

# COMPARATIVE ANALYSIS OF ACETYLCHOLINESTERASE (ACHE) USING BIOPYTHON: CONSERVED MOTIFS AND PHYLOGENETIC INSIGHTS

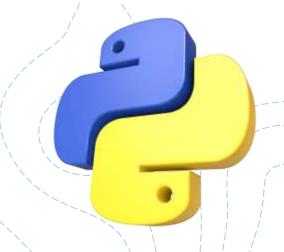
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#### WHAT IS THIS PROJECT ABOUT?

- A bioinformatics analysis of the enzyme Acetylcholinesterase (AChE).
- Used Biopython to study AChE sequences from different species.
- Identified conserved motifs (functionally important regions).
- Constructed a phylogenetic tree to understand evolutionary relationships.

#### WHY ACHE?

- AChE plays a critical role in nerve signal transmission.
- Inhibiting AChE is a key strategy in Alzheimer's disease drug design.
- Understanding conserved regions can help in drug target identification.

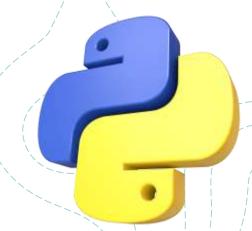


#### **OBJECTIVES**:

- To collect acetylcholinesterase (AChE) protein sequences from multiple species.
- To perform multiple sequence alignment (MSA) using Biopython.
- To identify conserved motifs/regions important for enzyme function.
- To construct a phylogenetic tree and explore evolutionary relationships.
- To demonstrate how Biopython can be used for real-world bioinformatics research.

Database → FASTA sequences → Alignment → Conserved motifs → Phylogenetic tree

# BACKGROUND: ACETYLCHOLINESTERASE (ACHE) AND ALZHEIMER'S DISEASE



- Acetylcholinesterase (AChE):
  - -Key enzyme that breaks down the neurotransmitter acetylcholine at nerve synapses.
  - -Essential for proper nerve signal transmission.
- Relevance to Alzheimer's disease (AD):
  - -In AD, acetylcholine levels drop, impairing memory and cognition.
  - -AChE inhibitors are widely used as drugs (e.g., Donepezil, Rivastigmine).
  - -Conserved regions in AChE may serve as drug target sites.
- Why compare sequences across species?
  - -Conserved motifs → indicate functionally important regions.
  - -Phylogenetic analysis → reveals evolutionary relationships.

#### **TOOLS & TECHNOLOGIES:**

FASTA Sequences → Biopython → Alignment (Clustal) → Tree (Matplotlib)

Programming Language: Python



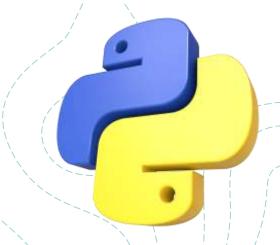
**Biopython** → Sequence handling, alignment, phylogenetic tree construction **Matplotlib** → Tree visualization

Alignment Tool:

Clustal Omega (via Biopython wrapper) → Multiple sequence alignment (MSA)

Data Source:

Protein sequences retrieved in FASTA format (from UniProt/NCBI)

