ZOO 4377L - VERTEBRATE MORPHOLOGY LAB

LAB 3: "HEMICHORDATES & PROTOCHORDATES"

Name: _____ SSN: _____

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N.B. Preparation for next week's lab (The Trunk Skeleton)

1) Walker & Homberger - Chapter 5 (pp. 95-110)

Preparation: Walker & Homberger - Chapter 1 (pp. 1-15)

INTRODUCTION

Last week we examined the anatomy of a "primitive" crantiate (or vertebrate), the lamprey. Now that we know a little bit about the general body plan of a craniate (i.e., bilateral symmetry, axial skeleton, segmental muscle, etc.), we will examine the anatomy of some representatives of the sister (i.e., most closely related) taxa to the craniates in order to obtain some idea as to the biology of the ancestor of all craniates (vertebrates). Again, you must remember that all organisms are mosaics of primitive and derived characteristics, and that the organisms you will examine today do not represent the ancestral organisms in all aspects.

In traditional taxonomy the phylum Chordata is subdivided into three subphyla: Urochordata (Tunicates), Cephalochordata, and Vertebrata (or more properly Craniata). In phylogenetic systematics (cladistics), the cephalochordates are the sister group to the craniates (vertebrates) and the urochordates are the sister group to the Notochorda (= Cephalochordates + Craniata; see the attached phylogeny (Nielsen, 1995)). Collectively the Urochordata, Cephalochordata and Craniata form the monophyletic clade Chordata. The synapomorphies (shared derived characters) which define the Chordata are as follows:

1) longitudinal muscles used in locomotion
2) endostyle
3) chorda (or urochord (Nielsen, 1995) - a dorsal, longitudinal stiffening rod the cellular composition of which varies among taxa
4) dorsal, unpaired, tubular nerve cord
5) mouth develops from dorsal side of apical pole - this is a developmental character and thus not visible *per se* in the life stages we will examine today

The synapomorphies uniting the cephalochordates and craniates into the Notochorda are:

1) notochord (true notochord; differs cellularly from chorda of Urochordates)
2) myomery (segmented longitudinal muscles)

The sister group to the Chordata is the Enteropneusta represented by acorn worms. This group shares with the Chordata pharyngeal ("gill") slits supported by a collagenous skeleton. Thus, with the Chordata, the Enteropneusta form the clade Cyrtotreta.

N.B. In traditional taxonomy the Enteropneusta and Pterobranchi are grouped together as the "Hemichordata." Nielsen (1995) holds that this grouping is polyphyletic (i.e., are derived from more than

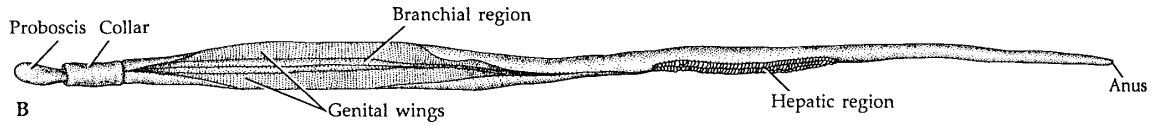
one source, thus not a single lineage). Similarly, the term "Protochordates" is a paraphyletic grouping (i.e., excludes some descendants, in this case the Craniata) of the Urochordates and Cephalochordates.

In today's lab we will examine the anatomy of three species representing the Enteropneusta, Urochordates and Cephalochordates. You will be responsible for knowing all structures whose name appear in **bold** for next week's quiz.

Station 1: **Acorn worm** (*Balanoglossus sp*? or *Dolichoglossus sp*?) - preserved specimen w/ dissecting scope.

The enteropneusta or acorn worms (phylum Enteropneusta) are marine organisms numbering approximately 80 species. These are worm-like creatures which rang in length from a few centimeters to about two meters and burrow in the sand and mud of tidal flats and shallow coastal waters.

Using your lab manual as a guide (pp. 2-4), attempt to identify the structures listed below. One animal has been pinned to expose the pharynx. The structures shown *italics* may be difficult to demonstrate; ask your instructor for assistance.



External morphology

proboscis
collar
mouth
trunk
pharynx
pharyngeal (gill) slits
genital ridges or wings
hepatic ridges
longitudinal mid-dorsal and mid-ventral ridges - expressions of underlying dorsal and ventral nerve strands

? Which of these structures is a derived trait shared with the Chordates?

Station 2: **tunicate larva** - whole mount slide w/ compound microscope

The tunicates (sub phylum Urochordata) are marine organisms numbering approximately 2000 species and divided into three classes: Ascidiacea, Thelaciea and Appendicularia. While the larvae are motile, the adults are sessile and encased in a leathery membrane called a tunic (L., coat), hence tunicates. Today we will examine the larval and adult anatomy of an ascidian.

