"CRISPR-associated protein 1 (Cas1)"

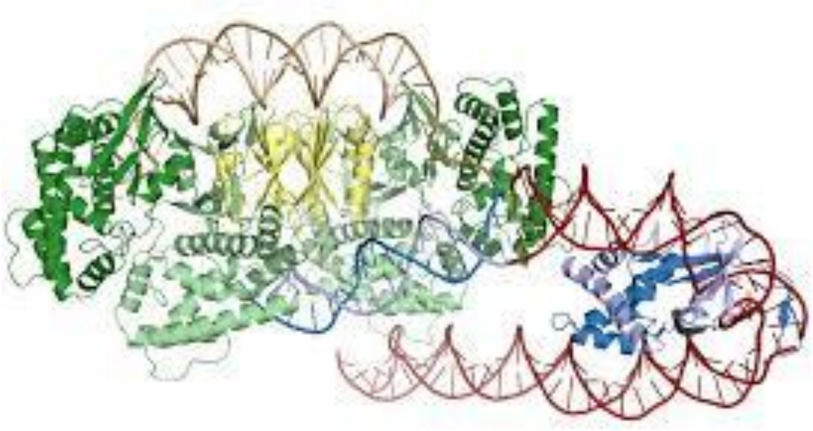
Molecular phylogenetics course project

Sedreh Nassirnia
Fall 2019

Objective

- Study evolutionary relationships among bacteria family based on cas1

- What percentage of organisms evolved according to viral strains



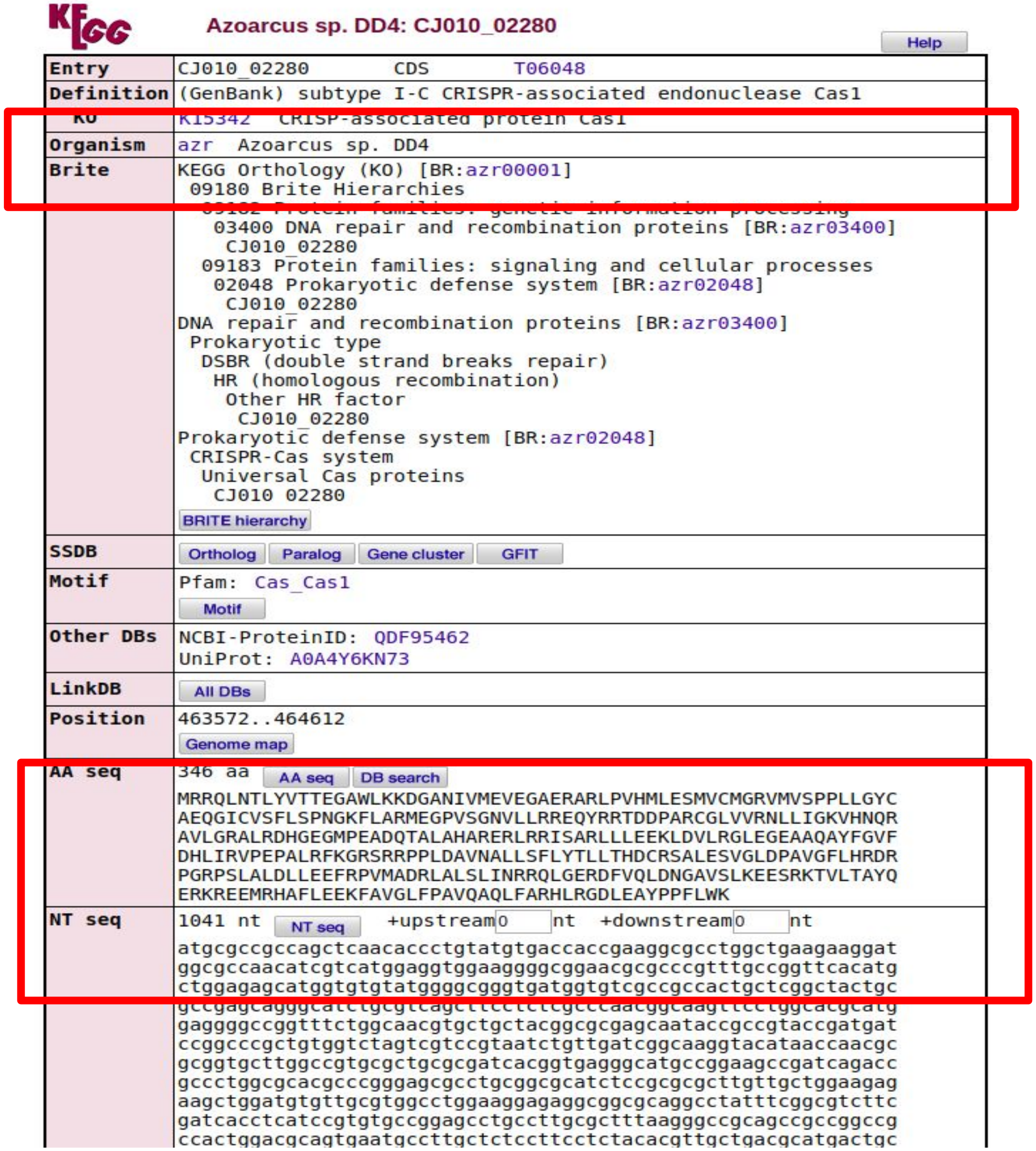
CRISPR-Cas Systems

- CRISPR (clustered regularly interspaced short palindromic repeat)
  - adaptive immune system that provides protection against mobile genetic elements
- Important for
  - clinical microbiologists, ecologists and evolutionary biologists
  - CRISPR-Cas system potential uses
    - Detection and genotyping of microbial pathogens
    - Host identification in metagenomes
    - Analysis of viral genomes
    - Targeted genome engineering in both prokaryotic and eukaryotic cells

CRISPR associated protein Cas1

- Cas1 responsible for the ability of the CRISPR immune system in bacteria to adapt to new viral infections
    - Identify the site in the genome where they insert viral DNA

