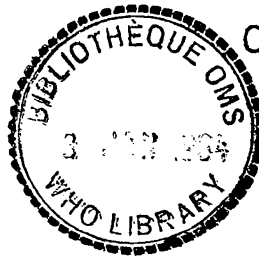


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RECENT INVESTIGATIONS OF THE A. GAMBIAE COMPLEX IN SOUTHERN AFRICA

CONTENTS

	<u>Page</u>
I. Records of the breeding of "salt-water <u>A. gambiae</u> " at inland localities in Southern Africa (H. E. Paterson, J. S. Paterson and G. J. van Eeden)	1
II. On the naming of the East African salt-water species of the <u>A. gambiae</u> complex (H. E. Paterson)	7
III. A preliminary report on a new member of the <u>A. gambiae</u> complex (H. E. Paterson, J. S. Paterson and G. J. van Eeden)	11

INTRODUCTION

There is now good evidence that the "A. gambiae" comprises four different mating types and an evidence of a fifth has recently come to light. No reliable and constant morphological differences between these forms have been found but crosses between them yield sterile F_1 males and often disturbed F_1 sex ratios. Field studies indicate that in areas where two of these forms exist they are prevented from cross-mating by different behaviour patterns. From the practical viewpoint, it seems that a reassessment of the status of "A. gambiae" is necessary. The three papers presented in this issue of WHO/Mal deal with this important problem.

I. RECORDS OF THE BREEDING OF "SALT-WATER ANOPHELES GAMBIAE"
AT INLAND LOCALITIES IN SOUTHERN AFRICA

by

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Recent work has shown that at least four genetically distinct forms comprise the A. gambiae complex. Paterson (1963) has outlined arguments for regarding them as separate species. In the eastern half of Africa, three species occur, "salt-water A. gambiae" and the two fresh-water breeding species, Species A and Species B (= group A and group B) (Davidson & Jackson, 1962). These forms are apparently morphologically inseparable, though the frequency of females bearing four white bands on their palps, as against three, is usually much higher in populations of "salt-water A. gambiae" than of the two fresh-water breeding species. Identification of the species is best achieved by performing crossing experiments with "type" colonies of the different species. Interspecific crosses produce sterile F_1 males (Paterson, 1962; Davidson, 1962) with testes and accessory glands reduced to varying degrees according to the cross and its direction. In crosses between "salt-water A. gambiae" and either Species A or Species B, F_1 sex ratios supply further information (Paterson, 1962; Kuhlow, 1962; Davidson, 1962), but crosses between the two fresh-water species usually produce normal sex ratios (Davidson, 1962). A physiological test (salinity-tolerance test) is available (Muirhead-Thomson, 1951) to distinguish first-stage larvae of "salt-water A. gambiae" from those of either Species A or Species B.

Although some records have been published (Haddow et al., 1951; Smith & Vail, 1959) of breeding by members of the A. gambiae complex in saline waters at inland localities, the only one investigated seemed to be due to either Species A or Species B. The purpose of this communication is to publish confirmed records of inland breeding by "salt-water A. gambiae" (Figure 1).

During investigations of the A. gambiae complex in Swaziland and Mozambique, collections of adult females and wild larvae were made near Big Bend, a settlement on the Usutu River near the Natal and Mozambique borders. Females were caught either in window-traps or resting outdoors. They were fed and some laid eggs. Adults raised from these eggs were crossed to type colony material to identify them (Table 1).

TABLE 1. RESULTS OF CROSSING EXPERIMENTS SET UP TO IDENTIFY WILD-CAUGHT MATERIAL OF THE A. GAMBIAE COMPLEX FROM BIG BEND (B.B.), SWAZILAND, AND TINONGANINE, MOZAMBIQUE (Ti), AS WELL AS CERTAIN CONTROL CROSSES

Cross	Source of wild material	Adult F ₁		Appearance of F ₁ testes and accessory glands
		Sex	Ratio	
		♂	♀	
Sp.A ₊ o x B.B. ₇ ♂	Window-trap	78	244	Both minute
B.B. ₃₊ o x Sp.A♂	Window-trap	218	37	Both moderately developed
Sp.A ₊ o x B.B. _B ♂	Resting outdoors	33	85	Both minute
<u>A. gambiae</u> "♂	Wild-caught larvae	76	78	Both quite normal; fertile
Sp.B ₊ o x B.B. _L ♂	Wild-caught larvae	7	8	Both very strongly reduced
Sp.A ₊ o x Ti♂	Cow-baited trap	110	115	Both minute
Ti ₊ o x Sp.A♂	Cow-baited trap	74	0	Both moderately developed
Sp.A ₊ o* x "salt-water <u>A. gambiae</u> "*♂	-	65	137	Both minute
"Salt-water <u>A. gambiae</u> "o x Sp.A♂	-	289	15	Both moderately developed

* Type colonies

Wild-caught larvae were kept until adults were obtained. These were pooled with a few superfluous adults from the window-traps, and a colony was started. Specimens from this colony were also crossed to material from type colonies (Table 1). It will be seen that these crossing experiments indicate that this material was "salt-water A. gambiae". This view is supported by the following information:

1. First-stage larvae from the Big Bend colony survive the salinity-tolerance test for three hours.
2. A second trip to the site yielded eggs from females caught in window-traps and calf-baited trap-nets, and larvae from both these sources survived the salinity test.
3. More larvae collected from pools in a nearby saltmarsh were used to obtain a second colony, first-stage larvae of which were also salt-water tolerant.

