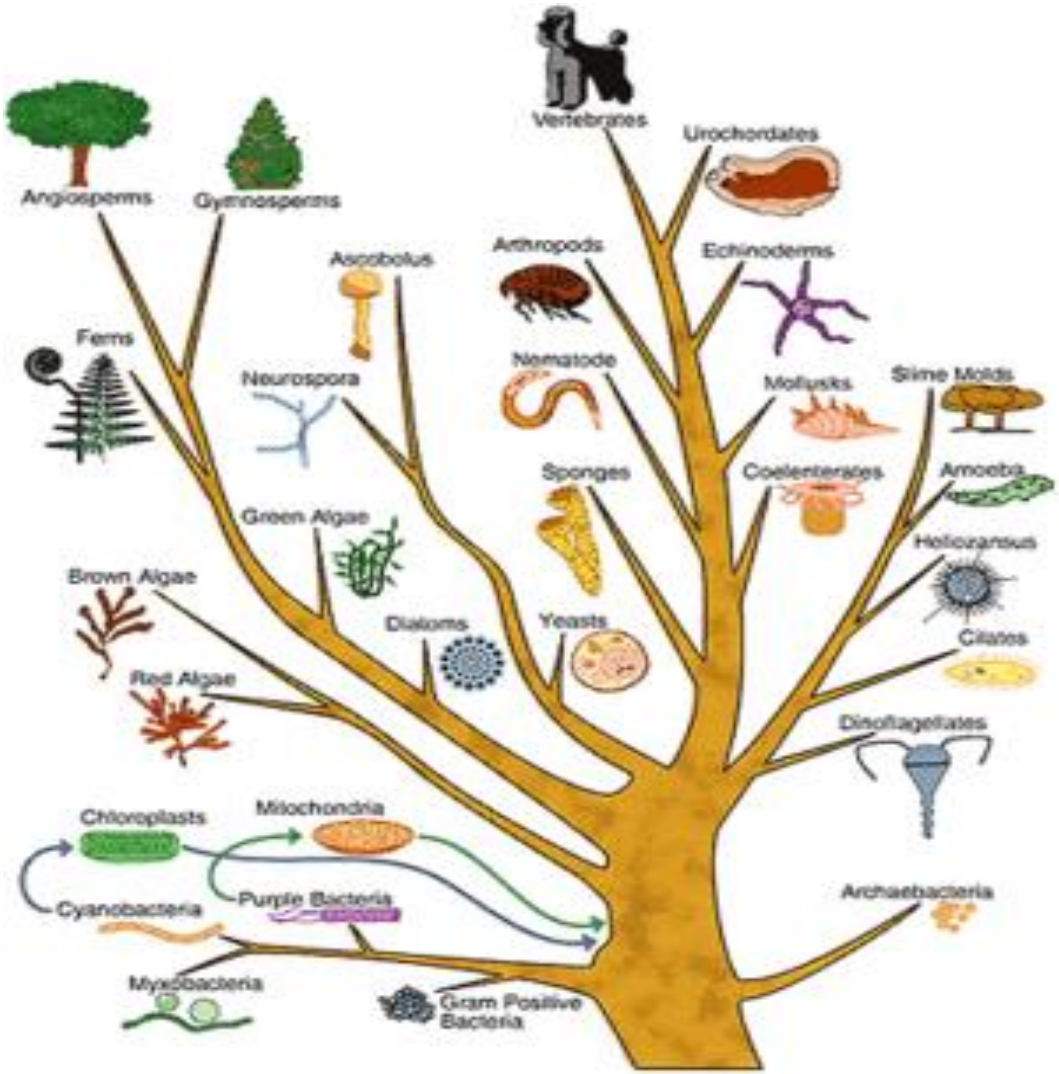
Phylogenetic trees

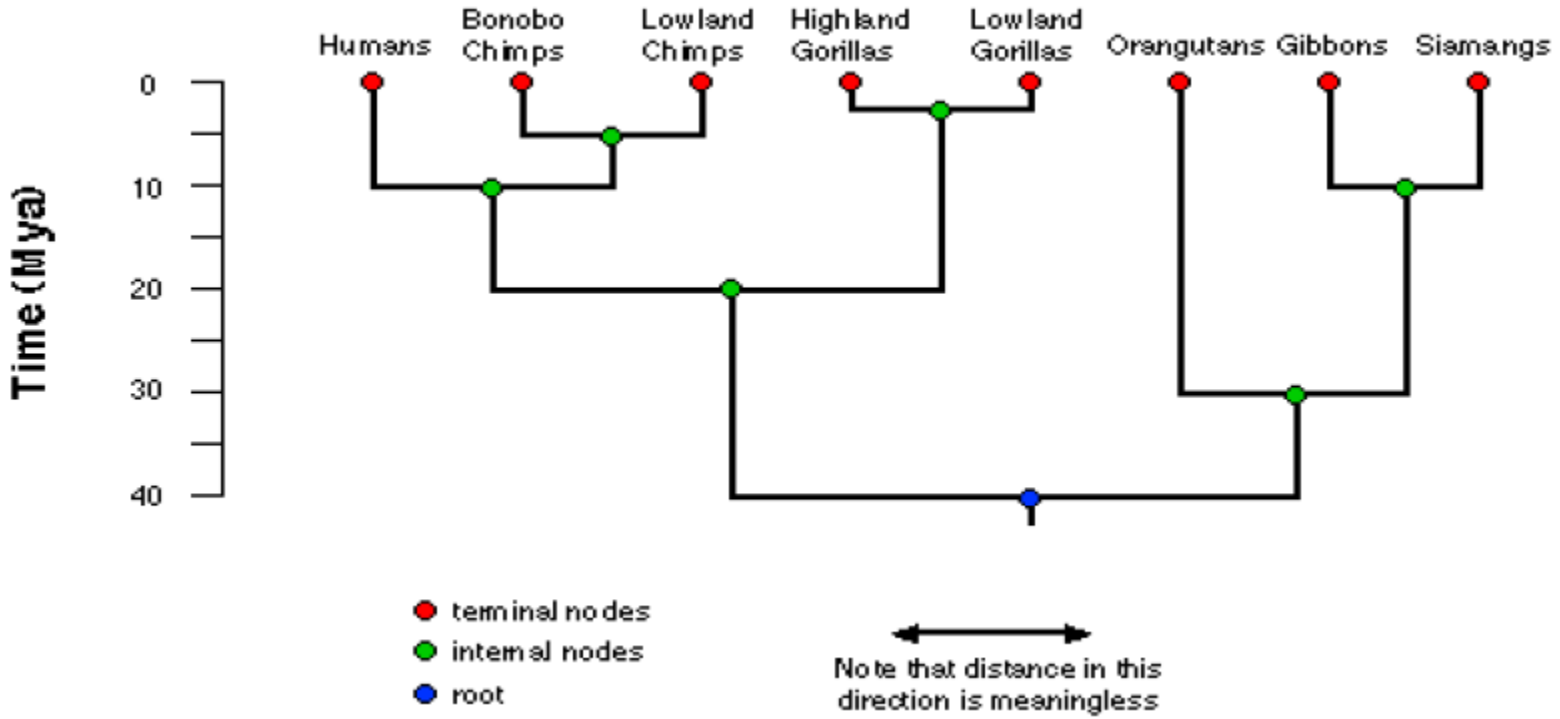


Phylogenetic tree

- The similarity of molecular mechanisms of the organisms that have been studied strongly suggests that all organisms on Earth had a common ancestor.
- Thus any set of species is related(evolutionary divergent), and this relationship is called a phylogeny.
- Usually the relationship can be represented by a phylogenetic tree.
- The task of phylogenetic is to infer this tree from observations upon the existing organisms.
- (Greek: phylon = race and genetic = birth)

Terminology

- Phylogenetic tree: Visual representation of evolutionary distances between species



Historical Note

- Approaches
  - Fossil Records , Phylogenetic Trees

- Until mid 1950's phylogenies were constructed by experts based on their opinion (subjective criteria)

- Since then, focus on **objective** criteria for constructing phylogenetic trees
  - Thousands of articles in the last decades

Morphological vs. Molecular

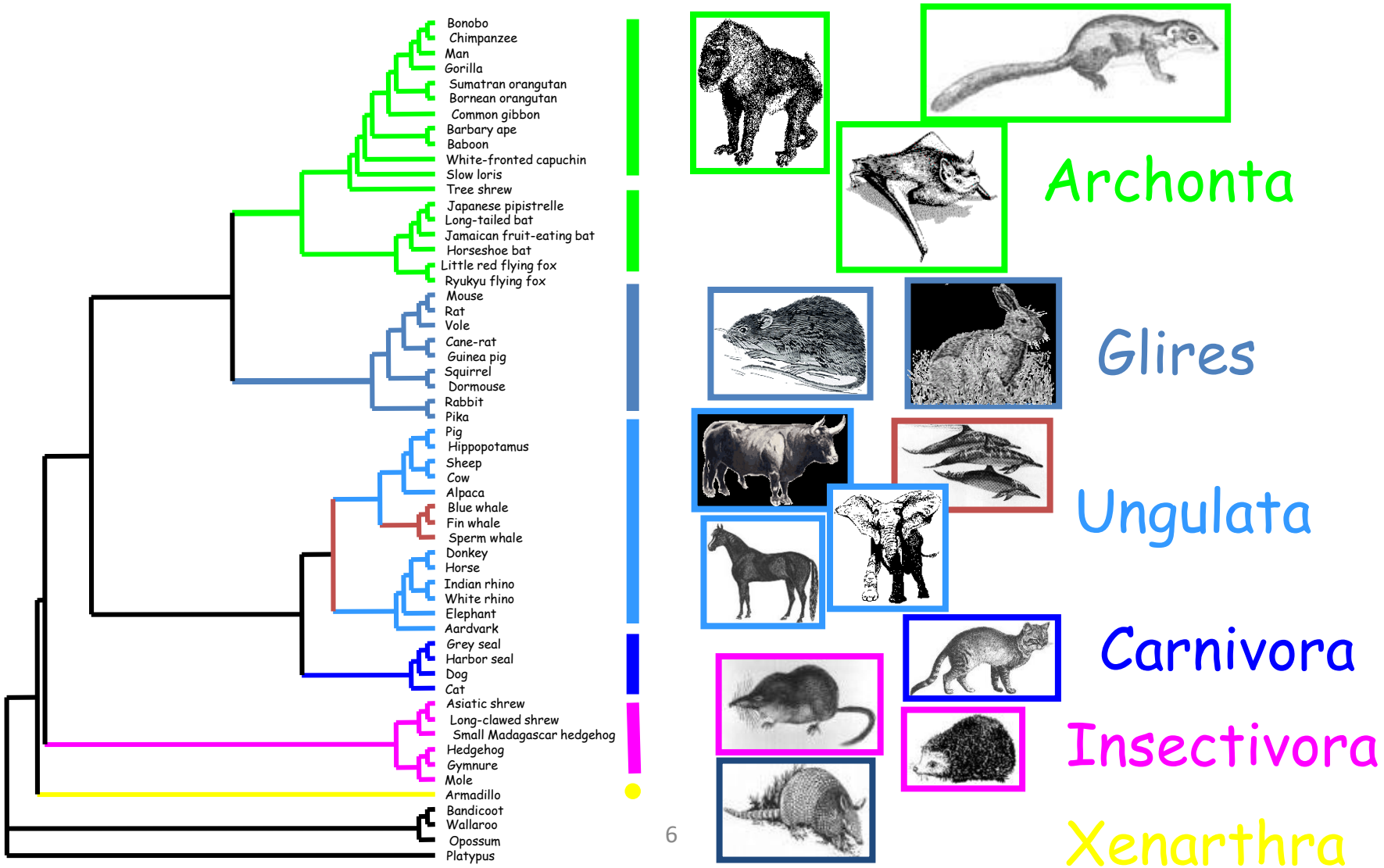
- Classical phylogenetic analysis: **morphological** features: number of legs, lengths of legs, etc.

- Modern biological methods allow to use **molecular** features
  - Gene sequences
  - Protein sequences

- Analysis based on homologous sequences (e.g., globins) in different species

Morphological topology

(Based on Mc Kenna and Bell, 1997)

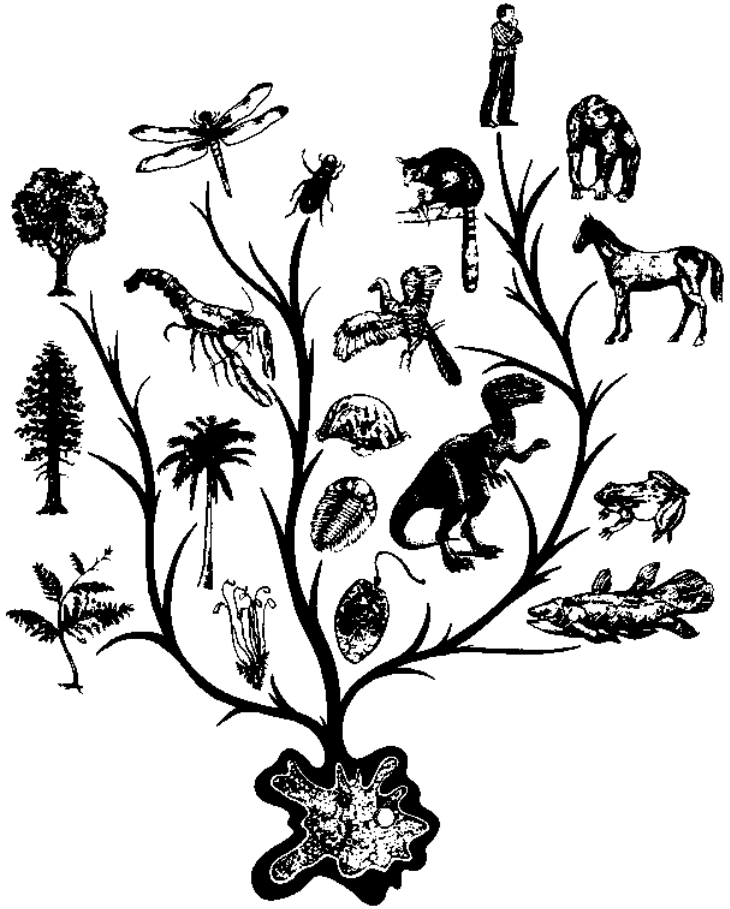


Morphological limitations

- fossil records have many limitations
- they may be available only for certain species
- Existing fossil data can be fragmentary and their collection is often limited by abundance, habitat, geographic range, and other factors.
- For microorganisms, fossils are essentially nonexistent.

From sequences to a phylogenetic tree

```
Rat       QEPGGLVVPPTDA
Rabbit    QEPGGMVVPPTDA
Gorilla   QEPGGLVVPPTDA
Cat       REPGGLVVPPTEG
```

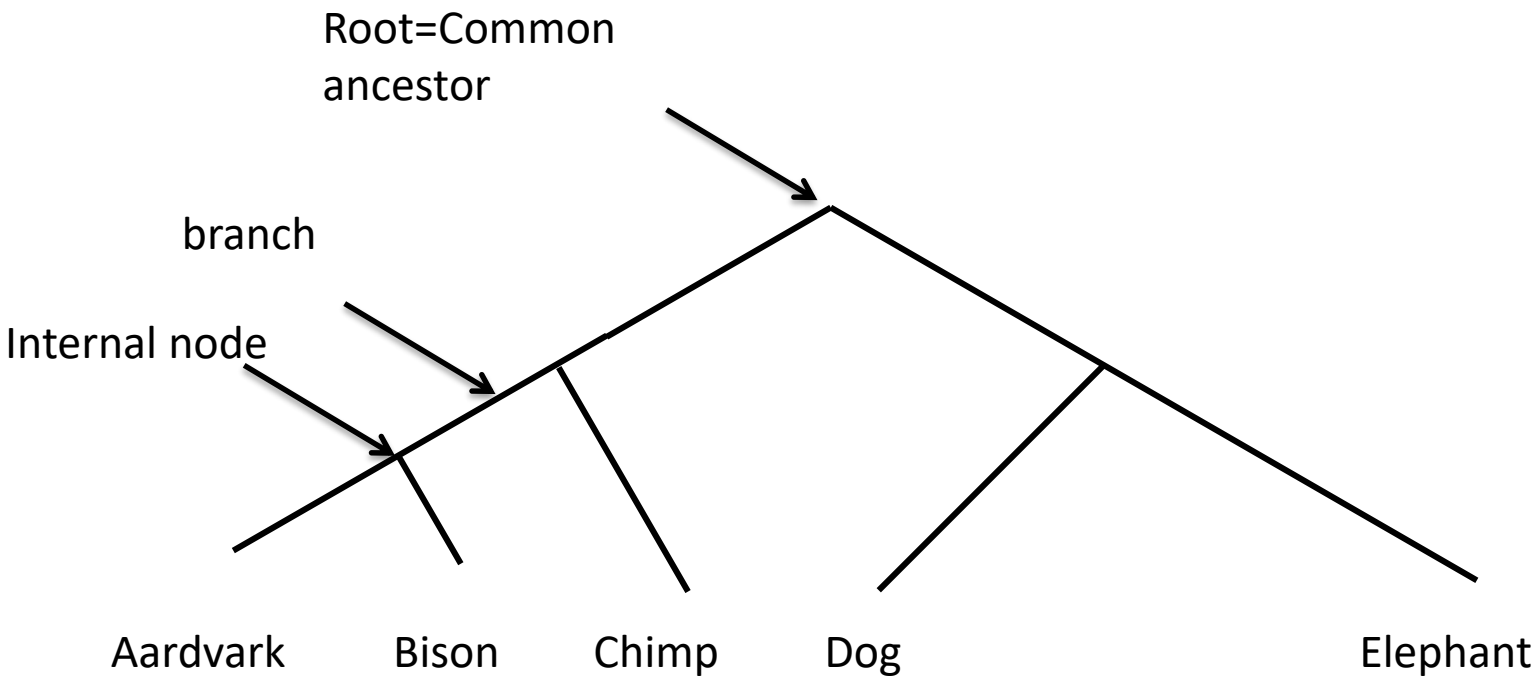


There are many possible types of sequences to use (e.g. Mitochondrial vs Nuclear proteins).

Basic Assumptions

- A universal ancestor exists for all life forms.
- Molecular difference in homologous genes (or protein sequences) are positively correlated with evolution time.
- each position in a sequence evolved independently.
- Phylogenetic relation can be expressed by a dendogram (a “tree”) .

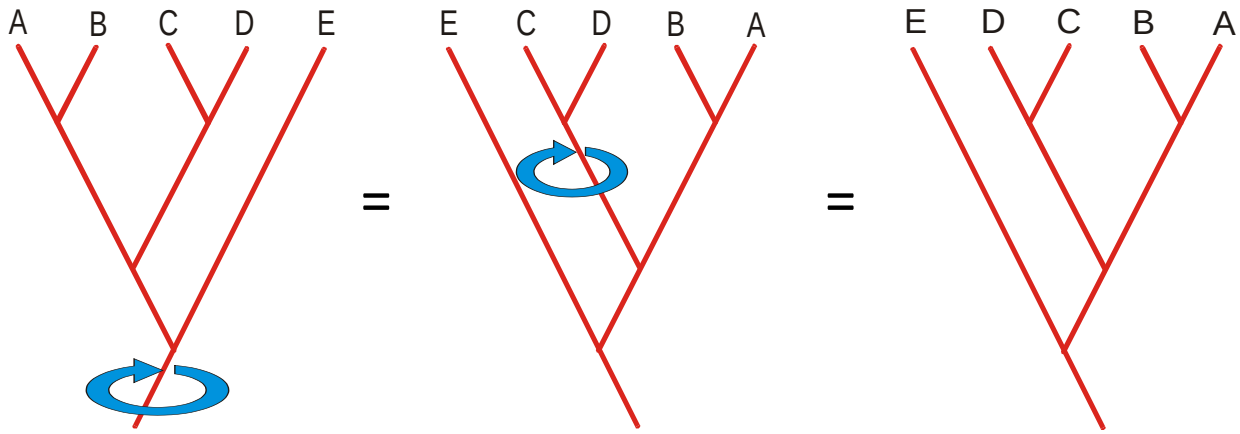
Phylogenetic trees



- **Leafs** - current day species(Taxa)
- **Nodes** - hypothetical most recent common ancestors
- **Edges length** - “time” from one speciation to the next

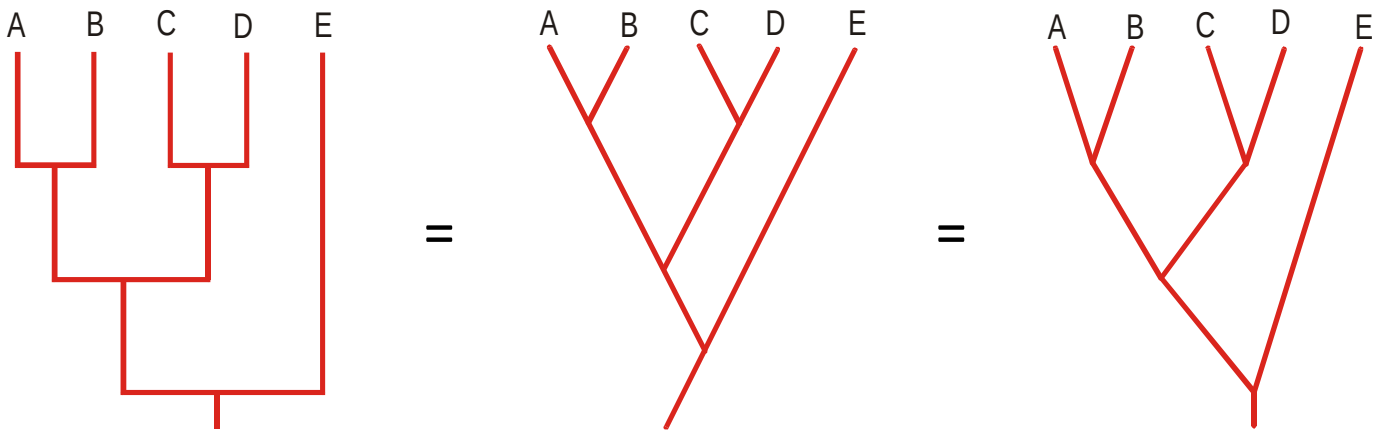
Phylogenetic trees

There are many ways of drawing a tree



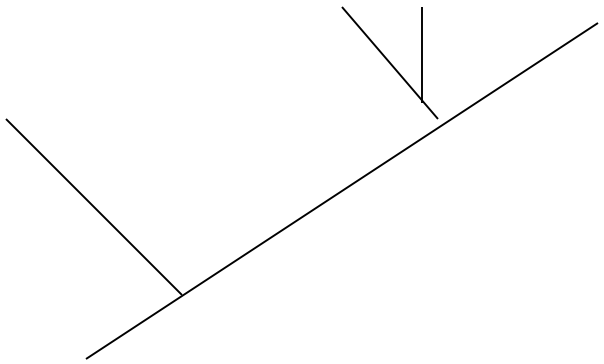
Phylogenetic trees

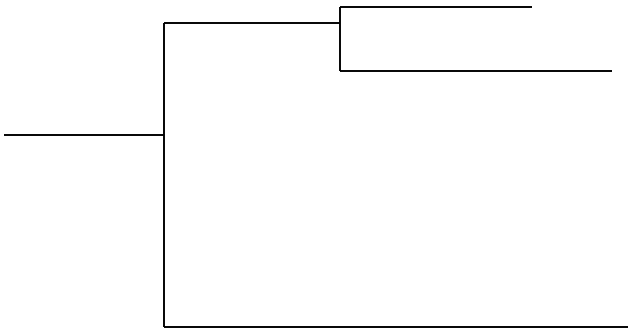
There are many ways of drawing a tree



The bifurcating tree

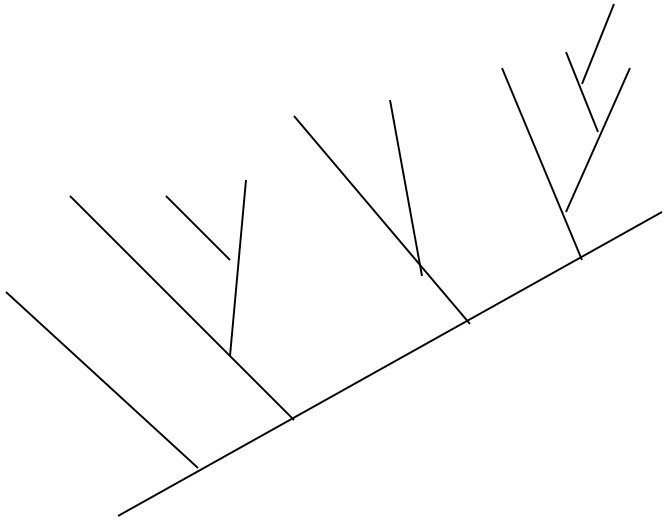
- A tree that bifurcates has a maximum of 2 descendants arising from each of the interior nodes.

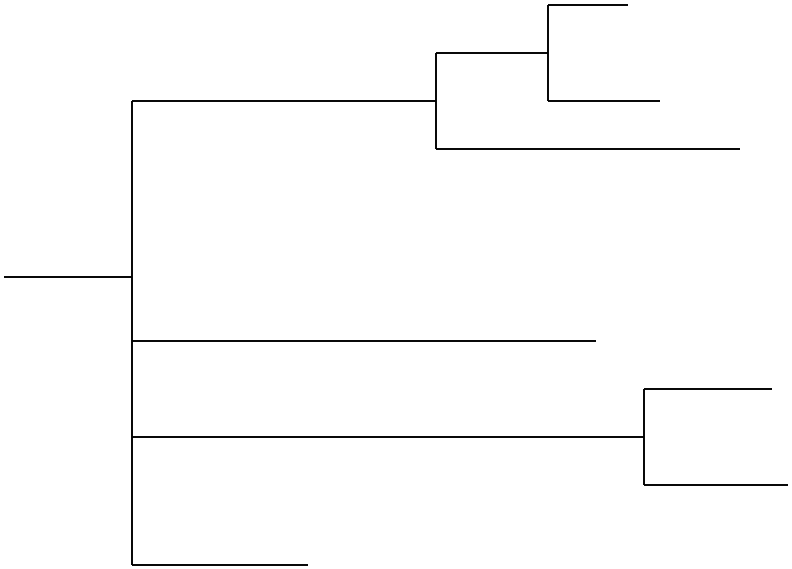




The multi-furcating tree

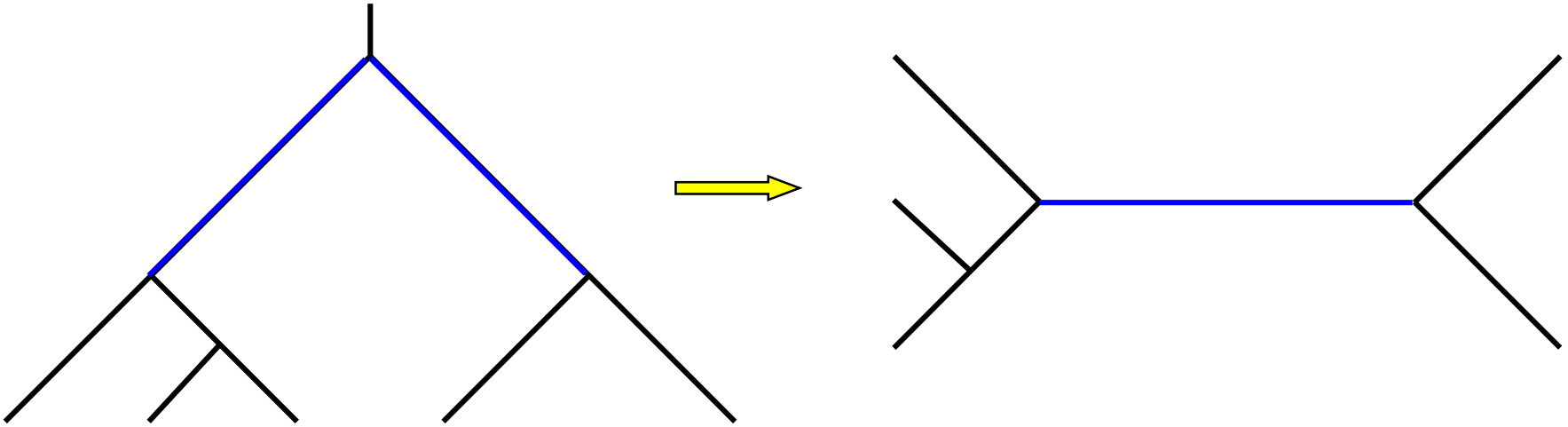
- A tree that multi-furcates has multiple descendants arising from each of the interior nodes.





Types of trees

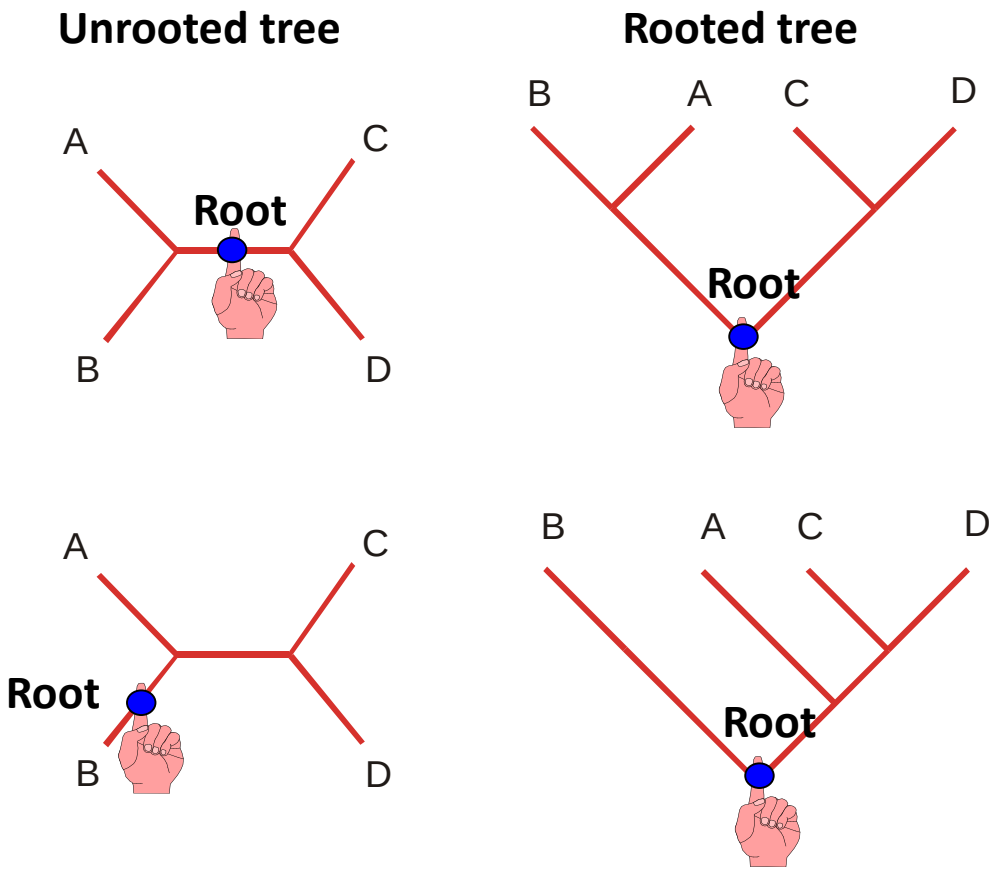
- A natural model to consider is that of **rooted** trees
- **Unrooted** tree represents the same phylogeny without the root node



Depending on the model, data from current day species does not distinguish between different placements of the root.

Phylogenetic trees

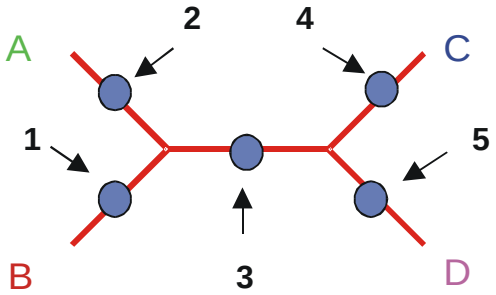
Trees can be unrooted or rooted



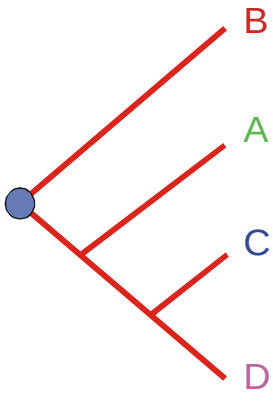
Phylogenetic trees

Trees can be unrooted or rooted

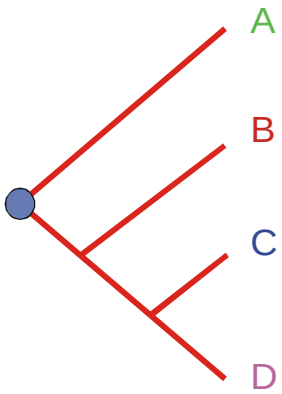
Unrooted tree



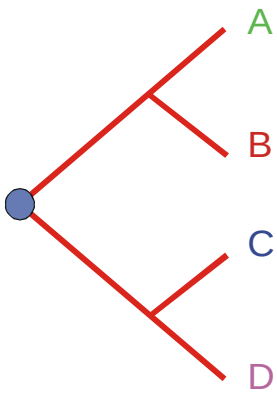
Rooted tree 1



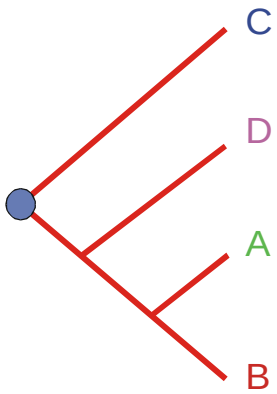
Rooted tree 2



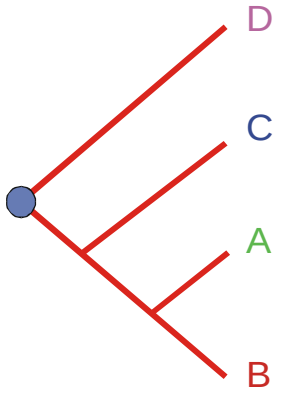
Rooted tree 3



Rooted tree 4



Rooted tree 5

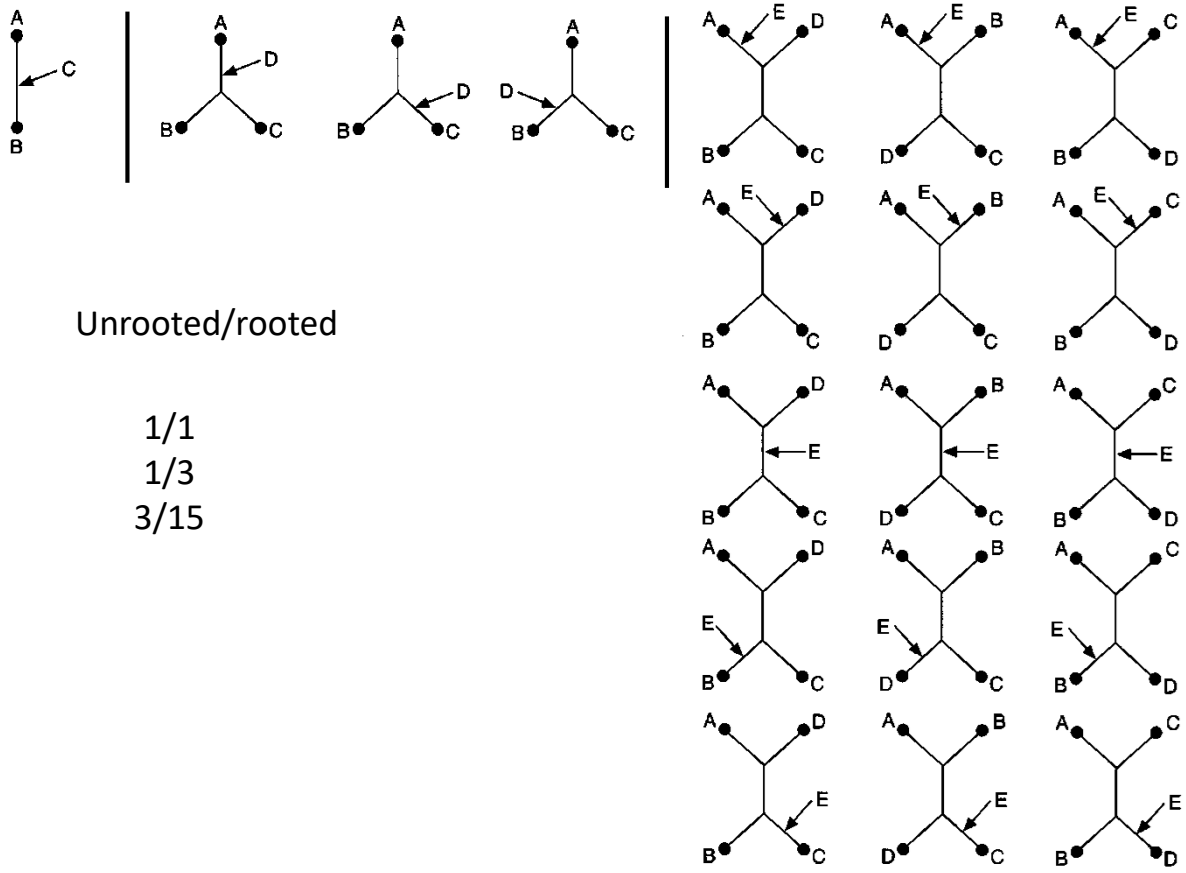


These trees show five different evolutionary relationships among the taxa!

Phylogenetic trees

Possible evolutionary trees

Taxa (n): 2 3 4



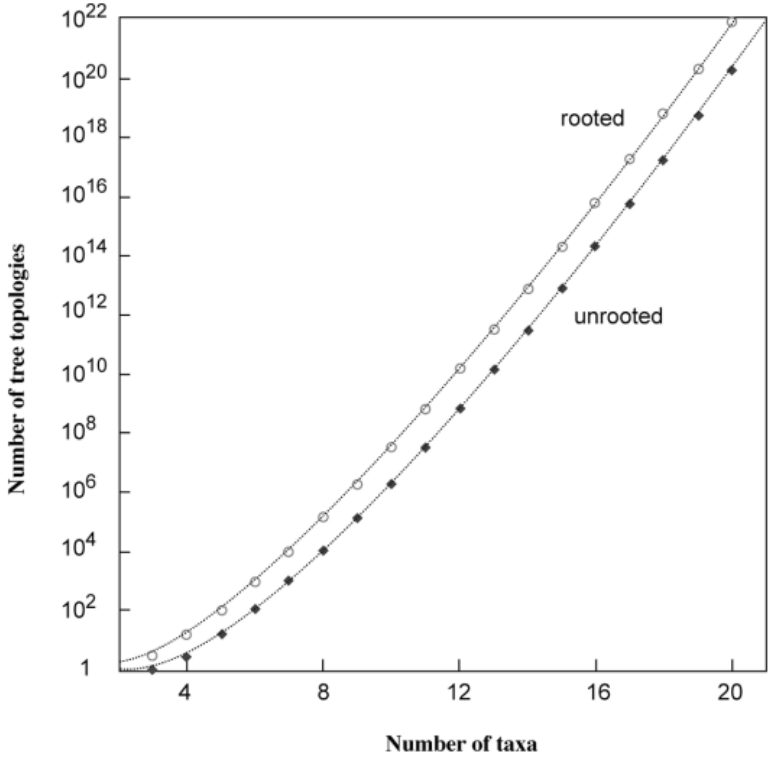
Taxa (*n*)	Unrooted/rooted
2	1/1
3	1/3
4	3/15

Phylogenetic trees

Possible evolutionary trees

Taxa (n)	rooted $(2n-3)!/(2^{(n-2)}(n-2)!)$	unrooted $(2n-5)!/(2^{(n-3)}(n-3)!)$
2	1	1
3	3	1
4	15	3
5	105	15
6	954	105
7	10,395	954
8	135,135	10,395
9	2,027,025	135,135
10	34,459,425	2,027,025

Number of Rooted VS Unrooted Trees



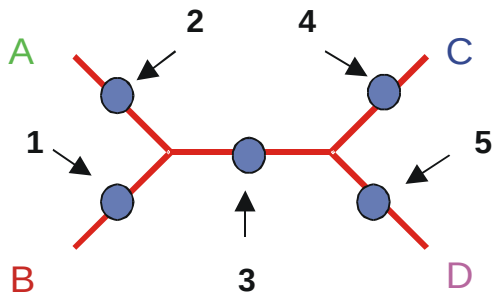
NR = (2n -3)!/ 2^(n-2) * (n – 2)!

NU = (2n – 5)!/ 2^(n-3) * (n – 3)!

But only one of these represents the true turn of events!
Most phylogenetic trees generated with molecular data are thus referred to as *inferred trees*.

Phylogenetic trees

How to root?

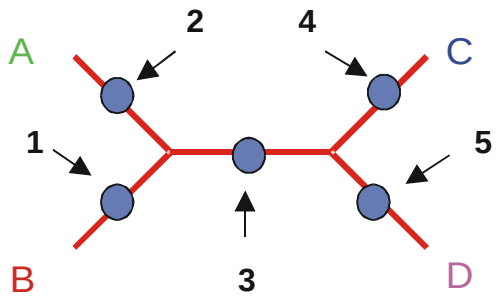


- **Use information from ancestors**

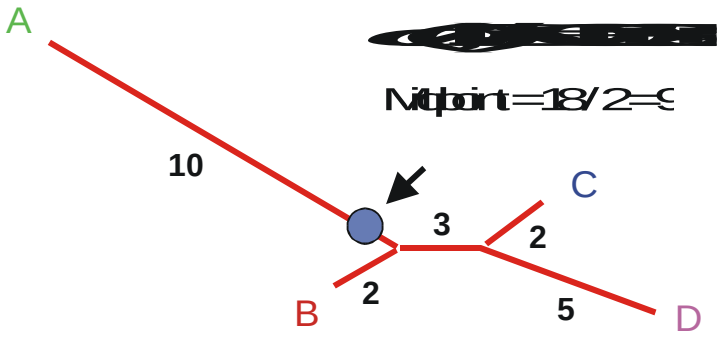
In most cases not available

Phylogenetic trees

How to root?



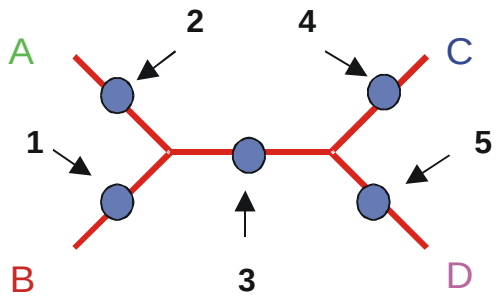
- **Use statistical tools will root trees automatically (*e.g.* mid-point rooting)**



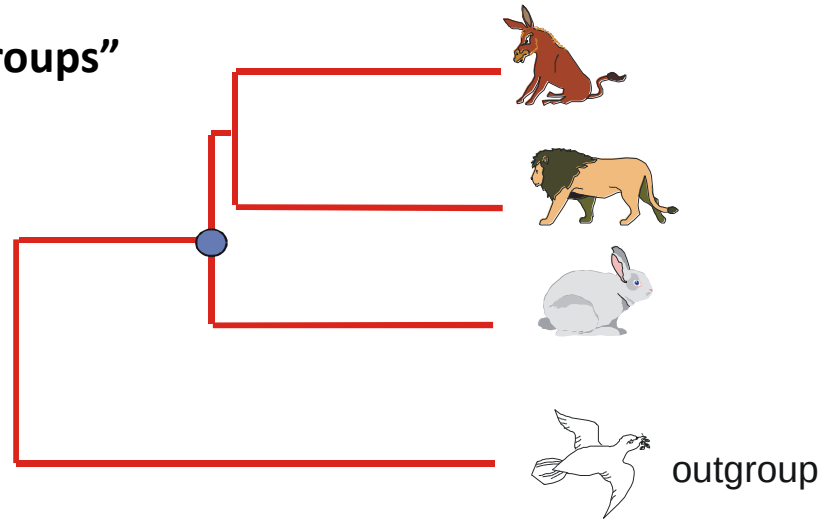
This must involve assumptions … BEWARE!

Phylogenetic trees

How to root?



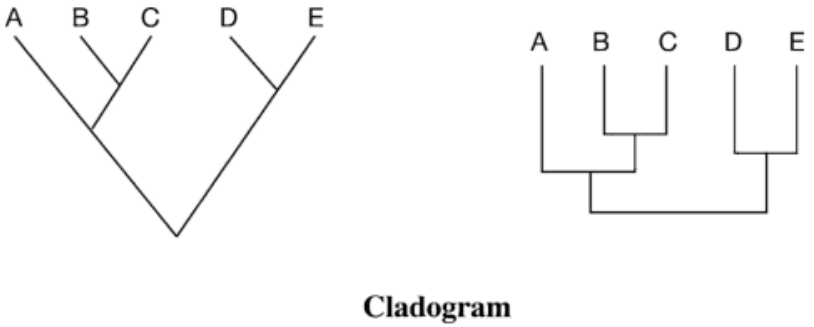
- **Using "outgroups"**



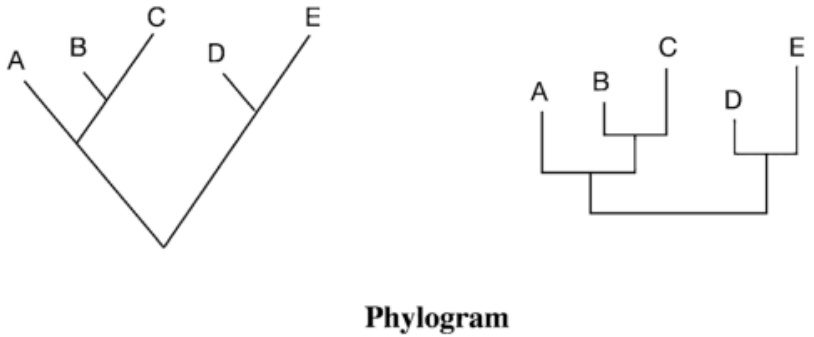
- the outgroup should be a taxon known to be less closely related to the rest of the taxa (ingroups)
- it should ideally be as closely related as possible to the rest of the taxa while still satisfying the above condition

Terminology

- Unrooted tree
- Rooted tree
  - Cladograms: Branch length have no meaning
  - Phylograms: Branch length represent evolutionary change

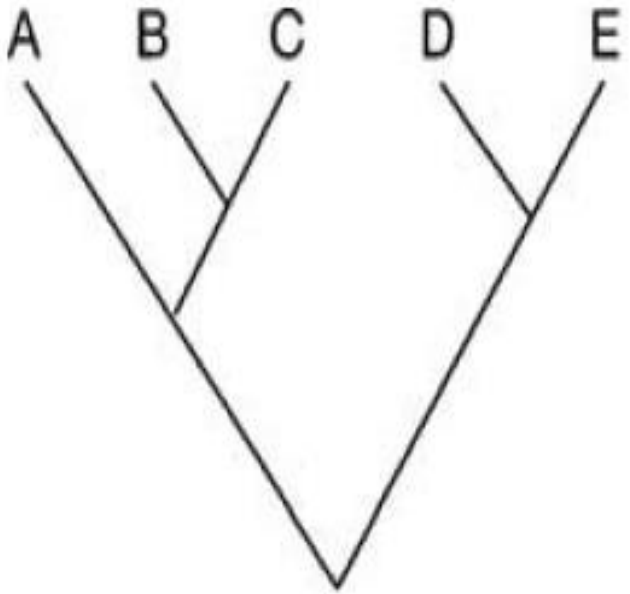


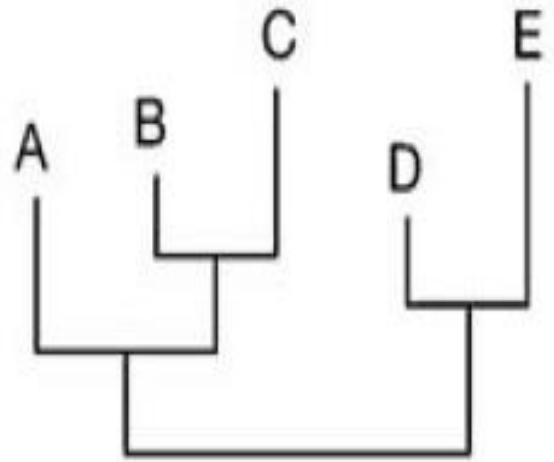
Cladogram



Phylogram

Formal Representation





(((B,C),A),(D,E))

(((B:1,C:2),A:2),(D:1.2,E:2.5))

Newick format

How to construct a phylogenetic tree?

- <u>Step1:</u>

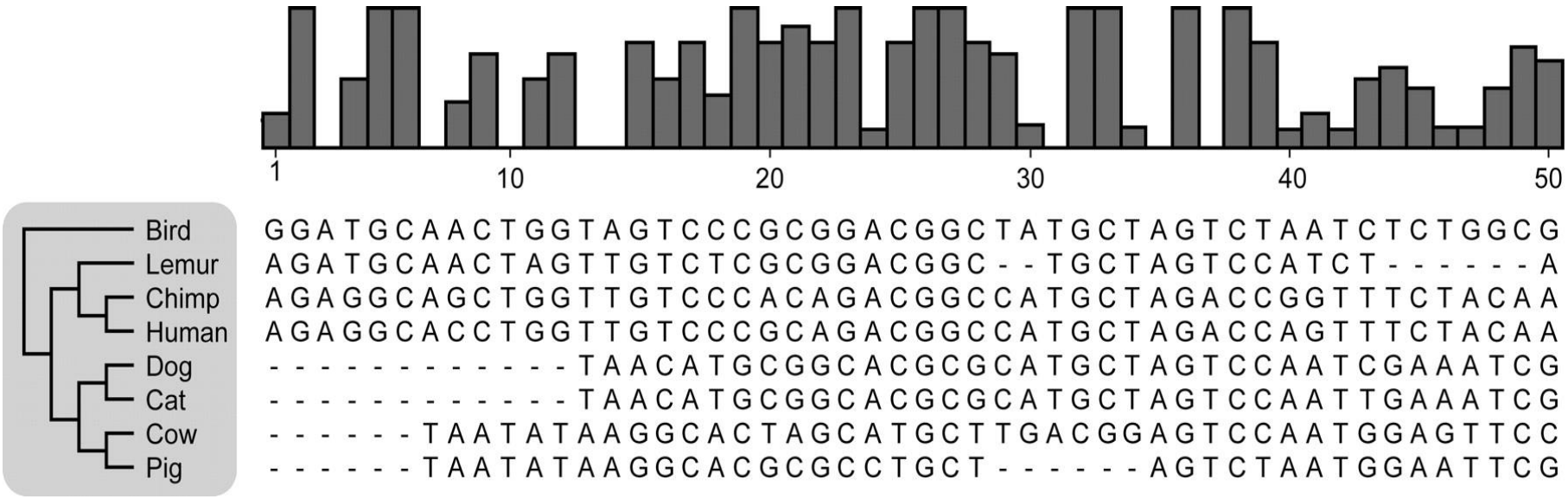
  Choice of Molecular Markers

  amino acid sequence

  DNA sequence

  RNA sequence

How to construct a phylogenetic tree?



- Step 2:

  Check the multiple alignment if it reflects the evolutionary process.

http://genome.cshlp.org/content/17/2/127.full

How to construct a phylogenetic tree? cont

- Step3:

  Choose what method we are going to use and calculate the distance or use the result depending on the method

- Step 4:

  Verify the result statistically.

Where do we get distances?

- commonly obtained from sequence alignments

$$f_{ij} = \frac{\#\text{mismatches}}{\#\text{matches} + \#\text{mismatches}}$$

in alignment of sequence i with sequence j

$$\text{dist}(i,j) = f_{ij}$$

- to consider evolutionary time between sequences:

$$\text{dist}_{\text{Jukes-Cantor}}(i,j) = -\frac{3}{4}\ln\left(1 - \frac{4}{3} f_{ij}\right)$$

Distance Matrix methods

- Calculate all the distance between leaves (taxa)
- Based on the distance, construct a tree
- Not very accurate
- Fastest method
  - UPGMA
  - Neighbor-joining

UPGMA

- Abbreviation of “Unweighted Pair Group Method with Arithmetic Mean”
- Originally developed for numeric taxonomy in 1958 by Sokal and Michener
- Simplest algorithm for tree construction, so it's fast!

How to construct a tree with UPGMA?

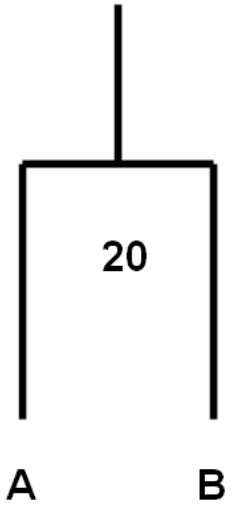
- Prepare a distance matrix
- Repeat step 1 and step 2 until there are only two clusters
- Step 1:

  Cluster a pair of leaves (taxa) by shortest distance
- Step 2:

  Recalculate a new average distance with the new cluster and other taxa, and make a new distance matrix

Example of UPGMA

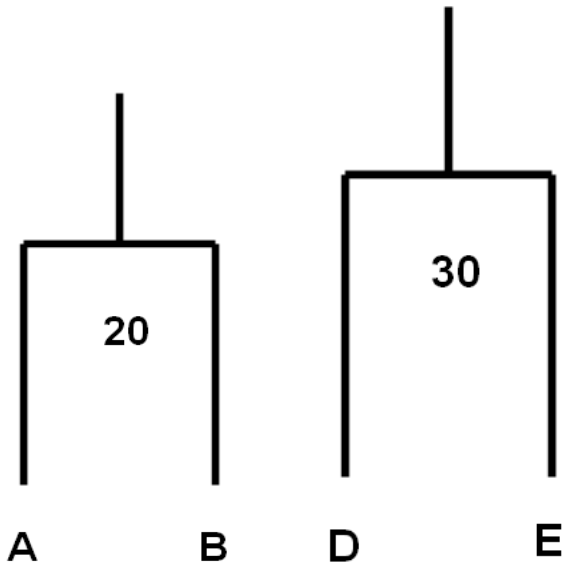
	A	B	C	D	E
A	0				
B	20	0			
C	60	50	0		
D	100	90	40	0	
E	90	80	50	30	0



- New average distance between AB and C is:
  - C to AB = (60 + 50) / 2 = 55
- Distance between D to AB is:
  - D to AB = (100 + 90) / 2 = 95
- Distance between E to AB is:
  - E to AB = (90 + 80) / 2 = 85

Example of UPGMA cont 1

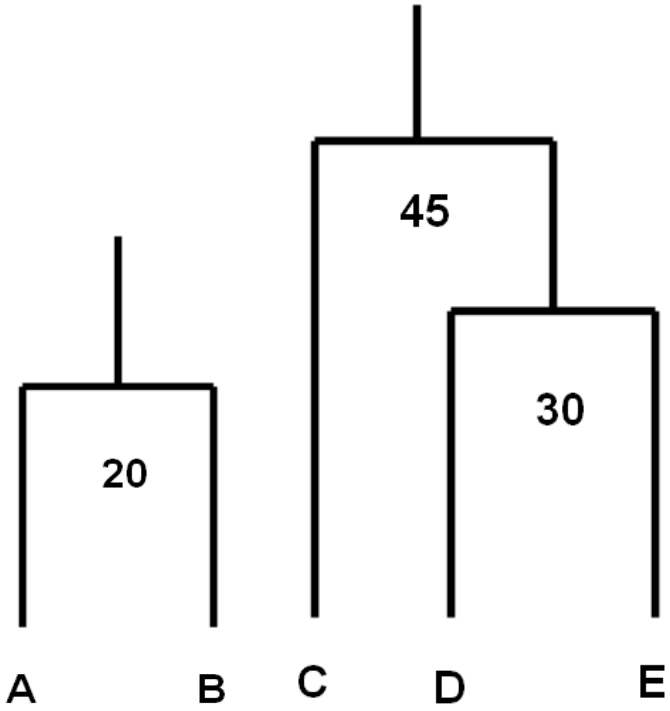
	AB	C	D	E
AB	0			
C	55	0		
D	95	40	0	
E	85	50	30	0



- New average distance between AB and DE is:
  - AB to DE = (95 + 85) / 2 = 90

Example of UPGMA cont 2

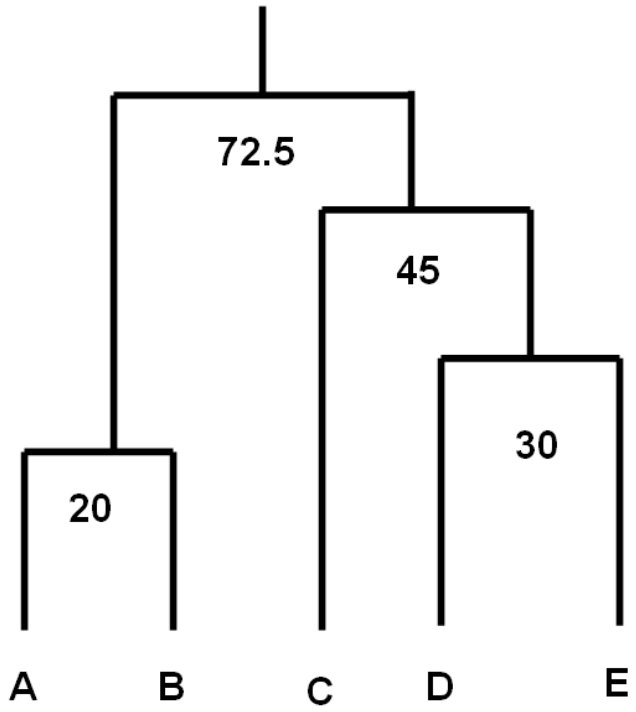
	AB	C	DE
AB	0		
C	55	0	
DE	90	45	0



- New Average distance between CDE and AB is:
  - CDE to AB = (90 + 55) / 2 = 72.5

Example of UPGMA cont 3

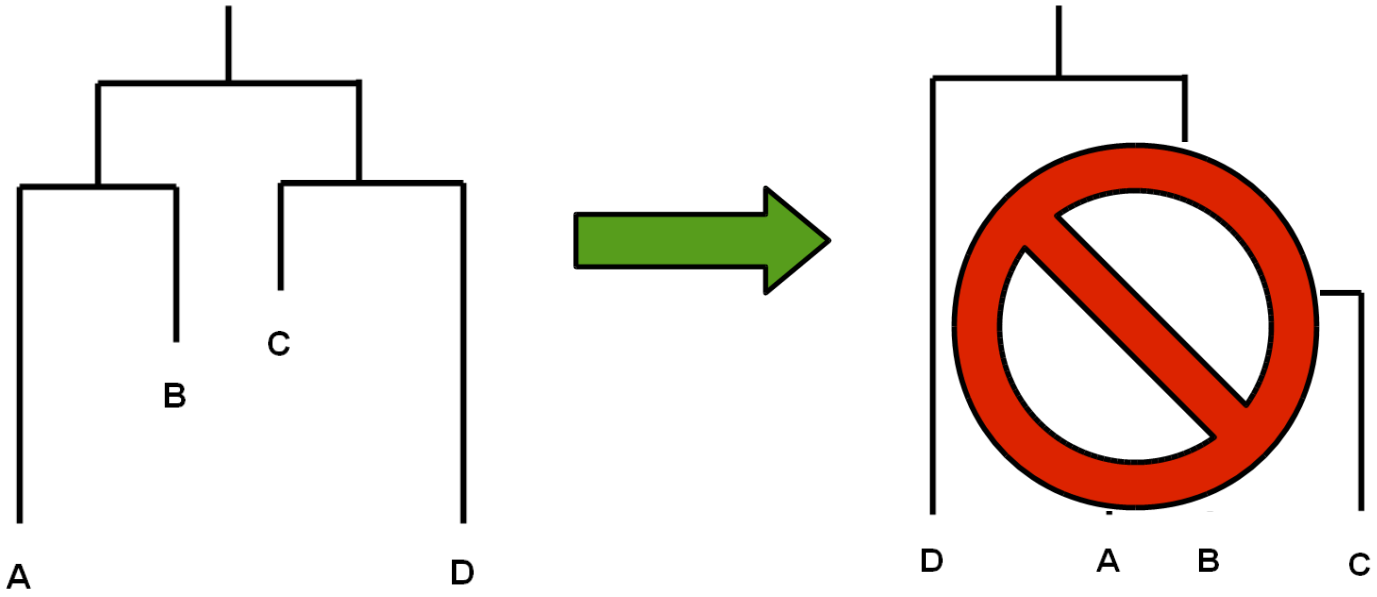
	AB	CDE
AB	**0**	
CDE	**72.5**	**0**



- There are only two clusters. so this completes the calculation!

Downside of UPGMA

- Assume molecular clock (assuming the evolutionary rate is approximately constant)
- Clustering works only if the data is ultrametric
- Doesn't work the following case:



Neighbor-joining method

- Developed in 1987 by Saitou and Nei
- Works in a similar fashion to UPGMA
- Still fast – works great for large dataset

How to construct a tree with Neighbor-joining method?

- Step 1:
  - Calculate sum all distance from x and divide by (leaves – 2)
    - Sx = (sum all Dx) / (leaves - 2)
- Step 2:
  - Calculate pair with smallest M
    - Mij = Distance ij – Si – Sj
- Step 3:
  - Create a node U that joins pair with lowest Mij
    - S1U = (Dij / 2) + (Si – Sj) / 2

How to construct a tree with Neighbor-joining method?

- Step 4:
  - Join I and j according to S and make all other taxa in form of a star
- Step 5:
  - Recalculate new distance matrix of all other taxa to U with:
    - $D_{xU} = D_{ix} + D_{jx} - D_{ij}$

Example of Neighbor-joining

	A	B	C	D	E
B	5				
C	4	7			
D	7	10	7		
E	6	9	6	5	
F	8	11	8	9	8

- Step 1: S calculation : Sx = (sum all Dx) / (leaves - 2)
  - S(A) = (5 + 4 + 7 + 6 + 8) / 4 = 7.5
  - S(B) = (5 + 7 + 10 + 9 + 11) / 4 = 10.5
  - S(C) = (4 + 7 + 7 + 6 + 8) / 4 = 8
  - S(D) = (7+ 10 + 7 + 5 + 9) / 4 = 9.5
  - S(E) = (6 + 9 + 6 + 5 + 8) / 4 = 8.5
  - S(F) = (8 + 11 + 8 + 9 + 8) / 4 = 11

Example of Neighbor-joining cont 1

- Step 2: Calculate pair with smallest M

  Mij = Distance ij – Si – Sj

- Smallest are
  - M(AB) = d(AB) – S(A) –S(B) = 5 – 7.5 – 10.5= -13
  - M(DE) = 5 – 9.5 – 8.5 = -13

	A	B	C	D	E
B	-13				
C	-11.5	-11.5			
D	-10	-10	-10.5		
E	-10	-10	-10.5	-13	
F	-10.5	-10.5	-11	-11.5	-11.5

Example of Neighbor-joining cont 2

- Step 3: Create a node U

  $S1U = (Dij / 2) + (Si - Sj) / 2$

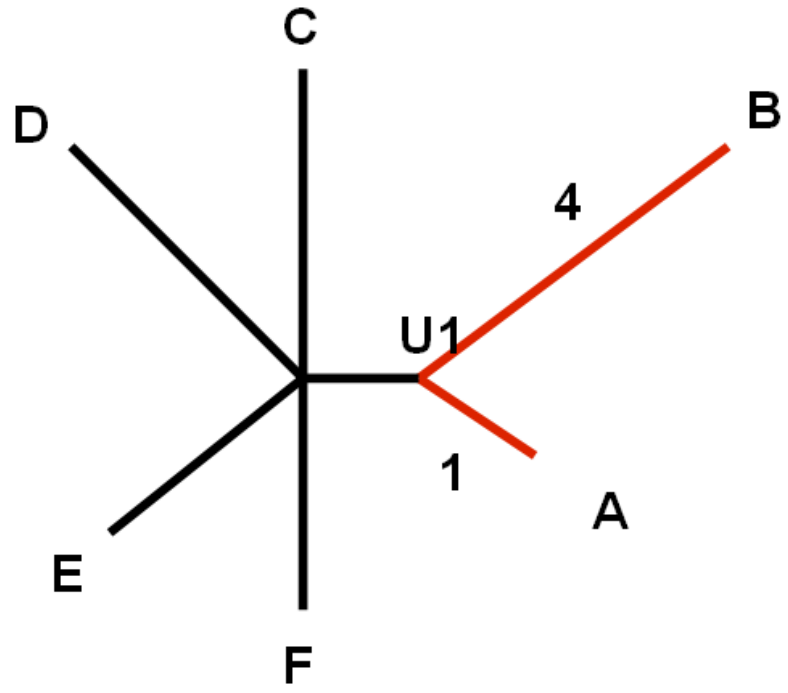
- U1 joins A and B:
  - $S(AU1) = d(AB) / 2 + (S(A) - S(B)) / 2$

    $= 5 / 2 + (7.5 - 10.5) / 2 = 1$
  - $S(BU1) = d(AB) / 2 + (S(B) - S(A)) / 2$

    $= 5 / 2 + (10.5 - 7.5) / 2 = 4$

Example of Neighbor-joining cont 3

- Step 4: Join A and B according to S, and make all other taxa in form of a star. Branches in black are unknown length and Branches in red are known length



Example of Neighbor-joining cont 4

- Step5: Calculate new distance matrix

  Dxu = (Dix + Djx – Dij) / 2

  - d(CU) = (d(AC) + d(BC) - d(AB)) / 2

    = (4 + 7 - 5) / 2 =3

  - d(DU) = d(AD) + d(BD) - d(AB) / 2 = 6

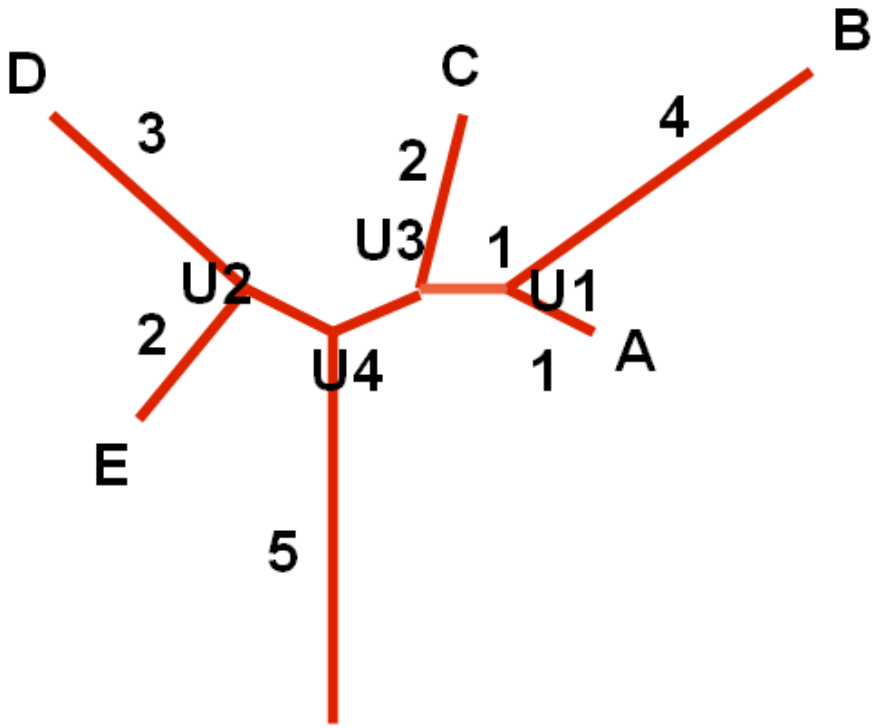
    Same as EU and FU

- Then we get the new distance matrix

	U1	C	D	E
C	3			
D	6	7		
E	5	6	5	
F	7	8	9	8

Example of Neighbor-joining cont 5

- Repeat 1 to 5 until all branches are done
- In this example, we will get this at the end

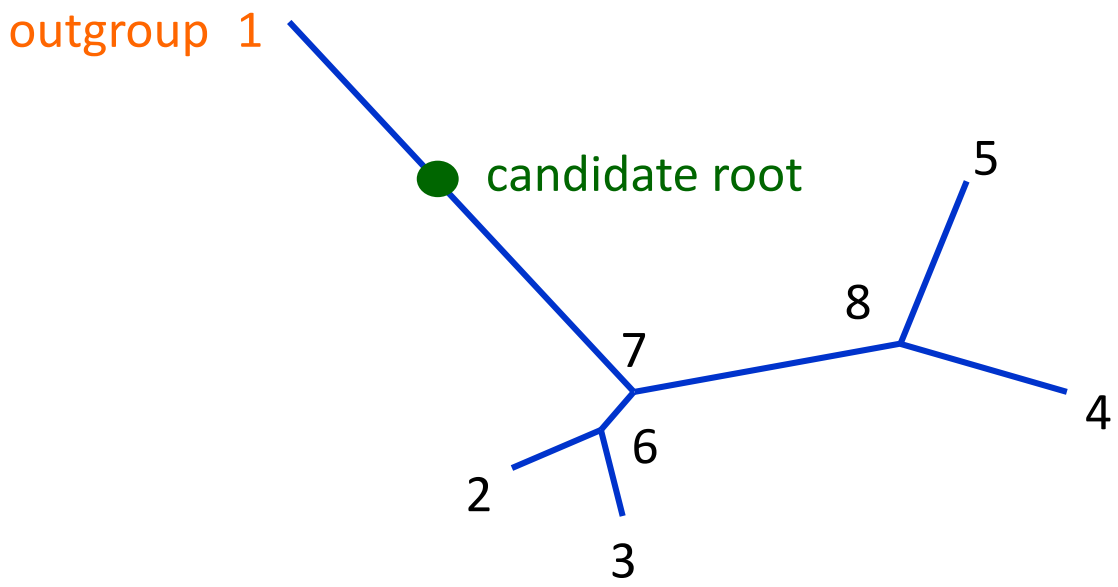


Downside of Neighbor-joining

- Generates only one possible tree
- Generates only unrooted tree

Rooting trees

- finding a root in an unrooted tree is sometimes accomplished by using an *outgroup*
- outgroup: a species known to be more distantly related to remaining species than they are to each other
- edge joining the outgroup to the rest of the tree is best candidate for root position



Character state methods

- Need discrete characters
  - Maximum likelihood
  - Maximum parsimony (will be covered by Kyle)

Maximum likelihood

- Originally developed for statistics by Ronald Fisher between 1912 and 1922
- Therefore, explicit statistical model
- Uses all the data
- Tends to outperform parsimony or distance matrix methods

How to construct a tree with Maximum likelihood?

- Step 1:

  Make all possible trees depending on the number of leaves
- Step 2: Calculate likelihood of occurring with the given data

  L(Tree) = probability of each tree.
  - optimizing branch length
  - generating tree topology
- Step 3:

  Pick the tree that have the highest likelihood.

Sounds really great?

Num of leaves	Num of possible trees
3	1
5	15
10	2027025
13	15058768725
20	8200794532637891559375

- Maximum likelihood is very expensive and extremely slow to compute

Topics

- Phylogenetic tree types
- Distance Matrix method
  - UPGMA
  - Neighbor joining
- Character State method
  - Maximum likelihood

Rooting trees

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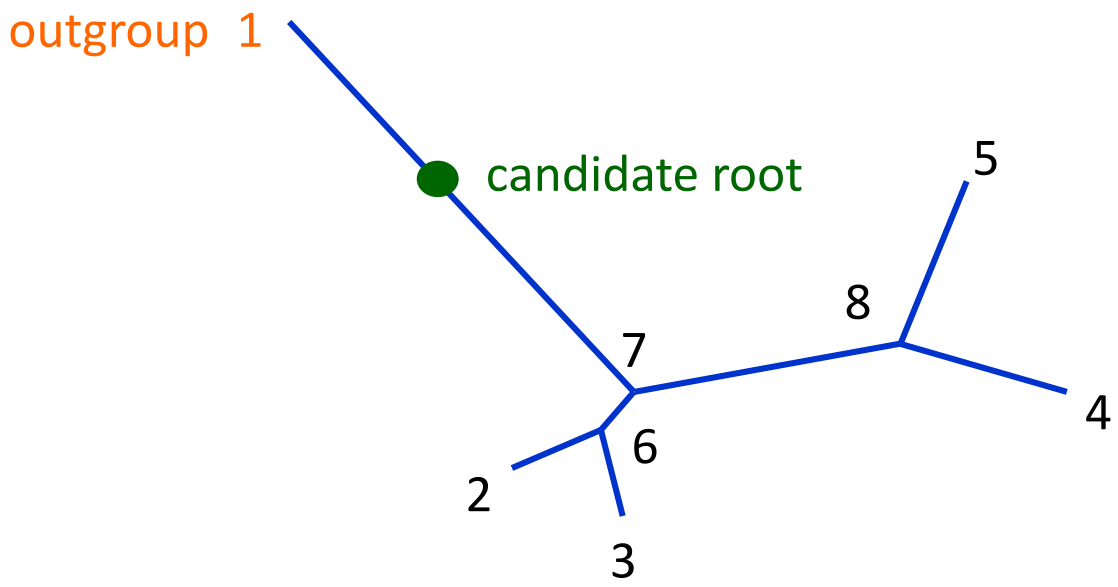
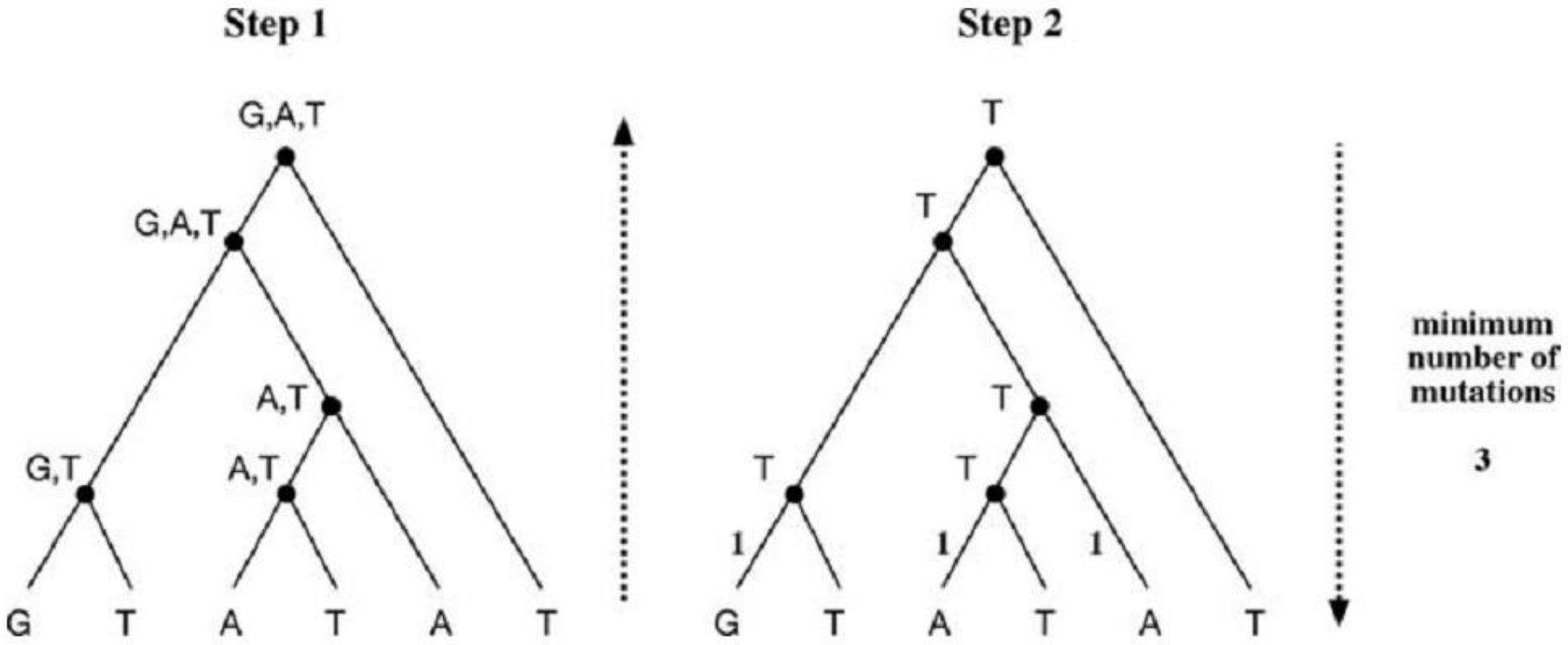
- outgroup: a species known to be more distantly related to remaining species than they are to each other
- edge joining the outgroup to the rest of the tree is best candidate for root position

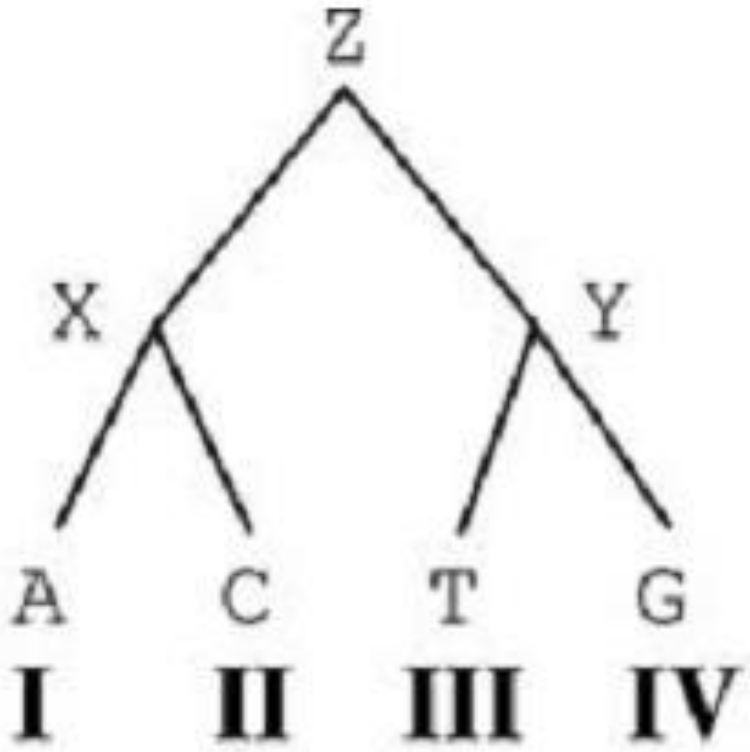


Topics

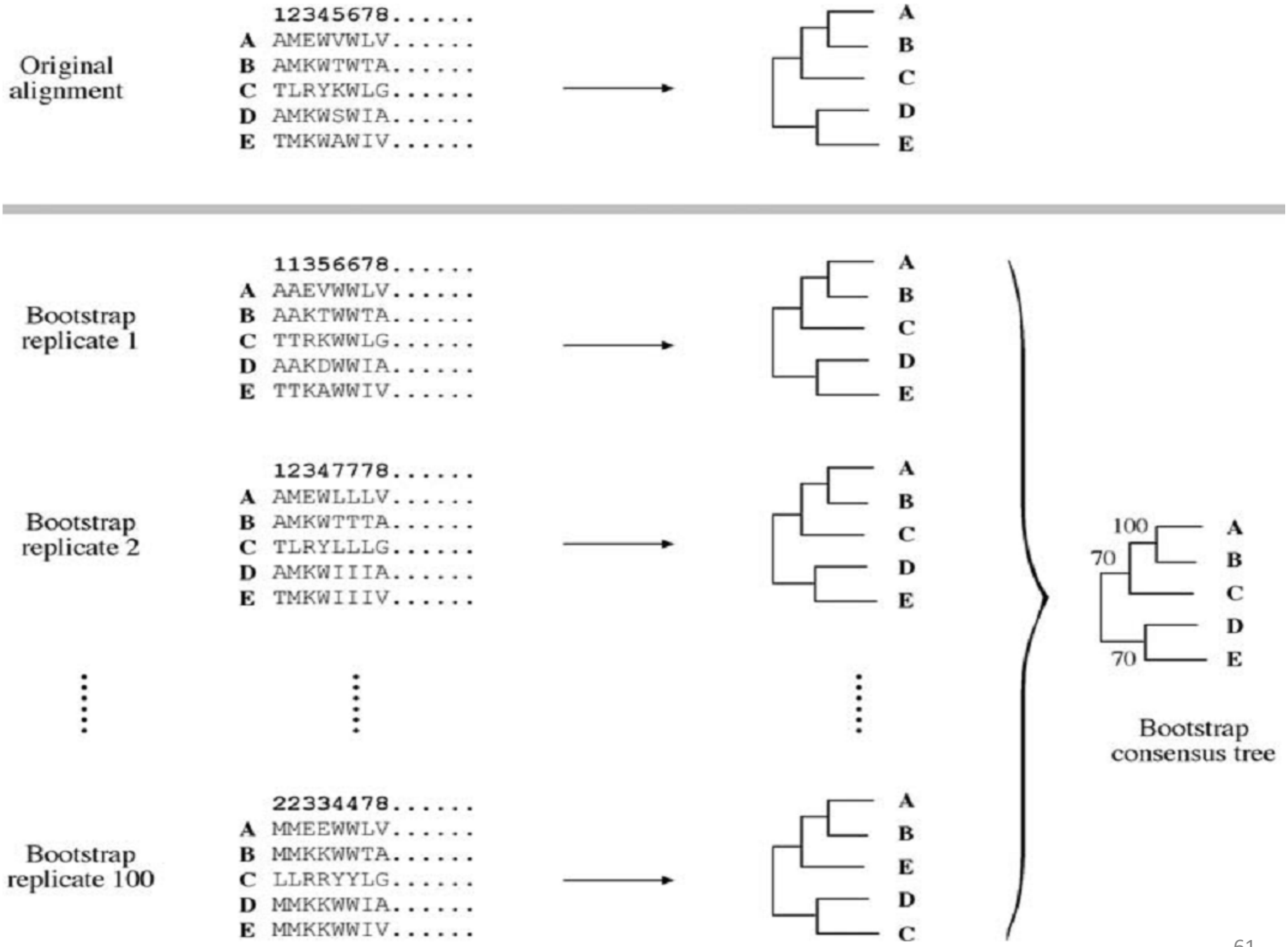
- Maximum parsimony is a character-based method that infers a phylogenetic tree by minimizing the total number of evolutionary steps required to explain a given set of data, or in other words by minimizing the total tree length

taxa \ sites	1	2	3	4	5	6	7	8
I	A	A	T	T	A	G	C	T
II	G	G	T	C	G	T	A	G
III	A	A	T	G	C	G	C	T
IV	A	G	T	A	A	G	C	A
V	A	C	T	T	C	G	C	G
VI	A	C	A	T	G	G	C	A

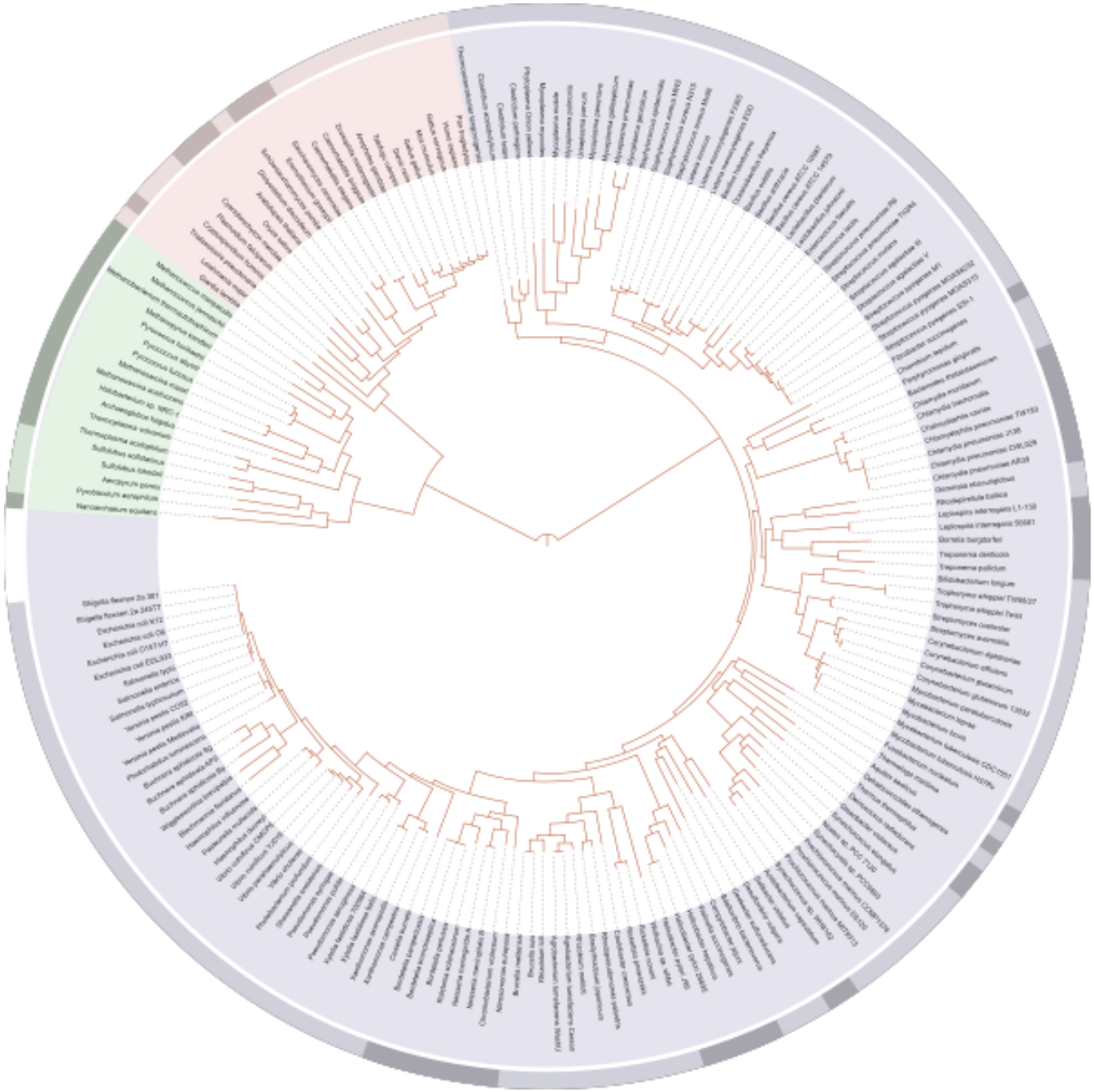

Step 1
G,A,T
G,A,T
A,T
G,T
A,T
G
T
A
T
A
T
Step 2
T
T
T
T
T
1
1
1
G
T
A
T
A
T
minimum number of mutations
3



$$L_{(4)} = Pr(Z \rightarrow X) * Pr(Z \rightarrow Y) * Pr(X \rightarrow A) * Pr(X \rightarrow C) * Pr(Y \rightarrow T) * Pr(Y \rightarrow G)$$


Original alignment
12345678.....
A AMEWVWLV.....
B AMKWTWTA.....
C TLRYKWLG.....
D AMKWSWIA.....
E TMKWAWIV.....
A
B
C
D
E
Bootstrap replicate 1
11356678.....
A AAEVWWLV.....
B AAKTWWTA.....
C TTRKWWLG.....
D AAKDWWIA.....
E TTKAWWIV.....
Bootstrap replicate 2
12347778.....
A AMEWLLLV.....
B AMKWTTTA.....
C TLRYLLLG.....
D AMKWIIIA.....
E TMKWIIIV.....
Bootstrap replicate 100
22334478.....
A MMEEWWLV.....
B MMKKWWTA.....
C LLRRYYLG.....
D MMKKWWIA.....
E MMKKWWIV.....
100
70
70
Bootstrap consensus tree

Tree of life constructed from all species for which their complete genome has been sequenced



https://en.wikipedia.org/wiki/Tree_of_life_(biology)