THE UNIVERSITY OF MELBOURNE

COMPUTATIONAL BIOLOGY RESEARCH

A Hallmark Research Initiative

Introduction to Pathogen Phylogenetic analyses

November 20 - 22
University of Melbourne

Contributors

21 workshops, 5 locations

Several contributors over the years

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Bayesian phylogenetic methods, Language evolution, BEAST2

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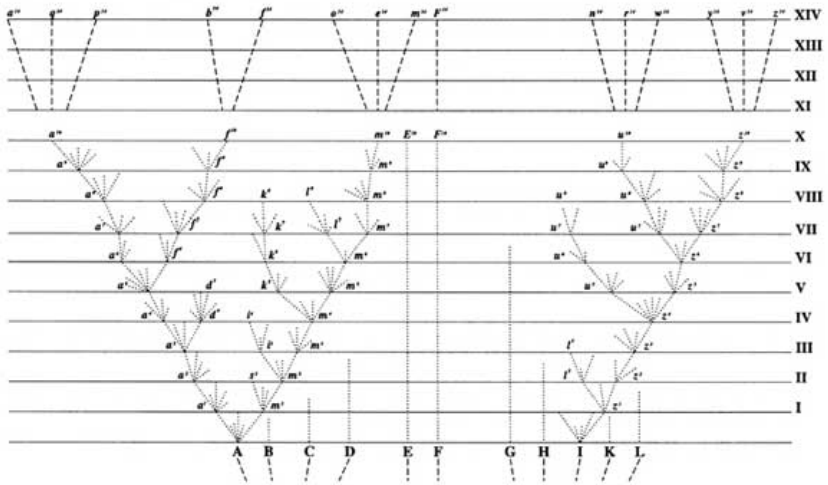
University of Sydney

Infectious diseases, virus evolution

Workshop overview

- Introduction to phylogenetic analysis
  - Interpreting phylogenetic trees
  - Phylogenetic methods
  - Estimating evolutionary rates and timescales
  - Bayesian phylogenetic analysis
  - Phylodynamics



MEGA

- *Molecular Evolutionary Genetics Analysis*
- Koichiro Tamura and Sudhir Kumar
- Population genetics and phylogenetics
  - Maximum parsimony
  - Distance-based methods
  - Maximum likelihood

PhyML

- Maximum likelihood phylogenetic tree inference
- Guindon S, Dufayard, JF, Lefort V, Anisimova M, Hordijk W, Gascuel, O.
- Command line interface
- Rapid branch support

TempEst

- Visualisation tool to assessing temporal structure
- Rambaut A, Lam T, de Carvalho L, Pybus O.
- Graphical interface

BEAST

- *Bayesian Evolutionary Analysis by Sampling Trees*
- Beast 1:Alexei Drummond & Andrew Rambaut
- **Beast 2:** Bouckaert et al.
- Bayesian phylogenetic analysis
  - Implements many evolutionary models – flexible but complex

BEAST 2 (and BEAST 1.6x)

BEAST 1

R

- Programming language, designed for statistics and graphics
- Ross Ihaka and Robert Gentleman
- We will only use it for a few demonstrations and programming knowledge is not necessary.

Workshop programme – Day 1

09:15 – 09:30	**Arrival**
09:45 – 10:30	**Lecture 1.1**: Introduction to molecular phylogenetics
10:30 – 11:00	**Practical 1a**: Sequence alignment and using *MEGA*
	– Coffee break –
11:30 – 12:30	**Lecture 1.2**: Substitution models
	– Lunch –
13:30 – 14:15	**Lecture 1.3**: Phylogenetic methods
14:15 – 14:45	**Practical 1b**: Model selection in *MEGA*
14:45 – 15:15	**Practical 1c**: Maximum likelihood analysis in *PhyML*
15:20 – 16:30	Public Seminar by JS Eden (Agar theatre)

Workshop programme – Day 2

09:50 – 9:30	Arrival
09:30 – 10:15	Lecture 2.1: The molecular clock
10:15 – 11:00	Practical 2a: Assessing temporal structure in *TempEst*
	– Coffee break –
11:30 – 12:30	Lecture 2.2: Bayesian phylogenetics
	– Lunch break –
13:30 – 14:30	Lecture 2.3: Priors in Bayesian phylogenetics
14:30 – 15:00	Practical 2b: Markov Chain Monte Carlo
15:00 – 15:30	Lecture 2.4: Demographic priors and model selection
15:30 – 16:30	Practical 3a: Molecular dating using *BEAST2*

Workshop programme – Day 3

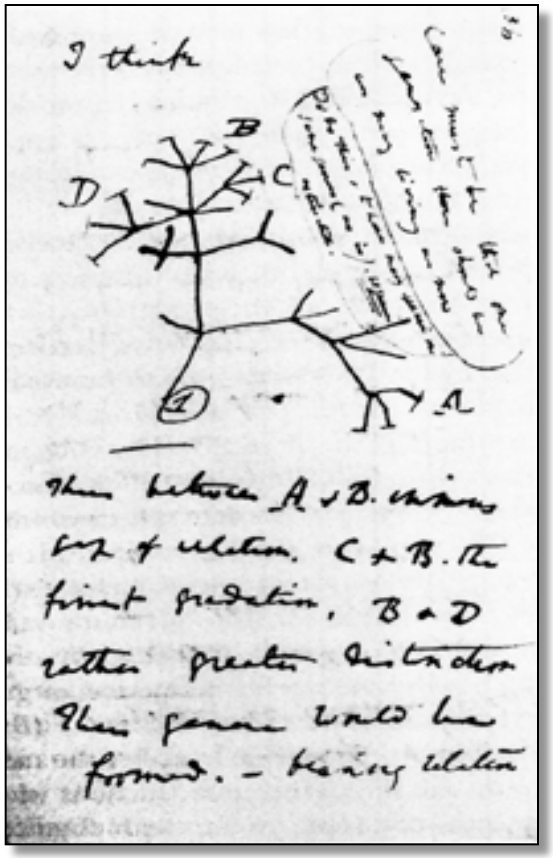
09:15 – 9:30	Arrival
09:30 – 10:00	A tour of *BEAST2* (Remco Bouckaert)
10:00 – 11:00	Lecture 3.1: Infectious disease phylodynamics
	– Coffee break –
11:30 – 12:30	Practical 3a: Phylodynamics in BEAST2 Coalescent and Birth-Death Models
	– Lunch break –
13:30 – 14:30	Practical 3b: Phylodynamics in BEAST Birth-Death SIR
14:30 – 15:30	BEAST2 Clinic (Remco Bouckaert)
15:45 – 16:45	Public seminar by Remco Bouckaert (Agar theatre)
17:30 – 19:00	Networking at Naughtons

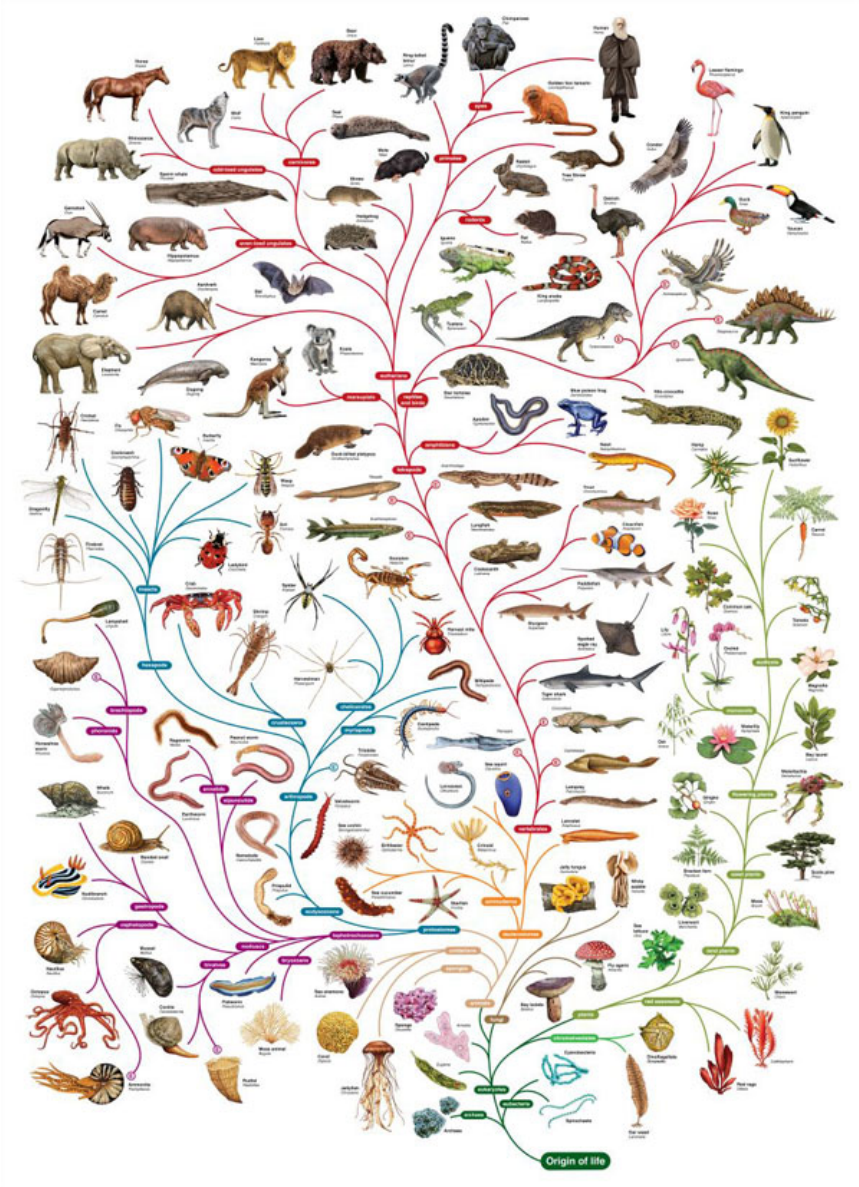
Lecture 1.1:
Introduction to Molecular Phylogenetics

