Brilliant Barnacles

Evidence for evolutionary relationships

Teacher's Pack

LinneanLearning

DARWIN INSPIRED LEARNING

Contents

Specification links

AQA AS and A-Level Biology: Module 3 Genetic information, variation and relationships between organisms

Section 3.4.4: Genetic Diversity and Adaptation

- Natural selection results in species that are better adapted to their environment. These adaptations may be anatomical, physiological or behavioural.

Section 3.4.5: Species and Taxonomy

- A phylogenetic classification system attempts to arrange species into groups based on their evolutionary origins and relationships. It uses a hierarchy in which smaller groups are placed within larger groups, with no overlap between groups. Each group is called a taxon (plural taxa).
- One hierarchy comprises the taxa: domain, kingdom, phylum, class, order, family, genus and species.
- Each species is universally identified by a binomial consisting of the name of its genus and species, eg, Homo sapiens.
- Recall of different taxonomic systems, such as the three domain or five kingdom systems, will not be required.

OCR A-Level Biology: Module 4 Biodiversity, Evolution and Disease

Section 4.2.2 - Classification and Evolution

- Evolution has generated a very wide variety of organisms. The fact that all organisms share a common ancestry allows them to be classified. Classification is an attempt to impose a hierarchy on the complex and dynamic variety of life on Earth. Classification systems have changed and will continue to change as our knowledge of the biology of organisms develops.

Edexcel A-Level Biology: Topic 3 Classification and Biodiversity

Topic 3.1: Classification.

- Discuss classification system consists of a hierarchy of domain, kingdom, phylum, class, order, family, genus and species.
- Understand the limitations of the definition of a species as a group of organisms with similar characteristics that interbreed to produce fertile offspring.
- Understand why it is often difficult to assign organisms to any one species or to identify new species.
- Understand how gel electrophoresis can be used to distinguish between species and determine evolutionary relationships.
- Know that DNA sequencing and bioinformatics can be used to distinguish between species and determine evolutionary relationships.
- Understand the role of scientific journals, the peer review process and scientific conferences in validating new evidence supporting the accepted scientific theory of evolution.
- Understand the evidence for the three-domain model of classification as an alternative to the five-kingdom model and the role of the scientific community in validating this evidence.

WJEC AS Biology Unit 2: Biodiversity and Physiology of Body Systems

Section 1: All organisms are related through their evolutionary history.

Section 2.1: All organisms are related through their evolutionary history

- The classification of organisms into groups based on their evolutionary relationships and that classification places organisms into discrete and hierarchical groups with other closely related species
- The need for classification and its tentative nature
  The three domain classification system as compared with the five Kingdom classification system
- The characteristic features of Kingdoms: Prokaryotae, Protoctista, Plantae, Fungi, Animalia
- The use of physical features and biochemical methods to assess the relatedness of organisms, including that DNA 'genetic fingerprinting' and enzyme studies show relatedness without the problem of morphological convergence
- The concept of species
- The use of the binomial system in naming organisms

SQA Higher Unit 2: DNA and the Genome

- Evolution.

Darwin-Inspired Learning

1. Encourages a sense of place and direct engagement with the natural world using local environments and the environment of Down House and other places Darwin worked.
2. Has a pedagogy of enquiry which places importance in:
   - Active learning - seeking out experiences and questions, solving problems and dialogue between teachers and pupils and between learners.
   - Teaching that facilitates imagination and thoughtful hands-on inquiry as well as the delivery of high quality engaging content.
   - Teaching that engages critical, creative thinking about how we know and how scientists work.
3. Encourages interdisciplinary studies, with Darwin as the context, between science, literature, writing and expression, history, religious studies, geography and horticulture, dance and drama, design and technology, numeracy, music and art.

How to use this pack

There are three lessons in this module. Each lesson is laid out in the suggested order of teaching.

Depending on when in the A Level course this module is used, more explanation of terms may be required as some elements use knowledge gained in other units of the specifications.

At the start of each lesson there is a timing guide. However, you may want to allow more or less time depending on what you would particularly like to focus on with your students. Although you can choose to miss certain activities, we recommend working through all of them to provide students with a broader understanding of the topic.

In the teacher's pack, the answers to the activities have been provided in green. Please bear this in mind if you choose to print this pack in black and white.

Powerpoint slide numbers are given at the start of the text they refer to.

The module is complemented by videos of Darwin Inspired Scientists which provide a case study of how scientists work today and the relevance of Darwin to their work and current research.

Some of the activities in this pack are practical-based, requiring the use of practical equipment. These are highlighted with a microscope symbol.

Darwin Inspired Scientists:

Video interviews with a contemporary scientist www.bit.ly/DarwinsBarnacles.

Further sources of information:

Invertebrate Zoology Online by Richard Fox, Lander University. This online laboratory manual has an extensive range of exercises that may be suitable for the classroom. It also features anatomical descriptions of species: http://bit.ly/LabInvert

The best UK book on all aspects of brine shrimps, *Brine Shrimp Ecology* by Michael Dockery and Stephen Tomkins, is available to download free from the British Ecological Society: http://bit.ly/BrineShrimpEcology

"The secret life of barnacles" written by Professor Phil Rainbow provides an excellent introduction to classification, structure and functioning of barnacles: http://bit.ly/SecretBarnacles

A very authoritative article by Marsha Richmond that gives more depth to the issues raised in this module about phylogeny and classification: http://bit.ly/DarwinsCirripedia

The Darwin on-line website provides free access to all his publications including those on barnacles: http://bit.ly/DarwinsPapers

The Microscopy UK website provides a general introduction to microscopes; including their history and how to use one: http://bit.ly/IntroMicroscopes

Module Overview

Learning objectives and outcomes

Students will:

- Develop an understanding of the relationship of taxonomy to phylogeny.
- Understand that phylogenetic relationships are based on theories and depend on the data sources used and that new phylogenies develop as new sources of evidence and analysis are available.
- Apply practical skills to use a light microscope and prepare slides.
- Interpret scanning electron micrographs and know their limitations.
- Develop an appreciation of how scientists have built on Darwin's work using DNA evidence.
- Appreciate the significance of Darwin's work on barnacles and how it affected his theory of evolution.
- Observe *Artemia*, the brine shrimp, and understand the arthropod body plan, structure and life history. Understand how *Artemia* is similar to the barnacle.
- Develop confidence in naming organisms and familiarise themselves with binomial nomenclature.

Introduction to module

During his voyage on *HMS Beagle* as a young man, Charles Darwin collected a vast variety of plants and animals amongst which was a minute barnacle that burrowed into the shells of sea snails. It didn't fit with the existing classification of barnacles. So he pickled it and brought it home in 1836 resolving, as soon as he had time, to study as many forms of the group as he could, to find out how the strange burrowing barnacle from the coast of Chile fitted.

By 1844 he had written his extended essay to sketch out his theory of evolution but then he broke off from getting it published and instead started to study barnacle taxonomy. It took him eight years to study every known fossil and living barnacle and his work forms the basis upon which the serious study of barnacle classification and evolution has been based. In this module students will study barnacle morphology, life histories and life styles as Darwin did and upon which he based his classification and search for a common ancestor.

Keywords

arthropods, crustacea, cirripedes, taxonomy, phylogeny, homology, segmentation, classification, body plans, species, genus, family, order, class, phylum, kingdom, binomial nomenclature, morphology, life histories, nauplius, cypris, DNA sequences, scanning electron microscopy

The theme of this module is the evidence base for evolutionary relationships using Darwin's barnacles as an example. Darwin studied barnacles to give an evidential basis for his theory of evolution. He set out to show how a single animal group had evolved through entirely natural means. The only tools he had available to study the structure of this diverse group of invertebrates were simple lenses and compound microscopes. He also observed the life histories of current species and studied barnacle fossils. Simple microscope work integrated with the binomial naming of species allows students to experience systematic work which is foundational to the study of biodiversity.

Recent work using genetic molecular evidence and scanning electron microscopy are used to show how some key difficulties in drawing the phylogenetic tree of barnacles have been clarified very recently by systematists (scientists who study of the diversification of living forms, both past and present, and the relationships among living things through time).

Darwin's ideas continue to inspire and inform today's scientists and research into barnacle classification and evolution, generating new and intriguing insights into evolutionary relationships.

This module will examine Darwin's legacy for contemporary science and our current understanding of

- taxonomy
- phylogeny
- classification
- life cycles

Darwin's questions and big ideas

- What's the relationship between taxonomy and phylogeny?
- Variation or new species?
- How do you search for a common ancestor?
- How do barnacles colonise new habitats?
- What is the importance of different sorts of evidence in building a family tree?

- Species change through geological time.
- Hidden aspects of the natural world can be very significant.
- The evolutionary tree.
- The common ancestor.

Darwin's barnacles

Darwin's interest in marine life began whilst he was at Edinburgh University, where he was studying medicine, through exploring the sea shore with naturalist Dr Robert Edmond Grant. In 1828, he moved to Cambridge to study theology and classics and accepted Captain Fitzroy's offer to join the survey ship, *HMS Beagle*, as a naturalist sailing on a voyage round the world from Plymouth, starting on 27th December 1831. During the trip he used a plankton net and simple microscope and, when near land, visited shores and estuaries. Early in the voyage at St Jago (now called Santiago), in the Cape Verde Islands, he made detailed notes on the behaviour of the barnacle *Pyrgoma anglicum* and later a minute unknown naked Chilean barnacle which burrowed into the shell of a gastropod mollusc. He named this unknown barnacle *Arthrobalanus* (meaning jointed barnacle) and thought it might be related to the acorn barnacles attached to rocks. Its parasitic nature and absence of shell plates was unusual. He also noticed that it had developing eggs at its base and recorded seeing four different larval stages as the eggs developed. Darwin, unlike many of his contemporaries, recognised his *Arthrobalanus* as a crustacean and a barnacle, but where did it fit into the family tree? He preserved it to study later.

When he returned to England in 1836 he had amassed thousands of specimens and notes. He distributed many specimens for identification by experts while he himself worked on publishing the results of his voyage and investigating two other invertebrate groups he had collected. In 1844, he began in earnest on the practical study of barnacles requesting specimens, living and fossilised, from his contacts across the world. Darwin was looking for an evolutionary interpretation of their classification that would support his arguments in *On the Origin of Species*, the book he had had in manuscript since 1842. He felt he had to provide overwhelming evidence of evolutionary relationships from one family to be scientifically credible. He chose the barnacles, little realising that it would take him eight years of close detailed work. He published his work on *Living Cirripedia* in two volumes in 1851 and 1854.

Darwin's ways of working

- Raising problems through observation and reflection.
- Deep engagement with the natural world, observing and noting.
- Collaborating with others - collectors and museums - to collect all known barnacles.
- Reflection on evidence and ideas and building of theories on evolutionary relationships.
- Collecting, comparing and classifying barnacles.
- Publishing *Living Cirripedia* and *Fossil Cirripedia* describing species.
- Eight years intensive work on one topic - barnacles.
- Attention to the very small internal structures and larvae using a microscope.
- Using existing theories, e.g. homologies, to guide observations.
- Predicting where and when new evidence was needed.

Scientific questions

This unit addresses the following questions:

- What sort of organisms are barnacles?
- What different sorts of barnacle are there and how much does each species vary?
- How do they feed, grow, move, protect themselves and reproduce?
- What morphological features can we use to classify barnacles?
- How can the evidence from DNA and other molecules be used to reveal relationships between barnacles?
- How can you decide what the common ancestor of the barnacles was like?

In addition, barnacles are used today in pollution monitoring and as indicators of global warming. These topics raise other questions:

- How are barnacles distributed in their habitats?
- How does their behaviour and life history affect their tolerance to disturbance?
- How do barnacles colonise new habitats?

Background information

Barnacles

Barnacles are sessile organisms, that is they are fixed to rocks and other objects. Through dissection, Darwin discovered that barnacles are hermaphrodite (that is they have both male and female reproductive organs).

The two most common types of barnacles are goose (or stalked) barnacles and acorn (or sessile) barnacles. Goose barnacles are the less common of the two varieties and live in the ocean, attached to floating debris or ships. At the end of the protruding stalk is a body with legs, held within shell plates. Acorn barnacles live on the seashore, where they are uncovered at low tide, and may also live on the underside of boats. There are also a small number of parasitic barnacles which live within another organism, such as a crab.

Many barnacles have very long penises and can reach over several times their own length to deposit sperm in the mantle cavity of a neighbouring barnacle. In *Semibalanus balanoides*, a common acorn barnacle found on British shores, fertilisation between neighbouring barnacles occurs in November. The sperm are deposited inside in the mantle cavity where the eggs have been released from the ovaries. The fertilised egg mass develops inside the mantle cavity (inside the shell but outside of the body). The barnacle lives in the intertidal zone of the rocky shore and has strong shell plates that shut up when the tide is out, trapping the water inside the mantle cavity to prevent desiccation.

The eggs use the energy of their yolk sacs to develop within the mantle cavity and hatch as nauplius larvae the following March. The nauplii swim out on the high tide and into the sea where they feed on phytoplankton (microscopic plant-like organisms, such as algae).

In the sea the nauplius goes through six stages of growth, moulting its exoskeleton each time and growing larger as its swims in the plankton. It feeds on small algae passing them to its mouth with the mandibles. This stage is free-swimming and enables the sessile barnacles to invade new habitats.

In April, the nauplius metamorphoses into the non-feeding cyprid stage. The cypris has a hard shell (or carapace) and its role is to find a suitable place to settle, assessing potential sites with modified attenules. As the cypris uses up its finite energy reserves, it becomes less selective in the sites it selects. At high tide, over about 3 days, it selects a settlement site on a rock and the antennae and the cement glands produce a glue so that the cypris fixes to a space on the rock by its head with its legs upmost.

Within 24 hours of gluing itself down the cypris metamorphoses into an tiny adult barnacle on the spot it will stay for the rest of its life.

Both goose and acorn barnacles are suspension feeders, staying protected inside their shells whilst extending feeding limbs called cirri into the water which they use to filter passing plankton or detritus which they draw into their shell to consume.

Brine shrimp

As accessing live specimens of living barnacles to look closely at their structure and life history may be difficult, we suggest using the brine shrimp *Artemia* as a typical arthropod crustacean allowing students to explore the body plan of arthropod crustaceans and understand how the taxonomic hierarchy is worked out.

Brine shrimps are crustaceans like barnacles and belong to the subclass *Branchiopoda*. Unlike brine shrimp, barnacles belong to the subclass *Cirripedia*. Adult brine shrimp also differ from adult barnacles in that they are free-swimming. However, the nauplius larval stage of the brine shrimp is almost identical to the nauplius larval stage of the barnacle and adult brine shrimp show all the features of the typical crustacean.

