

# INVESTIGATING A DEEP SEA MYSTERY

## KEY

For centuries, ichthyologists, scientists who study fishes, have been fascinated by what lives in the deep ocean. The fishes that live in this environment, where the pressure is high and sunlight does not reach, have some unusual shapes and interesting (even bizarre) adaptations. We still know very little about many of them, because they are so difficult to collect and essentially impossible to observe in their habitat. Even fundamental questions about how the fishes are related and how they should be classified are a source of ongoing investigations.

A team of scientists\* from the United States, Japan, and Australia undertook an investigation into one such question. What they discovered was amazing indeed!

**Your goal:** Follow in the footsteps of these ichthyologists and investigate a set of deep sea fishes to generate and test hypotheses about their relationships.

\* Dave Johnson (National Museum of Natural History, Smithsonian Institution), John Paxton (Australian Museum, Sydney), Tracey Sutton (Virginia Institute of Marine Science, Virginia), Takashi Satoh and Mutsumi Nishida (Ocean Research Institute-University of Tokyo), and Tetsuya Sado and Masaki Miya (Natural History Museum and Institute in Chiba, Japan)

## PART I - INTRODUCTION - WHAT IS A FISH?

### What is a fish?

Draw a picture of a fish. Be sure to include and label the features you think all fishes have and can be used to identify something as a fish. Make a list of other important behaviors or traits that all fishes have.

### Fish Checklist

After reading *What is a fish?*, create a checklist you could use to determine if something is a fish.

- \_\_\_\_\_ *Lives in water* \_\_\_\_\_
- \_\_\_\_\_ *Has a cranium* \_\_\_\_\_
- \_\_\_\_\_ *Has gills* \_\_\_\_\_
- \_\_\_\_\_ *Has fins* \_\_\_\_\_
- \_\_\_\_\_ *Cold blooded (ectothermic)* \_\_\_\_\_
- \_\_\_\_\_ *Has scales* \_\_\_\_\_

What are some exceptions to the general fish characteristics you listed?

*Some fish do not have scales*

*Some fish have lung structures*

*Some fish are essentially warm blooded (endothermic)*

## PART I - INTRODUCTION - WHAT IS A FISH?

**What is a Fish Notes:**

## PART II - CLASSIFYING SOME UNUSUAL FISH

### **Notes: Deep Sea Fish Classification**

What did you learn about the deep-sea fishes that you classified?

*Answers will vary.*

## PART III - LOOKING FOR PATTERNS IN COLLECTION DATA

### Fish Collection Data Table

Number of specimens collected	Cetomimidae Whalefishes	Megalomycteridae Bignose fishes	Mirapinnidae Tapetails
<b>Total</b>	<b>600</b>	<b>65</b>	<b>120</b>
<b>Adults</b>	<b>600</b>	<b>65</b>	<b>0</b>
<b>Immature</b>	<b>0</b>	<b>0</b>	<b>120</b>
<b>Females</b>	<b>600</b>	<b>0</b>	<b>?</b>
<b>Males</b>	<b>0</b>	<b>65</b>	<b>?</b>
<b>Size (mm)</b>	<b>26-408</b>	<b>34-68</b>	<b>5-56</b>
<b>Depth caught (m)</b>	<b>Below 1000</b>	<b>Below 1000</b>	<b>Above 200 1 caught below 200</b>

The table above is a summary of data from members of the three clades that has been collected by scientists over many years. Take a close look at the data. What patterns do you observe?

*Students should notice that all the collected Whalefish specimens are adult females, all Bignose specimens are adult males, and the only Tapetails caught are immature. They should also see that all but one Tapetail was collected above 200m, whereas individuals from the other two groups are all found at depths below 1000m. They may also observe many more Whalefishes have been collected than the other two groups. Tapetails have a much smaller minimum size than the other two groups and Whalefishes grow to be the largest.*

How does this data impact the Three Clade Hypothesis?

