Biology 337	NAME:	Lab worksheet
Biology of Invertebrates	LAB TIME:	Dr. Bob Podolsky

LAB J. Superph. Panarthropoda [Ph. Arthropoda (Subph. CHELICERATA and Subph. TRACHEATA); Ph. TARDIGRADA and Ph. ONYCOPHORA]

Today's lab is devoted to the two arthropod subphyla that include most of the terrestrial members of the phylum, as well as the two other members of the Superphylum Panarthropoda. As you reconsider the arthropod subphyla, think about how their radiations are related to habitat. (1) The **Crustacea** (covered in a previous lab), while mostly marine, include just a few terrestrial representatives among isopods and land crabs. (1) The **Chelicerata**, which are mostly terrestrial, include the two smaller marine classes Merostomata (horseshoe crabs) and Pycnogonida (sea spiders). (3) Even some of the terrestrial **Tracheata** have larval stages that live in freshwater. Nevertheless, it is striking that there are few to no truly marine insects, the most diverse taxon in the history of animal life. Whatever characteristics were responsible for the enormous radiation of insects on land seems to have constrained their ability to compete with the other group of mandibulates, the crustaceans, in marine habitats.

A. TAXONOMY

General characteristics of arthropods and taxonomic information on crustaceans were given earlier. You should **become familiar with the chelicerate classes and orders, as well as the tracheate classes (and the orders of myriapods)** as listed below. Also listed are a number of the **most common insect orders—you don't need to memorize these**, but they are all familiar groups and you should be able to sort your collections using these taxonomic categories.

Ph. Arthropoda

Subph. Chelicerata. Body divided into prosoma (= cephalothorax) and opisthosoma (= abdomen). In all members, the prosoma has 6 pairs of appendages: 1 pair of chelicerae, 1 pair of pedipalps, and 4 pairs of walking legs.

Cl. Merostomata. Four species in the Subcl. Xiphosura (the horseshoe crabs) plus the extinct Subcl. Eurypterida.

Cl. Arachnida. Several orders: scorpions, spiders, ticks/mites, harvestmen, pseudoscorpions, etc.

O. Scorpionida. Scorpions have chelate pedipalps with a telson that is modified into a venomous stinger.

O. Araneae. Spiders are remarkably common—one estimate is 50,000 spiders per acre of grassland. (What supports such high densities?) The prosoma and opisthosoma are distinct and connected by a thin stalk. The chelicerae are subchelate, forming a fang rather than a claw.

O. Acari. Ticks and mites have a prosoma and opisthosoma that are not well-defined. Ticks are the smaller group but are better known because they are typically larger in body size and feed on the blood of vertebrates. On the other hand, we all harbor hundreds of tiny little mites that live in the roots of our eyelashes and crawl out at night to eat dead skin and body oils.

O. Opiliones. Daddy long-legs have a fused prosoma and opisthosoma, slender chelate chelicerae, leg-like pedipalps, and 4 pairs of, well, long legs.

Cl. Pycnogonida. In sea spiders, the prosoma constitutes most of the small body and the opisthosoma is highly reduced. Males (and sometimes females) have an "additional" pair of "ovigerous" legs—really an outgrowth of other appendages—that are used to carry clusters of eggs after they have been collected from several females. Most pycnogonids feed by sucking body fluids out of cnidarians or urochordates.

Subph. Tracheata (= Uniramia). Typically with only one pair of uniramous antennae as well as uniramous thoracic appendages. As in crustaceans (the other group within the sometimes recognized Mandibulata), the third pair of head appendages are mandibles. Adult stages are terrestrial.

Cl. Myriapoda. The "many-legged" tracheates, including the millipedes and centipedes (as well as the orders Symphyla and Pauropoda), have two tagmata: a head and an elongated trunk. Many secrete noxious substances to deter predation.

O. Diplopoda. Millipedes appear to have *two* pair of appendages per segment, violating expectations about how an arthropod body is constructed, but in fact each apparent segment is two fused during development (hence the name Diplopoda).

