Article

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Contents

1 Phylogenetics Evaluation

For each of the three matrices (16S, RAG1 nucleotides, RAG1 amino acids) present:

- Best-fit substitution model.
- Maximum parsimony (MP) tree.
- Distances (NJ) tree.
- Maximum likelihood (ML) tree.
- For every tree, show bootstrap values as well.
- Brief discussion of the results: comparison of topologies, branch lengths, support of phylogenetic relationships and resolution.
- Compare the trees of the three matrices among them, and also with the reference phylogeny.

1.1 Best-fit substitution model

1.1.1 Selected models from jModelTest

We use **jModelTest** to obtain the most appropriate model of nucleotide evolution given the sequence data of RAG1 and 16S.

These are the versions involved:

```
Phyml version = 3.0
Phyml binary = PhyML_3.0_macOS_i386
jModelTest version = 2.1
OS:       Mac OS X (10.14.1)
Arch:     x86_64
Java:     1.8.0_144 (Oracle Corporation)
```

16S: GTR+I+G The models selected under AIC and BIC criterions are equivalent:

```
Model   GTR+I+G
partition   012345
-lnL    13398.4723
K   27
freqA   0.3697  R(a)    4.4083
freqC   0.2395  R(b)    6.5698
freqG   0.1687  R(c)    5.3153
freqT   0.2222  R(d)    0.3201
ti/tv   -   R(e)    20.8173
R(f)    1.0000
p-inv   0.1950  gamma   0.9680
```

RAG1: GTR+I+G The models selected under AIC and BIC criterions are equivalent:

```
Model   GTR+I+G
partition   012345
-lnL    10202.7853
K   27
freqA   0.2859  R(a)    2.8475
```

```
freqC   0.2349  R(b)    7.3488
freqG   0.2584  R(c)    3.1158
freqT   0.2208  R(d)    1.1479
ti/tv   -   R(e)    11.5334
R(f)    1.0000
p-inv   0.3840  gamma   1.2830
```

1.1.2 Selected model for Prottest

We use **ProtTest3** to infere the most appropriate model of protein evolution given the sequence data of RAG1 amino acid.

These are the versions employed:

```
Version:  3.4.2 : 8th May 2016
OS:       Mac OS X (10.14.1)
Arch:     x86_64
Java:     1.8.0_144 (Oracle Corporation)
```

RAG1 JTT+I+G Prottest results:

```
**************************************************************************
Best model according to BIC: JTT+G
Confidence Interval: 100.0
**************************************************************************
Model          deltaBIC       BIC            BICw           -lnL
--------------------------------------------------------------------------
JTT+G          0.00           8062.58        0.76           3975.25
JTT+I+G        2.33           8064.91        0.24           3973.30
...
*********************************************
Relative importance of parameters
*********************************************
  alpha       (+G):     0.763
  p-inv       (+I):     0.000
  alpha+p-inv (+I+G):   0.237
  freqs       (+F):     No +F models

*********************************************
Model-averaged estimate of parameters
*********************************************
  alpha (+G):           0.379
  p-inv (+I):           0.520
  alpha (+I+G):         1.190
  p-inv (+I+G):         0.380


**************************************************************************
Best model according to LnL: JTT+I+G
```

```
Confidence Interval: 100.0
*************************************************************************
Model          deltaLnL       LnL            LnLw           -lnL
-------------------------------------------------------------------------
JTT+I+G        0.00           3973.30        0.71           3973.30
JTT+G          1.95           3975.25        0.27           3975.25
LG+I+G         7.29           3980.59        0.02           3980.59
...
*********************************************
Relative importance of parameters
*********************************************
  alpha       (+G):     0.276
  p-inv       (+I):     0.000
  alpha+p-inv (+I+G):   0.724
  freqs       (+F):     No +F models

*********************************************
Model-averaged estimate of parameters
*********************************************
  alpha (+G):           0.379
  p-inv (+I):           0.520
  alpha (+I+G):         1.183
  p-inv (+I+G):         0.379
```

The models are estimated under Maximum Likelihood LnL information criterion and under Bayesian Information Criterion. Both criterion selected as the best substitution models: JTT+I+G and JTT+G, exchanging the rank between them.

We launch another run, now with the graphical interface, and seek for AIC criterion (the previous run is launched in console with this command line `java -jar prottest-3.4.jar -i HRSV-A_aa.phy -all-matrices -all-distribution`).

The result is:

```
*****************************************************
              AKAIKE INFORMATION CRITERION
*****************************************************

*************************************************************************
Best model according to AIC: JTT+I+G
Confidence Interval: 100.0
*************************************************************************
Model          deltaAIC       AIC            AICw           -lnL
-------------------------------------------------------------------------
JTT+I+G        0.00           7984.61        0.59           3973.30
JTT+G          1.89           7986.50        0.23           3975.25
...
```

So, by consensus, we choose the **JTT+I+G** model.

1.2 Maximum parsimony (MP) tree.

1.2.1 16S nucleotides

Bla bla bla

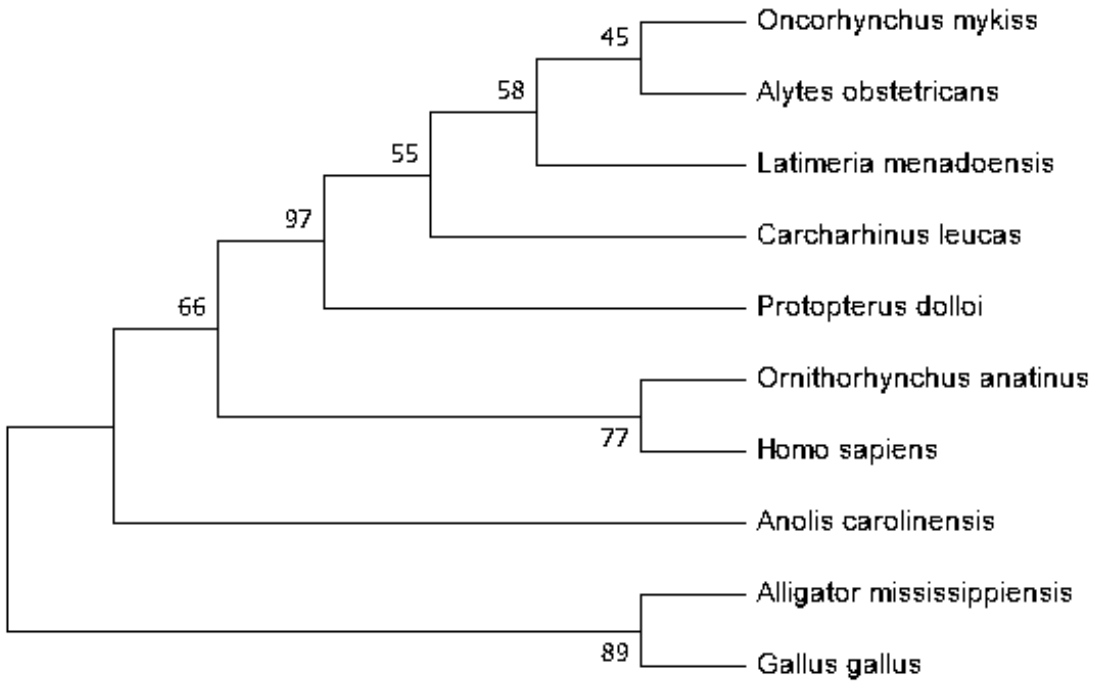

Figure 1: 16S. Maximum parsimony tree

Bla bla bla

1.2.2 RAG1 nucleotides

Bla bla bla

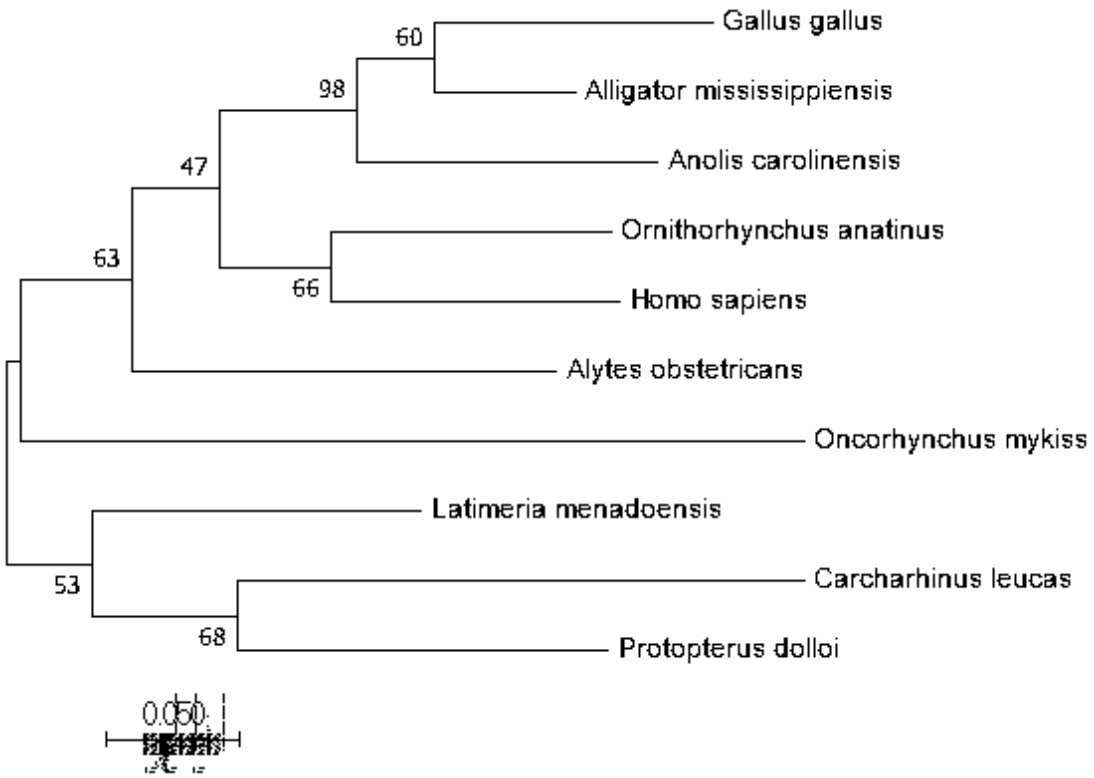

Figure 2: RAG1 nucleotides. Maximum parsimony tree

Bla bla bla

1.2.3 RAG1 amino acid

Bla bla bla

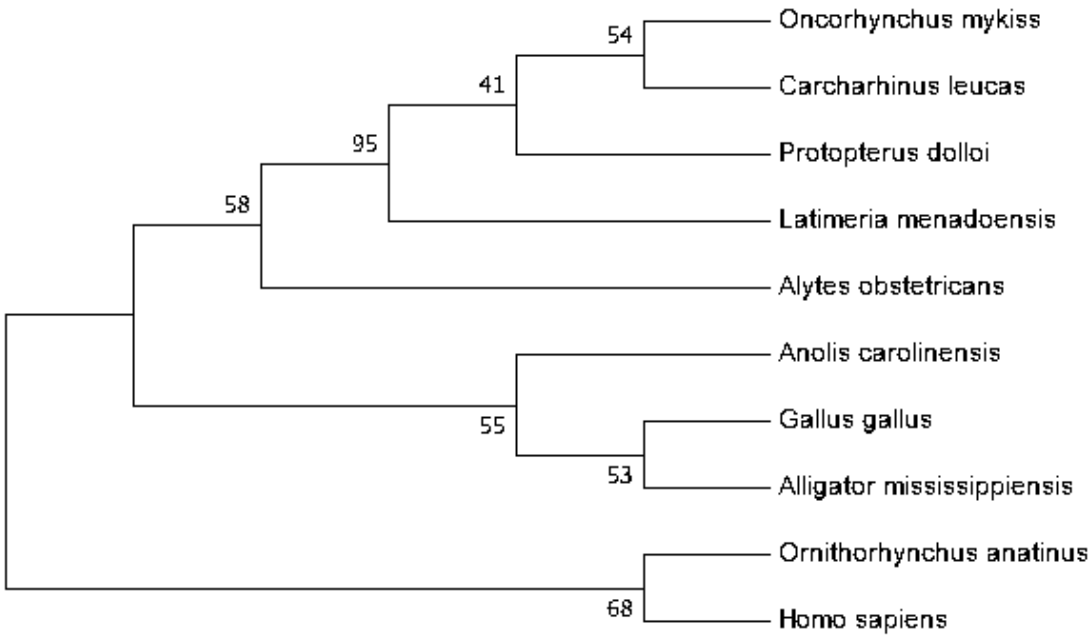

Figure 3: RAG1 AA. Maximum parsimony tree

Bla bla bla

1.3 Class AlignSequences

This class implements recursively three alignment algorithms: 1. Global alignment (Needleman-Wunsch inspired).

2. Local alignment (Smith-Waterman inspired).
3. Longest common substring.(the search for the longest common sequence can also be considered a type of alignment).

Script 1.3.1 (python)

```python
"""This module shows alternative recursive implementations of global sequence alignments:
    Global alignment (Needleman-Wunsch based)
    Local (Smith-Waterman based)
    Finding of the longest common substring.
Todo:
    * Return all the solutions of the alignments. Now it only returns one solution
    * Control of errors
    * Implement multi-alignments
    * Implement heuristic algorithms
"""
import re
from Bio import pairwise2
from Bio.pairwise2 import format_alignment
from Bio.SubsMat import MatrixInfo
```

```
import time
import sys

MIN = -sys.maxsize - 1
COMPAC = 100000
"""int: Constant to compact max score."""
SCORE_MATCH = 2
"""int: Default match score."""
SCORE_NO_MATCH = -3
"""int: Default no match score."""
SCORE_GAP_INI = -10
"""int: Default gap ini in affine gap penalty."""
SCORE_GAP_CONT = -2
"""int: Default gap continuation in affine gap penalty"""
DEFAULT_SUBST_MATRIX = {('A', 'A'): 0, ('A', 'C'): 1, ('A', 'G'): 1, ('A', 'T'): 1, ('C',
↪  'A'): 1, ('C', 'C'): 0, ('C', 'G'): 1, ('C', 'T'): 1, ('G', 'A'): 1, ('G', 'C'): 1,
↪  ('G', 'G'): 0, ('G', 'T'): 1, ('T', 'A'): 1, ('T', 'C'): 1, ('T', 'G'): 1, ('T', 'T'): 0}
"""dict: Default substitution matrix (for "ACGT" common nucleotide alphabet)"""

sys.setrecursionlimit(5000)

class AlignSequences:
    """Recursive implementation of global, local and long substring alignments methods.

    Attributes:
        sequences (list of str): Contains the wo sequences to align. The first
            one (index 0) is the query sequence (BLAST concept) or bottom sequence on
↪  alignment prints
            or vertical sequence in the common graphical representation of score matrix.
        len_seq0 (int): Sequence 0 length.
        len_seq1 (int): Sequence 1 length.
        mode (str): Computation mode:
            'GLOBAL'           Global Alignment
            'LOCAL'            Local Alignment
            'LONG_SUBSTRING'   Obtain long common substring
        score_match (int): Score of match characters.
        score_no_match (int): Score of no match characters.
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
        score (int): Score of last computed alignment.
        gaps (int): Number of gaps of the last computed alignment.
        matches (int): Number of matches of the last computed alignment.
        unmatches (int): Number of unmatches of the last computed alignment.
        align_seq0 (str): Sequence 0 with the gaps necessary for the alignment.
        align_seq1 (str): Sequence 1 with the gaps necessary for the alignment.
        matching (str): Printable line with the align relations ('|, '.', ' ') between
            both align_seq, necessary for printing the alignment.
        ini_time (int): Initial time of computation, for profiling purposes
        finish_time (int): Final time of computation, for profiling purposes
        score_store (dict of tuple int): Store of scores, for each calculated cell with
↪  tuple (i,j,g)
```

```
            where i is the coordinate of the bottom sequence, j the coordinate of the top sequence
            and g has the value 1 if the cell is a gap init cell and 0 if it's a gap continuation.
            For a explanation of calculared cell see align method.
        matches_store (dict of tuple int): Store of the number of matches in the calculated cell
        gaps_store (dict of tuple int): Store of the number of gaps in the calculated cell
        max_score_index (tuple of int): Cell coordinate tuple of the cell with the maximun score
        max_score (int): maximum computed score
        forward_arrow (dict of str): Store of the optimal displacements accomplished at a cell
            to guarantee an optimal score: 'v' vertical (down), 'h' horizontal (rigth),
            'd' diagonal.
        stacks (list of list of str): Stacks of sequences related to principal sequences in a msa
        stacks_indexes (list of str): Indexes of the sequences of stack relatives to original sequences
        stacks_refs (list of dict): References of the char in sequence os stack relatives to
        char positions on original sequences
        matrix_mode (str): If "SUBST" it's a substitution matrix, if not it's a weight matrix
            with the keys
                i = position of first sequence in stack
                j = position of second sequence on stack
                pos_i = coordinate of char on first sequence
                pos_j = coordinate of char on second sequence
            and the value is the weight to score this position
            if not match, the score is 0.
    """

    def __init__(self, sequences, mode="ALIGN", score_match=SCORE_MATCH, score_no_match=SCORE_NO_MATCH,\
                 score_gap_ini=SCORE_GAP_INI, score_gap_cont=SCORE_GAP_CONT, subst_matrix={}):
        """Init parameters of alignment"""
        self.set_sequences(sequences)
        self.set_stacks()
        self.len_seq0 = len(self.sequences[0])
        self.len_seq1 = len(self.sequences[1])
        self.init_stores()
        self.set_scores(score_match, score_no_match, score_gap_ini, score_gap_cont)
        self.set_mode(mode)
        self.score = 0
        self.matches = 0
        self.unmatches = 0
        self.gaps = 0
        self.align_seq0 = ""
        self.align_seq1 = ""
        self.matching = ""
        self.ini_time = 0
        self.finish_time = 0
```

```
105         self.set_subst_matrix(subst_matrix)
106         self.set_matrix_mode()
107 
108     def init_stores(self):
109         """Init dictionary that store temp data of the alignment"""
110         self.score_store = {}
111         self.matches_store = {}
112         self.gaps_store = {}
113         self.max_score_index = (0, 0, 0)
114         self.max_score = 0
115         self.forward_arrow = {}
116 
117     def set_sequences(self, sequences):
118         """Update the target sequences of the alignment"""
119         self.sequences = sequences
120 
121     def set_stacks(self, stack_0=[], stack_1=[],\
122                    stack_0_indexes=[], stack_1_indexes=[], stack_0_refs=[], stack_1_refs=[]):
123         """Update the stacks for msa"""
124         self.stacks = [stack_0, stack_1]
125         self.stacks_indexes = [stack_0_indexes, stack_1_indexes]
126         self.stacks_refs = [stack_0_refs, stack_1_refs]
127 
128     def set_matrix_mode(self, mode="SUBST"):
129         """Update matrix mode"""
130         self.matrix_mode = mode
131 
132     def set_subst_matrix(self, subst_matrix={}):
133         """Update the score matrix"""
134         self.subst_matrix = subst_matrix
135 
136     def set_scores(self, score_match=SCORE_MATCH, score_no_match=SCORE_NO_MATCH,\
137                    score_gap_ini=SCORE_GAP_INI, score_gap_cont=SCORE_GAP_CONT):
138         """Update the weigth scores of the alignment"""
139         self.score_match = score_match
140         self.score_no_match = score_no_match
141         self.score_gap_ini = score_gap_ini
142         self.score_gap_cont = score_gap_cont
143 
144     def set_mode(self, mode="ALIGN"):
145         """Set computation mode"""
146         self.mode = mode
147 
148     def forward_track(self, index):
149         """Calc alignments in forward direction.
150 
151             The alignment strings are calculated from init cell (0,0) in global
152             alignments or maximum score cell in local alignments.
153 
154             In local mode it's necessary to extend the alignments (local) to the total
    ↪  length of
155             the sequences to show the location of the alignment, and in order to compare with
```

```
            BioPython outputs.

            Args:
                index (tuple of int): Cell coordinates of the starting cell

            Returns:
                string: align sequence 0 (bottom) for printing purposes
                string: align sequence 1 (top) for printing purposes
                tuple of int: Coordinates of the last cell
        """
        ret_align_seq0, ret_align_seq1 = "", ""
        (i, j, gap_ini) = index
        ret_final_pos = (self.len_seq0, self.len_seq1)
        while i < self.len_seq0 or j < self.len_seq1:
            if self.mode == "LOCAL" and self.score_store[(i, j, gap_ini)] == 0:
                ret_final_pos = (i, j)
                break
            arrow = self.forward_arrow[(i, j, gap_ini)]
            if arrow == "d":
                ret_align_seq0 += self.sequences[0][i]
                ret_align_seq1 += self.sequences[1][j]
                i, j, gap_ini = i + 1, j + 1, 1
            elif arrow == "h":
                ret_align_seq0 += "-"
                ret_align_seq1 += self.sequences[1][j]
                i, j, gap_ini = i , j + 1, 0
            elif arrow == "v":
                ret_align_seq0 += self.sequences[0][i]
                ret_align_seq1 += "-"
                i, j, gap_ini = i + 1, j, 0
        #compute the complete align in local mode
        if self.mode == "LOCAL":
            ret_align_seq0 = self.sequences[0][0:index[0]] +\
                             ret_align_seq0 + self.sequences[0][ret_final_pos[0]:]
            ret_align_seq1 = self.sequences[1][0:index[1]] +\
                             ret_align_seq1 + self.sequences[1][ret_final_pos[1]:]
            diff_pos_ini = index[1] - index[0]
            if diff_pos_ini > 0:
                ret_align_seq0 = '-' * diff_pos_ini + ret_align_seq0
            else:
                ret_align_seq1 = '-' * -diff_pos_ini + ret_align_seq1
            diff_len = len(ret_align_seq1) - len(ret_align_seq0)
            if diff_len > 0:
                ret_align_seq0 += '-' * diff_len
            else:
                ret_align_seq1 += '-' * -diff_len
        return ret_align_seq0, ret_align_seq1, ret_final_pos

    def calc_matching(self, align_seq0, align_seq1, ini_pos=(), final_pos=()):
        """Calc matching string

            The matching string is the string line to print between the top and
```

```
208             bottom alignment strings. It contains the match (|), no match (.) and
209             gap ( ) indicators.
210 
211             Args:
212                 align_seq0 (string): Bottom sequence
213                 align_seq1 (string): Top sequence
214                 ini_pos (tuple of int): Initial cell coordinates
215                 final_pos (tuple of int): Final cell coordinates
216 
217             Returns:
218                 string: Matching string
219 
220         """
221         count = 0
222         ret_matching = ""
223         diff_pos_ini = ini_pos[1] - ini_pos[0]
224         if diff_pos_ini > 0:
225             delta_pos = diff_pos_ini
226         else:
227             delta_pos = 0
228         for n, (i, j) in enumerate(zip(align_seq0, align_seq1)):
229             if self.mode == "LOCAL" and not (n >= ini_pos[0] + delta_pos and n <
                ↪  final_pos[0] + delta_pos):
230                 ret_matching += ' '
231             else:
232                 if i == j: ret_matching += '|'
233                 elif i != j and i != '-' and j != '-': ret_matching += '.'
234                 else: ret_matching += ' '
235             count += 1
236         return ret_matching
237 
238     def store(self, key, score, matches, gaps):
239         """Store info related to a computed cell
240         The maximum score is computed having into account the number of matches, if there are
241         most than one solution. If the score are equal, the path with more matches is
    ↪  selected.
242              Args:
243                 key (tuple of int): Cell coordinates
244                 score (int): Cell score
245                 matches (int): Cell matches
246                 gaps (int): Cell gaps
247         """
248         self.score_store[key] = score
249         super_score = score * COMPAC + 10 * matches
250         if super_score > self.max_score:
251             self.max_score_index = key
252             self.max_score = super_score
253         self.matches_store[key] = matches
254         self.gaps_store[key] = gaps
255 
256     def calc_score_binary(self, seq_0, seq_1, i, j, seq_0_index=0, seq_1_index=1, pos_0=0,
        ↪  pos_1=0):
```

```
        """Compute alignment scores for two sequences
        If there are a substitution matrix (actually dictionary) defined,
        the scores are computed from the dictionary.
            Args:
                seq_0 (int): Sequence 0
                seq_1 (int): Sequence 1
                i (int): Sequence 0 char index
                j (int): Sequence 1 char index
                seq_0_index (int): Sequence 0 index on original sequences (MSA)
                seq_1_index (int): Sequence 1 index on original sequences (MSA)
                pos_0 (int): Sequence 0 index on stack 0
                pos_1 (int): Sequence 1 index on stack 0
        """
        if self.subst_matrix:
            if self.matrix_mode == "SUBST":
                #print("PAIR", i, j, seq_0[i], seq_1[j])
                subst_matrix_index = (seq_0[i], seq_1[j])
                subst_matrix_index_swap = (seq_1[j], seq_0[i])
                if subst_matrix_index in self.subst_matrix:
                    matrix_score = self.subst_matrix[subst_matrix_index]
                elif subst_matrix_index_swap in self.subst_matrix:
                    matrix_score = self.subst_matrix[subst_matrix_index_swap]
            else: #weight matrix
                if pos_0 in self.stacks_refs and i in self.stacks_refs[pos_0]:
                    i_orig = self.stacks_refs[pos_0][i]
                else:
                    i_orig = i
                if pos_1 in self.stacks_refs and i in self.stacks_refs[pos_0]:
                    j_orig = self.stacks_refs[pos_1][j]
                else:
                    j_orig = j
                if i_orig in self.subst_matrix[seq_0_index][seq_1_index] and \
                    j_orig in self.subst_matrix[seq_0_index][seq_1_index][i_orig]:
                    matrix_score = self.subst_matrix[seq_0_index][seq_1_index][i_orig][j_ori
g]
                else:
                    matrix_score = 0
        # Gaps in almost one of the sequences. This case only arises in MSA
        # There is no matrix related entry. If matrix is a weight matrix we compute
        # as zero (as defined in T-Coffee)
        if seq_0[i] == "-" or  seq_1[j] == '-':
            inc_matches = 0
            if self.subst_matrix:
                if self.matrix_mode == "SUBST":
                    inc_score = self.score_gap_cont
                else:
                    inc_score = 0
            else:
                inc_score = self.score_gap_cont
        else:
            if seq_0[i] == seq_1[j]:
                if self.subst_matrix:
```

```
308                     inc_score = matrix_score
309                 else:
310                     inc_score = self.score_match
311                 inc_matches = 1
312             else:
313                 if self.subst_matrix:
314                     inc_score = matrix_score
315                 else:
316                     inc_score = self.score_no_match
317                 inc_matches = 0
318 
319         return inc_score, inc_matches
320 
321     def calc_score(self, i, j):
322         """Compute alignment scores.
323         If there are stacks associated with the sequence, we compute the score weigthing the
324         scores of the stacks (SOP: Score of Pairs). Stacks contains also the guiding
    ↪  sequences.
325             Args:
326                 i (int): Sequence 0 index
327                 j (int): Sequence 1 index
328         """
329         if self.stacks == [[],[]]:
330             return self.calc_score_binary(self.sequences[0], self.sequences[1], i, j, 0, 1,
                ↪  0, 0)
331         else:
332             computed_score = 0
333             computed_matches = 0
334             nvalues = 0
335             for pos_0, (seq_0, index_0) in enumerate(zip(self.stacks[0],
                ↪  self.stacks_indexes[0])):
336                 for pos_1, (seq_1, index_1) in enumerate(zip(self.stacks[1],
                    ↪  self.stacks_indexes[1])):
337                     score, matches = self.calc_score_binary(seq_0, seq_1, i, j, index_0,
                        ↪  index_1, pos_0, pos_1)
338                     computed_score += score
339                     computed_matches += matches
340                     nvalues += 1
341             ret_score = computed_score /  nvalues
342             ret_matches = computed_matches / nvalues
343             return ret_score, ret_matches
344 
345     def align(self, i=0, j=0, ini_gap=1):
346         """Recursive align of sequences
347         For each cell, which coordinates are (i, j, ini_gap), calc the maximum score path
    ↪  from
348         three alternative displacements:
349 
350         1) To (i + 1, j + 1, 1), that is, matching or no matching the seq0(i) and seq1(i)
    ↪  characters.
351         This is a diagonal displacement.
```

```
        2) To (i, j + 1, 0), that is, seting a gap in seq0 and advance seq1. Horizontal displacement.
        3) To (i + 1, j, 0), that is, seting a gap in seq1 and advance seq0. Vertical displacement.

        The scores of these displacements are calculated adding the score ot the target cells
        (that are computed recursively) and the matrix, default of gap scores in each case.

        The score, matches, gaps and forward_arrow are stored at related dictionary entry based on
        coordinates (i, j, ini_gap), all of them asociated to the maximum score of
        the three possible paths starting from the cell, avoiding recomputation
        of the cell if it's called from another recuersive path.

        Each cell has a third score coordinate, because a cell could be called from a cell with yet
        has a gap (only from horizontal or vertical prior displacement) or from a cell with has a match/no match.
        Then we need to store two scores, matches, gaps and forward_arrows related to the two possible
        cell incarnations at coordinates (i, j, 0) and (i, j, 1).

        We store matches and gaps in order to have one aditional criterion to tiebreaker
        if some of the scores are equal. We are using this aproach in local alignment computation. If two
        scores are equal we choose the solucion with the greatest number of matches.

        We store the displacement directions in forward_arrow dict to compute the alignment.
        It's posible to avoid this, using only the score information, but we have let this aproach
        as proof of concept and for clarity in the algorithm.

        In this scenario we observe that the differences between the global,
        local and long substring algorithms are minimal.

        Local algorithm:

            Starting from the global algorithm, which would be the most general,
            the local algorithm only changes two aspects:
                1. Rejection of the roads with negative values of the score, equaling these values
                to 0, that is, not letting previous alignments of poor quality affect the final result.

                2. Use as cell of beginning of the alignment the one with the highest scores.
                In our implementation we also take into account the number of matches,
                as we have already mentioned.

        Finally, but outside the algorithm of alignment itself (at forward_track and matching methods)
        it only remains to extend the alignment obtained to show its location within the chains to be aligned.
```

```
392
393         Search algorithm of the long common substring:
394
395             Modify the global algorithm in the following aspects:
396                 1. Only computes matches between characters or gaps in one or another
    ↪  initial sequence.
397
398          Args:
399                 i (int): Sequence 0 index
400                 j (int): Sequence 1 index
401                 ini_gap (int): 1 if gap initiation, 0 if gap continuation
402         """
403         score_diag, score_hor, score_ver = MIN, MIN, MIN
404         matches_diag, matches_hor, matches_ver = MIN, MIN, MIN
405         gaps_diag, gaps_hor, gaps_ver = MIN, MIN, MIN
406         #align and advance seq0 and seq1
407         #in long_substring mode only matches are processed
408         if i < self.len_seq0 and j < self.len_seq1 and\
409         (self.mode != "LONG_SUBSTRING" or self.sequences[0][i] == self.sequences[1][j]):
410             inc_score, inc_matches = self.calc_score(i, j)
411             key = (i + 1, j + 1, 1)
412             if key in self.score_store:
413                 score_diag, matches_diag, gaps_diag = \
414                 self.score_store[key] + inc_score, self.matches_store[key] + inc_matches,
                    ↪  self.gaps_store[key]
415             else:
416                 score, matches, gaps = self.align(i + 1, j + 1, 1)
417                 self.store(key, score, matches, gaps)
418                 score_diag, matches_diag, gaps_diag = score + inc_score, matches +
                    ↪  inc_matches, gaps
419         #don't align and gap in seq0 (advance seq1)
420         if j < self.len_seq1:
421             gap_score = self.score_gap_cont + ini_gap * self.score_gap_ini
422             key = (i, j + 1, 0)
423             if key in self.score_store:
424                 score_hor, matches_hor, gaps_hor = self.score_store[key] + gap_score,\
425                 self.matches_store[key], self.gaps_store[key] + 1
426             else:
427                 score, matches, gaps = self.align(i, j + 1, 0)
428                 self.store(key, score, matches, gaps)
429                 score_hor, matches_hor, gaps_hor = score + gap_score, matches, gaps + 1
430         #don't align and gap in seq1 (advance seq0)
431         if i < self.len_seq0:
432             gap_score = self.score_gap_cont + ini_gap * self.score_gap_ini
433             key = (i + 1, j, 0)
434             if key in self.score_store:
435                 score_ver, matches_ver, gaps_ver =\
436                 self.score_store[key] + gap_score, self.matches_store[key],
                    ↪  self.gaps_store[key] + 1
437             else:
438                 score, matches, gaps = self.align(i + 1, j, 0)
439                 self.store(key, score, matches, gaps)
```

```
                score_ver, matches_ver, gaps_ver = score + gap_score, matches, gaps + 1
        #choose the high score path
        matcher_diag, matcher_hor, matcher_ver = score_diag, score_hor, score_ver
        if i < self.len_seq0 or j < self.len_seq1:
            if self.mode == "LOCAL" and matcher_diag < 0 and matcher_hor < 0 and matcher_ver
            ↪  < 0:
                score_diag, score_hor, score_ver = 0, 0, 0
                #matcher_diag, matcher_hor, matcher_ver  = 0, 0, 0
            if matcher_diag > matcher_hor and matcher_diag > matcher_ver:
                ret_score, ret_matches, ret_gaps, ret_arrow =\
                score_diag, matches_diag, gaps_diag, "d"
            elif matcher_hor > matcher_ver:
                ret_score, ret_matches, ret_gaps, ret_arrow =\
                score_hor, matches_hor, gaps_hor, "h"
            else:
                ret_score, ret_matches, ret_gaps, ret_arrow =\
                score_ver, matches_ver, gaps_ver, "v"
        else:
            ret_score, ret_matches, ret_gaps, ret_arrow =\
            0, 0, 0, ""
        self.forward_arrow[(i, j, ini_gap)] = ret_arrow
        if i == 0 and j == 0:
            self.store((0, 0, 1), ret_score, ret_matches, ret_gaps)
            if self.mode in ["GLOBAL", "LONG_SUBSTRING"]: self.max_score_index = (0, 0, 1)
            else: ret_score = self.max_score // COMPAC
            ret_matches = self.matches_store[self.max_score_index]
            ret_gaps = self.gaps_store[self.max_score_index]

        return ret_score, ret_matches, ret_gaps

    def compute(self, mode="LOCAL", silent=False):
        """Calc alignment
            Args:
                mode (str): Type of algorithm (local, global or long substring)
                silent (bool): If true don't show alignment output
        """
        self.ini_time = time.time()
        self.init_stores()
        self.set_mode(mode)
        self.score, self.matches, self.gaps = self.align()
        self.align_seq0, self.align_seq1, final_pos = self.forward_track(self.max_score_inde
x)
        self.matching = self.calc_matching(self.align_seq0, self.align_seq1,
        ↪  self.max_score_index, final_pos)
        self.unmatches = self.matching.count('.')
        self.gaps = self.matching.count(' ')
        self.finish_time = time.time()
        if not silent:
            self.view()

    def get_len_long_common_substring(self):
        """Getter for the len of the common substring
```

```
        That is equal to the number of matches of the alignment
        """
        return self.matches

    def get_long_common_substring(self):
        """Returns the longest common substring
        whitout alignment (positional) information
        """
        long_common_substring = ""
        for (char, match_char) in zip(self.align_seq1, self.matching):
            if match_char == '|':
                long_common_substring += char
        return long_common_substring

    def view(self):
        """Prints the alignment data"""
        #unmatches = self.matching.count('.')
        #gaps = self.matching.count(' ')
        if self.matching:
            gap_groups = self.matching.count('| ') + self.matching.count('. ') +
            ↪  self.matching[0].count(' ')
        else:
            gap_groups = 0
        print(" ")
        if self.mode == "LOCAL":
            print("### AlignSequences. Local alignment (Smith-Waterman)")
        elif self.mode == "LONG_SUBSTRING":
            print("### AlignSequences. Long substring finder")
        else:
            print("### AlignSequences. Global alignment (Needleman-Wunsch)")
        if self.subst_matrix:
            print("\tUsing score matrix with matrix mode",self.matrix_mode)
        print(self.align_seq1)
        print(self.matching)
        print(self.align_seq0)
        print("\tScore:", self.score)
        print("\tSimilarity (wo gaps):", self.matches / (self.matches + self.unmatches))
        print("\tDistance (wo gaps):", self.unmatches / (self.matches + self.unmatches))
        print("\tDistance:", self.unmatches / (self.matches + self.unmatches + self.gaps))
        print("\tInit index:", self.max_score_index)
        print("\tMatches:", self.matches, " Unmatches:", self.unmatches, " Gaps:",
        ↪  self.gaps, " Gap groups:", gap_groups)
        #simple scoring verification todo: apply to matrix
        if not self.subst_matrix:
            print("\tScore verified:", self.matches * self.score_match + self.unmatches *
            ↪  self.score_no_match \
                 + self.gaps * self.score_gap_cont + gap_groups * self.score_gap_ini)
        print("\tFinish. Execution milliseconds:", round((self.finish_time - self.ini_time)
        ↪  * 1000))
        print("\tScore Dictionary Size", len(list(self.score_store.keys())))

```

```
536     def edit_distance(self, score_match=0, score_no_match=-1, score_gap_ini=0,
        ↪  score_gap_cont=-1):
537         """Calculates an edit distance as requested in questions 1 and 3
538         It's the same computation as a global alignment with -1 penalities applied to
539         score_gap_cont and score_nomatch and 0 in score_match and score_gap_ini
540 
541             Args:
542                 score_match (int): Score of match characters.
543                 score_no_match (int): Score of no match characters.
544                 score_gap_ini (int): Score of gap init.
545                 score_gap_cont (int): Score of gap continuation.
546         """
547         self.set_scores(score_match, score_no_match, score_gap_ini, score_gap_cont)
548         self.compute("GLOBAL", True)
549         return abs(self.score)
```

1.4 MSA

In these functions, what is necessary to perform a multiple alignment of sequences is developed.

The method of progressive alignment based on a guide tree is used.

The guide tree can be obtained in two alternative ways: **Unweighted Pair Group Method with Arithmetic Mean (UPGMA)** and **Neighbor Join (NJ)**, the same options present in *CLUSTAL* software.

The alignment has three known phases. These are the particularities of my implementation on each phase:

1. Perform pairwise alignments between all the sequences involved and assign them a score.

In the case of **UPGMA** we use the proportion (in percentages) between matches and matches plus no matches (without taking gaps into account). That is, we use a measure of the identity between the two sequences involved. You can also use the distance, which would be the complement to 100 of identity, but we wanted to do so to be able to compare with the information that *CLUSTAL* throws at the beginning of his output. It does not affect the result, we simply have to look for maximum identities to build the guide tree, instead of minimum distances. In the case of **NJ**, we have chosen to use distances, computed also in percentages. Also not taking into account the number of gaps in the denominators.

2. Build the guide tree. As I said, we can do it using UPGMA or NJ. The NJ method generates an unrooted tree. As we need a root, for purposes of the subsequent alignment, we have chosen to root it by clustering the two nodes that have no relation. We do not know if it is the method used by *CLUSTAL* or similar programs, but it seems to work.

3. Multiple alignment. It is, as we know, a progressive alignment following the order indicated by the guide tree. In each step we need to align two groups of sequences, of length $n >= 1$ and $m >= 1$. Each group is aligned as a whole, in the sense that the gaps entered in one of the sequences of the group must be introduced in the same positions in the rest of the sequences of their group.

To assign a score to a position, the combined score of all the residuals of that position is used. To do this we produce the Cartesian product nxm of all the characters of that position and calculate the average of scores:

$$\frac{\sum_{\substack{0 \leq i < n \\ 0 \leq j < m}} matrix(i,j)}{nm}$$

If in any of the positions we have a gap, we have chosen to penalize it as the sum of penalties assigned to the start of the gap plus gap continuation penalty. It is a criterion, *CLUSTAL* we know that it uses another one.

If we already have a pairwise development, as it was my case, it would be easy to extend it to address MSA?. The answer is affirmative. With slight modifications in the class **AlignSequences**, we have managed to address an MSA, in the following way: 1. Generalize the one-position scoring algorithm to take into account all the sequences of both groups, averaging the scores as indicated above.

2. Take a sequence from each group (the first) to perform a simple pairwise alignment (but with the scores calculated as indicated in 1).

3. Compare the sequences resulting from the pairwise alignment with their originals from each group, compute where the gap is introduced and introduce the gap at the same positions in the rest of the sequences of each group.

1.5 MSA generic methods

Script 1.5.1 (python)

```
"""This methods shows alternative implementations of multiple sequence alignments, CLUSTAL,
↪  T-COFFEE.

TODO:
    * Many more tests. Create a test battery.

    * Achieve that the results obtained are more similar to those of CLUSTAL (if they have
↪  to be).
    Given the lack of detailed information it will be necessary to resort
    to the sources (in C++) of CLUSTAL.

    * Allow to configure the initial alignment and the final multialignments with different
↪  parameters.

    * Draw the alignments in a more standard way.

    * Draw the phylogenetic trees.

    * Include all new methods in AlignSequences or in another class.
"""
from ete3 import Tree, TreeStyle
MIN_SCORE = 0

def draw_guide_tree(tree):
    """
    Draw guide tree with ETE library
    """
    t = Tree(tree + ";")
    ts = TreeStyle()
    ts.show_leaf_name = True
    ts.show_branch_length = False
    ts.show_branch_support = False
    ts.scale = 160
    ts.branch_vertical_margin = 40
    print(t)
```

```
33      return t, ts
34
35  def readFasta(file):
36      """
37      Reads all sequences of a FASTA file
38      Args:
39          file (str): name of the imput FASTA file
40      Returns:
41          dict of str, str: sequences readed
42      """
43      ret_seqs = {}
44      seq = ""
45      key_found = False
46      with open(file, 'r') as f:
47          key = ""
48          for line in f:
49              line = line.replace('\n', '')
50              if len(line) > 0:
51                  if line[0] == ">":
52                      if key_found:
53                          ret_seqs[key] = seq
54                      key_found = True
55                      key = line[1:].split(" ")[0]
56                      seq = ""
57                  elif key_found:
58                      seq += line
59      if key_found:
60          ret_seqs[key] = seq
61      return ret_seqs
62
63  def pairwise_align(s1, s2, matrix, matrix_mode, mode, score_gap_ini=0, score_gap_cont=-8,\
64                     score_match=3, score_no_match=-2):
65      """
66      Performs initial pairwise alignments against the class AlignSequences
67      returning the %identity.
68
69      Args:
70          s1 (str): First sequence to compare.
71          s2 (str): Second sequence to compare.
72          matrix (dict of tuples of int): Substitution matrix, Biopython format
73          matrix_mode (str): Type of matrix
74              'SUBST'             Substitution matrix
75              'WEIGHT'            Weight matrix
76          mode (str): Computation mode:
77              'GLOBAL'            Global Alignment
78              'LOCAL'             Local Alignment
79              'LONG_SUBSTRING'    Obtain long common substring
80          score_gap_ini (int): Score of gap init.
81          score_gap_cont (int): Score of gap continuation.
82          score_match (int): Score of match characters (used if no matrix informed)
83          score_no_match (int): Score of no match characters (used if no matrix informed)
84
```

```
    Returns:
        (int): % identity between sequences
    """
    align = AlignSequences([s1, s2])
    align.set_scores(score_match, score_no_match, score_gap_ini, score_gap_cont)
    align.set_subst_matrix(matrix)
    align.set_matrix_mode(matrix_mode)
    align.compute(mode.upper(), silent = True)
    return round((align.matches + 1) * 100 / (align.matches + align.unmatches + 2))

def pairwise_align_distance(s1, s2, matrix, matrix_mode, mode, score_gap_ini=0,
 ↪  score_gap_cont=-8):
    """
    Performs initial pairwise alignments against the class AlignSequences
    returning the distance between 0 and 100.

    Args:
        s1 (str): First sequence to compare.
        s2 (str): Second sequence to compare.
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
        matrix_mode (str): Type of matrix
            'SUBST'            Substitution matrix
            'WEIGHT'           Weight matrix
        mode (str): Computation mode:
            'GLOBAL'           Global Alignment
            'LOCAL'            Local Alignment
            'LONG_SUBSTRING'   Obtain long common substring
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
        score_match (int): Score of match characters (used if no matrix informed)
        score_no_match (int): Score of no match characters (used if no matrix informed)

    Returns:
        (int): distance between sequences
    """
    align = AlignSequences([s1, s2])
    align.set_scores(0, 0, score_gap_ini, score_gap_cont)
    align.set_subst_matrix(matrix)
    align.set_matrix_mode(matrix_mode)
    align.compute(mode.upper(), silent = True)
    identity = round((align.matches + 1) * 100 / (align.matches + align.unmatches + 2))
    return 100 - identity

def guide_tree_UPGMA(sequences, matrix, matrix_mode, mode,\
                     score_gap_ini, score_gap_cont,\
                     score_match, score_no_match):
    """
    Performs initial pairwise alignments against the class AlignSequences
    returning the guide_tree derived from UPGMA method.

    Args:
        sequences (lit of str): Sequences to align
```

```
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
        matrix_mode (str): Type of matrix
            'SUBST'             Substitution matrix
            'WEIGHT'            Weight matrix
        mode (str): Computation mode:
            'GLOBAL'            Global Alignment
            'LOCAL'             Local Alignment
            'LONG_SUBSTRING'    Obtain long common substring
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
        score_match (int): Score of match characters (used if no matrix informed)
        score_no_match (int): Score of no match characters (used if no matrix informed)

    Returns:
        (list of 3-tuples of int): guide three, the third position of the tuple contains the root
                                of the other two nodes.
        (dict of int, boolean = True): contains all nodes

    """
    tree = {} #initial tree
    guide_tree = [] #guided tree, pairs to align in sequence
    max_score = MIN_SCORE
    max_score_position = ()
    for i in range(0, len(sequences)):
        for j in range(0 , i):
            if (i,j) not in tree:
                score = pairwise_align(sequences[i], sequences[j], matrix, matrix_mode, mode,\
                                score_gap_ini, score_gap_cont, score_match, score_no_match)
                tree[(i,j)] = score
                if score >= max_score:
                    max_score = score
                    max_score_position = (i,j)

    print(tree)
    len_tree = len(sequences)
    guide_tree_nodes = {}
    # Generate guide tree. At every step we compute another row averaging the
    # most closer rows and removing all their row coordinates from the tree
    while len(tree) > 0:
        (imax, jmax) = max_score_position
        guide_tree.append((imax, jmax, len_tree))
        guide_tree_nodes[imax] = True
        guide_tree_nodes[jmax] = True
        guide_tree_nodes[len_tree] = False

        # Average scores from i,j rows into new row in new_row_pos
        for j in range(0, len_tree):
            if j in [imax, jmax]:
                continue
            nscores = 0.0;
            for coordinate in [(imax, j), (j, imax), (jmax, j), (j, jmax)]:
```

```
                if coordinate in tree:
                    score = tree[coordinate]
                    nscores += 1
                    if (len_tree, j) not in tree:
                        tree[(len_tree, j)] = score
                    else:
                        tree[(len_tree, j)] += score
            if nscores > 0:
                tree[(len_tree, j)] = tree[(len_tree, j)] / nscores

        # Tree cleaning and calc max score
        max_score = MIN_SCORE
        max_score_position = ()
        for i in range(0, len_tree + 1):
            for j in range(0, len_tree + 1):
                if (i,j) in tree:
                    if i == imax or i == jmax or j == imax or j == jmax:
                        del(tree[(i,j)])
                    else:
                        if tree[(i,j)] >= max_score:
                            max_score =  tree[(i,j)]
                            max_score_position = (i,j)

        len_tree += 1

    return guide_tree, guide_tree_nodes

def q(i, j, nseq, n, dmatrix):
    """
    NJ method: calculate element of intermediate Q matrix.
    """
    d = (nseq - 2) * dmatrix[(i,j)]
    for k in range(0, n):
        if (i,k) in dmatrix:
            d -= dmatrix[(i,k)]
        if (j,k) in dmatrix:
            d -= dmatrix[(j,k)]
    return d

def calc_qmatrix(nseq, n, dmatrix):
    """
    NJ method: calculate intermediate Q matrix.
    """
    qmatrix = {}
    for (i,j) in dmatrix:
        qmatrix[(i,j)] = q(i, j, nseq, n, dmatrix)
    return qmatrix

def smallest_q(qmatrix):
    """
    NJ method: returns the coordinates of the minimun score in intermediate Q matrix.
    """
```

```
    sq = ()
    min_sq = - MIN
    for key in qmatrix.keys():
        if qmatrix[key] <  min_sq:
            min_sq = qmatrix[key]
            sq = key
    return sq

def djoin(joined_pair, nseq, n, dmatrix):
    """
    NJ method: returns distances of joined nodes to the rooted node, so, it returns the
    ↪ branch lengths
    """
    (i, j) = joined_pair
    d_i_1 = dmatrix[(i,j)] / 2.0
    d_i_2 = 0
    for k in range(0, n):
        if (i,k) in dmatrix:
            d_i_2 += dmatrix[(i,k)]
        if (j,k) in dmatrix:
            d_i_2 -= dmatrix[(j,k)]
    d_i = d_i_1 - d_i_2 / (2*(nseq - 2))
    d_j = dmatrix[(i,j)] - d_i
    return d_i, d_j

def dnjoin(k, joined_pair, dmatrix):
    """
    NJ method: returns distance of sequence k to the new nodetht routes the joined_pair.
    The distance is the mean of the distances ftom k to each of nodes joined.
    """
    (i, j) = joined_pair
    d_k = 0
    if (i,k) in dmatrix:
        d_k += dmatrix[(i,k)]
    if (j,k) in dmatrix:
        d_k += dmatrix[(j,k)]
    d_k = (d_k - dmatrix[(i,j)]) / 2.0
    return d_k

def recalc_dmatrix(joined_pair, n, dmatrix):
    """
    NJ method: recalc distance matrix taking into account the joined pair
    """
    (i, j) = joined_pair
    # Recalculate distances
    for k in range(0, n):
        if (i,k) in dmatrix and (j,k) in dmatrix:
            dmatrix[(n + 1, k)] = dnjoin(k, joined_pair, dmatrix)
            dmatrix[(k, n + 1)] = dmatrix[(n + 1, k)]
    # Remove joined rows from dmatrix
    for k in range(0, n + 1):
        for l in range(0, n + 1):
```

```
290             if k == i or k == j or l == i or l== j:
291                 if (k, l) in dmatrix:
292                     del(dmatrix[(k, l)])
293     return
294 
295 def guide_tree_NJ(sequences, matrix, matrix_mode, mode,\
296                   score_gap_ini, score_gap_cont,\
297                   score_match, score_no_match):
298     """
299     Performs initial pairwise alignments against the class AlignSequences
300     returning the guide_tree derived from NJ method.
301 
302     Args:
303         sequences (lit of str): Sequences to align
304         matrix (dict of tuples of int): Substitution matrix, Biopython format.
305         matrix_mode (str): Type of matrix
306             'SUBST'             Substitution matrix
307             'WEIGHT'            Weight matrix
308         mode (str): Computation mode:
309             'GLOBAL'            Global Alignment
310             'LOCAL'             Local Alignment
311             'LONG_SUBSTRING'    Obtain long common substring
312         score_gap_ini (int): Score of gap init.
313         score_gap_cont (int): Score of gap continuation.
314         score_match (int): Score of match characters (used if no matrix informed)
315         score_no_match (int): Score of no match characters (used if no matrix informed)
316 
317     Returns:
318         (list of 3-tuples of int): guide three, the third position of the tuple contains the
    ↪ root
319                                 of the other two nodes.
320         (dict of int, boolean = True): contains all nodes
321 
322     """
323     dmatrix = {} #initial distance matrix
324     n = len(sequences)
325     for i in range(0, n):
326         for j in range(0 , i):
327             if (i,j) not in dmatrix:
328                 distance = pairwise_align_distance(sequences[i], sequences[j], matrix,
                    ↪ matrix_mode,\
329                                 mode, score_gap_ini, score_gap_cont)
330                 dmatrix[(i,j)] = distance
331                 dmatrix[(j,i)] = distance
332     nseq = n
333     new_nodes = n - 2
334     guide_tree = [] #guided tree, pairs to align in sequence
335     guide_tree_nodes = {} #guided tree rooted nodes to complete
336     for i in range(0, n):
337         guide_tree_nodes[i] = False
338     while new_nodes > 0:
339         qmatrix = calc_qmatrix(nseq, n, dmatrix)
```

```
        (joined_i, joined_j) = smallest_q(qmatrix)
        #print("JOIN:", (joined_i, joined_j))
        guide_tree.append((joined_i, joined_j, n + 1))
        guide_tree_nodes[joined_i] = True
        guide_tree_nodes[joined_j] = True
        guide_tree_nodes[n + 1] = False
        recalc_dmatrix((joined_i, joined_j), n, dmatrix)
        n += 1
        nseq -= 1
        new_nodes -= 1
    # Root the tree
    #print("DMATRIX:", dmatrix)
    rooting_tuple = []
    for node in guide_tree_nodes:
        if not guide_tree_nodes[node]:
            rooting_tuple.append(node)
    rooting_tuple.append(n + 1)
    guide_tree_nodes[n + 1] = True
    #print("Rooting tuple:", rooting_tuple)
    if len(rooting_tuple) == 3:
        guide_tree.append(tuple(rooting_tuple))
    assert len(rooting_tuple) == 3
    return guide_tree, guide_tree_nodes

def gapeator(a, a_gapped, b_stack, b_stack_refs):
    """
    Introduces gaps in all the sequences of b_stack taking into account the positions
    and the gaps introduced in sequence a to obtain sequence a_gapped
    Args:
        a (str): template sequence not gapped
        a_gapped (str): template sequence gapped
        b_stack (list of str): stack of b sequences ungapped
        b_stack_refs (list of dict): stack of references to original positions
    Returns:
        list of str: stack b gapped as a does
        list of dict: stack b coordinates refered to original sequence
    """
    ini_a_gapped = a_gapped
    b_gapped_stack = []
    b_references_stack = []
    len_a_gapped = len(a_gapped)
    for b, b_refs in zip(b_stack, b_stack_refs):
        b_gapped = ""
        b_gapped_references = {}
        a_gapped = ini_a_gapped
        base_ref = 0
        for k, (i, j) in enumerate(zip(a, b)):
            index = a_gapped.index(i)
            a_gapped = a_gapped[index + 1:]
            #print("a_gapped", a_gapped )
            #print(i, j, index)
            b_gapped += "-" * index + j
```

```
            if k in b_refs:
                b_gapped_references[base_ref + k + index] =  b_refs[k]
            base_ref += index
            #print("b_gapped", b_gapped )
        b_gapped += b[k+1:]
        remaining_gaps = "-" * (len_a_gapped - len(b_gapped))
        b_gapped +=  remaining_gaps
        b_gapped_stack.append(b_gapped)
        b_references_stack.append(b_gapped_references)
    return b_gapped_stack, b_references_stack

def pairwise_align_msa_step(stack_0, stack_1, sequences, matrix, matrix_mode,\
                            mode, stack_0_indexes, stack_1_indexes, stack_0_refs,
                             ↪  stack_1_refs,\
                            score_match, score_no_match, score_gap_ini, score_gap_cont):
    """
    Performs msa alignment of sequence stack 0 and 1.

    Args:
        stack_0 (list of str): First stack of sequences to align.
        stack_1 (list of str): Secong stack of sequences to align.
        sequences (list of str): Sequences to align.
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
        matrix_mode (str): Type of matrix
            'SUBST'             Substitution matrix
            'WEIGHT'            Weight matrix
        mode (str): Computation mode:
            'GLOBAL'            Global Alignment
            'LOCAL'             Local Alignment
            'LONG_SUBSTRING'    Obtain long common substring
        stack_0_indexes(list of int) : Indexes of initial sequences related to stack
    ↪  sequences 0
        stack_1_indexes(list of int) : Indexes of initial sequences related to stack
    ↪  sequences 1
        stack_0_refs(list of dict) : stack_0 references to original sequences
        stack_1_refs(list of dict) : stack_1 references to original sequences
        score_match (int): Score of match characters (used if no matrix informed)
        score_no_match (int): Score of no match characters (used if no matrix informed)
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.

    Returns:
        list of str: stack_0 gapped (with the gaps necessary for the alignment)
        list of str: stack_0 gapped (with the gaps necessary for the alignment)
        list of dict: stack_0 references to original sequences
        list of dict: stack_1 references to original sequences
    """
    align = AlignSequences([stack_0[0], stack_1[0]])
    align.set_scores(score_match, score_no_match, score_gap_ini, score_gap_cont)
    align.set_subst_matrix(matrix)
    align.set_matrix_mode(matrix_mode)
```

```
440     align.set_stacks(stack_0, stack_1, stack_0_indexes, stack_1_indexes, stack_0_refs,
        ↪  stack_1_refs)
441     align.compute(mode.upper(), silent = True)
442     # align_seq0 align_seq1 are the seq0 and seq1 alignments
443     # we need to deduce the rest of alignments.
444     # what we do is perform the same gap insertions, if any, as the first sequence of the
        ↪  stacks
445     # the gap insertions where performed taken into account the initial sequence
446     # to compute the references to inital sequence in order to employ a weight matrix if
        ↪  informed
447     stack_0_gapped, stack_0_references = gapeator(stack_0[0], align.align_seq0, stack_0,
        ↪  stack_0_refs)
448     stack_1_gapped, stack_1_references = gapeator(stack_1[0], align.align_seq1, stack_1,
        ↪  stack_1_refs)
449     return stack_0_gapped, stack_1_gapped, stack_0_references, stack_1_references
450 
451 def get_name(index, sequence_names):
452     """
453     Obtain sequence name from index
454     """
455     name = ""
456     if index < len(sequence_names):
457         name = sequence_names[index]
458     else:
459         name = str(index)
460     return name
461 
462 def to_newick(tree, sequence_names):
463     """
464     Obtain guide tree in newick format
465     """
466     # Change format to intermediate roots
467     roots = {}
468     newick_tree = ""
469     for branch in tree:
470         (i, j, k) = branch
471         name_i = get_name(i, sequence_names)
472         name_j = get_name(j, sequence_names)
473         name_k = get_name(k, sequence_names)
474         if name_i in roots:
475             new_root_i = roots[name_i]
476         else:
477             new_root_i = name_i
478         if name_j in roots:
479             new_root_j = roots[name_j]
480         else:
481             new_root_j = name_j
482         roots[name_k] = [new_root_i, new_root_j]
483 
484     for root in roots.values():
485         s_root = str(root)
486         if len(s_root) > len(newick_tree):
```

```
            newick_tree = s_root.replace("[","(").replace("]",")").replace("'","")

    return newick_tree
```

1.6 T-COFFEE methods

Script 1.6.1 (python)

```python
# T-COFFEE specific methods
def pairwise_align_coffee(s1, s2, matrix, mode, score_gap_ini=0, score_gap_cont=-8,\
                  score_match=3, score_no_match=-2):
    """
    Performs initial pairwise alignments against the class AlignSequences
    returning the %identity and the alignments to construct the primary library

    Args:
        s1 (str): First sequence to compare.
        s2 (str): Second sequence to compare.
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
        mode (str): Computation mode:
            'GLOBAL'           Global Alignment
            'LOCAL'            Local Alignment
            'LONG_SUBSTRING'   Obtain long common substring
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
        score_match (int): Score of match characters (used if no matrix informed)
        score_no_match (int): Score of no match characters (used if no matrix informed)

    Returns:
         int: % identity between sequences
         str: sequence 1 aligned
         str: sequence 2 aligned
    """
    align = AlignSequences([s1, s2])
    align.set_scores(score_match, score_no_match, score_gap_ini, score_gap_cont)
    align.set_subst_matrix(matrix)
    align.compute(mode.upper(), silent = True)
    return round((align.matches + 1) * 100 / (align.matches + align.unmatches + 2)), \
            align.align_seq0, align.align_seq1

def get_pos(k, seq_i, align_i):
    """
    Obtain position of a character in the original sequence given the
    position in the alignment(k), the original sequence (seq_i)
    and the align_i (gapped) sequence
    """
    char = align_i[k]
    count_char = align_i[0:k+1].count(char)
    index = -1;
    for _ in range(0, count_char):
```

```
        index = seq_i.find(char, index + 1)
    return index

def update_weight_at_pos(weight_library, i, j, pos_i, pos_j, identity):
    """
    Update weight at pos i , j, pos_i, pos_j
    """
    if i not in weight_library:
        weight_library[i] = {}
    w_i = weight_library[i]
    if j not in w_i:
        w_i[j] = {}
    w_i_j = w_i[j]
    if pos_i not in w_i_j:
        w_i_j[pos_i] = {}
    w_i_j_pi = w_i_j[pos_i]
    if pos_j not in w_i_j_pi:
        w_i_j_pi[pos_j] = identity
    else:
        w_i_j_pi[pos_j] += identity

def update_weight_library(weight_library, i, j, identity,\
                  seq_i, seq_j, align_i, align_j):
    """
    Update weights library from alignments and %identity
    """
    for k, (c_i, c_j) in enumerate(zip(align_i, align_j)):
        if c_i != "-" and c_j != "-":
            pos_i = get_pos(k, seq_i, align_i)
            pos_j = get_pos(k, seq_j, align_j)
            #print("Position:", pos_i, pos_j)
            update_weight_at_pos(weight_library, i, j, pos_i, pos_j, identity)
            update_weight_at_pos(weight_library, j, i, pos_j, pos_i, identity)

def compute_library(sequences, matrix={}, weight_library={}, mode="GLOBAL",\
                  score_gap_ini=0, score_gap_cont=-8,\
                  score_match=3, score_no_match=-2):
    """
    Compute initial library of identities based on scores of PA
     Args:
        sequences (list of str): Sequences to compare.
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
        mode (str): Computation mode:
            'GLOBAL'           Global Alignment
            'LOCAL'            Local Alignment
            'LONG_SUBSTRING'   Long substring alignment
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
        score_match (int): Score of match characters (used if no matrix informed)
        score_no_match (int): Score of no match characters (used if no matrix informed)

    Returns:
```

```
        list of str: primary library of alignments
    """
    primary_library = {}
    for i in range(0, len(sequences)):
        for j in range(0 , i):
            if (i,j) not in primary_library:
                identity, align_i, align_j = pairwise_align_coffee(sequences[i],
                ↪  sequences[j],\
                                matrix, mode, score_gap_ini, score_gap_cont,\
                                score_match, score_no_match)
                update_weight_library(weight_library, i, j, identity,\
                               sequences[i], sequences[j], align_i, align_j)
                primary_library[(i,j)] = (align_i, align_j, identity)
    #print(weight_library)
    return primary_library

def extend_weigths(weight_library, i, k, j):
    """
    Extend weigths for pair of sequences (i,j) at pos (pos_i_posj)
    taken into account the routes using k as
    intermediate, by means of the alignments (i, k) and (k, j).
    """
    for pos_i, pos_i_j in weight_library[i][j].items():
        for pos_j in pos_i_j.keys():
            if pos_j in weight_library[j][k].keys():
                for pos_k in weight_library[j][k][pos_j].keys():
                    if pos_k in weight_library[k][i].keys():
                        for pos_i_new in weight_library[k][i][pos_k].keys():
                            if pos_i_new == pos_i:
                                #print("Extension", pos_i, pos_j, pos_k, weight_library[i][k
                                ↪  ][pos_i][pos_k],weight_library[j][k][pos_j][pos_k])
                                m = min(\
                                    weight_library[i][k][pos_i][pos_k],\
                                    weight_library[j][k][pos_j][pos_k])
                                #print("++", m, weight_library[i][j][pos_i][pos_j])
                                weight_library[i][j][pos_i][pos_j] +=  m

def extend_library_weigths(sequences, weight_library):
    """
    Extend library for all triplets of sequences
    Taken into account the simetry i -> k -> j
    """
    len_sequences = len(sequences)
    for i in range(0, len_sequences):
        for k in range(0, len_sequences):
             if k != i:
                for j in range(0, len_sequences ):
                    if j != k and j != i:
                        #print("Triplet:", i, k, j)
                        extend_weigths(weight_library, i, k, j)


```

```python
def compute_libraries(sequences, matrix,\
                      score_gap_ini, score_gap_cont, score_match, score_no_match):
    """
    Compute initial library of identities based on scores of pairwise alignments
     Args:
        sequences (list of str): Sequences to compare.
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
        score_match (int): Score of match characters (used if no matrix informed)
        score_no_match (int): Score of no match characters (used if no matrix informed)

    Returns:
        dict : primary library of alignments
        dict : weight matrix
    """
    weight_library = {}
    primary_library = compute_library(sequences, matrix,\
                weight_library, "GLOBAL", score_gap_ini, score_gap_cont,\
                score_match, score_no_match)

    _ = compute_library(sequences, matrix,\
                weight_library, "LOCAL", score_gap_ini, score_gap_cont,\
                score_match, score_no_match)

#     _ = compute_library(sequences, matrix,\
#                 weight_library, "LONG_SUBSTRING", score_gap_ini, score_gap_cont,\
#                 score_match, score_no_match)

    extend_library_weigths(sequences, weight_library)

    return primary_library, weight_library
```

1.7 Main MSA method

Script 1.7.1 (python)

```python
# Generic MSA method
def do_msa_from_fasta(file, main_alg="CLUSTAL", method="NJ", matrix={}, matrix_mode="SUBST",\
                      mode="GLOBAL", score_gap_ini =-10, score_gap_cont=-5, score_match=3,\
                      score_no_match=-2, verbose=False):
    """
    Performs MSA alignments from fasta file
    Args:
        file (str): Name of the FASTA file.
        main_alg (str): Main algorithm:
            "CLUSTAL"       Clustal like
            "T-COFFEE"      T-COFFEE like
        method (str): NJ neighbor join / UPGMA
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
```

```
        matrix_mode (str): Type of matrix
            'SUBST'             Substitution matrix
            'WEIGHT'            Weight matrix
        mode (str): Computation mode:
            'GLOBAL'            Global Alignment
            'LOCAL'             Local Alignment
            'LONG_SUBSTRING'    Obtain long common substring
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
        score_match (int): Score of match characters (used if no matrix informed)
        score_no_match (int): Score of no match characters (used if no matrix informed)
        verbose (bool): If True prints verbose info

    Returns:
        list of 3-tuples of int: guide three, the third position of the tuple contains the root
                                 of the other two nodes.
        list of str: alignments
        list of str: sequence_names
        list of int: sequence indexes relating strings in alignment to original sequences
    """
    seq_fasta = readFasta(file)
    sequences = list(seq_fasta.values())
    sequence_names = list(seq_fasta.keys())
    return do_msa(sequences, sequence_names,\
                  main_alg, method, matrix, matrix_mode,\
                  mode, score_gap_ini, score_gap_cont, score_match,\
                  score_no_match, verbose)

def do_msa(sequences, sequence_names, main_alg="CLUSTAL", method="NJ", matrix={}, matrix_mode="SUBST",\
                      mode="GLOBAL", score_gap_ini=-10, score_gap_cont=-5, score_match=3,\
                      score_no_match=-2, verbose=False):
    """
    Performs MSA alignments from sequences
    Args:
        sequences (list of str): Sequences
        sequence_names (list of str): Names of sequences
        main_alg (str): Main algorithm:
            "CLUSTAL"       Clustal like
            "T-COFFEE"      T-COFFEE like
        method (str): NJ neighbor join / UPGMA
        matrix (dict of tuples of int): Substitution matrix, Biopython format.
        matrix_mode (str): Type of matrix
            'SUBST'             Substitution matrix
            'WEIGHT'            Weight matrix
        mode (str): Computation mode:
            'GLOBAL'            Global Alignment
            'LOCAL'             Local Alignment
            'LONG_SUBSTRING'    Obtain long common substring
        score_gap_ini (int): Score of gap init.
        score_gap_cont (int): Score of gap continuation.
```

```
64          score_match (int): Score of match characters (used if no matrix informed)
65          score_no_match (int): Score of no match characters (used if no matrix informed)
66          verbose (bool): If True prints verbose info
67
68      Returns:
69          list of 3-tuples of int: guide three, the third position of the tuple contains the
    ↪   root
70                                   of the other two nodes.
71          list of str: alignments
72          list of str: sequence_names
73          list of int: sequence indexes relating strings in alignment to original sequences
74      """
75      if main_alg == "T-COFFEE":
76          primary_library, weight_library = compute_libraries(sequences, matrix,\
77                          score_gap_ini, score_gap_cont, score_match, score_no_match)
78          #print("Primary library", primary_library)
79          #print("Weight library", weight_library)
80          # Matrix mode and other MSA parameters
81          matrix = weight_library
82          matrix_mode = "WEIGHT"
83  #         score_gap_ini = 0
84  #         score_gap_cont = 0
85          # From here only we need is to compute a MSA with weight matrix as reference.
86      else:
87          matrix_mode = "SUBST"
88      if method == "NJ":
89          guide_tree, guide_tree_nodes =\
90              guide_tree_NJ(sequences, matrix, matrix_mode,\
91              mode, score_gap_ini, score_gap_cont,\
92              score_match, score_no_match)
93      else: #UPGMA
94          guide_tree, guide_tree_nodes =\
95              guide_tree_UPGMA(sequences, matrix, matrix_mode,\
96              mode, score_gap_ini, score_gap_cont,\
97              score_match, score_no_match)
98      sequences_store = {}
99      sequences_store_indexes = {}
100     sequences_store_refs = {}
101     print("Guide Tree",guide_tree, sequence_names)
102     #return guide_tree, "", sequence_names
103     # Create MSA
104     for i in guide_tree_nodes.keys():
105         if i < len(sequences):
106             sequences_store[i] = [sequences[i]]
107             sequences_store_indexes[i] = [i]
108             sequences_store_refs[i] = []
109             autorefs = {}
110             for k in range(0, len(sequences[i])):
111                 autorefs[k] = k
112             sequences_store_refs[i].append(autorefs)
113
114     if verbose: print(sequences_store)
```

```
    for (i ,j ,k) in guide_tree:
        stack_i = sequences_store[i]
        stack_j = sequences_store[j]
        stack_i_indexes = sequences_store_indexes[i]
        stack_j_indexes = sequences_store_indexes[j]
        stack_i_references = sequences_store_refs[i]
        stack_j_references = sequences_store_refs[j]
        if verbose: print("Stack i", i, stack_i)
        if verbose: print("Stack j", j, stack_j)
        if verbose: print("Stack i_indexes", i, stack_i_indexes)
        if verbose: print("Stack j_indexes", j, stack_j_indexes)
        stack_0, stack_1, stack_0_references, stack_1_references =\
            pairwise_align_msa_step(stack_i, stack_j, sequences, matrix, matrix_mode,\
                                    mode, stack_i_indexes, stack_j_indexes,\
                                    stack_i_references, stack_j_references,\
                                    score_match, score_no_match, score_gap_ini,
                                     ↪  score_gap_cont)
        sequences_store[k] = []
        sequences_store_indexes[k] = []
        sequences_store_refs[k] = []
        if verbose: print("==================")
        for s in stack_0:
            if verbose: print(s)
            sequences_store[k].append(s)
        for s in stack_1:
            if verbose: print(s)
            sequences_store[k].append(s)
        for seq_index in stack_i_indexes:
            sequences_store_indexes[k].append(seq_index)
        for seq_index in stack_j_indexes:
            sequences_store_indexes[k].append(seq_index)
        for seq_refs in stack_0_references:
            sequences_store_refs[k].append(seq_refs)
        for seq_refs in stack_1_references:
            sequences_store_refs[k].append(seq_refs)
        if verbose: print("==================")
        if verbose: print("Sequences store indexes",sequences_store_indexes[k])
        if verbose: print("Sequences store references",sequences_store_refs[k])
        if verbose: print("New stack:", k, sequences_store[k])
    alignment = sequences_store[k]
    newick_tree = to_newick(guide_tree, sequence_names)
    return newick_tree, alignment, sequence_names, sequences_store_indexes[k]

def score(alignment, matrix, score_gap_ini=0, score_gap_cont=0):
    """
    Score based on sum of pair scores (SOP) taking into account substitution matrix
    Derived from the objetive score of MUSCLE refinement stage
    """
    msa_score = 0
    for k in range(0, len(alignment[0])): #columns of msa
        score_column_k = 0
        nvalues = 0
```

```
        for i in range (0, len(alignment)):
            for j in range (i + 1, len(alignment)):
                if alignment[i][k] == "-" and alignment[j][k] != "-":
                    score_column_k += score_gap_cont
                    if k == 0 or alignment[i][k-1] != "-":
                            score_column_k += score_gap_ini
                if alignment[j][k] == "-" and alignment[i][k] != "-":
                    score_column_k += score_gap_cont
                    if k == 0 or alignment[j][k-1] != "-":
                            score_column_k += score_gap_ini
                elif (alignment[i][k], alignment[j][k]) in matrix:
                    score_column_k += matrix[(alignment[i][k], alignment[j][k])]
                    nvalues += 1
                elif (alignment[j][k], alignment[i][k]) in matrix:
                    score_column_k += matrix[(alignment[j][k], alignment[i][k])]
                    nvalues += 1
        if nvalues > 0:
            #score += score_column_k / nvalues
            msa_score += score_column_k
    return msa_score

def score_from_fasta(file, matrix, score_gap_ini=0, score_gap_cont=0):
    seq_fasta = readFasta(file)
    sequences = list(seq_fasta.values())
    return score(sequences, matrix, score_gap_ini, score_gap_cont)
```

1.8 T-COFFEE RAG1_AA

Script 1.8.1 (python)

```
matrix = MatrixInfo.blosum80
file = "files/RAG1_AA.fas"
quality_score_gap_ini = -10
quality_score_gap_cont = -5

guide_tree_upgma, align, sequence_names, indexes = do_msa_from_fasta(file,\
                main_alg = "T-COFFEE", method = "UPGMA", \
                matrix = matrix, matrix_mode = "SUBST",\
                mode = "GLOBAL", score_gap_ini = -10,\
                score_gap_cont = -5, score_match = 3, score_no_match = -2, verbose = False)

print("# Guide Tree:", guide_tree_upgma)
t_upgma, ts_upgma = draw_guide_tree(guide_tree_upgma)
print("# Alignment:")
for i, s in enumerate(align):
    print(">" + sequence_names[indexes[i]])
    print(s)
print()
print("Score", score(align, MatrixInfo.blosum62, quality_score_gap_ini,
↪  quality_score_gap_cont))
```

```
print()

guide_tree_nj, align, sequence_names, indexes = do_msa_from_fasta(file,\
                main_alg = "T-COFFEE", method = "NJ", \
                matrix = matrix, matrix_mode = "SUBST",\
                mode = "GLOBAL", score_gap_ini = -10,\
                score_gap_cont = -5, score_match = 3, score_no_match = -2, verbose = False)
print("# Guide Tree:", guide_tree_nj)
t_nj, ts_nj = draw_guide_tree(guide_tree_nj)
print("# Alignment:")
for i, s in enumerate(align):
    print(">" + sequence_names[indexes[i]])
    print(s)
print()
print("Score", score(align, matrix, quality_score_gap_ini, quality_score_gap_cont))
```

Output

```
{(1, 0): 72, (2, 0): 75, (2, 1): 79, (3, 0): 74, (3, 1): 77, (3, 2): 81, (4, 0): 73, (4, 1):
↪  79, (4, 2): 82, (4, 3): 80, (5, 0): 73, (5, 1): 79, (5, 2): 81, (5, 3): 79, (5, 4): 85,
↪  (6, 0): 73, (6, 1): 78, (6, 2): 82, (6, 3): 81, (6, 4): 88, (6, 5): 87, (7, 0): 73, (7,
↪  1): 79, (7, 2): 83, (7, 3): 82, (7, 4): 87, (7, 5): 91, (7, 6): 93, (8, 0): 75, (8, 1):
↪  79, (8, 2): 83, (8, 3): 82, (8, 4): 87, (8, 5): 87, (8, 6): 89, (8, 7): 91, (9, 0): 74,
↪  (9, 1): 80, (9, 2): 83, (9, 3): 84, (9, 4): 88, (9, 5): 87, (9, 6): 89, (9, 7): 91, (9,
↪  8): 93}
Guide Tree [(9, 8, 10), (7, 6, 11), (11, 10, 12), (12, 5, 13), (13, 4, 14), (14, 2, 15), (15,
↪  3, 16), (16, 1, 17), (17, 0, 18)] ['Oncorhynchus_mykiss', 'Carcharhinus_leucas',
↪  'Latimeria_menadoensis', 'Protopterus_dolloi', 'Alytes_obstetricans',
↪  'Anolis_carolinensis', 'Gallus_gallus', 'Alligator_mississippiensis',
↪  'Ornithorhynchus_anatinus', 'Homo_sapiens']
# Guide Tree: (((((((Alligator_mississippiensis, Gallus_gallus), (Homo_sapiens,
↪  Ornithorhynchus_anatinus)), Anolis_carolinensis), Alytes_obstetricans),
↪  Latimeria_menadoensis), Protopterus_dolloi), Carcharhinus_leucas), Oncorhynchus_mykiss)

                      /-Alligator_mississippiensis
                   /-|
                  |   \-Gallus_gallus
                /-|
               |  |   /-Homo_sapiens
             /-|   \-|
            |  |      \-Ornithorhynchus_anatinus
          /-|  |
         |  |   \-Anolis_carolinensis
       /-|  |
      |  |   \-Alytes_obstetricans
    /-|  |
   |  |   \-Latimeria_menadoensis
 /-|  |
|  |   \-Protopterus_dolloi
--|  |
  |   \-Carcharhinus_leucas
```

```
  |
   \-Oncorhynchus_mykiss
# Alignment:
>Alligator_mississippiensis
RTVKAVTGRQIFQPLHALRTAEKALLPGYHSFEWKPPLKNVSANTEVGIIDGLSGLPHTVDDYPIDTIAKRFRYDAALVSALMDMEEDILEGM
↪ KAHDLDDYLNG-PFTVVVKESCDGMGDVSEKHGCGPAVPEKAVRFSFTVMTIAI--THGNTNVRIFEEVKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAERETMKNSVLLLEMGGILRTFKFIFRGTGYDEKLVREVEGLEASGSTYICTLCDATRLEASQNLVLHSITRSHTEN
↪ LERYEVWRSNPYHESVDELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQFEIGEVYKNPDASKEERKRWQSALDKHLRKKMN
↪ LKPIMRMNGNFARKLMTKETVEAVCELIKCEERHEALKELMDLYLKMKPVWRSSCPAKECPELLCQYSFNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYEMEDVL
>Gallus_gallus
RTVKAVTGRQIFQPLHALRTAEKALLPGYHPFEWKPPLKNVSTNTEVGIIDGLSGLPLSIDDYPIDTIAKRFRYDTALVSALKDMEEEILEGM
↪ KAKNLDDYLNG-PFTVVVKECCDGMGDVSEKHGSGPAVPEKAVRFSFTVMNIAI--DHENERIRIFEEVKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKNSELLLEIGGILRTFKFIFRGTGYDEKLVREVEGLEASGSTYICTLCDATRLEASQNLVFHSITRSHAEN
↪ LERYEIWRSNPYHESVDELRDRVKGVSAKPFIETVPSIDALHCDIGNATEFYRIFQMEIGEVYKNPDATKEERKRWQLTLDKHLRKKMK
↪ LKPMMRMSGNFARKLMSKETVEAVCELIKCEERHEALKELMDLYLKMKPVWRSSCPAKECPELLCQYSYNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKFYEMEDVL
>Homo_sapiens
RTVKAITGRQIFQPLHALRNAEKVLLPGYHHFEWQPPLKNVSSSTDVGIIDGLSGLSSSVDDYPVDTIAKRFRYDSALVSALMDMEEDILEGM
↪ RSQDLDDYLNG-PFTVVVKESCDGMGDVSEKHGSGPVVPEKAVRFSFTIMKITI--AHSSQNVKVFEEAKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKSSELMLELGGILRTFKFIFRGTGYDEKLVREVEGLEASGSVYICTLCDATRLEASQNLVFHSITRSHAEN
↪ LERYEVWRSNPYHESVEELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQLEIGEVYKNPNASKEERKRWQATLDKHLRKKMN
↪ LKPIMRMNGNFARKLMTKETVDAVCELIPSEERHEALRELMDLYLKMKPVWRSSCPAKECPESLCQYSFNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYEMEDVL
>Ornithorhynchus_anatinus
RTVKAITGRQIFQPLHSLRTAEKVLLPGYHPFEWDPPLKNVSANTEVGIMDGLSGLPVSVDDYPVDTIAKRFRYDAALVSALMDMEEDILEGM
↪ KSQDLDDYLSG-PFTVVIKESCDGMGDVSEKHGSGPAVPEKAVRFSFTVMNITL--AYEQENVKIFEEAKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKDSELKLEMGGILRSFRFIFRGTGYDEKLVREVEGLEASGSVYICTLCDATRLEASQNLVLHSITRSHAEN
↪ LERYEVWRSNPFHESVEELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQLEIGEAYKNPNASKEERKRWQATLDKHLRKKMK
↪ LKPIMRMNGNFARKLMTKETVEAVCELVHCEERHEALRELMDLYLKMKPVWRSSCPAKECPESLCQYSFNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYEMEDVL
>Anolis_carolinensis
RTVKAVTGRQIFQPLHALRTAEKALLPGYHQFEWKPPLKNVSSNTEVGIIDGLSGIQHLVDDYPVDTIAKRFRYDAALASALMDMEEDILEGL
↪ KRQDLDDYFKG-PFTVVIKESCDGMGDVSEKHGCGPAVPEKAVRFSFTLMTISV--THGNASIRVFEECKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLVAEREAMKDSVLILDMAGIPRTFKFIFRGTGYDEKLVREVEGLEASGSTYICTLCDATRLEASQNLILHSITRSHAEN
↪ LERYELWRTNPYHETVDELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQFEIGEVYKNTDASKEERRRWQSTLDKHLRKKMN
↪ LKPMTRMNGNFARKLMTKETVEAVCELIKSEERHEALRELMDLYLKMKPVWRSSCPTKECPELVCQYSFNSQRFAELLATKFRYRYAGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKFYEMEDVL
>Alytes_obstetricans
RTVKATTGRQIFQPLHALRNAEKALLPGYHPFEWKPPLKNVSTCTDTGIIDGLSGLNRSIDEYPVEAISKRFRYDTALVSALKDMEEDILEGL
↪ RSHDMDDYLNG-PFTVVIKESCDGMGDVSEKHGSGPAVPEKAVRFSFTVMYISV--PNNNECVRIFDETKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKTSELMLEMGGILRNFKFIFRGTGYDEKLVREVEGLEASGSVYICTLCDSTRLEASQNLVFHSITRCHTEN
↪ LQRYETWRANPHHESVDELRDRVKGVSAKPFIETLPSIDALHCDIGNAAEFYRLFQLEIGEVYKNPNATKEERKRWQSTLDKHLRKKMN
↪ LKPIMRMNGNFARKLMSKETVEAVCELVHSEERQEILRELMDLYLKMKPVWRSSCPAKECPELLYQYSFHSQRFAELLSTKFKYRYAGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKFYEMEDVL
>Latimeria_menadoensis
```

```
RTVKATTGKQIFQPLHSLRNAEKALLPGFHPFEWQPPLKNVSSTTEVGIIDGMSGMTQFVDEYPLDTISKRFRYDAALVSALKDLEEELLKGL
↪  IEEDLEDYLSG-PFTVIIKESCDGMGDVSEKHGSGPAVPEKAVRYSFTIMTISV-ANSHNENVTIFEEGKPNSELCCKPLCLMLADESD
↪  HETLTAILGPVIAEREAMKNSELFLEMGGILRSFKFIFRGTGYDEKLIRDVEGLEASGSSYICTLCDSTRSEASQNFILHSITRSHKEN
↪  LERYEIWRSNPYQEPVEELRDRVKGVSAKPFIETLPSIDALHCDIGNATEFYKIFQDEIGEIYKNPNPSREEKKRWHSVLDKHLRKNMN
↪  LKPVMRMNGNYARKLMTKETVNAVCELIPSEERQEALKELVDLYLKMKPVWRSTCPAKECPELLCQYSFHSQRFAELLSTMYRYRYEGK
↪  ITNYLHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKYYELEDVL
>Protopterus_dolloi
RTVKAATGRQIFQPLHALRSAEKALLPGYHPFEWQPPLVGVSSSTDVGIINGLSGLTSSVDEYPVEALAKRFRYDAALVSALKDIEENILEGM
↪  KQNGLDEYLSG-PFTVVIKESCDGMGDVSEKHGSGPPVPEKAVRFSFTIMSISV-AMSDSENVQIFEEFKPNSELSCKPLCLMIADESD
↪  HETLTVILGPVIAEREAMKTSELMLELGGILRTFKFFFRGTGYDEKLVREVEGLEASGSHYICTLCDATRQEASRNLVLHSITRSHAEN
↪  LERYEVWRSNPYNESVDELRDRVKGVSAKPFIETRPCIDALQCDIGNATEFYKIFQDEVGEVYKRPNPSKEDRKRWHMTLDKHLRKKLS
↪  LKPVMRMNGNFARKLITKEAVDAVCELIPSEERRAAIRDLVHLYMLMKPVWRSTYPAKECPELLCQYSFNSQRFAELLSTKFQYRYEAK
↪  ITNYLHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYELEDVL
>Carcharhinus_leucas
KTVKAITGKQIFQPLHALRNAEKTLLPGYYSFEWQPPLANISTNTRVGIIDGLSGWVQCVDDYPMETISRRLSYDVALASAVKEMEDDILEGL
↪  RSQNVDEFVSG-PFTVVIKESCDGMGDVSEKHGCGPTVPEKAVRYSFTIMSISV-MNENNEKVKVFEEMKPNSELCCRPLCLMLADESD
↪  RETLTAILGPVIAERQSMKTSDLIVEIGDLYRSFQFIFRGTGYDEKLVREVEGLEASGSIYICTLCDSTRSEASKNMVLHSITRNHAEN
↪  LERYEIWRSNPYHETADELRDRVKGVSAKPFIETQPSIDALHCDIGNATEFYRIFQDEIGEVYKNSNSSKEERKRWQSMLDKHLRKKMN
↪  LKPIMRMNGNFARKLMTKETVEAVCELIPSEERREILRELMHLYLLMKPVWRSTFPTTECPDLLCQYSFNSQRFAELLHTEFSHRYEGK
↪  ITNYLHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKSYELEDIL
>Oncorhynchus_mykiss
RTVKATSGRQIFQPLHTLRTAEKELLPGYHPFEWQPALKSVSTSCHVGIIDGLSGWIASVDDSPADTVTRRFRYDVALVSALKDLEEDIMEGL
↪  RERGLEDSACTSGFSVMIKESCDGMGDVSEKHGGGPPVPEKPVRFSFTIMSVSIQAEGEDEAITIFREPKPNSEMSCKPLSLMFVDESD
↪  HETLTGVLGPVVAERNAMKHSRLILSVGGLSRSFRFHFRGTGYDEKMVREMEGLEASGSTYICTLCDSTRAEASQNMTLHSVTRSHDEN
↪  LERYELWRTNPHSESAEELRDRVKGVSAKPFMETQPTLDALHCDIGNATEFYKIFQDEIGEVYHKANPSREQRRSWRAALDKQLRKKMK
↪  LKPVMRMNGNYARKLMTREAVEAVCELVCSEERQEALRELMGLYIQMKPVWRSTCPAKECPDELCRYSFNSQRFAELLSTVFKYRYDGK
↪  ITNYLHKTLAHVPEIVERDGSIGAWASEGNESGNKLFRRFRKMNARQSKTFELEDVL

Score 99825

Guide Tree [(7, 6, 11), (11, 5, 12), (9, 8, 13), (12, 4, 14), (13, 3, 15), (14, 2, 16), (16,
↪  1, 17), (15, 0, 18), (17, 18, 19)] ['Oncorhynchus_mykiss', 'Carcharhinus_leucas',
↪  'Latimeria_menadoensis', 'Protopterus_dolloi', 'Alytes_obstetricans',
↪  'Anolis_carolinensis', 'Gallus_gallus', 'Alligator_mississippiensis',
↪  'Ornithorhynchus_anatinus', 'Homo_sapiens']
# Guide Tree: ((((((Alligator_mississippiensis, Gallus_gallus), Anolis_carolinensis),
↪  Alytes_obstetricans), Latimeria_menadoensis), Carcharhinus_leucas), (((Homo_sapiens,
↪  Ornithorhynchus_anatinus), Protopterus_dolloi), Oncorhynchus_mykiss))

                    /-Alligator_mississippiensis
                 /-|
              /-|   \-Gallus_gallus
             |  |
          /-|   \-Anolis_carolinensis
         |  |
       /-|   \-Alytes_obstetricans
      |  |
    /-|   \-Latimeria_menadoensis
   |  |
   |   \-Carcharhinus_leucas
   |
--|         /-Homo_sapiens
```

```
  |      /-|
  |   /-|   \-Ornithorhynchus_anatinus
  |  |  |
   \-|   \-Protopterus_dolloi
     |
      \-Oncorhynchus_mykiss
# Alignment:
>Alligator_mississippiensis
RTVKAVTGRQIFQPLHALRTAEKALLPGYHSFEWKPPLKNVSANTEVGIIDGLSGLPHTVDDYPIDTIAKRFRYDAALVSALMDMEEDILEGM
↪ KAHDLDDYLNG-PFTVVVKESCDGMGDVSEKHGCGPAVPEKAVRFSFTVMTIAI--THGNTNVRIFEEVKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAERETMKNSVLLLEMGGILRTFKFIFRGTGYDEKLVREVEGLEASGSTYICTLCDATRLEASQNLVLHSITRSHTEN
↪ LERYEVWRSNPYHESVDELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQFEIGEVYKNPDASKEERKRWQSALDKHLRKKMN
↪ LKPIMRMNGNFARKLMTKETVEAVCELIKCEERHEALKELMDLYLKMKPVWRSSCPAKECPELLCQYSFNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYEMEDVL
>Gallus_gallus
RTVKAVTGRQIFQPLHALRTAEKALLPGYHPFEWKPPLKNVSTNTEVGIIDGLSGLPLSIDDYPIDTIAKRFRYDTALVSALKDMEEEILEGM
↪ KAKNLDDYLNG-PFTVVVKECCDGMGDVSEKHGSGPAVPEKAVRFSFTVMNIAI--DHENERIRIFEEVKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKNSELLLEIGGILRTFKFIFRGTGYDEKLVREVEGLEASGSTYICTLCDATRLEASQNLVFHSITRSHAEN
↪ LERYEIWRSNPYHESVDELRDRVKGVSAKPFIETVPSIDALHCDIGNATEFYRIFQMEIGEVYKNPDATKEERKRWQLTLDKHLRKKMK
↪ LKPMMRMSGNFARKLMSKETVEAVCELIKCEERHEALKELMDLYLKMKPVWRSSCPAKECPELLCQYSYNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKFYEMEDVL
>Anolis_carolinensis
RTVKAVTGRQIFQPLHALRTAEKALLPGYHQFEWKPPLKNVSSNTEVGIIDGLSGIQHLVDDYPVDTIAKRFRYDAALASALMDMEEDILEGL
↪ KRQDLDDYFKG-PFTVVIKESCDGMGDVSEKHGCGPAVPEKAVRFSFTLMTISV--THGNASIRVFEECKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLVAEREAMKDSVLILDMAGIPRTFKFIFRGTGYDEKLVREVEGLEASGSTYICTLCDATRLEASQNLILHSITRSHAEN
↪ LERYELWRTNPYHETVDELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQFEIGEVYKNTDASKEERRRWQSTLDKHLRKKMN
↪ LKPMTRMNGNFARKLMTKETVEAVCELIKSEERHEALRELMDLYLKMKPVWRSSCPTKECPELVCQYSFNSQRFAELLATKFRYRYAGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKFYEMEDVL
>Alytes_obstetricans
RTVKATTGRQIFQPLHALRNAEKALLPGYHPFEWKPPLKNVSTCTDTGIIDGLSGLNRSIDEYPVEAISKRFRYDTALVSALKDMEEDILEGL
↪ RSHDMDDYLNG-PFTVVIKESCDGMGDVSEKHGSGPAVPEKAVRFSFTVMYISV--PNNNECVRIFDETKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKTSELMLEMGGILRNFKFIFRGTGYDEKLVREVEGLEASGSVYICTLCDSTRLEASQNLVFHSITRCHTEN
↪ LQRYETWRANPHHESVDELRDRVKGVSAKPFIETLPSIDALHCDIGNAAEFYRLFQLEIGEVYKNPNATKEERKRWQSTLDKHLRKKMN
↪ LKPIMRMNGNFARKLMSKETVEAVCELVHSEERQEILRELMDLYLKMKPVWRSSCPAKECPELLYQYSFHSQRFAELLSTKFKYRYAGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKFYEMEDVL
>Latimeria_menadoensis
RTVKATTGKQIFQPLHSLRNAEKALLPGFHPFEWQPPLKNVSSTTEVGIIDGMSGMTQFVDEYPLDTISKRFRYDAALVSALKDLEEELLKGL
↪ IEEDLEDYLSG-PFTVIIKESCDGMGDVSEKHGSGPAVPEKAVRYSFTIMTISV-ANSHNENVTIFEEGKPNSELCCKPLCLMLADESD
↪ HETLTAILGPVIAEREAMKNSELFLEMGGILRSFKFIFRGTGYDEKLIRDVEGLEASGSSYICTLCDSTRSEASQNFILHSITRSHKEN
↪ LERYEIWRSNPYQEPVEELRDRVKGVSAKPFIETLPSIDALHCDIGNATEFYKIFQDEIGEIYKNPNPSREEKKRWHSVLDKHLRKNMN
↪ LKPVMRMNGNYARKLMTKETVNAVCELIPSEERQEALKELVDLYLKMKPVWRSTCPAKECPELLCQYSFHSQRFAELLSTMYRYRYEGK
↪ ITNYLHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKYYELEDVL
>Carcharhinus_leucas
KTVKAITGKQIFQPLHALRNAEKTLLPGYYSFEWQPPLANISTNTRVGIIDGLSGWVQCVDDYPMETISRRLSYDVALASAVKEMEDDILEGL
↪ RSQNVDEFVSG-PFTVVIKESCDGMGDVSEKHGCGPTVPEKAVRYSFTIMSISV-MNENNEKVKVFEEMKPNSELCCRPLCLMLADESD
↪ RETLTAILGPVIAERQSMKTSDLIVEIGDLYRSFQFIFRGTGYDEKLVREVEGLEASGSIYICTLCDSTRSEASKNMVLHSITRNHAEN
↪ LERYEIWRSNPYHETADELRDRVKGVSAKPFIETQPSIDALHCDIGNATEFYRIFQDEIGEVYKNSNSSKEERKRWQSMLDKHLRKKMN
↪ LKPIMRMNGNFARKLMTKETVEAVCELIPSEERREILRELMHLYLLMKPVWRSTFPTTECPDLLCQYSFNSQRFAELLHTEFSHRYEGK
↪ ITNYLHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKSYELEDIL
>Homo_sapiens
```

```
RTVKAITGRQIFQPLHALRNAEKVLLPGYHHFEWQPPLKNVSSSTDVGIIDGLSGLSSSVDDYPVDTIAKRFRYDSALVSALMDMEEDILEGM
↪ RSQDLDDYLNG-PFTVVVKESCDGMGDVSEKHGSGPVVPEKAVRFSFTIMKITI--AHSSQNVKVFEEAKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKSSELMLELGGILRTFKFIFRGTGYDEKLVREVEGLEASGSVYICTLCDATRLEASQNLVFHSITRSHAEN
↪ LERYEVWRSNPYHESVEELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQLEIGEVYKNPNASKEERKRWQATLDKHLRKKMN
↪ LKPIMRMNGNFARKLMTKETVDAVCELIPSEERHEALRELMDLYLKMKPVWRSSCPAKECPESLCQYSFNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYEMEDVL
>Ornithorhynchus_anatinus
RTVKAITGRQIFQPLHSLRTAEKVLLPGYHPFEWDPPLKNVSANTEVGIMDGLSGLPVSVDDYPVDTIAKRFRYDAALVSALMDMEEDILEGM
↪ KSQDLDDYLSG-PFTVVIKESCDGMGDVSEKHGSGPAVPEKAVRFSFTVMNITL--AYEQENVKIFEEAKPNSELCCKPLCLMLADESD
↪ HETLTAILSPLIAEREAMKDSELKLEMGGILRSFRFIFRGTGYDEKLVREVEGLEASGSVYICTLCDATRLEASQNLVLHSITRSHAEN
↪ LERYEVWRSNPFHESVEELRDRVKGVSAKPFIETVPSIDALHCDIGNAAEFYKIFQLEIGEAYKNPNASKEERKRWQATLDKHLRKKMK
↪ LKPIMRMNGNFARKLMTKETVEAVCELVHCEERHEALRELMDLYLKMKPVWRSSCPAKECPESLCQYSFNSQRFAELLSTKFKYRYEGK
↪ ITNYFHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYEMEDVL
>Protopterus_dolloi
RTVKAATGRQIFQPLHALRSAEKALLPGYHPFEWQPPLVGVSSSTDVGIINGLSGLTSSVDEYPVEALAKRFRYDAALVSALKDIEENILEGM
↪ KQNGLDEYLSG-PFTVVIKESCDGMGDVSEKHGSGPPVPEKAVRFSFTIMSISV-AMSDSENVQIFEEFKPNSELSCKPLCLMIADESD
↪ HETLTVILGPVIAEREAMKTSELMLELGGILRTFKFFFRGTGYDEKLVREVEGLEASGSHYICTLCDATRQEASRNLVLHSITRSHAEN
↪ LERYEVWRSNPYNESVDELRDRVKGVSAKPFIETRPCIDALQCDIGNATEFYKIFQDEVGEVYKRPNPSKEDRKRWHMTLDKHLRKKLS
↪ LKPVMRMNGNFARKLITKEAVDAVCELIPSEERRAAIRDLVHLYMLMKPVWRSTYPAKECPELLCQYSFNSQRFAELLSTKFQYRYEAK
↪ ITNYLHKTLAHVPEIIERDGSIGAWASEGNESGNKLFRRFRKMNARQSKCYELEDVL
>Oncorhynchus_mykiss
RTVKATSGRQIFQPLHTLRTAEKELLPGYHPFEWQPALKSVSTSCHVGIIDGLSGWIASVDDSPADTVTRRFRYDVALVSALKDLEEDIMEGL
↪ RERGLEDSACTSGFSVMIKESCDGMGDVSEKHGGGPPVPEKPVRFSFTIMSVSIQAEGEDEAITIFREPKPNSEMSCKPLSLMFVDESD
↪ HETLTGVLGPVVAERNAMKHSRLILSVGGLSRSFRFHFRGTGYDEKMVREMEGLEASGSTYICTLCDSTRAEASQNMTLHSVTRSHDEN
↪ LERYELWRTNPHSESAEELRDRVKGVSAKPFMETQPTLDALHCDIGNATEFYKIFQDEIGEVYHKANPSREQRRSWRAALDKQLRKKMK
↪ LKPVMRMNGNYARKLMTREAVEAVCELVCSEERQEALRELMGLYIQMKPVWRSTCPAKECPDELCRYSFNSQRFAELLSTVFKYRYDGK
↪ ITNYLHKTLAHVPEIVERDGSIGAWASEGNESGNKLFRRFRKMNARQSKTFELEDVL

Score 107036
```

1.9 CLUSTAL

Script 1.9.1 (python)

```python
matrix = {}
file = "files/16S.fas"
quality_score_gap_ini = -10
quality_score_gap_cont = -5
guide_tree_upgma, align, sequence_names, indexes = do_msa_from_fasta(file,\
                main_alg = "CLUSTAL", method = "UPGMA", \
                matrix = matrix, matrix_mode = "SUBST",\
                mode = "GLOBAL", score_gap_ini = -10,\
                score_gap_cont = -5, score_match = 3, score_no_match = -2, verbose = False)
print("# Guide Tree:", guide_tree_upgma)
t_upgma, ts_upgma = draw_guide_tree(guide_tree_upgma)
print("# Alignment:")
for i, s in enumerate(align):
    print(">" + sequence_names[indexes[i]])
    print(s)
print()
```

```
#print("Score", score(align, MatrixInfo.blosum62, quality_score_gap_ini,
↪  quality_score_gap_cont))

# print()
# guide_tree_nj, align, sequence_names, indexes = do_msa_from_fasta(file,\
#                 main_alg = "CLUSTAL", method = "NJ", \
#                 matrix = matrix, matrix_mode = "SUBST",\
#                 mode = "GLOBAL", score_gap_ini = -10,\
#                 score_gap_cont = -5, score_match = 3, score_no_match = -2, verbose = False)
# print("# Guide Tree:", guide_tree_nj)
# t_nj, ts_nj = draw_guide_tree(guide_tree_nj)
# print("# Alignment:")
# for i, s in enumerate(align):
#     print(">" + sequence_names[indexes[i]])
#     print(s)
# print()
# print("Score", score(align, matrix, quality_score_gap_ini, quality_score_gap_cont))
```

Output

```
{(1, 0): 70, (2, 0): 71, (2, 1): 72, (3, 0): 69, (3, 1): 68, (3, 2): 69, (4, 0): 71, (4, 1):
↪  71, (4, 2): 70, (4, 3): 69, (5, 0): 68, (5, 1): 64, (5, 2): 67, (5, 3): 64, (5, 4): 65,
↪  (6, 0): 66, (6, 1): 65, (6, 2): 67, (6, 3): 65, (6, 4): 67, (6, 5): 67, (7, 0): 67, (7,
↪  1): 66, (7, 2): 68, (7, 3): 65, (7, 4): 66, (7, 5): 67, (7, 6): 69, (8, 0): 69, (8, 1):
↪  65, (8, 2): 67, (8, 3): 67, (8, 4): 67, (8, 5): 70, (8, 6): 67, (8, 7): 67, (9, 0): 66,
↪  (9, 1): 65, (9, 2): 68, (9, 3): 64, (9, 4): 67, (9, 5): 67, (9, 6): 67, (9, 7): 66, (9,
↪  8): 71}
Guide Tree [(2, 1, 10), (9, 8, 11), (4, 0, 12), (12, 10, 13), (7, 6, 14), (13, 3, 15), (11,
↪  5, 16), (16, 14, 17), (17, 15, 18)] ['Carcharhinus_leucas', 'Oncorhynchus_mykiss',
↪  'Latimeria_menadoensis', 'Protopterus_dolloi', 'Alytes_obstetricans',
↪  'Anolis_carolinensis', 'Alligator_mississippiensis', 'Gallus_gallus',
↪  'Ornithorhynchus_anatinus', 'Homo_sapiens']
# Guide Tree: ((((Homo_sapiens, Ornithorhynchus_anatinus), Anolis_carolinensis),
↪  (Gallus_gallus, Alligator_mississippiensis)), (((Alytes_obstetricans,
↪  Carcharhinus_leucas), (Latimeria_menadoensis, Oncorhynchus_mykiss)), Protopterus_dolloi))

            /-Homo_sapiens
         /-|
      /-|   \-Ornithorhynchus_anatinus
     |  |
   /-|   \-Anolis_carolinensis
  |  |
  |  |   /-Gallus_gallus
  |   \-|
  |      \-Alligator_mississippiensis
--|
  |         /-Alytes_obstetricans
  |      /-|
  |     |   \-Carcharhinus_leucas
  |   /-|
  |  |  |   /-Latimeria_menadoensis
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   \-|   \-|
     |      \-Oncorhynchus_mykiss
     |
      \-Protopterus_dolloi
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>Ornithorhynchus_anatinus
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>Carcharhinus_leucas
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>Oncorhynchus_mykiss
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>Protopterus_dolloi
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↪  TTTA------TACTTCTAGTATTGGAGAAAGAAAGAA--ATTCAAGCGCAT--------AAAAGTACCGCAAGGGAAAAC-TGAAAAAC
↪  TAGTGAAA------AATTAAGTTTTAAAAAGCAAAGACTAACACTTGTACCTTTTGCATCATGGTCTAGCTAGTCA-TGAAGGGCAGAA
↪  AG-AATTTTAGTCC-CACCCCCGAAA-CTAGGCGAGCTACTCCGAGACAGCCAA---ACGGGCCAACTCGTCCATGTGGCAAAATGGTG
↪  AGAAGAGCTCCGAGTAGCGGTGAAAAGCCAAACGAGCCTAGAGATAGCTGGTTGCGCGAGAAACGAATCTTAGTTCTACCCTAAATTTT
↪  TCACGGGCTACAGCCTTAAACCCCGTA------------ATTAAATTTAGAG-GCTACTCAAAGGGGGGACAGCCCCTTTGT-GAAAGG
↪  ACACAACCTCAACAAA-CGGATAATGATTAATCTACA---AG---GTAAAATTTAAGTGGGCCTAAAAGCAGCCACCAACAAAGAAA-G
↪  CGT-ATAGCTCCCC--CATAACTTTATCCATCAATTCAATTAAA-CAATCATAATCCATCTTAC-GTATCGAGCTGACTTATATCATTA
↪  TAAGTGCAAAAATGCTAGAATGAGT-AACAAGAAAAT-----ACAATTTTCTCCTCACATAGGTGTTAGTCAGAACAGATT-ACCTACT
↪  GACAATTACTGATAATGAACACAA-------TGTATTAACCTG-TCATAAGCAAGAAAACCCTACACTGATATATCATA-AATTCTACA
↪  CTGAAGTGTGCTT--GGAAAAATTAAGGGGGGGAGAAGGAATTCGGCAA--CTATGGCCTCGCCTGTTTACCAAAAACATCGCCTCCTG
↪  CC--------AAATATAGGAGGTACTGCCTGCCCTGTGACTC---TGTTTAACTGGCCGCGGTAT-TTTGACCGTGCGAAGGTAGCGTA
↪  ATCACTTGTCTCTTAATTAGGGACCTGTATGAATGGCAACACGAGGGTCCAACTGTCTCCTCCCCCAGATTAGTGAAATTGATCTATCC
↪  GTTCAAAAGCGGATATTTTTTCATAAGACGAGAAGACCCTGTGGAGCTTAAAGTTCTAATATTAAACAT--------------------
↪  -GAGCGTAAATATATACTTTAAGTTTTG-TAATATTAAATACTTTCGGTTGGGGCGACCACGGAGTATAAAAAACCCTCCGC-------
↪  ----------AATACAATTCTATTTTAAGAAGGACACTTCTCA-AACTAGAATATCTAGC-ACAATTGACCCAG----TCTAACTGAGC
↪  AATGAACCAAGTTACCCCAGGGATAACAGCGCAATCCCCTTTAAGAGTCCCCATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAG
↪  GGTATCCTGGTGGTGCAGCCGCTACCAAGGGTTTGTTTGTTCAACAATTAA-TATCCTACGTGATCTGAGTTCAGACCGGAGCAATCCA
↪  GGTCAGTTTCTATCTATGACTTC--TTTTTTCTAGTAC-GAAAGGACTGAAAAAGGGGGGCCTATAT-AAAAATATGCCCCA---CCCA
↪  CTACTACTGAATTTATATAAAGTAGCCAAGTGGGAAACCCCCCACACG-----GGAGAAAACCACACT--
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