Ascidians, or sea squirts (if a live adult specimen is available, you may wish to investigate the origin of the latter, colloquial name), are marine animals that are often brightly colored. Some species are solitary, others colonial. As in all urochordates, the larvae are planktonic, but adults are sessile.

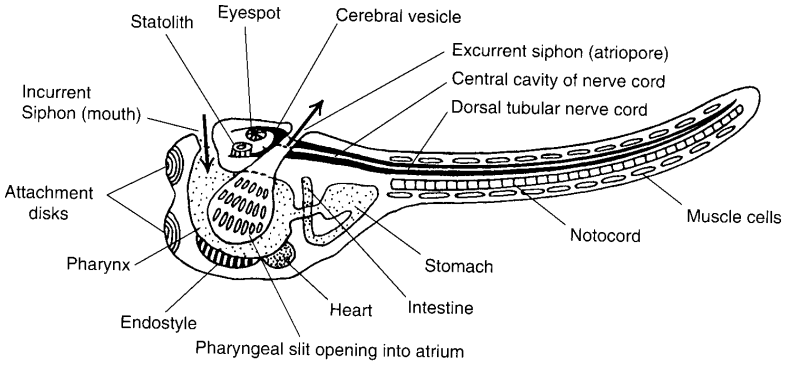
The larva, sometimes called the ascidian tadpole, does not feed during its short sojourn of a few days as a free-living member of the plankton. Instead, the larva disperses and selects the site at which it will undergo a dramatic metamorphosis and take up a permanent residence as an adult. Only the larval stage exhibits the four non-developmental chordate characteristics.

Under a compound microscope, examine the mounted larvae. Note the expanded anterior end (body) and slender tail which give the larva the appearance of a "tadpole". The tail is a locomotor organ

supported by a central notochord and surmounted by a dorsal tubular nerve cord and which extends into the body to form the cerebral vesicle. Surrounding the notochord and powering the tail movements are *unsegmented* longitudinal bands of muscles.

In the body, the branchial basket, an enlargement of the pharynx, is recognized by the presence of tiny, regular rows of perforations, the pharyngeal slits. Water is drawn in through an incurrent siphon and expelled through the excurrent siphon. The anus empties internally into the excurrent siphon and thus does not clearly mark the point beyond which the tail continues.

Other features can be identified. Several adhesive papillae on the anterior end of the body are involved in eventual attachment of the larva when it settles down. Usually two very black spots are present: the tiny, marble-shaped spot, the otolith, is a sensor of gravity; the irregular black spot, the ocellus, is a light-sensitive eyespot. Other organs are present but difficult to identify in most specimens.



C

Using the accompanying figure and Figure 1-2C of your dissector as a guide, attempt to identify the following structures:

notochord
dorsal nerve cord
longitudinal body muscles
adhesive papillae
otolith
ocellus

? Which of the above structures are synapomorphies which define the chordates? Hint: All three are found within the tail.

Those with extreme anatomical imaginations (or the proper mind-enhancing preparation) might wish to try and identify the following:

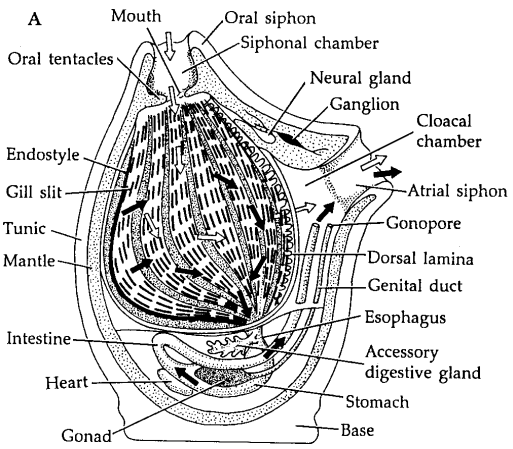
incurrent and excurrent openings
endostyle
pharynx with pharyngeal slits

Station 3: **adult tunicate** - live or preserved specimen with dissecting scope

In contrast to the larvae, the adult tunicates are sessile and enclosed in a leather membrane (tunic). Using your lab manual as a guide (pp. 4-6), attempt to identify the structures listed below. The structures shown in bold should be demonstrable while those in italics may be difficult to demonstrate.

N.B. In dissecting you will find the tunic to be easily removable whereas removal of the underlying mantle (without inflicting significant damage) can be challenging. In general, most of the organs can be viewed through the mantle and thus only a small portion need be removed in order to better observe the pharynx. It is recommended that you try to identify as many of the organs prior to dissecting the mantle.

One or two species may be available for investigation:



1) laboratory sea squirt (*Molgula occidentalis*)
- largest of all *Molgula* species, the tunic is thin and leathery with embedded sand and mud
- animal can be easily removed by spitting tunic between siphons
- gonads are visible with distinct large eggs and sperm; gravid year round.

