BIO233: Insect diversity and biology

Laboratory and field manual

BIO233 – Field and laboratory work, overview

Lab	Date	Topic
Field	22.-25. Aug.	**Field work Åse, Lindås 8.30-16** **Field work Rambjøra, Bergen 8.30-16** **Lab work 8.15-16; arthropod orders, sorting** **Lab work 8.15-16; arthropod orders, sorting**
1.	31. Aug.	**External morphology of insects (adults and larvae)**
2.	07. Sept.	**Insect preservation and curation** **Identification of field collections – orders and families**
3.	14. Sept.	**Introduction to taxon specialisation** **Identification of field collections – 10 specimens from 10 orders**
4.	21. Sept.	**DNA barcoding – introduction and protocol** **Identification to genus and species**
5.	28. Sept.	**Identification to genus and species** **DNA barcoding**
6.	05. Oct.	**Internal anatomy: neural system and reproductive organs in cockroaches, respiratory systems w/ trachea and spiracles**
7.	12. Oct.	**Introduction to spiders** **Identification to genus and species** **DNA barcoding**
–	19. Oct.	**Essay – no teaching**
–	26. Oct.	**Essay – no teaching**
8.	02. Nov.	**Adaptations to feeding:** - **digestive systems in fungus farmers, incl. proventriculus** - **specialized mouthparts in bees, mosquito, bed bug, butterfly**
9.	09. Nov.	**Reproductive systems: male and female genitalia** **How to produce identification keys**
10.	16. Nov.	**Databases (BOLD, Artsdatabanken, GBIF)** **evaluation of DNA barcoding results**
11.	23. Nov.	**Finalize and submit 25 samples for the entomological collections** **Write identification key for your 25 identified insects**

Lab equipment

Students provide:

- scalpel, scissors, fine forceps, lab journal, pen, pencil, pencil sharpener and eraser for drawing
- ideally a laptop for lab course 10 (databases & evaluation of DNA barcoding results)

Lab provides:

For collecting and sorting:

- Tubes and ZipLoc bags of various sizes, trays, glass jars, killing jar with ethyl acetate, collection boxes for pinned material, styrofoam and pins for pinning, 70% and absolute ethanol (C_2H_5OH), soft tweezers, stereomicroscope, identification literature, labelling paper, scissors

For internal anatomy:

- cockroach specimens (*Periplaneta americana*)
- dissection pins, dissection trays, 0.9% sodium chloride (NaCl) solution

For genitalia dissection:

- glass slides, cover glasses, 8% potassium hydroxide (KOH), Euparal mounting medium

Expected lab course outcome

- Sort material from the field work
- Ability to distinguish insect orders and suborders
- Knowledge about insect preservation
- Knowledge about external and internal insect anatomy
- Knowledge about the principle of DNA barcoding
- Knowledge on how to work with the databases introduced in the course
- A final collection of 20 identified insects per student, consisting of:
    - **specimens from 10 different arthropod orders or suborders**
    - **specimens of 10 additional species from your order of focus**
    - **DNA barcoding data for 3 of the 10 specimens from your order of focus**
    - **An identification key to all 20 identified arthropods**

Questions and tasks

Throughout this manual you will come across a number of questions and tasks. For convenience, these are marked as “TASK” in bold letters, and continuously numbered. You have to answer these questions and tasks in your lab journal.

Essay

The lecture in week 42 (on 18.10.) will include a training session on scientific writing that will prepare you for writing the **compulsory essay**. In this lecture you will also pick a topic for your essay.

Weeks 42 and 43 are reserved for writing the essay, and no BIO233 lab courses will take place in those two weeks; in week 43 there will be no lecture.
Extent of essay: 3 pages, about 1,500 words. Remember to include a reference section at the end of the essay where you cite all sources that you mention in your text.

Final oral exam

The final exam at the end of the course will examine your knowledge from the lectures and the identification of insects. You will have to identify two different insects to their order or family and explain your conclusion. Each specimen is accompanied by a question on the general biology or adaptations of the species or insect group.

22.08.-25.08.2017

Field work

Field work will take place in two nice locations near Bergen city. Insect traps have been installed in these localities the week before. Depending on the weather forecast, we will spend two days in the field (daytrips, Tuesday-Thursday), and two days with laboratory work to sort samples (Wednesday-Friday). If it rains all day, we will focus more on water insects and collect our traps, eventually work in the lab the second day if rain continues, and go into the field the third day.

Bring the following equipment: rain gear and rubber boots (if raining), lunch pack and plenty of water and/or hot drinks. Notebook and pencil/ ink-pen (not pen), forceps, paint brush (good for picking up tiny insects). Tip: bring a jacket or similar with pockets to carry collecting gear/tubes.

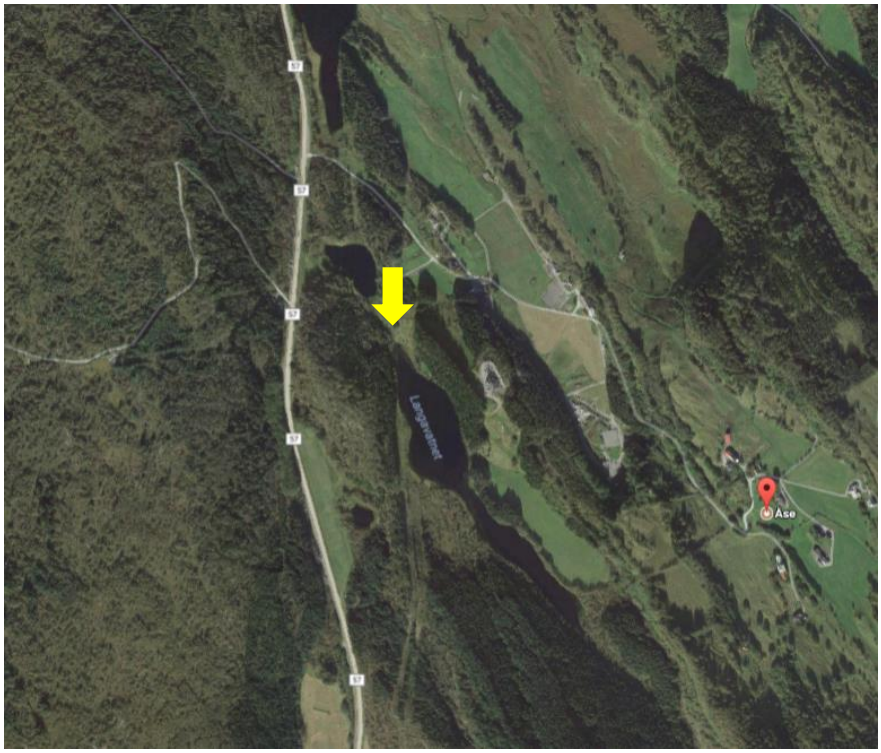
Equipment provided by the teaching staff in the field: plastic trays, ethanol tubes of various sizes, killing-jar, zip-lock bags, various nets.

Field procedures:

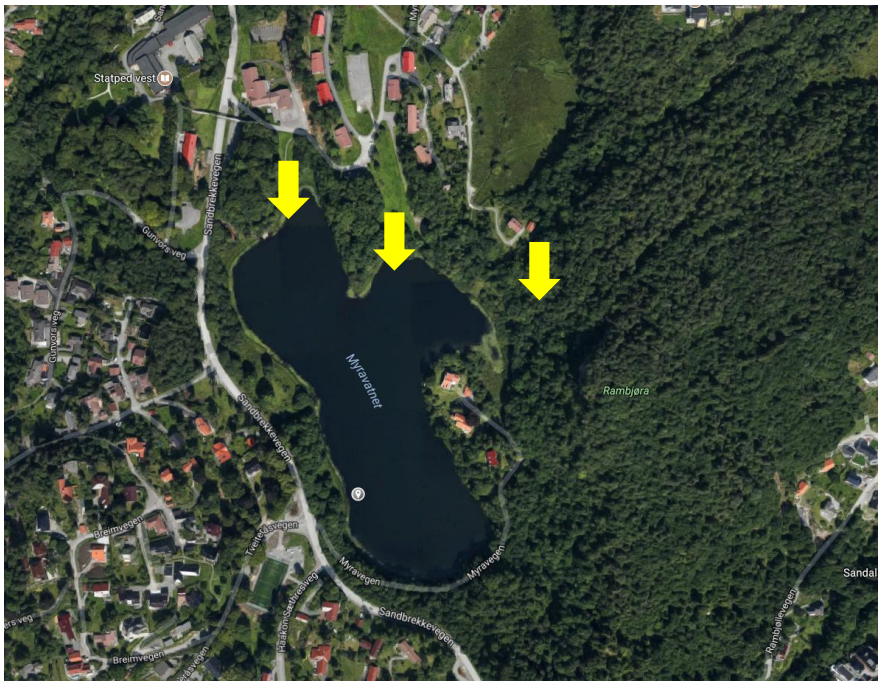
1. Collect trap samples:
    a. Malaise trap
    b. Yellow pans
    c. Barber traps
    d. Flight interception traps

Staff will explain and discuss the utility of each trap. Make your notes!

2. Collect insects and other arthropods by hand. Make sure to swap collecting gear between students:
    a. Sweep net (air)
    b. Sweep net (ground)
    c. Leaf litter sift
    d. Soil samples in plastic bag for Berlese funnel extraction (in lab)
    e. Aquatic net (stream and lake)
    f. Washing stones (streams) – use plastic tray
    g. Hand collecting directly from /under bark, logs, stones and moss
    h. Galls on plants (collect leaves, note plant name)



Locality 1: Åse, Lindås. Pine and mixed broadleaf forest, small streams and medium eutroph lake. Bus transportation.



Locality 2: Rambjøra & Myravatnet, Bergen. Eutrophic lake, small streams, broadleaf forest dominated by oak, ash, hazel and maple. Transport by city rail.

Field samples - laboratory part (two of the days Wednesday-Friday):

You will get a brief intro to the characteristics of the different orders of insects and other arthropods that can be expected in your samples.

TASK 1: Sort all major groups of arthropods. Make sure that all samples have been studied briefly, and that a plan for priorities is made for the lab work (Sept-Oct) is prepared.

TASK 2: Insects in killing jars must be preserved the next day to keep their DNA useful for DNA barcoding and to avoid mould growing on specimens. Pin butterflies, dragon flies and large beetles, consult lab assistants for further advice.

TASK 3: Associate nymphs and larvae in freshwater with adults of the same insect order.

Report the following in your field/laboratory journal:

TASK 4: Describe which collecting gear you have used in which habitats, with comments on their usefulness.

TASK 5: Give at least three examples of typical arthropod groups collected in the different habitats.

TASK 6: Describe the function of the demonstrated traps, and which insect or other arthropod group was most abundant in each trap type.

Thursday, 31.08.2017

Lab course 1 – **external morphology of insects (adults and larvae)**

Material required for this course. Lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. Today you will be introduced to the insect collection at the University Museum of Bergen. In the second part of the course you will study the external morphology of insects and familiarise yourself with the terms of the different body parts.

Visiting the insect collection (ca. 1h)

We meet at **12:15** (not later) in **Realfagsbygget, 1st floor**, in the big hall just below the cantina. From there we will go to the entomological collection of the University Museum of Bergen which is situated in the building's basement. Please have warm clothes with you as the collection room is rather chilly. Furthermore, the oxygen level in the collection is lowered to 15% (normal atmospheric proportion is 21%). We will be in the collection for about 20–30 minutes. Please tell us in advance if you have health issues that might interfere with this lowered oxygen level. We will introduce to you the structure and purpose of scientific collections. You will have the opportunity to see what your field-collected specimens will look like (prepared, pinned, and labelled) and the collection into which they will be integrated. Moreover, we will explain the necessity and means of protecting a scientific collection against a number of harms.

In context with this collection visit, answer the following questions in your lab journal:

TASK 7: Name and explain different kinds of harm that can threaten a scientific collection.

TASK 8: Discuss why insects are conserved (in collections), and archived for such a long time (decades, centuries).

TASK 9: Discuss why several specimens (per gender) per species are usually collected.

Studying insect morphology in the lab (1,5-2h)

After visiting the collection, we continue to the BIO building, lab room A (1C09). There you will study the general external morphology of larval stages and the adult stage of insects in order to answer the following tasks as part of your lab journal:

TASK 10: Make line drawings of the body structure of a fly, a hymenopteran and a third insect order from your material. Indicate the different major body parts (tagmata, Singular: tagma) on them. Derive the presumable main function(s) of the different tagmata from their structure and the associated appendages.

TASK 11: Draw sketches of the legs for at least two adult insects, name the different parts and homologize them among the insects.

TASK 12: Count the number of abdominal segments in the studied insects, and discuss the reasons for differences in the number of abdominal segments among your studied insects.

TASK 13: Name key features in which the larvae ('nymphs') of hemimetabolous insects differ from those of holometabolous insects. Furthermore, name main features in which the larvae differ from the adult stage in the two insect groups.

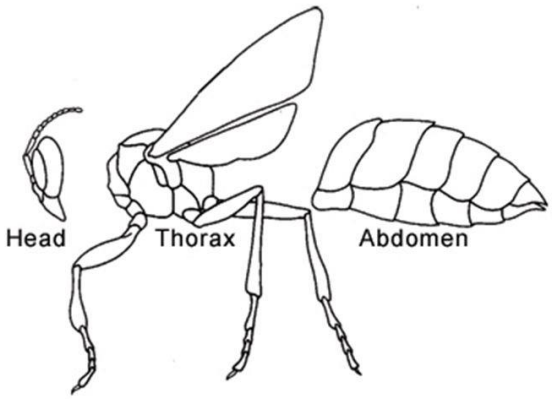


Fig. 1-1. Main body parts of an insect.

