ANNEX: The use of morphological characters for the identification of the adult female flies of the three groups: S. damnosum / S. sirbanum, S. squamosum, S. soubrense / S. sanctipauli.

The study of the vectorial capacity of different species depends heavily on the exact identification of the adult female flies. Garms et al. (1980, 1981, 1982) have described the characters they used for the differentiation of the flies and they insist on the particular use of the ratio thorax/antennae for the separation of the species. Additional information is provided by the colour of hairs at different body regions and by the colour and compression of the antennae (Dang and Peterson, 1980, Quillévéré et al., 1977), although these characters might be very variable.

From own experience, it seems to be necessary to give a detailed description of the way of examination of these morphological characters, since their aspects may change by different methods of examination. Garms et al. (1981) stress the advantages of alcohol-preserved flies, but in contrast to this we had to identify fresh flies for the subsequent histochemical identification of the enclosed filarial larvae (by Dr. Omar).

No attention has been paid to the identification of S. yahense, since it seems not to be an important vector in Togo or Benin. Larvae of S. yahense have only been identified very rarely in larvae samples collected from the two countries (less than 1 promille, report Fiagsorgbor, June 1982).

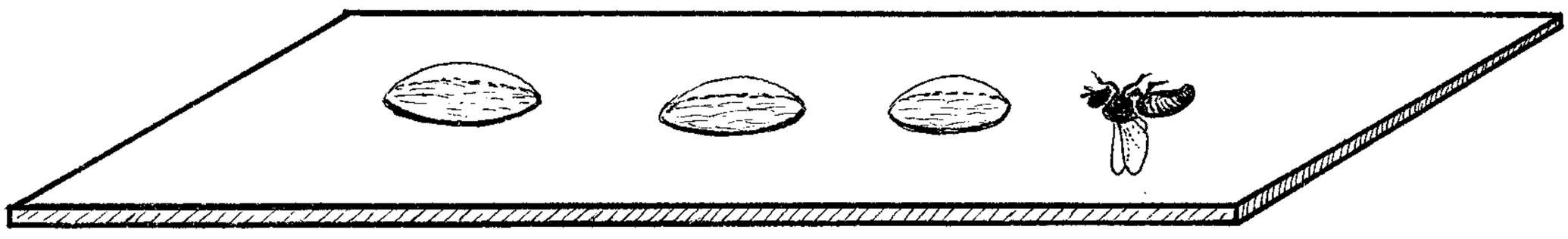
Material and Methods

- Wild M5 dissecting microscope, 10x oculars, with transmission-illumination
- 1 ocular micrometer, 12', 120''
- 1 Nachet-glasfiber lamp with two arms, fixed at the microscope
- 1 pipette with 70% alcohol
- dissecting needles, saline NaCl 0.4%, slides
- dissecting record forms "fiches 2A"
- Wild M11 transmission light compound microscope with ocular micrometer
- 1 stage micrometer
- 1 pocket calculator

Procedure of examination:

The fly is killed by chloroform-vapor (attention: no wetting by the liquid!) and placed on a slide with three drops of saline (0.4%, no detergent (!)) (Fig. 1):

Fig 1



Under the microscope, the following characters are examined at 50x magnification under illumination from above:

- colour of wing tufts
- colour of the basicostal hairs
- colour of the sculellar hairs
- colour of the postcranial hairs
- colour of the fore-coxae hairs

The colour observed was recorded on the "fiches 2A" adapted herefore using the following codings (Table 1):

00 = observation not possible (hairs missing)
01 = all hairs pale
02 = some black hairs intermixed (up to five for wing tufts)
03 = half black half pale mixed
04 = most hairs black, some pale (up to five for wing tufts)
05 = all hairs black

Only very dark hairs were considered to be black, not greyish ones. The fly must not be submerged under the saline at this stage of dissections, since the following measurement of the lengths of thorax and antennae can not be done accurately, if the fly is not dry.

Tab. 1

OCP/VCU

CAPTURES ET DISSECTIONS S. DAMNOSUM

FICHE 2 A (1-2) PAGE (3-4) DE

SECTEUR : .... S/SECTEUR : .... COURS D'EAU : ....

POINT DE CAPTURE : .... (5-10) AN : 19 (11-12) MOIS : (13-14) JOUR : (15-16)

NBR HEURES DE CAPTURE : (17-18) PARTIE DU JOUR : (19-20) NBR S.D. CAPTUREES : (21-24) DISSEQUEUR : .... DATE DISSECTION : ....

