CHAPTER-III

MATERIALS AND METHODS

MATERIALS AND METHODS

Any minor change in the materials and methods provide drastic results, either positive and negative hence, appropriate materials and methods have tremendous importance in scientific experimentation.

Materials and methods used in completion of the present work are given below.

MATERIALS:

1) Glass Troughs (Fig. 3):

Glass troughts of size 9 × 25 cm and size 10 × 25 cm (height and diameter) were used for rearing of crickets. During the process, the glass troughs were covered with muslin cloth.

2) Test Tubes (Fig. 4):

The test tubes of size 15 × 2.5 cm (length and diameter) were used for handling the nymphs and adults of cricket species.

3) Insect net (Fig. 5):

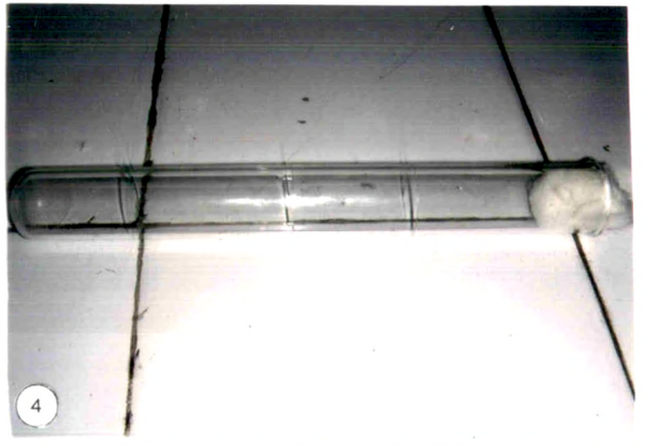
Insect hand net made up of aluminium handle 35 cm long, circular iron ring of 12 cm diameter and ordinary mosquito bag of 35 cm in depth was used for collecting nymphs and adults of both crickets i.e. mole cricket and house cricket.

4) Plastic containers (Fig. 6):

Plastic containers (fig.6) of capacity 1 litre and 1.5 litre were used for keeping / collecting the samples of nymphs/ adults of crickets from the natural habitat.

5) Cricket rearing cage (Fig.7):

The cricket rearing box (panpuda local name) made up of steel of size, length 16.5 cm × width 11.5 cm × height 6.5 cm has been used for rearing the crickets and keeping crickets live for longer period for the taxonomical study and photography. The sides of the box were ventilated with small open windows of size, length 20.00 mm × width 3.00 mm. 6 windows were into the width side and 12 into the length side.



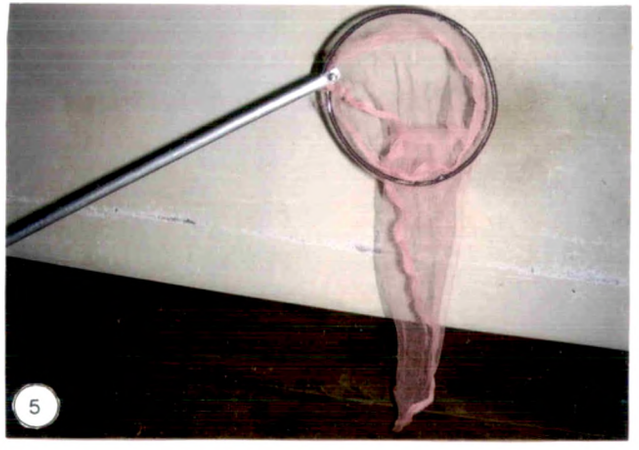


PLATE –1: Fig.3 : Glass trough Fig. 4: Test tubes Fig.5: Insect net Fig. 6: Plastic containers.

6) Slide boxes (Fig. 8):

Slide boxes were used for keeping the permanent slides safely. Slide boxes of size, 28 × 22 ×3.5 cm, 21 × 19 × 3.5 cm (length, width and height) were used for keeping the slides of various body parts of crickets.

7) Slides and cover slips (Fig. 9):

Ordinary slides and coverslips were used for preparing the body parts such as antenna, wings, cerci and genitalia of crickets.

8) Oven (Fig. 10):

Oven of size, 3.6 × 2.4 feet (height and width) have been used for drying the slides of crickets and drying killed specimens.

9) Insect store box (Fig. 11):

Insect store box of size, 47 cm length × 30 cm width was used for keeping insects for longer period.

10) Camera Nikon S4 (Fig. 12):

Nikon Coolpix S4 Camera, 10x optical zoom and 6 megapixal was used for taking photographs of crickets.

11) Compound microscope (Fig. 13):

Simple compound microscope with objectives 10x, 45 x, 100x were used for describing the cricket species.

12) Microphotographs:

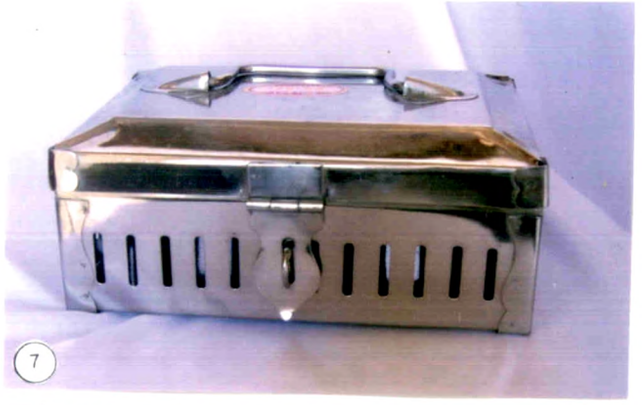
Microphotographs of whole mounts of the crickets and their various body parts namely antenna, wings, cerci and genitalia have been photographed by using microphotographic camera (Nikon S4).