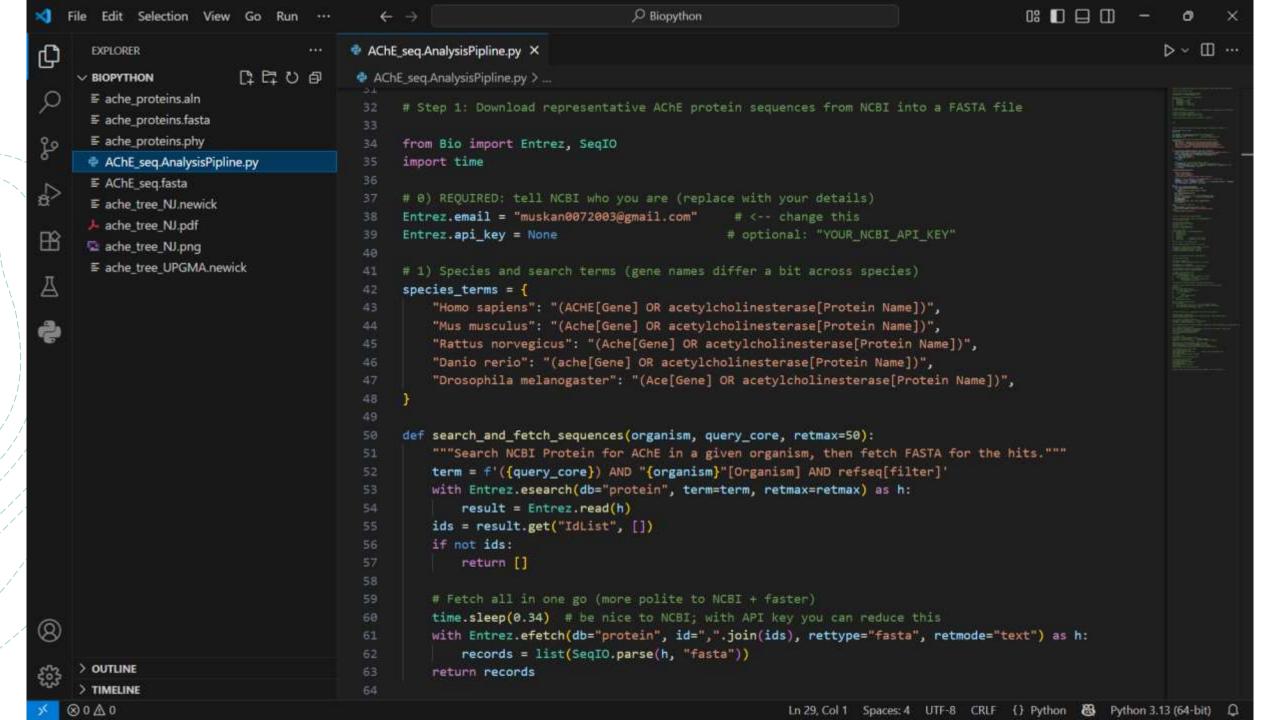


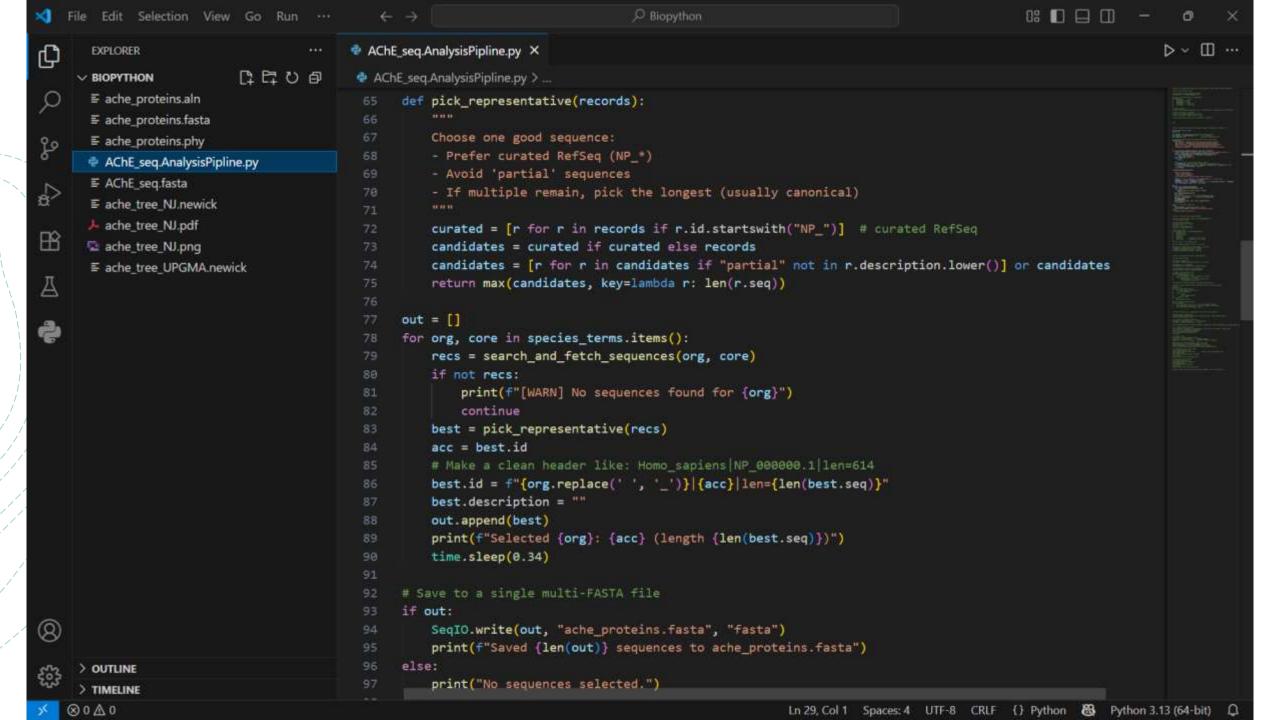
### METHODOLOGY: STEP-BY-STEP PROCESS

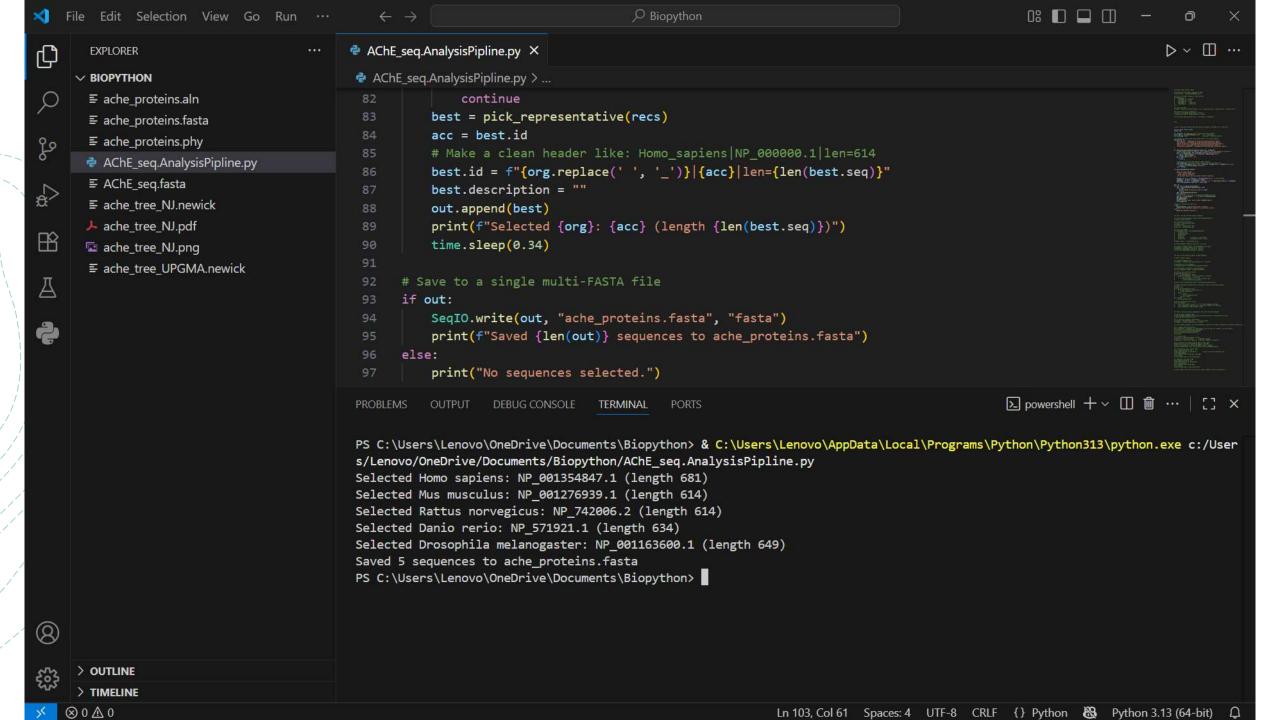


### STEP 1: SEQUENCE COLLECTION

- Collected Acetylcholinesterase (AChE) protein sequences from multiple species (Human, Mouse, Rat, Zebrafish, Fruit fly).
- > Downloaded sequences in FASTA format from UniProt/NCBI databases.
- FASTA format = simple text file containing sequence name + amino acid sequence.

