The Separation of the Immatures of Certain Mosquitoes of the Subgenus *Anopheles* in Southern Africa Using Computer Techniques

M. Coetzee and K. Newberry
National Institute for Tropical Diseases
P. O. Box 33
Tzaneen 0850
South Africa

ABSTRACT: Differences in the immature stages of *Anopheles (Anopheles) coustani*, *Anopheles (Anopheles) tenebrosus* and *Anopheles (Anopheles) ziemanni* from southern Africa are described. Belkin's (1962) system was used initially to differentiate between the larvae and pupae with computer analysis being utilized for the final discrimination. The usefulness of egg characters to discriminate between the three species, and the specific status of these taxa are discussed.

INTRODUCTION

For many years, *Anopheles (Anopheles) tenebrosus* Dönitz and *Anopheles (Anopheles) ziemanni* Grünberg were considered to be varieties of *Anopheles (Anopheles) coustani* Laveran (Evans 1938, De Meillon 1947). Gillies and De Meillon (1968) elevated these varieties to full specific rank on the basis that they coexist with *An. coustani* in nature without evidence of intergrades in hind leg markings and have differing geographic distributions and behaviors. However, Gillett (1972) suggested that perhaps they might equally well be regarded as members of a single polymorphic species, but nevertheless treated the group along the lines of Gillies and De Meillon (1968).

No differences in the pupae and larvae of these three species have been reported (Evans 1938, De Meillon 1947, Gillies and De Meillon 1968) though Evans (1938) does mention a pupal variation in some specimens of *An. ziemanni*.

Gibbins (1933) described the egg of *An. ziemanni* as having a continuous open deck between the floats and the exochorion as stippled but without polygonal markings. De Meillon (1937) described the eggs of *An. coustani* and *An. tenebrosus* as both having polygonal markings on the exochorion but *An. tenebrosus* has a continuous opening between the floats as in *An. ziemanni*.

MATERIALS AND METHODS

Egg batches were obtained from identified wild-caught females from various localities in South West Africa, Zimbabwe and South Africa. Families were

reared from egg to adulthood with larvae and pupae skins preserved in 80% alcohol. These pelts were later mounted in Canada Balsam. Setal counts of 300 pupae from 42 families and 100 larvae from 30 families were recorded using the Belkin (1962) system. The range of branching for each species is given in Tables 2 to 7. Eggs were examined using 100 times magnification to ascertain characteristic differences.

Illustrations (Figs. 1 to 6) were compiled from a few specimens of a single family of each of the three species which occur sympatrically at Grey Stones, Letaba district, Northern Transvaal. There is variation in length and number of branches within the family, so the illustrations merely represent the family and not a particular individual. A map of southern Africa has been included with each pupal illustration showing collection areas for that species (Figs. 1, 3 and 5).

The computer program used for the discriminant analysis was SPSS (Nie et al. 1975) utilizing the stepwise method and running all three groups at once.

RESULTS

PUPA:

Having recorded the branching of 100 pairs of setae from each of 66 pupae, it became apparent that eight of these setae could be of diagnostic significance. They are M-11, I-3, I-5, III-8, IV-4, IV-6 and V-1. The number of ribs on the trumpets was also a good character. All these characters were then recorded for 300 pupae (100 of each species and not more than 10 individuals per family).

Using computer analysis 99.33% discrimination was obtained with only two of the 300 specimens being misclassified. At a 95% level of probability, 91% of the specimens were correctly classified (Fig. 7).

A key was formulated using only two of the nine characters, M-11 and IV-6, whereby 89% of the 300 pupae could be correctly identified (Table 1).

LARVA:

The branching of 168 setae from 30 larvae was recorded and 11 salient characters extracted. They were C-8, A-1, M-1, M-4, I-2, I-9, II-5, II-9, III-8, III-9, and IV-11. These were then recorded for a total of 100 larvae. It was immediately apparent that the antennal shaft hair with 14 or more branches was highly characteristic of *An. tenebrosus; An. coustani* and *An. ziemanni* were more difficult to separate.

Again computer analysis was utilized and using all 11 characters, 99% discrimination, at 99.8% probability, was obtained (Fig. 8). Two of the 11 characters, A-1 and I-2, were used to construct a key whereby 91% correct identification was obtained for this material (Table 1).

EGG:

The descriptions by Gibbins (1933) of *An. ziemanni* and De Meillon (1937) of *An. coustani* and *An. tenebrosus*, showed differences which seemed to make the egg a good discriminating feature. However, 30 egg batches of *An. ziemanni* were examined and contrary to Gibbins, all showed characteristic polygonal markings on the exochorion, thus making them inseparable from the eggs of *An. tenebrosus*. The characteristic closed deck of *An. coustani* proved to be variable and both open and closed decks were seen in egg batches from single females.

DISCUSSION AND SUMMARY

Studies of the immature stages of *An. coustani*, *An. tenebrosus* and *An. ziemanni* by Evans (1938), De Meillon (1947) and Gillies and De Meillon (1968) suggested that they were virtually inseparable. By the use of modern techniques, i.e., Belkin's system in conjunction with computer analysis, these three stadia have been satisfactorily separated.

The heavily branched antennal shaft hair of the larva and the relatively few ribs on the pupal trumpets, make *An. tenebrosus* quite distinctive, *An. coustani* and *An. ziemanni* being more closely related. The keys to the pupae and larvae have been kept quite simple but Table 8 gives the branching of other significant setae.

The variation in pupal seta 5-V and VI with about nine branches (Evans 1938) has been seen in all three species (Tables 2, 4 and 6).

While the normally closed deck of the egg of *An. coustani* separates it from the other two species, it is not possible to distinguish between the three if the decks are open, and certainly, *An. ziemanni* and *An. tenebrosus* are inseparable in southern African material examined, both having distinct polygonal markings on the exochorion.

According to Gillies & De Meillon (1968) *An. tenebrosus* was often found in the Transvaal and Natal, while *An. ziemanni* had only been recorded from Mozambique. Regular catches made of mosquitoes biting man outside in the Tzaneen district, N.E. Transvaal, revealed quite large numbers of *An. ziemanni* but very few *An. tenebrosus* (Table 9). However, *An. tenebrosus* is very common in Zululand, Natal, while *An. ziemanni* is scarce.

No evidence has come to light during this study, to support the possibility of a single polymorphic species as suggested by Gillett (1972). The absence of intergrades of adult characters within families, correlated with pupal and larval characters, reinforces Gillies & De Meillon's (1968) decision to grant species status to *An. tenebrosus* and *An. ziemanni*. It is unlikely that a multiallelic gene is responsible for all this variation. Also, the data do not conform to Ford's (1940) well-known definition of a polymorphism. We therefore, prefer the simpler explanation that the covariation is a reflection of independent gene pools.

