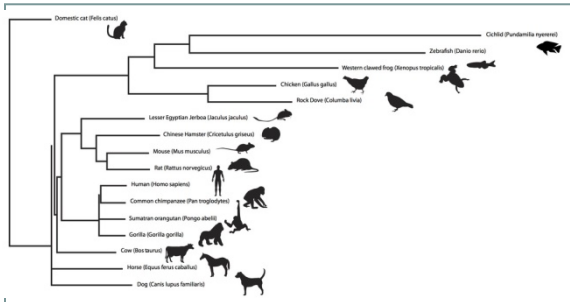
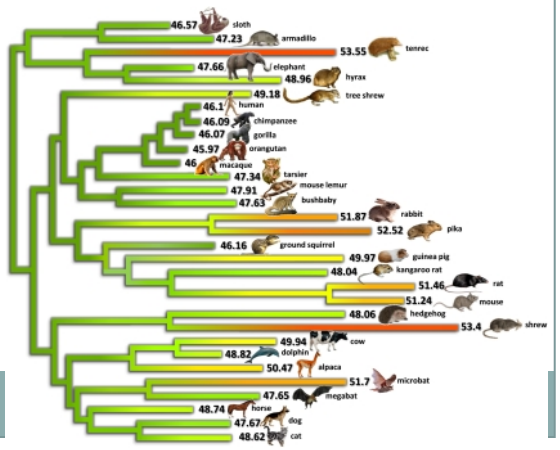
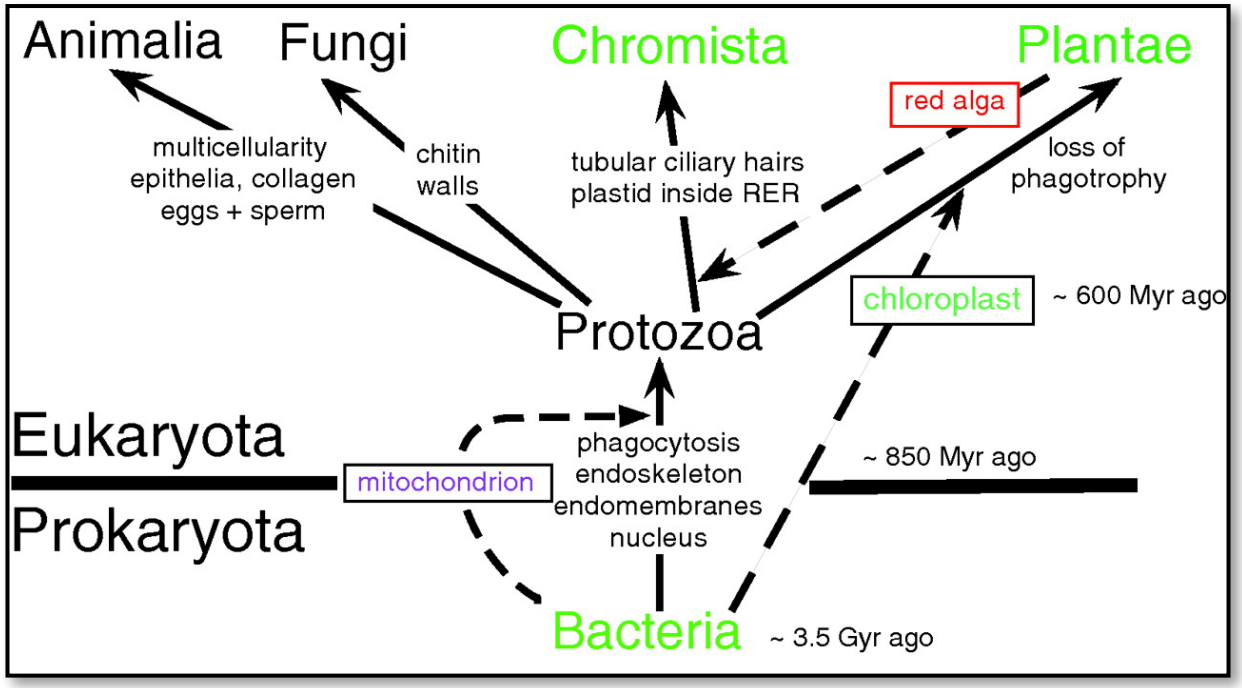


Phylogenetic Tree

By
Doaa Nassar
Morshed Kassouha



A phylogenetic tree or evolutionary tree is a branching diagram showing the evolutionary relationships among various biological species based upon similarities and differences in their physical or genetic characteristics.