Brine shrimp live in inland salt water bodies, such as Great Salt Lake in the USA. They are not found in the sea because there are too many predators at the lower salinity levels. They may also live in man-made salt evaporation ponds. They feed on algae, either filtering small particles with the fine spines on their legs as they swim or by grazing surfaces and scraping algae off with quick movements of their appendages. A feeding current, caused by the regular rhythm of the appendages, moves the algae towards the mouth via a central median food groove.

Reproduction occurs when a male grasps a female with his large second antennae (which have been modified into clasping organs) and fertilises her eggs. The eggs are then laid in a brood sac in the water. Reproduction can also occur without fertilisation (known as parthenogenesis). This happens when there are no males present. In this case females lay unfertilised eggs that will develop into female offspring.

Eggs will only hatch if environmental conditions (i.e. temperature, water supply, salt concentration) are right. When conditions are not suitable, eggs are deposited as cysts instead (eggs that are dried and surrounded by a thick shell). The cysts can remain in a dormant state for many years until conditions are suitable for hatching. Cysts are often laid in the autumn, and hatch in spring, as the salt water body habitats are often inhospitable over winter. Sexual reproduction is required for the production of cysts.

Eggs hatch into nauplii and go through several stages of growth, moulting their exoskeleton each time, before becoming adults.

Notes for technicians

It can be difficult to keep barnacles in the classroom as they require a seawater aquarium, but brine shrimps are readily available from aquarium suppliers and make excellent organisms for study in the classroom as they require very little equipment and maintenance.

Ready mixed dried eggs and non-iodised salt are added to 500 ml of tapwater. They require aeration by a small aquarium pump.

The eggs hatch within 24 hours in a warm place (longer if cold) in an aerated jar and the emerging nauplii can be closely observed. They use their antennae for swimming and moult 15 times, adding segments at each moult.

They can be grown on in two-litre clear plastic bottles with tapwater, 2 tablespoons of non-iodised salt and two pinches of bicarbonate of soda. They require sunlight and a moderately warm temperature but not blazing sun. Aeration also helps in the early stages. The third day after hatching they need feeding with an algal solution or a small pinch of brewer's yeast once a week.

Colonies can be maintained for many months to observe the whole life cycle many times.

There is further detailed information on setting up a brine shrimp culture in *Brine Shrimp Ecology* by Michael Dockery and Stephen Tomkins. This book is available to download free from the British Ecological Society: http://bit.ly/BrineShrimpEcology.

There are many aquarium suppliers which will supply ready-mixed brine shrimp eggs and salt. Blades Biological Ltd. are an educational supplier that also stock brine shrimp eggs and accessories:
www.blades-bio.co.uk.

Lesson 1: Morphology and life histories of barnacles

Overview and lesson plan

This lesson will use the context of Darwin's work on barnacles to explore the principles of classifying organisms based on morphology and life histories.

Students will investigate the brine shrimp, *Artemia*, and use it as a model for studying barnacle anatomy and life histories.

Activity	Timing	Description and Pedagogical Approach
Lesson starter [Worksheets]	10 min	Small group discussion: reasoning and argumentation Darwin's barnacle: what is it? Students are shown Sowerby's illustrations of the external and internal structure of barnacles. Then using the two diagrams in the Student Pack, students discuss the classification and structure of a sessile barnacle.
Activity 1 [Worksheets]	30 min	Peer to peer discussion: reasoning, causality, comparing Part 1: Students use the compound microsope at different magnifications to examine the nauplius larvae from the brine shrimp *Artemia*. Part 2: Students look at the body plan of the brine shrimp *Artemia* and compare with the body plan of a typical adult barnacle. Students use the compound microsope to examine living brine shrimps for their structure and behaviour.
Activity 1 - Extension [Worksheet]	10 min	Individual activity: reasoning, analytical thinking, problem solving Students consider the life cycle of a typical acorn barnacle and suggest how it is adapted.
Plenary	10 min	Small group task: discussion, reasoning, analytical thinking, problem solving, demonstrating/modelling A ranking exercise and discussion on why Darwin was interested in barnacles.

Introduction

(Slide 2) Darwin spent eight years investigating barnacles (pictured here with some limpets), with the aim of giving an evidential basis for his theory of evolution. He set out to show how a single animal group had evolved through entirely natural means.

(Slide 3) This video shows how barnacles filter feed when the tide is in.

(Slide 4) Darwin conducted his studies on barnacles at the family home Down House, Kent.

(Slide 5) The only tools he had available to study the structure of this diverse group of invertebrates were simple lenses and compound microscopes. He also observed the life histories of living species and studied barnacle fossils.

(Slide 6) Darwin first became interested in barnacles during his 5-year voyage on *HMS Beagle* when he found a barnacle which seemed to have a parasitic nature unlike most barnacles. He later studied this small Chilean barnacle he had brought home from his voyage and included it in his resulting publications on barnacles.

Lesson starter

Part 1:

- Show students the illustration of the external structure of a barnacle by George Sowerby (Slide 7).
- In groups of four, using the diagram in the Student Pack, ask the students to discuss the statements and questions which have been made about the classification and behaviour of barnacles. Which do they think are true? Which show reasoning that is scientific and which do not? Ask them to explain.

Part 2:

- Show students the illustration of the internal structure of a barnacle by George Sowerby (Slide 8).
- Working in the same groups, using the diagram in the Student Pack, ask the students to discuss the statements and questions which have been made about the classification and behaviour of barnacles. Ask them to revise their views in light of the evidence of the second drawing.
- Extension: class discussion: How would they classify the barnacle using their existing knowledge?

Lesson starter: Darwin's barnacle - what is it? - COMMON MISCONCEPTIONS

A number of common misconceptions may arise during these discussions:

- The belief that animals have to be able to move.
  Acorn barnacles are sessile, i.e. fixed to the rock as an adult though the larval forms are free-swimming in the sea.
- The classification of barnacles as molluscs because of their similarity to limpets.
  Internal morphology shows the barnacle to be an arthropod with jointed limbs which it uses to filter feed. Limpets graze the algae from rocks with the rasping mouth within its muscular foot and have no limbs.
- The thought that the arthropod exoskeleton is like our skin and flakes off.
  In arthropods, the ecdysis (shedding) of the cuticle happens at definite times of moulting to enable the animal to grow larger in spurts. We have an internal skeleton and have continuous growth.
- Many students do not imagine invertebrates having sex.
  Although many barnacles are hermaphrodite they do cross-fertilise and possess long penises. Some barnacles are female with parasitic males. Darwin thought that barnacles might show how sex evolved.

Introducing brine shrimps (*Artemia*)

(Slide 9) Brine shrimps are crustaceans like barnacles and belong to the subclass *Branchiopoda*. Unlike brine shrimp, barnacles belong to the subclass *Cirripedia*. Adult brine shrimp also differ from adult barnacles in that they are free-swimming. However, the nauplius larval stage of the brine shrimp is almost identical to the nauplius larval stage of the barnacle and adult brine shrimp show all the features of the typical crustacean.

(Slide 10) Brine shrimp live in inland salt water bodies, such as Great Salt Lake in the USA. They are not found in the sea because there are too many predators. They may also live in man-made salt evaporation ponds. They feed on algae, either filtering small particles with the fine spines on their legs as they swim or by grazing surfaces and scraping algae off with quick movements of their appendages. A feeding current, caused by the regular rhythm of the appendages, moves the algae towards the mouth via a central median food groove.

(Slide 11) Reproduction occurs when a male grasps a female with his large second antennae (which have been modified into clasping organs) and fertilises her eggs. The eggs are then laid in a brood sac in the water. Reproduction can also occur without fertilisation (known as parthenogenesis). This happens when there are no males present. In this case females lay unfertilised eggs that will develop into female offspring.

Eggs will only hatch if environmental conditions (i.e. temperature, water supply, salt concentration) are right. When conditions are not suitable, eggs are deposited as cysts instead (eggs that are dried and surrounded by a thick shell). The cysts can remain in a dormant state for many years until conditions are suitable for hatching. Cysts are often laid in the autumn, and hatch in spring, as the salt water body habitats are often inhospitable over winter. Sexual reproduction is required for the production of cysts.

Eggs hatch into nauplii and go through several stages of growth, moulting their exoskeleton each time, before becoming adults.

Barnacle life histories

Barnacles are sessile organisms, that is they are fixed to rocks and other objects. Through dissection, Darwin discovered that barnacles are hermaphrodite (that is they have both male and female reproductive organs).

(Slide 12) The two most common types of barnacles are goose barnacles and acorn barnacles. Goose barnacles are the less common of the two varieties and live in the ocean, attached to floating debris or ships. At the end of the protruding stalk is a body with legs, held within shell plates. Acorn barnacles live on the seashore, where they are uncovered at low tide, and may also live on the underside of boats. There are also a small number of parasitic barnacles which live within another organism, such as a crab.

(Slide 13) Many barnacles have very long penises and can reach over several times their own length to deposit sperm in the mantle cavity of a neighbouring barnacle. In *Semibalanus balanoides*, a common acorn barnacle found on British shores, fertilisation between neighbouring barnacles occurs in November. The sperm are deposited inside in the mantle cavity where the eggs have been released from the ovaries. The fertilised egg mass develops inside the mantle cavity (inside the shell but outside of the body). The barnacle lives in the intertidal zone of the rocky shore and has strong shell plates that shut up when the tide is out, trapping the water inside the mantle cavity to prevent desiccation.

(Slide 14) The eggs use the energy of their yolk sacs to develop within the mantle cavity and hatch as nauplius larvae the following March. The nauplii swim out on the high tide and into the sea where they feed on phytoplankton (microscopic plant-like organisms, such as algae).

In the sea the nauplius larva goes through six stages of growth, moulting its exoskeleton each time and growing larger as its swims in the plankton. This stage is free-swimming and enables the sessile barnacles to invade new habitats.

(Slide 15) In April, the nauplius metamorphoses into the non-feeding cyprid stage - its role is to find a suitable place to settle. As the cypris uses up its finite energy reserves, it becomes less selective in the sites it selects.

The cypris has a hard shell (or carapace) and assesses potential sites with modified attenules. At high tide, over about 3 days, it selects a settlement site on a rock and the antennae and the cement glands produce a glue so that the cypris fixes to a space on the rock by its head with its legs upmost.

Within 24 hours of gluing itself down the cypris metamorphoses into a tiny adult barnacle on the spot it will stay for the rest of its life.

Arthropod classification

(Slide 16) Barnacles are a subclass of the Crustacea which are a subphylum of Arthropoda, which from part of the Kingdom Animalia.

Background on body plans

The body plan of the brine shrimp *Artemia* is typical of the Arthropod phylum.

All arthropods have:

- A hard outer body covering called an exoskeleton
- Segmented body
- Paired, jointed legs
- Compound eyes

In addition, all crustaceans have:

- Mandibles
- Two pairs of antennae
- A number of jointed legs

Activity 1

Darwin studied the life histories of various barnacles and found their larval stages to be very similar though the adults were very different.

Examine a brine shrimp larva (nauplius) using a hand lens and a microscope (refer to the methods outlined below), then answer the questions in Part 1, using the diagrams and live specimens to help you.

Examine a brine shrimp adult using a hand lens and a microscope (refer to the methods outlined below), then answer the questions in Part 2, using the diagrams and live specimens to help you.

Equipment

- Hand lenses of various magnifications (e.g. x8, x15)
- Compound microscope (10x - 200x)
- Glass slides and coverslips
- Pipettes with inside diameter of at least 5mm and entry diameter of 3-5mm
- Brine shrimp (*Artemia*) nauplii
- Petri dish
- Brine shrimp (*Artemia*) adults

Part 1:
Method - observation with a hand lens

1. If necessary, use a pipette to transfer some larvae with sufficient water into a petri dish. Otherwise, observe the larvae within their existing container.
2. Use the hand lenses to observe the nauplii in greater detail: make a note of how they move, how they feed and any other behaviours you observe.

Method - observation with a microscope

1. Place the glass slide on a tissue and with a pipette suck up a small sample of the eggs and larvae.
2. Place a single drop on the slide and gently lower the glass coverslip onto the drop starting at one side and letting it trap the water and sample underneath.
3. Making sure that the slide is dry underneath, place the slide on the stage of the microscope.
4. Engage the lowest power objective lens and lower the objective lens to the lower limit (close to the slide) whilst looking from the side to avoid squashing the specimen.
5. Look down the eyepiece and wind the objective upwards using the focus knob until you see the image come into focus.

6. Repeat steps 4 and 5 using the higher power objective lenses.
7. Dispose of used slides according to agreed procedures.

Part 2:
Method - observation with a hand lens

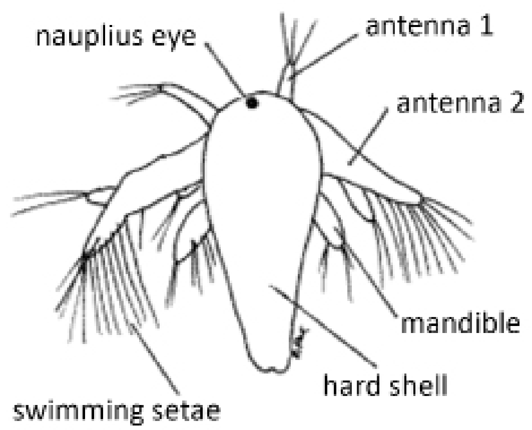
1. If necessary, use a pipette to transfer a brine shrimp adult with sufficient water into a petri dish. Otherwise, observe the adults within their existing container.
2. Use the hand lenses to observe the adults in greater detail: make a note of how they move, how they feed and any other behaviours you observe.

Method - observation with a microscope

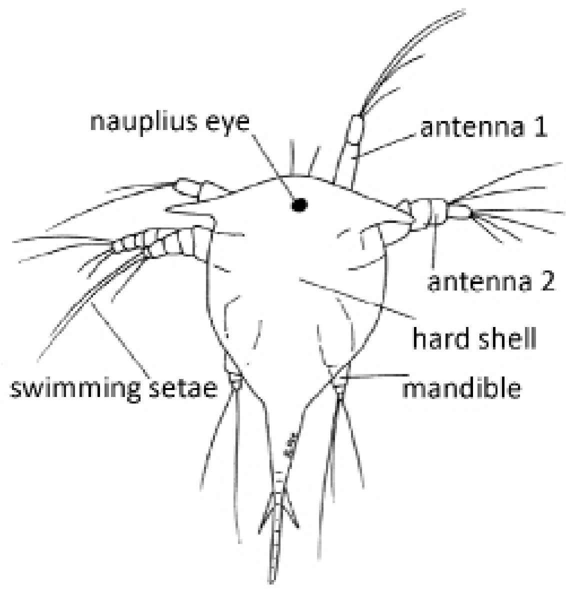
1. Use a pipette to transfer a brine shrimp adult with sufficient water into a petri dish.
2. Place the petri dish on the stage of the microscope.
3. Engage the lowest power objective lens and lower the objective lens to the lower limit (close to the slide) whilst looking from the side to avoid getting the lens in the water in the petri dish.
4. Look down the eyepiece and wind the objective upwards using the focus knob until you see the image come into focus.