Understanding Phylogenetic Trees

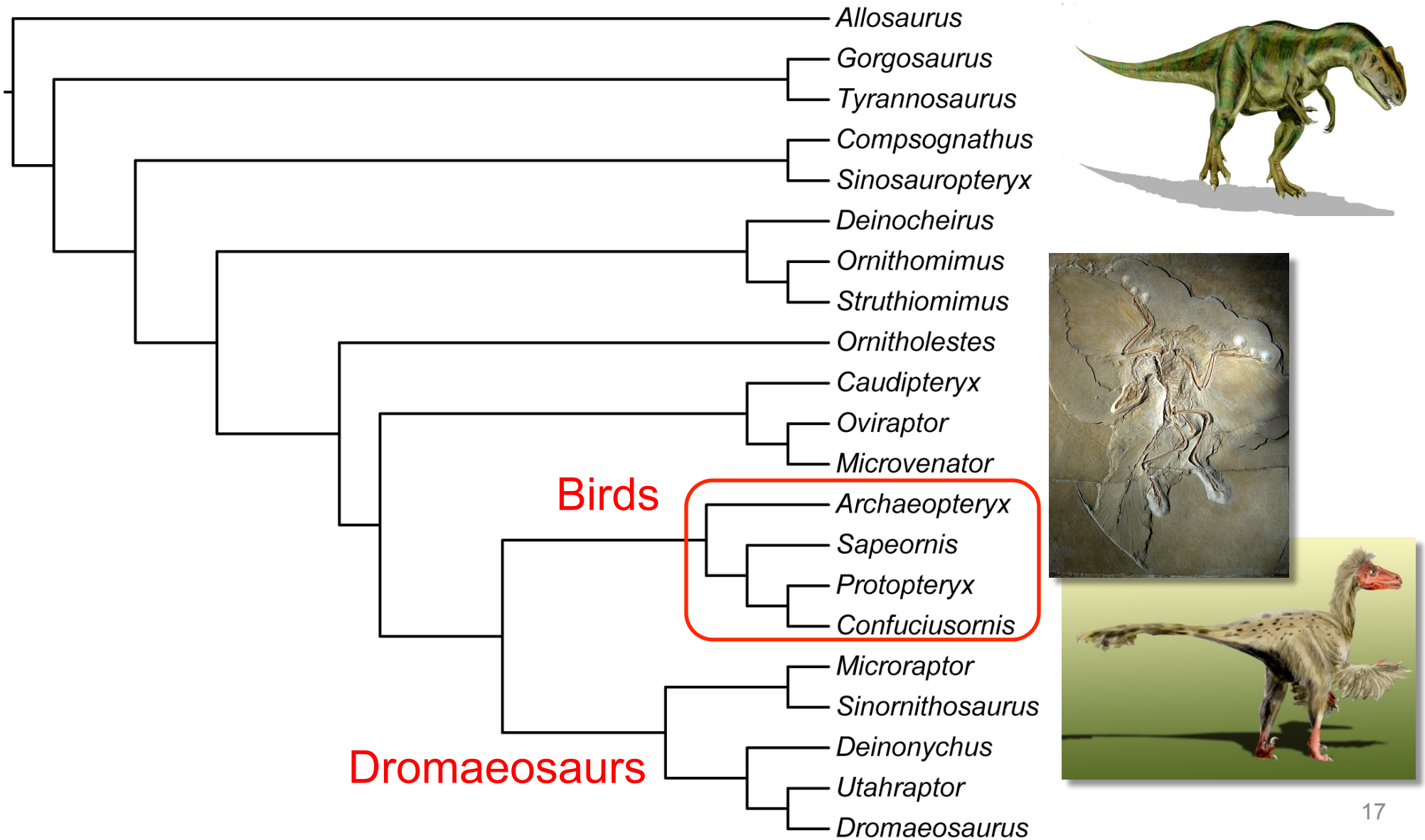
What is a phylogenetic tree?

- The phylogeny refers to the true evolutionary relationships among a set of organisms