ACKNOWLEDGMENTS

We wish to thank Dr. B. de Meillon, Dr. M. Gillies, Professor H. Paterson, Mr. C. A. Green and Mr. R. Hunt for their comments on the manuscript; Mr. D. Lambert and Professor H. Paterson for their assistance with the computer analysis; the Director and field staff of the National Institute for Tropical Diseases for the collection of wild material; and the Secretary for Health for permission to publish.

REFERENCES

Belkin, J. N. 1962. The mosquitoes of the South Pacific. Univ. California Press. 2 vols.

De Meillon, B. 1937. The eggs of some South African anophelines. Publ. S. Afr. Inst. Med. Res. 7:305.

_____. 1947. The Anophelini of the Ethiopian Geographical Region. Publ. S. Afr. Inst. Med. Res. 49.

Evans, A. M. 1938. Mosquitoes of the Ethiopian Region. II. British Museum (Natural History).

Ford, E. B. 1940. Polymorphism and Taxonomy. The New Systematics (ed. Julian Huxley). Clarendon Press. Oxford. pp. 493-513.

Gibbins, E. G. 1933. Eggs of some Ethiopian *Anopheles* mosquitoes. Bull. Ent. Res. 24:257.

Gillett, J. D. 1972. Common African Mosquitos and their Medical Importance. William Heinemann Med. Books Ltd. London.

Gillies, M. T. and B. De Meillon. 1968. The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region). Publ. S. Afr. Inst. Med. Res. 54.

Nie, N. H., C. Hadlai Hull, J. G. Jenkins, K. Steinbrenner and D. H. Bent. 1975. SPSS Statistical Package for the Social Sciences. 2nd Ed. McGraw-Hill Book Co. New York.

Table 1. Keys to the larvae and pupae of *Anopheles coustani*, *Anopheles tenebrosus* and *Anopheles ziemanni*, whereby 91 and 89% correct identification respectively was obtained.

Larva:

1. The sum of seta A-1, 23 or more branches . . . . . *tenebrosus*
   The sum of this seta with 22 or less branches 2

2. The sum of seta I-2 with 14 or more branches . . . . . *coustani*
   The sum of seta I-2 with 13 or less branches . . . . . *ziemanni*

Pupa:

1. The sum of seta M-11 with 8 or more branches . . . . . *tenebrosus*
   The sum of seta M-11 with 7 or less branches 2

2. Seta IV-6 simple . . . . . *coustani*
   Seta IV-6 with either or both hairs split . . . . . *ziemanni*

Table 2. The range of branches for the pupae of *Anopheles coustani*.

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Cephalothorax		Abdomen	I	Abdomen	III	Abdomen	IV
1	1-2	2	4-11	0	3-7	0	3-5
2	2-3	3	4-11	1	9-18	1	6-14
3	2-5	4	4-8	2	5-12	2	6-13
4	2-4	5	2-4	3	3-6	3	3-6
5	3-6	6	1-2	4	2-4	4	2-4
6	1-2	7	2-5	5	3-15	5	8-20
7	2-4	9	1-2	6	2-4	6	1-2
8	1-2			7	2-5	7	2-4
9	1-3	Abdomen	II	8	2-4	8	2-3
10	2-5	0	2-5	9	1	9	1
11	2-3	1	4-10	10	1-2	10	1
12	1-3	2	5-12	11	1-2	11	1
		3	2-5	14	1-2	14	1-2
		4	3-5				
		5	2-5				
		6	1-2				
		7	2-4				
		8	1-4				
		9	1				
		10	1-3				

Table 2 cont.

Table 2 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	V	Abdomen	VI	Abdomen	VII	Abdomen	VIII
0	2-5	0	2-5	0	1-5	0	1-3
1	3-11	1	1-4	1	1-3	4	1-3
2	5-13	2	5-11	2	3-7	9	12-20
3	1-5	3	1-3	3	2-4	14	1-3
4	2-4	4	1-2	4	1-2		
5	5-14	5	4-10	5	2-5		
6	1-2	6	1-2	6	2-3	Paddle	
7	2-3	7	1	7	1-2	1	1-3
8	2-3	8	2-3	8	2-4	2	2-5
9	1	9	1	9	1		
10	1	10	1	10	1-3	47 - 61 hairs	
11	1	11	1-3	11	1-2	beyond P-1	
14	1-2	14	1-2	14	1-3		

Table 3. Range of setal branching for the larvae of *Anopheles coustani*.

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Head		Prothorax		Mesothorax		Metathorax	
0	1	0	1	1	20-24	1	2-5
1	1	1	3-7	2	4-6	2	1
2	1-2	2	8-12	3	1	3	16-22
3	Many	3	1	4	4-10	4	3-6
4	2-5	4	12-18	5	1	5	21-36
5	13-19	5	29-33	6	4-8	6	2-4
6	14-19	6	1	7	3	7	21-29
7	16-21	7	24-34	8	14-22	8	22-32
8	8-12	8	20-29	9	1	9	1
9	7-11	9	1	10	1	10	1
10	3-4	10	1	11	1	11	1
11	Plumose	11	3-5	12	1	12	2-3
12	2-5	12	1	13	5-12	13	3-4
13	7-10	13	8-13	14	12-20		
15	6-12	14	6-9				
Antennae							
1	4-9						
4	11-16						

Table 3 cont.

Table 3 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	I	Abdomen	II	Abdomen	III	Abdomen	IV
1	9-16	0	4-6	0	3-6	0	3-6
2	6-11	1	12-20	1	19-23	1	16-25
3	2-3	2	6-14	2	6-11	2	3-5
4	6-11	3	1	3	1	3	2-7
5	5-7	4	5-8	4	3-5	4	4-7
6	19-27	5	8-17	5	8-15	5	5-9
7	17-26	6	19-31	6	18-27	6	2-4
9	5-10	7	22-30	7	4-6	7	3-6
10	1	8	2-4	8	3-5	8	4-6
11	2-4	9	7-16	9	8-19	9	10-17
12	2-3	10	2-4	10	1	10	1
13	6-17	11	1	11	2-4	11	2-4
		12	1	12	2-4	12	3-4
		13	9-18	13	8-18	13	5-9
		14	1-2	14	1-3	14	1-2

Table 3 cont.