NO. SEQ.	HORAIRE DE CAPTURE	ESPECE		AGE	NOMBRE DE LARVES				AUTRES PARASITES			REPAS DE SANG	
					Ier STADE	2ème STADE	3ème STADE TETE	3ème STADE TH. & ABD.	MERMITHIDES	AUTRES FILAIRES	FONGIDES		
25-26	27-28		30-31	32	33-34	35-36	37-38	39-40	41-42	43-44	45		
01		1											
02		1											
03		1											
04		1											
05		1											
06		1											
07		1											
08		1											
09		1											
10		1											
11		1											
12		1											
13		1											
14		1											
15		1											
16		1											
17		1											
18		1											
19		1											
20		1											

TOTAUX	DISSEQUEES	PARES	INFECTEES	AVEC LARVES 3ème ST. TETE	AVEC LARVES 3ème STADE	NBR LARVES 3ème ST. DANS LA TETE

			POSTCRANIAL HAIRS		FORE-COXAL HAIRS	BASI-COSTAL HAIRS	SCUTELLAR HAIRS	ANTENNAE COMPRESSION	LENGTH THORAX	LENGTH ANTENNA	RATIO THORAX/ANTENNA		SPECIES IDENTIFICATION

OBSERVATIONS

WHO 5385.1 OCP (8/79)

Measurement of the lengths of thorax and antenna

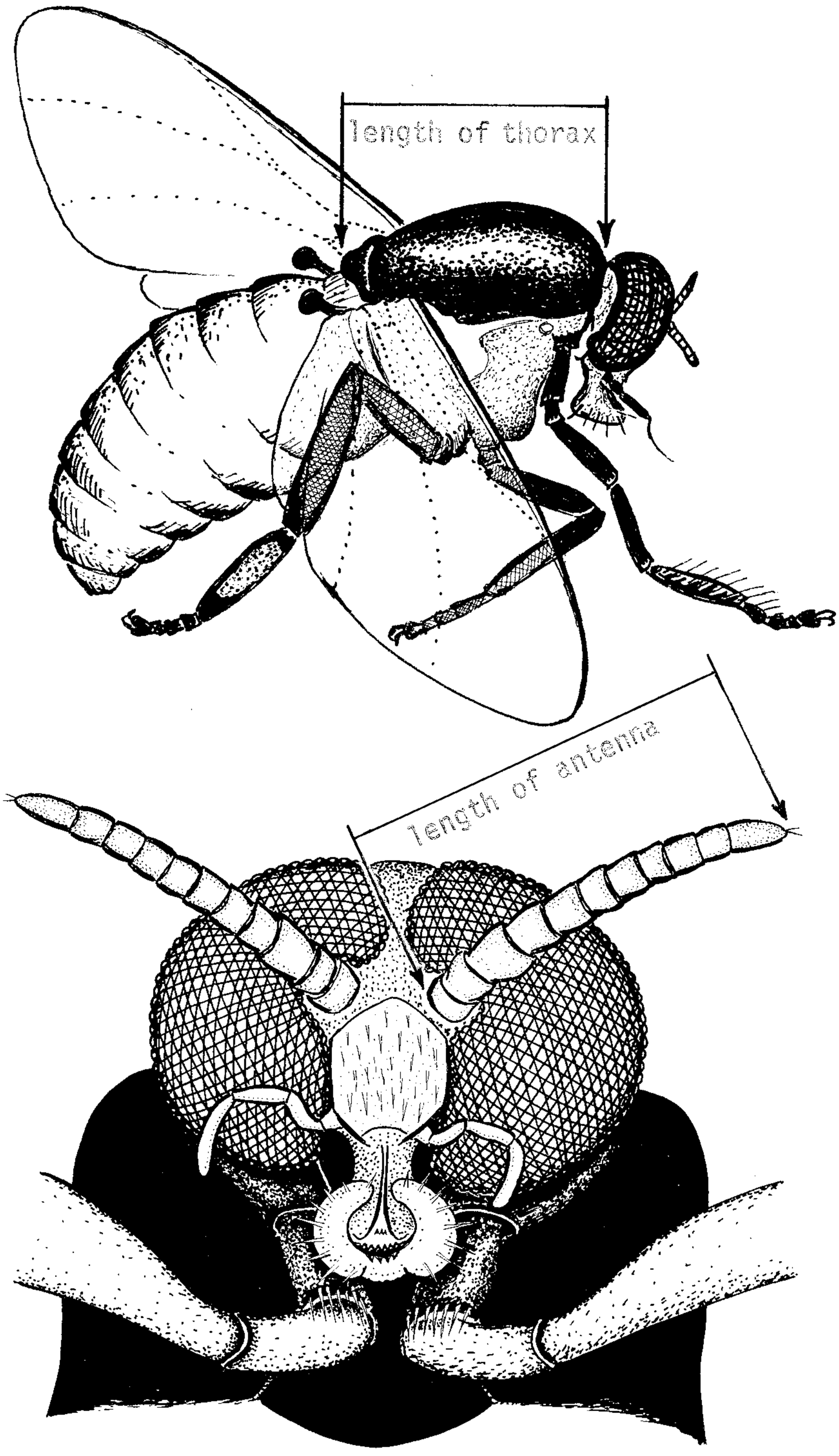
Thorax:

The fly is turned to one side and the length of the thorax is measured from the beginning of the prothorax to the tip of the scutellum under 50 x magnification (Fig. 2). In case the tip of the scutellum is hidden behind the wings, the fly is brought into the first drop of saline and the two wings are folded to the left and right side and are tipped into the saline for fixation. On the micrometer scale, the length of the thorax varied between 42 to 65 units (840 - 1300 /um).

