INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

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27th International Biology Olympiad

July 17-23, 2016
Hanoi, Vietnam



Practical Exam 2

ANIMAL SYSTEMATICS AND ANATOMY

Total points: 100
Duration: 90 minutes

DEAR PARTICIPANTS,

In this practical test, do the following two parts:

EXPERIMENT 1. CLASSIFICATION OF BUTTERFLIES (65 points)

- Task 1. Identify all the butterfly specimens provided
- Task 2. Compile the character matrix
- Task 3. Calculate the distance matrix based on the characteristic matrix provided
- Task 4. Resolve the phylogenetic relationship of all the specimens
- Task 5. Draw a phylogenetic tree (dendrogram)

EXPERIMENT 2. ANATOMY OF EARTHWORM *Amynthas aspergillum* (35 points)

- Task 6. Identify the external structures of *Amynthas aspergillum*
- Task 7. Dissect and identify the internal structures of *Amynthas aspergillum*

Important Information:

- Please remember to write your **Country** and **Student code** in the given box.
- Write your answers in the separate **Answer Sheet**. Only the answers given in the **Answer Sheet will be evaluated**.
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise the **Red card** immediately.
- During experiments, ensure to handle equipment properly. Any spilled solutions or broken equipment will not be replenished.
- Stop answering and put down your pen immediately when the bell rings at the end of the exam. Enclose the **Question Paper** and **Answer Sheet** in the provided envelope.
- No paper, materials or equipment should be taken out of the laboratory.

Good luck!!!

Materials and Equipment

Experiment 1. Classification of Butterflies

Name	**Quantity**
Box containing 8 butterfly specimens	1 box
Mask	1 piece
Forceps	1 pair
Magnifier glass	1 piece
Ruler	1 piece
Pen	1 piece
Calculator	1 piece
Scratch papers for calculating	1 set
Gloves	2 pairs
Tissue papers	1 box

Name	Quantity
Alcohol pre-treated earthworm	1 specimen
Stereomicroscope	1 piece
Tray	1 piece
Forceps	1 pair
Mounted needle	1 piece
Scissors	1 piece
Knife	1 piece
Petri disc	1 piece
Glass slide	1 piece
Pipette	1 piece
Plate with pins	1 plate
Plate with 5 colour-headed pins	1 plate
Magnifier glass	1 piece
Gloves	2 pairs
Mask	1 piece
Student Code Sheet	1 piece
Pen	1 piece
Tissue papers	1 box

* Participants carefully check the materials and equipment. If any of these items is missing or damaged or unable to distinguish the colour-headed pins, please raise the RED card immediately.

EXPERIMENT 1. CLASSIFICATION OF BUTTERFLIES (65 POINTS)

Introduction

Vietnam has rich and diverse fauna and flora. There are more than 1,200 species of butterflies. However, due to habitat disturbance and destruction, some species are threatened and endangered, thus learning about butterflies may contribute to preserving their diversity. The purpose of this practical task is to identify some butterfly species in Vietnam and compile the phylogenetic relationship of these species based on their morphological characters.

Note: Butterfly box will be a gift for the participants after completing all the practical (*Please, write your name in the label on the box*).

Task1. Identify all the butterfly specimens provided

Identify all butterfly specimens (A to H) using the following identification keys. Consult the figures (1, 2, 3) below to identify the required morphological characters.

Morphological characters

The following figures describe the required morphological characters.