The procedure

1- DNA extraction
2- PCR

Material	Quantity
2x Taq Ready Mix	125 µl
Forward primer: LshCytoD: TTG TAT GCA GAT AAT ATG TGG TGT GTG TTT AGC	10 µl
Reverse primer: LshCytoR: CCA TCT GAA CTC ATA AAA TAA TGT AAAC	10 µl
dd H2o	55 µl

Thermal cycler program:

- 5 min at 95 °C.
- 35 cycles: each composed of
- 30 seconds at 95 °C.
- 30 seconds at 56 °C.
- 1 min at 72 °C.
- A final elongation step at 72 °C for 10 min.

3- Gel electrophoresis

4- PCR Product Purification

Purification of PCR amplified DNA fragments for sequence analysis

Procedure:

1- Increase the volume of the PCR reaction up to 100 µl (add about 85 µl of DDW to each PCR tube to be purified).

2- Add 5 volumes of binding buffer PB to 1 volume of the PCR sample and mix. For example, add 500 µl of Buffer PB to 100 µl PCR sample.

3- Check that the color of the mixture is yellow. If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

4- Place a QIAquick spin column in the 2 ml collection tube provided.

5- To bind DNA, apply the sample to the QIAquick column and centrifuge for 30– 60s.

6- Discard flow-through. Place the QIAquick column back into the same tube.

7- To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30– 60s.

8- Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min. IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

9- Place QIAquick column in a clean 1.5 ml microcentrifuge tube. PCR Product Purification Protocol using Qiagene kit.

10- To elute DNA, add 30 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0– 8.5) to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min.

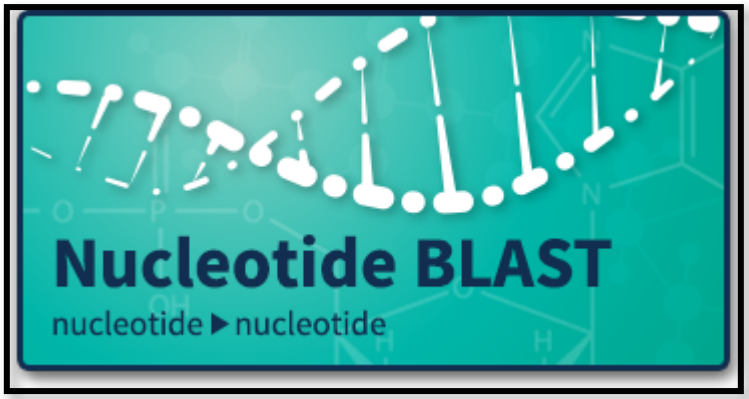
DNA sequence analysis

1- Purify ITS1-PCR and Leishmania cyto-PCR products that need to be identified. PCR cleaning can be carried out using the Qiagene PCR purification protocol. In the current example only purify the positive PCR clinical samples. There is no need to purify the Leishmania reference samples.