Antenna:

For the measurement, the fly is turned to the back and the wings and the abdomen are fixed by the contact with the first drop of saline. Then the fly can be orientated so that one antenna is in a horizontal position for all its length (Fig. 2). It is important, that the antenna must not get wetted by water or saline. When the length is measured, adjustments of the lengths have to be made in case the antenna is curved or if the first segment (Scapus) is not visible. The length is taken from the base of the antenna to the tip of the last segment (11th), but the hairs at its tip are not included. It is impossible to measure the length of an antenna accurately, once it is cut from the head during the dissection of the fly, since the saline causes the antenna to swell up considerably. Similarly, a fly cannot be measured when submerged in a drop of saline, due to the optical activity of the drop which acts like a convex (or concave) lense. In our measurements, the length of the antenna varied between 20 and 33 units (400 - 660 /um).

Fig. 2: The measurement of the length of thorax and antenna



The ratio length of thorax / length of antenna

Both the length of the thorax and the length of the antenna were recorded in micrometer-units on the record forms (fiches 2A). The ratio thorax/antenna was calculated on a pocket calculator and the result is recorded. Since the ratio is dimensionless, it is not necessary to convert the micrometer units into microns or mm. In case, that the ratio so calculated did not conform with the other morphological characters of the fly, the measurements were repeated with the same or the other antenna to verify the result. Ratios between 1.75 and 2.71 were observed, and 95% of 2.300 measurements were between 1.88 and 2.35.

The dissection of the fly

For the measurement of the length of thorax and antenna, the fly was already placed into the first drop of saline at the right side. The subsequent dissection of the fly was done as follows:

The spermatophora, if present, is detached by a gentle press with the dissection pin on the female genital orifice. Then the abdomen is opened for the separation of nulliparous and parous flies.

If the fly is parous, the abdomen is cut off and the thorax-head part is picked up by the dissecting pin and brought to the left drop of saline, where the head is cut off. The remaining thorax is then dissected in the drop in the middle. The head is dissected at last.

The recording of the age, the filarial infections and other parasites of the flies, found during dissection, was made according to OCP standards on the left column of the fiches 2A (Tab. 2). However, it should be mentioned, that it is impossible, to make separate recordings on these forms for non-infective (preinfective) larvae L_3 and infective larvae L_3 found in the thorax and in the abdomen.

During the dissection of the head, the antennae can be detached for the examination of their colour and compression of the segments 4-6.

If the antennae get not wetted by the saline, as it happens usually, when no soap or detergent is used, they are drawn out of the drop of saline and a small drop of 70% alcohol is added, which immediately submerges the antenna and clears it up beautifully. Records of the compression of the segments 4-6 is taken as follows:

- : no compression
(+) : slight compression (only segment 4)
+ : obvious compression (segments 4-6, often more)

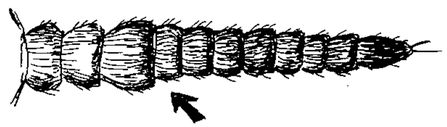
The antennae should always be looked on from the same side (from below).

Identification of the species group:

After the measurement of the flies and the examination of the colour of the various hairs, the fly is classified into one of the three groups S. da/si, S. sq and S. so/sa according to the combination of their main morphological caracters, given in Tab. 2 . This table was compiled from the reports of GARMS et al. (1980, 1981). As they have pointed out in their papers, there is much overlapping of these caracters in the three groups, especially for the colour of the hairs. Therefore, the ratio of the thorax/antenna length is considered to be the most selective parameter, in case that the other parameters differed.

Tab. 2

I D E N T I F I C A T I O N O F A D U L T F E M A L E S O F T H E S. damnosum K O M P L E X

	S. damnosum/sirbanum	S. sqamosum	S. soubrense/sanctipauli
A N T E N N A E - Form :			
- Description :	segments 4 and 5 always compressed, often other segments also	segment 4 (and 5) distinctly compressed	very seldom any compression
- Colour :	first 4 segm. normaly pale	first 2 (3) segments pale	all segments dark
Antennae / Thorax Ratio	2.10 - 2.40 (median 2.2-2.3)	2.04 - 2.20 (median 2.15)	1.80 - 2.04 (median 1.96)
W I N G T U F T S	almost all pale	most pale, some mixed	some pale, many mixed and dark
- colour class	01 02 03 04 05	01 02 03 04 05	01 02 03 04 05
- % observed	97.7 2.1 0 0.2 0	86.2 6.6 3.2 3.9 0.2	16.0 13.9 17.1 30.1 22.9
POSTCRANIAL HAIRS	typically all pale, lying flat on the head. Few dark	typically many dark or greyish hairs, but often most hairs pale. Sometimes protruding	almost all hairs very black, few pale hairs intermixed
FORE-COXAE HAIRS	all pale	seldom few dark hairs intermixed	usually some very black hairs visible

RESULTS

Thorax and antennae lengths of the flies:

A total number of 2,300 measurements of thorax and antennae were available from the identification and dissection of flies during the study on the vectorial capacity of different groups of the S. damnosum complex in Togo and Benin 1982. In this number, measurements of flies, found infected by mermithids at the subsequent dissection, were not included, since this parasite obviously changed the morphology of the flies due to abnormal growth.