It is thus certain that a focus of breeding by "salt-water A. gambiae" is present in Swaziland some 56 miles inland from the sea, and over 75 miles up the Usutu River. Salinities in the saltmarsh in three pools containing "salt-water A. gambiae" larvae were determined by conductivity measurements to be equivalent to 63.1%, 2.9% and 3.2% of sea water (31.7 g NaCl/litre). Titration of chlorides as sodium chloride gave a figure of 60.3% of sea water for the most concentrated sample, thus chlorides comprised most of the salts present. Dried patches of crystals on the mud in the marsh were salty to the taste.

Members of the Malaria Eradication Scheme in Mozambique supplied us with 12 batches of eggs from Tinonganine, all but one coming from females trapped in a net baited with a cow and situated near houses from which females of Species B had earlier been obtained.

The adults from these egg batches were crossed to Species A to identify them, and all the successful crosses gave results strongly suggesting that the females from the trap-nets were all "salt-water A. gambiae". In Table 1, two representative results are presented. No salinity tests have been done so far to support these findings, but it seems fairly certain that the identification is correct.

Tinonganine lies about 23 miles inland on the Maputo River into which the Usuto River flows. Much breeding by members of the A. gambiae complex occurs in the rice fields flanking the river. No information is available on the salinity of waters in the area.

At both these places it has been known for some time (since 1961 at Big Bend) that "A. gambiae" females with four-banded palps are common but the populations were, nevertheless, thought to be of aberrant fresh-water breeding forms. It would thus seem necessary that all populations with a high frequency of four-banded females should in future be investigated by the salinity-tolerance test, whether from inland sites or not.

Very recently salt-water A. gambiae larvae have been found in pools at the edge of Nyamithe Pan in the Ndumu Game Reserve, Northern Natal. Three of the pools which contained larvae had salinities of 108‰, 108‰ and 123‰ sea water as determined by the conductivity method. These waters were very salty to the taste. The females which were reared from these larvae were mostly four-banded ($35/41 = 85.4\%$). Crossing experiments with the Tanga colony yielded fertile F_1 males in both directions.

It is known from elsewhere in Africa (Muirhead-Thomson, 1951; Iyengar, 1962) that "salt-water A. gambiae" is a less efficient vector than the sympatric fresh-water breeding species due to differences in their behaviour patterns. No doubt Species A and Species B will also be found to differ from each other in behaviour. It can thus be seen to be unjustifiable to ignore the existence of these component species of the A. gambiae complex when entomological studies are made during antimalaria campaigns.