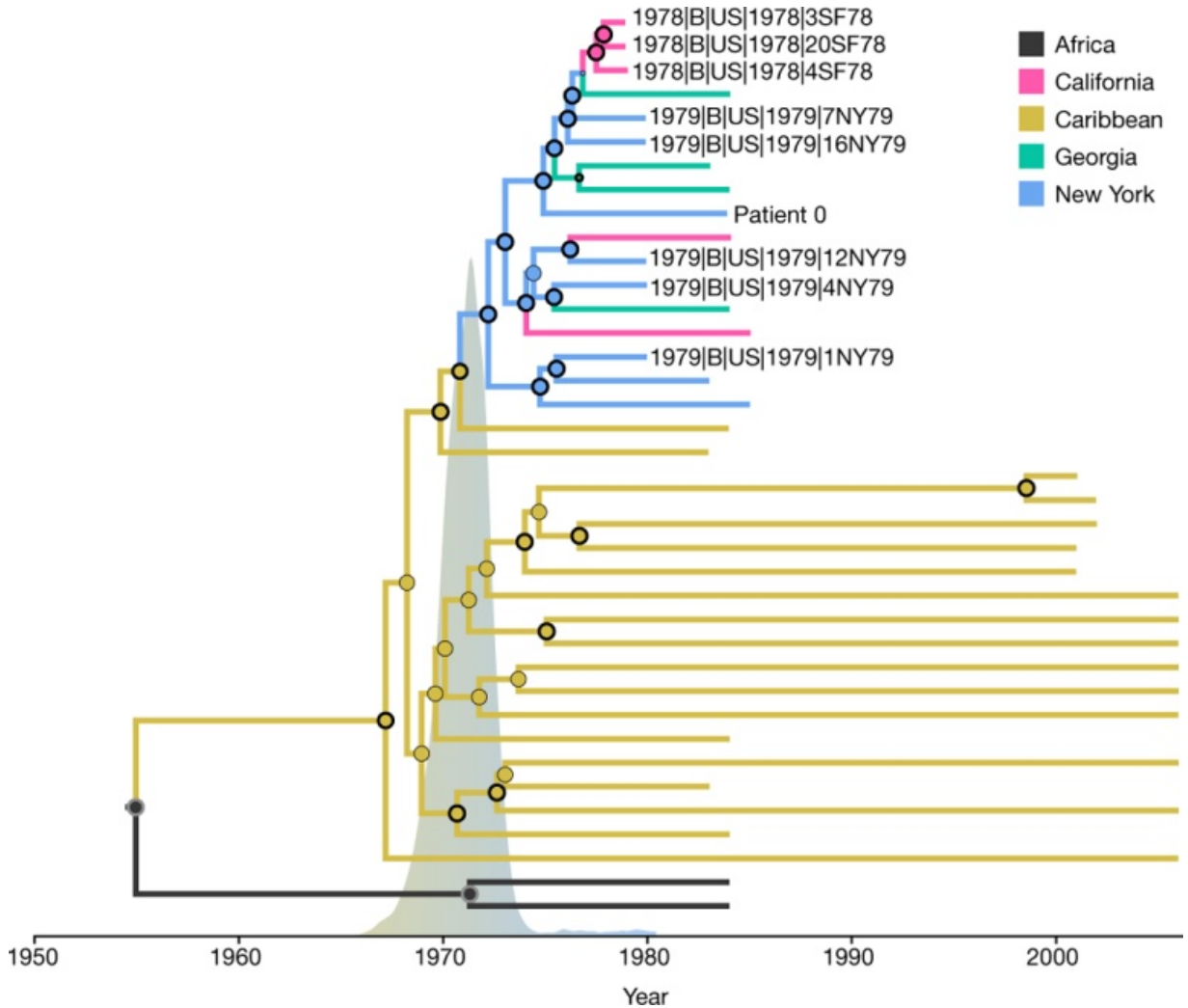




Evolutionary relationships



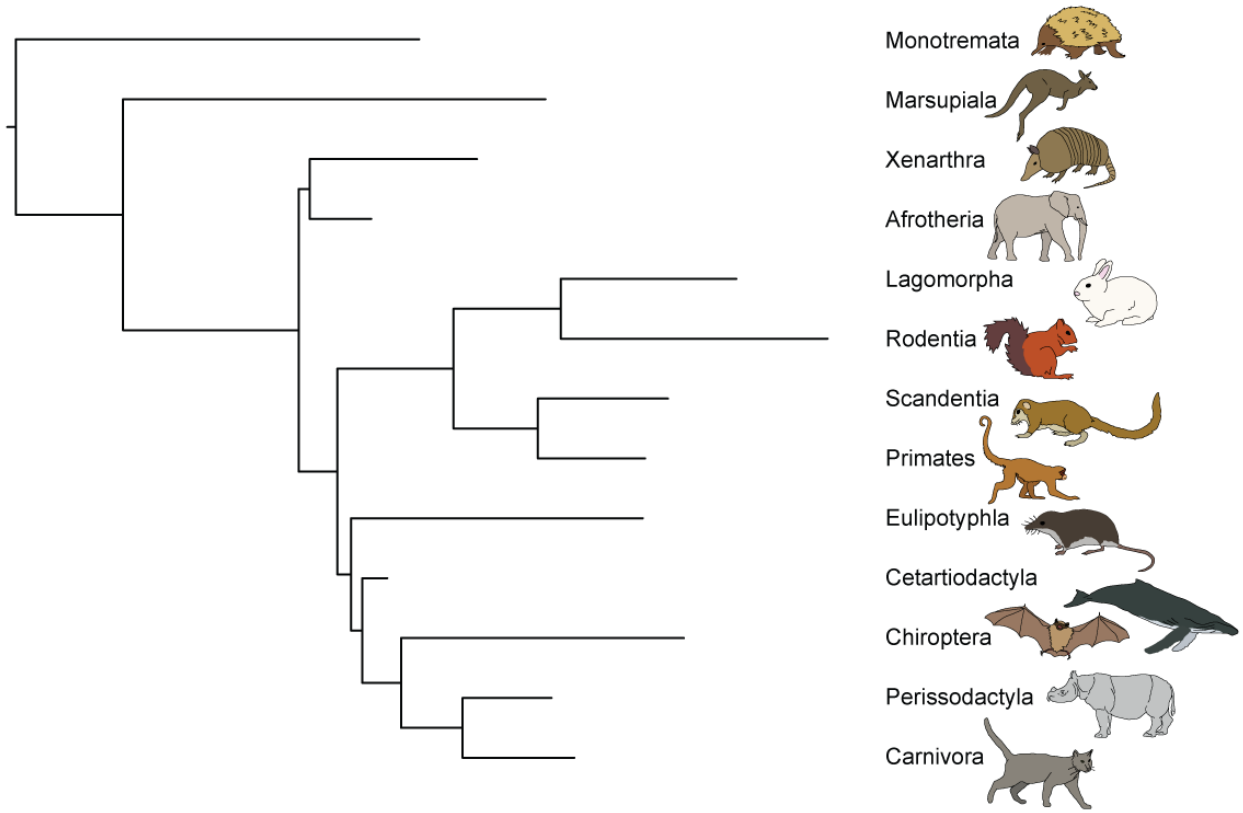
Evolutionary radiations



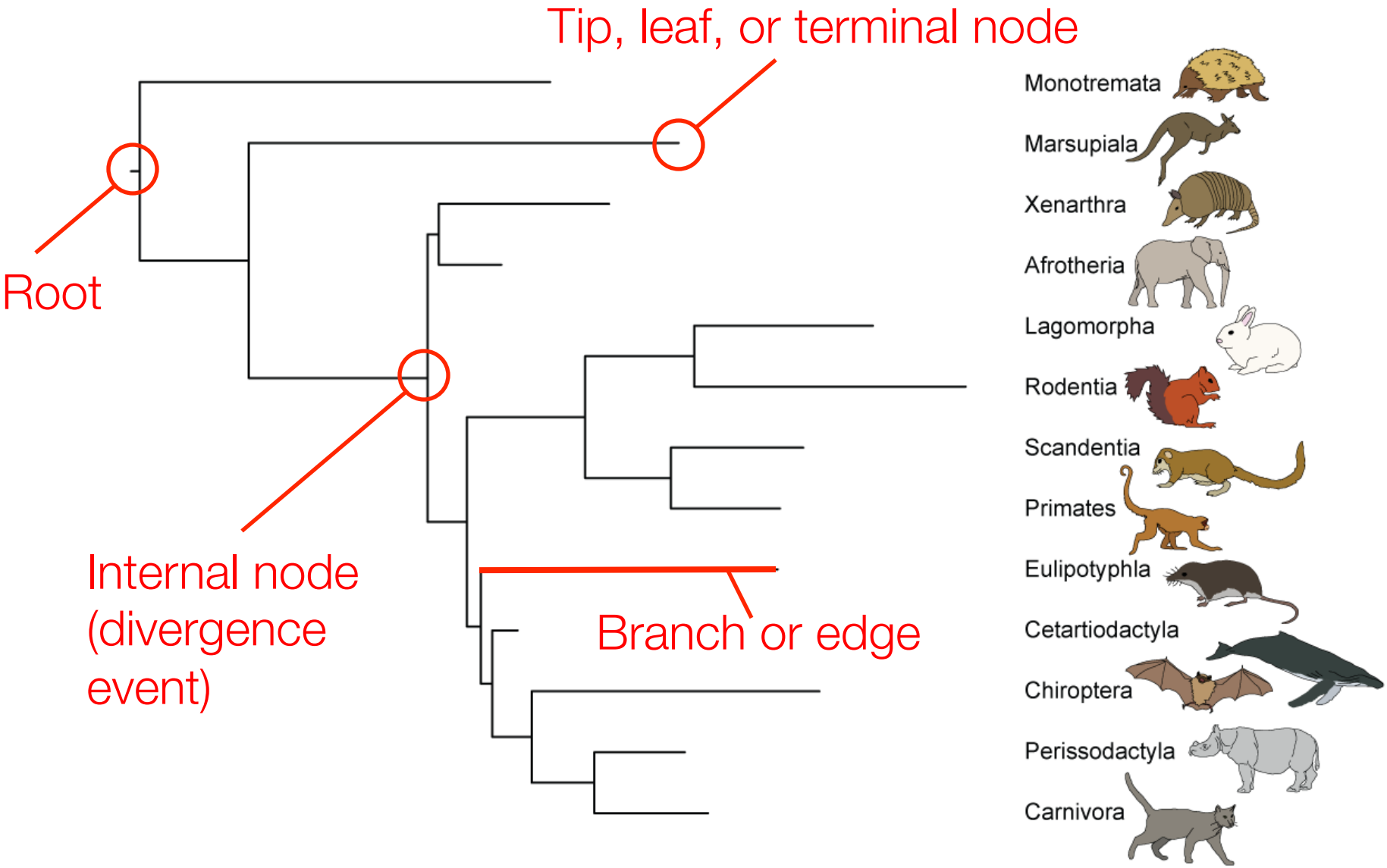
From Worobey et al. 2016 *Nature*

What is a phylogenetic tree?

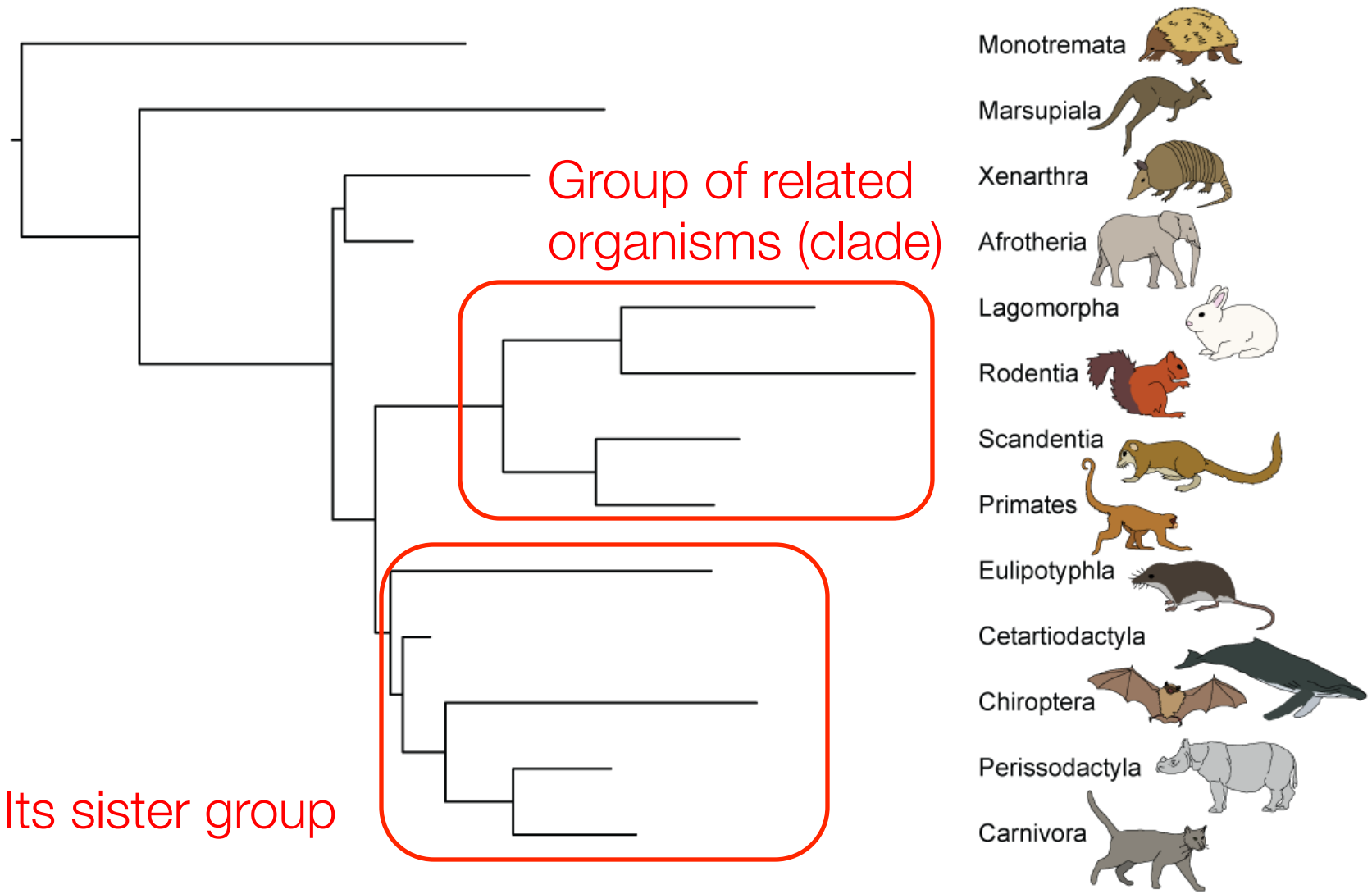
- A phylogenetic tree has two major components
  - Topology (relationships)
  - Branch lengths (amount of evolutionary change or time)