O. Chilopoda. Centipedes have one leg pair per segment, and are equipped with claws that can inject poisons into their prey or into a potential predator.

Cl. Hexapoda. Nearly all of the "six-legged" hexapods—which constitute the vast majority of animal species diversity (approx. 1 million described)—belong to the subclass **Insecta**. Insects have three distinct tagmata—head, thorax, and abdomen—with a pair of legs on each of the three thorax segments. At least 28 orders have been described. You already likely know taxonomic diversity in insects at the level of orders, and should become familiar with the scientific names of those listed below. The four big orders come first, followed by the rest in very rough order of how often they are likely to be encountered. Two main divisions related to life cycle complexity and flight deserve special mention:

<u>Life cycles</u>. Note that the orders with asterisks (*) below are all **holometabolous**—the larval stage that is distinct from the adult, and the transition involves a complete **metamorphosis**, sometimes referred to as **indirect development**. This type of development characterizes the largest orders, suggesting that the evolution of this type of complex life cycle has enabled a great deal of adaptive radiation in these groups. The remaining orders are **hemimetabolous**, where the early stages (nymphs) look like smaller versions of adults and undergo **direct development**.

<u>Flight</u>. A few insect orders are grouped in the Apterygota, the primitive wingless insects, such as Springtails (O. Collembola) and silverfish (O. Thysanura). *See Fig. 1*. All of the orders listed below belong to the Pterygota, a derived clade that includes all the winged insects (some, like fleas and lice, have secondarily lost their wings). Note that the ending of many of the order names, "-ptera", refers to wings, e.g. "di-" [two-wings], "hymeno-" [membrane-wing], "lepido-" [scale-wing], "coleo-" [sheath-wing]). *Can you figure out the meaning of the remaining names*?

Beetles (**O. Coleoptera***)—at 360K species—are twice as diverse as all non-arthropod species *combined*. Adults have hard forewings, called elytra (recall a similar term from the polynoid polychaetes), that protect the membranous rear flight wings. *See Fig. 6.*

Moths and butterflies (**O. Lepidoptera***) boast about 160K species. The large, sailing wings are covered with tiny scales and are often brightly colored. *See Fig. 9.*

Flies (**O. Diptera***), including the "two-winged" houseflies, fruit flies, mosquitoes, gnats, greenflies, no-see-ums, and blackflies, boast about 150K species. The hindwings are highly reduced "halteres" or balancers that help to stabilize flight. *See Fig. 8*.

Bees, wasps, and ants (**O. Hymenoptera**^{*}) include about 130K species (still more than any other single non-arthropod phylum). Most have venom that is injected via a stinger at the tip of the abdomen. All are haplo-diploid and many are eusocial. *See Fig. 11.*

Termites (O. Isoptera) are superficially similar to ants, but the abdomen is broadly joined to the thorax, not constricted. Most have symbiotic bacteria in their guts that digest cellulose, and they are important in the breakdown of wood. Like ants and many bees, termites exist in complex societies with specialized castes. *See Fig. 5.*

Cockroaches (O. Blattaria) have membranous hindwings that can be folded underneath the forewings. Although similar to orthopterans (see below), the females of this group do not have a well-developed ovipositor. *See Fig. 10.*

Mantids (O. Mantodea), which are similar to cockroaches (and sometimes grouped with them in the O. Dictyoptera), have raptorial appendages for spearing prey. *See Fig. 10.*

Walking sticks and leaf insects (O. Phasmida) are often cryptic, slow moving mimics of plant parts. More typically found in the tropics, all herbivorous.

"True" bugs (O. Hemiptera) include stink bugs, assassin beetles, squash bugs, chinch bugs, and water striders (Subo. Heteroptera) as well as cicadas, aphids, treehoppers and leafhoppers (Subo. Homoptera). Hemipterans have piercing-sucking mouthparts that they use for drinking plant juice or the body fluids of their insect prey. *See Fig. 4, 7.*

Grasshoppers, locusts, and crickets (O. Orthoptera) have long hind legs specialized for leaping. Females have a well-developed ovipositor. *See Fig. 3*.