Acknowledgements

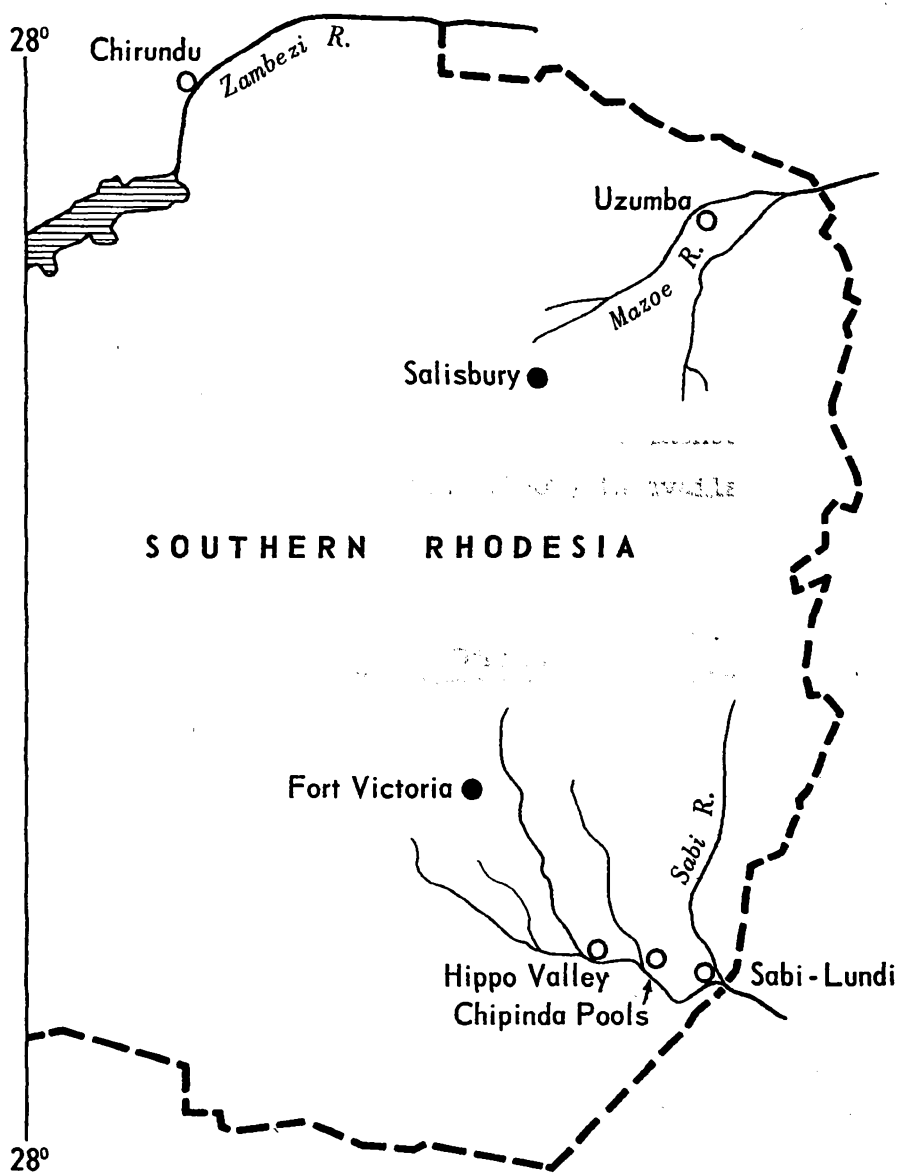
Our thanks are due to Dr R. D. Gauldie, Acting-Director of Medical Services, Swaziland, Mr J. Clarke and Mr C. Ramsdale, World Health Organization, and to Dr Botha de Meillon who originally suggested to us the investigations which led to this work. These studies have been supported by the World Health Organization, South African Institute for Medical Research and the Swaziland Government.

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FIG. 1

Localities ● in which salt water A. gambiae have been found away from the sea in Southern Rhodesia.



Open circles = Sp. C. localities in Rhodesia

WHO 3913

II. ON THE NAMING OF THE EAST AFRICAN SALT-WATER SPECIES
OF THE ANOPHELES GAMBIAE COMPLEX

by

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The argument for regarding as a good species the salt-water breeding form of the Anopheles gambiae complex which occurs along the east coast of Africa between Natal and Somalia, as well as on the islands of Pemba, Mauritius, the Comores and Aldabra, has recently been presented (Paterson, 1961, 1962, 1963). This view has been supported by Kuhlöw (1962b) who proposed the name Anopheles tangensis Kuhlöw for the species. The purpose of this note is to present evidence that it is very highly probable that the name Anopheles merus Dönitz (1902) is available for this species, and that A. tangensis is therefore a synonym, and should not be used in the literature until the matter is finally settled.

Notes on the type series of Anopheles merus Dönitz

A. merus Dönitz (1902) is the earliest name in the synonymy of A. gambiae which might be available as a name for "salt-water A. gambiae", and the possibility that its lectotype might have belonged to the same species as the members of Tanga colony and other populations of the East African salt-water breeding species of the A. gambiae complex does not seem to have been taken into account by Kuhlöw.

Through the kindness of Dr H. Schumann of the Humboldt Museum, Berlin, I have been able to examine the four remaining specimens, all females, from Dönitz's type series of A. merus. A single female from Dar-es-Salaam is missing and apparently lost. While I had the types in front of me I knew of no morphological character which would unambiguously distinguish females of the East African salt-water breeding species from the other members of the complex. However, I wished to ascertain the condition of banding on the female palps in the specimens from Dar-es-Salaam since it is known that a greater proportion of the females of the salt-water species has four (as opposed to three) white bands on their palps than is usually the case in populations in either of the two fresh-water breeding forms (Muirhead-Thomson, 1951). This polymorphic character becomes particularly important in the absence of other morphological differences.

At Mr Alan Stone's request, Professor Dr F. Peus selected as a lectotype one of the females from Dar-es-Salaam. This designation has not been published until now, except by implication, when Mr Stone published the restricted type locality (Stone et al., 1959). The female selected as lectotype was collected at Dar-es-Salaam in December 1899, and is in quite good condition except that it lacks the abdomen and most of the right hind leg. The other female from Dar-es-Salaam lacks all its legs except the right front leg and right middle femur, and has had its abdomen glued in place, which leaves one with the possibility that it is not its own. Both these females have four-banded palps.

The para-lectotypes from Franzfontein, South-West Africa, and "Mballa-Ebene", Tanganyika, are not considered further here since the argument which follows turns on the identity of the lectotype only.

Discussion

We thus know two pertinent facts about the lectotype of A. merus: that it has four-banded palps, and that it was caught at Dar-es-Salaam.

During 1947 and 1948, Muirhead-Thomson (1951) studied "A. gambiae" at Dar-es-Salaam and published figures on the frequency of the four-banded condition in the local fresh-water breeding and salt-water breeding populations. If the minor assumption is made that the frequencies in these populations were similar to the corresponding frequencies in 1899, when the lectotype was collected, we can estimate the probability of the lectotype having been drawn from either of the two populations.

In 1947 Muirhead-Thomson identified 231 members of the A. gambiae complex caught at Dar-es-Salaam by means of his salinity-tolerance test. He showed that 128 belonged to the salt-water breeding form and 103 to the fresh-water breeding population. In this sample all four-banded females were found to belong to the salt-water species. During the 1948 season Muirhead-Thomson, therefore, treated all four-banded females as members of this species, and applied the salinity test only to the three-banded specimens. A test of the reliability of this method of identification of part of the salt-water breeding population can be made as follows. If the four-banded group which he attributed to the salt-water species actually comprised a mixture of the fresh-water and salt-water breeding forms, one would expect that the four-banded group would have a

sporozoite rate intermediate between the rate in the three-banded salt-water breeding group and the rate in the three-banded fresh-water breeding group (see Muirhead-Thomson, 1951, Table III). In fact, the rate in the four-banded group is lower than the rate in the three-banded salt-water breeding group, though not significantly so ($P = 0.08$);¹ it is, moreover, highly significantly lower than in the three-banded fresh-water group ($P < 0.0001$).

It is thus apparent that the four-banded palp character accurately identified this segment of the population of the salt-water breeding species.

From Muirhead-Thomson's figures for 1948 (accepting all four-banded females as of the salt-water species) it can be seen that four-banded females comprised about 35% of the sample from the salt-water breeding population. Elsewhere in this paper Muirhead-Thomson states that the four-banded rate may reach 50% in Dar-es-Salaam. No record is available of any four-banded fresh-water "A. gambiae" ever having been found at Dar-es-Salaam. Further evidence of the rarity of four-banded fresh-water breeding females in the coastal areas of Tanganyika is that Gillies, after 12 years' work at Muheza, near Tanga, cannot recall having seen a four-banded female. It is thus clear that it is very highly probable that the lectotype of A. merus Dönitz was drawn from the salt-water breeding species population at Dar-es-Salaam.

The question now arises as to whether the salt-water breeding species at Dar-es-Salaam is conspecific with the salt-water breeding species found at Tanga which has been named Anopheles tangensis Kuhlöw, and which has been colonized ("Tanga Colony"). By crossing the offspring of wild-caught females with members of the Tanga colony, I have been able to demonstrate that the salt-water breeding populations from Pemba Island, Mauritius and Big Bend (Swaziland) are all conspecific with the Tanga colony (Paterson, 1963; Paterson et al., 1963). In other words, all the salt-water breeding populations within the A. gambiae complex from the eastern coastal areas of Africa and islands off Africa, which have been genetically identified, have proved to be conspecific with the "Tanga Colony". Since Tanga and Pemba lie north of Dar-es-Salaam, and Big Bend south of it, it appears virtually certain that the Dar-es-Salaam salt-water breeding species is also conspecific with the "Tanga Colony". It thus seems fair to conclude that Anopheles tangensis Kuhlöw (1962) is almost certainly a synonym of Anopheles merus Dönitz (1902).

¹ For method used see Bailey (1959, p. 61)

Despite these strong probabilities, I have, for the present, decided to refrain from formally reviving the name A. merus Dönitz and consequently sinking A. tangensis Kuhlou into synonymy. This is because I understand that Mario Coluzzi, who has been studying intensively the morphology of the members of the complex, may soon be in a position to confirm even more definitely that the lectotype of A. merus was drawn from a population of salt-water A. gambiae.

Conclusion

For the present it should be most strongly urged that the name Anopheles tangensis Kuhlou should not be used in the literature, but only the non-committal name "salt-water A. gambiae".

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III. A PRELIMINARY REPORT ON A NEW MEMBER
OF THE ANOPHELES GAMBIAE COMPLEX

by

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This preliminary note is to report the existence in Southern Africa of a fifth member of the Anopheles gambiae complex and to point out its probable significance in relation to so-called behaviour changes in "A. gambiae" following antimalarial spraying operations in the area.

Identification

Routine identifications of wild-caught females of "A. gambiae" are made by crossing their offspring to members of stock colonies of known identity. The following points are considered in making the identification:

- (a) F_1 sex ratio
- (b) Fertility of F_1 males
- (c) Appearance of testes and accessory glands of F_1 males

This information is considered together with information on the size of eggs laid by the wild females, and, in some crosses, the tolerance of first-stage larvae of these females to a solution of sodium chloride roughly equivalent to 75% sea water (Muirhead-Thomson, 1951).

For convenience, our routine crosses have usually been made to members of colonies of Species A (= group A of Davidson & Jackson, 1962; see also Paterson, 1963). In Table 1 are presented some typical results of crosses between the known East African members of the complex.

TABLE 1. TYPICAL CROSSES BETWEEN SPECIES A, SPECIES B
AND SALT-WATER A. GAMBIAE

P ₁		F ₁ adult sex ratio			F ₁ male fertility	F ₁ male ^a internal genitalia
♀ ++	♂	n	% ♂	% ♀		
Species A	Species B	1 014	48.2	51.8	Very low	2
Species B	Species A	270	49.6	50.4	Very low	2
Species A	S.W. <u>A. gambiae</u>	269	33.5	66.5	Nil	4
S.W. <u>A. gambiae</u>	Species A	187	>99.0	<1.0	Very low ^b	3
Species B	S.W. <u>A. Gambiae</u>	237	38.4	61.6	Nil	4
S.W. <u>A. gambiae</u>	Species B	156	100	0	Nil ^c	3

^a Numbers refer to illustrations in Figure 1

^b As judged by backcrosses

^c Sperms (all abnormal?) in testes

TABLE 2. RESULTS WHICH INDICATE THE EXISTENCE
OF A NEW FORM IN THE A. GAMBIAE COMPLEX

P ₁		F ₁ adult sex ratio			F ₁ male fertility	F ₁ male ^a internal genitalia
oo ++	♂♂	n	% ♂	% ♀		
Hippo Valley	Species A	22	100	0	+ ^b ₋	5
Chipinda Pools	Species A	20	100	0	+ ^b ₋	5
Sabi/Lundi R.	Species A	43	97.7	2.3	+ ^b ₋	5
Species A	Sabi/Lundi R.	222	43.2	56.8	Nil	6
Uzumba	Species A	7	100	0	+ ^b ₋	5
Chirundu 1	Species A	33	100	0	+ ^b ₋	5
Chirundu 3	Species A	78	100	0	+ ^b ₋	5
Chirundu 6	Species A	102	100	0	+ ^b ₋	5
Species B	Chirundu 6	20	40.0	60.0	Nil ^c	+2
Species A	Mzimpofo R.	212	22.2	77.8	Nil ^b ₋	6
Mzimpofo R.	Species A	138	100	0	+ ^b ₋	5
Balegane	Species A	173	80.9	19.1	+ ^b ₋	5
Balegane	Species B	18	100	0	Nil ^c	5
Sipofaneni	Species A	34	79.4	20.6	+ ^c ₋	5

^a - Numbers refer to illustrations in Figure 1

^b - As judged by backcrosses

^c - As judged by examining the vasa deferentia and testes

In Table 2 are gathered certain results from Swaziland and Southern Rhodesia which do not agree with any of these known crosses, but which agree well with each other. This information, when considered with the following facts, indicates that we are dealing with a new member of the A. gambiae complex, form C.

(a) The larvae from the two populations tested (Balegane and Mzimpofo River) were found to be susceptible with the salinity-tolerance test.

(b) The eggs of the three populations examined (Chirundu, Mzimpofo River and Balegane) have a mean size comparable to the means of the known fresh-water species. Thirty eggs from Balegane females, for example, had a mean size of 0.472 ± 0.003 mm.

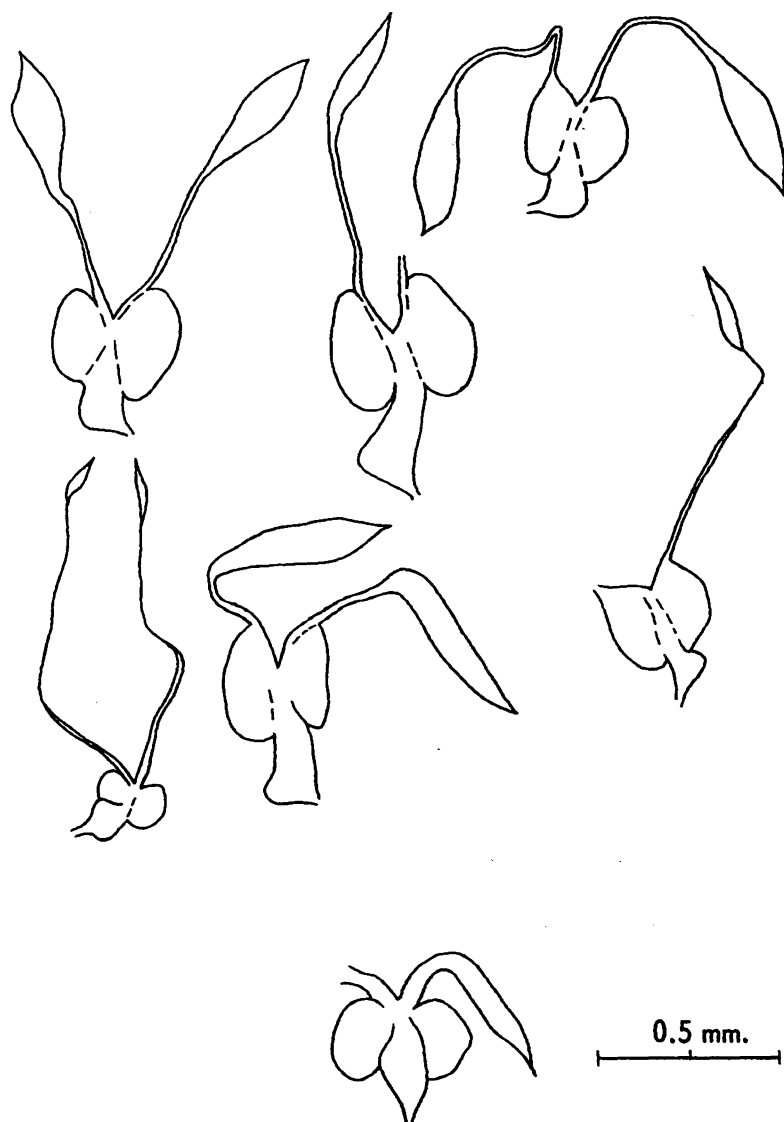
(c) The four-banded palp phenotype had a low frequency in small samples from four localities: 0/26 at Chipinda Pools, 3/29 at the junction of the Sabi and Lundi Rivers, 0/8 at Balegane, and 1/25 at Mzimpofo River.

To summarize the known differences between form C and the other forms of the A. gambiae complex:

1. From "salt-water A. gambiae" it can be distinguished by its small mean egg-size, the susceptibility of its first stage larvae to 75% sea water, by the relatively low frequency of the four-banded phenotype in natural populations, by the relatively high fertility of F_1 males from the cross form $C\text{♀} \times \text{Sp. A}\text{♂}$, and by the relatively large accessory glands in the F_1 males of the reciprocal cross.
2. From A. melas it is known to differ in that its first stage larvae are susceptible in the salinity-tolerance test. Further differences will appear when crossing experiments between the two forms are made.
3. The cross form $C\text{♀} \times \text{Sp. A}\text{♂}$ gives an F_1 generation composed largely of males, which distinguishes form C from Species A or Species B. The size of the testes and accessory glands in the F_1 males of the reciprocal cross further distinguishes form C from the other two known fresh-water breeding forms.

FIG. 1

Testes of F₁ males of certain crosses.



1. Sp. A ♀ x Sp. A ♂.
2. Sp. B ♀ x Sp. A ♂.
3. S.W. A. gambiae ♀ x Sp. B ♂.
4. Sp. A ♀ x S.W. A. gambiae ♂.
5. Sp. C ♀ x Sp. A ♂.
6. Sp. A ♀ x Sp. C ♂.
7. Balagane ♀ x S.W. A. gambiae ♂.

Biological notes

The little which is known of the new form's behaviour can be summarized as follows:

Breeding places. At Sipofaneni and Balegane it was found to utilize pools in muddy stream beds. At Mzimpofo River, pools in sand at the edge of the river and also rock pools were used. At Chipinda Pools breeding was found to occur in rock pools and in elephant hoofprints in mud.

Host preferences. At Uzumba, Mazoe Valley and in Swaziland the forms seem to strongly prefer bovines to man (see Mattingly, 1963). Window traps at the Swaziland localities gave no results and man-baited nets seemed ineffective, whereas cattle-baited nets often yielded specimens.

At Chirundu, an unsprayed area, form C was not found in catches from houses or huts in a small effective sample, but three or four of six females caught in the unique sheep shed were found to be form C. At Hippo Valley, Chipinda Pools, and Sabi/Lundi junction, our specimens were caught out of doors on human bait at sites well away from houses. At Chipinda Pools, the only resident humans within the immediate area were two tsetse workers, and there were no cattle at all. Here, and probably at Sabi/Lundi junction, the form probably is not dependent on man or his animals for blood. It is thus hardly significant that the few females caught along the Lundi River were caught on human bait, for the attack rate was always low, and alternative hosts few.

Exophily. The form seems to be very strongly exophilic in Swaziland and the Uzumba Reserve, Mazoe Valley, and it seems likely that this is true for Sabi/Lundi area, too. At Chirundu it was caught in a sheep shed which suggests that it will enter buildings if domestic animals are the attractant.

Sympatry with other members of the complex. Evidence is available for the presence of Species A and Species B at Chirundu (Table 3). The specimen of Species B, which is identified by the results in Table 3, was caught, together with the specimens of Species-C mentioned above, in a sheep shed.