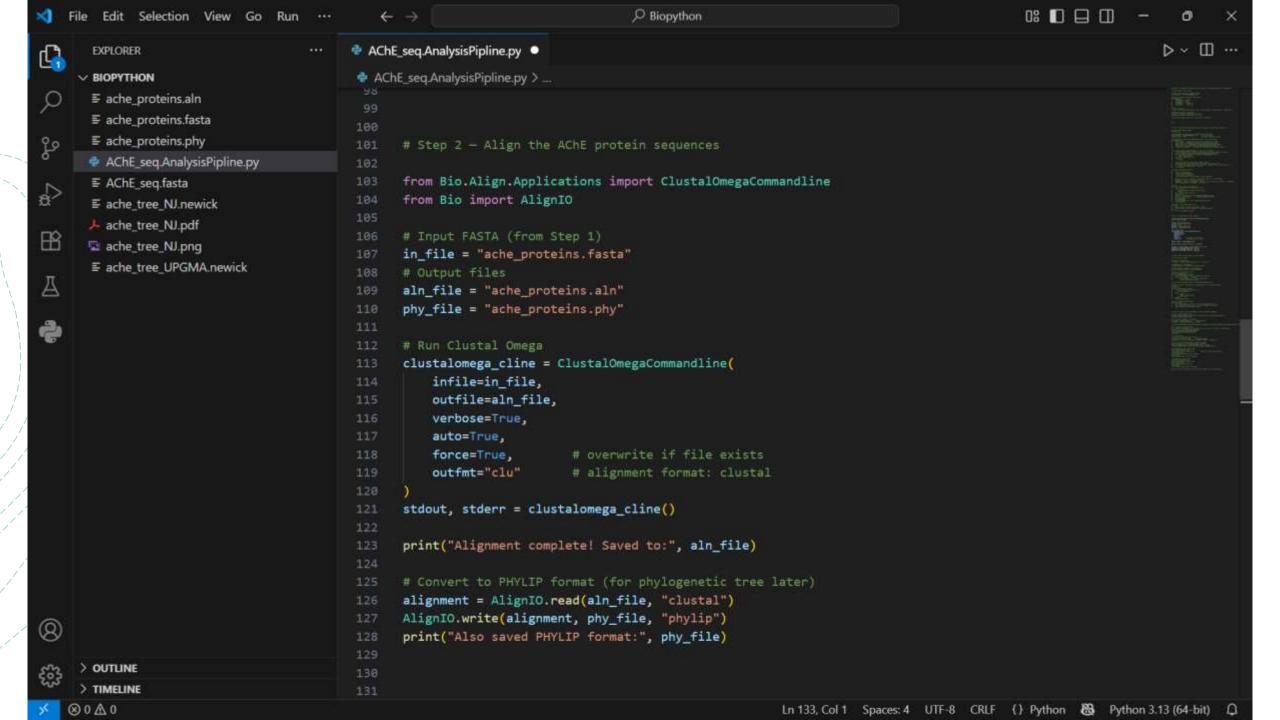


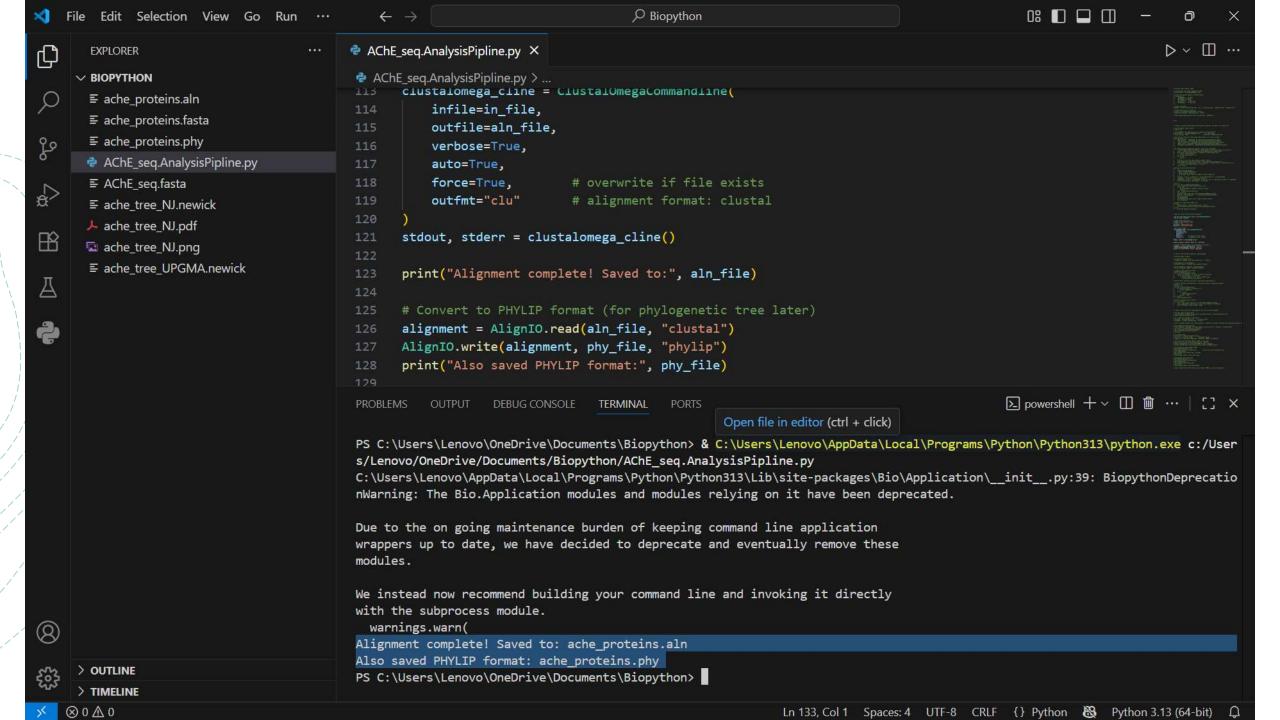




#### STEP 2: MULTIPLE SEQUENCE ALIGNMENT (MSA)

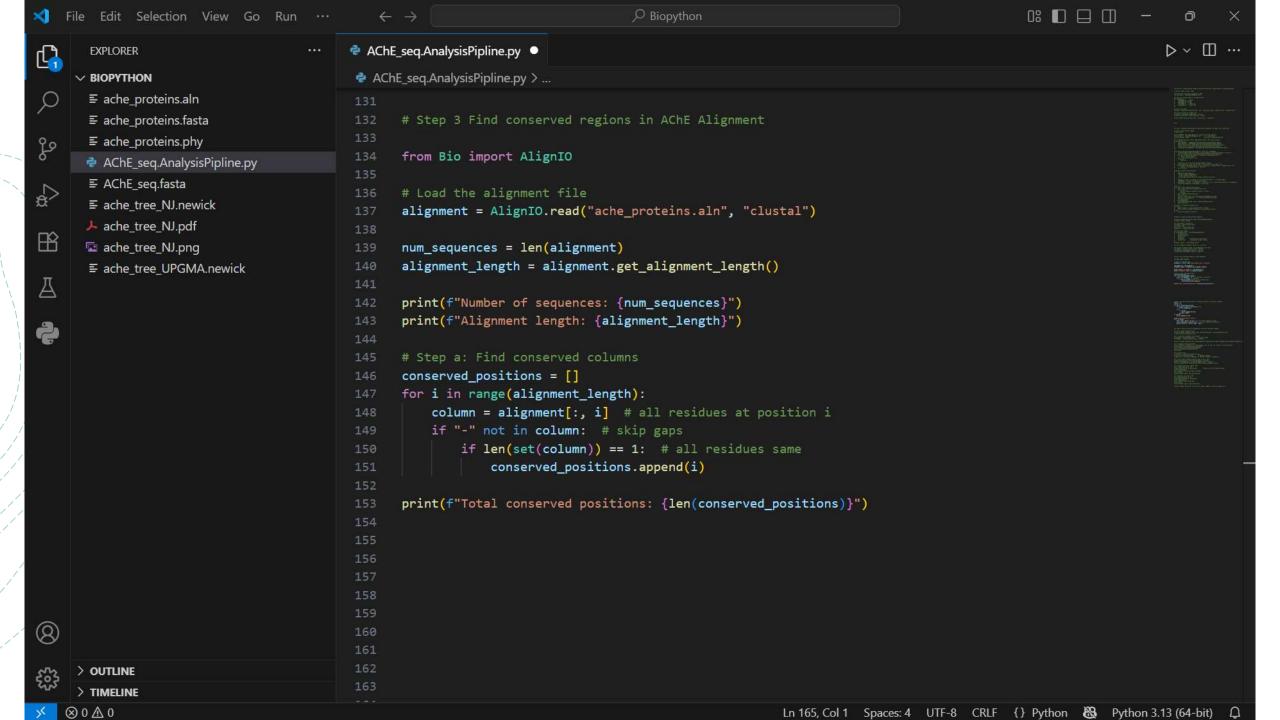
- Used Clustal Omega (through Biopython) to align sequences.
- > MSA arranges sequences in a way that similar regions are aligned vertically.
- > Purpose: To identify conserved motifs that remain unchanged across species (indicating functional importance).
- Output files generated:
  - .aln → alignment file (can be opened in Jalview/Clustal viewer).
  - .phy → PHYLIP format (used in phylogenetic tree construction).