Part 1: Looking closely at free-swimming larvae of crustaceans, the nauplius of the brine shrimp

Brine shrimp larva, the nauplius



Barnacle larva, the nauplius



Original drawings © Richard Fox

1. State the identity of the limbs that your brine shrimp nauplius uses for swimming.
Antenna 1 and 2 (which have setal hairs/setae).

2. After hatching, the brine shrimp nauplius uses the egg yolk as a nutrient source for three days, then its gut opens up and it starts to feed. Try to identify the gut in the living specimen. How do you think it feeds?
It passes food to its mouth with its mandibles.

3. In what ways do you think the barnacle nauplius is well adapted to its role as a free-swimming larval form? (Bear in mind that adult barnacles are fixed to a substrate).
It swims fast and has sensory organs such as the eye. This stage enables the barnacle to enter/invade new habitats.

Question to discuss

You have seen that the first larval stage (the nauplius) of barnacles and brine shrimps are very similar. This is true for *all* crustacean nauplii. Why do you think the first larval forms of all the crustacea are so similar when the adults are so different?

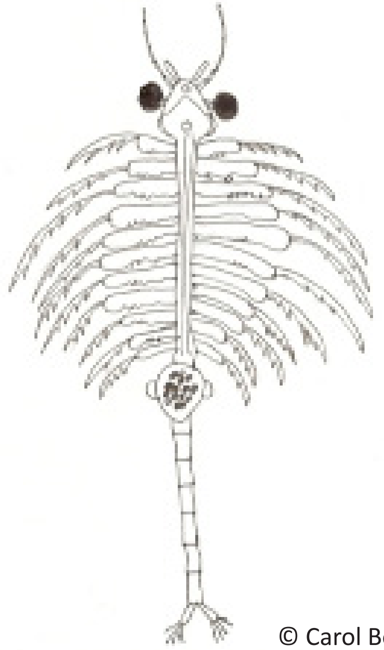
The nauplius of all crustacea are very similar because they are well adapted to providing dispersal in an aquatic environment.

Part 2: Looking closely at the adult brine shrimp as an example of the crustacean body plan - ANSWERS

Schematic chart of a typical adult barnacle

Head segment 1	Pair of stalked eyes and central eye
Head segment 2	Pair of unsegmented antennules
Head segment 3	Pair of segmented branched antennae
Head segment 4	Pair of mandibles/jaws
Head segment 5	Pair of mouthparts
Head segment 6	Pair of mouthparts
Thoracic segment 1	Pair of jointed limbs (biramous - each limb has two parts)
Thoracic segment 2	Pair of biramous jointed limbs
Thoracic segment 3	Pair of biramous jointed limbs
Thoracic segment 4	Pair of biramous jointed limbs
Thoracic segment 5	Pair of biramous jointed limbs
Thoracic segment 6	Pair of biramous jointed limbs
Abdominal segment 1	
Abdominal segment 2	
Abdominal segment 3	
Abdominal segment 4	
Abdominal segment 5	
Telson	Often forked

Adult female brine shrimp



1. Looking at the schematic chart of a typical adult barnacle and the diagram of the adult brine shrimp, identify any differences in the number and structure of the segments within the thoracic and abdominal regions. (Note that the brood sac is 2 segments).

The brine shrimp has 11 thoracic segments whilst the barnacle has 6.

The typical barnacle has biramous limbs which means that there is a base segment and then two separate jointed parts. *Artemia* does not have two distinct parts.

The brine shrimp has 7 abdominal segments whilst the barnacle has 5.

Observe the brine shrimp under a low power magnification (referring to the methods outlined at the start of the worksheet) and then answer the following questions.

2. How does the adult brine shrimp swim? Which way up is it?

It swims on its back and uses the feathery swimming limbs which beat rhythmically.

3. How does you think the adult brine shrimp feeds?

The beating limbs draw microscopic food towards the mouth along the food grove and it is taken in with the mandibles.

Activity 1 - Extension

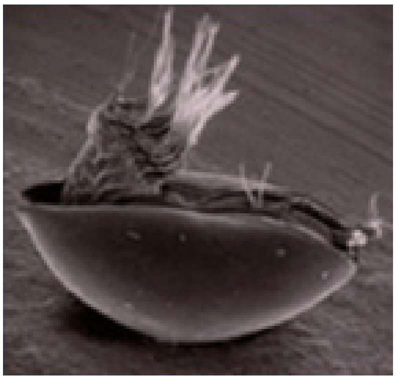
The life cycle of barnacles - ANSWERS

The images below show the two larval stages of the barnacle life cycle.

Barnacle larva, the nauplius

© P S Rainbow

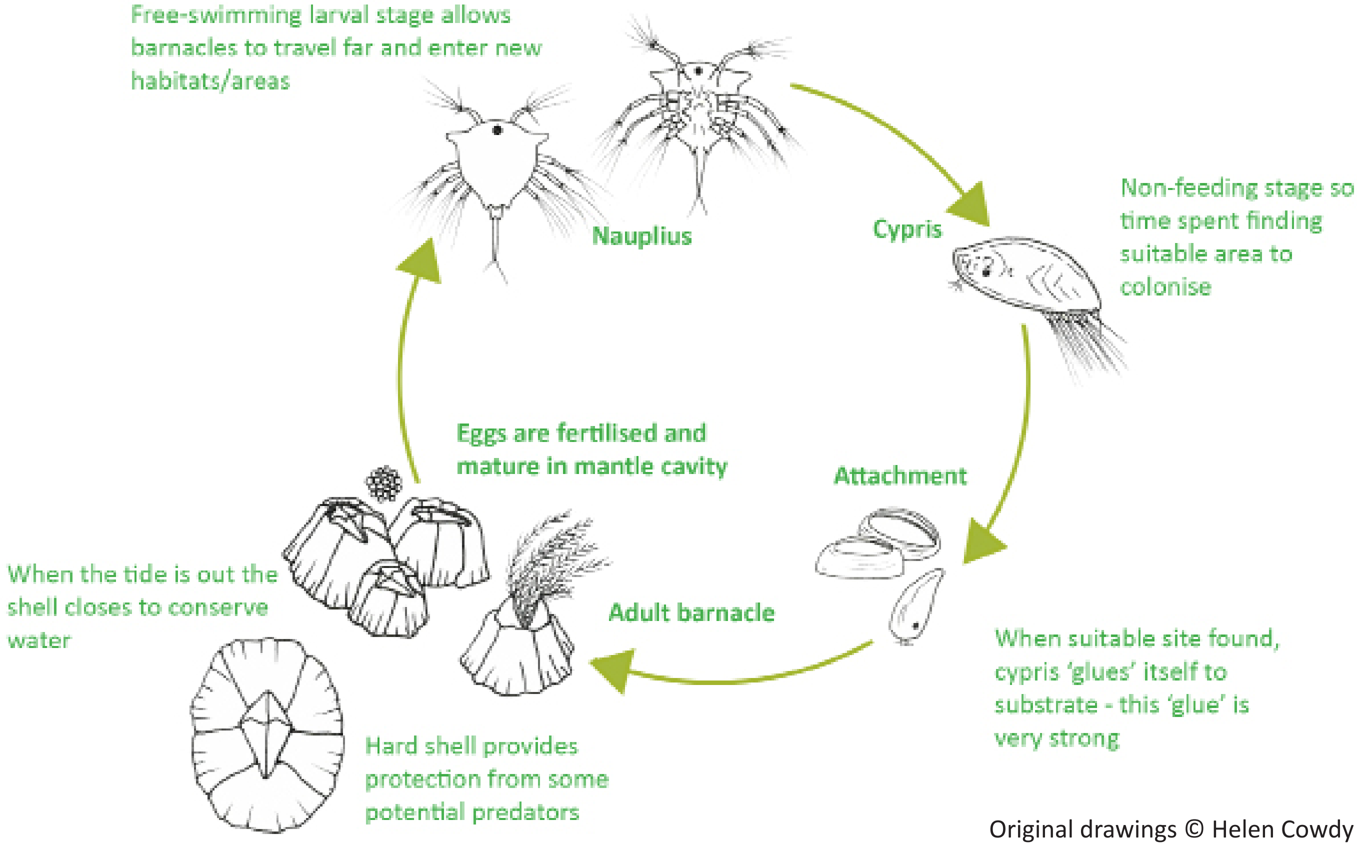
Barnacle larva, the cypris



© P S Rainbow

Label the diagram below to illustrate the life cycle of *Semibalanus balanoides* (a common acorn barnacle found on British shores). Make notes on your diagram to illustrate the adaptive features which allow this invertebrate to survive.

Students may include any of the following detail:



Original drawings © Helen Cowdy

Barnacle body plans and looking for homologies

(From Part 2 of Activity 1, students will have seen that *Artemia* has more limbs than the acorn barnacle. This should enable students to discuss Darwin's ideas of homology and the segmentation of the arthropod body plan.)

(Slide 17) When looking at body plans for homologies, Darwin's method was to look at the external plates of the barnacles (if they were present) and through dissection at the internal morphology and the larval development. This was painstaking practical work.

Darwin was the first to name the plates of the barnacle shell that are used to this day. His method of working out relationships was to look for homologies, that is for structures that had arisen from the same embryological parts or segments of the body.

(Slide 18) The idea of homologies is based on the segmentation of arthropods. The basic number of segments is 21 which is divided into head, thorax and abdomen. Barnacles seemed to Darwin to have 17 and were missing the 4 last segments of most arthropods.

In Darwin's evolutionary view of morphology, new structures arise from pre-existing ones but take on different functions. For example, legs become feeding appendages and segments are often lost. So it was important to work out which segments gave rise to which structures as evolution progressed. Structures arising from the same ancestral segments he called homologous.

Plenary - Why was Darwin interested in barnacles?

(Slide 19) Show students the quote from Darwin's autobiography.

Ask students to cut out the suggested answers (from page 35 of the Student Pack) and rank them in order of importance by placing them in rows, with the most important answer/s placed in the top row and the least important in the bottom row. Those judged to be of equal importance will go on the the same row.

Ask students to share and discuss their ranking and reasoning as a class.

Lesson 2: Building the tree of life - molecular evidence

Overview and lesson plan

This lesson will use the context of Darwin's work on barnacles to explore concepts of classification and evolutionary relationships.

Students are asked to use their knowledge of classification systems in order to investigate the problems of classification and to act as an introduction to phylogeny. Students are introduced to how the use of newer molecular evidence can lead to a revision of the classification of organisms.

Activity	Timing	Description and Pedagogical Approach
Lesson starter [Worksheets] Lesson starter [Worksheet]	10 min	Small group discussion: reasoning and argumentation Part 1: Students discuss questions relating to Linnaeus' binomial naming system and the problems that may arise from classifying in this manner. Part 2: Representing evolution: Darwin's tree of Life. Students examine and interpret Darwin's representation of the tree of life from his book *On the Origin of Species*.
Activity 1 [Worksheet]	20 min	Peer to peer discussion: reasoning, causality and comparing Using information cards of barnacles known to Darwin, students attempt to classify them and make a phylogenetic tree of them using morphological and life history evidence.
Activity 2 [Worksheet]	20 min	Individual task: reasoning, assessing and evaluating evidence New molecular evidence: Students explore a maximum likelihood consensus tree to understand how molecular evidence increases reliability of phylogenetic trees.
Plenary [Worksheet]	10 min	Small group task: discussion, reasoning, analytical thinking, problem solving, demonstrating/modelling Students complete a concept map of relationships between classification and phylogeny.

Classifying barnacles

During Darwin's "barnacle years" (1846-1854) there was great interest in the nature of the difference between plants and animals and how to classify the immense variety of the natural world. Darwin's contemporaries were uncertain about which phylum this animal should be in. Barnacles had originally been classified in the 18th century by Linnaeus and Cuvier as molluscs, like limpets. It was the naturalist John Vaughan Thompson who first suggested, in 1830, the similarities between the barnacle life cycle (with its larval stages) and that of crustaceans. Darwin was part of these later discussions about barnacle classification.

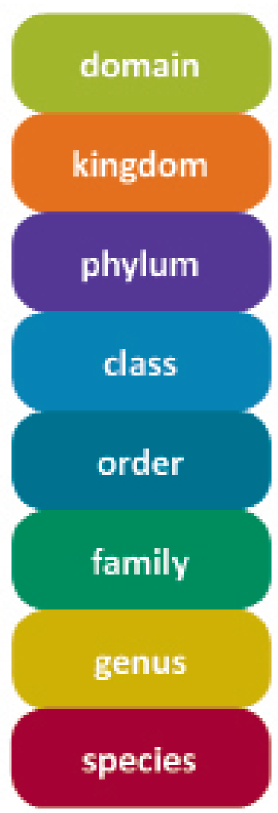
(Slide 2) The Swedish botanist, Carl Linnaeus (1707-1778), had devised a method for classifying and naming living things involving a binomial (two name) system for naming species with the genus name followed by the species name. Linnaeus' system relies on the observable characteristics of the organisms, especially their structural features and works by grouping like with like to impose order on the natural world. His system of binomial naming has remained in place until today and provides a universal means of identification and, coupled with the use of branching keys, enables accurate identification.

(Slide 3) The species named in this way was the lowest rank and above it the genus, through family, order, class, phylum, kingdom, and domain. The hierarchical system of ranks has also remained although the groups that are included within each rank have changed as new insights are gained, especially from developments in microscopy and molecular biology. Darwin worked within a three kingdom system of Protista, Plantae and Animalia. Today the system most commonly used has three domains and five kingdoms. In Darwin's time, bacteria (single-celled organisms without nuclei) and fungi were not placed in separate kingdoms.

(Slide 4) The five kingdoms
Prokaryotes: Organisms with no true nuclei (DNA is not enclosed by a membrane). Also known as bacteria.
Protoctists (also known as Protista): True nuclei present. Many are single-celled and simple in structure, though some are multicellular. Plants, animals and fungi are thought to have evolved from protoctists.
Plants: True nuclei, multicellular, cellulose cell walls. Photosynthetic. Autotrophic.
Fungi: True nuclei. Many have multinucleate cytoplasm. Chitin commonly present in cell walls. Body is typically a mycelium of thread-like hyphae.
Animals: True nuclei, multicellular, heterotrophic, no cell walls, motile.

Lesson starter part 1

Linnaeus' hierarchical classification: making order of the natural world - ANSWERS



1. Suggest one reason why Linnaeus' system of ordering the world and his binomial naming has stood the test of time.
It uses observable features and looks for similarities to group organisms together.
It is an international language (the same binomial is used by everyone worldwide).
It avoids ambiguities when organisms have more than one common name or when the same common name is applied to more than one species.

It is sometimes difficult to decide what is a species or what is a genus. For example, some scientists believe that chimpanzees are so closely related to humans that they should be included with humans in the genus *Homo*.

2. Why is it easier to decide which organisms should be included in a given species than in a given genus?
Definition of a species is that members of a species can interbreed (to produce fertile offspring).
However, there is not such a clear definition for genus (and so standards used may vary between taxonomists).

Darwin's Tree of Life

(Slide 5) The only illustration in his great work *On the Origin of Species* is a model phylogenetic tree which Darwin uses to discuss how genera might evolve through time as natural selection acts through thousands of generations. Notice how this tree shows time on the vertical axis, A-L are a range of closely related species at the start. A and I have a lot of variation in their offspring and diverge to form new species each with a different pattern. All the other species become extinct. This phylogenetic tree was one of the first evolutionary illustrations and branching tree diagrams continue to dominate our thinking about evolutionary change and how we represent evolution.

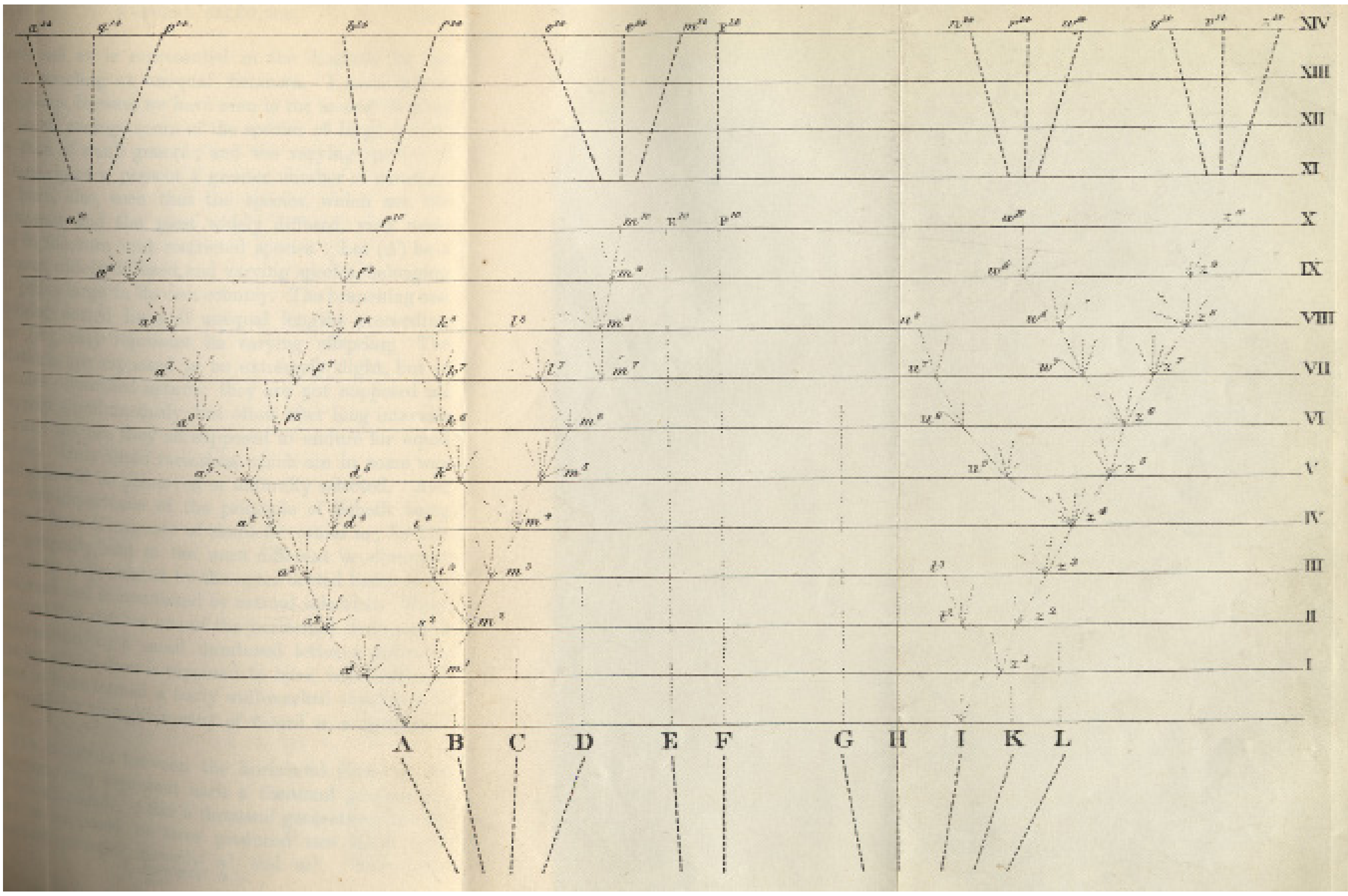
(Slide 6)

> “The affinities of all the beings of the same class have sometimes been represented by a great tree. I believe this simile largely speaks the truth. The green and budding twigs may represent existing species...”
>
> “As buds give rise by growth to fresh buds and these if vigorous branch out and overtop on all sides many a feebler branch, so by generation, I believe, it has been with the great Tree of Life which fills with its dead and broken branches the crust of the earth and covers the surface with its ever branching and beautiful ramifications”
>
> Charles Darwin, *On the Origin of Species*, 1859

Lesson starter part 2

Representing evolution: Darwin’s tree of life - ANSWERS

Look closely at this diagram and the notes below it which summarise Darwin’s explanatory text of his ‘Tree of Life’ from *On the Origin of Species*. Then answer the questions.



- A-L are a range of closely-related species in a large genus.
- The letters are not equal distances from each other showing that they do not all resemble each other to the same degree; there is considerable variation in this large genus.
- Time is shown on the vertical axis, each section being 1,000 generations.

- A and I are common, widespread and both have varying offspring over time.
- The most different offspring are preserved through natural selection.
- At a^1 and m^1 enough variation has accumulated for these to be well marked varieties.
- The process continues with more divergence to A2.
- After 10,000 generations species A has produced three forms a10, f10 and m10 which may be either varieties, subspecies or species - depending on how much they differ.
- There is a break in the diagram to represent more generations and 8 new species are represented - A14 to m14 at 14,000 generations.
- The grouping of these new species represents new genera.

1. Which species continue with unaltered descendants for 14,000 generations?
F.

2. Which species have become extinct?
B, C, D, E, G, H, K, L.

3. What might the dotted lines below the original species letters represent?
The connection to the common ancestral species.

How did Darwin study barnacles?

Darwin was seeking to show the descent of all the barnacles he had studied, their evolutionary relationships and how they might have had a common ancestor so that he could use this to illustrate his theory of evolution through natural selection.

(Slide 7) He enlisted the help of his contacts worldwide and requested specimens as well as using the collections he had made on the voyage of *HMS Beagle*. In common with all those involved with classifying the millions of organisms of the living world at that time he used what he could see with the naked eye and a simple light microscope both of the outside of the organisms and their internal anatomy. This process involved painstaking observation of the external features and dissection of the internal organs of each of the barnacles he studied together with studies of their life histories.

He dissected the barnacles to ascertain similarities of their internal systems and paid special attention to their reproductive systems and life histories. Most barnacles are hermaphrodite but he discovered a number of sexual combinations. He found that some hermaphrodite species had tiny complimentary males embedded internally or externally, some female species had tiny dwarf males. This made him theorise about the evolution of separate sexes.

He hatched the eggs and watched as the nauplius larvae emerged and swam about and developed and typically turned into cyprid larvae before settling down to a sessile life.

(Slide 8) Darwin used all the sources of evidence available to him:

- Fossils
- External morphology e.g. number of plates
- Internal morphology e.g. oviducts
- Life histories e.g. larval stages of development, number of plates as adult develops
- Distribution e.g. location and type of habitat

Activity 1

Ask students to cut out the picture cards of barnacles found at the end of the Student Pack and use them to answer the questions on the Lesson 2: Activity 1 worksheet.

Following in Darwin's footsteps: making a phylogenetic tree of barnacles using his morphological and life history evidence - ANSWERS

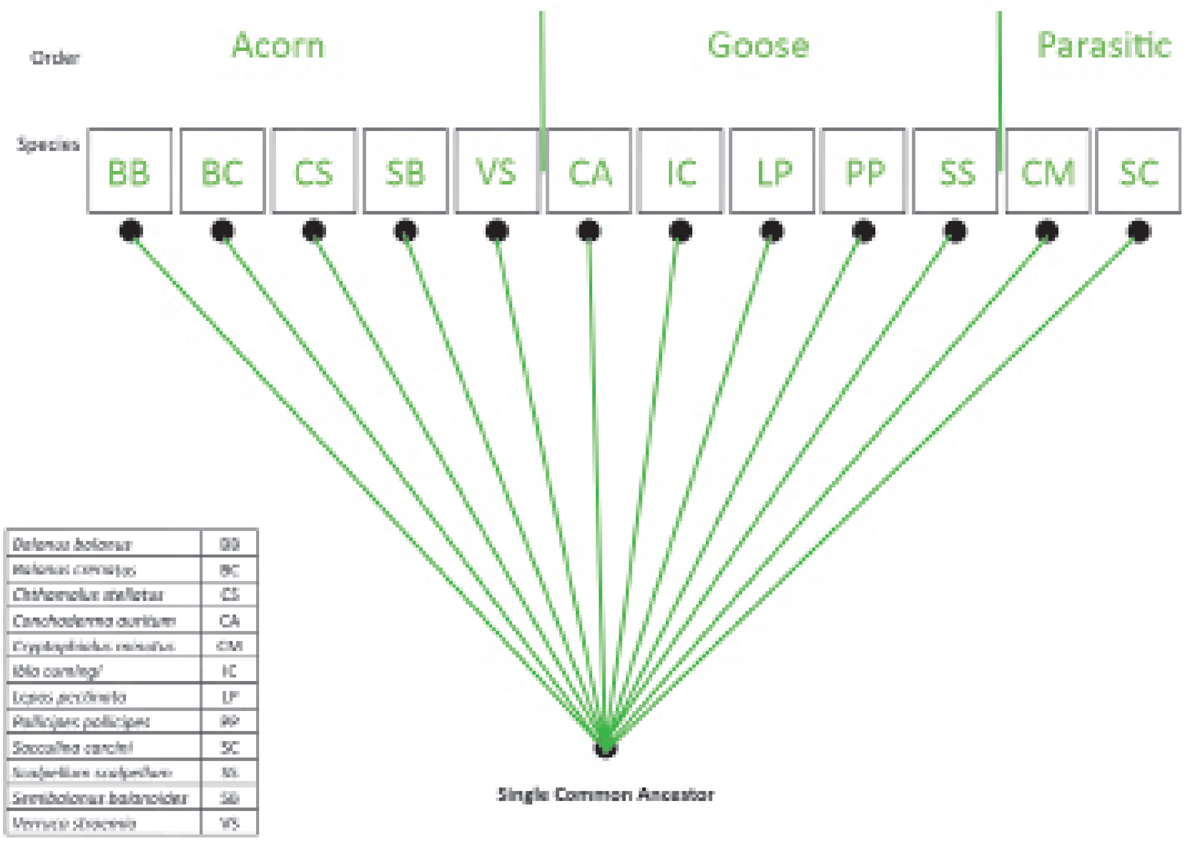
Darwin was sent many barnacle species through the post, from his many friends and contacts around the world. Imagine you have been sent the set of barnacles illustrated on the cards at the back of this Student Pack.

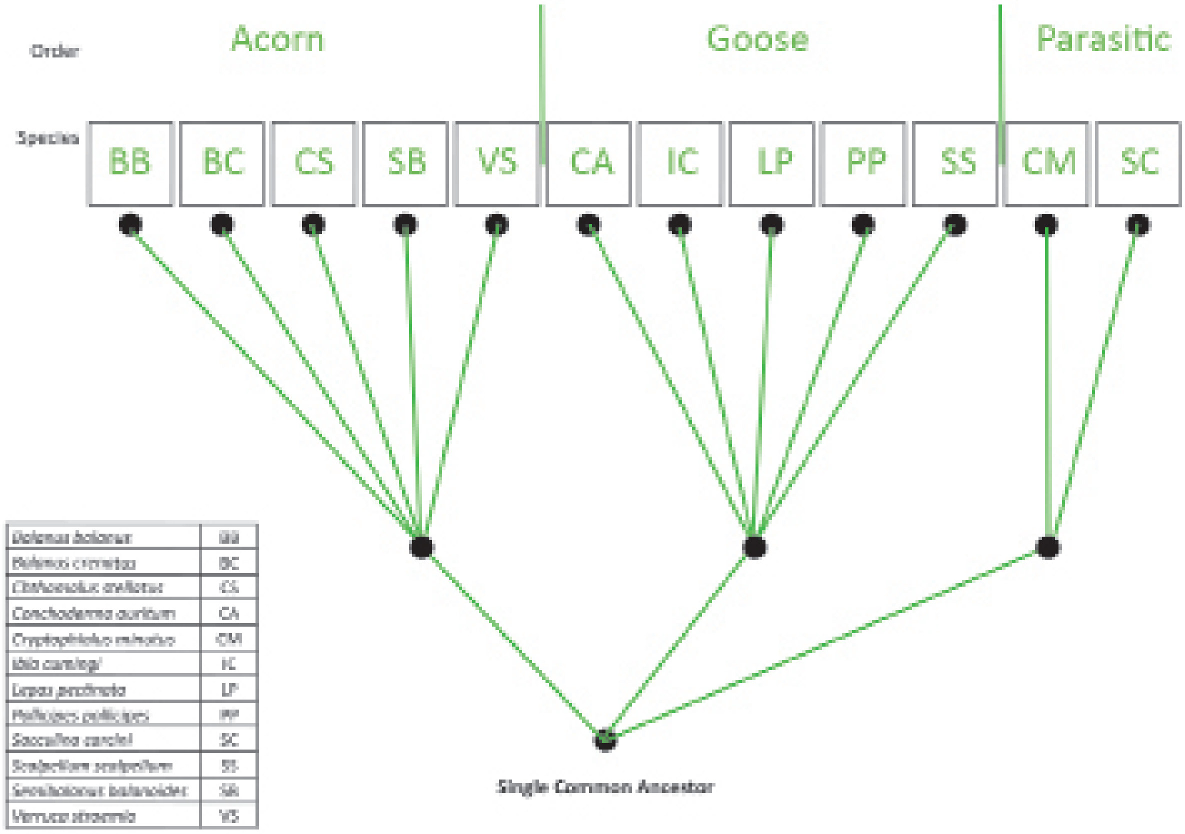
In this activity, you will be trying to create a phylogenetic tree using the template on the following page.

