II. MATERIAL AND METHOD

MATERIAL AND METHODS

The present study is based on the imaginal forms of Culicoides biting midges, collected from different parts of West Bengal during the period 1978-1984, and on similar materials received by exchange and donations from some concerns, 1. Dr.A. V. Gutsevich, Zoological Institute, Leningrad, U.S.S.R., 2. Dr. W. W. Wirth, U. S. National Museum, Washington, D.C., U.S.A., 3. Dr. Y. Braverman, Kimron Veterinary Institute, Israel, 4. Dr. D. H. Messersmith, Dept. of Entomology, University of Maryland, U.S.A., 5. Dr. P. Basu, All India Institute of Public Health and Hygiene, Calcutta, India, 6. Miss N. Jayalakshmi, Madras Veterinary College, Madras, 7. Dr. U. V. Shastri, Professor of Parasitology, College of Veterinary and Animal Sciences, MAU, Parbhani, Maharastra, India, 8. Dr. J. N. Misra, Field Lab., R. & D. organisation, Ministry of Defence, Govt. of India, Tezpur, Assam, India, at home and abroad, through the endeavour of my guide Dr. S. K. Dasgupta. Many specimens including types or paratypes of genus Culicoides were also received as contribution from the work of Dr. B. C. Majumder of Sree Chaitanya College, Habra. My collections covered almost all districts of West Bengal and a substantial portion was from Darjeeling, Burdwan and Calcutta districts and their surroundings.

A) Method of collection :

Several devices as follows were adopted in collecting Culicoides specimens from nature (after Dasgupta and his collaborators 1956-1972):

1. Light traps : The different types of light traps used during the present study were :

   i) Chinsurah light trap (Fig.1A) - designed by Banerjee and Basu (1956), it is essentially a stationary device fixed on a stand equipped with the provisions for fitting to it an electric lamp of 100 to 200 watts or a 'Petromax'. The device enclosed in a glass case to avoid rains. The bottom of this device is a cylindrical glass/plastic jar where the trapped insects are collected. Glacial Acetic Acid was used as the killing agent in a trap.

   ii) Open-tray type light trap (Fig.1B) - this trap was used by Sinha Ray et al. (1969), in course of their collection work on biting midges of Darjeeling, it consists of two or three ordinary table lamps, each of 100-200 watts, with their head bent down to the water level in a metallic tray measuring 30 cm x 25 cm x 4 cm covered with a fine wire-net of 8 mesh per sq.cm., the water in the tray having traces of Glacial Acetic Acid as the killing agent in the trap. This type of light trap was operated in Darjeeling area.

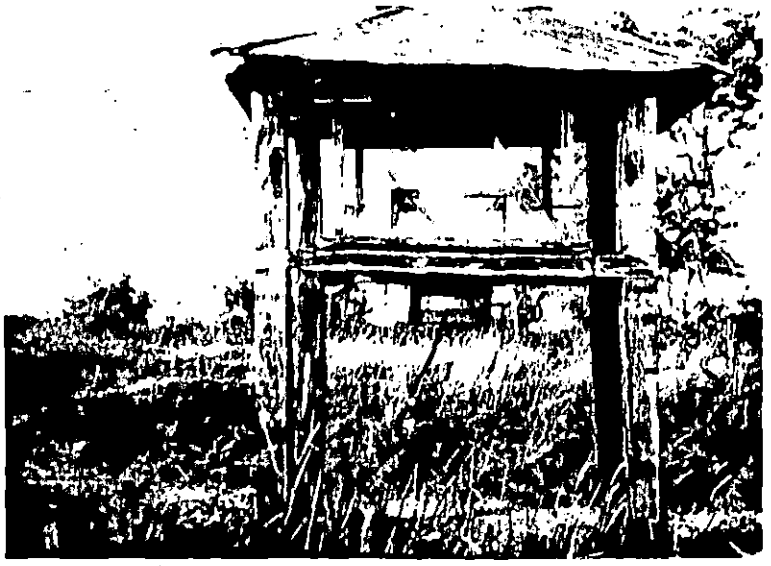

Fig. 1A : 'Chinsurah' light trap.

Fig. 1B : Open-tray type light trap.

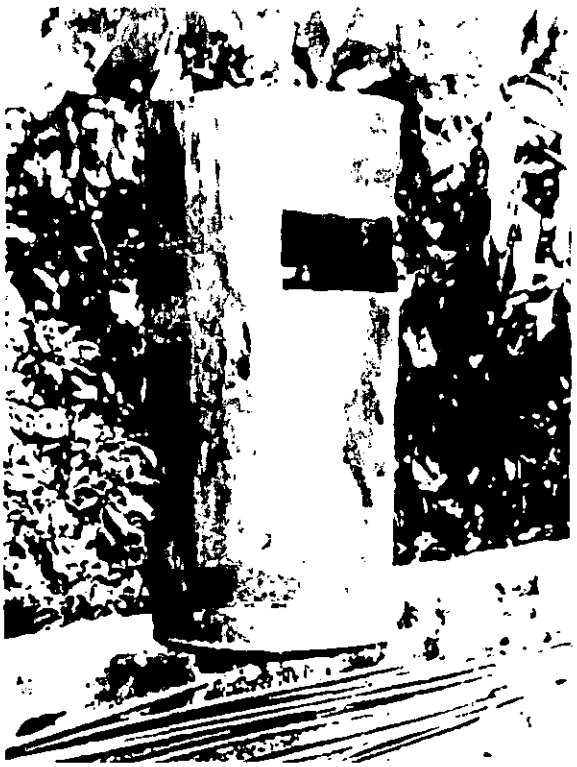

Fig. 1C : Closed-cylinder type light trap.

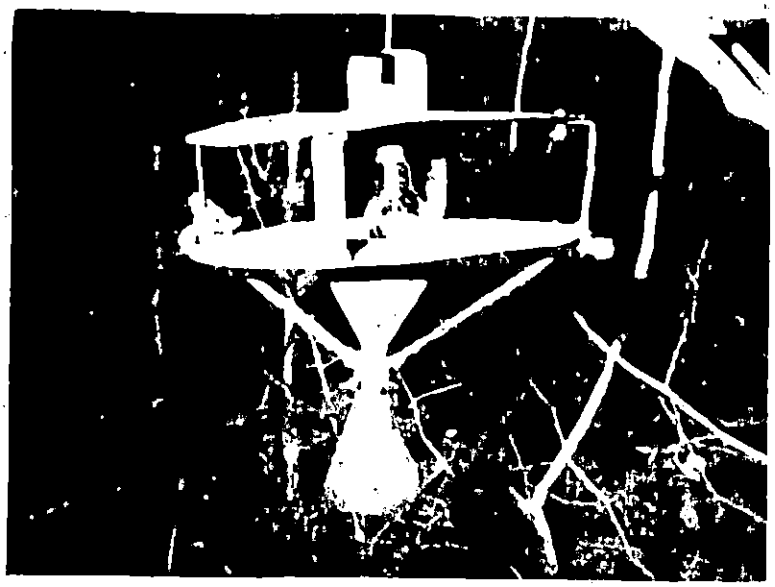

Fig. 1D : Disc-type open light trap.