Data



Azoarcus sp. DD4: CJ010_02280

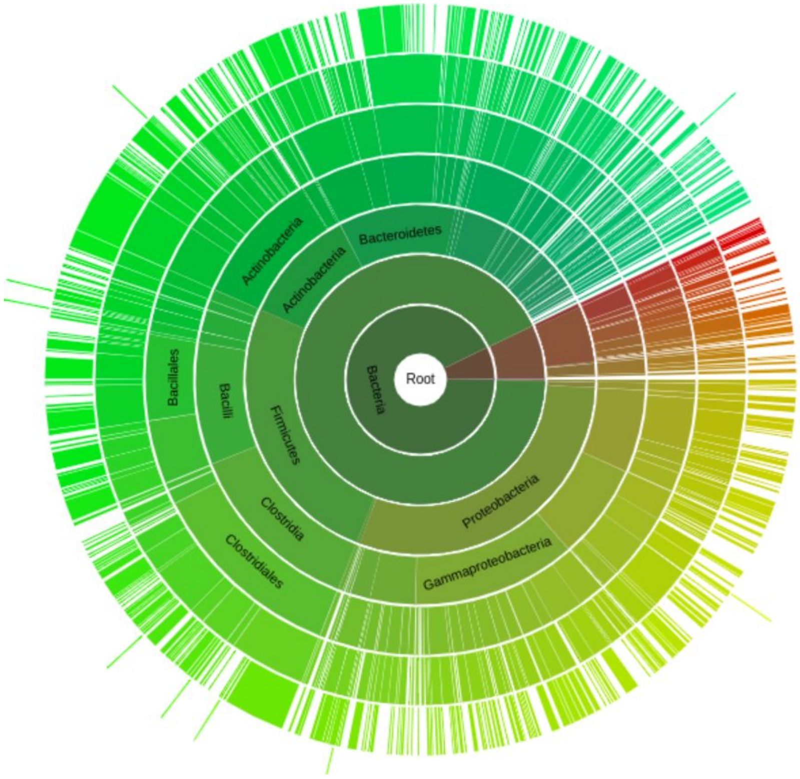
```
Entry       CJ010_02280        CDS       T06048
Definition  (GenBank) subtype I-C CRISPR-associated endonuclease Cas1
  KO        K15342  CRISP-associated protein Cas1
Organism    azr  Azoarcus sp. DD4
Brite       KEGG Orthology (KO) [BR:azr00001]
             09180 Brite Hierarchies
              09182 Protein families: genetic information processing
               03400 DNA repair and recombination proteins [BR:azr03400]
                CJ010_02280
              09183 Protein families: signaling and cellular processes
               02048 Prokaryotic defense system [BR:azr02048]
                CJ010_02280
            DNA repair and recombination proteins [BR:azr03400]
             Prokaryotic type
              DSBR (double strand breaks repair)
               HR (homologous recombination)
                Other HR factor
                 CJ010_02280
            Prokaryotic defense system [BR:azr02048]
             CRISPR-Cas system
              Universal Cas proteins
               CJ010 02280
SSDB
Motif       Pfam:  Cas_Cas1
Other DBs   NCBI-ProteinID:  QDF95462
            UniProt:  A0A4Y6KN73
LinkDB
Position    463572..464612
AA seq      346 aa
            MRRQLNTLYVTTEGAWLKKDGANIVMEVEGAERARLPVHMLESMVCMGRVMVSPPLLGYC
            AEQGICVSFLSPNGKFLARMEGPVSGNVLLRREQYRRTDDPARCGLVVRNLLIGKVHNQR
            AVLGRALRDHGEGMPEADQTALAHARERLRRISARLLLEEKLDVLRGLEGEAAQAYFGVF
            DHLIRVPEPALRFKGRSRRPPLDAVNALLSFLYTLLTHDCRSALESVGLDPAVGFLHRDR
            PGRPSLALDLLEEFRPVMADRLALSLINRRQLGERDFVQLDNGAVSLKEESRKTVLTAYQ
            ERKREEMRHAFLEEKFAVGLFPAVQAQLFARHLRGDLEAYPPFLWK
NT seq      1041 nt     +upstream0      nt  +downstream0      nt
            atgcgccgccagctcaacaccctgtatgtgaccaccgaaggcgcctggctgaagaaggat
            ggcgccaacatcgtcatggaggtggaaggggcggaacgcgcccgtttgccggttcacatg
            ctggagagcatggtgtgtatggggcgggtgatggtgtcgccgccactgctcggctactgc
            gccgagcagggcatctgcgtcagcttcctcttcgcccaacggcaagttcctggcacgcatg
            gaggggccggtttctggcaacgtgctgctacggcgcgagcaataccgccgtaccgatgat
            ccggcccgctgtggtctagtcgtccgtaatctgttgatcggcaaggtacataaccaacgc
            gcggtgcttggccgtgcgctgcgcgatcacggtgagggcatgccggaagccgatcagacc
            gccctggcgcacgcccgggagcgcctgcggcgcatctccgcgcgcttgttgctggaagag
            aagctggatgtgttgcgtggcctggaaggagaggcggcgcaggcctatttcggcgtcttc
            gatcacctcatccgtgtgccggagcctgccttgcgctttaagggccgcagccgccggccg
            ccactggacgcagtgaatgccttgctctccttcctctacacgttgctgacgcatgactgc
```

Downloading directly from Pfam doesn’t give information about Genus and Species

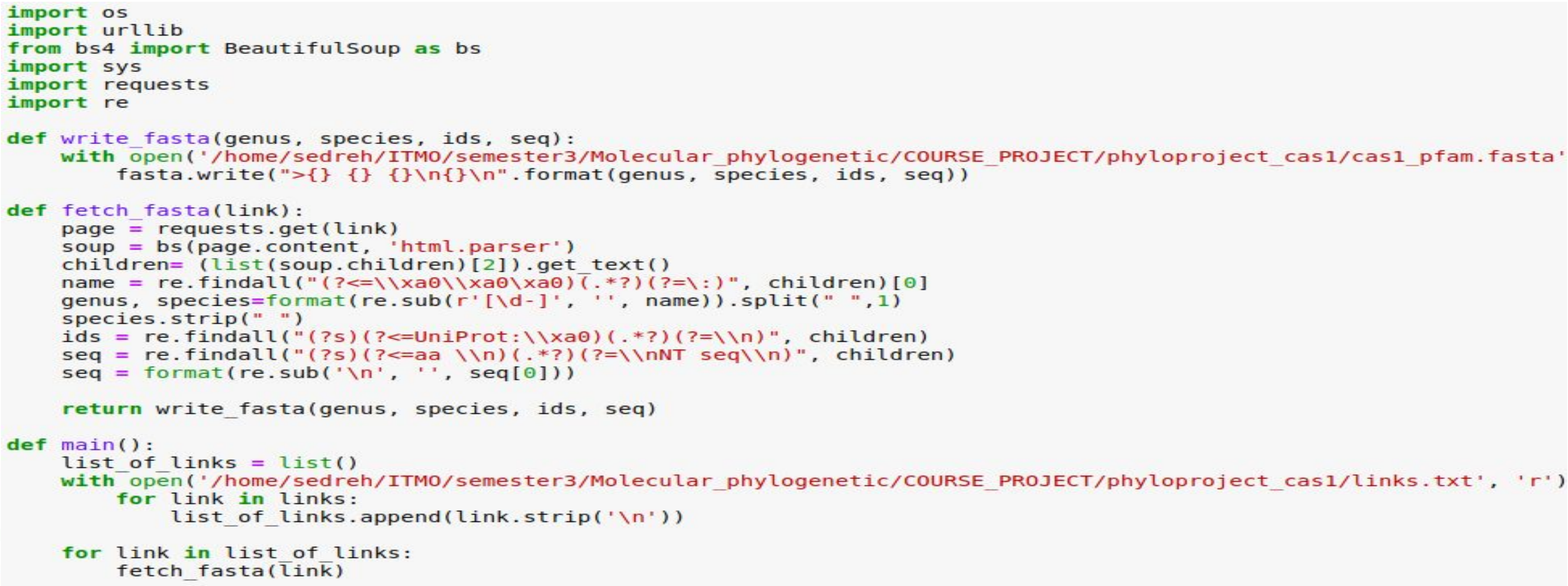
Link database was used to fetch this information.

Example:

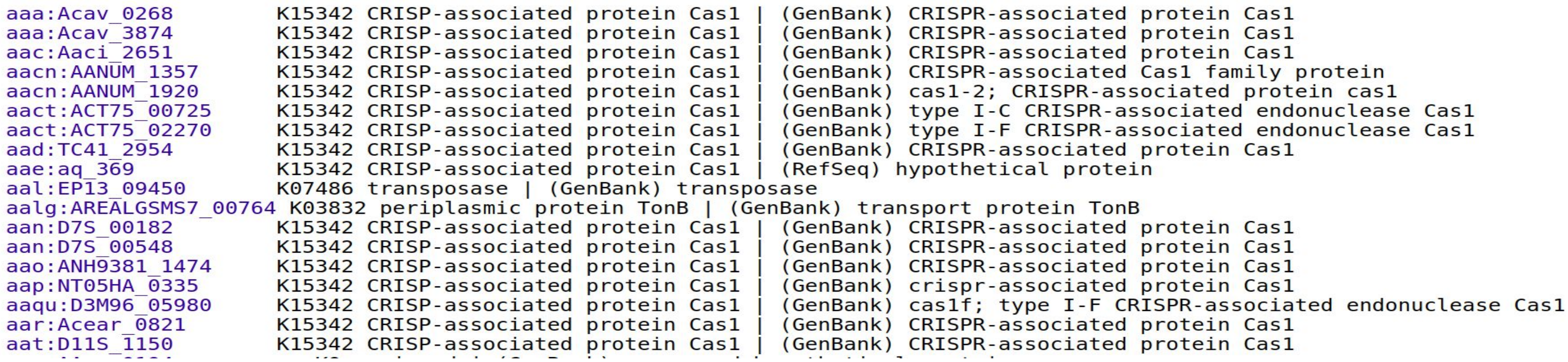
https://www.kegg.jp/entry/azr:CJ010_02280