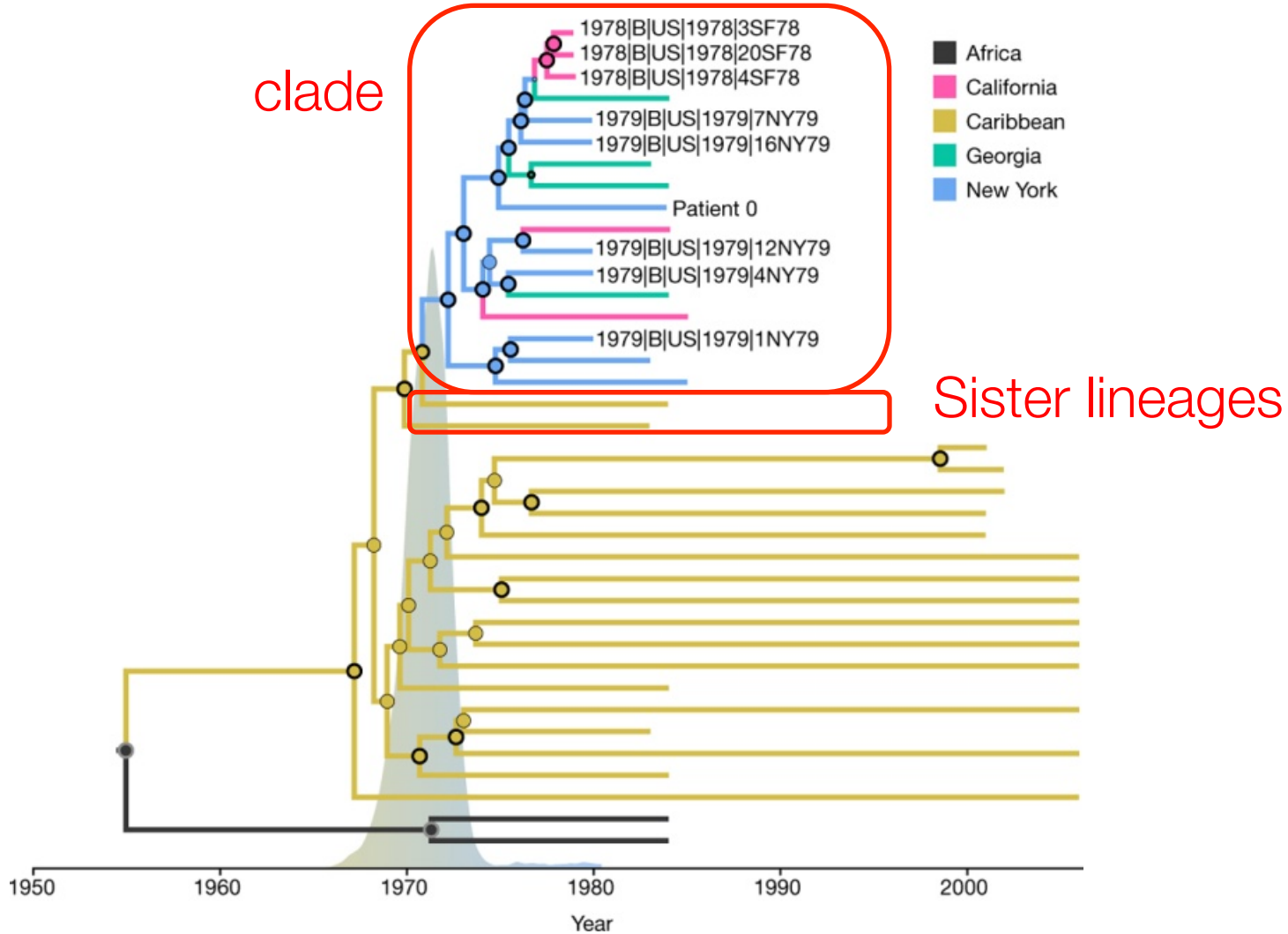
Phylogenetic trees



Phylogenetic trees

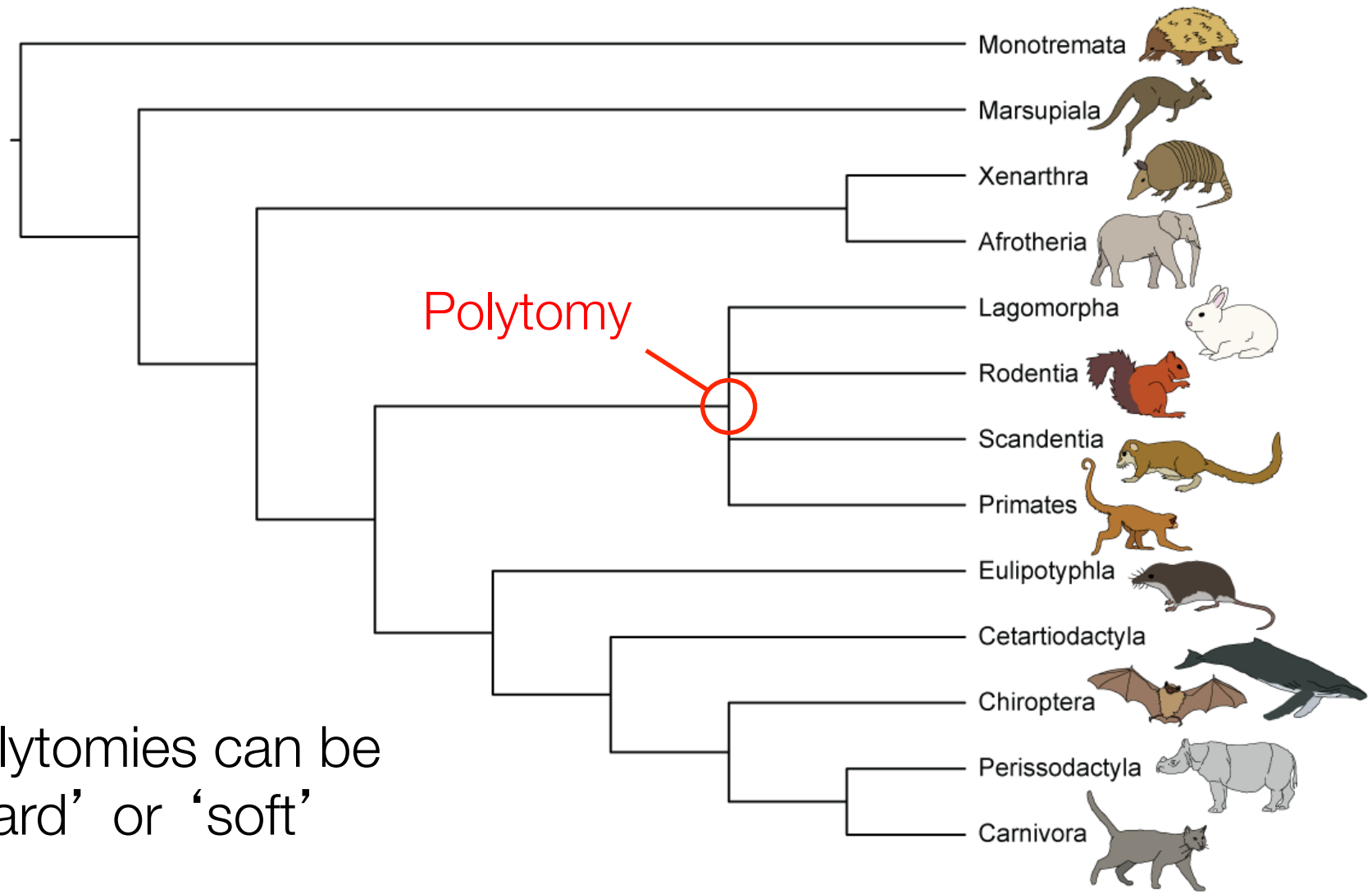


Phylogenetic trees



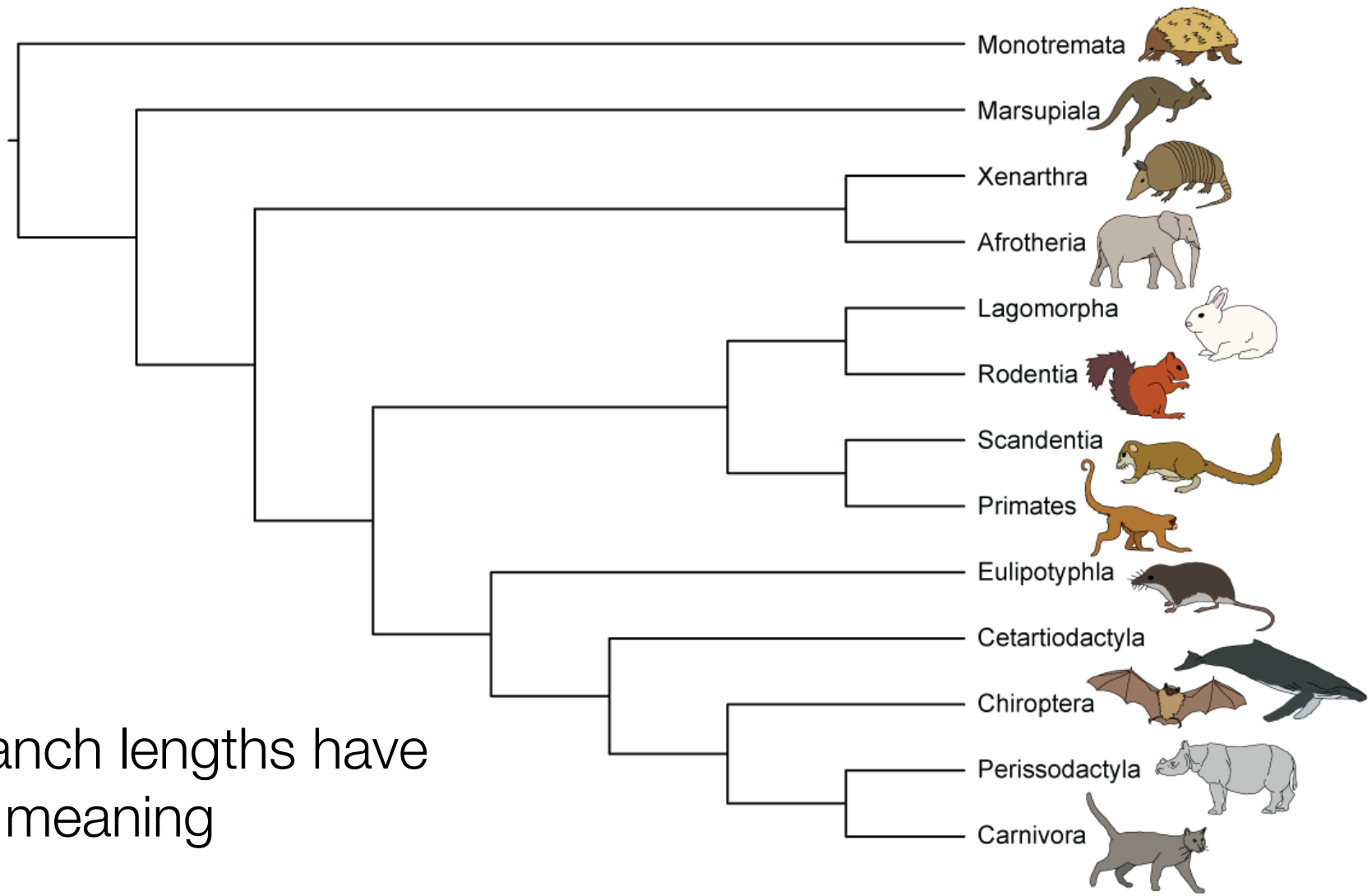
From Worobey et al. 2016 *Nature*

Phylogenetic trees



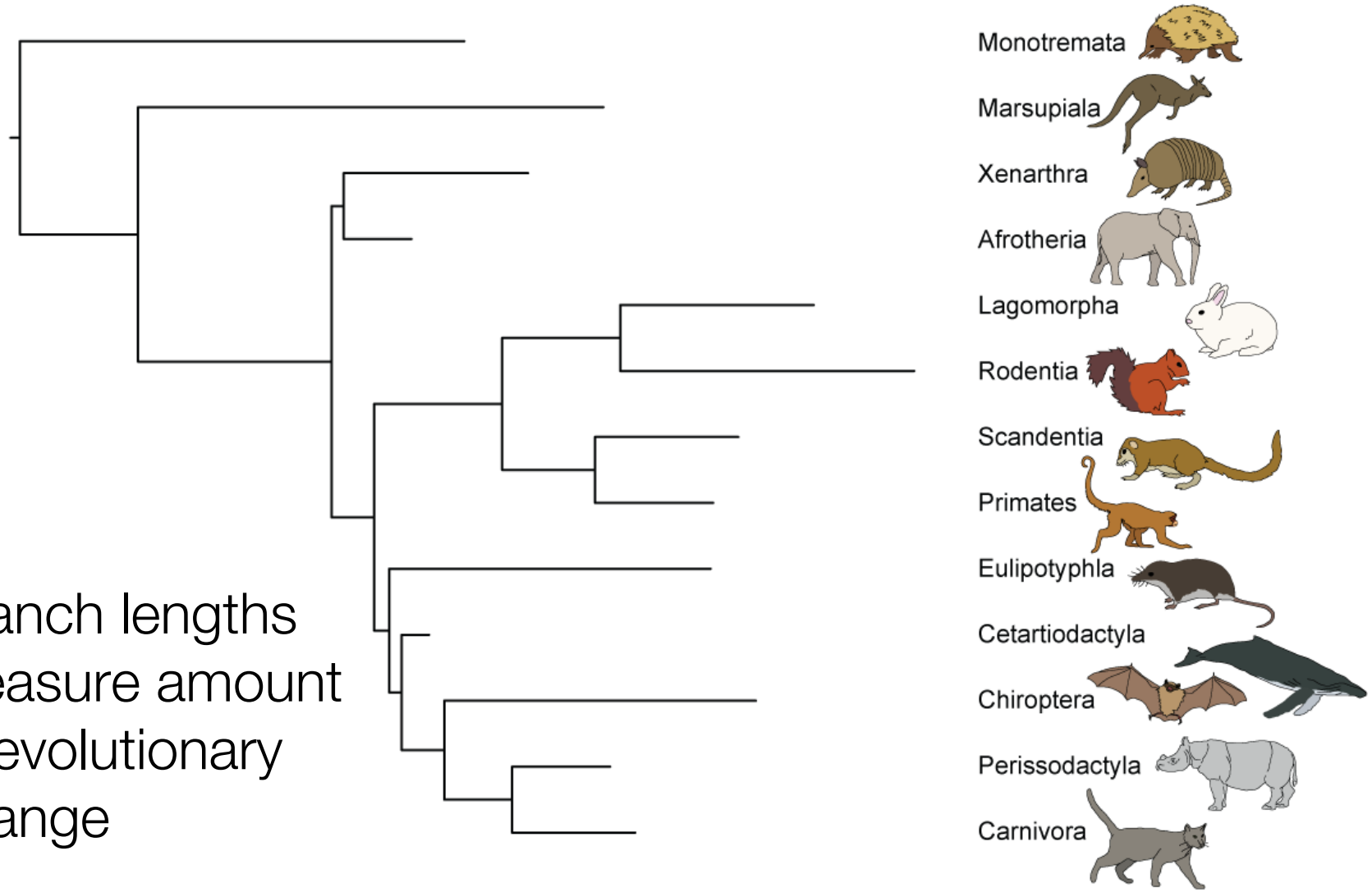
Polytomies can be ‘hard’ or ‘soft’

