CHAPTER 9

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

9.1 INTRODUCTION

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

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

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

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

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

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

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

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

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

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

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

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

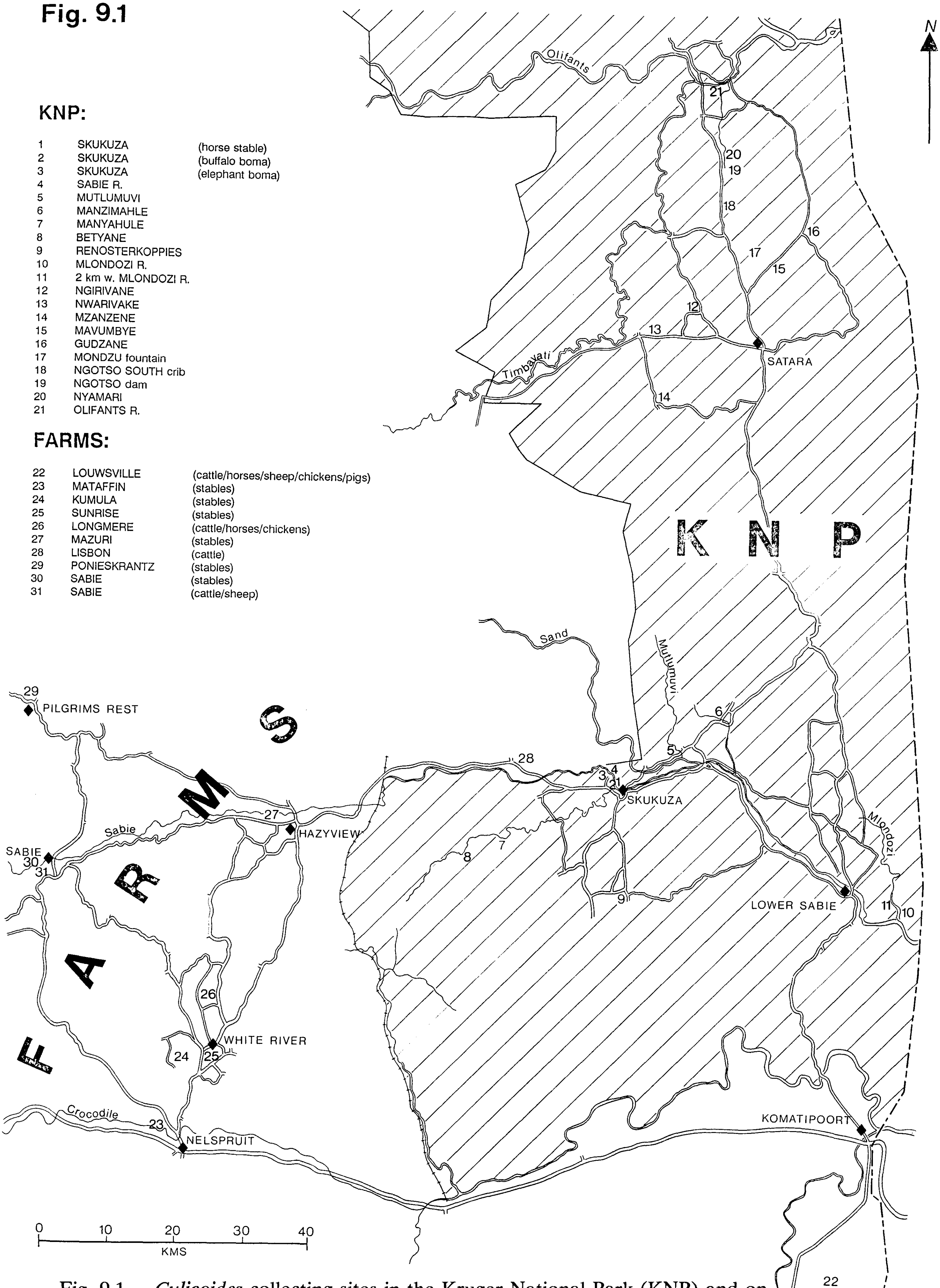
9.2 MATERIALS AND METHODS

9.2.1 Light-trap collections

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

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

Fig. 9.1



KNP:

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

FARMS:

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

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

9.2.2 Classification of sites

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

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

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

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

9.2.3 Subsampling and classification of species

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

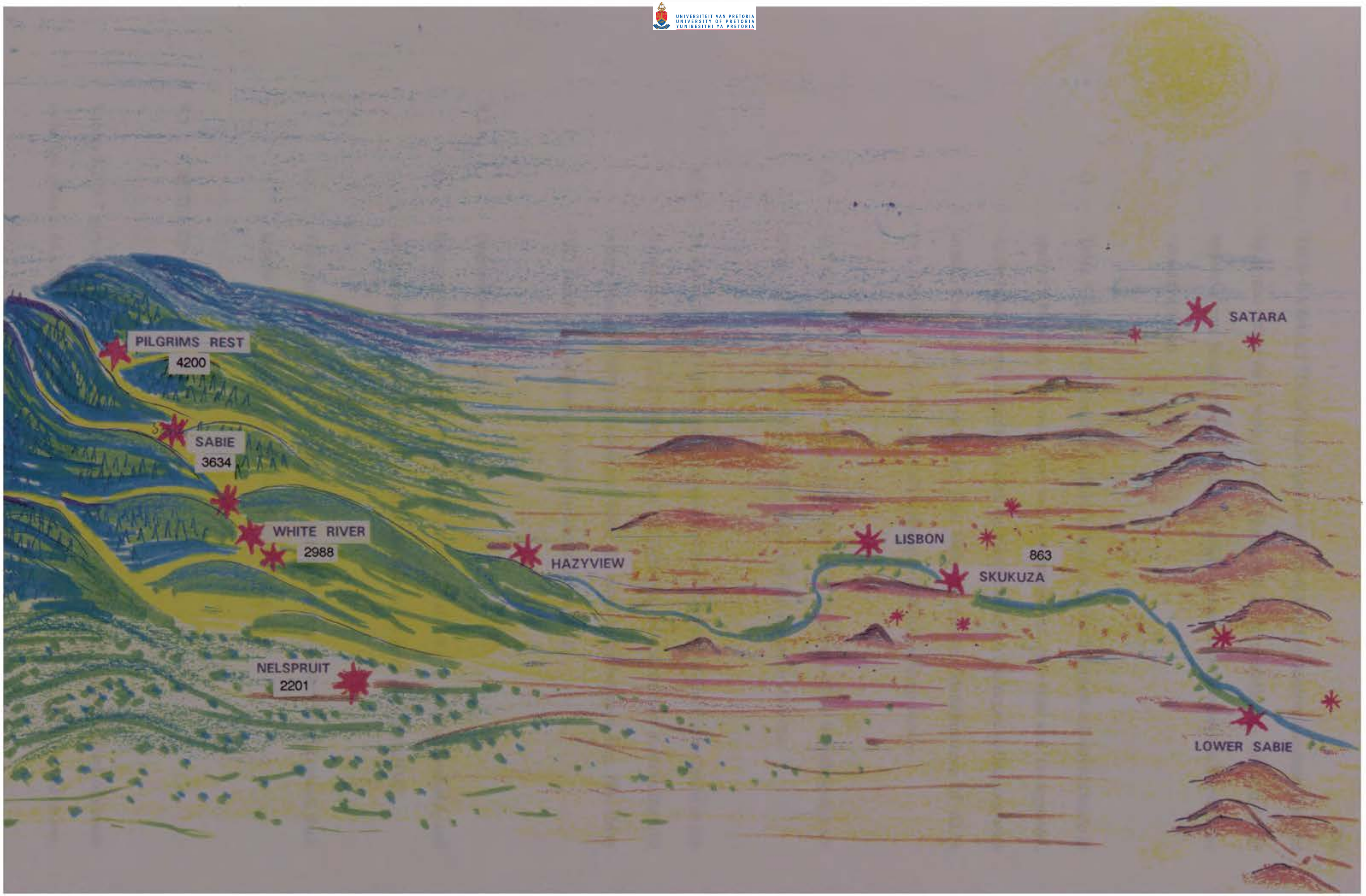


Fig. 9.2 Panoramic view of the Transvaal lowveld commencing with escarpment (FARMS) on the left and the low-lying KNP to the right. Not all light-trap sites shown; altitudes given in feet.

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

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

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

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

9.2.4 Discussion of species

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

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

9.3 RESULTS

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

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

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

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

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

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

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

inhibit the development of *Culicoides* foci.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

9.4 *CULICOIDES* IN THE SATARA AREA

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

a) *Culicoides nivosus, C. schultzei* and C. sp. # 110 (Schultzei grp.): These were the first-, second- and fourth-most abundant species, and made up 56 % of all *Culicoides* collected. Only three sites produced 94–97 % of the specimens; these were the Mondzu and Nyamari springs, and Mavumbye (a spring-fed, clay-bottomed lake). The development of such large, but localized, foci is due to the preference of these species for sunlit, muddy habitats, especially those of mature lakes undergoing changes in pH values and salinity. While nothing is known of the ability of these three species to transmit viruses in Africa, their lower prevalence (both spatial and temporal), and larval habitat specificity, would seem to weaken their potential to vector AHSV as rapidly and widely as has been documented in the Satara zebras (Barnard 1993).

b) *Culicoides kobae, C. similis, C. ravus* and *C. tropicalis* (20 %): These species are restricted to the warmer parts of South Africa, and are abundantly found along flat-bottomed rivers with sandy beds where they breed in the moist, sunlit margins. Such rivers, both large and small, drain the Satara area and explains the high abundances and widespread occurrences of these four species. Nothing is known as regards their feeding habits. However, it is notable that they all possess a swollen third palpal segment; this indicates ornithophily, but it remains to be proven. If true, this would limit their role in the transmission of AHS as there is no evidence that this virus persists in birds.

c) *Culicoides imicola* (0,94 %): This remains the only species proven to transmit African horsesickness in the continent. The low numbers in the Satara area at first glance suggest it not responsible for the rapid seroconversion rates seen in local zebra. However, *C. imicola* not only turned out to be the fifth-most prevalent species, but was also found in all habitats sampled, and in the presence of a variety of game animals throughout the year. In Table 9.10 the 'total *imicola*: total *Culicoides*' ratio reveals that *C. imicola* assumes some measure of dominance in the driest habitats where zebra occur, e.g. Ngotso south. It was also significantly more numerous in the company of the Nwarivake buffaloes, despite this 60-strong herd being 'in transit', and 5 km from the nearest potential breeding sites of *C. imicola*. This species was found to be dominant only at Bangu, known to be a congregation point for large numbers of zebras (and other game) in the drier months. During the summer, grass-margined rainpools form around Bangu, and, depending on their size, persist into the winter months. These provide ideal breeding sites for *C. imicola*. At certain times similar pools occurred at Gudzane, Ngirivane, Mondzu and Nyamari where the highest numbers of *C. imicola* were found. Although 1992 was the driest year on record in the KNP this century, and most of these pools had dried out, *C. imicola* still maintained a broad prevalence. Although its numbers were low, it was still found in the late winter/early spring months (August to October) when Barnard (1993) showed zebras to be seroconverting to AHS. While it is unknown how large a population of *C. imicola* is needed to transmit the virus, its high vector capacity implies it may have been plentiful enough to cycle virus amongst zebras. Furthermore, the data amply demonstrate that *C. imicola* can persist under the driest conditions, and that such small core populations would fairly rapidly spread and grow with the onset of rains. Limited breeding areas in a predominantly sandy terrain would preclude *C. imicola* from ever achieving abundance in the Satara area.

9.5 CONCLUSIONS

Marked differences and interesting similarities characterize the *Culicoides* faunas of the KNP and adjoining FARMS, and would affect the underlying dynamics of the epidemiology of African horsesickness in the area. In the farming community AHS is generally considered to be a 'late summer — early autumn' disease, whereas in the KNP Barnard (1993) found that zebras seroconverted rapidly to all nine serotypes of AHS in the late winter — early spring. This may simply be a coincidence, as winter is the period in the KNP when five- to six-month old foals lose their colostral immunity and so become fully susceptible. Whether transmission at this time is effected by a number of *Culicoides* species, and so accounting for rapid seroconversion, or is due to the increased prevalence of a single species such as *C. kwagga* (which is closely associated with zebras), remains unknown. The poor overlap in *Culicoides* species composition, seasonal prevalence and abundances in the KNP and on FARMS, must contribute further to the difference in incidence of AHS. There are also major disparities in the kind of hosts available (game vs. domesticated livestock), their AHS reservoir status, and in the timing of birth and recruitment rates of foals. All are essential elements for a virus such as AHS that needs to cycle continously. Finally, vaccination of horses against AHS further obscures the true epidemiology of this disease in the African context.

The overwhelming dominance of *C. imicola* on FARMS implies that it is the sole vector of AHS there. Although *C. imicola* is quite prevalent in the KNP veld, the average number of specimens/light-trap is half that found on FARMS in the middle/upper escarpment where AHS is rare to absent. At first glance this suggests that other species of *Culicoides* are involved in AHS transmission in the KNP veld, but the far higher prevalence of reservoir hosts, and the absence of vaccination, likely render this inference erroneous. In the final analysis, we simply do not know what population levels are required for *C. imicola* to effect transmission. However, the low numbers of this species in the driest KNP veld, where zebras persist with the help of artificial water supplies, and show rapid seroconversion rates against all nine serotypes of AHS, do suggest that other species of *Culicoides* may be involved in its transmission.

Eighteen of at least 52 species of *Culicoides* found in the KNP were, at one or other time, the dominant species in a light-trap collection. This is attributable to seasonal changes in the quality or availability of niches occupied by *Culicoides* species, and to the perpetual movement of game animals into and out of areas. For instance, members of the Similis and Schultzei groups can become abundant along the margins of broad-bottomed sand rivers, but their populations will crash when these rivers flood; the same species will not be found on FARMS as the character of a river changes as one ascends the escarpment; they become colder, and flow fast through rocky gorges that largely lack sunlit, sandy margins. Similarly, a handful of *Avaritia* species that breed rapidly and abundantly in elephant dung (constituting 6 % of all KNP *Culicoides*) are extinct on FARMS where elephants have been exterminated. Indeed, if light-trap data are depicted graphically, the seasonal and abundance patterns of one species seldom correlates with that of the next. Sensible comparison of data from various sites is further impeded when it is realized that a number of *Culicoides* species are found only where a particular animal resides. The daily and seasonal movements of these hosts fundamentally influence *Culicoides* density at a particular site in the KNP, and can even affect species prevalence and composition within the hour. The hosts referred to are the elephant, rhino, zebra, buffalo and wildebeest, and in the KNP veld, their dung is the larval habitat of 13 % of all specimens of *Culicoides* specimens captured. There are seven species of the subgenus *Avaritia* known to also feed exclusively on these herbivores, some of which are implicated as, or proven to be, reservoir hosts for African horsesickness. This close relationship between insect and animal may include the transmission of viruses, and for this reason the capacity of these *Avaritia* species to vector AHS merits investigation. The epidemiology of AHS in the KNP will be better understood only once virus transmission is unequivocally linked to one or more species of *Culicoides*, and the reservoir status of all suspected herbivores clarified.

9.6 AHS EPIDEMIOLOGY IN SOUTH AFRICA

It was hoped that this investigation would throw further light on the epidemiology of African horsesickness, and perhaps provide a better understanding as to why the eastern Transvaal lowveld is the AHS ‘hotspot’ of South Africa. Perhaps it is pertinent to re-examine the comments made by Theiler (1930) who was particularly intrigued by the disease. His knowledge was built on 40 years’ experience and, in the epidemiological sense, is the most accurate available. Although it was developed at a time when vaccines did not mask the natural course and spread of outbreaks in South Africa, the indiscriminate hunting of game may have had considerable impact on the geographic distribution of AHS due to elimination of reservoir hosts. Theiler concluded: ‘Horse sickness is only found in certain localities and under certain orographic, telluric and climatic conditions. During the late summer months, after the rains, it occurs regularly with more or less severity in enzootic form in the low-lying coast lands, valleys and swamps. The appearance of the disease and the severity of the outbreak are governed by the amount and distribution of the rainfall. When the rains appear late, horse sickness also appears late. Enzootics usually terminate with the first frosts. Continuous rains do not favour the spread of the disease, but heavy rains alternating with hot spells, or followed by drought, furnish optimal conditions for its dissemination. In South Africa, March and April constitute the usual horse-sickness season for the middle and high veld, whereas in low-lying lands it may occur during May and June, and in years with very late rains may be found throughout the winter.' As regards the comments surrounding the occurrence of AHS in 'low-lying lands', it is useful also to quote the remarks of Stevenson-Hamilton (1905), the first warden of the Sabi Reserve (later to grow and become the Kruger National Park): 'Horse-sickness is another obstacle ... It is no exaggeration to say that ... a horse brought down from the high country will, as a rule, not survive for a longer period than a month, while from November until the following May a fortnight is as long as he can be depended upon.' Elsewhere, Stevenson-Hamilton (1937) notes that horse-sickness is present 'almost everywhere at altitudes below 4 000 feet in the wet season ...' In the following discussion an attempt will be made to correlate current findings with these past experiences, and so pinpoint the species of *Culicoides* most likely to be involved in AHS transmission.

As regards the eastern Transvaal lowveld, the above quotes reveal that AHS can occur throughout the year and is twice as prevalent during the rainy summer season, and that the onset of cold reduces, but does not necessarily eliminate, incidence. This study adequately shows that many *Culicoides* species, including *C. imicola*, will be caught in light-traps throughout the winter. During winter a significant nulliparous rate amongst females proves that breeding is continuous; for this to happen, adults must be actively seeking and obtaining blood, and laying eggs. With reference to the seasonal transmission of AHS, Barnard (1993) showed that young zebra foals in the KNP seroconvert very rapidly to all nine serotypes of AHS, and, surprisingly, that seroconversion peaked in winter after an unexpected dip in the summer months (December — February). How can this be explained? These months mark the start of more regular rains, and soon the larger rivers are in flood or flowing wider than normal. This depresses *Culicoides* populations, especially the sand river species, as rushing waters scour out their larval habitats. Furthermore, these rivers would take some time to settle (if repeated rains fell in the escarpment) and so would prevent *Culicoides* from establishing significant new foci. With special reference to *C. imicola*, this study reveals that it seldom, if ever, becomes dominant along the margins of larger rivers (see 'wet veld' Table 9.3). Instead it does better in the 'dry veld' (Table 9.3), but only after the summer rains have commenced. This is ascribed to the creation of rainpools in the open veld, principally where clayey depressions occur. While this remains to be more conclusively proven, it is probable that such pools are rapidly colonized by *C. imicola*, and if visited by game, foci of this species would soon develop.

Therefore while rivers are flowing and rainpools are 'raw' (December — March), the growth in *Culicoides* populations would be inhibited; the subsequent climb in AHS seroconversions in the later months correlates positively with the stabilization of larval habitats and the increase in *C. imicola* numbers. By mid- to late-winter, many of the pools will have vanished and then game animals, especially zebras, will begin to concentrate at more permanent watering points, some natural, some man-made. These late-season gatherings of herbivores would lead to the build-up of *Culicoides* at the same sites. The stage would now be set for the transmission of African horsesickness.