13) Chemicals:

Following chemicals were used for preparation of slides and preserving insects.

i) 10 % KOH.

ii) 30 % to 100 % Ethyl alcohol grades.



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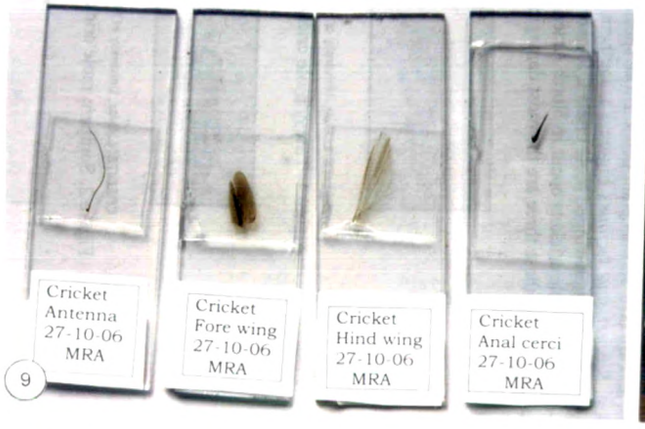

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PLATE – 2 Fig.7: Cricket rearing cage cover slips Fig.10: Oven.

Fig. 8: Slide boxes

Fig.9: Slides and

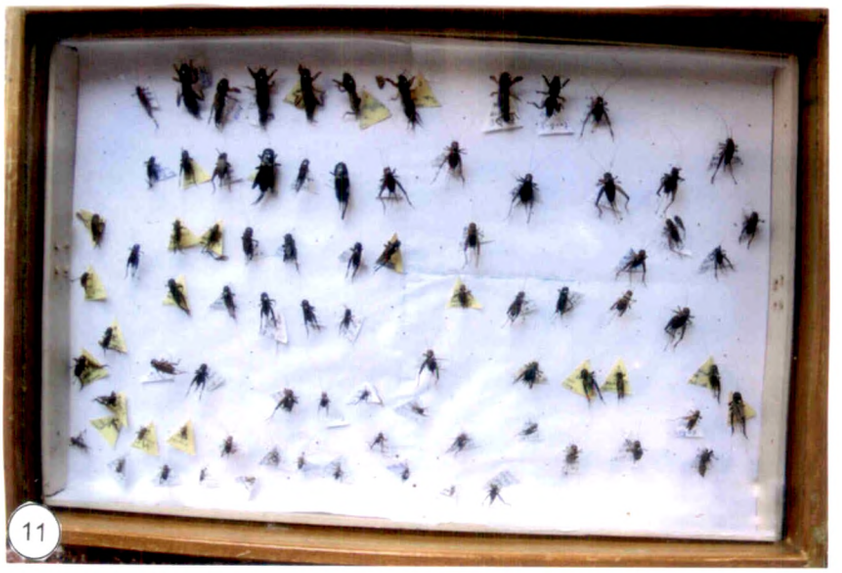

PLATE – 3 Fig.11: Insect store box

Fig.12: Camera Nikon S4

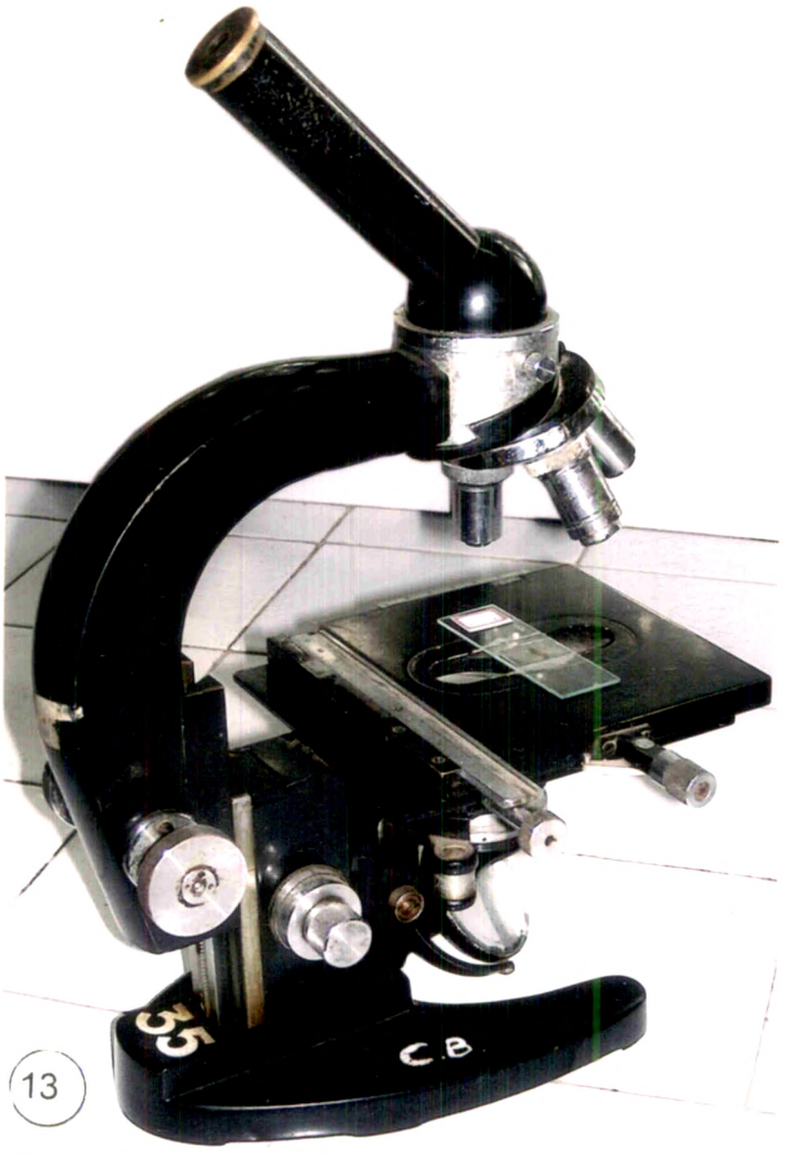

PLATE – 4 Fig. 13 Compound microscope.

iii) Glacial acetic acid.

iv) Xylene.

v) DPX / Canada Balsum.