Table 3 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	V	Abdomen	VI	Abdomen	VII	Abdomen	VIII
0	4-6	0	4-6	0	3-6	0	3-5
1	14-26	1	17-24	1	17-24	1	1
2	3-5	2	6-10	2	9-17	2	10-13
3	1	3	1-2	3	3-5	3	7-11
4	4-6	4	1	4	1	4	1
5	5-10	5	7-12	5	7-13	5	4-5
6	2-4	6	4-9	6	3-6	6	2-4
7	3-5	7	3-5	7	5-9	8	7-14
8	5-7	8	5-8	8	4-7	9	7-12
9	9-14	9	9-12	9	4-10	14	1-2
10	1	10	2-3	10	4-8		
11	2-4	11	1-3	11	1-2	Pecten	
12	3-5	12	1	12	1	1	3-6
13	3-5	13	10-18	13	3-4	2	6-10
14	1-3	14	1-2				
						Saddle hair simple.	

Table 4. Range of setal branching for the pupae of *Anopheles tenebrosus*.

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Cephalothorax		Abdomen	I	Abdomen	III	Abdomen	IV
1	1-2	2	6-10	0	3-8	0	2-5
2	2-3	3	2-8	1	10-19	1	7-15
3	2-4	4	5-8	2	4-11	2	4-12
4	2-4	5	4-13	3	3-9	3	3-7
5	2-5	6	1-4	4	2-4	4	2-4
6	2	7	2-5	5	7-19	5	6-19
7	1-3	9	1-3	6	2-4	6	1-4
8	1-3			7	2-4	7	2-3
9	2-4	Abdomen	II	8	1-3	8	1-2
10	2-6	0	2-5	9	1	9	1
11	4-7	1	5-10	10	1-2	10	1
12	2-6	2	4-12	11	1	11	1
		3	1-3	14	1-3	14	1-2
		4	3-6				
		5	2-6				
		6	1-3				
		7	2-6				
		8	1				
		9	1				
		10	1				

Table 4 cont.

Table 4 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	V	Abdomen	VI	Abdomen	VII	Abdomen	VIII
0	2-5	0	2-4	0	1-4	0	1-3
1	2-12	1	1-4	1	1-2	4	1-2
2	5-10	2	4-8	2	2-5	9	8-16
3	1-2	3	1-3	3	2-4	14	1-2
4	2-3	4	1-2	4	1-2		
5	4-14	5	3-9	5	1-6		
6	1	6	1	6	1-3	Paddle	
7	2-3	7	1-2	7	1-2	1	1-3
8	1-2	8	1-3	8	1-3	2	1-4
9	1	9	1	9	1		
10	1	10	1	10	1-2	17 - 54 hairs	
11	1	11	1-2	11	1	beyond P-1	
14	1-2	14	1-2	14	1-2		

Table 5. Range of setal branching for the larvae of *Anopheles tenebrosus*.

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Head		Prothorax		Mesothorax		Metathorax	
0	1	0	1-2	1	25-33	1	3-5
1	1	1	4-6	2	4-5	2	1
2	1	2	6-12	3	1	3	13-16
3	Many	3	1	4	4-5	4	3-8
4	2-5	4	11-16	5	1	5	21-27
5	12-17	5	19-30	6	4-6	6	2-3
6	12-16	6	1	7	3	7	16-22
7	14-22	7	22-26	8	12-17	8	17-22
8	10-16	8	18-23	9	1	9	1
9	5-8	9	1	10	1	10	1
10	2-4	10	1	11	1	11	1
11	Plumose	11	2-4	12	1	12	1-2
12	2-5	12	1	13	4-11	13	2-3
13	5-10	13	7-12	14	11-18		
15	3-7	14	5-8				
Antennae							
1	12-18						
4	7-11						

Table 5 cont.

Table 5 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	I	Abdomen	II	Abdomen	III	Abdomen	IV
1	7-12	0	4-6	0	4-6	0	3-6
2	6-10	1	8-15	1	14-21	1	17-24
3	3-5	2	5-12	2	5-8	2	4-7
4	8-12	3	1-4	3	1	3	2-4
5	4-7	4	5-8	4	2-5	4	2-4
6	15-24	5	7-14	5	7-14	5	5-8
7	12-18	6	17-24	6	15-22	6	2-3
9	3-5	7	16-23	7	2-4	7	2-4
10	1	8	2-3	8	2-4	8	2-5
11	2-3	9	5-10	9	7-11	9	10-14
12	2-3	10	1-3	10	1	10	1
13	6-12	11	1	11	1-2	11	1-2
		12	1	12	2-3	12	1-3
		13	8-12	13	7-11	13	5-12
		14	1-2	14	1-2	14	1-2

Table 5 cont.

Table 5 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	V	Abdomen	VI	Abdomen	VII	Abdomen	VIII
0	4-6	0	4-6	0	3-6	0	3-6
1	17-21	1	15-21	1	14-21	1	1
2	4-6	2	6-10	2	7-13	2	7-10
3	1	3	1	3	3-6	3	6-9
4	4-6	4	1	4	1	4	1
5	5-8	5	7-10	5	7-10	5	3-5
6	2-3	6	4-13	6	4-10	6	1-3
7	2-5	7	2-3	7	4-7	8	4-8
8	3-6	8	4-7	8	6-9	9	7-10
9	9-12	9	7-10	9	6-13	14	1-2
10	1	10	2-4	10	4-8		
11	2-3	11	1-2	11	1-2	Pecten	
12	1-5	12	1	12	1	1	4-5
13	3-4	13	9-15	13	2-5	2	5-9
14	1-2	14	1-2				

Saddle hair simple.

Table 6. Range of setal branching for the pupae of *Anopheles ziemanni*.

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Cephalothorax		Abdomen	I	Abdomen	III	Abdomen	IV
1	2-3	2	3-6	0	2-6	0	3-5
2	2-4	3	2-6	1	7-17	1	6-14
3	2-6	4	3-11	2	4-11	2	5-15
4	2-3	5	2-4	3	3-6	3	3-5
5	3-5	6	1-3	4	2-4	4	1-4
6	2-3	7	2-5	5	6-14	5	7-23
7	1-4	9	1-2	6	2-7	6	1-3
8	1-2			7	2-4	7	2-4
9	2-3	Abdomen	II	8	2-4	8	1-3
10	1-4	0	2-5	9	1	9	1
11	2-4	1	4-10	10	1-3	10	1-2
12	2-4	2	4-10	11	1-2	11	1
		3	1-4	14	1-2	14	1-2
		4	2-6				
		5	2-5				
		6	1-3				
		7	2-5				
		8	2				
		9	1				
		10	1-2				

Table 6 cont.