iii) Closed cylinder type light trap (Fig.1C) - designed by Dr. S. K. Dasgupta, it consists of a cylinder (30 cm.in length and 16 cm. in diameter) with 3-4 alternating rectangular-sized holes for letting out light-beams from light-source inside the top end of the cylinder. A detatchable metallic lid at the bottom acted as receptacle for the trapped insects. It is specially used for collecting insects in dry condition. Sometimes liquid Paraffin was placed in the receptacle and insects were collected therein. This instrument is handy and easily movable. Collections by this type of light trap were made at Chinsurah, Burdwan, Raiganj and other places.

iv) iv) Disc-type light trap (Fig.1D) - this type of light trap, adopted by Dr. B. Majumdar (1972), in his work on Culicoides insects, consists essentially of two circular plates, each of 35 cm. diameter. The upper plate is provided with arrangements for fitting an electric lamp of high wattage and the lower part with a large hole through which a funnel is fitted to a collecting bottle containing chloroform-soaked cotton. This device is easily portable. Collections by this type of light trap were made at Habra, Sodepur and other places of the District 24-Parganas.

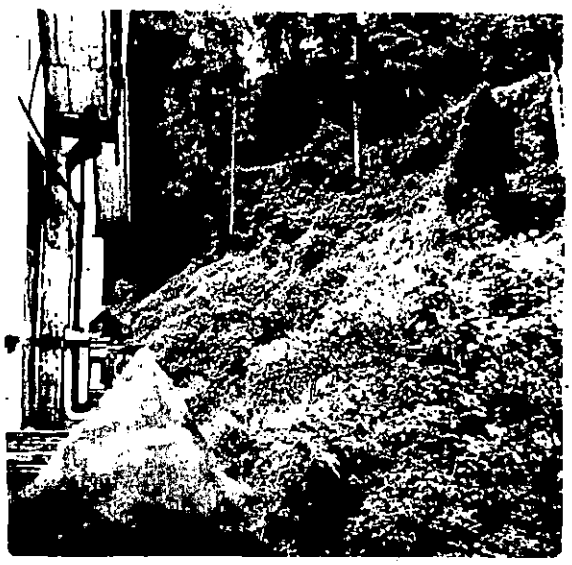

Fig. 1E : Site of collection at Darjeeling.

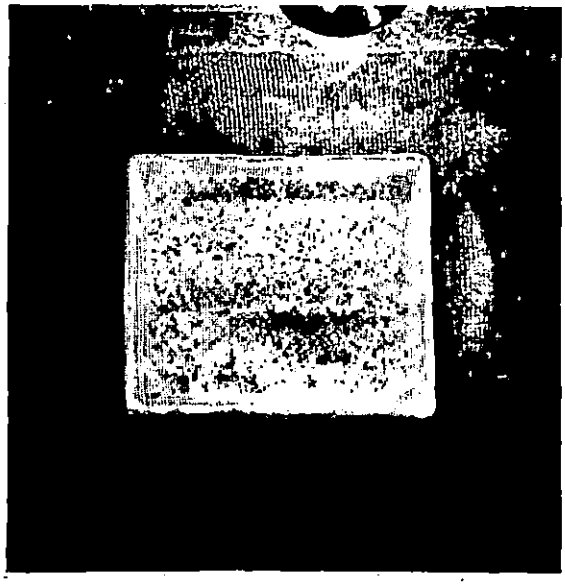

Fig. 1F : Light-trap collection at Pallaroad.

B) Sites and other data :

The light trap were operated in 65 localities (Fig.3) for 734 trap-nights in the districts of Bankura, Birbhum, Burdwan, Coochbehar, Calcutta, Darjeeling, Hooghly, Howrah, Jalpaiguri, Malda, Midnapur, Murshidabad, Nadia, Purulia, 24-Parganas and West-Dinajpur. Excepting Darjeeling, Calcutta and Burdwan, collections in other places were, however, stray and random. In these four districts of West Bengal of which six localities such as Calcutta, Darjeeling, Chinsurah, Habra, Palharoad and Raiganj, collections were made consistantly for a period from January to December each year during 1968-1981. An enormous number of specimens were assembled as a result of these repeated endeavours.

A total of 36,916 fulgoroidea specimens could be collected combining the field collection made in course of the present work as well as specimens obtained from different concerns, mentioned above. The data of field collections were shown in tables 1-4.