METHODS:

The cricket species were collected from the district Kolhapur (fig.14) from the years 2006 to 2007. Kolhapur lies between 74°.13' East longitude and 16°.41' North latitudes; Kagal lies between 74°.18' and 16°.34' N ; Ajara lies between 74°.28' E and 16°.51' N; Radhanagari lies between 74°.35' and 16°.60' N; Panhala lies between 74°.07' E and 16°.48' N and Shahuwadi lies between 74°.40' E and 16°.58' N . A large number of species were collected by visiting various places of Tahasils of district Kolhapur (Ajra, Radhanagari, Kagal, Kolhapur, Shahuwadi, Panhala, Gaganbawada, Karveer, Hatkangale, Shirol, Bhudargad, Gadhinglaj and Chandgad at 15 days interval. The crickets were collected with the help of insect net (fig. 5) and containers (fig. 6). The nymphs collected from various study spots were reared in the laboratory for their adult formation by using glass troughs (Fig. 3) and cricket rearing cages (fig. 7). The adult crickets were narcotized in ether and killed in

cyanide killing bottle /chloroform. The crickets were pinned with entomological pins from the dorsal of mesothorax and dried in drying chamber / oven at 55 ^{0}C. The dried specimens then kept in wooden insect box. Few naphthol bolls were placed at the bottom of the box for preventing fungal attack. Holotypes and paratypes are pinned, labelled and kept in insect box. The records were made on locality, date of collection and identification. For the taxonomical studies antenna, wings, cerci, genitalia, were mounted on slides in D.P.X. For preparation of slides of genitalia following method (Vasanth, 1993) was adopted. The abdominal extremity was cut open and the genitalia removed. The dissected genitalia was then kept overnight in 10 % cold potassium hydroxide solution to clear the muscles. (Keeping for a longer period of time in the KOH solution results in desclerotisation of the genitalic parts, making it extremely difficult for study). Muscles were cleared with the help of needles, the cleared genitalia was then washed thoroughly in distilled water and preserved in 90 % alcohol in a microvial. This was either kept along with the specimen (if the specimen were preserved in spirit) or in case of a pinned specimen, it was kept separately and an index-numbered label was given to both the