Table 6 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	V	Abdomen	VI	Abdomen	VII	Abdomen	VIII
0	2-5	0	2-4	0	2-4	0	1-3
1	3-8	1	1-5	1	1-2	4	1-3
2	5-13	2	4-10	2	2-7	9	11-17
3	1-2	3	1-3	3	2-4	14	1-2
4	2-4	4	1-2	4	1-2		
5	5-17	5	5-12	5	2-5		
6	1-2	6	1-2	6	1-3	Paddle	
7	2-3	7	1-2	7	1	1	1-3
8	1-3	8	1-3	8	2-4	2	1-3
9	.1	9	1	9	1		
10	1	10	1-2	10	1-3	27 - 55 hairs	
11	1-2	11	1	11	1-2	beyond P-1	
14	1-2	14	1-2	14	1-2		

Table 7. Range of setal branching for the larvae of *Anopheles ziemanni*.

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Head		Prothorax		Mesothorax		Metathorax	
0	1	0	1	1	20-27	1	2-4
1	1	1	2-5	2	2-4	2	1
2	1	2	6-11	3	1	3	14-25
3	Many	3	1	4	3-7	4	3-4
4	2-5	4	13-17	5	1	5	20-29
5	11-17	5	24-30	6	4-6	6	2-4
6	14-18	6	1	7	2-4	7	15-27
7	14-20	7	21-24	8	13-17	8	18-26
8	6-11	8	19-23	9	1	9	1
9	5-9	9	1	10	1	10	1
10	2-3	10	1	11	1	11	1-2
11	Plumose	11	2-4	12	1	12	1-3
12	2-4	12	1	13	6-13	13	2-3
13	8-11	13	8-14	14	10-17		
15	3-7	14	5-8				
Antennae							
1	5-11						
4	8-13						

Table 7 cont.

Table 7 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	I	Abdomen	II	Abdomen	III	Abdomen	IV
1	11-17	0	3-5	0	3-5	0	3-6
2	3-8	1	10-21	1	17-22	1	18-22
3	2-4	2	6-10	2	5-8	2	3-6
4	7-11	3	1	3	1-2	3	3-4
5	4-6	4	5-8	4	2-4	4	3-4
6	14-24	5	8-10	5	7-12	5	5-8
7	14-22	6	17-27	6	14-21	6	2-3
9	5-8	7	17-24	7	2-4	7	3-4
10	1	8	3-4	8	4-5	8	4-6
11	3-4	9	7-10	9	9-12	9	9-15
12	2-3	10	1-3	10	1-2	10	1
13	6-14	11	1	11	1-2	11	1-3
		12	1	12	3	12	2-3
		13	7-12	13	8-13	13	5-8
		14	1-2	14	1-3	14	1-3

Table 7 cont.

Table 7 (cont.)

Seta	Branches	Seta	Branches	Seta	Branches	Seta	Branches
Abdomen	V	Abdomen	VI	Abdomen	VII	Abdomen	VIII
0	2-5	0	3-5	0	2-5	0	3-5
1	16-25	1	15-23	1	17-22	1	1
2	3-6	2	5-8	2	8-11	2	8-11
3	1	3	1	3	3-6	3	5-9
4	3-6	4	1	4	1	4	1
5	6-8	5	7-9	5	6-10	5	3-5
6	2-3	6	6-9	6	3-7	6	1-3
7	3-5	7	3-4	7	4-6	8	7-12
8	5-7	8	5-7	8	4-7	9	7-12
9	7-14	9	7-11	9	5-11	14	1-2
10	1	10	2-3	10	4-7		
11	2-3	11	1-4	11	1-2	Pecten	
12	2-5	12	1	12	1	1	4-6
13	3-4	13	10-15	13	2-3	2	7-11
14	1-3	14	1-2			Saddle hair simple.	

Table 8. Mean number of branches for setae of significance other than those used in the keys.

Pupa

Seta	*An. coustani*	*An. tenebrosus*	*An. ziemanni*
I-3	6.0	4.1	3.4
I-5	3.5	6.1	2.4
III-5	11.4	10.6	9.0
trumpet ribs	12.2	7.0	12.5

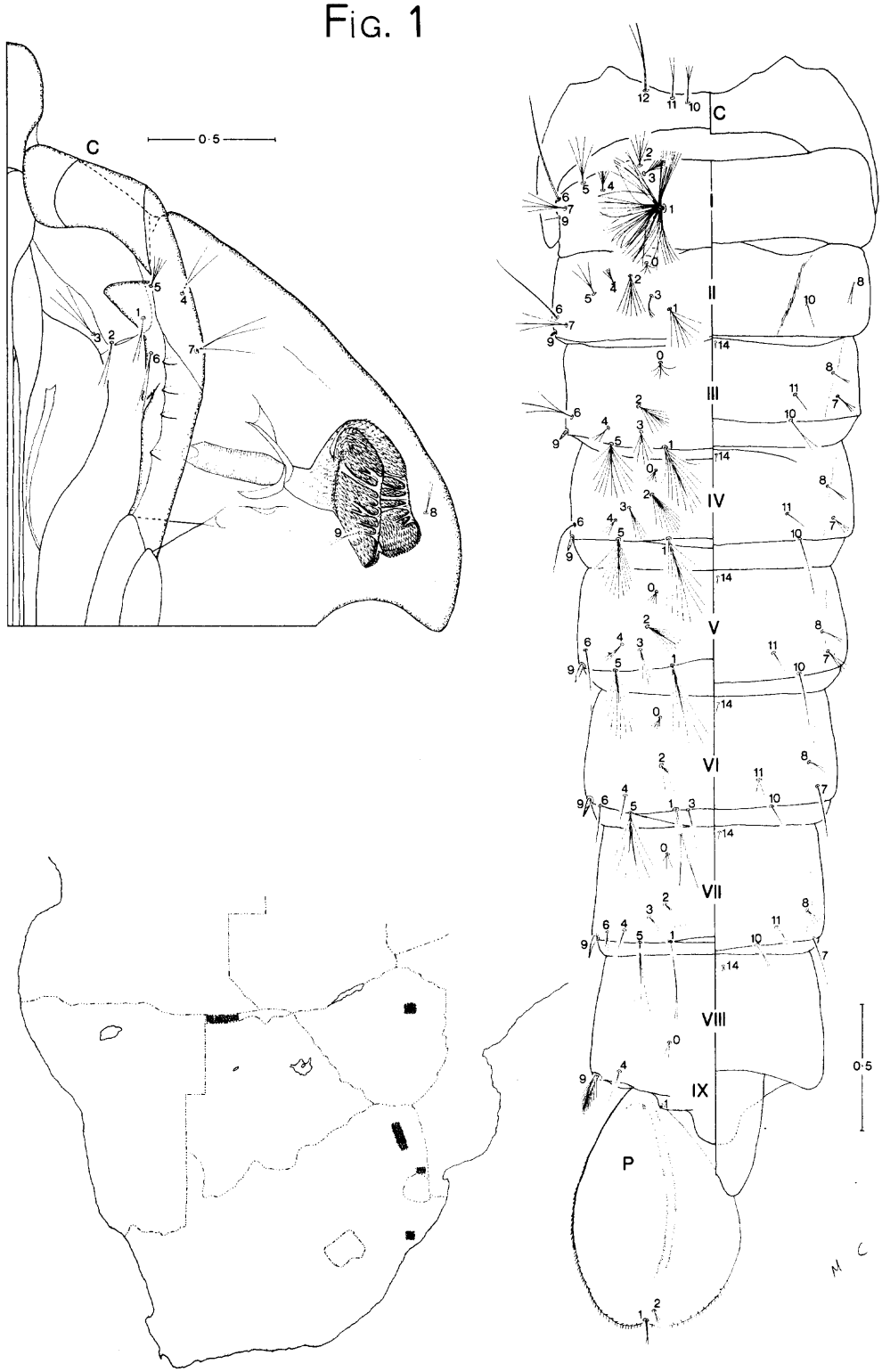
Larva

Seta	*An. coustani*	*An. tenebrosus*	*An. ziemanni*
C-8	9.7	12.4	8.6
M-1	23.9	28.2	23.7
I-9	7.7	4.2	5.5
II-5	12.7	10.5	9.3
II-9	11.6	7.1	8.3
III-9	12.8	9.2	10.8
IV-11	2.7	1.3	1.9

Table 9. Man-biting catches from three localities in the Transvaal for the period November, 1978 to October, 1979.

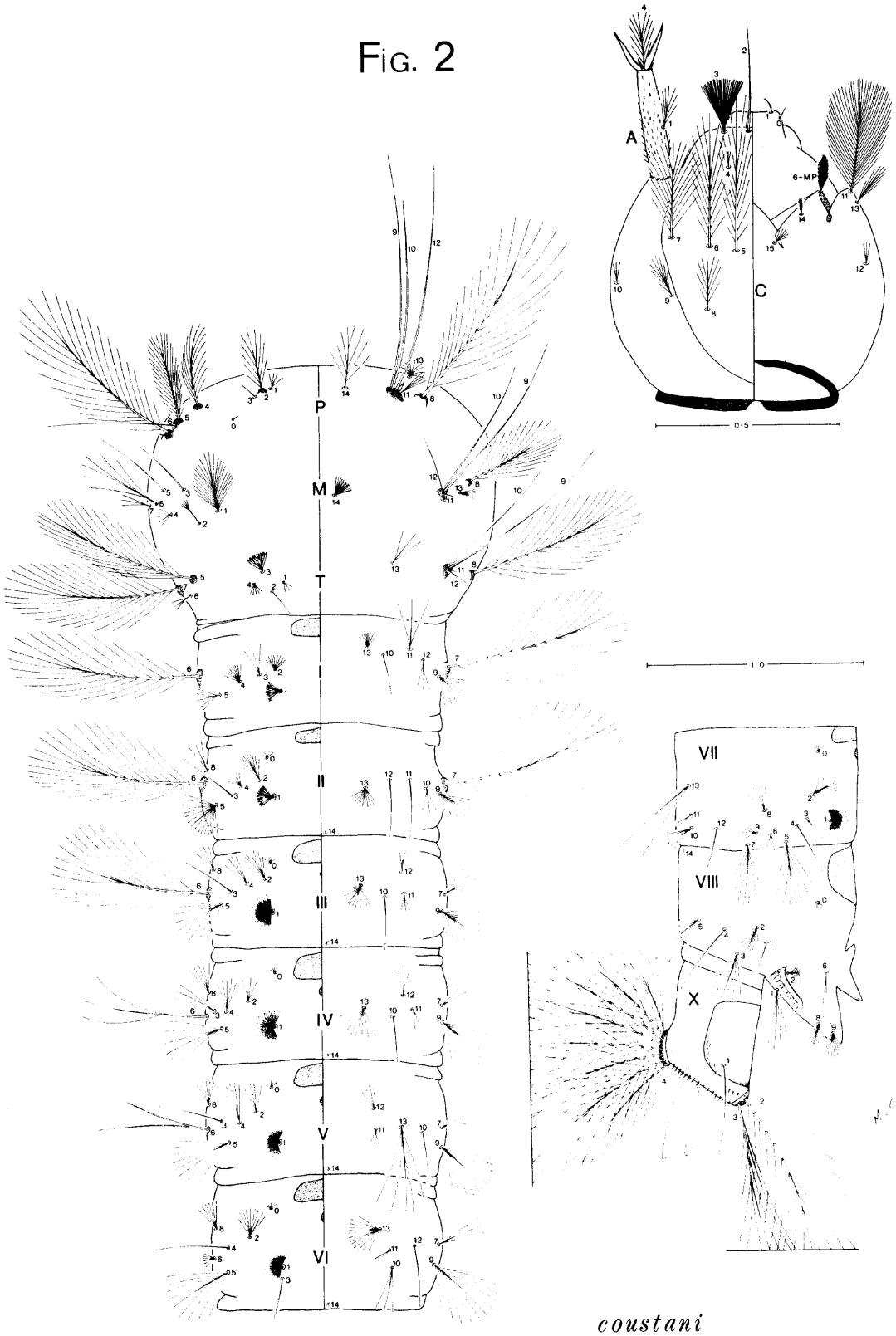
Locality	Jaffray			Pusela			Grey Stones		
Species	*An. coustani*	*An. tenebrosus*	*An. ziemanni*	*An. coustani*	*An. tenebrosus*	*An. ziemanni*	*An. coustani*	*An. tenebrosus*	*An. ziemanni*
Nov. '78	20		1	133		3	37		20
Dec.	21		4	65		5	36	2	52
Jan. '79	26		1	38		10	7		3
Feb.	26		1	49		20	14	3	13
Mar.	90		5	31		24	8		3
Apr.	103		1	35		31	13	4	35
May	57		1	21		12	4	1	3
June	20		2	15		16	8	2	1
July	3			24		10	10		2
Aug.	12			33	1	8	4		
Sept.	59		1	115		15	5	1	
Oct.	115			242		16	4		1
Totals	552	0	17	801	1	170	150	13	133

Fig. 1



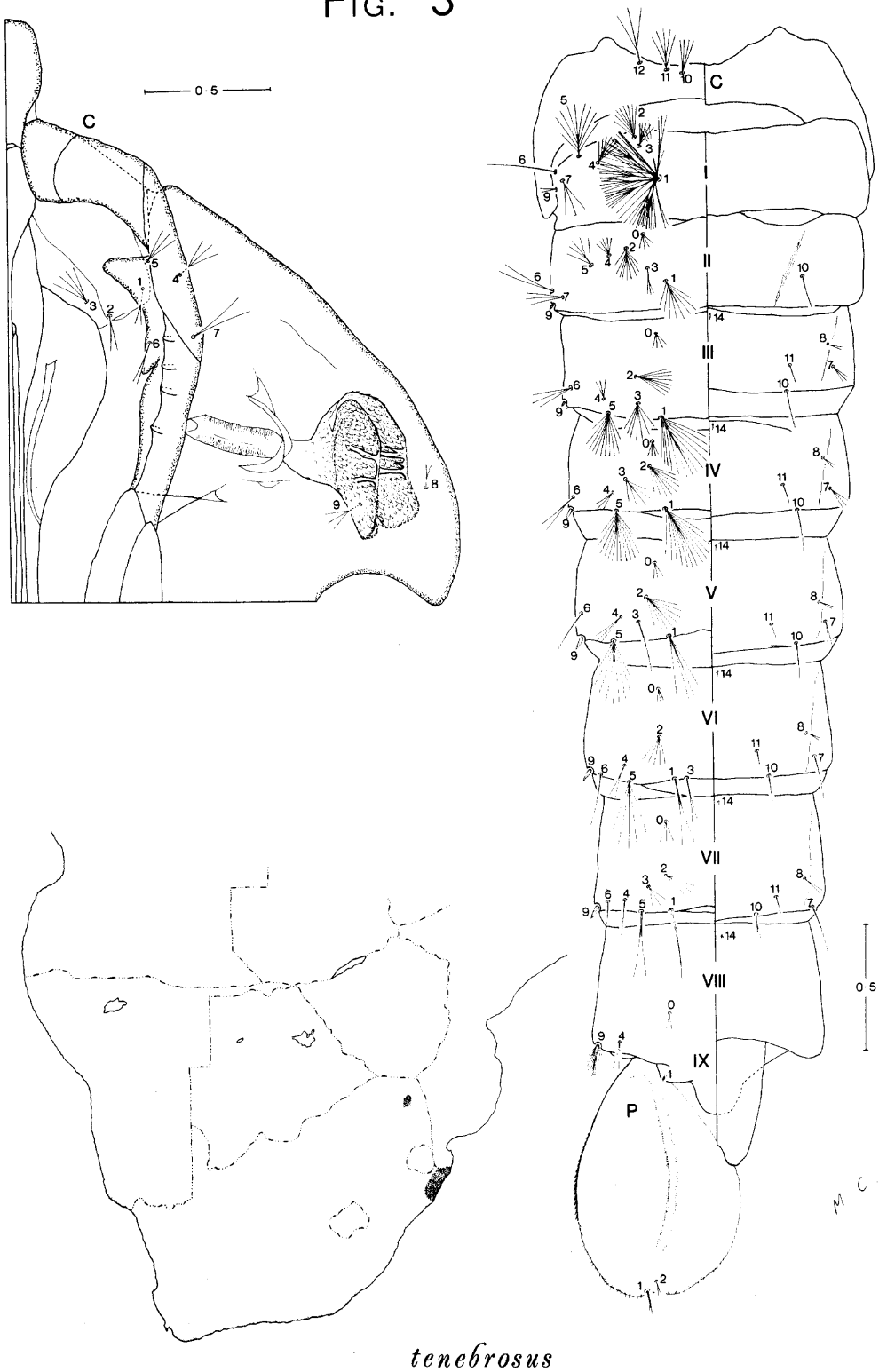
coustani

FIG. 2



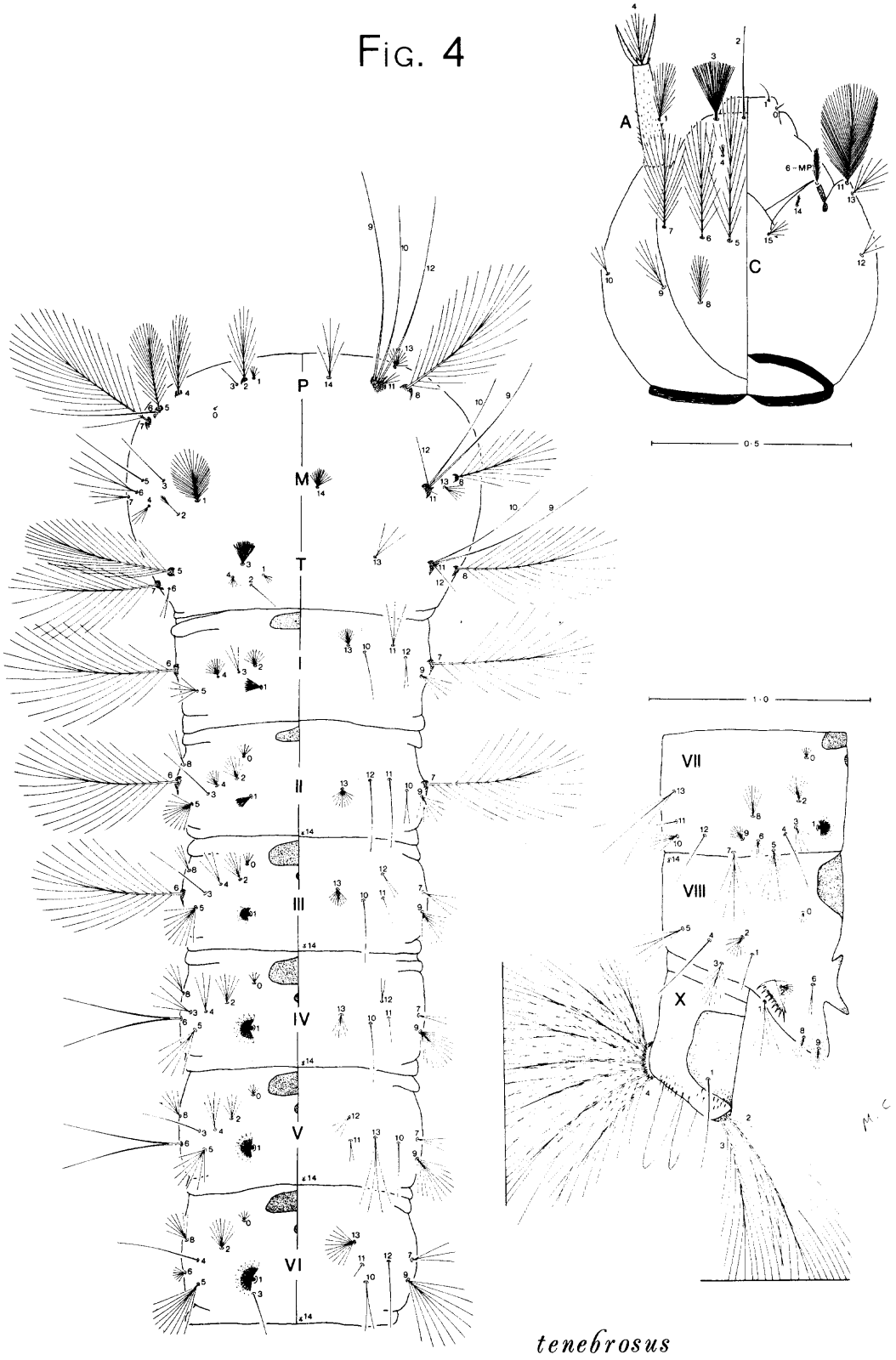
coustani

Fig. 3



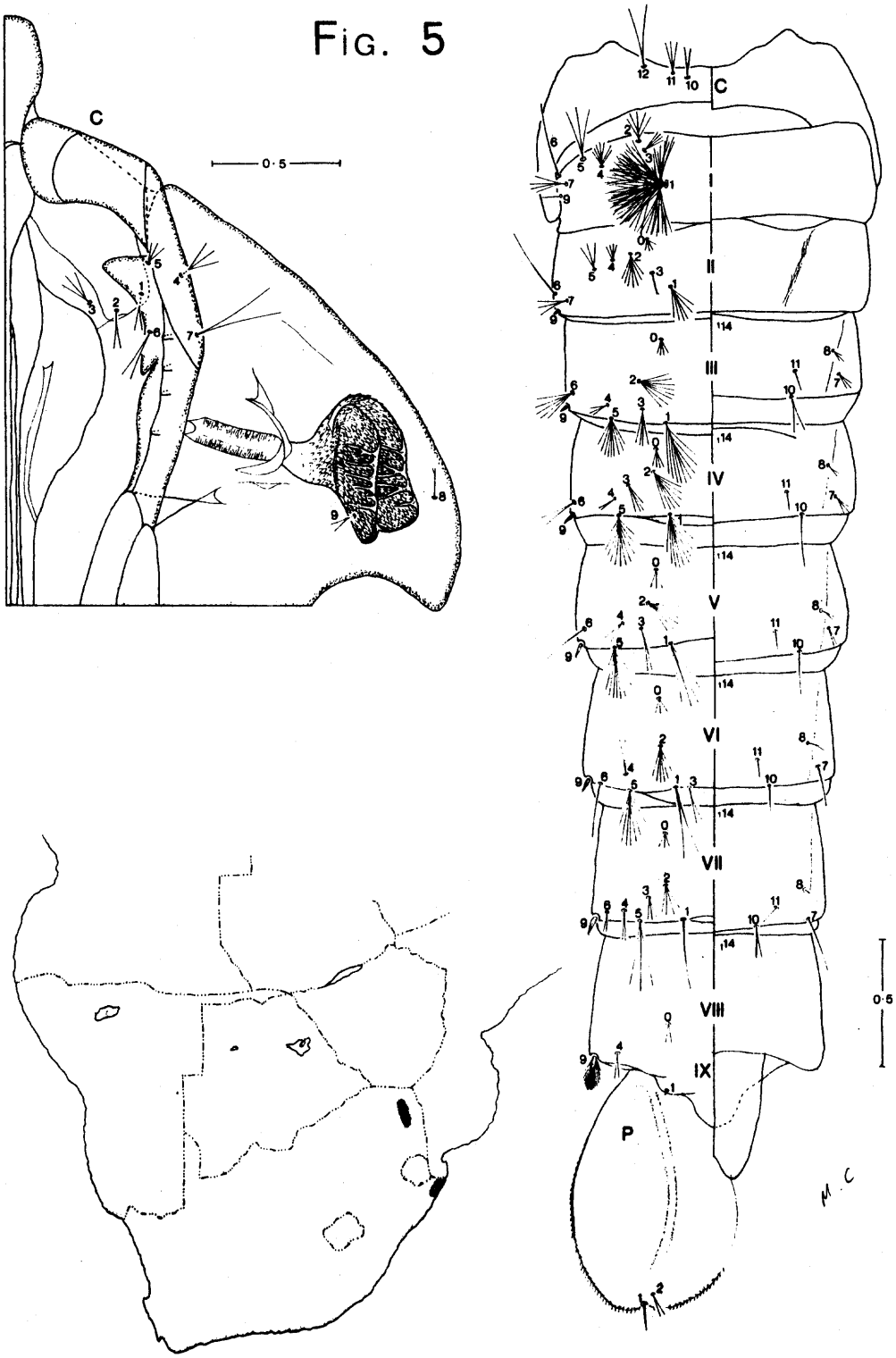
tenebrosus

Fig. 4



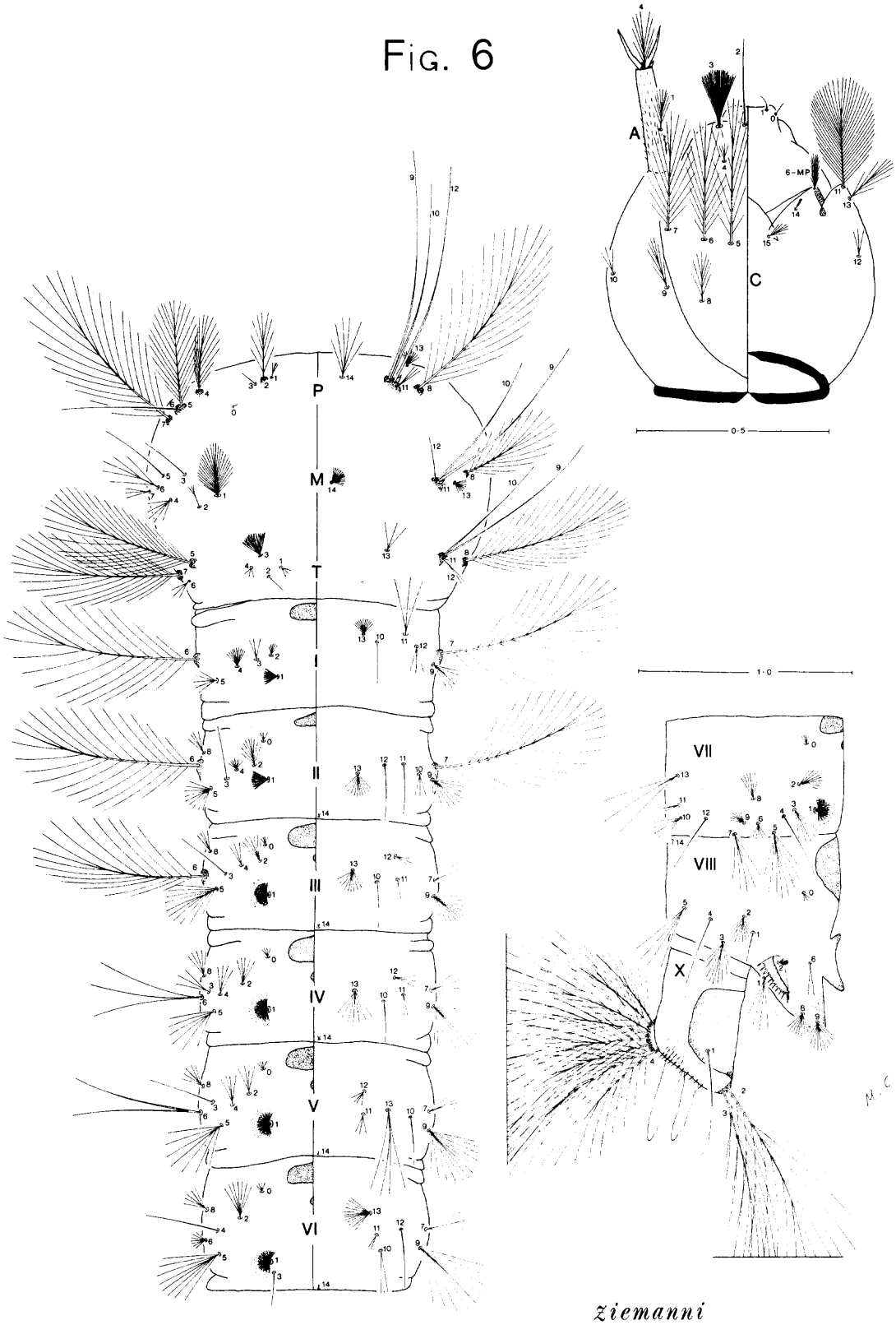
tenebrosus

Fig. 5



ziemanni

Fig. 6



ziemanni

Fig. 7. SCATTERPLOT OF THE COMPUTER ANALYSIS OF THE PUPAE

1. *Anopheles coustani*
2. *Anopheles tenebrosus* * indicates a group centroid
3. *Anopheles ziemanni*

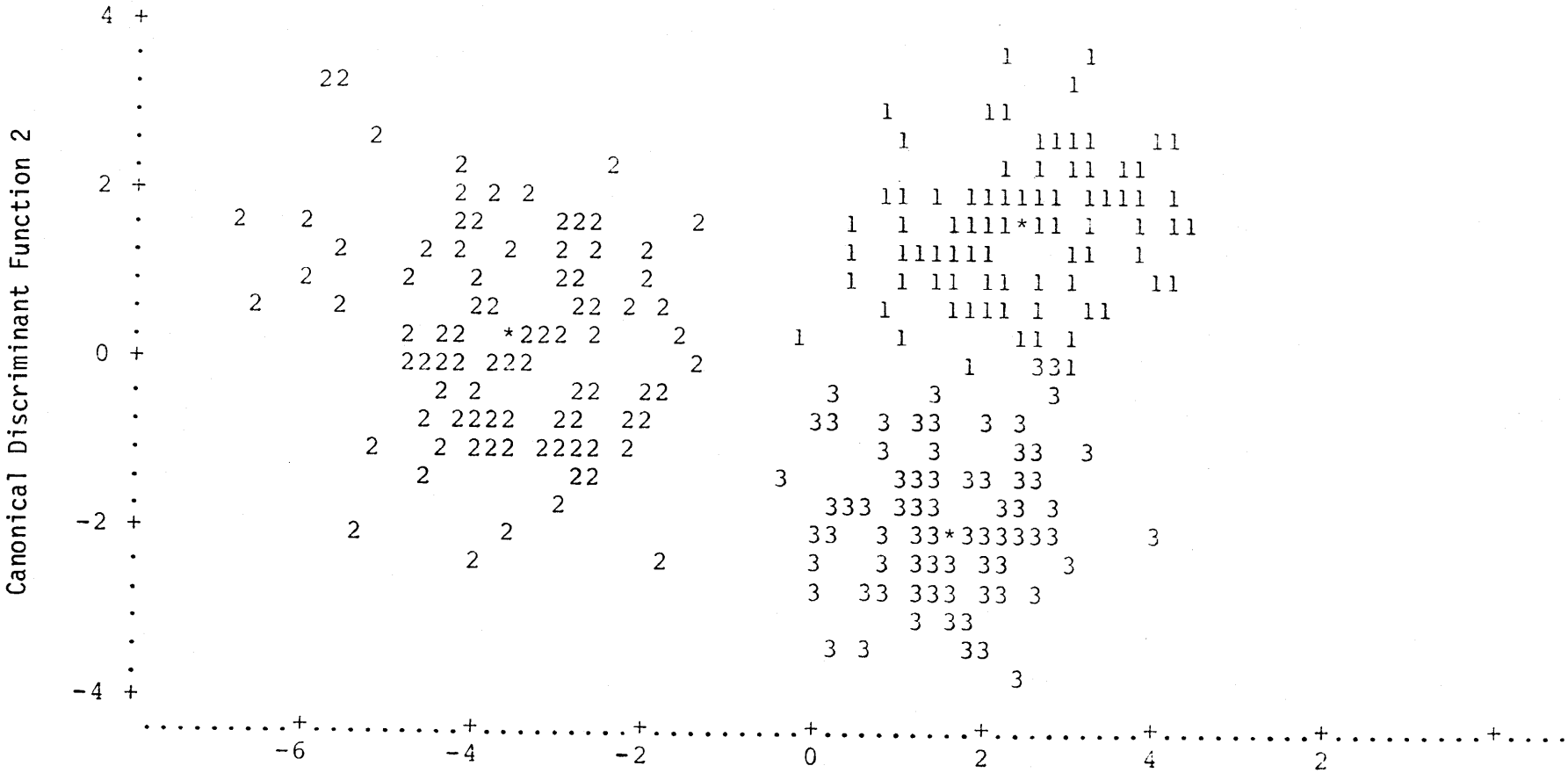


Fig. 8. SCATTERPLOT OF THE COMPUTER ANALYSIS OF THE LARVAE

1. *Anopheles coustani*
2. *Anopheles tenebrosus* * indicates a group centroid
3. *Anopheles siemanni*