Fetching IDs



```python
import os
import urllib
from bs4 import BeautifulSoup as bs
import sys
import requests
import re

def write_fasta(genus, species, ids, seq):
    with open('/home/sedreh/ITMO/semester3/Molecular_phylogenetic/COURSE_PROJECT/phyloproject_cas1/cas1_pfam.fasta'
        fasta.write(">{} {} {}\n{}\n".format(genus, species, ids, seq))

def fetch_fasta(link):
    page = requests.get(link)
    soup = bs(page.content, 'html.parser')
    children= (list(soup.children)[2]).get_text()
    name = re.findall("(?<=\\xa0\\xa0\xa0)(.*?)(?=\:)", children)[0]
    genus, species=format(re.sub(r'[\d-]', '', name)).split(" ",1)
    species.strip(" ")
    ids = re.findall("(?s)(?<=UniProt:\\xa0)(.*?)(?=\\n)", children)
    seq = re.findall("(?s)(?<=aa \\n)(.*?)(?=\\nNT seq\\n)", children)
    seq = format(re.sub('\n', '', seq[0]))

    return write_fasta(genus, species, ids, seq)

def main():
    list_of_links = list()
    with open('/home/sedreh/ITMO/semester3/Molecular_phylogenetic/COURSE_PROJECT/phyloproject_cas1/links.txt', 'r')
        for link in links:
            list_of_links.append(link.strip('\n'))

    for link in list_of_links:
        fetch_fasta(link)
```



```
aaa:Acav_0268        K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aaa:Acav_3874        K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aac:Aaci_2651        K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aacn:AANUM_1357      K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated Cas1 family protein
aacn:AANUM_1920      K15342 CRISP-associated protein Cas1 | (GenBank) cas1-2; CRISPR-associated protein cas1
aact:ACT75_00725     K15342 CRISP-associated protein Cas1 | (GenBank) type I-C CRISPR-associated endonuclease Cas1
aact:ACT75_02270     K15342 CRISP-associated protein Cas1 | (GenBank) type I-F CRISPR-associated endonuclease Cas1
aad:TC41_2954        K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aae:aq_369           K15342 CRISP-associated protein Cas1 | (RefSeq) hypothetical protein
aal:EP13_09450       K07486 transposase | (GenBank) transposase
aalg:AREALGSMS7_00764 K03832 periplasmic protein TonB | (GenBank) transport protein TonB
aan:D7S_00182        K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aan:D7S_00548        K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aao:ANH9381_1474     K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aap:NT05HA_0335      K15342 CRISP-associated protein Cas1 | (GenBank) crispr-associated protein Cas1
aaqu:D3M96_05980     K15342 CRISP-associated protein Cas1 | (GenBank) cas1f; type I-F CRISPR-associated endonuclease Cas1
aar:Acear_0821       K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
aat:D11S_1150        K15342 CRISP-associated protein Cas1 | (GenBank) CRISPR-associated protein Cas1
```

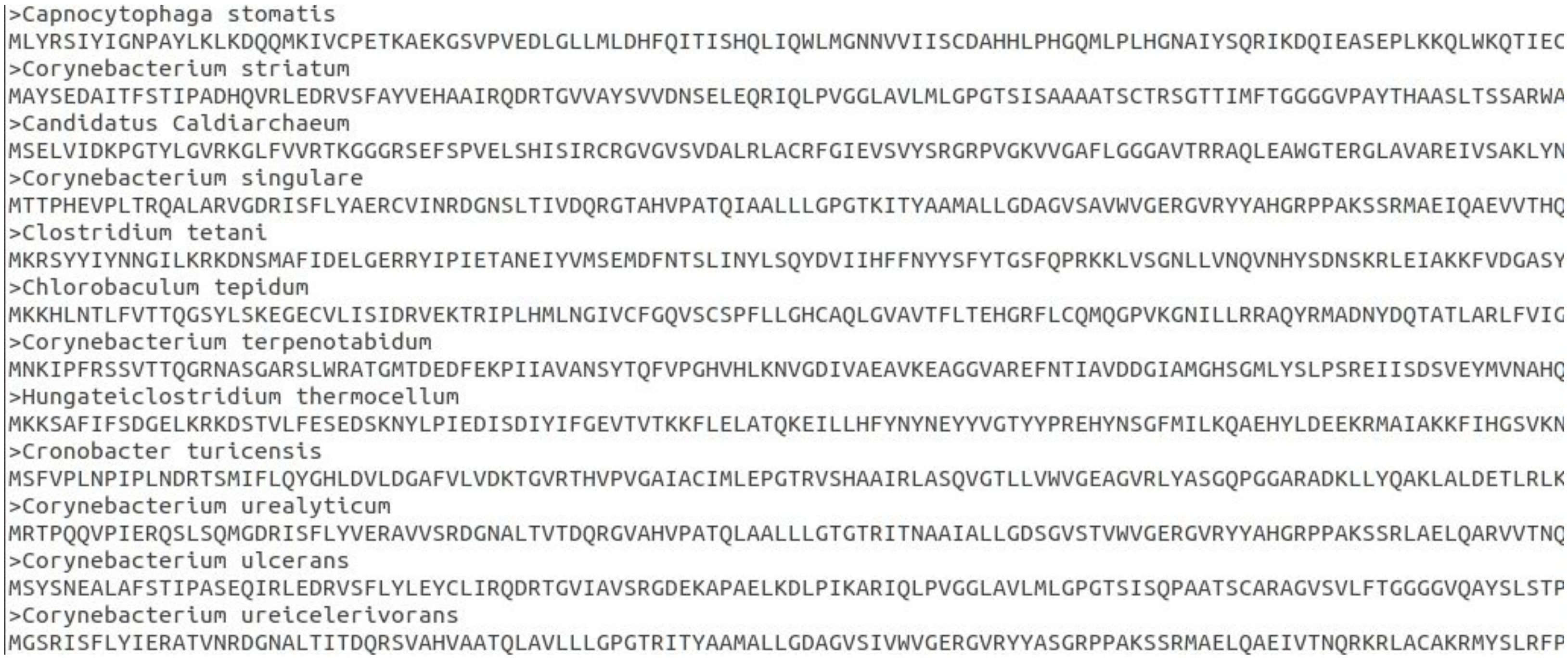
Cleaning data in R

- Raw data
  - 1413 organisms
  - More than 4000 sequences

- Clean and filtered data
  - Each genera must contain at least 3 entries

Final dataset:

- 47 genera
- 320 entries



```
>Capnocytophaga stomatis
MLYRSIYIGNPAYLKLKDQQMKIVCPETKAEKGSVPVEDLGLLMLDHFQITISHQLIQWLMGNNVVIISCDAHHLPHGQMLPLHGNAIYSQRIKDQIEASEPLKKQLWKQTIEC
>Corynebacterium striatum
MAYSEDAITFSTIPADHQVRLEDRVSFAYVEHAAIRQDRTGVVAYSVVDNSELEQRIQLPVGGLAVLMLGPGTSISAAAATSCTRSGTTIMFTGGGGVPAYTHAASLTSSARWA
>Candidatus Caldiarchaeum
MSELVIDKPGTYLGVRKGLFVVRTKGGGRSEFSPVELSHISIRCRGVGVSVDALRLACRFGIEVSVYSRGRPVGKVVGAFLGGGAVTRRAQLEAWGTERGLAVAREIVSAKLYN
>Corynebacterium singulare
MTTPHEVPLTRQALARVGDRISFLYAERCVINRDGNSLTIVDQRGTAHVPATQIAALLLGPGTKITYAAMALLGDAGVSAVWVGERGVRYYAHGRPPAKSSRMAEIQAEVVTHQ
>Clostridium tetani
MKRSYYIYNNGILKRKDNSMAFIDELGERRYIPIETANEIYVMSEMDFNTSLINYLSQYDVIIHFFNYYSFYTGSFQPRKKLVSGNLLVNQVNHYSDNSKRLEIAKKFVDGASY
>Chlorobaculum tepidum
MKKHLNTLFVTTQGSYLSKEGECVLISIDRVEKTRIPLHMLNGIVCFGQVSCSPFLLGHCAQLGVAVTFLTEHGRFLCQMQGPVKGNILLRRAQYRMADNYDQTATLARLFVIG
>Corynebacterium terpenotabidum
MNKIPFRSSVTTQGRNASGARSLWRATGMTDEDFEKPIIAVANSYTQFVPGHVHLKNVGDIVAEAVKEAGGVAREFNTIAVDDGIAMGHSGMLYSLPSREIISDSVEYMVNAHQ
>Hungateiclostridium thermocellum
MKKSAFIFSDGELKRKDSTVLFESEDSKNYLPIEDISDIYIFGEVTVTKKFLELATQKEILLHFYNYNEYYVGTYYPREHYNSGFMILKQAEHYLDEEKRMAIAKKFIHGSVKN
>Cronobacter turicensis
MSFVPLNPIPLNDRTSMIFLQYGHLDVLDGAFVLVDKTGVRTHVPVGAIACIMLEPGTRVSHAAIRLASQVGTLLVWVGEAGVRLYASGQPGGARADKLLYQAKLALDETLRLK
>Corynebacterium urealyticum
MRTPQQVPIERQSLSQMGDRISFLYVERAVVSRDGNALTVTDQRGVAHVPATQLAALLLGTGTRITNAAIALLGDSGVSTVWVGERGVRYYAHGRPPAKSSRLAELQARVVTNQ
>Corynebacterium ulcerans
MSYSNEALAFSTIPASEQIRLEDRVSFLYLEYCLIRQDRTGVIAVSRGDEKAPAELKDLPIKARIQLPVGGLAVLMLGPGTSISQPAATSCARAGVSVLFTGGGGVQAYSLSTP
>Corynebacterium ureicelerivorans
MGSRISFLYIERATVNRDGNALTITDQRSVAHVAATQLAVLLLGPGTRITYAAMALLGDAGVSIVWVGERGVRYYASGRPPAKSSRMAELQAEIVTNQRKRLACAKRMYSLRFP
```