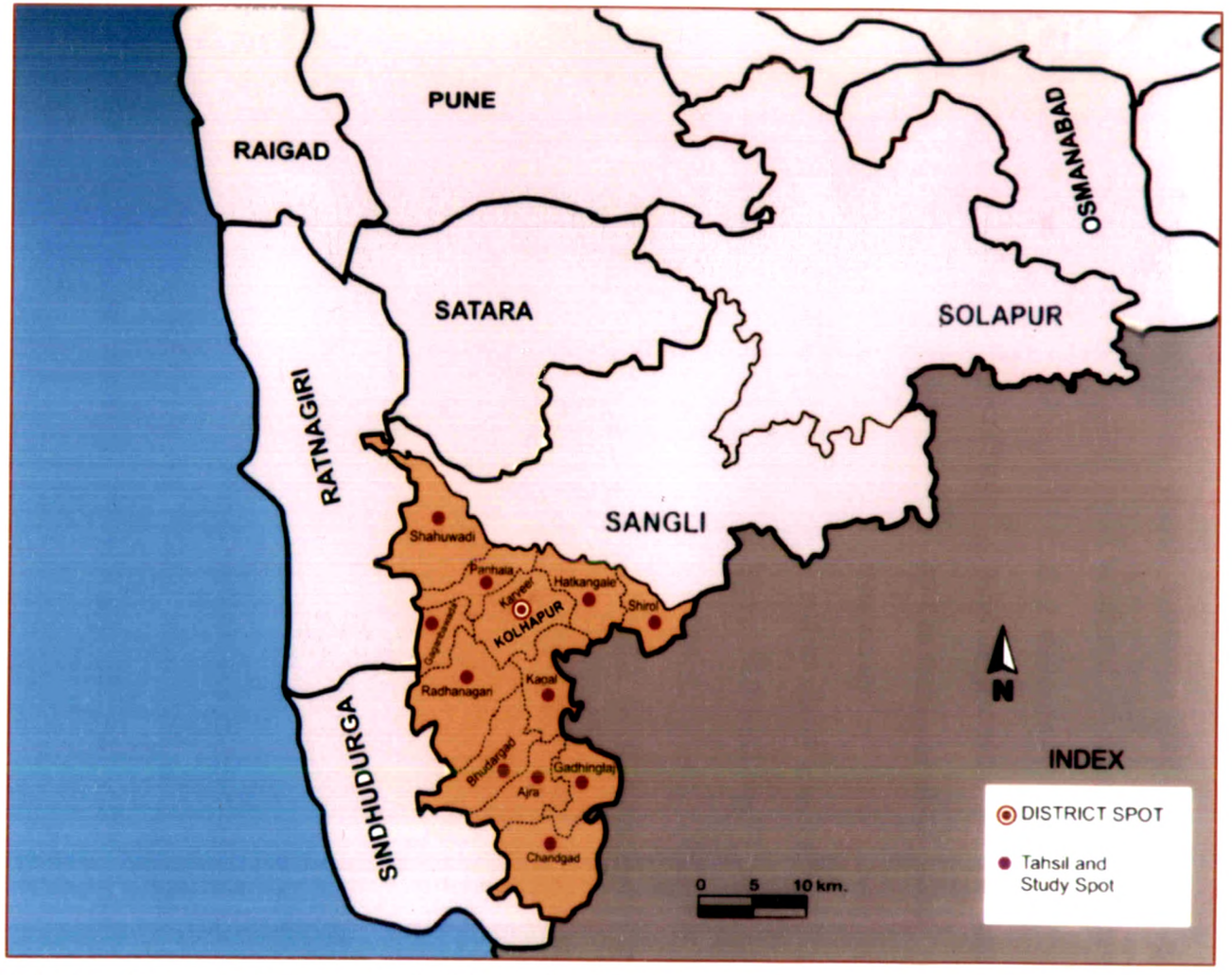

Fig. 14: Map of Kolhapur district showing study area

genitalia and specimen from which it was dissected out. Illustrations of the genitalia were made with the help of a camera lucida. The procedure of affixing the genitalia to a piece of paper kept with the pinned specimen was not adopted since this makes it impossible to study the ventral view of the genitalia.

Morphological studies were carried out with the help of monocular microscope. Comparative measurements of body parts of specimens were taken with the help of ocular micrometer and calculated with the help of graduated mechanical stage. All measurements were made in millimeter.

The terminology adopted for description of species is the same as that of Chopard (1928a, 1933a, 1969) and Vasanth (1993) which is represented in the figures 15 to 24. A large number of references were consulted for the present work and cited in bibliography.

PHOTOGRAPHY:

The whole crickets and their various body parts such as antenna, wings, cerci and genitalia were considered for microscopic photography. The coloured photography of whole insects and different

body parts such as antenna, wings, cerci and genitalia was made with the help of Nikon S4 camera (6.0 megapixal, 10x optical zoom). Whole mount figures have been enlarged 16 times to its original size, while other body parts have been enlarged to 60 times to its original size.

Fig.15 ADULT MALE (♂)

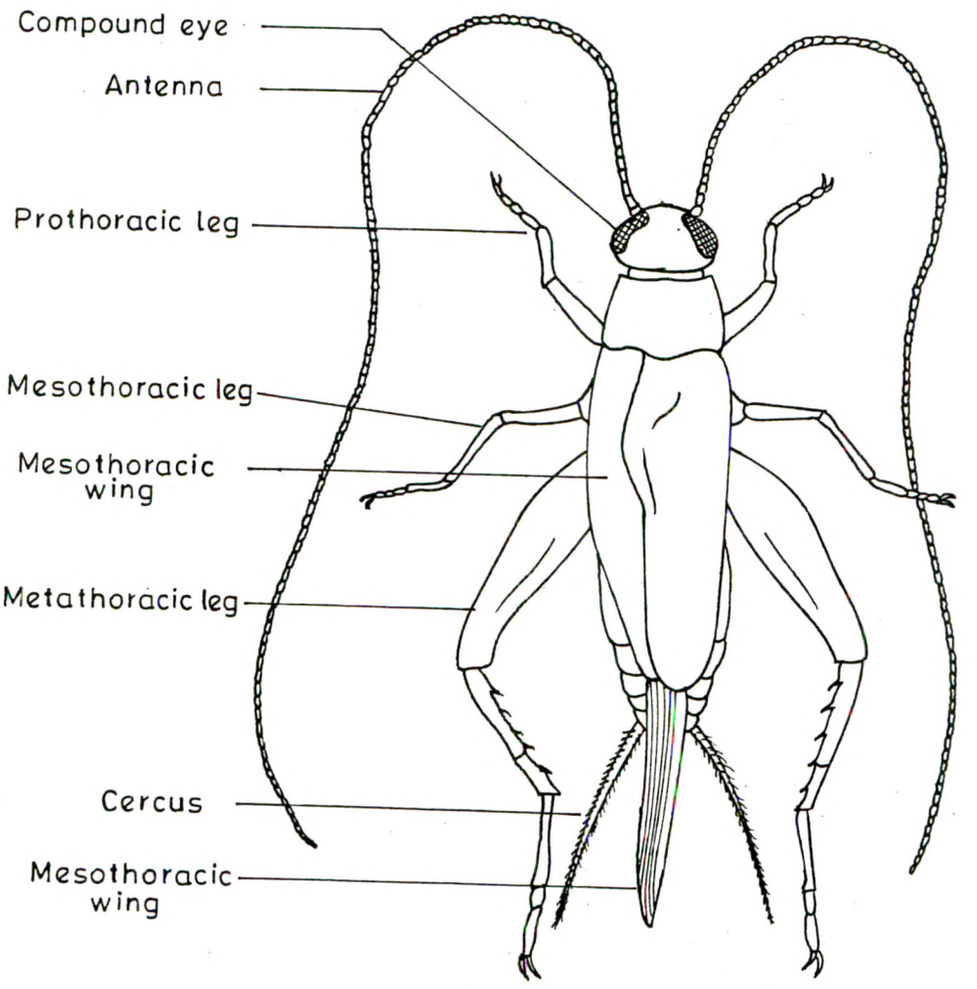

Fig.16 : ADULT FEMALE (♀)