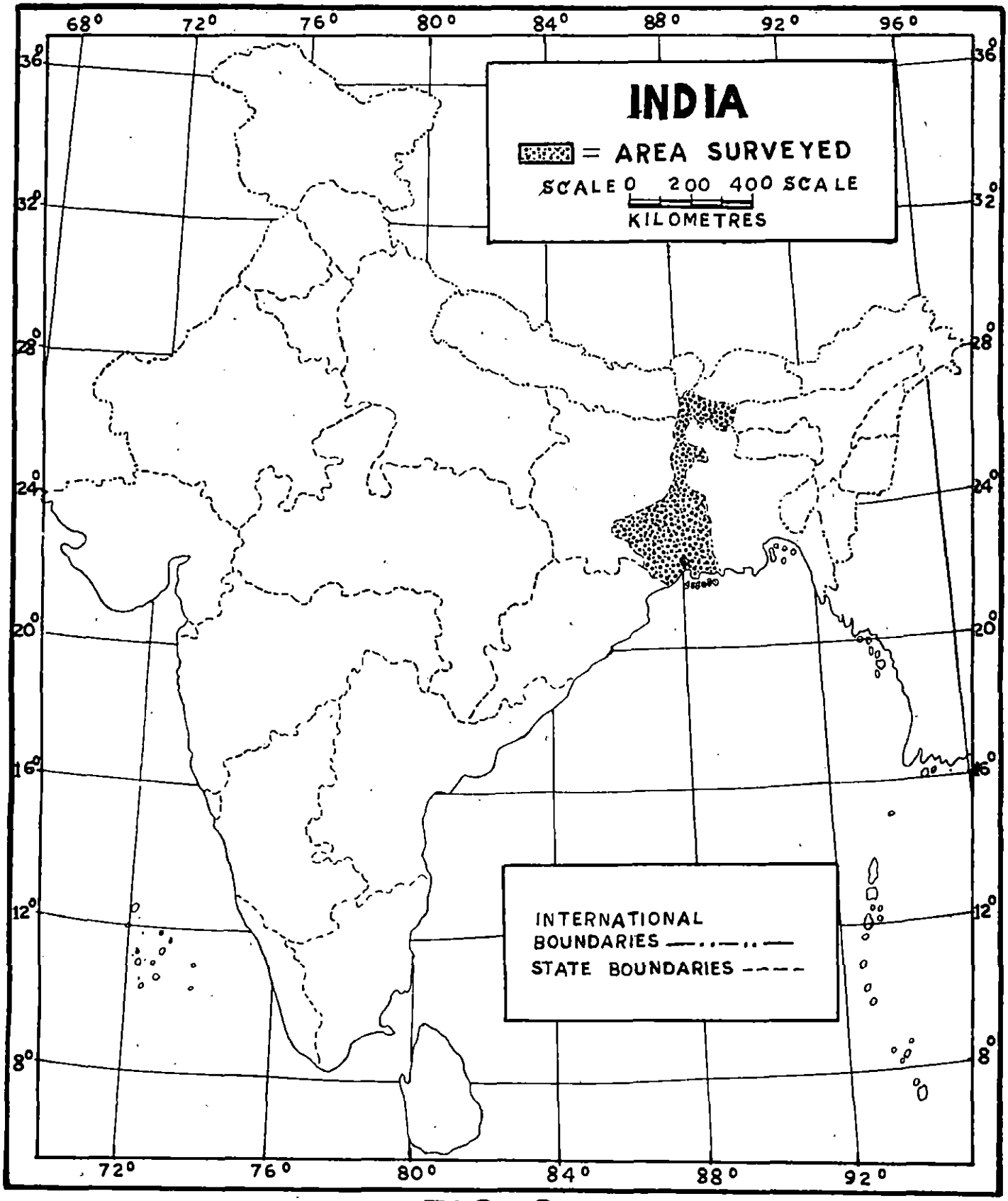

FIG.-2

MAP OF INDIA SHOWING THE LOCATION OF WEST BENGAL

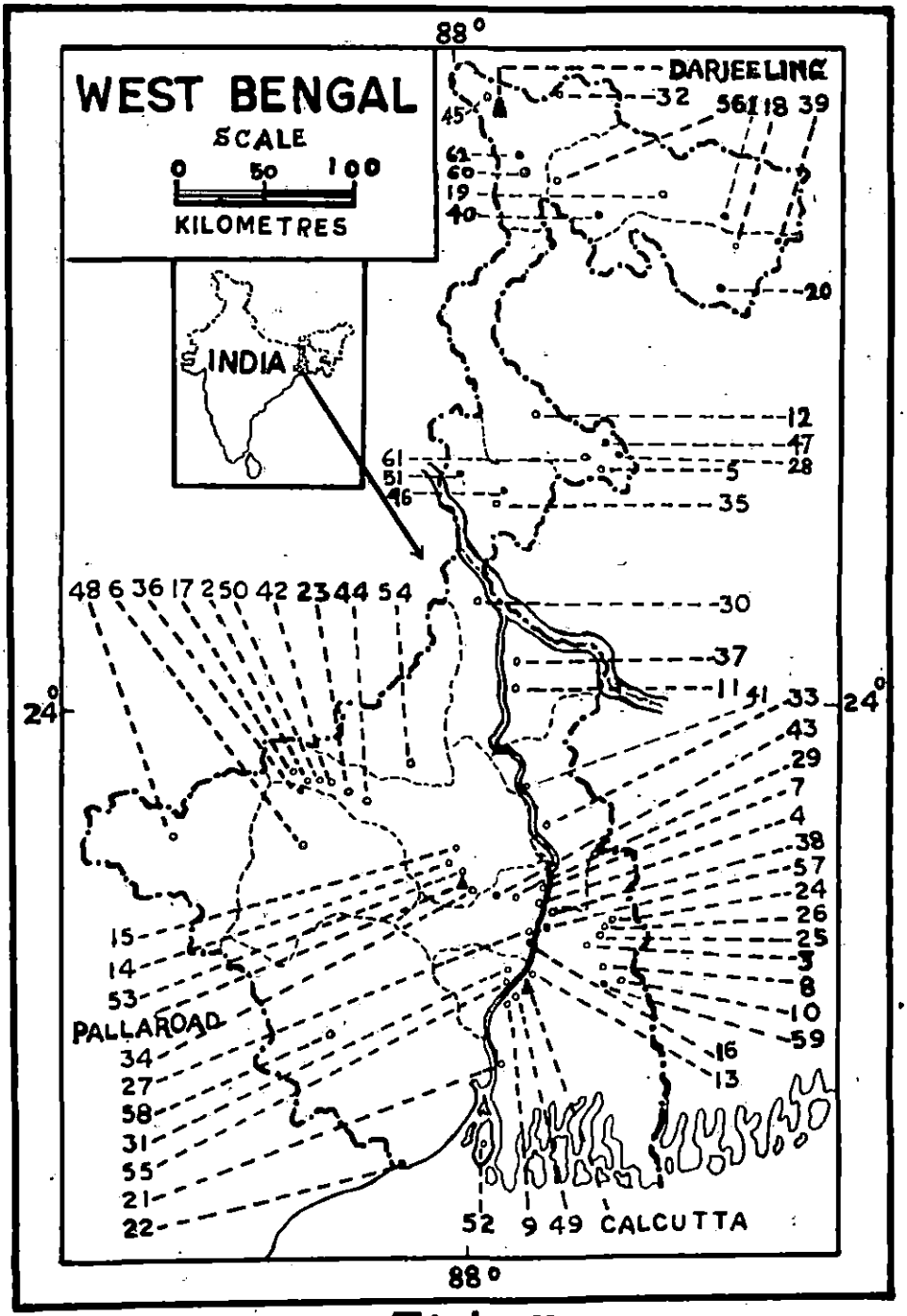

FIG.-3

MAP OF WEST BENGAL SHOWING THE SITES OF TRAPPINGS

Explanation of Map of West Bengal (Fig.3)
showing the sites of collection as follows:

1. Alipurduars
2. Asansol
3. Asholnagar
4. Bagati
5. Balurghat
6. Bankura Town
7. Baneshberia
8. Basirhat
9. Batanagar
10. Berachampa
11. Berhampore
12. Bolnadpur
13. Bon Hooghly
14. Burdwan Town
15. Burdwan farm area
16. Chinsurrah
17. Churulia
18. Coochbehar Town
19. Dhupguri
20. Dinhata
21. Diamond Harbour
22. Digha
23. Durgapur
24. Gobordanga
25. Guma
26. Habra
27. Hooghly Town
28. Hili
29. Itachuna
30. Jangipur
31. Kadamtala
32. Kalimpong
33. Krishnanagar
34. Madanpur
35. Malda Town
36. Mejia
37. Murshidabad Town
38. Naihati
39. Nimtijhar
40. New Jalpaiguri
41. Nakasipara
42. Ondal
43. Pandua
44. Panagarh
45. Pulbazar
46. Pandua
47. Patiram
48. Purulia
49. Rabindranagar
50. Raniganj village
51. Ratua
52. Sagar (Kakdwip)
53. Saktigarh
54. Santiniketan
55. Sibpur
56. Siliguri
57. Sodepur
58. Sonakania
59. Taki
60. Tindharia
61. Tapan
62. Tung