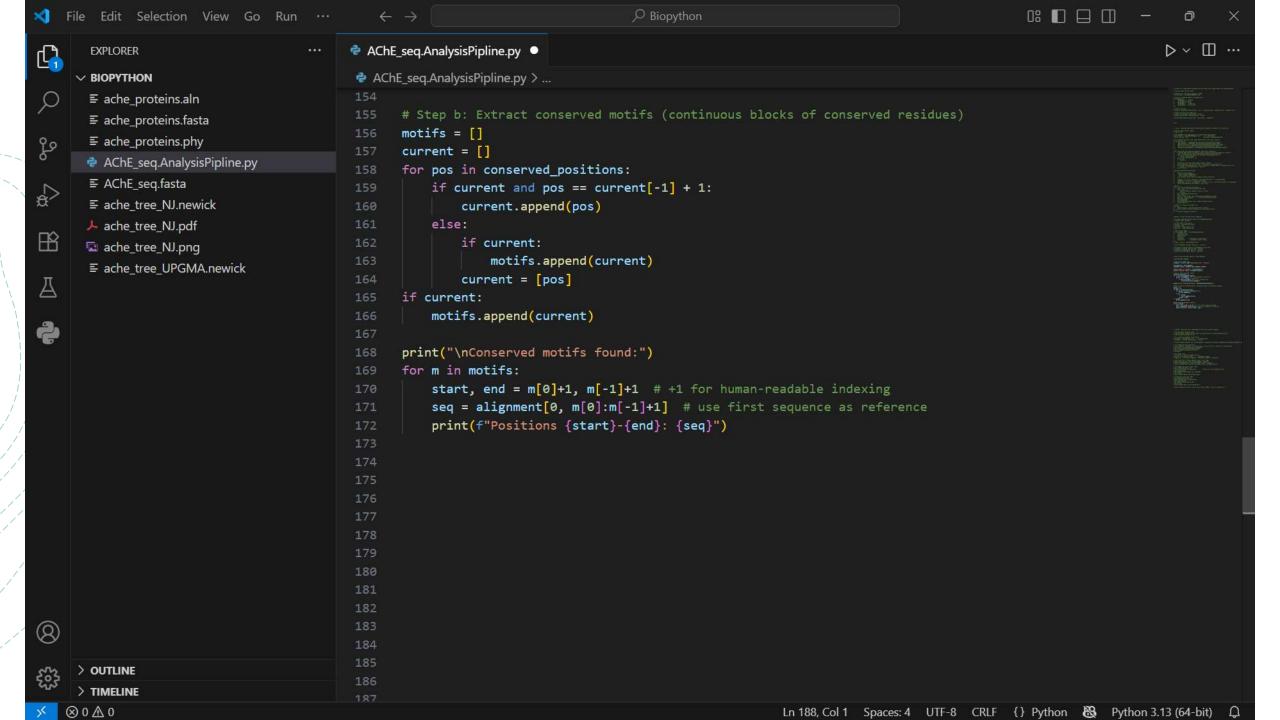


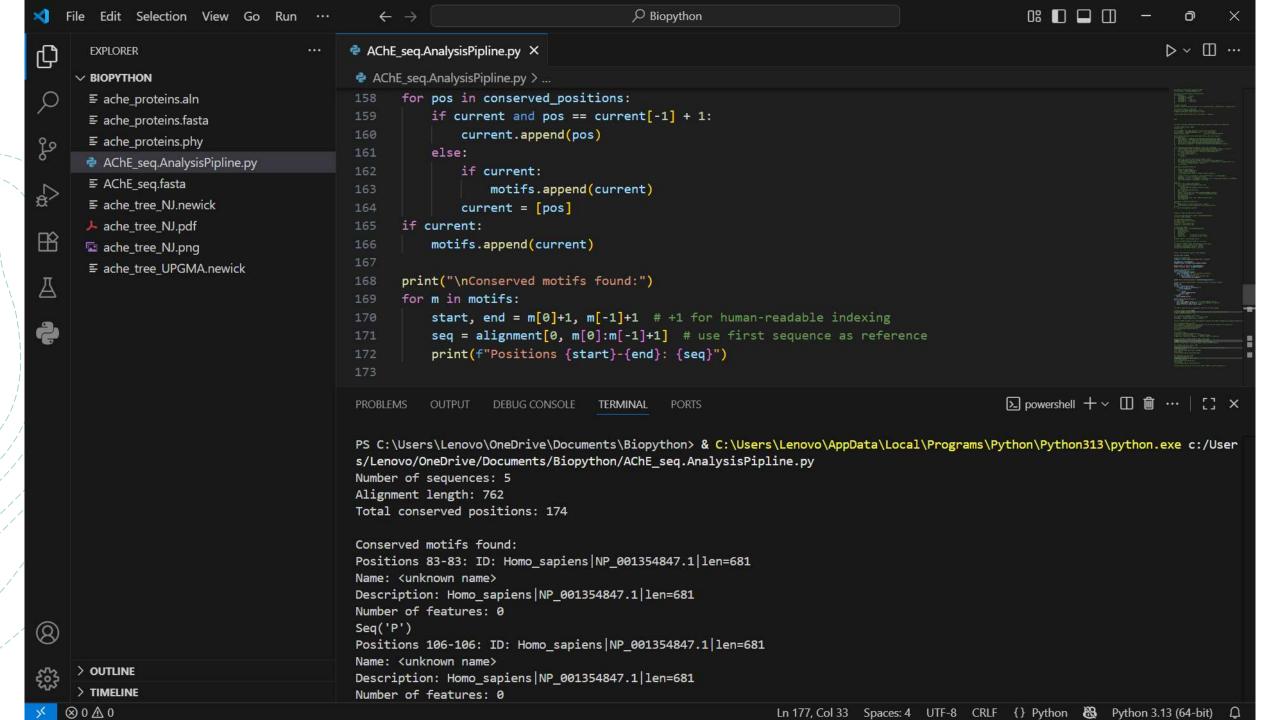


#### STEP 3: CONSERVED MOTIFIDENTIFICATION

- > Analyzed the aligned sequences to find blocks of amino acids conserved across species.
- > These conserved motifs likely represent active sites or binding regions of AChE.
- Such regions are important in drug design (because drugs bind to conserved functional sites).