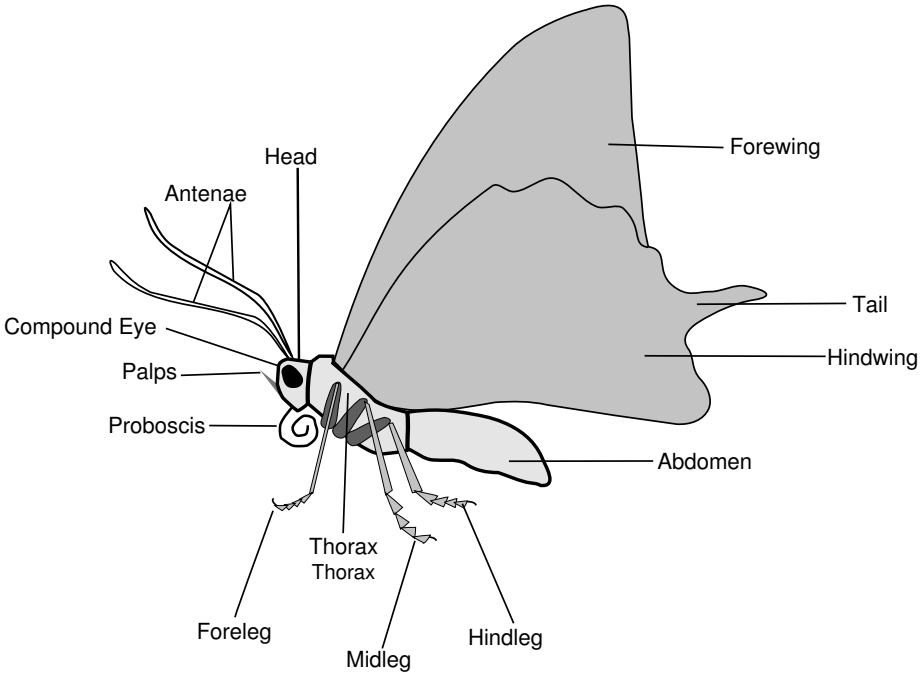


Figure 1. External structure of butterfly.

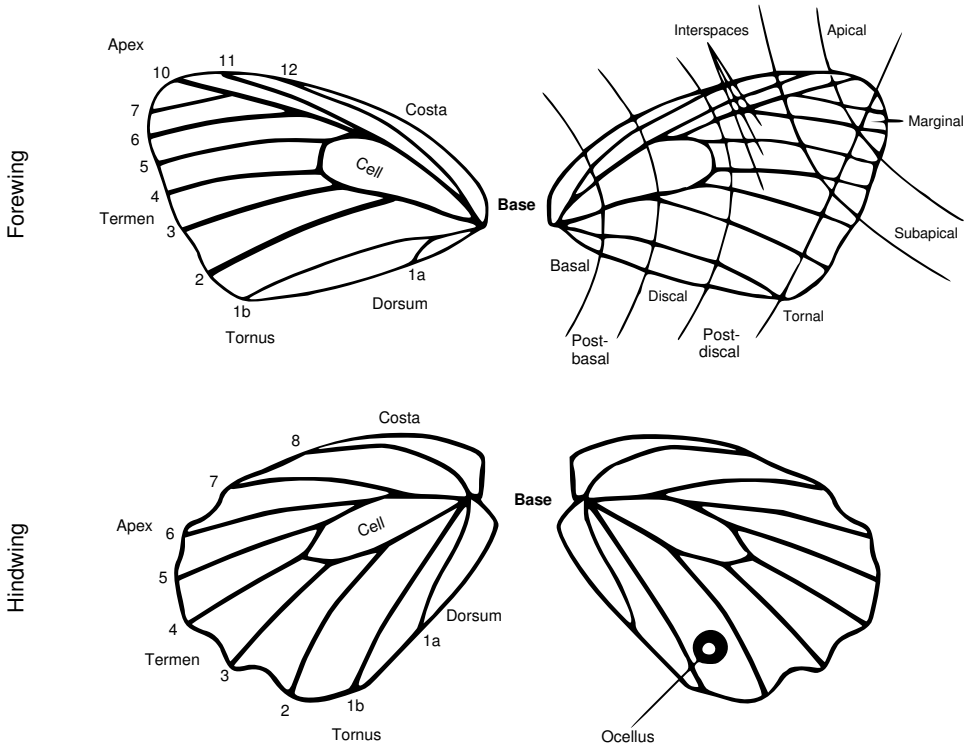


Figure 2. Butterfly wing terms.