The offspring of another specimen from this sheep shed collection when crossed to Species A females gave a sex ratio of 42.4% males, and, when crossed to Species B males, a sex ratio of 51.4% males. The F_1 males of the latter cross had normal-appearing testes.

and the obtaining of an F_2 generation showed them to be fertile. The F_1 males of the reciprocal cross had testes which varied in size from small to moderate, and some at least appeared to contain sperm. The obtaining of a small F_2 generation showed that these males were at least moderately fertile. These findings, which suggest that yet another form was involved, clearly require further support before any firm decisions can be taken.

Discussion

Evolution status of form C. Paterson (1963) has recently outlined the case for regarding all of the then recognized members of the A. gambiae complex as separate species. Since we have found that form C co-exists with Species A and Species B at Chirundu, we must conclude that it, too, is most likely a separate species. It should be remembered, in this regard, that the fact that the F_1 generations of laboratory crosses between Species A and Species B and the new form are semi-fertile, has little bearing on the argument for specific status, which depends on whether gene exchange occurs in nature.

Significance of the new form. The programme, of which these studies form a part, has as its main object, the elucidation of the apparent behaviour changes which occurred in Swaziland and in the Mazoe Valley following antimalaria spraying campaigns using the insecticide BHC.

The two alternative hypotheses which are being tested are:

TABLE 3. RESULTS DEMONSTRATING THE PRESENCE OF SPECIES A AND SPECIES B AT CHIRUNDU, ZAMBEZI VALLEY

P_1		F_1 adult sex ratio			F_1 male fertility	F_1 male ^a internal genitalia
♀♀	♂♂	n	%♂	%♀		
Species A	Chirundu	259	42.1	57.9	+ ^b	1
Chirundu	Species A	6	50.0	50.0	+	1
Species B	Chirundu 5	82	43.9	56.1	+ ^b	1
Chirundu 5	Species A	112	34.8	65.2	very low ^b	2

^a Numbers refer to illustrations in Fig. 1.

^b As judged by backcross; rest by observing vasa deferentia.

(a) That in these two areas the original vector was a separate sibling species of the A. gambiae complex from the present exophilic, zoophilic species, and that it was eradicated by the BHC because of its endophilic behaviour.

(b) That the change was a real change in behaviour within a single species due to powerful selection by the BHC against endophily.

It will be noted that the work described above has the greatest pertinence in deciding between these alternatives, for it shows that in both areas where the apparent switch to exophily and zoophily occurred, Species C, which appears to be strongly zoophilic even in unsprayed areas (Chirundu, Chipinda Pools), is the form which has survived spraying. Evidence from Chirundu suggests that either or both Species A and Species B were the original vector or vectors in the Mazoe Valley. In Swaziland it is probable that Species B was the autochthonous vector for we have proved it to enter houses and bite man at Tinonganine, Mozambique, which lies between Swaziland and the sea, and at Palmeira just north of Lourenço Marques, at both places before spraying began. If hypothesis (b) is to receive support, it must be shown that Species C in unsprayed areas enters houses to bite man and is a good vector. The evidence available does not support this view, though it is naturally desirable to extend it. Hypothesis (a), on the contrary, receives powerful support from this work.

A word should be said at this point about the recent recrudescence of malaria transmission in Swaziland along its border with the then unsprayed Mozambique. This was interpretable on either hypothesis as the invasion of either the vector species which had been eradicated from Swaziland, or as the invasion of an unselected population from Mozambique. We have recently found that at this place "salt-water A. gambiae" is present (Paterson et al. 1963), but we have also identified a single female caught in a window-trap on a hut as Species B. The identification is based on the sex ratio in the F_1 generation of the cross Big Bend ♀ x Sp. B♂, and the presence of sperm in the vasa deferentia of the F_1 males. This finding supports the first alternative and adds further strength to hypothesis (a) above.

It would be most interesting to follow up a spraying campaign at Chirundu, especially if BHC were the insecticide used, to see whether Species A and Species B are eradicated leaving only Species C, thus replicating the situation in Swaziland and Mazoe Valley.

The naming of Species C. It is not possible at this stage to name Species C formally as we are at present unable to recognize the type specimens of Anopheles gambiae Giles and other types bearing names which, up till now, have been considered synonyms of A. gambiae.

Correction. In Paterson 1963, Species A was recorded from Hippo Valley on the Lundi River, Southern Rhodesia and from Sipofaneni, Swaziland. These records were actually the first two of Species C but were attributed to Species A because too much weight was placed on the fertility of F_1 males in the crosses Sp. C ♀ x Sp. A ♂, and not enough on the F_1 sex ratios.

The results mentioned by Mattingly (1963, p. 41), refer to the same material which was collected at Hippo Valley in May, 1962.

It is also possible that some of the tentative identifications of "salt-water A. gambiae" from Tinonganine, Mozambique (Paterson et al. 1963) referred, in fact, to Species C, which gives somewhat similar results in its crosses to Species A and Species B.

Summary

1. Evidence is presented for the existence of a new member of the Anopheles gambiae complex of sibling species.
2. What is known of its distribution and behaviour patterns is summarized.
3. It is pointed out that these data lend strong support to the hypothesis that the "behaviour changes" displayed by A. gambiae following antimalarial spraying with the insecticide BHC in Swaziland and Southern Rhodesia are due to the eradication of the local anthropophilic vector sibling species of the A. gambiae complex, and leaving unscathed the strongly zoophilic, and, apparently, morphologically identical sibling species here reported on.

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FIG. 2
Known distribution of Sp. C in Swaziland

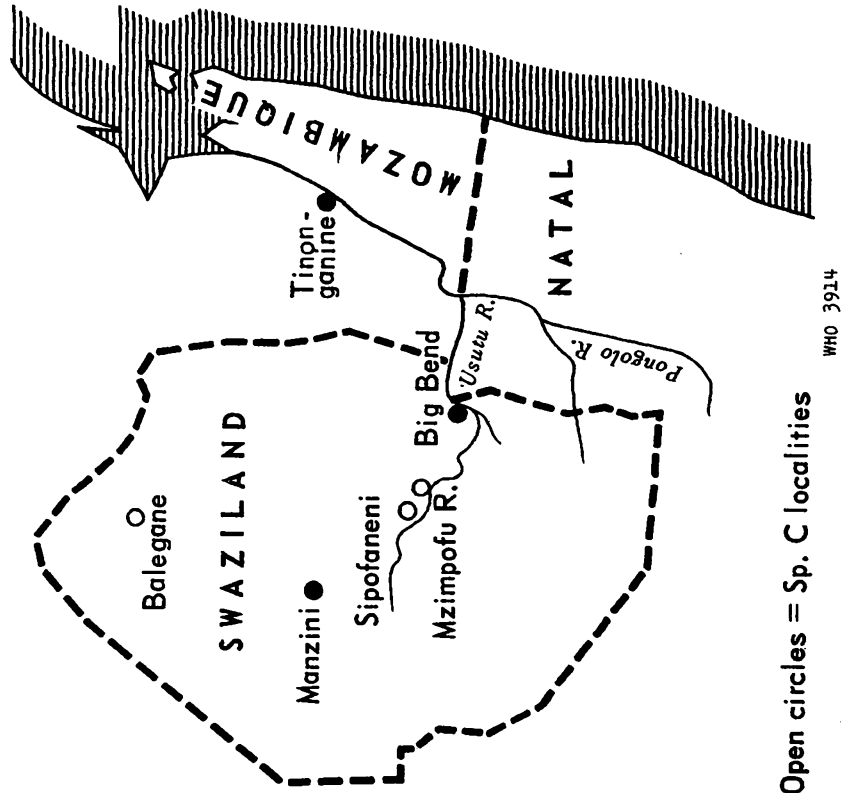
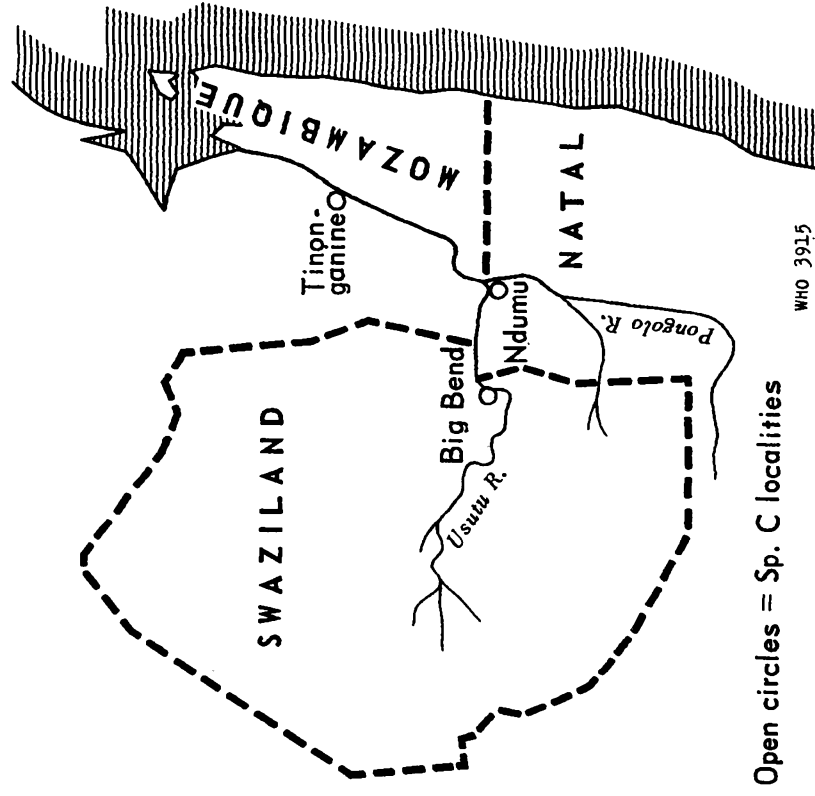


FIG. 3
Known distribution of Sp. C in Mozambique and Natal



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