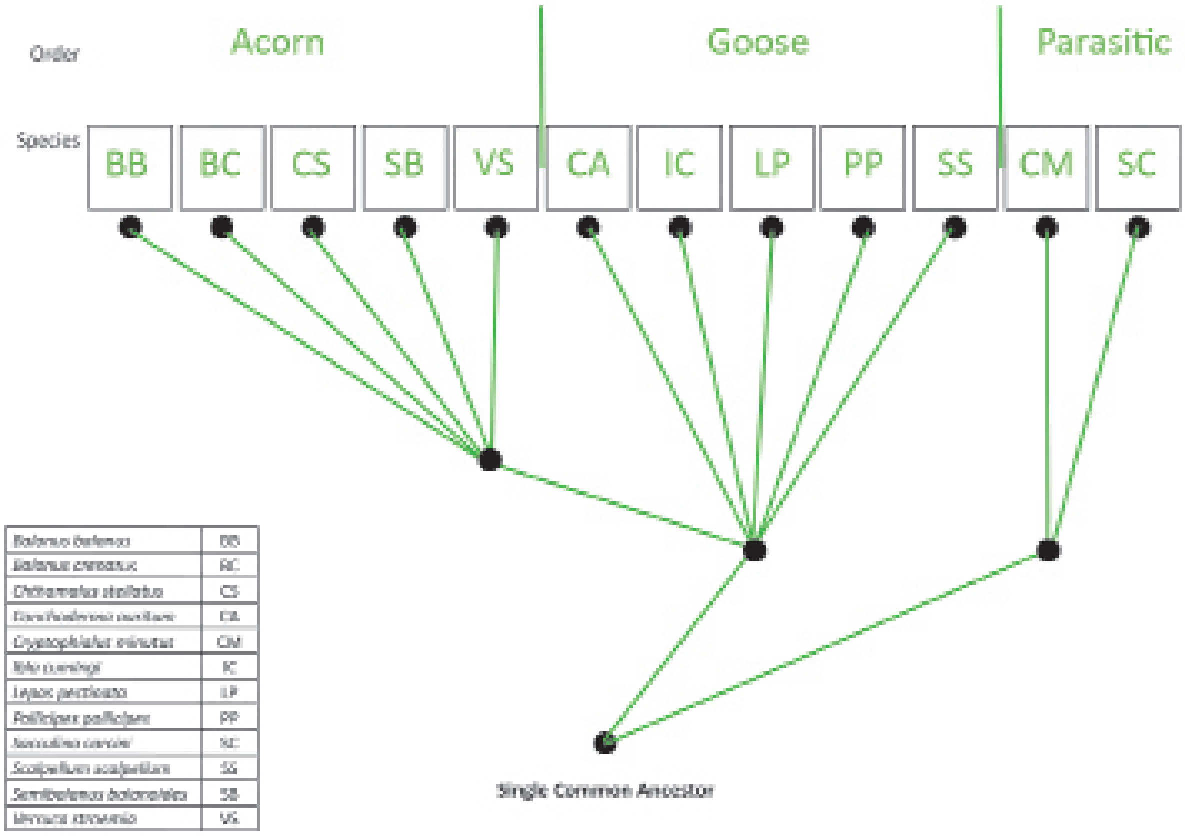
Look carefully at the external features of the barnacles and the information about them. Try to classify them by placing them into possible groups (orders) of species that you think might be related. You may want to think about stalks, plates, lifestyle or other features the species have in common.

1. Complete the top of the diagram by adding your orders and adding the species for each order.
Students should readily be able to sort them into acorn barnacles, goose barnacles and parasitic barnacles. These are the main orders of Sessilia, Pedunculata and Rhizocephala respectively.
Students may try to further arrange/classify the species within these grouyps by the number of plates in the shell.

2. Now try to make a tree (like Darwin's tree in the previous activity) by adding lines to connect the different species to the single common ancestor at the bottom of the diagram, to show how they might have evolved from a single common ancestor. When drawing the connecting lines, think about how the species and orders may be related to each other in terms of evolution - for example, did one order or species evolve from another or did they all evolve directly from the common ancestor?
There are 3 possible versions that students may come up with depending on their understanding of the topic:







3. What features have you used to help you decide where to place the species and where to place the lines on the tree?

Students are likely to suggest stalked/not stalked, lifestyle and perhaps number of plates.

4. If you also had fossil barnacles like Darwin, how might that help you to draw the tree?

In the absence of fossils you have to infer evolutionary relationships from the morphological and life history evidence you have. However, if fossils were available you would be able to see which order of barnacles appeared first in the fossil record and this might help you decide where to connect species or orders with lines.

Compare your decisions with Darwin's classification using the diagram below - taken from a 1973 paper by Michael Ghiselin. The diagram illustrates Darwin's work, showing the hierarchical relationships between the existing groups of Cirripedia and some inferred evolutionary relationships. It is a mixture of classification and a phylogenetic tree. (Note that this diagram includes more genera than you were given on your cards.)

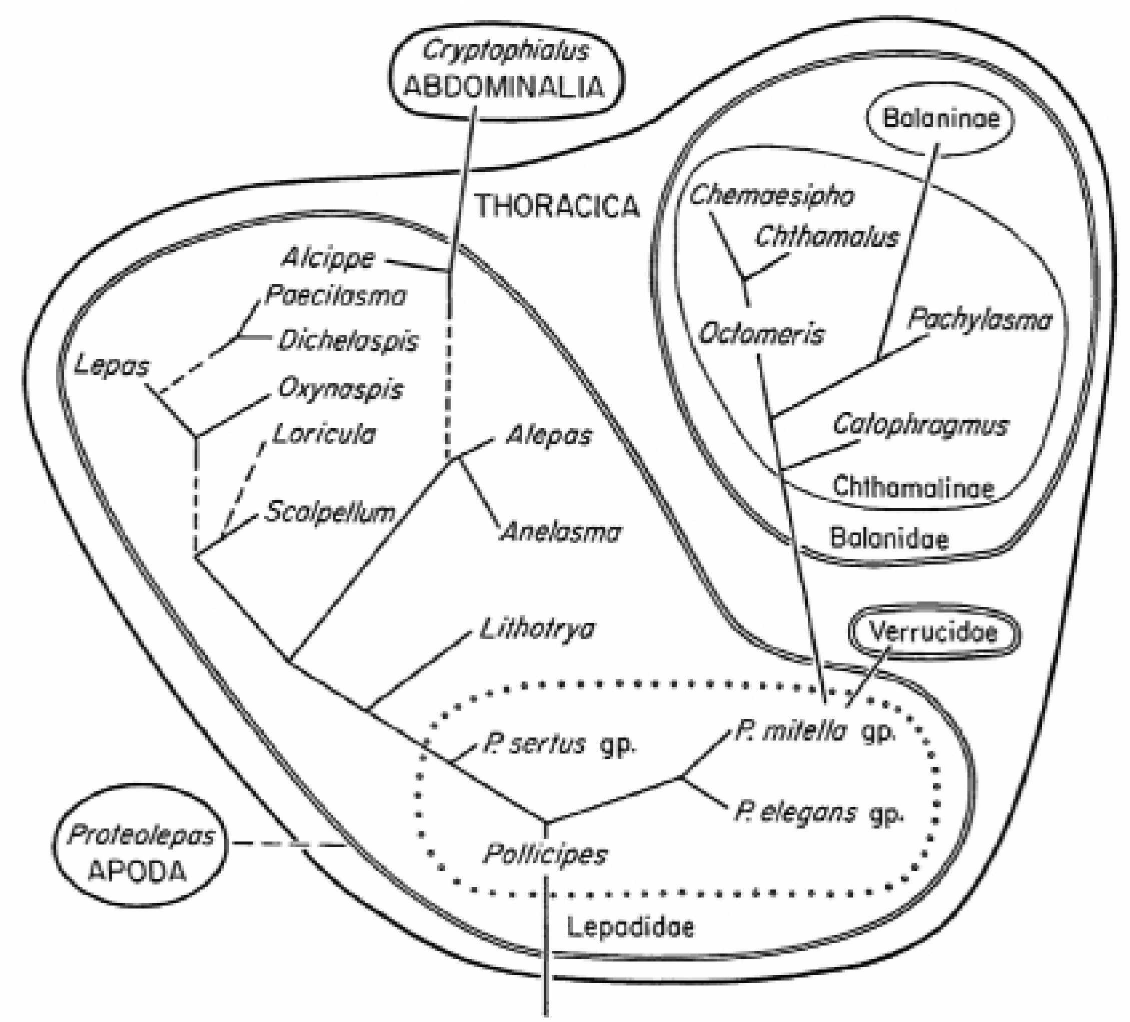


Figure from Ghiselin, M.T., Phylogenetic Classification in Darwin's Monograph on the Sub-Class Cirripedia, *Systematic Biology*, 1973, Vol 22, Issue 2, p 137, by permission of Oxford University Press.

Straight lines indicate branching sequences, whilst broken ones are uncertain. Genera are grouped within circles. Subfamilies are grouped by thin single lines. Families are grouped by double lines. Orders are grouped by thick lines.

Students will recognise the names of some of the barnacles they have grouped, enough to compare their branching tree with Darwin's ideas. The aim of this exercise is to have first hand experience of making a tree and asking the sort of questions that Darwin and any evolutionary systematist has to ask.

Criticisms of Darwin's classification

As soon as Darwin's *Living Cirripedia* was published, in two volumes (1851 and 1854), together with the monographs on fossil barnacles in 1851 and 1854 taxonomists started to check Darwin's assumptions and his observational data and the conclusions he had drawn from them. Darwin had described 154 living species, of which 61 were new to science.

(Slide 9) Looking at Ghiselin and Jaffe's interpretation of Darwin's classification, we can see he had made two new orders *Abdominalia* and *Apoda* each of which contained only one species. Both of these new orders were eventually discredited. The *Cryptophialus* turned out to have a thorax bent like a hairpin and no abdominal segments and so was renamed *Acrothoracica*. *Protolepus* has not been found since and in 1976 was shown to be an Isopod crustacean parasitic on cirripedes. Strangely Darwin never collected and studied the parasitic barnacles now in the *Rhizocephala*.

Phylogenetic trees

Darwin never produced a drawn phylogenetic tree of barnacles. All those involved in making phylogenetic trees have to make decisions as to the meaning of the evidence they have collected, starting with the similarities of form as you have done. Any of the branches below the leaf nodes are inferred from the evidence and therefore open to alternatives. Who was to say if the similarities were due to homology i.e. that they were based on similarity of inherited parts e.g. organs arising from the same segments of the body or to convergent adaptations originating in different structures in response to the environment? Using as many sources of evidence when looking at current species strengthens the likelihood that the internal nodes represent the course of evolution.

Deducing relationships from living organisms requires making assumptions about which characteristics evolved first and how evolutionary selection has acted since. The evidence is messy - the existing organisms only represent a fraction of what has been and the fossil record is incomplete. We try to make sense of the data we have by suggesting and using theories of how evolution might have proceeded. But the same morphological characteristics can arise or be lost more than once through selection and species can hybridise after periods of time.

Darwin's barnacle work recognised that the process of classification and building phylogenetic trees is theory-based and also changes when new evidence arises. His classification was based on his understanding of homologies i.e. that structures that develop from the same position or in the same way in embryology show early relationship and this theory guided his thinking over the 8 years he studied barnacles. His classification of barnacles has largely stood the test of time and is a magnificent achievement.

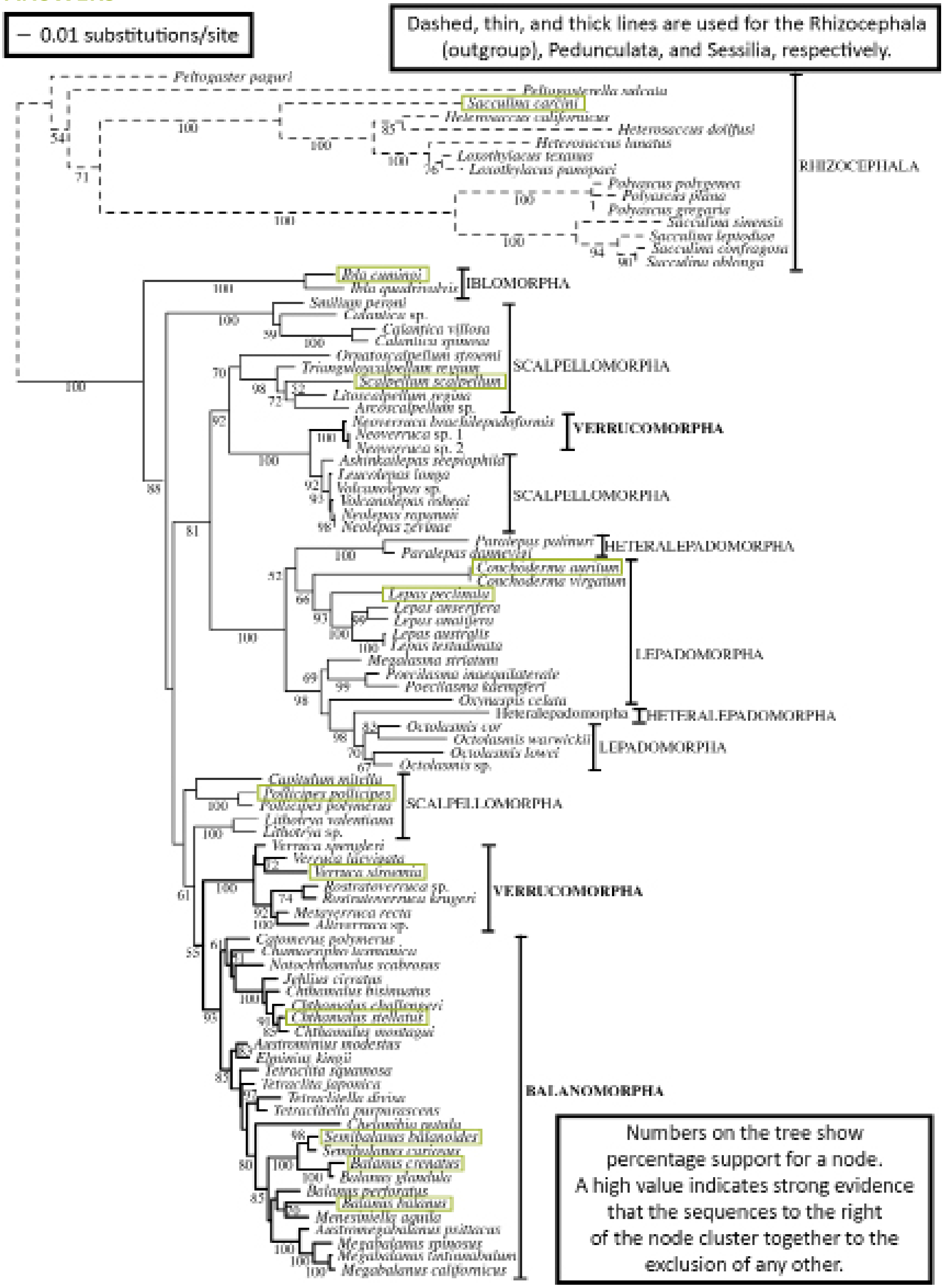
New molecular evidence: New trees, new suggested evolutionary relationships

(Slide 10) The rapid technological advances in sequencing DNA, RNA and proteins in the last 30 years has led to the use of molecular sequences to construct phyologenetic trees. The nucleotide or base pair is sequenced and analysed by computer. Computers also enable the evidence to be freely available online. This evidence is generally considered to give a good basis for inferring evolutionary relationships since the effects of evolution are reflected in the genetic sequences. These methods have suggested that some radical rethinking of the taxonomy and phylogeny of many groups may be necessary, especially amongst the single-celled animals. This has made the study of taxonomy a fascinating and rapidly expanding field.

Activity 2

If these topics have not been covered give simple explanations at an appropriate level

Interpreting a maximum likelihood consensus phylogenetic tree from molecular data - ANSWERS



The figure shows a phylogenetic tree of the DNA sequences of 76 thoracican (one of the superorders of barnacles) species and 15 rhizocephalan (one of the classes of barnacles) species. The rhizocephalan are the parasitic barnacles.

The species highlighted in green boxes are the ones that appeared on your cards in the previous activity (with the exception of *Cryptophialus* sp.).

The names to the right of the tree are the current taxonomic suborders to which these species belong.

- Nodes, where lines branch are common ancestors
- The dashed line is used to show the outgroup. An outgroup is a related group of organisms from a different taxon (here the rhizocephalans) from which the same genes were sequenced. These species are expected not to be part of the branches of the studied organisms. The outgroup is necessary to root the tree, and makes the tree easier to interpret.
- The lengths of the branches (horizontal parts) are shown proportional to the amount of differences in the sequences.

This phylogenetic tree is formed by using a computer programme that compares the sequences of three different genes (a total of 4,040 base pairs). These three genes are selected on the rate of nucleotide substitutions and deletions. The sequences of these genes are well conserved, but in time part of the DNA has mutated. The number of substitutions and deletions gives an estimation of the time passed since the species was formed. The computer uses the base pair data to calculate many possible trees. The computer programme, in this case using the criterion of "Maximum likelihood" makes hundreds of trees. The one you see is a consensus tree, and shows the commonalities based on all those trees. This is the tree from the molecular data that best fits the existing theory of the evolution of the barnacles.

1. According to the molecular tree, which is the most primitive barnacle (the most ancient in origin)?
Ibla is the most primitive barnacle. (This is the species that the first branch leads to.)

2. A monophyletic taxon is defined as one that includes the most recent common ancestor of a group of organisms and all of its descendents, whilst a polyphyletic taxon is composed of unrelated organisms descended from more than one ancestor and so does not include the common ancestor of all members of the taxon. Using these definitions and the phylogenetic tree of DNA sequences, are the acorn barnacle species shown on your cards monophyletic or polyphyletic?
All the acorn barnacles emerge from one branch and share a common ancestor, therefore they are monophyletic.

3. Why do phylogenetic trees, like this consensus tree, not give the final word on evolutionary relationships?
They do not necessarily represent evolutionary relationships because convergent evolution may have occurred.
(Students might also suggest horizontal gene transfer as a reason.)

Limitations of phylogenetic trees

(Slide 11) When using morphology to draw a phylogenetic tree it is important to remember that the same morphological feature can evolve independently (convergent evolution) so it does not necessarily show phylogenetic relationships. Particular features, such as plates, can also be lost during evolution. Therefore new evidence from molecular analysis is vital to revising existing trees.

Whilst molecular evidence does improve upon a classification solely based on morphological features, it does have some limitations. One of the main limitations is that there may have been horizontal gene transfer - this would not show in the gene sequence data. For example, if two distantly related bacteria have exchanged a gene, a phylogenetic tree including those species will show them to be closely related even though most other genes have diverged substantially.

What is drawn is actually a gene tree rather than a phylogeny of the group. For the most reliable trees a combination of sequences is interpreted using genes from different sources e.g. mitochondrial DNA and nuclear DNA are used and sometimes combined with morphological data.

Plenary: Concept map of relationships between phylogeny and classification

This exercise will allow you to consider how the different elements of classification, taxonomy and evolutionary relationships relate to each other.

(Slide 12) Classification is a process that sorts things into groups. Natural classification groups according to the closeness of relationship. The basic unit is the species. The nested nature of classification produces a hierarchy. Taxonomy is the study of the principles behind classification and phylogeny is the study of evolutionary relationships between organisms. The classification should match the tree produced to show relationships to a common ancestor. Modern trees show the evolutionary distance between species since they separated.

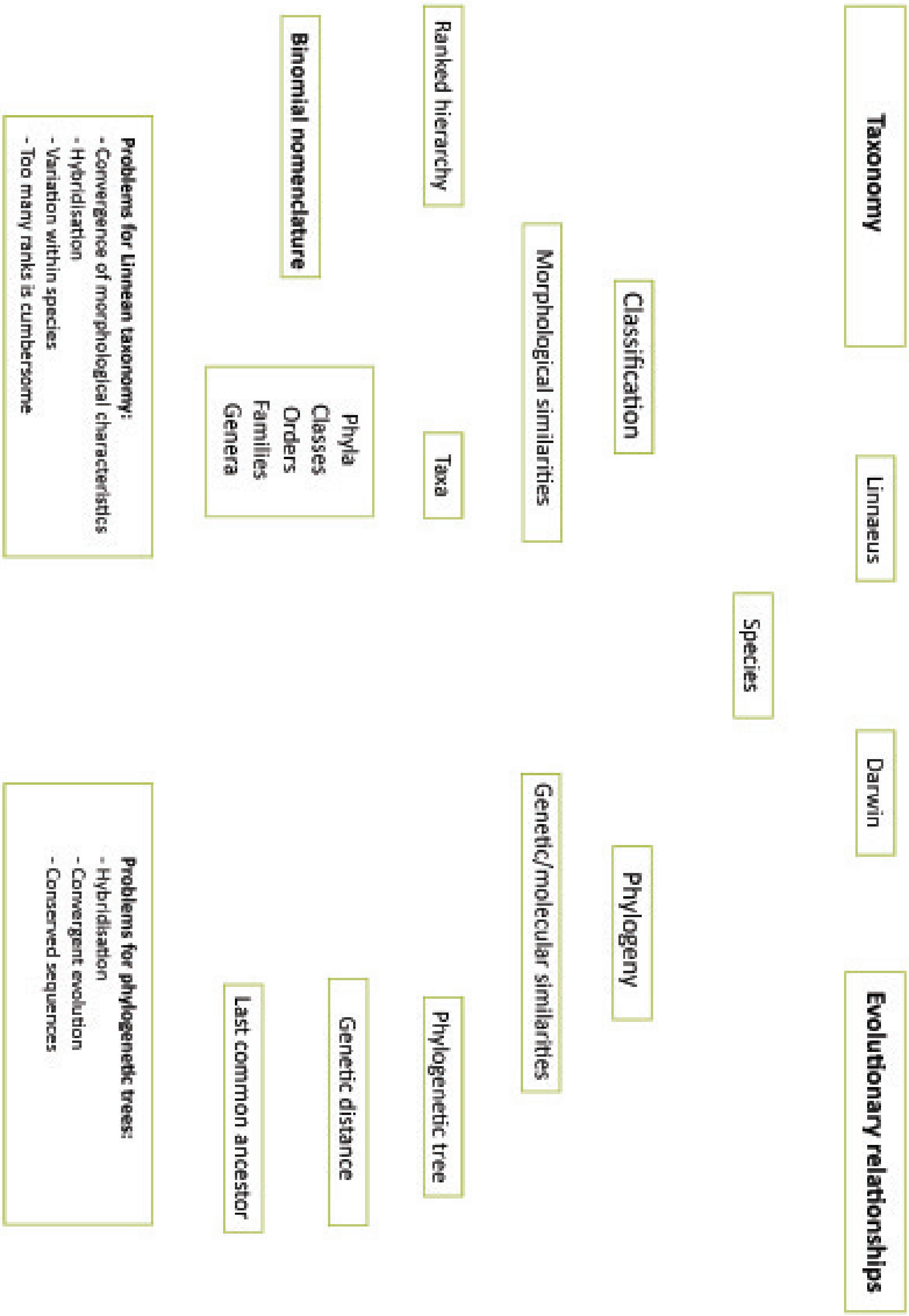
(Slide 13) Pupils will hopefully be familiar with making concept maps by linking key concepts with connecting lines and adding words to show the relationships. This exercise suggests relating two concepts to make clear their differences and connections. An outline map is provided for students to add connections and further concepts. Maps can then be reviewed as part of a class discussion.

A note on making concept maps:

- List the key concepts.
- Arrange the concepts in order of importance, and draw boxes around each concept.
- Connect the concepts by lines and add words to explain their relationships.

Concept map of relationships between classification and phylogeny

Link the key concepts in the concept map below with connecting lines to show the relationships. Add text to explain what these relationships are. Consider both differences and similarities, as well as any other connecting factors, when thinking about the relationships between concepts. You may also wish to add other relevant concepts to the map.



Lesson 3: New evidence from scanning electron microscopy (SEM)

Overview and lesson plan

This lesson will compare Darwin's microscope work with modern microscope techniques to explore how scientific knowledge is dependent on technology.

Students are asked to use information and data from a research paper to explore the limits of Darwin's equipment and analyse the results obtained.

Activity	Timing	Description and Pedagogical Approach
Lesson starter [Worksheet]	10 min	Small group discussion: reasoning and argumentation Students use a Sowerby's illustration of Darwin's barnacle dissected to discuss parts of its arthropod body and how it might feed.
Activity 1	15 min	Teacher-led discussion: observation, reasoning, causality, comparing Students look at nauplius at different magnifications of the microscope to ascertain the limitations of light microscopy
Activity 2 [Worksheet]	20 min	Small group discussion: reasoning, assessing, evaluating evidence and hypotheses Students interpret findings from a research paper that uses SEM evidence to study barnacle morphology. Students then consider how the evidence may affect phylogeny.
Plenary	15 min	Teacher-led feedback: discussion, reasoning, analytical thinking, problem solving Students discuss electron microscopes, resolution and scale and compare to light microscopes.

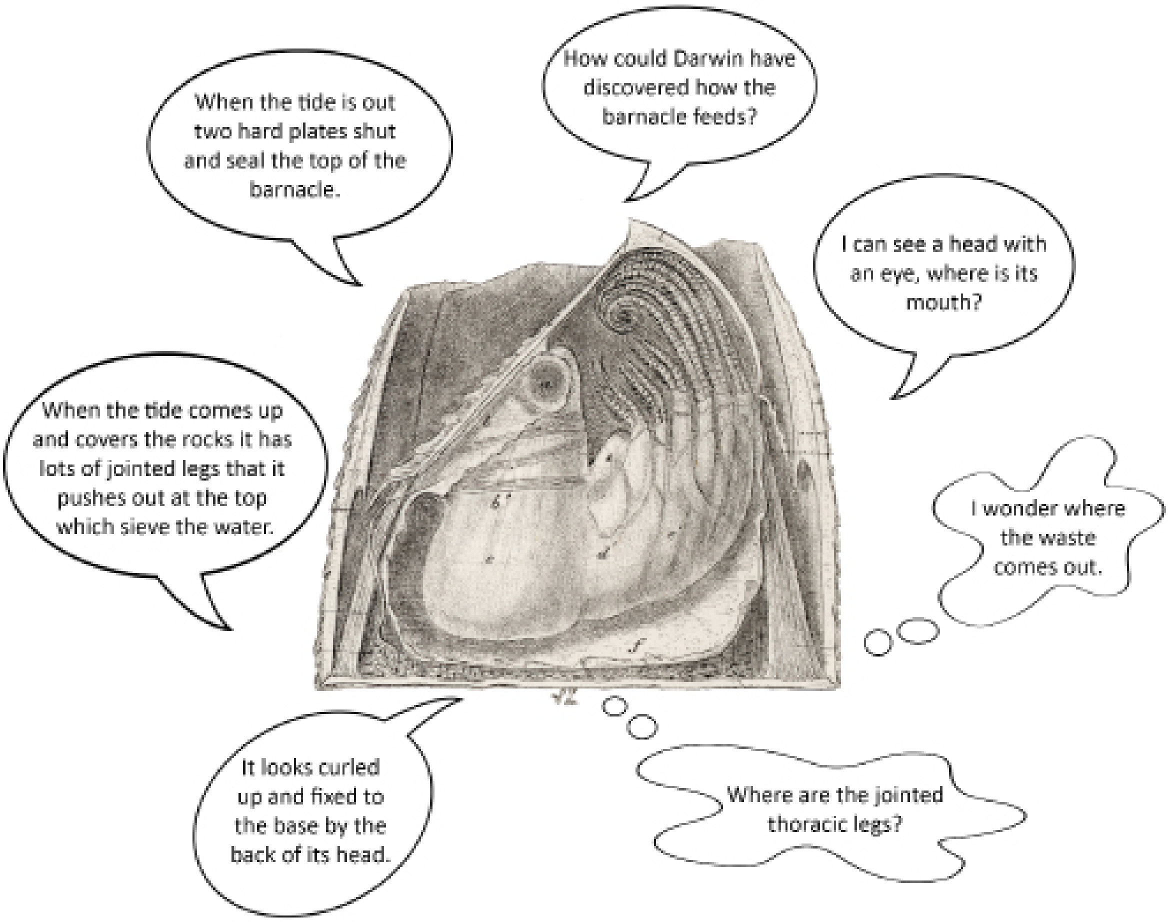
Lesson starter

- Show students the illustration of the internal structure of a barnacle by George Sowerby (Slide 2).
- In groups of four, using the diagram in the Student Pack, ask the students to discuss the statements and questions which have been made about barnacle nutrition. Then ask them to summarise how barnacles feed.

(If necessary, you can remind students of how barnacles use their cirri by showing the video on Slide 3 of the Lesson 1 PowerPoint).

Lesson starter: Barnacle nutrition - ANSWER

Below is a drawing by Sowerby of the inside of a typical barnacle. Discuss the statements and questions which have been made about barnacle nutrition.



Using the information in the diagram, summarise how barnacles feed.

Students will hopefully realise that the cirri are used for feeding and therefore essential for survival. Barnacles are suspension feeders and extend the cirri into the water to filter plankton before pulling into the shell to their mouth.

The compound light microscope: What Darwin saw

Charles Darwin was the first scientific naturalist to make a complete study of all the barnacles known at that time, both living and fossil. Through this work he perfected his skill in comparative anatomy and tested his developing understanding of how species evolve through natural selection, the foundational argument of *On the Origin of Species* (1859).

(Slide 3) For this task he ordered a new compound microscope from James Smith in London and it arrived in 1847 when it cost £34. He published his findings in two volumes entitled *Living Cirripedia* (*Cirripedia* being the Latin name for barnacles) and two volumes on *Fossil Cirripedia*. The backbone of his work, in his eight years of study, was to describe in great detail the features of each barnacle - both the external anatomy and the internal organs.

(Slide 4)

> ”I have purchased an 1/8inch object glass and it is grand – I have been getting on well with my beloved cirripedia and get more skilful in dissection”
>
> Charles Darwin, [in a letter to the botanist Joseph Hooker], May 1848

A compound microscope has both an eyepiece and objective lens and the magnification is calculated by multiplying the power of one by the other. In modern terms the 1/8 inch lens would have magnified nearly 70x and he could have achieved a maximum magnification of approaching 1,000x. However, at this magnification the resolution would have been low (i.e. he couldn’t clearly distinguish the fine detail because the lenses distorted the viewed image). Most of his high power work reported was at about 850x.

(Slide 5) This was a huge improvement over the magnification achieved with the simple single lens travelling microscope, that he took on *HMS Beagle*, which magnified about 10x. The development of high quality lenses for compound microscopes had helped Darwin to see more detail than anyone else had done before. It had extended his vision through improved technology.

Activity 1

To show the limits of the light microscope, students could make temporary mounts of nauplii of the brine shrimp *Artemia* (see Lesson 1 Activity 1) and work out the magnification and size of the larvae and experience the magnification and limits of resolution of the compound microscope.

Using technology to extend our vision today

Modern scientists have access to improved microscope technology allowing them to look more closely at the feeding mechanisms of barnacles, the larvae and the cementing mechanism. The level of detail needed for these studies is beyond the limit of resolution of the compound microscope.

(Slide 6) One research paper has used scanning electron microscopy (SEM) to look closely at the setae (limb hairs) on the cirri (thoracic limbs) on a range of barnacles.

They hypothesised that:

- The more diverse the range of setal types that a species has the more complex its feeding
- The setae can help to establish phylogenetic relationships between barnacles.

They studied seven species of barnacle, representing a range from the stalked and acorn classes. They adapted previous research that had shown that decapods (crabs, prawns, lobsters) had seven different types of setae on feeding limbs and each type had a different function.

(Slide 7) Setal type and function

- Serrulate = gentle handling of prey.
- Serrate = rough handling of prey.
- Plumose = generation of water currents and creating a barrier to stop ‘food’ escaping.
- Pappose = generation of water currents and filter feeding.
- Simple = rough handling of prey.
- Cuspidate = rough handling of prey.
- Multicuspidate = rough handling of prey.

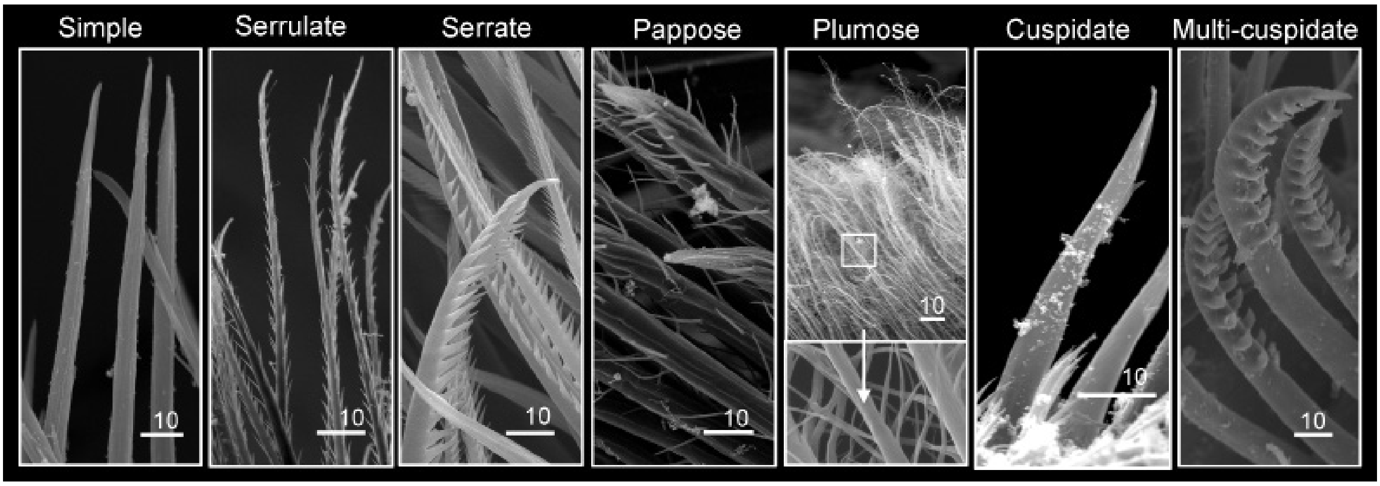
Electron microscopy and taxonomy - ANSWERS

This activity is based on findings from the research paper “Setal morphology and cirral setation of thoracican barnacle cirri: adaptations and implications for thoracican evolution” (2008) Chan *et al*. *Journal of Zoology*. Vol 275 issue 3. pp 294 - 306.

The authors hypothesised that:

- The more diverse the range of setae or limb hairs a barnacle has the more complex its feeding habits.
- The setae can help establish evolutionary relationships between barnacles.

They examined 7 species of barnacle representing a range within the barnacle class. They dissected out the limbs, coated them in platinum and examined them using a scanning electron microscope (SEM). A number of types of setae were found and using observations from other marine organisms like crabs and prawns a function was given to each of the 7 types:

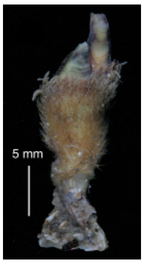
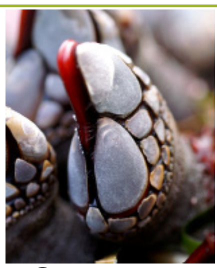
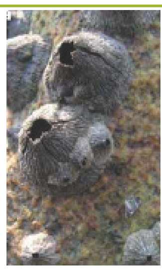


From Setal morphology and cirral setation of thoracican barnacle cirri: adaptations and implications for thoracican evolution, Chan *et al.* (2008) *Journal of Zoology*. Vol 275 issue 3. pp 294 - 306. With author’s permission.

Scale bars in µm

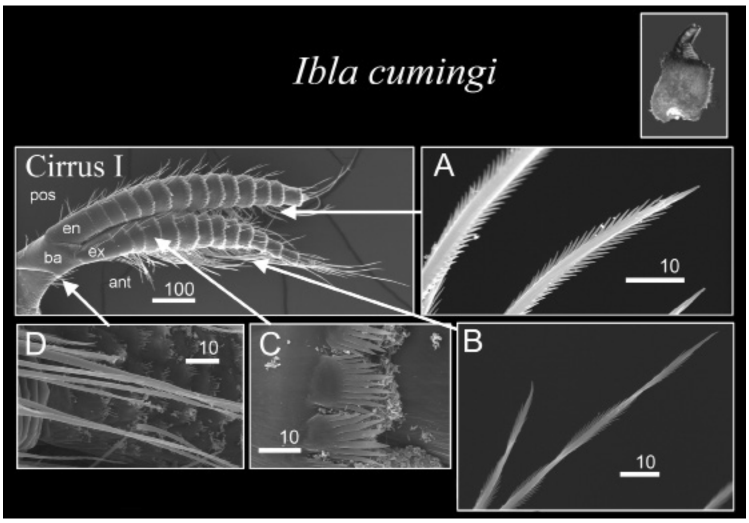
- Simple: rough handling of prey
- Serrulate: gentle handling of prey
- Serrate: rough handling of prey
- Pappose: generation of water currents and filter feeding
- Plumose: generation of water currents and creating a barrier to stop ‘food’ escaping
- Cuspidate: rough handling of prey
- Multicuspidate: rough handling of prey

Further information for 3 of the barnacle species is given in the following table.

Species	Illustration from Darwin's monograph (Sowerby)	Photograph	Diet	Habitat
Ibla cumingi		 © Benny Chan	Diatoms (single-celled algae) and tiny crustaceans	Inter-tidal rock crevices
Pollicipes polymerus		 © Minette Layne via Wikimedia	Diatoms (single-celled algae), small crustaceans and large crustaceans	Exposed rocky shores
Tetraclita japonica	N/A	 © Benny Chan	Diatoms (single-celled algae) and small crustaceans	Exposed rocky shores

Use this table and the scanning electron microscopy (SEM) images to answer the following questions.

1. Looking at Cirrus I of *Ibla cumingi* in the image below.



Scale bars in µm

From Setal morphology and cirral setation of thoracican barnacle cirri: adaptations and implications for thoracican evolution, Chan *et al*. (2008) *Journal of Zoology*. Vol 275 issue 3. pp 294 - 306. With author's permission.

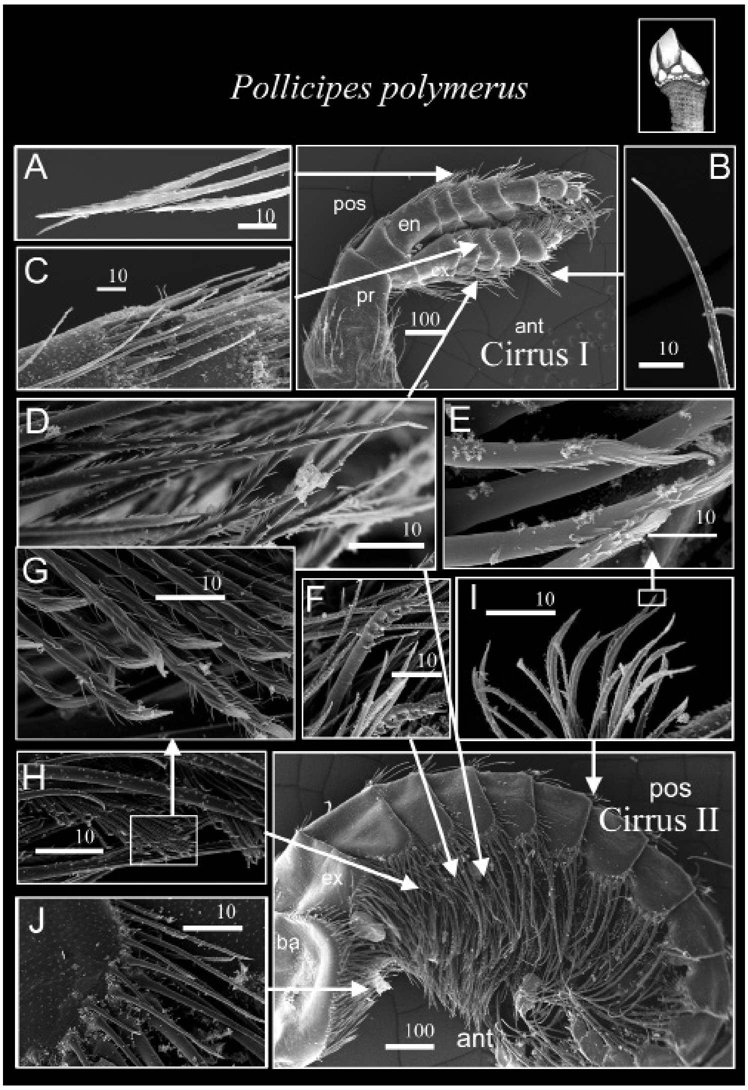
a) Count the number of segments in the upper branch of the limb. How many are there?
14.

b) How long is each branch of the limb? (there are 2)
Branches are 600µm and 500µm.

c) How might the pattern of the setae of the barnacle *Ibla* relate to its lifestyle?
Ibla lives in crevices where there is little disturbance and where the prey are small, so fine serrulate hairs are sufficient for handling of small prey type.

d) *Ibla* is considered to be one of the oldest forms of barnacle and this is demonstrated by its position in the phylogenetic tree studied in Lesson 2. Do you think the pattern on the setae is consistent with *Ibla* being the ancestral species? Why?
The serrulate setae appear on the others and are simple in structure.

2. Looking at the cirri on *Pollicipes polymerus* in the image below.



Scale bars in µm

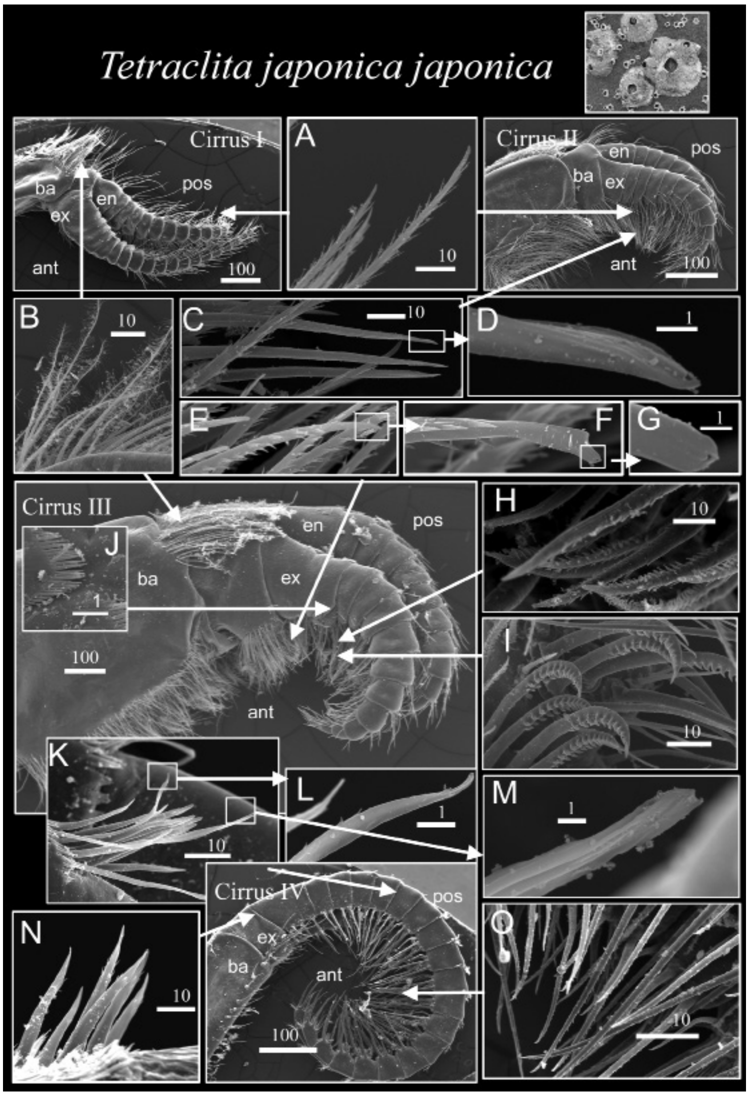
From Setal morphology and cirral setation of thoracican barnacle cirri: adaptations and implications for thoracican evolution, Chan *et al*. (2008) *Journal of Zoology*. Vol 275 issue 3. pp 294 - 306. With author's permission.

a) Identify the multicuspidate setae. How wide are they?
2.6µm. (F shows the multicuspidate setae).

b) How might the pattern of the setae of the barnacle *Pollicipes* relate to its lifestyle?
Pollicipes has serrulate (A, B, C, D, E, I, J), multicuspidate (F) and pappose (G, H) setae. *Pollicipes* lives on exposed shores and feeds on a wide variety of available prey of different sizes and mobility, and the

multicuspidate limbs allow rough handling of prey. It is a pedunculate barnacle and thus can wave in the flow, generating a water current.

3. Looking at the cirri on *Tetraclita japonica* in the image below.



Scale bars in µm

From Setal morphology and cirral setation of thoracican barnacle cirri: adaptations and implications for thoracican evolution, Chan *et al*. (2008) *Journal of Zoology*. Vol 275 issue 3. pp 294 - 306. With author's permission.

a) What types of setae can be seen?

Serrulate (seen in A, D, E, F, G, K, L, M, O), plumose (seen in B), simple (seen in C), serrate (seen in H), multicuspidate (seen in I) and cuspidate (seen in N). (J just shows detail on Cirrus III)

b) Using the information given in the table and the descriptions of setae functions, explain why *Tetraclita japonica* (an acorn type barnacle) might have a wider variety of setae than *Ibla cumingi* and *Pollicipes polymerus* (both goose type barnacles).

Their sessile lifestyle is compensated for by the wide variety of setae which enable them to feed and breathe effectively. Their hard cases close when the tide is out and prevent desiccation and protect the soft inner parts from damage by waves.

Tetraclita also inhabits rocky shores but is sessile and requires pappose and plumose setae to generate the flow of water for respiration and simple serrulate and cuspidate setae to deal with different prey.

Plenary
Resolution and scale in light and electron microscopes

There are limits to the magnification and resolution of light microscopes due not only to the quality of the lenses but due to the nature of light itself. Structures closer together than 200nm cannot be distinguished because the wavelength of visible light is 400nm (maximum resolution is half the wavelength of the radiation used).

(Slide 8)

	Human eye	Compound light microscope	Transmission electron microscope	Scanning electron microscope
Specimen preparation	-	Mounted alive or prepared in natural state or stained	Dry samples due to vacuum chamber Samples must be cut into very thin cross-sections	Dry samples due to vacuum chamber Samples require a coating of heavy metals
Magnification possible	-	Up to 1,500x	500,000x	10x - 500,000x
Wavelength used	Light 400-750nm	Light 400-750nm	Electron beam .004nm Passes through specimen (transmitted)	Electron beam .004nm Reflects off surface of specimen
Resolution possible	100,000nm	200nm	0.2nm	0.2nm
Viewed	Directly	Viewed directly by eye/screen based	Via micrographs or on fluorescent screen	Via micrographs or on fluorescent screen

(Slide 9) 1 m = 1,000 mm (millimetres), 1 mm = 1,000 µm (micrometres), 1 µm = 1,000 nm (nanometres)

Viruses range in size from 40 - 100nm
Ribosomes are around 20nm
Bacteria range in size from 500 - 5,000nm

(Slide 10) Discussion:
How many µm in 1 metre?
What fraction of a metre is 1nm?
Would it have been possible for Darwin to see bacteria and viruses with his compound light microscope?

The future of barnacle taxonomy

(Slide 11) There is much that we still don't know about the feeding behaviour of many barnacles, the structures they use and how these structures evolved from simple undifferentiated jointed thoracic limbs.

The use of the new technology of scanning electron microscopy is adding a new extension to our vision just as the development of effective compound microscopes did for Darwin in the 1860s.

The family tree of the barnacle is far from settled as scientists marry morphological, physiological and molecular evidence from the known barnacles.

Large groups of barnacles, such as those that attach to whales, those in deep sea vents and those in the deep ocean have yet to be investigated.

Extension Activity - Barnacle distribution on the rocky shore

This exercise uses an article from *Catalyst* magazine called Battle of the Barnacles, written by Gary Skinner, that can be downloaded from:
http://www.nationalstemcentre.org.uk/elibrary/resource/2670/battle-of-the-barnacles

In tidal regions with rocky shores the organisms that live in the intertidal zones are distributed in bands from low water to high water. Barnacles are no exception and data have been collected around British shores on their distribution since the 1950s. These show the effect of global warming over the period since then, with the warmer water species of *Chthamalus montagui* being found much further north than it used to be.

There are two species of *Chthamalus* and they typically occur higher up the shore than *Semibalanus*. One of the first experiments in ecology suggested that this was due to competition from *Semibalanus* preventing *Chthamalus* from colonising lower down.

Q: Can you suggest how the experiment might have been conducted?
The experiment is described in detail in the *Catalyst* article. It involved transplanting rocks with *Chthalamus* lower down the shore and removing the *Semibalanus* if they started to settle nearby. Where they were removed, *Chthalamus* did well.

Q: How do barnacles colonise other areas?
With free-swimming nauplii larvae (see lesson 1) which metamorphose into cyprid larvae to settle and glue to the rock.

It is possible to practice surveying barnacles by using photographs when access to the rocky shore is limited. The article mentioned above contains an exercise to survey barnacles from detailed photos of a vertical rocky wall encrusted with British barnacle species.

For this exercise remember that:
Chthamalus spp have lines across the kite shaped central area that cross at right angles.
Semibalanus spp have lines that cross the diamond shaped central area forming a V shape and tend to be whiter.

- Print the 3 large close up photos of the barnacles on a vertical rock wall. Stick the A4 photos together end on in the order shown and mark the top of the rocky wall.
- Make a quadrat by cutting a 10 cm square hole in a piece of card.
- Place your quadrat at equal intervals along a vertical line on the photos.
- Record the number of *Chthamalus* and *Semibalanus* species in each quadrat.
- Plot a kite diagram for each genus (see notes below).

Decide from the results if the hypothesis that the two species of *Chthamalus* occur higher up the shore than *Semibalanus* is supported.

Q: What other data would it be useful to have that in a real situation would be easier to collect?

Note on constructing kite diagrams:
- Kite diagrams show the distribution along the transect of particular species.
- The x axis shows the distance along the transect.
- The y axis records the number of individuals.
- Record half the number of individuals above and half below at each sampling point.
- If the scales of the x axes match, species distributions can be compared.

Exam-style question

1 (a) (i) Explain what is meant by the term taxonomy.

(1 mark)

Defining groups of organisms on the basis of similar characteristics and giving names to those groups/ the science dealing with the description, identification, naming, and classification of organisms;

1 (a) (ii) What is the word used to describe the study of evolutionary relationships between organisms?

(1 mark)

Phylogenetics;

1 (b) Naming living things depends on a hierarchical system with the domain at the top and the species at the bottom. Complete the boxes to show the missing levels in this hierarchy.

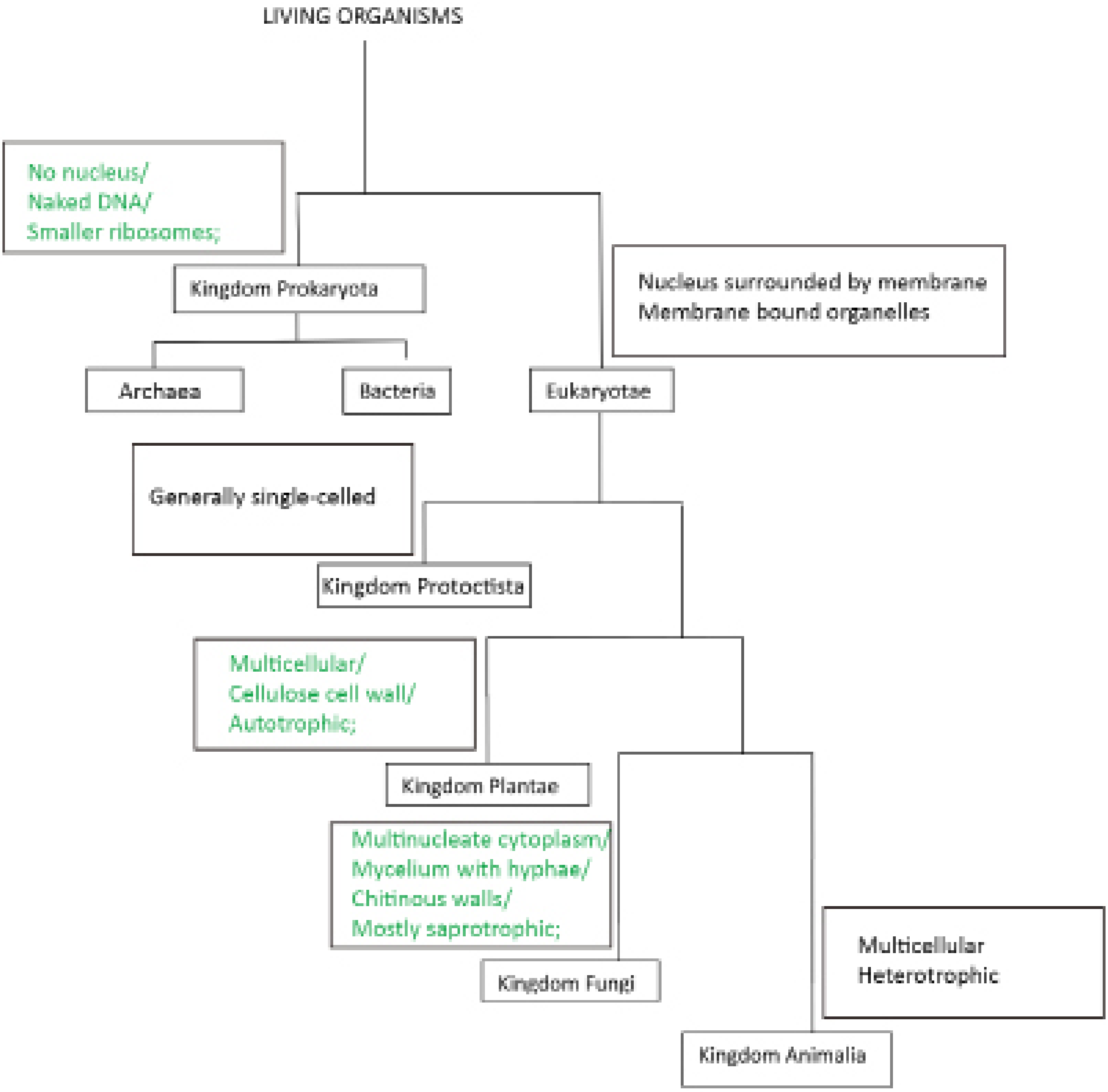
(3 marks)

Domain
Kingdom
Phylum;
Class
Order;
Family
Genus;
Species

QUESTION CONTINUES ON NEXT PAGE

1 (c) The diagram shows the 5 kingdoms of living things. Label the diagram to show the features used in constructing the tree. Some boxes have already been completed.

(3 marks)



8

2 The recent three domain theory is based on the study of RNA and the cell membrane, with Bacteria, Archaea and Eukaryotes as the three domains. The chart below shows some of the features used in this domain classification.

	Bacteria	Archaea	Eukaryotes
Ribosomes	Small ribosomes	Small ribosomes	Large ribosomes
*RNA polymerase	Simple. 5 proteins	8-10 proteins	12 proteins
Features of RNA transcription: **Promoter sites	2 sites before the start of the transcription site	***TATA sequence at start of transcription site	***TATA sequence at start of transcription site
Cell wall structure	Peptidoglycan present Have ester bonds with fatty acids and glycerol backbone	No peptidoglycan Have ether linkages in their fatty acids and glycerol backbone making them very stable	No peptidoglycan Have ester bonds with fatty acids and glycerol backbone
Nucleus?	No nucleus within a membrane	No nucleus within a membrane	Nucleus has membrane

**The primary RNA polymerase of all organisms is responsible for creating messenger RNA that is then translated into proteins at the ribosome.*
***Promoter sites are segments of DNA usually occurring upstream from a gene coding region and acting as a controlling element in the expression of that gene.*
****The TATA box has the core DNA sequence 5'-TATAAA-3' or a variant, which is usually followed by three or more adenine bases. It is usually located 25 base pairs upstream of the transcription site.*

2 (a) Explain why scientists think that Archaea are more closely related to Eukaryotes than they are to Bacteria.

(2 marks)

similarites in ribosomes/nucleus between Archaea and Bacteria;
Archaea and Eukaryotes both have the same feature of RNA transcription/TATA sequence at start of transcription site (whereas Bacteria do not);

2 (b) Suggest why ribosomal RNA is a good feature to use in domain classification?

(2 marks)

ribosomal RNA/rRNA carry out the same function (throughout nature)/their structure changes very little over time;
(therefore) similarities in rRNA are a good indication of how related cells or organisms are/differences in rRNA are a good indication of how unrelated different cells or organisms are;

4

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