Let us ignore *C. imicola* and consider whether AHS may be transmitted by other species of *Avaritia*, as suggested in previous chapters. Those meriting examination first are the coprophilic species that can become locally dominant where animals gather, and can also be among the most prevalent species in the KNP veld. Their complete reliance on the blood and dung of larger herbivores indicates them as potential vectors of viruses. Furthermore, their ability to use dung as a larval habitat serves to isolate them from the vagaries of droughts and floods, and means that they will always be found in the vicinity of the appropriate herbivore. Another consequence of coprophily is that *Avaritia* population levels are more stable, and always proportionate to the amount of dung dropped locally. However, the fact that this stability in numbers is not mirrored by an equally consistent seroconversion rate suggests that these *Avaritia* species are indeed not involved in AHS transmission. This conclusion may be erroneous, as the mid-summer dip in seroconversions noted by Barnard (1993) amongst zebras may simply be the result of a more prevalent colostral immunity. The majority (75 %) of foals in the KNP are born in November/December, and so would be largely refractory to infection during the following 5–6 months.

Another aspect of the life-cycle of coprophilic *Avaritia* species that would seem to interfere with their ability to disseminate AHSV, is that they are very host-specific. For example the elephant-associated species will never be found in the dung of buffaloes and, with rare exceptions, are also not found in the dung of the zebra, and *vice versa*. It thus seems reasonable to assume that these *Avaritia* species will also not feed regularly on alternative hosts. Subsequent to European colonization, hunting led to a swift decline in numbers of the big-dung-five (elephant, rhino, buffalo, wildebeest and zebra) in South Africa. This study provides ample evidence that dung-dependent *Culicoides* disappear when their hosts become locally extinct. Despite this, AHS has remained as widespread in South Africa as two centuries ago, and

Could any species other than *C. imicola* be involved in the transmission of AHS? As noted in Chapter 7 the new species, *C. kwagga*, was found to be closely associated with zebras, and because of this, and its close taxonomic relationship to *C. imicola*, it must be considered a potential vector of African horsesickness. A remarkable feature of its occurrence is that *C. kwagga* is found only in 'zebra country' in the KNP, and secondly, appears only in winter (June/July), disappearing in November/December. This interrupted prevalence is directly the result of its dependence on zebra dung; in the summer this dries out within three days, too soon for the larvae of *C. kwagga* which require moisture for a minimum of six days. What happens to this species during the hotter summer months remains unknown. While there is no evidence yet that *C. kwagga* will at this time utilize coarser elephant dung, it does seem to thrive in the finer-textured dung of the white rhinoceros. As regards its potential to transmit AHS, light-trap collections reveal that numbers of *C. kwagga* in the KNP do climb between July to November, the months in which Barnard (1993) noted a peak in AHS seroconversions amongst zebras. However, once again, this could merely be coincident with the simultaneous loss of colostral immunity in zebra foals, and, furthermore, would not eliminate *C. imicola* as being also involved, or solely responsible, for AHS transmission. At least the later summer (February — May) preponderance of AHS in most parts of South Africa, which coincides with the increased prevalence of *C. imicola*, would be achieved in the absence of *C. kwagga*. It needs to be stressed, though, that *C. kwagga*, on the cooler highveld, can be collected during the summer months, and, remarkably, has been found in every isolated pocket sampled where zebras and/or the white rhino are kept. Importantly, it has on one occasion been reared from horse dung at Onderstepoort; its appearance in the winter/spring months in the eastern Transvaal lowveld, at horse stables only, further confirms its close association with equines. Even if *C. kwagga* is ever shown to be a competent vector of AHS, it is highly unlikely that it be involved in the swift spread of epizootics; its presence is too limited and patchy for it ever to rival *C. imicola*. It seems that elephant-associated *Avaritia* species are even less likely to be involved in the epidemiology of African horsesickness; it is inescapable that the distribution of AHS in South Africa is far wider than that of the elephant, and its associated biting midges.

As regards the local distribution of AHS, it was remarked by Edington (1893) that 'certain conditions of locality seem, most markedly, to favour the production of the disease; thus it prevails mostly or more generally in low-lying districts. In connection with this statement, however, it must be noted that *the* *absolute altitude* of the district has not so much to do with its production, as its *comparative altitude* with regard to the district immediately around. Thus it may occur in the kloofs and vleys of a district which may stand at an altitude of 2 000 feet above the seas level.' It is notable how accurately this 100-year-old observation reflects the situation as regards *C. imicola* in the escarpment areas of the eastern Transvaal lowveld today. Although the great majority of specimens (94 %) were taken in the hotter, flatter, lower-lying areas, *C. imicola* still remained the dominant species (Table 9.2), but in far lower numbers, in the foothills and escarpment, where moist depressions rarely form due to efficient water runoff on a sharply sloping topography. Pilgrims Rest, a small community at 4 200 feet, and completely encircled by mountains, lies in a small, flat valley where two streams converge. At this marshy corner is sited the local Ponieskrantz stable (60 horses), fronted by an acre of kikuyu pasture; here *C. imicola* was the dominant species throughout summer, and in February 1993 (peak season) comprised 86 % of 3 300 *Culicoides* collected. A high nulliparous rate of 38 %, and numerous males (3%), attest that *C. imicola* is breeding locally. It seems clear that only the virus of AHS needs to be introduced into the Pilgrims Rest valley for an outbreak to occur in the absence of vaccination. The introduction in 1987 of just five infective zebra into central Spain, and the resultant deaths of some 2 000 horses over the next three years, illustrates how effectively *C. imicola* can cycle the virus in any locale. Before it was known that AHS was caused by a virus, and that it was transmitted by blood-sucking insects, these patterns of introduced infection were already being noted in the previous century. Edington (1895) observed: 'It also made its appearance in areas which had formerly been supposed to be safe, areas in which ... persons were in the habit of sending their horses ... during the season, as being there out of reach of it. One such area is known as the Bontebok flats, and I have been informed that several horses were sent there, of which one died of the sickness very soon after its arrival, and some weeks later the disease made its appearance among others. I am of opinion that this is to be accounted for by the fact of one horse dying there as a result of infection acquired elsewhere, and thus infecting the area.'

From the foregoing it is patently clear that of 112 species of *Culicoides* found in South Africa, *C. imicola* still remains the only one able to fulfil all requirements needed for the transmission of African horsesickness. Indeed, it seems quite fortuitous that this species, the most competent at vectoring AHS, is also the one that thrives best under man's hand: its catholic choice of hosts, and its ability to breed in areas saturated by consistent irrigation, means it can become superabundant in the presence of a variety of domesticated livestock. Furthermore, peak populations of *C. imicola* occur between February to May, the period when most cases of horsesickness are recorded in South Africa. While it is true that *C. imicola* is also the most widespread species, and so further mirroring the widespread occurrence of the disease, three factors appear seriously to limit its prevalence in the subregion:

1. Low mean temperatures in the central highlands restrict *C. imicola* to low populations; these areas are also free of AHS.
2. A sloping topography with good water-runoff also inhibits *C. imicola*; moist depressions are found intermittently in these escarpment areas, and can harbour *C. imicola*. Here the occurrence of AHS is similarly sporadic.
3. Limited data reveal that well-drained, sandy soils are remarkably free of *C. imicola*; the larvae cannot survive the lack of moisture and nutrients. While parts of the Cape Peninsula are considered to be horsesickness-free, these are likely to be only the sand flats and narrow coastal strips. Along the peninsular foothills and further inland, a change in soil type benefits *C. imicola* and large populations will then be found. This coincides remarkably well with severe outbreaks of AHS recorded this century in Ceres, Porterville, Clanwilliam, Wellington, Robertson, Montagu and Worcester. In support of soil type being influential, it is to be noted that the poorly drained black cotton soils of Onderstepoort support large populations of *C. imicola*.

Further north in equatorial Africa, there is some evidence that *C. imicola* declines in the higher- rainfall forested regions, dense shade and waterlogging of habitats are unfavourable conditions. It thus seems reasonable to postulate that *C. imicola* is restricted to the moderate rainfall (300–700 mm) savannas that surround the equatorial block. Its distribution is thus centered amongst the greatest concentration and diversity of herbivores found in Africa.

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Table 9.1 Percentage prevalence of *C. imicola* around vertebrate hosts (domestic and wild) in Africa, the Mediterranean and the Middle East according to authors

Authors	Country	Site	Hosts present (secondary hosts in brackets)	*C. imicola* % prevalence
Khamala 1971	Kenya, Tanzania, Uganda	Countrywide	Not listed	11 %
Nevill 1971	South Africa	Onderstepoort		97,4 %
Walker & Davies 1971	Kenya	Various	Sheep, cattle	0–30 %
Nevill & Anderson 1972	South Africa	Onderstepoort	Cattle Mules & horses Sheep Poultry	97,8 % 97,1 % 94,2 % 47,1 %
Braverman & Galun 1973	Israel	Bet Dagan	Sheep	60–100 %
Dipeolu, Durojaiye & Sellers 1974	Nigeria	Ibadan	Cattle (sheep & goats) Pigs	18 % 7,4 % (8,7 %)
Davies & Walker 1974	Kenya			1 %
Boorman 1974	s.e. Cyprus	Various	Sheep	Dominant
Dipeolu 1976a	Nigeria	Ibadan	'wild' game	36,9 %
Dipeolu 1976b	Nigeria	Various	Poultry, cattle; sm. ruminants	17,8 %
Dipeolu & Ogunrinade 1976	Nigeria	Eruwa	?	12–13,6 %
Dipeolu 1977	Nigeria	Countrywide	Various livestock	37,6 %
Dipeolu & Sellers 1977	Nigeria	Countrywide?	Various	38 %
Walker 1977	Kenya	Ruiru Kiboko Makindu	Cattle Sheep, goats, cattle Sheep, goats	18,1 % 12,9 % 37,4 %
Dipeolu 1978	Nigeria	Ibadan Univ.	Cattle (sheep, goats)	36,3% (44,8%)
Mellor & Pitzolis 1979	Cyprus	Various	Sheep	< 10 %
Jupp, McIntosh & Nevill 1980	South Africa	Bethulie; s. OFS	Sheep	< 1 %
Braverman & Phelps 1981	Zimbabwe	Harare area	Poultry (horses, pigs, cows nearby)	53,7 %
Phelps, Blackburn & Searle 1982	Zimbabwe	Harare	Horses, cattle	61,7 – 96,8 %
Randall 1982	South Africa	Pietermaritzburg; Natal	Cattle Sheep	53,9 – 80,8 % 57,7 – 67,8 %
Boorman & Mellor 1982	Sudan	Soba Nishishiba 7km s. Khartoum	Cattle, horse pens Cattle ?	12,0 % 18,0 % 2,1 %
Jennings, Boorman & Ergün 1983	Western Turkey	Various	Cattle, sheep	7,1 %
Herniman, Boorman & Taylor 1983	Nigeria	Vom	Cattle	35,7 %
Boorman & Wilkinson 1983	Greece	Island of Lesbos	Sheep?	24 %
Mellor, Jennings, Wilkinson & Boorman 1985	Spain/Portugal	Lisboa area	Sheep, cattle	9,0 %
Birley & Braverman 1987	Israel	Various	Sheep Cow Horse Turkey Zoo Mixed: pigs, donkey, horse	33 – 97 % 5 – 55 % 25 – 60 % 84 % 71 % 90 %

Authors	Country	Site	Hosts present (secondary hosts in brackets)	*C. imicola* % prevalence
Nevill *et al.* 1988	South Africa	Stellenbosch; w. Cape	Cattle (11,2%) Sheep (41,2%) Horse (29,4%) Goats (13,1%) Pigs (13,6%) Poultry (16,7%)	19,6 %
Venter & Sweatman 1989	Lesotho	Roma	Cattle, sheep, chickens, pigs	< 2 %
Kitaoka & Zulu 1990	Zambia	Lusaka	Guinea-pigs, cattle, chickens, rabbitry	34,8 %
Mohammed & Mellor 1990	Sudan	Khartoum (Shambat)	Cattle	2,0 %
Taylor, Al Busaidy & Mellor 1991	Oman	Rumais	Cattle	27,4 %
		Salalah	Cattle	12,4 %
		Salalah	Horses	5,0 %
		Seeb	Horses	35,3 %
Meiswinkel 1991	Côte d'Ivoire	Yamoussoukro	Zebu cattle	0,8 %
Venter 1991	South Africa	Countrywide	Various	71 %
Van Ark & Meiswinkel 1992	South Africa	Skukuza, KNP	400m from game bomas and horse stables	13,8 %
Van Ark & Meiswinkel 1992	Malawi	Kawalazi, e. of Mzuzu	Small game; birds	14,3 %
Burgu, Urman, Akca, Yonguc, Mellor & Hamblin 1992	Turkey	Countrywide	Various domesticates	10 %
Nevill, Venter & Edwardes 1992	South Africa	Countrywide	Various domesticates	
			E. Cape	> 10 %
			Central Cape	< 2 %
			N. Cape	> 50 %
			N.W. Cape	2 – 10 %
			Natal	> 10 %
			Transvaal	> 50 %
			Onderstepoort	> 50 %
Capela, Sousa, Pena & Caeiro 1993	Portugal	17 localities widespread	Various Pigs	57,4 %
			Horses	29,6 %
			Goats	5,8 %
			Cattle	3,8 %
			Sheep	3,4 %
Meiswinkel & Braack 1994	South Africa	n. KNP	Elephants & other game	2,0 %

Table 9.2 Total *Culicoides* captured in 878 light-trap collections made between VIII.1991 – VIII.1993 at 65 sites in the KNP (2 zones) and on FARMS (3 zones), with detailed breakdown of the prevalence and abundance of *C. imicola*

	KNP		**FARMS**			**TOTAL**
Zone	Bomas/stables	Veld	Lowveld	Lower escarpment	Middle/upper escarpment	
No. catches made	68	627	68	74	41	878
Total *Culicoides*	2 274 770		2 141 502			4 416 272
Subtotal *Culicoides* % No. *Culicoides*/trap between site ratio	742 135 32,6 10 914 30	1 532 635 67,4 2 444 7	2 014 885 94,1 29 631 81	110 573 5,2 1 508 4	15 044 0,7 367 1	
no. catches analysed total *Culicoides* analysed % analysed	36 602 205 81,1	139 465 924 30,4	36 1 845 453 91,3	37 98 252 88,1	21 9 523 63,3	269 (31%) 3 021 357 68
total *C. imicola* % no. imicola/trap between site ratio	297 329 49,4 8 259 56	21 166 4,5 152 1	1 494 160 89,1 41 504 273	55 695 56,7 1 505 10	7 001 735 333 2	1 875 351
no. traps *imicola* absent no. traps *imicola* < 10 range in no. *imicola*	0 0 91 – 96 429	12 (8%) 58 (40%) 0 – 7 714	0 0 69 – 482 807	0 2 (5%) 3 – 16 066	2 (9%) 9 (43%) 0 – 2 845	

Table 9.3 The dominant *Culicoides* species in the 20 largest light-trap collections made in each of 5 zones (3 in the KNP, 2 on FARMS). *C. imicola* (shaded) is clearly dominant where sedentary animals are kept year-round either on pastures or in bomas. In the natural veld *C. imicola* has to compete with 12 other species for dominance; roman numerals indicate months of dominance. C. sp. # 3 and C. sp. # 110 are both undescribed species of the Schultzei group

KNP			FARMS	
I–V, VIII, IX, XI, XII 587 – 4 981 **dry veld**	I–V, VII–X, XII 13 884 – 157 760 **wet veld**	I–VI, X–XII 8 294–190 456 **bomas/stables**	I–VI, X–XII 24 208–533 984 **lowveld**	I–V, X, XI 1 522–17 072 **escarpment**
bolitinos	*micheli*	*imicola*	*imicola*	*imicola*
imicola	*micheli*	*imicola*	*imicola*	C. sp. # 3
imicola	*nivosus*	*imicola*	*imicola*	*imicola*
tropicalis	*micheli*	*imicola*	*imicola*	*zuluensis*
bolitinos	*schultzei*	C. sp. # 3	*imicola*	*imicola*
loxodontis	*kobae*	C. sp. # 3	*imicola*	*imicola*
imicola	*kobae*	*imicola*	*imicola*	*imicola*
loxodontis	*schultzei*	*imicola*	*imicola*	*imicola*
tropicalis	*kobae*	C. sp. # 3	*imicola*	*imicola*
kobae	*nivosus*	C. sp. # 110	*imicola*	*bolitinos*
loxodontis	*micheli*	C. sp. # 3	*imicola*	C. sp. # 3
kobae	*kobae*	C. sp. # 54 p.f.	*imicola*	*imicola*
C. sp. # 54 d.f.	C. sp. # 54 p.f.	*imicola*	*imicola*	*imicola*
imicola	C. sp. # 54 p.f.	*imicola*	C. sp. # 110	*imicola*
imicola	*micheli*	*imicola*	*imicola*	*imicola*
schultzei	*schultzei*	*imicola*	*imicola*	*imicola*
loxodontis	*imicola*	*bolitinos*	*imicola*	*imicola*
imicola	*bolitinos*	*imicola*	*imicola*	*imicola*
imicola	C. sp. # 54 d.f.	*imicola*	*imicola*	*imicola*
C. sp. # 3	*kobae*	*imicola*	*imicola*	*imicola*

Table 9.4 Relative abundance and distribution of 75 *Culicoides* species in the eastern Transvaal lowveld

KNP 'endemics'		Shared			Escarpment 'endemics'	
***	*nigeriae*	***	*imicola*	*****	*onderstepoortensis*	***
**	*kanagai*	***	*bolitinos*	**	*neavei*	**
**	*pretoriensis*	***	sp. # 3	**	*engubandei*	**
*	*miombo*	***	sp. # 54 p.f.	*	*zuluensis*	**
*	*dutoiti*	***	sp. # 54 d.f.	*	*ovalis*	**
*	*meeserellus*	***	*kobae*	*	*gulbenkiani*	**
*	*moreli*	***	*tropicalis*	*	*brucei*	**
*	*remerki*	**	*exspectator*	**	*angolensis*	*
*	sp. # 61	***	sp. # 110		sp. # 35	*
*	*accraensis* sp. # A	***	*schultzei*		sp. # 48	*
*	*accraensis* sp. # B	***	*loxodontis*		*kibatiensis*	*
*	*nigripennis* sp. # A	**	*micheli*	*	sp. # 75	*
*	*nigripennis* sp. # B	**	*nivosus*	*	*magnus*	*
	moucheti	**	*kwagga*	*	*stercorarius*	
	trifasciellus	**	sp. # 50	*	*brucei* p.f.	
	distinctipennis		*leucostictus*	**	sp. # 69	
	sp. # 59	*	*tuttifrutti*	**	*dekeyseri*	
		*	*pycnostictus*	**	*huambensis*	
		**	*nigripennis*	*	*milnei*	
			sdp. # 9	**	*citroneus*	
		*	*ravus*	*	*kerichoensis*	
		*	*similis*	*	*olyslageri*	
		*	*bedfordi*	*	*gambiae*?	
		*	*coarctatus*	*		
		*	*accraensis*	*		
		*	*tororoensis*			
		*	sp. # 56			
		*	*eriodendroni*			
		*	*perettii*			
		*	*punctithorax*			
			sp. # 16	*		
		*	*africanus*			
		*	*walkeri*			
		*	*glabripennis*			
		*	*quinquelineatus*			

superabundant *****; v. common ****; common ***; uncommon **; rare *; extremely rare

Table 9.5 Subgenus *Avaritia*: comparative abundances and prevalence of six species of the Imicola group (and other species of the subgenus) in the KNP and on adjoining FARMS of the eastern Transvaal lowveld; VIII.1991–VIII.1993

