

Multiple Sequence Alignment (MSA) di sequenze SARS-CoV-2

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1 Abstract

Date le variazioni sulle sequenze rilevate e catalogate nelle parti precedenti, costruiremo una matrice binaria di caratteri, costruita appositamente per evitare la generazione della "matrice proibita", per realizzare la filogenesi perfetta delle sequenze.

Successivamente effettueremo un confronto dell'albero ottenuto rispetto a quello prodotto con i tool di allineamento.

2 Algoritmo

2.1 Matrice delle variazioni

Inizialmente, vengono recuperati gli identificativi delle 14 sequenze usate e caricati i file delle variazioni degli allineamenti generati in output nella prima parte del progetto:

Listing 1: Caricamento dei file necessari per l'elaborazione

```
1 reference_id = load_fasta_id(  
2     os.path.join('..', '..', 'project-1', 'input', 'reference.fasta')  
3 )  
4 sequence_ids = read_sequence_ids(paths=[  
5     os.path.join('..', '..', 'project-1', 'input', 'GISAID'),  
6     os.path.join('..', '..', 'project-1', 'input', 'ncbi'),  
7 ])  
8 sequence_ids.insert(0, reference_id) #insert reference no variations  
9  
10 clustal_output = load_output('Clustal-NC_045512.2.json')  
11 variations = clustal_output['unmatches'].items()
```

L'algoritmo a partire dai file di output della prima parte del progetto genera una matrice binaria utilizzando come indici di riga gli identificativi delle sequenze e come colonne un identificativo univoco assegnato ad ogni variazione.

La matrice binaria così impostata contiene il valore 1 qualora la variazione identificata dalla colonna sia presente nella sequenza identificata dalla riga, altrimenti il valore della cella sarà pari a 0. Questa è salvata nel file `character_table.csv`.

Listing 2: Generazione della matrice binaria di caratteri

```
1 for key, value in variations:  
2     row = np.zeros(len(sequence_ids))  
3     indexes.append('C{}'.format(counter))  
4     for sequence in value['sequences']:  
5         row[sequence_ids.index(sequence)] = 1  
6     rows.append(row)  
7     counter += 1  
8  
9 trait_matrix = pd.DataFrame(rows, index=indexes, columns=  
10     ↪ sequence_ids, dtype=np.uint8).transpose()  
11 trait_matrix = phylogeny.reorder_columns(trait_matrix, axis=0)  
12 trait_matrix.to_csv(os.path.join('..', 'output', 'character_table.  
13     ↪ csv'))
```

2.2 Filogenesi perfetta

Prima di procedere con la creazione dell'albero è necessario verificare che la matrice binaria generata in precedenza sia valida per una filogenesi perfetta.

Il metodo riportato nel listato 3 riporta il codice utilizzato per costruire la matrice binaria più grande che non sia "matrice proibita".

Listing 3: Funzione di generazione della matrice binaria per filogenesi perfetta

```
1 def get_perfect_phylogeny_character_matrix(df):
2     columns = df.columns
3     candidate_matrix = df[columns[0:1]]
4     for i in range(1, len(columns)):
5         candidate_matrix = candidate_matrix.join(df[columns[i:i+1]])
6
7     if phylogeny.is_forbidden_matrix(candidate_matrix):
8         candidate_matrix = candidate_matrix.drop(labels=
9             ↪ candidate_matrix.columns[-1], axis=1)
10    return candidate_matrix
```

	C0	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29
H40_000102.2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPL_05_442523	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPL_05_442524	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPL_05_442540	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPL_05_442544	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPL_05_442546	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPL_05_442547	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.2	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.5	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.7	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.9	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.11	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.13	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.15	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.17	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.19	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.21	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.23	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.25	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.27	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.29	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.31	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.33	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.35	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.37	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.39	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.41	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.43	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.45	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.47	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MF200000.48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Figura 1: Albero di output del nostro script

2.3 Generazione dell'albero

A questo punto è possibile procedere con la ricostruzione dell'albero filogenetico a partire dalla `candidate_matrix` e utilizzando funzioni definite nel file `phylogeny.py` per costruire e visualizzare in output l'albero della filogenesi.

Listing 4: Chiamata alla generazione dell'albero

```
1 candidate_matrix = get_perfect_phylogeny_character_matrix(
2     ↪ trait_matrix)
3 phylogeny.build_tree(candidate_matrix)
```

2.3.1 Creazione dell'albero

La generazione dell'albero viene svolta come segue:

1. Ordinamento decrescente delle colonne del dataframe in base al numero di valori 1 presenti.

Listing 5: Ordinamento decrescente delle colonne

```
1 sorted_axis = df.sum(axis=0).sort_values(ascending=False)
2 return df[sorted_axis.index]
```

2. A partire dal nodo `root`, per ogni sequenza genera una sequenza di nodi uno per ogni variazione presente, collegandola alla variazione precedente o al nodo `root`.

Durante l'elaborazione delle sequenze successive, se esiste già un figlio del `current_node` rappresentante la variazione attuale, questo viene riutilizzato, altrimenti viene creato un nuovo nodo e collegato come figlio di `current_node`.

Listing 6: Funzione di creazione dell'albero

```

1 root = Node('root', edges={})
2 for i, row in df.iterrows():
3     current_node = root
4
5     for j in range(len(row)):
6         # If alteration is present in the current sequence
7         if row.iloc[j]:
8             # If current_node has a link to the variation with label=j
9             if j in current_node.edges:
10                 # Follow the same path without creating new nodes
11                 current_node = current_node.edges[j]
12             else:
13                 u = Node('U-{}'.format(row.index[j]), edges={})
14                 current_node.parent = current_node
15                 current_node.edges[j] = u
16                 current_node = u
17
18 Node(i, parent=current_node)

```

MC_005022.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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Figura 2: Albero di output del nostro script

3. Conversione dell'albero precedentemente generato in un `newick_tree` elaborabile dalla libreria `biopython.Phylo` usata per la visualizzazione.

Listing 7: Funzione di conversione in Newick Tree

```
1 def to_newick_tree(node):
2     if node.is_leaf:
3         return node.name
4
5     return '({})'.format(', '.join([ to_newick_tree(child) for child in
6         ↪ node.children ]))
7
8 newick_string = to_newick_tree(root)
9 tree = Phylo.read(io.StringIO(newick_string), 'newick')
```

4. Inserimento delle informazioni aggiuntive sulle sequenze nelle foglie dell'albero. Questo step non può essere effettuato durante la conversione a `newick_tree` perché alcuni caratteri usati verrebbero interpretati come parte della struttura dell'albero, alterando il risultato finale.

Listing 8: Funzione per l'inserimento dei dati nelle foglie dell'albero

```
1 def merge_sequences_data(node, sequences_data):
2     if node.is_terminal():
3         data = sequences_data[node.name]
4         node.name = '{}\n {} in {}'.format(node.name, data['date'], data
5         ↪ ['location'])
6     return
7
8 [ merge_sequences_data(child, sequences_data) for child in node.
9     ↪ clades ]
10
11 root = newick_tree.clade
12 merge_sequences_data(root, sequences_data)
```

5. Salvataggio e visualizzazione dell'albero filogenetico finale.

Listing 9: Salvataggio e visualizzazione dell'albero filogenetico finale

```
1 fig = plt.figure(figsize=(10, 8))
2 ax = fig.add_subplot(1, 1, 1)
3
```

```
4 | Phylo.draw(tree, do_show=False, axes=ax)
5 |
6 | ax.set_xlabel('Number of alterations')
7 | ax.set_ylabel('Sequences')
8 | plt.tight_layout()
9 | plt.savefig(os.path.join '..', 'output', 'phylogenetic-tree.png'))
10| plt.show()
```

3 Conclusioni

L'albero generato con ClustalW in fig. 2, rispetto all'albero da noi prodotto (fig. 1), riesce a ottenere un livello di dettaglio maggiore arrivando a scindere i rami terminanti con tre foglie da noi identificati.

Il nodo israeliano etichettato con MT276597.1 in entrambi gli alberi risulta distante dalle altre due sequenze israeliane. Questo potrebbe far presupporre che derivi da un ceppo diverso del virus o da un'area geografica differente.

Il nodo MT262993.1 presenta alterazioni differenti rispetto alle altre sequenze rilevate in Pakistan. Come già descritto in precedenza nella seconda parte del progetto, alcune di queste alterazioni risultano essere cancellazioni di porzioni di basi per noi riconducibili ad un errore in fase di sequenziamento.

I rami restanti dell'albero in fig. 1 mostrano come nella stessa area geografica i genomi siano simili tra loro il che ci porta a ipotizzare che la diffusione all'interno del paese si sia sviluppata a partire da un singolo paziente per nazione e non da pazienti contagiati da mutazioni diverse del virus.

Analizzando l'albero, i ceppi *iraniani* e *turchi* presentano mutazioni comuni. Inoltre, le sequenze di entrambi i paesi condividono una variazione con il ceppo *pakistano*. Il ceppo di virus rilevato in *Israele* sembra avere un'origine differente e non condivide mutazioni simili con le sequenze degli altri paesi analizzati.

In conclusione, gli alberi ottenuti in fig. 1 e 2 risultano abbastanza simili, tuttavia presentano alcune differenze.

Ipotizziamo che queste derivino principalmente dal fatto che l'albero in fig. 1 è costruito basandosi sul numero di alterazioni rispetto alla sequenza reference. Al contrario, l'albero in fig. 2 è generato in base alla distanza tra le *intere* sequenze a partire da un allineamento.

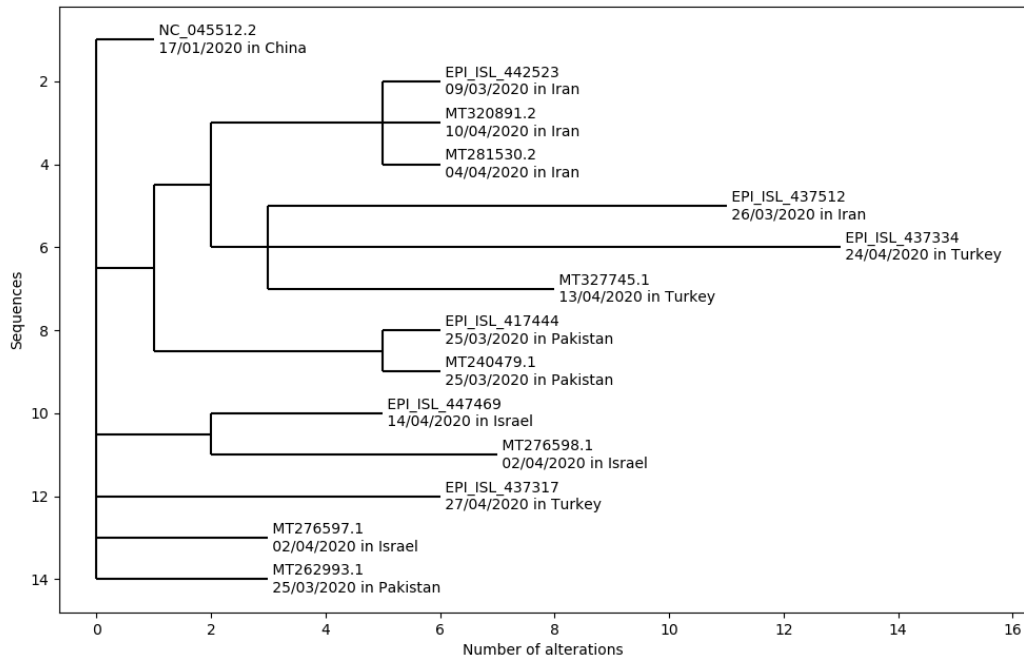


Figura 3: Albero di output del nostro script

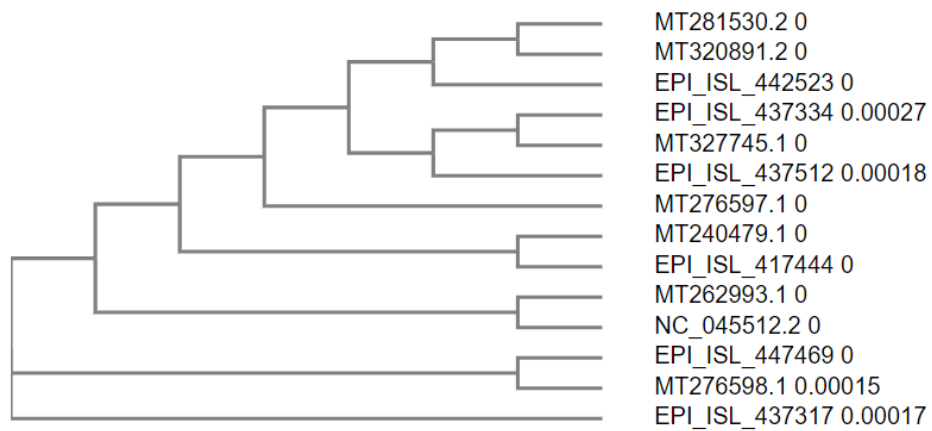


Figura 4: Albero di output derivato dall'allineamento con ClustalW