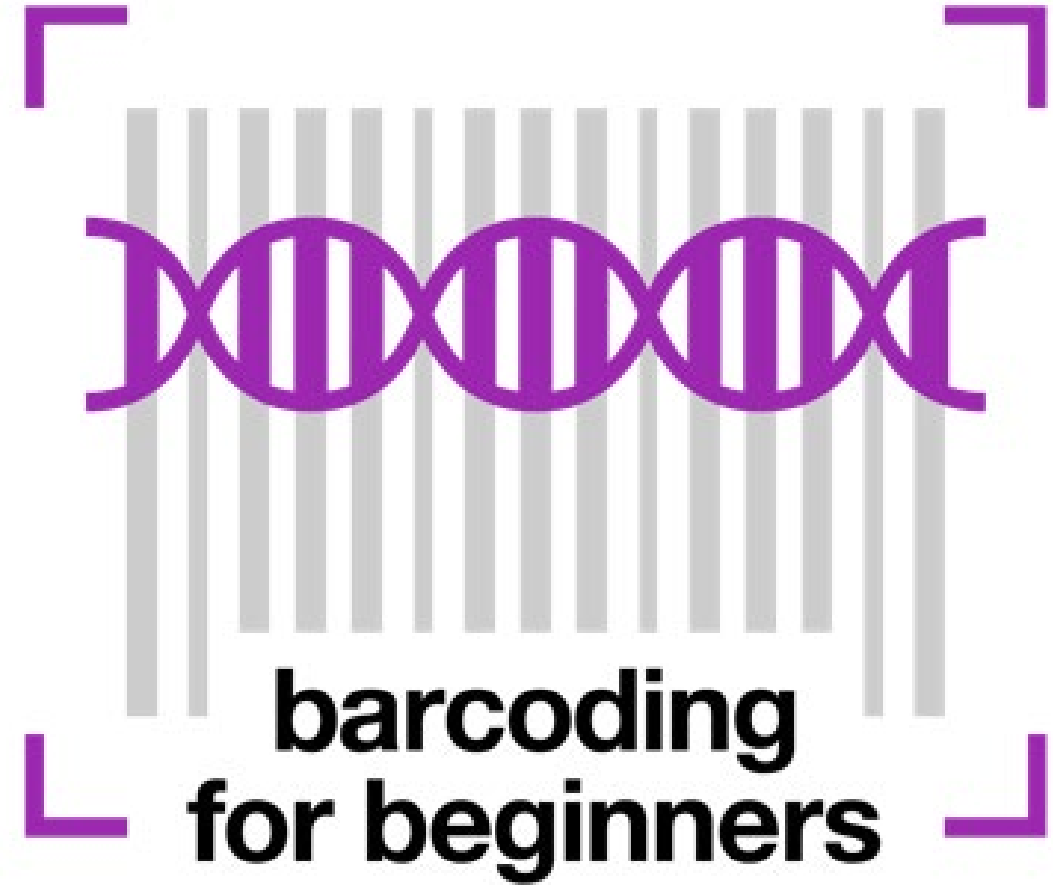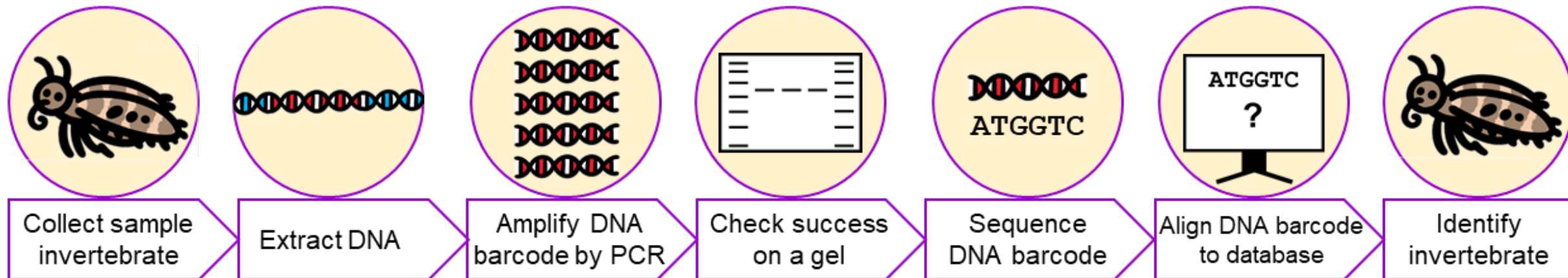# Overview of practical activities

Next practical activities

Use some pre-sequenced invertebrate DNA to develop skills in bioinformatics as you interpret chromatogram quality and identify unknown invertebrates from their DNA barcodes.