▲ Sites of constant collection

Table No. 1

Data of fixed light-trap collections at Calcutta during 1980 - 1981

Collecting period	No. of collecting nights	Total Diptera	Total Ceratopogonidae	Total Culicoidae	Average Culicoidae per night (in no.)
May, 1980	4	60	35	14	3.5
June, 1980	6	90	39	18	3.00
July, 1980	8	98	46	33	4.12
August, 1980	2	30	16	8	4.00
October, 1980	7	1,212	510	272	38.50
November, 1980	3	1,165	625	248	82.60
December, 1980	5	1,520	782	190	38.00
January, 1981	9	129	25	9	1.00
February, 1981	10	899	270	44	14.40
March, 1981	9	1,475	618	192	21.33
April, 1981	11	414	190	102	9.27
Grand Total	74	7,088	3,156	1,030	

Table No. 2

Data of fixed light-trap collections at Darjeeling town during 1968 and 1978-1979

Collecting period	No. of collecting nights	Total Diptera	Total Corotopogonidae	Total Culicoides	Average Culicoides per night (in no.)
April, 1968	1	224	2	0	0.00
May, 1968	24	7,401	476	157	6.54
June, 1968	20	20,616	2,923	1,890	94.70
July, 1968	21	68,117	9,690	3,511	167.19
August, 1968	21	34,275	3,322	1,200	57.14
September,1968	16	36,316	4,207	1,112	69.50
April, 1978	11	444	188	64	5.81
May, 1978	9	2,555	506	102	11.33
June, 1978	6	4,691	892	399	66.50
July, 1978	4	10,353	633	240	60.00
April, 1979	12	201	40	5	3.33
May, 1979	27	2,307	901	201	7.44
June, 1979	11	3,435	987	388	35.27
Grand Total	183	1,90,939	20,767	9,269	

Table No. 3

Data of fixed light-trap collections at Palharoad (Burdwan Dt.) during 1978-1980

Collecting period	No. of collecting nights	Total Diptera	Total Ceratopogonidae	Total Culicoides	Average Culicoides per night (in no.)
November,1978	2	36	16	4	2.00
December,1978	2	23	6	0	0.00
May, 1979	5	72	34	17	3.40
July, 1979	4	420	260	81	20.25
October, 1979	2	824	266	88	44.00
November,1979	1	254	92	32	32.00
April, 1980	3	360	66	24	8.00
May, 1980	3	57	24	8	2.66
June, 1980	4	1,377	403	131	32.75
August, 1980	2	599	291	93	46.50
September,1980	1	491	169	54	54.00
October, 1980	3	1,090	351	105	35.00
November,1980	3	1,840	282	97	32.33
Grand Total	35	7,443	2,260	734	

Table No. 4

Data of random light-trap collections at different places of West Bengal during 1966-1968 and 1977-1981

Collecting sites	Collecting period	Collecting nights(No.)	Collected Culicoides(No.
1. Alipur duars, Dist. Jalpaiguri	December,1972	3	5
2. Asansol,Dist.Burdwan	September,1962	3	67
3. Ashokenagar, Dist. 24 Parganas	April-Nov.,1966	7	103
4. Bagati,Dist.Hooghly	September, 1966	1	6
5. Balurghat, Dist. West Dinajpur	July, 1966	1	3
6. Bankura Town, Dist. Bankura	October, 1966	3	41
7. Banshberia, Dist. Hooghly	August, 1966	4	142
8. Bashirhat, Dist. 24 Parganas	October, 1965	1	11
9. Batanagar, Dist. 24 Parganas	October, 1966	7	45
10. Berachampa, Dist. 24 Parganas	October, 1966	1	37
11. Berhampur, Dist. Murshidabad	September, 1967	2	4
12. Buniadpur, Dist. West Dinajpur	September, 1968	3	21
13. Bonhooghly, Dist. 24-Parganasx	November, 1966	2	4
14. Burdwan Town Dist. Burdwan	April-Sept.,1967	27	106

contd...

Table No.4 contd.:

Collecting sites	Collecting period	Collecting nights(No.)	Collected Culicoides(No)
15. Burdwan farm area, Dist. Burdwan	April-Sept.,1967	11	421
16. Churrulia, Dist.Burdwan	August, 1967	5	148
17. Chinsurah, Dist. Hooghly	July-August,1966	27	2,071
18. Coochbehar Town, Dist. Coochbehar	November, 1966	4	71
19. Dhupguri, Dist. Jalpaiguri	November, 1967	6	227
20. Dinhata, Dist. Coochbehar	November, 1967	1	9
21. Diamond Harbour Dist. 24 Parganas	March, 1967	1	16
22. Digha,Dist.Midnapur	December, 1967	2	0
23. Durgapur, Dist. Burdwan	April-Dec.,1967	?	72
24. Gobardanga Dist.24 Parganas	Apri,'66-March'67	31	3668
25. Guma,Dist.24 Parganas	October, 1966	4	31
26. Habra,Dist.24 Parganas	August'66-July'67	141	14,706
27. Hooghly Town, Dist. Hooghly	August, 1966	2	3
28. Hili,Dist.West Dinajpur	April,1967	2	5
29. Itachuna, Dist. Hooghly	April-December,1967	8	188
30. Jangipur, Dist. Murshidabad	September, 1967	1	49

contd.....

Table No.4 contd.:

Collecting sites	Collecting period	Collecting nights(No.	Collected Culicoides (No.)
31. Kadamtala, Dist.Howrah	July, 1966	2	5
32. Kalimpong, Dist. Darjeeling	April, 1968	2	110
33. Krishnanagar, Dist. Nadia	October, 1966	4	40
34. Madanpur, Dist.Burdwan	April-December,1967	4	66
35. Maldah Town Dist. Maldah	October, 1968	2	133
36. Majia,Dt.Bankura	April-December,1968	9	48
37. Murshidabad Town, Dist. Murshidabad	November, 1966	1	4
38. Naihati, Dist. 24-Parganas	June, 1966	4	11
39. Nintijhar T.E., Dist. Coochbehar	November, 1966	2	8
40. New Jalpaiguri,Dist. New Jalpaiguri,	July, 1966	4	8
41. Naknaipara,Dist.Nadia	June, 1967	2	36
42. Ondal,Dist. Burdwan	April, 1967	2	19
43. Pandua,Dist. Hooghly	November, 1966	4	5
44. Panagarh,Dist.Burdwan	April, 1967	1	2
45. Pandua, Dist.Maldah	October, 1966	1	4
46. Patiram, Dist.West Dinajpur	February, 1967	3	64
47. Pulbazar, Dist. Darjeeling	April, 1978	1	100

contd.....

