼L c L½L c	L½L c L½L c	L c L c	L c L c	L c L c	c c	c c
Thailand 737	sp.# H	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 738	"	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 815	sp.# I	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 816	"	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 1055	sp.# J	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 228	sp.# K	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	–

Table 11.5 Subgenus *Avaritia*: presence/absence and modalities of 10 character states examined in 25 male specimens of 11 south-east Asian species of the Orientalis group. Arrows indicate tendencies and fractions a reduction in size

Character state	Specimen number and species																								
	T66	T155	T585	P24	P25	T27	T633	T712	T1032	T1054	T736	T1058	P15	T55	T227	T676	T353	T354	T355	T737	T738	T815	T816	T1055	T228
palps long	*	*	*								↑		*	*	*	*	*	*	*						
palps mod. long				*	*		*	*	*	*	*	*								*	*	*	*	*	
palps mod. short						*						↓													*
wing: anal angle dark	*	*	*	*	*	*							*	*	*	*	*	*	*						
anal angle pale							*	*	*	*	*	*								*	*	*	*	*	*
genitalia: parameres feathered	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*		*
parameres simple																				*	*			*	
sternital spicules in 2 groups	*	*	*										*	*	*	*	*	*	*						
spicules continuous				*	*	*	*	*	*	*	*	*		↓						*	*	*	*	*	*
spicules sparse/mod. abundant				*	*	*	*	*	*	*	*	*								*	*	*	*		
spicules abundant/very abundant	*	*	*										*	*	*	*	*	*	*					*	*
spicules short/mod. short	*	*	*										*	*	*	*	*	*	*					*	
spicules mod. long						*	*	*	*	*	*	*										*	*	↓	*
spicules long/very long				*	*															*	*				
distimeres slender	*	*	*	*	*	*	*	*	*	*	*	*								*	*	*	*	*	*
distimeres swollen											↓	↓	*	*	*	*	*	*	*	↓	↓				
apicolateral bristles present			* ↓	*							*	*	*	*		*	* ↓			*	*			*	*

apicolateral bristles absent	*	*			*	*	*	*	*	*					*			*	*			*	*		
apices hind tibiae pale	*	*	*				*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	
apices hind tibiae dark				*	*	*					↓	*													*
antenna: L trichodea short				↑	↑		*	*	*	*															*
L trich. mod. long	*	*	*	*	*	*					*	*					*	*	*	*	*			*	
L trich. very long			↓										*	*	*	*						*	*		
2 L trich. on VII	*		*										*	*	*	*	*	*	*	*	*	*	*	*	
1 L trich. on VII		*	↓	*	*	*	*	*	*	*	*	*													*
2 L trich. on IX																				*	*	*	*	*	
1 or other trich. ¾, ½ or ¼ developed														¼ *		½ *	¼ *								
1 L absent on IX	*	*	*	*	*	*	*	*	*	*	*	*	*	↓	*			*	*						*
	A	A	A	B	B	C	D	D	D	D	E	E	F	F	F	F	G	G	G	H	H	I	I	J	K
	jacobsoni			*dumdumi*		*pun-gens*	*fulvus*				*maculatus* ?		*orientalis* ?				?			?		?		?	?

Table 11.6 Subgenus *Avaritia*: the female antennal ratio (AR) of 17 species of the Orientalis and Imicola groups according to various authors and this study

Author	Orientalis group	Imicola group	Range in AR	Average value	Deviation	n
Arnaud 1956	*jacobsoni* (as *kitaokai*)	-	-	1,15	-	?
Tokunaga 1959	" (as *unisetiferus*)	-	-	1,14	-	?
Tokunaga 1959, 1962	" (as *buckleyi*)	-	0,95-1,17	-	22	?
McDonald & Lu 1972	"	-	-	1,21	-	?
Lee 1978	"	-	-	1,15	-	-
Kitaoka 1985	"	-	-	1,16	-	-
Wirth & Hubert 1989	"	-	1,13-1,28	1,21	15	10
this study	" (as sp. #A)	-	-	1,10	-	1
Kitaoka 1985	*dumdumi*	-	-	1,26	-	?
Wirth & Hubert 1989	"	-	1,31-1,39	1,35	8	10
this study	" (as sp. #B)	-	-	1,25	-	1
Wirth & Hubert 1989	*pungens*	-	1,27-1,47	1,37	20	10
this study	" (as sp. #C)	-	-	1,28	-	1
Wirth & Hubert 1989	*fulvus*	-	1,19-1,26	1,23	7	7
this study	" (as sp. #D)	-	-	1,09	0	2
Tokunaga 1959, 1962	*orientalis*	-	1,04-1,24	1,08	20	?
Lee 1978	"	-	-	1,15	-	?
Dyce 1980	"	-	-	1,14	-	?
Wirth & Hubert 1989	"	-	-	1,13	-	?
this study	" (as sp. #F)	-	-	1,12	0	2
Arnaud 1956	*maculatus* (as *sigaensis*)	-	-	1,09	-	1
McDonald & Lu 1972	"	-	-	1,20	-	?
Lee 1978	"	-	-	1,20	-	?
Kitaoka 1985	"	-	-	1,04	-	?
Wirth & Hubert 1989	"	-	1,07-1,14	1,10	7	2
Kitaoka 1975	*flavipunctatus*	-	1,25-1,37	1,32	12	12
Kitaoka 1985	"	-	-	1,22	-	?
Wirth & Hubert 1989	"	-	-	1,30	-	?
this study	(as sp. #K)	-	1,19-1,26	1,23	7	5
McDonald & Lu 1972	*hui*	-	-	1,17	-	?
Lee 1978	"	-	-	1,20	-	?
Kitaoka 1985	"	-	-	1,12	-	?
Wirth & Hubert 1989	"	-	1,12-1,13	1,12	1	2
this study	"	-	1,00-1,07	1,04	7	4
Delfinado 1961	-	*brevitarsis* (as *radicitus*)	-	1,10	-	?

Kitaoka 1985	-	"	-	1,14	-	?
Wirth & Hubert 1989	-	"	1,17-1,25	1,22	8	5
this study	-	"	1,06-1,18	1,12	12	27
Delfinado 1961	-	*nudipalpis*	1,09-1,18	1,14	9	4
Wirth & Hubert 1989	-	"	-	1,20	-	?
this study	-	"	1,06-1,12	1,07	6	5
Wirth & Hubert 1989	-	*imicola*	1,17-1,19	1,18	2	2
Boorman & Dipeolu 1979	-	"	1,01-1,22	1,10	21	20
Glick 1990	-	"	0,95-1,13	1,05	18	15
Boorman 1989	-	"	1,08-1,19	1,14	11	?
Khamala & Kettle 1971	-	" (as *pallidipennis*)	-	1,17	0	1
Meiswinkel 1989	-	"	0,95-1,10	1,01	15	167
Meiswinkel 1989	-	*bolitinos*	0,96-1,12	1,04	16	54
Meiswinkel 1991	-	*miombo*	1,09-1,25	1,15	16	66
Meiswinkel 1992	-	*loxodontis*	0,92-1,08	0,99	16	80
this study	-	*pseudopallidipennis*	1,13-1,24	1,19	11	45
"	-	*tuttifrutti*	0,98-1,13	1,06	15	104
"	-	*kwagga*	0,96-1,12	1,06	16	31

CHAPTER 12

Subgenus *Avaritia* (*Culicoides*; Ceratopogonidae): key to nine world species of the Imicola group

12.1 INTRODUCTION

The purpose of a key is to unlock knowledge simply. The best keys utilize characters that unambiguously distinguish one species from the next; these may be distilled from intensive study of many hundreds of specimens. Specialist students usually spend a lifetime assembling these data, but the current trend to fragment research into short-term projects does not favour long taxonomic 'journeys'. As regards the Imicola group, the journey is not complete — I am aware of three undescribed species known only from a few individuals or a single sex.

Despite being small in size (0,6–2,0 mm), *Culicoides* can exhibit some notable differences in coloration, especially in the wing pattern. However, it is inevitable that other species will be more similar to one another, and the danger now exists that small differences will be labelled as intraspecific variation. This is a problem that lies at the heart of the identification of all living biota, and likely is the reason why, on average, only one new species of the Imicola group is described every 12 years.

An important point to be made is that in the Afrotropical Region as many as six species of the Imicola group can be found in a single light-trap collection, and may occur in sympatry with 30 other species of *Culicoides*. However, such a high number of Imicola group species is the exception rather than the rule, as species diversity in any area is dependent upon the heterogeneity of hosts and larval habitats available; climate, soil and altitude further induce or reduce species turnover. Seldom are two or more species of the Imicola group equally represented in a complex community; this variance in abundance and prevalence patterns further complicates identification of individual specimens.

In the knowledge that the true size of the Imicola group remains unknown, the strategy adopted in its study was to slide-mount phenotypically heterogeneous collections (especially if a new locality was sampled), and to then score each individual carefully. Over time, these population data were coupled to those obtained from reared series, the most diagnostic features extracted, and the key built.

It may be argued that some couplets of the key are overly long, but it is beyond question that distinct species pairs or subgroups constitute the Imicola group; this implies recent divergence in evolutionary terms, which may or may not be well expressed in the phenotype. Careful scrutiny is thus essential. Therefore, if a couplet demands agreement on four or more characters, the specimen must comply fully before an identification can be accepted as correct. As all species of the Imicola group are very similar, it is recommended that more than one specimen of each sex in any given population be examined.

A major shortcoming of the key must be mentioned: it does not inform as to whether a particular specimen belongs to the Imicola group. As indicated in Chapter 1, at least 33 species (20 undescribed) of *Avaritia* are to be found in Africa; another 50 known species occur elsewhere. All fall into 10 or more groups. Until these groups are defined, it will remain problematical to use a key as precisely aimed as the one given below for the Imicola group which deals with only 12 % of the known species of world *Avaritia*.

Finally, a prominent non-taxonomic feature of the Imicola group is that each species has a specific life-cycle which, if known, is a most useful aid to identification. However, biological features are not utilized in the key, as the capture of free-flying individuals in light-traps, the principal method used for surveying *Culicoides* worldwide, provides no data on their biology.

12.2 Key to slide-mounted males and females of nine world species of the Imicola group

1. Male 2
Female 8

2. Anal angle of wing with dark smudge 3
Anal angle pale 4

3. Scutum with two prominent yellow vittae; halteres dark; sternum nine
densely spiculate; apex of aedeagus smoothly rounded . . . . ***miombo***
Scutum entirely brown; halteres pale; sternum nine bare or sparsely
spiculate; apex of aedeagus minutely papillate or sclerotized . . . . ***kwagga***

4. Membrane of sternum nine moderately to densely spiculate
(Africa, S. Europe and mainland Asia) . . . . ***imicola***
(s.e. Asian islands e. of Wallace line) . . . . ***nudipalpis***
Membrane bare or with < 20 spicules 5

5. Scutellum with two median bristles; terminal projection of aedeagus
rather short, apex minutely papillate . . . . ***loxodontis***
Scutellum with one median bristle; terminal projection of aedeagus
moderately long, apex smoothly rounded 6

6. Scutum with > 80 bristles; proximal margin of distal pale spot in
wing cell R_5 markedly pointed . . . . ***tuttifrutti***
Scutum with < 80 bristles; pale spot in R_5 gently rounded or
straightish 7

7. One of the two long (L) blunt-tipped sensilla trichodea on flagellar
segments V and/or VI reduced in size or absent; third palpal segment
moderately inflated, longer than wide . . . . ***pseudopallidipennis***
Both long (L) trichodea present and equal in size; third palpal
segment smaller and roundish (Afrotropical Region) . . . . ***bolitinos***
(Oriental/Australasian Regions) . . . . ***brevitarsis***

8. Third palpal segment swollen; sensilla coeloconica on flagellar segments III, XI–XV; long blunt-tipped sensilla trichodea inflated 9

Third palpal segment short and roundish, or moderately long and slender, coeloconica on segments III, XII–XV; blunt-tipped trichodea slender 10

9. Antennal ratio (AR) 0,98–1,13, average 1,06; Afrotropical woodlands and tree savannas . . . . ***tuttifrutti***

AR 1,13–1,24, average 1,19; equatorial regions . . . . ***pseudopallidipennis***

10. Anal angle of wing with obvious, but variable-sized, dark smudge 11

Anal angle pale 12

11. Scutum brown with two yellow admedian vittae; haltere knobs brown; eyes sparsely pubescent; wing darker . . . . ***miombo***

Scutum entirely brown; halteres pale; eyes bare; wing paler . . . . ***kwagga***

12. Scutellum with two median bristles; flagellar segments VI and VIII with four sensilla chaetica . . . . ***loxodontis***

Scutellum with one median bristle; segments VI and VIII with three chaetica 13

13. Wing vein M_2 with a broad, well-defined preapical excision; third palpal segment moderately long and slender; proximal margin of distal pale spot in R_5 moderately to noticeably pointed 14

Vein M_2 without preapical excision; third palpal segment shortish, almost round; proximal margin of distal spot in R_5 straight or gently rounded 15

14. Proboscis/head (P/H) ratio 0,66–0,73; restricted to sout-east Asian islands east of the Wallace line . . . . ***nudipalpis***

P/H ratio higher (0,82–1,02); restricted to Africa, southern Europe, and mainland Asia . . . . ***imicola***

15. Antennal segments VI–IX shorter, approximately 1,4 x longer than wide; restricted to Oriental/eastern Palaearctic and Australasian Regions . . . . ***brevitarsis***

Antennal segments VI–IX longer, ranging between 1,4–1,7 x longer than wide; restricted to Afrotropical Region . . . . ***bolitinos***