Collect sample invertebrate → Extract DNA → Amplify DNA barcode by PCR → Check success on a gel → Sequence DNA barcode → Align DNA barcode to database → Identify invertebrate

Create a phylogenetic tree

Explaining phylogenetic trees

A phylogenetic tree is a diagram that represents evolutionary relationships among organisms.

In the same way that a family tree shows who has descended from whom, a phylogenetic tree shows which organisms have descended (evolved) from other organisms.
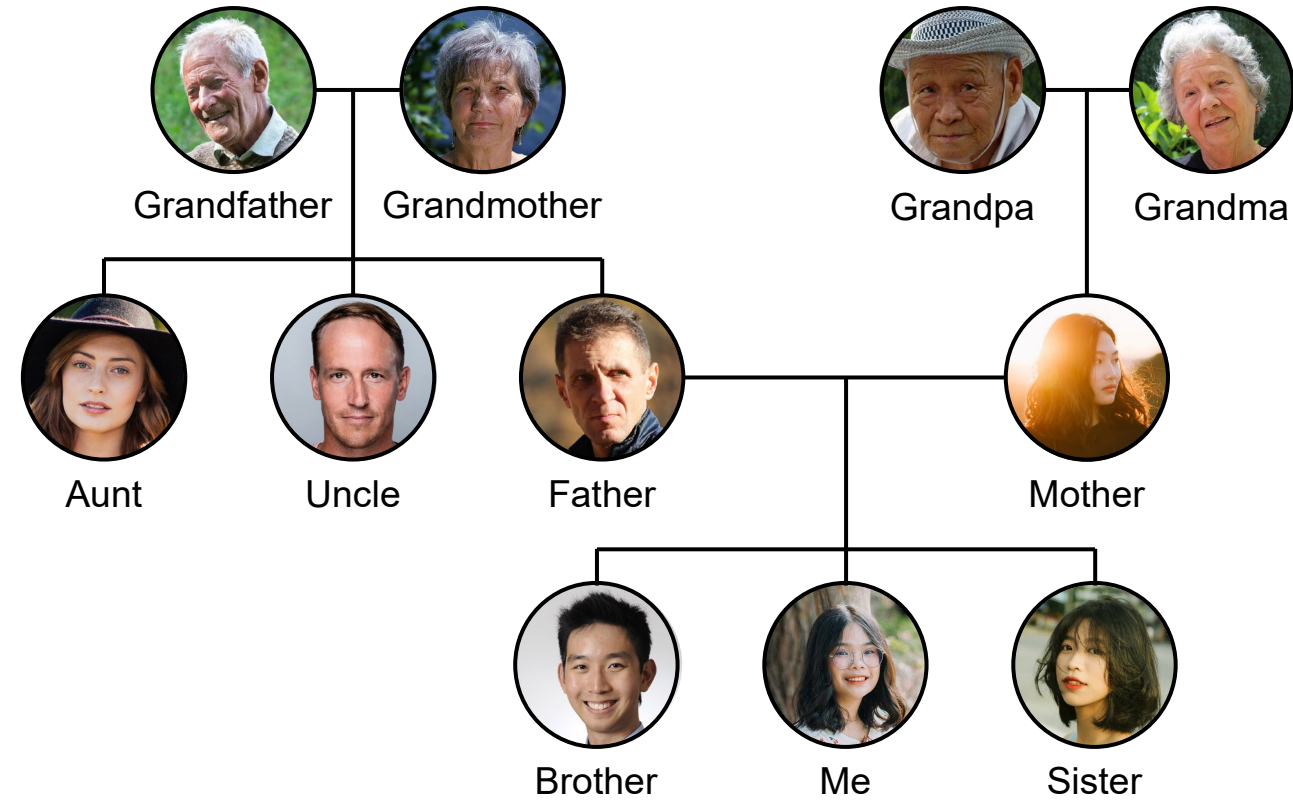
Explaining phylogenetic trees

Charles Darwin was the first to propose that all species on earth are related and that they had evolved from a common ancestor.
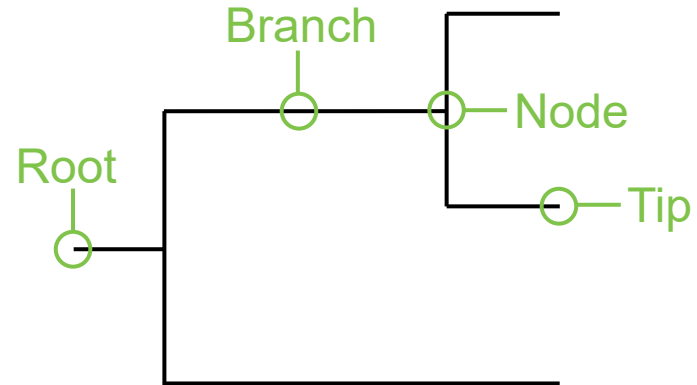
He illustrated this with a 'tree of life' sketch in 1837 in his First Notebook on Transmutation of Species.
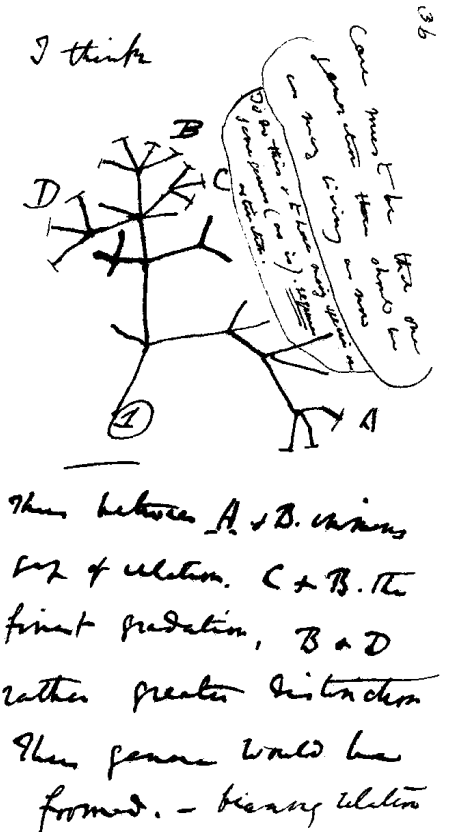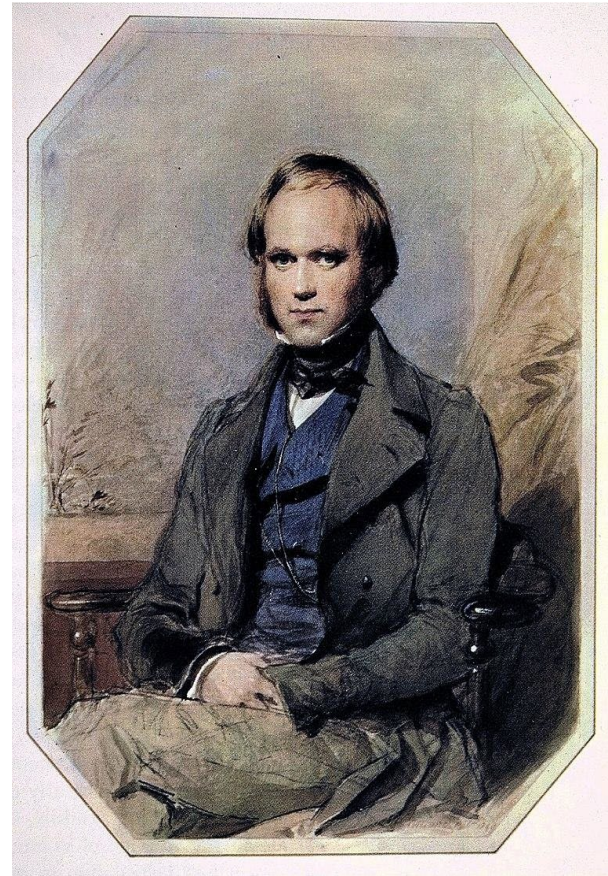
Explaining phylogenetic trees

Since the work of Charles Darwin:

- the way that we represent phylogenetic trees has been standardised.

Root - a common ancestor for the whole tree

Branch length - the relative time since which species diverged

Node - where speciation occurred from a common ancestor

Tips - the species represented in the phylogenetic tree

Explaining phylogenetic trees

Since the work of Charles Darwin:

- the way that we represent phylogenetic trees has been standardised.

- the characteristics that we use to classify have become more sophisticated.

The system for classification, proposed by Carl Linneaus in 1758, recognised 2 primary kingdoms: Plantae and Animalia based on structure and function.

| The living being		
Kingdom: Plantae		Kingdom: Animalia
x	Locomotion	✓
x	Response to external stimuli	✓
do not eat	Mode of nutrition	eat
x	Conductile and contractile system	✓
✓	Cell wall	x

Explaining phylogenetic trees
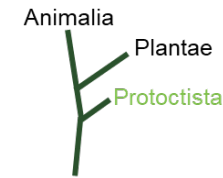
Since the work of Charles Darwin:

- the way that we represent phylogenetic trees has been standardised.

- the characteristics that we use to classify have become more sophisticated.

As the field of classification developed, the morphology (shape and structural features) and mode of nutrition of organisms continued to be used by taxonomists.
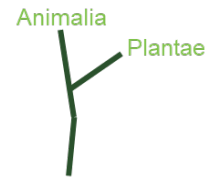
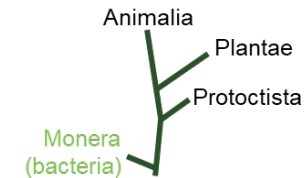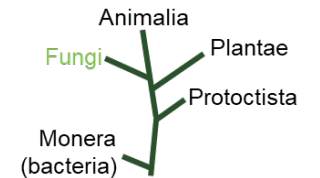
Carl Linnaeus (1758)

2 kingdoms

Ernst Haeckel (1866)

3rd kingdom

Herbert Copeland (1938)

4th kingdom
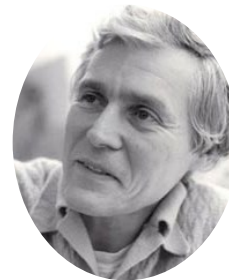
Robert Whittaker (1969)

5th kingdom

Explaining phylogenetic trees

Since the work of Charles Darwin:

- the way that we represent phylogenetic trees has been standardised.

- the characteristics that we use to classify have become more sophisticated.

Carl Woese was the first to use molecular analysis of genetic material in classification.

He sequenced 16S ribosomal RNA and classified microorganisms based on sequence similarity, rather than morphological similarity.

Carl
Woese
(1977)

6 kingdoms
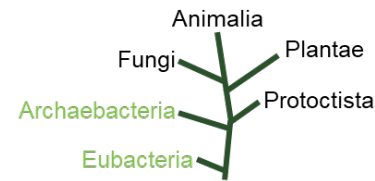

The 3D structure of rRNA.

Explaining phylogenetic trees

Since the work of Charles Darwin:

- the way that we represent phylogenetic trees has been standardised.

