4.5.2 Scanning electron microscope technique

Several complicated fixation methods were tried, to preserve the eggs without distortion, but none was successful. The eventual method employed was simply to fix the hatched eggs in 80% alcohol for several weeks and then to air dry them. Very little distortion was apparent.

Eggs were then placed on glued stubs and gold coated for SEM examination.

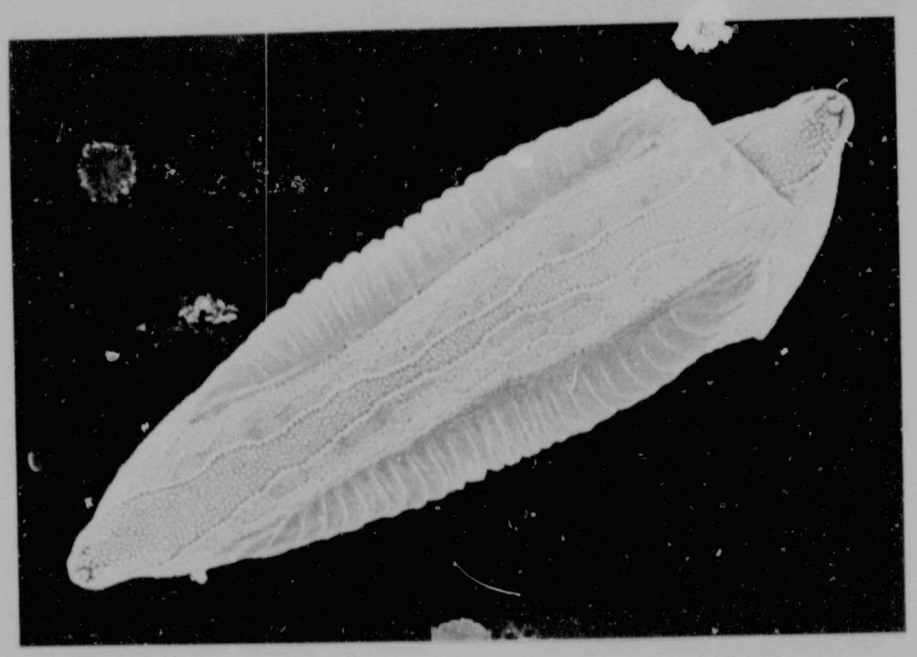
4.5.3 Results

The polygonal markings on the dorsal surface of the exochorion between the floats of tenebrosus were not apparent but this may be due to damaged or old specimens as they have been observed on fresh specimens under a light microscope (X100 magnification). Polygonal markings on the other four species are all obvious (figs. 22, 23, 24, 25 & 26).

The only possible difference was that tenebrosus had one to three rosettes at each pole on the dorsal surface while the others had four or five (fig. 27). No differences were observed in either the micropyles or the air ducts in the floats.

Fig. 22. The egg of A.coustani A.

Fig. 23. The egg of A.coustani B.



