In [1]:
```
%pip install biopython
```

```
Requirement already satisfied: biopython in ./.local/share/pipx/venvs/note
book/lib/python3.12/site-packages (1.86)
Requirement already satisfied: numpy in ./.local/share/pipx/venvs/noteboo
k/lib/python3.12/site-packages (from biopython) (2.3.5)

[notice] A new release of pip is available: 25.2 -> 25.3
[notice] To update, run: /home/naushin_parveen/.local/share/pipx/venvs/not
ebook/bin/python -m pip install --upgrade pip
Note: you may need to restart the kernel to use updated packages.
```

In [1]:
```
from Bio import Entrez, SeqIO
print("Biopython import OK")
```

```
Biopython import OK
```

In [2]:
```
from Bio import Entrez, SeqIO
Entrez.email = "naushin.mansuri@iitgn.ac.in"
query = "globin[Protein Name] NOT partial[Title] NOT fragment[Title]"
handle = Entrez.esearch(db="protein", term=query, retmax=200)
record = Entrez.read(handle)
handle.close()
print("IDs Found:", len(record["IdList"]))
ids = record["IdList"]
handle = Entrez.efetch(db="protein", id=",".join(ids), rettype="fasta", r
sequence_data = handle.read()
handle.close()
print("\nSample Output (first 400 characters):\n")
print(sequence_data[:400])
with open("globin_sequences_raw.fasta", "w") as file:
    file.write(sequence_data)
print("\nSaved all sequences to 'globin_sequences_raw.fasta'")
```

```
IDs Found: 200

Sample Output (first 400 characters):

>WP_447037499.1 globin [Streptomyces sp. DSM 118878]
MDSVKEIPHGTVQEQTYYEQVGGEETFRRLVHLFYQGVAEDPLLRPMYPEGDLGPAEERFALFLMQYWGG
PRTYSDNRGHPRLRMRHAPFTVDRAAHDAWLKHMRAAVDQLGLSEEHERTLWNYLTYAAASMVNSEG

>WP_447029335.1 globin [Streptomyces hypolithicus]
MNEIPIGTLQEQTFYEQVGGEETFRRLVHRFYQGVAEDPLLKPMYPEEDLGPAEERLALFLMQYWGGPRT
YSDERGHPRLRMRHAPFTVDKAAHDAWLQHMRVAVDELGLSEDHERQLWNYLTYAAASMVNKTG

>WP_447006119.1 glo

Saved all sequences to 'globin_sequences_raw.fasta'
```

In [4]:
```
from Bio import SeqIO
print("Total sequences in file:", sum(1 for _ in SeqIO.parse("globin_sequ
rec = next(SeqIO.parse("globin_sequences_raw.fasta","fasta"))
print("First ID:", rec.id, "Length:", len(rec.seq))
```

```
Total sequences in file: 200
First ID: WP_447037499.1 Length: 137
```

In [4]:
```
from Bio import SeqIO
lengths = [len(r.seq) for r in SeqIO.parse("globin_sequences_raw.fasta","
```

```
print("Total sequences:", len(lengths))
print("Shortest:", min(lengths))
print("Longest:", max(lengths))
print("Example lengths:", lengths[:10])
```

```
Total sequences: 200
Shortest: 53
Longest: 166
Example lengths: [137, 134, 128, 154, 133, 139, 139, 139, 139, 130]
```

In [5]:
```
from Bio import SeqIO

input_file = "globin_sequences_raw.fasta"
output_file = "globin_sequences_filtered.fasta"

filtered_sequences = []

for record in SeqIO.parse(input_file, "fasta"):
    if len(record.seq) >= 100:
        filtered_sequences.append(record)

SeqIO.write(filtered_sequences, output_file, "fasta")

print("Total sequences after filtering:", len(filtered_sequences))
print("Saved filtered sequences to 'globin_sequences_filtered.fasta'")
```

```
Total sequences after filtering: 196
Saved filtered sequences to 'globin_sequences_filtered.fasta'
```

In [6]:
```
mv -f globin_sequences_filtered.fasta final_globin_sequences.fasta
```

In [7]:
```
from Bio import SeqIO
count = sum(1 for _ in SeqIO.parse("final_globin_sequences.fasta","fasta"
print("Total sequences in final file:", count)
```

```
Total sequences in final file: 196
```