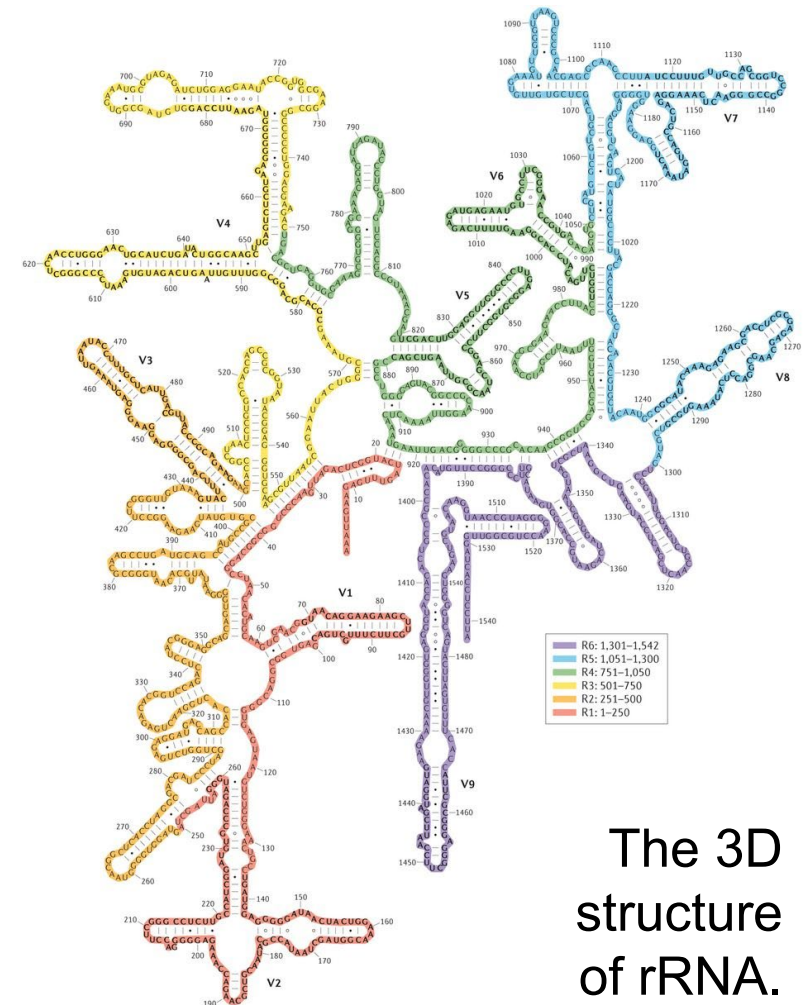- the characteristics that we use to classify have become more sophisticated.

- The use of DNA sequence in phylogeny has become more widespread.

Cletus Kurtzman compared DNA sequence similarity to classify yeast.

Comparing the short DNA barcode could contribute to phylogenetic analysis.

ATGGTC   ATGGTC

Same DNA barcode = same species

ATGGTC   ATTGTC

Similar DNA barcode = more closely related species

ATGGTC   GTTGCG

Dissimilar DNA barcode = less closely related species

Explaining phylogenetic trees

- Comparison of complete genomes gives the greatest information for phylogenetic analysis, but takes a lot of time and computing power.

- The shorter DNA barcode sequence can be used to look at the relatedness of organisms more rapidly and using less computing power. However, the data produced will be less accurate.

ATGGTC   ATGGTC

Same DNA barcode = same species

ATGGTC   ATTGTC

Similar DNA barcode = more closely related species

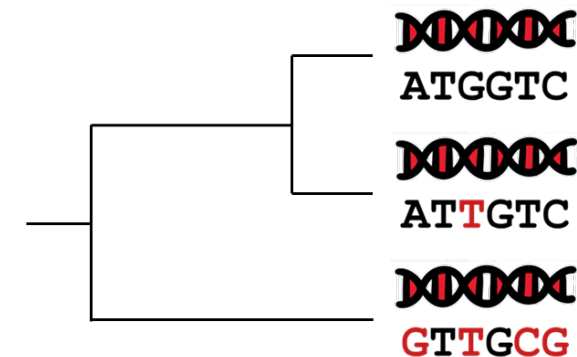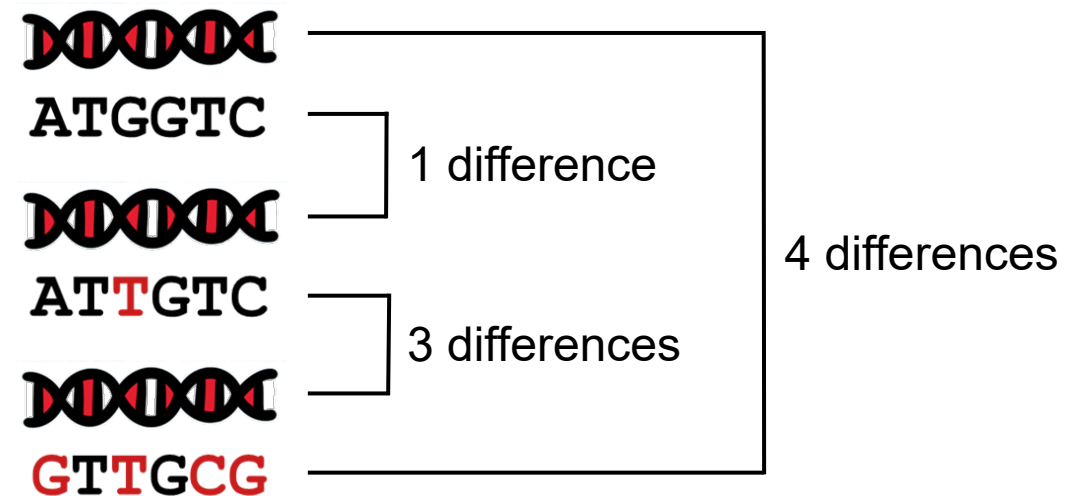ATGGTC   GTTGCG

Dissimilar DNA barcode = less closely related species

Creating a phylogenetic tree using DNA barcode sequence

To create a phylogenetic tree using DNA barcode sequence:

- use DNA barcode sequences from multiple organisms

- align multiple DNA barcode sequences using a bioinformatics tool called Clustal Omega

- this will give the number of differences in DNA sequence between each pair

- use this data to draw a phylogenetic tree using a bioinformatics tool called EMBL Interactive Tree of Life

Creating a phylogenetic tree using DNA barcode sequence

To create a phylogenetic tree using DNA barcode sequence:

- use DNA barcode sequences from multiple organisms

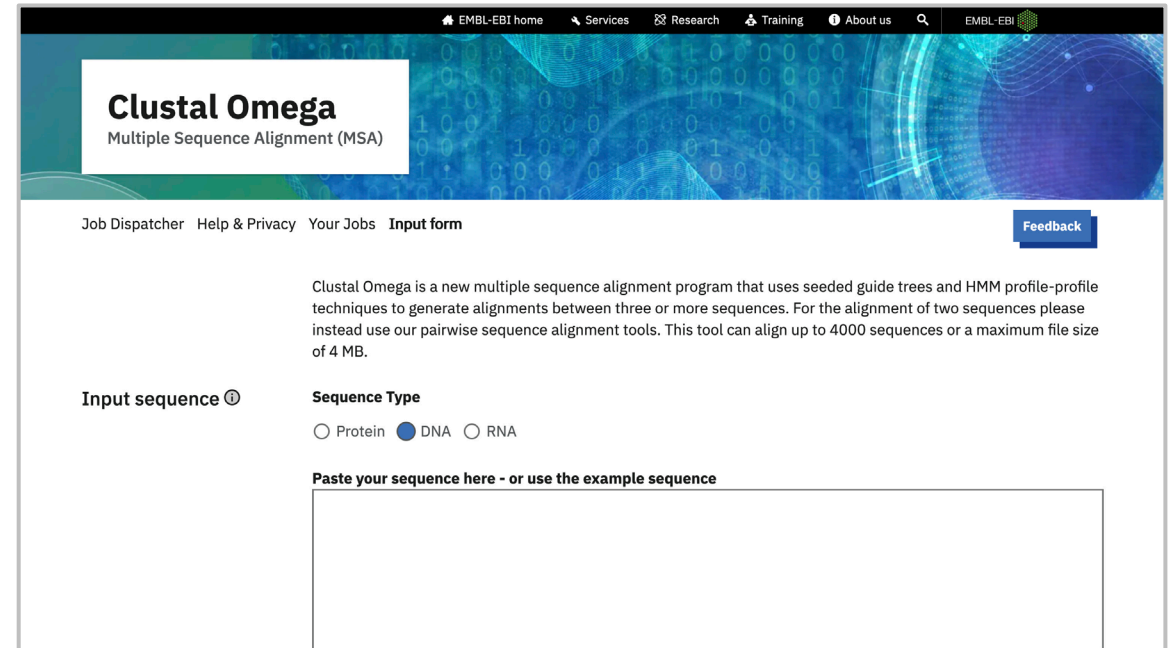- Find the DNA barcodes in a file named 'KnownBarcodes'

```
>7 spot ladybird
AACATTATATTTCTTATTCGGAATATGAGCCGGAATAATTGGGACCTCTTTAAGAATTTT
AATTCGTCTTGAATTAGGAACTACTAATAGATTAATTGGAAATGACCAAATTTATAATGT
AATTGTAACAGCTCATGCCTTCATTATAATTTTTTTTATAGTTATACCAATTATAATTGG
AGGATTTGGAAATTGACTTGTTCCTTTAATAATTGGAGCACCTGACATAGCTTTCCCTCG
ATTAAATAATATAAGATTTTGACTACTCCCACCTGCCTTAACCTTACTTATTATTAGAAG
ATTAGTGGAAATAGGTGCAGGAACTGGATGAACTGTCTACCCTCCTTATCCTCTAACTT
AGCTCATAATGGACCTTCAGTAGATTTAGTAATTTTTAGTTTACACTTAGCAGGTATCTC
ATCTATTTTAGGAGCCGTAAATTTTATTTCAACTATTATAAATATACGACCATTTGGCAT
AAACCTTGATAAGACACCTCTTTTTGTATGATCAGTACTAATTACTGCTATTTTACTTTT
ATTATCATTACCTGTATTAGCCGGGGCAATTACAATATTATTAACAGATCGTAATATTAA
TACTTCTTTTTTTTGATCCAATAGGAGGGGAGATCCCATCCTTTATCAACATTTATTT
```

Creating a phylogenetic tree using DNA barcode sequence

To create a phylogenetic tree using DNA barcode sequence:

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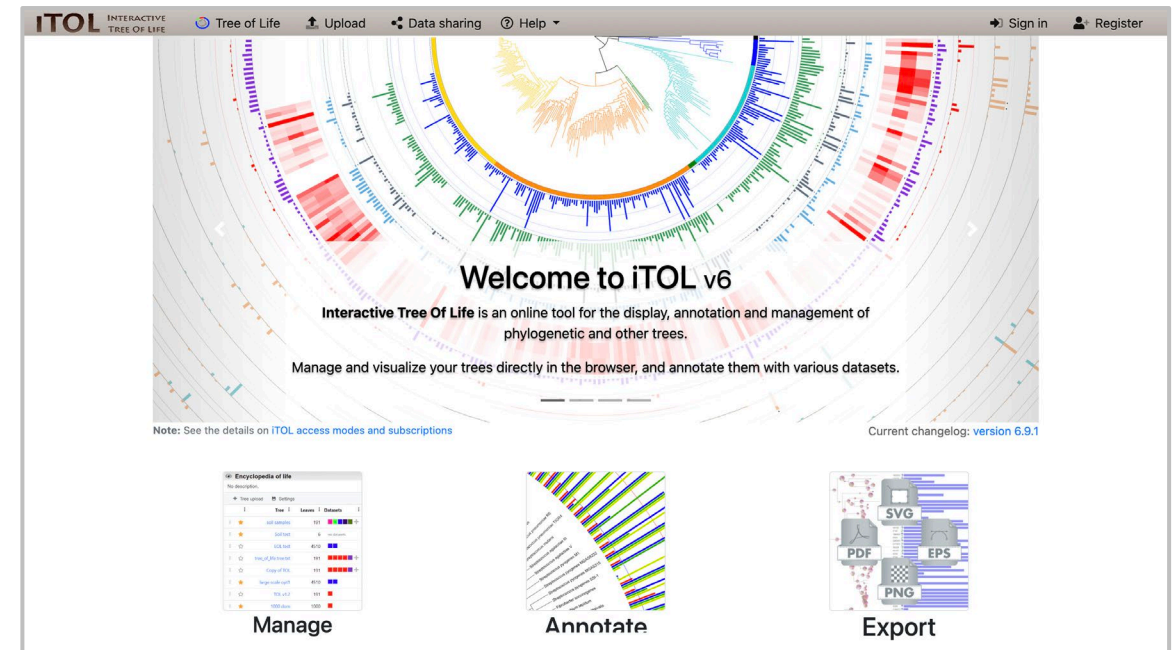Follow the printed or digital instructions to align the barcode sequences from the invertebrates using Clustal Omega

Creating a phylogenetic tree using DNA barcode sequence

To create a phylogenetic tree using DNA barcode sequence:

- collect DNA barcode sequences from multiple organisms

- align multiple DNA barcode sequences using a bioinformatics tool called Clustal Omega

- this will give the number of differences in DNA sequence between each pair

- use this data to draw a phylogenetic tree using a bioinformatics tool called EMBL Interactive Tree of Life

Follow the printed or digital instructions to display information as a phylogenetic tree using the Interactive Tree of Life website
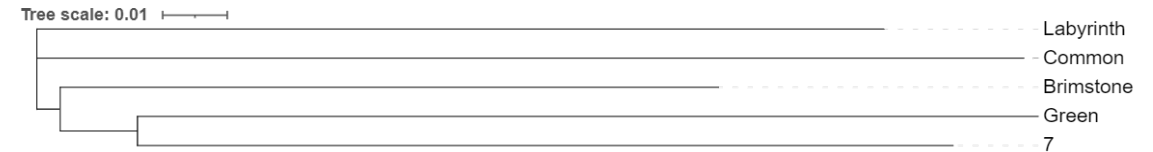
Creating a phylogenetic tree using DNA barcode sequence

💬 Compare your phylogenetic tree to others in the class.

✎ A. Which barcodes (and therefore organisms) are most closely related from this phylogenetic tree?

You should see a phylogenetic tree like the one opposite. On this phylogenetic tree the green peach aphid and 7-spot ladybird are most closely related, then the brimstone butterfly. The labyrinth spider and common woodlouse are less closely related to the other invertebrates.

Tree scale: 0.01

Labyrinth
Common
Brimstone
Green
7

Creating a phylogenetic tree using DNA barcode sequence

B. Look at the organisms most closely related – does this make sense with your knowledge of the invertebrates sampled?

Certainly the spider and woodlouse have different numbers of legs to the butterfly, aphid and ladybird, so the fact that they are less related is not surprising. The ladybird and aphid both have a rounded body shape with head, thorax and abdomen and each has 6 legs, so the fact that they are most related is also believable.

Tree scale: 0.01

Labyrinth
Common
Brimstone
Green
7
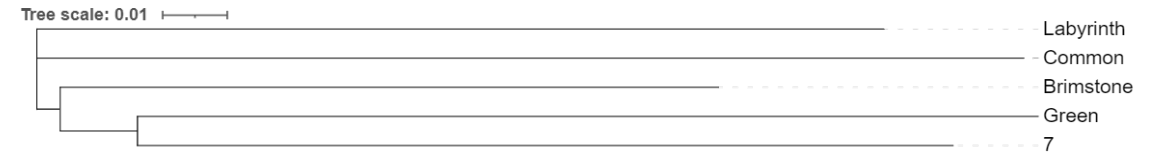
Creating a phylogenetic tree using DNA barcode sequence

C. How could you make the phylogenetic tree more accurate?

To make the phylogenetic tree more accurate, a longer DNA sequence should be used. This will give greater ability to examine similarity at the genetic (or even genomic) level between species.

Tree scale: 0.01

Labyrinth
Common
Brimstone
Green
7
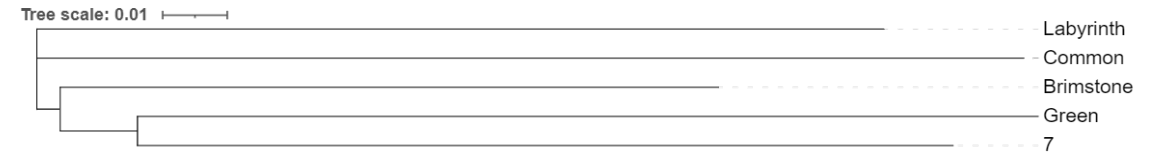
Genome = 799 million bases

DNA barcode amplicon = 710 bases