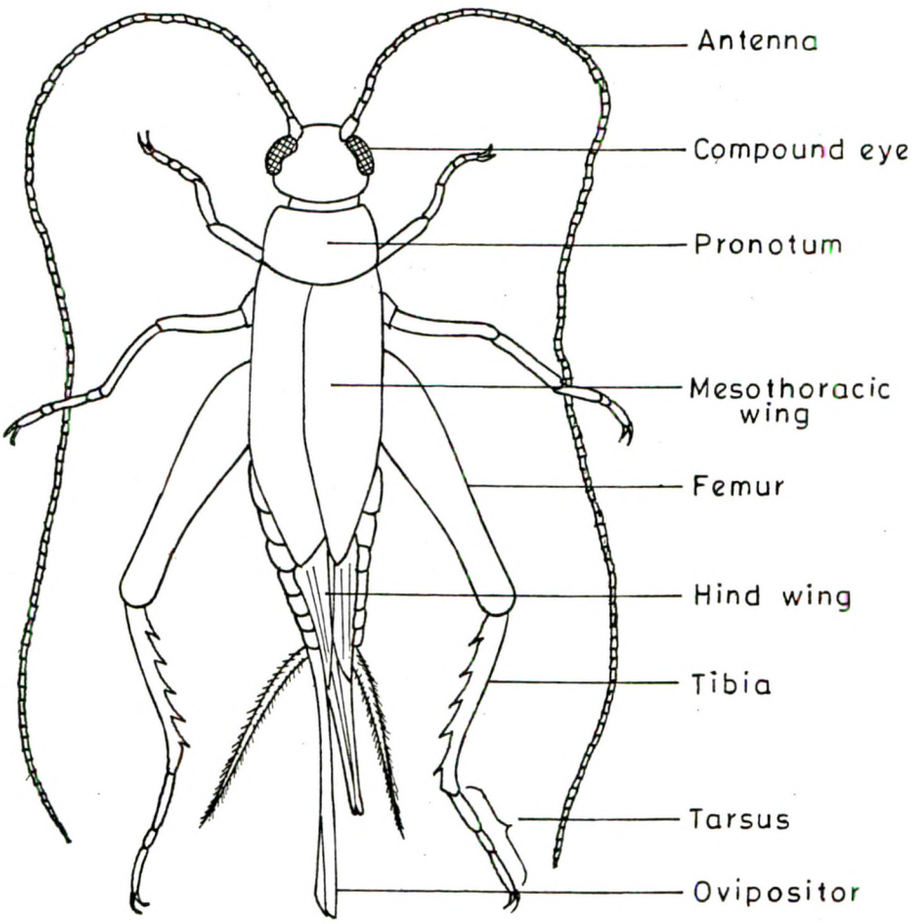

Fig.17 LATERAL VIEW OF FIELD CRICKET

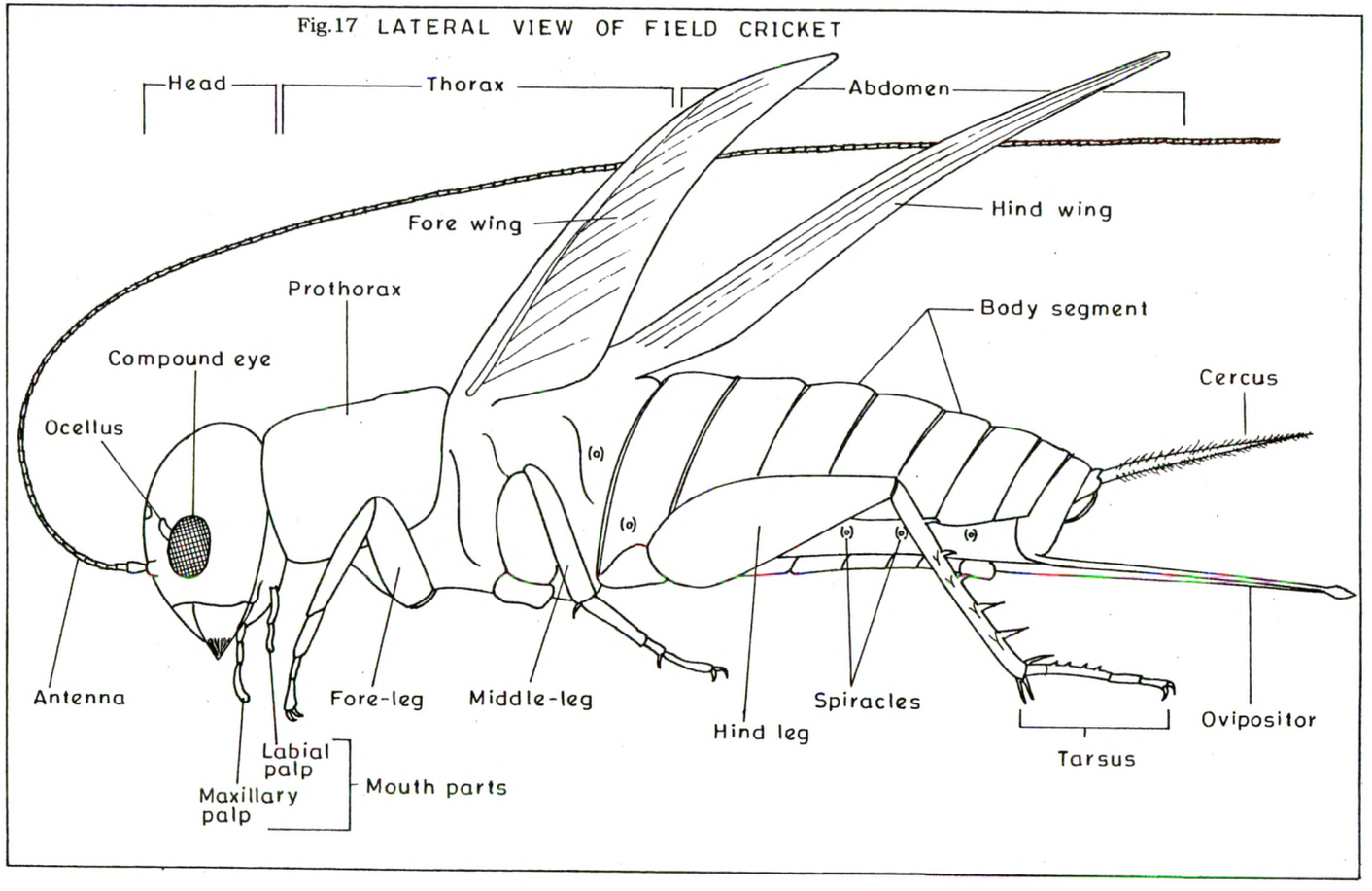