Table No.4 contd.:

Collecting sites	Collecting period	Collecting nights (No.)	Collected Culicoiden (No.)
48. Purulia Town, Dist. Purulia	November, 1967	2	26
49. Rabindranagar, Dist. Calcutta	September, 1966	3	26
50. Raniganj Dist.Burdwan	April-December,1967	7	105
51. Ratua,Dist.Maldah	August, 1967	2	64
52. Sagar, Dist.24-Parganas	March, 1984	2	66
53. Saktigarh, Dist. Burdwan	April, 1980	8	16
54. Santiniketan Dist. Birbhum	November, 1966	3	13
55. Sibpur, Dist. Howrah	November, 1966	1	1
56. Siliguri, Dist. Jalpaiguri	May-June, 1968	15	473
57. Sodepur, Dist. 24-Parganas	May'80-April'81	24	530
58. Sonakania, Dist. Midnapur	November, 1968	2	26
59. Taki, Dist. 24-Parganas	October, 1967	3	1106
60. Tindharia, Dist. Darjeeling	April, 1978	1	8
61. Tapan, Dist. West Dinajpur	June, 1979	1	10
62. Tung[illegible],Dist.Darjeeling	April, 1978	2	70
Total		442	25,483

It should be mentioned that 36,516 Culicoides collected by light trappings over years were daily examined and sorted out into species those belonged to either in dry state or in alcohol (70%) preserved state or in slide mounts. While the majority could be sorted out duly as per synoptic tables provided in the present thesis, a minor fraction of the collected specimens defied identification for various reasons. While we rejected outright such specimens as were found badly damaged after collection, it was seen that the fraction defying identification belonged either to variable category of specimens, or stray 'aberrant' forms or 'teneral' forms. As male-female collation in such cases was not found feasible and as there was lack of a concistance in the variability, it was preferred to keep such specimens collected now out of the present study. Only, more collections at the very locations of their availability may make it possible in future to categorise these 'difficult' specimens.

As for the speciswise quantum of the identified material, a further separate list/table and concomitant significance of the same were not provided now since the same were actually beyond the scope of the present study. It is, however, clarified that an indication of the numerical abundance of the species expressed in the text by commenting as "a scarce element", "less common species", "very extencively distributed", "limited distributions", "very uncommon in its place of occurance", "a common form", "most abundant" element", "prevail abundantly", "colaterally distributed", "very wide geographical distribution" etc. were a pointer to the turn-up frequency of the species in our collection.

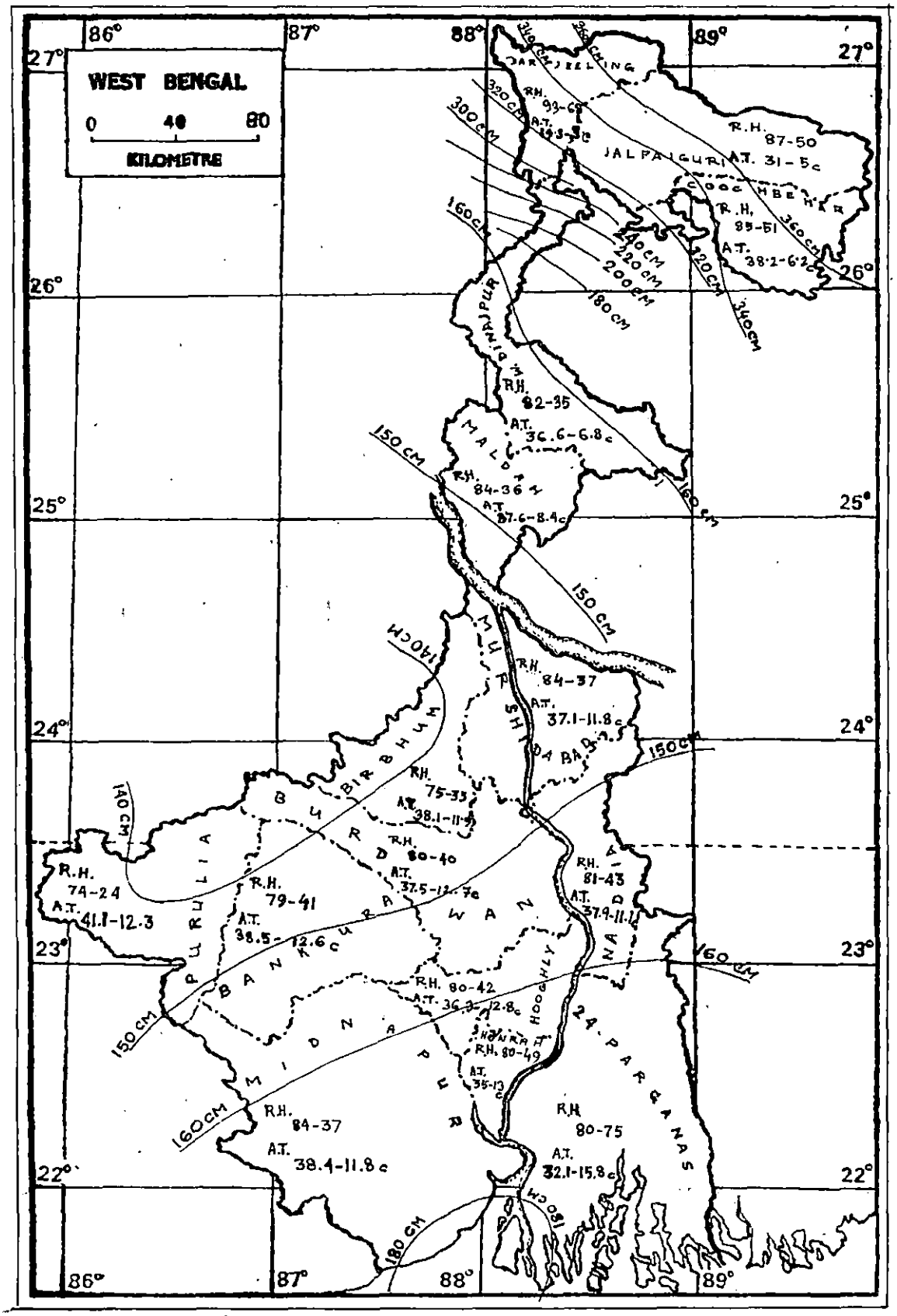

FIG.-4 Composite Map of West Bengal showing districtwise relative humidity (R.H.) temperature range (A.T.) and avarage annual rainfall (CM)

In the text, for a species, a reference was only made to such samples which actually were based in preparing illustrated, taxonomic descriptions of the species.

The locations cited in above tables and also in subsequent pages of the thesis are all shown in the physical map of the State of West Bengal, India (Figs.2-3).

The 65 locations whose Culicoidea specimens are now studied belonged to 16 districts of the West Bengal State. The edaphic and topographic nature alongwith average annual rainfall and range of temperature and relative humidity in those districts were illustrated in figs. 4-5. It could follow from the data provided therein that the State of West Bengal altitudinally is highly variable, being over 2000 m height from sea level in subhimalayan region of Darjeeling in the north to almost zero level in the coastal areas in the south. On basis of edaphic factors, the state is divisible to some 9 zones, from extreme north southward the zones being - Hill soil area (major part of Darjeeling district and minor part of Jalpaiguri district), Lateritic area (major parts of Birbhum and Malda districts and minor parts of Bankura, Burdwan and Midnapur districts), Red soil area (major parts of Bankura and Burdwan districts, and minor part of Birbhum, Malda, Midnapur, Murshidabad and West Dinajpur districts), Marshy soil area (minor part of Malda, Nadia and 24-Parganas districts), Deltaic and saline soil area (major part of 24-Parganas district and minor part of Midnapur

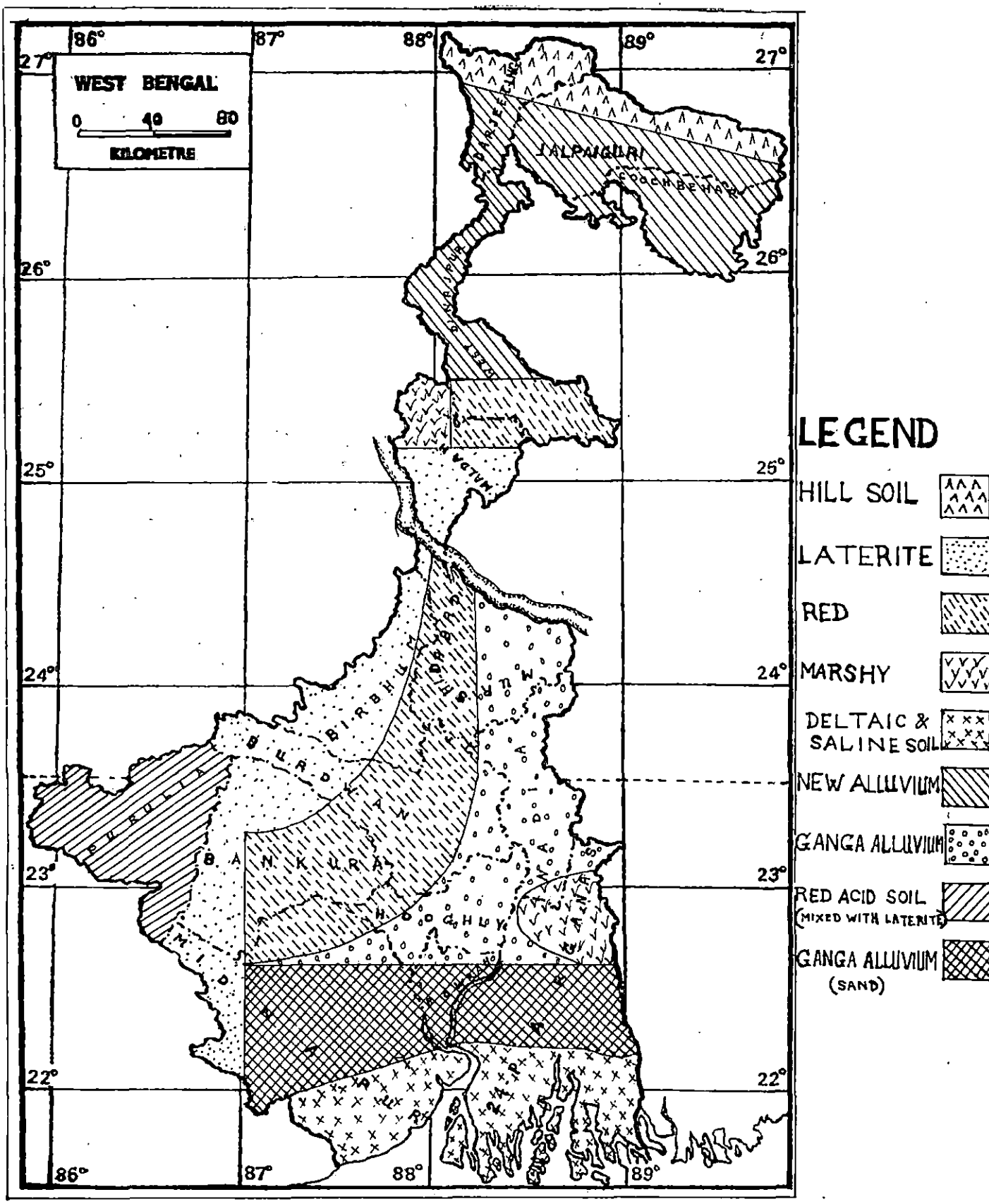

FIG-5 Soil map of West Bengal.

district), New alluvium area (major part of Jalpaiguri and West Dinajpur districts, whole of Coochbehar district and minor part of Darjeeling district), Ganga alluvium area (major part of Hooghly and Nadia districts, and minor part of Burdwan, Howrah, Midnapur, Murshidabad and 24-Parganas districts), Red acid soil area mixed with laterite (whole of Purulia district) and Ganga alluvium sand area (rest of Calcutta, Howrah, Midnapur and 24-Parganas districts). Large forest areas prevailed in Bankura, Coochbehar, Darjeeling, Jalpaiguri and 24-Parganas districts.

C) Procedures and terminologies :

In the present study, the Culicoides populations of the subgenus Trithecoides were given the emphasis since in past studies (Dasgupta and his collaborators 1956-'72), it received scant or no attention. The species belonging to that subgenus found as new to science were all fully described.

In preparing the specimens for taxonomic examination and study, the method of Wirth and Blanton (1959) has been mainly followed. The specimens were first placed in the saturated solution of pure phenol in absolute alcohol and kept in a warm oven for 12-15 hours for clearing. The treatment made the specimens clear and somewhat transparent. The well cleared specimens were then taken on micro-slides, one on one slide, and a drop of mountant previously prepared by making a homogeneous mixture of canada balsam and phenol in equal

proportion was put over it. The specimen was then suitably dissected into several parts, the female body into wings, head, legs, thorax and abdomen, and the male body into wings, genitalia and the rest. The dissected parts were suitably oriented in the mountant and a cover-slip was placed over it. The prepared slides were then left in hot oven for complete drying and hardening of the mountant. Additional canada balsam was added from time to time according to the necessity to fill up the gaps in the slides, if any.

The unmounted specimens were preserved in 70% alcohol in the laboratory where the work was executed.