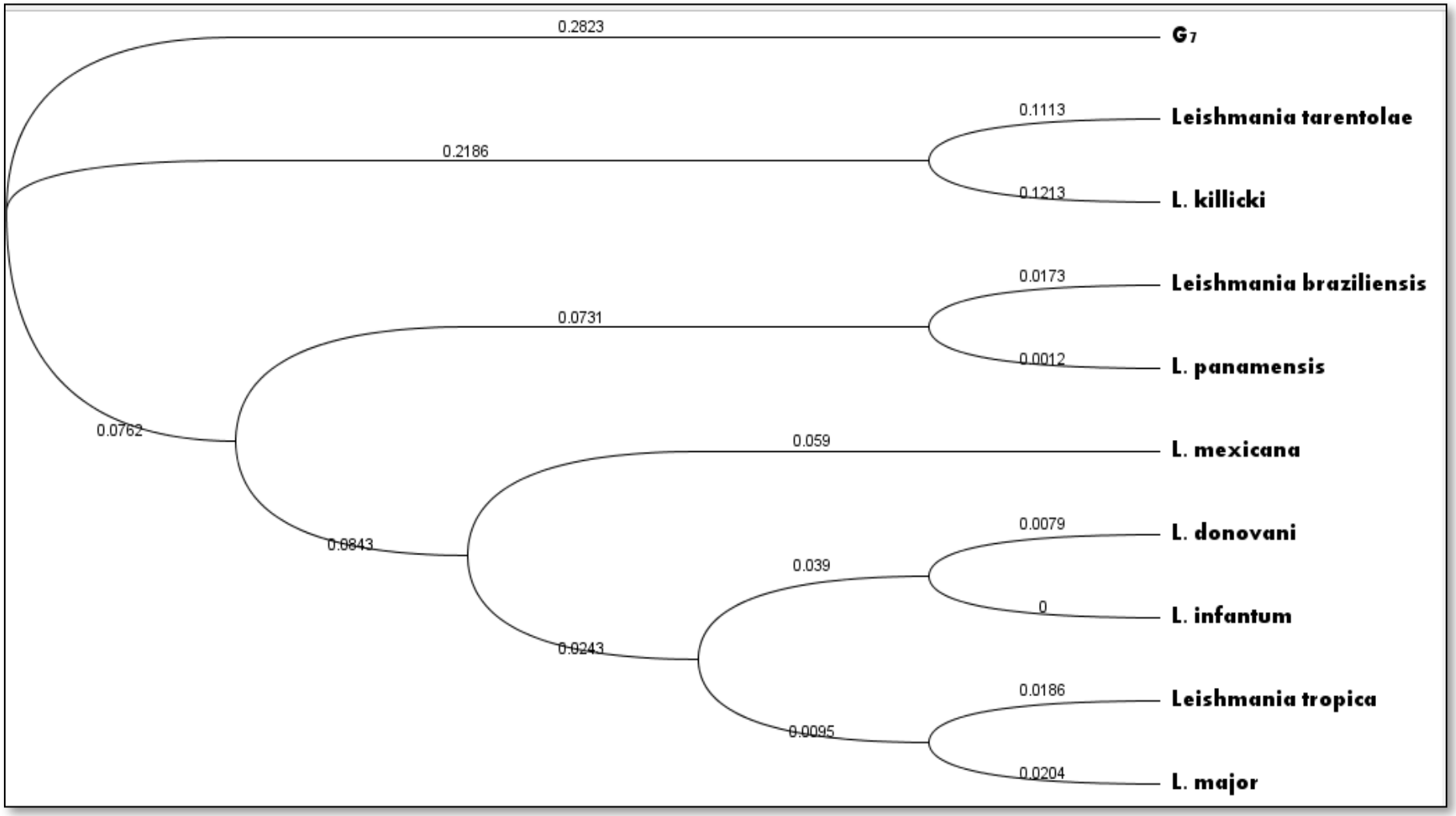
2- Samples are eluted in low volume (about 30 µl) of elution buffer or DDW. Do not dilute as high concentrations of purified DNA give better results.

3- For DNA sequencing of PCR products you need to use one of the two oligonucleotide primers in the amplification reaction (if only one strand is to be sequenced). Dilute your primers to 5 pmoles/µl, or according to the instructions of the DNA sequencing service provider.

4- Once the sequence is received it is possible to carry out BLAST (Basic Local Alignment Search Tool) DNA sequence comparison on (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and generate phylogenic trees.