Phylogenetic trees: Cladogram



Branch lengths have no meaning

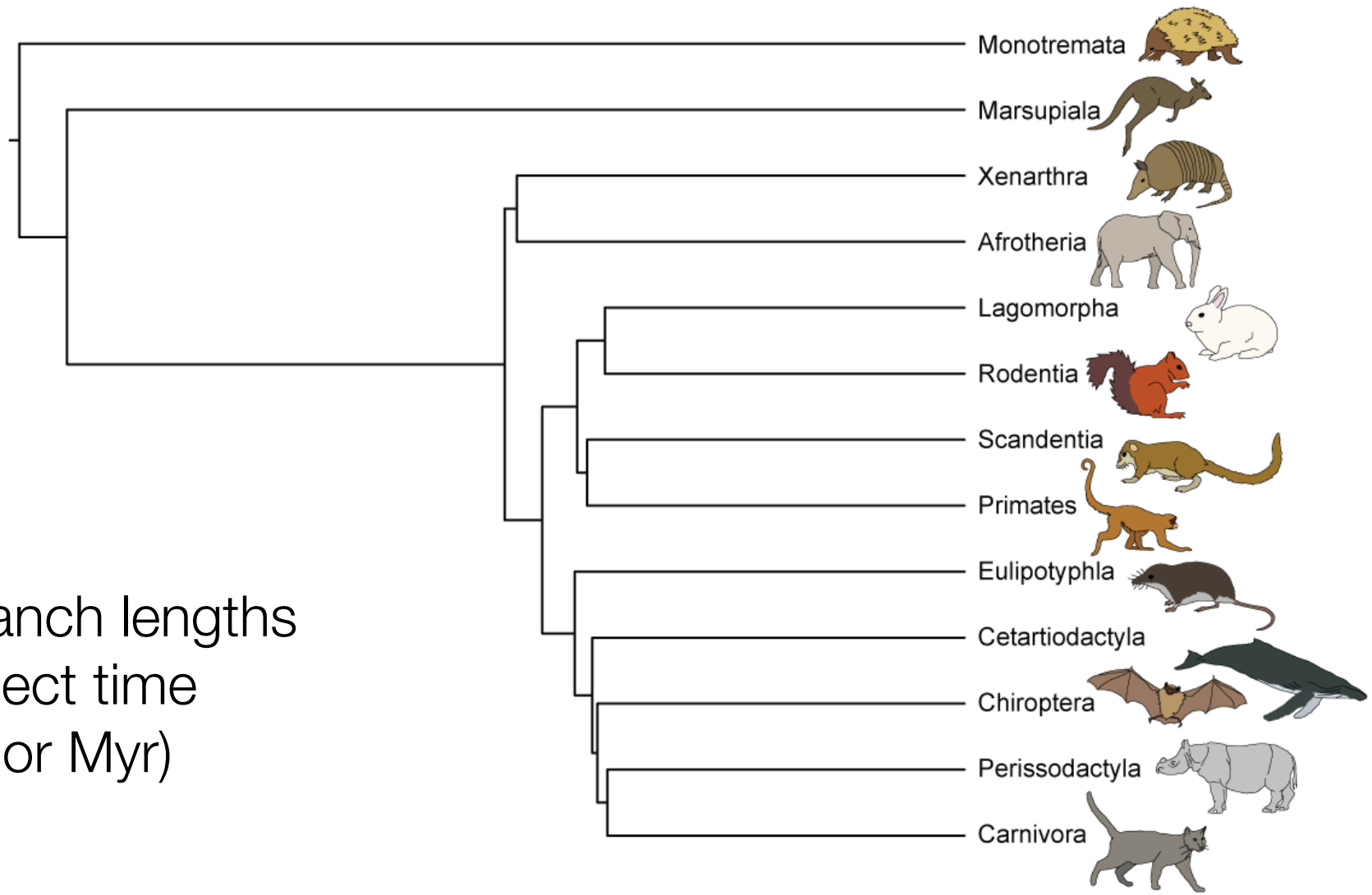
Phylogenetic trees: Phylogram



Branch lengths measure amount of evolutionary change

Phylogenetic trees: Chronogram

Ultrametric

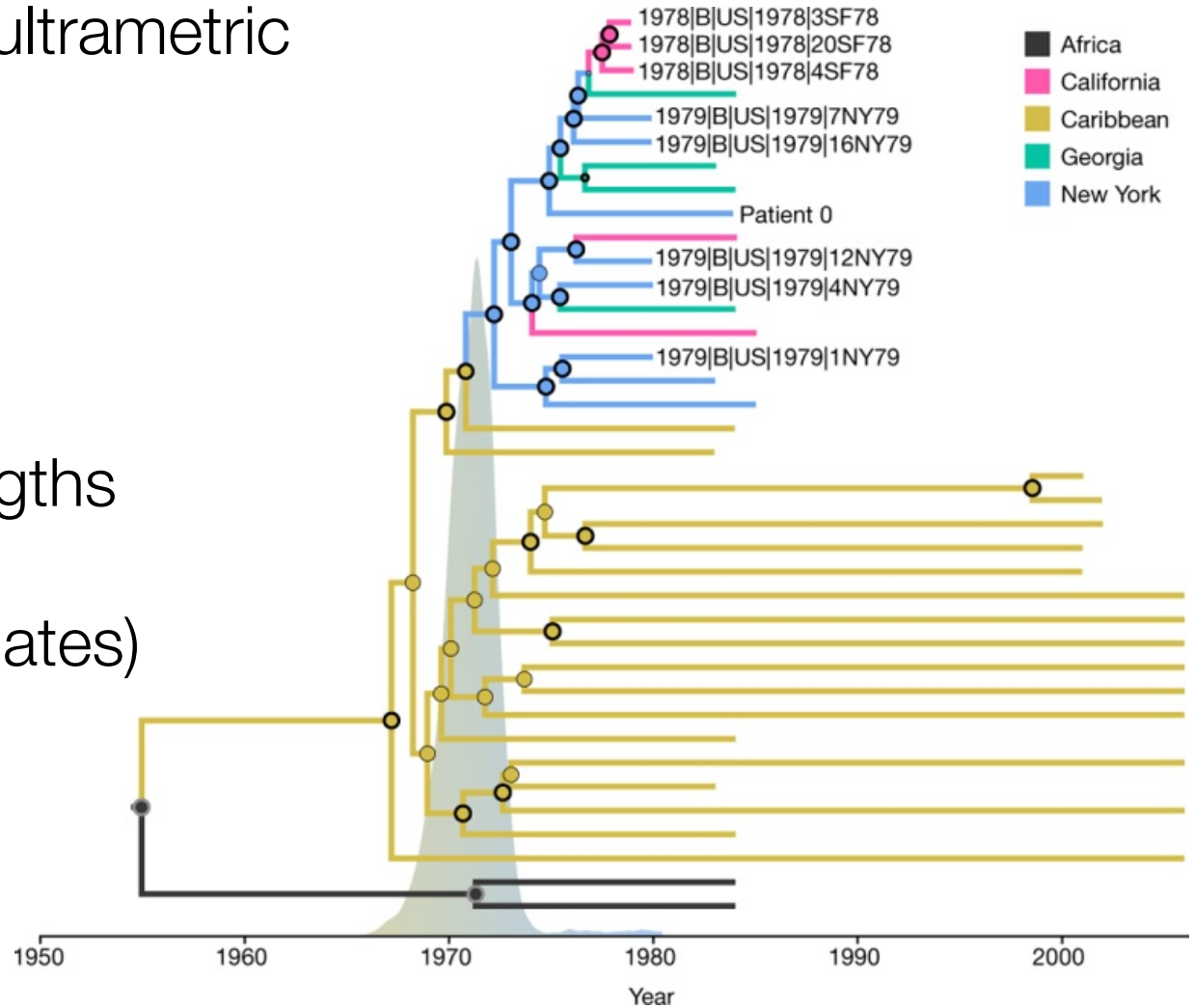


Branch lengths reflect time (yr or Myr)

Phylogenetic trees: Chronograms

Non-ultrametric

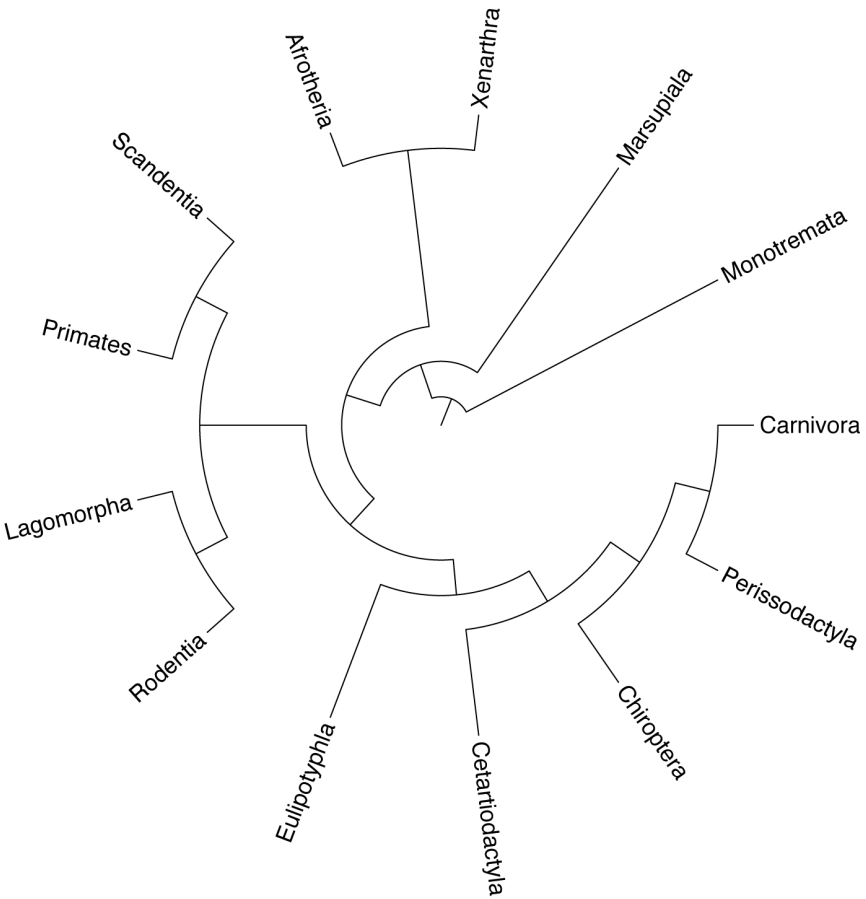
Branch lengths reflect time (calendar dates)



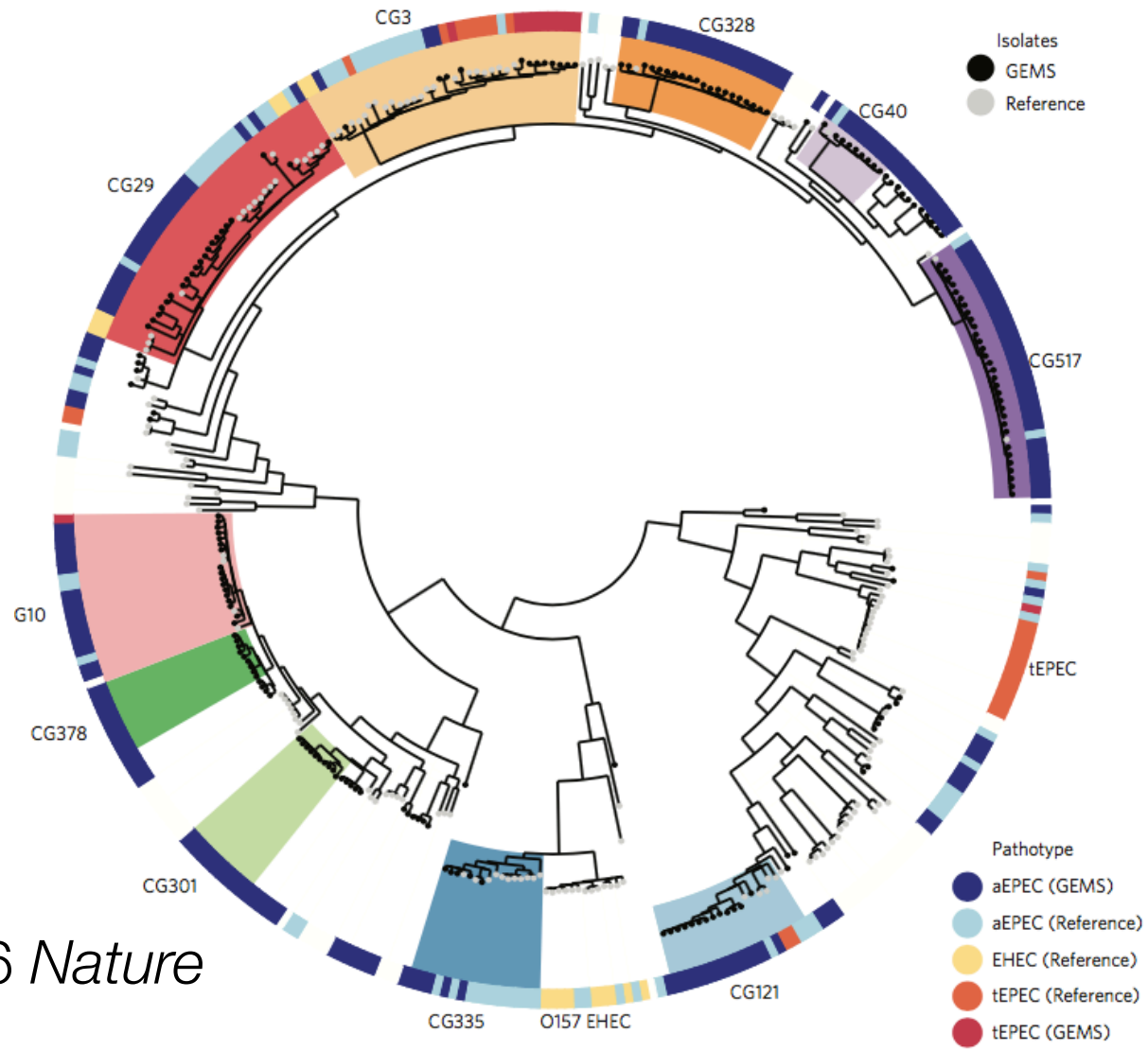
From Worobey et al. 2016 *Nature*

Phylogenetic trees: Circular

- Root is placed in centre
- Cladogram, phylogram, or chronogram
- Often used to visualise large trees
- Can be difficult to interpret
- Visually appealing



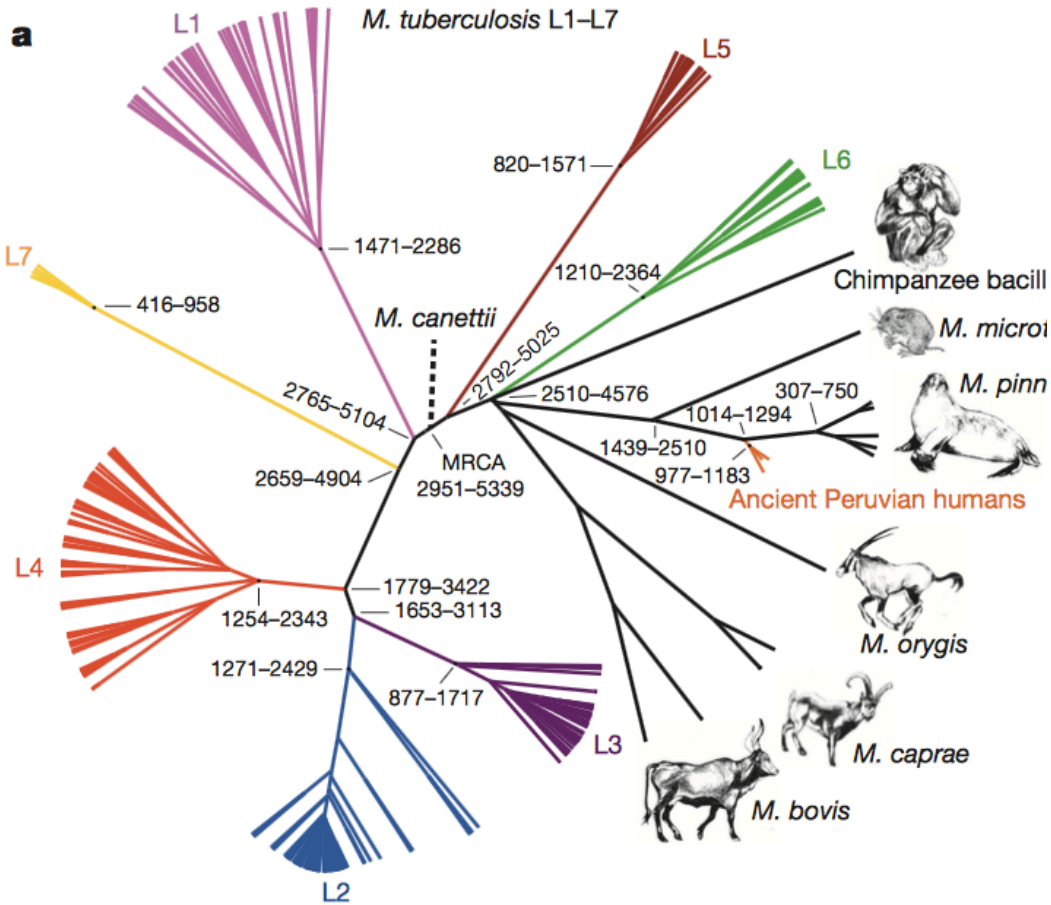
Phylogenetic trees: Circular



From Ingle et al. 2016 *Nature Microbiology*

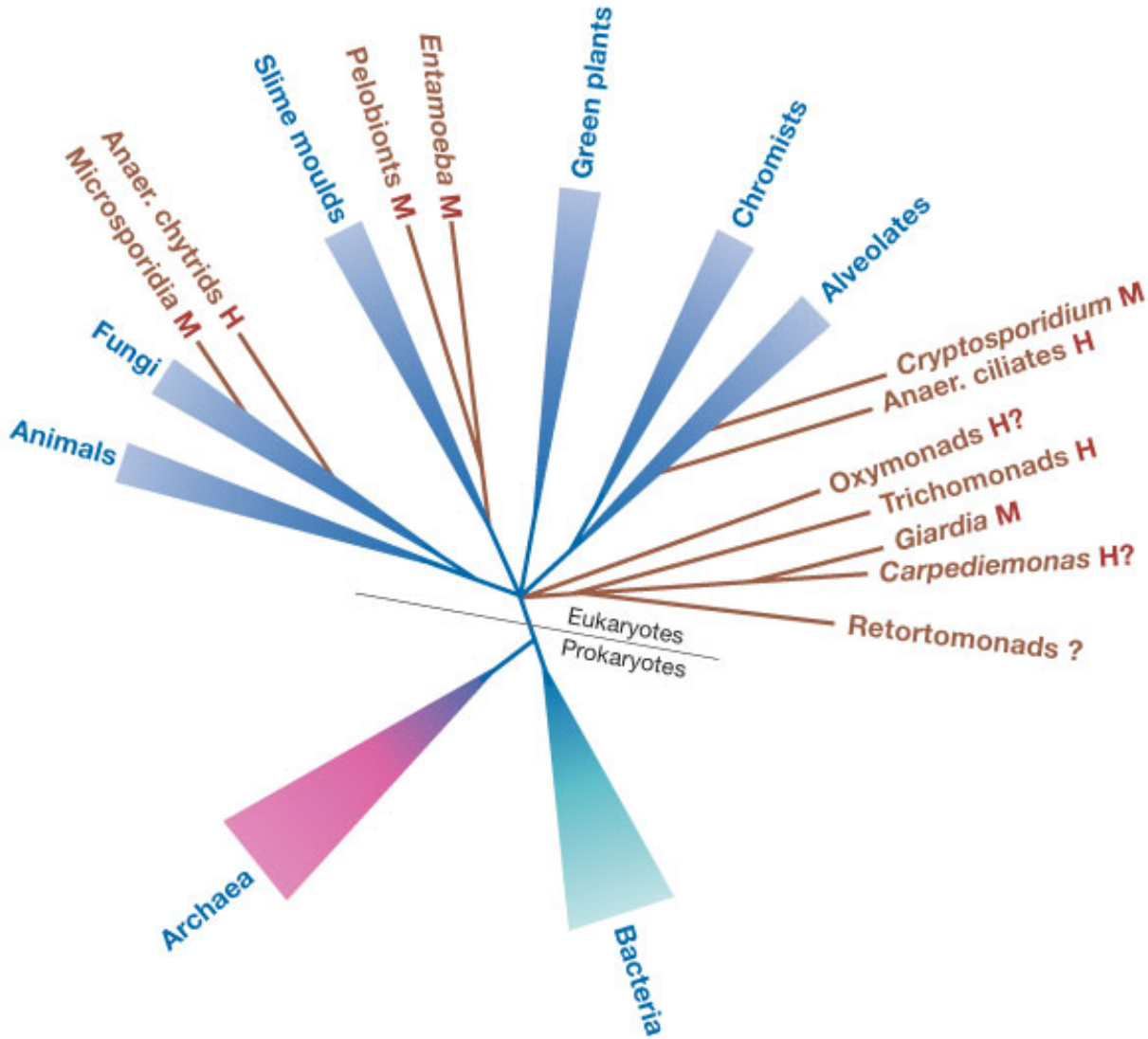
Phylogenetic trees: Unrooted

- Position of root is unknown
- Branch lengths usually represent amount of genetic change (substitutions/site)



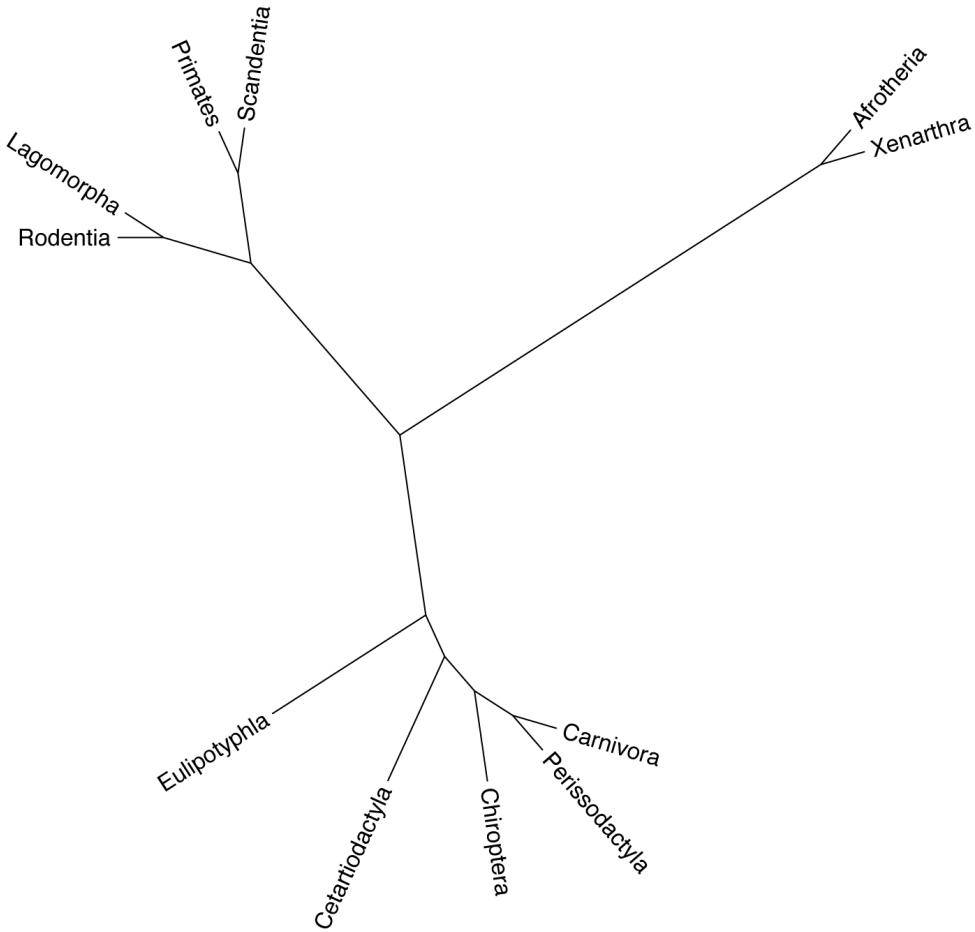
From Bos et al. 2014 *Nature*

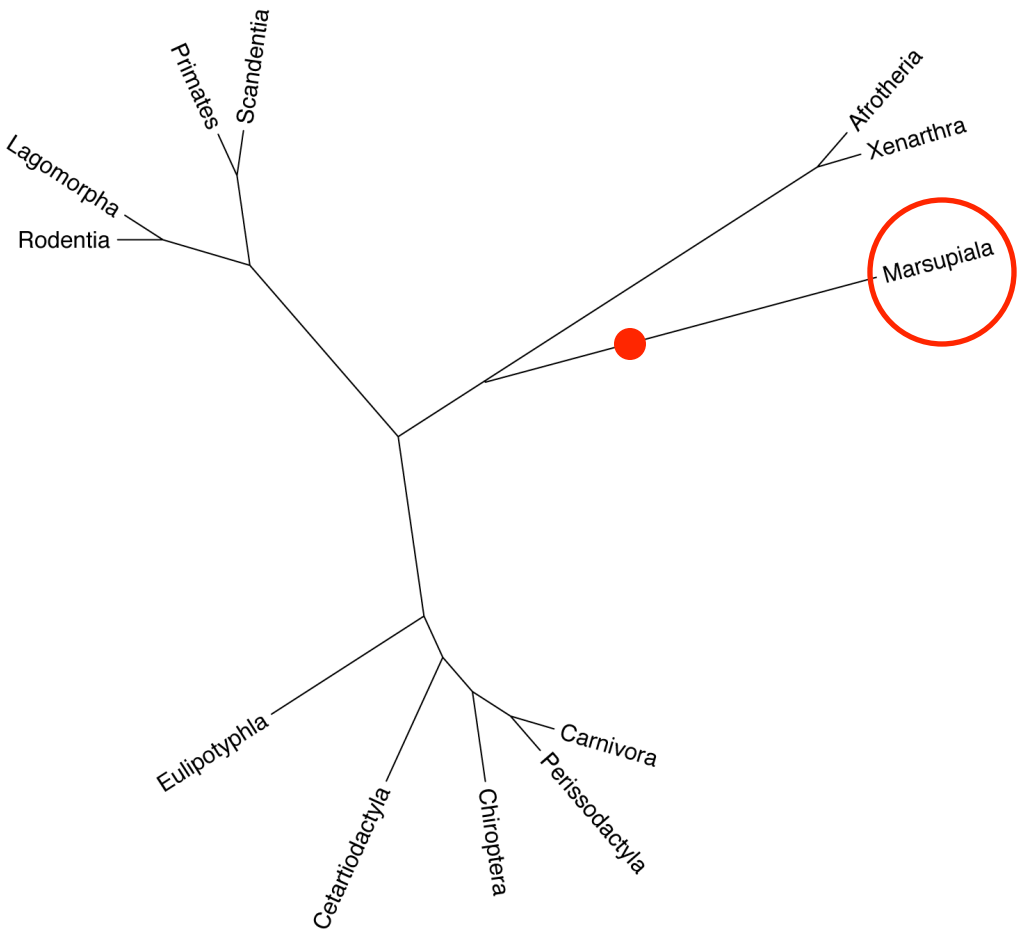
Phylogenetic trees: Unrooted



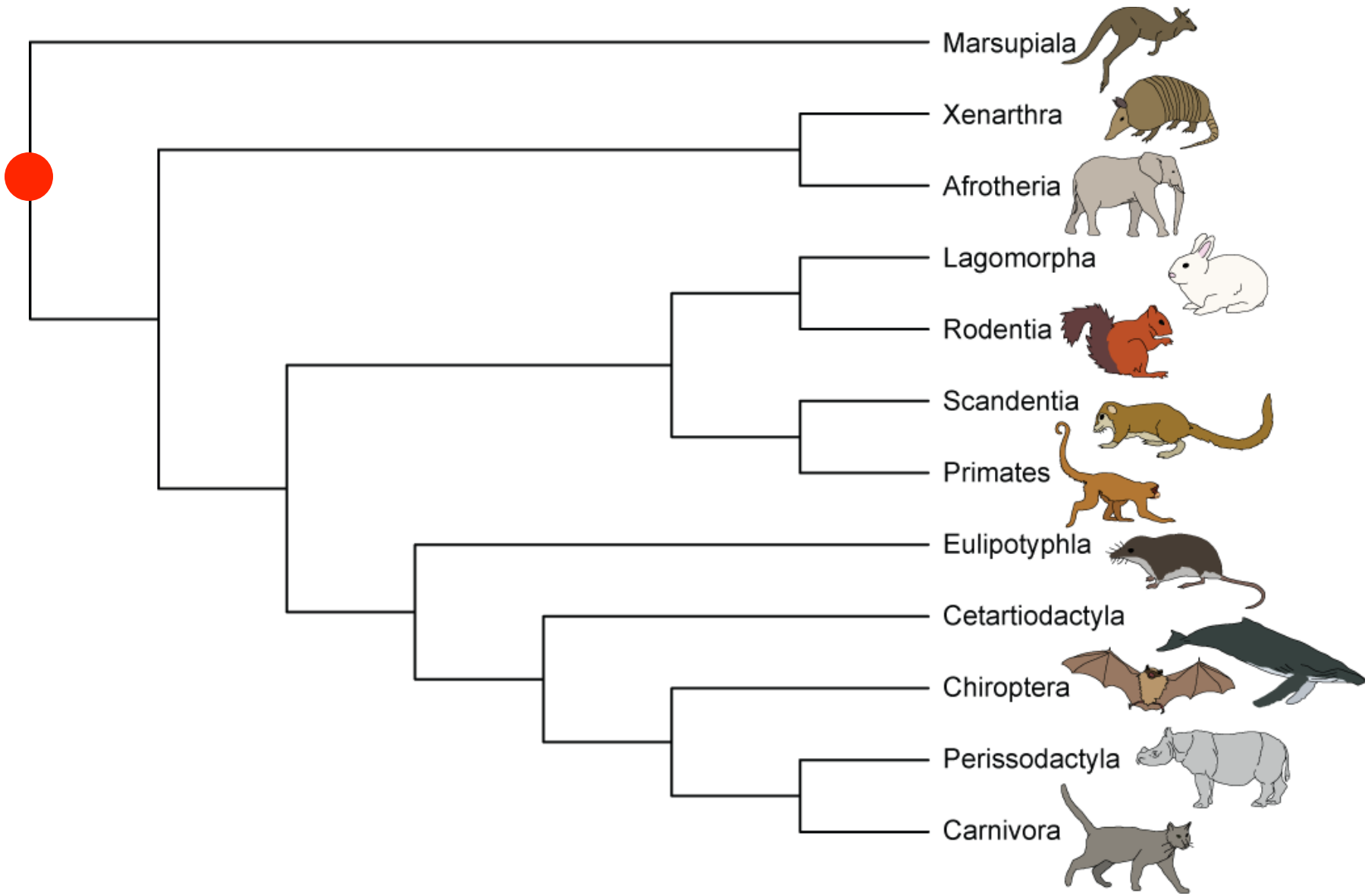
Rooting

- Can root a tree by including an outgroup taxon





Rooting


Marsupiala
Xenarthra
Afrotheria
Lagomorpha
Rodentia
Scandentia
Primates
Eulipotyphla
Cetartiodactyla
Chiroptera
Perissodactyla
Carnivora

Rooting

- Three methods for estimating the root of the tree
  1. Include an outgroup sequence
  2. Root at the midpoint of the tree
  3. Use a molecular clock

Phylogenetic trees: Newick format

- Without branch lengths (cladogram):
  - (Monotremata,(Marsupiala,((Afrotheria,Xenarthra),(((Rodentia,Lagomorpha),(Primates,Scandentia)),(Eulipotyphla,(Cetartiodactyla,(Chiroptera,(Carnivora,Perissodactyla))))))));

- With branch lengths (phylogram/chronogram):
  - (Monotremata:12.0,(Marsupiala:11.0,((Afrotheria:1.0,Xenarthra:1.0):9.0,(((Rodentia:1.0,Lagomorpha:1.0):2.0,(Primates:1.0,Scandentia:1.0):2.0):5.0,(Eulipotyphla:4.0,(Cetartiodactyla:3.0,(Chiroptera:2.0,(Carnivora:1.0,Perissodactyla:1.0):1.0):1.0):1.0):4.0):2.0):1.0):1.0);

Phylogenetic Analysis and Sequence Alignment

Phylogenetic analysis

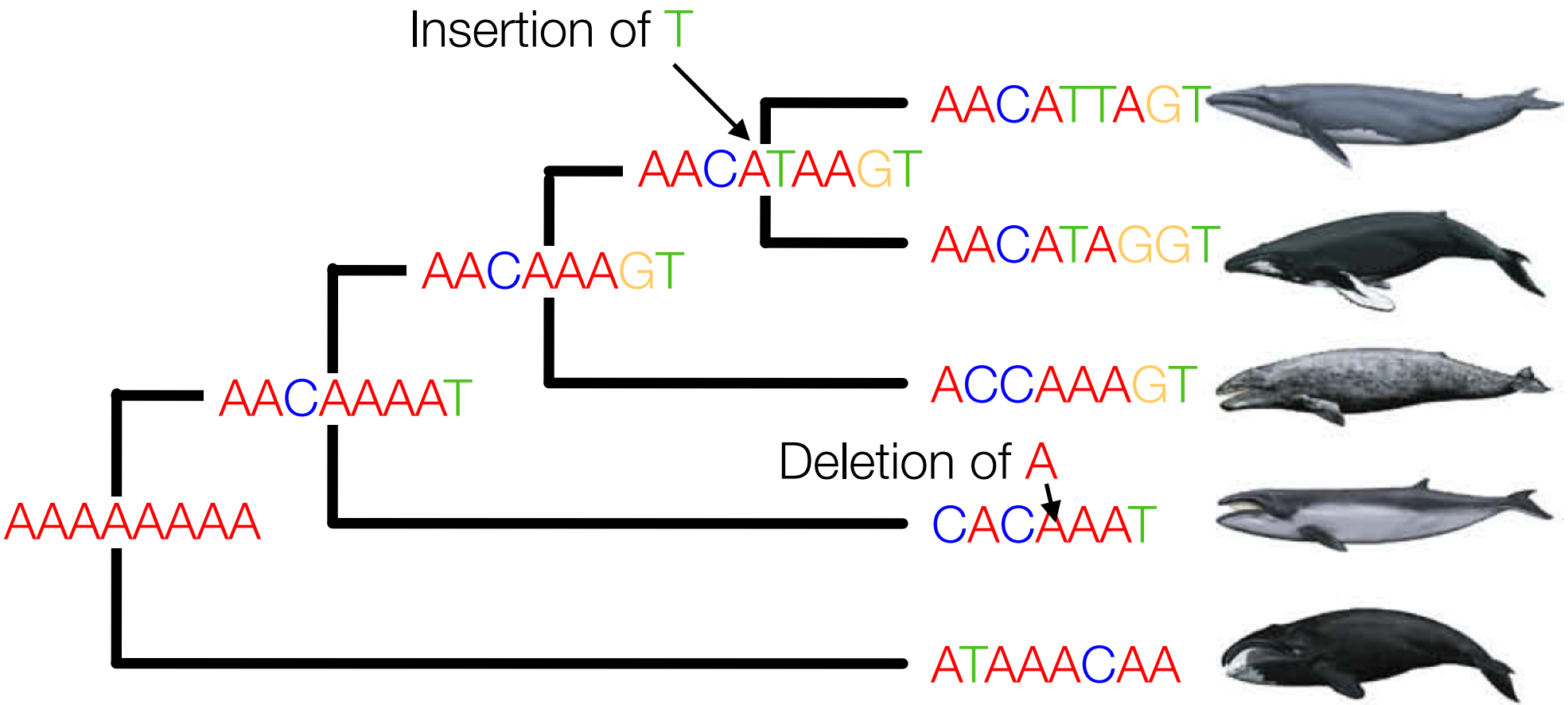
- Sometimes we know the phylogeny
  - Viral transmission histories
  - Documented pedigrees (family histories – humans, domesticated animals, lab organisms, etc.)
- Usually we do not know the phylogeny but we can estimate it
  - Morphological data
  - Molecular data
- Two fundamental results:
  - Estimate of evolutionary relationships
  - Estimate of evolutionary rates and time-scales

Fundamental assumptions

- Phylogenetic methods make several fundamental assumptions:
  - Each aligned site represents a set of orthologous characters
  - Sites in an alignment evolve independently
  - Lineages evolve independently
  - The relationships among the sequences can be represented by a bifurcating (binary) tree
  - Our models of evolution are accurate

blue whale	CGTTAGTACACT
humpback whale	CGATAGTTCACT
gray whale	CGTTAGTTTACC
right whale	CATTGGTTTACT

Example: Whales



DNA sequence alignment

- Homologous sites need to be aligned
- Inferring insertions and deletions "indels"

AACATTAGT

AACATAGGT

ACCA-AAGT

CACA--AAT

ATAA-ACAA

Nucleic Acids Research, 1994, Vol. 22, No. 22 4673–4680

CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice

Julie D.Thompson, Desmond G.Higgins+ and Toby J.Gibson*
European Molecular Biology Laboratory, Postfach 102209, Meyerhofstrasse 1, D-69012 Heidelberg, Germany

BMC Bioinformatics

BioMed Central

Software

Open Access

MUSCLE: a multiple sequence alignment method with reduced time and space complexity

Robert C Edgar*

prank-msa

Phylogeny-aware progressive sequence alignment method

DNA sequence alignment

- Homologous site
- Inherited from the common ancestor of all sequences in the alignment
- The aim of sequence alignment is to maximise the number of sites for which you can infer homology

```
AACATTAGT
AACATAGGT
ACCA-AAGT
CACA--AAT
ATAA-ACAA
```

DNA sequence alignment

- Groups together the first 3 sequences
- Groups together the last 2 sequences
- Informative for all phylogenetic methods

```
AACATTAGT
AACATAGGT
ACCA-AAGT
CACA--AAT
ATAA-ACAA
```

DNA sequence alignment

- Does not group any sequences
  - Not useful for maximum parsimony
- But informative for estimating amount of evolutionary change
  - Useful for other methods

```
AACATTAGT
AACATAGGT
ACCA-AAGT
CACA--AAT
ATAA-ACAA
```

DNA sequence alignment

- Indel – insertion or deletion
- Potentially informative
- Most phylogenetic methods do not really use indel data
- Maximum-likelihood and Bayesian methods typically treat them in the same way as missing data

```
AACATTAGT
AACATAGGT
ACCA-AAGT
CACA--AAT
ATAA-ACAA
```

MEGA

- *Molecular Evolutionary Genetics Analysis*
- Koichiro Tamura and Sudhir Kumar
- Population genetics
- Phylogenetics
  - Sequence alignment
  - Model selection
  - Maximum parsimony
  - Distance-based methods
  - Maximum likelihood

Go to Practical 1: Sequence alignment in *MEGA*

MoPad, Data and prac in github

https://public.etherpad-mozilla.org/p/phyloworkshop_melbourne

https://github.com/sebastianduchene/Phyloworkshop_Melbourne_2017