*The patterns in the data call into question that these fishes belong in three different clades. One would expect to see males and females, larva and adults among the specimens of each group if they were indeed separate clades.*

What is an alternative explanation or hypothesis to explain the patterns in the data?

*The members of the different fish groups are really members of the same clade and represent different sexes and life stages. This represents the Single Clade Hypothesis that the students will be investigating.*

## PART III - LOOKING FOR PATTERNS IN COLLECTION DATA

### Two Hypotheses to Test

#### 1. Three Clade Hypothesis

*The three types of fishes are in three different clades. The patterns in the data could be a result of incomplete sampling (i.e. we have not yet caught representatives of each of the sexes and growth forms).*

#### 2. Single Clade Hypothesis

*The fishes are all part of the same clade. Whalefishes, Bignose fishes and Tapetails are the female, male and larval forms of species within the same clade. The huge differences seen between Whalefishes and Bignose fishes are the result of extreme sexual dimorphism. Tapetails are the larval forms that transform into the adult forms (Whalefishes and Bignose fishes).*

What types of data would you like to collect to help you test the hypotheses?

*Answers will vary.*

## PART IV - TESTING HYPOTHESES – USING MORPHOLOGY

### Testing Hypotheses Using Morphology

Testing hypotheses involves using different types of data to evaluate each explanation. You will use three different types of morphological data to test the two hypotheses that explain the classification and relatedness of the deep sea fish specimens: gut, muscle, and gill.

### Gut Morphology

Use the Gut Morphology Card to make observations about each fish group's internal anatomy associated with feeding. In the table below, use a checkmark "✓" to indicate which fishes have a normal presence of the feature. If a fish does not have a feature, leave it blank. If the feature is an unusual size or shape make a note in the table.

Feature	Whalefishes	Bignose fishes	Tapetails
Stomach	✓		<i>distended</i>
Esophagus	✓		✓
Liver	✓	<i>enlarged</i>	✓
Intestine	✓	✓	✓
Other observations		<i>enlarged testes</i>	

What inference might you make about the feeding habits of each fish?

*Whalefishes have normal gut structures so they must have typical feeding habits of fish. The lack of an esophagus and stomach in Bignose fishes suggests that these fishes do not feed. They do have a very large liver that could provide a nutrient reserve. Therefore, it could be inferred that these adults must obtain food reserves in an earlier stage of development, such as in the larval stage, which are then stored in the enlarged liver. The enlarged testes suggest that they are males that are able to reproduce. Since some Tapetails are found with distended guts full of more copepods than could be digested in a short timeframe, one could infer that this serves as a food reserve for later use.*

What do these data suggest about the accuracy of each hypothesis?

*This evidence does not impact the **Three Clade Hypothesis**.*

*This line of evidence best supports the **Single Clade Hypothesis**.*

*If Bignose fishes were a separate clade, they would not be able to survive without a stomach and an esophagus to obtain food. However, if Tapetails are larvae, then it is possible that the nutrients in the distended stomachs of Tapetails are transformed into the large liver seen in Bignose fishes or adult males. These adult males could live off this reserve and would not need to search for food. The enlarged testes suggest that these fish have a sole mission to reproduce.*

## Muscle Morphology

Use the Muscle Morphology Card to make observations about the relative proportion of red muscle tissue visible in each type of fish. Use a checkmark “✓” to record your observations.

Visible Red Muscle	Whalefishes	Bignose fishes	Tapetails
Proportionally high	✓	✓	
Proportionally low			✓

What inference could you make about the swimming capabilities of each group of fish?

*Large amounts of red aerobic muscles in Whalefishes and Bignose fishes suggest that they are strong swimmers. The Tapetails have proportionally less red muscle, and so more white anaerobic muscle visible, suggesting that they are relatively weak swimmers.*

What do these data suggest about the accuracy of each hypothesis?

*This line of evidence does not impact the **Three Clade Hypothesis**; these three groups could have different lifestyles.*

*This line of evidence supports the **Single Clade Hypothesis**.*

*The weak swimming ability of the Tapetails suggests that they could be a larval form, because many larval forms live high in the water column and feed on plankton, e.g. they are drifters. Their long tails also make them unable to swim well. The proportionally high amount of red muscle in Whalefishes suggests that they are strong swimmers, typical of many adult fishes. The dominance of red muscle in Bignose fishes suggests a strong sustained swimming ability that would allow them to search for mates, which can be particularly challenging given the deep, dark environment in which they live.*

## Gill Arch Morphology – Looking for transitional specimens

Use the Gill Arch Morphology Cards to make observations about the structures and locations of specific features in the gill arches of these fishes. In the table below, record whether each specimen has a **tongue** or no tongue; the **orientation of the bone** (horizontal, vertical, or the angle from horizontal); and the **shape of the gill rakers** (e.g. thick or thin, forked or conical, smooth or toothed).

Feature	Specimen 1 Whalefish	Specimen 2 Tapetail	Specimen 3 Young Whalefish	Specimen 4 Young Whalefish
Tongue	<i>no tongue</i>	<i>tongue</i>	<i>tongue</i>	<i>no tongue</i>
Bone 1	<i>vertical</i>	<i>horizontal</i>	<i>at 10° angle from horizontal</i>	<i>at 45° angle from horizontal</i>
Gill Rakers	<i>thick, conical, toothed</i>	<i>thin, forked, smooth</i>	<i>cannot determine</i>	<i>thick, conical, toothed</i>

Is it possible to order the specimens in a way that shows a transition from a larval form to adult (e.g. Tapetail to Whalefish)? If so, list the specimens in the order you suspect represents how they would develop.

*Yes. The changes in the features support a transition in the following order (from larva to adult): Specimen 2 – Specimen 3 – Specimen 4 – Specimen 1.*

What do these data suggest about the accuracy of each hypothesis?

*This line of evidence does not impact the **Three Clade Hypothesis**.*

*This line of evidence supports the **Single Clade Hypothesis**. Both of the transitional specimens have structures that show a change from Tapetail to Whalefish.*



## External Morphology Revisited

### Summary of Observations

*Tapetails (larvae), Whalefishes (adult females) and Bignose fishes (adult males) have very different anatomy. Larvae tend to live in the upper 200 meters of water and have large eyes for finding food and navigating in the light. Adults live in deep water where sunlight does not penetrate, and so do not rely on vision to navigate.*

What inferences can you make about how the fishes are related? What do these data suggest about the accuracy of each hypothesis?

*Students should be able to use the different lines of evidence to claim that the **Single Clade Hypothesis** is best supported from the morphological data. It appears that the three fish groups are members of the same clade but represent the male, female, and larval forms. This is an example of extreme metamorphosis from larva to adult and sexual dimorphism.*

What other lines of morphological evidence would you seek in order to further test the two hypotheses?

*Answers will vary.*

What new questions should be explored?

*Answers will vary.*

## PART V - TESTING HYPOTHESES – USING PHYLOGENETIC TREES

### An Exercise in Tree Interpretation

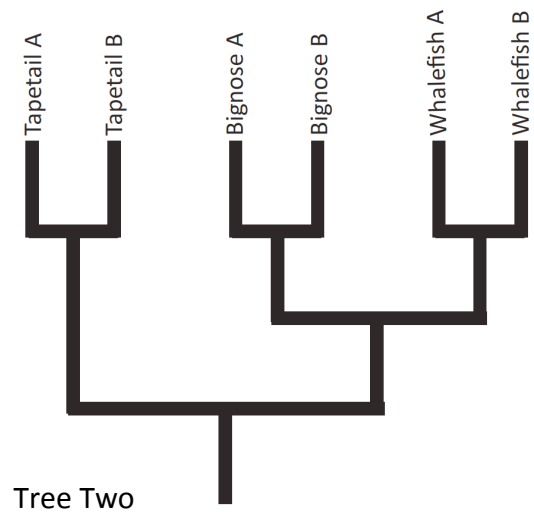
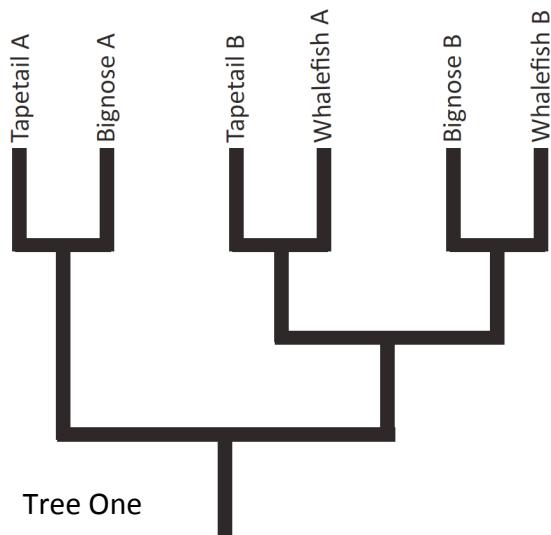
Phylogenetic trees are another line of evidence that can be used to test hypotheses about the classification and evolution of organisms. Before looking at the real fish phylogenetic data, practice your skill at tree interpretation using the two below.

Which tree best supports the Three Clade Hypothesis?

*Tree Two*

Which tree best supports the Single Clade Hypothesis?

*Tree One*

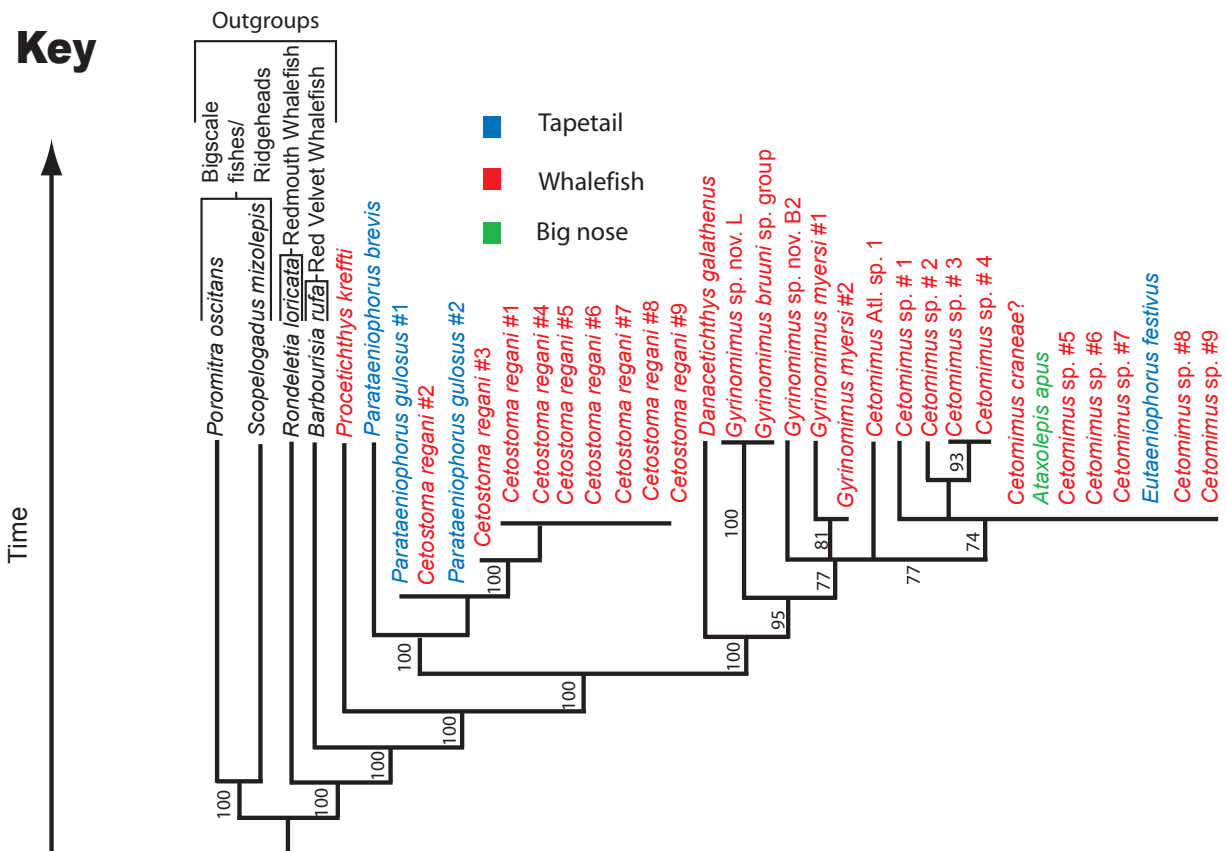


## PART V - TESTING HYPOTHESES – USING PHYLOGENETIC TREES

### Phylogenetic Trees

The following is a real phylogenetic tree based on ribosomal DNA. Create a color-coded key for each of the fish groups; Whalefishes, Bignose fishes, and Tapetails, and then use the Deep Sea Specimen Key to identify each fish species in the tree by color.

*NOTE: Outgroups include Bigscale Fishes/Ridgeheads, Redmouth Whalefishes and Red Velvet Whalefishes (these groups are not true Whalefishes).*



Maximum Likelihood tree based on partial 16s ribosomal DNA sequences. Numerals beside internal branches indicate bootstrap values (only 50% and above are shown) based on 1000 replicates.

Look closely at the patterns that appear on the tree. Which hypothesis does this tree best support?

*The molecular tree supports the **Single Clade Hypothesis**.*

## PART VI – WRAPPING IT UP

### Summary Report

Summarize your findings

#### **How can three different looking fishes be one?**

*Three different looking fishes can be the same when they are different sexes and are in different stages in the fish life cycle. With the help of molecular and morphological analyses, collection of new specimens, and an understanding of fish life cycles and sexual dimorphism, Johnson and his colleagues have gained a more complete understanding of the fishes commonly referred to as Tapetails, Whalefishes and Bignose fishes. The original family Mirapinnidae (Tapetails) are larval Whalefishes and live in the upper 200 meters of the ocean and have small upturned mouths that help them gorge on their food—copepods. These larvae metamorphose into juveniles and then become sexually mature (adults). Female Tapetails metamorphose into the fishes originally considered to be Whalefishes, whereas male Tapetails metamorphose into Bignose fishes. Males and females live in deeper waters and have drastically different morphologies because of their different feeding regimes. The original Cetomimidae (Whalefishes) was composed of adult females with large gaping mouths and long horizontal jaws. This type of mouth allows females to capture larger prey than the males or larval forms. The original Megalomycteridae (Bignose fishes) are adult male Whalefishes. Before the males are sexually mature (i.e. become adults), they stop eating—the stomach and esophagus are lost and the fish convert the energy from the huge amount of copepods they ate as larvae into a large liver that will support them for the length of their adult life (the timing of these changes are not well understood). Their main goal is to find females. This is aided by the presence of the large nasal organ that is so distinct on Bignose fishes. Today, we know that Tapetails, Bignose fishes, and Whalefishes all belong to the same family—the Cetomimidae—this name is used because it is the oldest family name applied to these fishes. The changes in the skulls, body shapes, and organ systems between the larvae, and male and female adults makes members of the Cetomimidae an extreme example of metamorphism and sexual dimorphism in vertebrates.*

Questions for future investigations

*Answers will vary.*