	KNP				**FARMS**	
	bomas/stables	prevalence in traps	veld	prevalence in traps		prevalence in traps
no. catches made	68		627		183	
total *Culicoides*	**2 274 770**				**2 141 502**	
no. catches analysed	36		139		94	
subtotal *Culicoides* (%)	742 135 (32,6)		1 532 635 (67,4)		2 141 502 (100)	
total analysed (%)	602 205 (81,1)		465 924 (30,4)		1 953 228 (91,2)	
total *C. imicola* (%)	297 329 (49,4)	36/36	21 166 (4,5)	127/139	1 556 856 (79,7)	92/94
total *C. bolitinos* (%)	37 442 (6,2)	33/36	16 292 (3,3)	112/139	17 565 (0,9)	79/94
total *C. loxodontis* (%)	4 511 (0,75)	28/36	19 801 (4,25)	119/139	468 (0,02)	10/94
total *C. tuttifrutti* (%)	1 372 (0,23)	25/36	214 (0,05)	30/139	6 628 (0,34)	61/94
total *C. kwagga* (%)	1 262 (0,21)	12/36	699 (0,15)	44/139	254 (0,01)	14/94
total *C. miombo* (%)	98 (0,02)	4/36	27 (0,006)	8/139	0 (–)	–
total Imicola group (%)	342 373 (56,9)		57 191 (12,3)		1 581 771 (81,0)	
no. dung-breeders (%) (Imicola group)	43 574 (12,7)		35 784 (62,6)		18 287 (1,16)	
no. C. sp. # 54 s.l. (%)	14 955 (2,48)	19/36	25 967 (5,57)	130/139	894 (0,05)	30/94
no. C. sp. # 50 (%)	1 244 (0,21)	18/36	437 (0,09)	41/139	185 (0,009)	2/94
no *C. kanagai* (%)	251 (0,04)	7/36	342 (0,07)	43/139	0	
no *C. tororoensis* (%)	111 (0,02)	1/36	77 (0,02)	8/139	2	1/94
no. *C. trifasciellus* (%)	2 (0,0003)	1/36	0 (0)	0	0	
no. dung-breeders (other groups)	16 561		26 823			
total dung-breeders	**122 742 (11,5%)**				**19 366 (0,99)**	
	60 135 (9,99%)		62 607 (13,4%)			
total subg. *Avaritia*	358 936 (59,60%)		84 014 (18,0%)		1 582 850 (81,0%)	

Table 9.6 Nwarivake grasslands 20 km w. Satara, central KNP; analysis of species and age-grading of *Culicoides* caught in light-trap set near herd of 60 buffalo, 24.V.1993. Collection no. KNP 93.67a

Culicoides species	FEMALES				MALES	TOTAL	%
	nullipar	parous	b/f	gravid			
C. bolitinos	123	242	3*	94	17	479	84,78
C. loxodontis	6	5	-	5	-	16	2,83
C. imicola	8	2	-	4	-	14	2,48
C. sp. # 54 p.f.	2	3	-	7	-	12	2,12
C. tropicalis	1	1	-	9	1	12	2,12
C. leucostictus	1	-	-	7	1	9	1,59
C. kanagai	1	2	-	1	4	8	1,42
C. ravus	3	3	-	1	-	7	1,24
C. exspectator	3	-	-	-	-	3	0,53
C. kobae	2	-	-	1	-	3	0,53
C. accraensis grp.	-	-	-	1	-	1	0,18
C. similis	-	1	-	-	-	1	0,18
						= 565**	

* one of the bloodfeds fresh, remaining two dark red (± 10 h old)

** 11,34 % of entire catch of 4 981 *Culicoides* analysed; subgenus *Avaritia* = 5 species = 93,64 %; dung-breeders = 4 species = 91,16 %

Table 9.7 The known larval habitat preferences of the commonest *Culicoides* species in the eastern Transvaal lowveld

Subgenus or species group	Species	Larval habitat	Extent of availability of larval habitat
subg. *Avaritia* (Imicola group)	*C. imicola*	organically enriched, sunlit, shortly vegetated areas on saturated clays	widespread on flat terrain; localized pockets of irrigated man-made pasture
	C. bolitinos	buffalo/wildebeest/cattle dung	widespread but localized to presence of these animals
	C. loxodontis	elephant dung	only found in elephant country
subg. *Avaritia* (group unknown)	C. sp. # 54 p.f.	elephant dung	only found in elephant country
subg. *Remmia*	*C. schultzei*	restricted to saline waters; seepages and pools in calcareous rock; tolerant of high temps. and high pH	more widespread and abundant in dry western regionsof S.A.; occasionally abundant but very localized in the east
	C. sp. # 3	sunlit, bare, sandy clays	widespread
	C. sp. # 110	?	widespread in arid western regions of S.A.; occasionally abundant but localized in east
Similis group	*C. tropicalis*	sunlit margins of broad-bottomed sandrivers	restricted to sand rivers and their tributaries
	C. kobae	"	"
	C. micheli	?	appears to be more prevalent along forested margins of sand rivers
Group unknown	*C. nigeriae*	?	"
subg. *Meijerehelea*	*C. nivosus*	sunlit, unvegetated putrefying sites in clay soils	locally abundant; restricted to contaminated habitats, ‘dying’ lakes, etc.

Table 9.8 Number of *Culicoides* and light-trap collections (in brackets) made at 17 sites in the Satara area, central KNP (August 1991 — May 1993).

	VIII 91	IX 91	X 91	XI 91	XII 91	I 92	II 92	III 92	IV 92	V 92	VI 92	VII 92	VIII 92	IX 92	X 92	XI 92	XII 92	I 93	II 93	III 93	IV 93	V 93	Total
Ngotso-south	123 (3)						103 (3)	6178 (3)	10784 (21)	5554 (3)	936 (9)	1712 (3)	1268 (3)		442 (6)	1929 (3)					17 (3)	383 (4)	(64)
Ngirivane		2319 (3)					27220 (1)	214 (2)	19695 (3)	1692 (3)	2308 (3)	2830 (3)	976 (4)		2624 (3)								(25)
Ngotso dam									52064 (3)	2038 (3)	384 (3)	1821 (3)	6735 (2)		525 (1)	186 (3)							(18)
Mzanzene							37356 (2)	210 (2)	15923 (3)	555 (3)	598 (1)	511 (2)	599 (2)		1074 (2)								(17)
Gudzane							5124 (1)	11972 (3)	27016 (3)	491 (3)	206 (3)	438 (1)			2197 (3)								(17)
Nyamari									4393 (3)		6923 (2)	13395 (1)	38680 (2)		8550 (2)	9599 (2)					408 (2)		(14)
Ntomeni															8051 (12)							434 (3)	(15)
Olifants R										1260 (1)	11251 (1)	26459 (1)			3485 (1)	6750 (1)					19271 (1)	2924 (1)	(7)
Mondzu															160699 (3)								(3)
Mavumbye															32195 (2)								(2)
Nwarivake																						6799 (2)	(2)
Bangu																						281 (2)	(2)
Kambana											4 (2)												(2)
Sweni											1169 (3)												(3)
Rockvale												206 (1)											(1)
2 km s. of Ngotso south								320 (2)															(2)
Nsemani											137 (2)												(2)
Total collections	3	3	-	-	-	-	7	12	36	16	29	15	13	-	35	9	-	-	-	-	3	8	196
Total *Culicoides*							69803	18894	129875	11590	23916	47372	48258		219842	18464					19696	10821	
Average/trap	43	773	-	-	-	-	9972	1575	3608	724	825	3158	3712		6182	2052					6565	1353	

Table 9.9 Number of *Culicoides* collected and number analysed in 200 light-trap collections; Satara area, central KNP (August 1991 — May 1993).

	No. light traps	No. analysed	Total *Culicoides*	Total analysed
Ngotso south (trough)	65	29	29 329	11 561
Ngotso dam	18	2	63 753	17 290
Mzanzene	17	0	40 042	-
Gudzane	17	1	31 521	1 530
Ntomeni	15	1	8 485	814
Nyamari fountain	14	1	81 948	25 480
Olifants R.	7	2	71 400	22 756
Mondzu fountain	3	1	160 699	99 720
Sweni	3	0	1 169	-
Nwarivake	2	2	6 799	6 799
Mavumbye	2	1	32 195	26 486
Bangu	2	1	281	213
Nsemani dam	2	0	137	-
Kambana	2	0	4	-
2 km s. Ngotso south	2	2	320	320
Rockvale	1	0	206	-
Ngirivane	28	6	62 434	2 564
	200	48	590 722	231 540
		24% subsampled		39,2% analysed

Table 9.10 Total number, percentage and prevalence ranking of 35 species of *Culicoides* collected in the Satara area, central KNP (August 1991 — May 1993).

Species	(larval habitat)	Total	%	Prevalence (rank)
nivosus	(mud lakes)	65 881	28,24	28/47 (9)
schultzei	(mud lakes)	43 386	18,59	21/47 (12)
kobae	(rivers)	31 285	13,41	38/47 (3)
C. sp. # 110 (Schultzei grp)	(mud lakes)	21 265	9,11	18/47 (14)
C. sp. # 54 p.f.	(elephant dung)	13 285	5,69	40/47 (1)
loxodontis	(elephant dung)	12 099	5,19	40/47 (2)
bolitinos	(buffalo dung)	11 960	5,13	25/47 (10)
similis	(rivers)	9 556	4,10	33/47 (6)
leucostictus		5 135	2,20	32/47 (7)
C. sp. # 3 (Schultzei grp)		4 841	2,07	14/47 (17)
ravus	(rivers)	3 968	1,70	37/47 (4)
tropicalis	(rivers)	3 424	1,47	30/47 (8)
imicola	(grass-margined rain pools)	2 198	0,94	34/47 (5)
bedfordi		1 096	0,47	8/47
nigeriae	(rivers)	756	0,32	11/47 (20)
pycnostictus		752	0,32	25/47 (11)
walkeri		394	0,17	5/47
C. sp. # 54 d.f.	(elephant & zebra dung)	390	0,17	17/47 (15)
exspectator		355	0,15	15/47 (16)
pretoriensis	(rock pools)	348	0,15	9/47
micheli		198	0,08	9/47
C. sp. # 30	(rotting fruits)	179		20/47 (13)
kanagai	(elephant dung)	147		12/47 (19)
neavei		131		2/47
dutoiti		99		7/47
C. sp. # 61		62		14/47 (18)
C. sp. # 107	(zebra dung)	44	0,02	4/47
coarctatus		31		2/47
remerki	(tree holes)	17		8/47
C. sp. # 56		16		5/47
eriodendroni	(tree holes)	13		3/47
Accraensis grp.	(tree holes)	10		2/47
C. sp. # 50	(elephant dung)	6		3/47
africanus		1		1/47
punctithorax	(tree holes)	1		1/47
		233 330		
			= 39,5% of total captured	

Table 9.11 Numbers of, localities, and months in which *C. imicola* was collected in the Satara area, central KNP (August 1991 — May 1993).

Collection No.	Date	Locality	Total *imicola*	Total *Culicoides*	*imicola*: *Culicoides* ratio	Rank	Months dominant
KNP 91.2	13.VIII.1991	Ngotso south trough	-	28	-		
KNP 91.16 a	18.IX.1991	Ngirivane	7	697	1:100	18	
KNP 91.16 b	"	"	15	317	1:21	4	September
KNP 92.33	26.II.1992	"	163	27 220	1:67		
KNP 92.34 abc	27.II.1992	Ngotso south	-	103	-		
KNP 92.49 a	25.III.1992	2 km s. Ngotso south	1	259	1:259		
KNP 92.49 b	"	"	2	56	1:28	6	March
KNP 92.50 a	"	Ngotso south	4	3 132	1:783		
KNP 92.50 b	"	"	5	555	1:111		
KNP 92.70 a	20.IV.1992	"	5	529	1:106	20	
KNP 92.70 c	"	"	18	2 390	1:133		
KNP 92.73 a	21.IV.1992	"	-	96	-		
KNP 92.73 b	"	"	1	245	1:245		
KNP 92.76 a	23.IV.1992	Ngotso dam	160	14 000	1:88	17	
KNP 92.79 a	24.IV.1992	Ngotso south	-	53	-		
KNP 92.79 b	"	"	-	52	-		
KNP 92.79 c	"	"	-	72	-		
KNP 92.80 B	26.IV.1992	"	6	712	1:119		
KNP 92.81 c	27.IV.1992	"	6	1 030	1:172		
KNP 92.109 b	26.V.1992	"	31	2 563	1:83	16	
KNP 92.113 c	28.V.1992	Ngirivane	8	842	1:105	19	
KNP 92.144 a	16.VI.1992	Ngotso south	3	179	1:60	13	
KNP 92.144 b	"	"	2	92	1:46	10	June
KNP 92.144 c	"	"	2	141	1:70	14	
KNP 92.176 a	20.VII.1992	"	3	392	1;131		
KNP 92.206 a	24.VIII.1992	"	7	199	1:28	5	August

Collection No.	Date	Locality	Total *imicola*	Total *Culicoides*	*imicola*: *Culicoides* ratio	Rank	Months dominant
KNP 92.207 a	"	Ngotso dam	20	3 290	1:165		
KNP 92.208 b	"	Nyamari fountain	479	25 480	1:53	11	August
KNP 92.209 b	27.VIII.1992	Ngirivane	-	115	-		
KNP 92.225 abc	13.X.1993	Ngotso south	7	81	1:12	3	October
KNP 92.227	14.X.1992	Olifants R.	90	3 485	1:39	7	October
KNP 92.230 a	"	Ngotso south	-	158	-		
KNP 92.230 c	"	"	-	116	-		
KNP 92.231 a	15.X.1992	Gudzane	213	1 530	1:7	2	October
KNP 92.232 b	"	Mavumbye	85	26 486	1:312		
KNP 92.235 a	16.X.1992	Ngirivane	4	566	1:142		
KNP 92.236 a	18.X.1992	Ntomeni	10	814	1:81	15	
KNP 92.237 a	17.X.1992	Mondzu fountain	151	88 720	1:588		
KNP 92.261 a	23.X.1992	Ngotso south	12	543	1:45	9	November
KNP 93.50	26.IV.1993	Olifants R.	364	19 271	1:53	12	
KNP 93.67 a	24.V.1993	Nwarivake	124	4 981	1:40	8	May
KNP 93.67 b	"	"	88	1 818	1:21	4	May
KNP 93.66 a	23.V.1993	Bangu	103	213	1:2	1	May
			2 198	233 621			
			0,94%				

CHAPTER 10

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

10.1 INTRODUCTION

Culicoides midges (Diptera, Ceratopogonidae) are among the most plentiful biting insects; up to 500 000 can be caught at a horse stable on a warm summer night.

In South Africa, *C. imicola* is without doubt the commonest and most prevalent of all *Culicoides* species. In a countrywide study it was found on 33 of 34 farms surveyed and comprised 71 % of > 3 000 000 midges captured (Venter 1991). A second study (see Chapter 9) done in the eastern Transvaal lowveld, the African horsesickness 'hotspot' of South Africa, confirmed these data; its superabundance appears to be largely artificial as the maintenance of cattle, horses, sheep, and other livestock on irrigated pastures exactly suits the needs of this midge. Under more natural conditions, where drought, floods and the constant movement of animals are the norm, large populations of *C. imicola* are unable to develop.

The occurrence of *C. imicola* throughout the farming community of South Africa means that animals always run the risk of contracting any one of the following viruses: bluetongue (BT), African horsesickness (AHS), three-day stiffsickness (BEF), equine encephalosis (EE), and Akabane (AKA); *C. imicola* has a proven involvement in the transmission of some of these diseases (du Toit 1944). South Africa thus stands classified as an enzootic zone, with the result that free trade in live animals, semen and embryos is disallowed, and participation in overseas equestrian events is complicated as horses have to undergo lengthy quarantine periods.

The re-acceptance of South Africa into the international arena has meant that current embargoes on animal exports needed reappraisal and modification. In the case of African horsesickness, the European community has developed the concept of regionalization; low-risk zones, known to be relatively free of disease, are selected for quarantine purposes. The Cape Peninsula to some extent fulfils the requirements, and so is under consideration as a possible horsesickness-free zone (Bosman, Brückner & Faul 1995). However, it must subscribe to a restrictive protocol. Not only does this protocol rely on the integrity of involved persons/owners/farmers/officials, but the controls also include a ban on vaccination, strict monitoring of animals into and out of the zone, constant surveillance of insect populations, obligatory notification of mortalities, and mandatory post-mortems (Bosman 1994). This protocol is, however, necessary as *Culicoides* surveys in the s.w. Cape have shown that not only is *C. imicola* present at all sites but at > 20 % of these it is also the dominant species (Nevill *et al.* 1988; Nevill *et al.* 1994), and probably explains the rare intrusion of AHS into the area. It is thus clear that in the presence of *C. imicola* animals are at risk in this subregion; in the absence of vaccination, only a virus needs to be introduced to result in losses.

Most aspects of the complex protocol outlined above could be eliminated if an *imicola*-free zone were to be found in South Africa. Such a zone would not only have obvious benefits as regards the quarantining of animals, but their safety also would be assured at a far lower cost in both man-hours and money.

New data, indicating that an *imicola*-free area does exist in South Africa, are presented here. Not only is the area well-suited for horses and other livestock, but it is also accessible and possesses an excellent infrastructure that includes a harbour and an airport. This area is the sandy dune field immediately west of Port Elizabeth. Possible reasons for the absence of *C. imicola* are advanced and the potential quarantine area shown on a general and a detailed map. A four-part research plan, designed to resolve remaining questions, is presented.

10.2 MATERIALS AND METHODS

Culicoides were collected with a single U-V light-trap site set on most nights between 21–31.XII.1993. Eight plots or farms were sampled — the majority were situated in the Sardinia Bay area west of Port Elizabeth; four of the sites had only horses, two only cattle, and two had a mixture of both (Table 10.1). The sites (Fig. 10.2) and weather prevailing on the day and night of trapping are respectively indicated at the top and bottom of Table 10.1. Each specimen of 4 749 *Culicoides* captured was identified and sexed (Table 10.1).

A brief resumé of the climate and geomorphology of the Port Elizabeth area is necessary to allow for a more sensible evaluation of the collected data (adapted from Marker 1988).

10.2.1 Climate:

- Frost-free; the average monthly minimum temperature ranges between 7,3–16,9 °C; maximum averages between 19,5–25,5 °C.
- Rainfall year-round; classified as a winter maximum rainfall area; precipitation ranges from a low of 33,4 mm (February) to a high of 65,1 mm (June). Mean annual rainfall (1960–1980): 613,7 mm. On average rain falls every 3,5 days.
- Winds: dominated by westerlies and south-westerlies; hot winds from the north are rare and of short duration; on average, only one in five days is calm. Known as one of the windiest parts of southern Africa.

10.2.2 Geomorphology:

- Most of the coastal belt is rural, and intensive agriculture is scarce, the soils being poor. The dominant coastal landform is of dunes and shelving arcuate sandy beaches separated by low rocky promontories fronting a stepped coastal plain. The underlying geology of this coastal belt is complex as can be seen in Fig. 10.1. The most recent events occurred during the Pleistocene when multiple northern hemisphere glaciations caused repeated drops in sea level. This created a straight coast from Cape St. Francis to East London and resulted in the bays becoming dry land. Calcareous sands were then blown onshore from the exposed sea floor and deposited against any uneven ground. The resultant dunes are of various ages; the oldest are the inland aeolianite solified dunes and the youngest are the low fore-dunes on the present beaches. The scale of these events can be appreciated when one examines the extent of the yellow areas (sand) in Fig. 10.1; Fig. 10.2 details the exact extent of these dune fields west of Port Elizabeth, an area of ± 400 km^2 in which collections of *Culicoides* were made. To the north and north-west of Port Elizabeth are found the Alexandria dune fields, the largest in southern Africa.