Fig.18

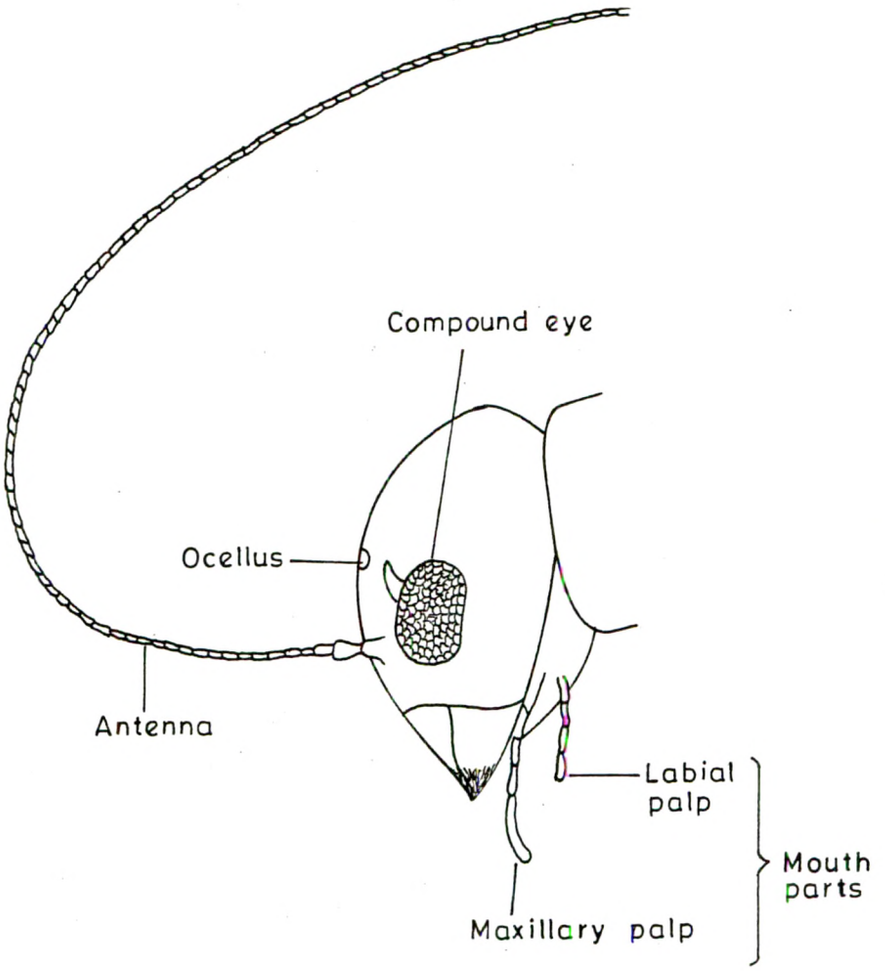
Compound eye
Ocellus
Antenna
Labial palp
Mouth parts
Maxillary palp

Fig.19 HEAD OF GRYLLUS

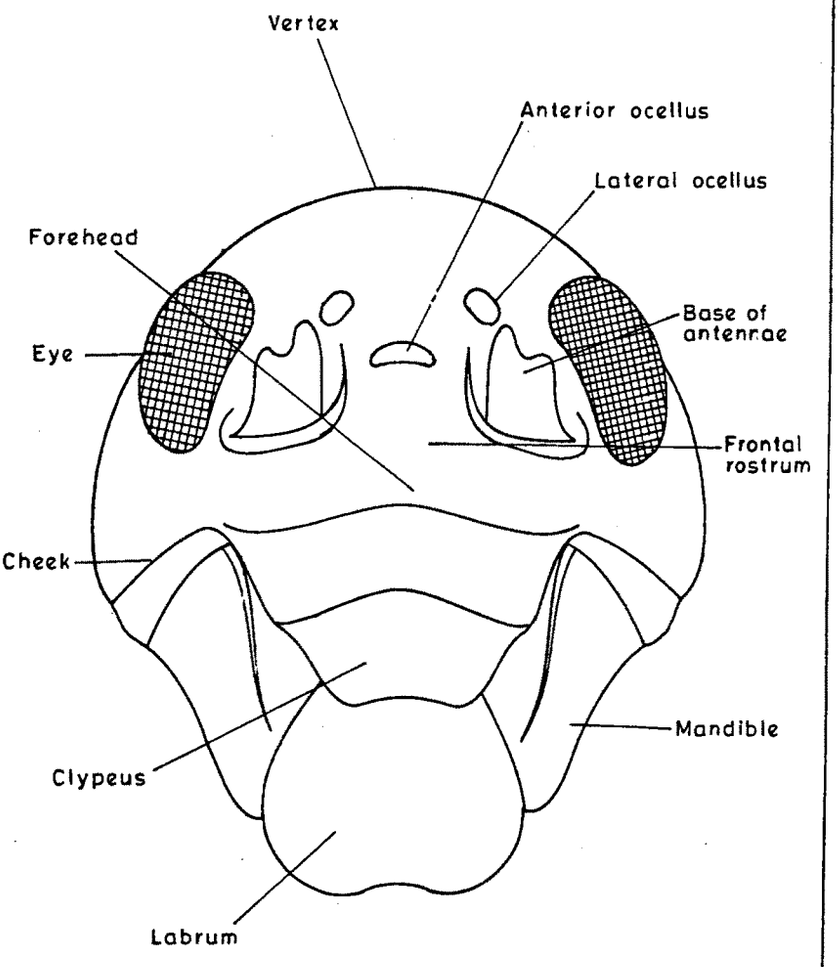

Fig.20 ANTENNA

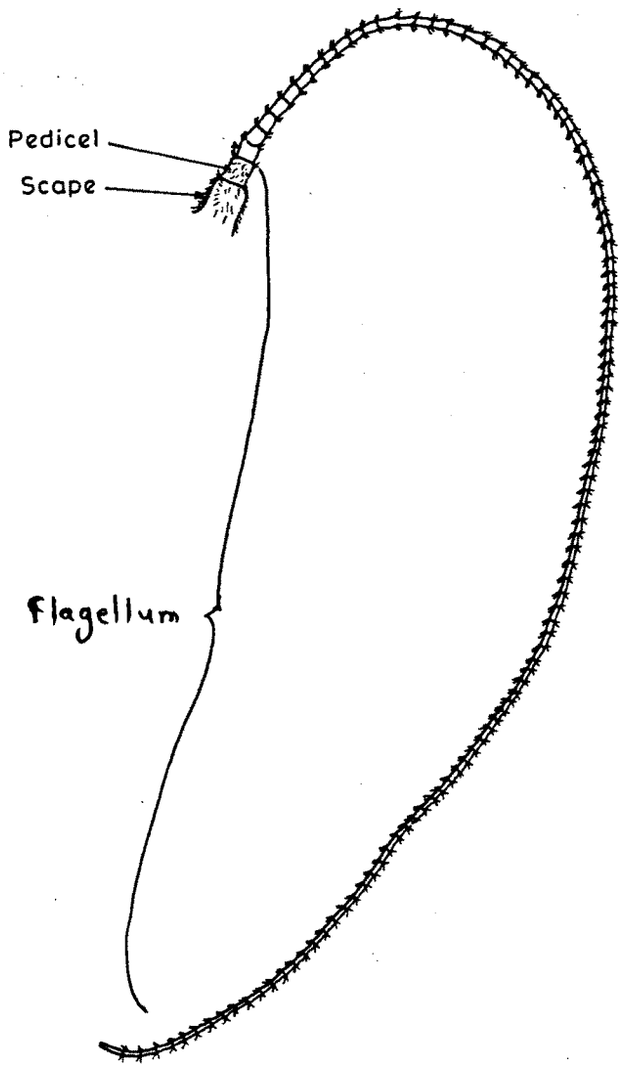

Fig.21 THORAX

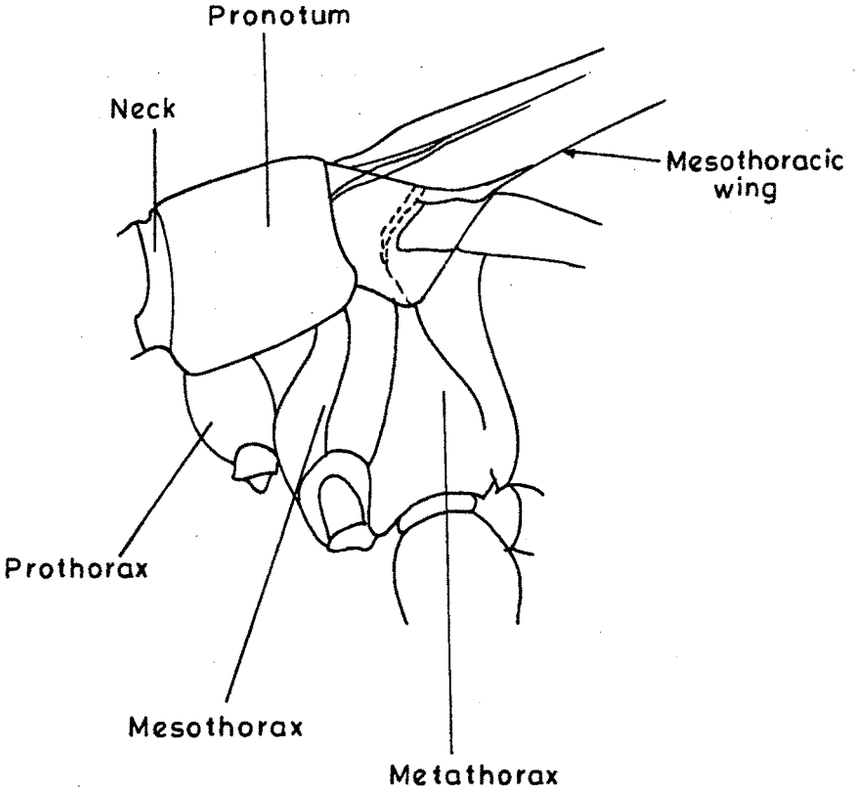

Fig.22 MALE TEGMINA OF GRYLLUS

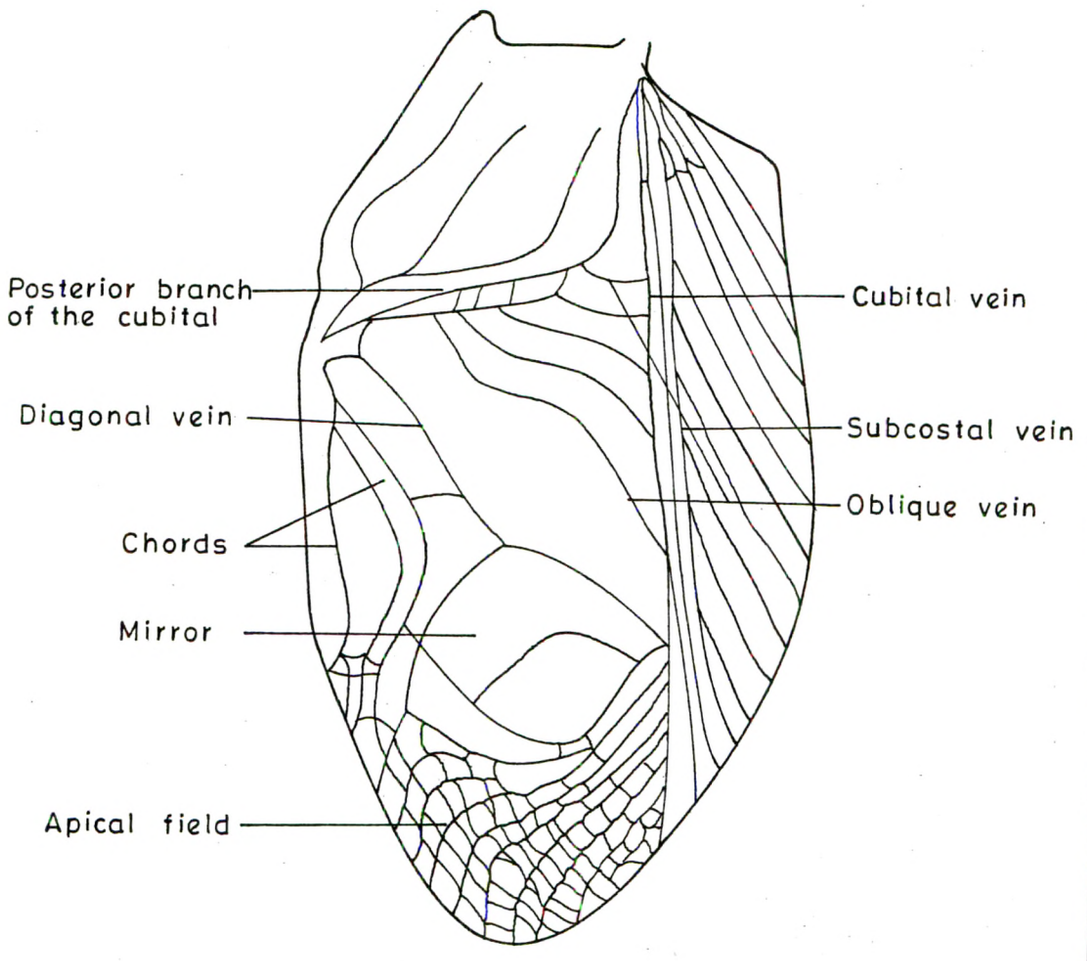

Fig.23 HIND LEG

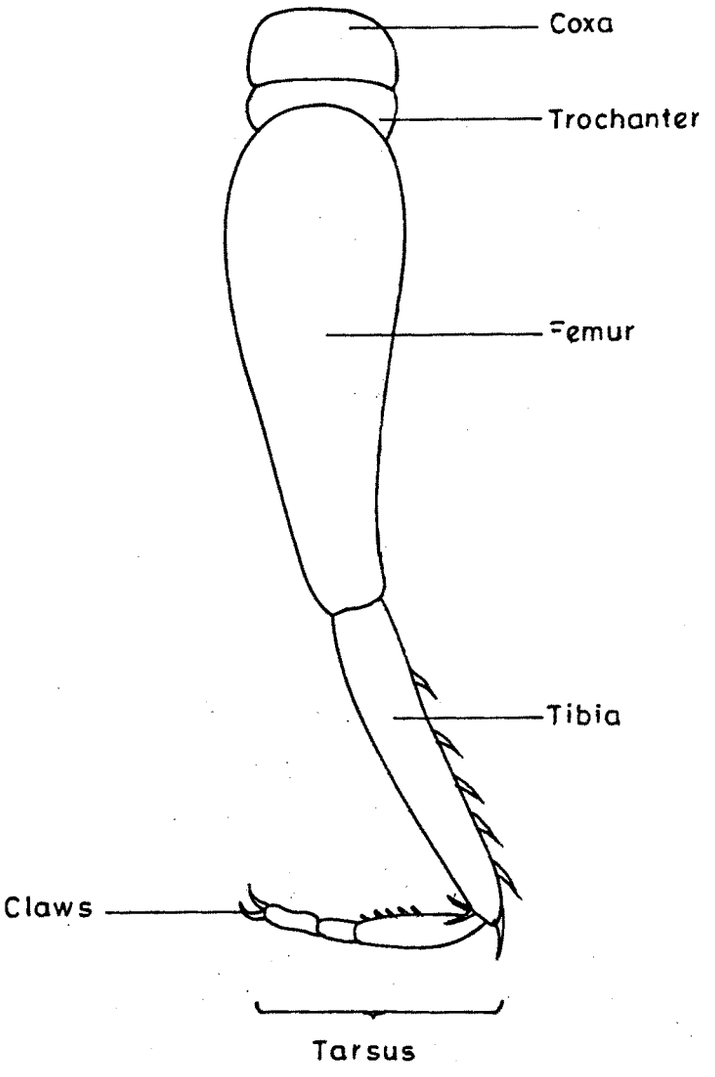

Fig.24 ABDOMEN