

In [6]:

```
import os
os.chdir('/home/sedreh/ITMO/semester3/Molecular_phylogenetic/homework_5/homework_5/iqtree_output')
```

### **1. Build an ML tree (500 bootstrap replicates) for the whole sequence using RAxML or IQ-Tree with GTRCAT model.**

I will try both RAxML and IQ-Tree to build the trees. Also in homeworks i used different ways of drawing tree to learn!

RAxML (Randomized Axelerated Maximum Likelihood) is a program for sequential and parallel Maximum Likelihood based inference of large phylogenetic trees. It can also be used for postanalyses of sets of phylogenetic trees, analyses of alignments and, evolutionary placement of short reads

#### **RAxML**

## **finalcommand for RAxML**

```
raxmlHPC -m GTRCAT -b 12345 -# 500 -p 12345 -s
/home/sedreh/ITMO/semester3/Molecular_phylogenetic/homework_5/data/SUP35_aln.best.fas -n
raxmltree_500
```

## **finalcommand for RAxML with partition**

```
raxmlHPC -m GTRCAT -b 12345 -# 500 -p 12345 -s
/home/sedreh/ITMO/semester3/Molecular_phylogenetic/homework_5/data/SUP35_aln.best.fas -q
/home/sedreh/ITMO/semester3/Molecular_phylogenetic/homework_5/data/partitions_for_raxml.txt -n
raxmltree_partition
```

#### **IQ-Tree**

```
iqtree -s /home/sedreh/ITMO/semester3/Molecular_phylogenetic/homework_5/data/SUP35_aln.best.fas -m
GTR -bb 1000
```

```
iqtree -s /home/sedreh/ITMO/semester3/Molecular_phylogenetic/homework_5/data/SUP35_aln.best.fas -spp
/home/sedreh/ITMO/semester3/Molecular_phylogenetic/homework_5/data/partitions_for_raxml.txt -m GTR -
bb 1000
```

#### **Also web version of IQtree**

<http://iqtree.cibiv.univie.ac.at/> (<http://iqtree.cibiv.univie.ac.at/>)

***what is the alignment file?***

In [1]:

```

from Bio import AlignIO
aln = AlignIO.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/homework_
5/data/SUP35_aln.best.fas', 'fasta')
print (aln)

```

SingleLetterAlphabet() alignment with 10 rows and 2460 columns

```

ATGTCA-----GACCAA---.AAT SUP35_Kla_AB03974
9
ATGTCGGAGGAAGATCAAATTCAATCGCAAGGCAACGACCAAGG..... SUP35_Agos_ATCC_1
0895_NM_211584
ATGTCG-----GAT----- SUP35_Scer_74-D69
4_GCA_001578265.1
ATGTCG-----GAT----- SUP35_Sbou_unique
28_CM003560
ATGTCG-----GAT----- SUP35_Scer_beer07
8_CM005938
ATGTCG-----GAT----- SUP35_Spar_A12_Li
ti
ATGTCT-----GAT----- SUP35_Smik_IF0181
5T_30
ATGTCT-----GAT----- SUP35_Sarb_H-6_ch
rXIII_CM001575
ATGTCA-----GAT----- SUP35_Skud_IF0180
2T_36
ATGTCT-----GAT----- SUP35_Seub_CBS123
57_chr_II_IV_DF968535

```

In [3]:

```
from Bio import Phylo
iQtree_bootstrap = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/homework_5/homework_5/iqtree_output/bootstrap/SUP35_aln.best.fas.contree', 'newick')
print(iQtree_bootstrap)
```

```
Tree(rooted=False, weight=1.0)
  Clade()
    Clade(branch_length=0.1516534253, name='SUP35_Kla_AB039749')
    Clade(branch_length=0.1842978575, name='SUP35_Agos_ATCC_10895_NM_211584')
      Clade(branch_length=0.1640239505, confidence=100)
        Clade(branch_length=0.0204648048, confidence=90)
          Clade(branch_length=0.0318391609, confidence=100)
            Clade(branch_length=0.043144188, confidence=100)
              Clade(branch_length=2.9866e-06, confidence=59)
                Clade(branch_length=0.0004872062, name='SUP35_Scer_74-D694_GCA_001578265.1')
                Clade(branch_length=0.0004860338, name='SUP35_Sbou_unique28_CM003560')
                  Clade(branch_length=0.0020071022, name='SUP35_Scer_beer078_CM005938')
                  Clade(branch_length=0.0287843445, name='SUP35_Spar_A12_Liti')
                  Clade(branch_length=0.0620711868, name='SUP35_Smik_IF01815T_30')
                  Clade(branch_length=0.0134741159, confidence=45)
                    Clade(branch_length=0.0181625478, confidence=55)
                      Clade(branch_length=0.0668424392, name='SUP35_Sarb_H-6_chrXIII_CM001575')
                      Clade(branch_length=0.0800730359, name='SUP35_Sub_CBS12357_chr_II_IV_DF968535')
                      Clade(branch_length=0.0788922395, name='SUP35_Skud_IF01802T_36')
```