There is a strong correlation between the length of the antennae and the length of the thorax (Fig. 3): large flies have longer antennae than small ones. No clear separation of different species groups can be observed in this figure, although one might suspect two different clusters, the one above representing the groups S. sq and S. da/si and the other below S. so/sa.

The frequency distribution of the thorax lengths resembles to a normal distribution (Fig. 4), whereas the distribution of the antennae length (Fig. 4) shows two maxima, one at 480 /um and a second at 540 /um.

If one assumes, that the thorax length of the flies is more or less normaly distributed in the three species groups, with rather similar means for each group, then it is interesting to examine, whether the length of the antenna is at a certain constant ratio within the three species groups, i.e, whether the ratio thorax/antenna length shows a three-modal pattern (Fig. 5). Due to the fact, that only integer values were recorded for the length measurements, certain values (like 1.98, 1.99, 2.01 ...) can not be obtained by a division of two numbers within the range of lengths observed (42-64 units for the thorax, 20-33 units for the antenna, minimum ratio 1.75, maximum ratio 2.50). This phenomenon can be overcome by forming larger frequency classes (histogramm II).

The length of thorax and antenna of a mixed population of
S. damnosum, S. sirbanum, S. squamosum, S. soubrense and S. sanctipauli

(2,300 flies measured)

