ARPPIS

Insect Functional
Morphology
A Laboratory Manual

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Department of Biological Sciences
University of Zambia
Lusaka, Zambia

Preface

When the African Regional Postgraduate Programme in Insect Science (ARPPIS) was established at the International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya in 1983, the main objective was to strengthen the capabilities of African universities in postgraduate training in arthropod science, using technical expertise and the advanced research facilities already existing at the ICIPE. The multidisciplinary setting of ICIPE's research programme enables ARPPIS to admit students from a variety of training backgrounds, not limited to entomology. Thus, the programme also admitted graduates of basic sciences such as mathematics and chemistry, who wished to apply their disciplines to the study of arthropod populations and behaviour.

In order to prepare ARPPIS students for research in arthropod science, it became apparent that the students should first be brought to a reasonable and more uniform level of understanding of basic arthropod science before they embarked on their thesis work. It was, therefore, decided that the ARPPIS course-work should include a mandatory taught semester containing six graduate-level courses, to be taught by some of the best-known authorities in each discipline. The selected six courses were: insect functional morphology; insect physiology and biochemistry; insect taxonomy; insect ecology; biological control of arthropod pests; and biomathematics and experimental design.

Experience from the ARPPIS taught semesters revealed the deficiency of relevant text books and manuals for the intensive teaching programme. Most urgent was the need for a laboratory teaching manual on insect functional morphology, which is the backbone to the understanding of all other aspects of insect science and its application. In the early 1990s, the ARPPIS Academic Board took a decisive stand; that the programme had to produce its own training manuals and reference text books relevant to the special needs of its curriculum. The ICIPE promptly took this challenge and commissioned professional experts in the various disciplines to start work on training materials; and there could be no better choice of experts than those who had hands-on experience with the ARPPIS taught courses.

The author of *Insect Functional Morphology Laboratory Manual*, Dr Keith Japhet Mbata, is head of Biological Sciences Department at the University of Zambia, Lusaka. His association with ARPPIS programme started in 1989 when he took over responsibility for teaching insect functional morphology to ARPPIS Ph.D. students. His predecessor was Professor El Amin El Rayah of the Department of Zoology, Faculty of Science at the University of Khartoum, who coordinated the course from 1985 to 1988. Another lecturer on the course during Dr Mbata's time was Dr Walter Jura, who was a research scientist at ICIPE; he also assisted in reviewing the initial manuscripts of this manual.

Work on the preparation of this manual started in 1991, when Dr Mbata accepted the assignment from Professor Thomas R. Odhiambo, then director of ICIPE. The intention was for Dr Mbata to complement his vast knowledge of the subject with specific experiences on the ARPPIS programme, and to incorporate into the manual suitable instruction materials which had been accumulated by the programme over a long period. This was no mean task, however; for a good quality manual the information had to be thoroughly researched, and fresh laboratory preparations and displays of morphological structures had to be undertaken in order to produce suitable illustrations for the manual. We note with great satisfaction that the work has been expertly executed, and an instruction guide of rare quality has been assembled.

This manual is intended specifically for graduate students of tropical entomology, although the depth and breadth of its coverage also makes it appeal to young scientists from other parts of the world. It is a laboratory manual designed for use in practical instruction, but could also be a useful contribution to a reference collection for researchers and diagnostic laboratories. In the ARPPIS programme, the material covered in the manual forms a mandatory course-work in insect functional morphology, required at the M.Sc. level. The manual will be of particular use in the ARPPIS Sub-Regional M.Sc. Programme which is currently hosted by three Sub-Regional Centres: Biological Sciences Department, University of Zimbabwe, Harare (*M.Sc. in Tropical Entomology - MTE*); Department of Crop Science, University of Ghana, Legon (*M. Phil. Insect Science*); and Department of Biology, Addis Ababa University (*M.Sc. Insect Science*). Other universities with graduate-level course-work in insect science are welcome to use the manual in their programmes.

Financial support for the production of the manual was provided by the Netherlands Government, through their "Direct Support to Training Institutes in Developing Countries (DSO)", as part of a training project grant to ARPPIS through ICIPE. We are grateful to the DSO for their unwavering support to the ARPPIS programme; another manual of this series produced with DSO support is *Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies*, Volumes 1 and 2 (Edited by J.P.R. Ochieng-Odero). Both manuals have been widely circulated to universities in Africa for use in graduate training programmes; inquiries for additional copies may be sent to ICIPE Science Press.

The ARPPIS programme is indebted to all individuals and institutions whose efforts and all forms of contribution made it possible to produce *Insect Functional Morphology Laboratory Manual*. We congratulate Dr Keith Mbata on this excellent achievement.

Vitalis O. Musewe, Ph.D.
Head, Capacity Building Programme
ICIPE

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General Introduction

A. Why Study Insect Functional Morphology?

Entomology is the study of insects. Since time immemorial, man has been interested in the insects around him for several reasons, some of which even conflict with each other. Insects have interested man as a source of food, materials and services, on one hand, and as his "natural enemies", on the other. In several regions of the world today, especially in the third world countries, several species of grasshoppers, crickets, termites, cicadas, caterpillars, wasps and beetles including some aquatic bugs, are highly prized as food. Though usually consumed in small quantities, these delicacies serve as alternative sources of protein, free amino acids and minerals, especially to the poor rural communities. Some insects like the African honeybee, *Apis mellifera adansonii* (L.) or *A. m. capensis* (L.) provide man with honey and wax, while others like the silkworm, *Bombyx mori* (L.) provide him with silk. Other products by insects important to man include certain dyes by some scale insects (order, Homoptera) and although still to be proven here in Africa and elsewhere in the Tropics, some insects are a source of medicines and various charms.

Other benefits man derives from insects which he may or may not be aware of include: the pollination of agricultural crops by bees and wasps (order, Hymenoptera), the two-winged flies (order, Diptera) and butterflies and moths (order, Lepidoptera); the natural regulation of populations of a few insects he considers his own "natural enemies" by some wasps and two-winged flies; the decomposition of organic wastes in the environment by some insect groups such as dung and chorion beetles (order, Coleoptera); and the improvement of soil conditions by termites (order, Isoptera). In addition, at present , man has developed a very lucrative trade in both live and dead insect stocks, for research or simply for their aesthetic value. Dead insect specimens are displayed in museums, offices and homes. Some countries in Africa such as Kenya, for example, are involved in such business. In Kenya, live insect stock trade involving exportation of lepidopterous pupae is linked to the country's strong forest conservation programmes. Local communities participating in the forest conservation programme east of the country, north of Mombasa, benefit from the programme by being permitted to harvest from those forests lepidopterous pupae that they export overseas for cash.

As a natural enemy of man, several insect species are answerable to many ills man faces today. In the field of agriculture, many insect pests do much damage to crops. In fact, crop damage starts from the time the seed is put into the soil and continues into the storage facilities after harvest. Consequently man incurs much pre- and postharvest losses, all to the detriment of his ever increasing population. Notable among crop pests are: locusts such as the desert locust, *Schistocerca gregaria* Forskal; the African migratory locust, *Locusta migratoria migratorioides* (Reiche & Fairm); and the red locust, *Cyrtacanthacris septemfasciata* (Serv.): various leaf, flower, fruit, stalk and root feeders such as; the spotted stalk borer (= sorghum stem-borer), *Chilo partellus* (Swinhoe); the maize stalkborer, *Busseola fusca* (Fuller); the African armyworm, *Spodoptera exempta* Wlk; the banana weevil, *Cosmopolites sordidus* (Germ.); the corn earworm (= cotton bollworm), *Heliothis zea* (Boddie); the bean pod moth (= cowpea pod-borer), *Maruca testulalis* (Geyer); the maize weevil, *Sitophilus zeamays* Motsch; the rice weevil, *S. orizae* L.; and the American bollworm, *Heliothis armigera* (Hb.).

Some insects are pests by virtue of the fact that they transmit to man and his livestock disease-causing agents. Medically, anopheline mosquitoes such as *Anopheles gambiae* (Giles), *A. funestus* (Giles) and *A. arabiensis* (Patton), are important vectors of protozoal parasites that cause malaria in man. Tsetse flies like, *Glossina pallidipes* Austen, *G. morsitans morsitans* Westwood and *G. fuscipes fuscipes* Newstead, are of both medical and veterinary importance. They transmit trypanosome protozoal parasites that cause sleeping sickness in man and an equivalent disease in livestock called nagana.

Other insect pests of man and his livestock directly infest both and either live as external parasites (e.g. lice, fleas and sheep keds) or as internal parasites (e.g. screwworm and botfly larvae causing myiasis). Other insect pests simply cause much discomfort to man and his livestock, when they visit them to steal their blood, a little at a time. Others seek for body secretions around the eyes, ears, anus and genitalia and the mouth.

Due to the above outlined relationship of man to insects, man is left with no choice but to study insects around him. This is either for knowledge's sake, to enable him know his "six-legged neighbours" better or in his continuing quest to find ways and means of controlling insect pests. For beneficial insects, man may study them in order to develop ways of conserving this important insect natural resource and find ways of maximizing its sustainable use. Insect Functional Morphology is a subject of study that is basic to all other studies of insects. Before anything else can be fruitfully done about a given insect species by man, pest or not, he needs to identify the insect in question and know what makes it tick. The study of Insect Functional Morphology aims at elucidating the structure of the insect body, how it works and at creating an appreciation of the adaptive radiation that has occurred to it during the evolution of the class Insecta, to which all insects belong. What then is an insect? What is its relationship to other animals in existence today?

B.   Insects and Their Close Relatives

Insects belong to an invertebrate animal phylum called Arthropoda. All animals belonging to this phylum are bilaterally symmetrical and possess the following distinguishing features:

1.   a body that is made up of ring-like segments,
2.   jointed appendages,
3.   an exoskeleton,
4.   a ventral nerve cord, and
5.   a dorsal blood vessel.

Arthropods have maintained a segmented body-type of their millipede-like or annelid-like ancestor. During the evolution of the group, these segments have differentially combined with each other to give the different body types characteristic of the extant representative groups. The arthropod skeleton is external. It is made up of a combination of the polysaccharide chitin and several types of proteins, lipids, pigments and other materials to form a cuticle. The latter is usually hardened in its outer portions to form plates or sclerites.

The segmented nature of the appendages on the segmented insect body has with passage of time allowed the development of the many forms of appendages that we see today in different arthropod groups. This has partly enabled them, especially the insects, to occupy thousands of niches in the biosphere. In fact, the phylum's name derives from two Greek words meaning segmented feet (*Athros* = segmented, *podos* = foot:Gk). The blood vessel comprising of a segmented heart and an aorta is located in the dorsal aspects of the body in arthropods, while the ventral nerve cord is ventral.

Five classes are recognized in the phylum Arthropoda. Of the five and due to the fact that its members are the most successful in terms of numbers of species and individuals and due to their present occupation of a diversity of niches, the class Insecta, is the most commonly known. Close relatives of insects belong to; class Arachnida (e.g. scorpions, spiders, ticks and mites), class Crustacea (e.g. shrimps, cray fish, crabs and lobsters), class Diplopoda (millipedes) and class Chilopoda (centipedes).

Several features are unique to members of the class Insecta and these set them apart from the rest of the arthropods. These features are the possession of;

1.   a body that is divided into three regions namely; head, thorax and abdomen.
2.   three pairs of legs.
3.   one or two pairs of wings, when winged.
4.   compound and/or simple eyes, and
5.   a system of internal tubules called tracheae, used for respiration.

Unlike insects, the Crustacea have a body with an ill-defined head bearing two pairs of antennae, have four pairs of legs, are wingless and gaseous exchange in their bodies is through gills that arise as outgrowths of the legs or the body wall. The arachnids have a body divided into two regions, the prosoma and opisthosoma. They bear four pairs of legs, are wingless and gaseous exchange is effected through internal air spaces called "lungs" or "gills" or "book lungs". Although the millipedes (class Diplopoda) and the centipedes (class Chilopoda) have heads that closely resemble those of insects in structure, they lack antennae and their bodies are divided into only two regions, a head and the trunk. The trunk of millipedes bears two pairs of legs on each segment, while that of centipedes has a pair of legs to a segment.

C.   The Class Insecta

Members of the class Insecta are divided into two subclasses on the basis of the nature of the thorax, wing development and type of metamorphosis (i.e. changes in physical structure during postembryonic development). These subclasses are Apterygota and Pterygota.

Subclass Apterygota comprises the most primitive insects that lack wings and do not undergo metamorphosis. Due to the latter, except for size, both young and adults of these insects resemble each other. Presently, four orders of insects are recognized in the subclass Apterygota namely;

Order Protura (the proturans)
Order Diplura (the diplurans)
Order Thysanura (bristletails e.g. firebrat and silverfish)
Order Collembolla (springtails)

The subclass Pterygota, on the other hand, comprises insects that are winged or if wingless, they are secondarily so. All members of the subclass undergo some kind of metamorphosis during their postembryonic development. Among the Pterygote insect orders in existence today, two belong to a primitive category called the Paleopteroid orders. The two orders are Odonata and Ephemeroptera. Dragonflies and damselflies belong to the order Odonata, while the mayflies belong to the order Ephemeroptera. Members of these two orders lack wing-flexing mechanisms in their thorax. Consequently, they are unable to fold their wings over their bodies when at rest. Generally these insects either hold their wings outstretched horizontally (dragonflies and damselflies) or vertically (mayflies) over their abdomens when at rest. All Paleopteroid insects undergo simple metamorphosis.

The rest of the Pterygote orders have members with wing-flexing mechanisms at the bases of their wings in the thorax that enable them to fold the wings over their abdomens when at rest. They form the insect category termed Neopteroid orders.

The Neopteroid insect orders are further divisible into two categories on the basis of the type of metamorphosis they undergo and the mode of development of wings in their immatures. The category called Exopterygota comprise insect orders whose members undergo simple metamorphosis (i.e. development is from egg to nymph and then to adult or Egg ---> Nymph ---> Adult) and wing development in the immature insect is external. The immature exopterygotes called nymphs, resemble the adults except for size and the wings that appear as buds on the body. The second category called Endopterygota includes insect orders whose members undergo complete metamorphosis (i.e. Egg ---> Larva ---> Pupa ---> Adult) and wing development in the immature insect's body is internal. The immature endopterygote insects do not resemble the adults and may even occupy different niches from those of adults. The last immature stage called pupa is a quiescent stage in which the transition from larva to adult occurs.

The Exopterygota include those insect orders whose members possess biting and chewing mouthparts, the Orthopteroid orders (e.g. Orthoptera and Isoptera), and those whose members have haustellate (sucking) mouthparts, the Hemipteroid orders (e.g. Hemiptera and Anoplura).

The Endopterygota comprise the most advanced pterygote insects. They include the orders; Coleoptera, Stepsiptera, Diptera, Mecoptera, Siphonaptera, Lepidoptera, Trichoptera and Hymenoptera. Thus the overall classification of the class Insecta is as follows:

Class Insecta

    Subclass Apterygota (Primitive, wingless insects
                         without metamorphosis)
      Order Protura (Proturans)
      Order Diplura (Diplurans)
      Order Thysanura (Bristletails)
      Order Collembolla (Springtails).

    Subclass Pterygota (Winged insects with simple or
                        complete metamorphosis)

      Paleopteroid orders (Members without a
                        wing-flexing mechanism in the
                        thorax. With simple metamorphosis)
         Order Odonata (Dragonflies and damselflies)
         Order Ephemeroptera (Mayflies)
      Neopteroid orders (Members with a wing-flexing
                        mechanism in the thorax.
                        Metamorphosis simple or complete)
        Exopterygota (Wing development external.
                        Metamorphosis simple)

Orthopteroid orders (With biting and chewing
mouthparts)
Order Orthoptera (Locusts, grasshoppers, stick insects,
mantids, crickets and cockroaches)
Order Isoptera (Termites)
Order Dermaptera (Earwigs)
Order Plecoptera (Stoneflies)
Order Embioptera (Webspinners)

Hemipteroid orders (With haustellate mouthparts)
Order Hemiptera (Bugs)
Order Homoptera (Cicadas, aphids, hoppers, scale
insects, whiteflies and psyllids)
Order Mallophaga (Chewing lice)
Order Anoplura (Sucking lice)
Order Thysanoptera (Thrips)
Order Psocoptera (Psocids)
Order Zoraptera (Zorapterans)

Endopterygota (Wing development internal.
Metamorphosis complete)
Order Coleoptera (Beetles)
Order Strepsiptera (Twisted-winged parasites)
Order Neuroptera (Lacewings, antlions,
snakeflies etc.)
Order Diptera (Flies)
Order Siphonaptera (Fleas)
Order Trichoptera (Caddisflies)
Order Mecoptera (Scorpionflies)
Order Lepidoptera (Butterflies and moths)
Order Hymenoptera (Bees, wasps, ants, sawflies,
ichneumonids and chalcids)

D.   About This Laboratory Manual

The major objectives of the exercises in this laboratory manual are to, (1) get
you acquainted with the functional morphology of the marvellous insect body
and, (2) to familiarize you with and make you appreciate the adaptive radiation
that has occurred in structure and function, of the insect body in selected
examples from the generalized (primitive) condition, during the evolution of
the class Insecta, which has led to insects becoming the most successful
terrestrial animal group in the biosphere today. The grasshoppers (order,
Orthoptera) and several of their close relatives such as termites (order, Isoptera)
and earwigs (order, Dermaptera), bear generalized types of morphology and
anatomy and will be used throughout the exercises as a base on which

advanced structures exhibited by other insects, will be seen to have evolved. However, for the sake of comparison of variation in structure, even among insects considered primitive, the longhorned grasshoppers, *Enyaliopsis matebelensis* Peringuey, and *Acanthoplus speiseri* Brancsik (Orthoptera:Tettigoniidae), have been selected to serve as examples in the introductions to the various sections. The introductions to external morphology/endoskeletal processes and the insect organ systems, will draw examples from *E. matebelensis* and *A. speiseri* anatomy, respectively.

Enyaliopsis matebelensis and *A. speiseri* belong to the primitive tettigoniid subfamily called Hetrodinae, whose members are restricted in their geographical distribution to Africa, south of the Sahara. Members of the Hetrodinae are commonly called armoured ground crickets, Koringkrieks or dikpens (Skaife, 1979; Scholtz and Holm, 1989). Both sexes of the two species are heavy-bodied and sluggish, ground-dwelling insects. The term, "armoured" in the first common name refers to the presence of heavy spines on the pronota and legs of both sexes, while the term, "cricket" in the common name is a misnomer to the group. The latter term is said to have been erroneously coined to the group, due to their general resemblance to the true crickets of the orthopteran family Gryllidae. The males in both species are brachypterous and stridulate, using mesothoracic wings (tegmina) that have been reduced to the level of the stridulating organs (Figs 30, 31, 34, 40, 79, 82). The structures are held covered by the expanded pronotum when at rest (Figs 50, 65, 77). The females are apterous and therefore do not stridulate. Their tegmina are very reduced and scale-like (Figs 33, 35).

The laboratory exercises in this manual are divided into five sections, each of which is preceded by an introduction. The specimens and chemicals needed for the exercises in each section are detailed at the beginning of the sectional exercises. For all exercises however, you will need a good dissecting set, containing at least; a pair of fine scissors, a dissecting needle and a pair of fine forceps. You will also need to be provided with a dissecting dish or tray with some beeswax at the bottom, a binocular and compound microscopes.

At the end of each section are listed key references dealing with the material covered in the section, while at the end of the laboratory manual are given, supplementary reading materials that you are encouraged to consult for in-depth details of specific subjects covered in the laboratory manual.

Following each laboratory session, you will be expected to write a comprehensive report on the exercises you have conducted and hand it in the next time you go for practicals. The report is expected to show evidence of your having read on the subject(s) you studied in the previous practical on which you have reported, in the introduction, discussion and the reference sections. The laboratory report write-up should have the following main headings:

Introduction

Under this heading in your report, introduce the subject matter of the laboratory session that you are reporting on. With reference to the literature (journals, books and even lecture notes) explain what is known about the subject. Give any critical information from the literature that will make it clear to whoever reads your report, about the relevance of the laboratory exercises that you performed. Finally, also in the introduction, state the objectives of the laboratory exercises conducted.

Materials and Methods

All materials used in performing the laboratory exercises should be detailed here unless you are instructed not to do so by the supervisor. The methodologies followed in carrying out the exercises need also to be described here and any unique techniques employed highlighted.

Results

Here you append the drawings that you have made in the laboratory session you are reporting on. Remember that while you are conducting these lab exercises, you are at the same time being trained to be very observant as a biologist in general and as an insect scientist in particular, in preparation for your own future postgraduate research. In the lab, draw only what you see on your specimens and this is what is to be presented in your laboratory report under this heading. NOTE; illustrations found in textbooks and published articles are often summary diagrams that may include structural details only seen when using sophisticated study techniques and equipment. With the type of equipment available to you in these laboratory sessions, it may not be possible for you to see all the expected detail of structure. The rule of thumb, as regards lab drawings, is to draw only what you see under the microscope or otherwise and not what you imagine should be on your specimen from your knowledge acquired either from lectures or books. **"DOCTORED" drawings with imaginary structures of detail not seen under the type of microscope you are using will not be tolerated.**

Discussion

Under this heading, relate your results to the objectives set for the laboratory exercises conducted. Have your objectives been fulfilled and if not, why? Also relate the results to what is known in the literature that you have reviewed in the introduction to the report. If there are any deviations from what is known, attempt at explaining those but again using the literature as the basis for your arguments. Indicate whether the methods used were adequate to give you the expected results that

meet the set objectives. If not, state how they could be improved upon to give better results.

References

All references quoted from periodicals (e.g. journals, bulletins, newsletters etc.) and books appearing in your report in the introduction and elsewhere, should be listed in full under this heading, using the standard format recommended for entomology. The references must be listed alphabetically by authors names. In case any single author is quoted more than once in your report, overall the references should be arranged alphabetically as stated above but in addition articles by a given author quoted, must be arranged chronologically beginning with the oldest first.

In the laboratory exercises designed for each section that follows in this laboratory manual, selected insect species are suggested for use. Where the suggested specimens are not available, it is recommended that the instructor for the Insect Functional Morphology practicals, choose specimens from other species that are closely related to those suggested. The specimens suggested for the exercises in this laboratory manual are from 9 insect orders. These are, Odonata, Orthoptera, Isoptera, Dermaptera, Hemiptera, Coleoptera, Diptera, Lepidoptera and Hymenoptera.

E.   Features of the Insect Orders from which Specimens Used or Suggested for Use in the Laboratory Manual were Drawn

Paleopteroid Orders

These comprise primitive pterygote insects that lack a wing-flexing mechanism in their thorax and hence when not flying, the wings are either held vertically or horizontally over the body. These insects have two pairs of membranous wings with many veins. Wing development in the immatures is external. They undergo simple metamorphosis and the immatures are aquatic. Both nymphs and adults are predaceous.

Order Odonata

To this order belong the dragonflies and damselflies. These are medium to large insects that spend most of their time as adults on the wing, near water bodies. They bear large compound eyes that cover most of the head and in both nymphs and adults, the mouthparts are the biting and chewing type. There are four membranous wings each with many veins. The immatures are aquatic and spend 2–3 years in water before emerging as adults. The adults, on the other hand, spend only about one to eight weeks

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Fig. 1. Order Odonata
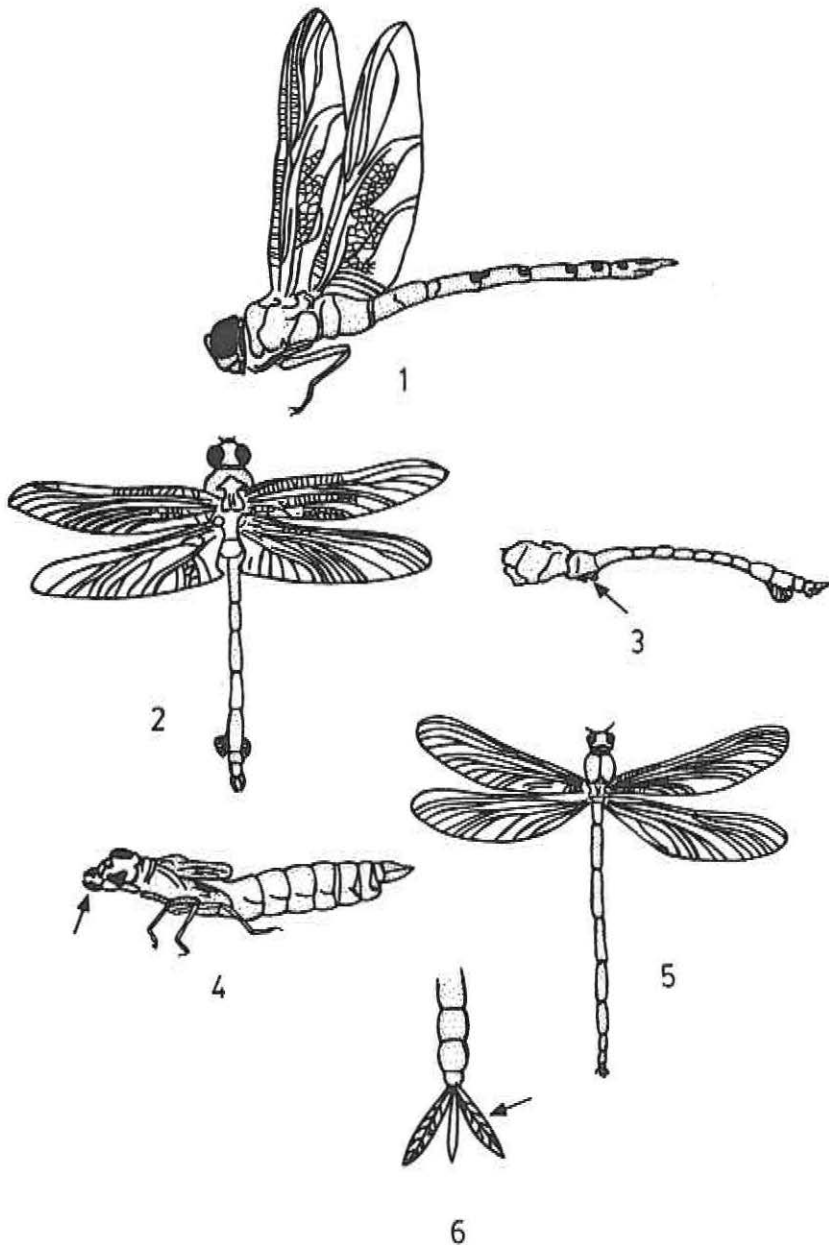
1. A female darner (Dragonfly).
2. A male darner (Dragonfly).
3. Abdomen of a male darner showing position of the accessory sex organs on the sternum of the second abdominal segment (see arrow).
4. Dragonfly nymph (family, Aeshinidae), the arrow points to a mask, the grasping organ.
5. A damselfly.
6. Abdominal tip of a damselfly nymph showing the terminal tracheal gills (see arrow).

before dying. During this period, they mate and deposit eggs in water. Nymphs like the adults are predaceous. They have a specially adapted mouthpart below the mandibles called a mask for grasping prey. Nymphal dragonflies (suborder, Anisoptera) utilize rectal respiration, while nymphal damselflies (suborder, Zygoptera) respire through three leaf-like tracheal gills located at the tip of the abdomen (Fig. 1).

Unique in the class Insecta, is the presence of accessory reproductive structures on the sternum of the second abdominal segment in male Odonata. The genital pore in these insects, as is the case with males of all other insects, opens on the sternum of the 9th abdominal segment. However, for male Odonata, no external reproductive organs associate with this aperture. Instead, the accessory external reproductive structures occur on the sternum of the second abdominal segment (Fig. 1, 3). In order to inseminate females, the males transfer sperm from their genital pores on the 9th abdominal segments to the accessory reproductive structures. Copulation occurs in flight. The male holds the female by the neck using cerci at its abdominal tip, which are modified as claspers. Held in that position, the female bends her abdomen downwards and forward to attach her subgenital chamber to the male's accessory sex organs to receive sperm. Many male Odonata can be seen holding females in tandem while in flight. They tend to spend most of their time this way and even assist the females when ovipositing their eggs in water or on aquatic vegetation.

Neopteroid Orders

The rest of the pterygote insects belong to these orders. Every member of the neopteroid pterygote insect orders bears a wing-flexing mechanism in its thorax. Consequently, neopteroid pterygote insects are able to flex and fold their wings over their abdomens when not in use. The type, structure and form of wings are varied in these groups but wing development in the immatures is either external or internal. Metamorphosis is simple or complete and the immatures occupy various habitats, some of which may be totally different from those occupied by the adults of the same species.

Exopterygota

This includes neopteroid pterygote insect orders in which wing development in the immatures is external. All have biting and chewing mouthparts or the mouthparts may be sucking (haustellate) type. Metamorphosis is simple. There are few to many malpighian tubules on the alimentary canal.

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Orthopteroid Orders

In these orders, members bear biting and chewing (mandibulate) mouthparts. Their alimentary canals have many malpighian tubules.

Order Orthoptera

The order includes locusts and grasshoppers, crickets, stick insects, praying mantids and cockroaches. All have well-developed mandibles. They may be winged, brachypterous or wingless. When winged, two pairs of wings are present. The front wing pair is modified into leathery protective sheaths for the hind wings called tegmina (singular, tegmen). The hindwings are the proper flight wings. They are membranous and bear many veins and cross-veins.

Many Orthoptera are phytophagus, some are predaceous, while others are scavengers or omnivorous. Among the phytophagus ones, some are important pests of crops. These include locusts and grasshoppers (family, Acrididae) (Figs 2, 3).All orthopteran nymphs usually occupy the same habitat as adults and except for size and developing wings, which appear as pads on the thorax, resemble adults.

Many Orthoptera produce sound by rubbing one modified body part against another, a process called stridulation. Songs and/ or sounds may be produced by rubbing a hardened vein on one tegmen against a file of cuticular teeth underneath the other tegmen (Gryllidae and Tettigoniidae)(Fig. 2, 3) or by rubbing a hardened vein at the edge of one tegmen against a row of cuticular pegs on the hind femur (some Acrididae). In a few other acridids, sounds are produced by snapping the tegmina together while in flight. Songs and sounds produced by the Orthoptera are significant in pair formation during mating (calling songs), announcing to others of disturbances in the population (alarm sounds or songs) and in warding off intraspecific competitors for territory and/or mates (aggressive songs).

Order Isoptera

This order is composed of insects called white ants or termites. These are small to medium sized, soft-bodied and light-coloured insects that live in colonies in well-organized social groupings. In each of their colonies is a well-organized caste system comprising a queen, reproductives, workers and soldiers (Fig. 4). The worker and soldier castes involve both sexes. The workers include nymphs and sterile adult males and females. Adult workers lack wings,
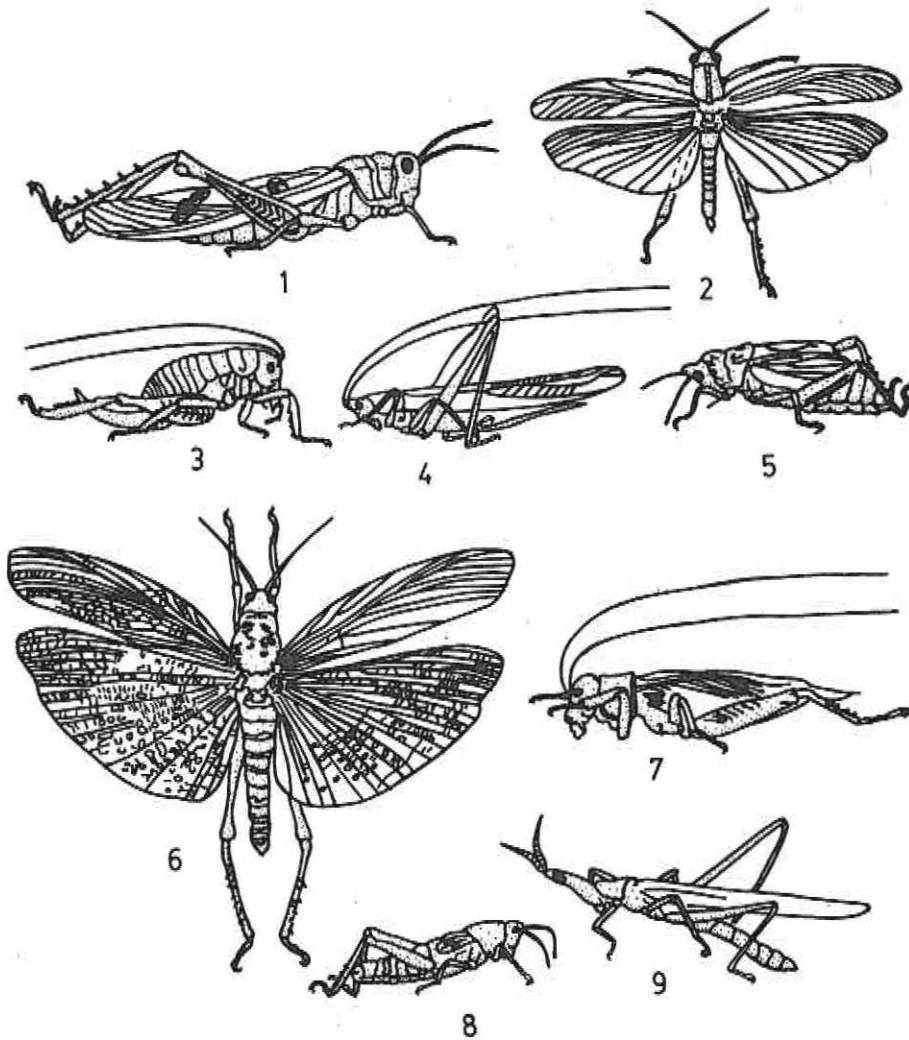
Fig. 2. Order Orthoptera 1

1. Lateral view of the common garden locust, *Acanthacris ruficornis* Fabricius (Acrididae).
2. Dorsal view of the common garden locust, *Acanthacris ruficornis* Fabricius with wings spread.
3. Ground cricket (Stenopelmatidae).
4. Edible grasshopper, *Homorocoryphus nitidulus vicinus* Walker (Tettigoniidae).
5. *Dictyophorus griseus* (Rieche & Fairmaire) (Pyrgomorphidae).
6. *Phymateus viridipes* male (Pyrgomorphidae).
7. Tobacco cricket, *Brachytrypes membranaceous* (Drury)(Gryllidae).
8. Elegant grasshopper nymph, *Zonocerus elegans* (Pyrgomorphidae).
9. Remarkable grasshopper, *Acrida sulphuripennis* (Acrididae).
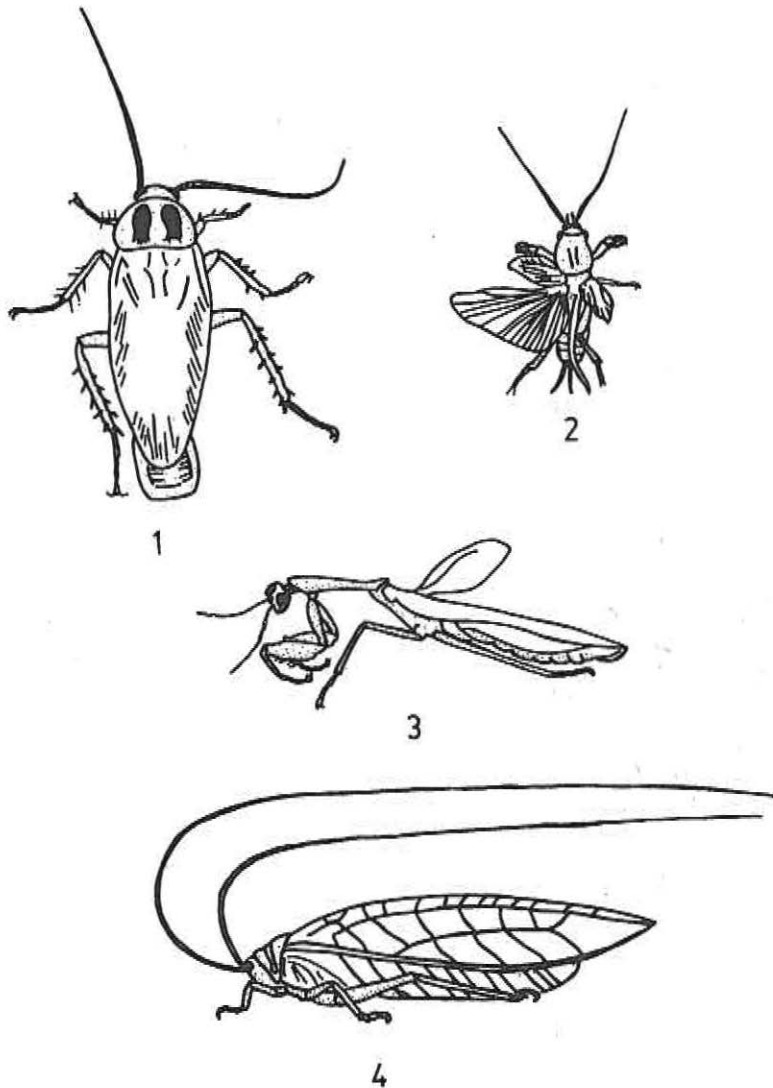
Fig. 3. Order Orthoptera 2

1. German cockroach, *Blattella germanica* (L.) (Blattellidae).
2. African mole cricket, *Gryllotalpa africana* (Beauv.) (Gryllidae).
3. Praying mantis (Mantidae).
4. *Zabalius aridus* (Walk.) (Tettigoniidae).

compound eyes and have reduced mandibles. Soldiers are sterile adult males and females with large heads and mandibles. They also lack compound eyes.

The reproductives (future kings and queens) are winged. They possess two pairs of same-sized wings with reduced venation

Fig. 4. Order Isoptera
(Adapted from, Scholtz and Holms 1989. *Insects of Southern Africa*. Butterworth Co. Durban, South Africa).

1. Winged adult harvester termite, *Hodotermes mossambicus* Hagen (Hodotermidae).
2. Soldier harvester termite, *H. mossambicus*.
3. Worker harvester termite, *H. mossambicus*.
4. Primary reproductive of the harvester termite, *H. mossambicus*.

each. Once a year, usually at the beginning of the rainy season here in the tropics, the reproductives leave the parent colony, fly out of the ground in swarms, pair up, mate and then each pair or

so digs into the soil to establish new colonies. The reproductives shed off their wings prior to or soon after mating and before digging into the soil to establish new colonies.

Termites have chewing mouthparts. Many are phytophagus, some of which are pests of crops, timber and timber products.

Order Dermaptera

Members of this order are commonly called earwigs. They are elongated, slender and flattened, beetle-like nocturnal insects bearing unique forceps-like cerci at their abdominal tips in both sexes (Fig. 5). They have chewing mouthparts and may be winged or wingless. When winged, the front wing pair called tegmina or elytra (like in beetles), is short, leathery and veinless. The hind wings are membranous, rounded and with radiating veins.
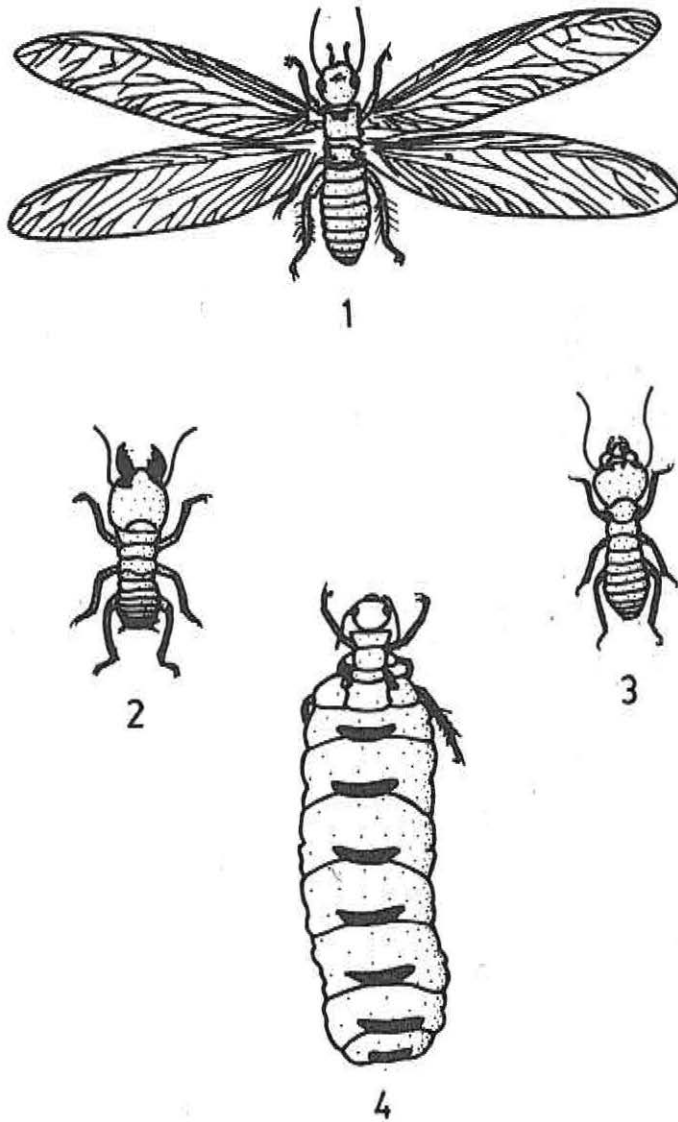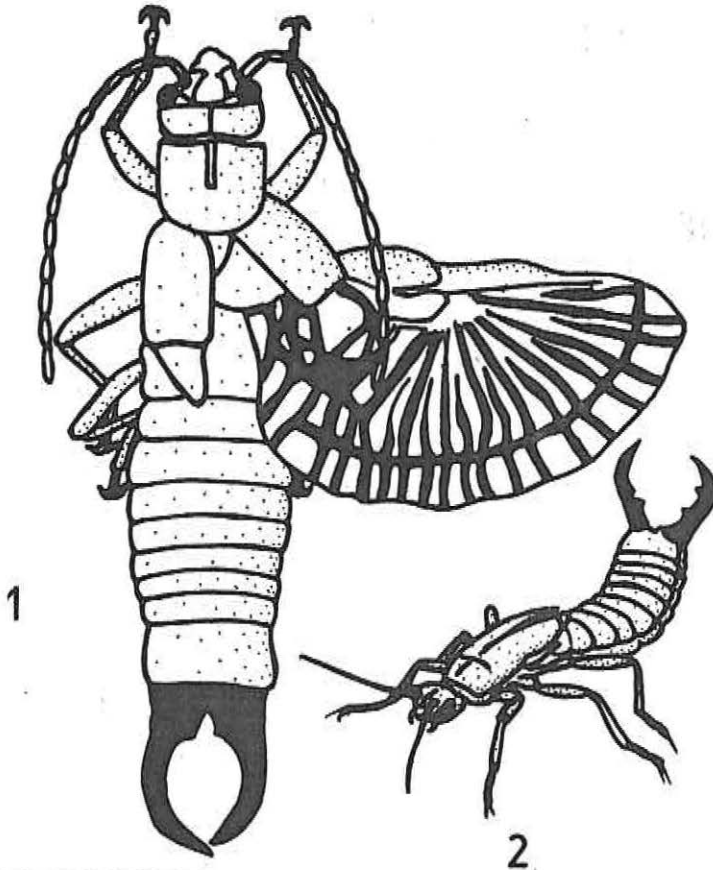

Fig. 5. Order Dermaptera
(Adapted from, Scholtz and Holms 1989. *Insects of Southern Africa*. Butterworth Co. Durban, South Africa).

1. Adult earwig (Forficulidae)
2. Threatening posture of a male with cerci raised.

Earwigs feed on dead or decaying plant matter though a few feed on live plant parts. Some are carnivorous. Female earwigs practice parental care of the eggs from the time they are deposited to the time they hatch.

Hemipteroid Orders

The mouthparts of the members of these orders are the haustellate or sucking type. Their alimentary canals have few malpighian tubules on them.

Order Hemiptera

All true bugs belong to this order. The majority are small to medium-sized insects, although a few, especially the aquatic forms like the giant waterbug (family, Belostomatidae) and the water scorpion (family, Nepidae) may get very large (Fig. 6). All possess piercing and sucking mouthparts that are in the form of a segmented beak. The beak is usually held downwards and backwards between the legs, when not in use.

Hemiptera may be winged, brachypterous or wingless. This order of insects derives its name from the nature of the forewings in winged forms. The forewings serve as protective sheaths for the hindwings. They are leathery at their bases and membranous at their tips. Such wings are called hemielytra (singular, hemielytron). The membranous tip of a hemielytron has very few veins in it. The hindwings are shorter than the forewings and are completely membranous with few veins in them. Hemiptera are both aquatic and terrestrial. Many feed on plant juices, an act that makes a few, important pests of crops. Others are haematophagus, while others are predaceous. Some haematophagus species of Hemiptera transmit disease to man.

Endopterygota

These are pterygote insect orders whose members have their wings developing internally in the immatures. They may have chewing or sucking mouthparts and all undergo complete metamorphosis. There are few malpighian tubules on their guts.

Order Coleoptera

Members of this order are called beetles. They are the most abundant insects in the biosphere in terms of number of species and individuals. Forty per cent of all known insect species today
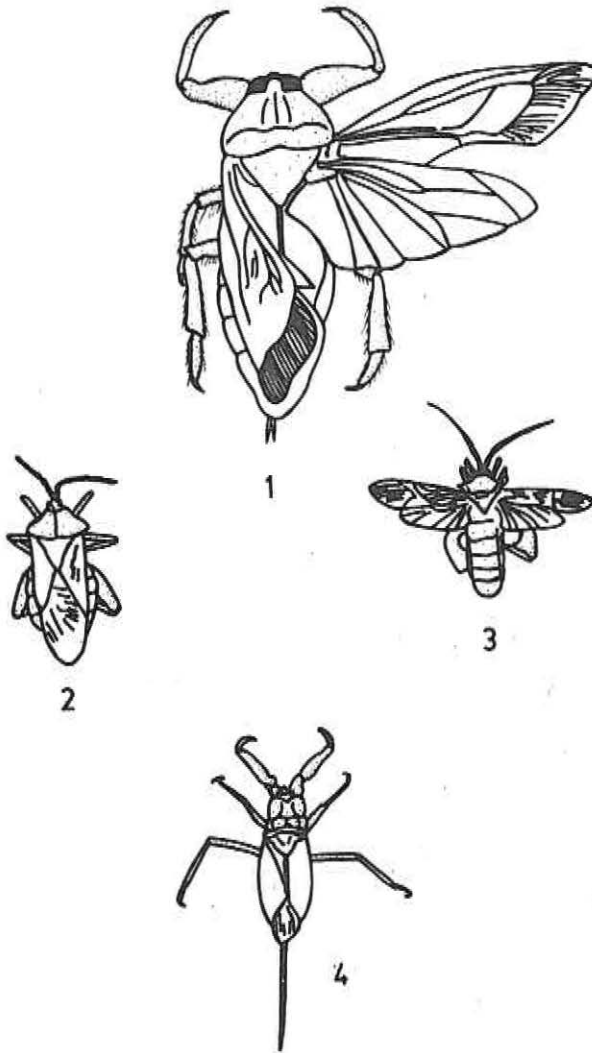
Fig. 6. Order Hemiptera

1. Giant water bug, *Lethocerus* sp. (Belostomatidae).
2. Coreid plant bug.
3. Twig wilter male, *Anoplocnemis curvipes* (Coreidae).
4. Water scorpion (Nepidae).

(or over 250,000 species) are beetles. They occupy every known habitat where insects are to be found (i.e. in the soil, water and semiaquatic environments). They are tiny to very large insects (Fig. 7). They may be winged, brachypterous or wingless. For winged forms, two pairs of wings are present. The forewings are called elytra (s i n g u l a r, elytron).These are not used in flight and are hard and brittle structures that serve as protective sheaths for the hind-wings. The latter are the proper flight wings. They

are membranous and possessing few veins. Beetles bear chewing type mouthparts with well-developed mandibles. Many beetles are phytophagus. Some of these being important pests of crops. Other beetles are predaceous, while others are scavengers or feed on mould and fungi. A few beetles are important as pests of stored agricultural products.
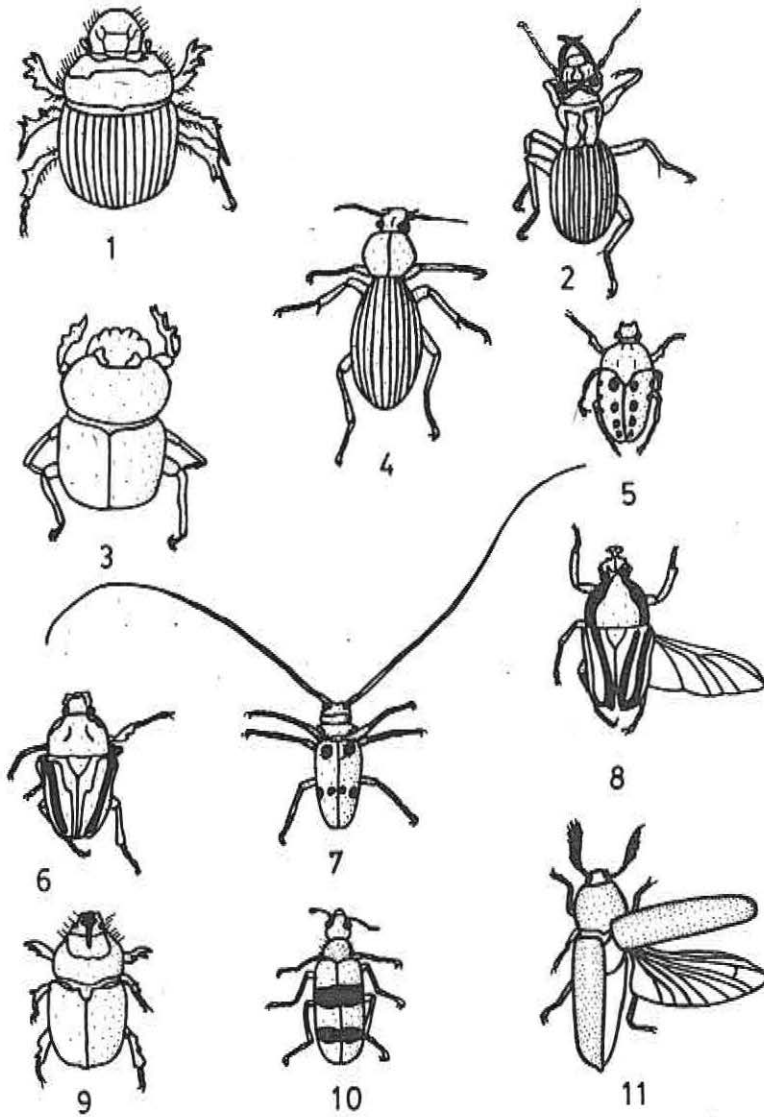
Fig. 7. Order Coleoptera

1. Dung beetle, *Heliocopris japetus* Klug (Scarabaeidae).
2. Ground beetle, *Anthia* sp. (Carabidae).
3. Dung beetle (Scarabaeidae).
4. Ground beetle, *Tefflus violaceus* (Carabidae).
5. Plant chaffer, *Amaurodes passerini* (Scarabaeidae, Cetoniinae).
6. Plant chaffer, *Dicronorhina derbyana* female (Scarabaeidae).
7. Longhorned beetle, *Batocera* sp. (Cerambycidae).
8. Plant chaffer, *Dicranorhina derbyana* (Scarabaeidae, Cetoniinae).
9. Dung beetle (Scarabaeidae).
10. Blister beetle, *Mylabris* sp.
11. Click beetle, *Tetralobus rotundifrons* Guer (Elateridae).

Order Diptera

Members of this order are the true flies. They are also sometimes referred to as the two-winged flies. They are usually small, soft-bodied insects. They include important haematophagus insects like mosquitoes, black flies, tsetse flies, sandflies, horse flies and deer flies. Many of these transmit disease agents to man and livestock such as malaria, river blindness, sleeping sickness/nagana, sandfly fever, yellow fever, filariasis, etc. Other Diptera such as houseflies are scavengers. They are important for the transmission of diseases like typhoid fever, dysentery and diarrhoea.

Fig. 8. Order Diptera
(Re-drawn from 1992 poster entitled, "Insects of Public Health", by Sumitomo Chemical Co., Ltd., Pesticides Division, Osaka, Japan).