10.2.3 Vegetation: - The Sardinia Bay area is characterizedby abundant dune thicket, and stretches of coastal fynbos; coastal forest is occasionally present; coastal grasslands and acacia savanna are absent. Extensive areas have been planted to kikuyu pasture.

10.3 RESULTS AND DISCUSSION

Three facts emerge from the light-trap data given in Table 10.1:

a) In South Africa the concurrence of a number of factors causes large populations of *Culicoides* midges to develop. These factors occur in the Port Elizabeth area and are:

- a frost-free regime
- good rainfall
- extensive irrigation of kikuyu pastures, and
- the presence of large groups of animals providing blood for biting *Culicoides*.

Although these preconditions are to be found in the Port Elizabeth dune field, *Culicoides* numbers, and species diversity, are low. The most immediate reasons appear to be:

- windy circumstances: only one in five days is calm. Windspeeds of more than 4 m/sec are known to suppress flight in *Culicoides*, and
- sandy soils, being poor in organic matter, and having excellent drainage, would inhibit the development of *Culicoides* larvae.

b) The great majority of *Culicoides* caught (92,54 %) in the Port Elizabeth area belong to only two species, which are known to inhabit the dung of cattle elsewhere in South Africa. These are *C. bolitinos* and *C. gulbenkiani*; neither of these has yet been found to transmit viruses. This is only one of three sites known in the country where dung-loving *Culicoides* overwhelmingly dominate other species. Not only is dung largely independent of the soil type on which it is deposited, but it carries its own suite of micro-organisms on which *Culicoides* larvae, of a few species only, will feed. It seems improbable that the currently recorded catches will increase markedly if sampling were to be done at any other time of the year, as these data clearly show that the dominant *Culicoides* in the dune fields are coprophilic; their population levels are thus determined by the amount of dung available. This, in turn, is governed by cattle density. It is unknown what role horse-dung plays in the maintenance of local *Culicoides*.

c) Twelve of the remaining 13 species (7,42 %) of *Culicoides* captured are 'groundwater' breeders; their larvae and pupae develop on the edges of water bodies, either large or small. These would include lakes, streams, drainage furrows, overflows from leaking cement dams, and similar places. The low prevalence of groundwater *Culicoides* suggests a lack of these larval habitats, but this is certainly not the case; it is more likely the result of the sandy substrate not remaining water-logged for sufficiently long periods (10–20 days). Rapidly drying soils would adversely affect moisture-loving *Culicoides* larvae, and if the soils are also poor in organic matter, would further exacerbate conditions for a species such as *C. imicola* so dependent upon prey organisms which thrive in organically enriched, semi-moist situations. As moisture is essential for *Culicoides* larvae, aridity would depress overall species abundance and diversity. The total number of groundwater *Culicoides* (355 individuals), and the number of species (12), is the lowest recorded for any group of sites yet sampled over 15 years in South Africa; even the cold eastern Orange Free State has three times this number of groundwater species (Venter & Meiswinkel 1994). This reinforces the notion that it is not climate but organically poor, single origin, young soils that are responsible for the short *Culicoides* species list in the Port Elizabeth area. Wind would affect *Culicoides* numbers too, but not overall species diversity.

10.4 CONCLUSIONS

a) The total absence of *C. imicola* in a particular area surveyed is unprecedented in South Africa. Only once previously in 15 years of sampling throughout the Republic has *C. imicola* been found to be absent at a site. This was at Rhodes — a small high-altitude enclave in the southern Drakensberg — but it was sampled for one night only (Venter 1991). Its absence in the Port Elizabeth area is all the more suprising when we consider that elsewhere in South Africa *C. imicola* is by far the commonest *Culicoides* found in association with cattle, horses or sheep (Venter 1991). For instance, in the eastern Transvaal lowveld where African horsesickness is most prevalent, up to 500 000 *C. imicola* females can be caught on a single summer night near horses (R. Meiswinkel, personal observation 1992).

b) The absence of *C. imicola* in the Port Elizabeth area is a cause for celebration, as it is still the only species proven to be an efficient vector of the viruses of bluetongue and African horsesickness (du Toit 1944; Venter, Hill, Pajor & Nevill 1991). The sandy dune field area would thus serve as an excellent natural quarantine station, possessing not only an excellent infrastructure including a harbour and an airport. Furthermore, if South Africa were in future to host the Olympic Games, the Port Elizabeth area would seem to be the ideal venue for all gymkhana events; in the absence of vaccination, valuable horses would not be exposed to *C. imicola* and thus African horsesickness. In addition, recent efforts to export breeding stock of goats and sheep to Australia and America necessitated huge enclosures that could only be insect-proofed with difficulty and at great cost. A vector- or disease-free area in South Africa would be invaluable for such ventures.

c) *Culicoides bolitinos* was by far the most common species collected in the Port Elizabeth area. Its role in the transmission of AHSV is unknown, but it is thought not to be a vector since *C. bolitinos* also dominates in the eastern Orange Free State where African horsesickness is absent (Venter & Meiswinkel 1994). Nevertheless, it remains essential that this species be tested in the laboratory for its ability to multiply AHSV and other arboviruses.

d) As discussed, the total absence of *C. imicola* appears to be the result of nutrient-poor, well-drained sandy soils being unable to sustain the immature stages. This suggests that many groundwater *Culicoides* persist in slow-drying clay-based soils that not only retain moisture but are generally richer in nutrients and micro-organisms. Some evidence does exist that *C. imicola* predominates in regions with clayey soils: (i) it is the most abundant and prevalent species in the greater Onderstepoort area, much of which is situated on dense black cottonsoils (Edwardes, personal communication 1989); and (ii), of 1 568 *Culicoides* of nine species reared by Nevill *et al.* (1988) in the Stellenbosch area, 18 (1,1 %) were *C. imicola*; while the total is very low, half were reared from the only one of five sites sampled that had the soil 'clay-like and medium moist'. At the remaining four sites no *C. imicola* or few individuals were reared from situations that were either 'well-drained', 'dry and sandy' or 'with fine particle size (silt)'. It remains to be proved that the highly variable prevalence of *C. imicola* in light-traps in the 540 km^2 area surveyed by Nevill *et al.* (1988) correlates positively with latitudinal changes in soil types.

10.5 RECOMMENDATIONS

a) Three two-week light-trap surveys should be carried out: one each in spring, summer and autumn. During each visit, five north-south transects, each 50–100 km in length, and bisecting various ecozones and/or soil types, should be surveyed. Ten to 20 farms should be sampled along each transect, and seven to 10 farms sampled per night. Random samples should also be done between transects where any sizeable groups of livestock are encountered, so as to improve the chances of finding *C. imicola* amongst any *Culicoides* foci. Each of the two-weekly surveys should yield 150–200 light-trap collections; this large number is needed to give adequate coverage of a large area, to reduce the effects of poor weather which will significantly depress the size of four of every five catches made, and to more accurately define the geographic limits of *C. imicola*. Collections should be made near all kinds of livestock.

b) After the first transect survey has been completed, two farms should be chosen at each of three well-separated points along one transect, and the traps run weekly by farmers. This year-round sampling in three zones where *C imicola* is abundantly present, sporadically present, and absent, will help establish:

(i) whether *C. imicola* is truly absent throughout the year in a 'clean' area, namely the sandy dune field;

(ii) when, for how long, and in what numbers, *C. imicola* intrudes into the sporadic zone;

(iii) just how abundant *C. imicola* is on those farms where it occurs permanently, to compare with abundance data collected elsewhere in South Africa; and finally,

(iv) compare in greater detail, overall *Culicoides* abundances and species composition on different soil types.

c) On 35 farms, blood should be drawn from six animals and screened for antibodies to BT, AKA, BEF and AHS. Not only will this reveal the geographic distribution of these viruses but, if present, will also indicate:

(i) Farm-specific and recent circulation of viruses. On 'positive' farms the dominant *Culicoides* should be artificially infected with the relevant virus/es, and attempts made to re-isolate the virus/es in the laboratory seven to 10 days later.

(ii) Whether higher levels of virus transmission are occurring on one or other soil type. If a distinct pattern emerges, it will give an indication as to which *Culicoides* species are involved, and what numbers are needed to effect transmission.

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TABLE 10.1: Numbers and species of *Culicoides* midges collected at eight horse stables and dairy farms west of Port Elizabeth (21.XII. — 31.XII.1993)

Site No.	1	2	3	4	5	6	7	8			
Site:	Hustlers' six horses, Sardinia Bay, P.E. 21.XII.1993	El Paso, two cattle, horses. Sardinia Bay 22.XII.1993	G & P Scallen; Farm Broadlands, Sardinia Bay, nine horses 23.XII.1993	Farm Avonlea, Sardinia Bay, P.E. — David Emslie 24 horses 26.XII.1993	B & N Bartlett, Under Milkwoods, Sardinia Bay. 30 horses & cattle 28.XII.1993	Yellow Sea dairy, P.E. 160 Jersey herd, blacklight 29.XII.1993	Fairview Racecourse, Green bushes, P.E. 360 horses 30.XII.1993	Bushy Park Dairy ± 400 cattle, Sardinia Bay 31.XII.1993	Male	Total (Male + female)	%
C. bolitinos	2 916	1	416	218	635	95	1	16	56	4 354	91,72
C. pycnostictus	2	1	2	4	86	16	3	16	16	146	3,08
C. nivosus	1		1	5	20	6		9	1	43	0,91
C. leucostictus					9				1	10	0,21
C. zuluensis						3			18	21	0,44
C. gulbenkiani	1					24			14	39	0,82
C. magnus	1			1		61	19	1	13	96	2,02
C. neavei	1					12	1		8	22	0,46
C. angolensis						1				1	0,02
C. ondersteportensis				2	1	1			1	5	0,11
C. sp. # 69						1				1	0,02
C. bedfordi	2							3	2	7	0,15
C. similis				1						1	0,02
C. tropicalis	1									1	0,02
C. accraensis group		1		1						2	0,04
	2 925	3	419	232	751	220	24	45	130	4 749	
Weather conditions:	Warm/calm	Very windy	Coolish/calm	Cool/misty/calm	Coolish/calm	Intermittently windy	Windy	Windy			

Entire catches analysed: subgenus *Avaritia* = two species = 92,54 % = dung-breeders
C. imicola: 0 %

Identified by: R. Meiswinkel and M.E. Botha

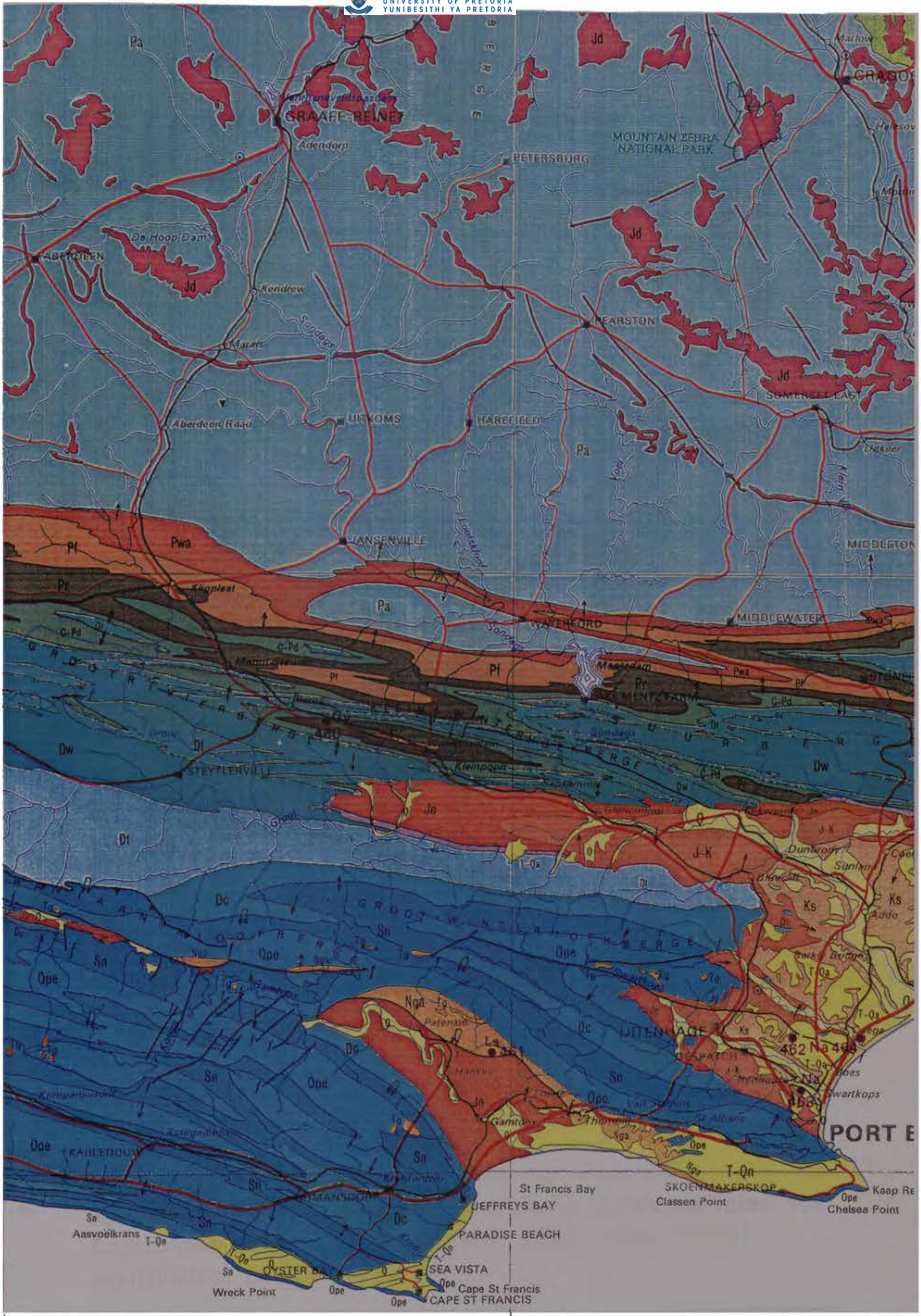


Fig. 10.1 Southern Cape coast: extent of ancient, nutrient-poor sea sands (yellow) blown onland during past eons.

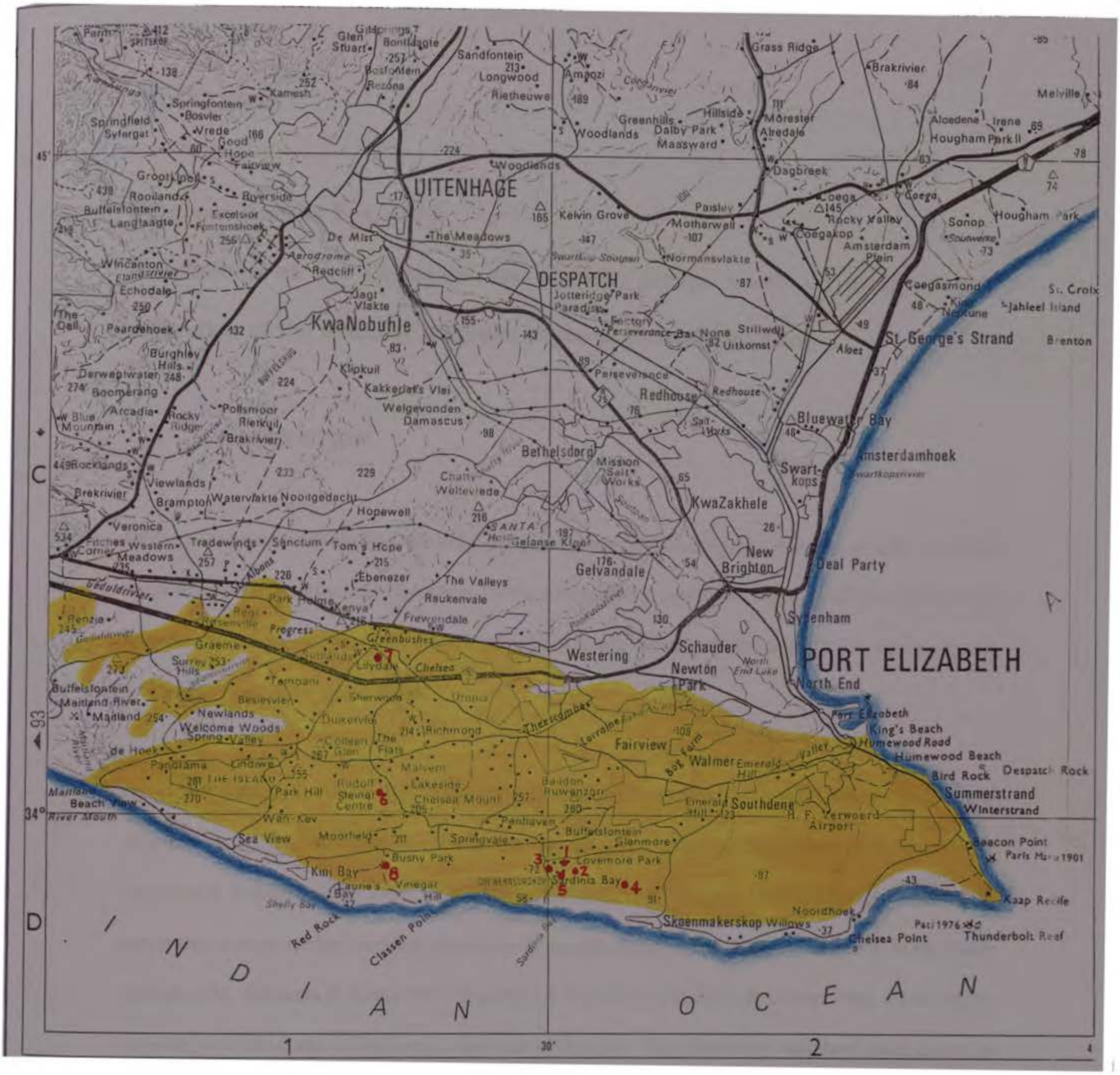


FIG.10.2 Location of 8 horse stables and dairy farms sampled for *Culicoides* midges on the sandy coastal strip (▭) south-west of Port Elizabeth

CHAPTER 11

Subgenus *Avaritia* Fox, 1955:

Redefinition of the Orientalis and Imicola groups based on morphological separation of the adults (*Culicoides*; Diptera: Ceratopogonidae)

11.1 INTRODUCTION

Of the 109 names available for *Culicoides* species belonging to the subgenus *Avaritia*, 63 are considered valid and 46 to be synonyms (Boorman 1991). Allowing for omissions, subsequent descriptions and resurrection from synonymy, the number of valid *Avaritia* species stands nearer to 70, approximately 5 % of the *Culicoides* world fauna.

Fox (1955) created *Avaritia* and chose the Holarctic species *C. obsoletus* (Meigen) as subgenotype. While *Avaritia* was adopted by researchers worldwide, its usage was rather random (Wirth & Blanton 1959; Campbell & Pelham-Clinton 1960; Vargas 1960; Delfinado 1961; Atchley 1967; Blanton & Wirth 1979) and in time a more refined tendency developed to use species-groups instead (Jamnback & Wirth 1963; Kremer 1965; Khamala & Kettle 1971; Boorman & Dipeolu 1979; Itoua & Cornet 1986; Glick 1990); however, it resulted also in the more limited use of *Avaritia*. This dichotomy may have been due to an emergent realization that no *Avaritia* species in other regional faunas has the complex, almost flamboyantly aberrant, genitalia that characterize *C. obsoletus* and seven other species of the Obsoletus group. Vargas (1962, 1973) also sent mixed signals when he incorrectly excluded taxa from *Avaritia*, while assigning other, clearly unrelated, ones to it.