In [4]:

```
#Root the tree at SUP35_Kla_AB039749 as an outgroup
iQtree_bootstrap.root_with_outgroup({'name': 'SUP35_Kla_AB039749'})
Phylo.write(iQtree_bootstrap, 'rooted_iQtree_bootstrap.newick', 'newick')
```

Out[4]:

1

In [5]:

```
#read rooted tree
rooted_iQtree_bootstrap = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/homework_5/homework_5/iqtree_output/bootstrap/rooted_iQtree_bootstrap.newick', 'newick')
```

In [6]:

```
print("Total branch length %0.2f" % rooted_iQtree_bootstrap.total_branch_length())
```

Total branch length 0.95

*I draw the tree using ete toolkit and attached in iqtree results folder as a pdf*

**Collapse all the descendents of this tree, leaving only terminals. this will safely collapse nodes with poor bootstrap support**

In [9]:

```
# "collapse" clades with bootstrap support < 70%.
rooted_iQtree_bootstrap.collapse_all(lambda c: c.confidence is not None and c.confidence < 70)
print("Total branch length %0.2f" % rooted_iQtree_bootstrap.total_branch_length())
```

```
#I wrote collapsed rooted tree in new file and draw it using ete3.
Phylo.write(rooted_iQtree_bootstrap, 'collapsed_rooted_iQtree.nwk', 'newick')
```

Total branch length 0.99

Out[9]:

1

In [10]:

```
#reading tree with partition file (is done with iqtree web server)
iQtree_partition = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogeneti
c/homework_5/homework_5/iqtree_output/partition/SUP35_aln.best.fas.contree', 'ne
wick')
print (iQtree_partition)
```

```
Tree(rooted=False, weight=1.0)
  Clade()
    Clade(branch_length=0.2465794068, name='SUP35_Kla_AB039749')
    Clade(branch_length=0.3123320332, name='SUP35_Agos_ATCC_1089
5_NM_211584')
      Clade(branch_length=0.3043830659, confidence=100)
        Clade(branch_length=0.0196157323, confidence=50)
          Clade(branch_length=0.0182767691, confidence=56)
            Clade(branch_length=0.0341467522, confidence=97)
              Clade(branch_length=0.0355792096, confidence
=100)
            Clade(branch_length=0.047585444, confide
nce=100)
          Clade(branch_length=2.9947e-06, conf
idence=53)
        Clade(branch_length=0.000512076,
name='SUP35_Scer_74-D694_GCA_001578265.1')
          Clade(branch_length=0.000511083
5, name='SUP35_Sbou_unique28_CM003560')
            Clade(branch_length=0.0021310603, na
me='SUP35_Scer_beer078_CM005938')
              Clade(branch_length=0.0343559735, name
='SUP35_Spar_A12_Liti')
                Clade(branch_length=0.0773001405, name='SUP3
5_Smik_IF01815T_30')
                  Clade(branch_length=0.0995792047, name='SUP35_Sk
ud_IF01802T_36')
                    Clade(branch_length=0.0785497518, name='SUP35_Sarb_H
-6_chrXIII_CM001575')
                      Clade(branch_length=0.084094937, name='SUP35_Seub_CBS123
57_chr_II_IV_DF968535')
```

In [11]:

```
#Root the tree at SUP35_Kla_AB039749 as an outgroup
iQtree_partition.root_with_outgroup({'name': 'SUP35_Kla_AB039749'})
Phylo.write(iQtree_partition, 'rooted_iQtree_partition.newick', 'newick')
```

Out[11]:

1

In [19]:

```
#reading rooted tree
rooted_iQtree_partition = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/homework_5/homework_5/iqtree_output/partition/rooted_iQtree_partition.newick', 'newick')
print (rooted_iQtree_partition)
```

```
Tree(rooted=False, weight=1.0)
  Clade(branch_length=0.0)
    Clade(branch_length=0.24658)
      Clade(branch_length=0.31233, name='SUP35_Agos_ATCC_10895_NM_211584')
        Clade(branch_length=0.30438, confidence=100.0)
          Clade(branch_length=0.01962, confidence=50.0)
            Clade(branch_length=0.01828, confidence=56.0)
              Clade(branch_length=0.03415, confidence=97.0)
            0)
              Clade(branch_length=0.03558, confidence=100.0)
                Clade(branch_length=0.04759, confidence=100.0)
                  Clade(branch_length=0.0, confidence=53.0)
                    Clade(branch_length=0.00051, name='SUP35_Scer_74-D694_GCA_001578265.1')
                      Clade(branch_length=0.00051, name='SUP35_Sbou_unique28_CM003560')
                        Clade(branch_length=0.00213, name='SUP35_Scer_beer078_CM005938')
                          Clade(branch_length=0.03436, name='SUP35_Spar_A12_Liti')
                            Clade(branch_length=0.0773, name='SUP35_Smik_IF01815T_30')
                              Clade(branch_length=0.09958, name='SUP35_Skud_IF01802T_36')
                                Clade(branch_length=0.07855, name='SUP35_Sarb_H-6_chrXIII_CM001575')
                                  Clade(branch_length=0.08409, name='SUP35_Seub_CBS12357_chr_II_IV_DF968535')
                                    Clade(branch_length=0.0, name='SUP35_Kla_AB039749')
```

In [20]:

```
print("Total branch length %0.2f" % rooted_iQtree_partition.total_branch_length())
```

Total branch length 1.40

In [21]:

```
#cutt_off <70

rooted_iQtree_partition.collapse_all(lambda c: c.confidence is not None and c.confidence < 70)
print("Total branch length %0.2f" % rooted_iQtree_partition.total_branch_length())
Phylo.write(rooted_iQtree_partition, 'collapsed_rooted_iQtree_partition.nwk', 'newick')
```

Total branch length 1.45

Out[21]:

1

**Based on the trees constructed, describe the relationship between the taxa *S. cerevisiae*, *S. boulardii*, and *S. paradoxus* .**

In IQtree with partition and without partition:

As we know phylogenetic trees illustrate degrees of relationship among taxa. In the constructed trees, Taxon *S. cerevisiae* and Taxon *S. boulardii* are more closely related to one another than either is to Taxon *S. paradoxus*! because they share a shallower node. It means they have more similarity with each other than Taxon *S. paradoxus*.

**As I read about using partitioning model, the choice of partitioning scheme will change the topology of the tree. length of branches and bootstrap support are also affected by the choice of partitioning scheme. But if all partitions have similar substitution model, the branch lengths may not be differed.**

about position of outgroup based on my trees, after collapsing it is the same.

**visualizing RAxML\_tree. for more practice i did this part in R and visualize tree using Densitree**

In [22]:

```
#Reading RAxML_tree.
from Bio import Phylo
RAXML_pure = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/home
work_5/homework_5/RAXML/RAxML_bestTree.raxmltree_pure', 'newick')
print (RAXML_pure)
```

```
Tree(rooted=False, weight=1.0)
  Clade(branch_length=0.0)
    Clade(branch_length=0.3458628526845534, name='SUP35_Agos_ATC
C_10895_NM_211584')
      Clade(branch_length=0.33429047059705164)
        Clade(branch_length=0.007575626547297922)
          Clade(branch_length=0.031881927345970514)
            Clade(branch_length=0.07402835493614932, name='S
UP35_Smik_IF01815T_30')
              Clade(branch_length=0.0349570157575185)
                Clade(branch_length=0.04606946044381412)
                  Clade(branch_length=0.001955791814969358
6, name='SUP35_Scer_beer078_CM005938')
                    Clade(branch_length=1.00000050002909e-0
6)
                      Clade(branch_length=0.00047362586657
263514, name='SUP35_Scer_74-D694_GCA_001578265.1')
                        Clade(branch_length=0.00047331342081
471836, name='SUP35_Sbou_unique28_CM003560')
                          Clade(branch_length=0.03178901268921656, nam
e='SUP35_Spar_A12_Liti')
                            Clade(branch_length=0.09811363581516733, name='SUP35
_Skud_IF01802T_36')
                              Clade(branch_length=0.01050267129655633)
                                Clade(branch_length=0.09982132820352614, name='SUP35
_Seub_CBS12357_chr_II_IV_DF968535')
                                  Clade(branch_length=0.07643015587494675, name='SUP35
_Sarb_H-6_chrXIII_CM001575')
                                    Clade(branch_length=0.2636648350210716, name='SUP35_Kla_AB03
9749')
```