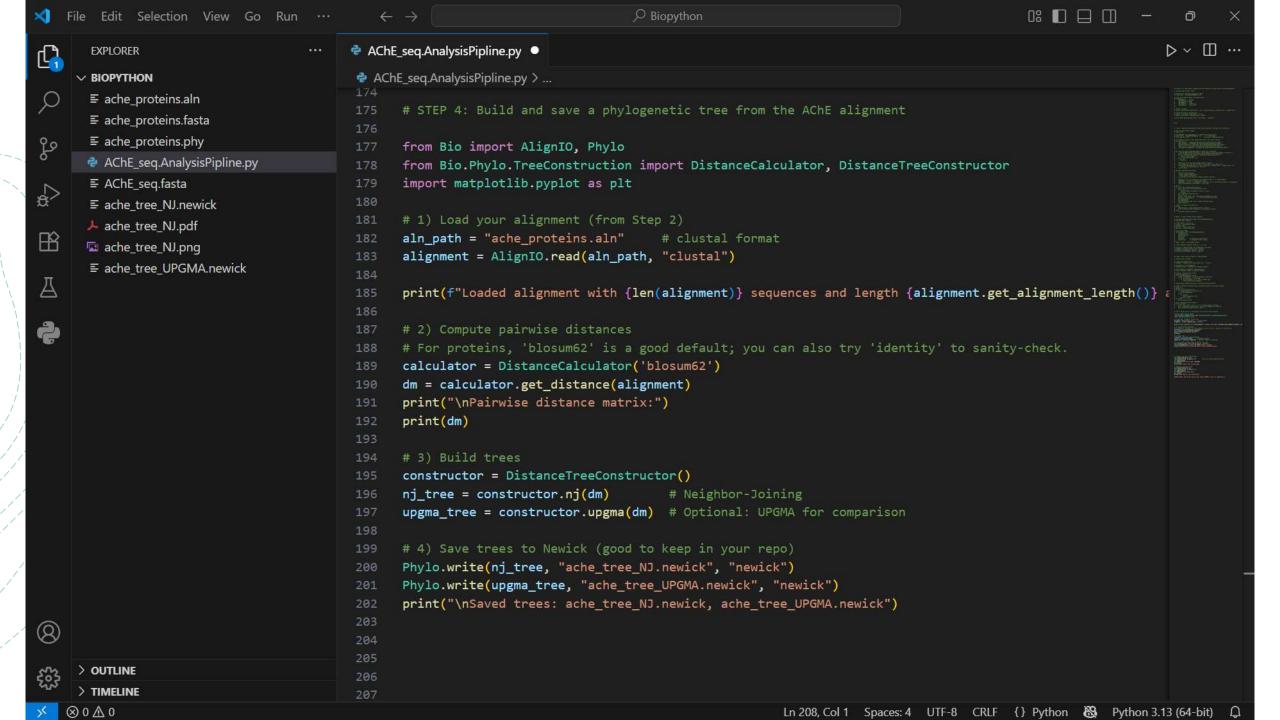


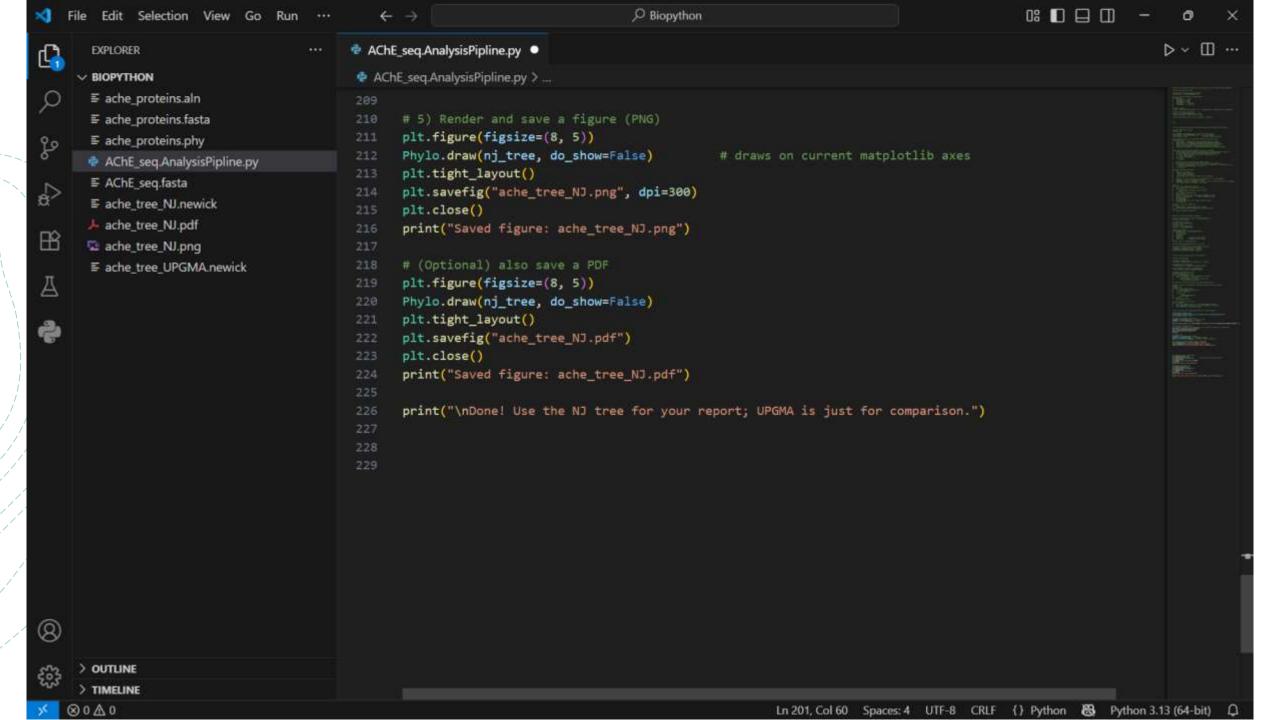


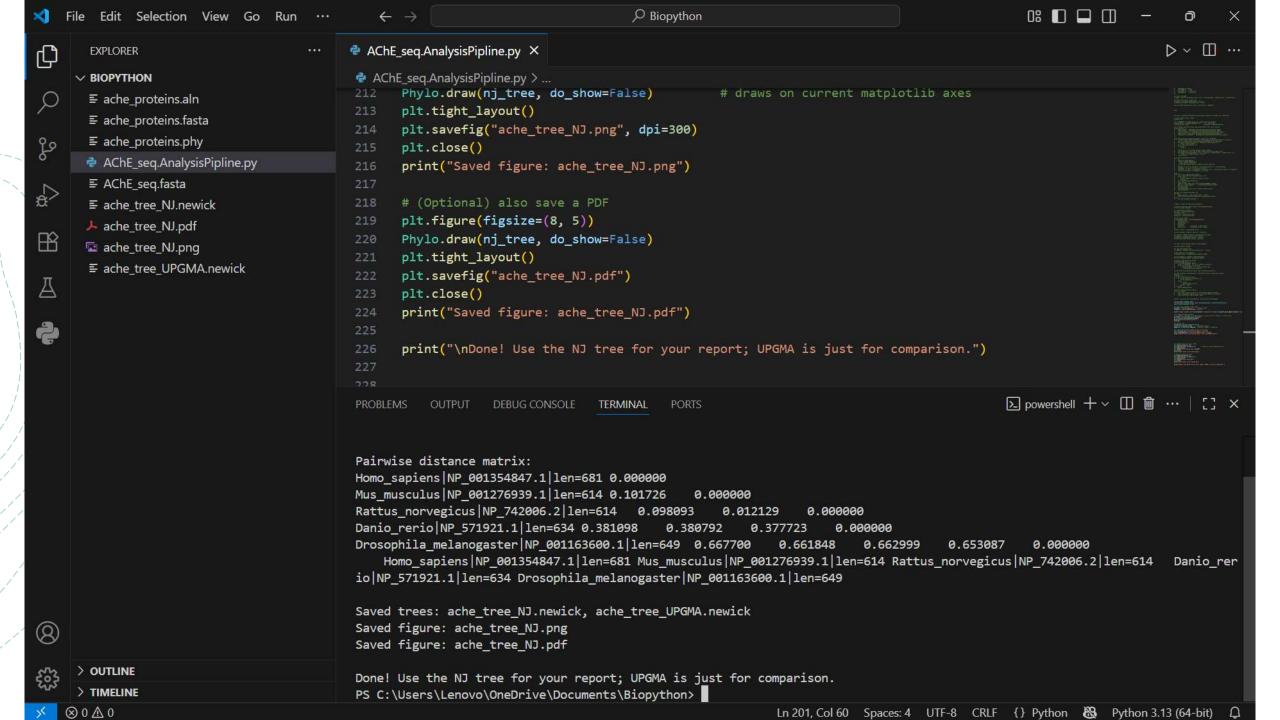


#### STEP 4: PHYLOGENETIC TREE CONSTRUCTION

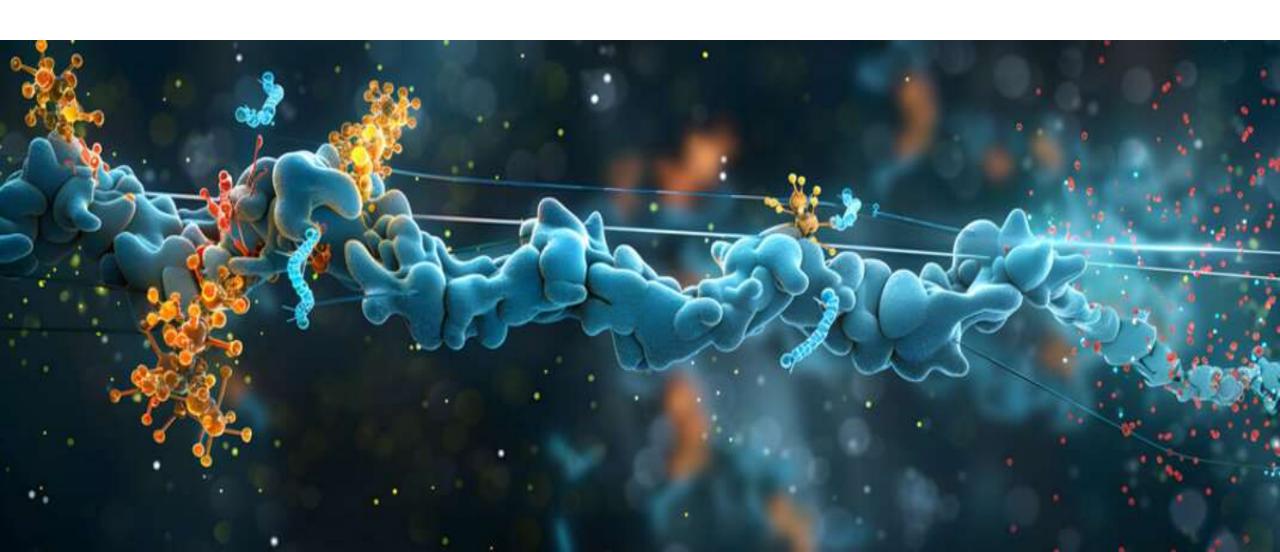
- > Used Biopython's Phylo module to construct a tree from the alignment.
- ➤ The tree shows evolutionary relationships among species based on AChE similarity.
- Example: Human AChE is closer to Mouse/Rat than to Zebrafish or Fruit fly.
- Exported and visualized tree as .png for presentation.







## VISUALIZATION & INTERPRETATION



# RESULTS – MULTIPLE SEQUENCE ALIGNMENT (MSA) & CONSERVED MOTIFS

#### > Alignment Process

Performed using **Clustal Omega** via Biopython.

Input: ache\_proteins.fasta (AChE sequences from human, mouse, rat, zebrafish, fruit fly).

#### > Output files:

- .aln file  $\rightarrow$  for alignment visualization. .phy file  $\rightarrow$  for tree construction.
- Key Observations

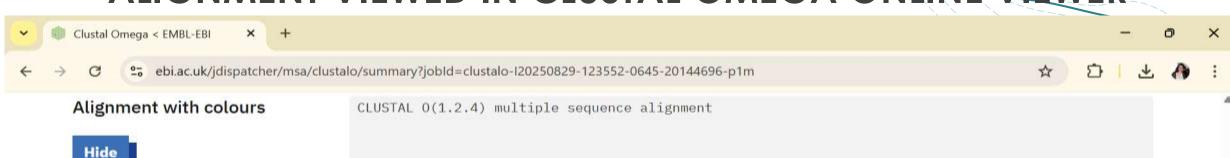
Fully conserved motifs detected → identical residues across all species.

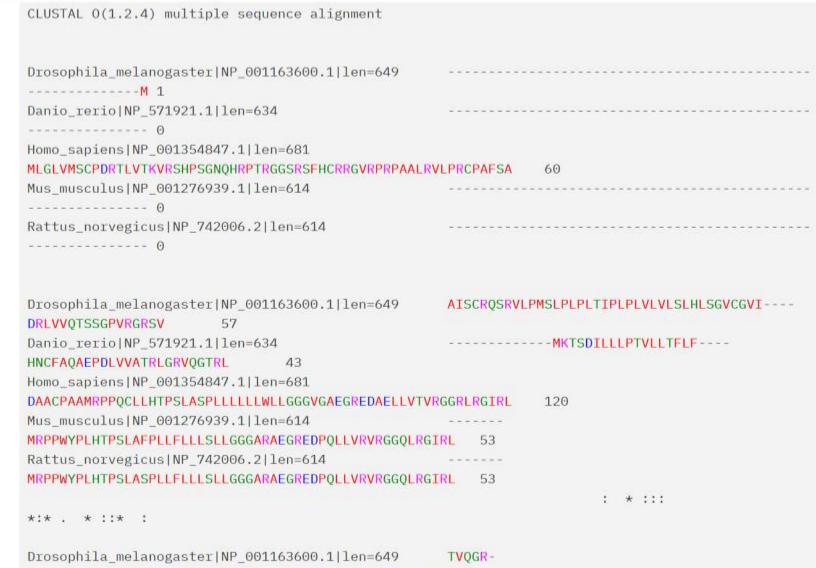
Semi-conserved regions → substitutions with chemically similar amino acids.

Variable regions → species-specific differences.

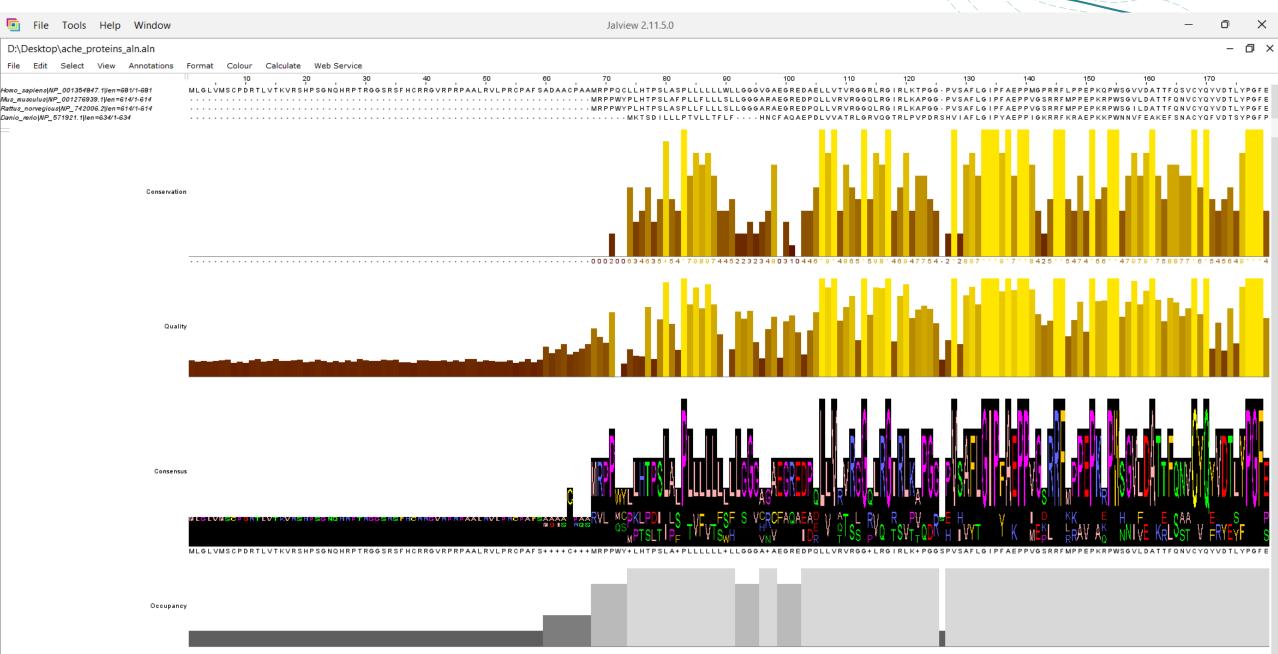
Conserved motifs suggest functional importance (likely active or binding sites).

#### ALIGNMENT VIEWED IN CLUSTAL OMEGA ONLINE VIEWER





#### ALIGNMENT VIEWED IN JALVIEW



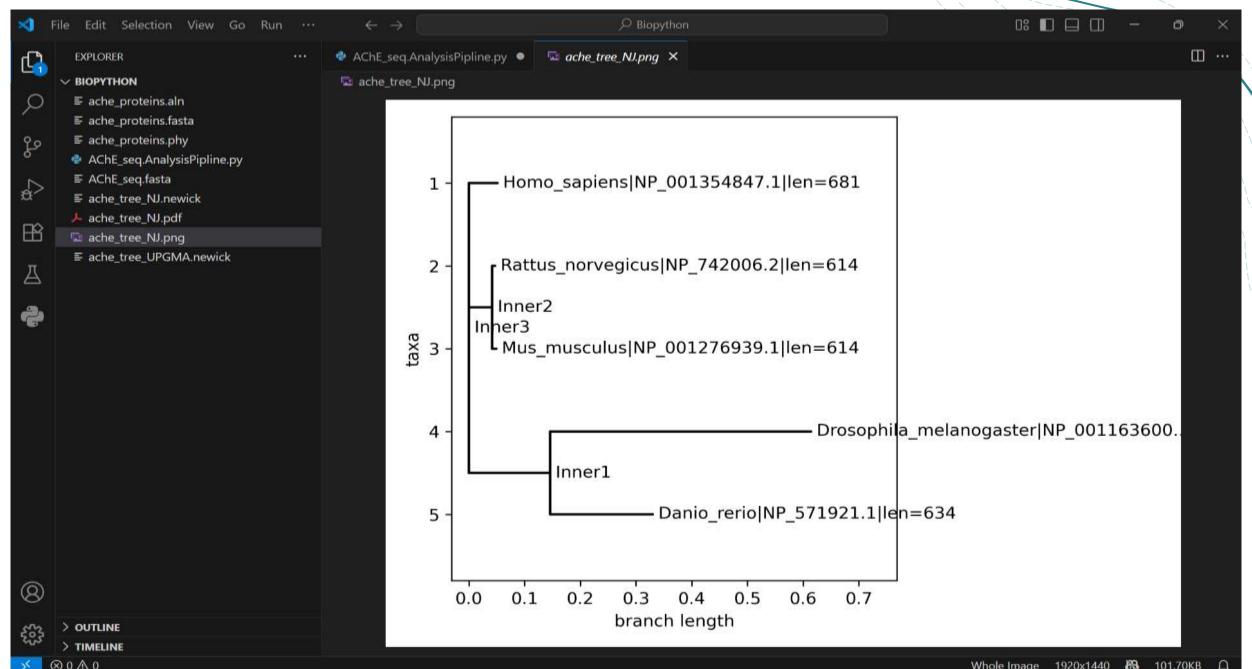
# RESULTS - PHYLOGENETIC TREE (BIOPYTHON + MATPLOTLIB)

Tree Construction Used Biopython (Phylo module) with the .phy alignment file. Tree visualization performed using Matplotlib. Exported tree as an image (tree.png).

#### Key Observations

- -Human AChE clustered closely with **mouse and rat**, reflecting mammalian evolutionary relationship.
- -Zebrafish formed a separate branch → closer to mammals than insects but still distinct.
- -Fruit fly (insect) appeared as the **most distant branch**, confirming evolutionary divergence.

#### PHYLOGENETIC TREE VISUALIZED WITH MATPLOTLIB + BIOPYTHON



#### **DISCUSSION & BIOLOGICAL SIGNIFICANCE:**

#### √ Key Findings

- Multiple Sequence Alignment (MSA):
  - -Conserved motifs identified across all 5 species.
  - -Suggest these residues are functionally essential (active/binding sites).

#### Phylogenetic Tree:

- -Mammals (human, mouse, rat) grouped together.
- -Zebrafish formed a separate branch but still closer to mammals than insects.
- -Fruit fly diverged earliest, showing largest evolutionary distance.

#### ✓ Biological Significance

Conserved motifs → potential drug target regions for Alzheimer's therapies.

Mammalian clustering → validates mouse/rat as suitable models for AChE research.

Evolutionary insights → highlights structural conservation of AChE across species.

#### **CONCLUSION:**

- ✓ Successfully analyzed **Acetylcholinesterase** (**AChE**) **sequences** from multiple species using Biopython.
- ✓ Multiple Sequence Alignment (MSA): revealed conserved motifs, indicating essential functional regions.
- ✓ Phylogenetic Tree: showed evolutionary relationships mammals grouped closely, insect (fruit fly) most distant.
- ✓ Demonstrated how bioinformatics tools (Biopython, Jalview, Matplotlib) can provide insights into protein conservation and evolution.

#### **FUTURE WORK:**

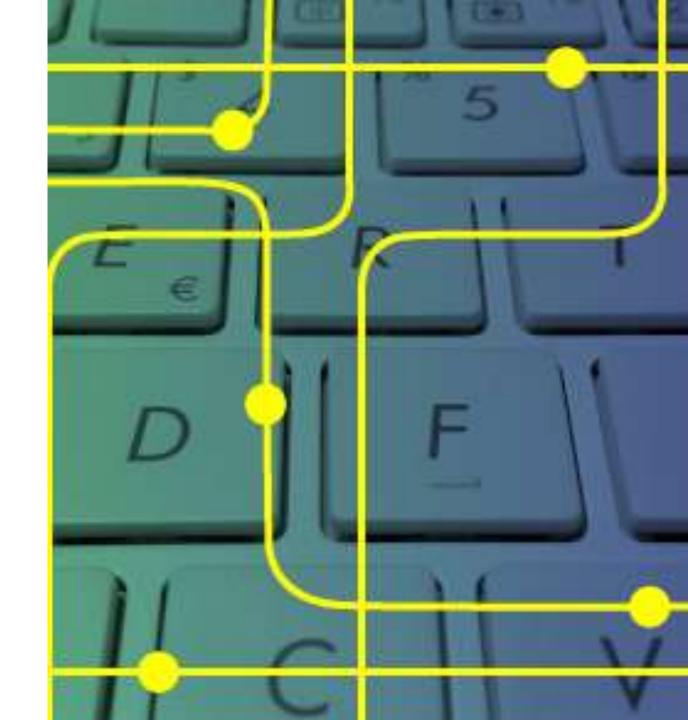
- **❖Expand dataset** → include more species for broader evolutionary analysis.
- **♦ Structural modeling** → map conserved motifs onto 3D AChE protein structure.
- ❖ Drug docking studies → test interactions of conserved sites with potential inhibitors (e.g., curcumin, donepezil).
- ❖Automated pipeline → build a Biopython script to handle retrieval, alignment, tree building, and visualization in one go.

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#### **ACKNOWLEDGEMENTS**

- Biopython developers for providing opensource bioinformatics tools.
- ☐ Clustal Omega & Jalview for multiple sequence alignment visualization.
- ☐ Matplotlib for phylogenetic tree plotting.
- □ **NCBI** for sequence data retrieval.



#### REFERENCES

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# **THANK YOU!**