Although the pendulum has recently swung back to using *Avaritia*, this seems to be more a case of nomenclatorial legalism (Wada & Kitaoka 1977; Dyce 1980, 1983; Dyce & Wirth 1983; Howarth 1985; Kitaoka 1985; Wirth, Dyce & Peterson 1985; Glukhova 1989; Mirzaeva 1989; Boorman 1989; Kitaoka & Zulu 1990). While there is some merit in rejecting the informal species-group category, its *ad hoc* replacement with *Avaritia* cannot be seen as progressive as this only means that new species are now simply being gathered under a 'named' umbrella, one that is growing ever larger and more disparate. Furthermore, by 'securely' placing a species within a formal subgenus we may neglect to explore the relationships that undoubtedly exist between its constituent taxa.

In the last eight years, however, a small group of workers has begun to recognize that *Avaritia* is a polyphyletic assemblage, and that it can be split (Itoua & Cornet 1986; Meiswinkel 1987, 1989, 1991; Wirth, Dyce & Spinelli 1988; Wirth & Hubert 1989; Boorman 1991). To date seven species groups have been proposed. These are the Actoni, Andicola, Imicola (= Pallidipennis), Montanus, Obsoletus, Orientalis and Pusillus groups (Wirth, Dyce & Spinelli 1988; Wirth & Hubert 1989; Glick 1990; Boorman 1991). While all except the Montanus group of Boorman (1991) appear to be valid, they are either not delineated (Andicola and Pusillus groups), have been established in the female only (Actoni and Orientalis groups), or are too broadly defined (Imicola and Orientalis groups). As a result their usage is confused, especially as regards the Orientalis and Imicola groups.

While some authors have begun to establish species groups within *Avaritia*, others do not recognize the subgenus; they prefer to use the group caterogy in lieu of *Avaritia* (Itoua & Cornet 1986; Glick 1990). This complicates levels of ranking, however, as discrete lineages subsequently discovered must be accommodated by further subdivision of the said group, a reality that led Itoua & Cornet (1986) to present, but not define, the Afrotropical Grahamii, Trifasciellus and Imicola subgroups of the Imicola group. Not only do subgroups at this level hamper further fragmentation involving species-pairs, but also the discordance in hierarchy obscures the fact that their first two subgroups are, in part, synonymous with the south-east Asian Actoni and Orientalis groups of Wirth & Hubert (1989).

Before a proper cladistic analysis is done to clarify the various levels of relatedness that appear to exist between the taxa comprising each group, I suggest that the three subgroups of Itoua & Cornet (1986) be recognized as groups. This would bring studies on the Afrotropical *Avaritia* fauna in line with those done in other Regions; the Orientalis group is here used instead of the Trifasciellus group as the nominal species of the former was described earlier. The Orientalis and Imicola groups are here understood to clearly belong within the subgenus *Avaritia*, and are not equal to or meant to replace it.

Although there may be problems with hierarchial ranking, it is more important to note that the undisputed presence of closely related species in two or more regional faunas is tacit acknowledgement that discrete lineages exist within *Avaritia*, and that current species-group definitions are failing to expose them clearly enough. As mooted in Chapter 4, I believe that some 10 species groups consitute *Avaritia* worldwide. In this chapter two of these, the Orientalis and Imicola groups, are redefined and separated. Not only have they been used by authors as a catchall for a miscellany of species, but they also contain the most important vectors of bluetongue, African horsesickness and Akabane viruses currently known to occur in the Old World. This analysis has consequences and recommendations that include:

(i) adjustments being made to current species group lists;

(ii) new and seldom-used characters are presented that should be employed in future species descriptions and group definitions;

(iii) in future studies, larger series of both sexes should be scored to better understand inter- and intra-specific variation;

(iv) the accurate observation of small but important character states also requires that *Avaritia* specimens be more carefully prepared during slide-mounting;

(v) a combination of (ii) — (iv) has left the systematics of the south-east Asian Orientalis group in some disarray, and is discussed.

11.2 MATERIALS AND METHODS

Nearly 600 slide-mounted males and females of nine species of the Imicola group were examined, and provided a better understanding of the taxa than can be claimed for the Orientalis group. Fifty males and females of the latter group, collected in Thailand and the Philippines, were studied. As the males provide more group-specific data, they were intensively studied and are used to spearhead arguments that the systematic study of the Orientalis group in south-east Asia is in some disarray. Data on the male antennal trichodea distributions of the three known Afrotropical species of the Trifasciellus subgroup (synonymous with the Orientalis group) were taken from Itoua & Cornet (1986), and supplemented by examination of 20 specimens of *C. trifasciellus* in the Onderstepoort collection.

The 11 Orientalis group species are lettered sp. #A — sp. #K; only four have been identified with confidence, namely *jacobsoni* (sp. #A), *dumdumi* (sp. #B), *pungens* (sp. #C) and *fulvus* (sp. #D). It is probable that sp. #E is *maculatus* and sp. #F *orientalis*. Each putative species was identified using either keys, illustrations or descriptions found in the works cited.

Illustrations were made at 400 x magnification from slide-mounted material in which the genitalia were clearly and symmetrically displayed. Each one of numerous spicules on the ninth sternal membrane has been illustrated as observed, to faithfully reproduce their size, position and density of distribution accurately. In the same figures the aedeagus and sternum have been fractionally displaced anteriad of the tergum so that the sclerotised median cleft of the tergum can be clearly depicted. Excepting for the short bristle found at the base of the apicolateral processes of most species, the spiculation and setation of the basimere and tergum nine have been omitted.

The long and short blunt-tipped sensilla trichodea on male and female flagellar segments III–XII are labelled L and c respectively, following the nomenclature of Itoua & Cornet (1986). Sensilla that are atrophied or under-developed are shown as a ¾L, ½L or ¼L in Tables 11.4 and 11.5; Table 11.2 is less detailed, giving only presence/absence data. Derivation of the wing costal ratio (CR), antennal ratio (AR), and antennal trichodea ratio (AtR) are given in the text where appropriate.

11.3 RESULTS AND DISCUSSION

Despite problems with specific identifications, it is clear that the Orientalis and Imicola groups represent two lineages within *Avaritia*, and can be quite easily separated on the 18 character states listed in Table 11.1; the nine that appear in bold type are considered the more important. Each of the characters is discussed below, and comments are made on its reliability and variability both within and between species. In the female two are compared in Figs. 11.1a–11.1d, in the male seven are shown in Figs. 11.3a–h, while the eighth, the antennal blunt-tipped trichodea distribution on flagellar segments III–XII, is presented in Table 11.2. The data on the Orientalis group in Table 11.2 (including the three African species of the Trifasciellus group) summarize what is given in greater detail in Table 11.4, and is provided to demonstrate that species of the Orientalis group mostly have more trichodea than those of the Imicola group. In Table 11.3, all Old World species are assigned to their respective groups; synonyms are included following Wirth & Hubert (1989) and Boorman (1991). The south-east Asian species *C. hui* is included under the Orientalis group; reservations as to its true position within *Avaritia* are submitted and discussed. Eleven of the 19 species included by Wirth & Hubert (1989) in the Orientalis group are omitted; they belong either in the Imicola group (i.e. *C. brevitarsis, C. imicola, C. nudipalpis* and *C. pseudopallidipennis*), or to as yet undefined groups (i.e. *C. boophagus, C. brevipalpis, C. dikhros, C. fragmentum, C. pastus, C. suzukii* and *C. wadai*).

The broader group definitions are followed by a more detailed appraisal of slide-mounted specimens of the Orientalis group species, with special emphasis on the male; detailed data on their antennal trichodea distributions are given in Table 11.4. In Table 11.5 other male character states are listed that correlate with species-specific differences indicated in Table 11.4, and support the contention that at least 11 species (12 if *C. hui* is included) of the Orientalis group exist in south-east Asia. The more obvious differences, such as the shape of posterior margin of tergum nine and spiculation of sternal membrane, are illustrated (Figs. 11.4a–k). The data are used to explore incongruities in the literature on the south-east Asian Orientalis group, and help to explain why it is difficult to identify individual specimens held in collections. As regards females of the Orientalis group, Table 11.6 summarizes data on the antennal ratio (AR) as given by various authors, and is compared with data on the Imicola group. While there is a paucity of data, the great variability in recorded values reinforces the notion that the Orientalis group embraces more species than currently recognized.

11.3.1 **Character states defining the Orientalis and Imicola groups** (Table 11.1)

♀: 1. **Abdomen: dorsal pigmented tergites**

As shown in Fig. 11.1a, species of the Orientalis group always have large, rectangular tergites dorsally on abdominal segments II–VII whereas in the Imicola group they are reduced on segments III–V being smaller and appear almost round at low magnifications (Fig. 11.1c). This feature is the most reliable for assigning a specimen to its respective group when viewed under a dissecting microscope. Unfortunately, the tergites are only clearly visible in nulliparous females as the burgundy pigmentation laid down in the abdomens of older parous and gravid females tends to obscure them. Similarly, during slide-mounting, these tergites fade in material treated in KOH, and are further obscured in females whose abdomens are mounted ventral side up for better examination of the spermathecae and sclerotized plates surrounding the gonopore. While the shape of these tergites remains unknown for other species groups, it is probable that some will share the conformations described here.

2. Abdomen: sclerotized plates surrounding gonopore

As illustrated (Fig. 11.1b, 11.1d), the precise form of these plates differs between the two groups. In the Imicola group they are simpler i.e. lack the forefinger and thumb-like projections that partially embrace the gonopore opening, and seen in all Orientalis group species. Their precise delineation depends strongly on specimens being carefully prepared, especially cleared of pigment and the abdomen stretched as the distal segments can be telescoped. To my knowledge the shape of these plates has only been used once before as a taxonomic character in the genus *Culicoides* (Wirth & Hubert 1989), and its usefulness for defining other species-groups still needs to be ascertained.

3. Legs: hind tibial comb

The first, and longest, of five spines comprising the hind tibial comb is equally long in both species groups. While it appears to be more darkly pigmented and more robust in the Orientalis group, it must be noted that degree of pigmentation can be affected by the clearing process during slide-mounting. Also, due to its relatively small size, this spine is difficult to measure accurately and thereby establish the degree of difference that may exist between the two groups.

4. Wing: Costal ratio (CR)

This is obtained by dividing the length of the costa (measured from the arculus to the tip of the second radial cell) by the total wing length (also measured from the arculus). As shown in Table 11.1, this value is higher in the Orientalis group, i.e. the costa in each species of this group is fractionally longer than that found in members of the Imicola group.

5. Antenna: Antennal ratio (AR)

As indicated in Table 11.1, the antennae of species of the Orientalis group are more slender than those of the Imicola group; this may create the impression, falsely, that they are also longer. However, there is little exact data on total antennal length in the literature as they are either omitted by authors (Kitaoka

FIG.11.1

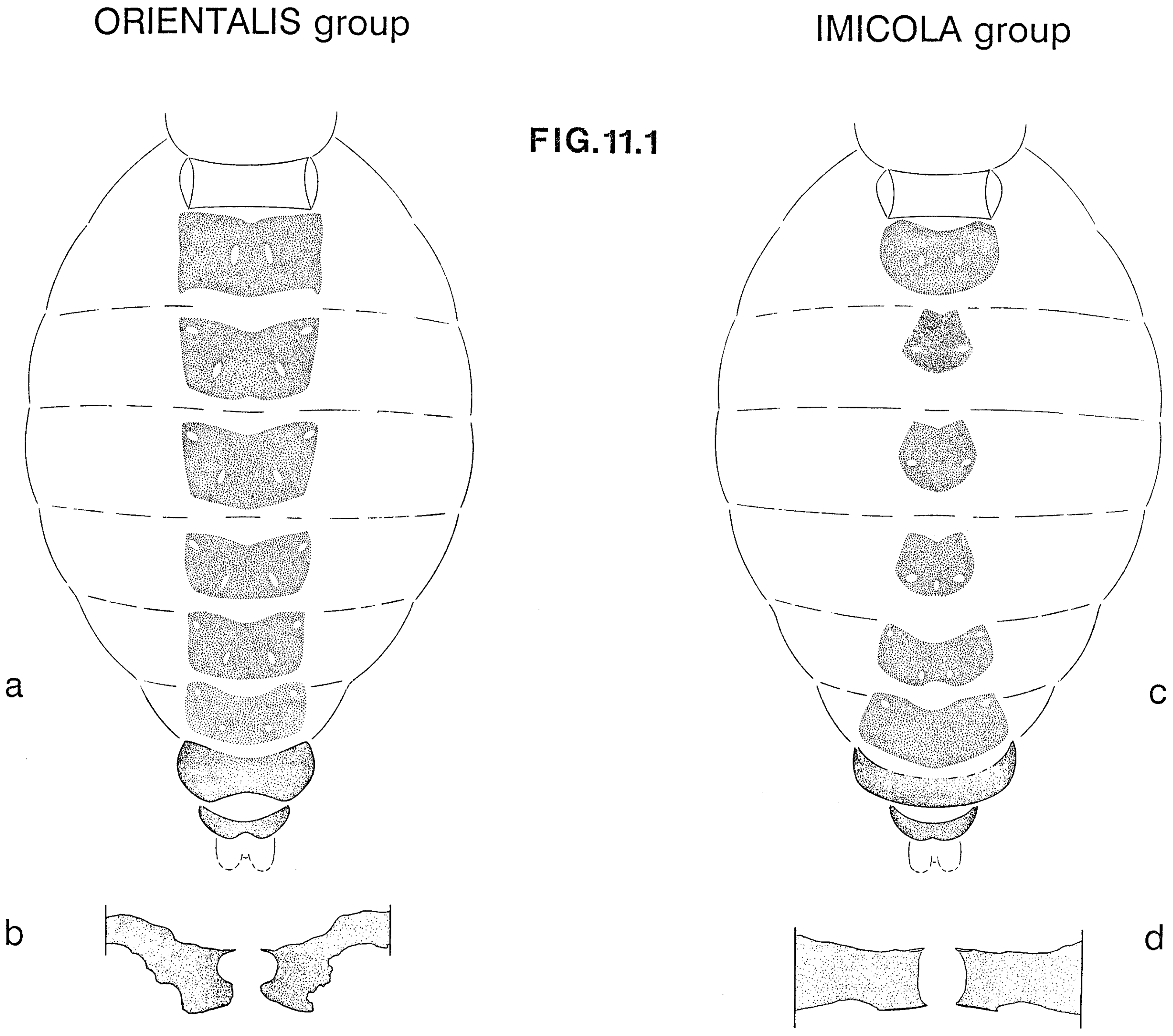


Fig. 11.1a Female abdomen (Orientalis group): dorsal pigmented plates

Fig. 11.1b Female abdomen (Orientalis group): sclerotized plates surrounding gonopore

Fig 11.1c Female abdomen (Imicola group): dorsal pigmented plates

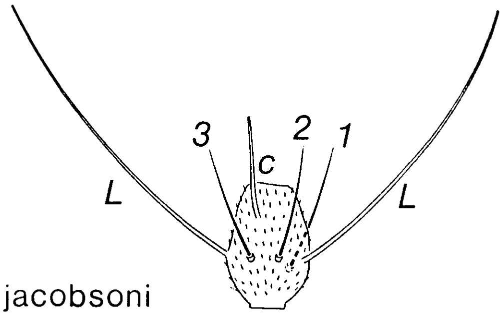
Fig. 11.1d Female abdomen (Imicola group): sclerotized plates surrounding gonopore

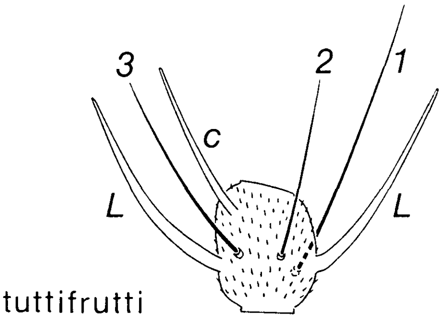
1985), or flagellar segment lengths are only given as proportions (Wirth & Hubert 1989). To overcome the range in variation that measurements of small to large specimens of a species would give, the antennal ratio (AR) has been widely adopted by researchers working in *Culicoides*; the AR is obtained by dividing the sum of the lengths of the five apical segments (XI–XV) by the sum of the basal eight segments (III–X). As remarked upon by Campbell & Pelham-Clinton (1960), the AR must be based on precise measurements if it is to be of any value. In Table 11.6, the AR data on 17 species of the Imicola and Orientalis groups are presented according to 12 sets of authors. If perused carefully, it reveals discrepancies between authors describing the same species. For example, Wirth & Hubert (1989) give an antennal ratio for eight Orientalis group species (43 specimens) that ranges between 1,07–1,47; for five of these species, Kitaoka (1985) gives a narrower range of 1,04–1,26 (number of specimens unknown), which is comparable to the 1,09–1,28 generated in this study for seven species (10 specimens).

The Imicola group AR data generated here from 562 specimens (nine species; Table 11.6) show a lower group-ratio, ranging between 0,92–1,25. This does, however, overlap considerably with ratios published on the Orientalis group. While it can be argued that far too few specimens of Orientalis group species were examined by the cited workers, it must be pointed out that the range in their values should thus have been smaller, and not larger, as shown in Table 11.6. For example, Wirth & Hubert (1989) derived from 10 specimens of *C. pungens* an AR that ranged between 1,27–1,47. In this study, 167 specimens of *C. imicola* gave a more conservative range of 0,95–1,10. Indeed, fairly large samples of the remaining Imicola group species (Table 11.6) revealed a consistent deviation between upper and lower values. This consistency is entirely absent in the Orientalis group data, and either indicates mixed species or technical problems. As discussed under Materials and Methods (Chapter 1) only antennae removed from the head, mounted straight and flat, were used to obtain the Imicola group measurements presented in this study. Antennae left on a rounded head and/or lying curled, and at varying angles in thick mountant, would contribute to the type of inconsistent data that currently exist for the Orientalis group.

Despite apparent shortcomings in the data available for the Orientalis group, it seems justified to conclude that its AR is higher than that found in the Imicola group. However, considerable overlap nullifies its value as a group character. These two groups can be separated on other features of the

FIG. 11.2





		C. jacobsoni		*C. tuttifrutti*
length segments 3-10	:	213		209
length segments 11-15	:	194		224
total antennal length	:	407		433
antennal ratio (AR)	:	0,91	*	1,07
length segment VI	:	20,5		23
width segment VI	:	14,5		18
no. chaetica segment VI	:	3		3 or 4
no. trichodea segment VI	:	LLc		LLc
length short trichodea (c)	:	19	*	29
length long trichodea (L)	:	61	*	38
length chaetica 1	:	22,5	*	50
antennal trichodea ratio (AtR)	:	2,98	*	1,65

Fig. 11.2a Female antenna (Orientalis group): Various sensilla on flagellar segment VI of *C. jacobsoni*

Fig. 11.2b Female antenna (Imicola group): various sensilla on flagellar segment VI of *C. tuttifrutti* antenna as will be shown for the male. Figs. 11.2a and 11.2b are of the sixth antennal segment of *C. jacobsoni* (Orientalis group) and *C. tuttifrutti* (Imicola group) respectively. Accompanying the illustrations are various measurements and ratios; those values showing significant differences are indicated with an asterisk. These reveal that, in spite of the two species having antennae of similar length and the same sensilla coeloconica distributions, they can be easily separated on the antennal ratio (AR) and the respective lengths and thicknesses of the long trichodea (L) and chaetica (1, 2 and 3). The large difference in long trichodea length explains the discrepancy in antennal trichodea ratios (AtR), where the length of L is divided by the length of segment VI; in *C. jacobsoni*, these trichodea are also very slender. In the same species, the flagellar segment is also narrower than that of *C. tuttifrutti* and so makes for an antenna that is more slender than, but nearly equal in length to, that of the more robust *C. tuttifrutti*.