Dragonflies and damselflies (O. Odonata) are flying/gliding insects that live near water where they prey on insects and deposit eggs. As in several other orders, including stoneflies (O. Plecoptera), caddisflies (O. Trichoptera*), dobsonflies (O. Megaloptera*), and mayflies (O. Ephemeroptera), larvae develop in freshwater. *See Fig. 2*.

Earwigs (O. Dermaptera) typically have large, pincer-like cerci used for defense and food capture.

Ph. Tardigrada and Ph. Onychophora. Like the arthropods, these phyla have an external cuticle that is periodically molted, but the cuticle is thin and flexible, and the lobe-shaped appendages ("lobopods"), with small clawed hooks, are non-jointed

B. OBSERVING TERRESTRIAL ARTHROPODS

We will start with a brief (1.25-h) trip to the Fort Johnson forest to collect terrestrial arthropods. You will use various approaches (see below) to seek out diverse myriapods, hexapods, and arachnids that are often hidden. In addition to your hands and intuition, you will have **scraping tools**, insect **sweep nets**, **aerial nets** and **collection jars** to capture and subdue as many different types as you can. The collection jars have a base saturated with ethyl acetate, which will kill the organisms through inhalation (try not to inhale it yourself). In addition, earlier this week I set out a number of insect **pitfall traps** at ground level. Upon returning to lab, complete the exercises on the last page of this handout.

<u>Approaches to collecting insects</u>. To visualize where to find insects, think like an insect-eating bird or mammal. Look inside and under...leaf litter, curled up leaves, bark on fallen trees, rocks. Insects are occasionally found on tops of surfaces, but are more often below surfaces or inside of plant materials.

 \rightarrow Leaf litter, soil, and fallen trees are sources of decaying material and as a result are a good source of terrestrial arthropods. Dig under the surface of leaf litter, and pull away the bark of fallen trees, to see what emerges.

 \rightarrow Areas beneath **rocks, sticks, boards, and logs** are typically cool and hospitable for moisture seeking arthropods. Turn or roll these over to find some of the highest diversity of terrestrial arthropods. **Important**: whether in a tidepool or a forest, always be sure to turn these structures back to their original position, to minimize disturbance to the community.

 \rightarrow Flowering plants are an excellent source of flying insects, while brushy vegetation is a good source of crawling/herbivorous insects. Use sweep nets to sweep through the surface of vegetation, and pursue butterflies and other flying insects with butterfly nets.

→ Pitfall traps are useful for sampling ground-dwelling beetles and spiders. Each trap is a small plastic cup flush to the ground, shielded by a cover, that contains saturated salt solution (as a preservative) and a drop of dish soap (to break the surface tension). Each group of 3-4 students will retrieve samples from 5 cups along a path. Be sure to retrieve all materials (flags, cups, covers, stakes) at each site.

 \rightarrow Most insects are harmless. If you are allergic to bee stings, **do not** attempt to capture a bee, wasp, hornet, yellow jacket, or ant. If you do capture a stinging insect, shake the insect down to the very end of the net, then place the end of the net into the collection jar.

Be sure to share material and to study animals collected by the entire class. You will very likely not collect members of all groups covered in the taxonomic descriptions. You should make use of preserved specimens for any groups missing from your collections.

C. ADDITIONAL NOTES ON ARTHROPOD TAXA

Life cycles

• Direct developing stages, which resemble adults, are often called **nymphs**. Indirect developing larval stages sometimes have special names, such as grubs (coleopteran larvae), maggots (dipteran larvae), and caterpillars (lepidopteran larvae). Look for examples of early stages in preserved material for some of the groups below.

SUBPH. TRACHEATA CL. HEXAPODA, SUBCL. INSECTA O. Orthoptera (grasshoppers)

<u>External</u>: distinct head, thorax, and abdomen. Head: single pair of antennae, compound eyEs and simple eyes (may be hard to see), labrum (upper lip), labium (lower lip formed from fused second maxillae), mandibles, palps arising from maxillae and from the labium. Body: membranous hindwings covered by protective, thickened forewings, three pairs of uniramous legs, tympanum (for hearing), spiracles opening into the tracheae, and posterior genitalia (including the retracted ovipositor in the female).