Method

Step 1: All sequences aligned using Muscle multiple sequence alignment

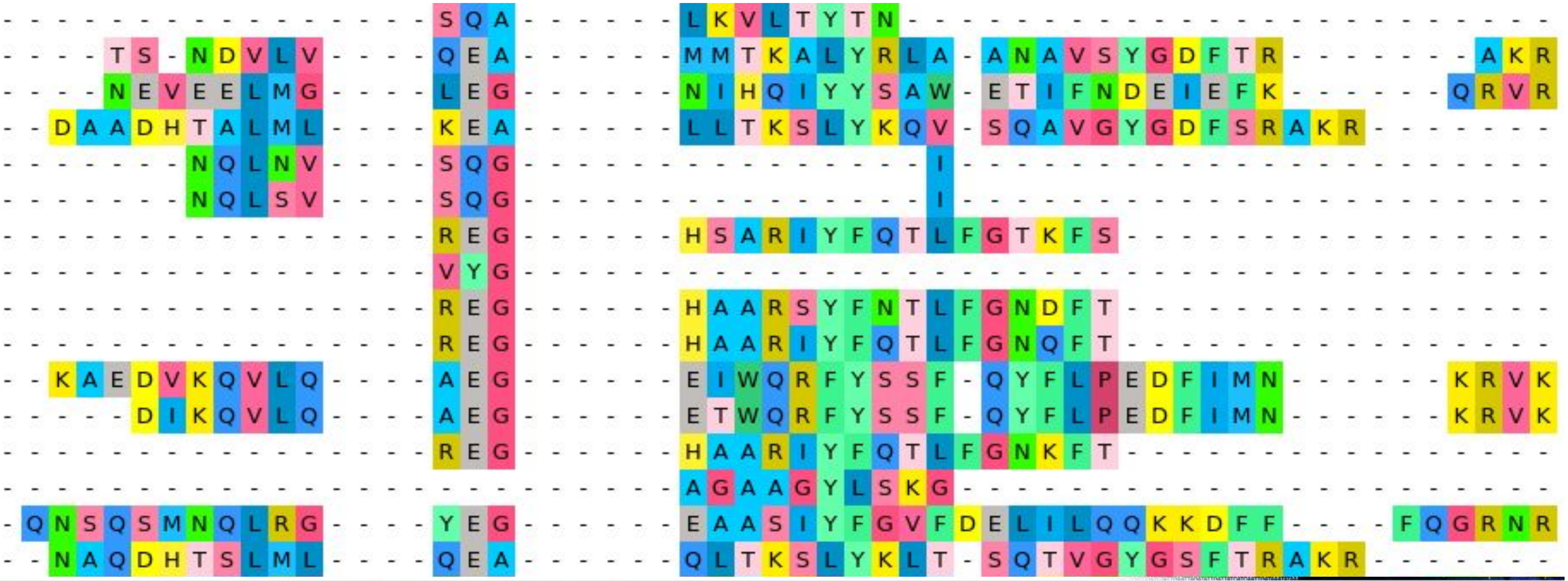
Step 2: Model selection for aligned data using IQ-tree

Step 3: Bootstrapping for 1000 trees in IQ-tree

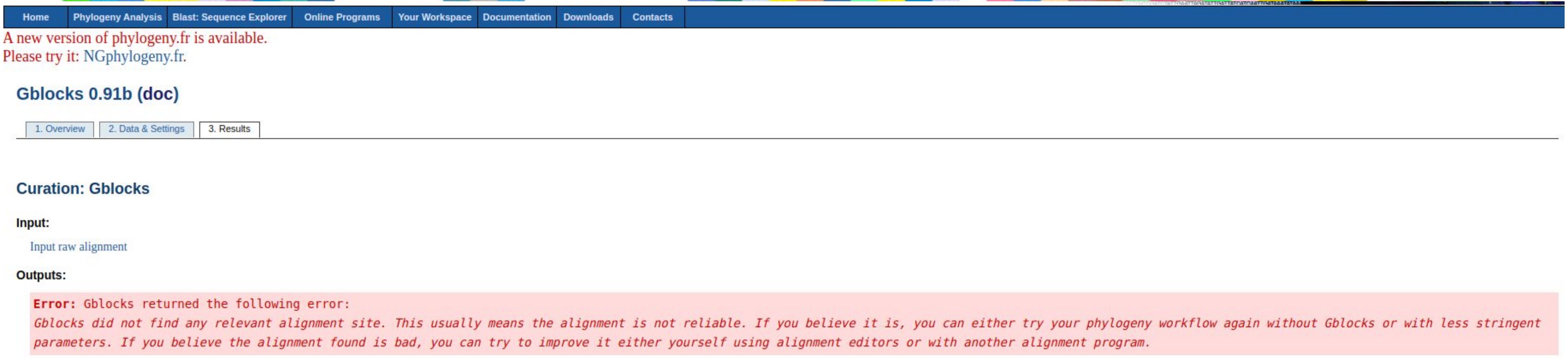
Step 4: Root the tree and collapse clades with bootstrap support < 95%

Step 5: Analysing the results

Results-Alignment and GBLOCKS



```
- - - - - - - - - - - - - - - - S Q A - - - - - - L K V L T Y T N - - - - - - - - - - - - - - - - - - - - - - - -
- - - - T S - N D V L V - - - - Q E A - - - - - - M M T K A L Y R L A - A N A V S Y G D F T R - - - - - - - A K R
- - - - N E V E E L M G - - - - L E G - - - - - - N I H Q I Y Y S A W - E T I F N D E I E F K - - - - - - Q R V R
- - D A A D H T A L M L - - - - K E A - - - - - - L L T K S L Y K Q V - S Q A V G Y G D F S R A K R - - - - - - -
- - - - - - - N Q L N V - - - - S Q G - - - - - - - - - - - - - - - I - - - - - - - - - - - - - - - - - - - - - -
- - - - - - - N Q L S V - - - - S Q G - - - - - - - - - - - - - - - I - - - - - - - - - - - - - - - - - - - - - -
- - - - - - - - - - - - - - - - R E G - - - - - - H S A R I Y F Q T L F G T K F S - - - - - - - - - - - - - - - -
- - - - - - - - - - - - - - - - V Y G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
- - - - - - - - - - - - - - - - R E G - - - - - - H A A R S Y F N T L F G N D F T - - - - - - - - - - - - - - - -
- - - - - - - - - - - - - - - - R E G - - - - - - H A A R I Y F Q T L F G N Q F T - - - - - - - - - - - - - - - -
- - K A E D V K Q V L Q - - - - A E G - - - - - - E I W Q R F Y S S F - Q Y F L P E D F I M N - - - - - - K R V K
- - - - - D I K Q V L Q - - - - A E G - - - - - - E T W Q R F Y S S F - Q Y F L P E D F I M N - - - - - - K R V K
- - - - - - - - - - - - - - - - R E G - - - - - - H A A R I Y F Q T L F G N K F T - - - - - - - - - - - - - - - -
- - - - - - - - - - - - - - - - - - - - - - - - - A G A A G Y L S K G - - - - - - - - - - - - - - - - - - - - - -
- Q N S Q S M N Q L R G - - - - Y E G - - - - - - E A A S I Y F G V F D E L I L Q Q K K D F F - - - - F Q G R N R
- - N A Q D H T S L M L - - - - Q E A - - - - - - Q L T K S L Y K L T - S Q T V G Y G S F T R A K R - - - - - - -
```



A new version of phylogeny.fr is available.
Please try it: NGphylogeny.fr.