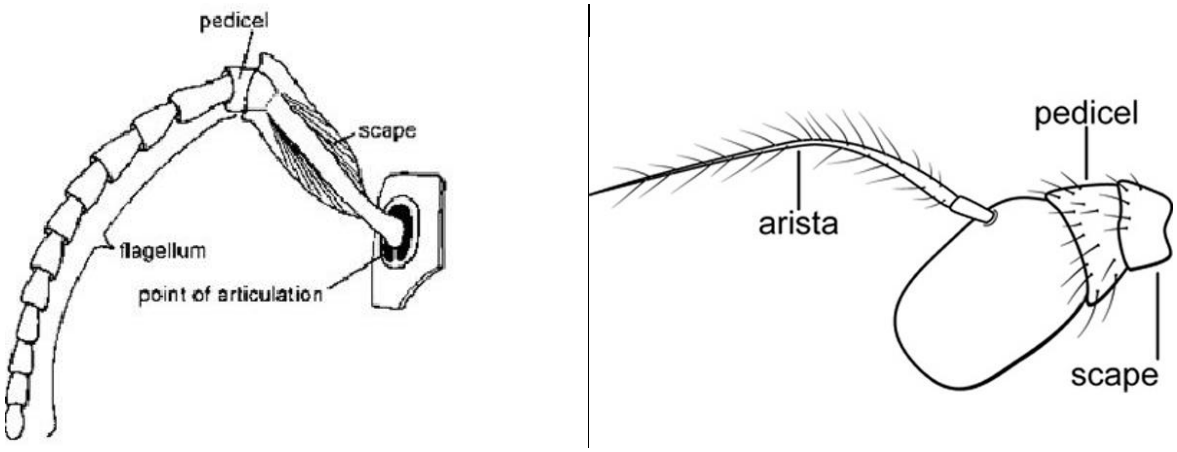


Fig. 1-2. Groundplan of the insect antenna; left: a geniculate antenna, right: an aristate antenna.

Study the structure of the mouthparts (see Fig. 6-12) and of the leg (see Figs 6-13 and 6-14).

TASK 14: Sketch and name the main parts of the mouthparts as well as of the leg, including the tarsus.

Fig. 1-3. Mouthparts of *P. americana*. a) labrum, b) mandible, c) maxilla, d) hypopharynx, e) labium. *a* posterior pivot of the mandible, *Bsk* basal sclerite, *Ca* cardo, *Ga* galea, *Gl* glossa, *I* incisivi, *Lc* lacinia, *M* mola, *Mt* mentum, *Pl* palpiger, *Pgl* paraglossa, *Prmt* praementum, *s* anterior pivot of the mandible, S_1 adductor sinew, S_2 abductor sinew of the mandible, *Smt* submentum, *St* stipes, *Sus* suspensorium.

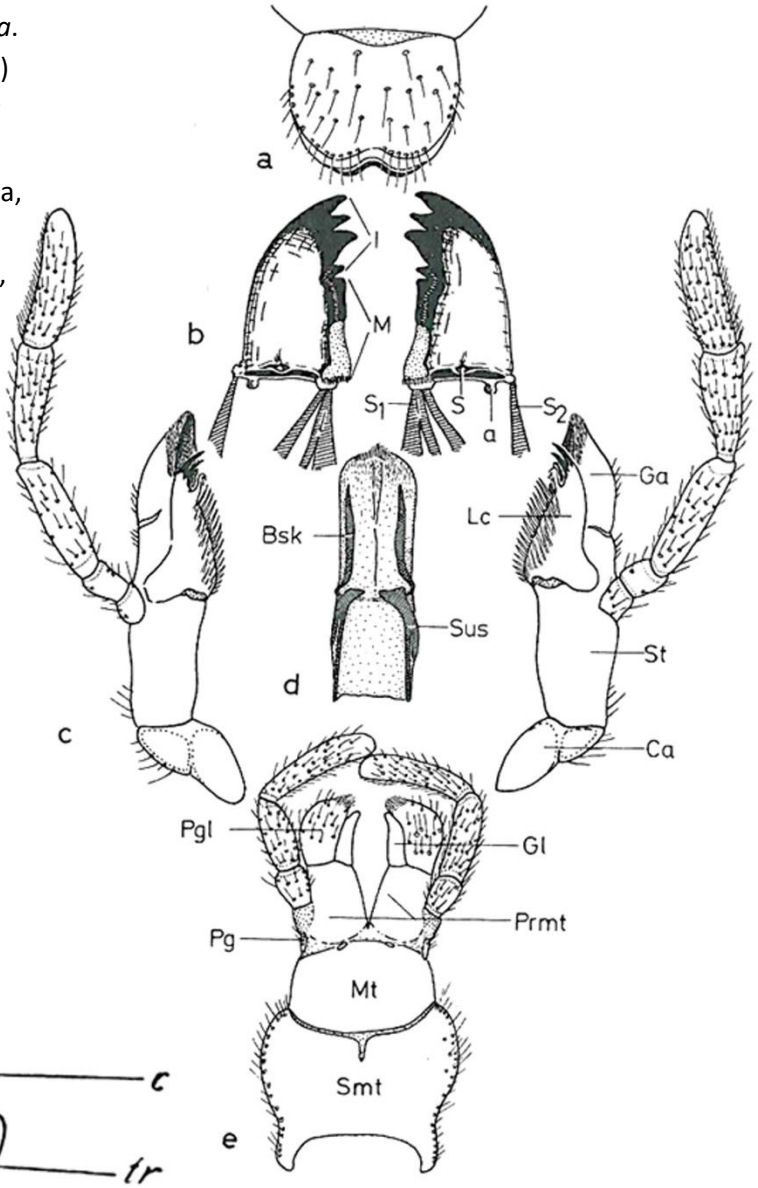
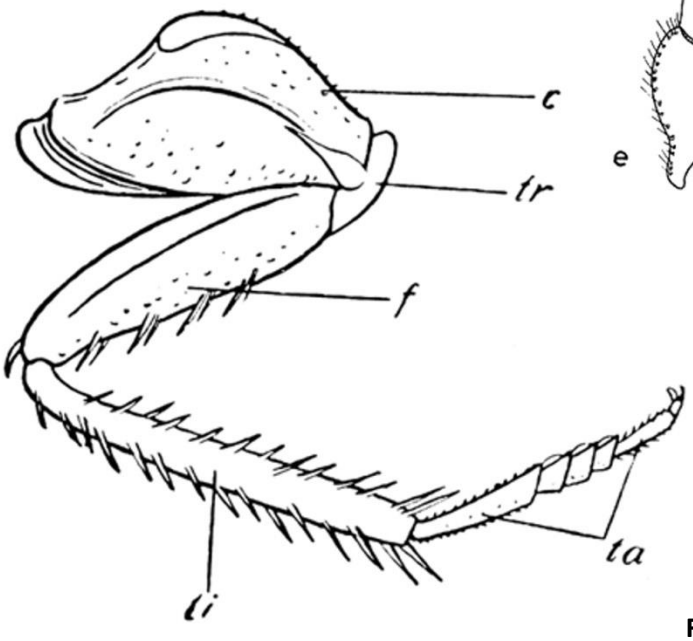


Fig. 1-4. Insect leg. *c* coxa, *f* femur, *ta* tarsus, *ti* tibia, *tr* trochanter.



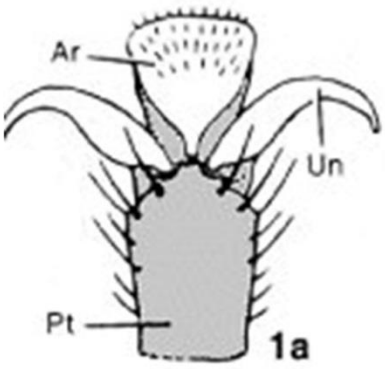


Fig. 1-5. Praetarsus of *P. americana*. *Ar* arolium, *Pt* praetarsus, *Un* unguis (claw).

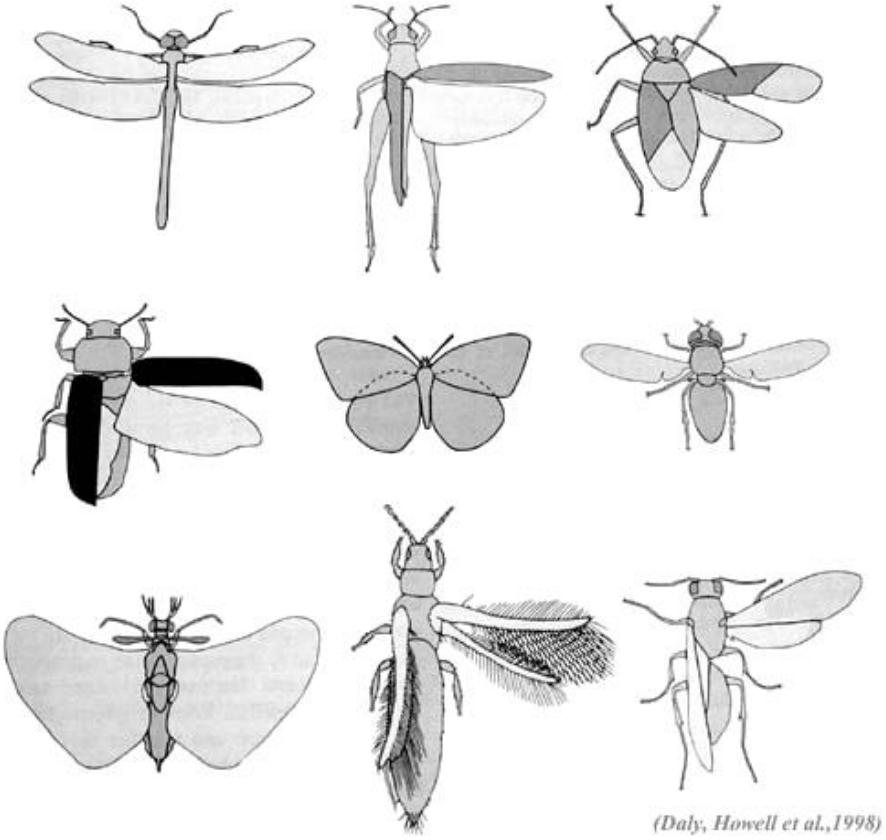


Fig. 1-6. Different kinds of insect wings in various insect orders.

Thursday, 07.09.2017

Lab course 2 – **Identification of field collections to orders; Insect preservation and curation**

Material required for this course. Fine forceps, lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. Today you will learn about the different ways of preserving and curating insects and other arthropods. Also, you will start sorting your collected material to orders or families.

Preservation techniques

Insects can and should be preserved in different ways, although several methods can work for the same insect group. Read **chapter 18** in the textbook carefully.

This lab will demonstrate the following:

- Ethanol preservation (80% EtOH) in vials
- Pinned preservation (larger specimens)
- Pointed preservation (insect glued to a pinning paper tip, for specimens shorter than 1cm)
- Spreading the wings of Lepidoptera
- Preservation of dissected genitalia

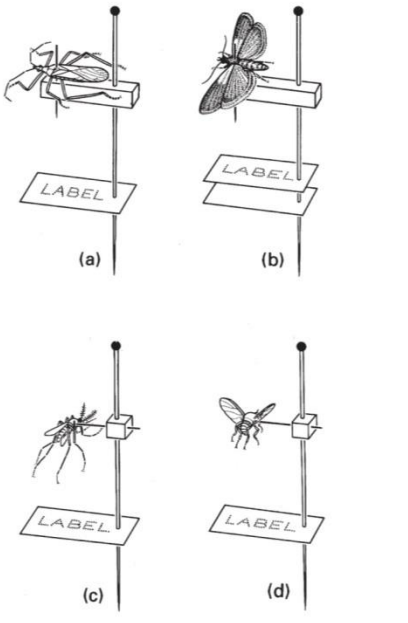


Fig. 18.4 Micropinning with stage and cube mounts: (a) a small bug (Hemiptera) on a stage mount, with position of pin in thorax as shown in Fig. 18.2h; (b) moth (Lepidoptera) on a stage mount, with position of pin in thorax as shown in Fig. 18.2d; (c) mosquito (Diptera: Culicidae) on a cube mount, with thorax impaled laterally; (d) black fly (Diptera: Simuliidae) on a cube mount, with thorax impaled laterally. (After Upton 1991.)

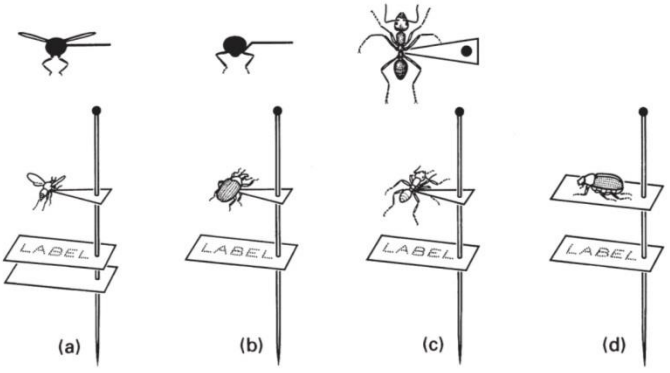


Fig. 18.5 Point mounts: (a) a small wasp; (b) a weevil; (c) an ant. Carding: (d) a beetle glued to a card mount. (After Upton 1991.)

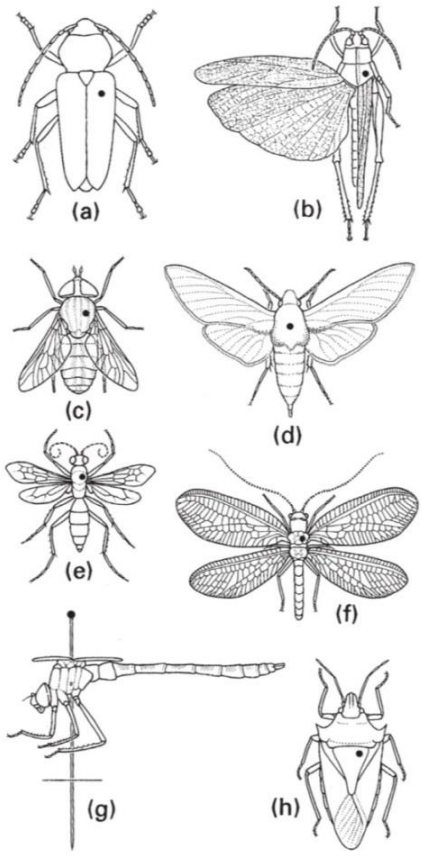


Fig. 18.2 Pin positions for representative insects: (a) larger beetles (Coleoptera); (b) grasshoppers, katydids and crickets (Orthoptera); (c) larger flies (Diptera); (d) moths and butterflies (Lepidoptera); (e) wasps and sawflies (Hymenoptera); (f) lacewings (Neuroptera); (g) dragonflies and damselflies (Odonata), lateral view; (h) bugs, cicadas, leafhoppers and planthoppers (Hemiptera: Heteroptera, Cicadomorpha and Fulgoromorpha).

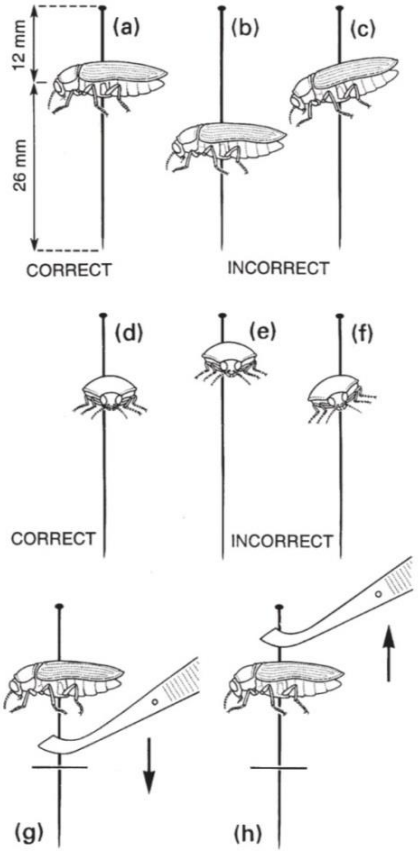


Fig. 18.3 Correct and incorrect pinning: (a) insect in lateral view, correctly positioned; (b) too low on pin; (c) tilted on long axis, instead of horizontal; (d) insect in front view, correctly positioned; (e) too high on pin; (f) body tilted laterally and pin position incorrect. Handling insect specimens with entomological forceps: (g) placing specimen mount into foam or cork; (h) removing mount from foam or cork. ((g,h) After Upton 1991.)

Dry insects are fragile, and appendages of the exoskeleton (legs, antennae etc.) can break off easily. Therefore, if you are going to pin an insect, make sure to use a soft surface to place the specimen upon in order to minimize damage will pushing the pin through the thorax. It will also help you with adjusting the angle of the pin so that it runs vertically through the insect thorax.
Later in the course we will look at slide preparation and how to relax insects like Lepidoptera or Odonata for pinning on a setting board.

For your final collection, please consult the lab staff for the correct preservation- and mounting methods.

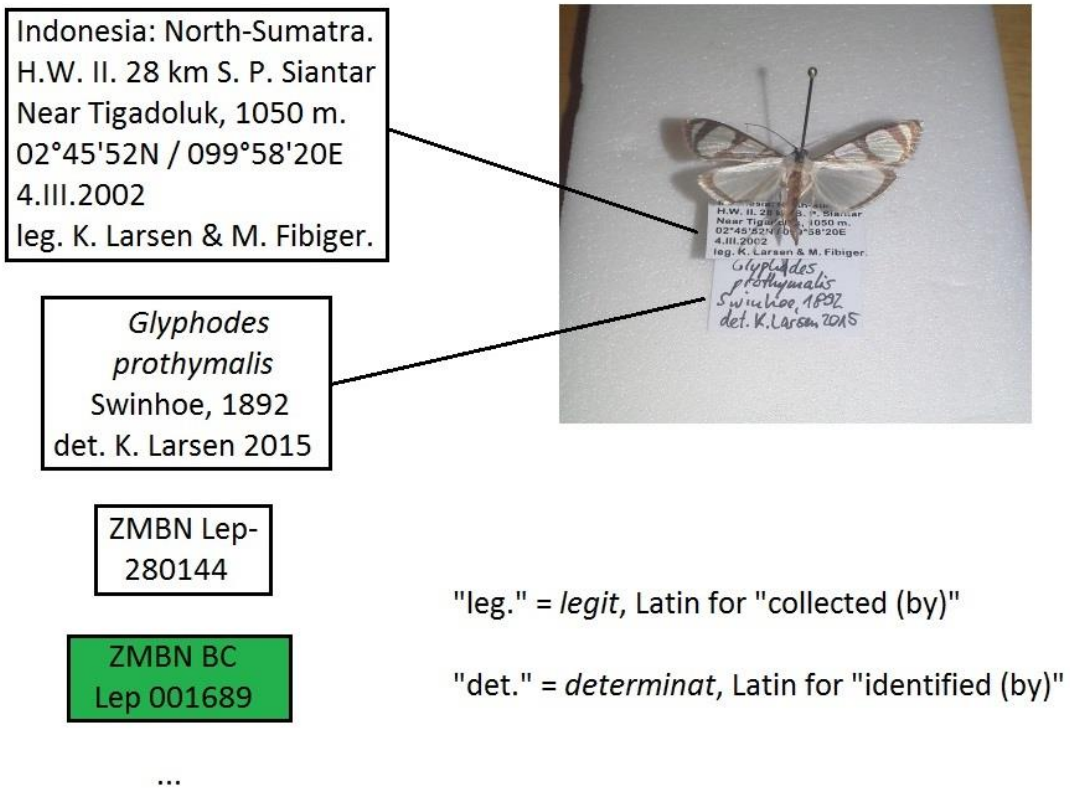
Labelling

Scientific material is labelled in a certain way, and the labels may be organised in the following manner.

The 'origin' label (the uppermost label in pinned specimens) contains the geographical origin where the specimen was collected. The information should be as precise as possible, e.g. containing coordinates and elevation in addition to the name of the location. This label also contains the date of

collecting and the name(s) of the collector(s), indicated by “leg.” or “legit”, which is Latin for “collected (by)”.

If the specimen is identified e.g. to genus or species level, then this information should be added to the specimen on a separate ‘identification’ label. It should also contain the name of the identifier and the year of identification. The collector is indicated by “det.”, which is Latin for “identified (by)”. If the specimen was misidentified, or the species was transferred to a different genus, then this identification label can be easily replaced with one containing the correct name. If needed, additional labels can be added to the specimen, e.g. to indicate the specimen as voucher specimen for DNA extraction or to associate a genitalia dissection number with the specimen. A museum number is always attached to the pinned specimen or the ethanol vial when finally deposited in the collection.



TASK 15: Pin 10 specimens from your collected material.
TASK 16: Glue 5 specimens (true bugs, small beetles) from your collected material.
TASK 17: Label the pinned and glued specimens accordingly.

You may attach a photo of your training session collection.

Sorting of insects and arthropods to order

You will need several lab sessions to sort a sufficient number of insects for further selection of specimens. Please keep everything sorted to order, and **remember to label each new vial.**

Please keep in mind that your collection should comprise **10 orders of arthropods**, primarily insects. In addition, you are required to **specialize on one of these 10 orders** and include additional 10 specimens, **identified to genus or species level** (depending on the difficulty of the group), from that order into your collection.

TASK 18: Document in your lab journal the arthropod orders that you have identified so far, along with their origin and preservation method.

Thursday, 14.09.2017

Lab course 3 – **Identification of field collections to 10 orders; Beginning of taxon specialisation**

Material required for this course. Fine forceps, lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. Today you will continue with identification of your material to orders. You will also pick an order of interest on which you would like to focus in your identification work.

By now you should have a good idea about the diversity of your collected material on the order level. Arthropod orders of which you likely encountered specimens in your collected material are:

- Diptera – flies, mosquitos and midges
- Coleoptera – beetles
- Hymenoptera – wasps, bees, ants
- Lepidoptera – moths and butterflies
- Trichoptera – caddisflies
- Hemiptera – true bugs
- Odonata – dragon- and damselflies
- Dermaptera – earwigs
- Symphypleona – globular springtails (Sminthuridae and similar families)
- Araneae – spiders
- Opiliones – harvestmen
- Isopoda – woodlice
- Myriapoda (subphylum) – millipedes, centipedes and others
- Group Acari, mites – order Oribatida (moss mites)

You should now start to decide which arthropod order you would like to specialise on. In case you choose spiders (Araneae) or harvestmen (Opiliones), keep in mind that in lab course 7 (on 12.10.) a guest researcher (Dr Nils Hein, University of Bonn) will give an introduction to spiders and will provide assistance with these groups; this will give you the opportunity to ask him questions and address difficulties that you came across in the identification process.

TASK 19: Document in your lab journal the final ten arthropod orders that you have identified, along with their origin and preservation method. Write a brief summary (2 sentences) of the key characteristics (apomorphies) for each of the ten orders – this information will be useful for producing the identification key later in the course.

TASK 20: Discuss with the lab staff on what arthropod group you would like to specialise. Once you have chosen a group, explicitly state this group in your lab journal.

Thursday, 21.09.2017

Lab course 4 – **DNA barcoding protocol; Identification to genus and species**

Material required for this course. Fine forceps, lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. Today and in lab courses 5 and 7, you will provide three specimens for DNA barcoding. Furthermore, you will continue identifying and preparing your specimens for your final collection of specimens from 20 different species. You should now have picked your order of interest on which you would like to specialise, and start identifying the specimens of this order to genus and ideally to species.

Introduction to DNA barcoding

In this lab we will familiarise you with the protocol of the so-called DNA barcoding approach. Katrine Kongshavn (University Museum) will give a presentation on the topic.

"DNA barcoding is a taxonomic method, that uses one or more standardized short genetic markers in an organism's DNA to identify it as belonging to a particular species. Through this method unknown DNA samples are identified to registered species based on comparison to a reference library."

(from the Barcode of Life Data Systems homepage, v4.boldsystems.org)

During your identification efforts you will potentially come across specimens that are difficult to identify to species, or with some ambiguity remaining. Such cases might be used for DNA barcoding, where a standardized DNA barcode sequence is amplified, sequenced and eventually compared with the entries of the DNA barcode library on the Barcode of Life Database (BOLD). The DNA barcode sequence is unique for most species that have been investigated. Therefore, there is a high chance of getting a match with sequences from identified specimens of your species.

In order to obtain the DNA barcode sequences for your specimens there are a number of steps necessary:

- Documentation of collection data in a spreadsheet
- Photo of your specimen
- Tissue sample from your specimen, e.g. a leg

The genetic and collection data of your specimen will become part of a global, public database. Your specimen will become a voucher specimen, with an additional label attached to it that assigns a unique DNA barcode number to it. If in the future doubts arise about the identity of your specimen (e.g. it could turn out to belong to an undescribed species), then researchers can easily find it in the collection and re-investigate it.

The BOLD Database will be introduced to you in lab course 10, when we analyse and evaluate the results of the DNA barcoding.

TASK 21: Choose three specimens from your group of focus for DNA barcoding.

Fill the collection- and identification data of your specimens in the DNA barcoding spreadsheet that is available here:
https://www.dropbox.com/sh/p2rkm4qyh5laxax/AABdimbXulgh7fpq0IcpDella?dl=0

Send the filled-out spreadsheet as an email attachment to richard.mally@uib.no along with a good-quality, sharp photo of each of the three specimens. Specimens for DNA barcoding can be brought to the lab staff in lab courses 4, 5 and 7. After that, the DNA barcode plate will be sent to Guelph (Canada) for sequencing.

Identification to genus and species

You should begin to identify the material of your arthropod order of interest. Familiarise yourself with the available identification keys and consult the lab staff if you have questions.

Remember to always label your material so that there is never any doubt from which collecting locality a particular specimen originates.

TASK 22: Document your current stage of identification for the specimens from your group of focus.

Thursday, 28.09.2017

Lab course 5 – **Identification to genus and species; DNA barcoding**

Material required for this course. Fine forceps, lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. Continue to identify the material of your arthropod order of interest. Remember to always label your material so that there is never any doubt from which collecting locality a particular specimen originates.

If you have specimens that you would like to have DNA-barcoded, please approach the lab staff about this.

TASK 23: Identify three specimens to species. These will obtain a museum catalogue number and will be entered into the MUSIT database.

Thursday, 05.10.2017

Lab course 6 – **Anatomy**

Circulatory, neural, and reproductive system in cockroaches respiratory systems with tracheae and spiracles

Material required for this course. Scalpel, scissors, fine forceps, Lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. Today you will dissect a cockroach in order to study different aspects of its anatomy.

General preparation of a cockroach (*Periplaneta americana*):

1. Decapitate the cockroach with your scissors.
2. Cut off all legs and wings close to their basis.
3. Fix the cockroach, with the ventral side facing down, with some dissection pins to the surface of the dissection tray. **Flush with saline (sodium chloride) solution whenever you made an opening in the body.**

Part 1: Circulatory system

4. Dissect the dorsal vessel ("aorta") of the thorax and the dorsal vessel ("heart") of the abdomen.

TASK 24: Count the number of "heart beats" per minute.

5. Open a small window in the central part of the pronotum, and carefully remove it. Now the dorsal vessel should be visible, still beating.
6. Cut the tergites of the abdomen close to its right-hand side edge, starting at the posterior end and continuing to the pronotum. Now, at each of the end points of that longitudinal cut, make a horizontal cut left edge of the tergites (see Fig. 6-1). Now you should be able to flip the abdominal and thoracic tergites open to the left, like opening a book. The dorsal vessel should be visible now; it might still be connected to the inner side of the tergites.

Fig. 6-1. Dorsal view on *Periplaneta*; the white lines on the body indicate the cuts described in step 6 of the dissection.

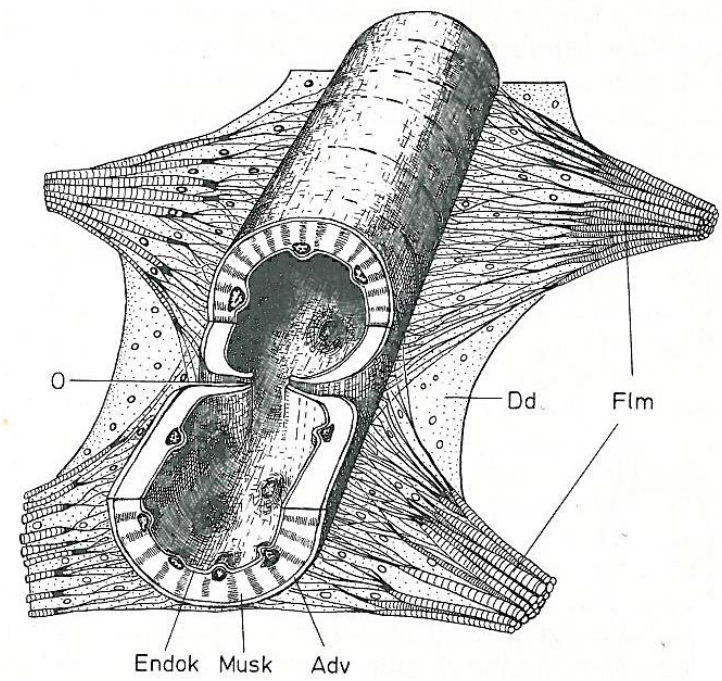


Fig. 6-2. Block diagramme of a partially opened section of the heart with the dorsal diaphragm. After Weber, with major alterations. *Adv* adventitia, *Dd* dorsal diaphragm, *Endok* endocard, *Flm* flight musle, *Musk* Muscularis, *O* ostium.