In [8]:
```
%pip install matplotlib
```

```
Requirement already satisfied: matplotlib in ./.local/share/pipx/venvs/not
ebook/lib/python3.12/site-packages (3.10.7)
Requirement already satisfied: contourpy>=1.0.1 in ./.local/share/pipx/ven
vs/notebook/lib/python3.12/site-packages (from matplotlib) (1.3.3)
Requirement already satisfied: cycler>=0.10 in ./.local/share/pipx/venvs/n
otebook/lib/python3.12/site-packages (from matplotlib) (0.12.1)
Requirement already satisfied: fonttools>=4.22.0 in ./.local/share/pipx/ve
nvs/notebook/lib/python3.12/site-packages (from matplotlib) (4.60.1)
Requirement already satisfied: kiwisolver>=1.3.1 in ./.local/share/pipx/ve
nvs/notebook/lib/python3.12/site-packages (from matplotlib) (1.4.9)
Requirement already satisfied: numpy>=1.23 in ./.local/share/pipx/venvs/no
tebook/lib/python3.12/site-packages (from matplotlib) (2.3.5)
Requirement already satisfied: packaging>=20.0 in ./.local/share/pipx/venv
s/notebook/lib/python3.12/site-packages (from matplotlib) (25.0)
Requirement already satisfied: pillow>=8 in ./.local/share/pipx/venvs/note
book/lib/python3.12/site-packages (from matplotlib) (12.0.0)
Requirement already satisfied: pyparsing>=3 in ./.local/share/pipx/venvs/n
otebook/lib/python3.12/site-packages (from matplotlib) (3.2.5)
Requirement already satisfied: python-dateutil>=2.7 in ./.local/share/pip
x/venvs/notebook/lib/python3.12/site-packages (from matplotlib) (2.9.0.pos
t0)
Requirement already satisfied: six>=1.5 in ./.local/share/pipx/venvs/noteb
ook/lib/python3.12/site-packages (from python-dateutil>=2.7->matplotlib)
(1.17.0)

[notice] A new release of pip is available: 25.2 -> 25.3
[notice] To update, run: /home/naushin_parveen/.local/share/pipx/venvs/not
ebook/bin/python -m pip install --upgrade pip
Note: you may need to restart the kernel to use updated packages.
```

In [10]:
```python
from Bio import SeqIO
import matplotlib.pyplot as plt

input_file = "final_globin_sequences.fasta"
```

In [11]:
```python
##Collect IDs and lengths
lengths = []
ids = []

for record in SeqIO.parse(input_file, "fasta"):
    ids.append(record.id)
    lengths.append(len(record.seq))

#Save ID + length
with open("sequence_id_length.txt", "w") as f:
    for seq_id, length in zip(ids, lengths):
        f.write(f"{seq_id}\t{length}\n")
```

In [12]:
```python
import os

print("File exists:", os.path.exists("sequence_id_length.txt"))
```

```
File exists: True
```

In [13]:
```python
with open("sequence_id_length.txt") as f:
    for i in range(10):
        print(f.readline().strip())
```

```
WP_447037499.1  137
WP_447029335.1  134
WP_447006119.1  128
WP_446888964.1  154
YBV26126.1      133
YBV21917.1      139
YBV18343.1      139
YBV09626.1      139
YBV14740.1      139
BGO70610.1      130
```

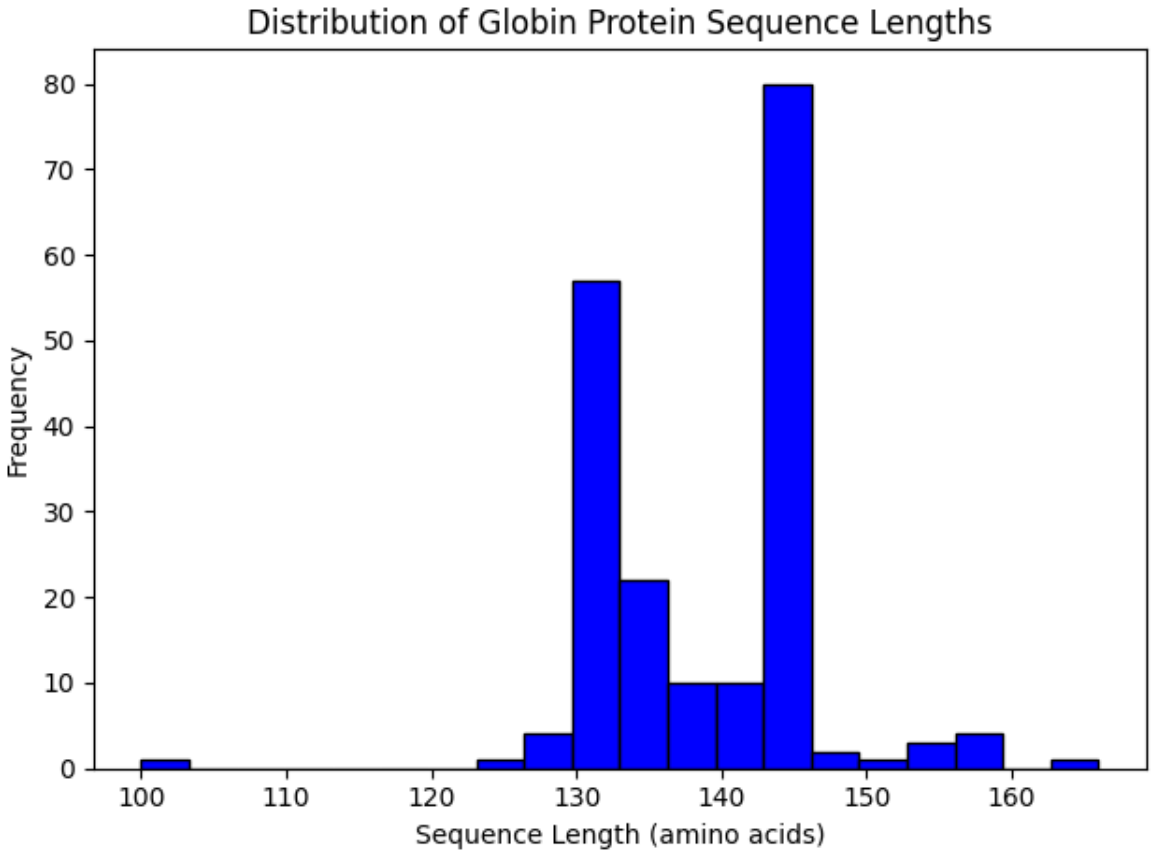
In [14]:
```
##summary
print("Total sequences:", len(lengths))
print("Minimum length:", min(lengths))
print("Maximum length:", max(lengths))
print("First 10 lengths (sorted):", sorted(lengths)[:10])
```

```
Total sequences: 196
Minimum length: 100
Maximum length: 166
First 10 lengths (sorted): [100, 126, 128, 129, 129, 129, 130, 130, 130, 1
30]
```