<u>Internal</u>: If there is time and interest, these animals are good for a brief dissection. Make a middorsal incision the length of the body and slowly pull down the lateral body walls. As you do so, notice clear, flexible **tracheal tubes** coming in from **spiracles** on the body wall. Now pin your animal down in a dissecting tray. If you have a female, a series of large, elongate ovary components will be immediately evident above the intestine. If you have a male, a pair of flat, ovoid testes can be seen. Abundant, very small, thread-like **Malpighian tubules** may be seen coming off the intestine. Look for the **ventral nerve cord**.

O. Hemiptera (true bugs)

The "true bugs" have piercing and sucking mouth parts that form a noticeable beak. Often found in freshwater habitats swimming under the surface in search of prey, they still breathe air through spiracles. Most will carry a bubble of air attached to hydrophobic hairs beneath the abdomen when diving. Look for this bubble in living specimens. *Halobates*, a "water strider" that skates on the surface tension of water, is one of the very few oceanic insects. Also, examine a prepared slide of *Cimex*, the "bed bug," and note the piercing beak.

O. Homoptera

On demonstration may be the shed exoskeletons of the final nymphal stage (called an instar) of a cicada, found attached to the trunks of trees. What are the ways that nymphal morphology differs from adult morphology?

O. Coleoptera (beetles)

Note the characteristic leathery, protective forewings (elytra) that provide a covering for the more delicate hindwings. Terrestrial beetle larvae (grubs) are commonly found in plant roots or rotten logs, whereas other beetle larvae with more varied morphologies are common in freshwater habitats.

O. Lepidoptera (butterflies and moths)

Under a dissecting scope, note the tubular mouth parts used for nectar feeding. Also note the distinctive scales that cover the wings. This order includes some of them most damaging herbivorous larval ecological pests (as caterpillar larvae).

O. Diptera (true flies)

Compare the sponging mouthparts of houseflies to the piercing mouthparts of mosquitos. Only female mosquitos feed on blood; males feed on nectar. If both male and female mosquitos are available, notice that the antennae show greater development in the male. This sexual dimorphism is due to the male having to locate the female for mating.

For any available dipterans, carefully observe the thorax, noting the single larger pair of wings. If you look closely, however, you will see a second, degenerate pair of wings called **halteres**. These sensory structures aid in stabilizing the animal during flight.

O. Odonata (dragonflies and damselflies)

Note the two pairs of membraneous wings and the large eyes. The large number of ommatidia in each eye increases the sharpness of the visual image, an obvious benefit in flying predators (imagine capturing a mosquito on the wing). In the field you may be fortunate to watch three characteristic types of behavior: predatory behavior (swooping and rapid maneuvering to catch prey), dipping behavior by females (touching the abdomen to water to release eggs), and territorial behavior (males competing to "control" areas where females will mate and deposit their eggs). The larval nymphs are aquatic but otherwise look like adults without wings. If you have living larvae, you can see them move water in and out of the anus for gas exchange (in which two other phyla have you seen this before?)

O. Hymenoptera (ants, bees, wasps)

Note the narrow "waist" connecting the thorax and abdomen, characteristic of this order. As in the termites, many hymenopterans live in complex societies with distinct castes (morphologically and functionally distinct morphs). Development to a particular caste is not genetic but rather determined by environmental influences like diet.

If available, examine a honeybee. Note the two pairs of membraneous wings, antennae, large compound eyes, mouthparts modified for gathering nectar, and the terminal stinger on the abdomen. The stinger is a modified ovipositor found in all members of the colony except males. If you examine the last pair of legs on a worker honeybee you will notice that two of the segments are very wide and heavily bristled. Pollen, a valuable protein-rich food, is brushed off the body by the legs and ultimately ends up being pushed up into a depression called the pollen basket next to the joint between these two enlarged segments.

>Although you are unlikely to have collected the following two orders, they figure prominently in the lives of humans and pets as well as other animals, on which they are ectoparasites.

O. Anopleura (sucking lice)

All have sucking mouthparts have secondarily lost their wings, and are parasites on birds and mammals. Those that are ectoparasitic on humans are the crab louse and body louse. As is true with mosquitos, biting flies, and fleas, of particular concern is the role of lice as disease vectors. Examine a prepared slide of *Pediculus*, the human body louse. Note the absence of wings and the presence of gripping claws on the legs. You will also be able to see the branching tracheal system through the body wall. Note how the tracheae branch inward from the spiracles.

O. Siphonaptera (fleas)

Like the lice, fleas have secondarily lost wings and are blood-sucking ectoparasites. Examine a prepared slide of *Pulex* and note the laterally flattened body, lack of wings, and the piercing mouthparts. You should be able to see the ringed tracheae branching in from the spiracles.

SUBPH. TRACHEATA CL. MYRIAPODA O. Chilopoda (centipedes)

Note the single pair of antennae, large poison fangs, numerous trunk segments, and uniramous walking legs (one pair per segment). In a large species, note the large maxillae and tracheal spiracles (dorso-lateral on each segment).

O. Diplopoda (millipedes)

Note the single pair of antennae, compound eyes, mandibles, "lower lip" made of fused maxillae, absence of poison fangs, pores from repugnatorial glands (lateral on each segment), and two pairs of walking legs per segment (formed by fusion of adjacent segments in embryos). Modified copulatory legs called gonopods are a short distance behind the head. Note the variety of form and size: some are round-bodied, others have a flattened dorsal scutum.

SUBPH. CHELICERATA

CL. ARACHNIDA O. Araneae (spiders)

Note the following: prosoma, opisthosoma, dorsal carapace on the prosoma, multiple eyes, chelicerae (poison fangs), leg-like pedipalps, and walking legs. On the ventral side, silk-secreting spinnerets and slit-like spiracle openings into the book lungs and tracheae. In males, the tip of each pedipalp is bulbous or otherwise complex, and serves as a receptacle for sperm before transfer to the females gonopore.

If a silken cocoon is available, note that larval stages ("spiderlings") look like small adults. Spiders use silk threads to disperse at early stages through the process of ballooning.

O. Scorpiones

Put a preserved scorpion in a dish. Note the prosoma, carapace, eyes, opisthosoma (posterior part extended as a "tail"), sting, small chelate chelicerae used to tear up food in front of the mouth, larger chelate pedipalps used to capture prey, and four pairs of legs. Locate the spiracles opening into internal gas exchange structures. Why are the gas exchange structures internal?

O. Opiliones (harvestmen or "daddy long-legs")

Note the fusion of the prosoma with the opisthosoma and the four pairs of very long legs. Identify the slender, chelate chelicerae, leg-like pedipalps, and eyes. These rather slow moving, easily seen animals do not have large chelate claws, fangs or poison glands. How do you suppose they reduce the chance of being eaten by potential predators? In what other groups have you seen such a strategy?

O. Acari (mites and ticks)

Mites include species that are herbivorous, carnivorous, or parasitic, while all ticks are bloodsucking parasites. Examine a prepared slide of mites or ticks. Note the fused prosoma and opisthosoma, the four pairs of legs with terminal claws, and the chelicerae and pedipalps. In the acari the chelicerae and pedipalps are joined together with a body extension to form one structure, the capitulum. This is the "head" of the tick that you do not want left under your skin when removing the animal. If you look at the tip of a tick chelicera closely, you will see the hooks used to dig into and hang onto the host. On demonstration may be an engorged, swollen dog tick. Identify the capitulum and legs.