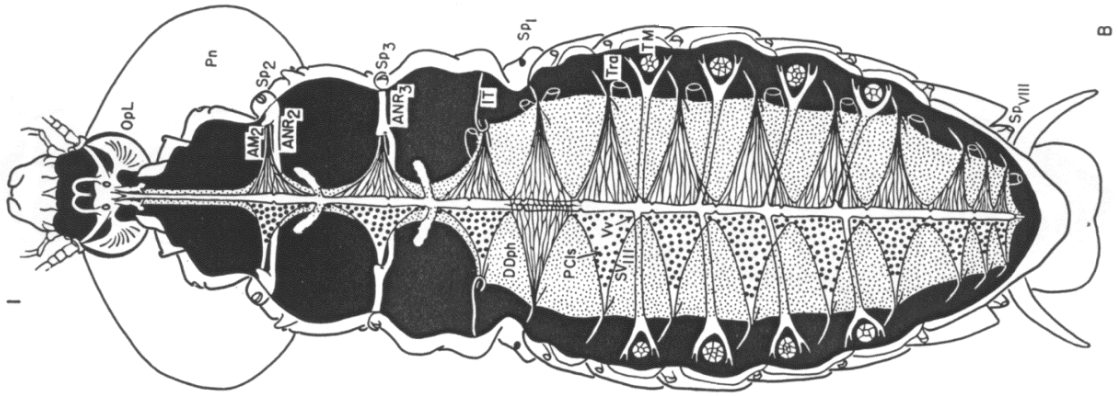


Fig. 6-3. Dorsal view on *P. americana*, with the tergites removed.

Part 2: Digestive and excretory systems

7. Dissect the gut system of the cockroach. **TASK 9:** Sketch and name the different parts.
8. Open the proventriculus. **TASK 10:** Describe its structure in your own words and make a sketch.

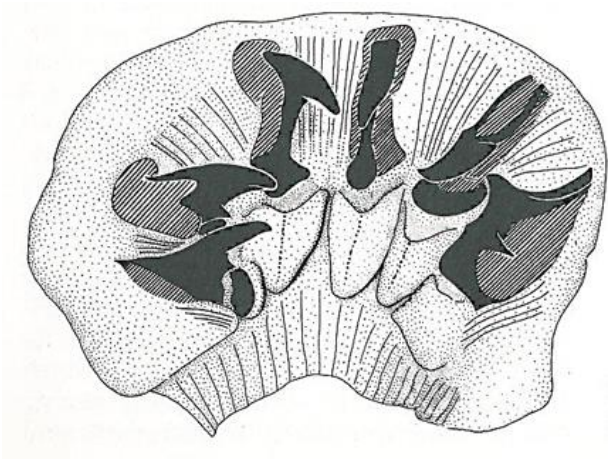


Fig. 6.4. Internal view of the opened-up proventriculus of *P. americana* with the six cuticula teeth.

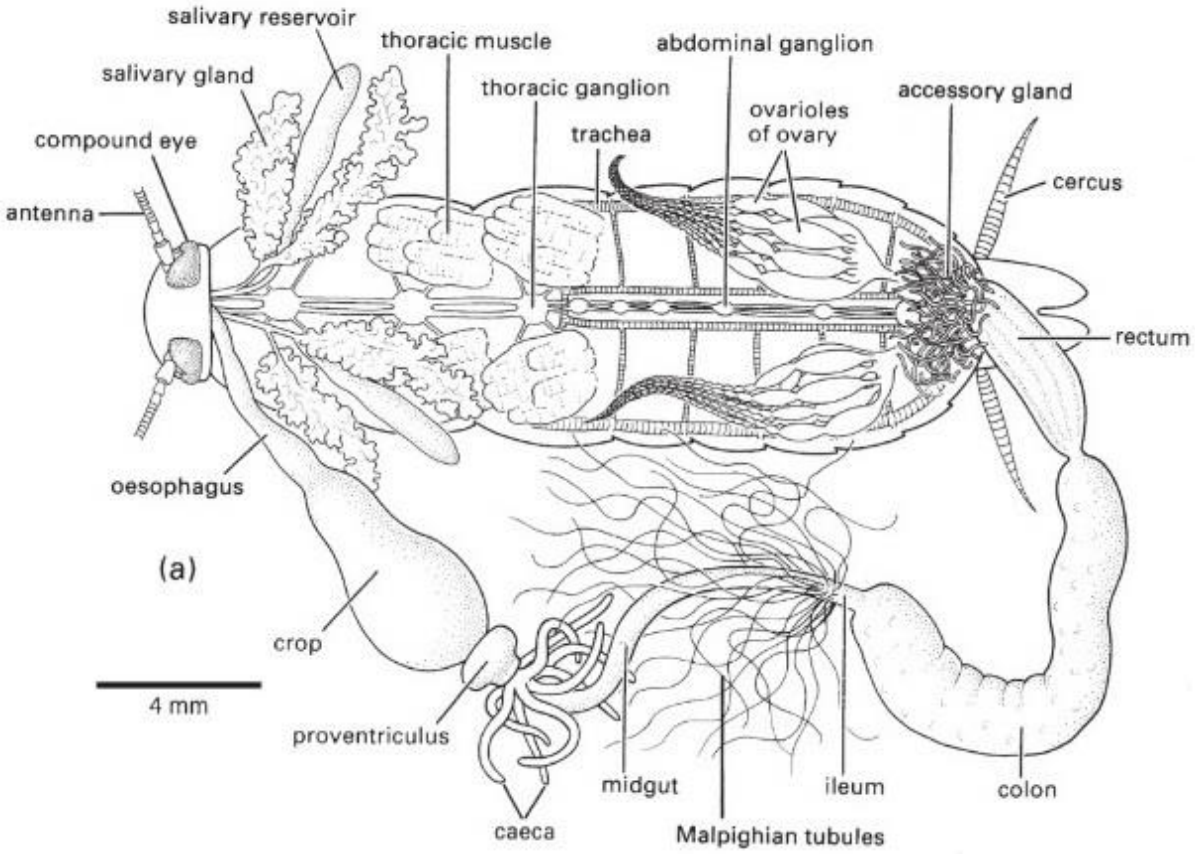


Fig. 6-5. Dissection of a female *P. americana*, with the fat body and most of the trachaea removed; most details of the nervous system are not shown.

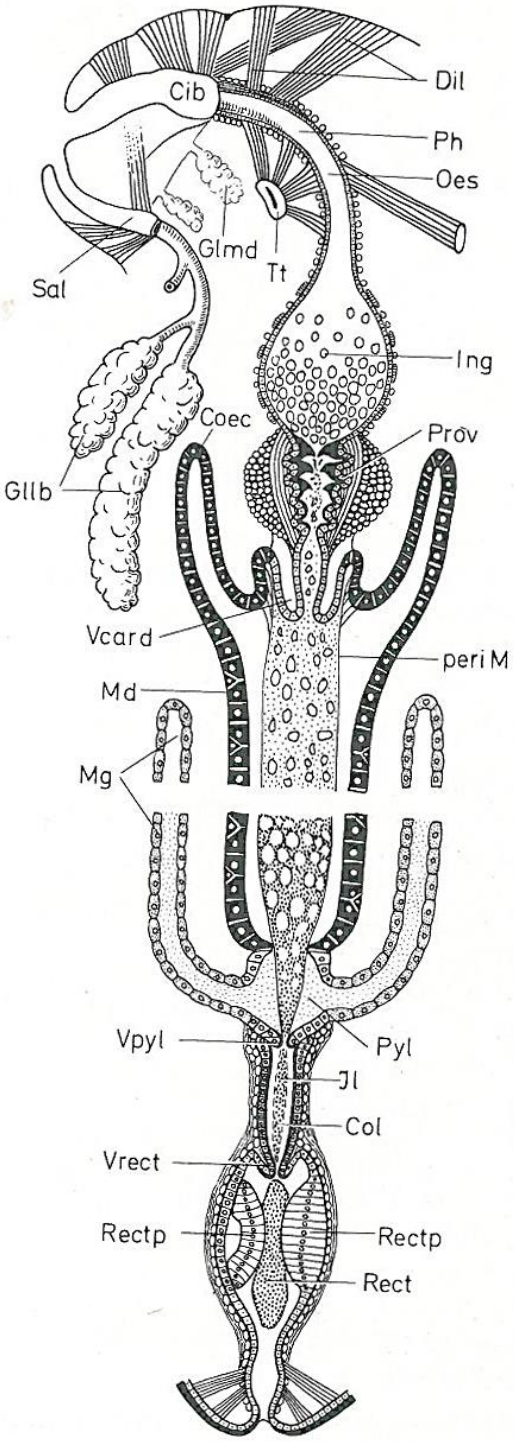


Fig. 6-6. Bauplan of the insect intestinal tract. After Weber, modified. *Cib* cibarium, *Coec* coecum, *Col* colon, *Dil* dilatator, *Gllb* labial gland, *Glmd* mandibular gland, *Il* ileum, *Ing* ingluvies, *Md* mid gut, *Mg* Malpighi tubule, *Oes* oesophagus, *periM* peritrophic membrane, *Ph* pharynx, *Prov* proventriculus, *Pyl* pylorus, *Rect* rectum, *Rectp* rectal papilla, *Sal* salivarium, *Tt* tentorium, *Vcard* valvula cardiac, *Vpyl* valvula pylorica, *Vrect* valvula rectalis.

Part 3: Central nervous system

9. Dissect the ventral nervous system both of the abdomen and the thorax.

TASK 25: Sketch the terminal ganglion, one abdominal ganglion and one thoracal ganglion. Note different sizes of these ganglions, of the descending nerves and the length of connectives.

TASK 26: Why are both terminal ganglion and thoracal ganglions larger than the abdominal ganglions?

10. Fix the head with the antennae facing upwards by piercing a pin into the mouthpart. Open the head capsule with a pair of scissors by cutting a line on the back of the head from one eye to the other. Raise the dorsal head capsule and clean the preparation with saline solution. The brain should now be visible. Try to find the paired corpora cardiaca (CC) and corpora allata (CA).

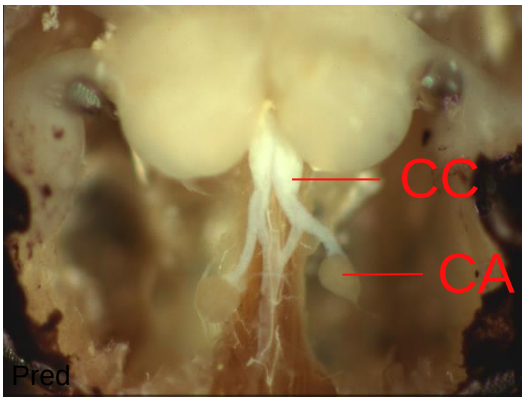


Fig. 6-7. The retrocerebral complex. *CA* corpus allatum, *CC* corpus cardiacum.

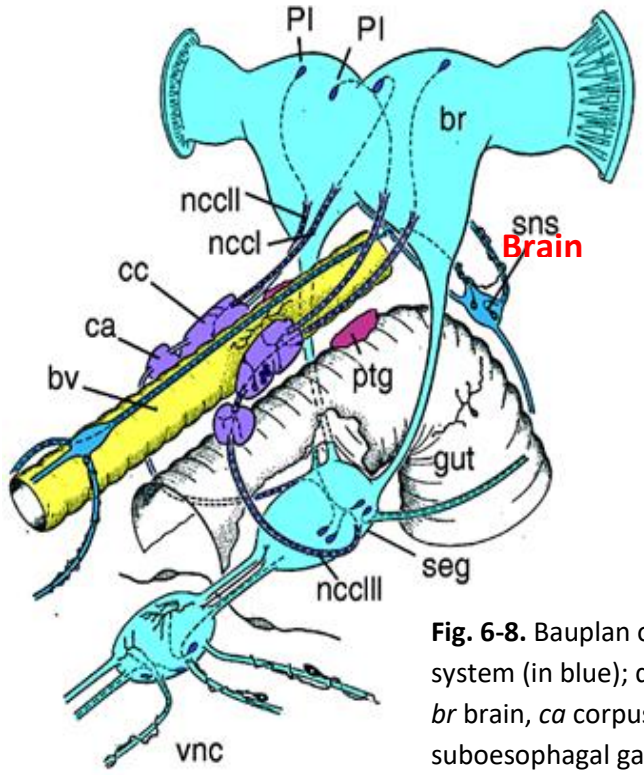


Fig. 6-8. Bauplan of the brain and stomatogastric nervous system (in blue); digestive tract in white, aorta (*bv*) in yellow. *br* brain, *ca* corpus allatum, *cc* corpus cardiacum, *seg* suboesophagal ganglion.

11. Remove the brain and if possible the suboesophageal ganglion (*seg* in Fig. 6-8) and transfer both in a separate dissection dish with saline solution. Dissect the brain and try to find the proto-, deuto-, and tritocerebrum (see Fig. 6-9).