In [16]:
```
plt.savefig("sequence_length_histogram.png", dpi=300)
```

```
<Figure size 640x480 with 0 Axes>
```

In [25]:
```
##Plot histogram
plt.hist(lengths, bins=20, edgecolor='black', color='blue')
plt.title("Distribution of Globin Protein Sequence Lengths")
plt.xlabel("Sequence Length (amino acids)")
plt.ylabel("Frequency")
plt.tight_layout()
plt.show()
outpng = "length_distribution.png"
fig.savefig(outpng, dpi=300)
print("Saved histogram to", outpng)
```




```
Saved histogram to length_distribution.png
```

In [26]:
```
import os
print(os.path.exists("length_distribution.png"))
```

```
True
```

In [30]:
```
for org in organisms:
    for r in records:
        if org.lower() in r.description.lower():
            outfile = org.replace(" ", "_") + ".fasta"
            SeqIO.write(r, outfile, "fasta")
            print("Saved:", outfile)
            break
```

```
Saved: Bacillus.fasta
```

In [37]:
```
from Bio import SeqIO

records = list(SeqIO.parse("final_globin_sequences.fasta", "fasta"))

organisms = [
    "Streptomyces hypolithicus",
    "Saccharothrix isguenensis",
    "Pseudoalteromonas sp. SaAl2",
    "Sphingomonas sp. CJ20",
    "Leptospira interrogans",
    "Gordonia sp. J1A",
    "Azospira sp. I13",
    "Sphingopyxis sp.",
    "Streptomyces spiroverticillatus",
    "Pseudomonas aeruginosa"
]

counter = 1   # start numbering files
```

```
for org in organisms:
    for r in records:
        if org.lower() in r.description.lower():
            outfile = f"{counter}_" + org.replace(" ", "_") + ".fasta"
            SeqIO.write(r, outfile, "fasta")
            print("Saved:", outfile)
            counter += 1   # increase number after saving
            break
```

```
Saved: 1_Streptomyces_hypolithicus.fasta
Saved: 2_Saccharothrix_isguenensis.fasta
Saved: 3_Pseudoalteromonas_sp._SaAl2.fasta
Saved: 4_Sphingomonas_sp._CJ20.fasta
Saved: 5_Leptospira_interrogans.fasta
Saved: 6_Gordonia_sp._J1A.fasta
Saved: 7_Azospira_sp._I13.fasta
Saved: 8_Sphingopyxis_sp..fasta
Saved: 9_Streptomyces_spiroverticillatus.fasta
Saved: 10_Pseudomonas_aeruginosa.fasta
```

In [2]:
```
# Cell 1: checks and paths
import shutil, subprocess, sys
from pathlib import Path

# EDIT if your fasta has a different name or location
fasta_in = "combined_sequences.fasta"     # <-- your 10-sequence FASTA
align_out = "sequences_aligned.fasta"
clustal_out = "sequences_aligned.clustal"
tree_out = "sequences_tree.dnd"

print("Current working directory:", Path.cwd())
print("Project PDF (uploaded): /mnt/data/Project_assignment2.pdf\n")

# check fasta exists
if not Path(fasta_in).exists():
    print(f"ERROR: Input FASTA not found at: {fasta_in}")
    raise SystemExit("Place your combined FASTA in the notebook folder or

# check clustalo on PATH
clustalo_path = shutil.which("clustalo")
if clustalo_path:
    print("clustalo found at:", clustalo_path)
    try:
        out = subprocess.run(["clustalo", "--version"], capture_output=Tr
        ver = out.stdout.strip() or out.stderr.strip()
        print("clustalo version info:", ver)
    except subprocess.CalledProcessError:
        print("clustalo exists but version query failed; may still run.")
else:
    print("clustalo NOT found in PATH.")
    print("Install and start Jupyter from the same conda env, for example
    print("  conda activate MSA")
    print("  conda install -c bioconda clustalo")
    print("  jupyter notebook")
    raise SystemExit("Install clustalo in the env you run Jupyter from, t
```

```
Current working directory: /home/naushin_parveen
Project PDF (uploaded): /mnt/data/Project_assignment2.pdf

clustalo found at: /home/naushin_parveen/miniconda3/bin/clustalo
clustalo version info: 1.2.3
```

In [3]:
```
# Cell 2: run clustalo to produce aligned FASTA and guide-tree
import subprocess
from pathlib import Path

cmd = [
    "clustalo",
    "-i", fasta_in,
    "-o", align_out,
    "--guidetree-out", tree_out,
    "--outfmt=fasta",
    "--force"
]

print("Running Clustal Omega:")
print(" ".join(cmd))
try:
    proc = subprocess.run(cmd, check=True, capture_output=True, text=True
    print("Clustal Omega finished (returncode=0).")
    if proc.stdout:
        print("STDOUT snippet:", proc.stdout[:600])
    if proc.stderr:
        # some versions print version/info to stderr
        print("STDERR snippet:", proc.stderr[:600])
    # sanity: confirm files created
    print("\nOutput files created:")
    for p in (align_out, tree_out):
        print(" -", Path(p).resolve(), "(exists)" if Path(p).exists() els
except FileNotFoundError:
    print("ERROR: clustalo executable not found. Please install and resta
    raise
except subprocess.CalledProcessError as e:
    print("clustalo failed. Return code:", e.returncode)
    print("stdout (head):", e.stdout[:1000])
    print("stderr (head):", e.stderr[:1000])
    raise
```

```
Running Clustal Omega:
clustalo -i combined_sequences.fasta -o sequences_aligned.fasta --guidetre
e-out sequences_tree.dnd --outfmt=fasta --force
Clustal Omega finished (returncode=0).

Output files created:
 - /home/naushin_parveen/sequences_aligned.fasta (exists)
 - /home/naushin_parveen/sequences_tree.dnd (exists)
```

In [4]:
```
# Cell 3: read alignment, print summary, write Clustal format, compute co
from Bio import AlignIO
from Bio.Align import AlignInfo
from Bio.Seq import Seq
from Bio.SeqRecord import SeqRecord
import numpy as np
from pathlib import Path

# Read alignment
```

```
alignment = AlignIO.read(align_out, "fasta")
nseq = len(alignment)
L = alignment.get_alignment_length()
print(f"Number of sequences in alignment: {nseq}")
print(f"Alignment length (columns): {L}\n")

# Preview first two sequences
for rec in alignment[:2]:
    print(f">{rec.id}\n{str(rec.seq)[:200]}...\n")

# Write Clustal format copy (useful for viewers)
AlignIO.write(alignment, clustal_out, "clustal")
print(f"Wrote Clustal-format alignment to: {Path(clustal_out).resolve()}"

# Consensus (majority rule) using Bio.Align.AlignInfo.SummaryInfo
summary = AlignInfo.SummaryInfo(alignment)
consensus = summary.dumb_consensus(threshold=0.5, ambiguous='X')  # thres
print("Consensus (dumb_consensus, 50% threshold):")
print(str(consensus)[:200] + ("..." if len(consensus) > 200 else ""))

# Pairwise percent identity matrix
def pairwise_pid(rec_a, rec_b):
    # percent identity ignoring columns where both are gaps
    a = str(rec_a.seq)
    b = str(rec_b.seq)
    matches = 0
    compared = 0
    for x, y in zip(a, b):
        if x == '-' and y == '-':
            continue
        compared += 1
        if x == y:
            matches += 1
    if compared == 0:
        return 0.0
    return 100.0 * matches / compared

ids = [rec.id for rec in alignment]
pid_mat = np.zeros((nseq, nseq), dtype=float)
for i in range(nseq):
    for j in range(i, nseq):
        pid = pairwise_pid(alignment[i], alignment[j])
        pid_mat[i, j] = pid_mat[j, i] = pid

# print a small table (rounded)
print("\nPairwise % identity matrix (rounded):")
# header
hdr = "ID".ljust(15) + " " + " ".join([f"{i+1:>6}" for i in range(nseq)])
print(hdr)
for idx, seqid in enumerate(ids):
    row = seqid[:14].ljust(15) + " " + " ".join([f"{pid_mat[idx, j]:6.1f}
    print(row)

# Save the pid matrix to CSV for later use
import csv
with open("pairwise_pid_matrix.csv", "w", newline="") as fh:
    writer = csv.writer(fh)
    writer.writerow(["id"] + ids)
    for i, seqid in enumerate(ids):
        writer.writerow([seqid] + list(pid_mat[i]))
```

```
print("\nSaved pairwise % identity matrix to pairwise_pid_matrix.csv")
```

```
Number of sequences in alignment: 10
Alignment length (columns): 265

>WP_447029335.1
----------------------------------------------------------MN-EIPIGTLQEQTFYEQV
GGEETFRRLVHRFYQGVAEDPLL---KPMYPEEDLGPAEE
R-----------------------------------------------------------------LALFLMQY
WGGPRTYSDERG...

>WP_447006119.1
------------------------------------------------------------MTTPPENFYEAV
GGYETFHKIVARFYEEVAHDPVL---RPMYPEEDLGPAEE
R-----------------------------------------------------------------FRLFLMQY
WGGPHTYSDTRG...

Wrote Clustal-format alignment to: /home/naushin_parveen/sequences_aligne
d.clustal
Consensus (dumb_consensus, 50% threshold):
MXXXXXXXXXLXXSXXIXXXXXXXFXXXFXXXLKXNHTKXXXXFXXXXLXXVPXXMNXXXXXXXXXXXTXYEXX
GGXETFRXLVXRFYXXVAXDPXLAXLRPMXPXXDLXPXEXRLRAGIMNLVMYARXMXDXXLXXLXXXAAGEXXX
XXXXXELVVXXRXKLXLXAEEXDLLXDAXLXALXXFLXXYWGGPXXXSDXRG...

Pairwise % identity matrix (rounded):
ID                   1      2      3      4      5      6      7      8
9     10
WP_447029335.1   100.0   59.7    5.8   22.5    3.6   51.8   31.4    9.0
88.8    7.0
WP_447006119.1    59.7  100.0    5.3   19.1    2.3   61.1   26.9    9.0
59.7    5.4
WP_446888964.1     5.8    5.3  100.0    4.2   16.5    6.8    3.6    6.1
5.3    5.6
YBV26126.1        22.5   19.1    4.2  100.0    2.7   19.0   28.5   10.8
23.9    6.8
YBV21917.1         3.6    2.3   16.5    2.7  100.0    3.2    3.6    4.7
2.3    5.2
BGO70610.1        51.8   61.1    6.8   19.0    3.2  100.0   28.0    8.4
55.5    6.5
BHH87803.1        31.4   26.9    3.6   28.5    3.6   28.0  100.0    7.2
31.4    6.9
CAO3294171.1       9.0    9.0    6.1   10.8    4.7    8.4    7.2  100.0
9.0   13.6
CAM5329635.1      88.8   59.7    5.3   23.9    2.3   55.5   31.4    9.0  1
00.0    7.5
YBU08153.1         7.0    5.4    5.6    6.8    5.2    6.5    6.9   13.6
7.5  100.0

Saved pairwise % identity matrix to pairwise_pid_matrix.csv
```

```
/home/naushin_parveen/miniconda3/lib/python3.13/site-packages/Bio/Align/Al
ignInfo.py:62: BiopythonDeprecationWarning: The `dumb_consensus` method is
deprecated and will be removed in a future release of Biopython. As an alt
ernative, you can convert the multiple sequence alignment object to a new-
style Alignment object by via its `.alignment` property, and then create a
Motif object. You can then use the `.consensus` or `.degenerate_consensus`
property of the Motif object to get a consensus sequence. For more control
over how the consensus sequence is calculated, you can call the `calculate
_consensus` method on the `.counts` property of the Motif object. This is
an example for a multiple sequence alignment `msa` of DNA nucleotides:
>>> from Bio.Seq import Seq
>>> from Bio.SeqRecord import SeqRecord
>>> from Bio.Align import MultipleSeqAlignment
>>> from Bio.Align.AlignInfo import SummaryInfo
>>> msa = MultipleSeqAlignment([SeqRecord(Seq('ACGT')),
...                             SeqRecord(Seq('ATGT')),
...                             SeqRecord(Seq('ATGT'))])
>>> summary = SummaryInfo(msa)
>>> dumb_consensus = summary.dumb_consensus(ambiguous='N')
>>> print(dumb_consensus)
ANGT
>>> alignment = msa.alignment
>>> from Bio.motifs import Motif
>>> motif = Motif('ACGT', alignment)
>>> print(motif.consensus)
ATGT
>>> print(motif.degenerate_consensus)
AYGT
>>> counts = motif.counts
>>> consensus = counts.calculate_consensus(identity=0.7)
>>> print(consensus)
ANGT

If your multiple sequence alignment object was obtained using Bio.AlignIO,
then you can obtain a new-style Alignment object directly by using Bio.Ali
gn.read instead of Bio.AlignIO.read, or Bio.Align.parse instead of Bio.Ali
gnIO.parse.
  warnings.warn(
```

In [5]:
```
from Bio import AlignIO
alignment = AlignIO.read("sequences_aligned.fasta", "fasta")
print("SeqID\tAlignedLen\tNumGaps\tGapFraction\tUngappedLen")
for rec in alignment:
    seq = str(rec.seq)
    L = len(seq)
    gaps = seq.count('-')
    print(f"{rec.id}\t{L}\t{gaps}\t{gaps/L:.3f}\t{L-gaps}")
```

```
SeqID   AlignedLen      NumGaps GapFraction     UngappedLen
WP_447029335.1  265     131     0.494   134
WP_447006119.1  265     137     0.517   128
WP_446888964.1  265     111     0.419   154
YBV26126.1      265     132     0.498   133
YBV21917.1      265     126     0.475   139
BGO70610.1      265     135     0.509   130
BHH87803.1      265     112     0.423   153
CAO3294171.1    265     114     0.430   151
CAM5329635.1    265     131     0.494   134
YBU08153.1      265     135     0.509   130
```

In [6]:
```
# --- Replace dumb_consensus with this manual majority-rule consensus ---
from collections import Counter

def majority_consensus(alignment, threshold=0.5, ambiguous='X', gap_char=
    """
    Return a majority-rule consensus string for a MultipleSeqAlignment.
    threshold: fraction (0..1) of non-gap counts needed to call a residue
    ambiguous: char when no residue reaches threshold.
    """
    L = alignment.get_alignment_length()
    cons_chars = []
    for col in range(L):
        col_str = alignment[:, col]
        counts = Counter([c for c in col_str if c != gap_char])
        if not counts:
            cons_chars.append(gap_char)
            continue
        top_res, top_count = counts.most_common(1)[0]
        if top_count / sum(counts.values()) >= threshold:
            cons_chars.append(top_res)
        else:
            cons_chars.append(ambiguous)
    return "".join(cons_chars)

# usage (matching previous 50% behavior)
consensus_manual = majority_consensus(alignment, threshold=0.5, ambiguous
print("Consensus (manual majority, 50% threshold):")
print(consensus_manual[:300] + ("..." if len(consensus_manual)>300 else "
```

```
Consensus (manual majority, 50% threshold):
MKFNTENKKQLLKSINIIKPNFHCFTFTFQMQLKRNHTKYENIFSRIQLEDVPXXMNXXXXXXXXXXXTXYEXX
GGXETFRXLVXRFYXXVAXDPXLAGLRPMXPXXDLXPXEXRLRAGIMNLVMYARRMTDETLQILFGLAAGEPFI
XXXXXELVVTHRXKLXLXAEEIDLLXDAXLXALXXFLXXYWGGPXXXSDXRGHPRLRMRHAPFXIDXXXRDAWX
XXMXXAXXXXXXXLLXXXHXXQLXXYXXXAAXSMVNXEGVAAE
```

In [7]:
```
# Cell 1: consensus statistics and top residues per column
from Bio import AlignIO
from collections import Counter
from pathlib import Path

aln = AlignIO.read("sequences_aligned.fasta", "fasta")
L = aln.get_alignment_length()
consensus = ""  # build consensus same way to ensure alignment with colum
from collections import Counter
def majority_consensus_str(alignment, threshold=0.5, ambiguous='X', gap_c
    L = alignment.get_alignment_length()
    cons = []
    for col in range(L):
        col_str = alignment[:, col]
        counts = Counter([c for c in col_str if c != gap_char])
        if not counts:
            cons.append(gap_char)
            continue
        top_res, top_count = counts.most_common(1)[0]
        if top_count / sum(counts.values()) >= threshold:
            cons.append(top_res)
        else:
            cons.append(ambiguous)
    return "".join(cons)
```

```
consensus = majority_consensus_str(aln, threshold=0.5, ambiguous='X')
num_X = consensus.count('X')
pct_X = 100.0 * num_X / len(consensus)
print(f"Consensus length = {len(consensus)} columns")
print(f"Number of ambiguous positions (X): {num_X} ({pct_X:.1f}%)")

# compute per-column top residue frequency and list top conserved columns
top_fracs = []
for col in range(L):
    col_str = aln[:, col]
    counts = Counter([c for c in col_str if c != '-'])
    if not counts:
        top_fracs.append((col, None, 0.0))
    else:
        top_res, top_count = counts.most_common(1)[0]
        frac = top_count / sum(counts.values())
        top_fracs.append((col, top_res, frac))

# show columns with top residue >= 0.8 (80% conserved)
conserved80 = [(c+1, r, round(f*100,1)) for c,r,f in top_fracs if f >= 0.
print(f"Columns with >=80% same residue: {len(conserved80)}")
if conserved80:
    print("First 20 conserved cols (index, residue, %):")
    for item in conserved80[:20]:
        print(item)
else:
    print("No columns reach >=80% identity across sequences.")
```

```
Consensus length = 265 columns
Number of ambiguous positions (X): 76 (28.7%)
Columns with >=80% same residue: 59
First 20 conserved cols (index, residue, %):
(1, 'M', 100.0)
(11, 'L', 100.0)
(14, 'S', 100.0)
(17, 'I', 100.0)
(25, 'F', 100.0)
(29, 'F', 100.0)
(33, 'L', 100.0)
(34, 'K', 100.0)
(36, 'N', 100.0)
(37, 'H', 100.0)
(38, 'T', 100.0)
(39, 'K', 100.0)
(44, 'F', 100.0)
(49, 'L', 100.0)
(56, 'M', 85.7)
(86, 'R', 87.5)
(87, 'F', 100.0)
(95, 'P', 87.5)
(98, 'A', 100.0)
(101, 'R', 80.0)
```

In [8]:
```
# Cell 2: extract conserved blocks (contiguous columns) for possible mode
from Bio import AlignIO
from collections import Counter
from Bio.Seq import Seq
from Bio.SeqRecord import SeqRecord
from Bio import SeqIO
```

```
aln = AlignIO.read("sequences_aligned.fasta", "fasta")
L = aln.get_alignment_length()

# compute top residue fraction per column
top_ok = []
top_residues = []
for col in range(L):
    col_str = aln[:, col]
    counts = Counter([c for c in col_str if c != '-'])
    if not counts:
        top_ok.append(0.0)
        top_residues.append('-')
    else:
        top_res, top_count = counts.most_common(1)[0]
        frac = top_count / sum(counts.values())
        top_ok.append(frac)
        top_residues.append(top_res)

# find contiguous runs where frac >= threshold
threshold = 0.7
min_len = 8
runs = []
start = None
for i, frac in enumerate(top_ok):
    if frac >= threshold:
        if start is None:
            start = i
    else:
        if start is not None:
            end = i-1
            if (end - start + 1) >= min_len:
                runs.append((start+1, end+1, end-start+1))  # 1-based coo
            start = None
# tail
if start is not None:
    end = L-1
    if (end - start + 1) >= min_len:
        runs.append((start+1, end+1, end-start+1))

print("Conserved runs (1-based start, end, length) with threshold >= %.2f
if runs:
    for r in runs:
        print(r)
else:
    print("No conserved runs found with the chosen thresholds. Try lower

# If runs found, produce ungapped sequences of those regions (per sequenc
if runs:
    for idx, (s,e,l) in enumerate(runs, 1):
        records = []
        for rec in aln:
            seg = str(rec.seq)[s-1:e].replace('-', '')
            records.append(SeqRecord(Seq(seg), id=rec.id, description=f"B
        outname = f"conserved_block_{idx}_{s}_{e}.fasta"
        SeqIO.write(records, outname, "fasta")
        print("Wrote", outname)
```

```
Conserved runs (1-based start, end, length) with threshold >= 0.70 and min
_len 8:
(116, 128, 13)
Wrote conserved_block_1_116_128.fasta
```

In [9]:

```
# Cell 3 (Tree display): show guide tree in ASCII (use the tree you produ
from Bio import Phylo
from pathlib import Path
tree_path = "sequences_tree.dnd"   # or "sequences_tree_filtered.dnd" if 
try:
    tree = Phylo.read(tree_path, "newick")
    print("Guide tree (ASCII):\n")
    Phylo.draw_ascii(tree)
except Exception as e:
    print("Could not parse/display tree as Newick. Error:", e)
    try:
        raw = Path(tree_path).read_text()
        print("\nRaw tree head:\n", raw[:1200])
    except Exception as e2:
        print("Failed to read tree file:", e2)
```

```
Guide tree (ASCII):

      _______________________________________________________ WP_44688896
4.1
  ___|
 |   |_______________________________________________________ YBV21917.1
 |
 |          __________________________________________________ YBV26126.1
 |         |
_|  _______|   ______________________________________________ BHH87803.1
 | |       |  |
 | |       |__|                  ____________________________ BGO70610.1
 | |          |                 |
 | |          |_________________|                      _______ WP_44702933
5.1
 |_|                            |   _________________|
   |                            |__|                 |_______ CAM5329635.1
   |                               |
   |                               |_________________________ WP_44700611
9.1
   |
   | ________________________________________________________ CAO3294171.1
   ||
    |________________________________________________________ YBU08153.1
```

In [11]:

```
from Bio import Phylo

Phylo.write(tree, "my_saved_tree.nwk", "newick")
```

Out[11]: 1

In [12]:

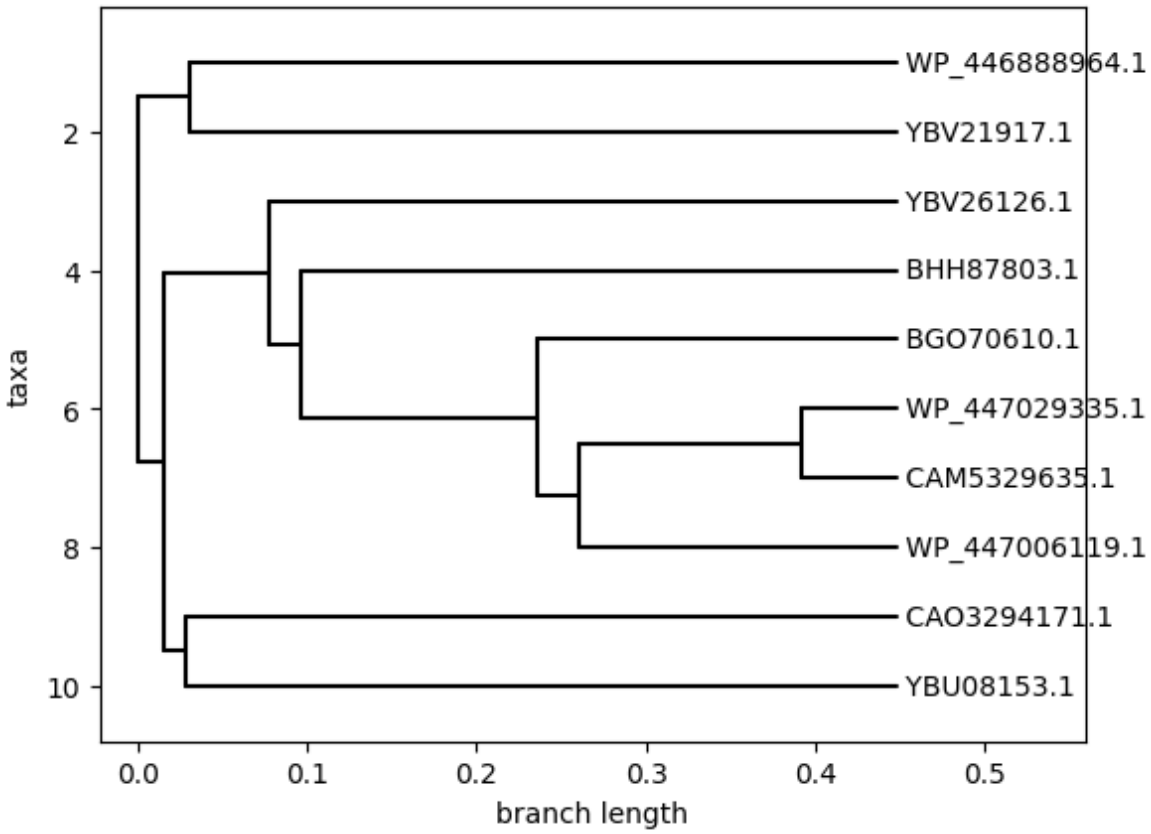
```
from Bio import Phylo
import matplotlib.pyplot as plt

Phylo.draw(tree)                          # draw in notebook
plt.savefig("my_tree.png", dpi=300, bbox_inches="tight")
plt.close()
```

```
---------------------------------------------------------------------------
-
ModuleNotFoundError                       Traceback (most recent call las
t)
Cell In[12], line 2
      1 from Bio import Phylo
----> 2 import matplotlib.pyplot as plt
      4 Phylo.draw(tree)                          # draw in notebook
      5 plt.savefig("my_tree.png", dpi=300, bbox_inches="tight")

ModuleNotFoundError: No module named 'matplotlib'
```