The wing is divided into several areas and interspaces. The wing vein are numbered. The forewing veins are numbered from 1 (1a, 1b) to 12; the hindwing veins are numbered from 1 (1a, 1b) to 8.

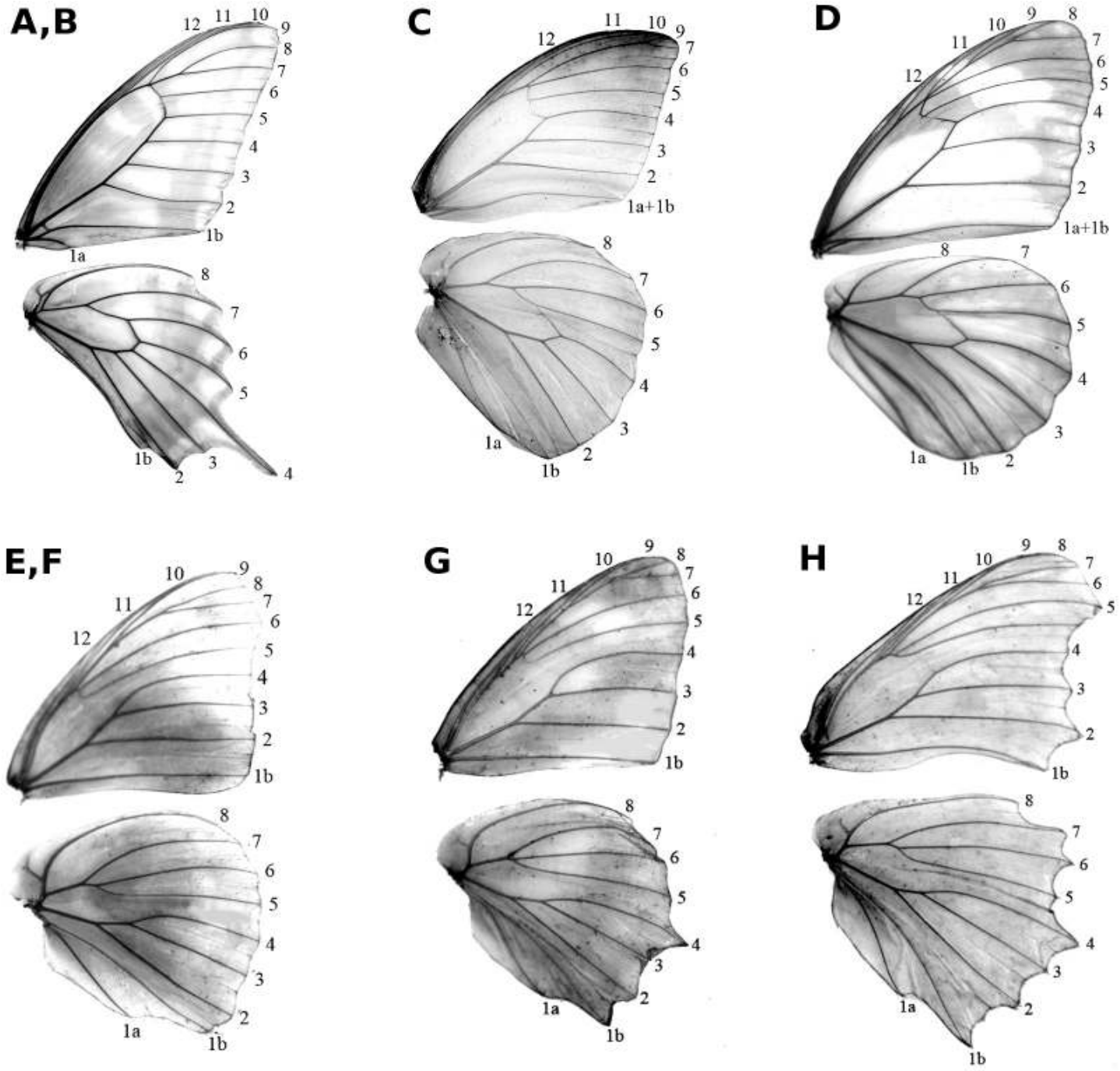


Figure 3.