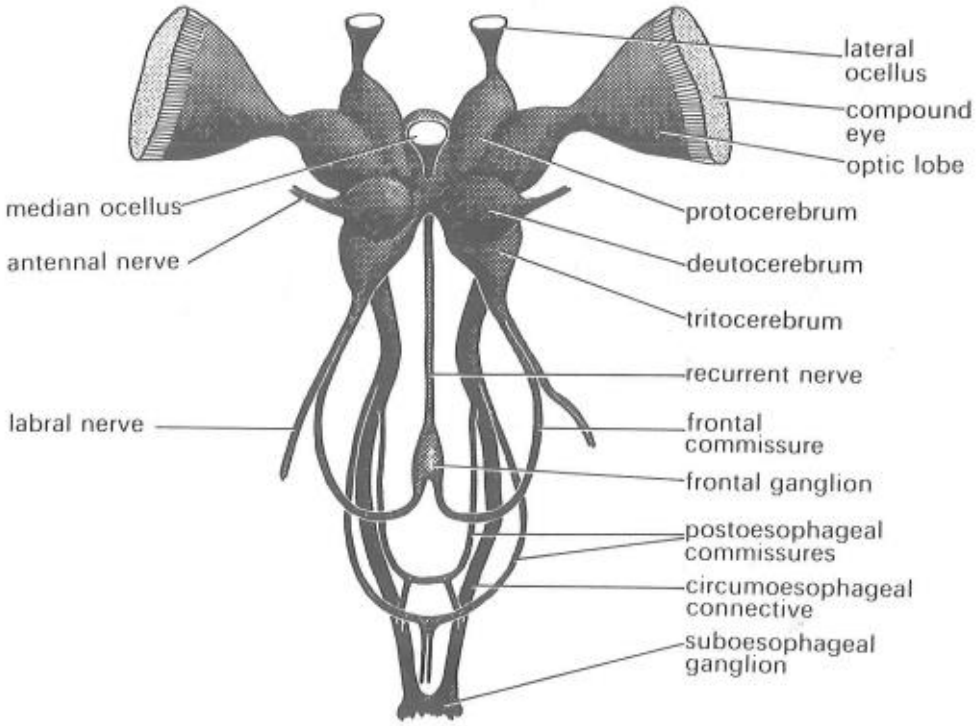


Fig. 6-9. Anterior view of the brain and stomatogastric nervous system of *Locusta* (Orthoptera).

Part 4: Genital system

12. Dissect the genital system of a male (Fig. 6-11) or female (Fig. 6-10) cockroach.

TASK 27: Sketch and name the main parts. If time, sketch and name the genital part of the other sex as well.

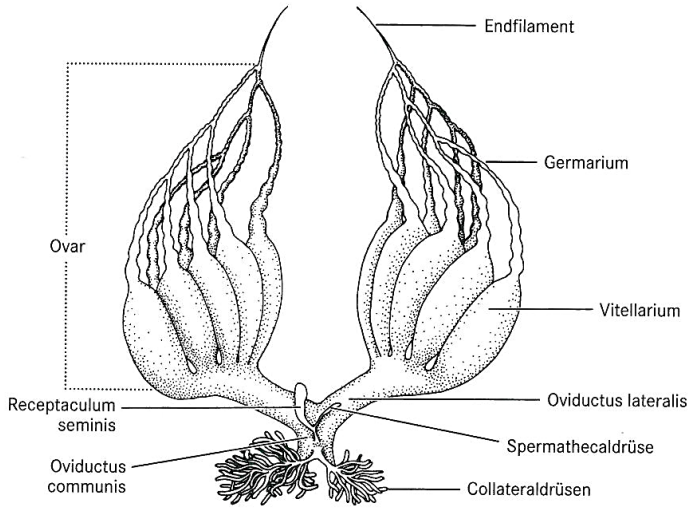


Fig. 6-10. Female genital system.

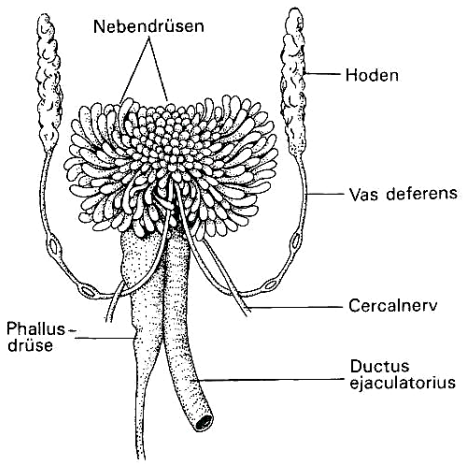


Fig. 6-11. Male genital system.

Thursday, 12.10.2017

Lab course 7 – **Introduction to spiders; Identification to genus and species; DNA barcoding**

Material required for this course. Fine forceps, Lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. An introductory presentation to spiders is given by Dr. Nils Hein (Univ. Bonn). You continue with identification of your material to genus and species. Today is the last opportunity to provide specimens for DNA barcoding, as the barcoding plate containing the tissue samples will be sent to the DNA Barcoding facility in Guelph in Canada, which takes some time. The DNA barcoding results will be evaluated in lab course 10.

TASK 28: Find and define diagnostic features for the following spider families:
Salticidae (jumping spiders)
Thomisidae
Lycosidae (wolf spiders)
Araneidae (orb-weaver spiders)
Linyphiidae

Distinguish males from females, and make a simple sketch of the sex-specific characters for each sex.

Thursday, 02.11.2017

Lab course 8 – **Adaptations to feeding**

Digestive systems in fungus farmers, incl. proventriculus
Specialized mouthparts in bees, mosquito, bed bug, butterfly

Material required for this course. Scalpel, scissors, fine forceps, Lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. Today you will study slide preparations of insect mouthparts. You will observe the feeding of bed bugs with blood. Furthermore, you will dissect a bark beetle and an ambrosia beetle in order to study their mouthparts and their proventriculus. These have been macerated in KOH over night to remove fat and proteins.

Part 1: Insect mouthparts

Use the microscope (not the stereoscope) to study the provided slide preparations of insect mouthparts.

TASK 29: Sketch the mouthparts and name the visible structures. Indicate for each studied insect the type of foraging that the mouthparts are adapted for.

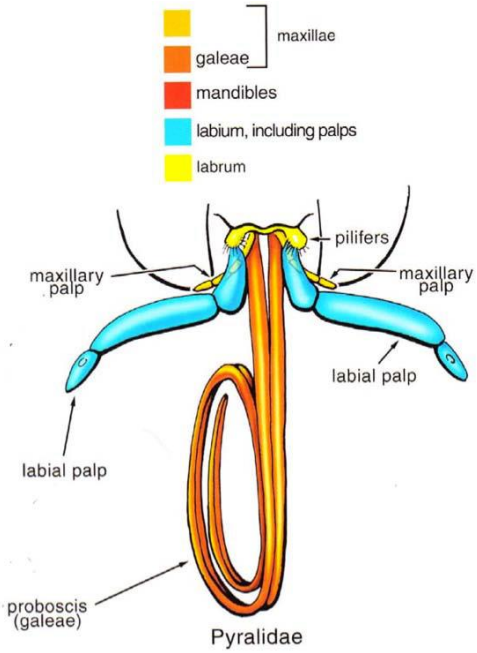


Fig. 8-1. Schematic structure of the mouthparts of Lepidoptera.

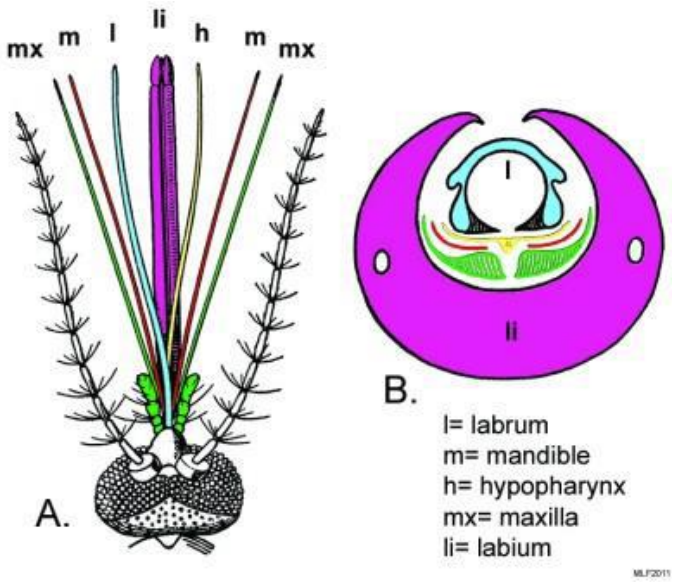


Fig. 8-2. Schematic structure of the mouthparts of a mosquito (Diptera). A dorsal view, B cross-section.

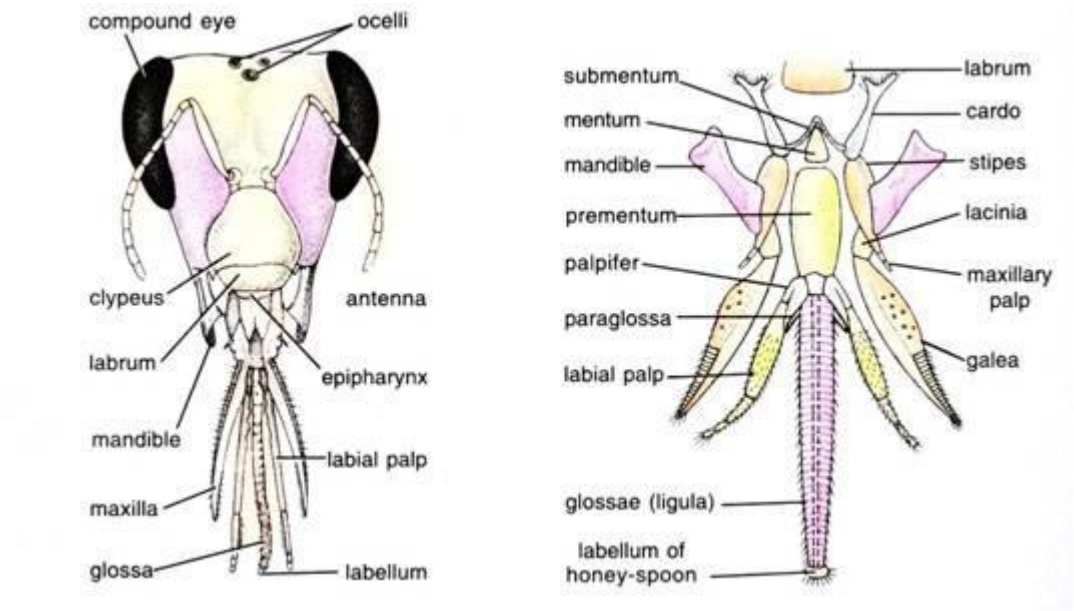


Fig. 8-3. Head (left) and mouthparts in detail (right) of the honey bee, frontal view.

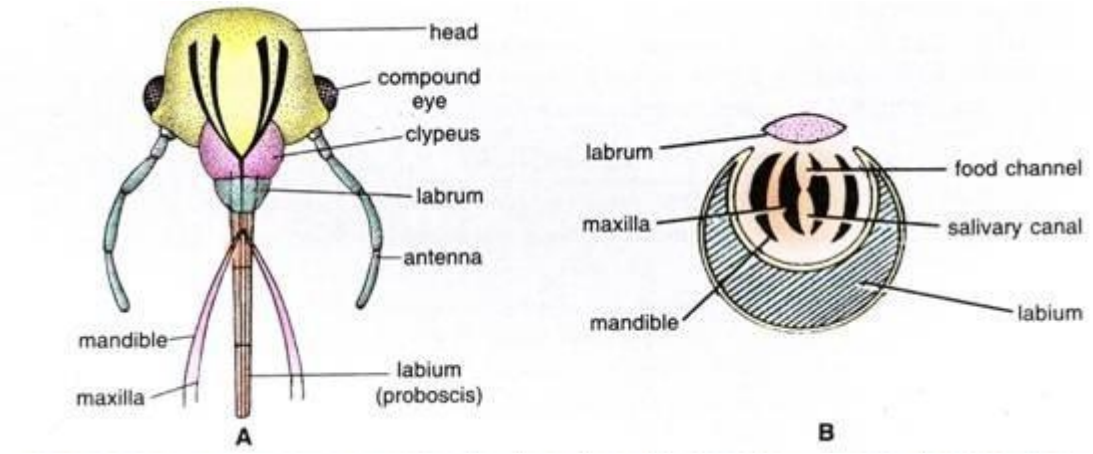


Fig. 8-4. Head (left) and cross-section through mouthparts (right) of the bed bug *Cimex*.

Live bedbugs will be fed on blood during this lab. These insects are shy and dislike light, but try to observe the bite and length of feeding.

Part 2: Mouthparts and proventriculus of bark and ambrosia beetles

In this section, you will dissect one bark beetle and one ambrosia beetle to observe the following:

a) Mouthparts
b) Proventriculus

The beetles have been macerated in 8% KOH, washed in water and placed in Ethanol ready for dissection (this procedure takes 2-3 hours in 90 degrees KOH). Use the stereoscope and dissecting equipment, i.e. fine forceps and dissecting pins.

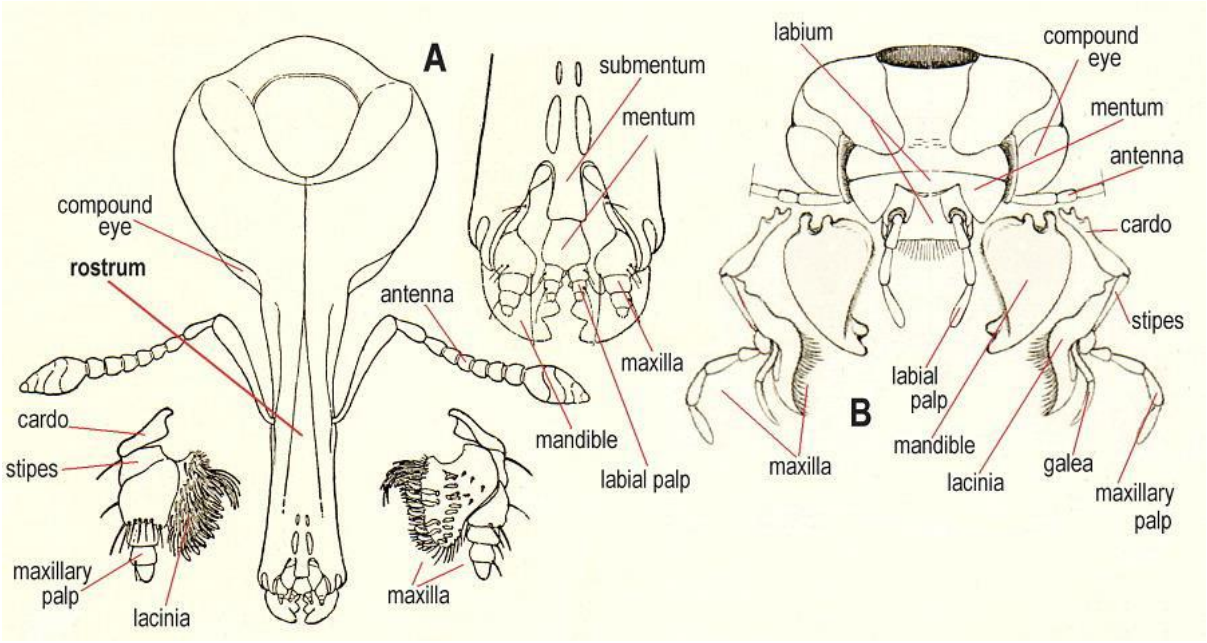


Fig. 8-5. Insect mouthparts of beetles. Left: ventral view on the head of a weevil, *Pissodes strobi*; Centre: ventral view on weevil mouth; Right: ventral view on the head of the great diving beetle, *Dytiscus marginalis*.

Bark beetles: *Ips* typographus.

1. Place the bark beetle in a dissecting dish (embryo dish) with ethanol.

TASK 30: Observe the mouthparts in dorsal and ventral view. Which type of mouthparts do they have?

2. Open the mandibles. Force the dissecting needle at the base of the labium (submentum area) and pull forward. Stop when the mouthparts are spreading.

TASK 31: Make a sketch of the mandible, maxillae and labium. Note in particular the thickness and placement of the lacinial setae.

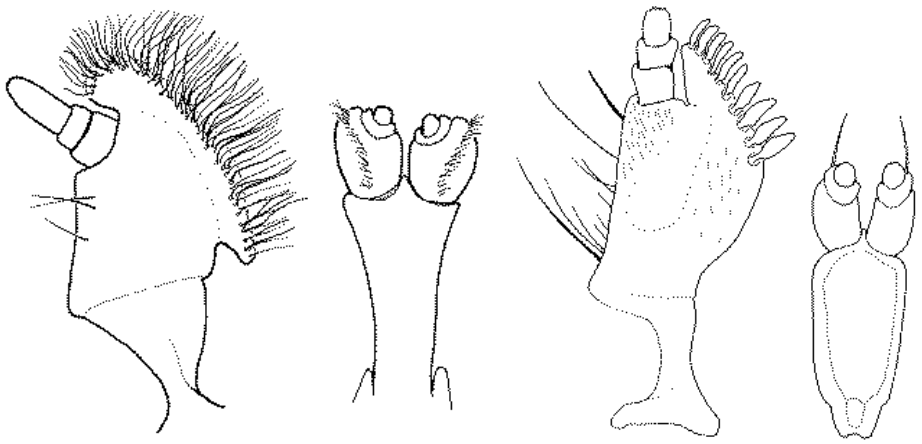


Fig. 8-6. Inner (dorsal) view of the left maxilla and labium of the ambrosia beetle genus *Xyleborus* (left) and the bark beetle genus *Dolurgocleptes* (right).

3. Detach the pronotum from the mesonotum. Now the proventriculus is visible as a barrel-shaped structure inside the pronotum. Pull the proventriculus out, stick a dissecting pin through its centre and break it open. Use the forceps to fix the proventriculus, and wash the interior gently as there are usually food particles inside.

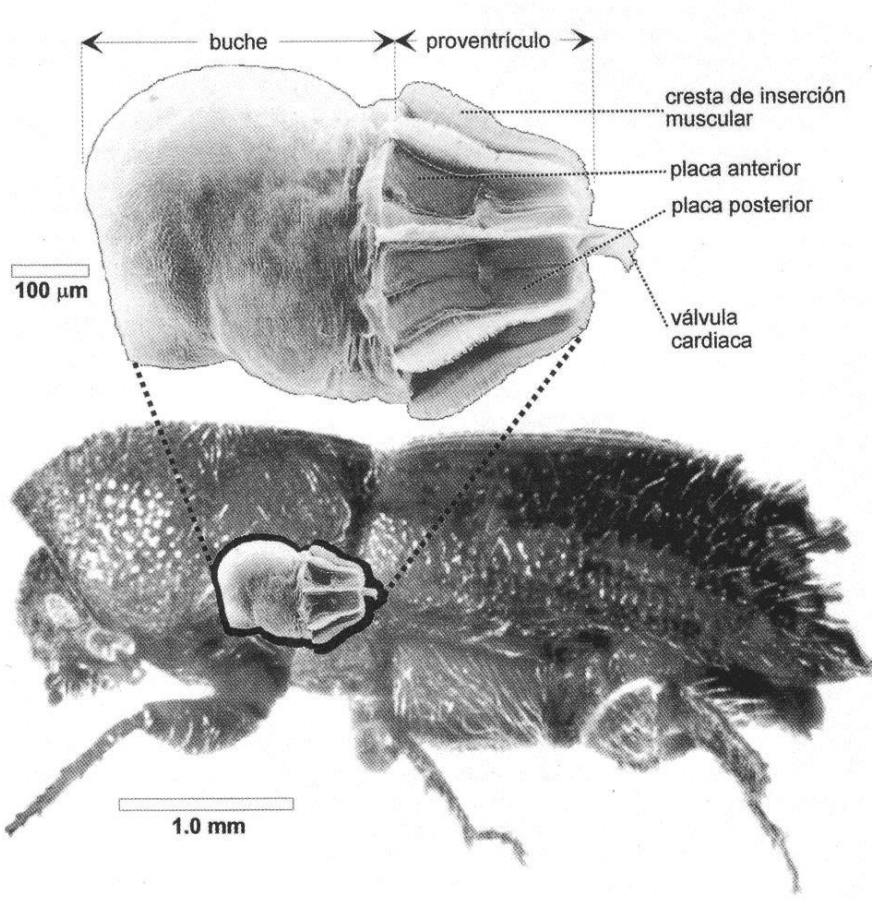


Fig. 8-7. Position and structure of the proventriculus in *Ips integer*.

4. Unfold the proventriculus, observe the structures on its inner face. Make a sketch, name structures: apical plate (placa anterior), median suture (sutura media), sutural teeth (dientes suturales), mastigatory brush (dientes mastigatores).

Ambrosia beetles: *Trypodendron lineatum*

Repeat the dissection procedure for the ambrosia beetle as described for the bark beetle above.

TASK 32: Point out the differences between bark- and ambrosia beetles in the mouthparts and the proventriculus. What is the adaptive advantage of these differences?

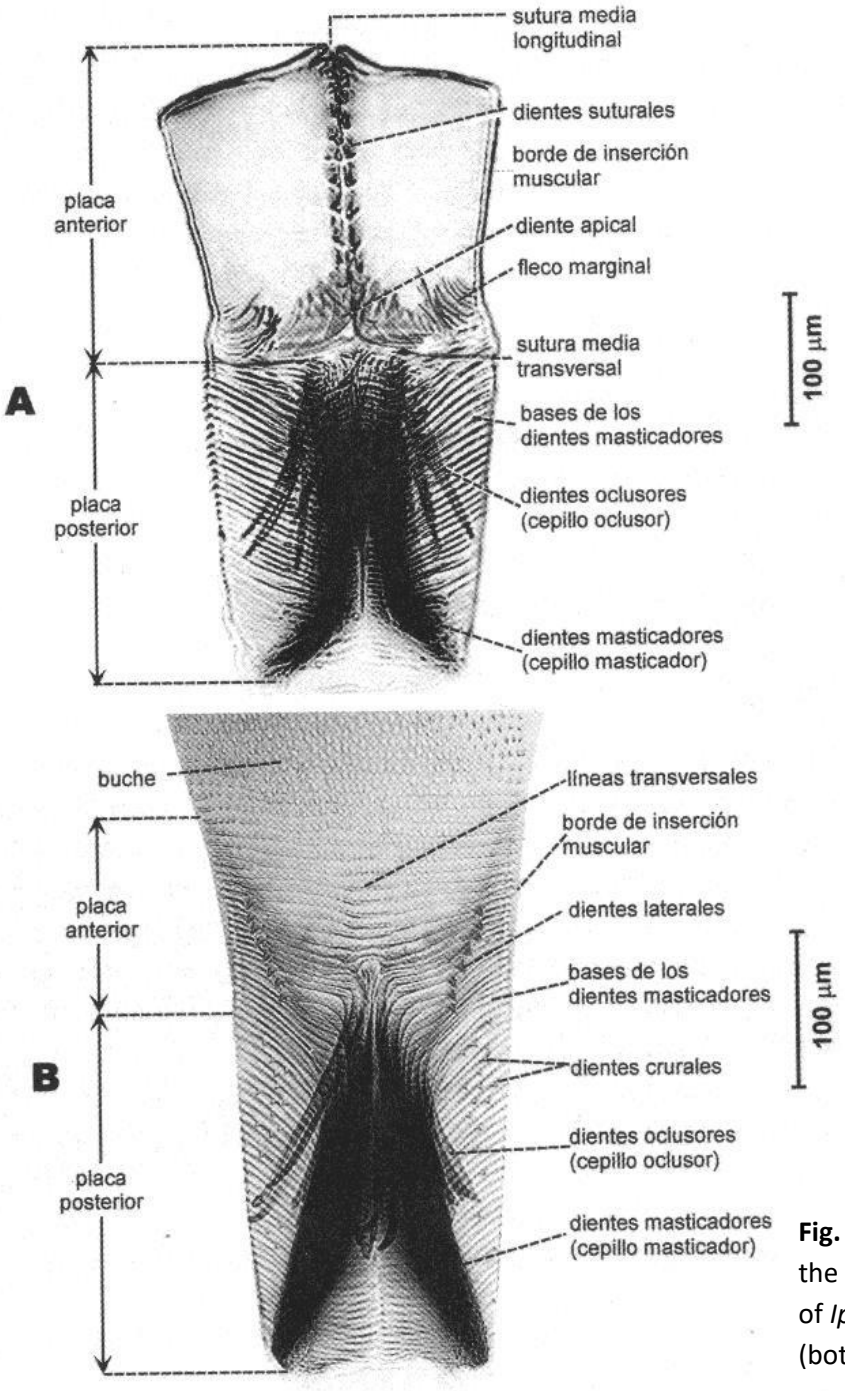


Fig. 8-8. Inner aspect of one of the eight proventricular blades of *Ips* sp. (top) and *Hylurgops* sp. (bottom).

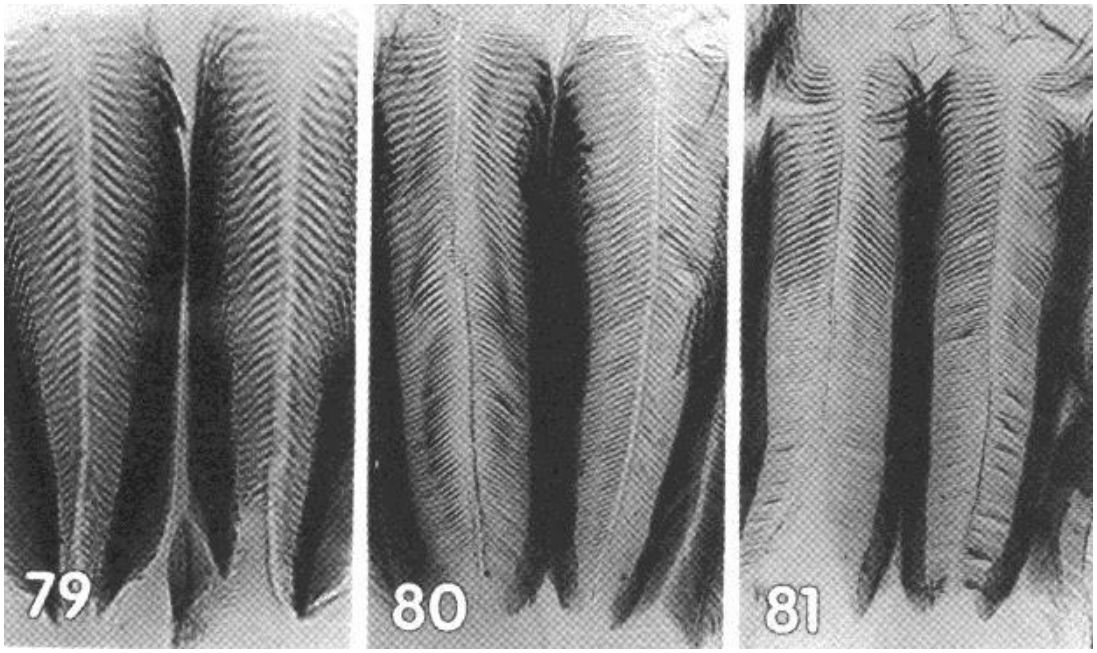


Fig. 8-9. Inner side of the proventriculus in ambrosia beetles of the genus *Corthylus*. Note the reduced apical plate.

Thursday, 09.11.2017

Lab course 9 – **Reproductive systems (male and female genitalia); preparation of an identification key**

Material required for this course. Fine forceps, Lab journal, pen, pencil, pencil sharpener and eraser for drawing

Summary. In the first part you will dissect the male genitalia of a Noctuidae moth in order to identify it based on genitalia features. In the second part, details on how to produce an identification key for your selection of specimens are given.

Part 1: Genitalia dissection

1. Detach the abdomen from the thorax of the adult specimen. (DONE)
2. Macerate the abdomen in warm 10% potassium hydroxide (KOH) for about 1 hour in order to dissolve the internal organs. Only the cuticular structures will remain. (DONE)
3. Transfer the macerated abdomen into water and clear it of the scales covering the abdomen's outer surface using a feather (on a stick). Try to carefully squeeze out the macerated remains from the inside of the abdomen.
4. Cut the abdomen open along the pleural membrane on one of the sides of the abdomen, starting from the anterior opening where the abdomen was attached to the thorax. End the cut at segment VII.
5. Take two fine forceps and carefully tear the pleural membrane of segment VIII apart, following your cut from step 4. After that, disconnect the genitalia from the segment VIII by tearing apart the vertical, intersegmental membrane on tergite and sternite VIII.
6. Transfer both the opened abdomen and the genitalia to 70% ethanol and continue cleaning it from remaining scales and intestines if necessary. The diluted ethanol will remove part of the water in the cuticle, which hardens the cuticle but also makes it more fragile. Carefully remove the phallus from the genital. Spread the valves of the genital to the sides so that the genital can be mounted flat on a microscope slide.
7. Transfer the abdomen to absolute ethanol and spread it out flat. The remaining water in the cuticle is now replaced with ethanol, making it even stiffer. Repeat this for the phallus and the genital. Make sure the valvae of the genital are properly spread by carefully pushing the genital flat for 20 to 30 seconds until it is hardened.

8. Clean a microscope slide and two cover glasses. Make sure the cover glasses are large enough to cover the spread abdomen and genital. Place a drop of Euparal on each the left and the central third of the slide. (The slide label will be placed on the right third.)
9. Transfer the abdomen with forceps from the absolute ethanol to the left drop of Euparal on your slide, and make sure to transfer as little ethanol as possible with it. (Absolute ethanol mixes very well with Euparal and would cause your viscous drop of Euparal to spread all over your slide.) Fully spread the abdomen in the Euparal and cover it with a cover glass. If necessary, add more Euparal from one side of the cover glass and let it be sucked under it.
10. Repeat step 9 for the genital and the phallus using the right drop of Euparal on your slide. Place the genital with the opened valvae facing to you, and the phallus below the genital.
11. Place a piece of adhesive label on the right third of the slide and annotate the species name, the specimen's origin, your name and your dissection number.
12. Let the slide dry for about two months. Avoid a dusty environment, and refill air bubbles under the cover glasses with Euparal (see step 9) if necessary.

Fig. 9-1. Genitalia slide with abdomen and male genitalia under the large cover slip, and the phallus under the small cover slip.

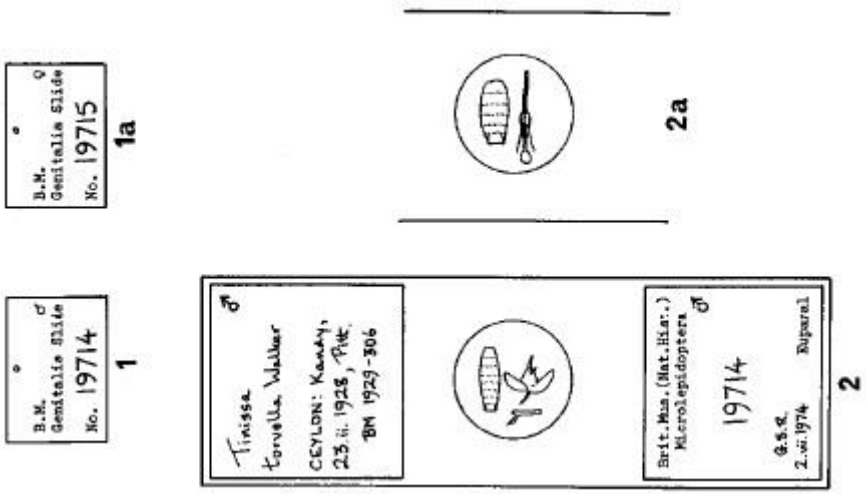


Fig. 9-2. Labelling and slide arrangement used for Microlepidoptera in the British Museum of Natural History, London.

TASK 33: Examine the abdomen. Do you find specialised structures on the segment plates or on the membranes connecting them? If so, draw a sketch of those structures, indicate the segment number and give a short description on what you can see.

TASK 34: Sketch the dissected genitalia and name the different sclerotised parts.

TASK 35: Try to identify the species by comparing the dissected genitalia to Figures 9-3 to 9-15. Take notes in your lab journal.

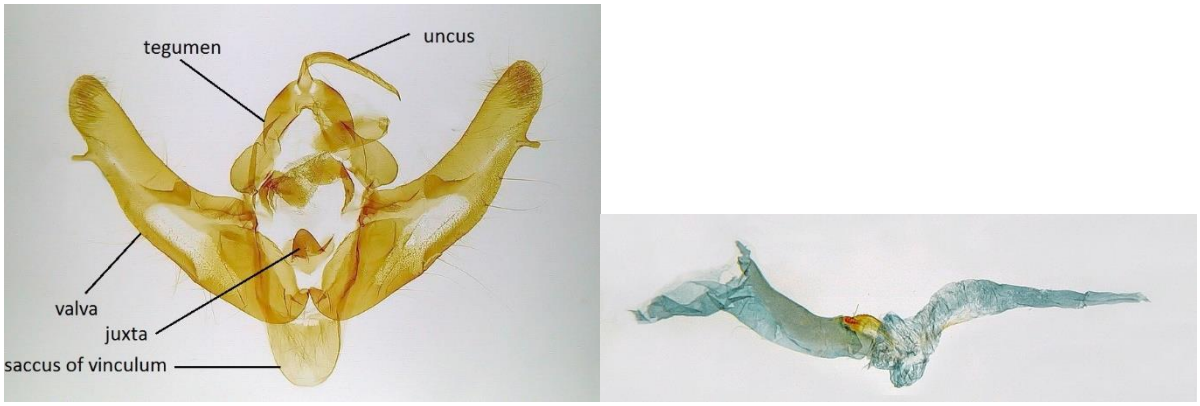


Fig. 9-3. Male genital (left) and phallus (right) of *Xestia triangulum*.

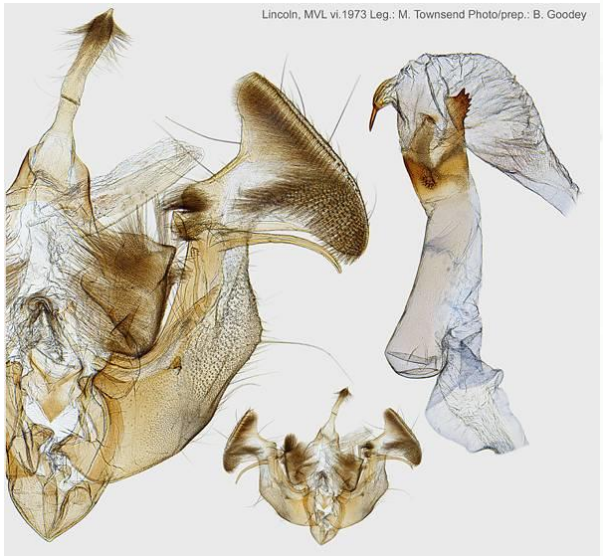


Fig. 9-4. Male genitalia of *Apamea remissa*.

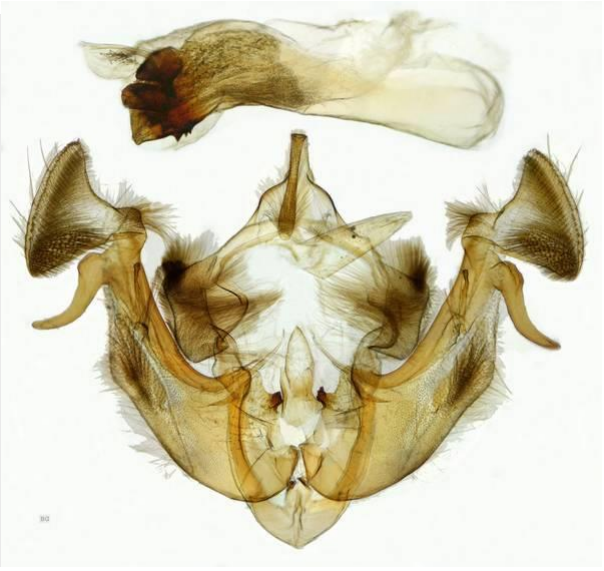


Fig. 9-5. Male genitalia of *Apamea sordens*.

Fig. 9-6. Male genitalia of *Peridroma saucia*.

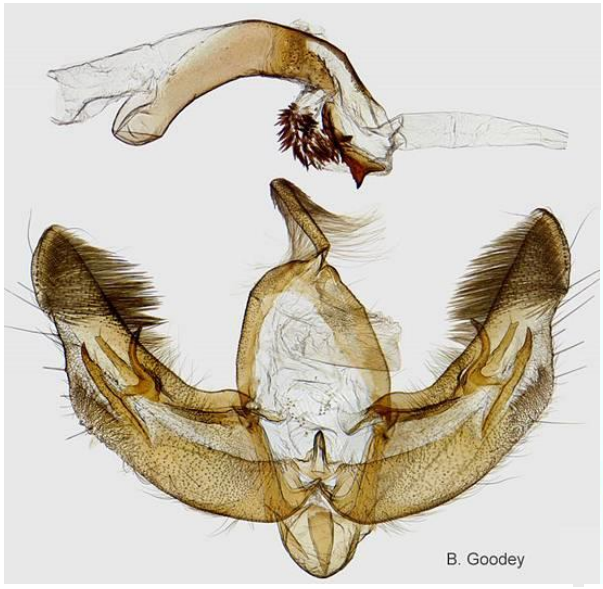


Fig. 9-7. Male genitalia of *Diarsia mendica*.

Fig. 9-8. Male genitalia of *Xestia ditrapezium*.

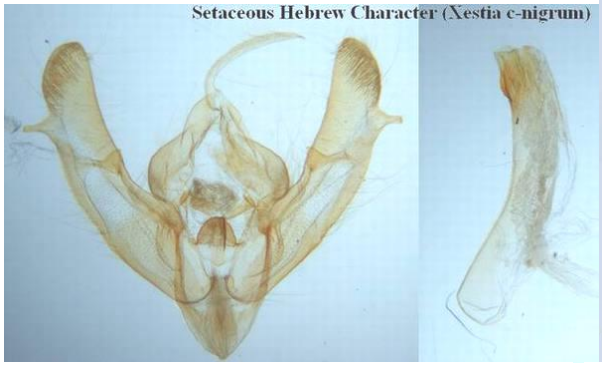


Fig. 9-9. Male genitalia of *Xestia c-nigrum*.

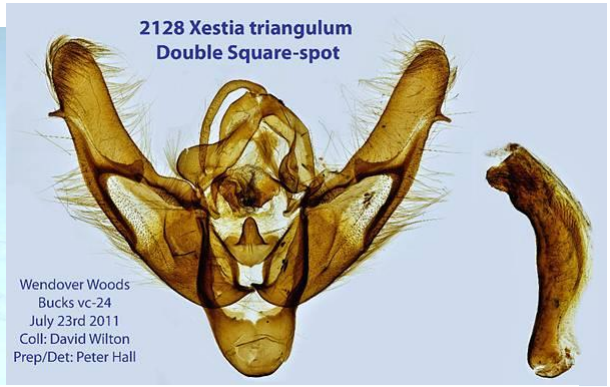


Fig. 9-10. Male genitalia of *Xestia triangulum*.

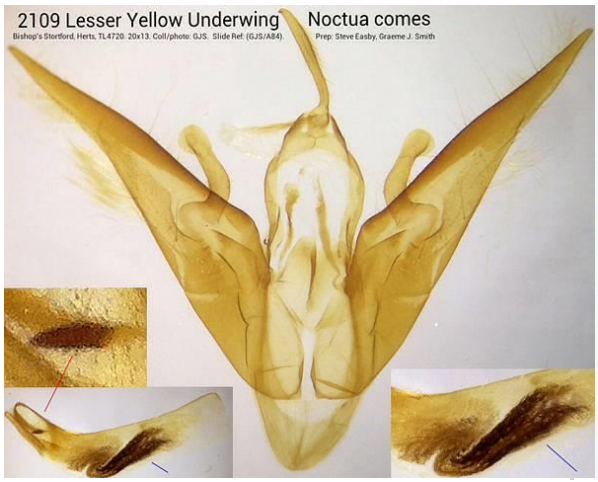


Fig. 9-11. Male genitalia of *Noctua comes*.

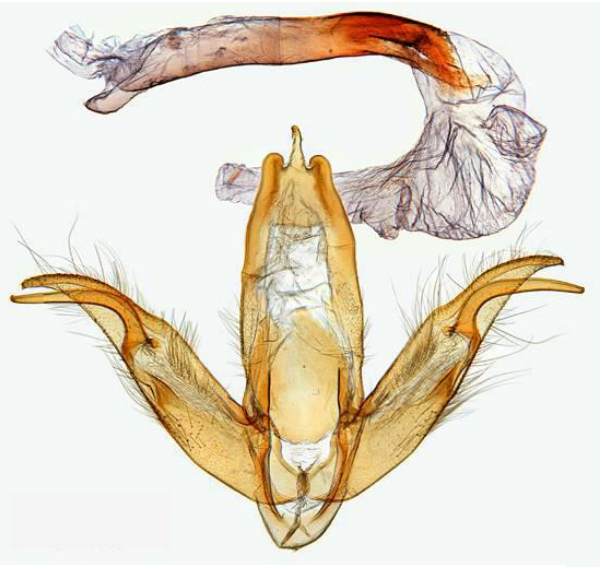


Fig. 9-12. Male genitalia of *Noctua fimbriata*.

Fig. 9-13. Male genitalia of *Lacanobia oleracea*.

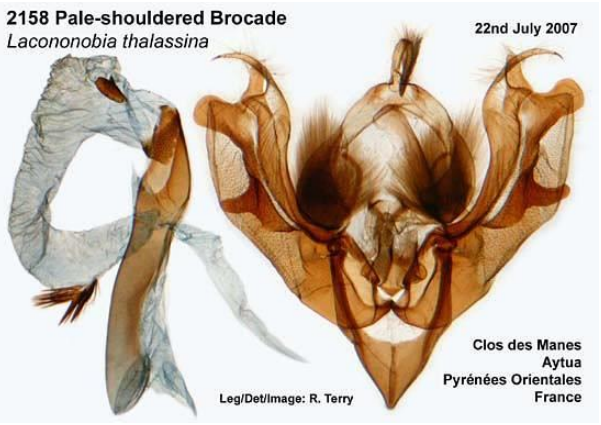


Fig. 9-14. Male genitalia of *Lacanobia thalassina*.

Fig. 9-13. Male genitalia of *Lacanobia w-latinum*.

Part 2: preparation of an identification key

Over the past lab course sessions, you intensely worked with identification keys so that by now you should be familiar with their structure. Here we will provide you with assistance and advice for producing the mandatory identification key for the 20 specimens of your final collection.