## MrBayse



In [71]:

# reading Mrbayse tree

```

from Bio import Phylo
MrBayse = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/homework_5/homework_5/MrBase/phylo_tree.tree', 'newick')
print (MrBayse)

```

```

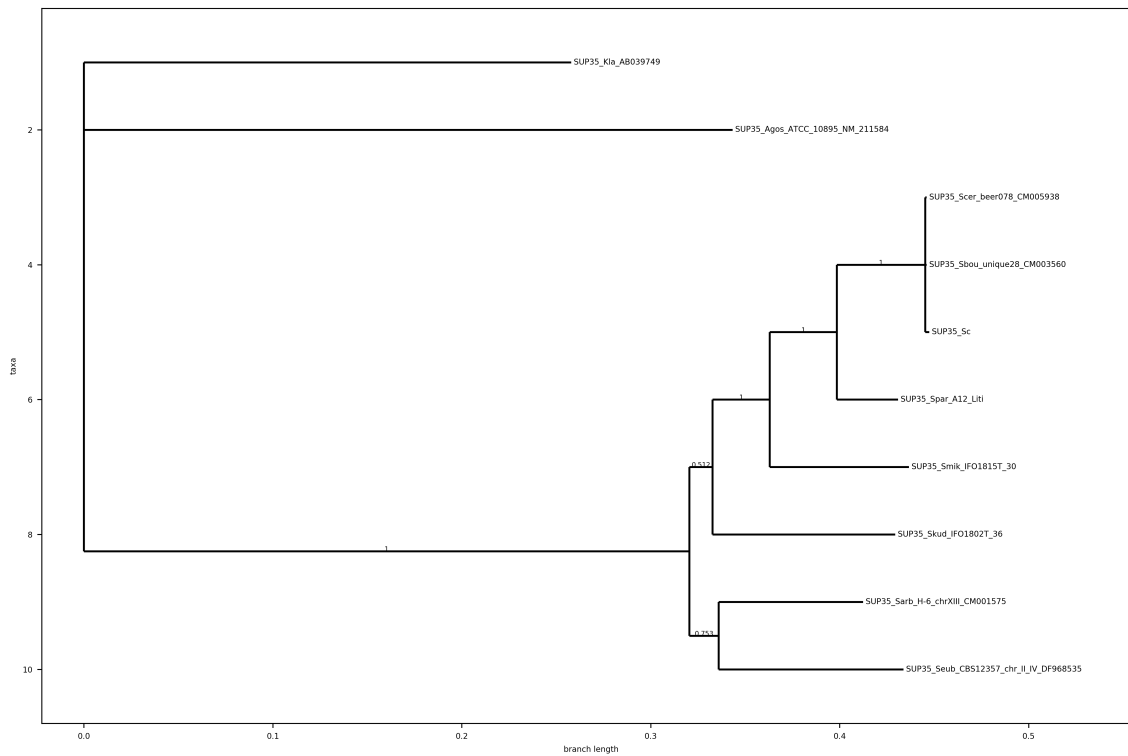
Tree(rooted=False, weight=1.0)
  Clade()
    Clade(branch_length=0.2578899, name='SUP35_Kla_AB039749')
    Clade(branch_length=0.3432715, name='SUP35_Agos_ATCC_10895_N
M_211584')
      Clade(branch_length=0.3203681, confidence=1.0)
        Clade(branch_length=0.0123637, confidence=0.512)
          Clade(branch_length=0.03026675, confidence=1.0)
            Clade(branch_length=0.03550824, confidence=1.0)
              Clade(branch_length=0.04669551, confidence=
1.0)
                Clade(branch_length=0.0007845279, name
='SUP35_Scer_beer078_CM005938')
                Clade(branch_length=0.0007929222, name
='SUP35_Sbou_unique28_CM003560')
                Clade(branch_length=0.002235554, name='S
UP35_Sc')
                Clade(branch_length=0.03238813, name='SUP35_
Spar_A12_Liti')
                Clade(branch_length=0.07363296, name='SUP35_Smik
_IF01815T_30')
                Clade(branch_length=0.09660268, name='SUP35_Skud_IF0
1802T_36')
                Clade(branch_length=0.01550912, confidence=0.753)
                Clade(branch_length=0.07650165, name='SUP35_Sarb_H-6
_chrXIII_CM001575')
                Clade(branch_length=0.09787922, name='SUP35_Seub_CBS
12357_chr_II_IV_DF968535')

```

In [34]:

```
import matplotlib
import matplotlib.pyplot as plt

matplotlib.rc('font', size=6) # set the size of the figure
fig = plt.figure(figsize=(15, 10), dpi=400)
axes = fig.add_subplot(1, 1, 1)
Phylo.draw(MrBayse, axes=axes)
```



In [35]:

```
#Root the MrBayse tree at SUP35_Kla_AB039749

MrBayse.root_with_outgroup({'name': 'SUP35_Kla_AB039749'})

Phylo.write(MrBayse, 'rooted_MrBayse.newick', 'newick')
```

Out[35]:

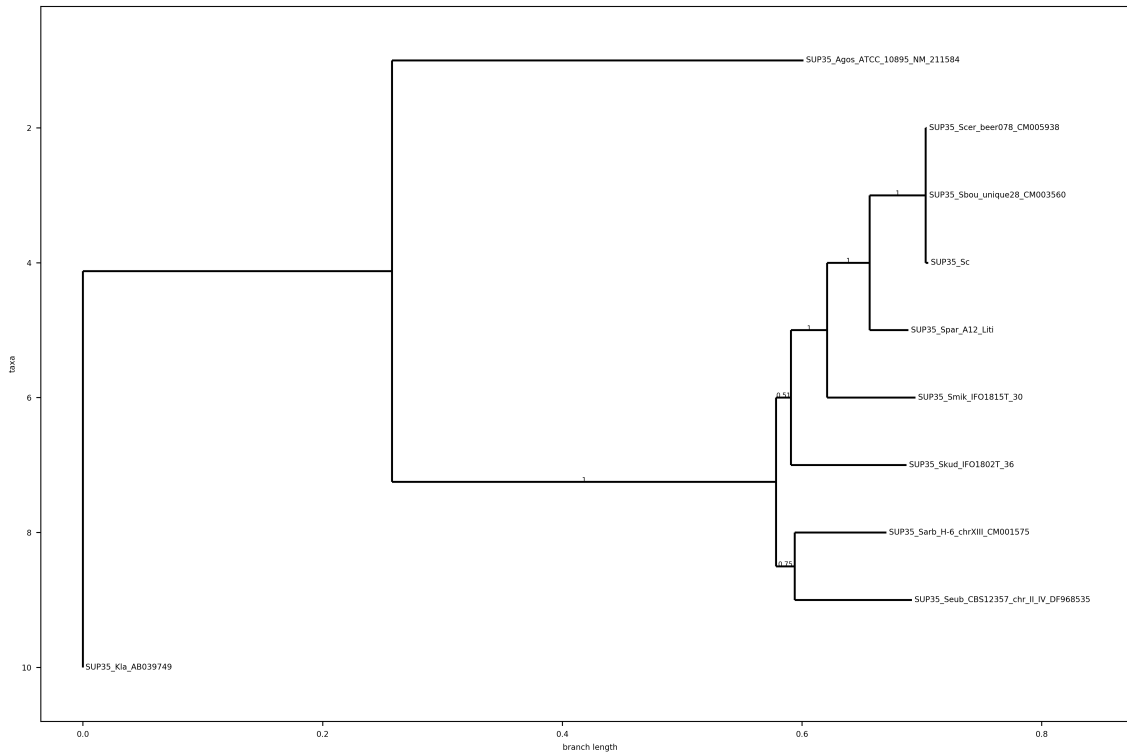
1

In [69]:

```
#reading rooted tree
rooted_MrBayse = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/
homework_5/homework_5/MrBase/rooted_MrBayse.newick', 'newick')
```

In [70]:

```
matplotlib.rc('font', size=6) # set the size of the figure
fig = plt.figure(figsize=(15, 10), dpi=400)
axes = fig.add_subplot(1, 1, 1)
Phylo.draw(rooted_MrBayse, axes=axes)
```



In [40]:

```
print("Total branch length %0.2f" % rooted_MrBayse.total_branch_length())
```

Total branch length 1.44

In [67]:

```
rooted_MrBayse.collapse_all(lambda c: c.confidence is not None and c.confidence
< 0.7)
print("Total branch length %0.2f" % rooted_MrBayse.total_branch_length())
Phylo.write(rooted_MrBayse, 'collapsed_rooted_MrBayse.nwk', 'newick')
```

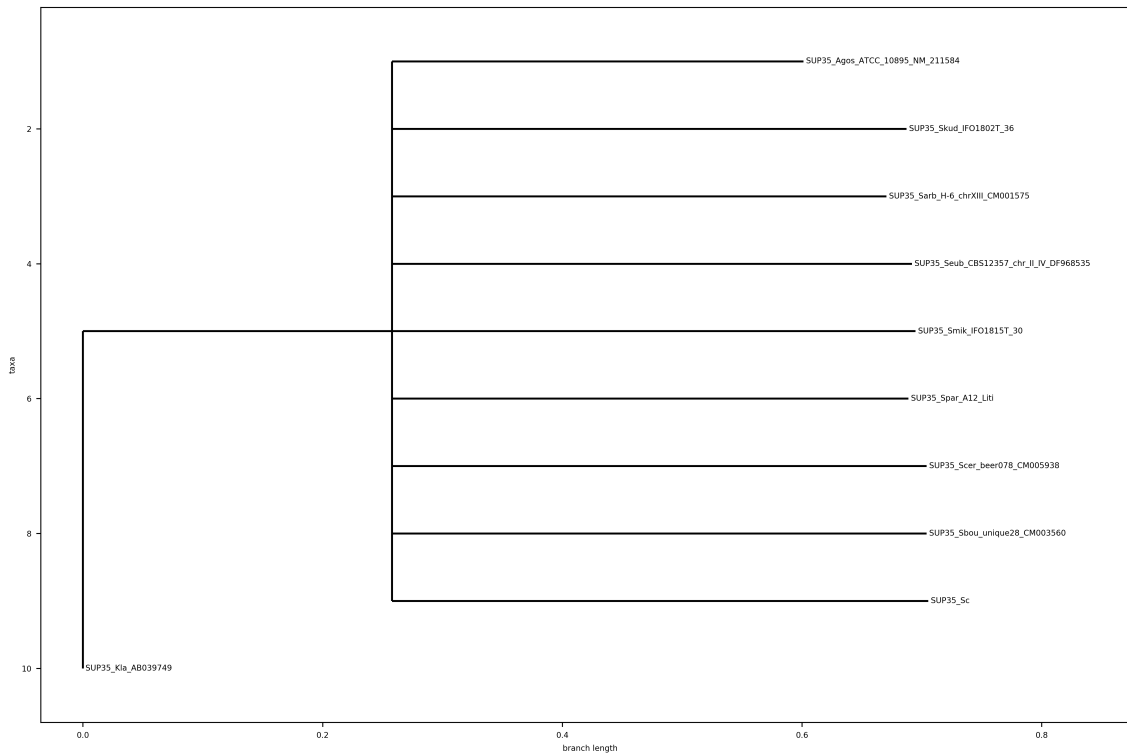
Total branch length 4.08

Out[67]:

1

In [68]:

```
collapsed_MrBayse = Phylo.read('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/homework_5/homework_5/MrBase/collapsed_rooted_MrBayse.nwk', 'newick')
matplotlib.rc('font', size=6) # set the size of the figure
fig = plt.figure(figsize=(15, 10), dpi=400)
axes = fig.add_subplot(1, 1, 1)
Phylo.draw(collapsed_MrBayse, axes=axes)
```



**The tree topology after collapsing is weird! I am collapsing less than 70 but it collapsed even branch with 75% bootstrap value! I can not understand why! for last question I should change the value of collapsing like less than 72% or 73% and so on and see in what value my tree will be the same! I understand the concept but do not know where I made mistake!**

**Is the chain converged (standard deviation of split frequencies <0.01)? If not, how many generations did it take for the chain to reach the convergence?**

**for part 2 of home work I have used MrBayse online version. Standard deviation till 10 generation was between 0.129448- 0.017060 but in generation 10 based on log pdf file that attached was 0.006784!**

***Summary statistics for partitions with frequency  $\geq 0.10$  in at least one run:***

Average standard deviation of split frequencies = 0.005098

Maximum standard deviation of split frequencies = 0.016026

Average PSRF for parameter values (excluding NA and  $>10.0$ ) = 1.001

Maximum PSRF for parameter values = 1.002

In [ ]: