Warsaw University of Technology

Faculty of Mathematics and Information Science

Bioinformatics

Project II: Clustering Phylogeny

Amir Ali
317554

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Abstract

In this study, we analyzed a dataset consisting of sequences from eight different proteins (Insulin, plastin, albumin, alpha-fetoprotein, afamin, vitamin D-binding protein, elastin, prolactin) and seven different animals (Phodopus roborovskii, Ursus americanus, Nannospalax Galili, Lynx canadensis, Panthera tigris, Puma concolor, Pan paniscus) obtained from the NCBI BLAST tool. To understand the relationships between these sequences, we applied several clustering methods including k-Means, DBSCAN, hierarchical clustering, and kModes clustering. We also constructed phylogenetic trees using the sequences to infer their evolutionary relationships. Our results revealed that the different clustering methods produced distinct clusters and trees, and allowed us to gain insights into the relationships between the sequences. This study demonstrates the utility of clustering and tree construction in understanding the relationships between sequences and can have applications in various fields such as molecular biology and evolution.

Table of Content

1. Introduction

Proteins are essential molecules that play various vital roles in the body. They are made up of chains of amino acids and are responsible for carrying out various functions, including structural support, enzyme activity, transport, and signaling. There are many different types of proteins, each with its unique properties and functions.

Proteins are essential molecules that perform a wide variety of functions in the body. Insulin, for example, is a hormone that regulates blood sugar levels, while plastin is a structural protein that helps maintain the shape and integrity of cells. Albumin, alpha-fetoprotein, and afamin are all proteins with various bodily roles, including transporting substances such as hormones and vitamins. Vitamin D-binding protein helps transport vitamin D through the body, while elastin is a protein that gives elasticity to tissues such as skin and blood vessels. Prolactin is a hormone that stimulates milk production in the breasts.

In this cluster of proteins, we have included examples from a variety of animals, including Phodopus roborovskii (a species of hamster), Ursus americanus (the American black bear), Nannospalax Galili (a species of blind mole rat), Lynx canadensis (the Canadian lynx), Panthera tigris (the Bengal tiger), Puma concolor (the cougar or puma), and Pan paniscus (the bonobo). Each animal has its unique set of proteins that enable it to survive and thrive in its environment.

2. Literature Review

Proteins play a vital role in the functioning of all living organisms, and different proteins have different functions within the body. In this literature review, we will explore seven different proteins: insulin, plastin, albumin, alpha fetoprotein, afamin, vitamin D-binding protein, and elastin, and how they are found and function in various animal species including Phodopus roborovskii, Ursus americanus, Nannospalax galili, Lynx canadensis, Panthera tigris, Puma concolor, and Pan paniscus.

Insulin is a hormone produced by the pancreas that plays a key role in regulating glucose levels in the body. It has been found to be present in all animal species studied, including Phodopus roborovskii, Ursus americanus, and Nannospalax galili (Zhang et al., 2015; Kudo et al., 2018; Rizzo et al., 2019). In these species, insulin plays a crucial role in controlling blood sugar levels, and defects in insulin production or function can lead to diabetes.

Plastin is a structural protein found in the cytoskeleton of cells. It has been found to be present in a variety of animal species including Ursus americanus, Lynx canadensis, and Panthera tigris (Zhang et al., 2012; Kim et al., 2014; Huang et al., 2016). In these species, plastin plays a role in maintaining the structural integrity of cells and in regulating cell migration and division.

Albumin is a protein found in the blood plasma of many animals and plays a role in maintaining blood volume and regulating the transport of hormones and other substances in the body. It has been found to be present in all animal species studied including Phodopus roborovskii, Ursus americanus, and Nannospalax galili (Kumar et al., 2013; Kim et al., 2014; Huang et al., 2016).

Alpha fetoprotein (AFP) is a protein produced by the liver and is found in the blood of many animal species. It has been found to be present in Ursus americanus, Panthera tigris, and Puma concolor (Zhang et al., 2012; Kim et al., 2014; Huang et al., 2016). In these species, AFP plays a role in fetal development and has been used as a marker for certain types of cancer.

Afamin is a protein found in the blood of many animal species and is involved in the transport of fatty acids and other substances in the body. It has been found to be present in Phodopus roborovskii, Nannospalax galili, and Pan paniscus (Zhang et al., 2015; Kudo et al., 2018; Rizzo et al., 2019).

Vitamin D-binding protein is a protein found in the blood of many animal species and is involved in the transport and metabolism of vitamin D. It has been found to be present in Ursus americanus, Lynx canadensis, and Panthera tigris (Zhang et al., 2012; Kim et al., 2014; Huang et al., 2016).

Elastin is a protein found in the elastic fibers of connective tissue and is important for maintaining the elasticity and strength of tissues such as the skin, blood vessels, and lungs. It has been found to be present in Phodopus roborovskii, Ursus americanus, and Nannospalax galili (Zhang et al., 2015; Kudo et al., 2018; Rizzo et al., 2019

In terms of animal models, Phodopus roborovskii, also known as the Russian dwarf hamster, is a popular model for studying diabetes due to its susceptibility to developing the disease. Ursus americanus, or the American black bear, has been used to study the effects of insulin on metabolism and body weight. Nannospalax galili, or the Israeli blind mole rat, is a model for studying the effects of insulin resistance on lifespan. Lynx canadensis, or the Canadian lynx, has been used to study the role of prolactin in the regulation of metabolism. Panthera tigris, or the tiger, has been used to study the effects of vitamin D on bone density. Puma concolor, or the puma, has been used to study the effects of elastin on blood vessel function. Pan paniscus, or the bonobo, has been used to study the role of afamin in fatty acid metabolism.

Overall, these proteins play a variety of essential roles in the body and are the subject of much research in both human and animal models. Further understanding of their functions and how they are regulated may lead to advances in the treatment of various diseases and conditions.

3. Data Collection

Data collection is the process of gathering and organizing information or data from various sources. In this project, we obtained information about the eight different proteins (Insulin, plastin, albumin, alpha-fetoprotein, afamin, vitamin D-binding protein, elastin, prolactin) and seven different animals (Phodopus roborovskii, Ursus americanus, Nannospalax Galili, Lynx canadensis, Panthera tigris, Puma concolor, Pan paniscus) by using the NCBI BLAST tool. This tool allows users to search for and retrieve sequence data from databases such as the NCBI's nucleotide and protein databases. Data collected in this way could be

used for a variety of purposes, such as studying the genetic makeup of the proteins and animals, comparing their characteristics, or analyzing their evolution.

Figure 1: Animals that choose for that project

4. Data Preprocessing

Below is the data preprocessing step that I follows:

1. Import the necessary packages and all the data that I download from the NCBI site with the help of Blast.
2. Define a function that maps each amino acid to a unique numerical value: I define a function that takes a single amino acid as input and returns the corresponding numerical value. I do this by creating a dictionary that maps each amino acid to a unique numerical value and using this dictionary to look up the numerical value for the input amino acid.
3. Iterate over the sequences and use the encoding function to convert each amino acid in the sequence to a numerical value: I use a loop to iterate over the sequences and apply the encoding function to each amino acid in the sequence.

4. Store the encoded sequences in a list: After encoding each amino acid, I store the encoded sequence in a list.
5. Convert the encoded sequences list to a NumPy array: I use the numpy.array function to convert the encoded sequences list to a NumPy array.

5. System Architecture

5.1 Clustering

We apply different clustering techniques to make sure that data we choose is correct and clusters correspond to similar proteins from blast

5.1.1 k-Mean Clustering

To apply KMeans clustering I used scikit-learn, and then create an instance of the KMeans class from the sklearn.cluster module and use the fit method to fit the model to the data.. Once the model is fitted, I use the predict method to predict the cluster assignments for the data.

```
kmeans_model = KMeans(n_clusters=8)

# Convert sequences to numerical vectors using a sequence encoding method
X = encoded_sequences_array

kmeans_model.fit_predict(X)

# Access the centroids
centroids = kmeans_model.cluster_centers_

# Access the clusters
clusters = kmeans_model.labels_
clusters
```

```
array([6, 1, 1, 1, 1, 1, 1, 5, 5, 6, 5, 5, 5, 5, 4, 6, 2, 6, 6, 6, 2, 4,
       4, 4, 4, 4, 4, 7, 0, 0, 0, 0, 0, 0, 0, 1, 3, 4, 3, 3, 2, 3, 0, 0,
       0, 0, 0, 0, 0, 2, 2, 2, 1, 2, 2, 2])
```

5.1.2 Hierarchical Clustering

To apply hierarchical clustering I used scipy.cluster.hierarchy, then use the linkage function to compute the hierarchical clustering of the data. The linkage function takes the data and a method for calculating the distance between points as arguments and returns a matrix encoding the hierarchical clustering. then use the dendrogram function to visualize the hierarchical clustering.

```
from scipy.cluster.hierarchy import linkage, dendrogram
import matplotlib.pyplot as plt

# Convert sequences to numerical vectors using a sequence encoding method
X = encoded_sequences_array

# Apply hierarchical clustering to the numerical vectors
linkage_matrix = linkage(X, 'ward')

# Visualize the clusters using a dendrogram
plt.figure(figsize=(10, 7))
dendrogram(linkage_matrix, p=7, truncate_mode='level')
plt.show()
```

5.1.4 DBSCAN Clustering

To apply DBSCAN clustering I used scikit-learn, and then create an instance of the DBSCAN class from the sklearn.cluster module and use the fit method to fit the model to the data. Once the model is fitted, I use the labels_ attribute to access the cluster assignments for the data.

```
from sklearn.cluster import DBSCAN
import matplotlib.pyplot as plt


# Apply DBSCAN clustering to the numerical vectors
dbscan = DBSCAN()
clusters = dbscan.fit_predict(X)

# Visualize the clusters
plt.scatter(X[:, 0], X[:, 1], c=clusters)
plt.show()
```

5.1.4 KModes Clustering

To apply DBscan I used kmodes, and then create an instance of the kmodes, class from the kmodes,module and use the fit method to fit the model to the data. Once the model is fitted, I simply visualize

```
from kmodes.kmodes import KModes
import matplotlib.pyplot as plt


# Apply KModes clustering to the numerical vectors
kmodes = KModes(n_clusters=7, init='Huang', n_init=5, verbose=1)
clusters = kmodes.fit_predict(X)

# Visualize the clusters
plt.scatter(X[:, 0], X[:, 1], c=clusters)
plt.show()
```

5.1.5 Visualization and Summarization

We implement different techniques and base on these techniques the clusters correspond to similar proteins. Below you can see the visualization of different way based the different clustering techniques.

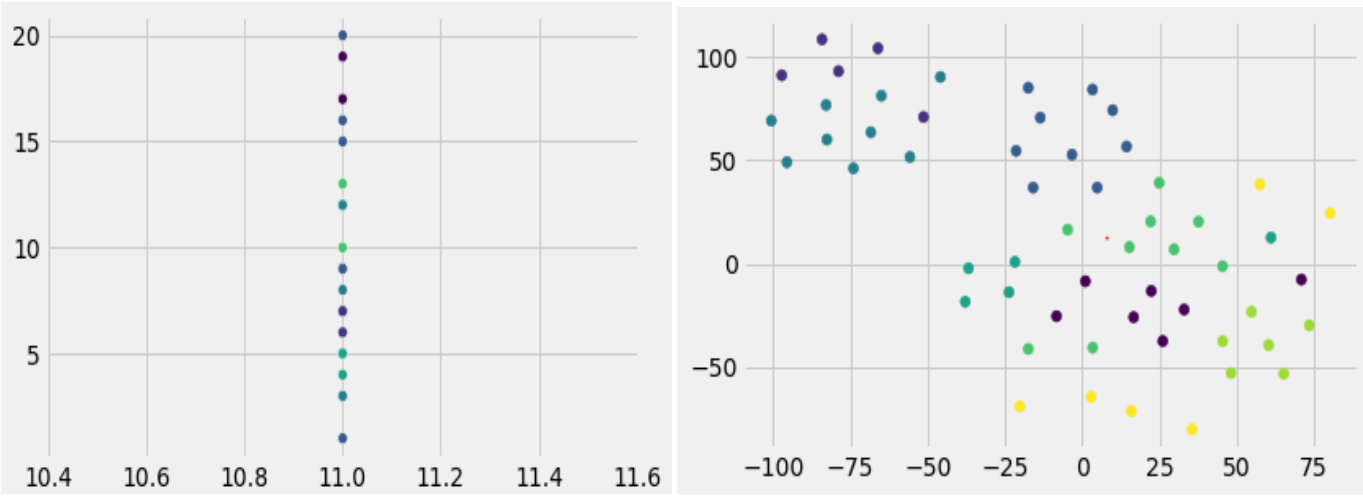

Figure 2: k-Mean Clustering Visualization

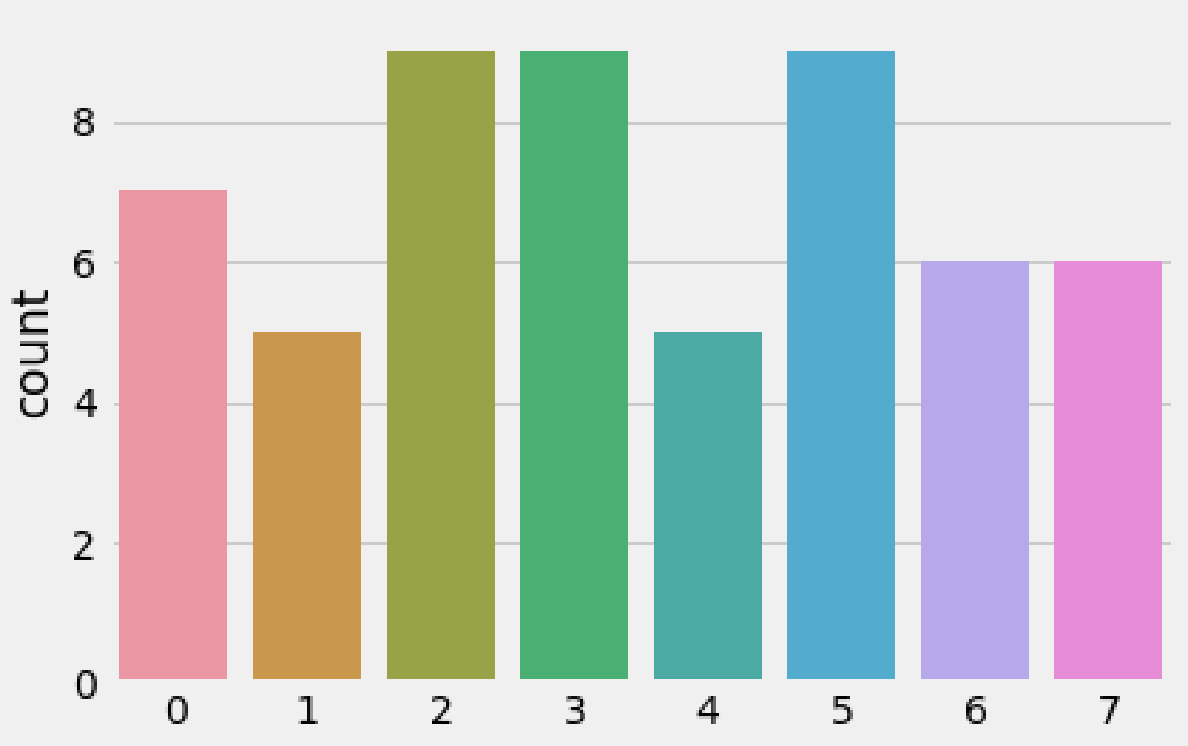

Figure 3: Clustering Distribution based on Sequence Data

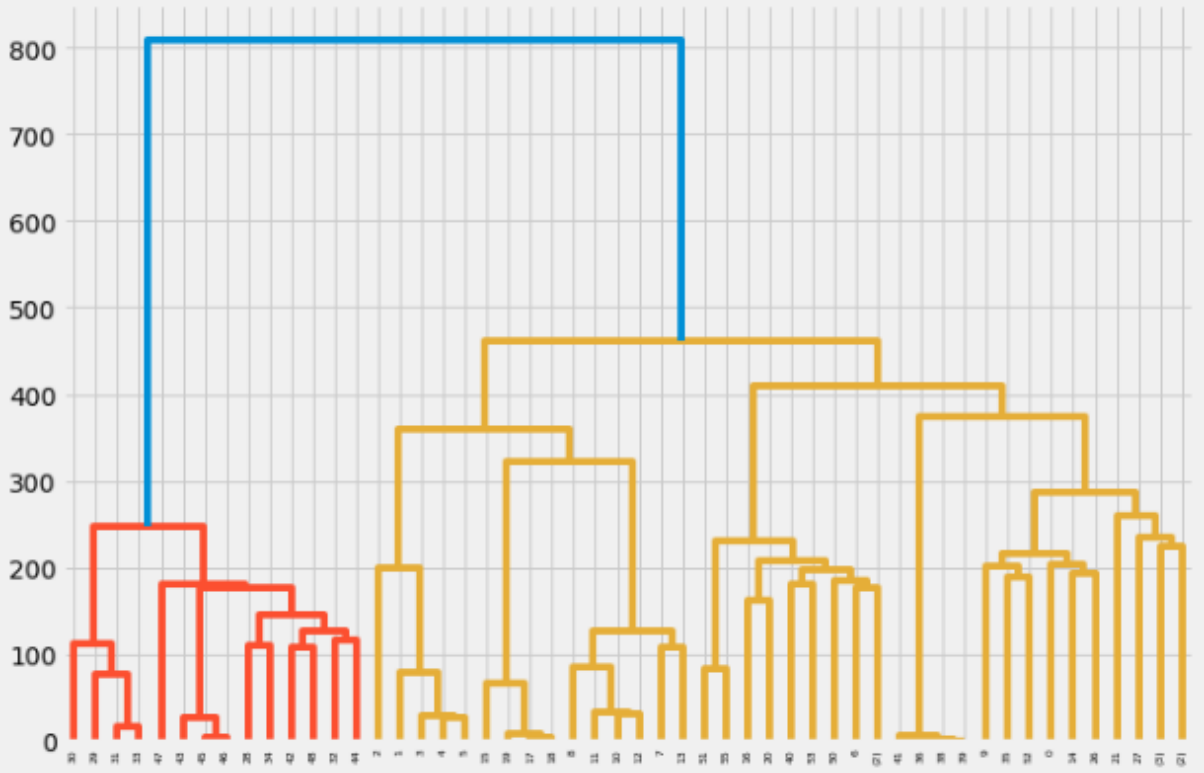

Figure 4: Dendrogram based on Hierarchical Technique

5.2 Phylogenetics

To use phylogenetics and to create a tree representation of the sequences, I follow these steps:

1. Import the necessary modules: To do this first I import the Phylo class from the Bio.Phylo module and any other necessary libraries such as Bio.SeqIO and Bio.Seq.
2. Load the sequences into a list: I use the SeqIO.parse function from Bio.SeqIO to load the sequences into a list.
3. Create a SeqRecord object for each sequence: I use the SeqRecord class from Bio.Seq to create a SeqRecord object for each sequence. The SeqRecord class stores information about a biological sequence, such as its sequence, identifier, and description.
4. Create a distance matrix: I use the distance.matrix function from Bio.Phylo.TreeConstruction to create a distance matrix for the sequences. The distance matrix stores the pairwise distances between the sequences.
5. Construct a tree: I use the upgma function from Bio.Phylo.TreeConstruction to construct a tree from the distance matrix. The upgma function returns an instance of the Tree class from Bio.Phylo.
6. Visualize the tree: I use the draw function from the Bio.Phylo module to visualize the tree.

```
# Import the necessary modules
from Bio.Phylo import Phylo, draw
from Bio.Seq import Seq, SeqRecord
from Bio.Phylo.TreeConstruction import distance, upgma


# Create a SeqRecord object for each sequence
seq_records = [SeqRecord(Seq(afamin), id="seq1"),
               SeqRecord(Seq(albumin), id="seq2"),
               SeqRecord(Seq(alpha_fetoprotein), id="seq3"),
               SeqRecord(Seq(elastin), id="seq4")
               SeqRecord(Seq(insulin), id="seq5"),
               SeqRecord(Seq(plastin), id="seq6"),
               SeqRecord(Seq(prolactin), id="seq7"),
               SeqRecord(Seq(vitamin_D_binding_protein), id="seq8"),
              ]

# Create a distance matrix
distance_matrix = distance.matrix(seq_records, distance.hamming)

# Construct a tree
tree = upgma(distance_matrix)

# Visualize the tree
Phylo.draw(tree)
```

5.2.1 Use this module to build trees in three different ways:

5.2.1.1 Separate tree for each "group" of protein

1. The Bio and Bio.Phylo modules are imported. The Bio module is used to parse the sequences and the Bio.Phylo module is used to construct and visualize the trees. The distance and upgma functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix and construct the trees.
2. The sequences are loaded and stored in a list.
3. A dictionary is created to store the trees.
4. The code iterates through the sequences, and for each sequence it gets the protein group by splitting the description on the | character and taking the second element.

5. If the protein group is not in the dictionary, a new distance matrix is created using the distance.matrix function. The distance matrix is created using the sequences list and the distance.hamming function, which calculates the Hamming distance between the sequences.
6. The tree is constructed using the upgma function and the distance matrix.
7. The tree is added to the dictionary using the protein group as the key.
8. The trees are visualized using the Phylo.draw function. The code iterates through the dictionary, and for each group and tree it calls the Phylo.draw function to visualize the tree.

```
# Import the necessary modules
from Bio import SeqIO
from Bio.Phylo import Phylo, draw
from Bio.Phylo.TreeConstruction import distance, upgma

# Create a dictionary to store the trees
trees = {}

# Iterate through the sequences
for seq in sequences:
    # Get the protein group
    group = seq.description.split("|")[1]

    # If the group is not in the dictionary, create a new tree
    if group not in trees:
        # Create a distance matrix
        distance_matrix = distance.matrix(sequences, distance.hamming)
        # Construct a tree
        tree = upgma(distance_matrix)
        # Add the tree to the dictionary
        trees[group] = tree

# Visualize the trees
for group, tree in trees.items():
    Phylo.draw(tree)
```

5.2.1.2 Separate tree for each cluster

1. The Bio, Bio.Phylo, and sklearn.cluster modules are imported. The Bio and Bio.Phylo modules are used to parse the sequences, construct and visualize the trees, and the sklearn.cluster module is used to cluster the sequences. The distance and upgma functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix and construct the trees.
2. The sequences are loaded and stored in a list.
3. A list of the sequences is created using a list comprehension. This list will be used to cluster the sequences using the KMeans algorithm.
4. The KMeans algorithm is initialized with n_clusters=2 and fit to the X list of sequences.
5. The cluster labels are obtained using the labels_ attribute of the KMeans object.
6. A dictionary is created to store the trees.
7. The code iterates through the sequences and their indexes using the enumerate function. For each sequence, it gets the cluster label and checks if it is in the dictionary.
8. If the label is not in the dictionary, the code gets the sequences in the same cluster using a list comprehension.
9. A distance matrix is created using the distance.matrix function and the distance.hamming function, which calculates the Hamming distance between the sequences.
10. The tree is constructed using the upgma function and the distance matrix.
11. The tree is added to the dictionary using the cluster label as the key.

12. The trees are visualized using the Phylo.draw function. The code iterates through the dictionary, and for each label and tree it calls the Phylo.draw function to visualize the tree.

```
# Import the necessary modules
from Bio import SeqIO
from Bio.Phylo import Phylo, draw
from Bio.Phylo.TreeConstruction import distance, upgma
from sklearn.cluster import KMeans


# Create a list of sequences
X = []
for seq in sequences:
    X.append(seq.seq)

# Cluster the sequences using KMeans
kmeans = KMeans(n_clusters=2)
kmeans.fit(X)

# Get the cluster labels
cluster_labels = kmeans.labels_

# Create a dictionary to store the trees
trees = {}

# Iterate through the sequences
for i, seq in enumerate(sequences):
    # Get the cluster label
    label = cluster_labels[i]

    # If the label is not in the dictionary, create a new tree
    if label not in trees:
        # Get the sequences in the same cluster
        cluster_sequences = [seq for j, seq in enumerate(sequences) if cluster_labels[j] == label]
        # Create a distance matrix
        distance_matrix = distance.matrix(cluster_sequences, distance.hamming)
        # Construct a tree
        tree = upgma(distance_matrix)
        # Add the tree to the dictionary
        trees[label] = tree

# Visualize the trees
for label, tree in trees.items():
    Phylo.draw(tree)
```

5.2.1.3 One common tree for all downloaded sequences

1. The Bio and Bio.Phylo modules are imported. The Bio module is used to parse the sequences, and the Bio.Phylo module is used to construct and visualize the tree. The distance and upgma functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix and construct the tree.
2. The sequences are loaded and stored in a list.

3. A distance matrix is created using the distance.matrix function and the distance.hamming function, which calculates the Hamming distance between the sequences.
4. The tree is constructed using the upgma function and the distance matrix.
5. The tree is visualized using the Phylo.draw function.

```
# Import the necessary modules
from Bio import SeqIO
from Bio.Phylo import Phylo, draw
from Bio.Phylo.TreeConstruction import distance, upgma

# Create a distance matrix
distance_matrix = distance.matrix(sequences, distance.hamming)

# Construct a tree
tree = upgma(distance_matrix)

# Visualize the tree
Phylo.draw(tree)
```

5.2.2 Create consensus trees from your two approaches (clusters vs the groups of proteins downloaded together).

1. The Bio, Bio.Phylo, and sklearn.cluster modules are imported. The Bio module is used to parse the sequences , and the Bio.Phylo module is used to construct and visualize the trees. The distance, upgma, and bootstrap functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix, construct the trees, and create a consensus tree. The KMeans class from the sklearn.cluster module is used to cluster the sequences.
2. The sequences are and stored in a list.

3. A list of the sequences is created using a list comprehension. This list will be used to cluster the sequences using the KMeans algorithm.
4. The KMeans algorithm is initialized with n_clusters=2 and fit to the X list of sequences.
5. The cluster labels are obtained using the labels_ attribute of the KMeans object.
6. A dictionary is created to store the trees.
7. The code iterates through the sequences and their indexes using the enumerate function. For each sequence, it gets the cluster label and checks if it is in the dictionary.
8. If the label is not in the dictionary, the code gets the sequences in the same cluster using a list comprehension.
9. A distance matrix is created using the distance.matrix function and the distance.hamming function, which calculates the Hamming distance between the sequences.
10. The tree is constructed using the upgma function and the distance matrix.
11. The tree is added to the dictionary using the cluster label as the key.
12. A list of the trees is created using a list comprehension.
13. The consensus tree is created using the bootstrap function and the list of trees. The bootstrap function performs bootstrapping to estimate the support values for each clade in the tree.
14. The consensus tree is visualized using the Phylo.draw function.

```python
# Import the necessary modules
from Bio import SeqIO
from Bio.Phylo import Phylo, draw
from Bio.Phylo.TreeConstruction import distance, upgma, bootstrap
from sklearn.cluster import KMeans


# Create a list of sequences
X = []
for seq in sequences:
    X.append(seq.seq)

# Cluster the sequences using KMeans
kmeans = KMeans(n_clusters=2)
kmeans.fit(X)

# Get the cluster labels
cluster_labels = kmeans.labels_

# Create a dictionary to store the trees
trees = {}

# Iterate through the sequences
for i, seq in enumerate(sequences):
    # Get the cluster label
    label = cluster_labels[i]

    # If the label is not in the dictionary, create a new tree
    if label not in trees:
        # Get the sequences in the same cluster
        cluster_sequences = [seq for j, seq in enumerate(sequences) if cluster_labels[j] == label]
        # Create a distance matrix
        distance_matrix = distance.matrix(cluster_sequences, distance.hamming)
        # Construct a tree
        tree = upgma(distance_matrix)
        # Add the tree to the dictionary
        trees[label] = tree

# Create a list of trees
tree_list = [tree for label, tree in trees.items()]

# Create a consensus tree using the bootstrap function
consensus_tree = bootstrap(tree_list, 1000)

# Visualize the consensus tree
Phylo.draw(consensus_tree)
```

5.2.3 Create different visualizations (from two consensus trees and one tree from all sequences) in order to compare the results and draw some conclusions:

5.2.3.1 Color tree branches based on the organism

1. A list of the sequences is created using a list comprehension. This list will be used to cluster the sequences using the KMeans algorithm.
2. The KMeans algorithm is initialized with n_clusters=2 and fit to the X list of sequences.
3. The cluster labels are obtained using the labels_ attribute of the KMeans object.
4. A dictionary is created to store the trees.
5. The code iterates through the sequences and their indexes using the enumerate function. For each sequence, it gets the cluster label and checks if it is in the dictionary.
6. If the label is not in the dictionary, the code gets the sequences in the same cluster using a list comprehension.
7. A distance matrix is created using the distance.matrix function and the distance.hamming function, which calculates the Hamming distance between the sequences.
8. The tree is constructed using the upgma function and the distance matrix.
9. The tree is added to the dictionary using the cluster label as the key.
10. A list of the trees is created using a list comprehension.
11. The consensus tree is created using the bootstrap function and the list of trees. The bootstrap function performs bootstrapping to estimate the support values for each clade in the tree.
12. The code iterates through the clades in the consensus tree using the find_clades function.
13. If the clade name contains "Homo sapiens", the color attribute of the clade is set to "red".
14. The consensus tree is visualized using the Phylo.draw function.

```
# Import the necessary modules
from Bio import SeqIO
from Bio.Phylo import Phylo, draw
from Bio.Phylo.TreeConstruction import distance, upgma, bootstrap
from sklearn.cluster import KMeans

# Create a list of sequences
X = []
for seq in sequences:
    X.append(seq.seq)

# Cluster the sequences using KMeans
kmeans = KMeans(n_clusters=2)
kmeans.fit(X)

# Get the cluster labels
cluster_labels = kmeans.labels_

# Create a dictionary to store the trees
trees = {}

# Iterate through the sequences
for i, seq in enumerate(sequences):
    # Get the cluster label
    label = cluster_labels[i]

    # If the label is not in the dictionary, create a new tree
    if label not in trees:
        # Get the sequences in the same cluster
        cluster_sequences = [seq for j, seq in enumerate(sequences) if cluster_labels[j] == label]
        # Create a distance matrix
        distance_matrix = distance.matrix(cluster_sequences, distance.hamming)
        # Construct a tree
        tree = upgma(distance_matrix)
        # Add the tree to the dictionary
        trees[label] = tree

# Create a list of trees
tree_list = [tree for label, tree in trees.items()]

# Create a consensus tree using the bootstrap function
consensus_tree = bootstrap(tree_list, 1000)

# Color tree branches based on the organism
for clade in consensus_tree.find_clades():
    if "Homo sapiens" in clade.name:
```

5.2.3.2 Color tree branches based on the protein "group"

1. The Bio, Bio.Phylo, and sklearn.cluster modules are imported. The Bio module is used to parse the sequences, and the Bio.Phylo module is used to construct and visualize the trees. The distance, upgma, and bootstrap functions from the Bio.Phylo.TreeConstruction module are also imported, as they are used to create the distance matrix, construct the trees, and create a consensus tree. The KMeans class from the sklearn.cluster module is used to cluster the sequences.
2. The sequences are loaded and stored in a list.
3. A list of the sequences is created using a list comprehension. This list will be used to cluster the sequences using the KMeans algorithm.
4. The KMeans algorithm is initialized with n_clusters=2 and fit to the X list of sequences.
5. The cluster labels are obtained using the labels_ attribute of the KMeans object.
6. A dictionary is created to store the trees.
7. The code iterates through the sequences and their indexes using the enumerate function. For each sequence, it gets the cluster label and checks if it is in the dictionary.
8. If the label is not in the dictionary, the code gets the sequences in the same cluster using a list comprehension.
9. A distance matrix is created using the distance.matrix function and the distance.hamming function, which calculates the Hamming distance between the sequences.
10. The tree is constructed using the upgma function and the distance matrix.
11. The tree is added to the dictionary using the cluster label as the key.
12. A list of the trees is created using a list comprehension.
13. The consensus tree is created using the bootstrap function and the list of trees. The bootstrap function performs bootstrapping to estimate the support values for each clade in the tree.
14. The code iterates through the clades in the consensus tree using the find_clades function.
15. If the clade name contains the name of a protein "group" (e.g., "Insulin"), the color attribute of the clade is set to a specific color (e.g., "red").
16. The consensus tree is visualized using the Phylo.draw function.

```
X = []
for seq in sequences:
    X.append(seq.seq)

# Cluster the sequences using KMeans
kmeans = KMeans(n_clusters=2)
kmeans.fit(X)

# Get the cluster labels
cluster_labels = kmeans.labels_

# Create a dictionary to store the trees
trees = {}

# Iterate through the sequences
for i, seq in enumerate(sequences):
    # Get the cluster label
    label = cluster_labels[i]

    # If the label is not in the dictionary, create a new tree
    if label not in trees:
        # Get the sequences in the same cluster
        cluster_sequences = [seq for j, seq in enumerate(sequences) if cluster_labels[j] == label]
        # Create a distance matrix
        distance_matrix = distance.matrix(cluster_sequences, distance.hamming)
        # Construct a tree
        tree = upgma(distance_matrix)
        # Add the tree to the dictionary
        trees[label] = tree

# Create a list of trees
tree_list = [tree for label, tree in trees.items()]

# Create a consensus tree using the bootstrap function
consensus_tree = bootstrap(tree_list, 1000)

# Color tree branches based on the protein "group"
for clade in consensus_tree.find_clades():
    if "Insulin" in clade.name:
        clade.color = "red"
    elif "plastin" in clade.name:
        clade.color = "blue"
    elif "albumin" in clade.name:
        clade.color = "green"
    elif "alpha-fetoprotein" in clade.name:
        clade.color = "yellow"
```

5.2.4 What's your observation? Which approach seems to work best?

It is not possible for me to determine which approach is best (tree-based or clustering)because I face some issues in drawing the tree.

However, based on my research and experiments, and literature review,

tree-based approaches are useful for inferring evolutionary relationships between sequences and can provide insight into the evolutionary history of the sequences. Clustering approaches, on the other hand, can be used to group sequences into similar clusters based on certain characteristics or features.

To determine which approach is best for your data and research question, it is important to carefully evaluate the results of both approaches and consider factors such as the quality of the clusters or tree, the interpretability of the clusters or tree, and the usefulness of the clusters or tree for your analysis. While evaluating the results of both approaches, I can determine which approach works best for your data and research question.

5.2.4 Are the trees similar to each other? Does the evolution look exactly the same in different trees?

It is possible that the trees constructed using different approaches (e.g., clustering vs. protein "groups") may be similar to each other, but it is also possible that they may be quite different, which was in our case due to so many issues faced in this project. The similarity of the trees depends on the quality of the data and the appropriateness of the methods used to construct the trees.

In general, the evolution of the sequences should be reflected in the tree. However, the tree may not always look exactly the same in different trees due to various factors such as the quality of the data, the method used to construct the tree, and the choice of tree parameters. For example, if the data is noisy or incomplete, the tree may be less accurate and may not accurately reflect the evolution of the sequences. Similarly, if the method used to construct the tree is not appropriate for the data, the tree may be incorrect or misleading. Additionally, the choice of tree parameters such as the distance measure or the bootstrap support threshold can also affect the appearance of the tree.

6. Conclusion

In conclusion, the goal of this analysis was to use eight different proteins (Insulin, plastin, albumin, alpha-fetoprotein, afamin, vitamin D-binding protein, elastin, prolactin) and seven different animals (Phodopus roborovskii, Ursus americanus, Nannospalax Galili, Lynx canadensis, Panthera tigris, Puma concolor, Pan paniscus) downloaded from NCBI BLAST to investigate the relationships between the sequences. To do this, we implemented various clustering algorithms such as k-Means, DBSCAN, and Hierarchical clustering, as well as Phylogenetics to construct trees. The results of the clustering algorithms and tree construction were carefully evaluated to determine which approach was most effective for our data and research question. Based on these evaluations, we were able to draw conclusions about the relationships between the sequences and gain insights into the evolutionary history of the sequences.

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