2) leathery sea squirt (*Stylea plicata*)
large, solitary; grows on wharf pilings; fertile year long; oblong with 4-lobed siphons

External Features:

incurrent siphon - note tentacles
excurrent siphon
tunic

? How can you distinguish the incurrent from the excurrent siphon? What might be the function of the tentacles around the incurrent siphon?

Internal Features:

tunic - attached to body wall only at siphons
mantle - body wall; thin (nearly transparent); muscular; deep to tunic
pharynx
- **longitudinal bars** - derived from pharyngeal bars
- **pharyngeal slits**
- **endostyle**

atrium - best observed at excurrent siphon
gut tract
- *esophagus*
- *"stomach"*
- *intestine*
- *anus*

gonads - bilateral
renal vesicle
neural gland complex -
- *neural ganglion* - remnant of dorsal nerve cord

? Although larval tunicates have all four of the non-developmental synapomorphies of chordates, which (hint: one) is retained in the adult organism? Why are the chorda, longitudinal muscles and dorsal nerve cord lost or atrophied?

? Describe the flow of water through an adult tunicate, i.e., list the structures through which water passes starting with the incurrent siphon and ending with the excurrent siphon.

? What two biological roles does the pharyngeal apparatus serve?

Station 4: **<u>lancelet or amphioxus</u>** (*Branchiostoma floridae)*) - live or preserved specimen with dissecting scope.

The subphylum Cephalochordata is comprised of 2 genera and 45 species. These marine organisms are mostly found in shallow waters burrowed vertically into the substrate, with their "head" extending into the water column to feed. Since cephalochordates are the sister group to the craniates, examination of their anatomy provides clues as to how the ancestral vertebrate may have appeared.

Using your lab manual as a guide (pp. 7-11), attempt to identify the structures listed below. The structures shown in bold should be demonstrable while those in italics may be difficult to demonstrate.

External Features

myomeres
myosepta
dorsal fin
ventral fin
caudal fin
metapleural folds
oral hood
    cirri
atrium - may be difficult to see in preserved specimens
    atriopore
anus - asymmetrical (left side)
post-anal tail
gonads - may be difficult to see in preserved specimens

? What is the function of the cirri?

? Given your knowledge of craniate (vertebrate) structure from Lab 2, and based on your observations in the enteropneusta, urochordates and cephalochordates, are external pharyngeal slits a primitive or derived condition in craniates? [Hint: Examine the cladogram.]

? Which characteristic in the above list is a synapomorphy uniting craniates and cephalochordates?

Station 5: **<u>lancelet or amphioxus</u>** (*Branchiostoma floridae)*) - whole-mount slide; compound microscope

Use pages 8-11 of your dissector to identify the following structures.

myomeres
ray fin boxes
metapleural folds
notochord
dorsal nerve cord
    photoreceptors
pigment spot

oral hood
- **cirri**
- **wheel organ**
  - *Hatschek's groove*
  - *Hatschek's pit*

velum
- **velar tentacles**

pharynx
- **pharyngeal slits**
- **pharyngeal bars**
- *endostyle*

atrium
- atriopore

gut tract
- esophagus
- midgut
  - cecum
- **ileocolic ring**
- hindgut - Be sure and examine the forming fecal pellets for the non-digestible skeletons of the protozoa (e.g., diatoms) that are the amphioxus' diet
- anus

? Recalling the lamprey, how does the rostral extension of the notochord differ between it and the lancelet?

? According to Kardong (and others), to which endocrine gland in the craniates might Hatschek's pit be homolous?

? What is the major site of gas exchange (blood aeration) in the lancelet?

Station 6: **lancelet or amphioxus** (*Branchiostoma floridae*) - Transverse sections w/ compound microscope. **N.B.** Each microscope slide has a complete set of cross-sections.

Use pages 11-15 of your dissector to identify the following structures.

Features common to all sections (i.e., be able to recognize in all!):

dorsal fin ray box
myomeres
myosepta
nerve cord
- *neurocoel*

notochord

Transverse section through oral hood:

walls of the oral hood
- wheel organ
  - Hatschek's groove

cirri

Transverse section through the pharynx:

metapleural folds
transverse muscle

pharynx
pharyngeal bars
pharyngeal slits
endostyle
atrium
gonads - may not be present (more caudal)
midgut cecum - may not be present (more caudal)

? Name two functions associated with the midgut cecum. What two organs handle these functions in craniates?

Transverse section through the gonads:
gonads
gut
coelom

? Are you looking at an ovary or testes? How can you distinguish an ovary from a testes? Are lancelets monoecious or dioecious?

Transverse section through the hindgut:
hindgut
coelom
ventral fin