In [13]:
```
!pip install matplotlib
```

```
Collecting matplotlib
  Downloading matplotlib-3.10.7-cp313-cp313-manylinux2014_x86_64.manylinux
_2_17_x86_64.whl.metadata (11 kB)
Collecting contourpy>=1.0.1 (from matplotlib)
  Downloading contourpy-1.3.3-cp313-cp313-manylinux_2_27_x86_64.manylinux_
2_28_x86_64.whl.metadata (5.5 kB)
Collecting cycler>=0.10 (from matplotlib)
  Using cached cycler-0.12.1-py3-none-any.whl.metadata (3.8 kB)
Collecting fonttools>=4.22.0 (from matplotlib)
  Downloading fonttools-4.60.1-cp313-cp313-manylinux1_x86_64.manylinux2014
_x86_64.manylinux_2_17_x86_64.manylinux_2_5_x86_64.whl.metadata (112 kB)
Collecting kiwisolver>=1.3.1 (from matplotlib)
  Downloading kiwisolver-1.4.9-cp313-cp313-manylinux2014_x86_64.manylinux_
2_17_x86_64.whl.metadata (6.3 kB)
Requirement already satisfied: numpy>=1.23 in ./miniconda3/lib/python3.13/
site-packages (from matplotlib) (2.3.4)
Requirement already satisfied: packaging>=20.0 in ./miniconda3/lib/python
3.13/site-packages (from matplotlib) (25.0)
Collecting pillow>=8 (from matplotlib)
  Downloading pillow-12.0.0-cp313-cp313-manylinux_2_27_x86_64.manylinux_2_
28_x86_64.whl.metadata (8.8 kB)
Collecting pyparsing>=3 (from matplotlib)
  Using cached pyparsing-3.2.5-py3-none-any.whl.metadata (5.0 kB)
Requirement already satisfied: python-dateutil>=2.7 in ./miniconda3/lib/py
thon3.13/site-packages (from matplotlib) (2.9.0.post0)
Requirement already satisfied: six>=1.5 in ./miniconda3/lib/python3.13/sit
e-packages (from python-dateutil>=2.7->matplotlib) (1.17.0)
Downloading matplotlib-3.10.7-cp313-cp313-manylinux2014_x86_64.manylinux_2
_17_x86_64.whl (8.7 MB)
   ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━ 8.7/8.7 MB 902.0 kB/s eta 0:0
0:00m eta 0:00:016m0:00:010m
Downloading contourpy-1.3.3-cp313-cp313-manylinux_2_27_x86_64.manylinux_2_
28_x86_64.whl (362 kB)
Using cached cycler-0.12.1-py3-none-any.whl (8.3 kB)
Downloading fonttools-4.60.1-cp313-cp313-manylinux1_x86_64.manylinux2014_x
86_64.manylinux_2_17_x86_64.manylinux_2_5_x86_64.whl (4.9 MB)
   ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━ 4.9/4.9 MB 788.8 kB/s eta 0:0
0:00 kB/s eta 0:00:01:02
Downloading kiwisolver-1.4.9-cp313-cp313-manylinux2014_x86_64.manylinux_2_
17_x86_64.whl (1.5 MB)
   ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━ 1.5/1.5 MB 510.1 kB/s eta 0:0
0:001m849.7 kB/s eta 0:00:01
Downloading pillow-12.0.0-cp313-cp313-manylinux_2_27_x86_64.manylinux_2_28
_x86_64.whl (7.0 MB)
   ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━ 7.0/7.0 MB 691.6 kB/s eta 0:0
0:001m710.3 kB/s eta 0:00:01
Using cached pyparsing-3.2.5-py3-none-any.whl (113 kB)
Installing collected packages: pyparsing, pillow, kiwisolver, fonttools, c
ycler, contourpy, matplotlib
   ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━ 7/7 [matplotlib] 6/7 [matplotl
ib]ourpy]
Successfully installed contourpy-1.3.3 cycler-0.12.1 fonttools-4.60.1 kiwi
solver-1.4.9 matplotlib-3.10.7 pillow-12.0.0 pyparsing-3.2.5
```

In [14]:
```python
from Bio import Phylo
import matplotlib.pyplot as plt

Phylo.draw(tree)
plt.savefig("my_tree.png", dpi=300, bbox_inches="tight")
plt.close()
```



In [15]:
```
%matplotlib inline
from pathlib import Path
from Bio import Phylo
import matplotlib.pyplot as plt

# create an explicit figure + axes and draw onto that axes
fig = plt.figure(figsize=(12, 25))
ax = fig.add_subplot(1, 1, 1)

# draw explicitly onto our axes; do_show=False prevents Biopython from op
Phylo.draw(tree, axes=ax, do_show=False, label_func=lambda n: n.name if n

# force the renderer to render the figure before saving
fig.canvas.draw()

# save using the figure object (safer than plt.savefig in some notebook b
out = Path("my_tree.png")
fig.savefig(out, dpi=300, bbox_inches="tight")
plt.close(fig)

print(f"Saved {out} ({out.stat().st_size} bytes)")
```

```
Saved my_tree.png (187041 bytes)
```

In [ ]: