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;

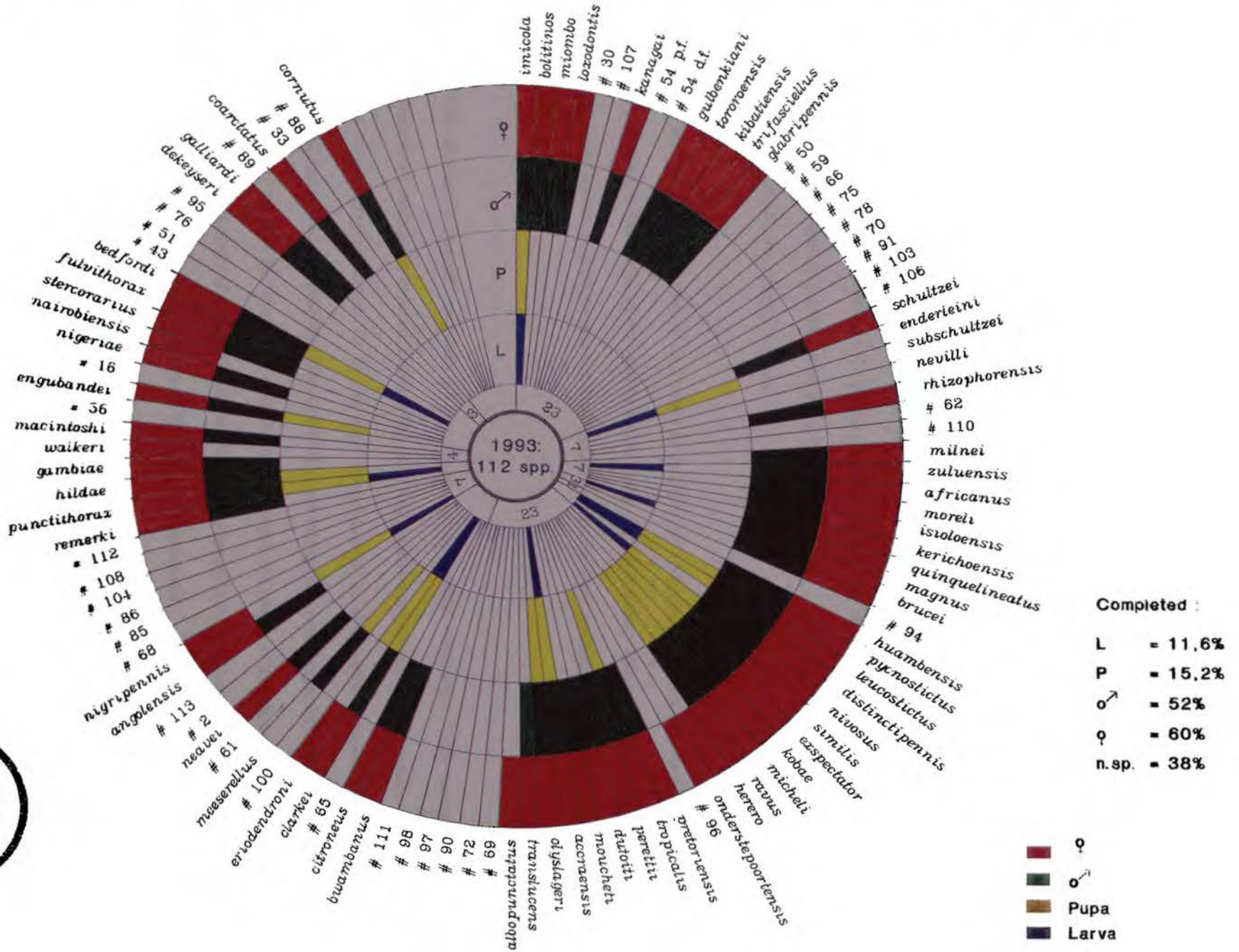
(ii) that the Imicola group, in the strict sense, is clearly separate from the Orientalis group, and that both groups are represented in Africa;

(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



Fiedler 1951:
22 species

Completed :