D. OBSERVATIONS OF MARINE CHELICERATES

Cl. Merastomata (horseshoe crabs)

Examine the dorsal surface of *Limulus*. Note the two body regions (prosoma and opisthosoma), the shield-like carapace covering the prosoma, large compound eyes, smaller simple eyes, and the terminal, spike-like telson. Although it looks like a formidable weapon, the telson is used instead to assist the animal in righting itself if turned over.

On the ventral prosoma, identify the chelate chelicerae, pedipalps (which look like other legs), chelate legs, a pair of "pushing" legs, and a final pair of very small appendages. Note the spined gnathobases at the "elbows" of the prosomal appendages. These elbows chew food, e.g. worms or clams, prior to ingestion. If a mature male is available, note that the swollen pedipalps, which are used to hold onto the female during mating.

On the ventral opisthosoma, note the flaplike appendages. The first flap covers the paired gonopores, but the rest (the gill opercula) have leaflike, delicate book gills beneath them. The opercula move constantly to ventilate the gills and to aid blood movement through the gills. Finally, look for anus is seen at the base of the telson.

Cl. Pycnogonida (sea spiders)

Note the very short body, long legs, proboscis, chelicerae, pedipalps (= "palps"), ovigerous legs, and eyes present on a small mound. A very highly-reduced, bulbous opisthosoma is present. Males may be carrying masses of fertilized eggs on their ovigerous legs. These appendages are frequently smaller or absent in females. If living pycnogonids are available, note their extremely slow climb over the substrate in search of sessile prey, such as hydroids and sponges.

E. OBSERVATIONS OF OTHER MEMBERS OF THE PANARTHROPODA

Ph. TARDIGRADA

Although many tardigrades are marine, interstitial organisms, others are terrestrial and can enter a cryptobiotic stage, called a tun, under deteriorating conditions. The tun stage can withstand pretty much any conditions it is naturally or unnaturally exposed to (absolute zero temperatures, ethanol, radiation, etc.). The day before lab I will collect some dried moss and hydrate it in spring water overnight. The moss should contain a large number of tuns, and hopefully you will be able to see live tardigrades that have "hatched" overnight.

Using a dissecting microscope, search for movement in the bottom of a Petri dish where moss has been hydrated. Identify the following: the plump, segmented body; four pairs of unjointed appendages with terminal claws; stylets inside the mouth for piercing plant or animal tissue; gut and ocelli. Note the method of movement and distinguish movement from a typical arthropod. Scan parts of the slide to look for shed cuticles, which act as a cocoon and may contain eggs.

Ph. ONYCHOPHORA

Look at a preserved specimen of *Peripatus* under a dissecting microscope. Note the segmented, worm-type body; the paired stubby unsegmented appendages with terminal claws; antennae; oral papillae with glue glands, used to subdue prey and predators; mandibles and mouth. Which characteristics led observers to mistakenly believe that onychophorans were the "missing link" between annelids and arthropods?

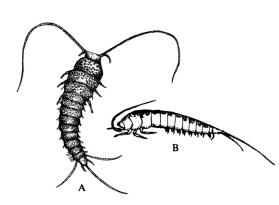
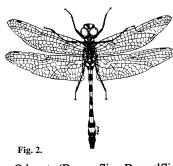


Fig. 1. Bristletails. A, silverfish, Lepisma saccharina (Lepismatidae); B, Machilis variabilis (Machilidae).



Odonata (Dragonflies, Damselflies)





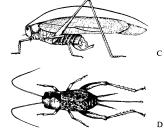
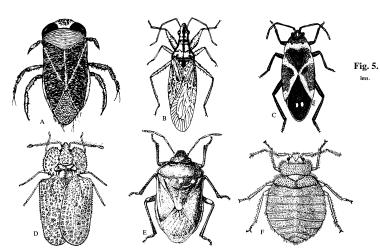
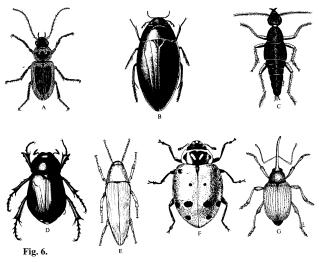


Fig. 3.

Orthoptera (Grasshoppers, Katydids, Crickets). A, pygmy grasshopper or grouse locust; B, grasshopper; C, katydid; D, crickets.



Hemiptera (Suborder Heteroptera—True Bugs). A, water boatman; B, damsel bug; C, seed bug; D, lace bug; E, stink bug; F, bed bug. Fig. 4.



Coleoptera (Beetles). A, ground beetle; B, water scavenger beetle; C, rove beetle; D, scarab beetle; E, click beetle; F, lady beetle; G, weevil.





Isoptera (Termites). A, winged; B, wing-







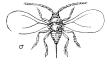


Fig. 7.

Hemiptera (Suborder Homoptera-Cicadas, Leafhoppers, Planthoppers, Aphids, Scale Insects, others). A, cicada; B, aphids; C, leafhopper; D, treehopper; E, scale insects.

E

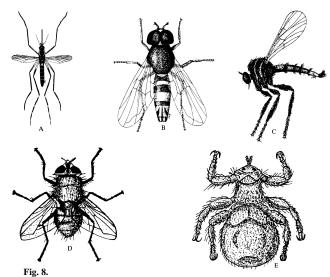


Fig. 8. Diptera (Flies). A, mosquito; B, flower fly; C, robber fly; D, blow fly; E, louse fly (a wingless species).

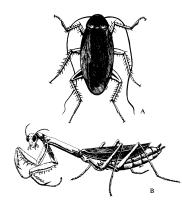


Fig. 10. Dictyoptera (Cockroaches, Mantids). A, cockroach; B, mantid.

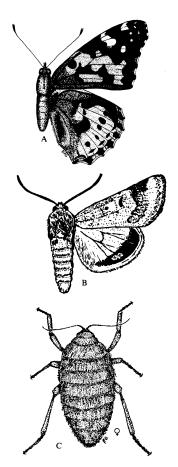
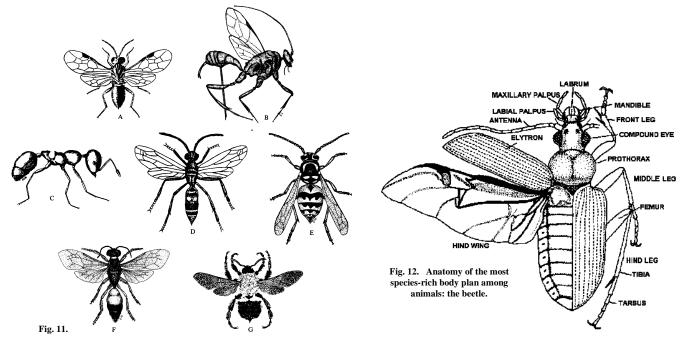


Fig. 9. Lepidoptera (Butterflies, Moths). A, brushfooted butterfly; B, noctuid or owlet moth; C, geometrid moth (a wingless species).



Hymenoptera (Ants, Bees, Sawflies, Wasps, others). A, sawfly; B, ichneumon; C, ant; D, wasp; E, yellowjacket; F, wasp; G, bumble bee.

EXERCISE

1) **TQ**: Failing to look <u>below</u> surfaces will result in much lower collection diversity. List three or more reasons why terrestrial arthropods are expected to be found in greater numbers below surfaces as compared to on surfaces. Which of these ideas apply to marine relatives?

2) Sort your collection of terrestrial arthropods according to subphylum, class, and order (if a hexapod). Record below the contents of your collection and any observations about what distinguishes the group from other organisms you collected.

Subphylum, Class, Order Common name Distinguishing features

3) Use the back of this page to make detailed sketches of <u>four</u> members of your collection (or parts of the permanent collection) to compare the following major aspects of body design. (Additional notes for any order you are likely to encounter are in this handout.)

- the shape, prominence, and differentiation of tagmata
- the size and shape of **mouthparts**
- the shape and relative prominence of wings

- the prominence of sensory structures (e.g., antennae, eyes)
- the location of **spiracles** (hexapods) or **book lungs** (arachnids)