Specimens A, B) With Veins 1a, 1b in the forewing; without Vein 1a on the hindwing; with long tail on the hindwing,

Specimen C) With Veins 1a, 1b in the forewing intersected; Veins 8 and 9 in the forewing overlapped.

Specimen D) With Vein 1a, 1b in the hindwing; Vein 1a and 1b on the forewing intersected.

Specimens E, F) With Vein 1a, 1b in the hindwing, without Vein 1a on the forewing, open wing cells ; apex of forewing cut

Specimen G), H) Without Vein 1a in the forewing, with Veins 1a and 1b in the hindwing, knob tail on the Vein 4 in the hindwing; open wing cells.

Specimen H) Apex of forewing cut.

Identification Key for Butterflies

1.	Hindwing **with** long tail	Continue at 2.
	Hindwing **without** long tail	Continue at 6.
2.	A white **patch** on the wing	Continue at 3.
	No white patch on the wing	Continue at 4.
3.	Forewing **with** white spots in interspace between 1a and 1b (Figure 2)	***Papilio noblei***
	Forewing **without** white spots in interspace between 1a and 1b	***Papilio helenus***
4.	Upper side of wings **with** a pale yellowish green macular band from the apex to the mid of dorsum of the forewing	***Papilio demolion***
	Upper side of wings **without** a pale yellowish green macular band from the apex to the mid of dorsum of the forewing	Continue at 5.
5.	Upper side of hindwings tornus with a **red spot** but **no black dot** inside	***Papilio machaon***
	Upper side of hindwings tornus with an **orange or pale yellow spot** and a **black dot** inside	***Papilio xuthus***
6.	White or yellow wings	Continue at 7.
	No white or yellow wings	Continue at 8.
7.	**Yellow** wings with a wide orange band in the forewings	***Ixias pyrene***
	White wings with a big red-orange patch on half of forewing	***Hebomoia glaucippe***
8.	Apex of forewing **rounded or pointed**	Continue at 9.
	Apex of forewing **cut**	Continue at 11.
9.	Hindwing with knob tail; upper side with orange bands on brown wings	***Symbrenthia lilaea***
	Hindwing **without** knob tail	Continue at 10.
10.	Wing veins **brown**; upper side of hindwings orange **without** black spots	***Danaus genutia***
	Wing veins **not brown**; upper side of hindwings orange **with** black spots	***Danaus chrysippus***
11.	**Ocelli** on wings	Continue at 12.
	No ocelli on wings	Continue at 13.
12.	**Blue** hindwing; **upper** side of hindwing with 2 ocelli	***Junonia orythia***
	No blue hindwing; **lower** side with darker brown transverse bands	***Junonia iphita***
13.	Black wings with white macular bands and spots	***Athyma asura***
	Orange wings with black spots	***Polygonia c–aureum***

Q.1.1. CLASSIFICATION OF BUTTERFLIES (16 POINTS)

Mark the correct species name for each **Specimen A-H** with a "✓" in the **ANSWER SHEET**.

Task 2. Compile the character matrix

Consider the following characters:

a. Long tail on hindwing
b. Knob tail on hindwing
c. Vein 1a on forewing
d. Vein 1a on hindwing
e. Wing vein 8 and 9 in forewing: overlapped = 1, not overlapped = 0
f. Wing vein 1a: "stretches the dorsum near the base of forewing " = 1, "other cases" = 0
g. Wing vein 1a and 1b in forewing: intersected = 1, other cases = 0
h. Wing cells: open = 1, close = 0
i. Orange hindwings with brown wing veins
j. A big red-orange patch on half of forewing
k. Upper side with orange bands on brown wings
l. A series of white spots on the marginal area of wings
m. White patch on hindwing
n. Upper side hindwing with an orange or a pale yellow tornal spot with a black dot inside
o. Ocelli on wings
p. Apex of forewing: cut = 1, rounded or pointed = 0

Q.2.1 CHARACTER MATRIX (25.6 POINTS)

Compile the character matrix for the character listed above in the **ANSWER SHEET.** Use “1” for present and “0” for absent.

Task 3. Calculate the distance matrix based on the characteristic matrix provided

For the remaining part of this experiment use the character matrix provided Table 1. This matrix is not related to Tasks 1 and 2.

Characters	A	B	C	D	E	F	G	H
1	1	1	0	0	0	0	0	0
2	1	0	0	0	0	0	0	0
3	0	1	0	0	0	0	0	0
4	0	0	1	0	0	0	0	0
5	0	0	0	1	0	0	0	0
6	0	0	1	0	0	0	0	0
7	0	0	0	0	1	0	0	0
8	1	1	1	0	0	0	0	0
9	1	1	0	0	0	0	0	0
10	0	0	0	0	0	0	1	1
11	0	0	1	0	0	0	0	0
12	0	0	1	1	0	0	0	0
13	0	0	0	1	1	1	1	1
14	1	1	0	0	0	0	0	0
15	0	0	0	0	1	1	1	1
16	0	0	0	1	0	0	0	0
17	0	0	0	0	1	1	0	0
18	0	0	0	1	1	1	1	1
19	1	0	0	0	0	0	0	0
20	0	0	0	0	1	1	1	1
21	0	0	0	0	1	1	0	1
22	0	0	0	0	0	0	0	1
23	0	0	0	1	0	0	0	0
24	0	1	0	0	0	0	0	0
25	0	0	0	0	0	1	0	0
26	0	0	0	0	0	0	1	0

Q.3.1. CALCULATE DISTANCE MATRIX

Calculate the distance matrix based on the character matrix provided in Table 1. The distance between two specimens is defined as the number of characters at which the two specimens show different character states (present: “1”; absent: “0”). Write the numerical results in the **ANSWER SHEET (8.4 points).**

Reconstructing Phylogenetic Realtionship using UPGMA

UPGMA (Unweighted Pair Group Method with Arithmetic Mean) is considered the simplest method for reconstructing phylogenetic trees with the assumption that the data provided have constant rates of evolution. In the method, the pair of clusters with the shortest distance is combined into a cluster of higher level at each iteration. To illustrate this concept, consider the numbers of character differences between the taxa (specimens) M, N, O, P, and Q.

Taxa	M	N	O	P	Q
M	0				
N	2	0			
O	6	6	0		
P	4	5	7	0	
Q	7	8	9	7	0

Iteration 1: The pair of clusters with the smallest distance is the pair M and N, which is thus combined into a higher–level cluster (M,N). The relative age of newly formed cluster is computed as half the distance between two original clusters. In this case, the relative age of the cluster is 1. Next, a new matrix of all distance is generated by computing the distance between clusters as the average distance between all taxa from one cluster to all taxa of the other cluster. The distance between Cluster (M,N) and Cluster (P), for instance, is computed as the average between d(M,P) and d(N,P) as (4+5)/2, where d(x,y) is a notation to indicate the distance between Clusters x and y. The result is presented as the table below:

Taxa	(M,N)			
(M,N)	0.0	O		
O	6	0.0	P	
P	4.5	7	0.0	Q
Q	7.5	9	7	0.0

Iteration 2: The pair of clusters with the smallest distance is now the pair of MN and P, which is thus combined into a higher–level cluster ((M,N),P) with a relative age of 2.25. Again, a new matrix is constructed by calculating all distances as indicated above. The distance between Cluster ((M,N),P) and Cluster (O), for instance, is computed as the average between d(M,0), d(N,O), and d(P,O) as (6+6+7)/3 = 6.33. The result is presented as the table below:

Taxa	((M,N),P)		
((M,N),P)	0.0	O	
O	6.33	0.0	Q
Q	7.33	9	0.0

*Iteration 3***:** The pair of clusters with the smallest distance is now the pair of MNP and O, which is thus combined into a higher–level cluster **(((M,N),P),O)** with a relative age of 3.17. Again, a new matrix is constructed by calculating all distances as indicated above. The result is presented as the table below:

Taxa	(((M,N),P),O)	
(((M,N),P),O)	0.0	Q
Q	7.75	0.0

Iteration 4: In the last cluster, the two remaining taxa are combined into the new cluster (**(((M,N),P),O),Q)** with a relative age of 3.88.

Task 4. Resolve the phylogenetic relationship of all the specimens

Resolve the phylogenetic relationship of all specimens (A–H), showed at the Table 1, iteratively using the UPGMA method and based on the distance matrix you compiled above (Task 3). Make sure to report the names of the clusters using the Specimen codes A to H. Write the numerical results in the **ANSWER SHEET (10.75 points**).

Q.4.1. UPGMA ITERATION 1 (3 POINTS)

Q.4.2. UPGMA ITERATION 2 (2.5 POINTS)

Q.4.3. UPGMA ITERATION 3 (2 POINTS)

Q.4.4. UPGMA ITERATION 4 (1.5 POINTS)

Q.4.5. UPGMA ITERATION 5 (1 POINT)

Q.4.6. UPGMA ITERATION 6 (0.5 POINTS)

Q.4.7. UPGMA ITERATION 7 (0.25 POINTS)

Task 5. Draw a phylogenetic tree (dendrogram)

Q.5. PHYLOGENETIC TREE (4.25 POINTS)

Draw a phylogenetic tree (dendrogram) based on the UPGMA result in the **ANSWER SHEET**. Indicate the relative length of each branch by writing the correct numbers next to it.

EXPERIMENT 2. ANATOMY OF EARTHWORM (*AMYNTHAS ASPERGILLUM*) (35 POINTS)

Introduction

The earthworm *Amynthas aspergillum* belongs to the Family Megascolecidae, Phylum Annelida and is a common species in Vietnam. It is rich in protein and suitable food for fish, poultry, and cattle. The species is experimentally raised and used for improving the quality of soil in several areas in Vietnam. This practical test is for you to dissect and identify the external and internal structures of *Amythas aspergillum*.

Task 6. Identify the external structure of *Amynthas aspergillum*.

Use a magnifier glass or stereomicroscope to observe the dorsal pores, clitellum, and chaetae (setae) of *Amynthas aspergillum*. Then, answer the following three questions the **ANSWER SHEET.**

Q.6.1. (3 POINTS)

Indicate in the Answer sheet with a "✓" which of the following statements is True.
Location of the clitellum is from (The segment number is counted from the position behind the labium)

Q. 6.2. (3 POINTS)

Indicate in the Answer sheet with a "✓" which of the following statements is True.
The chaetae distribution in each segment is

Q.6.3. (3 POINTS)

Indicate in the Answer sheet with a "✓" which of the following statements is True.
The number of dorsal pore on each segment just behind the clitellum is

Task 7. Dissecting and identifying the internal structure of *Amynthas aspergillum*

- Place the specimen in the dissecting tray, dorsal side up (Figure 4A).
- Locate the clitellum and insert the tip of the scissors about 3 cm from the clitellum posteriorly (Figure 4B).

A **B**

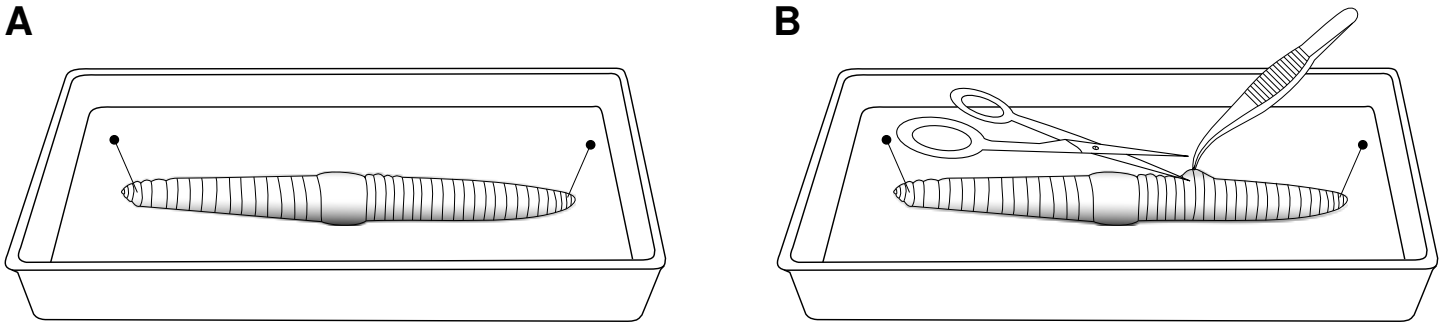


Figure 4.

- Cut skin carefully in both ways up to the head and to the anus. Try to keep the scissors pointed up, and only cut through the skin.
- Spread the skin of the worm out, use a knife to cut the septa (avoid damaging the internal organs).
- Place pins in the skin to hold it apart, angle the pins out so that they are not in your way.
- Pour water into the tray until the earthworm is submerged.

Q.7.1. (3 POINTS)

How many pairs of spermatheca are in *Amynthas aspergillum*? Write the correct number of spermatheca pair in the box in the **ANSWER SHEET**.

Q.7.2 (6 POINTS)

Observe the inside of the body wall and determine the presence/absence of septa between the following segments.

Indicate in the Answer sheet with a "✓" if septa are present or absent in the **ANSWER SHEET.**

Q.7.3. (10 POINTS)

- Identify the following organs by using the appropriate colour–headed pins.

Colour-headed pin	**Organ**
Blue	Stomach
White	Caecum
Red	Seminal vesicle
Yellow	Prostate gland
Purple	Nerve ganglion chain

- Write your student code on the "Student Code Sheet" and place it besides the tray.
- ***Raise the green card to inform the supervisor to take photographs and confirm the results on the "Dissecting result confirmation sheet".***

Q.7.4. (4 POINTS)

- Using the knife to make a cross section (about 0.5 – 1 mm) of the intestine at around segments 30th to 40th. Put this cross section into Petri dish containing water and gently move it in water to remove all remained food. Put the section on the glass slide, add a drop of water, observe the section under the stereomicroscope.
- ***Raise the green card to inform the supervisor to take photographs and confirm the results on the "Dissecting result confirmation sheet".***

Q.7.5 (3 POINTS)

Which of the following best describes the intestinal typhlosole observed in the cross section?

A. Intestinal typhlosole ≥ radius of intestine (Figure 5A)
B. Intestinal typhlosole branched (Figure 5B)
C. Intestinal typhlosole < 1/2 radius of intestine (Figure 5C)
D. No intestinal typhlosole (Figure 5D)

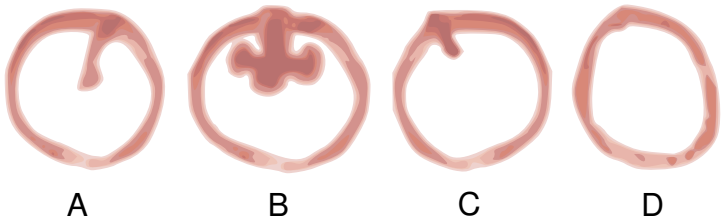


Figure 5.

Indicate in the Answer sheet with a "✓" the cross section that is observed.

End of Practical Exam 2