L	= 11,6%
P	= 15,2%
♂	= 52%
♀	= 60%
n.sp.	= 38%

♀
♂
Pupa
Larva

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

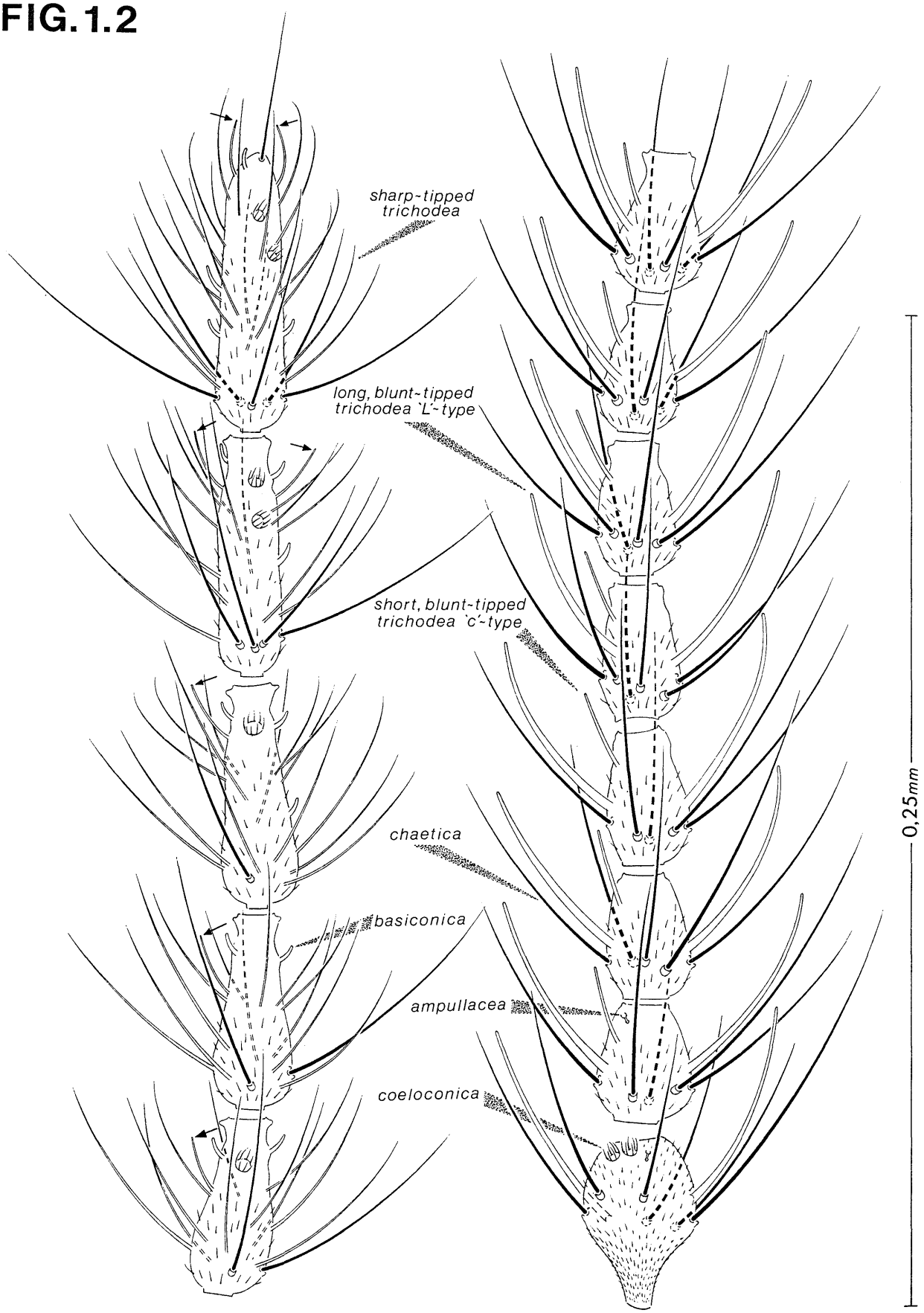


Fig. 1.2 *Culicoides*. Antenna, female: seven types of sensilla; arrows indicate short, blunt-tipped trichodea of the 'L'-type. Flagellar segments XI–XV on left, III–X on right

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.

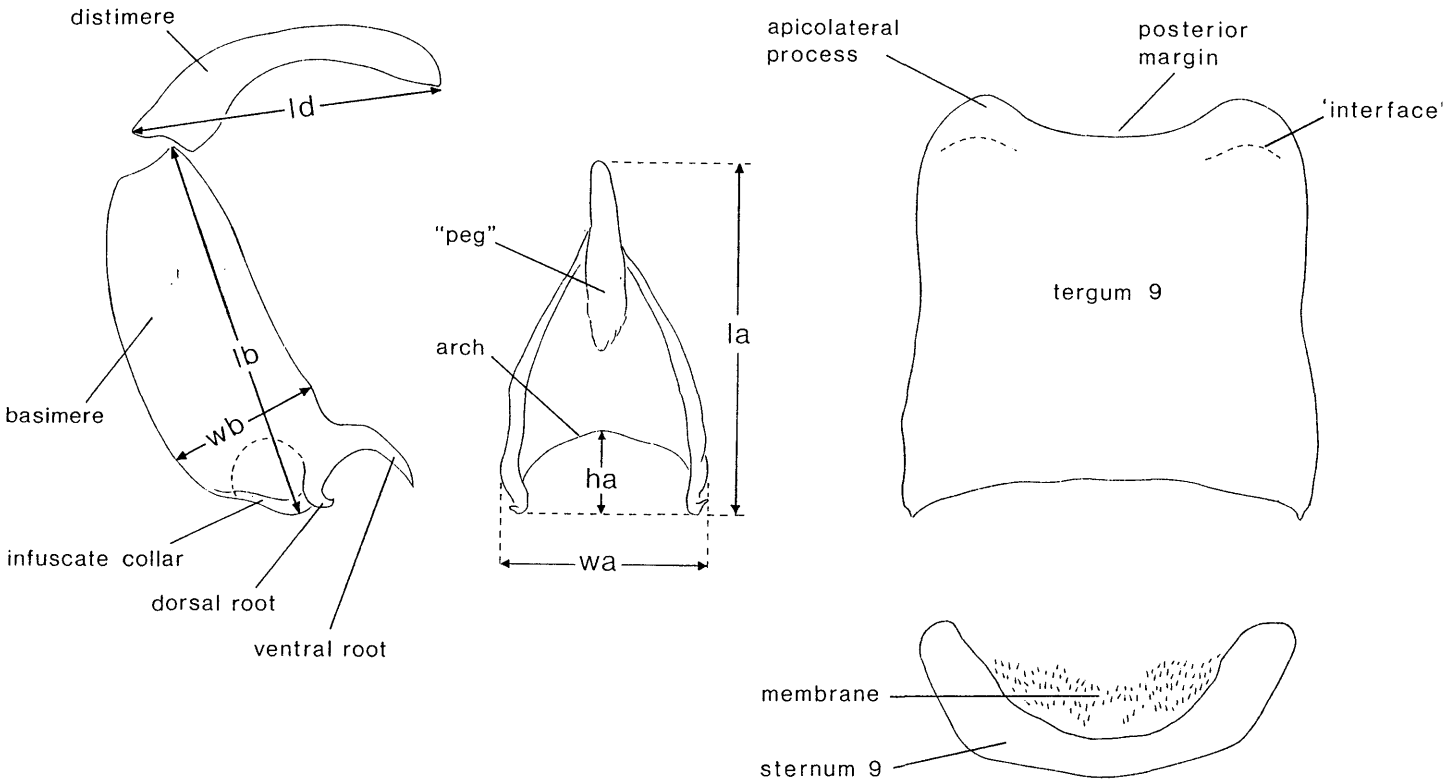


Fig.1.3

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.

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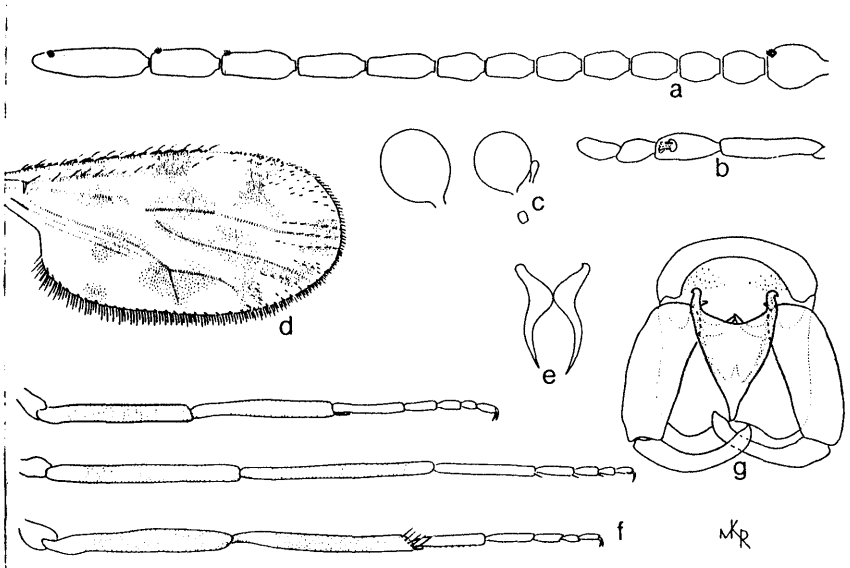


Fig. 119. *Culicoides imicola*: a. antenna; b. palpus; c. spermathecae; d. wing; e. parameres; f. legs; g. male genitalia, parameres omitted.

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

Species: C. sp. # 30

1.1

Specimen number:	Number of sensilla coeloconica on ♀ antennal flagellar segments: III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	Total number
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 99	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:	348	0	0	0	0	0	0	4	101	104	104	104	106	871
(n = 104)	3,35	0	0	0	0	0	0	0,04	0,97	1,00	1,00	1,00	1,02	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: III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	Total number
Renosterkoppies 83	5	3	2	4	2	4	2	4	–	–	–	–	–	26
	5	4	2	4	2	4	2	3	–	–	–	–	–	26
Renosterkoppies 89	5	4	2	4	2	4	2	3	–	–	–	–	–	26
	5	4	2	4	2	4	2	3	–	–	–	–	–	26
Renosterkoppies 90	5	3	2	4	2	4	2	3	–	–	–	–	–	25
	5	3	2	4	2	4	2	3	–	–	–	–	–	25
Renosterkoppies 91	4	3	2	4	2	4	2	3	–	–	–	–	–	24
	4	3	2	4	2	4	2	3	–	–	–	–	–	24
Renosterkoppies 92	5	3	2	4	2	4	2	3	–	–	–	–	–	25
	5	4	2	4	3	4	2	3	–	–	–	–	–	27
Renosterkoppies 93	5	4	2	4	2	3	2	3	–	–	–	–	–	25
	5	3	2	4	2	4	2	3	–	–	–	–	–	25
Renosterkoppies 94	5	3	2	4	2	4	2	3	–	–	–	–	–	25
	5	3	2	3	2	4	2	3	–	–	–	–	–	24
Renosterkoppies 95	5	3	2	3	2	4	2	3	–	–	–	–	–	24
	5	3	2	4	2	4	2	3	–	–	–	–	–	25
Renosterkoppies 96	4	4	2	4	2	4	2	3	–	–	–	–	–	25
	4	3	2	4	2	4	2	3	–	–	–	–	–	24
Renosterkoppies 97	5	3	2	4	2	5	2	3	–	–	–	–	–	26
	5	3	2	3	2	4	2	3	–	–	–	–	–	24
Renosterkoppies 98	5	3	2	4	2	3	2	3	–	–	–	–	–	24
	5	3	2	3	2	4	2	3	–	–	–	–	–	24
Renosterkoppies 100	5	3	2	4	2	4	2	3	–	–	–	–	–	25
	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

AR

	lengths of ♀ antennal flagellar segments III – XV :																
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 83	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
	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 89	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
	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 90	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
*	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 91	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
	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 92	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
	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 93	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
	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 94	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
	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 95	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
	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 96	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
	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 97	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
	16,25	10,5	10,25	11,25	11,5	11,5	11,5	12	–	18	18	18,5	18	30	–	206	20,9/20,3 = 1,03
Rnstrkpps → 98	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
	15,75	11	10,5	11,5	11,75	11,75	11,5	13	–	17,5	18	19,75	19,75	32	–	216,5	21,7/20,4 = 1,06
Rnstrkpps 100	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
	15	10	10,25	11	12	11,5	11	12	–	17,5	17	17,75	18	29,75	–	200	20,4/19,6 = 1,04
subtotal (n=24)	357,25	243,15	238,5	259,25	269,75	268,5	267,25	293,75	–	415,25	416,0	431,0	432,0	695,25		4751,5	25,14
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 (n=104)	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
	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
sum	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.

Species: C. tuttifrutti

♀ palp :

Specimen number :	length of palpal segments: I	II	III	IV	V	Total length	No. of sensilla chaetica/segment: I	II	III	IV	V	Total	PR: 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
Rnstrkppies 93	7	15	21,5	10	8,5	61	1	3	4	2	5	15	21,5 / 10	2,15
	←	— missing			——	>	←			—		>	/	/
Rnstrkppies 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	(n = 23) 23	13				428	248,0	49,67 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	rudimentary
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	26,3 + 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 = 21-33)	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			
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	AR: XIII–XV / III–XII	AR
White River 14 (Dodds)	lengths	29,5	←				138				→	167,5	37	30	36,5	103,5	271	103,5/167,5	0,62
		29,5	←				137,5				→	167	37,5	28,5	36,5	102,5	269,5	102,5/167	0,61
	coeloconica	2											1	1	2				
		2											1	1	2				
	chaetica	5											3	2	–				
		5											3	2	–				
	trichodea	LL	LLc	Lc	Lc	Lc	Lc	Lc	c	–	–								
		LL	LLc	L¼Lc	L¼Lc	Lc	Lc	Lc	c	–	–								
White River 18 (Dodds)	lengths	27,5	←				126				→	153,5	35	28	34,5	97,5	251	97,5/153,5	0,64
		27,5	←				126,5				→	154	36	27,5	33,5	97	251	97/154	0,63
	coeloconica	2											1	1	2				
		2											1	1	2				
	chaetica	5											3	2	0				
		5											3	2	0				
	trichodea	LL	Lc	L¾Lc	Lc	Lc	Lc	Lc	c	–	–								
		LL	LLc	L½Lc	Lc	Lc	Lc	Lc	c	–	–								
White River 19 (Dodds)	lengths	30	←				138				→	168	37	-29	37	103	271	103/168	0,61
		31,5	←				138				→	169,5	36,5	29,5	37,5	103,5	273	103,5/169,5	0,61
	coeloconica	2											1	1	2				
		2											1	2	2				
	chaetica	5											3	2	0				
		5											3	2	0				
	trichodea	LL	LLc	LLc	Lc	Lc	Lc	Lc	c	–	–								
		LL	LLc	L¾Lc	L⅛Lc	Lc	Lc	Lc	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.

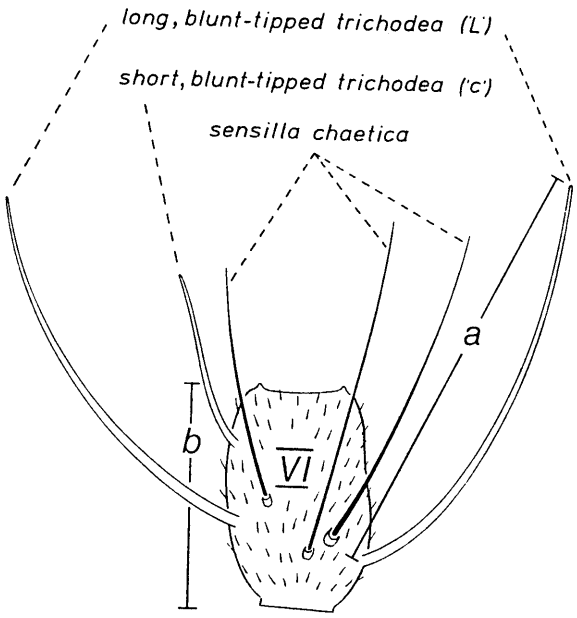
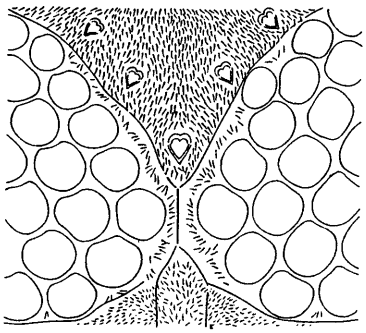
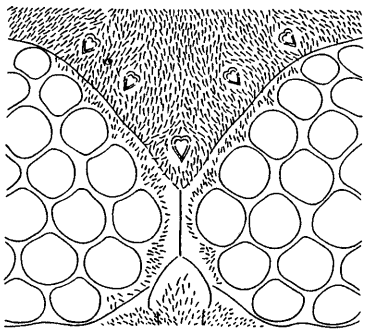


FIG. 2.1



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

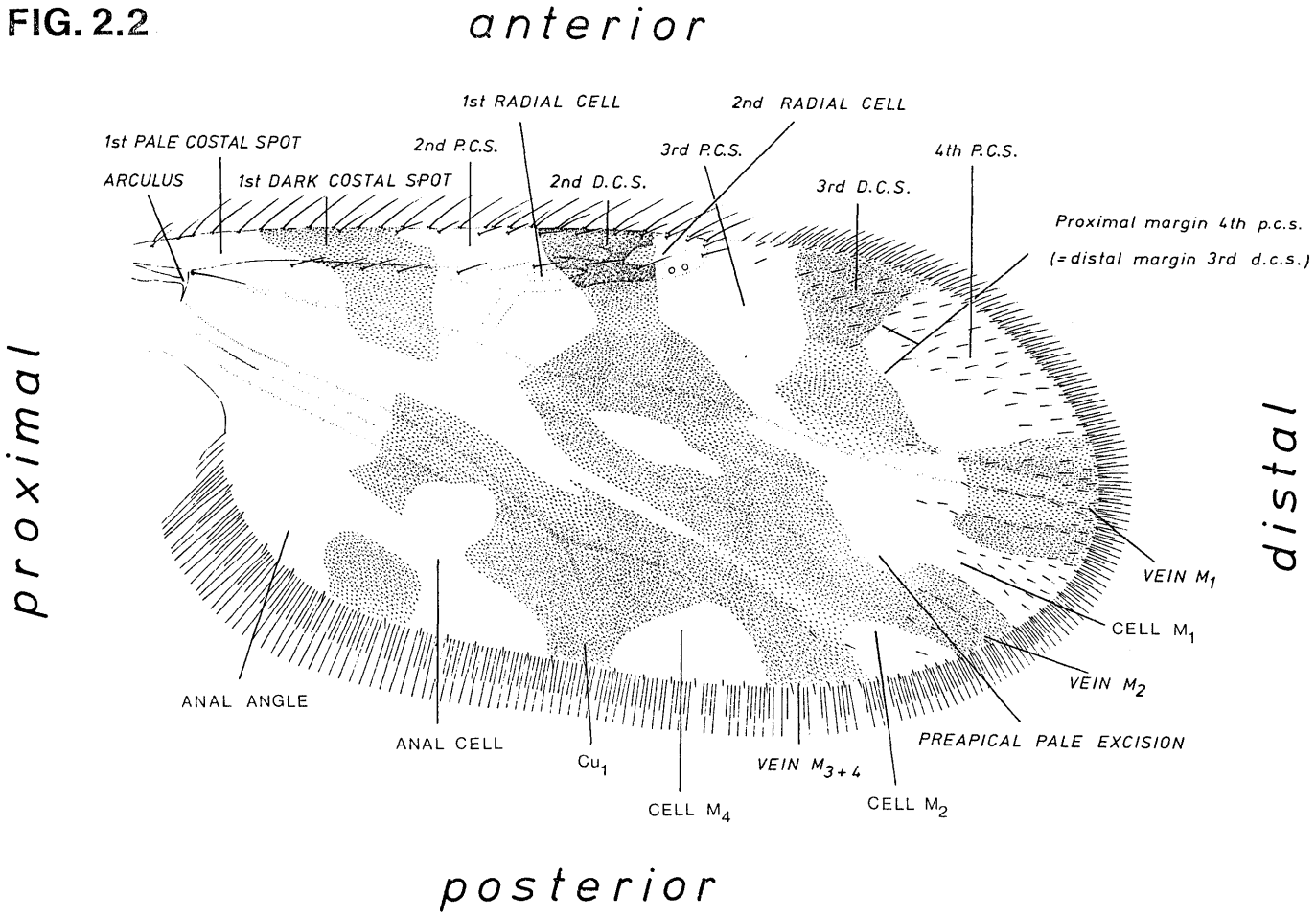


Fig. 2.2 *C. (Avaritia) imicola.* Wing, female: indicating important diagnostic characters (p.c.s. = pale costal spot; d.c.s. = dark costal spot).

Fig. 2.5

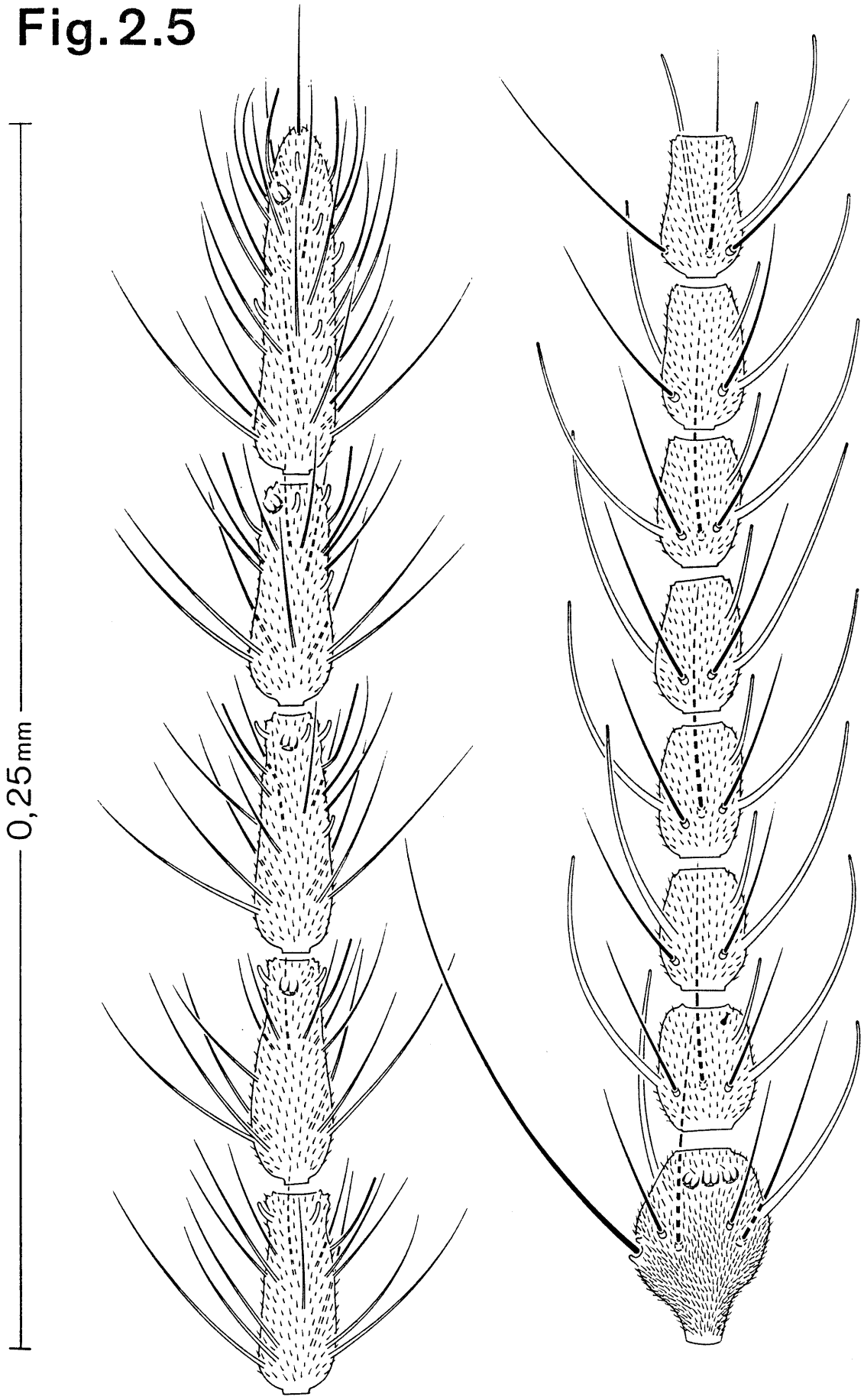


FIG. 2.5 *C. (Avaritia) imicola.* Antenna, female: segments XI–XV on left, segments III–X on right.

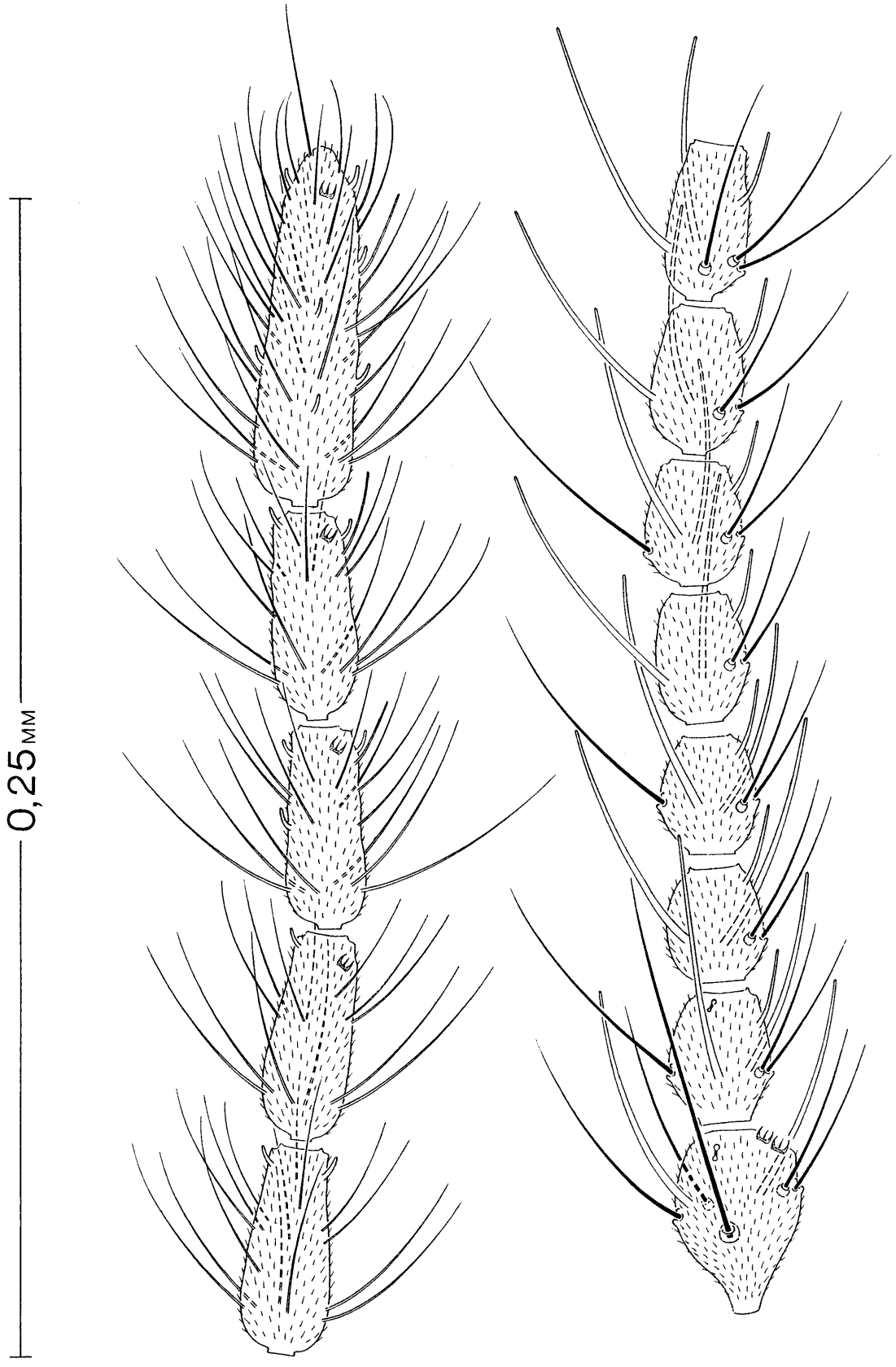


FIG. 2.6

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

Fig. 2.7

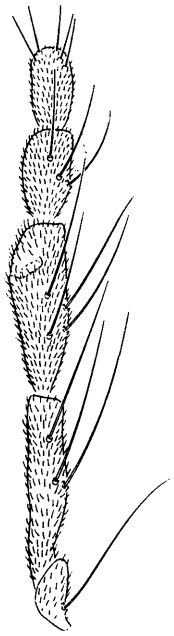
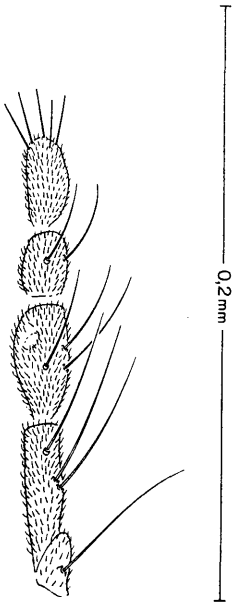


Fig. 2.8



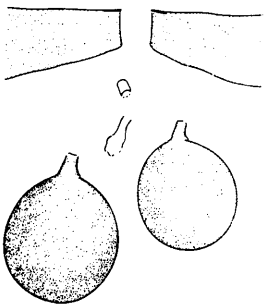


Fig. 2.13

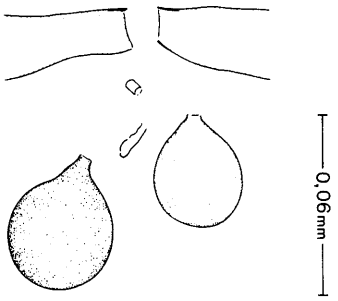


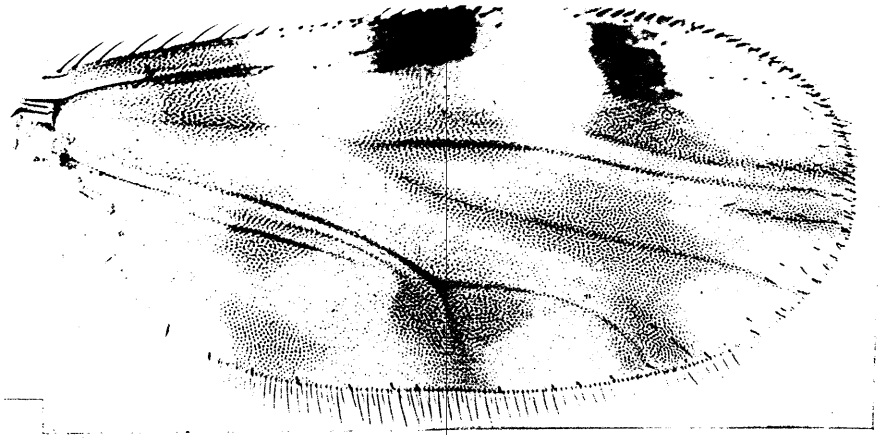
Fig. 2.14

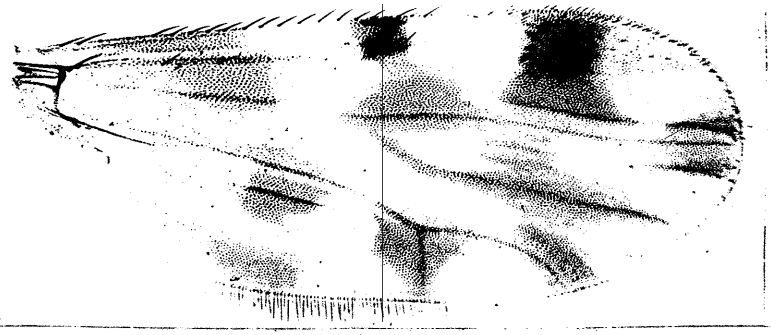
FIG. 2.7 *C. (Avaritia) imicola.* Palp, female.
FIG. 2.8 *C. (Avaritia) bolitinos*. Palp, female.

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*

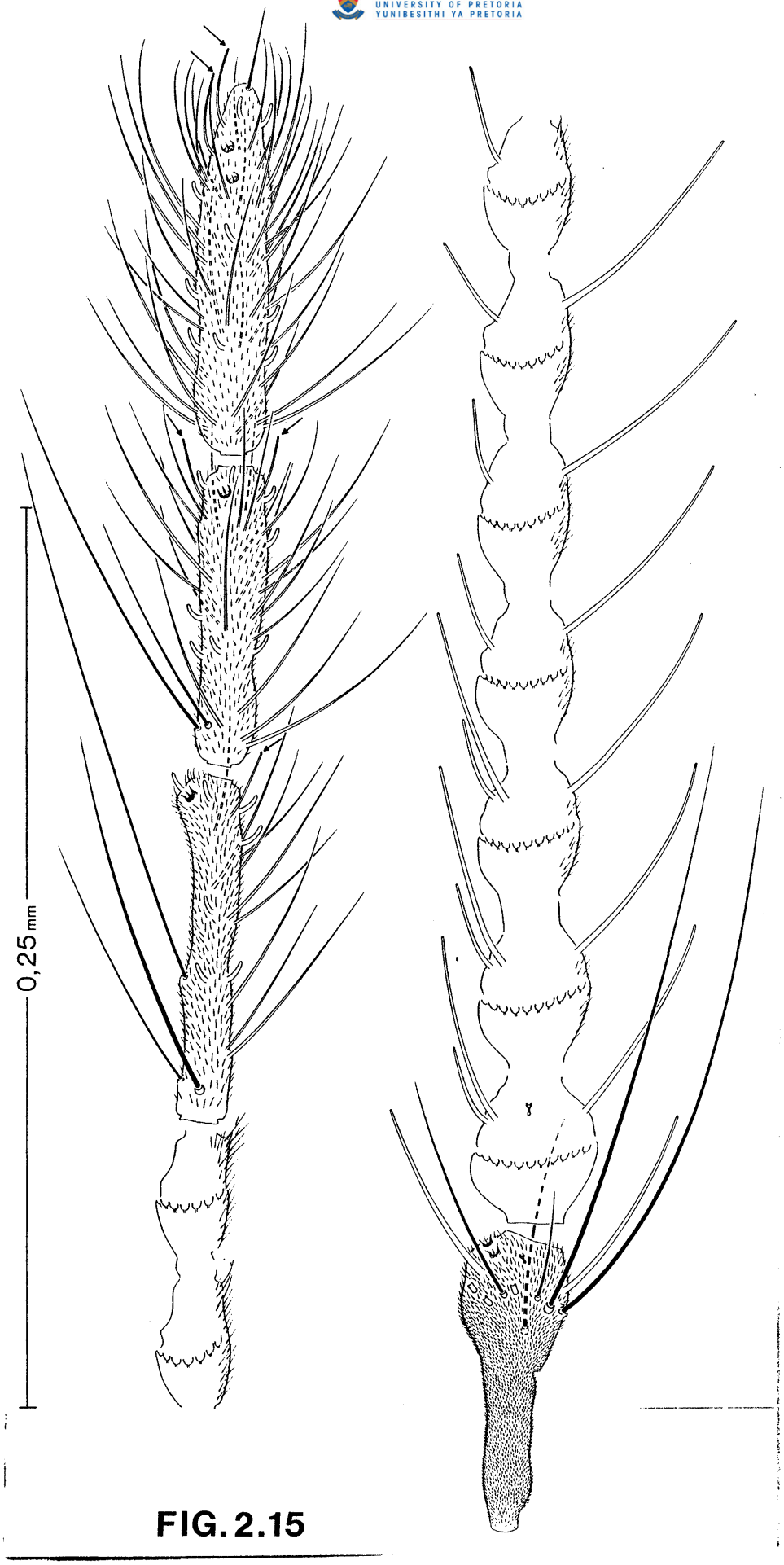


FIG. 2.15

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

Fig. 2.16

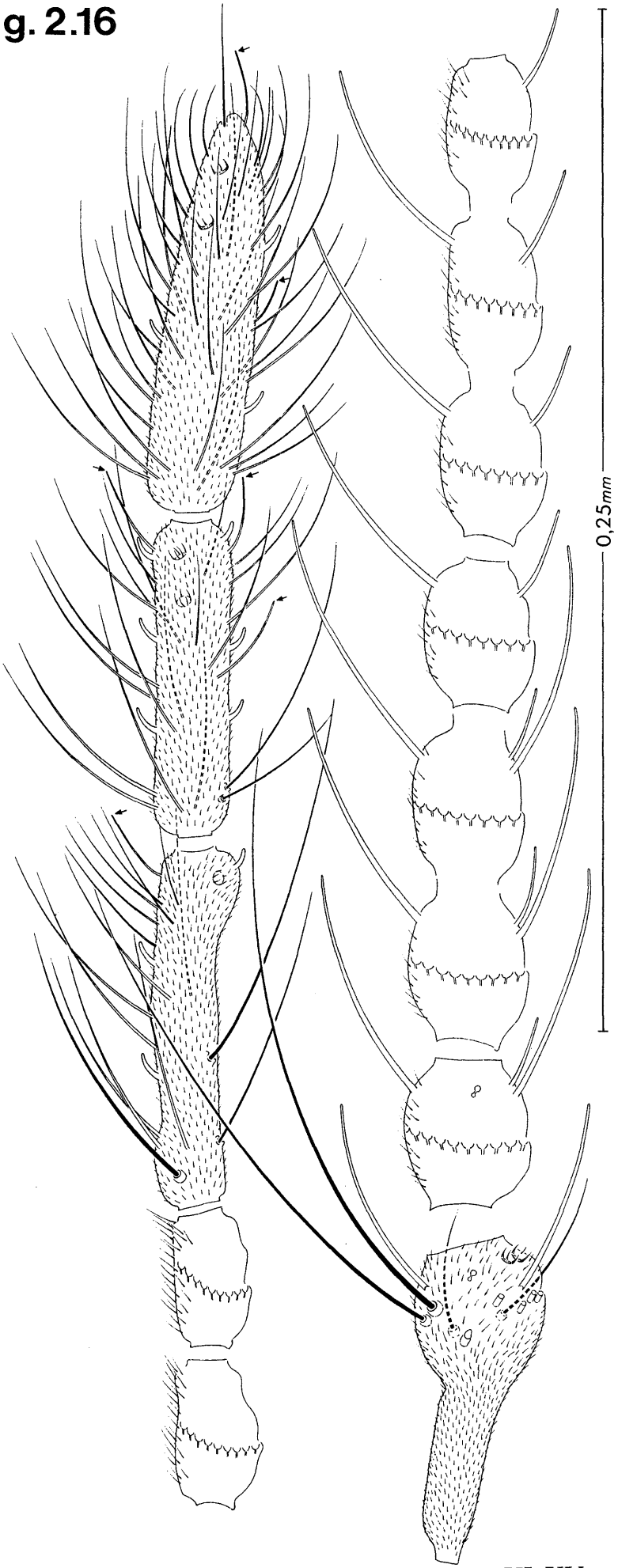


FIG. 2.16 *C. (Avaritia) bolitinos*. Antenna, male: segments XI–XV on left, segments III–X on right.

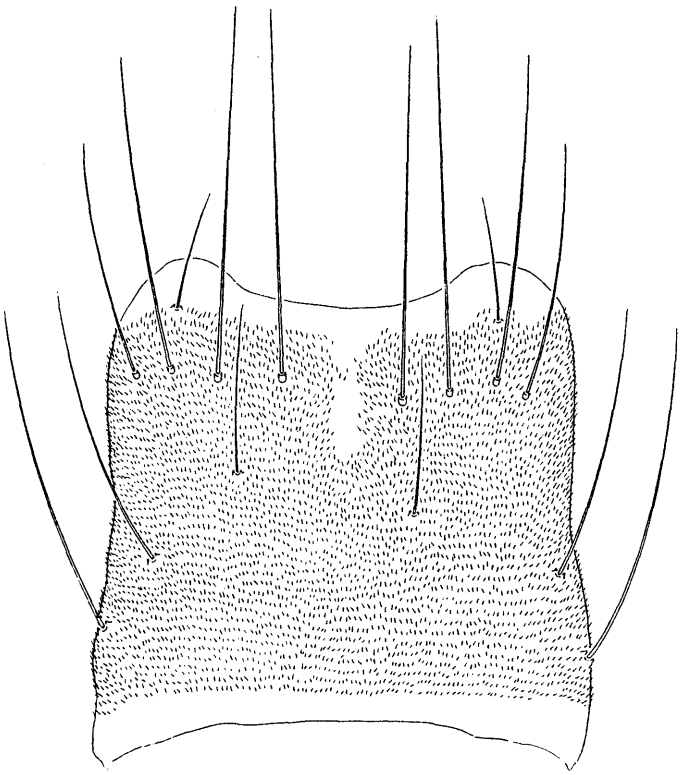


Fig. 2.17

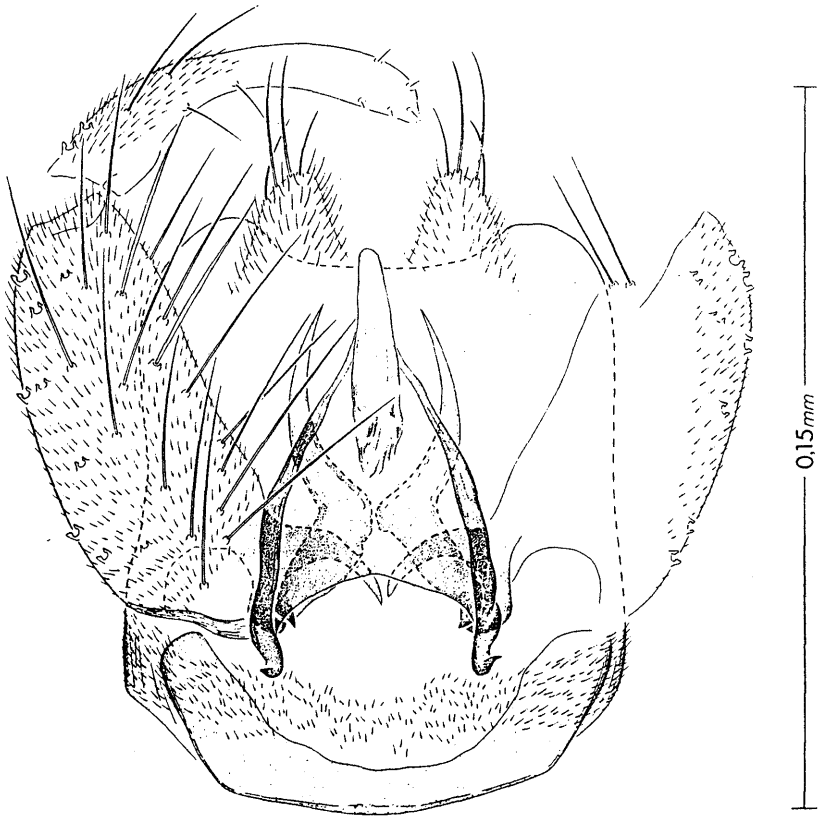


Fig. 2.19

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

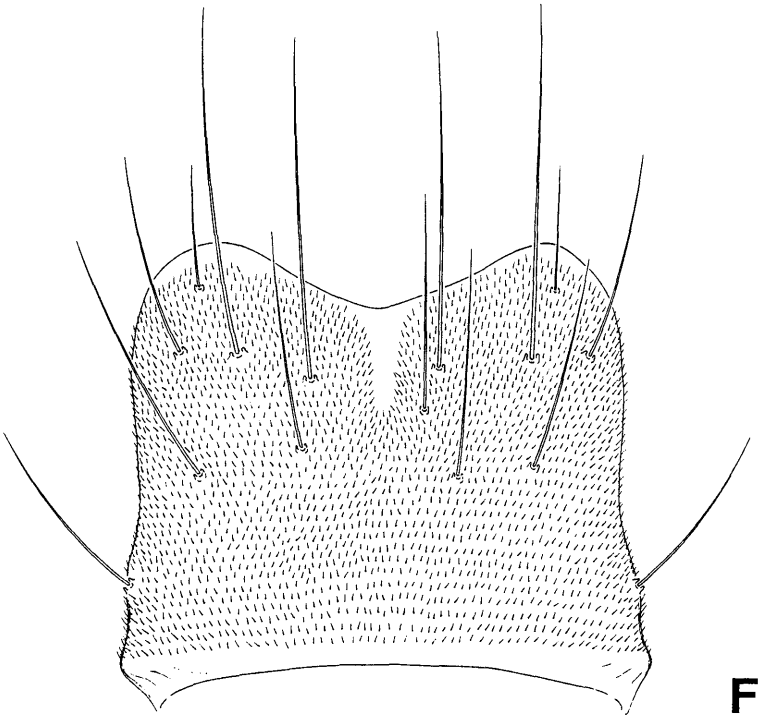


Fig.2.18

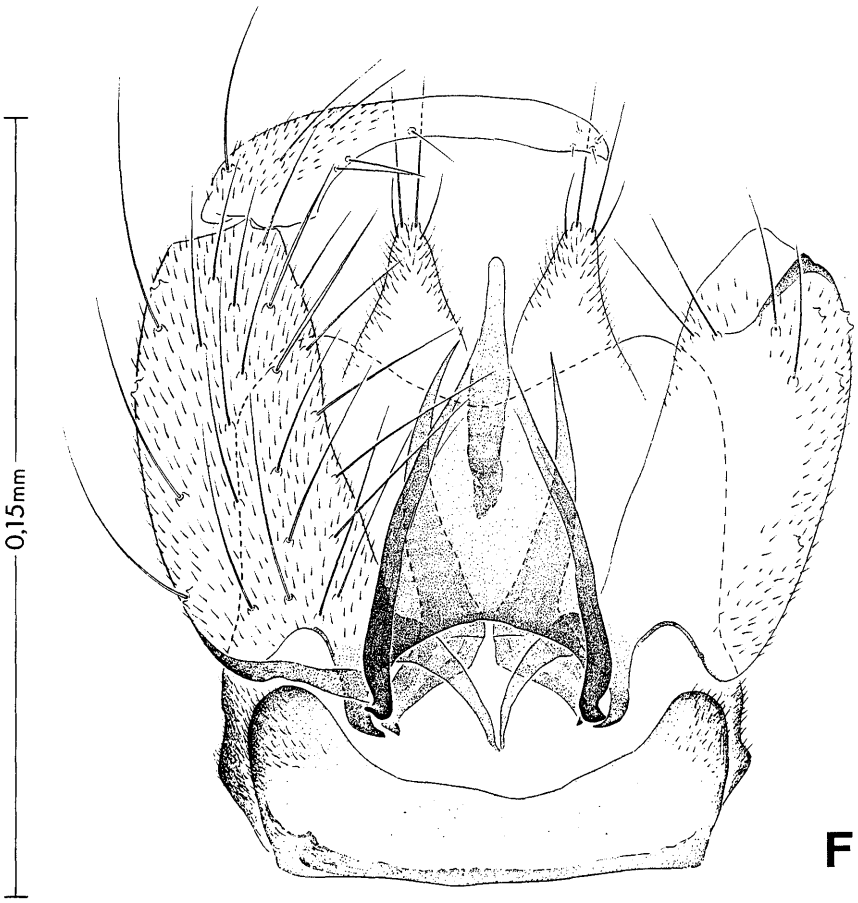


Fig.2.20

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

Fig. 2.21

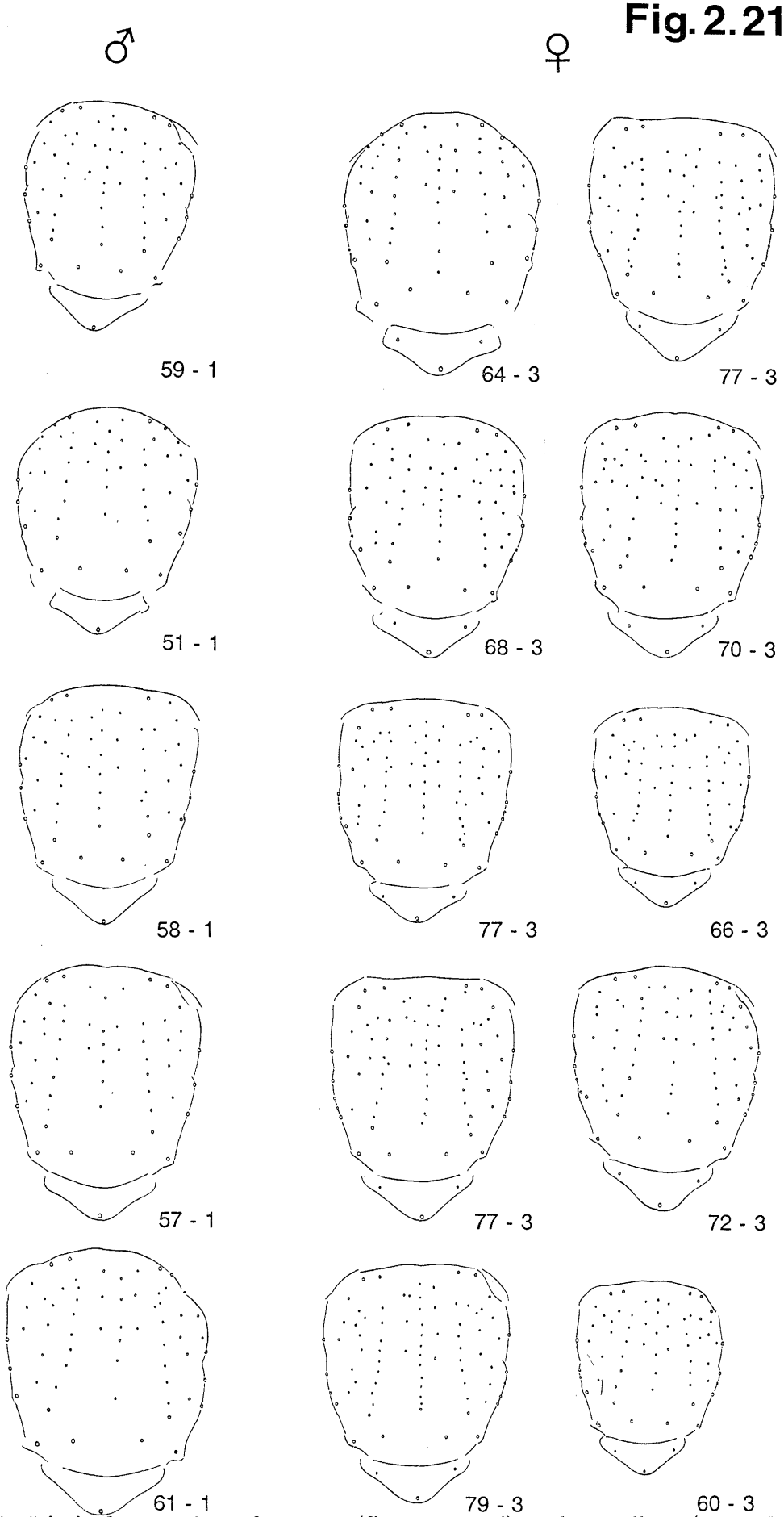


Fig. 2.21 *C. (A.) imicola*: setation of scutum (first numeral) and scutellum (second numeral); male (left column), female (two right columns)

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.

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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***