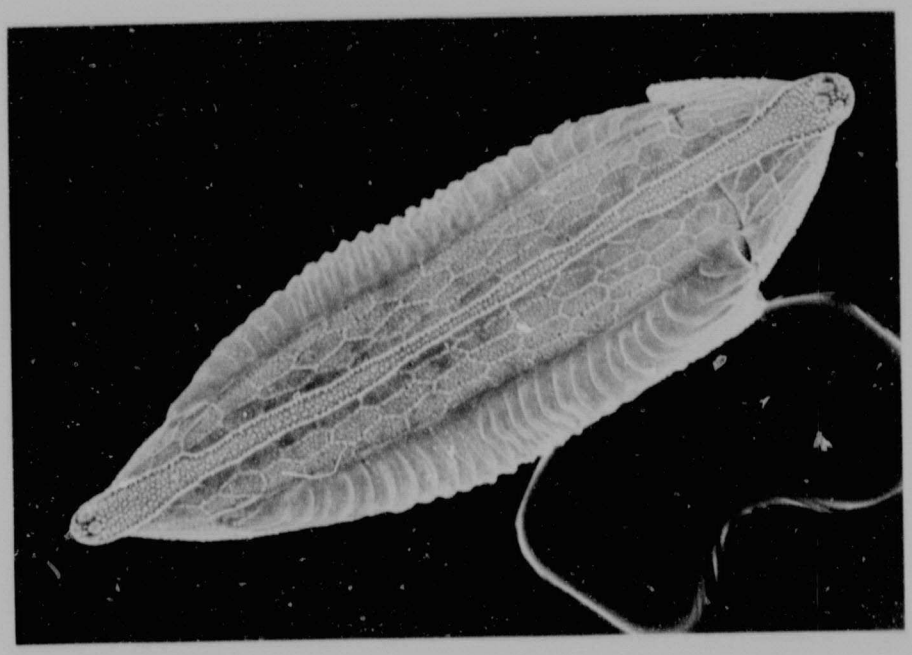


Fig. 24. The egg of A.ziemanni A.

Fig. 25. The egg of A.ziemanni B. This specimen has collapsed slightly at the one end.

Fig. 26. The egg of A.tenebrosus.

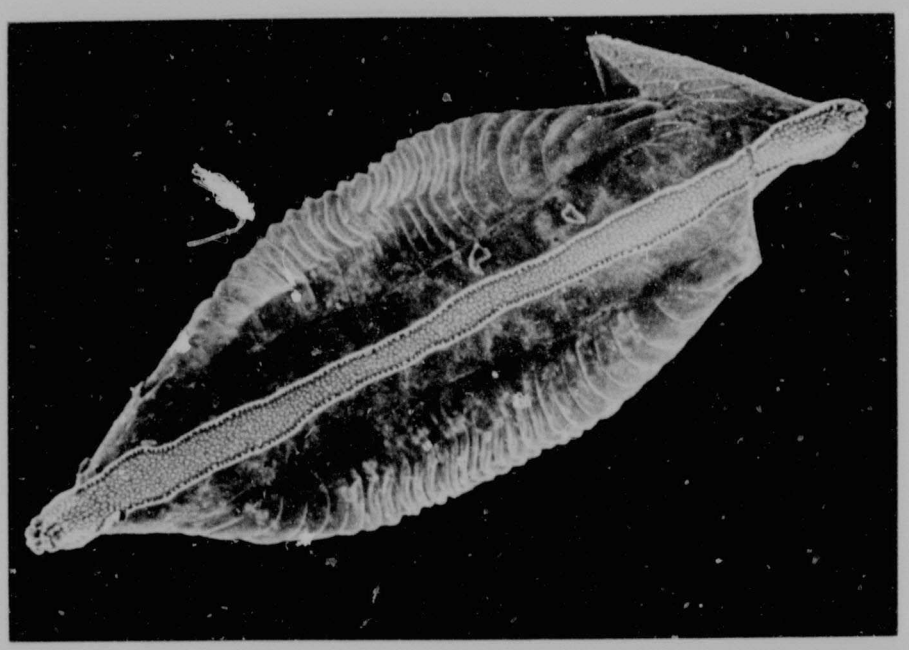
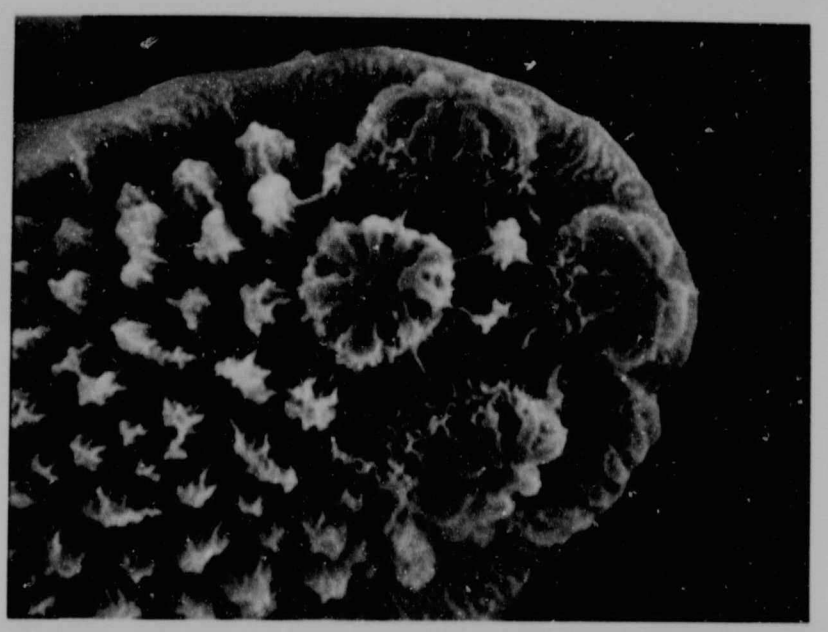


Fig. 27. a) The rosettes of A.tenebrosus and b) the rosettes of A.coustani B.

Fig. 27. a) The rosettes of A.tenebrosus and b) the rosettes of A.coustani B.

a



b

4.6 Discussion

In this morphology section, I have put forward a new key model for the identification of species complexes. The old method of keying out each developemental stage as a separate entity had to be abandoned as no significant discrimination resulted. The key presented in table 5 is based on correlated family material. However, it is possible to identify tenebrosus in any stage without breeding the material in the laboratory. The characters for tenebrosus are as follows:

adult: base of hind tarsomere one dark.

pupa: seta 11-C, 5 or more branches or
seta 5-I, 5 or more branches or
trumpets, 8 or less ribs.

larva: antennal shaft hair, 14 or more branches. This seta is a very good character for tenebrosus as no specimens of the other species examined so far, have had the sum of this seta with over 23 branches and tenebrosus invariably has the sum of 28 or more branches.

egg: one to three rosettes on dorsal poles.

The key is designed for the identification of the sibling species and requires the larva, pupa and adult to be correlated. By doing this, 100% positive identification can be made of the families.

Table 5. Combined key for identification of families of the five species tenebrosus, coustani A, coustani B, ziemanni A and ziemanni B.

1. Adult. Base of hind tarsomere 1 dark.....tenebrosus
   - Base of hind tarsomere 1 pale.....2

2. Adult. Apex of hind tibia with pale streak 3-5 times as long as broad; apical pale band on hind tarsomere 2, 1/8-2/5 length of segme.....3
   - Pale streak on tibia 1-3 times as long as broad; apical pale band on tarsomere 2, 1/15-1/8 length of segment.....4

3. Pupa. Total sum of branches of the three setae 6-I, 9-I and 6-II is 12 or less.
   Larva. Mean no. of branches of seta 9-V per family is 12.9 or less.....coustani A
   Pupa. Total sum of branches of 6-I, 9-I and 6-II is 13 or more.
   Larva. Mean no. of branches of 9-V is 13.0 or more.....coustani B

4. Pupa. Sum of seta 5-VI, 22 or less.
   Larva. Mean no. of branches per family of seta 2-I, 5.9 or less and 5-II, 9.9 or less.ziemanni A
   Pupa. Sum of 5-VI, 23 or more.
   Larva. Mean of 2-I, 6.0 or more and 5-II, 10.0 or more.....ziemanni B

Evans (1938) mentions a pupal variant of ziemanni where setae 5-V and 5-VI have about 9 branches each (not the sum). However, this variation has been seen in the four species coustani A and B, tenebrosus and ziemanni A. A.ziemanni B has very many more branches, about 30 for 5-V and 20 for 5-VI (not the sum). This character of Evans should be discarded.

The variation in pupal seta 5-VII mentioned by Vincke & Parent (in De Meillon, 1947) of tenebrosus having 12 branches is more important. A small number of ziemanni B from Namibia have this seta with up to 12 branches, but all specimens examined of tenebrosus from Natal and Transvaal, had this seta with not more than 6 branches. Unfortunately, no indication is given of the locality of the specimens examined by Vincke & Parent, but from the results of this study, the possibility exists that tenebrosus is also a species complex.

It is fortunate, due to the fact that the immature stages so closely resemble each other, that earlier workers have tended to describe them simply "as in coustani". This has left the larval and pupal sections completely uncluttered by descriptions of mixtures of sibling species. It is also a distinct advantage to base illustrations on specimens from a single family. Because the drawings for Appendix III were done this way, it enabled me to correlate the coustani illustrations with that of species A

and likewise, ziemanni with ziemanni A.

The naming of the new species will not present many problems as far as nomenclature goes, because only one previous synonym exists in the group, that is mauritianus for coustani. Efforts have been made to obtain museum material without results. Also, attempts to contact workers in the vicinity of the type localities of the different species have proved fruitless. It remains for someone with better access to the different institutions to follow up this part of the study and eventually to name the sibling species. In my opinion, ziemanni B is a completely new species never described before, but the coustani siblings are a more complicated problem, and live material from the type locality is essential to do chromosome and cross-mating studies initially, before coming to any decision about the classification i.e. which sibling species is coustani sensu strictu.

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CHAPTER FIVE

CONCLUDING DISCUSSION

5.1 Introduction

The role of the taxonomist in science can no longer be regarded as solely the function of classification by morphological criteria. It is now essential that the taxonomist has a rigorous training in and a very good understanding of basic genetics and evolutionary genetics, and that he applies this knowledge to any study he might undertake.

5.2 A recent example of the pitfalls of pure morphological taxonomy

A recent paper by Ribeiro *et al* (1979) describing a new sub-species *Anopheles quadriannulatus davidsoni*, graphically illustrates many of the pitfalls of applying morphological taxonomy without due regard to other relevant disciplines, so it is worth analysing in some detail here.

Firstly, as no genetic analysis of any kind was carried

out on the material collected, it is quite possible that more than one sibling species was being considered. Therefore, the reliability of all the biometric work done is uncertain.

Secondly, chromosomal studies place A.merus and A.melas closer to other members of the A.gambiae complex than to each other. The authors' decision to use salt-water breeding as a basic evolutionary criterion is therefore unjustified. It is also curious that no weight at all has been given to the very marked difference in feeding habits between their new sub-species and its supposed parent species.

Thirdly, the authors' relegation of species D of the gambiae complex to sub-specific status on the grounds of allopatry, ignores Davidson & Hunt (1973) who proved genetically that species D is a good species.

Fourthly, it would seem that Ribeiro et al (1979) used colony data for their comparisons with the wild-caught A.g.davidsoni. The strong artificial conditions and selection through frequent population crashes to "founder" numbers common in laboratory colonies, is certain to alter the genotype and phenotype of laboratory strains radically away from the wild condition. These comparisons are therefore unreliable and meaningless.

It is clear that the intensive biometric work on a presumed new species has in fact contributed nothing that will not have to be repeated with accompanying genetic work and reanalysed from a modern evolutionary standpoint.

5.3 Species-specific characters

While the techniques applied to the five species have been dealt with separately, actually, it is the species which are separate entities and the results of the individual techniques must be combined to form an intergrated whole.

A.coustani A. The commonest member of the group found in the Transvaal, seemingly confined to the low-lying eastern areas. This species was caught in large numbers biting man during the first four hours after sunset. While the adults are easily distinguished from the "ziemanni" species and tenebrosus on the hind leg banding patterns, it is necessary to have correlated larval and pupal pelts to be able to distinguish it from coustani B. The fixed inversion difference on the X chromosome of the salivary gland polytenes is a good character for separating the sibling species, but also necessitates the rearing of families in the laboratory and is as time-consuming as the morphological method. The quickest method of separating coustani A and B is by electrophoresis

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where the slow Est-2 seems to be species-specific for coustani A.

A.coustani B. As only four wild adults were obtained, little can be said about the biology of this species. One specimen was caught resting inside a house in the northern suburbs of Johannesburg. The other three were caught outdoors biting man near small-holdings outside Benoni on the East Rand. As the cattle kraals and sheds in the vicinity are regularly sprayed with insecticides, it is likely that this species is anthropophilic too. More sampling over a wider area is needed to establish whether this species is restricted to high altitudes or not. Similarly, a larger sample size will show if there is variation of the fast Est-2 electromorph or if it is species-specific and therefore a good character for discriminating between coustani A and B.

A.ziemanni A. An anthropophilic mosquito found in the lowveld (Tzaneen) and middleveld (Potgietersrus) areas of the northeastern Transvaal. The adults can be morphologically distinguished from all other members of the group by the hind leg banding patterns and the large pale apical wing spot. It is therefore unnecessary to go through the laborious procedure of laboratory rearing to examine other species-specific characters found in the immature stages and the polytene chromosomes, but this would probably be advisable when examining material

from localities other than those sampled here.

A.ziemanni B. Known only from the Kavango district in the northeastern parts of Namibia, this species was caught in large numbers in cattle kraals and is the only member of the group to show marked zoophilic tendencies. This in itself, is a species-specific character and distinguishes it from ziemanni A. The morphological species-specific characters are the small apical wing spot of the adult and the very bushy seta 5 on the pupa. The one family that had three adults looking like ziemanni A, had seta 5 on the correlated pupal pelts quite typical for ziemanni B. The larval characters were also typical of species B. As the specimens used here all come from a single village, it is recommended that when sampling other areas, eg. Angola, Botswana and Zimbabwe, correlated morphological and chromosomal species-specific characters be used to identify specimens. This ensures a correct estimate of the distribution range of this species.

A.tenebrosus. Rarely found in the Transvaal, Gillies & De Meillon (1968) report that it is widespread and abundant along the eastern side of the continent. De Meillon (1938) found it in large numbers in an animal shelter in Lourenco Marques. As some of the specimens used in this study were caught biting man, it appears that the species is both zoophilic and anthropophilic. Taxonomically and genetically this species is distinct

from all other members of the group. Morphologically it can be separated from the r[illegible] by the reduced pale areas on the hind legs of the [illegible], the fewer ribs on the pupal trumpets and the large, bushy antennal hair on the larva. Electrophoretically, the fast Sod. electromorph and the absence of Est-2 are unique to tenebrosus.

This study of the coustani group of mosquitoes illustrates very well the advantages of combining separate disciplines. While most of the species are readily recognised by morphological and biological characters, it is quite certain that the coustani sibling species would never have been discovered had genetical techniques not been applied. Future ecological studies of this mainly anthropophilic group will now have a sound basis.

5.4 On identification

"The search for sibling species and their routine identification is a fundamental task of the medical entomologist" (Coluzzi, 1970). The classical taxonomist uses morphological characters to identify a species taxon. In the event of sibling species, his techniques become mostly inoperative. The cytogeneticist, using chromosomal observations, may resolve the issue, but not always. Green & Hunt (1980) show that A.funestus and A.aruni? are homosequential species, that is, their polytene chromosomes are virtually identical. Cross-

mating experiments however, showed them to be different species. Here the geneticists could perhaps find species-specific characters by using electrophoresis.

H.J. Muller (1922) hit the nail on the head when he asked "Must we geneticists become bacteriologists, physiological chemists and physicists, simultaneously with being zoologists and botanists?" and his answer was "Let us hope so." If it takes the combination of all these disciplines to resolve a problem adequately, then indeed, let us hope that our zoologists will become geneticists and biochemists, mathematicians and anything else that provides the tools for us to resolve our biological problems.

Species	Locality	no. families bred	no. adults pinned	Collection methods
tenebrosus	Thabina, Tvl.	2	12	Biting man outdoors
	Grey Stones, Tvl	3	19	" " indoors, man-baited net
	Sautini, Tvl.	5	27	Biting man outdoors
	Zululand, Natal	2	10	Cattle kraal
	South Coast, Natal	1	5	Biting man outdoors
		13	73	
coustani A	Jaffray, Tvl.	11	66	Biting man outdoors
	Thabina, Tvl.	3	27	" " "
	Tarentaal, Tvl.	2	16	" " "
	Komatipoort, Tvl.	2	10	" " "
	Tzaneen, Tvl.	2	9	" " "
	Zangoma, Tvl.	2	6	" " "
	Sudwala Caves, Tvl.	2	10	" " indoors
	Hoogmoed, Tvl.	1	5	Cattle kraal
	Addington, Tvl.	1	9	Biting man outdoors
	Grey Stones, Tvl.	9	58	" " " , man-baited net
	Agatha, Tvl.	1	12	Inside house
	Sautini, Tvl.	1	9	Biting man outdoors
	Makonde, Tvl.	1	7	" " "
	Swaziland	6	49	" " " , man-baited net
		44	293	