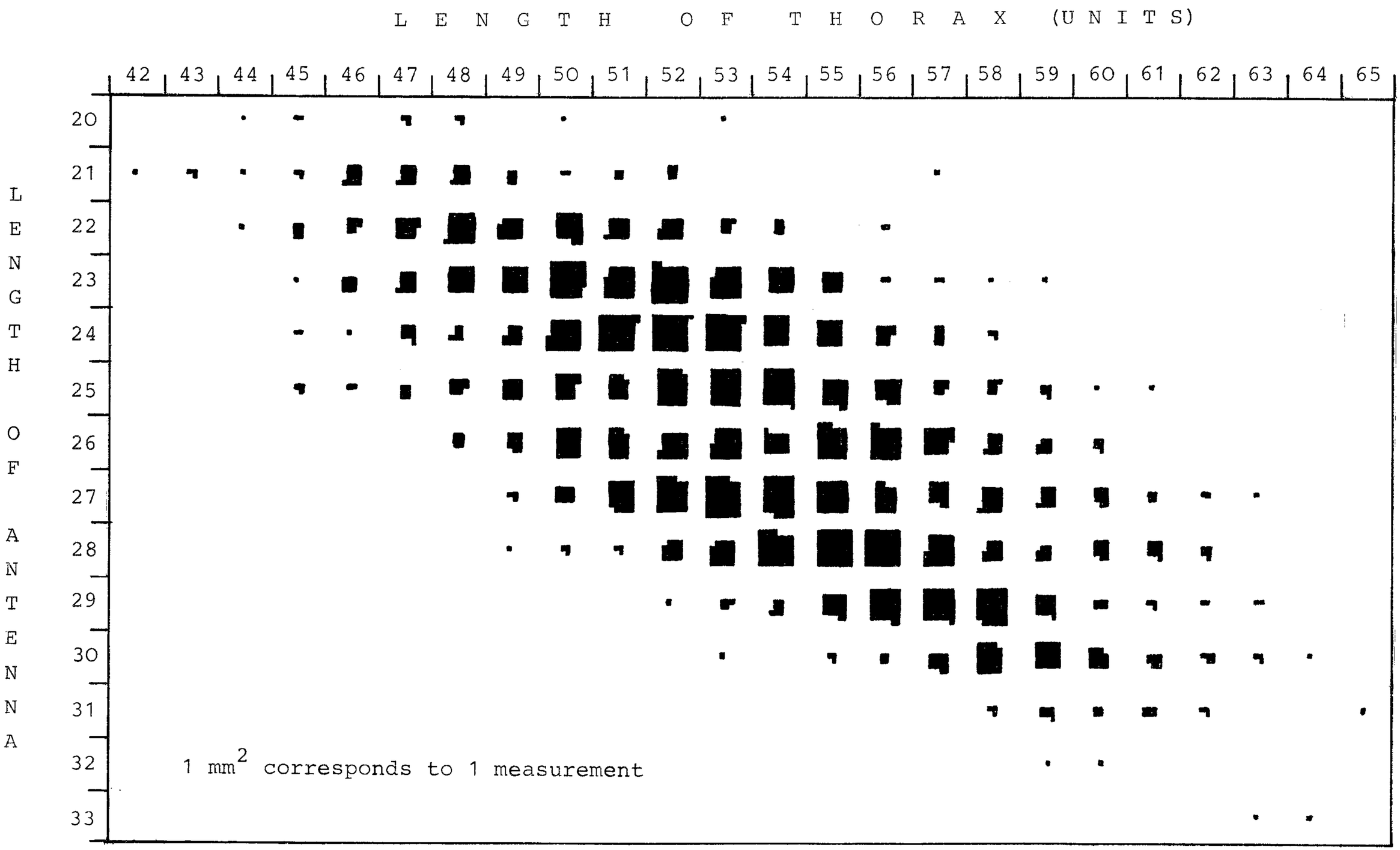
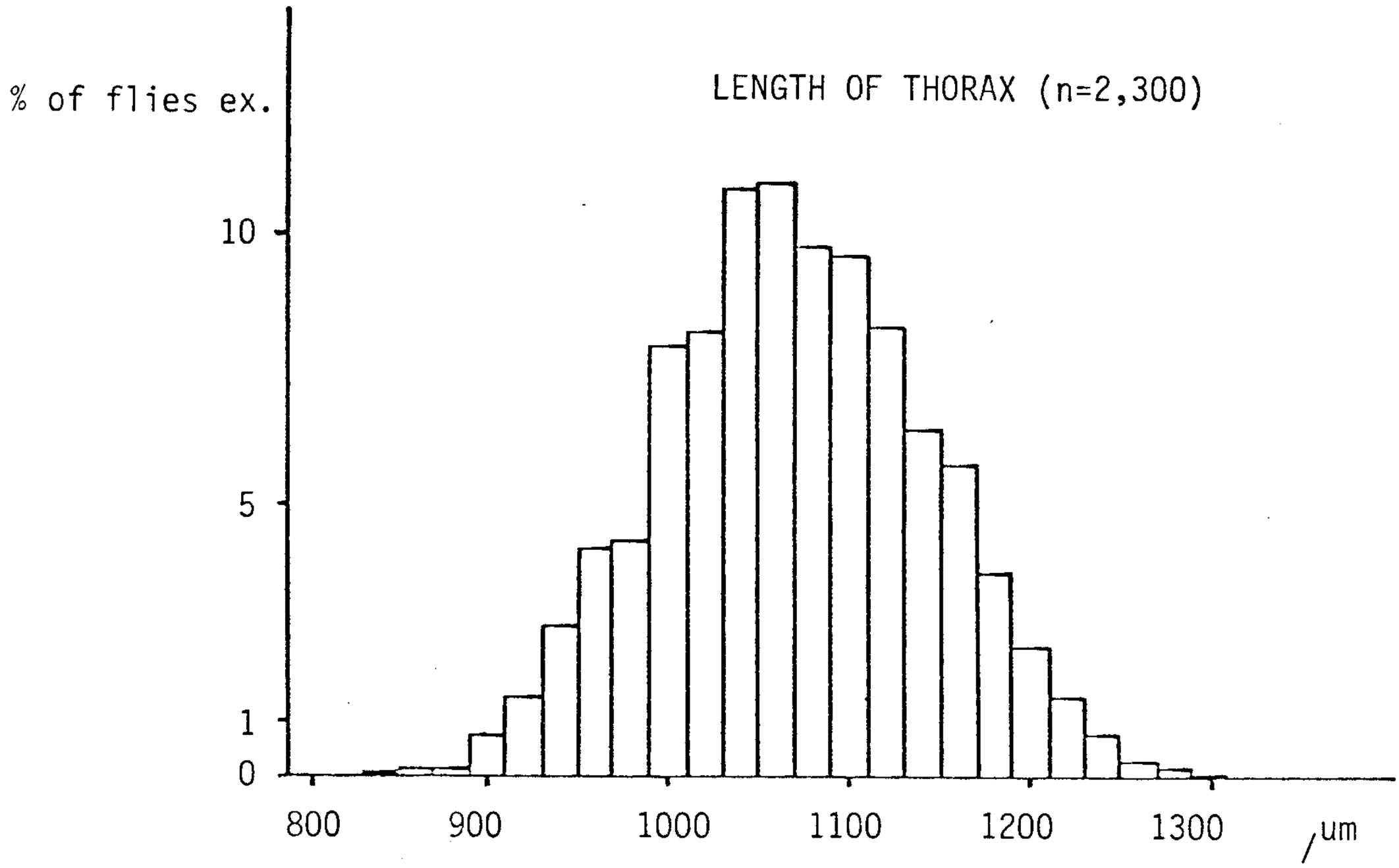


Fig. 4



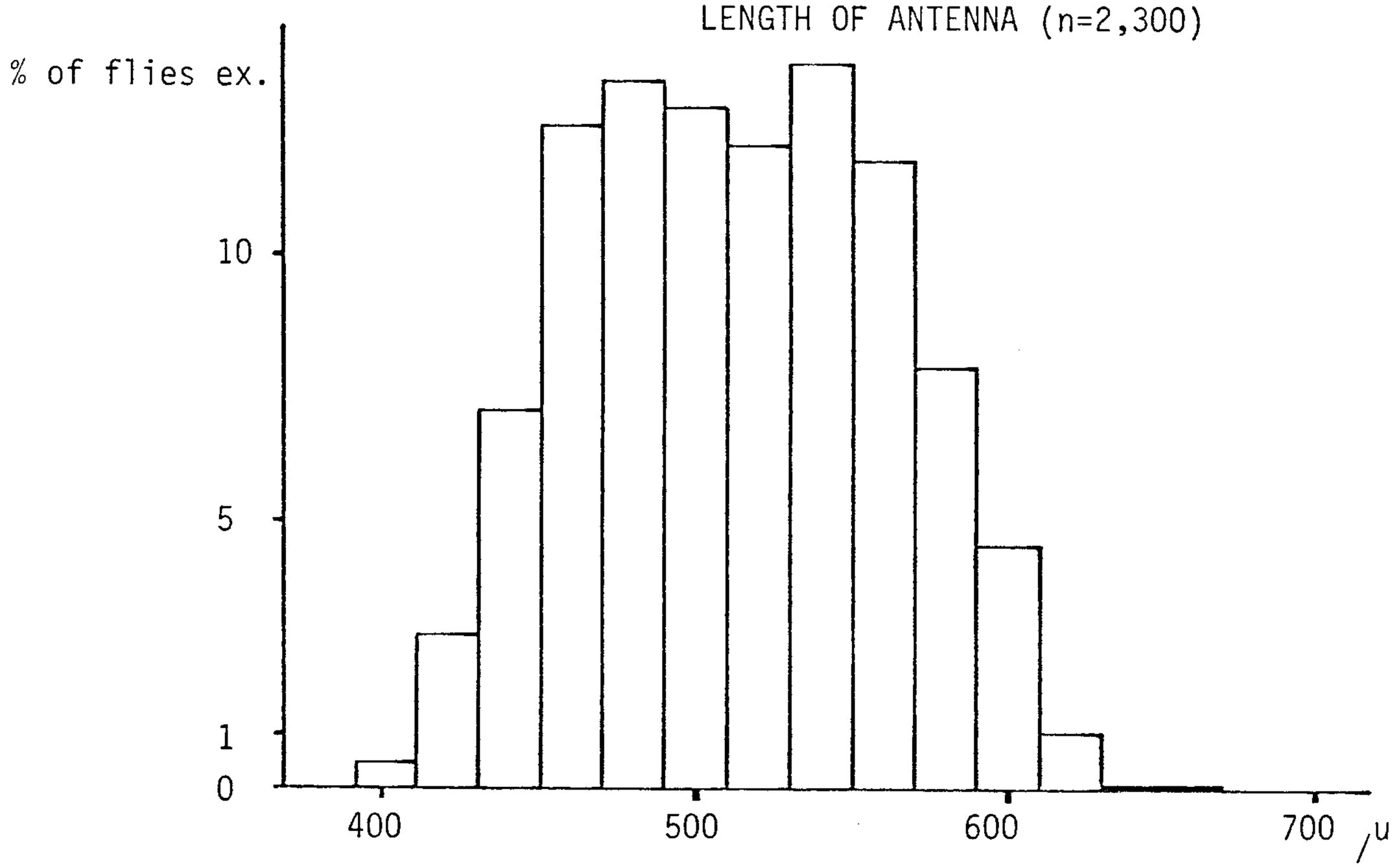
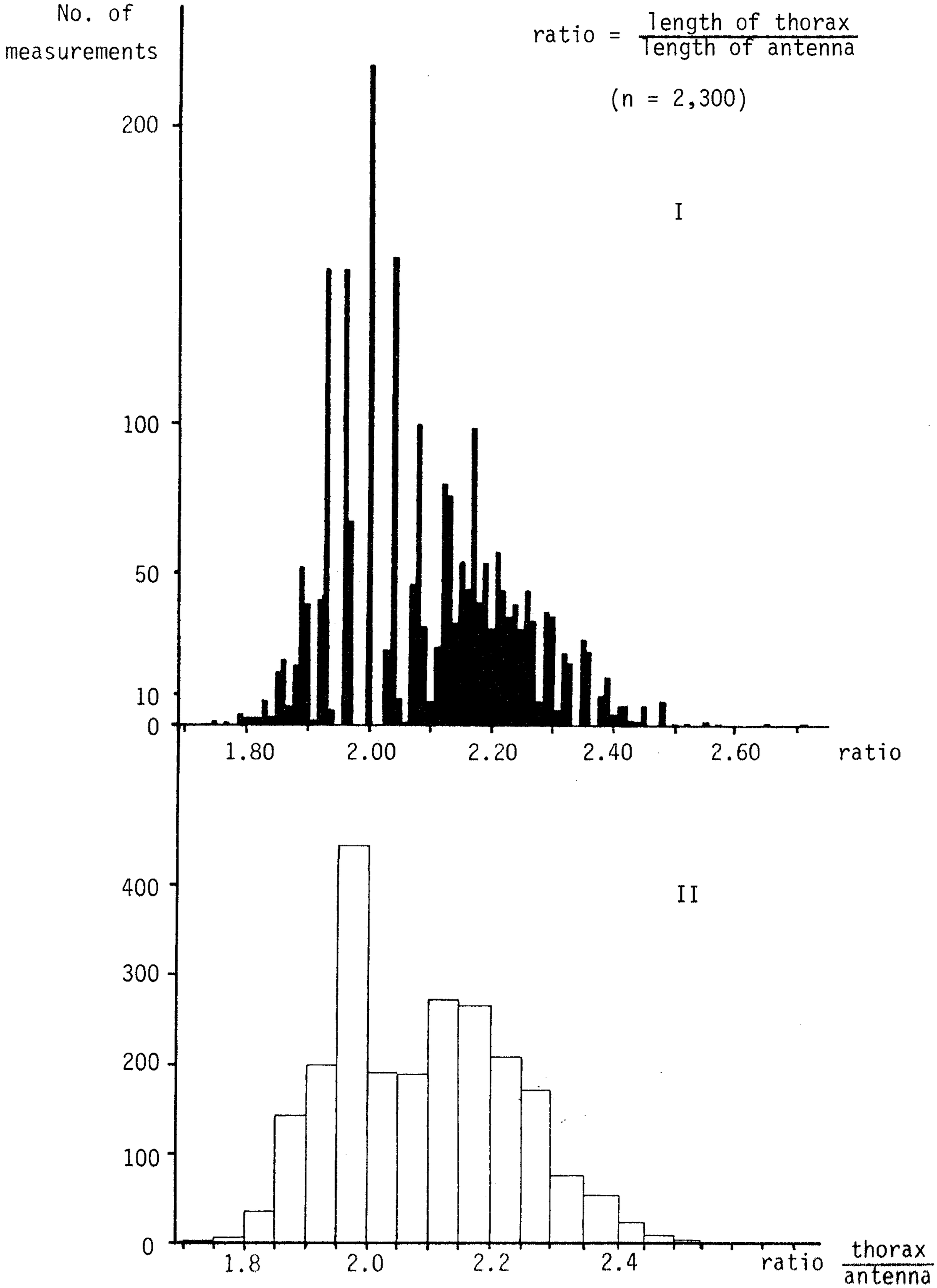


Fig. 4 : The frequency distribution of the length of the thorax and antenna of 2,300 S. damnosum s.l. flies from Togo and Benin, caught during the months August to October 1982. Of these flies, 963 were classified as S. soubrense, 741 as S. squamosum and 596 as S. sirbanum/S. damnosum s.str..

Fig. 5 : The frequency distribution of the measurement of the ratio length of thorax divided by length of antenna for 2,300 flies from catching sites in various bioclimatic zones of Togo and Benin

I) histogramm with ratio = 0.01
II) histogramm with ratio = 0.05



Two groups of flies can now clearly be distinguished by their ratio of thorax/antenna length, one with the maximum between 1.96-2.00, representing the S. so/sa group, the other group with the maximum in the range between 2.10-2.20, which is a mixture of the groups S. sq and S. da/si.

A better separation of the frequency distributions of the ratios for the different groups is obtained, when the results are presented separately for the five fly-catching sites and for nulliparous and parous flies (Fig.6). At Djodji, where the population is made up mainly by S. sq with some S. so/sa, but where there are no S. da/si, the maximum of the distribution is between 2.11-2.15 both for nulliparous and parous flies. At Landa Pozanda, the main species in the larvae from the local breeding site were S. da and S. si, and therefore, the ratio 2.26-2.30 in the nulliparous population can be attributed to this group, whereas in the parous population, an increased proportion of presumable invading S. sq flies resulted in a bimodal shape of distribution: a ratio of 2.16-2.20 for S. sq and 2.26-2.30 for S. da/si. The presence of S. so/sa in a population is indicated by the proportion of flies with a ratio of 1.96-2.00. At Djodji, the proportion of S. so/sa is considerably higher in the parous than in the nulliparous population. At Kaboua and Tététou, most of the flies belong to this group.

The colour of the wing tufts:

The large majority of S. da/si and S. sq from our catching sites in Togo and Benin had pale wing tufts (colour class 01 and 02, Tab.3), and almost all flies with dark tufts (04 and 05) were classified as S. so/sa. There are differences in the coloration of the wing tufts of the S so/sa group from the lower Mono (Tététou) in Togo as compared to those from the Okpara (Kaboua) and Ouémé (Bétérou) in Benin. S. so/sa were darker in the Benin (65.7% in the class 04-05 at Bétérou, 69.4% at Kaboua) than in Togo (42.8% in class 04-05 at Tététou).

Fig. 6 : Frequency distribution of the thorax/antenna ratio of nulliparous and parous flies from the savanna and rain-forest in Togo and Benin

Landa Pozanda
da/si: 61 %
sq : 35 %
so/sa: 4 %

Bétérou
da/si: 44 %
sq : 40 %
so/sa: 16 %

Djodji
da/si: 4 %
sq : 78 %
so/sa: 18 %

Kaboua
da/si: 11 %
sq : 13 %
so/sa: 76 %

Tététou
da/si: 2 %
sq : 2 %
so/sa: 96 %

Tab. 3

The colour of the wing tufts of S. so/sa, S. sq. and S. da/si

dissections 17.8. - 18.10. 82

Place	Number of flies examined with wing tuft colour-class: 00	01	02	03	04	05
Simulium soubrense/ S. sanctipauli						
Landa Pozanda	1	2	2	5	7	2
Djodji	1	10	3	16	34	16
Tététou	23	90	108	91	118	98
Bétérou	0	20	4	10	35	30
Kaboua	9	26	34	54	140	119
total flies ex.	34	148	151	176	334	265
% of flies ex.	/	13.8	14.1	16.4	31.1	24.7
Simulium squamosum						
Landa Pozanda	2	183	6	2	1	0
Djodji	3	292	36	10	6	0
Tététou	1	4	4	3	2	0
Bétérou	9	233	5	0	4	0
Kaboua	0	40	4	6	12	1
total flies ex.	15	752	55	21	25	1
% of flies ex.	/	88.1	6.4	2.5	2.9	0.1
Simulium damnosum/ S. sirbanum						
Landa Pozanda	1	334	2	0	0	0
Djodji	0	13	3	0	1	0
Tététou	0	6	2	0	1	0
Bétérou	11	256	4	0	0	0
Kaboua	0	56	1	0	0	0
Total flies ex.	12	665	12	0	2	0
% of flies ex.	/	97.9	1.8	0	0.3	0

DISCUSSION

The ratio of the thorax/antenna length was found to be the most distinctive parameter for the separation of the three species groups, together with the colour of the wing tufts and postcranial hairs. Less information is provided by the colour of the basi-costal, fore-coxae and scutellum hairs, and their examination could be omitted without loss of much information. Completely black scutellum hairs are of use to separate S. yahense from S. so/sa, which has pale or mixed hairs, but this is not of much help, since S. yahense is not a vector in Togo or Benin (GARMS, pers. comm.).

More attention should be paid to the colour and shape of the antennae, for which a more detailed form of recording the compression of the segments 4-6 should be used. The calculation of the compaction ratio (QUILLEVERE et al., 1977) would certainly promise the best results, but is very time-consuming and needs the mounting of the antenna for the measurement.

Our results from the identification of the species composition at the five fly-catching sites were in good agreement with the results from the cytotaxonomic identification of larvae samples from nearby breeding sites (cf. report pg. 16), and they were similar to the adult populations, observed during the last years at the same sites (GARMS et al. 1982).

However, there was no possibility to verify the results of identifications of single flies, and in view of the wide range of variation of the morphological parameters, misidentifications certainly do occur, especially in separating the group S. sq from S. da/si. Parasitism of flies by mermithids seemed to result in an abnormal growth, the length of the thorax of those flies being shorter as normal if compared to the length of the antenna. Mermithid infections were very common amongst nulliparous flies at Bétérou, and the high proportion of S. sq (40%) might be explained partially by this fact. The highest proportion of S. sq observed during the last year was 17% in September (GARMS et al. 1982).

The "Beffa" form of S. so/sa is frequently found with pale wing tufts (GARMS et al, 1980, MEREDITH et al., in press) and this distinguishes it from S. so/sa from the Ivory Coast, where the wing tufts are almost invariably dark (GARMS, pers. comm.). Nothing is known (?) about the colour of the wing-tufts of the other 'species' of the S. so/sa group (i.e. S. sa and S. so, as identified by their larvae) in Togo or Benin. Possibly, the higher proportion of dark wing tufts in the group S. so/sa from the Okpara and Ouémé rivers in Benin as compared to the flies from the lower Mono in Togo reflects the different proportion of the "Beffa" form in these rivers. In the lower Mono, 67% of the larvae were identified as "Beffa" (S. so/sa), and 33% were S. so. No S. sa were found (rainy season 1982). In the Ouémé, 34% were S. so/sa, 45% were S. so and 20% were S. sa during the same period. Therefore, the higher proportion of dark wing tufts in Benin could be explained by the higher proportion of S. so and S. sa, but more information, based on the identification of larger larvae samples from these sites, should be available to verify this question.

The frequency distribution of the measurements of the ratio thorax/antenna was very caracteristic for the different fly-populations at the five sites. It might be possible to develop a method for the calculation of relative proportions of the three fly-groups in a population on the base of the histogramm of the measurements of the ratio thorax/antenna.

Differences in the frequency distribution of the ratio thorax/antenna in the nulliparous as opposed to the parous population are an indicator of fly-migration. This can be shown at Landa Pozanda, where S. sq is more frequent in the parous flies, presumably due to reinvading parous flies in the Kara valley. On the other hand, the low parous rate of S. sq at Djodji, where the proportion of S. so/sa was increased in the parous population, might indicate, that S. sq leaves the area after its first blood-meal and travells North.