Gblocks 0.91b (doc)

Curation: Gblocks

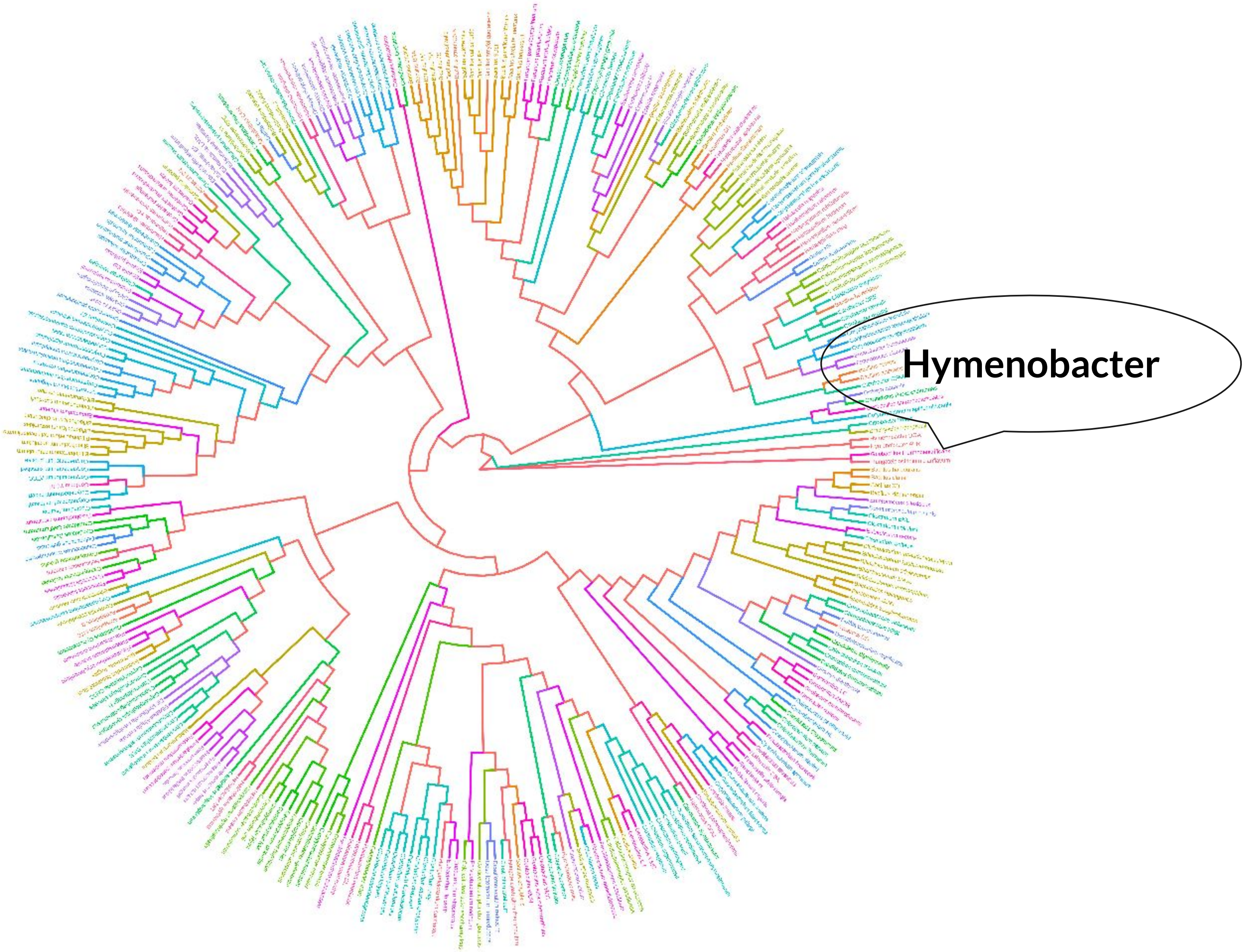
Input:

Input raw alignment

Outputs:

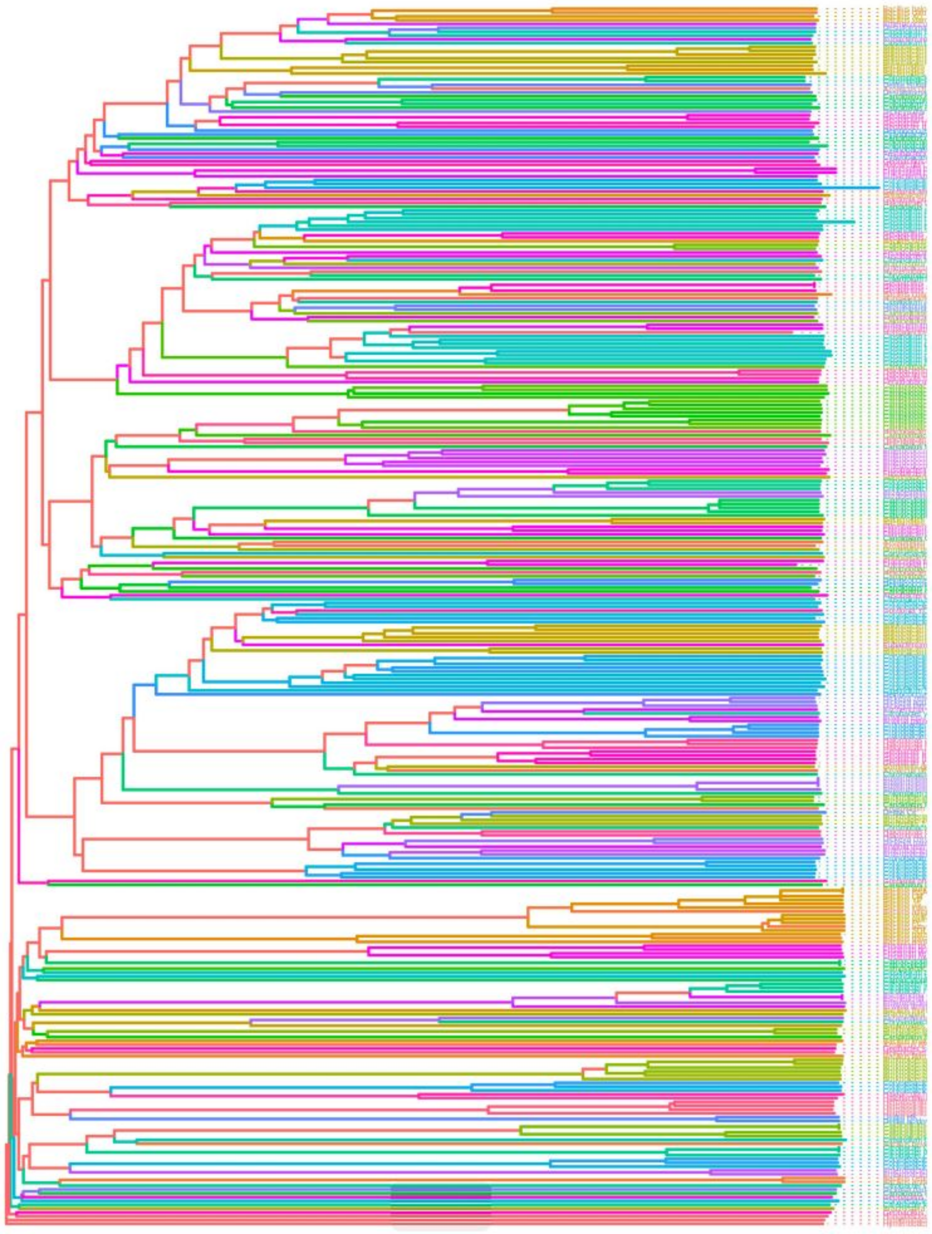
```
Error: Gblocks returned the following error:
Gblocks did not find any relevant alignment site. This usually means the alignment is not reliable. If you believe it is, you can either try your phylogeny workflow again without Gblocks or with less stringent parameters. If you believe the alignment found is bad, you can try to improve it either yourself using alignment editors or with another alignment program.
```