1. House fly, *Musca domestica* L.
2. Stable fly, *Stomoxys* sp.
3. Common mosquito, *Culex* sp.

The name of the order derives from the fact that members of the group bear a single pair of wings, the mesothoracic pair. These are the flight wings. The metathoracic wings are modified into knob-like structures called halteres important in balancing flight (Fig. 8).

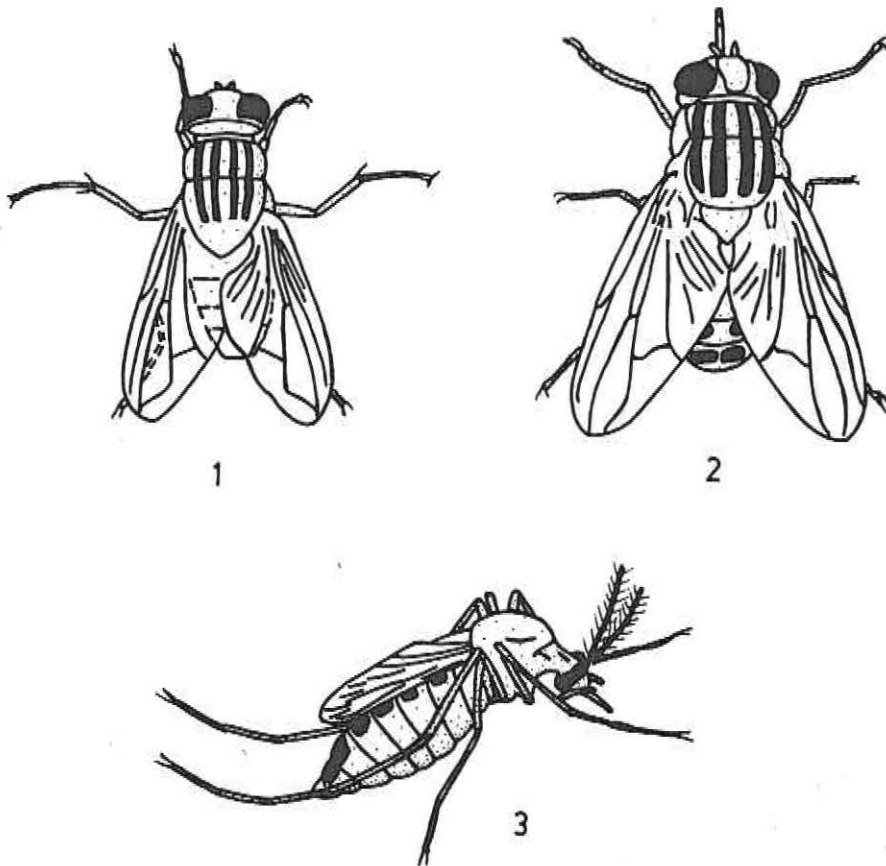Dipterous larvae are called maggots. They are legless and wormlike in structure and are to be found in water, soil, litter and in vegetation.

Some Diptera are important crop pests, while others are parasites and predators of insect pests and enemies of some weeds. Many Diptera, however, are important pollinators of plants.

Order Lepidoptera

Belonging to this order are butterflies and moths. In terms of colour and shape, some of the most beautiful insects known today belong to this order. All members bear scales on their wings and have large compound eyes.

The mouthparts are the sucking type made up of two prolonged and closely appressed galea of the maxillae forming a proboscis (Fig. 9). Wings are large, scaled and with few veins. Adults feed on various plant juices. Larvae are called caterpillars. They possess chewing mouthparts and many are phytophagus. A few are pests of crops, while others of stored food products.

Order Hymenoptera

This order comprises an assemblage of a wide array of insects. It includes bees, wasps, ichneumons, ants, sawflies and chalcids (Fig. 10). All possess mandibulate mouthparts except in bees which in addition to the mandibles, the labium is modified into a tongue-like structure for sucking nectar.

Hymenopterans may be winged, brachypterous or wingless. When winged, there are two pairs of membranous wings with few veins in them. The hindwings are smaller than the forewings and are provided at their leading edges with tiny hooks called hamuli, used in coupling them to the forewings. Coupled fore- and hindwings of each side of the body operate as a single unit during flight. The tiny hymenopterans, the chalcids, have very tiny veinless wings.

Larvae of Hymenoptera are grub-like, maggot-like or caterpillar-like. Many are parasites of insect pests, thus serve as important biological control agents. Some adult
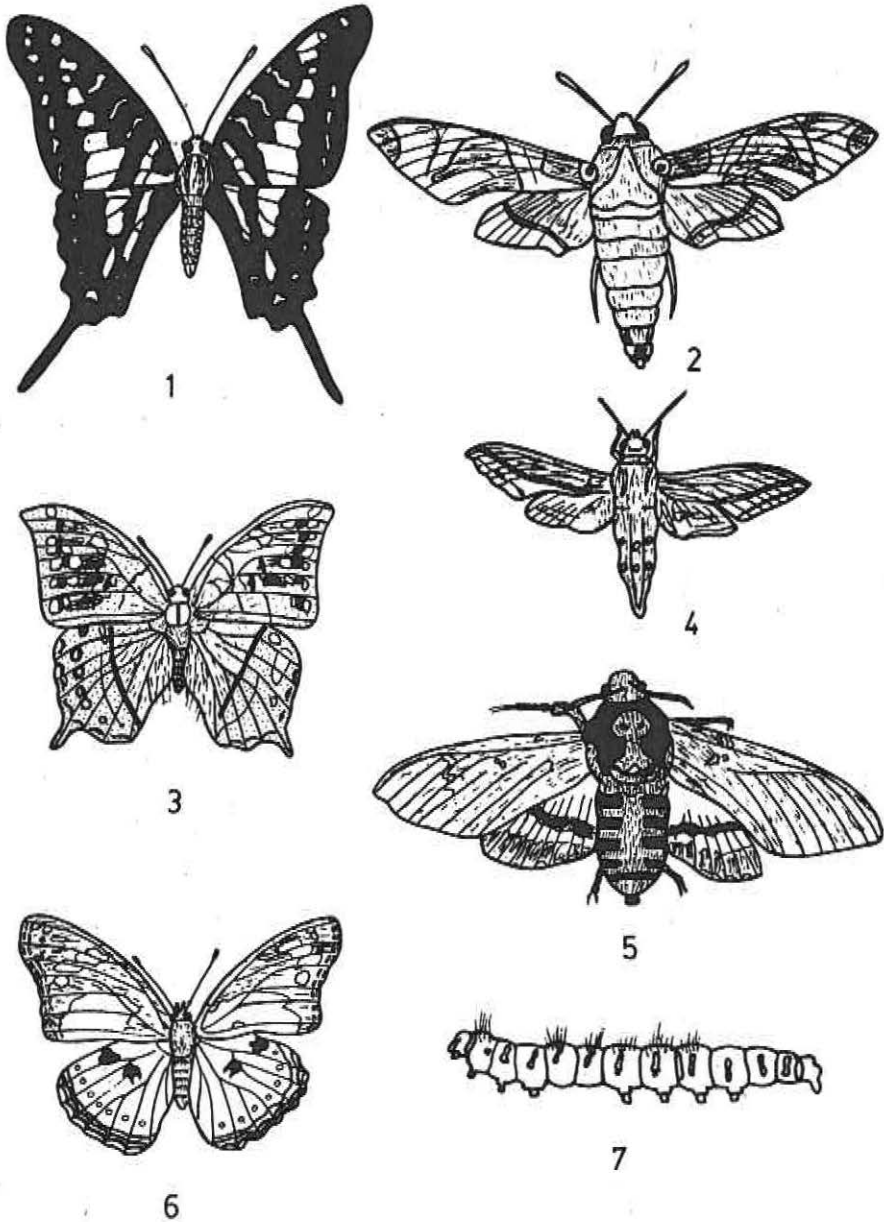
Fig. 9. Order Lepidoptera

1. Large striped swordtail male, *Graphium antheus* Cramer (Papilionidae).
2. Oleander hawk moth, *Deilephila nerii* (Linn.)(Sphingidae).
3. Pearl charaxes, *Charaxes varanes* Cramer (Charaxidae).
4. Silver striped hawk moth, *Hippotion celerio* (Linn.)(Sphingidae).
5. Death's head hawk moth, *Acherontia atropos* (Linn.).
6. Nymph, *Hypolimnas misippus* female (Nymphalidae).
7. Caterpillar.
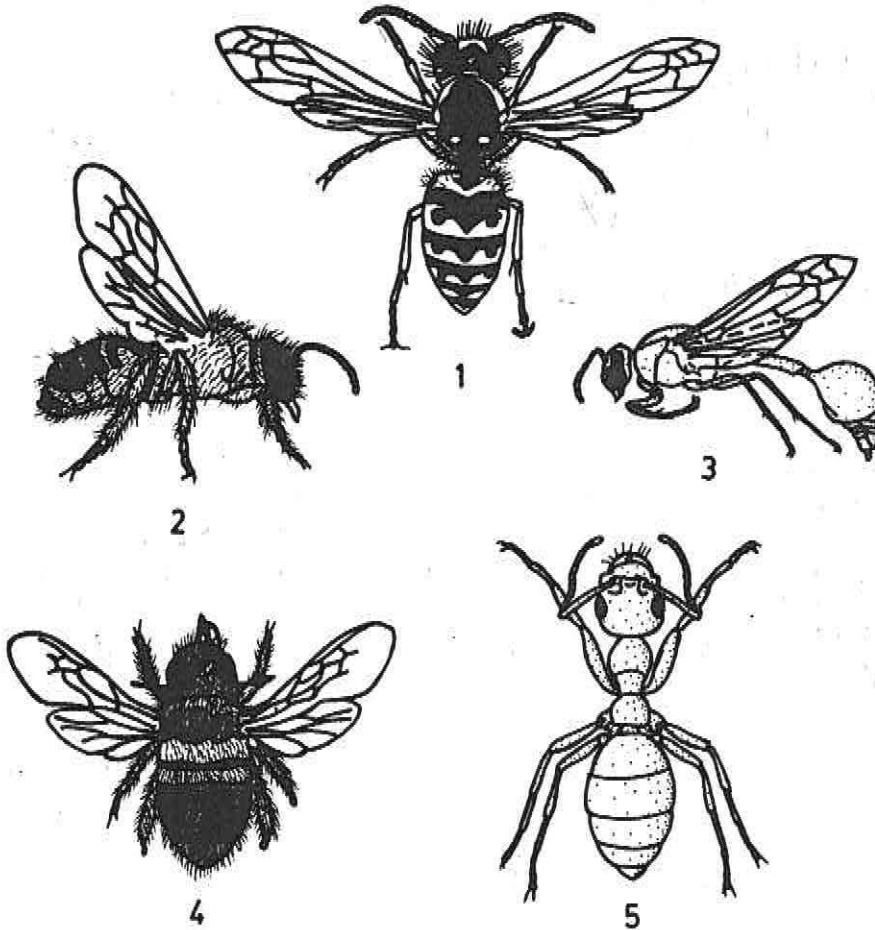
Fig. 10. Order Hymenoptera
(Adapted from, Scholtz and Holms 1989. *Insects of Southern Africa*. Butterworth Co. Durban, South Africa).

1. *Vespula germanica* (Vespidae).
2. *Megachile* sp. (Apoidea, Megachilidae).
3. *Eumenes* sp. (Eumenidae).
4. *Xylocopa caffra* (Apoidea, Anthophoridae).
5. An ant, *Plasiolepsis* sp. (Formicidae).

Hymenoptera are predators of pests, while others as bees are important pollinators of plants.

The ovipositor in female ants, wasps and bees is modified into a sting, an organ of offence and defence.

The Hymenoptera also exhibit various degrees of eusociality with the ants and bees being the most eusocial groups in the order. The ants have a complex caste system organization that

is comparable to that of the termites (order, Isoptera) described before. However, the caste system in ants differs from that of the termites in that worker and soldier ants are sterile adults of a single sex. This is the female sex. You will recall that in termites described above, these two castes comprise nymphs and sterile male and female individuals.

F.   Diagnostic Features of Insect Parts Used or Suggested for Use in the Laboratory Manual

Order, Odonata (Dragonflies and damselflies)

The unsegmented cerci of male *Anax speratus* Hagen a darner (Anisoptera, Aeshinidae) or *Brachythemis leucostica* (Burm), a common skimmer (Anisoptera, Libellulidae). The cerci are modified as claspers in male members of this order which aid in holding females by the neck, while copulating in flight. Since the accessory external reproductive organs in the male are located on the venter of the second abdominal segment, this enables the female held in tandem to push her abdomen down and forward, to come into contact with the male's accessory sex organs.

Order, Orthoptera (Locusts and grasshoppers, crickets, stick insects, praying mantids and cockroaches)

1.   External morphology of the armoured ground cricket, *Enyaliopsis* c.f. *matebelensis* Peringuey and internal anatomy of the armoured ground cricket, *Acanthoplus speiseri* Brancsik (both belonging to the family Tettigoniidae). Used as examples in the introductions to all sections in the laboratory manual.

2.   External morphology and internal anatomy of the desert locust, *Schistocerca gregaria* Forskal or the garden locust, *Acanthacris ruficornis* Fabricius (both belonging to the family Acrididae). Both have generalized external morphology and internal anatomy. They are suggested for use in the exercises in the laboratory manual.

3.   External morphology of the thorax and structure of the ovipositor of the bush cricket, *Liogryllus bimaculatus* (Orthoptera, Gryllidae) or the tobacco cricket, *Brachytrupes membranaceus* (Drury) or for those of any other member of the genus *Gryllus*. For comparison with those of the red or garden locust and the armoured ground crickets.

4. External morphology of the thorax of the stick insect, *Bractrododema* sp. (Phasmatidae). To study structural variation in the tagmata among insects.

5. Forelegs of the mole cricket, *Gryllotalpa africana* Pal. (Gryllotalpidae). Modified for digging (fossorial) into the ground where the insect lives in constructed tunnels. The femur and tibia are expanded and flattened, while the tarsus has highly sclerotized claw-like segments for digging.

6. Forelegs of the praying mantis, *Pseudocreobotra wahlbergi* (Stål)(Mantidae). The coxa of each foreleg is elongated to allow the spined femur and tibia linked to it to appose each other for grasping prey.

7. Leg structure and form and the structure of the ventral nerve cord of any of the following cockroaches: the American cockroach, *Periplaneta americana* (L.)(Blattidae); the German cockroach, *Blattella germanica* (L.)(Blattellidae); and the Oriental cockroach, *Blatta orientalis* (L.)(Blattidae). The legs are streamlined, an adaptation for running (cursorial), while the number of ganglia in the ventral nerve cord in the abdominal tagma is reduced.

8. Legs, cerci and the dorsal blood vessel of the wood cockroach, *Gyna maculipennis* (Schaum)(Blattidae). Suggested for study of structural variations in the morphology of insects.

9. Phallic organ and ovipositor of the longhorned grasshopper, *Homorocoryphus nitidulus vicinus* Wlk. (Tettigoniidae). There is no definite intromittent organ (aedegus) in the males of this species, as is the case with all other males of the family Tettigoniidae, instead there is a phallic organ comprising a dorsal, a ventral and a pair of lateral lobes. These mould spermatophores during copulation. The ovipositor in the female has two pairs of blades that are drawn out making the ovipositor sword-like in appearance.

Order, Isoptera (Termites)

1. Antennae and internal reproductive organs of alate harvester termite, *Hodotermes mossambicus* Hagen (Hodotermitidae). Antennae are moniliform, while the internal reproductive organs are suggested for comparison with those of the male and female desert locust.

2. Alimentary canal of alate bark-eating termite, *Macrotermes vatrialatus* (Termitidae). The rectum modified into a pouch housing cellulose-digesting protozoan flagellates.

Order, Dermaptera (Earwigs)

1. Cerci of the earwig, *Labidura riparia* (Beauv.) (Labiduridae). Cerci modified into forceps-like structures for offence and defence.

Order, Hemiptera (Bugs)

1. Head orientation, cervical sclerites' structure and external morphology of the thorax of the stink bug, *Nezara viridula* L. (Pentatomidae). The head orientation is opisthorhynchus type. External morphology of the thorax and structures of the cervical sclerites are compared to the generalized type.

2. Wing structure and venation of the coffee capsid, *Lamprocapsidea coffeae* (China)(Miridae) or the cotton lygus, *Taylorigus vosseri* (Popp.)(Miridae) or the leaf-footed plant bug (= squash bug), *Leptoglossus australis* (F.)(Lygaeidae). The forewings are hemielytra, i.e., are leathery on their basal portions and membranous at their tips. The hindwings are completely membranous and bear few veins in them.

Order, Coleoptera (Beetles)

1. Head orientation and nature of the mouthparts of the giant black tiger beetle, *Mantichora* sp. (Cicindelidae) or Burchell's anthia ground beetle, *Anthia burchelli* Hope (Carabidae) or the acacia wood beetle, *Acanthophorus maculatus* or *A. capensis* (Cerambycidae). Prognathous head orientation. Mouthparts directed forward for grasping prey or boring into wood.

2. Antennae, cervical sclerites, wing structure and venation of the common dung beetle, *Pachylomera femoralis* Kirby (Scarabaeidae). Antennae lamillate with three terminal segments of the flagellum expanded into plate-like structures. Cervical sclerites to be compared to those of the generalized condition. Forewings are elytra (singular, elytron), i.e. are hard and brittle. They serve as protective sheaths for the hind wings. The latter are the true flight wings and are membranous with few veins in them.

3. Internal reproductive organs of the fruit chafer, *Dicronorrhina derbyana* (Westw.) (Scarabaeidae). Suggested for comparison with those of the generalized insectan condition.

Order, Diptera (Flies or two-winged flies)

1. Antennae and mouthparts of any of the following mosquitoes: the anopheline mosquitoes; *Anopheles gambiae* (Giles), *A. funestus* (Giles) and *A. arabiensis* (Patton)(Culicidae); and the culicine mosquito, *Culex quinquefasciatus* (Culicidae). Antennae pilose in females and plumose in males. Mouthparts piercing-and-sucking types with the pair of mandibles, the pair of maxillae and the hypopharynx modified into needle-like piercing structures called stylets. The labia are modified into probosces serving as sucking tubes and protective sheaths for the stylets when not in use.

2. Antennae, mouthparts, ovipositor and the ventral nerve cord of the house fly, *Musca domestica* L. Antennae are aristate. Mouthparts are modified for sponging and lapping type of feeding, in which the mandibles and maxillae have been completely lost. The labium forms a proboscis at the tip of which there is a labellum with which food is lapped, sponged and sucked into the alimentary canal. The ovipositor in the female bears no sclerotized blades as obtains in the generalized insectan condition. Instead it is a completely new structure in the class Insecta comprising of telescoping terminal abdominal segments. Only a single ganglionic mass occurs in the ventral nerve cord and is located in the thorax. It supplies nerves to the thoracic and abdominal tissues and organs.

3. Mouthparts of the horse fly, *Tabanus* sp. (Tabanidae). Piercing and sucking type for comparison with other insects.

4. Mouthparts and internal reproductive organs of the tsetse fly, *Glossina pallidipes* Austen or *G. morsitans morsitans* Westwood (Glossinidae). Piercing and sucking type mouthparts. Internal reproductive organs suggested for comparison with those of other insects and the generalized types.

Order, Lepidoptera (Butterflies and moths)

1. Segment structure and segmentation of the spotted stalk borer (= sorghum stem-borer), *Chilo partellus* (Swinhoe)(Pyralidae) or the maize stalk borer, *Busseola fusca* (Fuller)(Noctuidae) or the cotton bollworm (= corn earworm), *Heliothis zea* (Boddie)(Noctuidae) or the African armyworm, *Spodoptera exempta* Wlk. (Noctuidae). All bear simple segment morphology and a primary type of segmentation as caterpillars.

2. Mouthparts and wing structure of the citrus swallowtail, *Papilio demodocus* Esper (Papilionidae). Mouthparts are the sucking type that form a long proboscis made up of apposing galea of the maxillae. The mandibles and maxillae as known in the generalized insectan condition are completely lost. The proboscis is coiled when not in use. The wings are covered with scales. Wing venation reduced. Hind wings with two eye spots each.

Order, Hymenoptera (Bees, wasps, ants, ichneumons, sawflies and chalcids)

1. Tentorium, mouthparts, cervical sclerites, pterothorax, wing structure and venation, hindleg structure, the ventral nerve cord and internal reproductive organs of the African honeybee, *Apis mellifera adansonii* (L.) or *A.m. capensis* (L.)(Apidae). Compared with those of the generalized insect.

2. Petiole of the stink ant, *Paltothyreus tarsatus* (Fabricius) or the harvester ant, *Messor barbarus* L. (Formicidae). The waist (petiole) is narrow, separating the rest of the abdomen from the thorax. The harvester ant has two nodes on its petiole.

3. Ovipositor of a parasitic wasp (Ichneumonidae). The ovipositor is drawn into a long structure for ovipositing eggs into hosts.
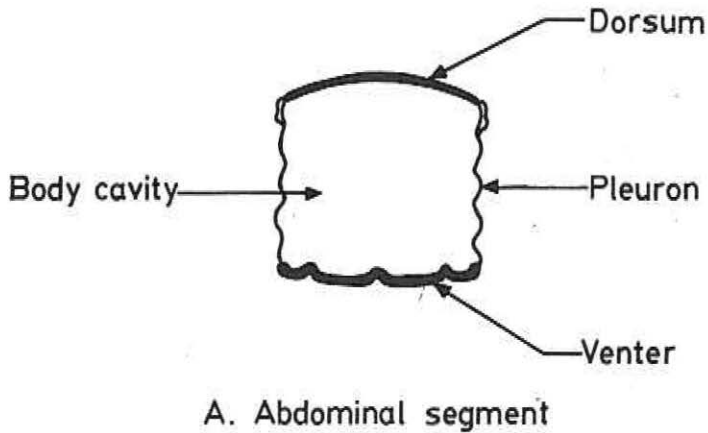
Section I

Segment Morphology and Segmentation

One characteristic that sets arthropods apart from the rest of the animal groups, is their possessing a body that is divided into ring-like segments and their bearing of jointed appendages. The evolutionary significance of such a body is that it allowed for specialization through differential fusion of the segments into body regions, a process termed tagmatization. Consequently the body of an arthropod may be divided into head and trunk tagmata like in millipedes (class, Diplopoda) and centipedes (class, Chilopoda) or cephalothorax and abdomen tagmata like in the crayfish (class, Crustacea) or head, thorax and · abdomen tagmata like in insects (class, Insecta).

In the majority of immature and adult insects, each body segment is a simple structure. A cross-section through the segment will reveal three major regions (Fig. 11A), a dorsal part called dorsum, a ventral part termed venter and two membranous lateral sides called pleura (singular, pleuron). The appendages (legs and wings) when present arise from the pleura, ventero-laterally and dorso-laterally, respectively, in those segments on the body in which they occur (Fig. 11B). In some immature insects and a majority of adult insects however, due to the secondary hardening (sclerotization) of the outer portions of the cuticular part of the body wall or integument, various plates of cuticle called sclerites are formed in the three regions of a segment named above. If the dorsum of a segment is covered by a single hardened plate, it is then referred to as a tergum or notum if it occurs in the thorax, while if the hardened plate occurs on the venter, it is called a sternum. However, often the dorsum bears a number of smaller sclerites called tergites. The pleural area bears small sclerites termed pleurites, while the venter bears sternites.

Two principal types of segmentation are recognized in insects, PRIMARY and SECONDARY SEGMENTATION. In most immature insects and many soft bodied adult insects, the FUNCTIONAL LINES on their bodies (i.e. lines on the body or areas of the body where movement is affected) coincide with areas of origin and attachment of the principal longitudinal muscles internally (Fig. 12). This is what is termed primary segmentation. It is characteristic of slow-moving insects. In some immatures and the majority of adult insects however, due to the sclerotization of the outer portions of the cuticle in their body walls, the functional lines in the form of unsclerotized cuticle called conjuctiva or simply membrane develop secondarily and occur anterad to the

A. Abdominal segment

B. Wing and leg bearing segment

Fig. 11. Cross-sections through an abdominal and a leg/wing bearing thoracic segment

points of origin and attachment of the principal longitudinal muscles internally (Fig. 13). This is what is called secondary segmentation. Body movement in this type of segmentation is affected through the conjuctiva or membranes.

Secondary segmentation confers certain advantages to the insect body namely, a) it allows for greater freedom of movement through the conjuctiva or articulating membranes, b) it allows for greater consolidation of

the body segments into tagmata (body regions) and c) it allows for the telescoping movements of segments, especially in the abdominal region, necessary for ventilation and stretching of the abdomen when the female is ovipositing.
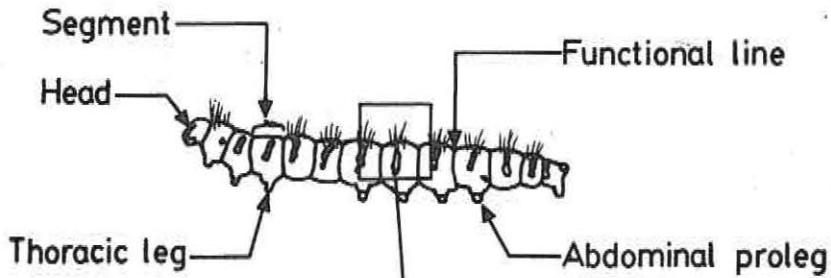
A. Lateral view of a caterpillar
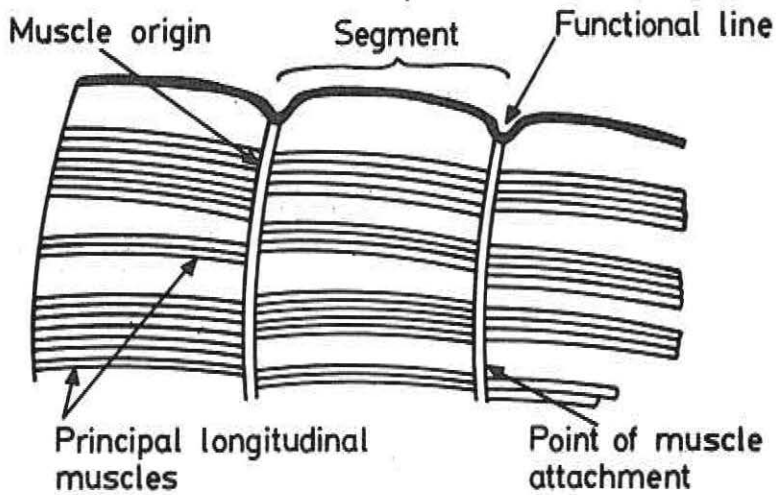
B. Longitudinal section through four caterpillar abdominal segments

Fig. 12. Primary segmentation in a caterpillar

Exercises

Materials Required:

Specimens:
1. Freshly anaesthetized or preserved (in 70% ethanol) late instar lepidopterous larvae of the spotted stalk borer (= sorghum stem-borer), *Chilo partellus* (Swinhoe)(Pyralidae) or the maize stalk borer, *Busseola fusca* (Fullar)(Noctuidae) or the cotton bollworm (= corn earworm), *Heliothis zea* (Boddie)(Noctuidae) or the African armyworm, *Spodoptera exempta* Wlk. (Noctuidae).

2. Freshly anaesthetized or preserved (in 70% ethanol) adult desert locust, *Schistocerca gregaria* Forskal or the garden locust, *Acanthacris ruficornis* Fabricius or any large grasshopper.

Apparatus:
1. Dissecting dish with some beeswax at the bottom.
2. Dissecting set.
3. Gas burner.
4. Minuten insect pins (No. 3).
5. Binocular microscope.

Chemicals:
1. 10% ethanol.

Thorax

Head

Abdomen

A. Adult male Armoured
   Ground Cricket

Articulating membrane
or conjuctiva
(functional line)

Antecostal suture

Tergite

Antecosta

Principal
longitudinal
muscles

Muscle origin

Point of muscle
attachment
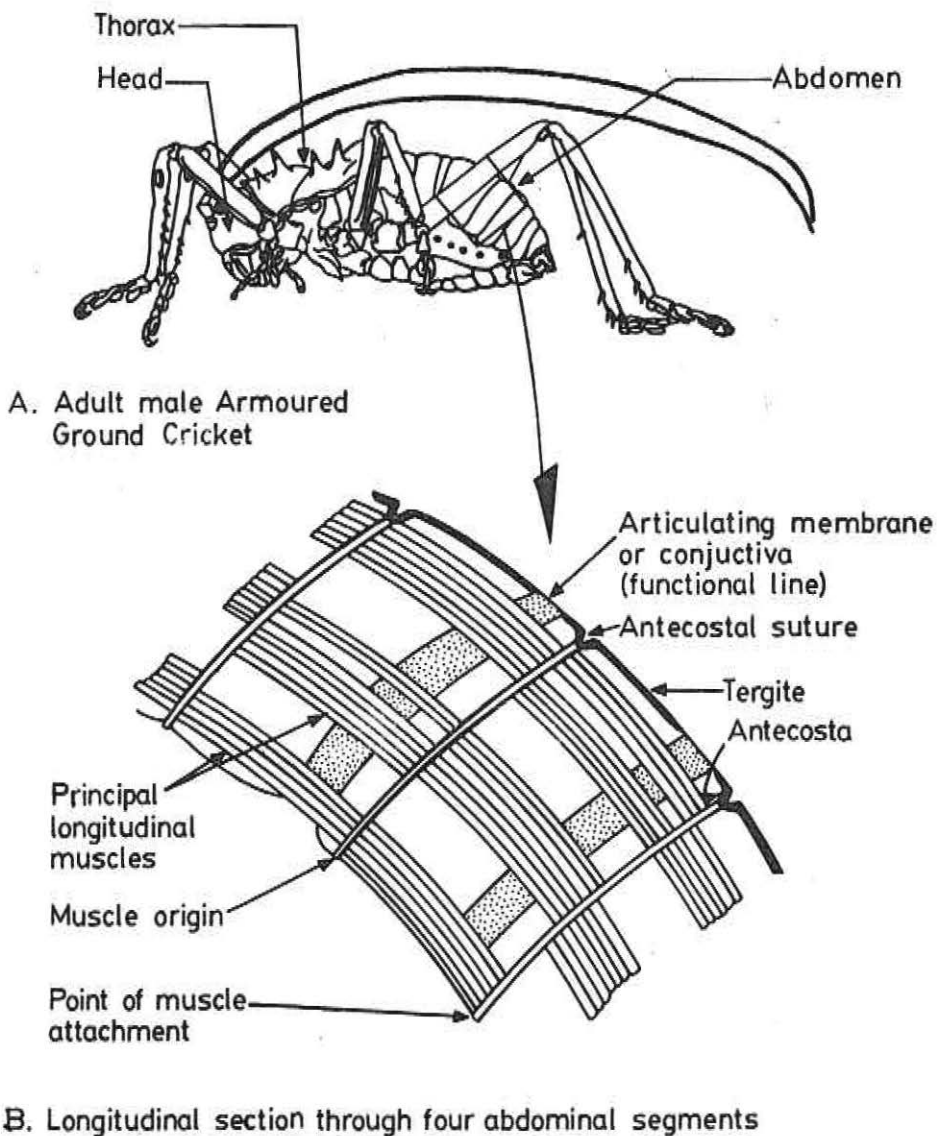
B. Longitudinal section through four abdominal segments

Fig. 13. Secondary segmentation

Exercise 1: **Segment Morphology.**

1. You are provided with specimens of freshly anaesthetized or preserved (in 70% ethanol) lepidopterous larva and an adult locust or grasshopper.

2. Using a pair of scissors, excise the mesothoracic segment of each specimen along the intersegmental membranes. The segment removed should include the appendages arising from it. In case of the adult locust or grasshopper, the wings may be too long for convenience. Clip off these wings at their bases.

3. Place each excised mesothoracic segment in the dissecting dish on the beeswax and pin it to the wax with the anterior side of the segment uppermost (see Fig. 11). Then submerge the specimens in 10% ethanol.

4. Examine the specimens under low and high powers of the binocular microscope.

5. Draw and fully label the cross-sections of the mesothoracic segments of your specimens.

6. How do the two specimens differ from each other in cross-section in terms of segment morphology?

Exercise 2: **Types of Segmentation.**

Primary Segmentation

1. Use a fresh or preserved (in 70% ethanol) late instar caterpillar of *Chilo partellus, Busseola fusca* or *Heliothis zea* or of any other species of Lepidoptera, for this exercise.

2. Melt a small portion of wax in the dissecting dish using a gas burner.

3. Before the molten wax solidifies, place the caterpillar right-side-up (i.e. with the dorsum uppermost) into the molten wax and then let the wax set (solidify) to fix the specimen to the wax (Fig. 14).

4. Once properly fixed to the wax, flood the dissecting dish with 10% ethanol until the specimen is submerged. The alcohol is to prevent your specimen from shrinking due to drying and will also improve the resolving power of the microscope when you examine the specimen under it.

Exercise 2: Continued

5. Using a pair of fine scissors, open up the specimen along the centre of the dorsum, from the abdomen and carefully work your way forward to the thorax by cutting through the terga.

6. In the thoracic area, make additional small cuts from the dorsum (i.e. from the point where you stopped in step 5 above) through the pleural region, on either side of the body, to the venter (Fig. 14).

Caterpillar   Dissecting dish   Main longitudinal cut
Bee's   Minuten insect pin
wax
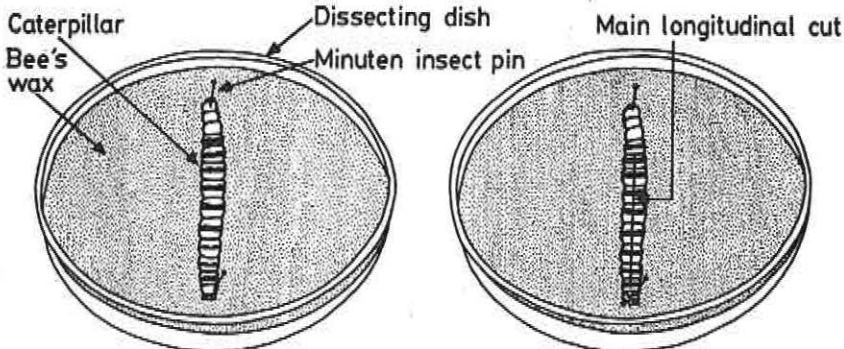
1. Place the specimen on molten wax right side up and then wait until the wax solidifies.

2. Make a longitudinal cut through the dorsum from the abdomen to the thorax and then submerge the specimen in 10% ethanol.
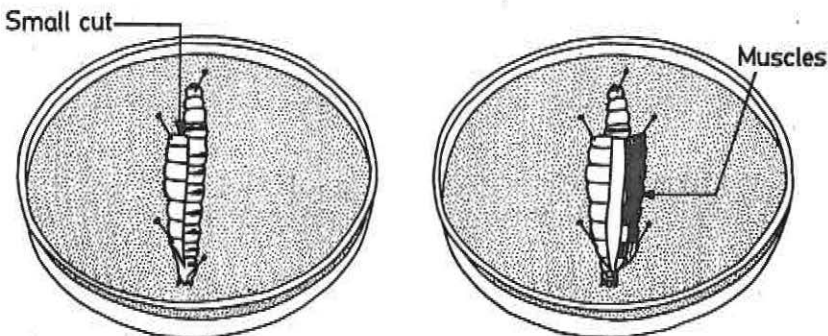
Small cut

Muscles

3. Make two additional small cuts in the thoracic region and pull back the body wall on one half and pin.

4. Pull back the other half of the body wall and pin. Remove any obstructing matter to expose muscles.

Fig. 14. Preparing a caterpillar to study primary segmentation

7. Separate the two halves of the body wall from around the thoracic region and pin them to the wax in the dissecting dish.

8. While examining your specimen under a binocular microscope, carefully clear off one half of the inside of the exposed body wall, of

Exercise 2: Continued

the fat body material and any connective tissues to expose the principal longitudinal muscles. Ensure that the muscle bands are not detached from their connections to the body wall.

9. Note the positions of the functional lines on the cleared segments in relation to the points of origin and insertion of the principal longitudinal muscles of the body.

10. Make a large, clear and well-labelled diagram of three segments of your specimen, from the cutup edge of the body wall to show the relationships of the points of origin and insertion of the principal longitudinal muscles to the functional lines.

11. How is movement effected in this type of segmentation?

Secondary Segmentation

1. You will need a fresh or preserved (in 70% ethanol) specimen of an adult desert locust, garden locust or any large type of grasshopper for this exercise.

2. Clip off the wings and legs of the specimen from their bases and then mount the specimen in wax in the dissecting dish as in the above exercise. Dissect the specimen along the dorsum midline from the abdomen to the thoracic region. Make additional small cuts in the thoracic region as you did with the caterpillar in the exercise above and then examine one half of the body wall from the cutup edge following the exposure of the principal longitudinal muscles (Fig. 15).

3. Identify the following structures from the cutup dorsal edge of one half of the body wall of your specimen and make a large, clear and well-labelled diagram of four abdominal segments illustrating them;
TERGITE
ACROTERGITE
ANTECOSTA
CONJUCTIVA
PRINCIPAL LONGITUDINAL MUSCLES

4. How is movement possible in this type of segmentation? What do you think would be the disadvantage of this type of arrangement if it occurred in the thorax especially the pterothorax? How has this been overcome during the evolution of the thorax of winged insects?
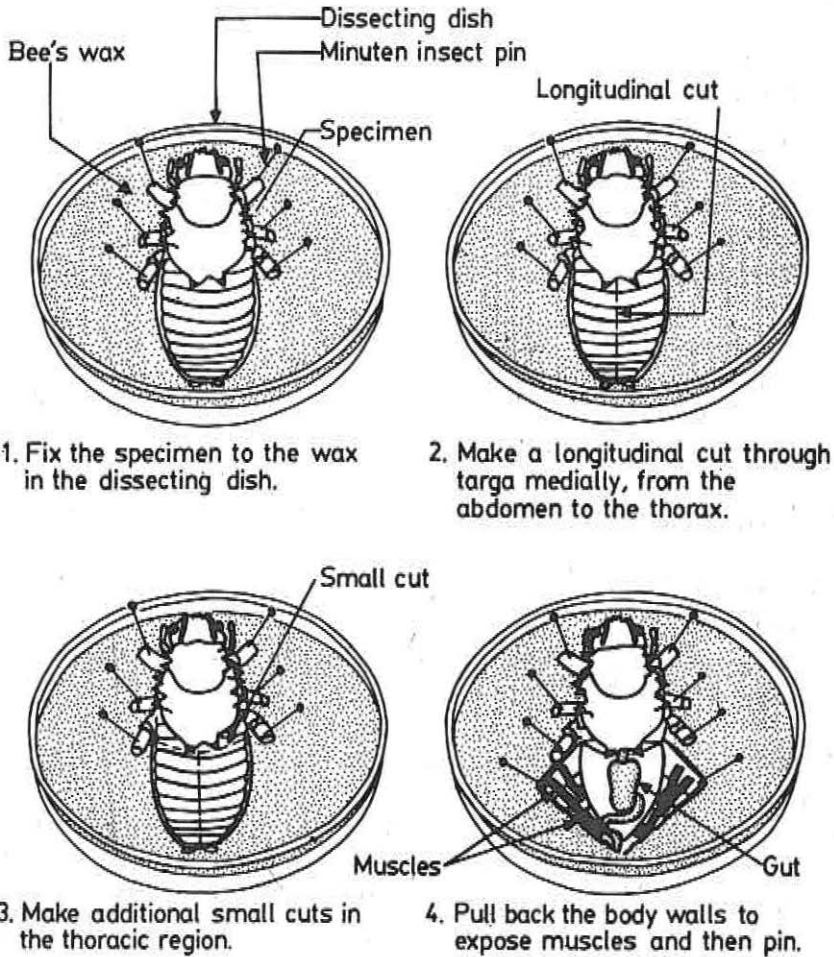
Fig. 15. Preparing the armoured ground cricket to study secondary segmentation

Supplementary Questions

1. In a generalized insect segment such as the ones obtaining in the abdominal region of the insect body, the pleural regions are membranous. However, it will be observed that the pleura of the thoracic segments of some immature insects and a majority of adult insects bear various sclerites. What is the origin of these plates and what functions do they serve in these segments?

2. What would you say are the disadvantages of primary segmentation and how have insects bearing this type of segmentation overcome them during their evolution?

3.   What kinds of variation occur in secondary segmentation in the class Insecta? Explain the functional morphological needs of such variations in named insects or insect groups.

4.   A caterpillar undergoes several moults during its development but until the last moult, retains primary segmentation. What do you think happens in the pupa that brings about change from primary to secondary segmentation characteristic of the adult insects?

References

Snodgrass, R.E. 1935b. *Principles of insect morphology.* McGraw-Hill Publishing Co. Ltd., London, i–ix, 667 pp.

Snodgrass, R.E. 1963. A contribution toward an encyclopedia of insect anatomy. *Smithson. Misc. Collect.* 146:1–48.

Section II

A. External Morphology

The insect head derives from 6–7 embryonic segments and comprises a highly sclerotized and compact capsule, a pair of antennae and the mouthparts. The degree of fusion of the precursor embryonic segments is such that, there is no definitive segmentation in the tagma. The only evidence of segmentation in certain regions of the tagma is a system of grooves or lines called sutures (NOTE: not all sutures on the insect cranium represent demarcations of the embryonic segments that form it). The head capsule houses the main sensory centre of the body, i.e. the brain. The head also serves as the ingestion centre of the body.

For convenience in its description, the insect head is divided into five areas namely, 1) fronto-clypeal, 2) parietals, 3) subgena, 4) occipital, and 5) post-occipital.

The Fronto-Clypeal Area (The Face)

This is the face area of the insect head. It is an area that one would see if they came "face-to-face" with an insect. This area of the head is variously defined by sutures in different insect groups. In the generalized (primitive) condition, it comprises the following sclerites and sutures; frons (the face) at the top, which is followed below by the clypeus, which in turn is linked ventrally, to the upper lip, the labrum. The frons is bounded dorsally by two frontal sutures, which are the arms of a large inverted Y-shaped suture called epicranial suture. The stem of this long suture is the coronal suture. It occurs at the centre at the top of the cranium (the vertex). The fronto-clypeal area is bounded on either side of the cranium by fronto-genal sutures. Finally, the frons is separated from the clypeus below by the epistomal suture, while the clypeus is joined to the labrum through the labro-clypeal suture.

Variations occur in this region of the face, even in the generalized condition. In the armoured ground cricket, *Enyaliopsis matebelensis* Peringuey, for example, the fronto-clypeal area is poorly defined owing to the presence of incomplete fronto-genal sutures (Fig. 16, fgs), resulting in the frons (Fr) merging with both cheeks or gena (Ge) laterally. The dorsal boundary of the frons is

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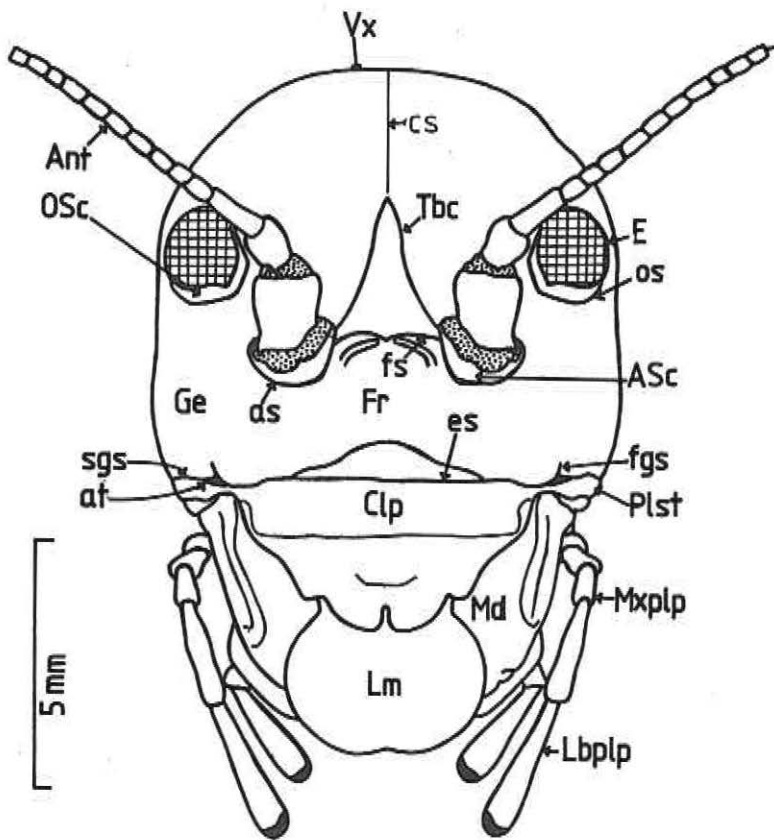
Fig. 16. Anterior view of the head of *Enyaliopsis matebelensis*

Ant, antenna; as, antennal suture; ASc, antennal sclerite; at, anterior tentorial pit; Clp, clypeus; cs, coronal suture; E, compound eye; es, epistomal suture; fgs, frontogenal suture; Fr, frons; fs, frontal suture; Ge, gena; Lbplp, labial palp; Lm, labrum; Md, mandible; Mxplp, maxillary palp; os, ocular suture; OSc, ocular sclerite; Plst, pleurostoma or subgena; sgs, subgenal suture; Tbc, tubercle; Vx, vertex.

Morphological Features of Taxonomic Importance

-   Very small compound eyes (E) when compared to the size of the rest of the cranium.
-   Antennae are inserted medially between and slightly below compound eyes (E).
-   Lack of ocelli on the cranium.
-   Presence of a large tubercle (Tbc) between the antennae.
-   Anterior tentorial pit (at) contained in both epistomal (es) and subgenal (sgs) sutures.
-   Incomplete fronto-genal sutures (fgs).

however, well demarcated by short frontal sutures (fs), occurring between the antennal sclerites (ASc). Ventrally, the frons is separated from an oblong clypeus (Clp) by a distinct epistomal suture (es) which connects anterior tentorial pits (at). The upper lip, or labrum (Lm) is oval in shape and projects ventrally from the clypeus over the biting jaws or mandibles (Md).

The Parietals (Cheek Areas)

The parietals extend from the top of the cranium on either side of the head, to small sclerites above the right and left mandibles called, subgena or pleurostomata. Each parietal bears a lateral ocellus, a compound eye and an antenna generalized in insects like the desert locust, *Schistocerca gregaria* Forskal. It is bounded at the top by the coronal suture, anteriorly by the fronto-genal suture, posteriorly by the occipital suture and ventrally by the subgenal suture.

In *E. matebelensis* (Fig. 17), the parietal (cheek) areas of the cranium are poorly delimited anteriorly, where the gena merges into the frons. Each parietal extends from the coronal suture on the vertex ventrally, to the subgenal suture, on either side of the cranium (Figs 16 & 17; cs, fgs, sgs, Vx). Posteriorly, each parietal is bounded by the occipital suture (Fig. 17, ocs). Each parietal supports a small hemispherical compound eye (E) and an antenna (Ant). The compound eyes are small in relation to the size of the cranium and the antennae are inserted medially below the compound eyes. Between the antennal sockets on the parietals in *E. matebelensis* arise a large spine (Figs 16 & 17, Tbc). There are no ocelli on the parietals in this species.
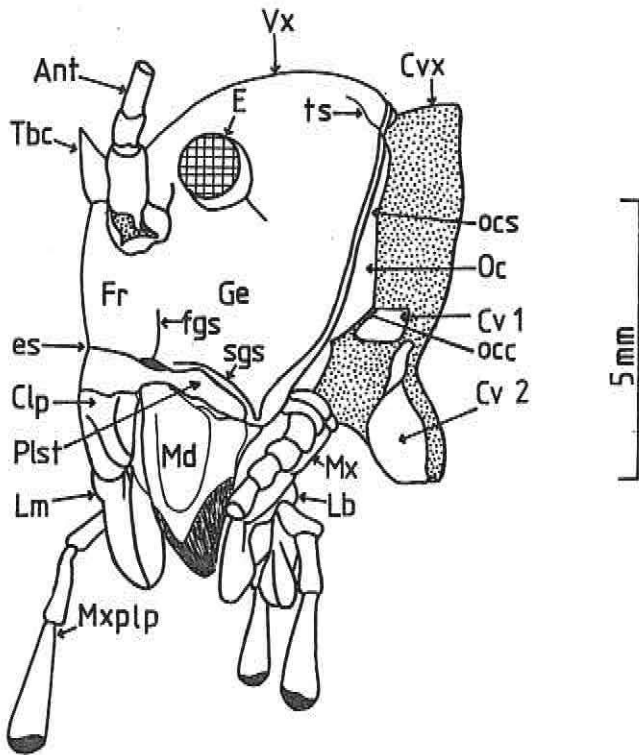
Fig. 17. Lateral view of the head of *E. matebelensis*

The Subgenal Areas

These are two narrow and elongated sclerites also known as the pleurostomata, that are located one below each parietal, above the points of attachment of the mandibles to the cranium. Each subgena is separated from the gena by the subgenal suture and articulates at two points ventrally with a mandible (Fig. 17, Plst, sgs).

The Occipital Arch

This area occurs at the back of the cranium and is variously modified especially, in higher insect orders. In the generalized condition as in *E. matebelensis* (Fig. 18, Oc), the area is C-shaped being very narrow dorsally and widening ventrally on both sides. It is bounded by the occipital suture (ocs) anteriorly and by the postoccipital suture (pos), posteriorly.
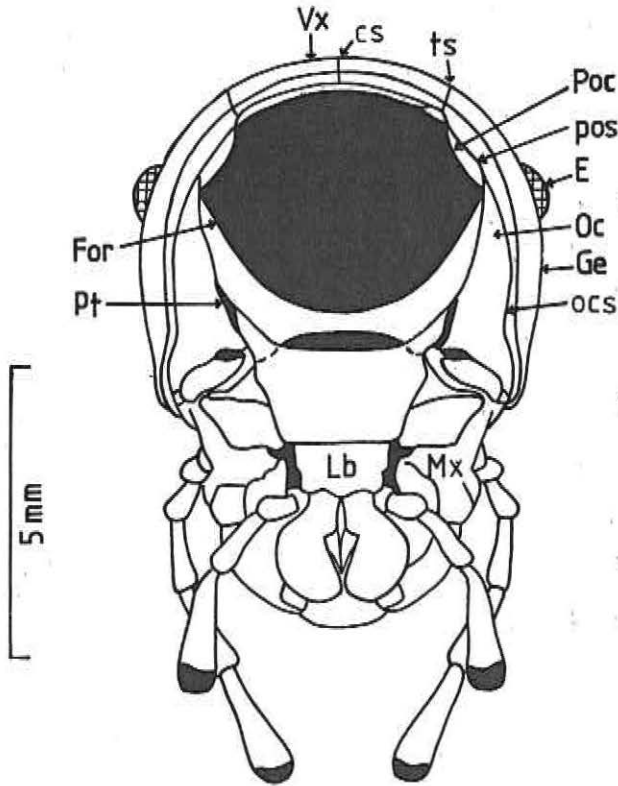
Postoccipital Arch

This is a very narrow area comprising a very narrow sclerite (postocciput), that forms a rim to the neck opening (foramen magnum). In *E. matebelensis* this sclerite is discontinuous, occurring mainly dorsally and dorso-laterally (Fig. 18, Poc). It is delimited from the occiput anteriorly, by the postoccipital suture (pos).

The appendages of the insect head are a pair of antennae and the mouthparts. In the generalized condition, the antennae are

Fig. 18. Posterior view of the head of *E. matebelensis*

Ant, antenna; Clp, clypeus; cs, coronal suture; Cv 1, first cervical sclerite; Cv 2, second cervical sclerite; Cvx, cervix or neck; E, compound eye; es, epistomal suture; fgs, frontogenal suture; For, foramen magnum; Fr, frons; Ge, gena; Lb, labium; Lm, labrum; Md, mandible; Mx, maxilla; Mxplp, maxillary palp; Oc, occiput; occ, occipital condyle; ocs, occipital suture; Plst, pleurostoma or subgena; Poc, postocciput; pos, postoccipital suture; Pt, posterior tentorial pit; sgs, subgenal suture; Tbc, tubercle; ts, temporal suture; Vx, vertex.

Morphological Features of Taxonomic Importance

·   Very small compound eyes (E) when compared to the rest of the cranium.
-    Presence of a tubercle (Tbc) between the antennae.
-    Incomplete fronto-genal suture (fgs).
-    Open ventral portion of the foramen magnum (For).

filiform. In *E. matebelensis*, each antenna bears a large basal segment (scape) that articulates with the cranium via a finger-like process, a second smaller segment (pedicel) and a whip-like flagellum (Fig. 23, Scp, ap, Pdc, Fl). The mouthparts in their primitive condition are biting type and comprise the labrum, a pair of mandibles, a pair of maxillae, a lower lip or labium and a tongue-like structure on which the mouth proper opens, the hypopharynx. Detailed structures of the generalized mouthparts of *E. matebelensis* are presented in Figures 19 –23.
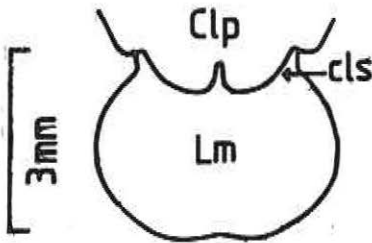
Fig. 19. The labrum
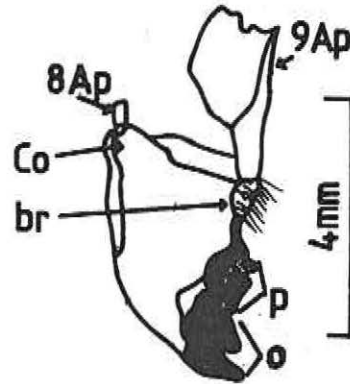
Fig. 20. The mandibles

a. Anterior view of the right mandible
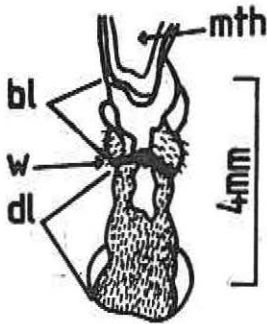
Fig. 21. Anterior view of the hypopharynx
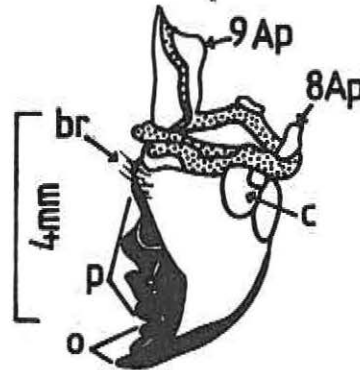
b. Posterior view of the right mandible

Occurring in the neck membrane (cervix) latero-ventally on either side of the body are small sclerites (cervical sclerites) (Fig. 17). The anterior cervical sclerite (Cv 1) articulates with the head anteriorly and with the second cervical sclerite (Cv 2) posteriorly. The latter in turn articulates with the presternum of the prothorax posteriorly.
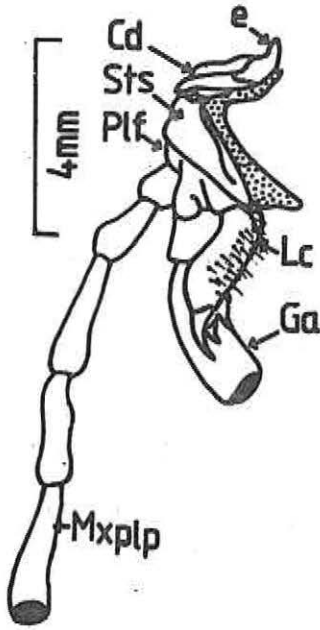
Fig. 22. The maxilla

a. Posterior view of the left maxilla
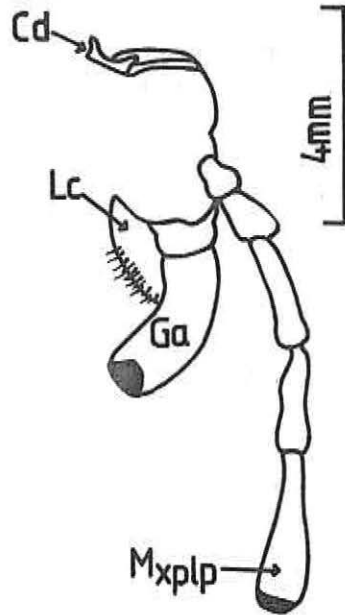
b. Anterior view of the left maxilla
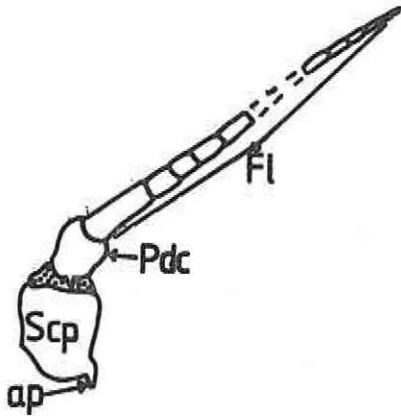
Fig. 23. The antenna
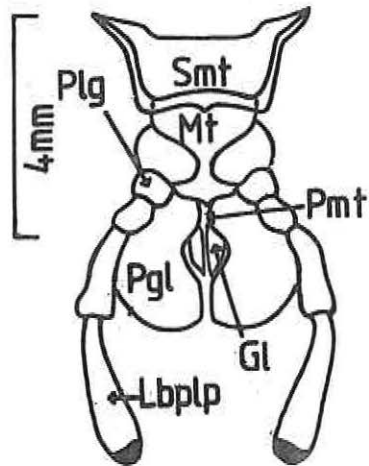
Fig. 23a. Posterior view of the labium
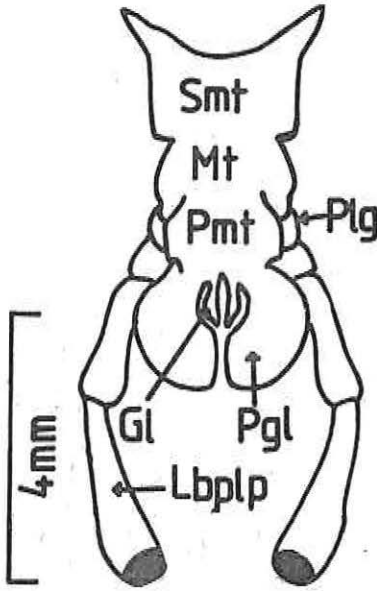
Fig. 23b. Anterior view of the labium

ap, antennal pivoting process; 8 Ap, abductor apodeme of the mandible; 9 Ap, adductor apodeme of the mandible; bl, basilingua; br, brustia; c, acetabulum; Cd, cardo; Clp, clypeus; cls, clypeolabral suture; Co, condyle; dl, distilingua; e, articulation of the maxilla with the cranium; Ga, galea; Gl, glossa; Lbplp, labial palp; Lc, lacinia; Lm, labrum; Mt, mentum; mth, mouth; Mxplp, maxillary palp; o, incisor lobe; p, molar lobe; Pdc, pedicel; Pgl, paraglossa; Plf, palpifer; Plg, palpiger; Pmt, prementum; Scp, scape; Smt, submentum; Sts, stipes; w, suspensorium of hypopharynx.

Morphological Features of Taxonomic Importance

- Biting and chewing mouthparts.
- Filiform antennae.

B.   Endoskeletal Processes

The main endoskeletal process of the insect cranium is the tentorium. This is a large, generally X-shaped structure occupying the lower margins of the cranium, just above the mouthparts. It is a compound structure resulting from the invagination of the cranial walls. The roots of these invaginations on the outside of the cranium appear as tentorial pits. From the anterior tentorial pits arise the anterior pair of tentorial arms and from the posterior pits, the posterior tentorial arms. These arm pairs fuse to form a central body, the corporo-tentorium. At or near the junction of the corporo-tentorium or as in some cases, at the bases of the anterior tentorial arms, arise a third pair of tentorial arms, the dorsal tentorial arms. The tentorium serves as an important brace for the cranium and also provides areas for muscle attachment.

In *E. matebelensis*, when viewed from the front, in a specimen in which the frons, vertex and parts of the genae have been excised (Fig. 24), the tentorium is seen as a large, horizontal, X-shaped frame, occupying the lower margins of the cranium. It consists of an expanded corporo-tentorium (Ct), from which three arm pairs diverge. Both the anterior (At) and posterior (Pt) tentorial arm pairs are hollow invaginations of the cranial walls, whose roots are represented on the outside of the cranium by the anterior- and posterior tentorial pits, respectively. The anterior tentorial pits (at) are wide and slit-like and are contained in both the subgenal (sgs) and epistomal (es) sutures. The anterior tentorial arms are wide at the cranial walls, where they arise at the anterior tentorial pits but narrow down progressively, posteriorly, before merging into the corporo-tentorium (Figs 24 & 25, At). The posterior tentorial arms are short and merge posteriorly with the postoccipital ridge at the back
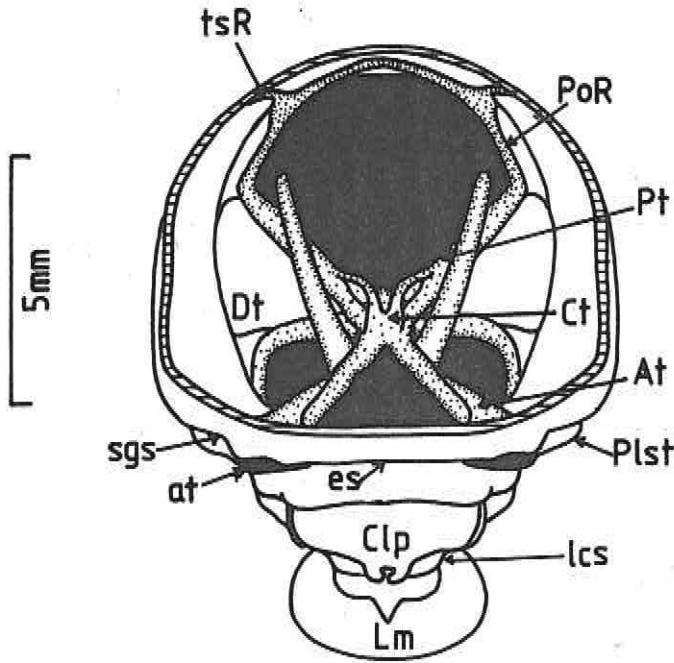
Fig. 24. Anterior view of the cranium with part of the anterior cranial walls excised to show the tentorium

of the cranium (Fig. 24, PoR). The posterior tentorial pits are wider and longer than the anterior tentorial pits (Fig. 25, pt). They occur at the base of the postoccipital suture, on either side of the head. The dorsal tentorial arms are solid and tendinous (Figs 24 & 25, Dt). Each dorsal tentorial arm arises dorsally on an anterior tentorial arm, near its junction with the corporotentorium. Each protrudes dorso-anteriorly to attach to the hypodermis, near the ventral margins of the antennal socket.

Fig. 25. Posterior view of the cranium showing the tentorium

a, primary cranial articulation of mandible; at, anterior tentorial pit; At, anterior arm of the tentorium; Clp, clypeus; cs, coronal suture; Ct, corporotentorium; Cv 1, first cervical sclerite; Dt, dorsal arm of the tentorium; es, epistomal suture; lcs, labroclypeal suture; Lm, labrum, Oc, occiput; occ, occipital condyle; Ocs, occipital suture; Plst, pleurostoma or subgena; PoR, postoccipital ridge; pt, posterior tentorial pit; Pt, posterior arm of the tentorium; sgs, subgenal suture; tsR, temporal ridge.
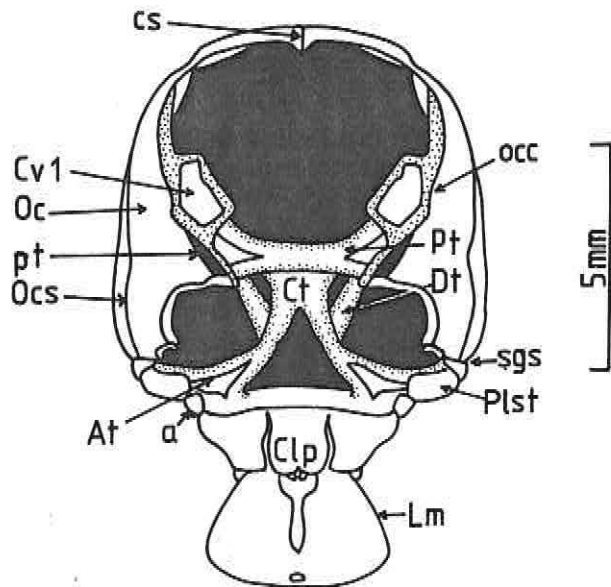
Morphological Features of Taxonomic Importance

Large X-shaped tentorium.
Anterior tentorial pits (at) contained in both the epistomal (es) and subgenal (sgs) sutures.

Exercises

Materials Required:

Specimens:
1. Live or mounted (on minuten insect pins) or preserved (in 70% ethanol) specimens of adult:

   a. Desert locust, *Schistocerca gregaria* Forskal or the garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper (Orthoptera, Acrididae).

   b. Stink bug, *Nezara viridula* L. (Hemiptera, Pentatomidae).

   c. Giant tiger beetle, *Mantichora* sp.(Coleoptera, Cicindelidae) or the groundbeetle, *Anthia burchelli* Hope (Coleoptera, Carabidae) or the acacia wood beetle, *Acanthophorus maculatus* or *A. capensis* (Coleoptera, Cerambycidae).

   d. African honeybee, *Apis mellifera adansonii* L. or *A.m. capensis* L. (Hymenoptera, Apidae).

   e. Coffee capsid, *Lamprocapsidea coffeae* (China) or the cotton lygus, *Taylorigus vosseri* (Popp.) (both, Hemiptera; Miridae) or the leaf-footed plant bug (= squash bug), *Leptoglossus australis* (F.)(Hemiptera, Coreidae).

   f. Tsetse fly, *Glossina pallidipes* Austen or *G. morsitans morsitans* Westwood (Diptera, Glossinidae).

   g. Common dung beetle, *Pachylomera femoralis* Kirby (Coleoptera, Scarabaeidae).

   h. Citrus swallowtail (= the orange dog), *Papilio demodocus* Esp. (Lepidoptera, Papilionidae).

   i. House fly, *Musca domestica* L. (Diptera, Muscidae).

   j. Horse fly, *Tabanus* sp. (Diptera, Tabanidae).

   k. Alate harvester termite, *Hodotermes mossambicus* Hagen (Isoptera, Hodotermitidae) or alate bark-eating termite, *Macrotermes vatrialatus* (Isoptera, Termitidae).

    1.   Stink ant, *Paltothyreus tarsatus* (Fabricius) (Hymenoptera, Formicidae).

    2.   Permanent prepared microscope slides of heads of male and female mosquitoes, *Anopheles gambiae* (Giles) or *A. funestus* (Giles) or *A. arabiensis* (Patton) or *Culex quinquefasciatus* (Diptera, Culicidae).

Apparatus:
    1.   Dissecting dish with beeswax at the bottom.
    2.   Dissecting set.
    3.   Razor blade or scalpel.
    4.   Minuten insect pins (Nos 3 & 5).
    5.   Binocular microscope.
    6.   Compound microscope.

Chemicals:
    1.   10% ethanol.

Exercise 1: Head Orientation.

    1.   You are provided with pinned or preserved (in 70% ethanol) adult specimens of a;
        a.   locust or grasshopper,
        b.   stink bug, and
        c.   ground beetle or tiger beetle or wood beetle.

    2.   Mount each specimen on wax in the dissecting dish with its head facing to the right side of the dissecting dish and with its left side uppermost, using minuten insect pins. For large specimens like the locust or grasshopper, you will need to clip off the legs and wings near their bases to facilitate the pinning of the specimen to the wax in the dissecting dish.

    3.   Examine each specimen under low power of the binocular microscope. Note the positioning of the head and mouthparts in relation to the longitudinal axis of the body and then draw.

    4.   What kinds of head orientations do your specimens have?

    5.   In what types of insects do we find the types of head orientations your specimens exhibit and what do you think are the advantages of these types of head orientations to the insects bearing them in terms of named specific niches they occupy?

Exercise 2: **Generalized Insect Head.**

1. Use a freshly anaesthetized or preserved (in 70% ethanol) locust or large grasshopper for this exercise.

2. Cut off the head of your specimen in the neck (cervix) region.

3. Place the head on wax in the dissecting dish with its frons uppermost.

4. Study the areas of the head and its appendages from this ANTERIOR VIEW, noting all visible sutures and then draw. **Remember**, not all sutures you have discussed in the lectures will be found on your specimen but draw all those present on your specimen.

5. Now turn the head on its side (LATERAL VIEW) and similarly study the sclerites and sutures present on this region of the head, including the mouthparts and other structures. Draw and label.

6. Next, draw the posterior view of the head, illustrating all sclerites, sutures and the appendages.

7. How does the head of your specimen differ from that of *E. matebelensis* described in the introduction to this section?

8. Both *E. matebelensis* and your specimen are considered to be generalized or primitive evolutionally. In your view, which of the two is more advanced and why?

Exercise 3 : **Generalized Mouthparts and Antennae.**

1. Using a forceps, ply out the mouthparts from the head of a freshly anaesthetized or a preserved (in 70% ethanol) adult locust or large grasshopper, beginning with the upper lip. Then remove one antenna from its articular membrane on the cranium.

2. Spread the mouthparts and antenna nicely on beeswax in the dissecting dish and then pin.

3. Submerge the specimens in 10% ethanol and then study them under the binocular microscope.

4. Make clean and large diagrams of the mouthparts and the antennae and label.

Exercise 3 : Continued

5.   How do the mouthparts and antennae of your specimen differ from those of *E. matebelensis*?

Exercise 4: **Modifications of the Head and its Appendages.**

Modifications of the Antennae
1.   You are provided with;

a.   Permanent prepared microscope slides of heads of adult male and female mosquitoes, and

b.   Pinned specimens of adult;
      i.   dung beetle
      ii.   house fly
      iii. alate termite

NOTE: If permanent mounted microscope slides of heads of mosquitoes are not available to you, use freshly anaesthetized or pinned specimens of adult mosquitoes.

2.   Examine the heads of male and female mosquitoes on permanent microscope slides under low power of a compound microscope (for pinned mosquitoes, examine the heads under a binocular microscope). Note the type, shape and segment structure of the antennae in the two sexes, then draw and label.

3.   Identify the types of antennae you have drawn. How do these antennae differ from those of a grasshopper you drew before?

4.   What are the functions of the antennae in insects? What do you think are the advantages of the antennae you have drawn to the insect groups bearing them?

Modifications of the Tentorium
1.   Using a forceps, pull out the mouthparts of;
      a.   a locust or grasshopper,
      b.   a honeybee, and
      c.   an ant.

2.   Cut off parts of the cranial walls above the anterior tentorial pits in each specimen, using a sharp razor blade or scalpel (Fig. 26).
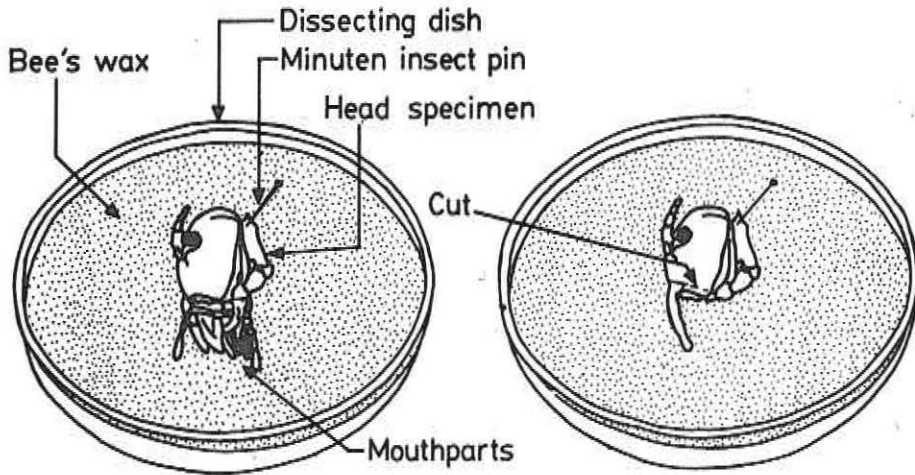
1. Cut off the head from the insect and place it in the dissecting dish.

2. Pull out the mouthparts using a forceps and then cut the cranium above the clypeus.
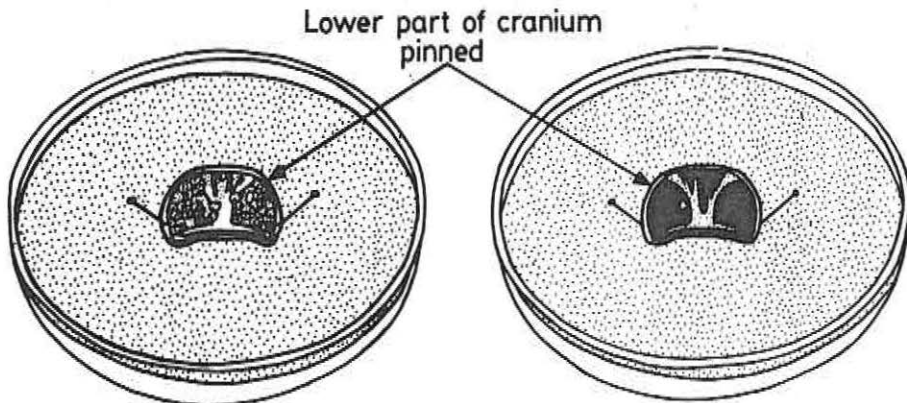
3. Discard the top part of the cranium and pin the lower part to the wax.

4. Remove all soft tissue using a forceps to expose the tentorium (see arrow).

Fig. 26. Preparing the insect cranium to study the tentorium

Exercise 4: Continued

    3.   Remove all muscle and connective tissue including the pharynx, brain and parts of the ventral nerve cord to expose the tentorium.

    4.   Make a dorsal view drawing of the tentorium for each specimen and label all the parts.

Exercise 4 : Continued

5. What differences in the structure of the tentorium occur in your specimens?

6. How do the tentoria of your specimens differ from those of *E. matebelensis*? What do you think are the reasons for the observed differences?

Modifications of the Mouthparts

1. Examine the mouthparts of an adult;
    a. female mosquito (pinned or on permanent microscope slide)
    b. house fly
    c. honeybee
    d. swallowtail butterfly
    e. tsetse fly
    f. horse fly

2. How do these mouthparts differ from each other and from the generalized condition of the armoured ground cricket, *E. matebelensis*?

3. What types of nutrition are they specialized for?

4. Explain the mechanism of nutrient ingestion by each type of mouthparts you have drawn.

Modifications of the Cervical Sclerites

1. Study the lateral sides of the cervical regions (necks) of the following insects under a binocular microscope;
    a. honeybee
    b. praying mantis
    c. stink bug, and
    d. dung beetle

2. Identify the cervical sclerites, noting their positions and interconnections. For each specimen, draw the lateral view of the cervix to show the cervical sclerites and label.

3. How many cervical sclerites occur in the neck region of each of your specimens?

4. What differences have you observed in the shape, structure and location of these small sclerites in your specimen?

5. What kinds of head movements do the muscles that insert on these sclerites produce?

Supplementary Questions

1. Why is it advantageous for carnivorous insects or those that burrow through the soil or plant material to possess prognathous type of head orientation? What kinds of cranial modifications were necessary to produce this kind of head orientation during the evolution of insects?

2. The ventral portion of the foramen magnum of the generalized insect head such as that of the garden locust, *Acanthacris ruficornis* Fabricius or the desert locust, *Schistocerca gregaria* Forskal is open. The evolutionary trend exhibited by members of the class Insecta is that towards the closure of this region of the cranium as the head orientation changed from hypognathous to prognathous type. Outline the major stages in the evolution of this part of the insect cranium in named insect groups and relate the modifications involved to the type of nutrition the insects adapted to.

3. How do the modified mouthparts of the following adult insect species operate in food ingestion? In your answer also indicate what type of modification it is from the generalized type of mouthparts and show which generalized structure or parts thereof gave rise to that part of the mouthparts in the species.
    a. the tsetse fly, *Glossina pallidipes* Austen,
    b. the mosquito, *Aedes aegypti* (L.) or *Anopheles gambiae* Giles,
    c. the sandfly, *Phlebotomus* sp.,
    d. the house fly, *Musca domestica* L., and
    e. the sorghum stemborer, *Chilo partellus* Swinhoe.

4. Antennal segments bear various types of sensilla on their surfaces that inform the insect of changes in various parameters of the external environment such as temperature, humidity and chemicals. Name the types of sensilla that are found on antennal segments. How are the sensilla distributed on the antennal surfaces considering that there exists more than 10 types of antenna in the class Insecta? How does a sensillum work? What roles does the Johnston's organ located in the second antennal segment (pedicel) play in different insect groups and finally, what other role does an insect antenna play in the life of an insect other than sensory?

5. Name the different types of functional units (ommatidia) of the compound eyes exhibited by insects. What kinds of insects bear them and how do they operate to produce vision?

6. Name an insect species that has highly reduced or no tentorium. How is the loss of this important endoskeletal process of the cranium compensated for in terms of cranial support and muscle attachment?

7. In addition to compound eyes and ocelli, how else do insects perceive light?

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Section III

A.   External Morphology

The insect thorax forms the middle tagma of the body. It comprises three segments namely, the pro-, meso- and metathorax. In a majority of insects, each thoracic segment bears a pair of legs and when wings are present, they are borne a pair each on the meso- and metathorax. If only a single pair of wings is present, this is always borne on the mesothorax. The prothorax never bears any wings in the class Insecta. The thorax of some immature insects (Holometabola) and a few adult insects, lack wings, while others may lack legs as well. The thorax is the principal locomotory centre of the insect body. A lot of variation occurs in this tagma that depend on the types and number of wings present, in the winged forms, and the leg adaptations evolved in the different groups.

In the generalized condition, the prothoracic segment is simple and articulates with the mesothoracic segment through ample membrane (conjuctiva). Its tergum (notum) usually forms variously shaped shields that may or may not be ornamented. In the Orthoptera, especially in the tettigoniid subfamily Decticinae, the pronotal shield may be large laterally and posteriorly. Lateral extension of the shield reduces the sizes of segment's pleural regions, while posterior extension of the shield results in the pronotum covering the meso- and metanota.

The mesothoracic segment is usually more closely linked to the metathoracic segment. This is even more so when wings are present on those thoracic segments. Due to the presence of wings in a majority of insects on these segments, the two are collectively called the pterothorax and individually as the pterothoracic segments.

Generally the pleural regions of the thoracic segments bear each two main sclerites, the episternum anteriorly and the epimeron posteriorly. The two plates are separated by the pleural suture. The latter in the pterothorax extends dorsally into the wing process, while ventrally, it terminates into the root (pit) of the internal pleural apophysial arm that projects into the thoracic cavity. Below this pit is the coxal articulation. In all thoracic segments, smaller sclerites occur below the episternum near the coxal articulation on either side of the body namely, the presternum and the trochantin. In the pterothoracic

region of winged insects, two epipleurites, the basalar and subalar occur on each segment. The basalar is located anterior to the wing process, while the subalar occurs behind it. These small sclerites serve as the origins of the similarly named direct flight muscles internally.

The sternal area of each thoracic segment bears two main sclerites, the basisternite anteriorly and the sternellum posteriorly. These sclerites are separated from each other by the furcal suture. The latter ends into a pit on either side of each segment. These are the roots of the internal sternal apophyses. In higher insect orders, additional sclerites may occur on the sternum of each thoracic segment derived from the two main sclerites named above or from the leg bases.

The notal areas of the pterothoracic segments are structurally complex, especially in winged insects. Generally, the notum is structured in such a way as to allow for wing articulation. Each notum is divisible into two main plates, the scutum anteriorly and the scutellum posteriorly. The two plates are
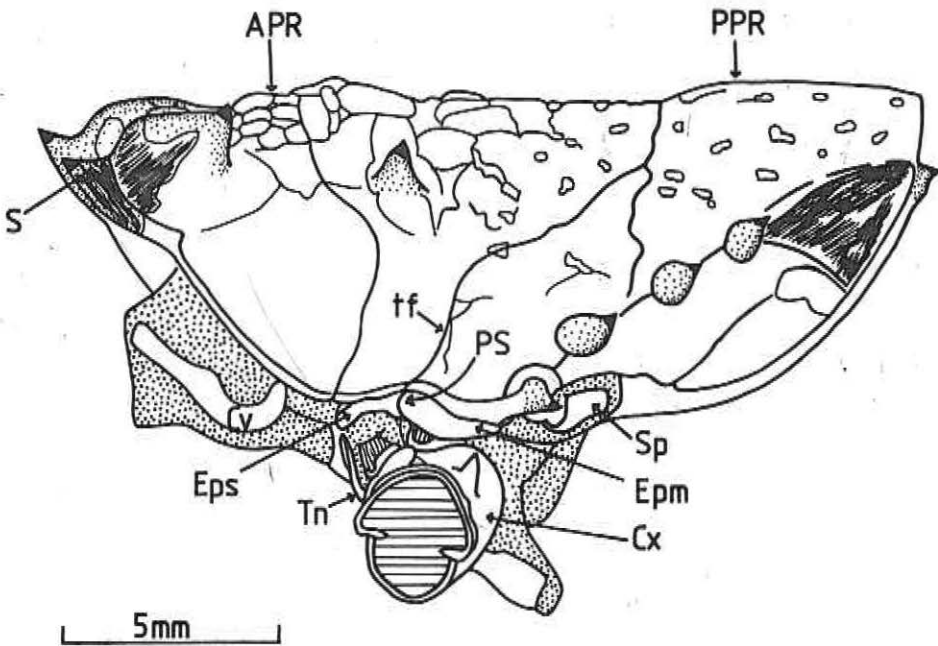
Fig. 27. Lateral view of prothorax

APR, anterior pronotal region; Cx, coxa; Epm, epimeron; Eps, episternum; PPR, posterior pronotal region; PS, pleural suture; S, spine; Sp, thoracic spiracle; tf, transverse pronotal furrow; Tn, trochantin.

Morphological Features of Taxonomic Importance

Large pronotal shield with large spines (S).

separated by a V-shaped suture. The latter is also referred to as the scuto-scutellar suture. Additional smaller sclerites may occur on the nota depending on the insect group and its level of evolutionary advancement.

In *E. matebelensis* (Figs 27–47), the pronotum of both sexes is a large shield that is adorned with large spines. It is divided into anterior and posterior regions by a transverse furrow (Fig. 27, APR, PPR, s, tf). The surface of the pronotal shield is punctate (Figs 27 and 28). The pleural regions of the prothorax bear small plates, the episternum and epimeron, separated by a short pleural suture (Fig. 27, Eps, Epm, PS). The sternal region is small and shield-shaped (Fig. 29). It bears a very narrow rim anteriorly, the presternum (Ps) which is cut off from the rest of the sternal region by a submarginal suture (j). The rest
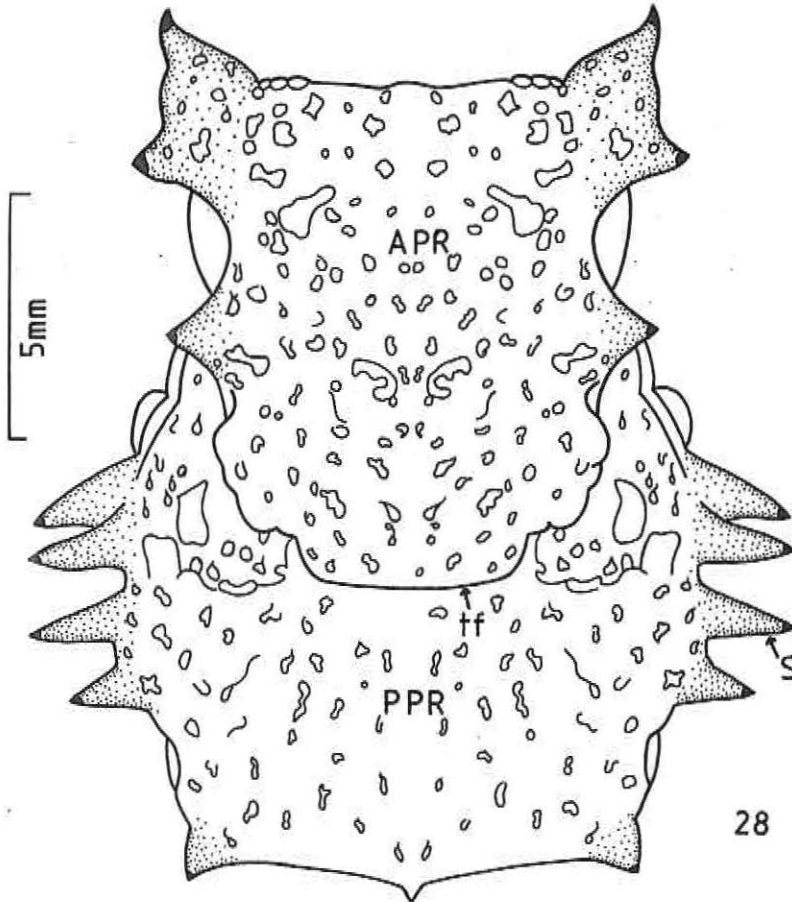
Fig. 28. Dorsal view of the pronotal shield

APR, anterior pronotal region; S, spine; PPR, posterior pronotal region; tf, transverse furrow.

Morphological Features of Taxonomic Importance

- Large pronotal shield with large spines (S).
- Punctate nature of the pronotal shield.
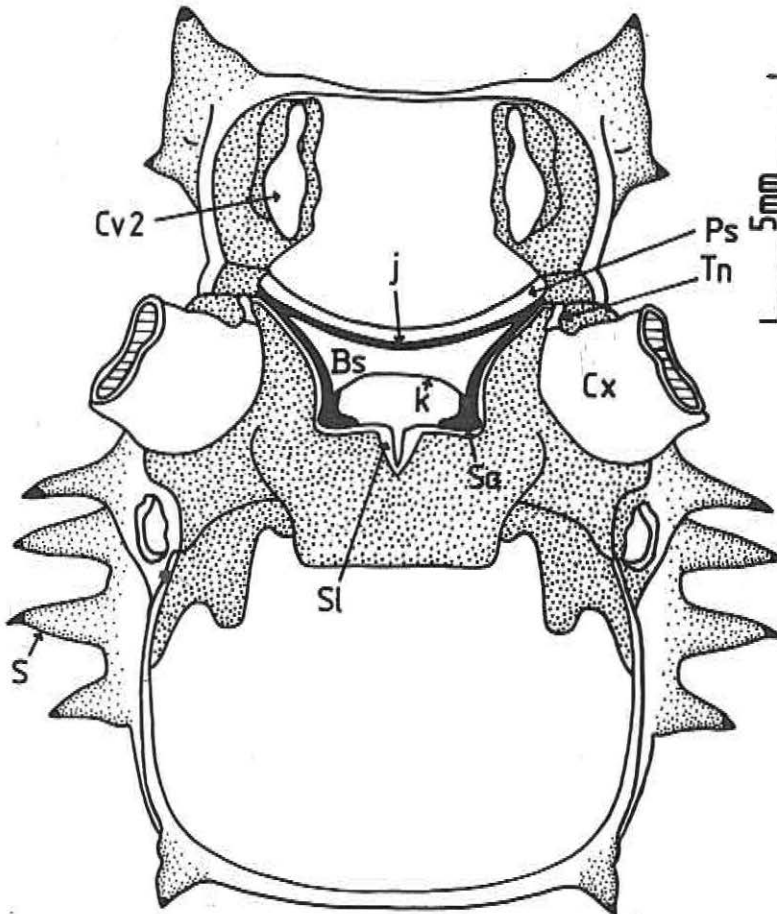
Fig. 29. Ventral view of the prothorax

Bs, basisternite; Cv 2, second cervical sclerite; Cx, coxa; j, submarginal suture; k, furcal suture; Ps, presternum; S, spine; Sa, sternal apophysial pit; Sl, sternellum; Tn, trochantin.

Morphological Features of Taxonomic Importance

Large membranous areas in the sternal region.

of the prothoracic sternum has an anterior basisternite (Bs) and a posterior sternellum (Sl) sclerites that are separated by a furcal suture (k). This suture terminates on either side of the segment laterally, into the roots of the sternal apophyses (Sa), the furcal pits.

Enyaliopsis matebelensis is sexually dimorphic as regards the structure of the pterothorax. Though brachypterous, the mesonotum of the male has the form of a generalized wing-bearing notum of winged insects. This is due to the fact that the mesonotum in the male supports highly reduced tegmina that serve only as sound producing structures (Fig. 30, LSO, RSO). The sound

producing organs are lacking in the females and consequently, the females do not stridulate in this species. The mesonota of the females are simple plates and the tegmina are represented by scale-like pads (Fig. 33).
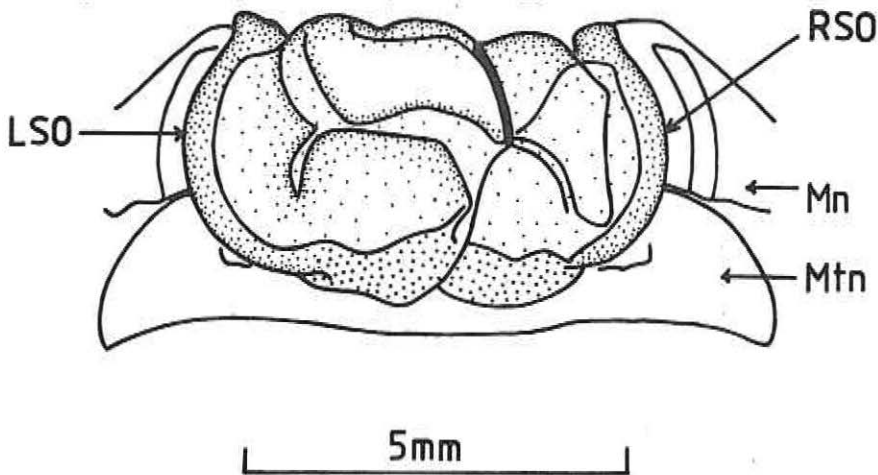
Fig. 30. Dorsal view of the male pterothorax showing the stridulating organs

Each stridulating organ in the male bears a file of cuticular teeth ventrally and a hardened edge called the scrapper or plectrum mesally (Fig. 31, F, Plm). The left stridulating organ overlaps the right at rest and during stridulation. Its cuticular file is longer (mean length, 2.94 mm) and bears a larger number of cuticular teeth (mean number of teeth, 62.1) than the right (mean length, 1.65 mm; mean number of teeth, 33.7).

The mesonotum of the male bears a narrow prescutum anteriorly that is bounded behind by the transverse notal suture (Fig. 32, Prsc, tns). The rest of the mesonotum is divided into a large scutal area and a narrower scutellum (scl). The scutal area is partially divided into two lateral areas (sct) by the scuto-scutellar — or V-shaped suture that separates the scutum and the scutellum. The orientation of this suture on the mesonotum is such that, it is curved anterad at its centre and comes to lie very close to the transverse notal
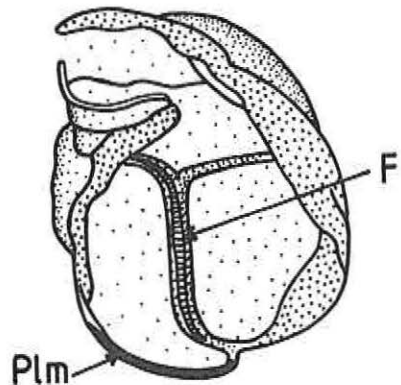
Fig. 31. Ventral view of the left stridulating organ

suture in front. Each lateral scutal area has an anterior (ANP) and a posterior notal process (PNP), with which a stridulating organ articulates with the notum. In winged insects, the notal processes serve as regions for wing articulation. The metanotum in both sexes of E. matebelensis is a simple plate (Figs 32 and 33).
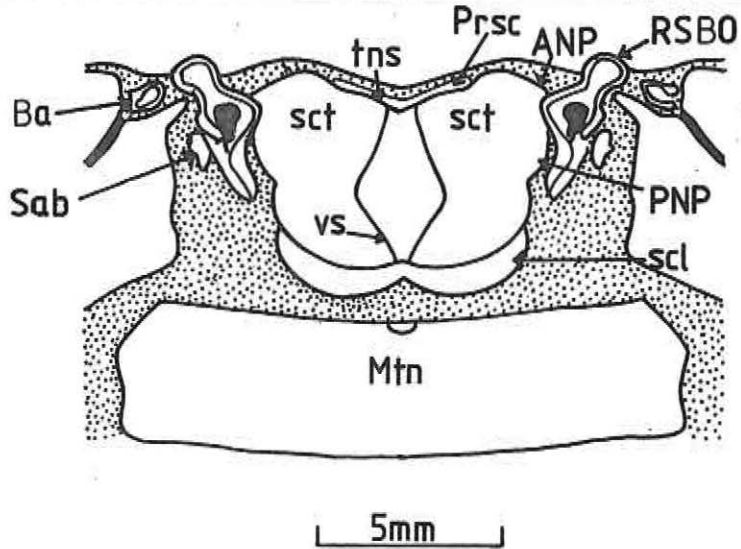
Fig. 32. Dorsal view of the male pterothorax with the stridulating organs excised

ANP, anterior notal process; Ba, basalar; F, tile; LSO, left stridulating organ, Mn, mesonotum; Mtn, metanotum; Plm, plectrum; PNP, posterior notal process; Prsc, prescutum; RSBO, right stridulating organ base; RSO, right stridulating organ; Sab, subalar; scl, scutellum; sct, scutum; tns, transverse notal suture; vs, scuto-scutellar or V-shaped suture.

Morphological Features of Taxonomic Importance

Mesothoracic wings (tegmina) reduced to the level of stridulating organs (RSO, LSO).
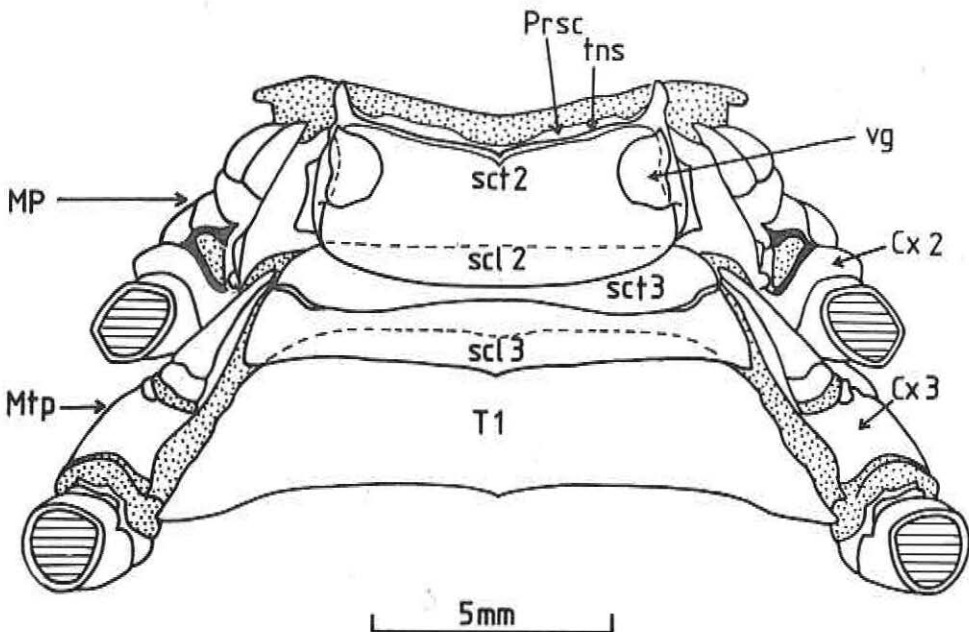
Fig. 33. Dorsal view of the female pterothorax

Cx 2, mesocoxa; Cx 3, metacoxa; Mp, mesopleuron; Mtp, metapleuron; Prsc, prescutum, scl 2, meso-scutellum; scl 3, meta-scutellum; sct 2, meso-scutum; sct 3, meta-scutum; T1, first abdominal tergite; tns, transverse notal suture.

Morphological Features of Taxonomic Importance

- Reduced, scale-like mesothoracic wings (tegmina).

Except for the presence of better developed epipleurites (i.e., the basalar and subalar) in male *E. matebelensis*, the pleural regions of the pterothoracic segments of both sexes are simple (Figs 34 and 35). The pleuron of each thoracic segment has a large episternum (Eps) in front and a large epimeron (Epm) behind which are separated by a pleural suture (PS). Dorsally, especially in the mesothorax, the pleural suture is continuous with the wing process (WP), while ventrally, it terminates into a root of the internal pleural apophysis (Pla). A coxal articulation occurs below this root. Smaller sclerites, the presternum (Ps) and the trochantin (Tn) occur below the episternum.

Fig. 34. Lateral view of the male pterothorax

The sternal regions of the pterothoracic segments in both sexes of *E. matebelensis* bear each a basisternite anteriorly and a sternellum (Fig. 36, Bs, Sl). The plates are separated by the furcal suture (k) and terminate into furcal pits (sa).
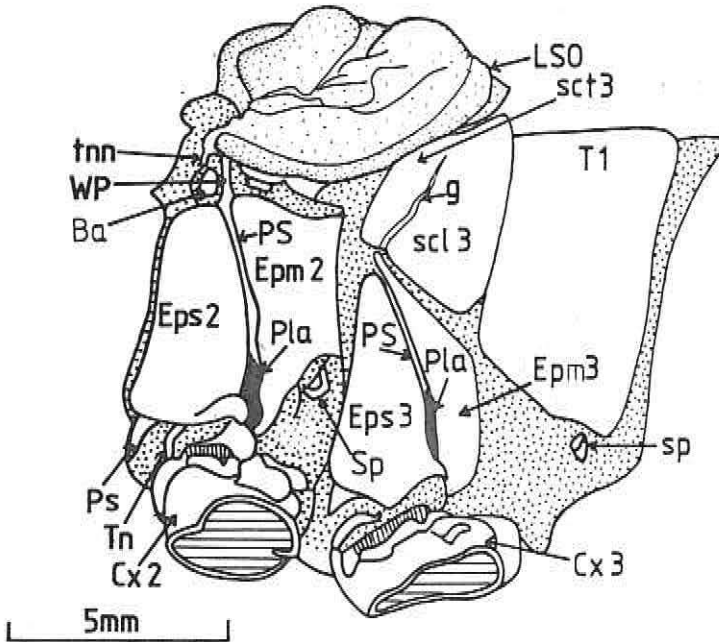
Though generalized in form, the prothoracic legs of the armoured ground cricket, *E. matebelensis* bear specialized structures on them. These are the hearing organs or tympana that are located on the proximal parts of the protibia (Fig. 37, Tym).

B.   Endoskeletal Processes

The principal endoskeletal processes of the thorax of insects are the pleural and sternal apophysial arms. These are special apodemes arising as
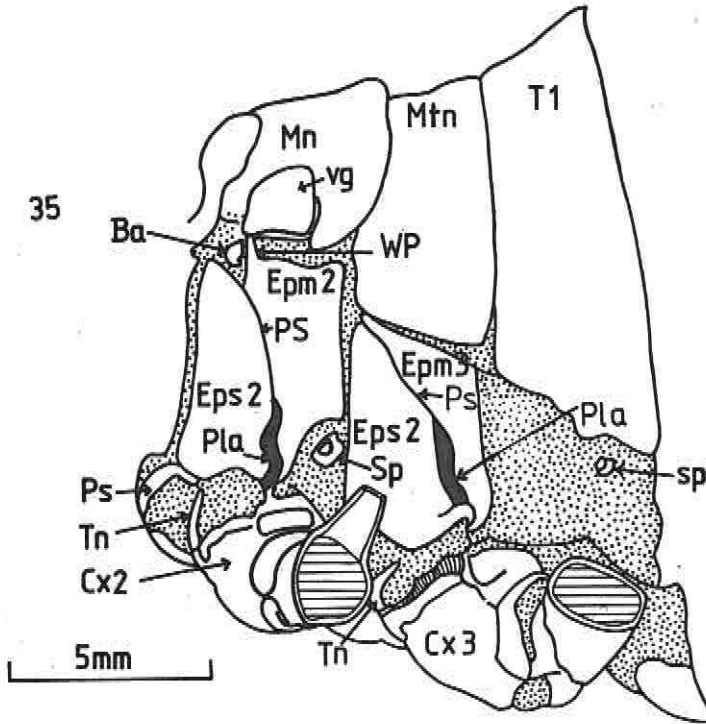
Fig. 35. Lateral view of the female pterothorax

Ba, basalar; Cx 2, mesocoxa; Cx 3, metacoxa; Epm 2, mesepimeron; Epm 3,
metepimeron; Eps 2, mesepisternum; Eps 3, metepisternum; g, line dividing
metanotum into two plates; LSO, left stridulating organ; Mn, mesonotum; Mtn,
metanotum; Pla, root of pleural apophysis; Ps, presternum; PS, pleural suture; scl
3, meta-scutellum; sct 3, metascutum; sp, abdominal spiracle; Sp, thoracic
spiracle; T 1, first abdominal tergite; Tn, trochantin; tnn, tendon; WP, wing process.

Morphological Features of Taxonomic Importance

-   Mesothoracic wings (tegmina) reduced to the level of stridulating organs (LSO)
    in the male.
-   Reduced, scale-like mesothoracic wings (vg) in the female.

invaginations of the thoracic body walls in the pleural and sternal regions,
respectively, of each thoracic segment. In *E. matebelensis* male, there are in
addition two large phragmata on the mesonotum laterally, that project into
the thoracic cavity (Fig. 39, Ph). These are regions on which the tergo-sternal,
tergo-coxal and tergo-pleural muscles that indirectly operate the stridulating
organs originate. The mesonotal phragmata are lacking in the females. In other
insects, especially the winged insects such as bees and wasps (order,
Hymenoptera), various phragmata arise from the meso- and metanota for the
attachment of the principal longitudinal indirect flight muscles.

In the prothorax of the armoured ground cricket (Fig. 38), each half of
the segment bears a large pleural apophysial arm (Pla 1) and a smaller spine-
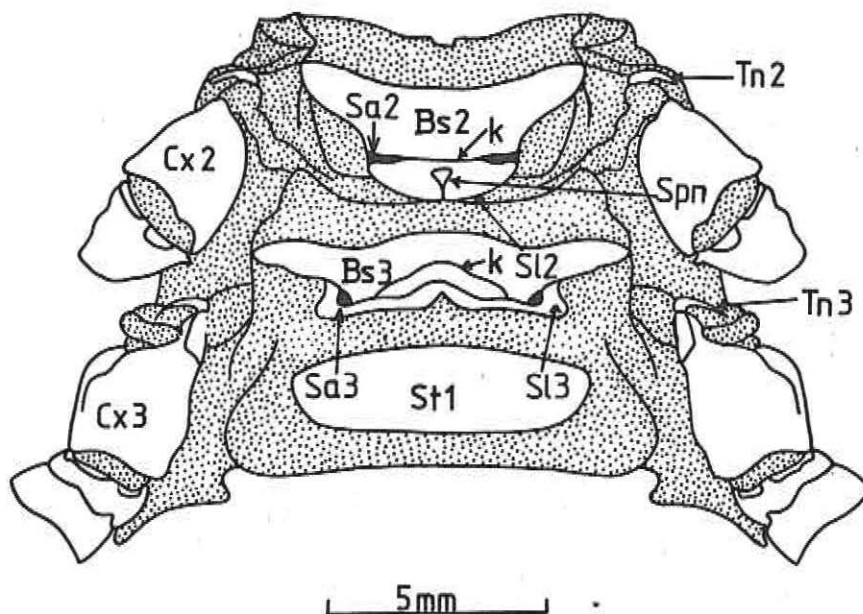
Fig. 36. Ventral view of the pterothorax

Bs 2, meso-basisternite; Bs 3, meta-basisternite; Cx 2, mesocoxa; Cx 3, metacoxa;
Sa 2, mesothoracic furcal pit; Sa 3, metathoracic furcal pit; Sl 2, meso-sternellum;
Sl 3, meta-sternellum; Spn, root of spina; St 1, first abdominal sternite; Tn 2,
mesotrochantin; Tn 3, metatrochantin.

Morphological Features of Taxonomic Importance

-    Large membranous areas in the sternal region.

like sternal apophysial arm (SA 1). The pleural apophysis arises above the
procoxal articulation. It is broad at the base, where it is fused to a short pleural
ridge. It tapers off dorsally into a free head within the thoracic cavity. The
sternal apophyses from the two halves of the segment are linked to each other
by a ridge that is represented externally by the furcal suture.

The pleural areas of the pterothoracic segments are generally similar to
each other, in both sexes of the armoured ground cricket except, for the sizes
of the apophysial arms (Figs 40 & 41). Each half of the meso- and metathoracic
segments has a pleural apophysis (PlA) that is continuous with the pleural
ridge (PlR) and abuts to the dorsal surface of the sternal apophysis (SA), across
the coxal opening. The mesothoracic pleural apophysial arms in the male (Fig.
40) however, are more expanded in the regions of their abutment than in the
female (Fig. 41). This is due to the presence in the male of large tergo-pleural
muscles that serve as the main closing muscles of the stridulating organs. The
tergo-pleural muscles originate from the mesonotal phragmata mentioned
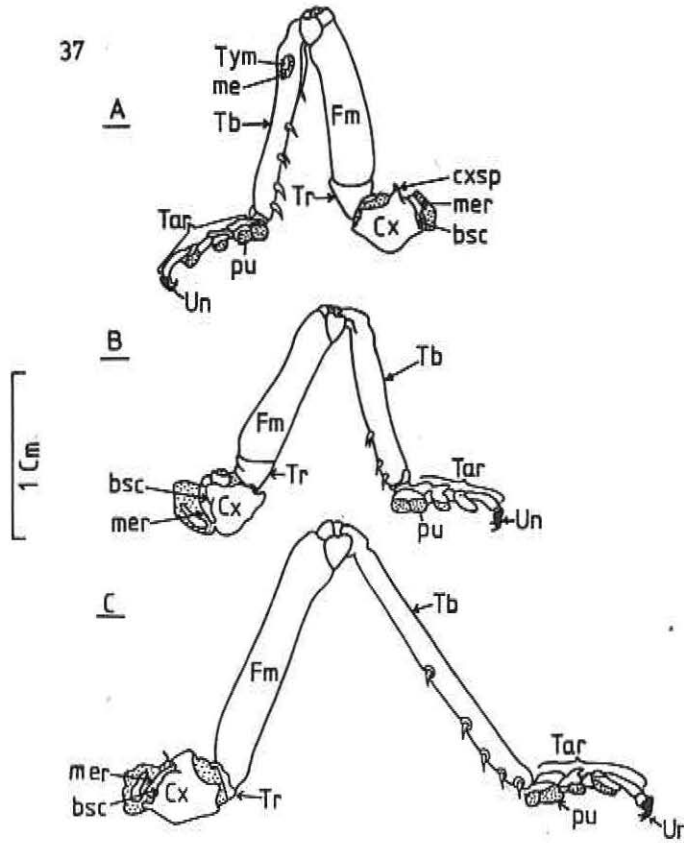
Fig. 37. The legs

a. The foreleg

b. The midleg

c. The hindleg

> bsc, basicoxal suture; Cx, coxa; cxsp, coxal spine; Fm, Femur; me, auditory membrane; mer, meron; pu, pulvillus; Tar, tarsus; Tb, tibia; Tr, trochanter; Tym, tympanum; Un, claw.

Morphological Features of Taxonomic Importance

- Presence of an ear or tympanal organ (Tym) on the tibia of the proleg (A).

before and insert on the dorsal expanded surfaces of the mesopleural apophyses. These muscles are wanting in the female.

The sternal region of each pterothoracic segment bears a pair of sternal apophysial arms that arise at the ends of the furcal ridge and abut to the pleural apophysial arms already described above.
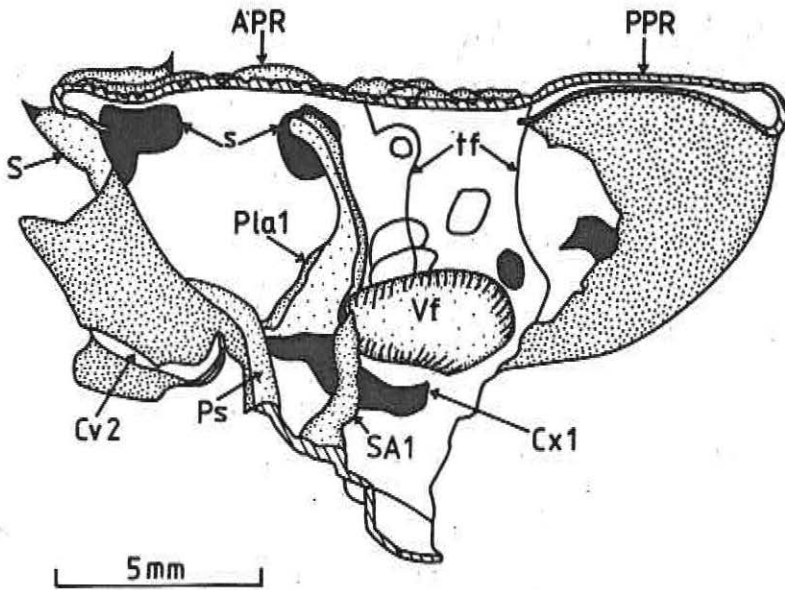
Fig. 38. Lateral internal view of the prothorax

Exercises

Materials Required:

Specimens:
Freshly anaesthetized or preserved (in 70% ethanol) adult:

1. Desert locust, *Schistocerca gregaria* Forskal or the garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper

2. Bush cricket, *Liogryllus bimaculatus* (Orthoptera, Gryllidae).

3. African honeybee, *Apis mellifera adansonii* or *A.m. capensis* (Hymenoptera, Apidae).

4. Stink bug, *Nezara viridula* L. (Hemiptera, Pentatomidae).

5. The citrus swallowtail or orange dog, *Papilio demodocus* Esper (Lepidoptera, Papilionidae) or the African armyworm, *Spodoptera exempta* Wlk. (Lepidoptera, Noctuidae).

6. Stick insect, *Bactrododema* sp. (Orthoptera, Phasmatidae).
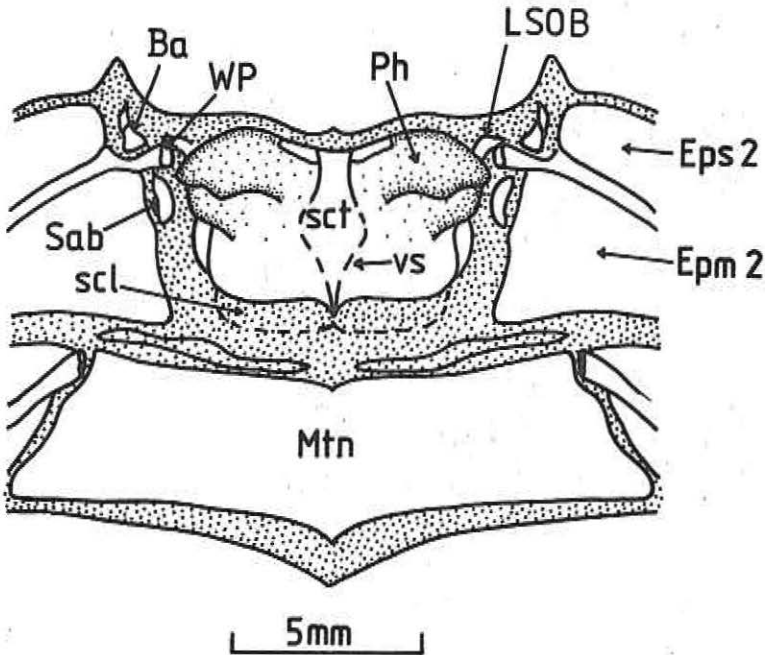
Fig. 39. Internal view of the male meso- and metanota

APR, anterior pronotal region; **Ba**, basalar; **Cv 2**, second cervical sclerite; **Cx 1**, procoxa; **Epm 2**, mesepimeron; **Eps 2**, mesepisternum; **LSOB**, left stridulating organ base; **Mtn**, metanotum; **Ph**, phragma; **Pla 1**, prothoracic pleural apophysis; **PPR**, posterior pronotal region; **Ps**, presternum; **s**, root of pronotal spine; **S**, spine; **SA 1**, prothoracic sternal apophysis; **Sab**, subalar; **scl**, scutellum; **sct**, scutum; **tf**, transverse ridge; **Vf**, vesicula femoralis; **vs**, scuto-scutellar or V-shaped suture; **WP**, wing process

Morphological Features of Taxonomic Importance

- Large air sac (Vf) and large pleural apophysial arm (Pla 1) in the prothorax.
- Large phragmata (Ph) projecting into the thoracic cavity on the mesonotum in the male.

7. Mole cricket, *Gryllotalpa africana* (Pal.) (Orthoptera, Gryllotalpidae).

8. Praying mantis, *Pseudocreobotra wahlbergi* (Stål) (Orthoptera, Mantidae).

9. American cockroach, *Periplaneta americana* (L.) or Oriental cockroach, *Blatta orientalis* (L.) (both belonging to Orthoptera, Blattidae) or the German cockroach, *Blattella germanica* (L.) (Orthoptera, Blattellidae).

10. Coffee capsid, *Lamprocapsidea coffeae* (China) or cotton lygus, *Taylorigus vosseri* (Popp.) (both belonging to Hemiptera, Miridae) or the leaf-footed plant bug (= squash bug), *Leptoglossus australis* (F.) (Hemiptera, Coreidae).
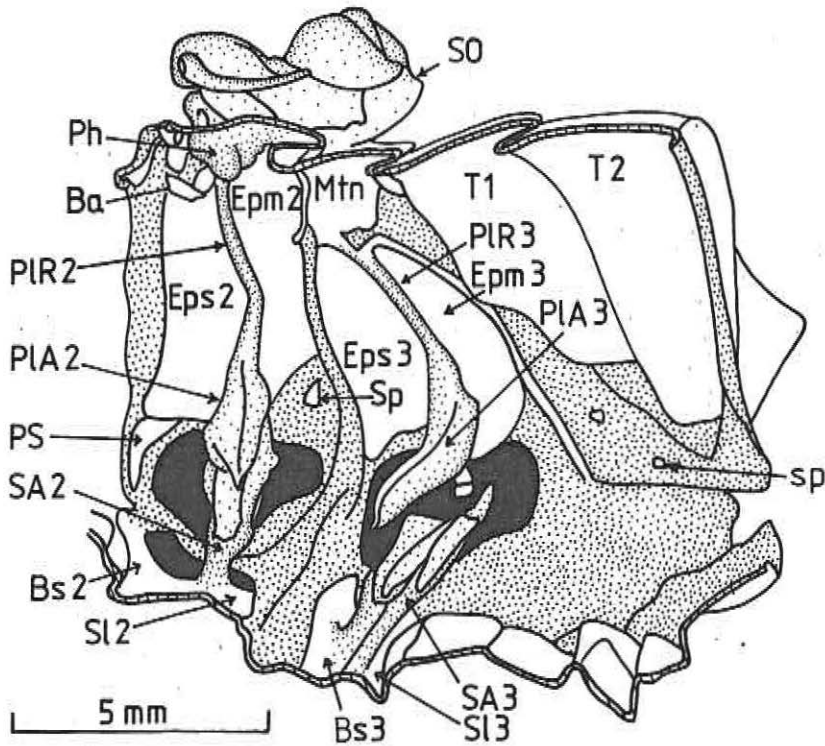
Fig. 40. Lateral view of the male pterothorax and the first two abdominal segments

Apparatus:
1. Dissecting dish with beeswax at the bottom.
2. Dissecting set.
3. Minuten insect pins (Nos 1 & 5).
4. Binocular microscope.

Chemicals:
1. 10% ethanol.

Exercise 1: **Lateral View of the Thorax.**

1. Clip off the wings of an anaesthetized or preserved locust or grasshopper near their bases, using a pair of scissors.

2. Cut off the legs and wings near their bases and then mount the specimen on beeswax in the dissecting dish with its left side of the body uppermost and its head directed to the right of the dissecting dish.
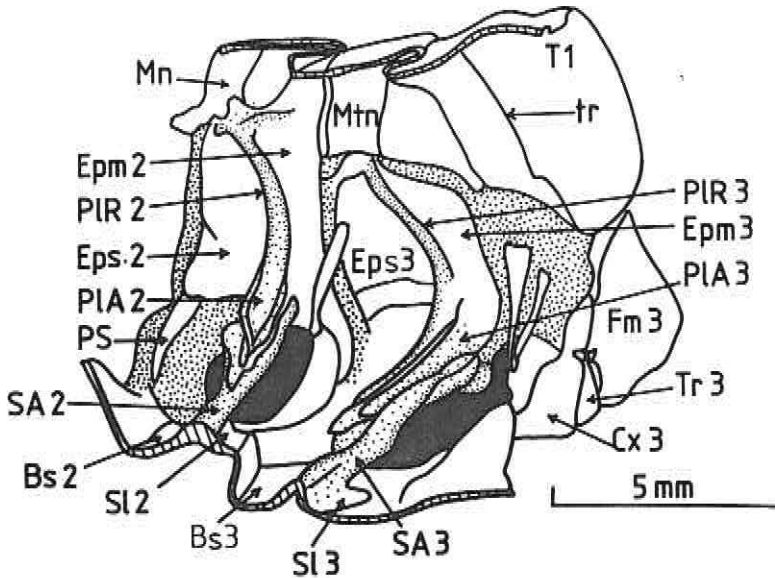
Fig. 41: Lateral view of the female pterothorax and the first abdominal segment

Ba, basalar; Bs 2, meso-basisternite; Bs 3, metabasisternite; Cx 3, metacoxa; Epm 2, mesepimeron; Epm 3, metepimeron; Eps 2, mesepisternum; Eps 3, metepisternum; Fm 3, metafemur; Mn, mesonotum; Mtn, metanotum; Ph, phragma; PIA 2, mesothoracic pleural apophysis; PIA 3, metathoracic pleural apophysis; PIR 2, mesothoracic pleural ridge; PIR 3, metathoracic pleural ridge; PS, presternum; SA 2, mesothoracic sternal apophysis; SA 3, metathoracic sternal apophysis; SI 2, mesothoracic sternellum; SI 3, metathoracic sternellum; So, stridulating organ; sp, abdominal spiracle; Sp, thoracic spiracle; T 1,T 2, abdominal terga.

Morphological Features of Taxonomic Importance

- More expanded pleural (Pla 2) and sternal (SA 2) apophysial arms in the mesothorax of the male than in the female.

Exercise 1: Continued

3. Flood the dissecting dish with 10% ethanol until the specimen is submerged.

4. Examine the pleural region of the thorax under the binocular microscope, noting the number, shapes and sizes of the sclerites, pits, conjuctiva and leg articulation of each segment.

5. Make a large, well-labelled diagram of the pleural region of the thorax of your specimen.

Exercise 2: Sternal Region of the Thorax.

1. Remount the specimen you have used in Exercise 1, right-side-down such that the sternal area is uppermost and the head is directed towards the top of the dissecting dish.

2. Study the sclerites constituting the venter of each thoracic segment, the sutures separating them and the positions of the furcal pits.

3. Draw and fully label the whole sternal area of the thorax of your specimen.

Exercise 3: The Notum of a Wing-Bearing Segment.

1. Use a fresh locust or grasshopper for this exercise.

2. Clip off the wings near their bases as in Exercise 1.

3. Cut off the notum of the mesothorax along the pleural regions of the segment on either side and through the dorsal intersegmental membranes.

4. Spread the excised mesonotum with its wing stubs uppermost on beeswax in the dissecting dish and pin.

5. Submerge the specimen in 10% ethanol and then examine it very carefully under the binocular microscope and identify the following structures and sutures;
    PRESCUTUM
    SCUTUM
    SCUTELLUM
    ANTERIOR NOTAL PROCESS
    POSTERIOR NOTAL PROCESS
    TRANSVERSE NOTAL SUTURE
    SCUTO-SCUTELAR SUTURE
    POSTALLAR BRIDGE

6. Make a large, clean and well - labelled drawing of your specimen illustrating these structures and sutures.

Exercise 4: **Thoracic Appendages.**

1. Remove the meso- and metathoracic wings of a locust or grasshopper by detaching them carefully from their membranous bases. Spread them on wax in the dissecting dish. Identify all major longitudinal veins and cross veins. Draw and label.

2. Remove the pro-, meso- and metathoracic legs of your specimen from one side of the body by detaching them from the body around their coxal articulations. Place the legs carefully on beeswax in the dissecting dish. Use minuten insect pins to hold them in preferred positions on the wax and then submerge them in 10% ethanol. Identify the different segments of each leg and then draw and label.

3. What are the differences in form, structure and function of the three legs?

4. How does the metathoracic leg of your specimen differ from that of the armoured ground cricket, *E. matebelensis* described in the introduction to this section? What is the significance of this difference in terms of the niches the two insect species occupy?

Exercise 5: **Pterothoracic Variations.**

1. Examine the pleural regions of the winged segments of the following insects under the binocular microscope noting the shapes and sizes of the sclerites, sutures and pits.
   a.   Bush cricket
   b.   Honeybee
   c.   Stink bug, and
   d.   Stick insect

2. Draw and fully label the pleural region of the thorax of each of the above specimens.

3. How do the pleural regions you have just drawn differ from each other and from that of a generalized insect such as *E. matebelensis?* What do you think is the biological significance of these differences?

Exercise 6: **Variations in Wing Structure and Venation.**

1. Remove both wings from one side of the body of a;
    a. Dung beetle
    b. Plant bug (coffee capsid, cotton lygus or leaf-footed plant bug)
    c. Butterfly or moth (citrus swallowtail or African armyworm), and
    d. African honeybee.

2. Spread both wings of each specimen on wax in the dissecting dish, in such a way that they assume a normal relationship to each other as they would *in situ* and then pin them.

3. Submerge the specimens under 10% ethanol and then examine each wing pair thus prepared under the binocular microscope. Observe the arrangements of the veins and distinguish longitudinal and cross-veins. Draw and label.

4. What variation do the wings of these insects exhibit? How do the wings differ from the generalized condition in terms of size, function and relationship to each other?

5. What unique structures occur on the leading edge of the hind wings of the honeybee and the anal angles of the fore wings or the humeral angles of the hind wings of the butterfly or moth that improve their efficiency in flight?

6. What specialized structures have you observed underneath the forewings of the bush cricket? What are their functions and how are these achieved?

Exercise 7: **Leg Variations.**

1. Examine under a binocular microscope a detached;
    a. Foreleg of a mole cricket,
    b. Foreleg of a preying mantis,
    c. Hindleg of a cockroach,
    d. Hindleg of a grasshopper or locust, and
    e. Hindleg of a honeybee.

2. Draw and label each leg specimen.

3. What kind of modification from the generalized condition does each of your specimens show?

Exercise 7: Continued

4.   How are the modifications you have noted in your specimens best suited for the modes of life that have been adapted by the insects from which the specimens were obtained?

Supplementary Questions

1.   In your view, why should there be variation in the shape, structure and location of sclerites in the pterothorax of insects?

2.   How does the nature of the insect thorax influence the efficiency of wings in flight?

3.   Using a selected group of insects, show how wing venation has been used taxonomically to separate its members.

4.   What kinds of specialized structures associate with legs of the following insects;
     a.   grasshoppers
     b.   the honeybee
     c.   longhorned grasshoppers belonging to the family Tettigoniidae and the true crickets (Orthoptera, Gryllidae).

5.   What is the origin of insect wings and how did the development of these structures in the class Insecta affect the ability of its members to occupy terrestrial niches in the biosphere?

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SECTION III

Section IV

The Insect Abdomen

A. External Morphology

The abdominal tagma comprises 11 definitive segments plus an end-piece termed the telson in the generalized condition. However, the 11th segment is usually reduced so that the maximum number of definitive segments in the generalized insect abdomen rarely appears more than 10. In other primitive insects such as the desert locust, *Schistocerca gregaria* Forskal and especially in the higher insect orders such as Diptera and Hymenoptera, this number is even further reduced by either the fusion that has occurred in some abdominal segments during their evolution or simply due to the telescoping of the more posterior abdominal segments into the anterior ones. For convenience in their description, the abdominal segments can be divided into three groups namely, the pregenital or visceral segments, the genital segments and the postgenital segments.

Abdominal segments 1–8 in the male insect and 1–7 in the female constitute the pregenital or visceral segments. As the collective names for the segments imply, these segments carry the principal viscera of the insect body and are located anterad to those abdominal segments on which the internal reproductive organs open to the outside and which bear the external reproductive organs. The pregenital segments are also important in the production of the main ventilatory movements of the body. Abdominal segments 8 and 9 in the female and only abdominal segment 9 in the male are the genital segments. Finally, abdominal segments 10 and 11 plus the telson are the postgenital segments in both sexes of insects.

In the armoured ground cricket, *Enyaliopsis matebelensis* Peringuey the visceral abdominal segments are generalized. Each bears a large tergum and a smaller sternum (Fig. 42, T, St). The pleural regions are membranous and abdominal segments 1–8 in both sexes bears each a pair of spiracles, one on either side of the segment, dorsolaterally.

The genital segment of the male bears a large tergum and its sternal region is modified into a large triangular subgenital plate that houses the external reproductive organs (Figs 42 & 43, sgp, Gc). The pleural regions of the male genital segment are membranous but form in the intersegmental regions between the 9th and 10th abdominal segments, the phallic organ. The latter

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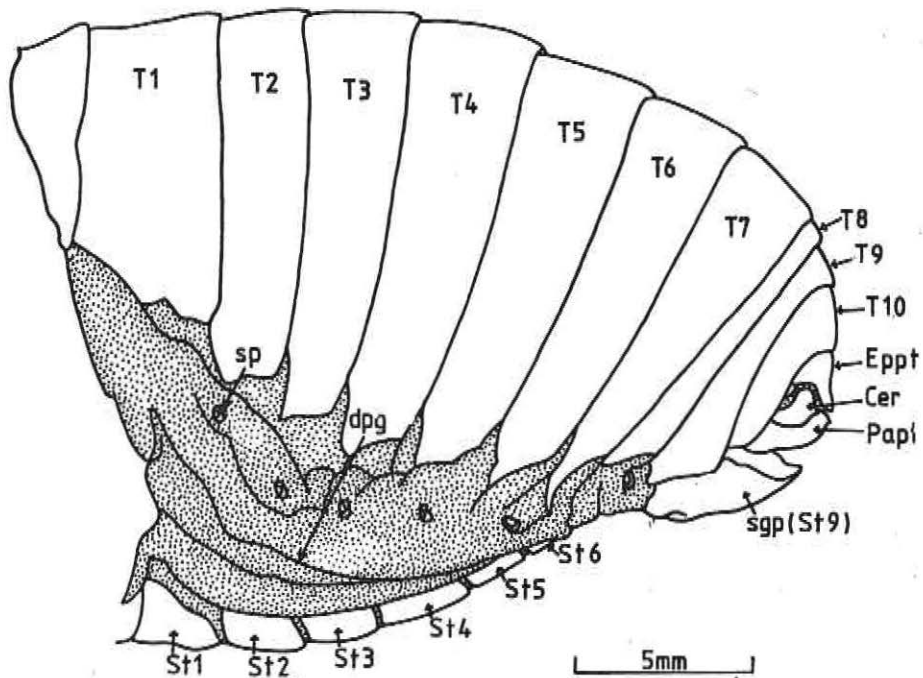
Fig. 42. Lateral view of the male abdomen

Cer, cercus; dpg, dorsopleural groove; Eppt, epiproct; Papt, paraproct; sgp (St 9), subgenital plate; St1–St6, abdominal sterna; T1 – T10, abdominal terga.

Morphological Features of Taxonomic Importance

-   Generalized structure of the abdominal segments each comprising a large tergum (T), sternum (St) and two membranous pleural regions.

(not illustrated) comprise four phallomeres that are separate phallic lobes. They include a pair of lateral phallic lobes, a dorsal and ventral phallic lobes. These phallomeres fold together around the opening to the internal reproductive organs, the phallotreme. They specialize in moulding spermatophores important in reproduction.

The prominent external feature of the female's genital segments is the ovipositor. In the female armoured ground cricket this comprises three pairs of blades or valves that work in unison to loosen soil during oviposition (Fig. 44). The ventral (Vl 1) and dorsal (Vl 3) ovipositor blade pairs are spined, while the inner blade pair (not shown in the diagram) is reduced and sandwiched between the dorsal pair.

The ovipositor blades arise from small triangular pleurites of the 8th and 9th abdominal segments. The ventral ovipositor blade pair arises from the 1st valvifer pleural sclerites of the 8th genital segment that are located one

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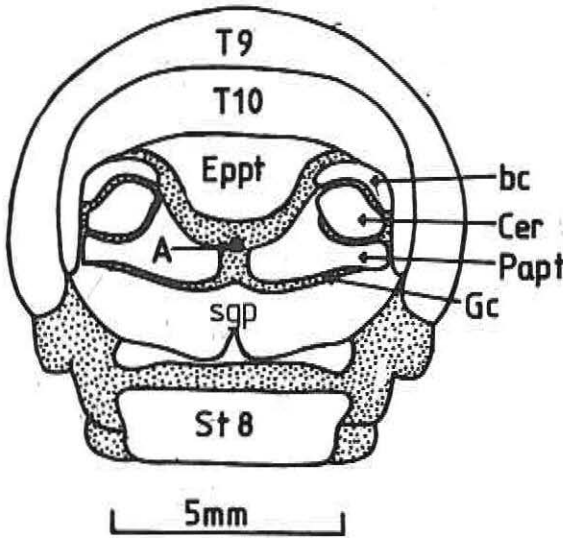
Fig. 43. Posterior view of the male genital and postgenital segments
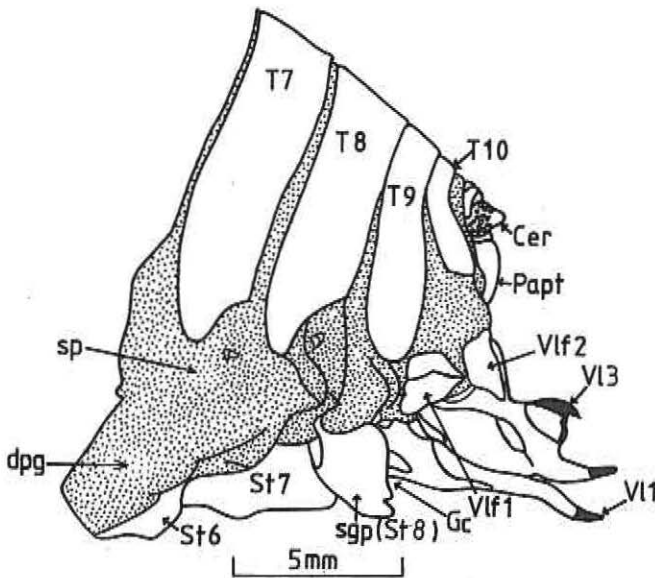
on either pleuron of the segment, while the inner and dorsal ovipositor blade pairs are both formed by the 2nd valvifer pleural sclerites of the 9th genital segment. Each 2nd valvifer, located one to each pleural region of the 9th abdominal segment forms one inner and one dorsal ovipositor blade. The arrangements of the valvifers on the female's body in the adult insects however, can be a bit confusing, especially to an amateur insect morphologist. This is so because the two small sclerites have moved backward on the body during the evolution of many insects such that the 1st valvifer now lies below the 9th tergum and the 2nd valvifer lies below the 10th tergum on either side of the body and they both appear to be parts of those segments. However, the shifting in positions of these pleurites can be observed during embryonic development.

The sternal region of the 8th abdominal segment in the female forms a subgenital plate (sgp), while that of the 9th segment is membranous and forms the dorsal wall of the genital chamber (Gc).

Fig. 44. Lateral view of the female terminal abdominal segments

The postgenital segments in both sexes bear membranous sternal areas. The 10th tergum is reduced and posteriorly fused with the 11th tergum. The latter is a triangular plate, called epiproct (Figs 43 & 45, Eppt). Two appendages arise from the pleural regions of the 11th segment namely the cerci (Cer) and paraprocts (Papt).
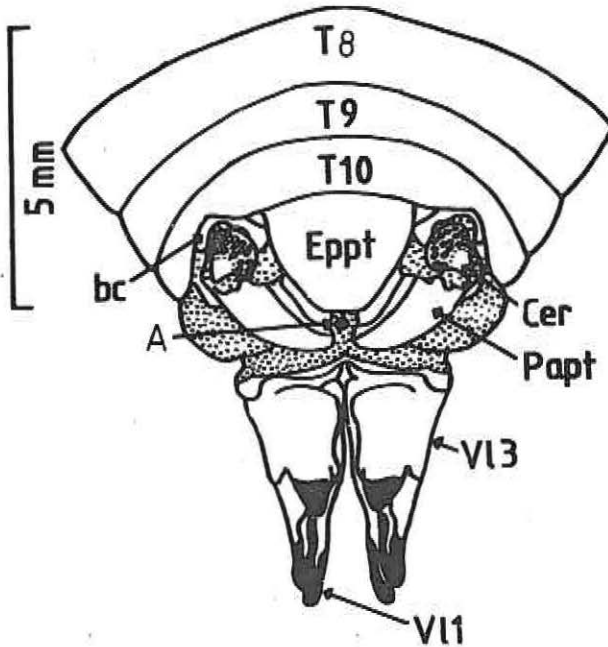
B.   Endoskeletal Processes

The visceral abdominal segments of both sexes in many insect groups including the armoured ground cricket, do not bear prominent endoskeletal processes except, for antecostas which provide attachment of the principal longitudinal muscles. The tergites of the 9th and 10th segments in male *E. matebelensis* (Fig. 46) and those of the 8th to the 11th abdominal segments in the female (Fig. 47) however, bears strongly developed antecostas.

Fig. 45. Dorsal view of the female terminal abdominal segments

A, anus; bc, basicercus; **Cer**, cercus; dpg, dorsopleural groove; **Eppt**, epiproct; Gc, genital chamber; **Papt**, paraproct; sgp, sgp (St 8), subgenital plate; sp, abdominal spiracle; St6 – St8, abdominal sterna; T7 – T10, abdominal terga; VI 1, first ovipositor blade; VI 3, third ovipositor blade; VIf 1, first valvifer; VIf 2, second valvifer.

Morphological Features of Taxonomic Importance

- The presence of highly sclerotized ovipositor in the female (VI 1, VI 2, VI 3).

Important muscles that operate the external reproductive organs, cerci, paraprocts and the epiproct in both sexes take their origins from these strongly developed antecostas.

In addition in the female, the antecosta of the 8th genital segment articulates ventrally on either side of the segment with the 1st valvifer (Fig. 47, a, VIf 1). The 1st and 2nd valvifers in the female bear cuticular processes that project anteriorly into the abdominal cavity venterolaterally (Fig. 48, VIf 1, VIf 2).
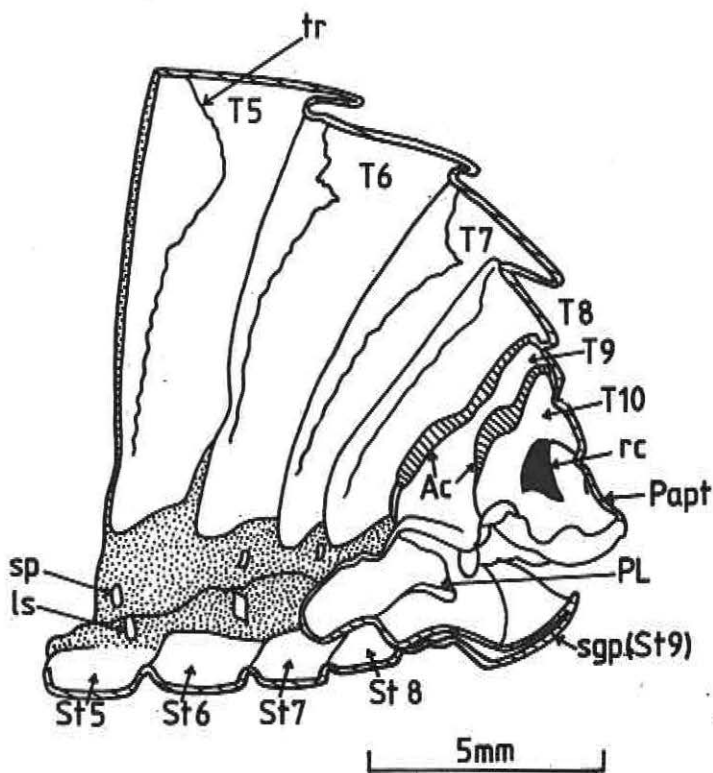
Fig. 46. Internal lateral view of male terminal abdominal segments

Exercises

Materials Required:

Specimens:
Freshly anaesthetized or preserved (in 70% ethanol) adult;

1. Desert locust, *Schistocerca gregaria* Forskal or garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper (Orthoptera, Acrididae).

2. Edible longhorned grasshopper, *Homorocoryphus nitidulus vicinus* Wlk (Orthoptera, Tettigoniidae).

3. Stink ant, *Paltothyreus tarsatus* (Fabricius) or harvester ant, *Messor barbarus* L. (Hymenoptera, Formicidae).
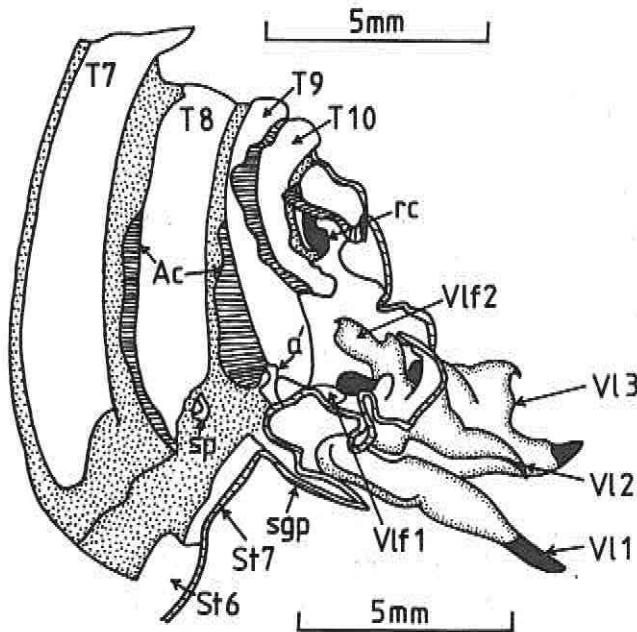
Fig. 47. Internal lateral view of the female terminal abdominal segments

a, articulation of antecosta with first valvifer; Ac, antecosta; ls, laterosternite; Papt, paraproct; PL, phallic lobe; rc, root of cercus; sgp, sgp (St 9), subgenital plate; sp, abdominal spiracle; St5 – St8, abdominal sterna; T5 – T10, abdominal terga; tr, tergal ridge; Vl 1, first ovipositor blade; Vl 2, second ovipositor blade; Vl 3, third ovipositor blade; Vlf 1, first valvifer; Vlf 2, second valvifer.

Morphological Features of Taxonomic Importance

- Strongly developed antecostas (Ac) in both sexes.

4. Darner dragonfly, *Anax speratus* Hagen (Odonata, Aeshinidae) or the skimmer dragonfly, *Brachythemis leucostica* (Burm.) (Odonata, Libellulidae).

5. American cockroach, *Periplaneta americana* (L.) (Orthoptera, Blattidae) or the Oriental cockroach, *Blatta orientalis* (L.) (Orthoptera, Blattidae) or the German cockroach, *Blattella germanica* (L.) (Orthoptera, Blattellidae) or the wood cockroach *Gyna maculipenis* (Schaum.) (Orthoptera, Blattidae).

6. Earwig, *Labidura riparia* (Beauv.) (Dermaptera, Labiduridae).

7. Parasitic wasp (Hymenoptera, Ichneumonidae).

8. Bush cricket, *Liogryllus bimaculatus* or the tobacco cricket, *Bracytrupes membranaceus* (Drury) (Orthoptera, Gryllidae).
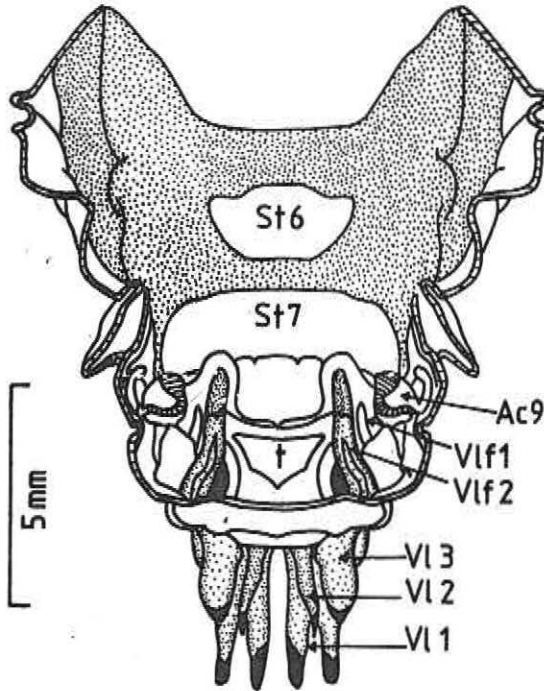
Fig. 48. Dorsal internal view of the female terminal abdominal segments

Ac 9, ninth antecosta; St6 – St7, abdominal sterna; t, dorsal wall of genital chamber; Vl 1, first ovipositor blade; Vl 2, second ovipositor blade; Vl 3, third ovipositor blade; Vlf 1, first valvifer; Vlf 2, second valvifer.

Morphological Features of Taxonomic Importance

- Strongly developed second valvifers (Vlf 2) projecting as apodemes into the abdominal cavity for muscle attachment.

9. African honeybee, *Apis mellifera adansonii* (L.) or *A.m. capensis* (L.) (Hymenoptera, Apidae).

10. House fly, *Musca domestica* L. (Diptera, Muscidae)

Apparatus:
1. Dissecting dish with beeswax at the bottom.
2. Dissecting set.
3. Minuten insect pins (No. 3).
4. Binocular microscope.

Chemicals:
1. 10% ethanol

Exercise 1: **Generalized Insect Abdomen.**

1. Mount a male and female locust or grasshopper, whose wings and legs have been clipped off, in wax with their left sides uppermost and the heads directed towards the right side of the dissecting dish.

2. Examine the abdomens of your preparations under a binocular microscope noting the sclerites, membranes and spiracles and then draw.

3. How do the abdomens of your specimens differ from those of the two sexes of the armoured ground cricket, *E. matebelensis*?

Exercise 2: **The Ovipositor.**

1. Using the female specimen in Exercise 1 above, cut off the posterior region of the abdomen around the 6th segment.

2. Bisect the posterior end of the abdomen longitudinally through the midlines of the dorsum and venter to expose a set of ovipositor blades from the internal side of each half of the body.

3. Mount one half of the abdomen on wax with its cutup face uppermost and pin.

4. Submerge the specimen in the dissecting dish in 10% ethanol and study the ovipositor blades under the binocular microscope noting their shapes and sizes.

5. What unique features occur on the blades of the ovipositor of your specimen? What are their functions?

Exercise 3: **The Male Reproductive Organs.**

1. Cut off the posterior part of the abdomen of a preserved or freshly anaesthetized male locust or grasshopper around the 6th segment.

2. Mount the excised abdominal tip on wax such that the posterior end of the abdomen is uppermost or place the abdominal tip on its side with the left side uppermost.

3. Study the external reproductive organs under the binocular microscope identifying all visible parts and draw.

Exercise 3: Continued

4. Repeat the exercise but this time use a bush cricket or the longhorned edible grasshopper *Homorocoryphus nitidulus vicinus.*

5. How do the external reproductive organs of the two specimens you have studied differ from each other?

6. How do these structures differ from those of the armoured ground cricket, *E. matebelensis*?

7. What is an aedegus? What are its functions? In what type of insects is it found and what structure in the longhorned grasshoppers performs a similar function and how?

Exercise 4: **Mode of Connection of the Abdomen to the Thorax.**

1. Examine the modes of connection of the abdomen to the thorax of the following insects under a binocular microscope and draw;
    a. a locust or grasshopper, and
    b. an ant

2. What differences are there in the two specimens?

3. What is the significance of a petiole in the ant?

4. What is a propodeum? What advantages does the presence of this structure confer in those insects bearing it?

Exercise 5: **Variations in Ovipositor Structure.**

1. Study ovipositors of the following insects under the microscope and then make large, clear diagrams of the structures to illustrate differences among them
    a. a parasitic wasp (Hymenoptera:Ichneumonidae)
    b. a bush cricket
    c. a house fly, and
    d. a honeybee.

2. What parts of the generalized ovipositor have been modified in your specimens?

3. What kind of substrata do you think your specimen is specialized to oviposit in?

Exercise 5: Continued

    4.   Is it really necessary for the ovipositor blades to be sclerotized in insects? Give reasons for your answer.

Exercise 6: **Variations in the Structure of Cerci.**

    1.   Examine the cerci of the following insects and then draw and label;
       a.   a dragonfly
       b.   a cockroach, and
       c.   an earwig.

    2.   What are the differences in the structure of the cerci in these insects?

    3.   What additional functions do the modified cerci of your specimens perform?

Supplementary Questions

1.   Males of primitive insects like those of longhorned grasshoppers bear phallic lobes as their external reproductive organs. Describe how these structures are used during spermatophore formation prior to or during copulation.

2.   What types of structural variations are found in the aedegus of a selected and named group of insects? How is this intromitent organ used to affect internal fertilization by the group?

3.   Why is the structure of the aedegus an important taxonomic feature in insects? Show how it has been used to separate individuals or groups of individuals in a selected insect order.

4.   The house fly has totally done away with the ovipositor as exhibited in the generalized condition as obtains in armoured ground crickets. What structure replaces the generalized ovipositor in the house fly and how does it function during oviposition?

5.   A bee sting is a modified ovipositor. How then do bees oviposit their eggs if the ovipositor is used as a stinging apparatus?

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Section V

A. The Dorsal Blood Vessel

Insects possess an open circulatory system comprising, a dorsal blood vessel and the haemocoelic cavities. The dorsal blood vessel consists of a segmented heart and an undifferentiated aorta. As the name implies, the dorsal blood vessel occurs on the dorsal aspects of the body. The segmented heart runs from around the 8th abdominal segment medially, to about the metathoracic segment just below the terga, while the aorta continues from there to the anterior side of the brain in the head. Blood entering the heart in the abdomen is pumped forward by the pulsating activities of the heart ampullae through fan-like alary muscles that are attached to them along the length of the segmented heart. In front of the brain, the blood is poured onto tissues where it percolates downwards into the haemocoelic cavities and moves backwards in the body until it is again picked up by the heart in the abdomen. The speed at which insect blood moves in the haemocoelic cavity depends on the general muscular activity of the insect body.

Unlike the vertebrates, insects can afford such a slow type of circulatory system, because insect blood or haemolymph is not involved in gaseous exchange. The latter in insects occurs through a system of internal tubules called tracheae. In addition to the segmented heart and the aorta, some insects have accessory circulatory structures in their circulatory systems. Phagocytic and segmental vessels are such accessory structures which help speed up the circulation of haemolymph in certain parts of the body in some orthopteroid subfamilies. In representatives of some higher insect orders, pulsating organs (another type of accessory circulatory structure) occur at the bases of the appendages (legs and wings) and in the thorax.

In the armoured ground cricket, *Acanthoplus speiseri* Brancsik, the dorsal blood vessel is associated with 12 pairs of alary muscles (Fig. 49, Am), that arise dorsolaterally on each tergite and attach to the heart medially between the ampullae (Amp). The heart extends from the 9th abdominal segment to the prothorax. It bears along its length, expanded intersegmental ampullae, each of which has a pair of lateral openings, one on either side called ostia (singular, ostium). Each ostium is a vertical slit-like opening of the lateral wall of an ampulla.

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The aorta (Ao) is a straight tube that is continuous with the segmented heart. It arises from a spherical ampulla in the prothorax and continues to the head, closely appressed to the dorsal medial outer surface of the crop of the alimentary canal. The aorta passes through the tritocerebral arch and ends up in front of the brain, where it has a ventral opening, through which the haemolymph is poured onto cranial tissues.

Throughout its length, the aorta is bordered by two dorsal pleurisegmental tracheal trunks (Dtra). The latter are connected to abdominal air sacs (As) laterally between the ampullae, and above the alary muscles.

Fig. 49. Gross external morphology of the dorsal blood vessel

Am, alary muscle; **Amp**, ampulla; **Ao**, aorta; **As**, tracheal air sac; **DCom**, dorsal tracheal commissure; **DTra**, dorsal plurisegmental tracheal trunk; **Ost**, ostia; **Papt**, paraproct; **Ph**, phragma; **w**, metathoracic tergal ridge; **I–III**, thoracic segments; **1–10**, abdominal segments.

Morphological Features of Taxonomic Importance

- Generalized condition of the structure of the dorsal blood vessel.

Exercises

Materials Required:

Specimens:
Freshly anaesthetized or preserved (in 70% ethanol) adult;

1. Desert locust, *Schistocerca gregaria* Forskal or the garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper (Orthoptera, Acrididae).

2.   Praying mantis, *Pseudocreobotra wehlbergi* (Stål).

3.   American cockroach, *Periplaneta americana* (L.) or the Oriental cockroach, *Blatta orientalis* (L.) or the wood cockroach, *Gyna maculipennis* (Schaum.)(Orthoptera, Blattidae) or the German cockroach, *Blattella germanica* (L.) (Orthoptera, Blattellidae).

Apparatus:
1.   Dissecting dish with beeswax at the bottom.
2.   Dissecting set.
3.   Minuten insect pins (Nos 3 & 5).
4.   Binocular microscope.

Chemicals :
1.   10% ethanol.

Exercise 1: **Dorsal Blood Vessel of a Locust or Grasshopper.**

1.   Using a freshly anaesthetized or preserved locust or grasshopper of any sex, clip off the wings and legs from their bases and mount the specimen on wax in the dissecting dish, right-side-up with the head directed towards the top of the dissecting dish.

2.   Remove the dorsum of your specimen by carefully cutting through the pleural regions on both sides of the abdomen and thorax from the epiproct to the cervix.

3.   Using a pair of forceps, lift up the excised dorsum of your specimen and then pin it up on wax in the dissecting dish with the inner surface uppermost and then submerge the specimen in 10% ethanol (see Fig. 59).

4.   If the dorsal blood vessel of your specimen is obscured by the fat body and connective tissue, remove this obscuring material carefully with a dissecting needle while examining the specimen under the binocular microscope.

5.   Study the dorsal blood vessel carefully under the binocular microscope. Identify the AORTA, HEART, AMPULLAE, OSTIA, ALARY MUSCLES, SURROUNDING TRACHEAE then draw and label.

6.   How does the dorsal blood vessel of your specimen differ from that of *A. speiseri* described in the introduction to these exercises?

Exercise 2: **Variation in the Structure of the Dorsal Blood Vessel.**

1. Study under the binocular microscope and draw the dorsal blood vessels of
    a. a praying mantis, and
    b. a cockroach.

2. What differences have you noted between these vessels and those of locusts and grasshoppers?

Supplementary Questions

1. How do the accessory circulatory structures such as the pulsating organs located at the bases of legs and wings of some Hymenoptera improve the efficiency of haemolymph circulation to the appendages?

2. Describe the structure of the segmented heart of a named insect species and explain how the haemolymph enters the organ and is propelled forward into the aorta and the haemocoelic cavities.

3. Distinguish between incurrent and excurrent ostia in the segmented heart of an insect. What structural differences are there in these two types of slits of the heart and how is this related to the uptake and release of blood from the dorsal blood vessel?

4. Describe the nature, innervation and operation of the alary muscles.

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B.   The Alimentary Canal

The alimentary canal in insects is an organ system derived from two embryonic germ bands, the ectoderm and the endoderm. It can be divided functionally into three regions namely, the foregut (stomodaeum), midgut (mesenteron) and the hindgut (proctodaeum). The stomodaeum and proctodaeum are ectodermal in origin, while the mesenteron is endodermal. Both the stomodaeum and proctodaeum are lined inside with very thin layers of cuticle called intima which is shed along with the rest of the cuticle when an insect

moults during postembryonic development, to allow for growth to take place. The mesenteron lacks cuticular intima, instead, it is either temporarily (each time food is introduced into it) or permanently lined by a peritrophic membrane which protects the underlying epithelial cells of the midgut (mesenteron) from damage by the digestive enzymes and protects these cells or the insect from infection by the microflora that is consumed together with the food. The peritrophic membrane is, however, permeable to the products of digestion.

Each region of the alimentary canal is divisible into a number of subregions that serve specific functions. The stomodaeum has the mouth, pharynx, oesophagus, crop and proventriculus. Its main function is ingestion, food storage and physical digestion of the food ingested (i.e., continues to physically break down the food consumed into finer pieces through the activities of the proventriculus). However a bit of digestion does occur in the stomodaeum since the food is mixed with saliva as it is being ingested. The saliva contains the digestive enzyme ptylin or amylase. Due to the presence of the cuticular intima, the stomodaeum is impermeable to materials passing through it.

The mesenteron is divided into gastric caeca and the ventriculus. The former produces the digestive enzymes, while the latter is the main seat of chemical digestion and absorption of the products of digestion. The number of gastric caeca vary in insects. In the desert locust, *Schistocerca gregaria* Forskal, there are six gastric caeca, while in longhorned grasshoppers (Tettigoniidae) and crickets (Gryllidae) the number is two. The peritrophic membrane mentioned above is either formed each time food enters the mesenteron and is later destroyed when the mesenteron is emptied of the food and digestive enzymes or as in some insects it is permanently present as the lining of the mesenteron.

The proctodaeum is divided into a pylorus, ileum, rectum and anus. This region of the alimentary canal is responsible for excretion and the reabsoption of mineral salts and amino acids. The principal excretory organs are the malpighian tubules. These blind-ending tubes arise from the pylorus and are scattered in the haemocoelic cavity where they absorb the nitrogenous wastes from the haemolymph. The latter are emptied into the proctodaeum where they are eliminated to the outside, together with the food wastes. The ileum is generally undifferentiated, though in termites (Isoptera) it is enlarged into a pouch housing symbiotic flagellates, which digest cellulose. The rectum bears rectal pads for mineral salt and amino acid reabsorption. In nymphal dragonflies (Odonata, Anisoptera), the rectum has rectal gills through which gaseous exchange is effected through rectal respiration.

The alimentary canal of the armoured ground cricket, *A. speiseri* (Fig. 50) is much longer than the insect's body. The stomodaeum includes a mouth (Mth) that is surrounded by a large buccal cavity (BuC). Following the mouth is a short pharynx (Phy) that leads into the oesophagus (Oe). The latter is continuous with a large crop (Cr) that opens posteriorly into a globular proventriculus (Pr), via a short neck. The proventriculus in turn opens into
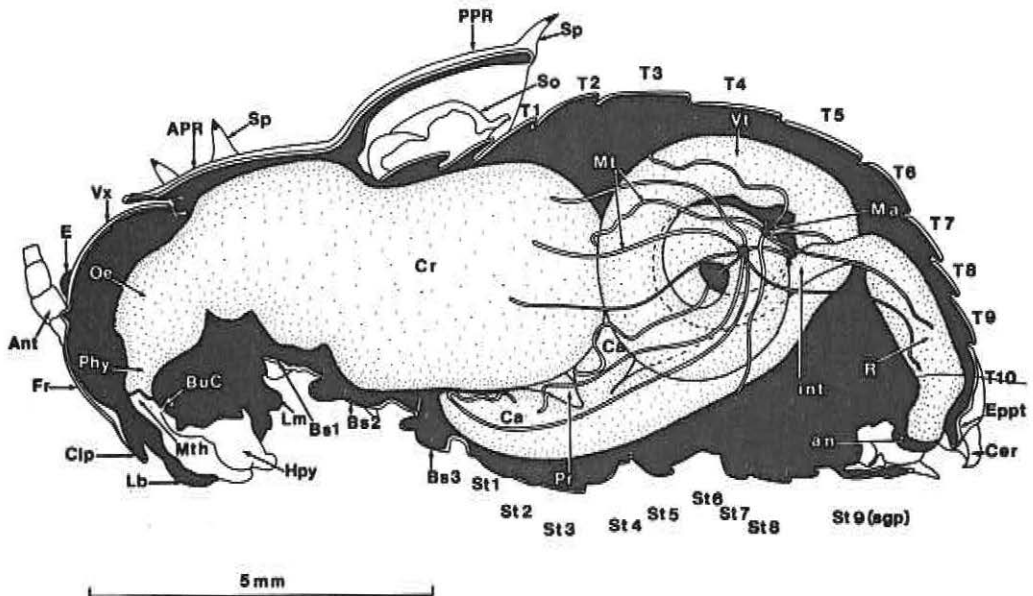
Fig. 50. The gross external morphology of the alimentary canal

an, anus; Ant, antenna; APR, anterior pronotal region; Bs, basisternite;
Ca, caecum; Cer, cercus; Clp, clypeus; Cr, crop; E, compound eye; Eppt, epiproct; Fr, frons; Hyp,
hypopharynx; int, intestine; Lb, labrum; Lm, labium; Ma, malpighian ampulla; Mt, malpighian tubule;
Mth, mouth; Oe, oesophagus; Phy, pharynx; PPR, posterior pronotal region; Pr, proventriculus; r,
rectum; So, stridulating organ; Sp, spine; St, abdominal sternite; T, abdominal tergite; Vt, ventriculus;
Vx, vertex.

Morphological Features of Taxonomic Importance

- Presence of two bulbous, and sac-like gastric caeca (Ca).

the mesenteron by way of a pyloric valve.

The mesenteron is bounded anteriorly by the pyloric valve and bears two bulbous, sac-like gastric caeca (Ca). The latter are typically located dorsally and ventrally; sandwiched between them, is the proventriculus of the stomodaeum. Caudad to the caeca is the ventriculus (Vt).

Anteriorly, the proctodaeum possesses six fascicles on which are borne the malpighian tubules (Mt). The fascicles discharge nitrogenous wastes into the same number of ampullae (Ma) that open into the ileum (Int). The last portion of the proctodaeum is the rectum (R) that opens to the outside through the anus (An).

The structure of the alimentary canal varies widely in the class Insecta depending among other things on the diet a given insect group is adapted to. Generally, the vegeterian species have longer alimentary canals than those

that eat meat. Other modifications may involve the development of what are termed filter chambers which facilitate the quick elimination of excess water in liquid food consumed by the fluid feeders, to concentrate it for digestion.

Exercises

Materials Required:

Specimens:
Freshly anaesthetized or preserved (in 70% ethanol) adult;

1. Desert locust, *Schistocerca gregaria* Forskal or the garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper (Orthoptera, Acrididae).

2. House fly, *Musca domestica* (L.) (Diptera, Muscidae).

3. African honeybee, *Apis mellifera adansonii* (L.) or *A. m. capensis* (L.) (Hymenoptera, Apidae).

4. Alate bark-eating termites, *Macrotermes vatrialatus* (Isoptera, Termitidae).

Apparatus:
1. Dissecting dish with beeswax at the bottom.
2. Dissecting set.
3. Minuten insect pins (Nos 3 & 5).
4. Binocular microscope.

Chemicals:
1. 10% ethanol.

Exercise 1: Alimentary Canal of a Locust or Grasshopper.

1. Cut off the dorsum of a grasshopper or locust provided along the pleural sides from the end of the abdomen to the cervix. In the head region cut the cranium across the gena on either side of the head and then discard the dorsum of the abdomen and thorax, including the top part of the cranium (see Fig. 59).

2. Mount the specimen in wax in the dissecting dish and using a dissecting needle, remove as much of the fat body around the alimentary canal as possible including some connective tissue (mainly tracheae) and some malpighian tubules.

Exercise 1: Continued

3. When the alimentary canal is distinctly exposed when viewed under a binocular microscope, submerge the specimen in 10% ethanol.

4. Study the specimen under the binocular microscope. Identify the PHARYNX, OESOPHAGUS, CROP, PROVENTRICULUS, GASTRIC CAECA, VENTRICULUS, MALPIGHIAN TUBULES, ILEUM, RECTUM and ANUS.

5. Draw and label.

6. What differences in structure have you observed between your specimen and the armoured ground cricket, *Acanthoplus speiseri* described in the introduction to these exercises?

Exercise 2: **The Proventriculus.**

1. Dissect out the proventriculus from the alimentary canal of your specimen in Exercise 1 above.

2. Bisect the proventriculus longitudinally to expose the inner walls.

3. Place one half of the proventriculus on wax in the dissecting dish with the inner wall of the organ uppermost, pin and then submerge the specimen under 10% ethanol.

4. Remove any obstructing material such as food that may be present making the wall of the proventriculus not clear, using a dissecting needle, while examining the specimen under the binocular microscope.

5. Study the inner wall of the proventriculus. What structures do you see on the wall? What are their functions? Draw and label.

Exercise 3: **Modifications of the Alimentary canal.**

1. Dissect and expose the alimentary canals of;
    a. a house fly,
    b. a honeybee, and
    c. a termite.

2. Examine the organs under the binocular microscope. Identify the parts and then draw and label.

Exercise 3: Continued

3.   How do the alimentary canals of the two insects differ from each other and from that of a grasshopper or locust you have studied in Exercise 1?

4.   What kind of nutrition are these two insects adapted to and how do the structures and lengths of their alimentary canals correlate to this?

Supplementary Questions

1.   Discuss the relationship between alimentary canal length and the type of nutrition a given insect is adapted to. In your discussion also explain the structural modifications in the system that are related to cellulose digestion in termites (Isoptera).

2.   Describe different types of filter chambers that associate with the alimentary canal in fluid feeders and explain how each type facilitates the quick elimination of excess water in the fluid food ingested by the insect bearing it.

3.   The cuticular armature of the inner lining of the proventriculus has been used as a taxonomic feature in the separation of certain insect groups and species. Discuss the variation shown by the cuticular spines lining the inner walls of the proventriculus in the Orthoptera and explain how these structures aid digestion.

4.   Describe the embryonic development of the alimentary canal in insects.

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C.   The Internal Reproductive System

The internal reproductive organs in both sexes of insects comprise a pair of gonads, a pair of lateral ducts, a common duct and accessory sex glands. In the male the gonads are the testes. Each testis is linked to a common duct, the ejaculatory duct via a lateral duct the vas deferens. The ejaculatory duct opens to the outside via modified cuticular parts forming the penis (aedegus) in some insects or the opening is simply flanked by specialized phallic lobes as in other insects, whose main function are to mould spermatophores (special sperm capsules). Various accessory sex glands that contribute materials to the semen or towards spermatophore formation are associated with the ejaculatory duct in many insects.

In the female, the paired gonads are the ovaries. Each is made up of several tubes called ovarioles in which the ova are produced meiotically from the oogonia. An ovary is linked up to the common oviduct by a lateral oviduct. The common oviduct or vagina opens to the outside on the floor of the subgenital plate (8th sternum) in most insects. Different types of accessory sex glands occur in association with the internal reproductive organs in the female insect. These contribute material to the developing eggs or produce material required for egg pod formation or simply for glueing the eggs together during oviposition. In addition, a unique structure that associates with the internal reproductive organs in the female that lacks a homologue in the internal reproductive structures of the male is the spermatheca. The latter is a special sperm receptacle, in which the female stores sperm following copulation, for future fertilization of her eggs. This is so because in certain insect groups, a female copulates once or only a few times in her lifetime.

In male armoured ground crickets (Fig. 51), the testes (Tes) are large, compact, bean-shaped bodies lying, one on either side of the alimentary canal and extending from the 7th to the 1st abdominal segments. Each testis has a large number of sperm tubes (ST).

Posteriorly, vas deferentia (Vd) emerge from the testes and are each thrown into complex convolutions immediately upon emerging from a testis forming compact epididymis-like bodies (Epdm). Beyond these bodies, each vas deferens is a short straight tube that opens into the median ejaculatory duct (Dej), together with a pair of stalks (AGS) bearing the accessory sex glands (AcGlds).

Two types of accessory sex glands are present on each accessory gland stalk in the male. One type consists of large long and numerous tubules that occupy most of the posterior and ventral regions of the male abdomen, being
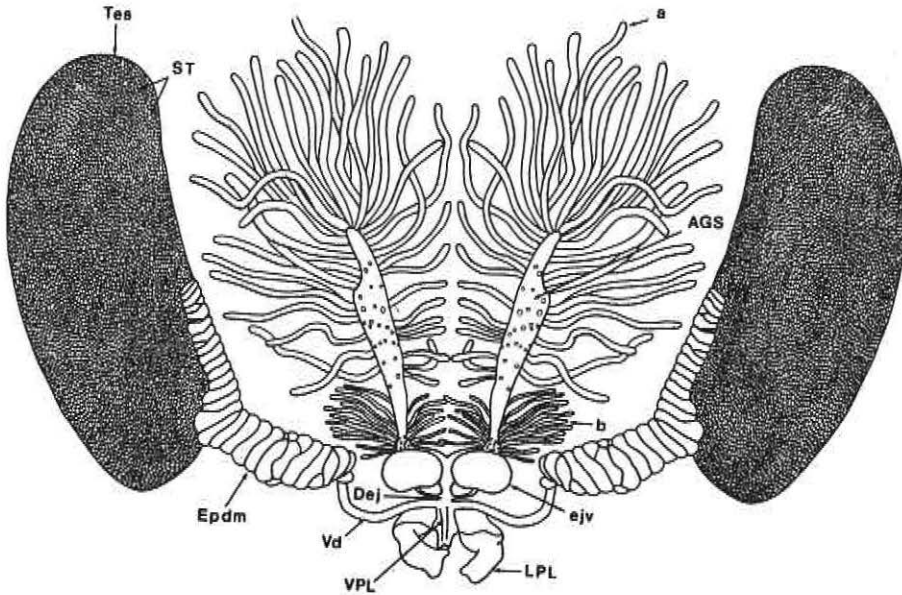
Fig. 51. Dorsal view of the male internal reproductive organs

a,b, two kinds of accessory glands; **AGS**, accessory gland shaft; **Dej**, ejaculatory duct; **ejv**, vesicle of the ejaculatory duct; **Epdm**; epididymis; **LPL**, lateral phallic lobe; **ST**, sperm tube; **Tes**, testis; **Vd**, vas deferens; **VPL**, ventral phallic lobe.

Morphological Features of Taxonomic Importance

- Presence of epididymis-like compact bodies (Epdm) on vas deferentia (Vd).
- Presence of two types of accessory sex glands (a & b).

intermingled with the alimentary canal and the fat body. They arise on about the distal two thirds of each accessory gland stalk (a). The second type of accessory sex glands are smaller, short and closely packed tubules that form compact globular bodies on the proximal third of each accessory gland stalk (b).

The ejaculatory duct opens to the outside via a wide chamber or the phalotreme that is flanked by phallic lobes (Fig. 51, VPL, LPL).

The female armoured ground cricket ovaries (Fig. 52) lie one on either side of the alimentary canal in the abdominal cavity. In gravid females, these are very large organs that occupy most of the abdominal cavity and due to the large sizes of the maturing eggs in them, the gravid females exhibit more highly distended abdomens than the males. The terminal ligament (Lg) of each ovary attaches to the tergal ridge of the metanotum dorsolaterally.

Posteriorly, each ovary gives off a large lateral oviduct (Odl). The latter from each ovary unite with the opposite number medially to form the common
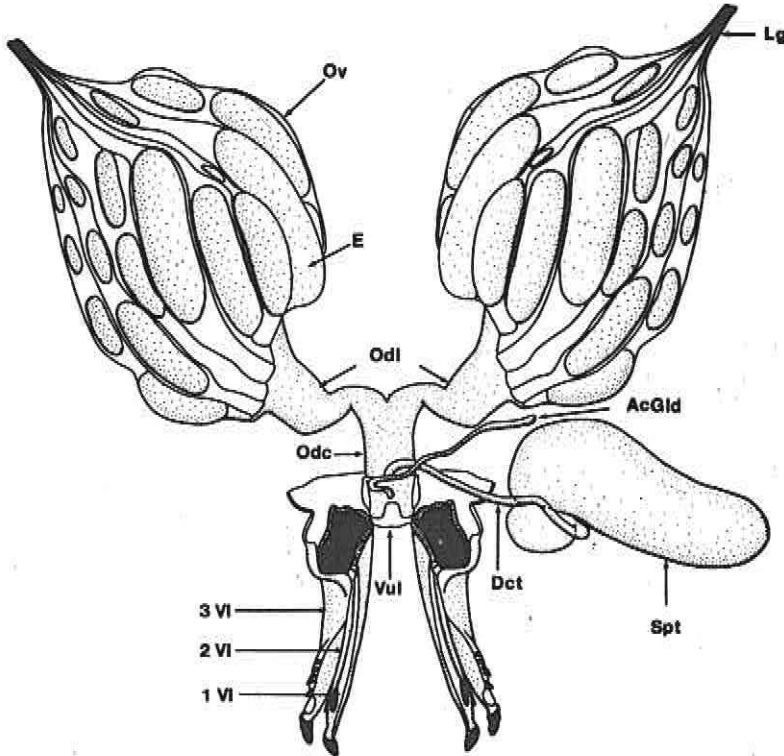
Fig. 52. Dorsal view of the female internal reproductive organs

AcGld, accessory gland; Dct, spermathecal duct; E, egg; Lg, ligament; Odc, median oviduct; Odl, lateral oviduct; Ov, ovary; Spt, spermatheca; Vul, vulva; VI, ovipositor blade.

Morphological Features of Taxonomic Importance

- Presence of a large shoe-shaped spermatheca.
- Presence of a single accessory sex gland.

oviduct (Odc). The common oviduct in turn opens to the outside on the floor of the genital chamber (GC) that leads to the vulva (Vul) or the external genital opening. The latter occurs between the ventral ovipositor blade pair and the subgenital plate.

A duct from a large shoe-shaped spermatheca opens on the dorsal wall of the genital chamber caudad to the opening of the common oviduct. A single unbranched tubular accessory sex gland (AcGld) opens behind the spermathecal aperture also on the dorsal wall of the genital chamber.

Exercises

Materials Required:

Specimens :
Freshly anaesthetized or preserved (in 70% ethanol) adult:

1. Desert locust, *Schistocerca gregaria* Forskal or garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper (Orthoptera, Acrididae).

2. Fruit chafer, *Dicronorrhina derbyana* (Westw.) (Coleoptera, Scarabaeidae).

3. Tsetse fly, *Glossina pallidipes* Austen or *G. morsitans morsitans* Westwood (Diptera, Glossinidae).

4. Alate harvester ant, *Hodotermes mossambicus* Hagen (Isoptera, Hodotermitidae).

5. African honeybee, *Apis mellifera adansonii* (L.) or *A.m. capensis* (L.) (Hymenoptera, Apidae).

Apparatus:
1. Dissecting dish with beeswax at the bottom.
2. Dissecting set.
3. Minuten insect pins (Nos 3 & 5).
5. Binocular microscope.

Chemicals:
1. 10% ethanol.

Exercise 1: **Generalized Internal Reproductive Organs.**

1. Using a male and female locust or grasshopper, dissect the specimen to expose the internal reproductive organs (see Figs 51 and 52).

2. To make the structures distinct, you may need to remove the alimentary canals from your specimens.

3. Examine the specimens under the binocular microscope.

    a)   in the male identify, draw and label the following structures;

Exercise 1: Continued

> TESTIS
> EJACULATORY DUCT
> VAS DEFERENS
> ACCESSORY SEX GLAND(S)
> AEDEGUS

b) in the female identify, draw and label the following structures;
OVARY
LATERAL OVIDUCT
MEDIAN OR COMMON OVIDUCT
SPERMATHECA
ACCESSORY SEX GLAND(S)
OVIPOSITOR

4. How do these structures differ from those of the armoured ground cricket described in the introduction to these exercises?

Exercise 2: **Variations in the Structures of the Internal Reproductive Organs.**

1. Dissect both sexes of each of the following insects and study, draw and label structures of their internal reproductive organs;
    a. a beetle
    b. a tsetse fly
    c. a termite, and
    d. a honeybee

2. Compare and contrast these structures with each other and with those of the generalized condition.

Supplementary Questions

1. Describe the variations in structure of ovarioles found in the class Insecta and indicate which insect group exhibits those types of ovarioles described.

2. Discuss the taxonomic significance of the male aedegus in a selected group of insects.

3. How do phallic lobes characteristic of the lower orders of insects such as the longhorned grasshoppers operate to produce spermatophores and what is the composition of a spermatophore?

4. Describe the histology of the accessory sex glands of the male and female of a selected insect group.

5. Ovaries of tsetse and mosquitoes can be used to determine the age of the gravid female. Describe how it is possible to use these organs to do so.

6. How does an ovariole produce eggs and the sperm tube sperms in insects?

7. Discuss the development of the ectodermal and endodermal parts of the reproductive system in male and female insects during embryonic development and indicate at which stage of development the two parts link up.

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D. The Nervous System

The insect nervous system is divisible into four regions namely, a) the brain or supraoesophageal ganglion, b) the ventral nerve cord (consisting of the suboesophageal, thoracic and abdominal ganglia), c) the stomatogastric system, and d) the peripheral nervous system.

In the generalized condition as seen in Orthoptera, the brain has three distinct subanatomical divisions, protocerebrum, deutocerebrum and tritocerebrum. The protocerebrum occurs at the top and is the largest subdivision of the brain. It bears the optic lobes. The latter may be broadly linked to it or are associated with it via stalks. Also arising from this subdivision are the ocellary nerves (*nervi ocellarii*) in those species bearing the simple eyes (ocelli).

Ventral to the protocerebrum and broadly linked to it is the deutocerebrum. The latter gives rise to the antennal nerves (*nervus antennalis*), the accessory antennal nerves (*nervus antennalis accessorius*) and the tegumentary nerves (*nervus tegumentalis*)

Below the deutocerebrum is the third subanatomical region of the brain, the tritocerebrum. Ventrally the latter bears a pair of circumoesophageal nerve connectives that link the brain to the ventral nerve cord. Also occurring ventrally on either side of this subanatomical division are a pair of labro-frontal nerves that ramify to form the *pars frontalis* nerve to the frontal ganglion and the *pars labralis* nerve to the labrum. The ventro-lateral regions of the tritocerebrum are interconnected below by a tritocerebral commissure.

The ventral nerve cord consists of a chain of ganglia that are interlinked by paired nerve connectives. The suboesophageal ganglion is the first and largest and is located below the oesophagus and coporo-tentorium. It supplies nerves to the mouthparts or gnathal appendages and the neck. There are three thoracic ganglia (one to a segment) and a number of abdominal ganglia. The number of ganglia occurring in the abdomen varies in different insect groups. It ranges from as few as zero in the house fly (in this species there is only one ganglionic mass in the ventral nerve cord which is located in the thorax. The ganglionic mass sends off nerves to all tissues and organs of the thorax and abdomen) to as many as seven in many Orthoptera.

The stomatogastric nervous system comprises a number of small ganglia including frontal ganglion, a pair of oesophageal ganglia, a hypocerebral ganglion, a pair of corpus allata and a pair of gastric or ingluvial ganglia. The frontal ganglion occurs anterior to the brain on the pharynx, to which it is tenaciously attached. The oesophageal ganglia and the hypocerebral ganglion occur immediately behind the brain above the oesophagus. The oesophageal ganglia communicate each with the protocerebrum via a short occipital nerve (*nervus occipitalis*) and with a corpus allatum via a much longer nerve, the *nervi corporum allatum*. The hypocerebral ganglion is linked to the oesophageal ganglia by short nerves and to the ingluvial ganglia (these are located in the region of the proventriculus on the crop wall) posteriorly by long nerves, the *nervi recurrens posterior externus*. Also issuing from the caudo-ventral portion of the hypocerebral ganglion in some orthoptera, are a pair of shorter nerves termed *nervi recurrens posterior internus*. The latter innervate the oesophagus.

The brain of the armoured ground cricket, *Acanthoplus speiseri* (Fig. 53) deviates from the typical orthopteran type in two important respects. It appears to bear only two subanatomical divisions externally and its optic lobes possess long stalks (LO, OS). However, all three subanatomical divisions of a typical generalized orthopteran brain, i.e. protocerebrum, deutocerebrum and tritocerebrum, are present.

The protocerebrum (P) is the largest subanatomical division. It occurs dorsally. It is cordate (heart-shaped) and bears stalked optic lobes (LO) dorsa-laterally. No ocellary nerves are present as this species lacks ocelli. Ventrad to the protocerebrum and strongly linked to it is the deutocerebrum (D). This
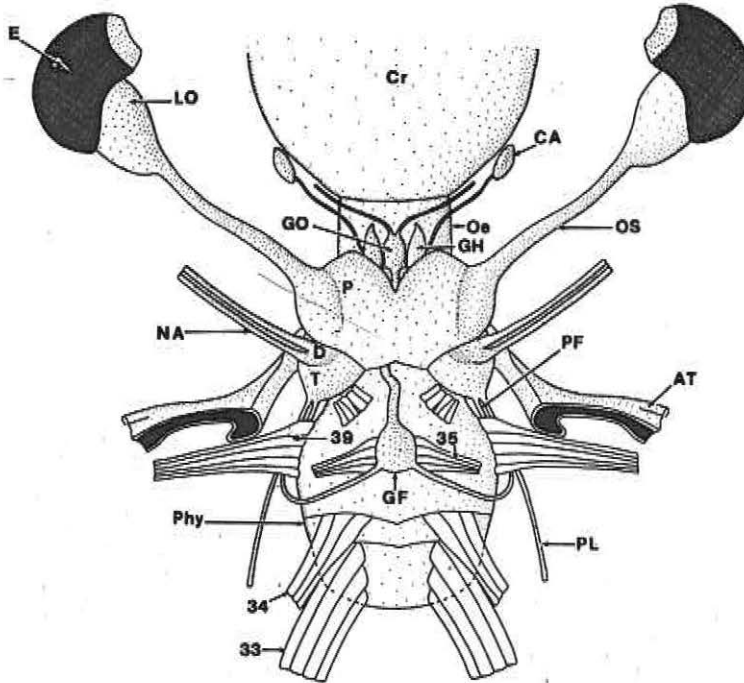
Fig. 53. Anterior view of the brain

At, anterior arm of the tentorium; CA, corpora allata; Cr, crop; D, deutocerebrum; E, compound eye; GF, frontal ganglion; GH, hypocerebral ganglion; GO, oesophageal ganglion; LO, optic lobe; NA, *nervus antennalis*; Oe, oesophagus; OS, optic stalk; P, protocerebrum; PF, *pars frontalis*; Phy, pharynx; PL, *pars labralis*; T, tritocerebrum; 33, 34, 35, 39, dilator muscles of the pharynx.

Morphological Features of Taxonomic Importance

- Stalked optic lobes (LO).
- Tiny compound eyes (E).

subanatomical division of the brain is small in the armoured ground cricket and is not distinctly demarcated externally. However, its relative position can be approximated by the position of the antennal nerves (NA) that arise from it.

Below the deutocerebrum is the tritocerebrum (T). Ventrally, the latter bears a pair of circumoesophageal nerve connectives that link the brain to the suboesophageal ganglion of the ventral nerve cord (Fig. 54, SOG). Also arising from the ventral margins of the tritocerebrum are the labro-frontal nerves and the tritocerebral commissure ventrally. The frontal nerves from the two sides of the tritocerebrum form a loop to the frontal ganglion on the pharynx (Fig. 53. PF, GF, Phy). The labral nerves project ventrad to innervate muscles of the
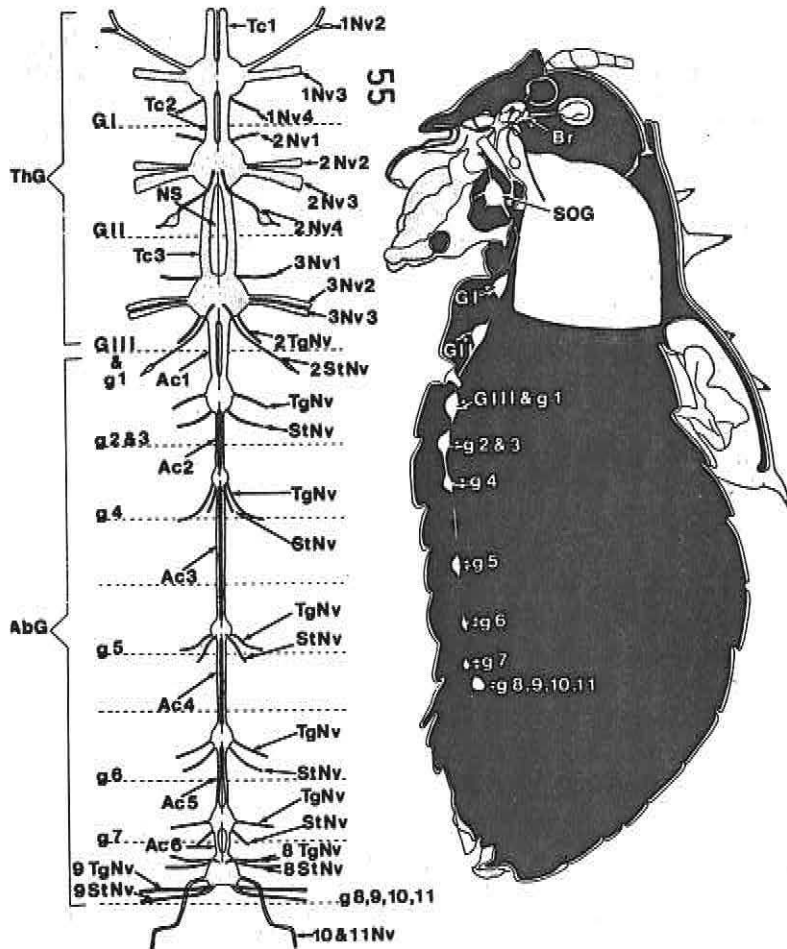
Fig. 54. The ventral ganglionic chain *in situ*

Fig. 55. Dorsal view of the male thoracic and abdominal ganglia

Ab, abdominal connective; AbG, abdominal ganglia; Ac, abdominal nerve connective; Br, brain; g, abdominal ganglion; G, thoracic ganglion; NS, median or subintestinal nerve; 1Nv2–1Nv4, nerves from the prothoracic ganglion; 2Nv1–2Nv4, nerves from the mesothoracic ganglion; 3Nv1–3Nv3, nerves from the metathoracic ganglion; TgNv, StNv, tergal and sternal nerves; 2TgNv, 2StNv, tergal and sternal nerves from the second definitive abdominal ganglion; Tc, thoracic nerve connective; ThG thoracic ganglia.

Morphological Features of Taxonomic Importance

- A tiny brain (Br) when compared to the size of the cranium.
- The presence of only 6 abdominal ganglia (g) in the ventral nerve cord.

labrum (PL). The tritocerebral nerve commissure loops around the oesophagus below, linking the two sides of the brain.

The first ganglionic mass in the ventral nerve cord is the suboesophageal ganglion (Fig. 54, SOG). It lies below the oesophagus under the corporo-tentorium. The circumoesophageal connectives linking the brain to it pass in front of the corporo-tentorium between its anterior arms. Nerves arising from this ganglion innervate the mandibles (Fig. 57, NM), hypopharynx, maxillae (NM1), labium (NM2) and the cervical region of the body. The suboesophageal ganglion is connected to the thoracic ganglionic masses by a pair of nerve connectives (TC1).

Of the three thoracic ganglia, those of the pro - and mesothorax are nearly equal in size. The metathoracic ganglion is larger due to incorporation into it of the first abdominal ganglion during the evolution of the armoured ground crickets. Emerging from the lateral margins of each thoracic ganglion are two principal nerve groups of the body (Fig. 55, Nv2, Nv3). The anterior nerve group (Nv2) provides nerves to the tergal promotors, sternal promotors (or the anterior rotators), basalar, subalar, axillary (in winged forms), sternal abductor and the tero-sternal muscles. The posterior nerve group (Nv3) ennervates the tergal remotors, sternal remotors (or the posterior rotators), sternal adductor and the ventral longitudinal muscles, in addition to sending a large nerve branch into the leg.

There are six free abdominal ganglia occurring in segments 1, 2, 4, 6, 7, and 8 (Fig. 55). The first abdominal ganglion is fused with the metathoracic ganglion. This is evidenced by the presence of a pair of nerves (1 TgNv, 1 StNv) that arise posteriorly from the metathoracic ganglion which ennervate the tergal, pleural and sternal muscles of the first abdominal segment.

The first definitive or the first free abdominal ganglion (g2) is in actuality the second ventral nerve cord ganglion. It is located in the first abdominal segment. This and the following four ganglia each bears two pairs of lateral nerve groups. The anterior nerve group (TgNv) innervates the tergal and pleural muscles and sends off nerves to the dorsal blood vessel. The posterior nerve group (StNv) innervates the sternal muscles.

The last or 8th definitive abdominal ganglion is much larger than the rest of the abdominal ganglia. It is heart-shaped and is a compound ganglionic mass supplying nerves to the 8th, 9th, 10th and 11th abdominal segments (Fig. 55, 8 TgNv, 8 StNv, 9 TgNv, 9 StNv, 10 Nv, 11 Nv). In the male, the nerves of the 9th abdominal segment innervate the phallic organs (not illustrated). In the female, the ventral (first) pair of ovipositor blades is innervated from nerves of the 8th nerve group, while the inner (second) and dorsal (third) ovipositor blade pairs are innervated from nerves of the 9th nerve group. In both sexes however, the nerves to the 10th abdominal segment innervate the cerci and the dilator muscles of the rectum. Nerves to the 11th abdominal segment supply motor filaments to the epiproct, paraprocts and the dilator muscles of the anus, in addition to supplying large sensory nerves to the cerci.

In the middle of the ventral nerve cord, between the paired nerve connectives, are very thin median or subintestinal nerves (Fig. 55, NS). These nerves supply branches to the spiracular muscles in those segments where spiracles are present.

The stomatogastric nervous system in the armoured ground cricket involves five ganglionic masses, the frontal ganglion, paired oesophageal ganglia or corpus allata, paired corpus cardiaca, a hypocerebral ganglion and paired ingluvial ganglia (Figs 56–58, GF, GO, GH). The frontal ganglion is located in front of the brain on the pharyngeal wall (Phy), to which it is firmly attached. The median oesophageal ganglia and the hypocerebral ganglion occur immediately behind the brain, attached above the oesophagus. The oesophageal ganglia are positioned above the hypocerebral ganglion and communicate with it via short nerves. Each oesophageal ganglion is shaped like an air-foil and is attached to the protocerebrum at its broad anterior end by the occipital nerve (NO). Ventrad on each of these ganglia, arises a large nerve that links it to the large oval-shaped corpus allatum (CA), that is abutted to the wall of the oesophagus laterally.

Posteriorly, the hypocerebral ganglion gives off two pairs of nerves. One pair are small, short median recurrent nerves (NRPI) that innervate the oesophagus. The other pair are large long nerves (NRPE) which run along the dorso-lateral
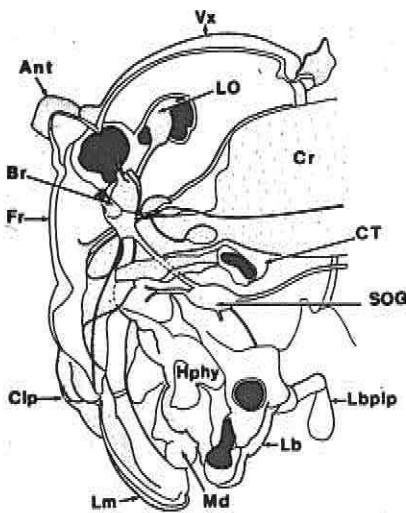
Fig. 56. Lateral view of the brain and the suboesophageal ganglion

walls of the crop to the ingluvial or gastric ganglia on the walls of the crop (not illustrated).

**Fig. 57. Lateral view of the brain,
    suboesophageal ganglion, and
    the stomatogastric ganglia**

Ant, antenna; Br, brain; CA, corpus allatum;
CC, circumoesophageal connectives; Clp,
clypeus; Cr, crop; CT, corporotentorium; D,
deutocerebrum; E, compound eye; Fr, frons;
GF, frontal ganglion; GH, hypocerebral gan-
glion; GO, oesophageal ganglion; Hphy, hy-
popharynx; Lb, labium; Lbplp, labial palp; Lm,
labrum; LO, optic lobe; Md, mandible; NA,
nervus antennalis; NM, *nervus mandibularis*;
NM1, *nervus maxillae*; NM2, *nervus labii*; NO,
nervus occipitalis; NRPE, *nervus recurrens
posterior externus*; NRPI, *nervus recurrens
posterior internus*; OS, optic stalk; P,
protocerebrum; PF, *pars frontalis*; Phy, phar-
ynx; PL, *pars labralis*; SOG, suboesophageal
ganglion; T, tritocerebrum; TCl, thoracic nerve
connective; TCom, tritocerebral commissure;
Vx, vertex.
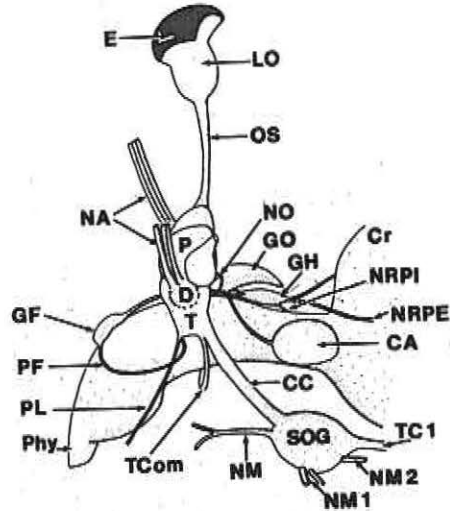
Morphological Features of Taxonomic Importance

- Stalked optic lobes (LO).
- Large corpus allata (CA).

Exercises

Materials Required:

Specimens:
Freshly anaesthetized or preserved (in 70% ethanol) adult:

1. Desert locust, *Schistocerca gregaria* or the garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper (Orthoptera, Acrididae).

2. American cockroach, *Periplaneta americana* (L.) or the Oriental cockroach, *Blatta orientalis* (L.) (Orthoptera, Blattidae) or the German cockroach, *Blattella germanica* (L.) (Orthoptera, Blattellidae).

3. African honeybee, *Apis mellifera adansonii* (L.) or *A.m. capensis* (L.) (Hymenoptera, Apidae).

4. House fly, *Musca domestica* L. (Diptera, Muscidae).

Apparatus:
1. Dissecting dish with beeswax at the bottom.
2. Dissecting set.
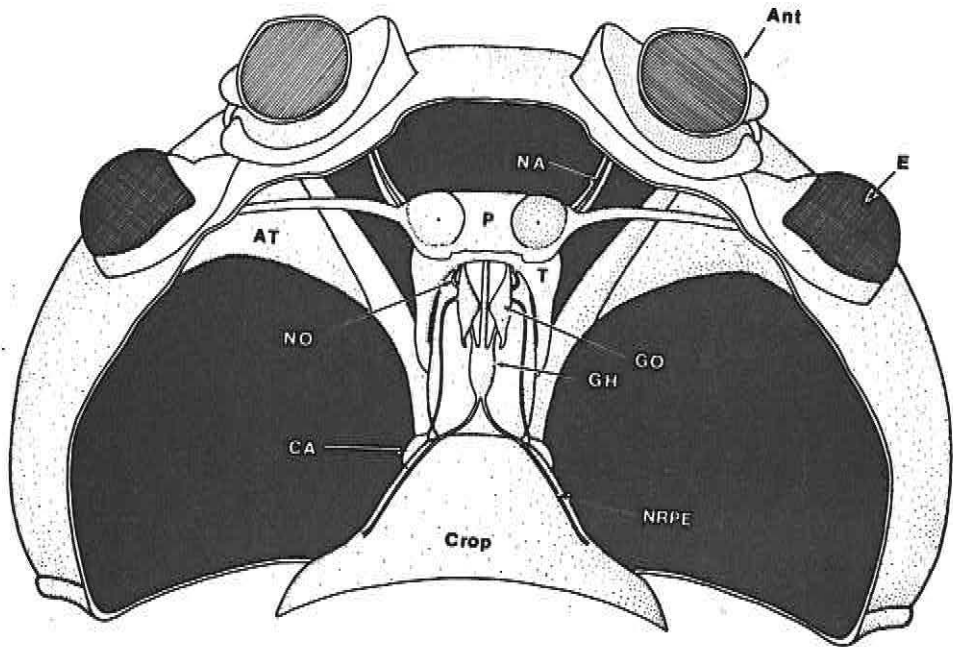
Fig. 58. Dorsal view of the brain and the stomatogastric ganglia

Ant, antenna; AT, anterior arm of the tentorium; CA, corpora allata; E, compound eye; GH, hypocerebral ganglion; GO, oesophageal ganglion; NA, *nervus antennalis*; NO, *nervus occipitalis*; NRPE, *nervus recurrens posterior externus*; P, protocerebrum; T, tritocerebrum.

Morphological Features of Taxonomic Importance

- Tiny brain (Br) when compared to the rest of the cranium.

3. Minuten insect pins (Nos 3 & 5).
4. Razor blade or scalpel.
5. Binocular microscope.

Chemicals:
1. 10% ethanol.
2. Basic fuchsin or methylene blue stain.

Exercise 1: The Ventral Nerve Cord.

1. Using a freshly anaesthetized or preserved grasshopper or locust of any sex, dissect out the alimentary canal and the internal reproductive organs (see Fig. 59).

1. Place insect specimen with clipped off wings and/or legs on wax and pin.

2. Remove the dorsum of the thorax and abdomen by cutting through the pleural regions.

3. Pin the dorsum to the wax with the inside uppermost.

4. Move the alimentary canal to to the side to expose the ventral nerve cord.
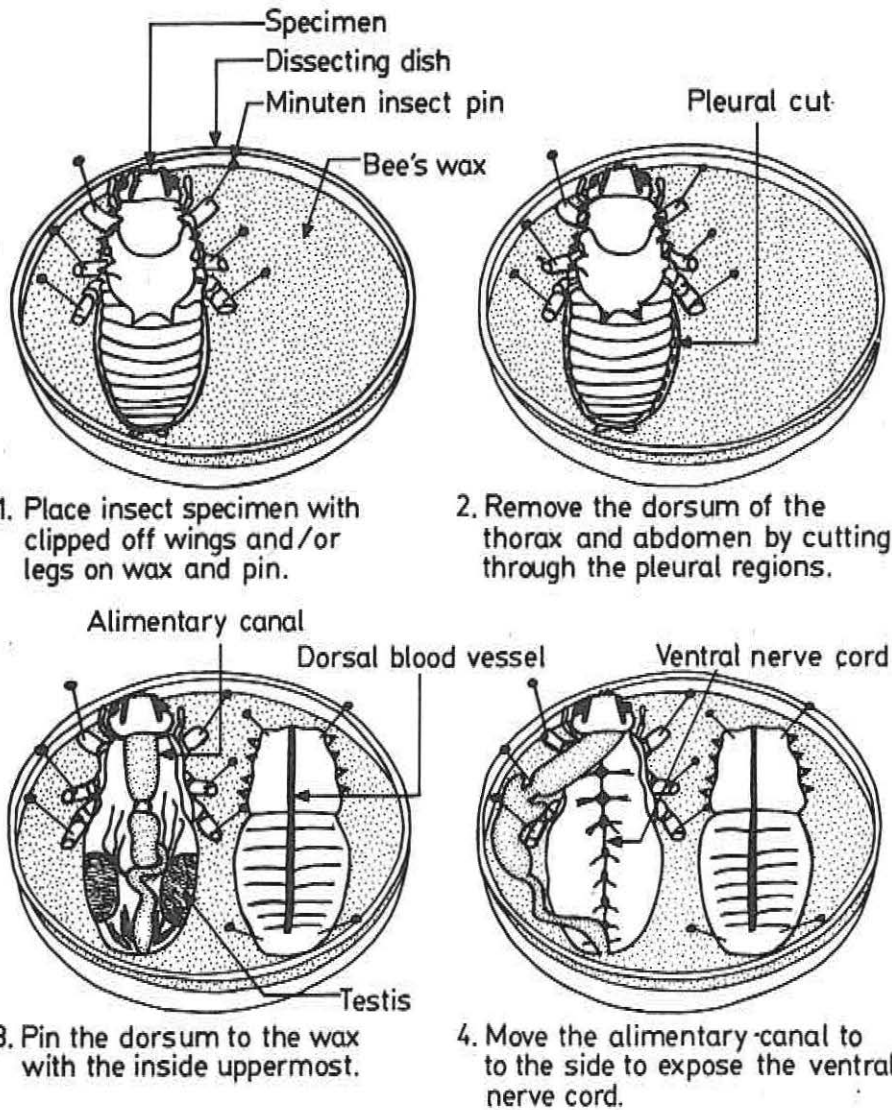
Fig. 59. Preparing an insect to study the ventral nerve cord

Exercise 1: Continued

    2.   Submerge your specimen in 10% ethanol and then examine the abdominal sterna in their midlines. You should be able to see a series of nerve ganglia that are linked to each other by paired nerve connectives. This is the ventral nerve cord.

Exercise 1: Continued

3. Trace the cord from the abdomen to the head by removing all obscuring fat and connective tissue using a dissecting needle and a forceps. In the head region, cut the cranium through the gena on either side of the head.

NOTE : Carry out all these operations while observing your specimen under a binocular microscope to avoid destroying the ventral nerve cord.

4. When the cord is clear, study it very carefully. Note the number of ganglia present and their relationship to the segments containing them then, make a large, clear drawing of the cord *in situ* and label.

5. How many ganglia are there in the ventral nerve cord of your specimen? What is the ratio of the number of ganglia to the number of the definitive segments in the thorax and abdomen of your specimen?

6. For those segments that may lack ganglia how are their tissues innervated?

Exercise 2: **The Brain or Supraoesophageal Ganglion.**

1. Use a fresh grasshopper or locust for this exercise. The larger the specimen, the better.

2. Cut off the head from the body through the cervix (neck membrane) using a pair of scissors.

3. Using a dissecting needle and a forceps, ply out the mouthparts from below. Carefully remove the walls of the cranium to expose the abductor and adductor muscles of the mandibles using a razor blade and forceps (see Fig. 60A).

4. Mount the excised head without the mouthparts on wax with the fronto-clypeal area uppermost (see Fig. 60B).

5. Again with a forceps, pull out single mandibular muscle bands exposed, a few at a time from their points of attachment on the mandibular apodemes to expose the brain (Fig. 60C). **Be very careful when doing so, as you could easily destroy such delicate nerves as the antennal, accessory antennal, ocellary and the labro-frontal nerves on the brain.**

-Dissecting dish
-Insect head specimen
-Minuten insect pin
-Bee's wax

Razor blade cuts

1. Pin the head of the insect with its mouthparts removed to the wax in the dissecting dish.

2. Cut the cranium above the clypeus and through the genae and vertex using a scalpel or razor blade.

Muscles and other tissues

Brain

3. Using a forceps ply out cranial walls of the frons and parts of the genae.

4. Carefully pull out muscles and other tissues to expose the brain with a forceps.

Fig. 60. Preparing an insect head to study the brain

Exercise 2: Continued

6. When the brain is clearly exposed, study it carefully under a binocular microscope. Identify the following structures and nerves in your specimen and make a large well - labelled diagram of the anterior view of the brain of your specimen;
   PROTOCEREBRUM
   DEUTOCEREBRUM
   TRITOCEREBRUM
   OPTIC LOBES
   ANTENNAL NERVES
   ACCESSORY ANTENNAL NERVES (if present)
   OCELLARY NERVES

Exercise 2: Continued

        FRONTAL GANGLION
        CIRCUMOESOPHAGEAL CONNECTIVES

7.   If details of the brain of your specimen are not clear, despite your having removed all obscuring matter, pour out the ethanol from the dissecting dish and then add a few drops of methylene blue or basic fuchsin stain on the brain. Let it stand for 10 min and then rinse it in 10% ethanol. Resubmerge the specimen in 10% ethanol and then examine your stained specimen under the binocular microscope and proceed with the lab exercise.

8.   In what ways is the brain of your specimen different from that of the armoured ground cricket described in the introduction to these exercises and illustrated in Fig. 60?

Exercise 3: **The Stomatogastric Nervous System.**

1.   Use a fresh specimen of a grasshopper or locust for this exercise.

2.   Cut off the head as in Exercise 2 above and ply out the mouthparts.

3.   This time however, pin the head on wax with its left parietal uppermost (see Fig. 61).

4.   Proceed to remove the sclerites of the cranium from the left parietal using a razor blade, a dissecting needle and a forceps. **Be very careful, especially when plying out the gena around the compound eye, to avoid destroying the optic lobe.**

5.   Peel out the mandibular muscle fibres a few at a time to expose the brain (Fig. 61C).

6.   Once the brain has become cleared, submerge it in 10% ethanol and then examine it under the binocular microscope. Identify the following structures and make a large, clear well - labelled diagram to show the lateral view of the brain;
        FRONTAL GANGLION
        FRONTAL NERVE
        OESOPHAGEAL GANGLION
        HYPOCEREBRAL GANGLION
        CORPUS ALLATUM

1. Pin the specimen to the wax in the dissecting dish left side uppermost.

2. Cut the cranium medially from the vertex to the epistomal suture and along the base of the gena.

3. Carefully remove cranial walls of the gena using a forceps.

4. Remove muscle and other tissues to expose part of the stomatogastric system.
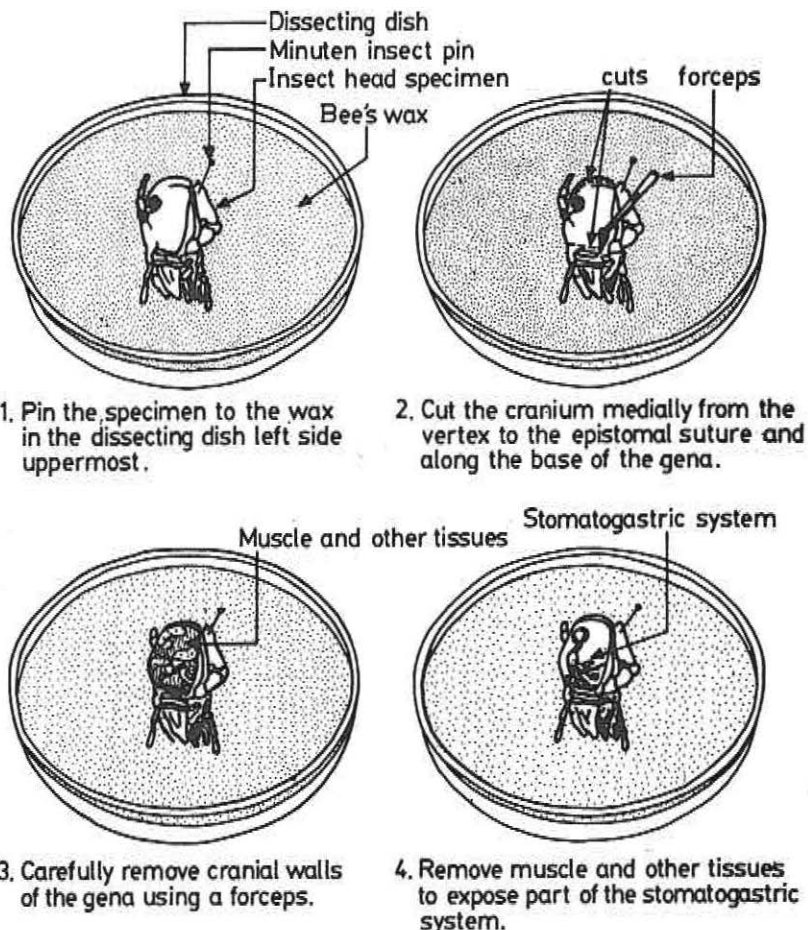
Fig. 61. Preparing an insect head to study the stomatogastric nervous system

Exercise 3: Continued

> NERVUS RECURRENS POSTERIOR EXTERNUS
> NERVUS RECURRENS POSTERIOR INTERNUS
> SUBOESOPHAGEAL GANGLION
> CIRCUMOESOPHAGEAL CONNECTIVES

NOTE: If details of structure are not clear on your specimen, stain the brain as you did in Exercise 2.

7. To examine the ingluvial ganglion, use the remaining part of your specimen in step 2 and dissect out the alimentary canal.

Exercise 3: Continued

8.   Examine the walls of the crop and identify the recurrent nerve to the ingluvial ganglion on one side of the crop. If the nerve is not distinct, stain your specimen with basic fuchsin or methylene blue and then re-examine.

9.   Once located, draw and label the ingluvial ganglion.

10. Where exactly on the crop wall does the ingluvial ganglion in your specimen abut?

11. What are the functions of the ingluvial ganglia or indeed the stomatogastric nervous system as a whole in insects?

Exercise 4: **Variation in the Nervous System.**

1.   You are provided with the following freshly anaesthetized or preserved adult insects:
   a.   cockroach
   b.   honeybee, and
   c.   house fly.

2.   Dissect the specimens for the ventral nerve cords following the steps outlined in Exercise 1. **Stain the specimens to make it easier for you to locate and identify the structures.**

3.   Compare and contrast the ventral nerve cords of your specimens. How do they differ from the generalized condition as in the desert locust?

4.   What would you say is the evolutionary tendency exhibited by the ventral nerve cord in the class Insecta ?

Supplementary Questions

1.   State the origin of the insect nervous system in the embryo and explain the relationship between the stomatogastric component of the system and growth, development and metamorphosis.

2.   What kinds of neurosecretory cell are present in the insect brain and in which of its subanatomical division are they located?

3.   What is the evolutionary trend in the organization of the brain and the ventral nerve cord in the class Insecta?

4.   The tendency of ganglia to fuse into each other is common in several insect groups. In your own words, what do you think is the advantage of having only a few ganglia in the abdominal tagma of insects?

5.   Some insect groups lack ocelli and others, compound eyes as well. What structural changes to the brain do you think are associated with loss of these visual organs?

6.   Describe the relationship between sensilla that usually are scattered throughout the cuticular surfaces of the insect body and the central nervous system (CNS).

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E.   The Respiratory System

The respiratory system in insects consists of a complex network of internal tubes (tracheae) that supply air to all parts of the body. Air enters the system via paired spiracular openings that are segmentally arranged, on the pleural regions of the thorax and abdomen.

The tracheal system originates as tubular invaginations of the integument in the embryo. The complex organization of the tracheae characteristic of the adult insect results from the ramifications of the invaginated tracheal tubes in the embryo, coupled with the formation of various interlinkages among the branches. Being ectodermal in origin, each tracheal tube, except for the finest branches, the tracheoles, is lined with cuticle internally, that is periodically shed during postembryonic growth. The cuticular lining called taenidia is spirally arranged in the tracheae.

The following sequence of events occur during the embryonic development of the tracheal system in insects. First a pair of spiracles develops in each spiracle-bearing segment by the invagination of the pleural body walls followed by further invagination of spiracular pits to form spiracular tracheae form. Each spiracular trachea then branches off to give rise to an anterior dorsal and a posterior dorsal connectives that associate with the muscles of the respective regions of a segment. At about this stage in the development, three additional tracheal branches form from the spiracular pit namely, an anterior spiracle connective, a posterior latero-tergal trachea and a leg trachea. The latter projects caudo-ventrally before subdividing to form an antero-tergal branch to the body wall, a pleural branch to the pleural fold, a ganglionic branch and a sternal branch.

The tracheal plan characteristic of an adult insect of a given insect group derives from the basic plan described above. For example, the lateral longitudinal tracheal trunk found common in some insect orders develops by the fusion of the anterior spiracular connective of one segment with the leg tracheal branch of the adjacent segment. The ventral longitudinal tracheal trunk results from the union of the sternal trachea with the ventral segmental trachea of the succeeding segment. Finally, the dorsal longitudinal tracheal trunk of some insect groups forms by the fusion of the anterior and posterior dorsal connectives of the dorsal segmental tracheae of adjacent segments.

The organization of the tracheae in the thorax is much more complex than in either the head or the abdominal body regions. This is due, among other things, to the absence of prothoracic spiracles, the occurrence of legs and in winged forms, the presence of wings in this tagma. The first thoracic (mesothoracic) spiracular pair is associated with tracheal trunks that not only supply air to the pro- and mesothorax but also to the head. The tracheae issuing from the second thoracic (metathoracic) spiracular pair, supply air to the metathorax in addition to forming anastomoses with the tracheal branches from the mesothorax and the abdomen.

The number of tracheal trunks entering the head from the mesothoracic spiracles varies in different insect groups. However, at least two primary tracheal pairs are known to enter the cranium from these spiracles in nearly all insect groups described to date. These are two dorsal and two ventral head tracheal trunks (one of each type from either spiracle). The head tracheal trunks may give off branches prior to or soon after entering the head. These ramifications supply air to the brain, muscles of the cranium and the mouthparts.

The spiracular openings are varied in shape and structure in insects. Two types of closing mechanisms that regulate the sizes of the aperture openings are recognized. The lip-type closing mechanism involve the presence of lip-like cuticular structure(s) at the spiracle, operated by muscles. In the valve type, the spiracular aperture size is varied by a special membranous fold in the spiracular atrium that controls the size of the opening to the trachea arising in the atrium (spiracular pit or chamber). The sizes and shapes of the

thoracic and abdominal spiracles differ in an individual and between species in many insect groups.

As an example of the organization of the tracheal system in the generalized condition, that of the armoured ground cricket *A. speiseri* is described below.

The Cephalic Tracheal Subsystem of the Armoured Ground Cricket (Figs 62–64)

The principal tracheal trunks supplying air to the head take their origin from the truncal apertures of the mesothoracic spiracles (Fig. 65, Sp2). The latter occur near the caudo-ventral margins of the pronotum (N1), above the procoxal articulations. Each mesothoracic spiracle gives off a dorsal head tracheal trunk (DHTra) and a ventral head tracheal trunk (VHTra), both of which enter the head through the foramen magnum.
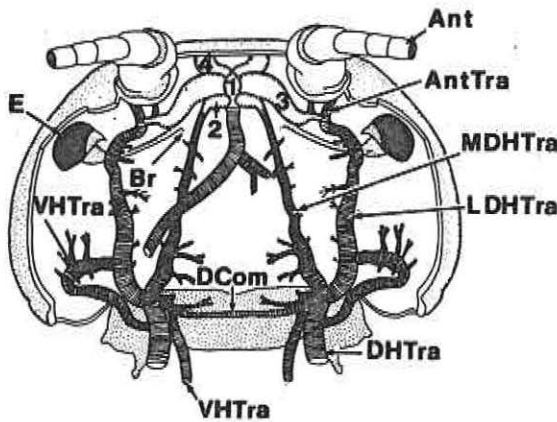
Fig. 62. Dorsal view of the head

The dorsal head tracheal trunk fuses with the dorsal longitudinal tracheal trunk (DTra) a short distance from spiracle before proceeding to the head. It divides soon after entering the cranium forming, 1) a large dorso-lateral trunk (LDHTra) that supplies branches to the adductor muscles of the mandible, the compound eye, and the antennae, 2) a smaller median dorsal branch (MDHTra) that serves the adductor muscles of the mandible, the brain and oesophagus, and 3) a short dorsal branch that fuses with an opposite number forming a dorsal commissure (DCom). The lateral and medial dorsal head tracheal trunks from the two halves of the head form an inosculation with each other and with the branches of the ventral head tracheal trunks, creating a system of air sacs in front of the brain called the **frontal-tracheal-air-sac-inosculation**. Branches issuing from below this inosculation are connected to chains of smaller air sacs in the labrum.

The ventral head tracheal trunk (VHTra) projects antero-ventrally shortly after emerging from the mesothoracic spiracle (Fig. 65). It gives off a small branch below that forms part of an X-shaped ventral commissure of the prothorax (X). In the cervical region, the ventral head tracheal trunk branches to form an arm that supplies the hypopharynx and labium via a number of small air sacs (Fig. 64, VHTra1, a, b, c, d, e). A second tracheal branch (VHTra2) is given off just prior to entering the cranium. This branch projects antero-
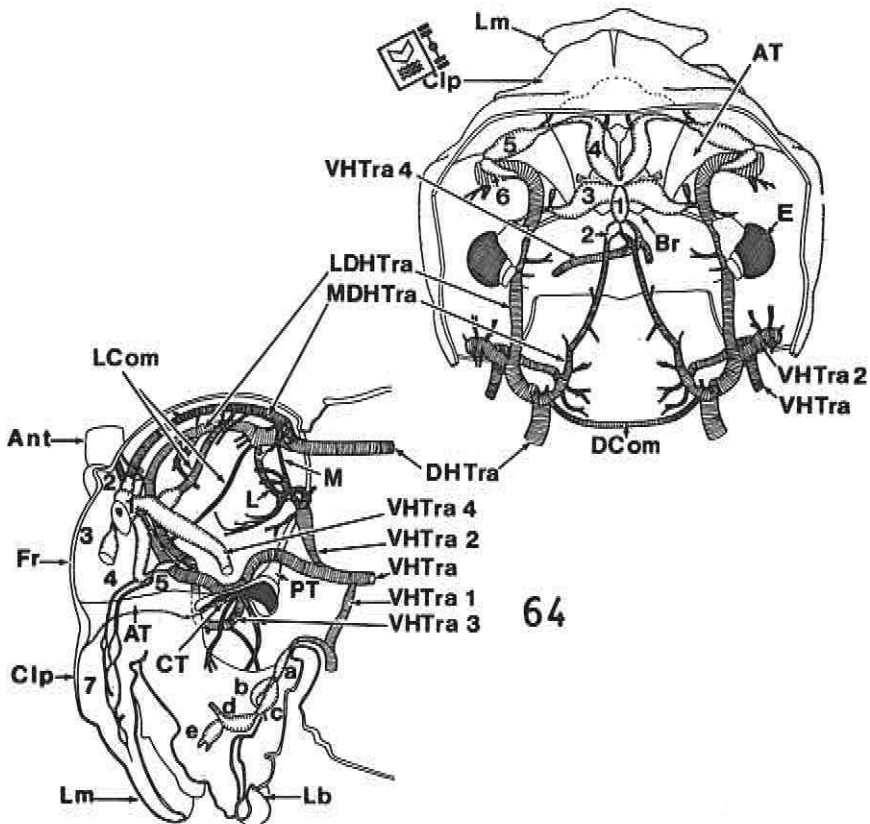
Fig. 63. Antero-dorsal view of the head showing the frontal-tracheal-air-sac-inosculation

Fig. 64. Lateral view of the dorsal and ventral head tracheal trunks

a,b,c,d,e, air sacs of the ventral head tracheal trunk; **Ant**, antenna; **AT**, anterior arm of the tentorium; **AntTra**, antennal trachea; **Clp**, clypeus; **CT**, corporo-tentorium; **DCom**, dorsal commissure; **DHTra**, dorsal head tracheal trunk; **E**, compound eye; **Fr**, frons; **Lb**, labium; **LCom**, lateral head commissure; **LDHTra**, lateral branch of the dorsal head tracheal trunk; **Lm**, labrum; **L,M**, dorso-ventral commissures of the second branch of the ventral head tracheal trunk; **MDHTra**, median branch of the dorsal head tracheal trunk; **PT**, posterior arm of the tentorium; **VHTra**, ventral head tracheal trunk; **VHTra1**, first branch of the ventral head tracheal trunk; **VHTra2**, first dorsal branch of the ventral head tracheal trunk; **VHTra3**, second ventral branch of the ventral head tracheal trunk; **VHTra4**, second dorsal branch of the ventral head tracheal trunk; **1–7**, cephalic tracheal air sacs.

Morphological Features of Taxonomic Importance

- Presence of a system of air sacs in front of the brain called frontal-tracheal-air-sac-inosculation.

dorsally and sends off smaller ramifications to the adductor and abductor muscles of the mandible and to the neck muscles. Two of its branches form anastomoses with the latero-dorsal branch (L, M). A third tracheal branch (VHTra3) is formed ventrally in the genal region. It supplies the mouthparts below and also has a ramus that fuses with the latero-dorsal head trunk. A fourth tracheal branch (VHTra4) is an air sac that, together with the main trunk of the ventral head trachea, fuses to the frontal-tracheal-air-sac-inosculation described above.

Two other lateral commissures are also found in the head. One links the latero-dorsal head trachea (LDHTra) to the third branch of the ventral head trachea (VHTra3). The second one connects the median head trachea (MDHTra) to the fourth branch of the ventral head trachea (LCom).

The Thoracic Tracheal Subsystem (Fig. 65).

The tracheation of the thorax in the armoured ground cricket is much more complex. In addition to its association with the dorsal and ventral tracheal trunks, the truncal aperture of the mesothoracic spiracle sends off tracheal branches to the pro- and mesothoracic organs, muscles and tissues. From the truncal stigma also issues, 1) a small tracheal branch (EPSVTra) that projects antero-ventrally, passing over a large prothoracic air sac (Vf) before fusing with the ventral head tracheal trunk (VHTra), and 2) an anterior supra-ventral trachea (ASVTra) which sends off fine branches of which extend to the mesothoracic tergo-sternal, tergo-pleural, tergo-coxal promotor, trochanteral depressor, subalar, basalar and the ventral longitudinal muscles. Below, it fuses with the ventral longitudinal tracheal trunk (VTra).

The anterior supra-ventral trachea (ASVTra) described before also forms an anastomosis with a shorter posterior dorso-lateral branch (PDLTra), a short distance from the truncal spiracle. This branch in turn, fuses with the posterior wing trachea (PWTra) or, as it is sometimes called, the dorso-lateral tracheal trunk (ADLtra), in the region above which another short dorsal anterior wing trachea originates. The latter unites with the dorsal longitudinal tracheal trunk (DTra) above. The posterior wing trachea (PWTra) is fused to the anterior middle leg trachea (ALTra) ventrally. It also sends off branches to the mesothoracic tergo-promotor, trochanteral depressor and other muscles occurring cephalad to the metathoracic pleural ridge. It then proceeds on to fuse with the metathoracic spiracle.

Above the dorsal head tracheal trunk (DHTra) in the prothorax and fused to it, is the paradorsal trachea (PDTra). It is expanded along its length into a number of air sacs. It also supplies branches to the prothoracic muscles.

The femoral stigma of the mesothoracic spiracle is linked internally to a large, bag-like, trumpet-shaped vesicle (Fig. 65, Vf). The latter is part of the acoustic or femoral tracheal trunk (ACTra) supplying air to the foreleg. The vesicle gives off a small communicating trachea (CTra) medially that fuses with a similar opposite number.
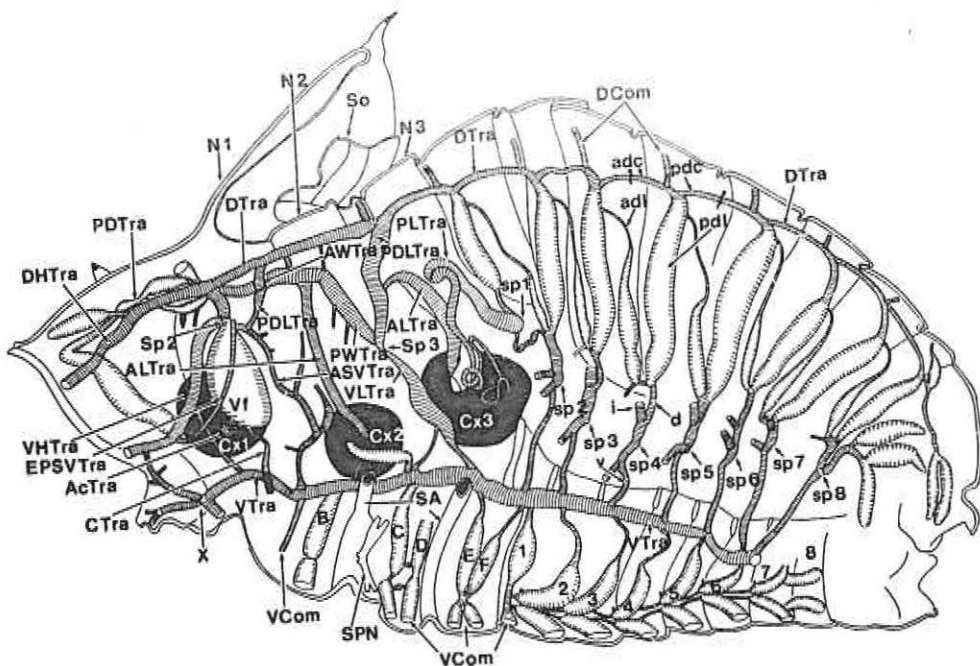
Fig. 65 Lateral view of the male thorax and abdomen showing the tracheal system

ACTra, acoustic or femoral trachea; adc, anterior dorsal connective; ALTra, anterior leg trachea; ASVTra, anterior supra-ventral trachea; AWTra, anterior wing trachea; CTra, communicating trachea: Cx, coxa; d, dorsal abdominal trachea; DCom, dorsal commissure; DHTra, dorsal head tracheal trunk; DTra, dorsal plurisegmental tracheal trunk; EPSVTra, external posterior supra-ventral trachea; i, intestinal trachea, N, notum; pdc, posterior dorsal connective; PDLTra, posterior dorso-lateral trachea; PDTra, paradorsal trachea; PLTra, posterior leg trachea; PWTra, posterior wing trachea; SA, sternal apophysis; SO, stridulating organ; sp, abdominal spiracle; Sp, thoracic spiracle; SPN, spina; v, ventral abdominal trachea; VCom, ventral commissure; Vf, vesicula femoralis; VHTra, ventral head tracheal trunk; VLTra, ventro-lateral trachea; VTra, ventral plurisegmental tracheal trunk; x, X-shaped ventral commissure; 1–8, ventral abdominal tracheal air sacs.

Morphological Features of Taxonomic Importance

- Presence of a large air sac, the vesicula femoralis (Vf) associated with the tympanal organ of the protibia on each side of the body.
- Presence of many abdominal tracheal air sacs.

The organization of the tracheae associated with the metathoracic spiracle is simpler than that of the mesothoracic spiracle. Three tracheal trunks arise from the spiracle, 1) a posterior wing trachea (PWTra) described before, 2) a ventro-lateral tracheal trunk (VLTra) that projects caudo-ventrally before fusing with the ventral longitudinal tracheal trunk below, and 3) a posterior dorso-lateral trachea (PDLTra) that forms an anastomosis with the dorsal longitudinal tracheal trunk above. The trachea gives off an anterior hind leg branch (ALTra). The posterior hind leg trachea (PLTra) originates from the first abdominal spiracle.

A number of ventral commissures occur in the thorax (Fig. 65, VCom, X). In the prothorax, there is an X-shaped commissure resulting from the fusion of the branches of the ventral head trachea and the ventral longitudinal tracheal trunk. A small tracheal ramus arises from each of the anterior arms of the commissure and enters the head. The ventral commissures of the meso- and metathoracic segments are paired tracheal air sacs that are fused medially (Fig. 65, B–F).

The Abdominal Tracheal Subsystem (Fig. 65).

The organization of the tracheae in the abdomen of the armoured ground cricket is generalized with only minor modifications (Fig. 65). Each half of the body bears two main longitudinal tracheal trunks namely, dorsal and ventral longitudinal tracheal trunks (DTra, VTra). These extend the length of the body from about the 7th abdominal segment to the prothorax, where the dorsal trunk is continuous with the dorsal head tracheal trunk and the ventral trunk terminates into the X-shaped ventral commissure.

The basic tracheal plan for each abdominal segment involves the following; a short dorsal trachea (d) arises from the spiracle and projects upwards. It forks to form anterior and posterior dorso-lateral branches (Fig. 65, adl, pdl). The anterior branch bears a long, narrow, tapering air sac that fuses above with the posterior branch of the dorsal trachea of the preceding segment. The posterior branch expands into a large sausage-shaped air sac whose narrowed dorsal end bears embryonic anterior and posterior connectives described before (Fig. 65, adc, pdc). The connectives fuse with each other in successive segments in a developing embryo to form the dorsal longitudinal tracheal trunk (DTra) characteristic of the adult.

The dorsal longitudinal tracheal trunks from the two halves of the body border the aorta and the heart (Fig. 49, DTra, Ao). They communicate with each other segmentally via short dorsal commissures (DCom) located between the ampullae (Amp) of the dorsal blood vessel, above the alary muscles (Am). A ventral trachea unites below with the ventral longitudinal tracheal trunk (Fig. 65, v, VTra). A pair of intestinal or visceral tracheae (i) ramify on the alimentary canal, the reproductive organs, fat body and other tissues.

A ventral air sac commissure occurs in the first abdominal segment (VCom). This commissure is linked to a chain of paired ventral air sacs caudally (one pair per segment) that communicate with each other via short tracheal connectives.

The thoracic and abdominal spiracles of the armoured ground cricket differ in shape and structure. The mesothoracic spiracle bears truncal and femoral stigmata that are closely situated (Figs 66, 67, ts, fs). The truncal stigma has a lip-type closing mechanism (L) externally, while the femoral stigma is open to the outside (a).

Internally (Fig. 67), the truncal stigma is associated with the roots of the dorsal and ventral head tracheal trunks (DHTra, VHTra), the extreme supra-
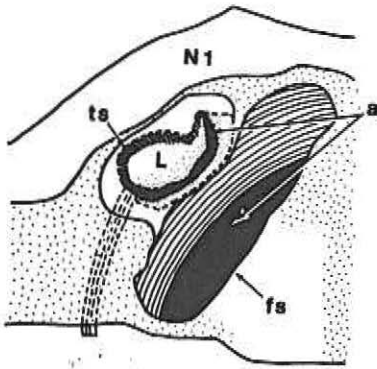
Fig. 66. External structure of the mesothoracic spiracle

ventral trachea (EPSVTra), and the anterior supra-ventral trachea (ASVTra). The opening of the truncal atrial pore is achieved through the contraction of the antagonistic spiracle muscle that arises on the mesosternum (Fig. 67, am, ts, L). The closure of the atrial pore occurs automatically when the antagonistic spiracle muscle relaxes, due to the elastic properties of the cuticle surrounding the truncal stigma.

The femoral stigma, on the other hand, is linked internally to a large horn-shaped air sac (Vf) that communicates with the acoustic or femoral tracheal trunk of the leg. The latter as the name implies, is associated with the auditory organ located on the proximal end of the foretibia.

The metathoracic spiracle (Figs 68, 69) has a
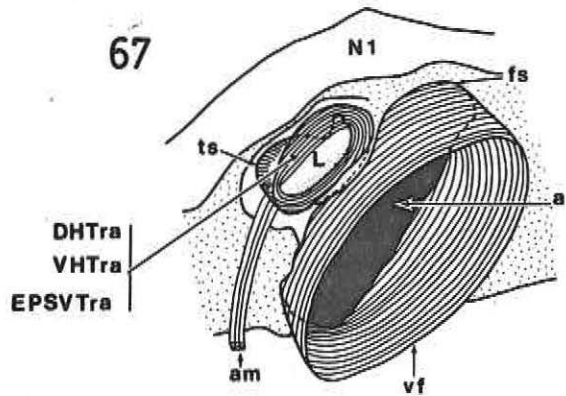
Fig. 67. Internal anatomy of the mesothoracic spiracle

Fig. 68. External view of the metathoracic spiracle

typical orthopteran structure consisting of a rigid anterior lip (c) and a movable C-shaped bar (d) that are linked ventrally by a cuticular lobe (q). The two components of the spiracle are surrounded by an oval peritreme (Ptr). Internally (Fig. 69), the spiracular opening is connected to the base of the posterior wing trachea (PWTra), the posterior dorso-lateral trachea (PDLTra) and the ventro-lateral trachea (VLTra). One branch of the spiracle antagonistic muscle inserts on the manubrium (m) of the movable bar, while another branch of nearly equal size, inserts on the peritreme (Ptr), antero-ventrally.

SECTION V
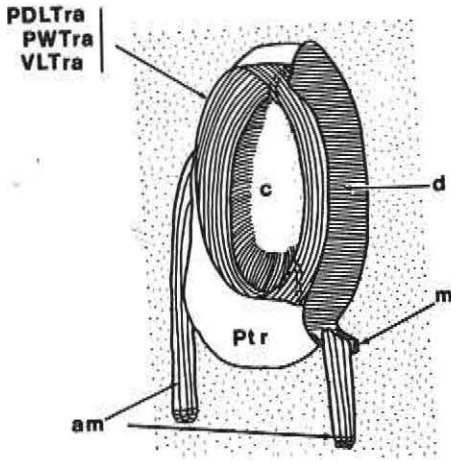
Fig. 69. Internal aspect of the metathoracic spiracle

The basic structure of the abdominal spiracles is the same although their sizes and shapes vary slightly. Each spiracle bears a thick, oval, elongated closing band and a C-shaped closing bow. Both of these are surrounded by a chitinous ring of peritreme (Figs 70, 72, 74, b, c, Ptr). Internally, the closing mechanism of the spiracle involves antagonistic and occlusor spiracle muscles (Figs 71, 73, 75, am, om).

The opening of the atrial pore is achieved through the contraction

a, aperture; am, antagonistic spiracle muscle; c, anterior spiracular lip; d, posterior spiracular lip; DHTra, dorsal head tracheal trunk; EPSVTra, external posterior supra-ventral trachea; fs, femora stigma; L, lip; m, manubrium; N1, pronotum; Ptr, peritreme; q, ventral lobe; ts, truncal stigma; Vf, vesicula femoralis; VHTra, ventral head tracheal trunk.

Morphological Features of Taxonomic Importance

- Presence of two types of apertures in the mesothoracic spiracle namely, truncal (ts) and femoral (fs) stigmata.
- Lip-type closing mechanism (c) on the mesothoracic spiracle.
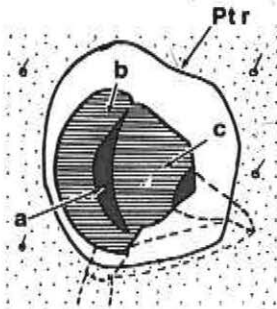
Fig. 70. External structure of the first abdominal spiracle
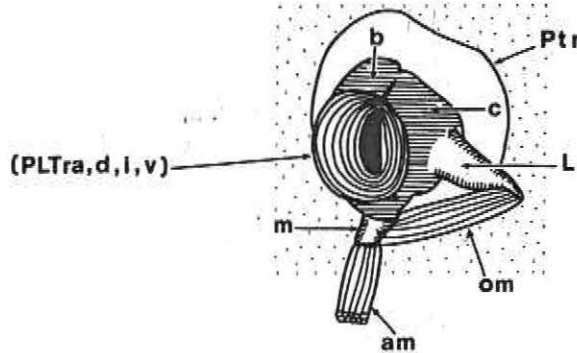
Fig. 71. Internal anatomy of the first abdominal spiracle

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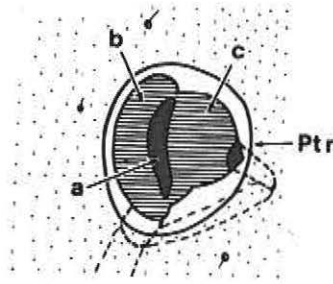
Fig. 72. External structure of the second abdominal spiracle

Fig. 73. Internal anatomy of the second abdominal spiracle

Fig. 74. External structure of the eighth abdominal spiracle
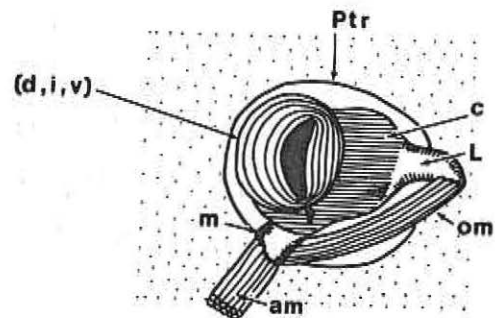
Fig. 75. Internal anatomy of the eighth abdominal spiracle

of the spiracle antagonistic muscle. The latter takes its origin on the posterior margin of the laterosternite of the segment bearing the spiracle and inserts on a manubrium (m) of the closing bow. Closure of the spiracle is the result of the relaxation of the antagonistic spiracle muscle, followed by the contraction of the occlussor spiracle muscle. The latter arises from a lever (L) on the closing band and inserts on the manubrium of the closing bow, adjacent to the point of insertion of the antagonistic spiracle muscle.

a, atrial aperture; am, antagonistic spiracular muscle; b, closing bow; c, closing band; d,i,v, dorsal, intestinal and ventral abdominal tracheae, respectively; L, closing lever; m, manubrium; om, occlussor spiracle muscle; PLTra, posterior leg trachea; Ptr, peritreme.

Morphological Features of Taxonomic Importance

- Lip-type spiracular closing mechanism (c).

Exercises

Materials Required:

Specimens :
1. Freshly anaesthetized or preserved (in 70% ethanol) adult desert locust, *Schistocerca gregaria* Forskal or garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper.

Apparatus:
1. Dissecting dish with beeswax at the bottom.
2. Dissecting set.
3. Minuten insect pins (No. 5).
4. Bunsen burner.
5. 250 ml beaker.
6. Binocular microscope.

Chemicals:
1. 3% KOH.
2. 10% ethanol.
3. Basic fuchsin or Congo red stain.

Exercise 1: Abdominal Tracheal Plan.

1. Using a razor blade or a pair of scissors clip off the legs and wings of a male or female grasshopper or locust and then bisect its body longitudinally along the dorsum and venter of the body (see Fig. 76).

2. Use one half of the body. Reserve the other half for the following exercises.

3. Pin the one half of the body to the beeswax in the dissecting dish with the cutup face uppermost.

4. The main tracheal trunks will be found close to the body wall. Remove as much of the tissues inside of the body half as possible while viewing it under the binocular microscope, i.e., the alimentary canal, internal reproductive organs, fat body and the connective tissue, including some minor tracheal branches.

5. If the main tracheae are not distinct, stain your specimen with Congo red or basic fuchsin for 10 min and then examine under the binocular microscope.

1. Bissect the specimen longitudinally through the midlines of the dorsum and venter.

2. Pin one half of the body to the wax in the dissecting dish with the cutup face uppermost.

3. Remove the alimentary canal and testis or ovary using a forceps.

4. Carefully remove the remaining fat body and connective tissues to expose the tracheal system.
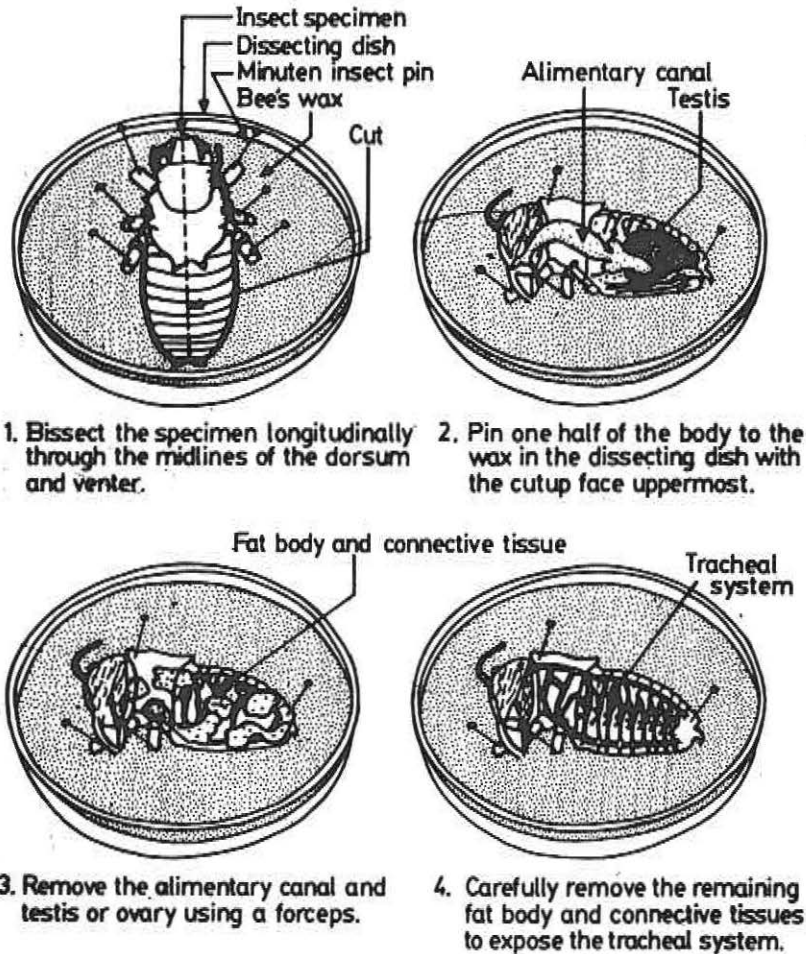
Fig. 76. Preparing an insect body to study the tracheal system

Exercise 1: Continued

6. Submerge the specimen in 10% ethanol. Study the arrangement of the tracheae in three abdominal segments. Draw and label.

7. How does the arrangement of the tracheae in your specimen differ from that described for the armoured ground cricket in the introduction to these exercises?

Exercise 2: **Thoracic Tracheal Plan.**

1. Continue using the specimen in Exercise 1 above.

2. Clear all unwanted material in the thoracic region to expose the main tracheae.

Exercise 2: Continued

3. Study the tracheal plan of this tagma noting the relationships of the tracheae to the spiracles.

4. Make a large, well - labelled diagram of the internal view of the thorax to show the tracheal arrangements.

5. What is the difference in the arrangement of the tracheae between your specimen and the armoured ground cricket?

Exercise 3: **Head Tracheal Plan.**

1. Again using the same specimen in exercises 2 and 3 above, now carefully work on the head. Trace the main tracheal trunks from the cervix into the cranium.

2. Note that you may have to remove a lot of tracheal branches and mandibular muscle fibres to expose the main tracheal trunks in the cranium.

3. When you are satisfied with your work, i.e., when most of the main tracheal trunks in the cranium are distinctly visible, submerge the specimen in 10% ethanol and then study the head tracheal plan under a binocular microscope.

4. Make a large, well - labelled diagram showing the tracheal plan of the one half of the head of the grasshopper.

5. How does this plan differ from that of the armoured ground cricket?

Exercise 4: **Thoracic and Abdominal Spiracles.**

1. Use the other half of the grasshopper or locust you put aside in Exercise 1 for this exercise.

2. Remove whatever remained of the alimentary canal, internal reproductive organs and the fat body from it using a forceps.

3. Place the specimen in a beaker containing 3% KOH and boil for 10 minutes to dissolve all remaining soft tissue.

4. Transfer the specimen to the dissecting dish with 10% ethanol. While examining it under the binocular microscope, remove whatever other soft tissue still remains from the body cavity using a dissecting needle

Exercise 4: Continued

and forceps to expose the thoracic and abdominal spiracles.

5. Study the external and internal structures of the following spiracles; MESOTHORACIC SPIRACLE, METATHORACIC SPIRACLE, ABDOMINAL SPIRACLES 1, 3, and 8.

6. How do the structures of these spiracles differ from that of the armoured ground cricket described in the introduction to these exercises?

Supplementary Questions

1. Describe types of respiratory systems found in; a. the caterpillar of the sorghum stem-borer, *Chilo partellus* Swinhoe; b. the pupa of the anopheline mosquito, *Anopheles gambiae* (Giles) or *A. funestus* (Giles); c. larvae of the screwworm, *Cochliomyia hominivorax* (Diptera).

2. How do the spiracular mechanisms of the desert locust, *Schistocerca gregaria* Forskal regulate gaseous exchange in the insect's body?

3. How is gaseous exchange effected in the tracheoles?

4. What are the functions of the air sacs in the tracheal system of insects?

5. In which insect groups are definitive prothoracic spiracles found and in what way does the existence of this extra pair of spiracles on the insect body affect the efficiency of ventilation in the insect group?

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Vinal, C.S. 1919. The respiratory system of the Carolina locust (*Dissosteria carolina* L.). *J.N.Y. Entomol. Soc.* 27:19–32.

F.   The Muscular System

Snodgrass (1935a,b) classified the thoracic muscles of an insect into 15 categories; dorsal, tergo-pleural, tergo-sternal, axillary, epipleural, lateral intersegmental, pleuro-sternal, ventral, tergo promotor(s) of the leg, tergal remotor(s) of the leg, sternal promotor(s) of the leg, sternal remotor(s) of the leg, pleuro-coxal, adductor(s) of the coxa and extracoxal depressor(s) of the trochanter. This categorization of the thoracic muscles is on the basis of their location, function and points of origin and insertion in the thorax. According to Snodgrass, not all muscles listed above are present in all insects, but many are in all insects described todate. Tiegs (1955) identified the tergal, dorso-ventral, pleuro-tergal, pleural and the axillary thoracic muscles as the important ones in the operation of the insect wing. The same muscles are important in the operation of the stridulating organs in the Tettigoniidae to which armoured ground crickets used throughout the introductions to the sections of this laboratory manual belong.

In the generalized condition as obtains in the Orthoptera, the abdominal musculature serves a respiratory function, except for specialized muscles that operate the genitalia. Three muscle groups, the tergal, sternal and pleural muscles are important in the abdomen. All three muscle groups plus their various combinations, occur in the genital segments of both sexes, but some muscles are so specialized that they are difficult to homologize with those of the visceral segments of the abdomen.

The general musculature of the armoured ground cricket *Acanthoplus speiseri* are described below. As closely as possible the terminology and numbering of the muscles follows that of Snodgrass (1935a,b).

The Head (Fig. 77)

The following muscles are seen in a sagittal (median) plane of the head of the armoured ground cricket (Fig. 77).

2.   **Anterior Retractor of the Labrum** — This is a large muscle that originates on the subantennal ridge and inserts medially on the anterior wall of the clypeo-labral region.

4.   **Elevator of the Antennae** — This muscle occurs in two groups (not illustrated in the diagram). The muscles arise from the anterior arm of the tentorium and insert on a single apodeme at the dorsal rim of the scape.

5a, 5b. **Depressors of the Antenna** — This muscle occurs in two groups. The muscles arise from the anterior arms of the tentorium and insert on an apodeme on the ventral rim of the scape.
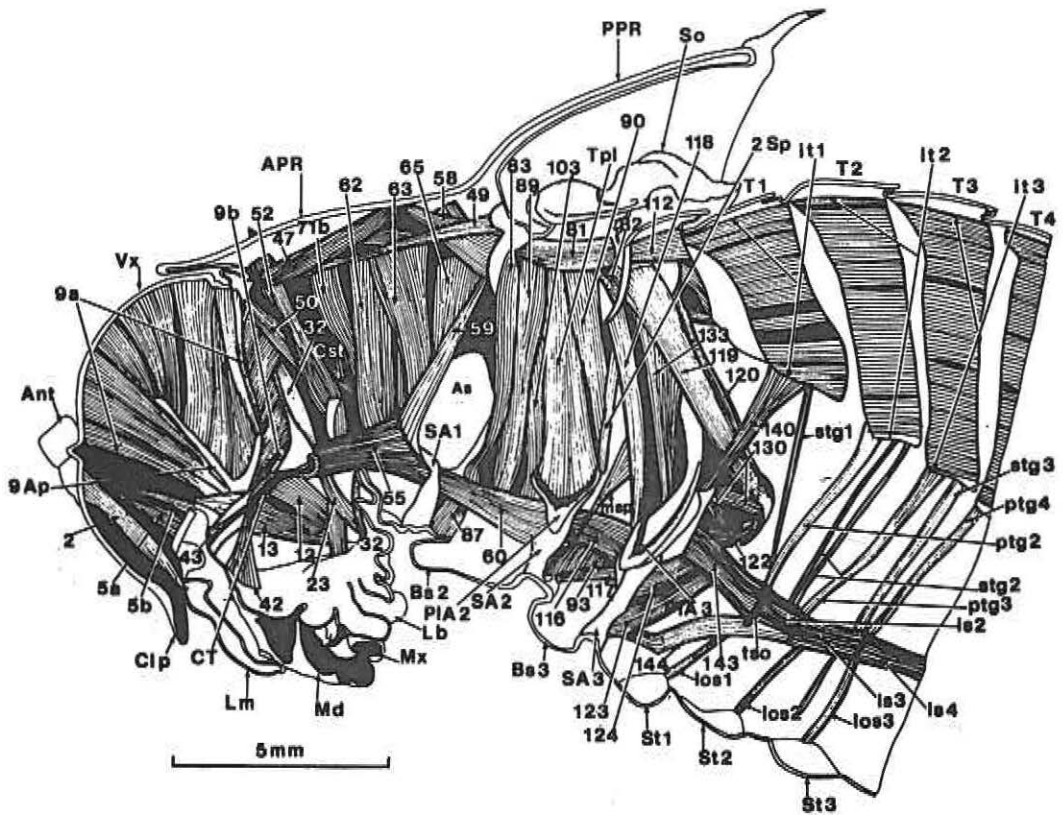
Fig. 77. Medial view of the masculature of the right side of the male head, thorax and the first three abdominal segments

Skeleton

Ant, antenna; 9 Ap, adductor apodeme of the mandible; APR, anterior promotal region; As, trachea air sac; Bs, basisternite; Clp, clypeus; CT, corporotentorium; Lb, labrum; Lm, labium; Md, mandible; Mx, maxilla; PIA, pleural apophysis; PPR, posterior pronotal region; SA, sternal apophysis; So, stridulating organ; St, abdominal sternite; T, abdominal tergite; Vx, vertex.

Musculature

2.	Anterior retractor of labrum.
4.	Elevator of the antennae.
5a, 5b.	Depressors of the antennae.
9a, 9b.	Adductors of the mandible.
12, 13.	Adductors of the stipes.
23.	Proximal retractor of the labium.
32.	Retractor of the hypopharynx.
42, 43.	Ventral dilators of the pharynx.
47.	First protergal muscle of the head.
49.	Dorsal longitudinal muscle of the neck and prothorax.
50.	Cephalic muscle of the neck sclerites.
Cst.	Cephalo-sternal muscle.
52.	Protergal muscle of the neck sclerites.
55.	First ventral longitudinal muscle.
58.	Tergo-pleural intersegmental muscle.
59.	Sterno-pleural intersegmental muscle.
60.	Second ventral longitudinal muscle.
62.	Tergal promotor of the pro-coxa.
63, 65.	First and third tergal remotors of the pro-coxa.
71b.	Depressor of the trochanter.
M sp.	Spina-pleural muscle.
Tpl.	Tergo-pleural muscle.
2 Sp.	Sterno-pleural intersegmental muscle.
81.	Median dorsal longitudinal muscle.
82.	Oblique dorsal muscle.
83.	First tergo-sternal muscle.
87.	Third ventral longitudinal muscle.
89.	Tergal promotor of the meso-coxa.
90.	First tergal remotor of the meso-coxa.
93.	Posterior rotator of the meso-coxa.
103.	Depressor of the trochanter.
112.	Median dorsal longitudinal muscle.

116, 117. Fifth and sixth ventral longitudinal muscles.
118. Tergal promotor of the meta-coxa.
119, 120. First and second remotors of the meta-coxa.
122, 123, 124. First, second and third rotators of the meta-coxa.
130. Adductor of the meta-coxa.
133. Depressor of the trochanter.
140. Sterno-pleural intersegmental muscle.

143, 144. Seventh and eighth longitudinal muscles.
is. Inner sternal muscle.
it. Inner tergal muscle.
los. Lateral outer sternal muscle.
ptg. Primary tergo-sternal muscle group.
stg. Secondary tergo-sternal muscle group.

9a, 9b. Adductors of the Mandible — These are the largest muscles in the cranium. They take their origin from the large mandibular apodeme (9 Ap) and insert on the cranial walls dorsally and posteriorly. 9a arises from the anterior branch of the mandibular apodeme, while 9b originates on a smaller posterior branch.

12, 13. Adductors of the Stipes — These are large muscles composed of many muscle fibres. They arise from the ventral face of the tentorium and insert on a ridge on the stipes. Muscle 13 arises anterior to 12 and some of its fibres are borne on the ventral surface of the anterior tentorial arms.

23. Proximal Retractor of the Labium — This muscle has its origin on the ventral surface of the posterior arm of the tentorium and inserts at the base of the prementum.

32. Retractor of the Hypopharynx — This is a long muscle that arises on the postoccipital ridge (PoR) dorsolaterally and inserts below, on the lateral sclerotized rods of the hypopharynx. Its point of origin is close to the base of the temporal ridge.

42, 43. Ventral Dilators of the Pharynx — Both muscles arise from the anterior ridge of the anterior tentorial arm, muscle 43 being dorsal to 42. They attach to the wall of the pharynx.

It should be noted, however, that many more muscles that could be visible in a dissection made along the sagittal (median) plane of the insect cranium have not been illustrated in Figure 57. These muscles include the compressor of the labrum, which originates below the anterior retractor muscle of the labrum (2) on the labral wall and inserts on the cranial wall below the subantennal ridge. Others are the posterior retractor of the labrum and several muscle groups that operate the stomodaeum in various capacities. The origins and insertions of these muscles in the armoured ground cricket *A. speiseri* are similar to those described for the migratory locust by Albrecht (1953).

The Neck (Cervical) and Prothoracic Musculature

47. **First Protergal Muscle of the Head (Figs 77, 78, 81)**— A median dorsal longitudinal muscle that originates from the tergal wall of the anterior pronotal region (APR) and attaches to the postoccipital ridge dorsolaterally.

49. **Dorsal Longitudinal Muscle of the Neck and Prothorax (Fig. 77)** — This muscle originates from the mesothoracic prescutal lobe and inserts on the postoccipital ridge, adjacent to muscle 47.
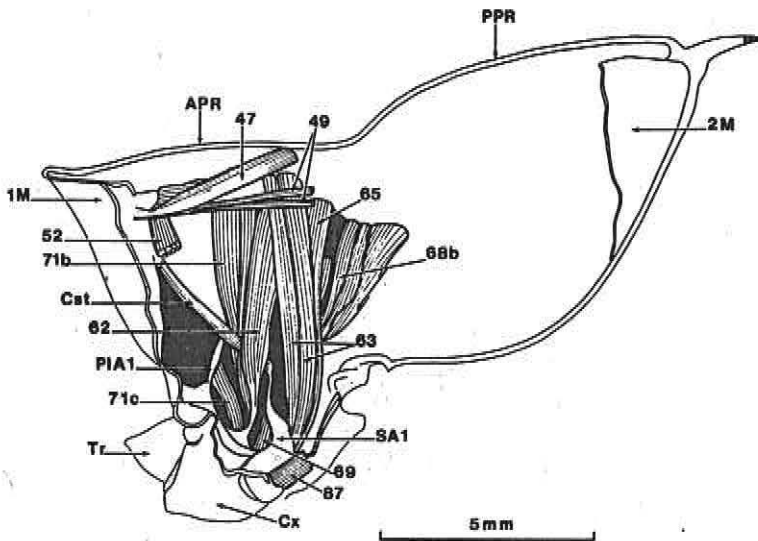
Fig. 78. Medial view of the male prothorax

Skeleton

APR, anterior pronotal region; Cx, coxa; 1M, 2M, intersegmental membranes; PIA, pleural apophysis; PPR, posterior pronotal region; SA, sternal apophysis; Tr, trochanter.

Musculature

47.	First protergal muscle of the head.	63, 65.	First and third remotors of the pro-coxa.
49.	Dorsal longitudinal muscle of the neck and prothorax.	68b.	Abductor of the pro-coxa.
52.	Protergal muscle of the neck sclerites.	69.	Adductor of the pro-coxa.
Cst.	Cephalo-sternal muscle.	71b, 71c.	Depressors of the trochanter.
62.	Tergal promotor of the pro-coxa.	87.	Third ventral longitudinal muscle.

Morphological Features of Taxonomic Importance

- Generalized masculature.

50. **Cephalic Muscle of the Neck Sclerites (Fig. 77)** — This muscle arises from the postoccipital ridge below muscle 49 and inserts on the first cervical sclerite.

Cst. **Cephalo-Sternal Muscle (Figs 77, 78)** — A slender muscle that arises from the postoccipital ridge below muscles 47 and 49. It projects posteroventrally, passing laterad of muscle 62 to insert on the tip of the prosternal apophysis. This muscle has not been mentioned by Snodgrass (1935a,b).

51. **Cephalic Muscle of the Neck Sclerites (Figs 85, 86)** — This muscle shares the same origin with muscle 50 on the postoccipital ridge but inserts on the second cervical sclerite.

52. **Protergal Muscle of the Neck Sclerites (Fig. 77)** — This muscle originates on the tergum in the anterior pronotal region (APR) and inserts at the posterior margin of the first cervical sclerite, near its junction with the second cervical sclerite.

52X. **Cephalic Muscle of the Neck Sclerites (Fig. 85)** — A short muscle that arises near the postoccipital condyle and inserts on the anterior margin of the first cervical sclerite.

54. **Prosternal Muscle of the First Neck Sclerite (Figs 85, 86)** — A horizontal, diagonal muscle that arises from the prosternal apophysis and inserts on the first cervical sclerite of the opposite side of the neck. The two muscles from the opposite sides of the neck cross each other medially.

55. **First Ventral Longitudinal Muscle (Fig. 77)** — A flat muscle composed of a number of fibre bundles. It arises from the base of the posterior arm of the tentorium and attaches to the anterior basal margin of the prosternal apophysis.

56. **Dorsal Lateral Neck Muscle (Fig. 82)** — A flat muscle possessing several fibre bundles that arise on the mesonotal antecosta and insert on the cervical membrane anteriorly.

57. **Ventral Lateral Neck Muscle (Fig. 85)** — A short, broad muscle that originates on the prothoracic episternum and inserts in the neck membrane.

58. **Tergo-Pleural Intersegmental Muscle (Fig. 77)** — A broad flat muscle that arises from the protergum and inserts on the mesonotal antecosta.
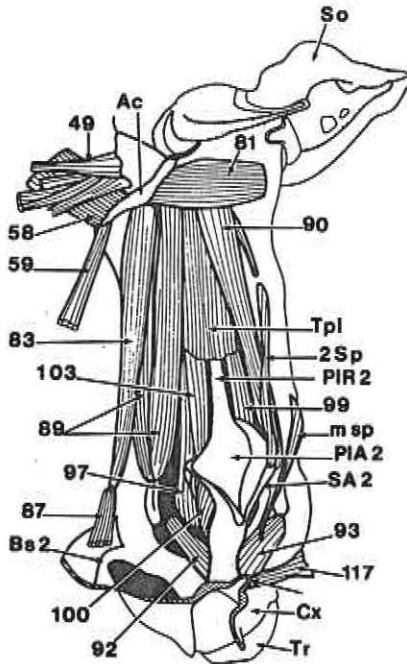
Fig. 79. Medial view of the male mesothorax
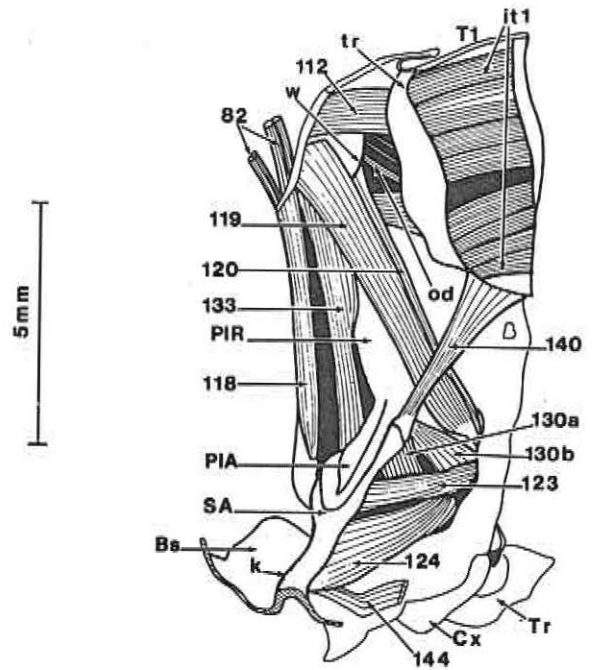
Fig. 80. Medial view of the male metathorax

Skeleton

Ac, antecosta; Bs, basisternite; Cx, coxa; k, sternal ridge; PIA, pleural apophysis; PIR, pleural ridge; SA, sternal apophysis; So, stridulating organ; Tr, trochanter; tr, abdominal tergal ridge; T, abdominal tergite; w, metathoracic tergal ridge.

Masculature

49.	Dorsal longitudinal muscle of the neck and prothorax.	100.	First adductor of the meso-coxa.
58.	Tergo-pleural intersegmental muscle.	103.	Depressor of the trochanter.
59.	Sterno-pleural intersegmental muscle.	M Sp.	Spina-pleural muscle.
81.	Median dorsal longitudinal muscle.	117.	Sixth ventral longitudinal muscle.
82.	Oblique dorsal muscle.	It 1.	First inner tergal abdominal muscle.
83.	First tergo-sternal muscle.	od.	Oblique dorsal muscle.
87.	Third ventral longitudinal muscle.	112.	Median dorsal longitudinal muscle.
Tpl.	Tergo-pleural muscle.	118.	Tergal promotor of the meta-coxa.
2 Sp.	Sterno-pleural intersegmental muscle.	119, 120.	First and second tergal remotors of the meta-coxa.
92.	Anterior rotator of the meso-coxa.		
93.	Posterior rotator of the meso-coxa.	123, 124.	Second and third posterior rotators of the meta-coxa.
97.	First pronator-extensor of the stridulating organ.	130a, 130b.	Adductors of the meta-coxa.
		140.	Sterno-pleural intersegmental muscle.
99.	Depressor-extensor of the stridulating organ.	144.	Ventral longitudinal muscle.
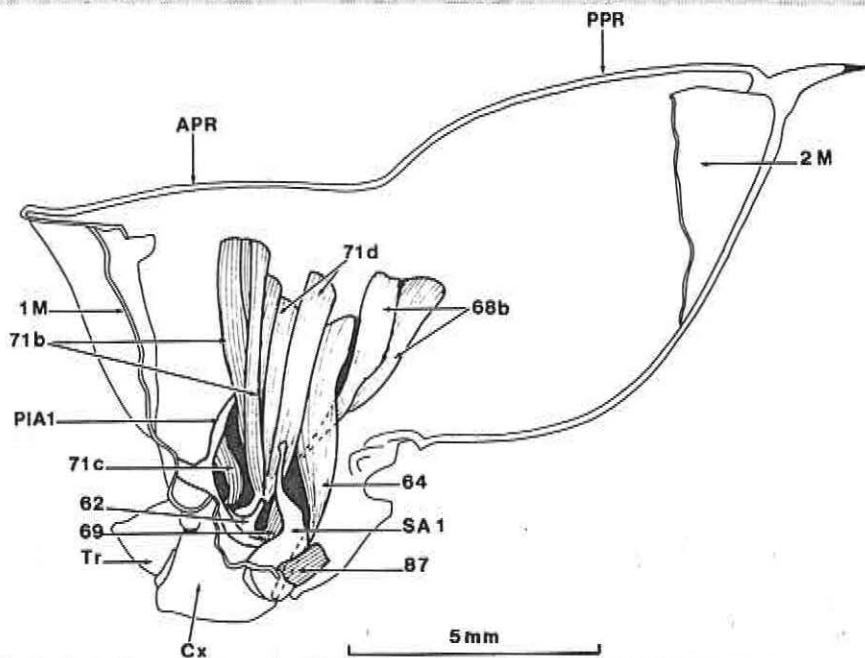
Fig. 81. Medial view of the male prothorax with the inner muscles removed

Skeleton

APR, anterior pronotal region; Cx, coxa; 1M, 2M, intersegmental membranes; PIA, pleural apophysis; PPR, posterior pronotal region; SA, sternal apophysis; Tr, trochanter.

Masculature

62.	Tergal promotor of the pro-coxa.	69.	Adductor of the pro-coxa.
64.	Second tergal remotor of the pro-coxa.	71b, 71c, 71d.	Depressors of the trochanter.
68b.	Abductor of the pro-coxa.	87.	Third ventral longitudinal muscle.

Morphological Features of Taxonomic Importance

- Generalized masculature.

59. **Sterno-Pleural Intersegmental Muscle (Figs 77, 79, 82, 85, 86)** — An oblique intersegmental muscle composed of many fibre bundles. It is broad at its origin on the antero-dorsal edge of the prosternal apophysis and gradually tapers towards its insertion point on the dorsal ridge of the mesothoracic episternum.

60. **Second Ventral Longitudinal Muscle (Figs 77, 85, 86, 87)** — A flat muscle arising from the posterior edge of the presternal apophysis. It tapers off posteriorly and inserts on the anterior edge of the mesosternal apophysis.

62. **Tergal Promotor of the Pro-Coxa (Figs 77, 78, 85, 86)** — A large tergo-sternal muscle that arises from the tergum, on the anterior
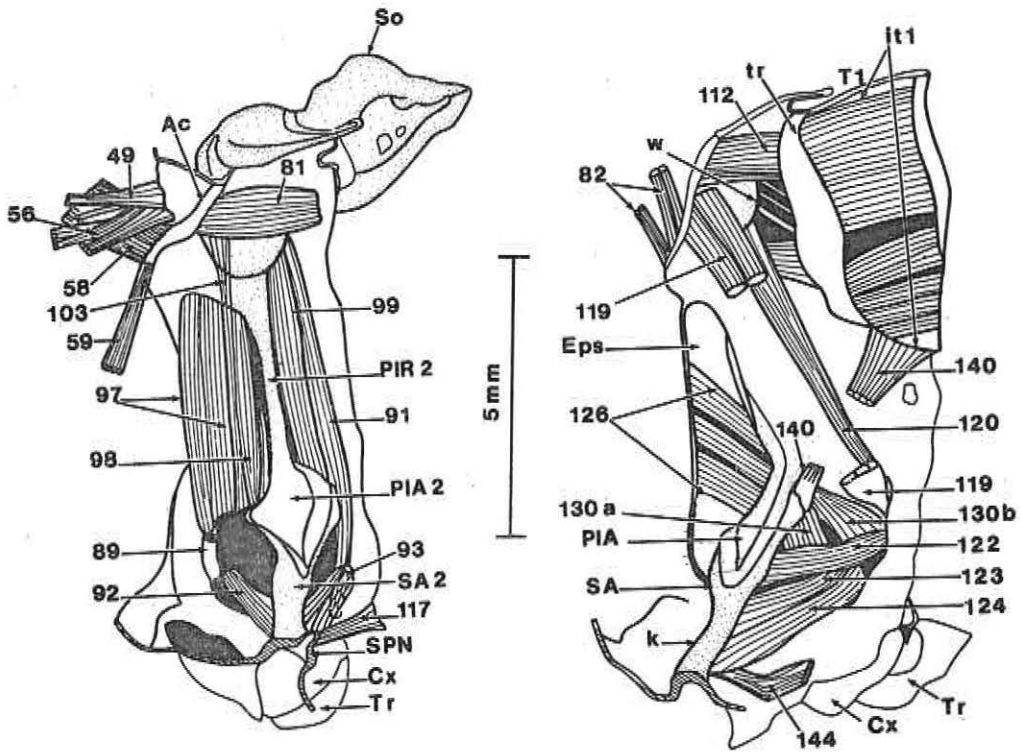
Fig. 82. Medial view of the male mesothorax with more inner muscles removed

Fig. 83. Medial view of the male metathorax with some inner muscles removed

Skeleton

Ac, antecosta; Bs, basisternite; Cx, coxa; Eps, episternite; k, sternal ridge; PIA, pleural apophysis; PIR, pleural ridge; SA, sternal apophysis; So, stridulating organ; SPN, spina; T, abdominal tergite; tr, abdominal tergal ridge; Tr, trochanter; w, metathoracic tergal ridge.

Masculature

49. Dorsal longitudinal muscle of the neck and prothorax.
56. Dorsal lateral neck muscle.
58. Tergo-pleural intersegmental muscle.
59. Sterno-pleural intersegmental muscle.
81. Median dorsal longitudinal muscle.
82. Oblique dorsal muscle.
89. Tergal promotor of the meso-coxa.
91. Second tergal remotor of the meso-coxa.
92. Anterior rotator of the meso-coxa.
93. Posterior rotator of the meso-coxa.
97, 98. First and second pronator-extensor of the stridulating organ.

99. Depressor-extensor of the stridulating organ.
103. Depressor of the trochanter.
112. Median dorsal longitudinal muscle.
117. Sixth ventral longitudinal muscle.
119, 120. First and second tergal remotors of the meta-coxa.
122, 123, 124. First, second and third posterior rotators of the meta-coxa.
126. Second abductor of the meta-coxa.
130a, 130b. Adductors of the meta-coxa.
140. Sterno-pleural intersegmental muscle.
144. Ventral longitudinal muscle.
it 1. First inner tergal abdominal muscle.

Morphological Features of Taxonomic Importance

- Well developed stridulatory muscles (97, 98 & 99)

pronotal ridge (APR) and inserts at the ventral end of the protrochantin near its junction with the coxa.

63. **First Tergal Remotor of the Pro-Coxa (Figs 77, 78, 85, 86)** — A large tergo-coxal muscle that arises medio-dorsally on the tergum and extends venterolaterally, passing behind the prosternal apophysis to insert at the inner posterior angle of the pro-coxa.

64. **Second Tergal Remotor of the Pro-Coxa (Figs 81, 85, 86)** — A second tergo-coxal muscle that shares the same point of insertion on the coxa with muscle 63 but lateral to it. The muscle also originates lateral to muscle 63 on the tergum.

M Sp. **Spina-Pleural Muscle (Figs 77, 79, 87)** — A slender transverse muscle that arises via a small apodeme on the mesothoracic spina and projects dorsolaterally to a wide insertion on the mesothoracic epimeron, near the second or metathoracic spiracle. This muscle is also not mentioned by Snodgrass (1935a,b). Ewer (1954) described a similar muscle in the tree locust *Acanthocris ruficornis* (Fab.) and Maki (1938) called a similar muscle the anterior transverse muscle and gave it the number 69.

65. **Third Tergal Remotor of the Pro-Coxa (Figs 77, 78, 85, 86)** — This muscle originates lateral to muscle 64 on the tergum and shares the same insertion point with muscles 63 and 64 at the posterior inner angle of the pro-coxa.

67. **Second Posterior Rotator of the Pro-Coxa (Figs 85, 86)** — A flat muscle arising mesally from a minute spinule and inserting at the posterior angle of the pro-coxa.

68a, 68b. **Abductors of the Pro-Coxa (Fig. 81)** — Two branches of an abductor muscle of the coxa. Number 68b is the largest, originating from a small ridge adjacent to the root of the third prothoracic spine and inserting at the outer rim of the pro-coxa just before the coxal articulation. Number 68a (not shown in the diagram) is a smaller branch that arises from the transverse ridge on the tergum. It shares the same insertion point with 68b.

69. **Adductor of the Pro-Coxa (Fig. 81)** — A flat muscle arising from the ventral surface of the prosternal apophysis and inserting at the ventral base of the pro-trochanter.

71b, 71c, 71d. **Depressors of the Trochanter (Figs 77, 78, 81)** — Three branches of a single five-branched muscle. The three arise on the

sides of the pronotum (b and d) and on the wall of the prosternal apophysis (c). They insert via a common apodeme on the ventral rim of the pro-trochanter.

The Mesothoracic Musculature

The musculature of the mesothorax is slightly different in the two sexes of the armoured ground cricket *A. speiseri*, due to the presence of the stridulating organs in the male in the segment, which are lacking in the female. The following muscles are identifiable in a specimen cut along the sagital (median) plane.

Tpi. **Tergo-Pleural Muscle (Figs 77, 79, 85, 87)** — This is the largest muscle in the mesothorax of the male. It is absent in the female. It arises from the ventral and posterior surfaces of the mesothoracic phragma and inserts on the dorsal flattened face of the meso-pleural apophysis. This muscle is a strong elevator of the wings in winged insects. In the armoured ground cricket, the muscle serves as the principal closing muscle of the stridulating organ.

2 Sp. **Sterno-Pleural Intersegmental Muscle (Fig. 77)** — An intersegmental muscle that takes its origin at the distal edge of the mesosternal apophysis and inserts at the anterolateral edge of the metatergum.

81. **Median Dorsal Longitudinal Muscle (Figs 77, 79, 82, 84)** — A flat muscle with many fibres. It arises medio-dorsally on the antecosta and inserts on the metathoracic antecosta.

82. **Oblique Dorsal Muscle (Fig. 77)** —A short muscle originating on the scutum, lateral of muscle 81. It extends postero-ventrally and attaches to the anterior edge of the metathoracic tergum.

83. **Tergo-Sternal Muscle (Fig. 77, 79)** — A long muscle that is broad at its region of origin on the lateral part of the prescutal lobe and narrow at its area of insertion on the mesosternum.

84. **Second Tergo-Sternal Muscle (Figs 85, 87)**— A large, long muscle lying lateral to muscle 83. It arises on the prescutum and inserts on the mesosternum before the inner margin of the meso-coxal cavity.

87. **Third Ventral Longitudinal Muscle (Figs 77, 78, 79)** — This muscle originates on the prothoracic spina and inserts on the mesepisternum adjacent to muscle 83.
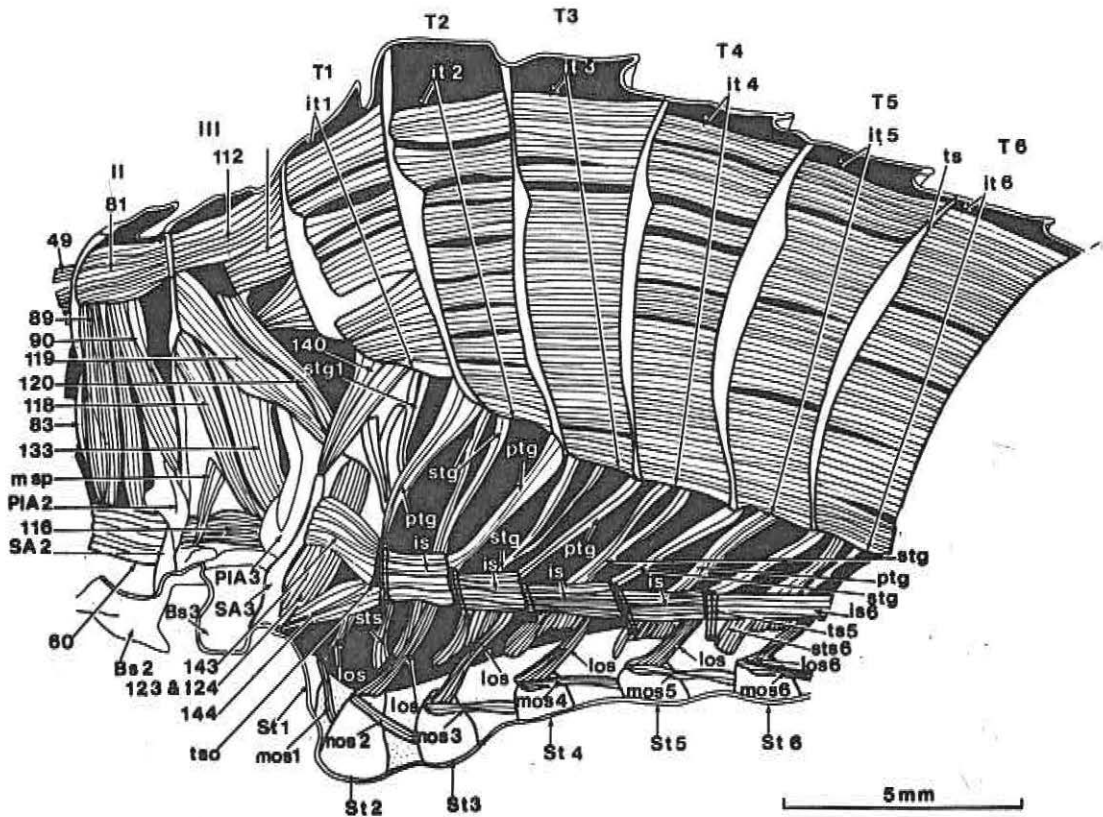
Fig. 84. Medial view of the female pterothorax and the first six abdominal segments

Skeleton

Bs, basisternite; PIA, pleural apophysis; SA, sternal apophysis; St, abdominal sternite; II, III, thoracic tergites; T, abdominal tergite; tr, abdominal tergal ridge.

Masculature

49.	Dorsal longitudinal muscle of the neck and prothorax.	133.	Depressor of the trochanter.
60.	Second ventral longitudinal muscle.	M Sp.	Spina-pleural muscle.
81.	Median dorsal longitudinal muscle.	140.	Sterno-pleural intersegmental muscle.
83.	First tergo-sternal muscle.	143, 144.	Ventral longitudinal muscles.
89.	Tergal promotor of the meso-coxa.	is.	Inner sternal muscle
90.	First tergal remotor of the meso-coxa.	it.	Inner tergal muscle.
112.	Median dorsal longitudinal muscle.	los.	Lateral outer sternal muscle.
116.	Fifth ventral longitudinal muscle.	mos.	Median outer sternal muscle.
118.	Tergal promotor of the meta-coxa.	ptg.	Primary tergo-sternal muscle group.
119, 120.	First and second tergal remotors of the meta-coxa.	stg.	Secondary tergo-sternal muscle group.
		sts.	Secondary transverse sternal muscle.
123, 124.	Second and third posterior rotators of the meta-coxa.	ts.	Transverse sternal muscle.
		tso.	Origin or insertion point of the transverse sternal muscle.

Morphological Features of Taxonomic Importance

- Lack of tergo-pleural muscles in the mesothorax of the female.

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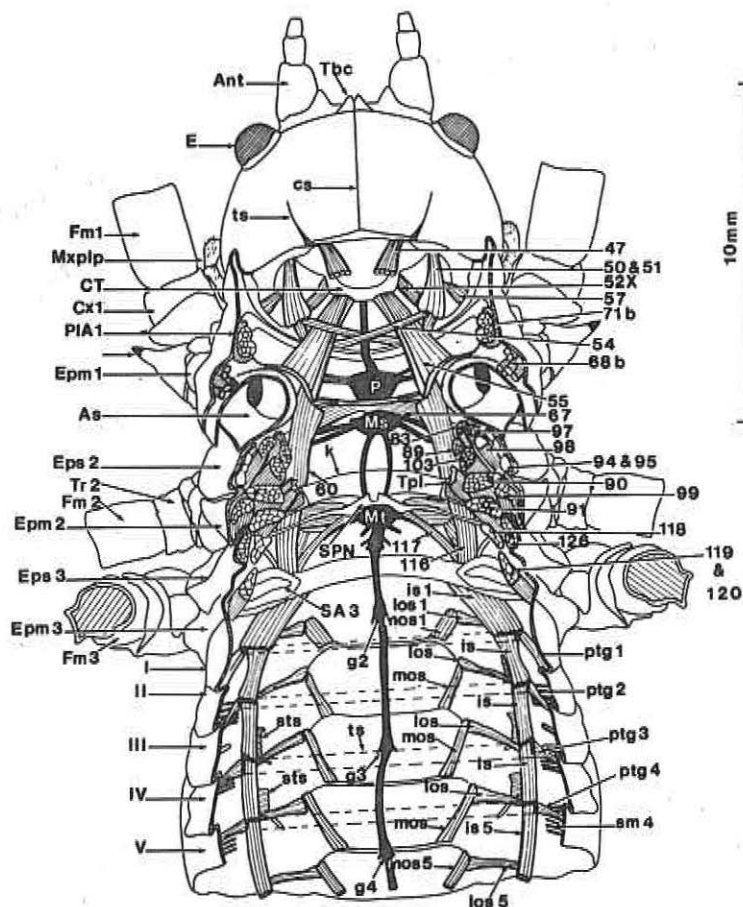
88. **T e r g a l Promotor of the M e s o - C o x a (Figs 77, 79, 84, 85, 87)** — A large tergo-coxal muscle that is divided into two branches. Both insert on a common stalk near the anterior angle of the meso-coxa, close to its junction with the trochantin. They arise on the anterior margin of the phragma on the mesoscutum.

90. **First Tergal Remotor of the M e s o - C o x a**

Fig. 85. Ventral view of the male cervix, thorax and first five abdominal segments

Skeleton

Ant, antenna; As, tracheal air sac; cs, coronal suture; CT, corporotentorium; Cx, coxa; E, compound eye; Epm, epimeron; Eps, episternum; Fm, femur; g, abdominal nerve ganglion; k, sternal ridge; Ms, mesothoracic nerve ganglion; Mt, metathoracic nerve ganglion; P, prothoracic nerve ganglion; PIA, pleural apophysis; SA, sternal apophysis; St, abdominal sternite; Tbc, tubercle; Tr, trochanter; ts, temporal suture; I–IV abdominal segments.

Musculature

47.	First protergal muscle of the head.	83.	First tergo-sternal muscle.
50, 51, 52X.	Cephalic muscles of the neck sclerites.	89.	Tergal promotor of the meso-coxa.
54.	Prosternal muscle of the first cervical sclerite.	90, 91.	First and second tergal remotors of the meso-coxa.
55.	First ventral longitudinal muscle.	94, 95.	First and second abductors of the meso-coxa.
57.	Ventral lateral neck muscle.	97, 98.	First and second pronator-extensors of the stridulating organ.
60.	Second ventral longitudinal muscle.	99.	Depressor-extensor of the stridulating organ.
67.	Second posterior rotator of the pro-coxa.		
68b.	Abductor of the pro-coxa.	Tpl.	Tergo-pleural muscle.
71b.	Depressor of the trochanter.	103.	Depressor of the trochanter.

116, 117. Ventral longitudinal muscles.
118.   Tergal promotor of the meta-coxa.
119, 120. First and second remotors of the meta-coxa.
126.   Second abductor of the meta-coxa.

is.   Inner sternal muscle.
los.   Lateral outer sternal muscle.
mos.   Median outer sternal muscle.
ptg.   Primary tergo-sternal muscle group.
sm.   Sterno-pleural muscle.

Morphological Features of Taxonomic Importance

-    Presence of tergo-pleural muscles in the mesothorax. These muscles are absent in the female.
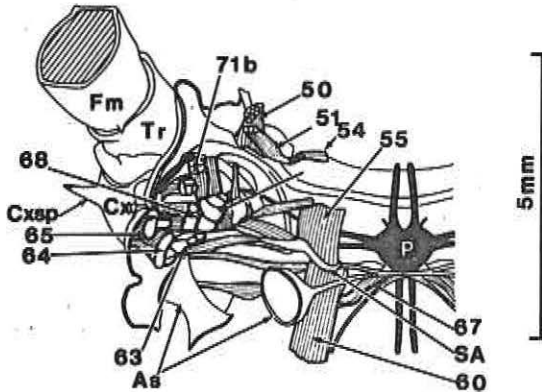
Fig. 86. Ventral view of the prothorax

(Figs 77, 79, 84, 85, 87)—A large, long muscle that acts antagonistically to muscle 89 (not illustrated). It arises from the meso-scutum at the posterior face of the phragma and inserts on a common stalk with another tergo-coxal remotor, muscle 91, on the posterior inner angle of the mesocoxa.

91. Second Tergal Remotor of the Meso-Coxa (Figs 81, 85, 87) — A smaller muscle that shares the same insertion stalk with muscle 90, on the posterior inner angle of the meso-coxa. It lies caudad to 90 and arises from the posterior surface of the phragma on the scutum.

92. **Anterior Rotator of the Meso-Coxa (Figs 79, 82)** — A short, broad muscle arising from the sternum adjacent to the insertion point of muscle 89. It attaches to the lateral face of the mesosternal apophysis.

93. **Posterior Rotator of the Meso-Coxa (Figs 77, 79, 82)** — Another short, broad muscle that originates from the spina and extends outward to insert on the posterior inner angle of the meso-coxa.

94, 95. **First and Second Abductors of the Meso-Coxa (Figs 85, 87)** — These muscles arise on the episternum and attach to the outer margin of the meso-coxa via short apodemes.

97. **First Pronator-Extensor of the Stridulating Organs (Figs 82, 85, 87)** — A basalar muscle that is broad in the male but slender in the female. It arises from the basalar sclerite, extending ventrally to attach to the wall of the sternum, before the meso-coxal cavity. In winged insects, this muscle serves as a direct flight muscle.

98. **Second Pronator-Extensor of the Stridulating Organs (Figs 82, 85, 87)** — A muscle arising from the basalar sclerite caudad of muscle 97, it inserts on the lateral wall of the meso-sternum, adjacent to the meso-coxal cavity. This is another direct flight muscle in winged insects.

99. **Depressor-Extensor of the Stridulating Organs (Figs 79, 82)** — A large muscle that originates from the subalar sclerite and inserts below the basicoxal ridge. This is a second group of direct flight muscle in winged insects.
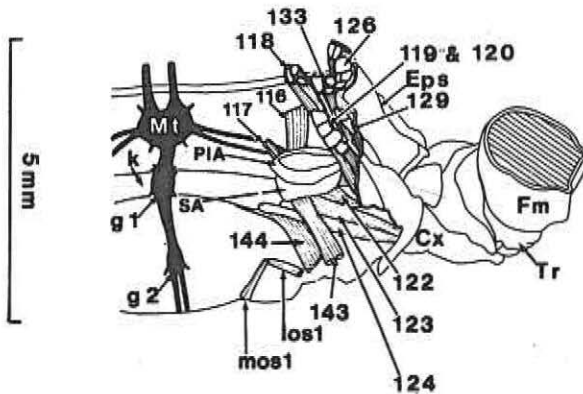
Fig. 87. Ventral view of the mesothorax

100. **First Adductor of the Meso-Coxa (Fig. 79)** — This muscle originates from the ventral margin of the mesosternal apophysis and insertion is on the inner rim of the meso-coxa.

103. **Depressor of the Trochanter (Figs 79, 85, 87)** — A long, broad muscle arising on the mesoscutum. It projects downward passing cephalad of the closely appressed meso-pleural and mesosternal apophyses and then bends caudally to attach at the ventral rim of the trochanter.

116. **Third Ventral Longitudinal Muscle (Figs 77, 84, 85, 87)** — A flat muscle that originates from the posterior edge of the mesosternal apophysis and inserts on the anterior apophysial margin of the metathorax.

117. **Fourth Ventral Longitudinal Muscle (Figs 77, 80, 83, 84, 85, 88)** — This is a slender muscle arising from the mesospina and inserting on the metathoracic apophysis.

The Metathoracic Musculature

The masculature of the metathoracic segment is similar to but simpler than that of the mesothorax. In both sexes, a tergo-sternal muscle homologous to muscle 84 of the mesothorax is lacking. The depressor of the trochanter (133) is inserted more distally in the trochanter than its homologue (103) in the
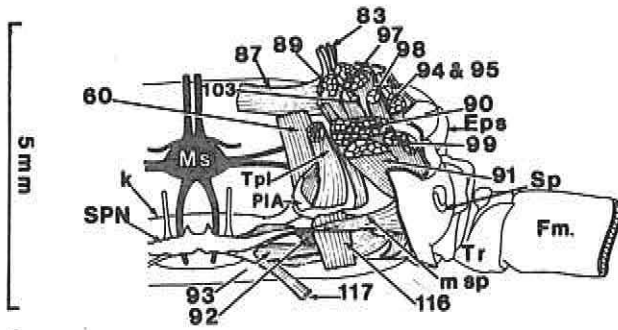
Fig. 88. Ventral view of the metathorax

Skeleton

As, tracheal air sac; **Cx**, coxa; **Cxsp**, coxal spine; **Eps**, episternite; **Fm**, femur; **g1, g2**, first and second abdominal nerve ganglia; **k**, sternal ridge; **Ms, Mt, P**, meso-, meta-, and prothoracic nerve ganglia; **PIA**, pleural apophysis; **SA**, sternal apophysis; **SPN**, spina; **Tr**, trochanter.

mesothorax. The following metathoracic muscles are seen in the internal longitudinal section of each half of the metathorax.

Od. Oblique Dorsal Tergal Muscle (Fig. 80) — The muscle originates on the metatergal ridge and inserts on the first abdominal tergal antecosta.

Musculature

50, 51. Cephalic muscles of the neck sclerites.
54. Prosternal muscle of the first neck sclerite.
55. First ventral longitudinal muscle.
60. Second ventral longitudinal muscle.
63, 64, 65. First, second and third tergal remotors of the pro-coxa.
67. Second posterior rotator of the pro-coxa.
68. Abductor of the pro-coxa.
71b. Depressor of the trochanter.
83. First tergo-sternal muscle.
87. Third ventral longitudinal muscle.
89. Tergal promotor of the meso-coxa
90, 91. First and second tergal remotors of the meso-coxa.
92, 93. Anterior and posterior rotators of the meso-coxa.
94, 95. First and second abductors of the meso-coxa.
97, 98. First and second pronator-extensor of the stridulating organ

99. Depressor-extensor of the stridulating organ.
103. Depressor of the trochanter.
116, 117. Fifth and sixth ventral longitudinal muscles.
M Sp. Spina-pleural muscle.
Tpl. Tergo-pleural muscle.
118. Tergal promotor of the meta-coxa.
119, 120. First and second tergal remotors of the meta-coxa.
122, 123, 124. First, second and third rotators of the meta-coxa.
126. Second abductor of the meta-coxa.
129. Depressor of the trochanter.
143, 144. Ventral longitudinal muscles.
los 1. Lateral outer sternal muscle of the first abdominal segment.
mos 1. Median outer sternal muscle of the first abdominal segment.

112. **Median Dorsal Longitudinal Muscle (Figs 77, 80, 83, 84)** —A first muscle arising from the antecosta of the metatergum and inserting at the anterior edge of the first abdominal tergum.

118. **Tergal Promotor of the Meta-Coxa (Figs 77, 80, 84, 85, 88)** — A muscle homologous to muscle number 83 of the mesothorax. It originates on the metascutum and inserts on the lateral part of the sternum, before the meta-coxal cavity.

119. **First Tergal Remotor of the Metathoracic Coxa (Figs 77, 80, 83, 84, 85, 88)** — A muscle homologous to muscle number 90 of the mesothorax and muscle number 63 of the prothorax. It is large with a number of fibre bundles. It arises from the metascutum dorsolaterally and projects ventero-posteriorly behind closely appressed metapleural and metasternal apophyses and inserts on an apodemal stalk, on the posterior inner angle of the meta-coxa.

120. **Second Tergal Remotor of the Metathoracic Coxa (Figs 77, 80, 83, 84, 85, 88)** — A moderately wide but long muscle. Its origin is on a ridge that separates the scutal and scetellar plates (sclerites) on the metatergum. The muscle tapers off ventrally and inserts on an apodemal stalk at the extreme posterior angle of the metacoxa.

122, 123, 124. **Posterior Rotators of the Metacoxa (Figs 77, 80, 83, 84, 85, 88)** — All arise from the posterior margin of the lateral arm of the metasternal apophysis. Number 122 inserts on the meral region of the metacoxa, 123 at the posterior angle of the metacoxa, and 124 on the meral ridge of the metacoxa.

126. **Second Abductor of the Metacoxa (Figs 83, 84, 88)** — A flat sheet of muscle arising from the inner wall of the metaepisternum and inserting at the base of the sternum above the metacoxal articulation.

130a, 130b. **Adductors of the Metacoxa (Fig. 83)** —Two short, flat muscle branches that originate from the posterior face of the metasternal apophysis and insert on the posterior part of the inner margin of the metacoxa.

133. **Depressor of the Trochanter (Figs 77, 80, 84, 88)** — A large muscle in both sexes of *A. speiseri* that arises from the metascutum. It is one of the three branches that share a commom insertion on the ventral rim of the metatrochanter.

140. **Sterno-Pleural Intersegmental Muscle (Figs 77, 80, 83)** — An oblique intersegmental muscle that arises from the distal tip of the metasternal apophysis and attaches to the first abdominal tergum antero-ventrally.

143. **Ventral Longitudinal Muscle (Figs 77, 84)** — A broad, flat muscle originating from the posterior face of the metasternal apophysis and inserting on the laterosternite of the first abdominal segment.

144. **Ventral Longitudinal Muscle (Figs 77, 80, 83, 84, 88)** — This muscle originates on the third spina and inserts on the first and third abdominal laterosternites.

The Abdominal Musculature

The musculature of the abdomen of the armoured ground cricket, *A. speiseri* is that of the generalized tettigoniid type. Morphologically in the Tettigoniidae, a family to which *A. speiseri* belongs, each abdominal segment bears a tergum, sternum and two pleural regions. The sternum is further subdivided into a medial eusternite and two laterosternites. However, the laterosternites of *A. speiseri* are membranous. The following muscles are distinguishable in dissections made in sagittal or medial plane of the abdomen.

The Tergal Musculature

Inner Tergal Muscles (Figs 77, 84, 89, 90, it) — These are flat muscles composed of many fibre groups. The number of fibres in each group varies and hence, also the group sizes are diverse. The muscles arise subanteriorly on anterior ridges of the tergum and insert on the anterior margin of the following tergum in segments 1–9 in the male and segments 1–8 in the female.

The Alary Muscles (Fig. 49, Am) — Nine pairs of alary muscles are present in the abdomen. They arise medio-laterally on the anterior margin of the tergites and insert medially on the heart between its ampullae. In all, 12 alary muscle pairs are present in the armoured ground cricket, nine pairs in the abdomen and three pairs are borne in the thorax.

The Sternal Muscles

Transverse Sternal Muscle (Fig. 89, ts) —A segmental muscle occurring in abdominal segments 1–8 in the male and segments 1–7 in the female. The muscle arises medio-laterally on the membranous laterosternite and inserts on the opposite laterosternite of the same segment. (Transverse sternal muscles are not shown in all diagrams). However, their relative areas of origin and/or insertion on the segment are indicated by the abbreviation, tso).

Secondary Transverse Sternal Muscle (Figs 84, 85, sts) — This muscle occurs in two groups, each composed of short muscle fibres that originate from the laterosternite and insert on the membrane, lateral to the eusternite.
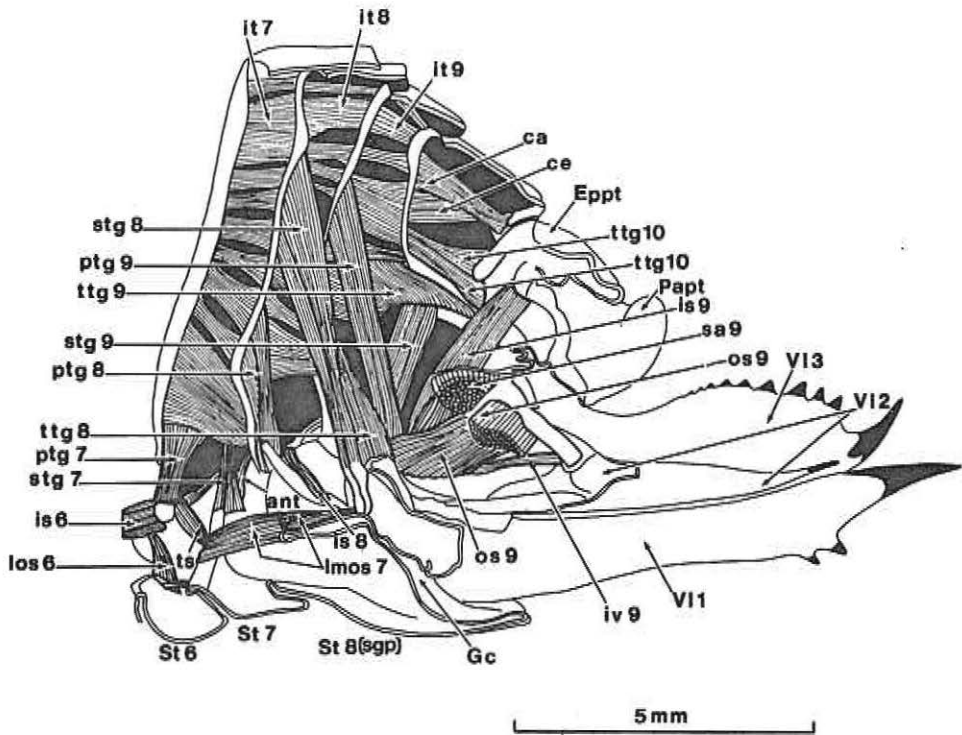
Fig. 89. Medial view of the female genital and postgenital abdominal segments

Skeleton

Eppt, epiproct; **Gc**, genital chamber; **Papt**, paraproct; **sgp**, subgenital plate; **St**, abdominal sternite; **T**, abdominal tergite; **Vl**, ovipositor valve (blade).

Musculature

ant.	Antigonistic spiracle muscle.	**sa.**	Superior apophysial muscle.
ca.	Cercal abductor muscle.	**stg.**	Secondary tergo-sternal muscle group.
ce.	Cercal elevator muscle.	**ttg.**	Tertiary tergo-sternal muscle group.
is.	Inner sternal muscle.	**lmos 7.**	Adductor of the subgenital plate.
it.	Inner tergal muscle.	**os 9.**	Protractor of the ovipositor.
iv.	Inner valvular muscle	**ptg 9.**	Third depressor of the ovipositor.
los.	Lateral outer sternal muscle.	**sa 9.**	Lateral abductor of the ovipositor blades.
os.	Outer sternal muscle.	**stg 8.**	First depressor of the ovipositor.
ptg.	Primary tergo-sternal muscle group.	**stg 9.**	First elevator of the ovipositor.
		ttg 8.	Second depressor of the ovipositor.

Morphological Features of Taxonomic Importance

-   Large ovipositor muscles (stg 8, ttg 8, ptg 9, stg 9).

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Inner Sternal Muscle (Figs 77, 84, 85, 89, 90, is) —These are intersegmental ventral longitudinal muscles. They are flat muscles composed of various numbers of fibre groups. They arise from the anterior margin of the laterosternite and insert posteriorly on the anterior margin of the following laterosternite. The muscles occur in abdominal segments 1–9 in both sexes.

Lateral Outer Sternal Muscle (Figs 77, 84, 85, 89, 90, los) — Arising on the antero-lateral angle of the eusternite, this muscle tapers off posteriorly to insert on the ventral margin of the laterosternite in segments 1–7 in the male and segments 1–8 in the female.

Median Outer Sternal Muscle (Figs 84, 85, 89, 90, mos) — This is a slender muscle sharing a common origin with the lateral outer sternal muscle (los) described above, but medial to it. It inserts on the anterior margin of the following eusternite, mediolaterally.

The Pleural Muscles

Primary Tergo-Sternal Group (Figs 77, 84, 85, 89, 90, ptg) — A long, slender muscle that is sometimes partially subdivided into two groups. This muscle arises anteroventrally on the tergum and inserts on the dorsal margin of the laterosternite in abdominal segments 1–9 in both sexes.

Secondary Tergo-Sternal Group (Figs 77, 84, 85, 89, 90, stg) — The muscle occurs in two groups both of which arise on the tergum, caudad to the primary tergo-sternal group (pts) described above. Both insert on the laterosternite.

Sterno-Pleural Muscle (Fig. 85, sm) — Composed of a number of short fibres that arise on the dorsal edge of the laterosternite and insert on the pleural membrane below the spiracle.

Antagonistic Spiracle Muscle (Figs 89, 90, ant) — This is a short muscle that originates on the posterior margin of the laterosternite and inserts on the closing bow of the spiracle.

Spiracle Occlusor Muscle (not illustrated) — This is another small muscle that arises on the closing band of the spiracle and inserts on the handle of the closing bow.

The Musculature of the Female Genitalia (Fig. 89)

The Sternal Musculature

Segment 8
Transverse Sternal Muscle (ts) —Absent in the adult.

Inner Sternal Muscle (is 8) — A flat muscle that arises on the subgenital plate antero-laterally. It inserts on the anterior intervalvular sclerite. The muscle is continuous with the inner sternal muscles of the 7th segment. Jointly, they serve as retractors of the ovipositor.

Segment 9
Inner Sternal Muscle (is 9) — A large paraproctal muscle that takes its origin from the 1st valvifer and inserts on the anterior margin of the paraproct.

Outer Sternal Muscle (os 9) — An inter-valvular muscle of the 1st valvifer. It arises from the median apodeme of the posterior intervalvular sclerite and inserts on the 1st valvifer. This is a protractor muscle of the ovipositor that pulls the ovipositor forward and backward.

Superior Apophysis Muscle (sa 9) — A large bundle of fibres originating from the dorsal blades of the ovipositor and inserting on the inner ovipositor blades.

Inner Valvular Muscle (iv 9) — A slender muscle that originates on the 1st valvifer and inserts on a ridge of the second valves, of the ovipositor.

The Pleural Musculature

Segment 8
Primary Tergo-Sternal Group (ptg 8) — This muscle is larger than its homologues in the preceding segments. It arises dorsally from the 8th antecosta and inserts on the subgenital plate antero-ventrally.

Secondary Tergo-Sternal Group (stg 8) — The largest muscle in the female genital abdominal segments. It arises from a highly developed antecosta of the 8th tergum, dorsolaterally and inserts on the antero-dorsal edge of the ventral ovipositor blade. The muscle functions as the first depressor of the ovipositor.

Tertiary Tergo-Sternal Group (ttg 8) — A large tergal muscle of the anterior intervalvular sclerite that arises from the antecosta of the 9th tergum and inserts medially on the anterior intervalvular sclerite. This muscle is the second depressor of the ovipositor.

> Antagonistic Muscle of the Spiracle (ant) — This muscle arises from the subgenital plate and inserts on the closing bow of the 8th abdominal spiracle.

Segment 9

> Primary Tergo-Sternal Group (ptg 9) — The second largest muscle group in the genital abdominal segments, it arises from the 9th antecosta and inserts on the anterior intervalvular sclerite, adjacent to its antecostal articulation. The muscle is the third depressor of the ovipositor and operates the dorsal blades.

> Secondary Tergo-Sternal Group (stg 9) — Originating from the dorsal margin of the posterior intervalvular sclerite apodeme, this muscle inserts on the anterior intervalvular sclerite and is the first elevator muscle of the ovipositor.

> Tertiary Tergo-Sternal Group (ttg 9) — This is also referred to as the posterior tergal muscle of the 2nd valvifer. It is a large flat muscle that arises from the anterior and ventral portions of the 9th tergum and attaches to the posterior dorsal apodeme of the 2nd valvifer.

> Superior Apophysis Muscle (sa 9) — A short, thick muscle arising from the median apodeme of the posterior intervalvular sclerite and inserting on the 2nd valvifer.

The Masculature of the Male Genitalia (Fig. 90)

The Sternal Musculature of the 9th Abdominal Segment

> Transverse Sternal Muscle — Absent in this segment.

> Inner Sternal Muscle (is 9) — A ventral longitudinal muscle that serves as a paraproct dilator. It originates from the 9th laterosternite and inserts on the 10th sternal area of the paraproct.

> Outer Sternal Muscle (os 9)
> This muscle occurs in two groups;

>> Lateral Outer Sternal Muscle (los 9)
>> Arising antero-laterally from the 9th sternum, this muscle inserts on the ventral phallic lobe.

>> Median Outer Sternal Group (mos 9) — Arising from the 9th sternum, anterolaterally. The muscle inserts on the genital pouch.

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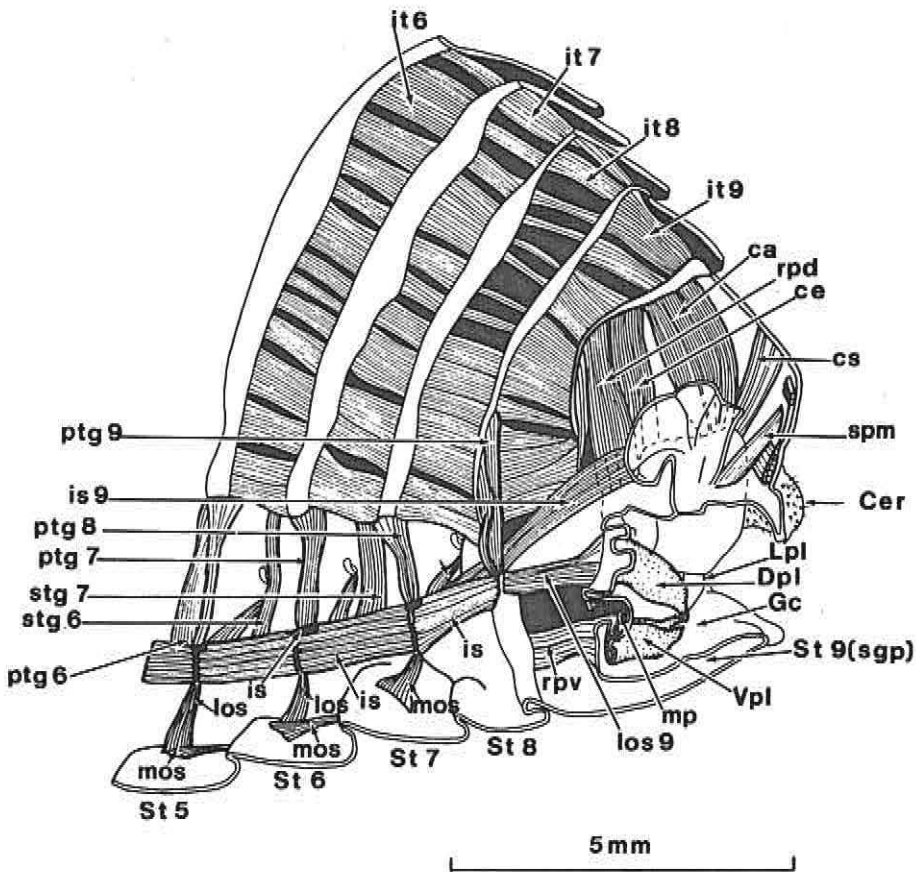
Fig. 90. Medial view of the male genital and postgenital abdominal segments

Skeleton

Cer, cercus; Dpl, dorsal phallic lobe; Eppt, epiproct; Gc, genital chamber; Lpl, lateral phallic lobe;
Papt, paraproct; sgp, subgenital plate; St, abdominal sternite; Vlp, ventral phallic lobe.

Musculature

ca.	Cercal abductor muscle.	mp.	Major constrictor of the spermatophore.
ce.	Cercal elevator muscle.	ptg.	Primary tergo-sternal muscle group.
cs.	Cercal muscle from the supra-anal plate.	rpd.	Dorsal retractor of the phallus (= ptg 10).
		rpv.	Ventral retractor of the phallus.
is.	Inner sternal muscle.	spm.	Supra-paraproctal muscle.
it.	Inner tergal muscle.	stg.	Secondary tergo-sternal muscle group.
los.	Lateral outer sternal muscle.	is 9.	Paraproctal muscle.
mos.	Median outer sternal muscle.	los 9.	Suspensory muscle of the spermatophore cup.

Morphological Features of Taxonomic Importance

- Presence of phallic lobes (Lpl, Dpl, Vlp) plus their associated muscles.

150

Major Protractor Muscle of the Genitalia (mp) — A cup-like muscle that envelops the genitalia and originates on the 10th antecosta.

Ventral Retractors of the Phallus (rvp) — A short muscle arising from the 9th sternum and inserting on the phallobase.

The Pleural Musculature of the 9th Abdominal Segment

Primary Tergo-Sternal Group (ptg 9)
Larger than its homologues in the pregenital abdominal segments of the male. This muscle arises from the 9th antecosta and inserts on the ninth sternum.

Dorsal Retractor of the Phallus (rpd)
This is a slender muscle that arises laterally from the 10th tergum and inserts on the phallobase.

The Cercal and Paraproctal Musculature in Both Sexes of the Armoured Ground Cricket (Figs 89, 90).

Cercal Abductor Muscle (ca)
This is a large muscle in the male originating from the 10th antecosta and attaching to the rim of the cercus medio-dorsally.

Cercal Elevator Muscle (ce)
A large muscle arising lateral to the cercal abductor muscle (ca) on the 10th antecosta and inserting on the rim of the cercus ventrally.

Cercal Muscle from the Supra-Anal Plate (cs)
A slender muscle originating from the 10th tergum medially and attaching to the rim of the cercus medially.

Paraproct Abductor Muscle (ttg 10)
A large muscle in the female arising from the antecosta of the 10th tergum and inserting on the rim of the paraproct, medio-dorsally.

Paraproct Dilator Muscle (is 9)
Arising from the subgenital plate, antero-laterally this muscle inserts on the 10th sternal area of the paraproct.

Exercises

Materials Required:

Specimens:
1. Freshly anaesthetized or preserved (in 70% ethanol) desert locust, *Schistocerca gregaria* Forskal or garden locust, *Acanthacris ruficornis* Fabricius or any large type grasshopper.

Apparatus:
1. Dissecting set with beeswax at the bottom.
2. Dissecting set.
3. Minuten insect pins (No. 5).
4. Binocular microscope.

Chemicals:
1. 10% ethanol.

Exercise 1: Abdominal Musculature.

1. You are provided with a fresh or preserved grasshopper or locust.

2. Bisect the specimen longitudinally along the dorsum and venter (see Fig. 76).

3. Mount one half on wax in a dissecting dish with the cutup face uppermost.

4. Submerge the specimen in 10% ethanol and then using a dissecting needle and forceps remove the alimentary canal, reproductive organs, fat body and other connective tissues to expose the muscles on the body walls.

5. Study the muscle plan of three visceral segments, the genital segment(s), and the postgenital segments in your specimen. Draw and label.

Exercise 2: Thoracic Musculature.

1. Using the same specimen, now work on the thorax and like before, remove all obstructing connective tissue with a forceps and dissecting needle. Do so while examining your work under the binocular microscope.

Exercise 2: Continued

2. Study the muscle plan of the whole thorax. Identify the different muscles seen in this medial (sagittal) plane of the thorax and then draw and label.

Exercise 3: **Head Musculature.**

1. Now study the muscles of the head of your specimen as seen from the sagittal view, draw and label.

2. To study the musculature of the gnathal appendages, excise an intact head from another fresh specimen of a locust or grasshopper.

3. Using a forceps and a dissecting needle, carefully remove the mouthparts one at a time so that they come out with their muscles still attached to them.

4. Lay the mouthparts thus removed on the beeswax in the dissecting dish. Submerge your specimens in 10% ethanol and then study them carefully under the binocular microscope.

5. Identify the muscles of the mouthparts. Draw each type of mouthpart completely with its muscles attached and then label.

    a. In what ways are the organizations of the muscles in the head, thorax and abdomen of your specimen different from each other?

    b. How are these organizations different from those of the armoured ground cricket described in the introduction to these exercises?

Supplementary Questions

1. What are antagonistic muscles and how do they operate to produce action in a given part of the insect body?

2. What is the basis for naming insect muscles and in what ways are insect muscles different from vertebrate muscles?

3. How are insect muscles attached to the insect body wall and other body parts?

4. Compare and contrast the musculature of the mouthparts of the following insects and explain how their muscles operate in food ingestion.
    a. the tsetse fly, *Glossina pallidipes*.
    b. the mosquito, *Culex quinquefasciatus* or *Anopheles gambiae*.

5. How do muscles of slow-flying insects like the red locust, *Cyrtacanthacris septemfasciata* (Serv.) or the African migratory locust, *Locusta migratoria migratorioides* (Rieche & Fairm.) physiologically compare with those of fast-flying insects like the Hymenoptera?

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Acknowledgements

Appendix 1

Basic Techniques for Collecting and Preserving Insects

Presently many techniques are available for collecting and preserving insects for study. The technique(s) that one selects to use, will very much depend on the objective(s) for making the insect collection and on a number of other factors related to the insects themselves to be collected. Important factors to consider as regards insects to be collected include; the type(s) of insects one is interested in (i.e. whether soft or hard bodied, winged or wingless etc.), their sizes, developmental stage(s) and the type(s) of habitat or ecological niches they occupy.

For insect functional morphological studies, where a whole range of representatives of the different insect orders need to be compared morphologically and anatomically, the techniques recommended are those that preserve the external and/or internal structures of insects. The collecting methods used range from hand-picking of insects to the use of various apparatuses, while insect preservation techniques range from pinning insects using minuten insect pins, for external morphological studies, to placing insect specimens in general or selective chemical preservatives that maintain their internal tissues and organs, for anatomical/histological studies.

Three activities are involved when collecting insects from the field namely; catching insects, killing the captured insects and, preserving the captured insects for later use. Hand-picking of insects is the simplest method of catching insects from various substrata in their habitats. However, this method is limited in its use in that not all insects can be hand-picked for various reasons, some of which are obvious to the reader. Alternatively, several types of special devices can be used to aid in catching insects. The selection of the kind of device to use is often dictated by the type of habitat and habits of the insect(s) to be collected. The simplest and most commonly used device for catching terrestrial insects, especially flying insects and those that dwell in vegetation, is the insect sweep (aerial) net (Fig. A1.1, 1). This is made up of a metal or plastic (sometimes other materials such as fibreglass are used) ring of about 30 cm diameter bearing a long handle and to which is attached a bag made from a mosquito net material.

For tiny terrestrial insects such as some ants, aspirators and small paint brushes are usually handy in catching them. A simple aspirator can be
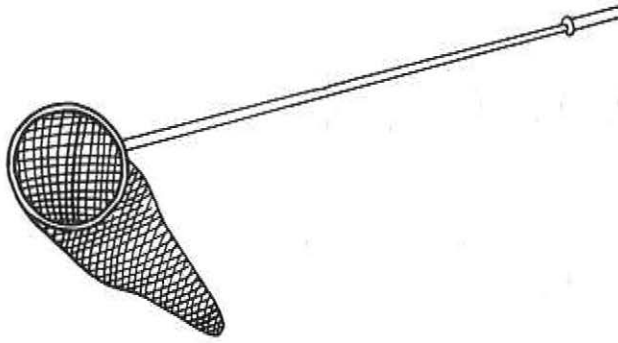
1. Insect Sweep (aerial) Net.
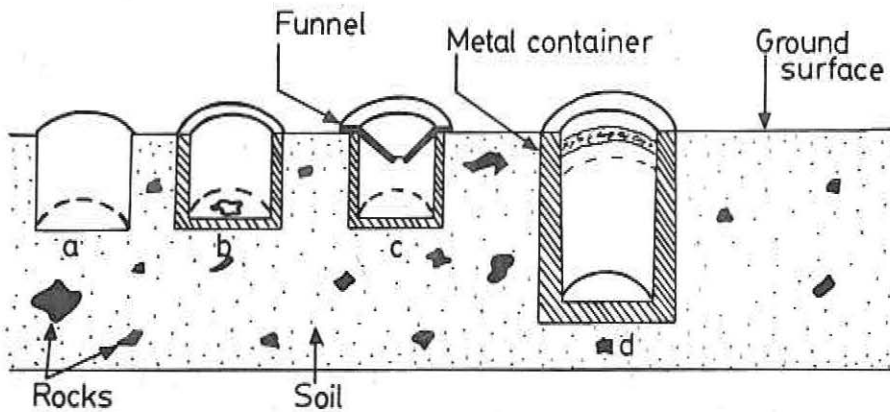
2. Pitfall Traps.

A1.1. Collecting apparatuses for terrestrial insects

constructed easily by a student of Insect Functional Morphology, by placing a small cotton wool plug in a short rubber tube (preferably a transparent one). The cotton plug is placed some distance into the rubber tubing, in such a way that the insect collector can suck and blow air through the rubber tube without dislodging the cotton plug. In other words, the plug should not be so tightly made such that air cannot be passed through it.

To catch tiny insects with such a simple aspirator, the collector applies one end of the rubber tube to his/her mouth and places the other end close to the tiny insect to be caught. He/she then sucks air through the tube resulting in the tiny insect being swept into the tubing together with the inhaled air. The cotton plug in the aspirator prevents the tiny insect from being swallowed by the collector! Insects thus caught are then dropped into a collecting bottle containing a chemical killing agent/preservative by reversing the collecting process, i.e., by blowing through the rubber tube or aspirator.

Other devices for catching insects from the terrestrial environment

include pitfall traps, light traps, traps that lure insects to them through chemical odours and those that do so by utilizing specific colours that are attractive to certain insect groups. Tsetse fly traps, some of which have been developed at the International Centre of Insect Physiology and Ecology (ICIPE) are examples of insect traps that lure insects to them by both chemical odours and specific colours of material selected for use in their construction. Pitfall traps (Fig. A1.1, 2) are of various types and are very useful for catching ground-dwelling and crawling arthropods such as millipedes, centipedes, ground beetles and caterpillars. They can be simple pits dug in the soil in the habitat of arthropods of interest or the pits could be supplemented with metal containers and can be operated with or without bait (Fig. A1.1, 2).

For insects that dwell in the soil or litter, the best technique for catching them involves collecting the soil or litter in which they are found from the

Electric cord

Electric bulb

Sieve wire

Funnel

Electric plug

Bottle with preservative

1. Simple Berlese Funnel.   2. Multiple Berlese Funnel Unit.
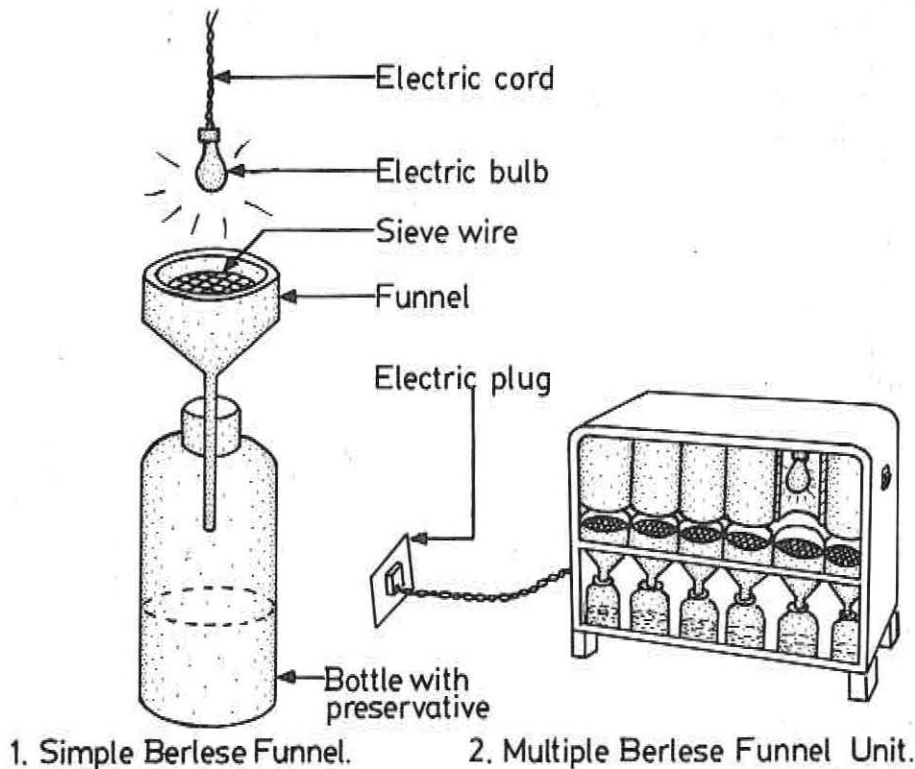
Fig. A1.2. Equipment for extracting insects from litter and soil

field. Later in the laboratory, special devices are used to extract the insects from the soil or litter. The Berlese funnel (Fig. A1.2, 1) is the commonest apparatus used in such extractions. This contraption can be easily constructed in the lab by Insect Functional Morphology students using simple materials (Fig. A1.2, 1) or it can be purchased from biological supply houses as a single or multi-unit structure (Fig. A1.2, 2). Basically a Berlese funnel comprises a bottle containing a chemical killing agent/preservative, above which is a funnel containing sieve wire and the funnel is overhang by an electric bulb (Fig. A1.2, 1). The field-collected soil or litter with insects is placed in the funnel at the top, the electric bulb switched on and then the setup left that way overnight. Heat from the electric bulb or bulbs in case of a multi-unit system, drives the insects that are in the soil or litter to the bottom where sooner or later they drop through the wire mesh in the funnel, into the chemical killing agent/ preservative at the bottom.

Collecting insects from an aquatic environment poses a different set of problems requiring other types of apparatuses. A special kind of insect net called aquatic insect net, is commonly used to catch insects found on water surfaces and those found submerged in water. Constructionwise the insect aquatic net is similar to the insect sweepnet in that there is a metal frame carrying a bag of netting material and a long handle. But the shape of the frame is often triangular to give it more strength to resist breakage while being dragged through water and the netting material used is water resistant (Fig. A1.3, 1).

Also used to catch insects from water bodies are devices that pick up insects from soil surfaces and in the soil at the bottom of water bodies. Such devices include grabbers and dredgers (Fig. A1.3, 2, 3).

Hand-picking or using a forceps for some aquatic insects, especially those of appreciable sizes is also possible. For example, immature stoneflies (order, Plecoptera), mayflies (order, Ephemeroptera) and caddisflies (order, Trichoptera) which tend to cling to the undersides of stones and other substrata that are submerged in still or running water, when these are turned up, can be picked by hand or by using a forceps. However, a word of caution on hand-picking insects, terrestrial or aquatic — many insects will inflict a very painful bite and sometimes even a poisonous one when mishandled. The collector, therefore, needs to be always on the lookout to avoid bites when hand-picking insects.

Once caught, insects then need to be comfortably put to sleep! (i.e., killed) before being preserved for later use. Various techniques are used to do this. For most adult insects from the terrestrial environment, chemical killing agents such as ethyl acetate, cyanide and chloroform are applied to tissue paper which is then placed in a stoppered "killing bottle". The insects are anaesthetized by dropping them into the killing bottle which is thus charged with a chemical killing agent. The stopper of the bottle should be applied when insects are dropped in. Again a word of caution to the collector. The chemicals used to kill insects can also cause man to go to sleep! Therefore, the collector's nose

1. Aquatic Insect Net.

3. Dredger

2. Grabber
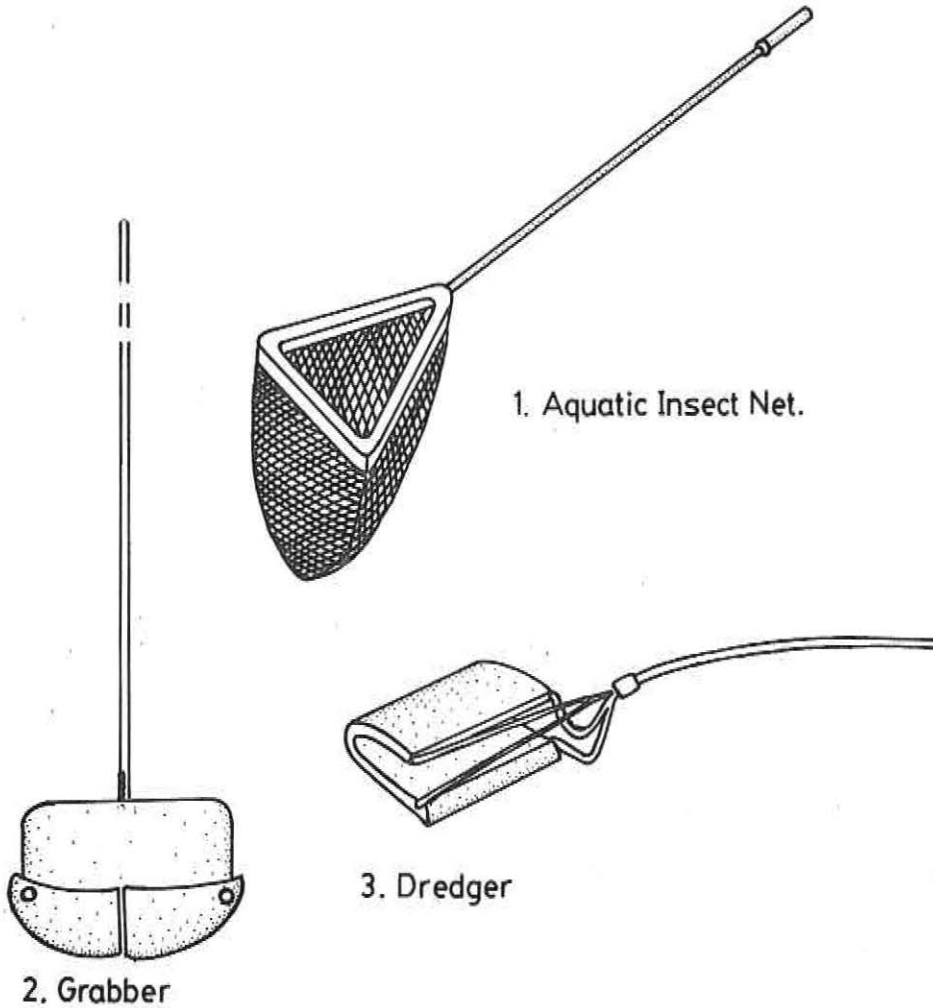
Fig. A1.3. Equipment for collecting aquatic insects

should be kept away (at all times) from the mouth of the killing bottle when it contains (i.e., is charged) with these chemicals. Alternatively, insects could be dropped in a killing bottle containing 75–85% ethyl or isopropyl alcohol. Many immature terrestrial insects are killed by dropping them in a liquid chemical killing agent such as 75–85% ethyl alcohol or in a solution called FAA (40% formalin, 95% ethyl alcohol and 5% glacial acetic acid mixed in a 1:4:1 ratio, respectively).

For large and fleshy immature insects, often with a lot of plant food material in their guts at the time they are caught such as lepidopterous larvae (caterpillars) and coleopterous larvae (e.g., grubs), the best killing method is using heat. Once collected these insects should be dropped into water that has been brought to a boil and then the container with the boiling water containing

insect specimens removed from the source of heat. The specimens should then be left in the water until it has completely cooled down. This heat treatment is to ensure that the microorganisms in the guts of the immature insects taken together with their food are destroyed before the specimens are preserved. Failure to destroy these microorganisms from the insect guts often results in specimens rotting in a chemical preservative. Live microflora/microfauna in the specimen's gut also often results in the discoloration of the specimen in the preservative. What all this implies is that fleshy immature insects from the terrestrial environment such as caterpillars and grubs need if possible to be collected live from the field and brought to the lab for heat treatment, that is if this is not possible to do in the field.

Both adult and immature aquatic insects are best killed by placing them in a chemical killing agent. Ethyl alcohol (75–85%) is the commonly used chemical killing agent for this. Live aquatic insects are simply dropped into a killing bottle containing this alcohol and depending on their sizes, die a few minutes later after swimming in the chemical killing agent. The beauty of using ethyl alcohol for this purpose is that the same chemical is the best preservative for aquatic insects. However, it should be noted that if these insects have to be preserved for long periods of time before use, it is imperative that after a few weeks, the alcohol is changed. The reason for this is that aquatic insects usually have a lot of water in them, which tends to dilute the alcohol preservative after some time in it. In order for the insects to be preserved properly there is need therefore to re-strengthen the ethyl alcohol after a few weeks. This is done by pouring out the alcohol in the preserving bottle(s) (i.e. the preservative) in which the insects were initially stored following collection and then replacing it with freshly prepared 75–80% ethyl alcohol.

Preservation of insects can be on minuten insect pins or in chemical preservatives. Large and hard-bodied insects are best preserved on minuten insect pins unless they are meant for anatomical studies. Minuten insect pins are available in various sizes on the market and the sizes used will depend on the sizes of insect specimens to be pinned. Even tiny but hard-bodied insects can also be mounted on insect pins although in this case pinning is done indirectly in that, the pin itself is not passed through the insect's body. Instead the pin is fastened near the base of a tiny isosceles paper triangle made by the collector to which the tiny insect is glued at its pointed tip.

For many soft-bodied insects, tiny insects and many immature insects as well as all aquatic insects (immature and adults), the best preservation is through chemical preservatives. In fact insects collected with the aim of studying the ultrastructure of their tissues and organs need to be preserved in chemical preservatives. This ensures that the tissues and organs remain intact for study.

Table A1 gives a summary of the techniques for catching, killing and preserving insects outlined above for quicker reference. The table also presents insect orders for which each technique is recommended. Of course, many

techniques unknown to the author may have been left out but those presented will, however, meet the requirements of the Insect Functional Morphology course such as this one. However, the student should feel free to try out other techniques that are recommended in the literature for collecting and preserving specific insect groups. Following below are examples of methods for collecting insects from selected insect habitats.

Table A1. Summary of techniques for collecting, killing and preserving insects for study. (Modified from Knudsen, J.N. 1966. *Biological techniques*. Harper & Row, New York. 525 pp).

Activity	Dev. stage	Technique	Insect group or order for which the technique is most appropriate
COLLECTING INSECTS	Adults	Hand-picking or with a forceps	All Apterygota and most pterygotes except, Odonata, Ephemeroptera, Mallophaga, Anoplura, Psochoptera, Siphonaptera and Lepidoptera
		Use of a brush or an aspirator	Mallophaga, Anoplura, Psochoptera, Dermaptera, Isoptera, Embioptera, Hemiptera, Homoptera Thysanoptera, Diptera and Coleoptera
		Use of an insect sweepnet or aquatic insect net	Orthoptera, Plecoptera, Odonata, Ephemeroptera, Hemiptera, Homoptera, Neuroptera, Mecoptera, Trichoptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera
	Immature insects	Hand-picking or with a forceps	All terrestrial insects and some aquatic insects
		Use of aquatic insect net, grabbers or dredgers	Many aquatic insects
		Dropping in alcohol	75–85% ethyl alcohol: All Apterygota and many aquatic and terrestrial pterygotes except, Orthoptera, Plecoptera, Odonata and Lepidoptera

Table A1: Continued

Activity	Dev. stage	Technique	Insect group or order for which the technique is most appropriate
KILLING INSECTS	Adults		75–85% isopropyl alcohol: Dermaptera, Embioptera, Psochoptera, Diptera & Coleoptera
		Killing with ethyl acetate or chloroform or cyanide	All terrestrial Pterygote insects
	Immature insects	Dropping them in 75–85% ethyl alcohol	All Pterygote insects except Orthoptera. All aquatic immature insects
		Use of xylene - ethyl alcohol in a 1:1 ratio	Lepidoptera and Coleoptera
		Use of FAA	All Apterygote and Pterygote insects
		Dropping them in boiling water	Lepidoptera and Coleoptera
PRESERVING INSECTS	Adults	In 75–85% ethyl alcohol with or without glycerine	All Apterygota and the terrestrial and aquatic pterygotes: Isoptera, Embioptera, Ephemeroptera, Psochoptera, Thysanoptera, Neuroptera, Mecoptera, Trichoptera, Siphonaptera, Coleoptera and Hymenoptera
		Pinning	Orthoptera, Dermaptera, Plecoptera, Odonata, Ephemeroptera, Hemiptera, Homoptera, Lepidoptera, Diptera, Coleoptera, Hymenoptera, including some aquatic pterygotes
		Mounting on microscope slides	Protura, Collembolla, Mallophaga, Anoplura, Psochoptera, some Homoptera, Diptera and Siphonaptera

Table A1: Continued next page

Table A1: Continued

Activity	Dev. stage	Technique	Insect group or order for which the technique is most appropriate
	Immature insects	In 75–85% ethyl alcohol with or without glycerine	All immature insects
		Pinning	Orthoptera, Dermaptera and Coleoptera
		On microscope slides	Apterygota and Hymenoptera

A.   Collecting Insects from the Air

The simplest device to use for this job is the insect sweep (aerial) net (Fig. A1.1, 1). Other apparatuses commonly used, especially in sampling insect pests aimed at controlling them, are various types of insect traps mentioned before.

To collect insects that are in flight using an insect sweepnet is an exciting and challenging venture. No specific technique for handling the net can be recommended as each individual insect collector sooner or later develops their own styles after a few trials with the sweepnet. However, the thing to remember is that the net has to be swung in such a way that its mouth is always directed towards the insect(s) to be caught. Once the insect(s) has been successfully captured in the net, the collector should then quickly flip the ring of the net such that the mosquito net bug opening is blocked to prevent the insect(s) from escaping.

Insects caught in the sweepnet are then transferred to a killing bottle charged with ethyl acetate, cyanide or chloroform as the killing agent or in case 75–80% ethyl alcohol is being used as the killing agent, transferred to the bottle containing this. The insects can be kept in the killing bottle throughout the collecting period in the field and then later preserved in the lab.

One word concerning the collection of butterflies and moths using an insect sweepnet. Once caught in the net, these insects tend to lose their scales as they flap their wings against the mosquito netting material of the sweepnet in their bid to escape from it. It should be borne in mind that lepidopteran scales and the colours they confer on the insects are very important for taxonomic purposes and in case of general insect collectors, for the aesthetic value they confer on the insects. Hence it is imperative that as much as possible the collector of butterflies and moths minimizes the loss of scales from his specimens in the field. To do this, the author has found it very useful that as soon as, especially when a butterfly is caught in a sweepnet, that its head is squeezed lightly from the sides between the collector's thumb and index finger.

This kills off the insect instantly and thus prevents unnecessary movements by the insect in the net which would result in scale loss, especially from the wings. Also while in the field, butterflies and moths should be stored separately in old envelops or between sheets of folded old newspapers to prevent further scale loss from the insects. These insects should not be mixed with other insects collected for the same reason. Ideally, each butterfly and moth collected should be kept separately and transported this way to the lab.

Insect traps such as the biconical, NG2B, NG2G, Vavoua and the Pyramidal trap attract and catch flying tsetse flies baited or unbaited. The colours of the material used to construct these traps are specially selected to attract flying tsetse from distances. Other insect traps like the common insect light trap, the UV-light trap, sticky trap, pheromone trap etc., utilized other means, chemical or physical, to lure flying insects to them. Many of these traps are generalist traps in that, they attract and catch a wide range of flying insects, while others are specialized in that they lure one type or a few but closely related insect groups.

B.   Collecting Insects from Vegetation

The easiest type of vegetation to deal with when collecting insects is grass. For this vegetation type, the insect sweepnet is the most ideal simple collecting aid. But to collect insects from shrubs and trees presents a big challenge to the collector. This is so because insects are to be found on various parts of shrubs and trees. As regards trees, insects to be collected may be too high up the branches and out of reach to the collector. However, the insect sweepnet could be used to collect insects from both shrubs and trees. To ensure that as many kinds of insects as possible are collected from shrubs and trees, catches by the insect sweep net can be supplemented by one other simple technique. A canvas or tarpaulin sheet could be spread below the shrub or tree and then the plant shaken vigorously to dislodge insects to the canvas or tarpaulin sheet below.

1.   To collect insects from grass, use an insect sweepnet. All that you are required to do is walk through the grass while using a sweeping motion on the net against the grass. Ensure that the mouth of the net is directed forward as you swing the net to and fro (i.e., the mouth of the net should always be directed in the direction of the swing). When enough plant material or insects have entered the net, remove the insects caught from the net and transfer them to the killing bottle.

2.   To collect insects from tall shrubs and trees, sweep the branches of the shrubs or trees, as high as you can reach using an insect sweepnet. The maximum height from which you can collect insects up the shrub or tree will be that reached with an outstretched hand. This of course can be slightly improved when you jump while holding the net in

an outstretched hand or when you stand on some kind of support.

Alternatively, to collect insects from shrubs and trees, spread a canvas or tarpaulin sheet on the ground below the shrub or tree of interest. Shake the shrub or tree or branches thereof vigorously. This will result in insects on the shrub or tree dropping to the canvas or tarpaulin sheet below. This is so because many insects when disturbed will either let go from wherever they are resting on the plant and/or feign death by dropping to the ground. When insects feign death by dropping to the ground, in this case onto the canvas or tarpaulin sheet, they remain motionless for some time before resuming their normal activities. This period of "death" can be taken advantage of by the collector. This is the time you transfer them to the killing bottle!

C.   Collecting Insects from the Soil and Litter

Soil- and litter-dwelling insects are best collected together with the soil and litter in which they are found in the field. In insect population density studies, this may require collecting a specific amount of soil or litter from randomly selected sampling units in the insect's habitat. However, for general insect collections and indeed those aimed at getting specimens for Insect Functional Morphology studies, knowing the habitat of an insect group of interest is enough. All that is required is to collect soil and/or litter from that habitat and then isolating the insects from the soil and litter, later in the lab. Collection of soils may require digging the soil to a certain depth and may require the use of what are called core samplers. Extraction of insects from the soil and litter later in the lab is done by using the Berlese funnels described before.

D.   Collecting Insects from Aquatic Environments

To collect water-dwelling insects, a variety of equipment can be used. The simplest of these is the aquatic insect net. Other equipment include grabbers and dredgers (Fig. A1.3). Aquatic insect nets are easy to use. Simply dip the net and move it in water or make sweeping movements with the net against aquatic vegetation or other substrata.

Grabbers and dredgers are for collecting those insects that are to be found on surfaces and in the soil under water bodies. These apparatuses can be operated by hand or through motorized boats. Insects caught by them are then extracted from the soil by hand or other means.

Appendix 2

Basic Techniques for Preparing Insect Material for Ultrastructural Studies

The microscopical techniques used and suggested for use throughout this laboratory manual are suitable for studying gross functional morphology of the insect body. They all require the employment of a less sophisticated optical equipment, the binocular microscope. In insect functional morphological studies however, it sometimes becomes imperative to study a given insect structure(s) up to the subcellular level of its tissues, in order to understand the basis of its function. The use of compound light microscopes and more especially the scanning and transmission electron microscopes, gives this kind of detail of structure required.

Preparatory techniques for light (using the compound light microscope) and for electron microscopy are more intricate than those for binocular light microscopy. Although the techniques for light microscopy may appear less complex than those for electron microscopy, in reality they are similar in many ways; it is really a question of degree. In both types of microscopy the following major procedures are recognized in specimen preparation; fixation, dehydration, embedding and/or mounting, sectioning, staining and observation of the material with the microscope. In between some of these procedures, other activities such as the cleaning of specimens to remove debris on them, may be included depending on the nature of the insect material being prepared. In some situations, the material may even require to be double fixed using the same type or different types of fixatives.

Fixation of tissue ensures that all cellular activity in it is arrested, i.e. most of the organelles (nucleus, chromosomes, mitochondria etc.) are preserved in their original states. Dehydration facilitates further processing of the material for microscopy, while embedding the specimens facilitates their later sectioning into fine sizes suitable for observation with a microscope. Staining enhances the contrast of the tissue when viewed with a microscope. For the scanning electron microscope (SEM), which is the best instrument todate for studying surface ultrastructure of tissues, organs and the body, the prepared specimens need to be finely coated with a thin layer of gold or of an alloy of gold and palladium etc., before being examined. For the transmission electron microscope (TEM), the types of stains used on the tissues are critical in improving the resolution of their structures by the microscope.

Several types of chemicals and materials are needed for fixation, dehydration, embedding/mounting, staining and coating specimens for light and electron microscopy. It is beyond the scope of this index to go through all these but those that are commonly used are named in the techniques outlined below, developed for preparing selected insect tissues for microscopy. Common stains for light microscopy and the insect tissues for which they are recommended are presented in tables A2.1 and A2.2, while recipes of some of the solutions named in the index are given in Appendix 3. At the end of this index are presented references from which the techniques outlined were extracted, as well as, additional readings which the student could consult for more detail on histological preparations of insect material.

A. Basic Techniques for Light Microscopy

(A superscript following a name of a technique is a reference number of the source of the technique as it appears in the reference section at the end of the appendix)

1. **Aubrey-Boudreaux-Grodner-Hammond Technique[2]**
    a. *Original Purpose of the Technique*
        -   To study sex pheromone-producing cells and the associated cuticle of a female moth.

    b. *Other Potential Uses of the Technique*
        -   Study of various insect glands with their associated cuticular components.

    c. *Procedures*
        i.   Excise the 8th and 9th abdominal segments of 2–4 days old female adult moths.
        ii. Fix the excised specimens in Bouin's fixative.
        iii. Embed the specimens in paraffin.
        iv. Section the material at 10 µm thickness using a ultramicrotome.
        v.   Stain the sections with Mallory's triple stain.
        vi. Observe under a compound light microscope.

2. **Pugh-King-Fordy Technique[24]**
    a. *Original Purpose of the Technique*
        -   To study the ultrastructure of a tick spiracle.

    b. *Other Potential Uses of the Technique*
        -   Ultrastructural studies of cuticular surfaces of insects.

c. *Procedures*
  i.   Fix tick specimens in Bouin's fixative.
  ii. Wash the specimens in absolute ethanol (EtOH).
  iii. Transfer the specimens through an ethanol/acetone series to pure acetone.
  iv. Infiltrate the specimens with Epon 812 epoxy resin.
  v.   Embed the specimens in fresh resin.
  vi. Cut 1.0 µm sections using a ultramicrotome with glass knives.
  vii. Stain the specimens with Malloy's azure II methylene blue.
  viii. Examine with a compound light microscope.

NOTE: Permanent mounts of the specimens can be made using the Epon resin as the moutant.

3. **Bland-Rentz Technique[4]**
  a. *Original Purpose of the Technique*
    -   To study the ultrastructure of the inner walls of the proventriculus of the Gryllacrididae for taxonomic purposes.

  b. *Other Potential Uses of the Technique*
    -   Ultrastructural studies of insect cuticular surfaces.

  c. *Procedures*
    i.   Dissect out the proventriculus from the alimentary canal of alcohol-preserved or freshly anaesthetized insects.
    ii. Make a single longitudinal slit through the proventriculus using a pair of scissors.
    iii. Place the specimen in 10% cold KOH for 18 hr to remove muscle and connective tissue.
    iv. Wash the remaining flap of sclerotized tissue with distilled water.
    v.   Sonicate the specimen in distilled water to remove debris.
    vi. Mount the specimen temporarily in Hoyer's medium, if need be.
    vii. Wash the specimen with distilled water.
    viii. Dehydrate in an ethanol (EtOH) series (e.g. 30, 50, 70, 90 & 100%).
    ix. Critical-point dry the specimen in CO_2.
    x.   Remove the remaining food debris on the specimen using a fine brush.
    xi. Mount the specimen in paraffin.
    xii. Cut the specimen into sections.
    xiii. Observe the specimen with a compound light microscope.

4. O'Connor-O'Brein-Salpeter Technique[23]

a. *Original Purpose of the Technique*
- To study leg muscles and their nerves in a cockroach.

b. *Other Potential Uses of the Technique*
- Study of muscles and their nerves in any other insect species.

c. *Procedures*
  i. Excise one leg from an anaesthetized cockroach.
  ii. Score the cuticle of the femur and place a drop of 5% glutaraldehyde fixative buffered with Sorensen's M/15 phosphate buffer, on the exposed muscle tissue to start the fixation of the muscle soonest.
  iii. Expose the rest of the muscle of the femur by dissection.
  iv. Fix the entire muscle set in 2% osmium tetroxide (OsO_4) buffered with M/15 phosphate buffer for 15 min.
  v. Soak in 2% aqueous uranyl acetate for 1 hr. (This procedure enhances tissue contrast when viewed with phase optics.)
  vi. Dehydrate the specimen in a graded ethanol series (e.g. 70, 80, 90 & 100%).
  vii. Embed in Epon.
  viii. Cut the specimen into 1 µm sections using a ultramicrotome.
  ix. Stain the sections with 0.5% toluidine blue in 1.0% borax stain or leave the specimen unstained.
  x. Examine the specimens with a phase contrast compound light microscope.

5. Spencer-Motara Technique[25]

a. *Original Purpose of the Technique*
- To study the relationship between follicle ultrastructure of ovaries of a mosquito and its diet.

b. *Other Potential Uses of the Technique*
- Ultrastructural studies of insect ovaries.

c. *Procedures*
  i. Fix dissected out ovaries in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 30 min.
  ii. Rinse the specimens in 0.1 M sodium cacodylate buffer for 5 min.
  iii. Fix the specimens in 1% osmium tetroxide (OsO_4) for 60 min.
  iv. Rinse 3 times with distilled water, each rinse lasting 2 min.
  v. Place the specimens in 0.5% uranyl acetate stain, in 80% acetone for 30 min.

vi.   Dehydrate in 95% acetone for 10 min followed by three changes of 100% acetone lasting 10 min each.

vii. Infiltrate the specimens with Spurr resin by adding equal amounts of Spurr resin and 100% acetone to them and by letting the specimens stay in this solution for 90 min.

viii. Place the specimens in two changes of pure Spurr resin each lasting 60 min.

ix.   Embed the specimens in Spurr resin and then leave them overnight at 70°C for the resin to polymerize.

x.   Cut 1 μm sections of the specimens using a ultramicrotome.

xi.   Stain the sections with uranyl acetate followed by lead citrate.

xii. Examine with a compound light microscope.

Table A2.1. Common differential stains and their effects on selected insect tissues, organs and organ systems. (Adapted with modification from, Barbosa, P. 1974. *Manual of basic techniques in insect histology.* Autumn Publishers. Amherst, Massachusetts 01002, U.S.A.).

Differential stain (1%)	Staining time (hr)	Tissue differentiation
Aniline blue	0.5	- Parts of fat body become light blue. - Thoracic muscles become grey. - Dorsal wall material become white. - Caeca and tracheae become blue. - Proventriculus becomes tinged blue. - Salivary glands become grey blue.
Azur II	0.5	- Fat body becomes light lavender. - Heart and tracheae in lavender outline. - Thoracic muscles become blue. - Malpighian tubules become greyish blue.
Brilliant cresyl blue	48.0	- Proventriculus, caeca, salivary glands, colon become lavender. - Malpighian tubules become yellowish. - Dorsal wall material becomes dark blue. - Fat body becomes bright blue with lavender marks. - Heart becomes tinged blue - Femurs become green. - Thoracic muscles become bluish green.

Table A2.1: Continued

Differential stain (1%)	Staining time (hr)	Tissue differentiation
Rose bengal	36.0	- Crop and proventriculus become deep red. - Caeca becomes orange red. - Thoracic muscles become streaked red. - Salivary glands become deep pink. - Dorsal wall material become light scarlet. - Heart appears deep wine.
Bordeaux red-toluidin blue	48.0	- Proventriculus and gut become deep purple. - Caeca become dark lavender. - Malpighian tubules appear deep wine red. - Heart becomes purple. - Dorsal wall material appear greenish blue. - Fat body becomes pale blue. - Leg muscles become dull green. - Ovaries become yellowish green. - Ventral nerve cord becomes blue.
Bordeaux red-azur II	24.0	- Caeca become tinged light green. - Gut becomes purple. - Malpighian tubules become faint pink. - Heart becomes brown. - Aorta becomes orange-pink. - Alary muscles become light tan. - Fat body becomes blue and pink.
Bjebrich scarlet-azure II	12.0	- Salivary glands become light to bright blue. - Malpighian tubules become blue or red. - Heart and wing muscles appear purple. - Leg muscles and testes appear light blue.

B. Basic Techniques for Scanning Electron Microscopy (SEM)

(A superscript following a name of a technique is a reference number of the source of the technique as it appears in the reference section at the end of the appendix)

1. **Cwikla-Freytag Technique[7]**
    a. *Original Purpose of the Technique*
        -   To study the external morphology of a bug.

    b. *Other Potential Uses of the Technique*
        -   Studies of external cuticle ultrastructure of many other insects.

c.   *Procedures*
   i.   Clear the specimens in KOH.
   ii.   Dry the specimens in a series of baths of 70% ethanol (EtOH), 90% EtOH and xylene. Leave the specimens for 10 min in each bath.
   iii.   Mount the specimens on scanning electron microscope (SEM) stubs.
   iv.   Coat the specimens thus mounted on stubs with 30 nm layer of gold with a sputter coater.
   v.   Examine the specimens with a SEM.

2.   **Webb Technique[30]**
   a. · *Original Purpose of the Technique*
      -   To study the ultrastructure of the ventromental plate and the external morphology of a chironomid larva (Diptera, Chironomidae).

   b.   *Other Potential Uses of the Technique*
      -   Ultrastructural studies of external cuticular surfaces of both immature and adult insects.

   c.   *Procedures*
      i.   Fix the material (chironomid larvae) in ethanol (EtOH):glacial acetic acid solution in a 3:1 ratio, respectively.
      ii.   Remove head capsules from the larvae and dissect out the ventromental plates from the specimens.
      iii.   Dehydrate whole head capsules and the dissected pieces containing ventromental plates through a graded series of ethanol (EtOH), e.g., 30, 50, 70, 90 & 100%.
      iv.   Air dry the specimens.
      v.   Attach specimens to scanning electron microscope (SEM) stubs.
      vi.   Coat specimens with a thin layer of gold in a sputter coater.
      vii.   Examine with a SEM.

3.   **Mbata Technique[21]**
   a.   *Original Purpose of the Technique*
      -   To study the ultrastructure of setal brushes and mesonotal evaporatoria of an assassin bug.

   b.   *Other Potential Uses of the Technique*
      -   Ultrastructural studies of the cuticle of many other insects.

c. *Procedures*

i. Dissect freshly anaesthetized bugs in 0.1 M Sórensen's buffer solution of pH 7.3–7.4 to excise cuticle containing structures of interest.

ii. Place the specimens (small pieces of cuticle with structures of interest) in a 7 cm³ screw-capped vial containing a detergent and then sonicate for 20 min to remove debris from the specimens.

iii. Replace the detergent with distilled water and again sonicate for 20 min.

iv. Transfer the specimens to the fixative, 6% glutaraldehyde solution for 2 hr at 25 °C.

v. Rinse the specimens in fresh 0.1 M Sórensen's buffer solution for 2 hr. Within this period, change the buffer solution 3 times.

vi. Post-fix the specimens in 2% osmium tetroxide (OsO_4) for 2 hr under a hood.

vii. Rinse the specimens in distilled water three times in 5 min under the hood.

viii. Again sonicate the specimens in a detergent water as in steps ii and iii above.

ix. Dehydrate the specimens in an ethanol (EtOH) series (i.e., 25, 50, 70, 90 & 100%) allowing the specimen to remain in each of the first three grades for 5 min and in each of the last two grades, 3 min.

x. Critical point dry the specimens with CO_2.

xi. Mount specimens on scanning electron microscope (SEM) stubs using silver conducting paint and then glue the stubs to their holders using the same paint.

xii. Coat the specimens thus mounted on stubs with gold-palladium by evaporating a 10 cm wire of the alloy in a sputter coater. Repeat this procedure 3 times on each specimen.

xiii. Observe the specimens with a SEM.

4. **Pugh-King-Fordy Technique[24]**

a. *Original Purpose of the Technique*
   - To study the ultrastructure of a tick spiracle.

b. *Other Potential Uses of the Technique*
   - Ultrastructural studies of cuticular surfaces of insects.

c. *Procedures*

i. Rinse the ticks (specimens) in ethanol (EtOH).

ii. Re-hydrate the specimens in a series of distilled water.

iii. Sonicate in an ultrasonic bath to remove debris from the specimens.

iv. Rinse specimens in distilled water.
v. Dehydrate in an acetone series to absolute acetone.
vi. Transfer to ether to remove the acetone.
vii. Air dry the specimens.
viii. Mount specimens on aluminum scanning microscope (SEM) stubs using Scotch tape or quick setting epoxy resin.
ix. Coat the specimens on SEM stubs with gold in a sputter coater.
x. View the specimens with a SEM.

5. **Bland Technique**[3]
    a. *Original Purpose of the Technique*
        - To study mouthparts sensilla and the ultrastructure of the mandibles of a weevil.

    b. *Other Potential Uses of the Technique*
        - Ultrastructural studies of mouthparts of insects.
        - Ultrastructural studies of external cuticular surfaces of insects.

    c. *Procedures*
        i. Place specimens in a solution of 0.1 mg crude pancreatic protease in 1 ml Tris buffer (pH 7.5) for 1 hr.
        ii. Rinse the specimens in distilled water.
        iii. Fix specimens in 4% glutaraldehyde in phosphate buffer (pH 7.2).
        iv. Again rinse in distilled water.
        v. Sonicate the specimens for 2 min in photoflo to remove debris on it.
        vi. Dehydrate the specimens in a graded series of ethanol (EtOH), e.g., 30, 50, 70, 75, 80, 90 & 100%.
        vii. Again sonicate the specimens for 1 min but this time in absolute ethanol.
        viii. Wash the specimens in acetone.
        ix. Critical-point dry the specimens in CO_2 or simply air dry them.
        x. Mount specimens on scanning electron microscope (SEM) stubs.
        xi. Coat the specimens with a 20 nm layer of gold with a sputter coater.
        xii. Observe with a SEM.

6. **Ghiradella Technique**[13]
    a. *Original Purpose of the Technique*
        - Study of the ultrastructure of lepidopterous scales.

    b. *Other Potential Uses of the Technique*
        - Study of scales of any other insect.

    c. *Procedures*
       i. Mount small bits of Lepidoptera wings from a dried up pinned insect on scanning electron microscope (SEM) stubs using silver conducting paint.
       ii. Sputter coat the specimens on the SEM stubs with gold/palladium alloy.
       iii. Observe with a SEM.

7. **Liu-Liu Technique[19]**
    a. *Original Purpose of the Technique*
      - To study sensilla on antennae of a noctuid moth.

    b. *Other Potential Uses of the Technique*
      - Study of ultrastructure of antennae of other insects.
      - Study of the ultrastructure of external surfaces of the cuticle of insects.

    c. *Procedures*
       i. Remove heads of adult moths and preserve in 5% glutaraldehyde in saline at 4°C until needed for use.
       ii. When ready, transfer the preserved specimens to freshly prepared 5% glutaraldehyde in 0.02 M phosphate buffer and leave overnight at 4°C.
       iii. Rinse the specimens in the same buffer.
       iv. Post-fix the specimens with 2% osmium tetroxide (OsO_4) in the buffer for 1 hr at room temperature.
       v. Dehydrate the specimens in 30, 50 & 70% ethanol for 30 min each with two changes.
       vi. Critical dry the specimens with CO_2.
       vii. Mount specimens on scanning electron microscope (SEM) stubs.
       viii. Coat the specimens with gold in a sputter coater.
       ix. Examine with a SEM.

8. **Aubrey-Boudreaux-Grodner-Hammond Technique[2]**
    a. *Original Purpose of the Technique*
      - Study of pheromone-producing cells and their associated cuticle in a moth.

    b. *Other Potential Uses of the Technique*
      - Study of insect sensilla.
      - Study of cuticular structures of insects.

   c. *Procedures*

     i. Excise the 8th and 9th abdominal segments of 2–4 days old adult female moths.

    ii. Fix the specimens in FAA (see Appendix 3).

   iii. Dehydrate in DMP (2,2-dimethoxypropane activated with a drop of concentrated HCl per 50 ml of DMP).

   iv. Critical-point dry the specimens with CO_2.

    v. Mount with Tube-Koat on scanning electron microscope (SEM) stubs.

   vi. Sputter with a gold-palladium alloy at 10 mg/min for 3–5 min.

  vii. Observe with a SEM.

9.   **Bland-Rentz Technique[4]**

   a. *Original Purpose of the Technique*

     - To study the ultrastructure of the inner walls of the proventriculus of the Gryllacrididae for taxonomic purposes.

   b. *Other Potential Uses of the Technique*

     - Studies of cuticular armature of the proventriculus of other insects.

     - Studies on ultrastructure of the internal cuticular surfaces in the insect body.

   c. *Procedures*

     i. Dissect out the proventriculus from the alimentary canal of alcohol preserved or freshly anaesthetized adult insects.

    ii. Make a single longitudinal slit through the proventriculus using a pair of scissors.

   iii. Place the specimen in 10% KOH for 18 hr to remove the unrequired muscle and connective tissues from the specimen.

   iv. Wash the remaining flap of sclerotized tissue with distilled water.

    v. Sonicate the specimen in distilled water to remove debris.

   vi. Dehydrate the specimen in an ethanol (EtOH) series such as 30, 50, 70, 90 & 100%.

  vii. Critical-point dry the specimen in CO_2.

 viii. Remove the remaining food debris from the specimen using a fine brush.

   ix. Mount the specimen on scanning electron microscope stubs.

    x. Sputter coat the specimen with 30 nm of gold.

   xi. Examine with a SEM.

10.   **Boppé Technique[5]**

   a. *Original Purpose of the Technique*

     - To study the androconial system (alar organs) of Lepidoptera.

b. *Other Potential Uses of the Technique*
  - Surface ultrastructural studies of the insect cuticular associations.

c. *Procedures*
  i. Dissect out the alary organs from the abdomens of butterflies.
  ii. Mount the specimens, together with small parts of surrounding cuticle of the abdominal segments on scanning electron microscope (SEM) aluminium stubs using acetone-celluoid glue or conductive carbon cement.
  iii. Desiccate the specimens thus mounted at room temperature.
  iv. Transfer to a saturated osmium tetroxide (OsO_4) atmosphere for 24–48 hr.
  v. Coat the specimens with gold for 8–10 min in a sputting chamber.
  vi. Examine with a SEM.

11. **LaChange Technique[18]**
  a. *Original Purpose of the Technique*
    - To study sperm production and their abnormalities in backcrosses of interspecific hybrids of two moths.

  b. *Other Potential Uses of the Technique*
    - Ultrastructural studies of testes and sperm tubes of insects.

  c. *Procedures*
    i. Dissect out the testes of 4–6 days old males in 0.1 M PO_4 buffer at pH 7.0.
    ii. Draw up the sperm tubes with a pipette and then drop them into a 1 µm nucleopore filter backed by a 0.45 µm millipore filter. The dual filter system should be placed in a millipore filter holder.
    iii. Gently and slowly force 3 ml of PO_4 buffer through the filter holder to the specimens with a syringe.
    iv. Pass through the filter holder to submerge the sperm bundles, a fixative mixture of 1:1 ratio, 8% glutaraldehyde and 0.1 M PO_4 buffer (pH 7.0). Fix the tissue for 2 hr.
    v. Remove the filters from the holder and the nucleopore filter holding the sperm bundles washed overnight with 0.1 M PO_4 buffer solution.
    vi. Run the filter with its contents through a graded series of ethanol (30, 50, 70, 90, & 100%), for 10 min in each grade.
    vii. Rinse the filter with its sperm bundles content with 100% ethanol.

   viii. Critical point dry the specimens in CO_2.
   ix. Attach the filter to a scanning electron microscope stub with double stick Scotch tape.
   x. Coat the specimens on the stub with gold/palladium alloy on a sputter coater.
   ·xi. Examine with a SEM.

12. Donaldson- Tonder Technique[9]

a. *Original Purpose of the Technique*
   - To study chorion ultrastructure of eggs of dung beetles.

b. *Other Potential Uses of the Technique*
   - Chorion ultrastructural studies of other insect eggs.

c. *Procedures*
   i. Place the eggs in a 15% solution of glycerol and water in sealed vials and store at -120°C in liquid nitrogen for 2–4 months in the vapour phase.
   ii. At the end of this period, thaw the specimens in stages, first by adjusting the temperature to -18°C for 2 hr and then to 4°C.
   iii. Fix the eggs overnight in 2.5 glutaraldehyde in a 0.2 M phosphate buffer (pH 7.2).
   iv. Post fix the specimens in 1% buffered osmium tetroxide (OsO_4) for 5 hr.
   v. Critical-point dry the specimens with CO_2.
   vi. Coat the specimens with a 20 nm layer of gold/ palladium in a sputter coater.
   vii. Examine the specimens with a SEM.

Table A2.2. Insect tissues and their recommended stains.(Adapted with modifications from Barbosa, P. 1974. *Manual of basic techniques in insect histology.* Autumn Publishers. Amherst. Massachusetts 01002, U.S.A.).

Insect tissue type	Stain (1% Conc.)	Staining time (hr)
Heart	Toluidin blue	0.5
	Trypan blue	24–36
Tracheae	Basic fuchsin	36
Ventral nerve cord	Borax carmine	0.5

Table A2.2: Continued next page

Table A2.2: Continued

Insect tissue type	Stain (1% Conc.)	Staining time (hr)
Gastric caeca	Aldehyde green	24
	Acid fuchsin	24
Salivary glands	Brilliant cresy blue	0.5–48
	Trypan blue	0.5
Ovaries	Trypan blue	0.5
	Aldehyde green	36
Fat body	Methyl violet B	36
	Azur II	0.5–48
Thoracic muscles	Toluidin blue	24–48
	Safranin O	24
Malpighian tubules	Azur II	24
	Light green SF yellowish	0.5–48
Digestive tract	Congo red	0.5
	Aldehyde green	0.5

C. Basic Techniques for Transmission Electron Microscopy (TEM)

(A superscript following a name of a technique is a reference number of the source of the technique as it appears in the reference section at the end of the appendix)

1. **Ghiradella Technique[13]**
    a. *Original Purpose of the Technique*
        - Study of the ultrastructure of lepidopterous scales and those of a weevil.

    b. *Other Potential Uses of the Technique*
        - Study of scales and setae of any other insect.

c. *Procedures*

    i. Soak briefly small bits of wing from dried up Lepidoptera specimen. Due to the difficulty of infiltrating weevil scales with embedding medium at a later stage, when compared to those of the Lepidoptera, strip off some scales from a weevil into ethanol, centrifuge them into a pellet, suspend the scale pellet and again centrifuge it with a change of solution.

    ii. Embed both, the bits of Lepidoptera wing and the weevil scales (separately of course) in Spurr low viscocity resin.

    iii. Cut the specimens into thin sections with glass knives, picking the sections on formvar-coated grids.

    iv. Double stain the sections on grids with uranyl acetate and lead acetate.

    v. Observe with a TEM.

2. **Elder Technique[10]**

  a. *Original Purpose of the Technique*

    - To study the ultrastructure of the stridulating (thoracic) muscles of a katydid (Orthoptera: Tettigoniidae).

  b. *Other Potential Uses of the Technique*

    - Ultrastructural studies of insect muscles in general.

  c. *Procedures*

    i. Dissect out the thoracic muscles of a katydid (stridulating muscles).

    ii. Fix the muscle in 3% glutaraldehyde in 0.1 M Millonig phosphate buffer solution with 3% sucrose at pH 7.4 for 2 hr at 4°C.

    iii. Rinse the specimens in distilled water.
NOTE: if not in a hurry, specimens could be stored at this stage following primary fixation with 3% glutaraldehyde. The specimens should be stored in 5% sucrose in the phosphate buffer at pH 7.4 and 4°C.

    iv. Post-fix the specimens in 1% osmium tetroxide (OsO_4) in 0.1 M PO_4 buffer at pH 7.4

    v. Embed in araldite.

    vi. Section the blocks.

    vii. Double stain with uranyl acetate and lead acetate.

    viii. Examine with a TEM.

3.   **O'Connor-O'Brein-Salpeter Technique[23]**
   a. *Original Purpose of the Technique*
       -    To study leg muscles and their nerves in a cockroach.

   b. *Other Potential Uses of the Technique*
       -    Study of muscles and their nerves in any other insect species.

   c. *Procedures*
       i.   Excise one leg from an anaesthetized cockroach.
       ii. Score the cuticle of the femur and place a drop of 5% glutaraldehyde fixative buffered with Sorensen's M/15 phosphate buffer, on the exposed muscle tissue to start the fixation of the muscle soonest.
       iii. Expose the rest of the muscle of the femur by dissection.
       iv. Fix the entire muscle set in 2% osmium tetroxide (OsO_4) buffered with M/15 phosphate buffer for 15 min.
       v.   Soak in 2% aqueous uranyl acetate for 1 hr. (This procedure enhances tissue contrast when viewed with phase optics.)
       vi. Dehydrate the specimen in a graded ethanol series (e.g., 70, 80, 90 & 100%).
       vii. Embed in epon.
       viii. Cut the specimen into 700µm thick sections using a ultramicrotome.
       ix. Stain the sections with an aqueous solution of uranyl acetate for 5 min followed by lead acetate for 15 min.
       x.   Examine the specimens with a transmission electron microscope.

4.   **LaChange Technique[18]**
   a. *Original Purpose of the Technique*
       -    To study sperm production and abnormalities in backcrosses of interspecific hybrids of two moths.

   b. *Other Potential Uses of the Technique*
       -    Ultrastructural studies of testes and sperm tubes of insects.

   c. *Procedures*
       i.   Collect sperm bundles from the testes of 4–6 days old adult moths by dissecting the insects in 0.1 M PO_4 buffer solution at pH 7.3.
       ii. Fix the specimens in 3% glutaraldehyde in 0.05% PO_4 buffer at pH 7.8.
       iii. Rinse in several 30 min changes of phosphate buffer, alternating with centrifugation at 8000 rpm to condense the specimen into a pellet.

iv. Post-fix the specimen pellet with osmium tetroxide (OsO_4) for 1 hr.

v. Wash in distilled water five times and repelletize following each wash by centrifugation.

vi. Suspend the pellet in 1% agar solution.

vii. Dehydrate the agar pellet in a graded series of ethanol (30, 50, 70, 90, 95 & 100%).

viii. Wash the pellet twice with propylene oxide (PPO) and then break into small pieces.

ix. Agitate the small pieces overnight in 1:1 PPO-Spurr mixture.

x. Embed the pieces in 100% Spurr plastic and cure at 65°C overnight.

xi. Section at 600–1000 μm sizes.

xii. Stain with saturated uranyl acetate in 70% EtOH and then counterstain with lead acetate.

xiii. Examine with a TEM.

5. **Davis-Herndon-Agee-Ellis Technique**[8]

   a. *Original Purpose of the Technique*

     - To study the ultrastructure of compound eyes of the Mediterranean fruit fly.

   b. *Other Potential Uses of the Technique*

     - Ultrastructural studies of insect compound eyes.

   c. *Procedures*

     i. Decapitate flies submerged in Karnovsky's fixative.

     ii. Cut the heads into small pieces.

     iii. Rinse the specimens in 0.1 M cacodylate buffer solution.

     iv. Select pieces bearing eye fragments and fix either in Karnovsky's fixative or glutaraldehyde - acrolein.

     v. Post-fix in osmium tetroxide (OsO_4).

     vi. En bloc stain in uranyl acetate.

     vii. Dehydrate in 2-2-dimetroxy propane to acetone.

     viii. Embed in a poly/bed 812 containing resin.

     ix. Cut very thin sections using a ultramicrotome.

     x. Stain the specimen with uranyl acetate and lead citrate.

     xi. Examine with a TEM.

6. **Wiederhold-Jupp-Alexander Technique**[31]

   a. *Original Purpose of the Technique*

     - To study salivary gland infection in a mosquito.

   b. *Other Potential Uses of the Technique*

     - Ultrastructural studies of salivary glands of other insects.

   c. *Procedures*
     i. Kill mosquitoes in CO_2.
     ii. Dissect out the salivary glands in 0.1 M cacodylate buffer.
     iii. Fix the specimens in buffered 2.5% glutaraldehyde for 2 hr at room temperature.
     iv. Rinse the specimens in buffer solution.
     v. Transfer the specimens to a molten drop of 1.5% noble agar and immediately chill on an ice bed.
     vi. Trim the agar blocks containing the specimens.
     vii. Post-fix the specimens in 1.0% buffered osmium tetroxide (OsO_4) for 30 min.
     viii. Dehydrate in a graded ethanol series containing uranyl acetate.
     ix. Replace ethanol with propylene oxide.
     x. Embed the specimens in araldite resin.
     xi. Section the specimens.
     xii. Stain the sections with lead acetate.
     xiii. Examine with a TEM.

7. Spencer-Motara Technique[25]
   a. *Original Purpose of the Technique*
     - To study the relationship between follicle ultrastructure of ovaries of a mosquito and its diet.

   b. *Other Potential Uses of the Technique*
     - Ultrastructural studies of insect ovaries.

   c. *Procedures*
     i. Fix dissected out ovaries in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 30 min.
     ii. Rinse the specimens in 0.1 M sodium cacodylate buffer for 5 min.
     iii. Fix the specimens in 1% osmium tetroxide (OsO_4) for 60 min.
     iv. Rinse 3 times with distilled water, each rinse lasting 2 min.
     v. Place the specimens in 0.5% uranyl acetate stain, in 80% acetone for 30 min.
     vi. Dehydrate in 95% acetone for 10 min followed by three changes of 100% acetone lasting 10 min each.
     vii. Infiltrate the specimens with Spurr resin by adding equal amounts of Spurr resin and 100% acetone to them and by letting the specimens stay in this solution for 90 min.
     viii. Place the specimens in two changes of pure Spurr resin each lasting 60 min.
     ix. Embed the specimens in Spurr resin and then leave them overnight at 70°C for the resin to polymerize.

x. Cut the specimens into 70 nm thick sections using a ultramicrotome.

xi. Stain the sections with uranyl acetate followed by lead citrate.

xii. Examine with a TEM.

APPENDIX 2

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APPENDIX 2

Appendix 3

Recipes of Some Reagents Mentioned in the Laboratory Manual

1. **Bouin's Fixative.**
   Mix: - Picrid acid (saturated aqueous solution) ..... 75 parts
         - Formalin (acid) .... 25 parts
         - Glacial acetic acid .... 5 parts

2. **FAA.**
   Mix: - 40% Formalin .... 1 part
         - 95% Ethyl alcohol .... 4 parts
         - 5% Glacial acetic acid .... 1 part

3. **Glutaraldehyde-Acrolein Fixative.**
   Mix: - 95% Acrolein .... 1 part
         - 2.5% Glutaraldehyde .... 50 parts

4. **Hoyer's Medium (moutant).**
   - Dissolve 30 parts of gum arabic (tear form) in 50 parts of distilled water with 200 parts of chlorate hydrate for 24–48 hr.
   - Place 20 parts of glycerine into the mixture and then filter.

5. **Poly/Bed 812 containing resin.**
   Mix: - Nonenyl succinic anhydride .... 0.5 parts
         - Polybed 8R .... 6 parts
         - Vinyl cyclohexene .... 100 parts

6. **Mallory's triple stain.**
   Solution A.
         - 5% Acid fuchsin (aqueous) solution.
   Solution B.
   Mix: - Brubler's water soluble anilin-blue .... 0.5 parts
         - Orange G .... 2.0 parts
         - Phosphomolybolic acid .... 100 parts

To process the specimens with the stain, first fix the tissue in a fixative and then place the specimen in solution A for 5 min followed by transfer to solution B for 20 min.

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