TASK 36: Begin drafting your key for 20 specimens (to be finalised later)

Thursday, 16.11.2017

Lab course 10 – **databases; evaluation of DNA barcoding results**

Material required for this course. Fine forceps, Lab journal, pen. If possible, please bring a **laptop** for the database work.

Summary. In the first part we introduce you to different online databases which contain useful information on the distribution of species in Norway and in a global scale. In the second part the results of the DNA barcoding are evaluated.

Part 1: Databases – Artsdatabanken, Global Biodiversity Information Facility (GBIF), Barcode of Life Database (BOLD)

Artsdatabanken - Artskart

1. Open the Norwegian Artskart: http://artskart.artsdatabanken.no/FaneArtSok.aspx
   - Find the distribution pattern in Norway for your 1-3 species recorded in MUSIT
   - Change the selection scheme to:
     - only records from Hordaland
     - only records from University Museum Bergen for whole Norway
2. Open GBIF (Global Biodiversity Information Facility): http://www.gbif.org/species

TASK 37: Describe the distribution pattern in Europe for your 1-3 species recorded in MUSIT. Discuss your findings.

Global Biodiversity Information Facility (GBIF)

A brief introduction is given on the global effort to feed distributional data for the earths biota.

Part 2: Evaluation of the DNA barcoding results

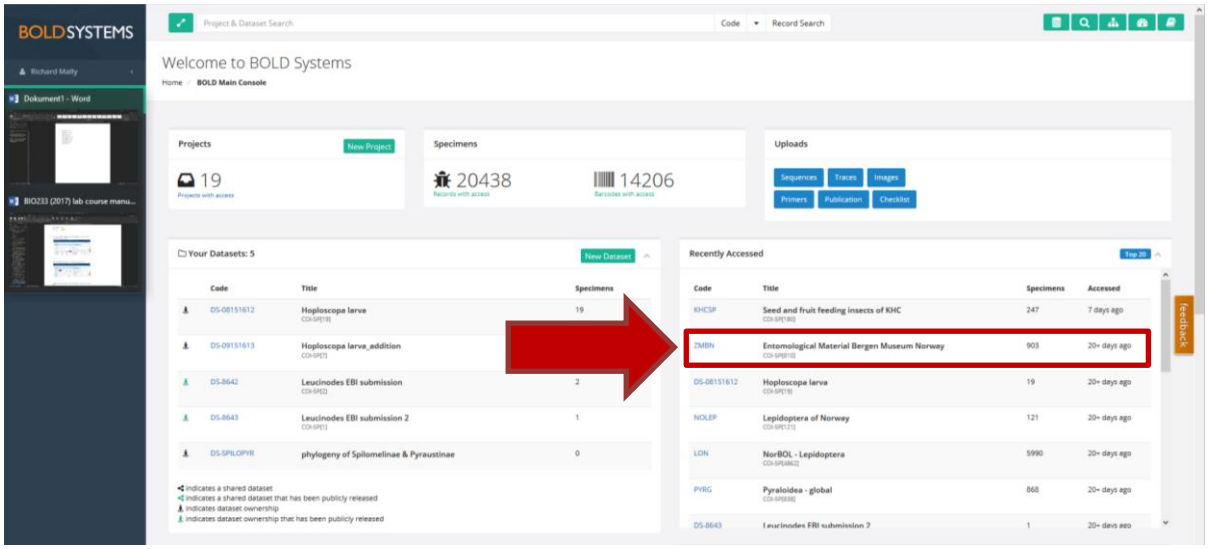
In this part we will have a look at the results of the DNA barcoding of the specimens that you selected for this purpose. We will consult the BOLD database and compare the

1. Open Barcoding databank: BOLD Systems 4
   http://v4.boldsystems.org/index.php/Login/page?destination=MAS_Management_UserConsole

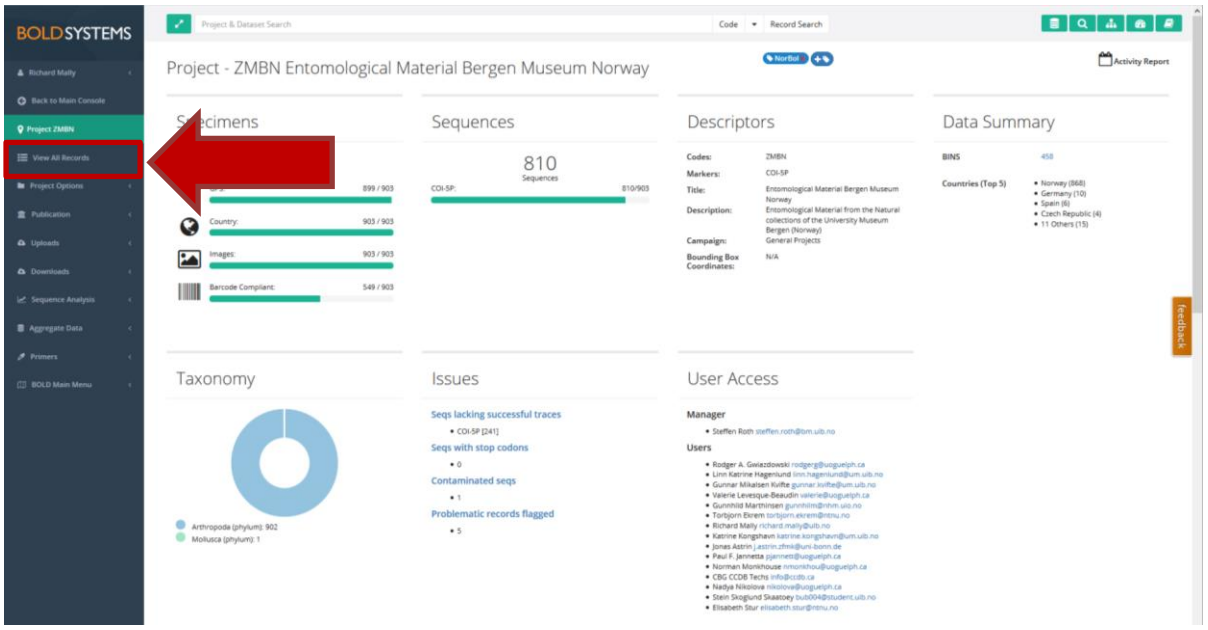
Login Username: **sroth**

Password: **Kurs2017**

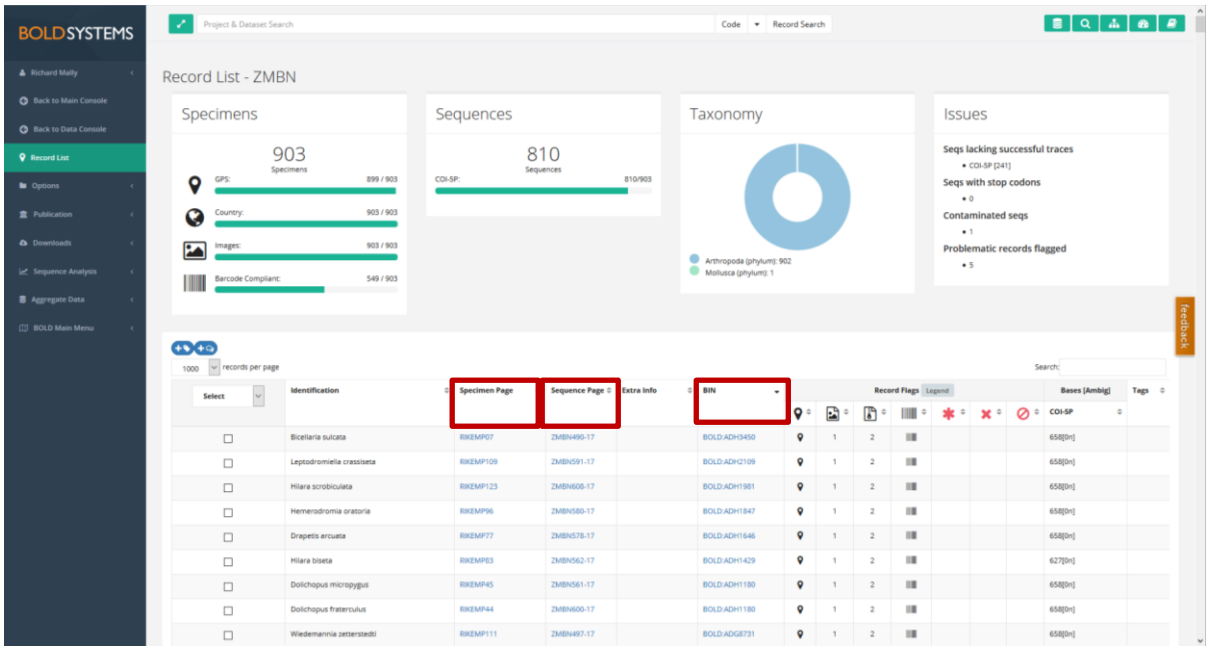
2. Open project “ZMBN Entomological Material Bergen Museum Norway” under "Recently Accessed Projects in Past 2 Months".



3. Open “View All Records” and find your own Barcode samples in the record list.



4. Analysis of results: open the BIN (Barcode Index Number) of your records (sixth column from the right).



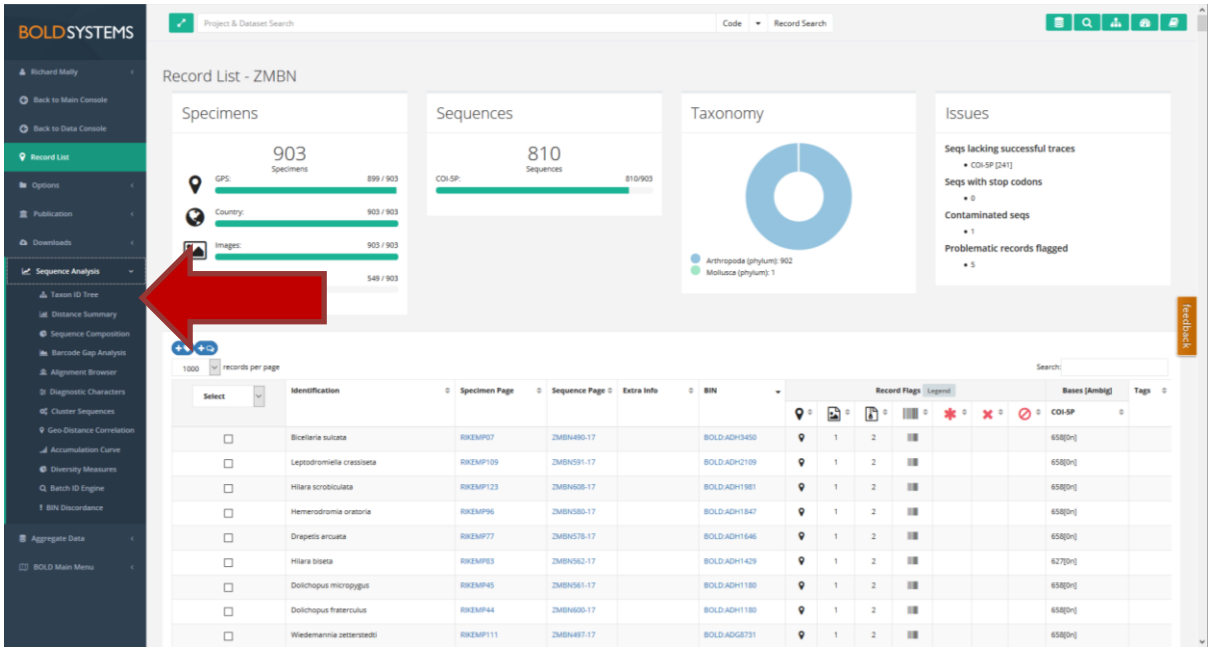
QUESTION 23: Find and evaluate the taxono**mic identification, distribution map and nearest neighbour distances** by using p-distances and tree reconstruction. Discuss why your record might have no BIN.

5. Open the **Specimen Page** (third column from the left in the record list) and **if needed, edit specimen's species name/taxonomic information.**

6. Open the **Sequence Page** (fourth column from the left in the record list).

QUESTION 24: Analyse the DNA Barcode sequence of your records; identify it by using the different options under "Identify Sequence Using:" (e.g. Full Length DB). Discuss the results and give reasons why the sequencing might have failed.

QUESTION 25: Create a Neighbor-Joining tree from all sample records using the "Taxon ID Tree" option under the “Sequence Analysis” heading (see screenshot below). Find your own samples in the tree and interpret their positions in that tree.


BOLD SYSTEMS
Record List - ZMBN
Specimens
903
Specimens
GPS: 899 / 903
Country: 903 / 903
Images: 903 / 903
549 / 903
Sequences
810
Sequences
COI-5P: 810/903
Taxonomy
Arthropoda (phylum): 902
Mollusca (phylum): 1
Issues
Seqs lacking successful traces
• COI-5P [241]
Seqs with stop codons
• 0
Contaminated seqs
• 1
Problematic records flagged
• 5
Sequence Analysis
Taxon ID Tree
Distance Summary
Sequence Composition
Barcode Gap Analysis
Alignment Browser
Diagnostic Characters
Cluster Sequences
Geo-Distance Correlation
Accumulation Curve
Diversity Measures
Batch ID Engine
BIN Discordance
feedback

Thursday, 23.11.2017

Lab course 11 – **Identification key and submission of your 20 insects**

Student collection

During your laboratory work you will prepare 20 samples to be submitted for evaluation. These shall include representatives from at least 10 arthropod orders, mainly insects. You will try to identify these to the level of species, if not, to genus. Occasional specimens may be too difficult to identify properly and we will accept specimens identified to family level.
In your laboratory journal, please include the following:

1. **List of 20 objects/specimens with information on:**
    a. Preservation method (ethanol, pinned, pointed)
    b. Classification
    c. Habitat
    d. Biology of the collected specimen (brief, 2-4 lines)

2. **Identification key to your 20 species or higher taxa**

3. **List of specimens which are given museum journal number and incorporated into the MUSIT database and visible in GBIF**

4. **List of barcoded specimens with information on:**
    a. your tentative ID
    b. your revised ID based on BOLD data
    c. eventual discrepancies in IDs