The morphological terminologies used in the text were based on Campbell and Pelham - Clinton (1960), Jaenback (1955) and Wirth & Blanton (1970), are fully explained in figs. 6-7. For convenience, the term "antennomere" or "segment" has been used for each of the 15 division of antenna and "palpomere" for a segment of the maxillary palp. Abbreviations H T C and A P used in the text meant hind tibial comb and apicolateral processes respectively. The technical terms and usages used in the text are amplified below :

Antennal ratio (AR) - is the ratio obtained by dividing the combined length of the distal five antennomeres by the combined length of the preceding eight antennomeres.

Costal ratio (C R) - is the value obtained by dividing the distance from the basal arculus to the end of the costa by the wing length.

Palpal ratio (L/W) - is the length to greatest breadth ratio of the third segment of the maxillary palp.

Proboscis to head index ratio (P/H) - is the value obtained by dividing the distance from torma to the tip of labrum-epipharynx by the distance from the interocular seta-base to the torma.

Tarsal ratio (T R) - is the value obtained by dividing the length of first tarsomere by the length of the second tarsomere of a hind leg.

The wing length has been measured from the basal arculus to the wing tip and spermatheca from one end to other excluding the sclerotized portion of the duct ("neck") and its maximum width was taken as its breadth. Measurements have usually been made of a series of 10-15 specimens when available and as far as possible presented as "Mean (minimum-maximum, n = number of specimens measured").

All figures has been drawn with the help of a camera lucida keeping error constant. To avoid clumsiness, aedeagus and parameres of males have been drawn separately.

The illustrations provided now in the thesis were of all the species described as new to science. The new species of Majumdar and Dasgupta (1972) which were re-studied now were now reviewed in important aspects only and illustrations of selected parts of the same were given. Of the previously described species, only the salient features were reviewed but no illustrations of those were given now as literature were replete with the same.

The types of the new species and other materials studied are in the custody of the Zoology Department (Entomology Section) of Presidency College Museum (abbreviated as "Cs PCZM). Representative specimens including paratypes, when available, will be sent in due course to the various depositories at home and abroad including the Zoological Survey of India in Calcutta, the U.S. National Museum in Washington, the British Museum (Natural History) in London and the Leningrad Zoological Institute, U.S.S.R.

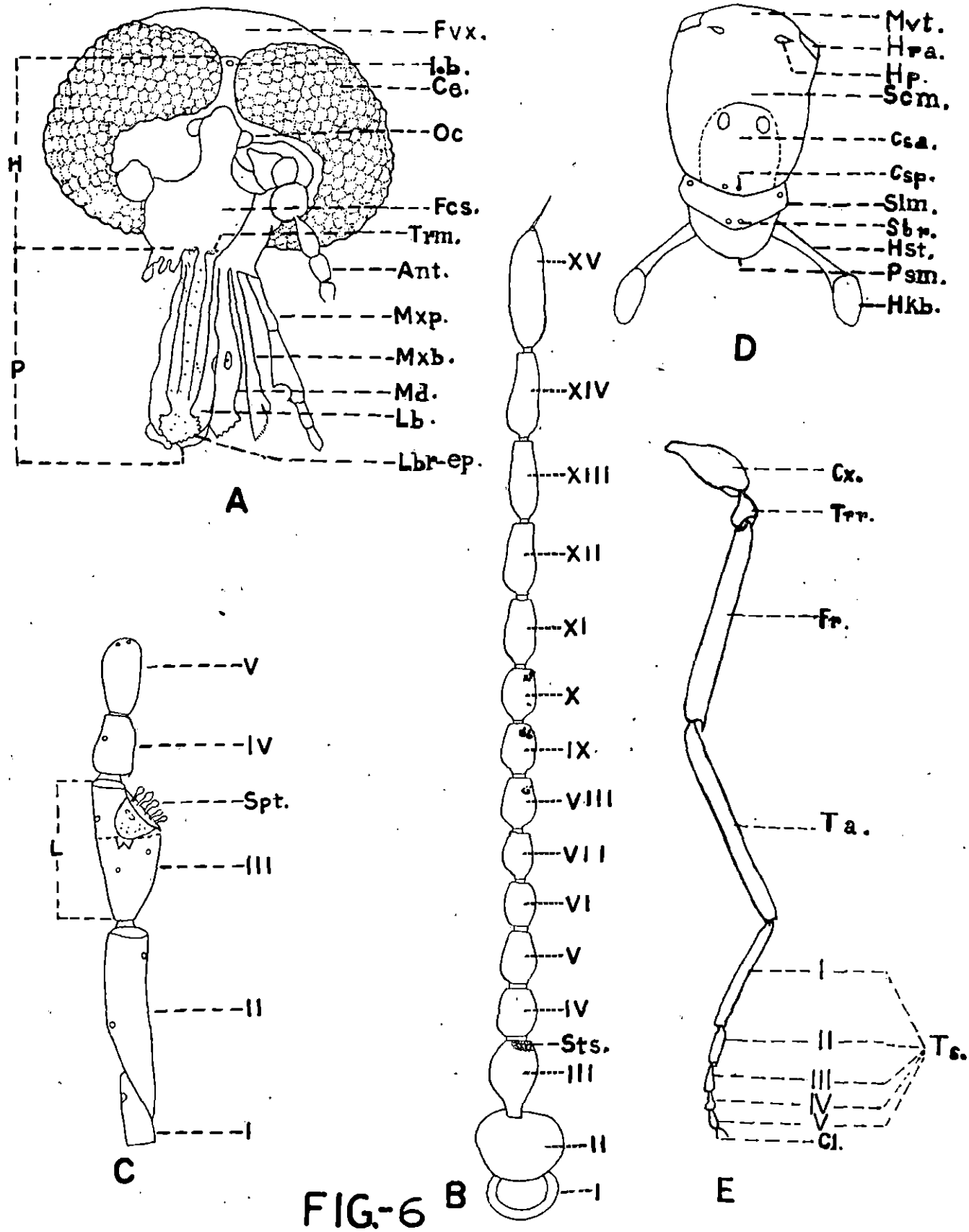
Fvx.
Lb.
Ce.
Oc
Fcs.
Trm.
Ant.
Mxp.
Mxb.
Md.
Lb.
Lbr-ep.
H
P
A
Mvt.
Hpa.
Hp.
Scm.
Csa.
Csp.
Slm.
Sbr.
Hst.
Psm.
HKb.
D
XV
XIV
XIII
XII
XI
X
IX
VIII
VII
VI
V
IV
Sts.
III
II
I
B
V
IV
Spt.
III
II
I
L
C
Cx.
Tr.
Fr.
Ta.
I
II
III
IV
V
Cl.
Ts.
E

FIG.-6

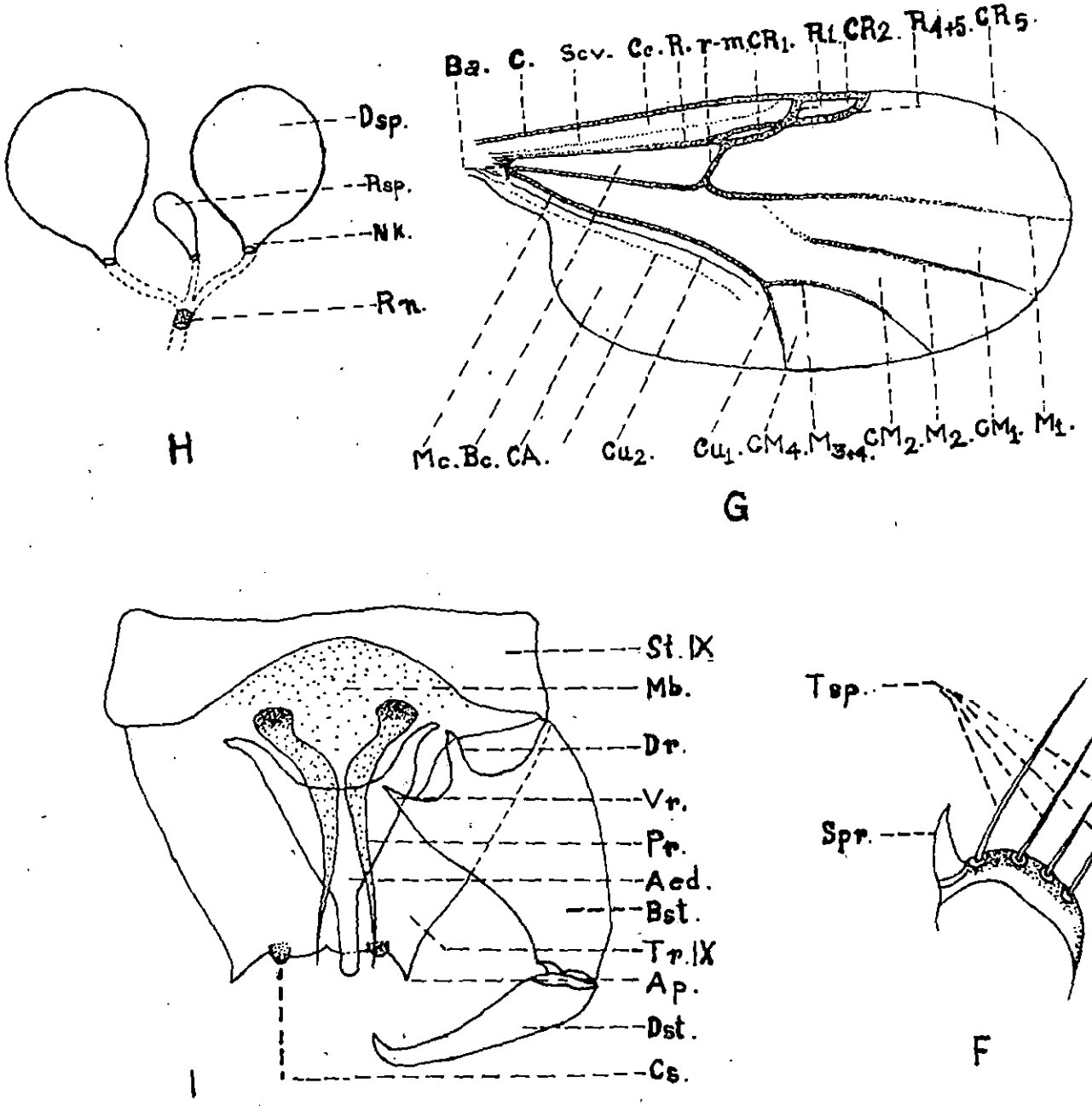
Dsp.
Rsp.
NK.
Rn.
H
Ba. C. Scv. Co. R. r-m. CR1. R1. CR2. R4+5. CR5.
Mc. Bc. CA. Cu2. Cu1. CM4. M3+4. CM2. M2. CM1. M1.
G
St. IX
Mb.
Dr.
Vr.
Pr.
Aed.
Bst.
Tr. IX
Ap.
Dst.
Cs.
I
Tsp.
Spr.
F

FIG.-7

Explanation of figs. 6 and 7

Morphological structures of Culicoides

A. Dorsal view of the head of a female
B. Antenna of a female
C. Maxillary palp of a female
D. Dorsal view of the thorax with haltere of a female
E. A female leg.
F. Tibial comb of hind leg of a female
G. A female wing
H. Spermathecal apparatus of a female
I. Ventral view of a male genitalia

Abbreviations

A_1 = First anal vein
Aed. = Aedeagus
Ant. = Antenna
Ap. = Apicolateral process
Ba = Basal arculus
Bc = Basal cell
Bst = Basistyle
C = Costal vein
CA = Anal cell
Cc = Costal cell
Ce = Compound eye
Cl = Tarsal claw
CM_1 = Cell M_1
CM_2 = Cell M_2
CM_4 = Cell M_4
CR_5 = Cell R_5
Csa = Caudoscutal area
Csp = Caudoscutellar pit
Cs = Circus
Cu_1 = Vein Cu_1
Cu_2 = Vein Cu_2
Cx = Coxa
Dr. = Dorsal root
Dsp = Developed spermatheca
Dst. = Dististyle
Fco = Fronto-clypeus
Fr = Femur
Fvx = Frontovertex
H = Distance from interocular seta-base to torma
Hkb = Halter knob
Hp = Humeral pit
Hra = Humeral area
Hst = Halter stem
Iob = Interorbital bristle
Lb = Labium
Lbr-ep = Labrum-epipharynx
M_1 = Vein M_1
M_2 = Vein M_2

M_{3+4} = Vein M_{3+4}

Ml = Ventral membrane

Mc = Mediocubital vein

Md = Mandible

Mvt = Median vitta

Mxb = Maxillary blade

Mxp = Maxillary palp

NK = Neck (spermathecal neck)

Oc = Ocellus

P = Distance from torma to tip of labrum-epipharynx

Pr = Paramere

Psm = Post scutellum

R = Radial vein

R_1 = Vein R_1

R_{4+5} = Vein R_{4+5}

CR_1 = Cell R_1

CR_2 = Cell R_2

r-m = Radio-medial cross-vein

Rm = Ring

Rs = Radial sector

Rsp = Rudimentary spermatheca

Sbr = Scutellar bristle

Scm = Scutum

Scv = Subcostal vein

Slm = Scutellum

Spr = Spur

Spt = Sensory pit

St.IX = Sternum IX

Sts = Sensory tuft (= distal sensory coeloconica)

Ta = Tibia

Tr.IX = Tergum IX

Trm = Torma

Trr = Trochanter

Ts = Tarsomere I-V

Tsp = Spines on hind tibial comb

Vr. = Ventral root

W = Width of segment III of the maxillary palp