Results-NJ tree construction

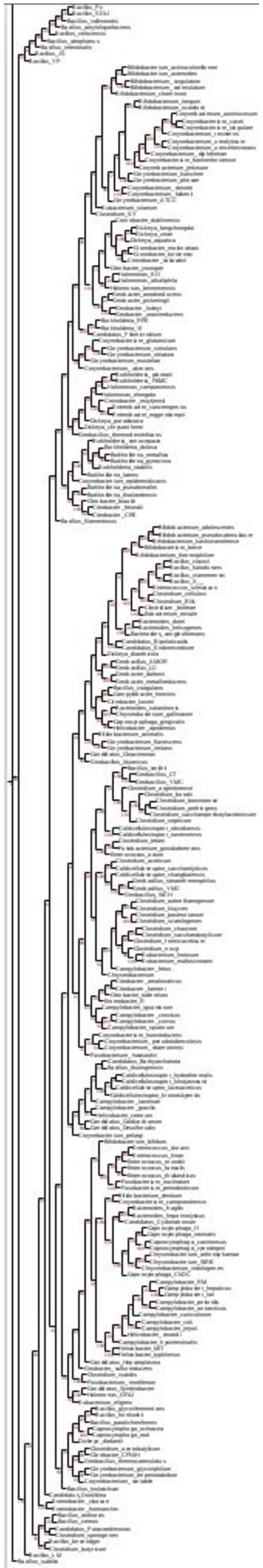


Hymenobacter

Results- Rooted NJ tree



Root tree using Hymenobacter



Results-Unrooted IQtree

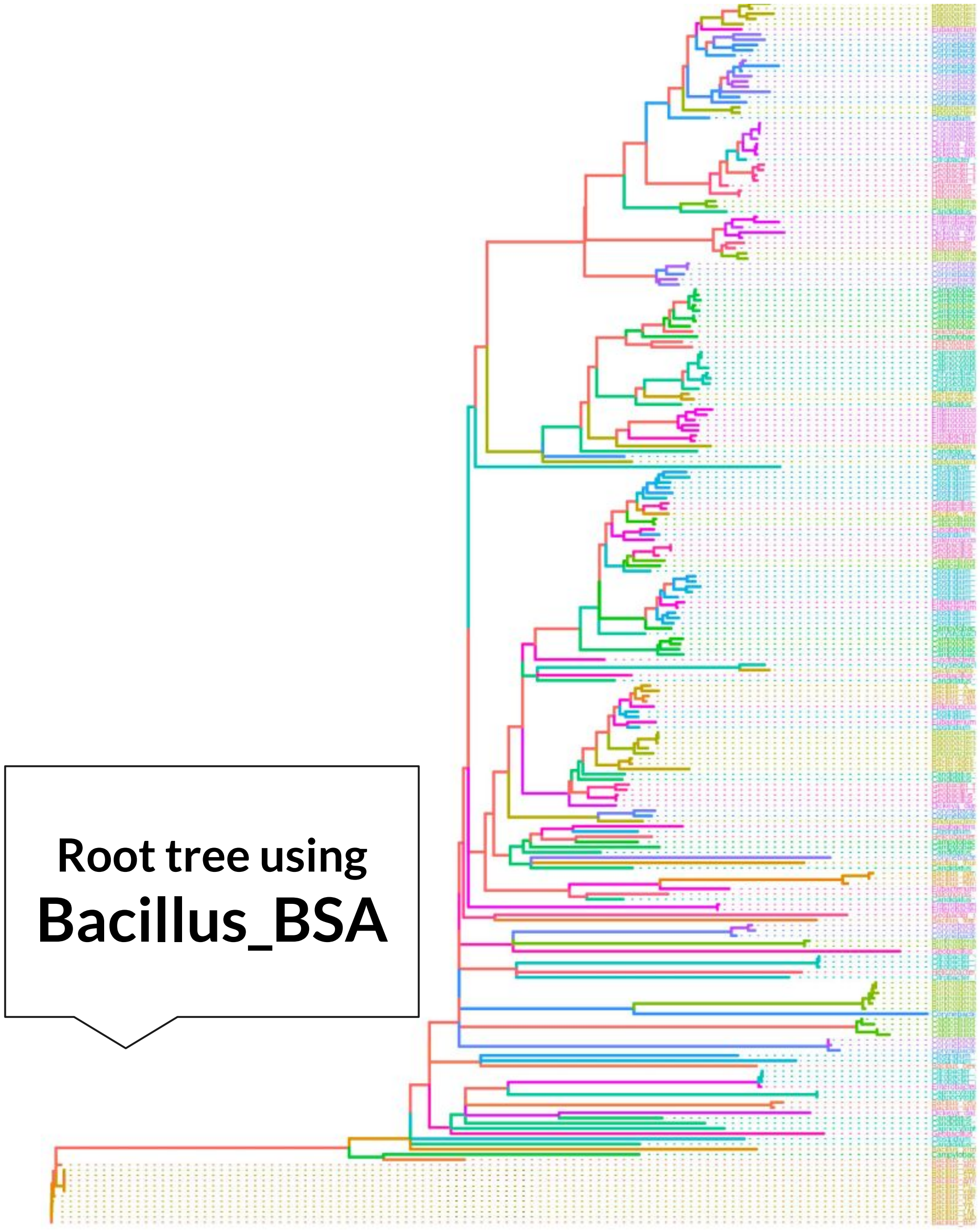
Bacillus_BSA ← **Outgroup**

Best-fit model:
VT+F+G4

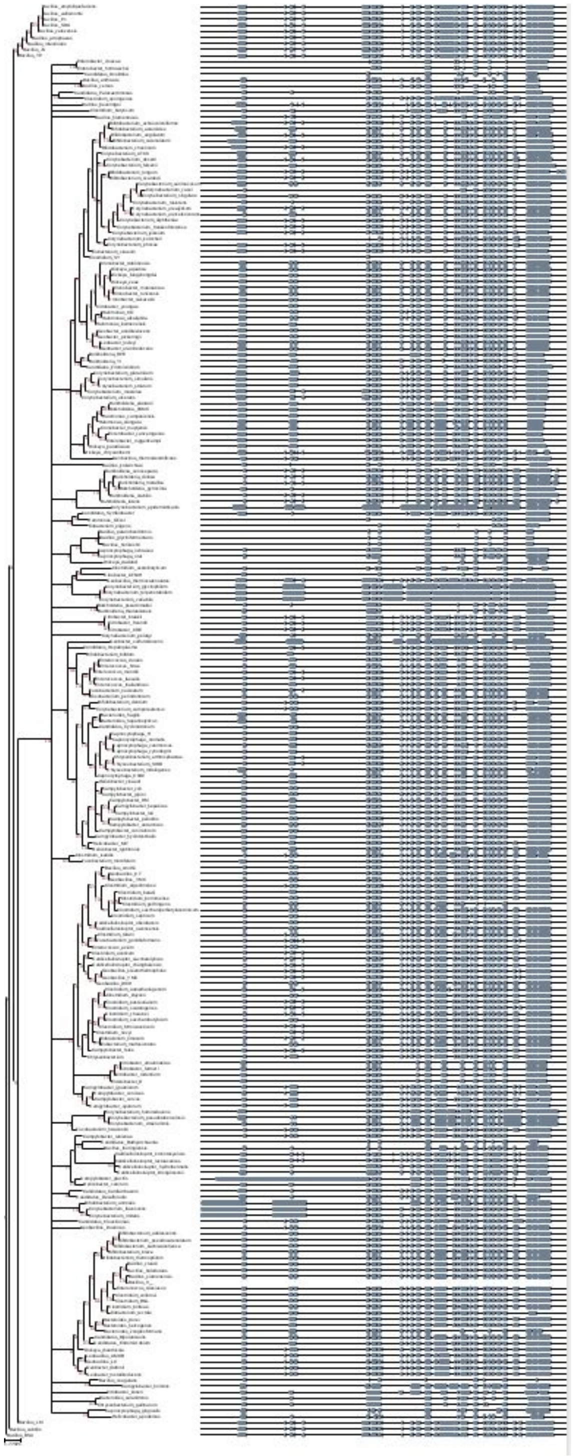
I got clear ml tree from
Phylogenetic tree (newick) viewer

http://etetoolkit.org/treeview/?treeid=ea0100092b9ba8d910322a09a4b64190&algid=f39106a4236c18b06913018514a73427

Results-Rooted IQtree

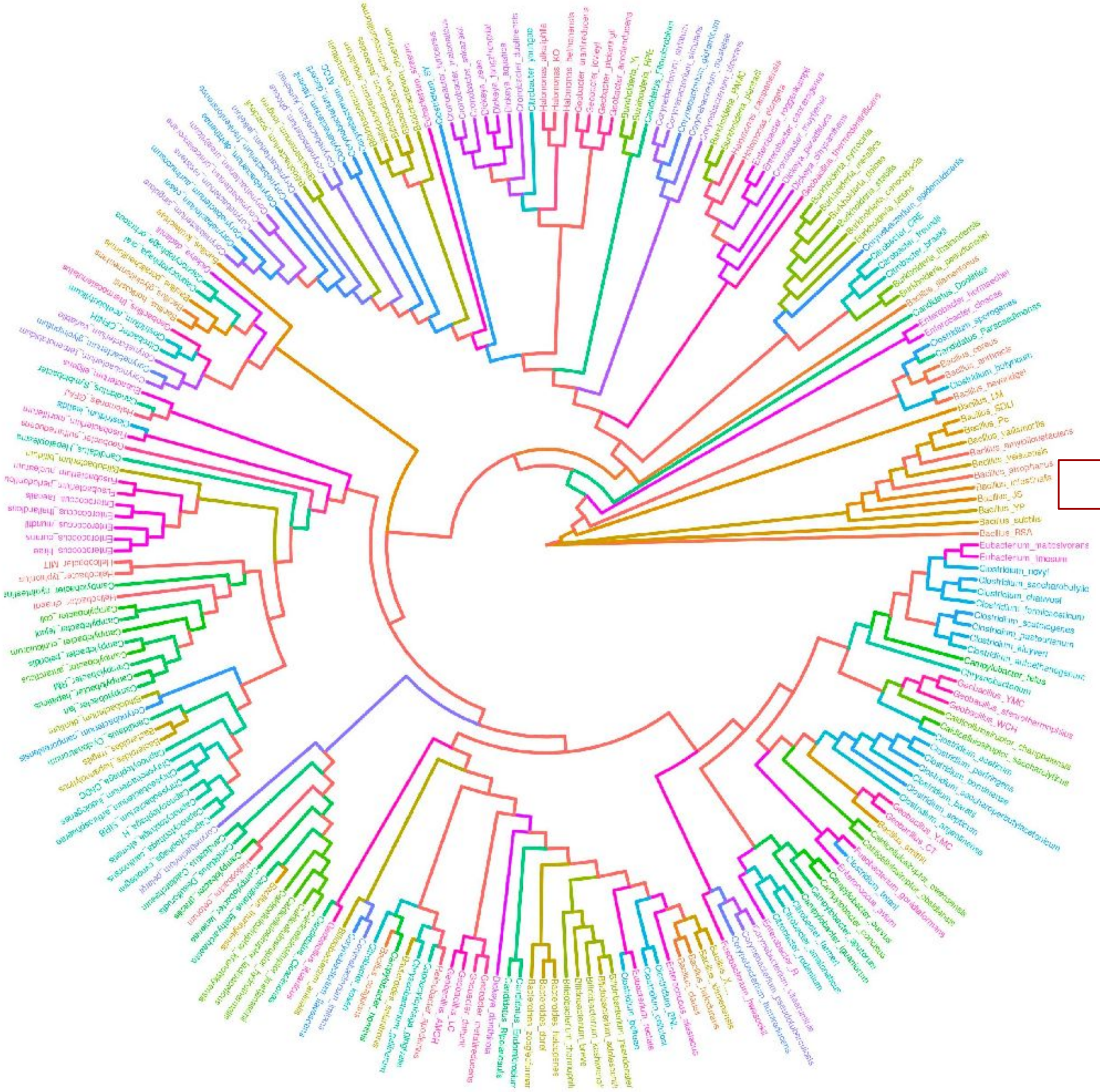


Root tree using
Bacillus_BSA



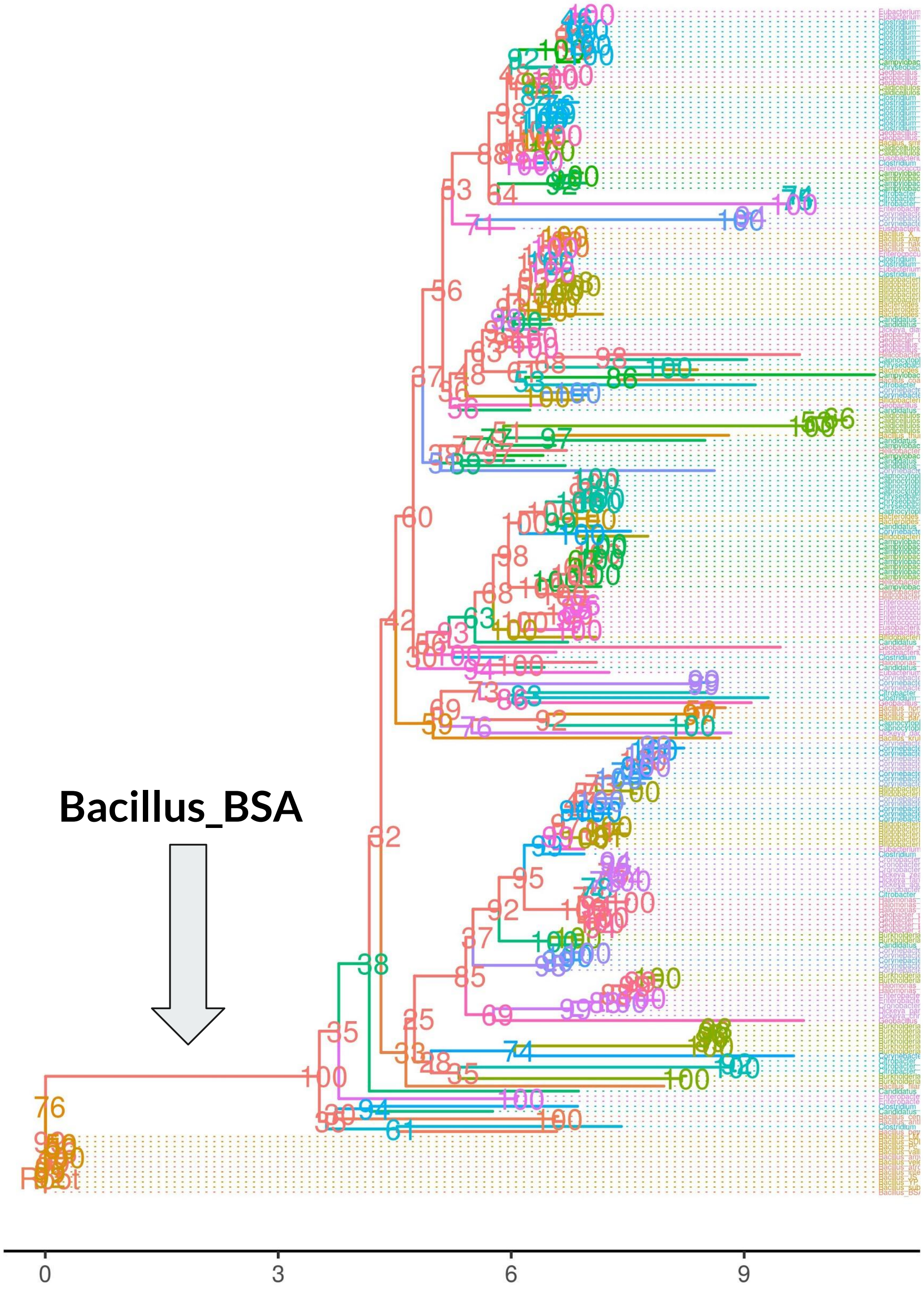
Rooted tree
with alignment

Results- IQtree with bootstrapping



Bacillus_BSA

Results- IQtree with bootstrapping

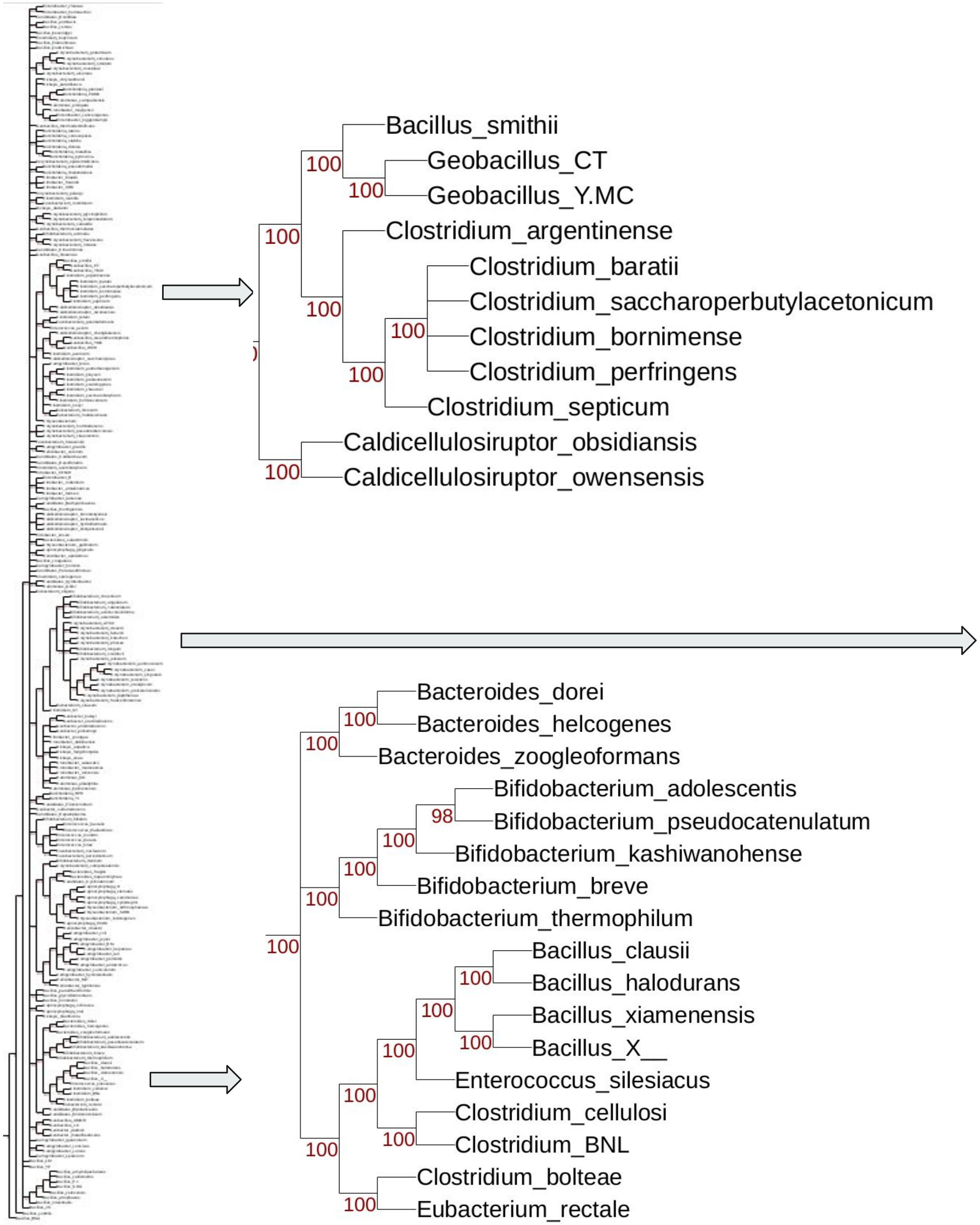


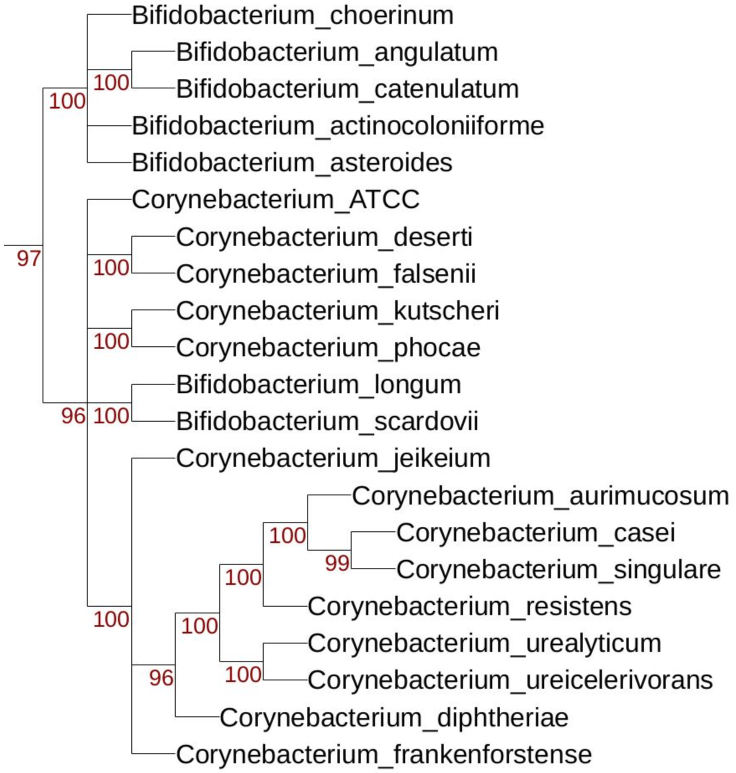
Bacillus_BSA

- Tree is huge and I have to compress it as much as possible!
- All clear photos are available on Github

After collapsing (Final tree)

- “collapse” clades with bootstrap support < 95%





Bifidobacterium_choerinum
Bifidobacterium_angulatum
Bifidobacterium_catenulatum
Bifidobacterium_actinocoloniiforme
Bifidobacterium_asteroides
Corynebacterium_ATCC
Corynebacterium_deserti
Corynebacterium_falsenii
Corynebacterium_kutscheri
Corynebacterium_phocae
Bifidobacterium_longum
Bifidobacterium_scardovii
Corynebacterium_jeikeium
Corynebacterium_aurimucosum
Corynebacterium_casei
Corynebacterium_singulare
Corynebacterium_resistens
Corynebacterium_urealyticum
Corynebacterium_ureicelerivorans
Corynebacterium_diphtheriae
Corynebacterium_frankenforstense
100
100
97
100
100
96
100
100
100
99
100
100
100
96

Discussion

1.) After bootstrapping “Bacillus_BSA” confirmed as an outgroup!

2.) It can be seen that cas 1 gene have been found scattered in most of the species from Bacilli (Bifidum group) and Clostridia (clostridium group) class!

   a.) both are gram positive species where bacillus are aerobic and clostridium are anaerobi

   b.) Bacillus mycoides is source organism of cas1

3.) most of the organisms evolved according to viral strains

4.) Also significant percentage of species from Actinobacteria class grouped with species from Bacilli and Clostridia class!

Source

All codes will be available on GitHub:

https://github.com/Sedreh/phylogenetics_semester3

THANK
YOU