6. **Antenna: sensilla coeloconica**

As shown in Table 11.1, all species of the Orientalis group have sensilla coeloconica distributed on flagellar segments III, XI–XV. Seven of the nine species of the Imicola group differ in having a III, XII–XV distribution; two, *C. pseudopallidipennis* and *C. tuttifrutti*, have coeloconica on III, XI–XV, while a third, *C. nudipalpis*, has this distribution in approximately 50 % of specimens. It is fair to say that more than 80 % of the 70 species of the subgenus *Avaritia* found worldwide will share one of these two distribution patterns, irrespective of species group, and therefore they have limited value as a group character.

♂: 7. **Genitalia: aedeagus**

In the Orientalis group, the infuscated 'peg', a prolongation of the distal process that projects into the aedeagus, narrows anteriorly to end in a sharp to blunt point (Figs. 11.3b, 11.4a–k), and is rather smoothly pigmented. In the Imicola group, the anterior end of the peg differs in that it usually expands in a more irregular manner (Figs. 11.3f, 2.20, 2.21, 4.12, 5.12, 6.9, 6.18) and, is not smoothly pigmented, but granular.

8. **Genitalia: aedeagus**

In the Orientalis group, the infuscated peg is connected to the lateral converging sclerotised arms of the

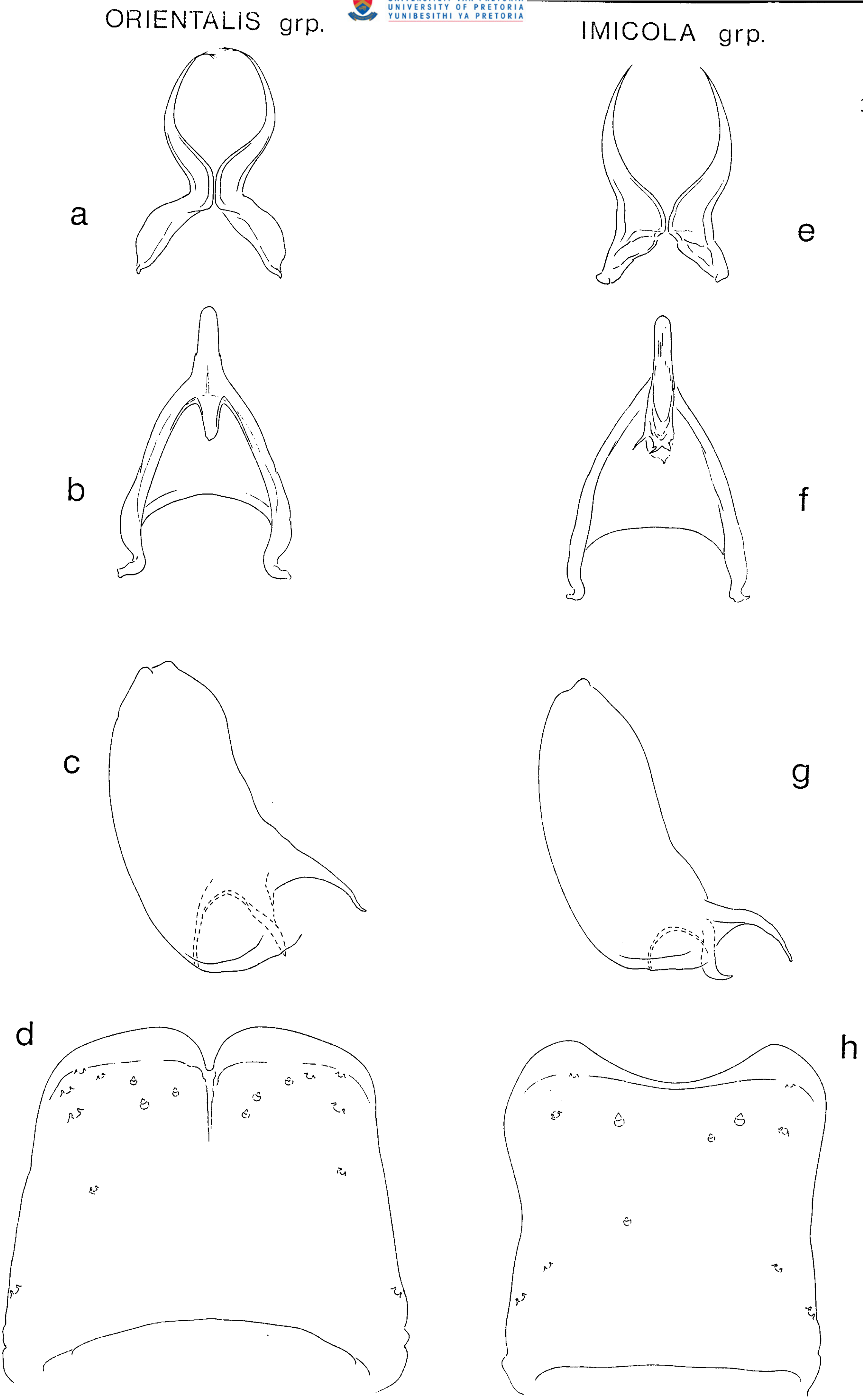


FIG. 11.3

Fig. 11.3a–d Male genitalia (Orientalis group: sp. #F); a: parameres; b: aedeagus; c: basimere; d: tergum nine

Fig. 11.3e–h Male genitalia (Imicola group: *C. imicola*); e: parameres; f: aedeagus; g: basimere; h: tergum nine aedeagus by a lightly pigmented membranous arch (Fig. 11.4a–k). This arch is better developed in some species (sp. #D, #E, #H, and #K) than in others; it is absent in all species of the Imicola group.

9. **Genitalia: parameres**

Comparison of the parameres in Fig. 11.3a and e indicates more than one difference to exist between the two species groups. These are the angle of projection, the degree of development, the shape of the anterior feet, and the configuration of the posterior tips. In the Orientalis group, the tips of the parameres are always sinuous, most often being limp and recurved, and can be bare (seldom) or inconspicuously to obviously filamentous or feathered (Fig. 11.3a; Table 11.5). In some species this tip has been described as bare but its precise observation depends on high magnification (800–1000 x) and clearly prepared material. For example Wirth & Hubert (1989) described the parameres of *C. flavipunctatus* and *C. orientalis* as 'tapering distally to a simple filamentous tip', but illustrated them as feathered. This could be the result of mixed series, or conflicting observations made by the taxonomist and co-operating illustrator. In the Imicola group, all species, without exception, have the tip of the parameres erect, sharp and simple (Fig. 11.3e).

10. **Genitalia: tergum nine**

Figs. 11.3d, 11.4a–k show that in the Orientalis group, the apicolateral processes are broad, subtlely triangular in shape, and, importantly, the apex of the process is found sublaterally. Furthermore, these processes are always narrowly and fairly abruptly separated medially by a deep and clearly pigmented subtriangular excision (Figs. 11.3d, 11.4a–k). In the Imicola group, the apicolateral processes are not as broadly developed, and arise on the posterolateral corners of the tergum. The processes are thus broadly separated by a gently concave posterior margin that is not infuscated but, at most, may be striated medially (Figs. 4.11, 5.11, 6.8, 6.17). While the configuration of the apicolateral processes described as characterizing the Imicola group may be found outside the group, that described for the Orientalis group does not seem to occur in other species of *Avaritia*.

11. **Genitalia: basimere**

Fig. 11.3c shows the basal half of the basimere of most species of the Orientalis group to be broader than the apical half, the ventral root arises at a shallower angle (45 °– 75 °), and the dorsal root is

Fig.11.4

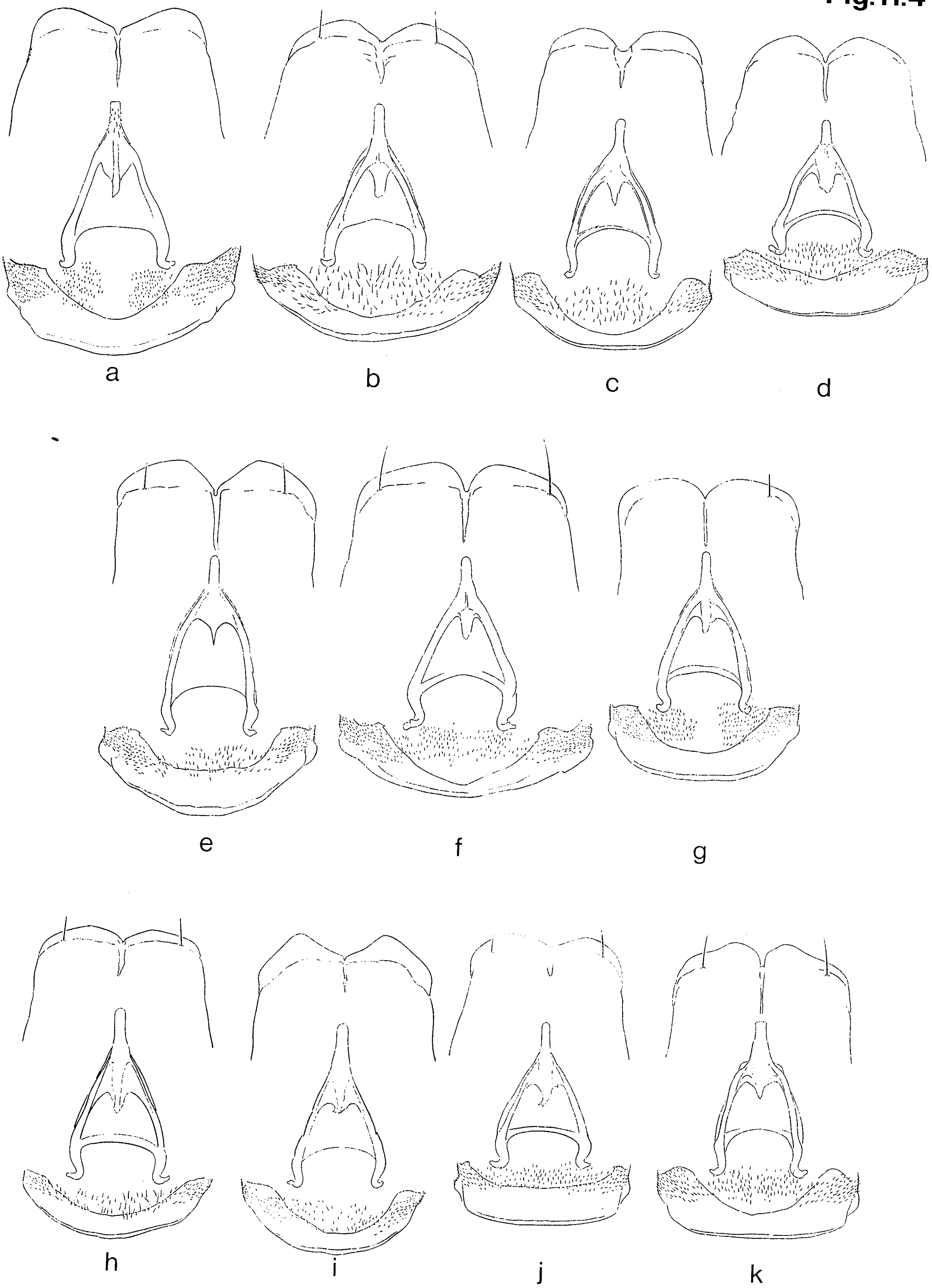


Fig. 11.4a–k Male genitalia (Orientalis group) showing posterior half of tergum nine, aedeagus and spiculation on membrane of sternum nine of 11 putative species.

almost straight. In the Imicola group (Fig. 11.3g), the basimere is nearly parallel-sided for its entire length, and the dorsal and ventral roots are distinctly bowed almost like a curled forefinger and thumb. Furthermore, the ventral root almost arises at right-angles (75 °–90 °) from the basimere. In all species of the Imicola group, the shorter dorsal root is rather broad and always curls around the base of the paramere.

12. **Genitalia: tergum nine**

In the Orientalis group, the tergum is trapezoidal in shape, gradually narrowing posteriorly (Fig. 11.3d, 11.4a–k); in the Imicola group, it is almost square, as wide anteriorly as posteriorly, and noticeably waisted medially (Fig. 11.3h). This character is best observed in specimens where the genitalia have not been squashed by coverslipping. In compressed specimens the anterior half of the tergum, in being three-dimensional where it is fused to the narrow strip that forms sternum nine, tends to splay outwards i.e. becomes trapezoidal, and so gives a false impression of its true shape.

13. **Genitalia: sternum nine**

In the Orientalis group, the membrane of sternum nine is never bare but always spiculate. In Figs. 11.4a–k it can be seen that this spiculation is fairly diagnostic for each species, being either sparse to moderately abundant (spp. #C, #E, #H and #I), or abundant to very abundant (spp. #A, #D, #G and #J), short (spp. #A, #F and #G), moderately long (spp. #C, #D, #E, #I and #K) to long or very long (spp. #H and #B), or distributed evenly (most species) or in two discrete patches (spp. #A and #G). In the Imicola group, two-thirds of the species i.e. *bolitinos, brevitarsis, loxodontis, pseudopallidipennis*, *tuttifrutti* and *kwagga*, have the membrane bare or with a few spicules found near the margin of the excavation (Figs. 2.20, 5.12, 6.9, 6.18). In the remaining species, *imicola, miombo* and *nudipalpis*, the spiculation ranges from being moderate to dense, but the spicules do not vary in size, being all short (Figs. 2.21, 4.12).

Although spicules on the sternital membrane can be present or absent in a group, and thus of limited value when assigning species, the degree, size and precise distribution of these spicules is indifferently treated in taxonomic studies. For example Wirth & Hubert (1989) describe the sternal membranes of *jacobsoni* and *maculatus* as spiculate but illustrate them as bare, while Kitaoka (1975), in his original description of *flavipunctatus*, stated the membrane to be bare, whereas his 1985 illustration of the same species shows it to be spiculate. In some instances the position of the spicules appear to be group- rather than species-specific. I am aware of *Avaritia* species not having spicules on the membrane but spread across the sclerotised anterior part of the sternum. As they do not lie exposed on the membrane in the excavated area, they may not be referred to, or noticed, by systematists. This rather important issue of spiculation is further complicated when the eighth abdominal segment is allowed to lie folded over sternum nine during preparation of slide-mounts. The dense spiculation of sternum eight thus overlies and obscures that which may be present on nine; while the spiculation is of a different type on these preceding segments, it is difficult to distinguish them as the respective membranes tend to subside onto a single plane during coverslipping.

14. Antenna: Long (L) and short (c) blunt-tipped sensilla trichodea

Comparison of the blunt-tipped trichodea distributions found in the Orientalis and Imicola species group (Tables 11.2 and 11.3) reveals that:

a) Despite some variation, the 14 Orientalis group species have significantly more trichodea on flagellar segments III–XII. These number 17–31/antenna as compared to the 16–18 found in the Imicola group (nine species). This is mainly due to most Orientalis group species having an additional long trichodea on X, and usually an additional short one on XI and XII.

b) While the trichodea distributions in the Imicola group are remarkably stable and similar, there exists a greater degree of interspecific variation in the Orientalis group. The trichodea distribution of *C. jacobsoni* is unusual, but this species must be regarded as an outlier as it is also the only one bearing retrorse spinules along the tip of the aedeagus (Fig. 11.4a). However, *C. jacobsoni* bears a number of Orientalis group distinctions that include the shape of the basimere, configuration of apicolateral processes, and 'arched' peg of the aedeagus. The same comments apply to *C. fulvus*, which also has a reduced number of antennal trichodea. This diversity in the Orientalis group implies an active and long evolutionary history and suggests that subgroups remain to be discovered.

c) In Table 11.2 the large number of trichodea encased in brackets suggests that a higher degree of intraspecific variation occurs within the Orientalis than in the Imicola group, but the more detailed data given in Table 11.4 indicates that this is not so. For example four specimens of sp. #F consistently had one of the L trichodea underdeveloped (exactly as recorded for *C. pseudopallidipennis* and *C. tuttifrutti* of the Imicola group). It can be seen that a similar pattern of reduction marks the antennae of sp. #G. Indeed, the two species (#F and #G) are very difficult to separate, differing only in the shape of the apicolateral processes and in that the former possesses a seta at the base of each process (Fig. 11.4f, g). Species #G is illustrated as having the spicules on the membrane in two distinct patches (Fig. 11.4g), whereas in #F they are shown as narrowly fused (Fig. 11.4f). However, they occur in two patches in a second specimen (Thailand 227; Table 11.5). Either one of those two species could be *C. orientalis* s.s.; it appears to be sp. #F if we compare the genitalia (Fig. 11.3a–d) to those given in its redescription by Dyce (1980). Along with sp. #E (Fig. 11.4e), which strongly resembles *C. maculatus* as illustrated by Kitaoka (1985) and Wirth & Hubert (1989), these two species indicate the presence of a subgroup. It is notable that the aforementioned *C. pseudopallidipennis* and *C. tuttifrutti* also have atrophied trichodea, and also form a distinct subgroup within the Imicola group. Various other characters justify the latter pair of species being split off as a subgroup. This aberration in the development of antennal trichodea has been consistently found in various populations of *C. tuttifrutti*, and has not been linked to cryptic speciation. Its unexpected existence underscores the necessity of examining long series of adults to obtain data that more accurately define a species.

11.3.2 Anomalies in the literature on the Orientalis group

As noted earlier, the systematics of this group in south-east Asia is in partial disarray. Incongruities in the published literature create doubt as to the exact identity of specimens, and this is exacerbated by descriptions being too brief, illustrations too stylized, and too few specimens being examined. Some of these difficulties are discussed below; notes are also given on the annectent taxonomic position of *C. hui*.

1. a) The key to species of *Culicoides* of south-east Asia, commencing on p. 57 in Wirth

& Hubert (1989), contains an error that leads the user astray: couplet 108 (p. 68) gives two choices, these leading to couplets 109 and 110; the latter should be numbered 111. Similarly, in couplet 109 the numbered choice should be '110' and not '111' as given.

b) In couplet 113 it is stated that 'sensilla chaetica (verticils) on segments XI–XV usually reduced' or 'well developed'. *Avaritia* species never have sensilla chaetica on these segments, only on segments III–X. Should read: 'sharp-tipped sensilla trichodea ...'

2. *C. fulvus* Sen & Das Gupta, 1959

Wirth & Hubert (1989) state:

a) 'ninth sternum with broad, shallow caudomedian excavation'. Fig. 117g (p. 268) shows it to be narrow if compared with all other Orientalis group species.

b) 'aedeagus 1,8 x as long as basal breadth ...' Measurement of Fig. 117g shows it to be 1,35.

c) 'aedeagus ... basal arch low, a fifth of total length ...' Fig. 117g shows it to be closer to a third.

d) parameres described (and illustrated) as 'tapering distally to a slender tip bearing minute fringing hairs'. In the discussion it is said that 'the long distal portion tapers to a fine tip.'

e) 'basistyle with ventral root long and slender ...' Fig. 117g shows ventral root entirely absent.

Similar discrepancies between the text and illustrations were noted above for other species of the Orientalis group described by Wirth & Hubert (1989). As observed, this could be the result of conflicting observations made by the taxonomist and the co-operating illustrator, or could be due to mixed series. It is pertinent to comment that Wirth & Hubert's material of many of the 168 species covered in their treatise of south-east Asian *Culicoides* came from a broad geographic area. While the localities are recorded, we are given hardly any information as to which specimens were described, or which were illustrated or photographed. It is thus impossible to link described material with a precise locality, or to establish whether the males were captured with the females or came from widely separated locales.

3. *C. flavipunctatus* Kitaoka, 1975 and *C. fulvus* Sen & Das Gupta, 1959

There appears to be some confusion as to the status of these two species. Wirth & Hubert (1989) separate them on central wing markings and shape of the apicolateral processes on tergum nine. They do not refer to, or use, the characters employed by Howarth (1985) who distinguishes them on colouration of the halteres and thorax, the intensity of darkness of the first costal wing spot, and the shape of the spermathecae. Unfortunately, Howarth did not redescribe these species but did note that *C. fulvus* 'is confusingly similar to' *C. flavipunctatus*, and 'except for the differently shaped spermathecae ... could be considered to represent pale specimens of *C. fulvus*. He goes on to say '... they occur together ... from widely scattered localities in Laos'. Wirth & Hubert (1989) echo the conclusions of Howarth in noting 'that the wing markings of *C. flavipunctatus* intergrade gradually into those of *C. fulvus* ... the two forms occur together in Indonesia.' Doubt is heightened when one refers to Kitaoka's original description of *C. flavipunctatus*. Judging from his wing photographs, the male and female are correctly associated; the ninth sternal membrane is described and illustrated as bare, but in a later publication it is figured as spiculate (Kitaoka 1985). If one accepts that Kitaoka was originally mistaken (as no members of the Orientalis group have bare membranes), one can then compare his 1985 illustration with that given by Wirth & Hubert (1989). Two differences become apparent: (i) the apicolateral processes on tergum nine appear to differ in shape, and (ii) the aedeagus illustrated by Wirth & Hubert (1989) has rather pronounced shoulders (but not described). The genitalia figured by Wirth & Hubert (1989) strongly resemble those illustrated here for C. sp. #K (Thailand 228); both have the unusual wavy shape of the posterior margin of tergum nine, shoulders on the aedeagus and same shape of basimere and distimere. Indeed, the posterior of tergum nine of C. sp. #K is very similar to that illustrated by Tokunaga (1951) in his original description of *C. suborientalis*. The females collected in southern Thailand in association with the C. sp. #K male nearly key out to *C. flavipunctatus* in Wirth & Hubert (1989), but fail as they do not have the dark spot at the base of wing vein M_2 isolated from, but have them broadly joined to, the dark area over base of vein M_1. In Kitaoka (1975, 1985), this spot also appears to be isolated but is too indistinctly figured, and not described. It is relevant to note that the narrow membranous area between the base of vein M_1 and the subbasal part of M_2 is concave or sunken; when photographed, this area is usually out of focus and can create the impression of being fractionally paler.

As noted earlier, Howarth (1985) separated *C. flavipunctatus* from *C. fulvus* on spermathecal size being 'about 1,4 x as long as wide' in the former and 'about 1,3 x ...' in the latter. The measurements for both spermathecae of *C. flavipunctatus* given by Kitaoka (1975) and Wirth & Hubert (1989) range between 1,24–1,31, thus matching those given for *C. fulvus* by Howarth (1985). The latter species was originally described from four males (Sen & Das Gupta 1959), and the female later described by Das Gupta (1962). Dyce & Wirth (1983) examined this material, noting that the 'unusual asymmetrical enlargments on the terminal sector of both parameres' illustrated by Sen & Das Gupta were in fact 'droplets of an oily residue.' While Dyce & Wirth (1983) recognized *C. fulvus* as a valid species, they did not redescribe it, and it is unknown whether Wirth & Hubert (1989) used part of the type series housed in the USNM in their later redescription. Furthermore, they do not refer to Howarth's distinguishing features, but instead compare *C. fulvus* with *C. brevitarsis*, a more simple exercise when one considers that they belong to two species groups.

As regards the biology of *C. flavipunctatus*, Howarth (1985) reared two males from 'old decomposed elephant feces collected ... in a shaded damp woodland ... Laos.' In the Afrotropical Region, five *Avaritia* species have been reared from elephant dung (see Chapter 8). These species are resource specific, and furthermore only utilize dung for oviposition when it is absolutely fresh. Howarth does not estimate the age of the elephant dung from which he reared *C. flavipunctatus*; we only know the two males emerged 12 days post-harvest. It is therefore unknown whether *C. flavipunctatus* utilizes fresh dung only, or will lay its eggs in any type of damp decomposing plant material. The distinction is important: is *C. flavipunctatus* primarily associated with elephants and their dung (as has been shown for five African species), or is it not? If it is, then one may go on to ask: are all mainland south-east Asian records conspecific with Kitaoka's original type series of *C. flavipunctatus* which was collected on the Nansei island where no elephants occur? It is notable that two other species of the Orientalis group have been reared: *C. dumdumi* from rotting seaweed, and *C. jacobsoni* from decaying fruits and mushrooms, ginger flowers and banana stems. These data suggest that *C. flavipunctatus* could utilize a range of moist vegetative materials for development of the immatures. The five species reared from elephant dung in Africa belong to four species groups within the subgenus *Avaritia*, but not to the Orientalis group. However, occasional specimens of a species resembling *C. trifasciellus* of this group have been collected in South Africa, and found to carry *Myianoetus* mites, a sure sign that the adults had emerged from dung. Unfortunately, this species is rare; like *C. flavipunctatus*, its precise association with dung also remains to be discovered.

This resumé indicates a clear need for *C. flavipunctatus* and *C. fulvus* to be redescribed and carefully compared. As conceded by Wirth & Hubert (1989), '... more data need to be gathered and checked' as '... it is possible that differences exist in proboscis length and antennal ratio.' It is also possible that more than two closely related species exist.

4. *C. pastus* Kitaoka, 1980

Examination of Wirth & Hubert's 1989 redescription of the female reveals that they have taken the numerical data directly from Kitaoka's original description of *C. pastus*, but introduced minor changes and made omissions, in the description of the palpus, legs and spermathecae. For the male they have added the note that the ninth tergum has a 'median cleft present, caudal margin with broad low rounded lobes.' This is reflected in the illustrated genitalia and is as typically seen in the Orientalis group. However, their tergum is distinctly different from that shown by Kitaoka (1980, 1985) who figures the apicolateral processes widely separated, much as those found in the Imicola group. There is also confusion as to whether the ninth sternal membrane of *C. pastus* is spiculate or not. Wirth & Hubert (1989) describe it as bare but illustrate it as spiculate; Kitaoka (1980, 1985) consistently illustrates and describes it as bare.

It seems clear that Wirth & Hubert did not have the male of *C. pastus* before them; I follow the original author, as his male and female are correctly associated judging from the wing photographs. However, his description of *C. pastus* is too superficial for it to be assigned to the Imicola group. Indeed, *C. pastus* probably belongs in an as yet undefined group, as it has large spermathecae with irregular margins and the apices of the parameres limp; the apices have been described both as feathered (Kitaoka 1980) and simple (Kitaoka 1985).

5. *C. hui* Wirth & Hubert, 1961

As noted elsewhere, I have been reluctant to include this species in the Orientalis group (Table 11.3) for the following reasons:

♀: a) Wing pattern unusual, but more like that found in species of the Imicola group. Pale spot in the base of cell M_1 does not align with those found at the apex of the second radial cell, medially in cell M_2 and M_{3+4}. It thus creates the overt 'zigzag' typifying species of the Imicola group.

b) Costal ratio (CR) of 0,61–0,64 is at the bottom end of the Orientalis group range, and at the top of the Imicola group range.

c) Pigmented terga on dorsum of abdomen appear to be large and rectangular as in the Orientalis group.

d) Pigmented sclerites surrounding the gonopore are simple, much as seen in the Imicola group.

e) First spine of hind tibial comb not markedly pigmented or robust like that seen in Imicola group.

f) Basal antennal segments IV and V shortish (Imicola group), VI–X slightly longer and barrel-shaped (Imicola/Orientalis group).

g) Antennal ratio (AR) ranges between 1,00–1,07, average 1,04, in two specimens available (1,12 according to Wirth & Hubert 1989); overlaps the Imicola group range of 0,92–1,25, rather than that of 1,04–1,47 recorded for the Orientalis group (Table 11.6).

h) Sensilla coeloconica III, XI–XV like that of the Orientalis group but not excluding Imicola group.

i) Blunt-tipped sensilla trichodea on basal flagellar segments IV–X moderately long and robust (Imicola group).

♂ j) Ninth tergum: illustrated as subtlely trapezoidal by Kitaoka (1985) and Wirth & Hubert (1989), like that of the Orientalis group.

k) Ninth tergum: posterior margin 'broadly rounded caudad' but illustrated as straight (Wirth & Hubert 1989); in Kitaoka (1985) the margin is also shown as almost straight, and though it appears to be fractionally indented medially, it is obscured by parameres and tip of aedeagus (Imicola group).

l) Ninth tergum: according to Wirth & Hubert (1989) posterior margin 'with only slight trace of caudomedian indentation' (Imicola group).

m) Parameres: in Wirth & Hubert (1989) described 'as tapering and curving ventrally to ... slender filament; due to poor preparation presence or absence of minute hairs at tip not determined.' Their illustration (Fig. 118e), however, shows the parameres as erect (Imicola group) but with hairs at tip (Orientalis group). Kitaoka (1985) illustrates the tips of the parameres as recurved (Orientalis group) but bare (either group).

n) Parameres: in both Kitaoka (1985) and Wirth & Hubert (1989) the anterior feet of the parameres do not diverge widely and are also shaped much as seen in the Imicola group.

o) Sternum nine: both sets of authors describe and/or illustrate the membrane as bare (Imicola group).

p) Basimere: both sets of authors illustrate the ventral roots as not arising at right angles (Orientalis group).

q) Aedeagus: both sets of authors illustrate the 'peg' as not being joined by an arch to lateral converging margins of aedeagus (Imicola group).

This examination suggests that *C. hui* falls within the Imicola group, but half the characters scored are too poorly described in the literature for more definite conclusions to be drawn. Furthermore, certain characters are likely to be shared by other, as yet undefined species groups of *Avaritia*. For the interim, *C. hui* is retained in the Orientalis group.

11.4 CONCLUSIONS

The Orientalis and Imicola groups of the subgenus *Avaritia* are redefined. Both contain species widely distributed in, but restricted to, the Old World. The former group is more tropical in its distribution, with most species found in the Oriental Region; it includes the three Afrotropical species comprising the Trifasciellus subgroup of Itoua & Cornet (1986). Only eight of the 19 species assigned by Wirth & Hubert (1989) to the Orientalis group are retained; four are moved to the Imicola group, while the remaining seven belong to unknown species groups. Nine species compose the Imicola group; seven are African and are found in abundance outside the equatorial forest block in the lower rainfall subtropical woodlands and savanna grasslands. The Orientalis and Imicola group definitions *sensu* Wirth & Hubert (1989) and Glick (1990) respectively, are here considered too broad. Only three characters in the Wirth & Hubert (1989) diagnosis indicate that they were dealing with species in the Orientalis group. These are (i) high antennal ratio (AR), (ii) sensilla coeloconica on III, XI–XV, and (iii) costa moderately long. These and remaining characters occur widely outside the two groups under review. Not one of the characters listed by Glick (1990) defines the Imicola group; his diagnosis is only appropriate for the subgenus *Avaritia*.

Under the dissecting microscope, adult females of the two groups are easily separated on the abdominal tergal pattern. However, its use is largely limited to nulliparous females, and furthermore, it is unknown whether these tergal patterns are also found in other species groups. Although the wing patterns offer some group-specific clues, they are too interspecifically variable to be used reliably. Three features deserve mention: (i) as a general rule, the pale spot found at the tip of the second radial cell in all species of the Orientalis group combines with those found at the base of cell M_1, and medially in cells M_2 and M_4, to form a broadly pale and straight line that bisects the wing longitudinally. In the Imicola group, the small, but prominent, pale spot in the base of cell M_1 is positioned more proximally and thus a subtle zigzag of pale areas bisects the wing. (ii) In the Orientalis group, the short, slanting vein that forms the proximal boundary of the second radial cell is always thickened where it meets the costa. This thickening does not occur in the Imicola group. (iii) Most species of the Orientalis group have the anal angle dark; in the Imicola group seven of the nine species have it pale. It seems probable that each of these characters will also find expression in other species groups of *Avaritia*, and so lose their diagnostic value. As for slide-mounted specimens, the two groups can be easily separated on nine character states and, to a lesser degree, on another nine. When these are assessed in combination they will ensure correct assignation of a species. While both sexes are not essential for grouping, the male does offer more distinguishing features. It is likely that some of the parameters given here also occur outside the Orientalis and Imicola groups.

Evidence is presented that the systematics of the Orientalis group is in disarray. A preliminary study indicates that at least 11 species of this group exist in south-east Asia, three more than the number currently recognized. The admission by Wirth & Hubert (1989) that 'due to poor preparation' of slide-mounted specimens certain characters were 'not determined' to a great extent sums up problems surrounding the systematics of world *Culicoides*. For example, the males of 28 % of the 168 south-east Asian species treated by Wirth & Hubert (1989) remain unknown. The use of insufficient numbers of specimens of both sexes, and the lack of attention to detail as regards their preparation, description and illustration, are hampering progress in a genus, and specifically the subgenus *Avaritia*, that can only be described as very challenging taxonomically.

Finally, the descriptive format used worldwide on the genus is considered too superficial and stylized. New and seldom-used characters are presented here, and discussed. Workers are urged to adopt them if our understanding of *Avaritia* is to grow and attain precision. Correct identifications are essential for studies on the phylogeny, biology and biogeography of each species; in turn, these form the foundation for more detailed studies on exactly which species are most competent at vectoring viral diseases such as bluetongue and African horsesickness.

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Table 11.1 Subgenus *Avaritia*: 18 male and female character states defining and separating the Orientalis and Imicola groups (most important features in **bold** type)

Character state	Orientalis group	Imicola group
♀: abdomen	dorsal pigmented plates on segments II–VII more or less equal in size and shape i.e. large and rectangular (Fig. 11.1a)	pigmented plates vary in size, those on segments III–V significantly smaller and roundish (Fig. 11.1c)
genitalia	sclerotized plates partly surrounding gonopore with finger-like projections both anteriad and posteriad of opening (Fig. 11.1b)	sclerotized plates simpler, lacking pronounced finger-like projections, and thus not partly embracing gonopore opening (Fig. 11.1d)
legs	first spine of hind tibial comb moderately darkly pigmented, and longer and more robust than second spine	first spine less robust and not as darkly pigmented
wing	costal ratio (CR) higher ranging between 0,56–0,67, average 0,63 (eight species)	CR lower; 0,54–0,61, average 0,57 (nine species)
antenna	sensilla coeloconica distributed on flagellar segments III, XI–XV	sensilla coeloconica III, XII–XV except for *C. pseudopallidipennis* and *C. tuttifrutti* (III, XI–XV)
antenna	antennal ratio (AR) higher: 1,07–1,47; all segments more slender with basal ones elongate barrel- or flask-shaped (Fig. 11.2a)	AR lower: 0,92–1,25; all segments stouter with basal ones most nearly spherical to barrel-shaped (Fig. 11.2b
antenna	blunt-tipped trichodea on segments III–X moderately long to long and always slender (Fig. 11.2a)	trichodea vary in length from short to moderately long and may be slender to rather swollen (Fig. 11.2b)
♂: paramere	apex sinuous usually limp and recurved with tip inconspicuously to obviously feathered (Fig. 11.3a)	apex always erect with tip sharp and simple (Fig. 11.3e)
aedeagus	anterior end of infuscate 'peg' somewhat V-shaped and smoothly pigmented (Figs. 11.3b, 11.4a–k)	infuscate 'peg' irregularly globular and pigmentation granular (Fig. 11.3f)
aedeagus	infuscate 'peg' joined to lateral converging margins of aedeagus by a roundly arched, window-like pigmented area (Figs. 11.3b, 11.4a–k)	this arched connection absent (Fig. 11.3f)
tergum nine	apicolateral processes broad and overtly triangular in shape with apex of process usually positioned mesally along posterior margin of tergum (Figs. 11.3d, 11.4a–k)	apicolateral processes narrower and arising on postero-lateral corners of tergum (Fig. 11.3h)
tergum nine	apicolateral processes narrowly separated by a clearly infuscate median cleft or ravine (Figs. 11.3d, 11.4a–k)	processes broadly separated by a gently concave posterior margin that is not infuscated (but may be striated) medially (Fig. 11.3h)
tergum nine	trapezoidal in shape gradually narrowing posteriorly (Fig. 11.3d)	square in shape noticeably waisted medially (Fig. 11.3h)

Character state	**Orientalis group**	**Imicola group**
basimere	basal half broader than apical half (Fig. 11.3c)	more parallel-sided with basal half fractionally broader (Fig. 11.3g)
basimere	ventral root arising at 45° and moderately bowed; dorsal root straight and not 'cupping' base of paramere (Fig. 11.3c)	ventral root most nearly arising at right-angles and noticeably bowed; dorsal root broader and curls around base of paramere (Fig. 11.3g)
sternum nine	membrane adorned with long or short spiculae, never bare (Fig. 11.4a–k)	membrane bare in most species, or adorned with short spicules only
antenna	all species (excepting *jacobsoni*) with one long trichodea on segment X and most with a short trichodea on XI and XII	all species without these long and short trichodea on segments X–XII
antenna	bearing a total of 17–31 long and short blunt-tipped trichodea on flagellar segments III–XII (Table 11.2)	with a lower total of 16–18 trichodea (Table 11.2)

Table 11.2 Subgenus *Avaritia*: presence/absencedistribution of long (L) and short (c) blunt-tipped trichodea on male flagellar segments III–XII of Old World species of the Orientalis and Imicola groups

Species group	Species	Flagellar segments										Total trichodea
		III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Orientalis:	*jacobsoni* (#A)	LL	LLc	LLc	LLc	(L)Lc	c	Lc	c	-	-	17-18
Southeast Asia	*dumdumi* (#B)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	-	20
	pungens (#C)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	c	21
	fulvus (#D)	LL	LLc	(L)Lc	(L)Lc	Lc	Lc	Lc	Lc	(c)	(c)	18-20
	maculatus? (#E)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	c	21
	orientalis? (#F)	LL	LLc	LLc	LLc	LLc	(L)Lc	(L)Lc	Lc	c	(c)	21-24
	? (#G)	LL	LLc	LLc	LLc	LLc	(L)Lc	(L)Lc	Lc	c	c	22-24
	? (#H)	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	c	24
	? (#I)	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	c	24
	? (#J)	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	c	24
	flavipunctatus? (#K)	LL	LLc	LLc	LLc	Lc	Lc	Lc	Lc	c	-	20
Africa	*brosseti*	LL	LLc	LLc	(L)Lc	Lc	Lc	Lc	Lc	c	c	20-21
	trifasciellus	LL	LLc	LLc	LLc	LLc	LLc	LLc	Lc	c	(c)	23-24
	dubitatus	LLc	LLc	LLc(c)	LLc	LLcc	LLc	LLcc	Lc(c)	c(c)	(cc)	26-31
Imicola:	*brevitarsis*	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
Southeast Asia	*nudipalpis*	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
Africa	*bolitinos*	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	imicola	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	loxodontis	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	miombo	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18
	pseudopallidipennis	LL	LLc	(L)Lc	(L)Lc	Lc	Lc	Lc	c	-	-	16-18
	tuttifrutti	LL	LLc	(L)Lc	(L)Lc	Lc	Lc	Lc	c	-	-	16-18
	kwagga	LL	LLc	LLc	LLc	Lc	Lc	Lc	c	-	-	18

Table 11.3 Subgenus *Avaritia*: revised listing (alphabetical) of 21 *Culicoides* species constituting the Old World Orientalis and Imicola groups including author, original date and country of description, and synonyms (valid species in **bold** type, synonyms indented)

Orientalis group	Imicola group
Oriental/Palaearctic Regions:	Oriental/Palaearctic/Australasian Regions:
dumdumi Sen & Das Gupta, 1959: India	***brevitarsis*** Kieffer, 1917: Australia
flavipunctatus Kitaoka, 1975: Nansei Islands	*radicitus* Delfinado, 1961: Philippines
fulvus Sen & Das Gupta, 1959: India	*robertsi* Lee & Reye, 1953: Australia
hui Wirth & Hubert, 1961: Taiwan	*superfulvus* Das Gupta, 1962: India
jacobsoni Macfie, 1934: Sumatra	***nudipalpis*** Delfinado, 1961: Philippines
buckleyi Macfie, 1937: Malaysia	***imicola*** Kieffer, 1913: Kenya
kitaokai Tokunaga, 1955: Japan	*iraqensis* Khalaf, 1957: Iraq
unisetiferus Tokunaga, 1959: Papua New Guinea	*minutus* Sen & Das Gupta, 1959: India
maculatus (Shiraki), 1913: Taiwan	*pseudoturgidus* Das Gupta, 1962: India
kii Tokunaga, 1937: Japan	
kyotoensis Tokunaga, 1937: Japan	
sigaensis Tokunaga, 1937: Japan	
suborientalis Tokunaga, 1951: Java	
tainana Kieffer, 1916: Taiwan	
orientalis Macfie, 1932: Malaysia, India, Java (syntype series)	
nayabazari Das Gupta, 1963: India	
pungens de Meijere, 1909: Sumatra	
obscurus Tokunaga & Murachi, 1959: Caroline Islands	
Afrotropical Region:	Afrotropical Region:
brosseti Vattier & Adam, 1966: Gabon	***bolitinos*** Meiswinkel, 1989: South Africa
dubitatus Kremer, Rebholtz-Hirtzel & Delecolle, 1976: Angola	***imicola*** Kieffer, 1913: Kenya
trifasciellus Goetghebuer, 1935: Zaire	*pallidipennis* Carter, Ingram & Macfie, 1920: Ghana
	loxodontis Meiswinkel, 1992: South Africa
	miombo Meiswinkel, 1991: South Africa
	pseudopallidipennis Clastrier, 1958: Senegal
	tuttifrutti sp. nov
	kwagga sp. nov.

TABLE 11.4 Subgenus *Avaritia*: variation in size and presence of long (L) and short (C) blunt-tipped trichodea on male flagellar segments III-XII of south-east Asian Orientalis group species; unidentified specimens grouped under alphabet symbol

Specimen no.	**Species**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
Thailand 66	*jacobsoni* (# A)	L L	L L c	L L c	L L c	L c	c	L c	c	–	–
Thailand 145	"	L L L L	L L c L c	L L c L c	L L c L c	L L c L c	c c	L c L c	c c	– –	– –
Thailand 585	*jacobsoni* (# A)	L L L L	L½L c L¾L c	L½L c L¾L c	L½L c L¾L c	L c L½L c	c c	L c L c	c c	– –	– –
Philippines 25 Philippines 24	*dumdumi* (# B) *dumdumi* (# B)	L L L L	L L c L L c	L L c L L c	L L c L L c	L c L c	L c L c	L c L c	L c L c	c c	c –
Thailand 27	*pungens* (# C)	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	c
Thailand 633	*fulvus* (# D)	L L L L	L L c L L c	L L c L c	L½L c L¼L c	L c L c	L c L c	L c L c	L c L c	– c	– c
Thailand 712	"	L L L L	L L c L L c	L L c L L c	L½L c L¼L c	L c L c	L c L c	L c L c	L c L c	c –	– –
Thailand 1032	"	L L L L	L L c L L c	L L c L¾L c	L c L c	L c L c	L c L c	L c L c	L c L c	– –	– –
Thailand 1054	"	L L L L	L L c L L c	L L c L L c	L c L½L c	L c L c	L c L c	L c L c	L c L c	– –	– –
Thailand 736	sp.# E (*maculatus*?)	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	c
Thailand 1058	"	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	c
Philippines 15	sp.# F (*orientalis* ?)	L L L L	L½L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L½L c L c	L c L c	L c L c	c c	– –
Thailand 55	"	L L L L	L¾L c L¾L c	L½L c L½L c	L½L c L½L c	L½L c L½L c	L½L c L½L c	L c L¼L c	L c L c	c c	c c
Thailand 227	"	L L L L	L¾L c L½L c	L½L c L½L c	L½L c L½L c	L½L c L½L c	L c L½L c	L c L c	L c L c	c c	c c

Thailand 676	"	L L L L	L¾L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L¾L c L¾L c	L½L c L½L c	L½L c L c	L c L c	c c	c c
Thailand 353	sp.# G	L L L L	L L c L L c	L L c L L c	L L c L¾L c	L L c L¼L c	L c L¼L c	L c L¼L c	L c L c	c c	c c
Thailand 354	"	L L L L	L L c L L c	L L c L L c	L L c L¾L c	L¾L c L¼L c	L½L c L½L c	L c L c	L c L c	c c	c –
Thailand 355	"	L L L L	L½L c L¾L c	L½L c L¾L c	L¼L c L½L c	L½L c L½L c	L c L c	L c L c	L c L c	c c	c c
Thailand 737	sp.# H	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 738	"	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 815	sp.# I	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 816	"	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 1055	sp.# J	L L	L L c	L L c	L L c	L L c	L L c	L L c	L c	c	c
Thailand 228	sp.# K	L L	L L c	L L c	L L c	L c	L c	L c	L c	c	–

Table 11.5 Subgenus *Avaritia*: presence/absence and modalities of 10 character states examined in 25 male specimens of 11 south-east Asian species of the Orientalis group. Arrows indicate tendencies and fractions a reduction in size

Character state	Specimen number and species																								
	T66	T155	T585	P24	P25	T27	T633	T712	T1032	T1054	T736	T1058	P15	T55	T227	T676	T353	T354	T355	T737	T738	T815	T816	T1055	T228
palps long	*	*	*								↑		*	*	*	*	*	*	*						
palps mod. long				*	*		*	*	*	*	*	*								*	*	*	*	*	
palps mod. short						*						↓													*
wing: anal angle dark	*	*	*	*	*	*							*	*	*	*	*	*	*						
anal angle pale							*	*	*	*	*	*								*	*	*	*	*	*
genitalia: parameres feathered	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*		*
parameres simple																				*	*			*	
sternital spicules in 2 groups	*	*	*										*	*	*	*	*	*	*						
spicules continuous				*	*	*	*	*	*	*	*	*		↓						*	*	*	*	*	*
spicules sparse/mod. abundant				*	*	*	*	*	*	*	*	*								*	*	*	*		
spicules abundant/very abundant	*	*	*										*	*	*	*	*	*	*					*	*
spicules short/mod. short	*	*	*										*	*	*	*	*	*	*					*	
spicules mod. long						*	*	*	*	*	*	*										*	*	↓	*
spicules long/very long				*	*															*	*				
distimeres slender	*	*	*	*	*	*	*	*	*	*	*	*								*	*	*	*	*	*
distimeres swollen											↓	↓	*	*	*	*	*	*	*	↓	↓				
apicolateral bristles present			* ↓	*							*	*	*	*		*	* ↓			*	*			*	*

apicolateral bristles absent	*	*			*	*	*	*	*	*					*			*	*			*	*		
apices hind tibiae pale	*	*	*				*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	
apices hind tibiae dark				*	*	*					↓	*													*
antenna: L trichodea short				↑	↑		*	*	*	*															*
L trich. mod. long	*	*	*	*	*	*					*	*					*	*	*	*	*			*	
L trich. very long			↓										*	*	*	*						*	*		
2 L trich. on VII	*		*										*	*	*	*	*	*	*	*	*	*	*	*	
1 L trich. on VII		*	↓	*	*	*	*	*	*	*	*	*													*
2 L trich. on IX																				*	*	*	*	*	
1 or other trich. ¾, ½ or ¼ developed														¼ *		½ *	¼ *								
1 L absent on IX	*	*	*	*	*	*	*	*	*	*	*	*	*	↓	*			*	*						*
	A	A	A	B	B	C	D	D	D	D	E	E	F	F	F	F	G	G	G	H	H	I	I	J	K
	jacobsoni			*dumdumi*		*pun-gens*	*fulvus*				*maculatus* ?		*orientalis* ?				?			?		?		?	?

Table 11.6 Subgenus *Avaritia*: the female antennal ratio (AR) of 17 species of the Orientalis and Imicola groups according to various authors and this study

Author	Orientalis group	Imicola group	Range in AR	Average value	Deviation	n
Arnaud 1956	*jacobsoni* (as *kitaokai*)	-	-	1,15	-	?
Tokunaga 1959	" (as *unisetiferus*)	-	-	1,14	-	?
Tokunaga 1959, 1962	" (as *buckleyi*)	-	0,95-1,17	-	22	?
McDonald & Lu 1972	"	-	-	1,21	-	?
Lee 1978	"	-	-	1,15	-	-
Kitaoka 1985	"	-	-	1,16	-	-
Wirth & Hubert 1989	"	-	1,13-1,28	1,21	15	10
this study	" (as sp. #A)	-	-	1,10	-	1
Kitaoka 1985	*dumdumi*	-	-	1,26	-	?
Wirth & Hubert 1989	"	-	1,31-1,39	1,35	8	10
this study	" (as sp. #B)	-	-	1,25	-	1
Wirth & Hubert 1989	*pungens*	-	1,27-1,47	1,37	20	10
this study	" (as sp. #C)	-	-	1,28	-	1
Wirth & Hubert 1989	*fulvus*	-	1,19-1,26	1,23	7	7
this study	" (as sp. #D)	-	-	1,09	0	2
Tokunaga 1959, 1962	*orientalis*	-	1,04-1,24	1,08	20	?
Lee 1978	"	-	-	1,15	-	?
Dyce 1980	"	-	-	1,14	-	?
Wirth & Hubert 1989	"	-	-	1,13	-	?
this study	" (as sp. #F)	-	-	1,12	0	2
Arnaud 1956	*maculatus* (as *sigaensis*)	-	-	1,09	-	1
McDonald & Lu 1972	"	-	-	1,20	-	?
Lee 1978	"	-	-	1,20	-	?
Kitaoka 1985	"	-	-	1,04	-	?
Wirth & Hubert 1989	"	-	1,07-1,14	1,10	7	2
Kitaoka 1975	*flavipunctatus*	-	1,25-1,37	1,32	12	12
Kitaoka 1985	"	-	-	1,22	-	?
Wirth & Hubert 1989	"	-	-	1,30	-	?
this study	(as sp. #K)	-	1,19-1,26	1,23	7	5
McDonald & Lu 1972	*hui*	-	-	1,17	-	?
Lee 1978	"	-	-	1,20	-	?
Kitaoka 1985	"	-	-	1,12	-	?
Wirth & Hubert 1989	"	-	1,12-1,13	1,12	1	2
this study	"	-	1,00-1,07	1,04	7	4
Delfinado 1961	-	*brevitarsis* (as *radicitus*)	-	1,10	-	?

Kitaoka 1985	-	"	-	1,14	-	?
Wirth & Hubert 1989	-	"	1,17-1,25	1,22	8	5
this study	-	"	1,06-1,18	1,12	12	27
Delfinado 1961	-	*nudipalpis*	1,09-1,18	1,14	9	4
Wirth & Hubert 1989	-	"	-	1,20	-	?
this study	-	"	1,06-1,12	1,07	6	5
Wirth & Hubert 1989	-	*imicola*	1,17-1,19	1,18	2	2
Boorman & Dipeolu 1979	-	"	1,01-1,22	1,10	21	20
Glick 1990	-	"	0,95-1,13	1,05	18	15
Boorman 1989	-	"	1,08-1,19	1,14	11	?
Khamala & Kettle 1971	-	" (as *pallidipennis*)	-	1,17	0	1
Meiswinkel 1989	-	"	0,95-1,10	1,01	15	167
Meiswinkel 1989	-	*bolitinos*	0,96-1,12	1,04	16	54
Meiswinkel 1991	-	*miombo*	1,09-1,25	1,15	16	66
Meiswinkel 1992	-	*loxodontis*	0,92-1,08	0,99	16	80
this study	-	*pseudopallidipennis*	1,13-1,24	1,19	11	45
"	-	*tuttifrutti*	0,98-1,13	1,06	15	104
"	-	*kwagga*	0,96-1,12	1,06	16	31

CHAPTER 12

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

12.1 INTRODUCTION

The purpose of a key is to unlock knowledge simply. The best keys utilize characters that unambiguously distinguish one species from the next; these may be distilled from intensive study of many hundreds of specimens. Specialist students usually spend a lifetime assembling these data, but the current trend to fragment research into short-term projects does not favour long taxonomic 'journeys'. As regards the Imicola group, the journey is not complete — I am aware of three undescribed species known only from a few individuals or a single sex.

Despite being small in size (0,6–2,0 mm), *Culicoides* can exhibit some notable differences in coloration, especially in the wing pattern. However, it is inevitable that other species will be more similar to one another, and the danger now exists that small differences will be labelled as intraspecific variation. This is a problem that lies at the heart of the identification of all living biota, and likely is the reason why, on average, only one new species of the Imicola group is described every 12 years.

An important point to be made is that in the Afrotropical Region as many as six species of the Imicola group can be found in a single light-trap collection, and may occur in sympatry with 30 other species of *Culicoides*. However, such a high number of Imicola group species is the exception rather than the rule, as species diversity in any area is dependent upon the heterogeneity of hosts and larval habitats available; climate, soil and altitude further induce or reduce species turnover. Seldom are two or more species of the Imicola group equally represented in a complex community; this variance in abundance and prevalence patterns further complicates identification of individual specimens.

In the knowledge that the true size of the Imicola group remains unknown, the strategy adopted in its study was to slide-mount phenotypically heterogeneous collections (especially if a new locality was sampled), and to then score each individual carefully. Over time, these population data were coupled to those obtained from reared series, the most diagnostic features extracted, and the key built.

It may be argued that some couplets of the key are overly long, but it is beyond question that distinct species pairs or subgroups constitute the Imicola group; this implies recent divergence in evolutionary terms, which may or may not be well expressed in the phenotype. Careful scrutiny is thus essential. Therefore, if a couplet demands agreement on four or more characters, the specimen must comply fully before an identification can be accepted as correct. As all species of the Imicola group are very similar, it is recommended that more than one specimen of each sex in any given population be examined.

A major shortcoming of the key must be mentioned: it does not inform as to whether a particular specimen belongs to the Imicola group. As indicated in Chapter 1, at least 33 species (20 undescribed) of *Avaritia* are to be found in Africa; another 50 known species occur elsewhere. All fall into 10 or more groups. Until these groups are defined, it will remain problematical to use a key as precisely aimed as the one given below for the Imicola group which deals with only 12 % of the known species of world *Avaritia*.

Finally, a prominent non-taxonomic feature of the Imicola group is that each species has a specific life-cycle which, if known, is a most useful aid to identification. However, biological features are not utilized in the key, as the capture of free-flying individuals in light-traps, the principal method used for surveying *Culicoides* worldwide, provides no data on their biology.

12.2 Key to slide-mounted males and females of nine world species of the Imicola group

1.	Male	2
	Female	8
2.	Anal angle of wing with dark smudge	3
	Anal angle pale	4

3. Scutum with two prominent yellow vittae; halteres dark; sternum nine densely spiculate; apex of aedeagus smoothly rounded . . . . ***miombo***
Scutum entirely brown; halteres pale; sternum nine bare or sparsely spiculate; apex of aedeagus minutely papillate or sclerotized . . . . ***kwagga***

4. Membrane of sternum nine moderately to densely spiculate
(Africa, S. Europe and mainland Asia) . . . . ***imicola***
(s.e. Asian islands e. of Wallace line) . . . . ***nudipalpis***
Membrane bare or with < 20 spicules 5

5. Scutellum with two median bristles; terminal projection of aedeagus rather short, apex minutely papillate . . . . ***loxodontis***
Scutellum with one median bristle; terminal projection of aedeagus moderately long, apex smoothly rounded 6

6. Scutum with > 80 bristles; proximal margin of distal pale spot in wing cell R_5 markedly pointed . . . . ***tuttifrutti***
Scutum with < 80 bristles; pale spot in R_5 gently rounded or straightish 7

7. One of the two long (L) blunt-tipped sensilla trichodea on flagellar segments V and/or VI reduced in size or absent; third palpal segment moderately inflated, longer than wide . . . . ***pseudopallidipennis***
Both long (L) trichodea present and equal in size; third palpal segment smaller and roundish (Afrotropical Region) . . . . ***bolitinos***
(Oriental/Australasian Regions) . . . . ***brevitarsis***

8. Third palpal segment swollen; sensilla coeloconica on flagellar segments III, XI–XV; long blunt-tipped sensilla trichodea inflated 9
Third palpal segment short and roundish, or moderately long and slender, coeloconica on segments III, XII–XV; blunt-tipped trichodea slender 10

9. Antennal ratio (AR) 0,98–1,13, average 1,06; Afrotropical woodlands and tree savannas . . . . ***tuttifrutti***
AR 1,13–1,24, average 1,19; equatorial regions . . . . ***pseudopallidipennis***

10. Anal angle of wing with obvious, but variable-sized, dark smudge 11
Anal angle pale 12

11. Scutum brown with two yellow admedian vittae; haltere knobs brown; eyes sparsely pubescent; wing darker . . . . ***miombo***
Scutum entirely brown; halteres pale; eyes bare; wing paler . . . . ***kwagga***

12. Scutellum with two median bristles; flagellar segments VI and VIII with four sensilla chaetica . . . . ***loxodontis***
Scutellum with one median bristle; segments VI and VIII with three chaetica 13

13. Wing vein M_2 with a broad, well-defined preapical excision; third palpal segment moderately long and slender; proximal margin of distal pale spot in R_5 moderately to noticeably pointed 14
Vein M_2 without preapical excision; third palpal segment shortish, almost round; proximal margin of distal spot in R_5 straight or gently rounded 15

14. Proboscis/head (P/H) ratio 0,66–0,73; restricted to sout-east Asian islands east of the Wallace line . . . . ***nudipalpis***

P/H ratio higher (0,82–1,02); restricted to Africa, southern Europe, and mainland Asia . . . . ***imicola***

15. Antennal segments VI–IX shorter, approximately 1,4 x longer than wide; restricted to Oriental/eastern Palaearctic and Australasian Regions . . . . ***brevitarsis***

Antennal segments VI–IX longer, ranging between 1,4–1,7 x longer than wide; restricted to Afrotropical Region . . . . ***bolitinos***