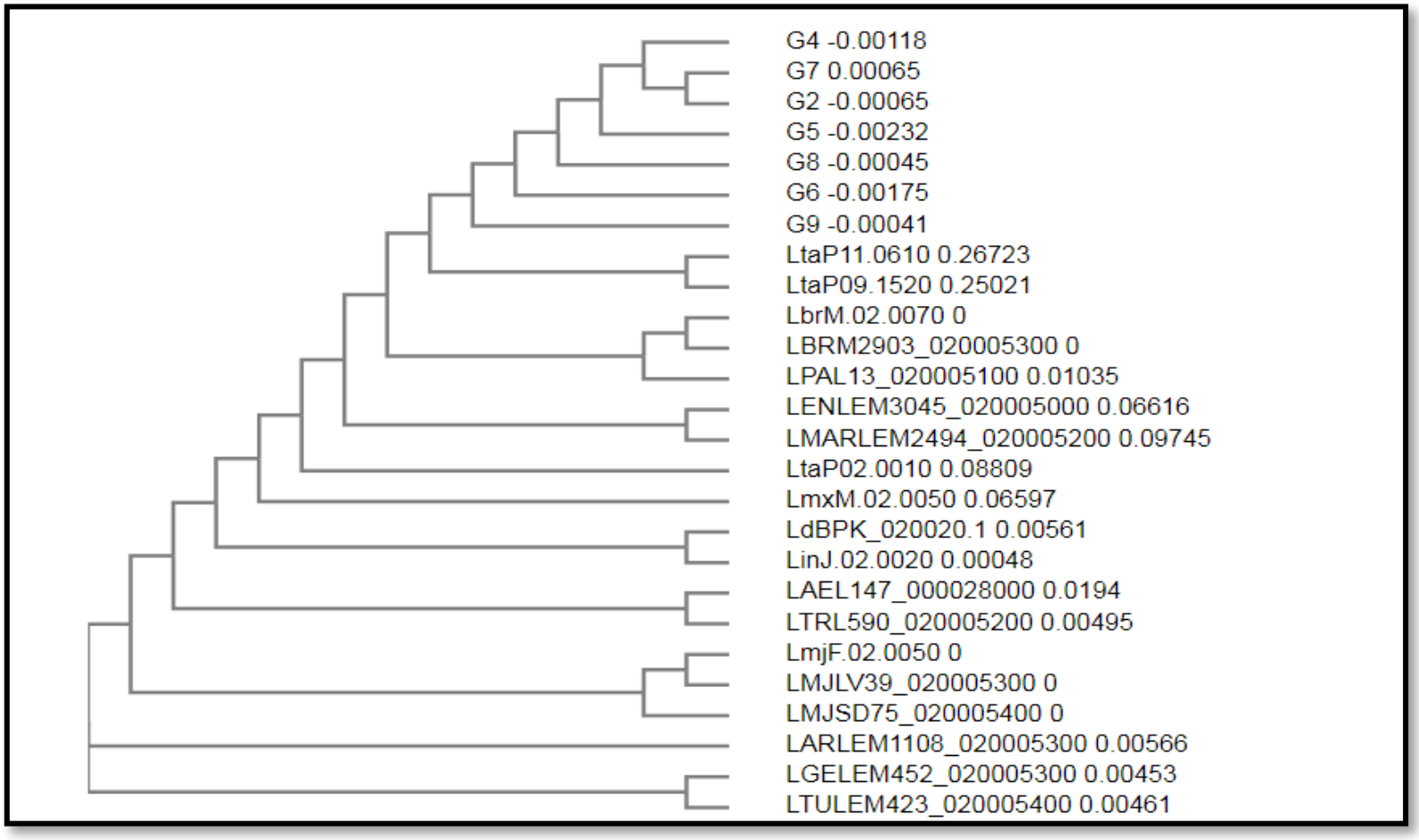
EBI Bioinformatic Tools (ie. sequence alignment and phylogenetic tree building).



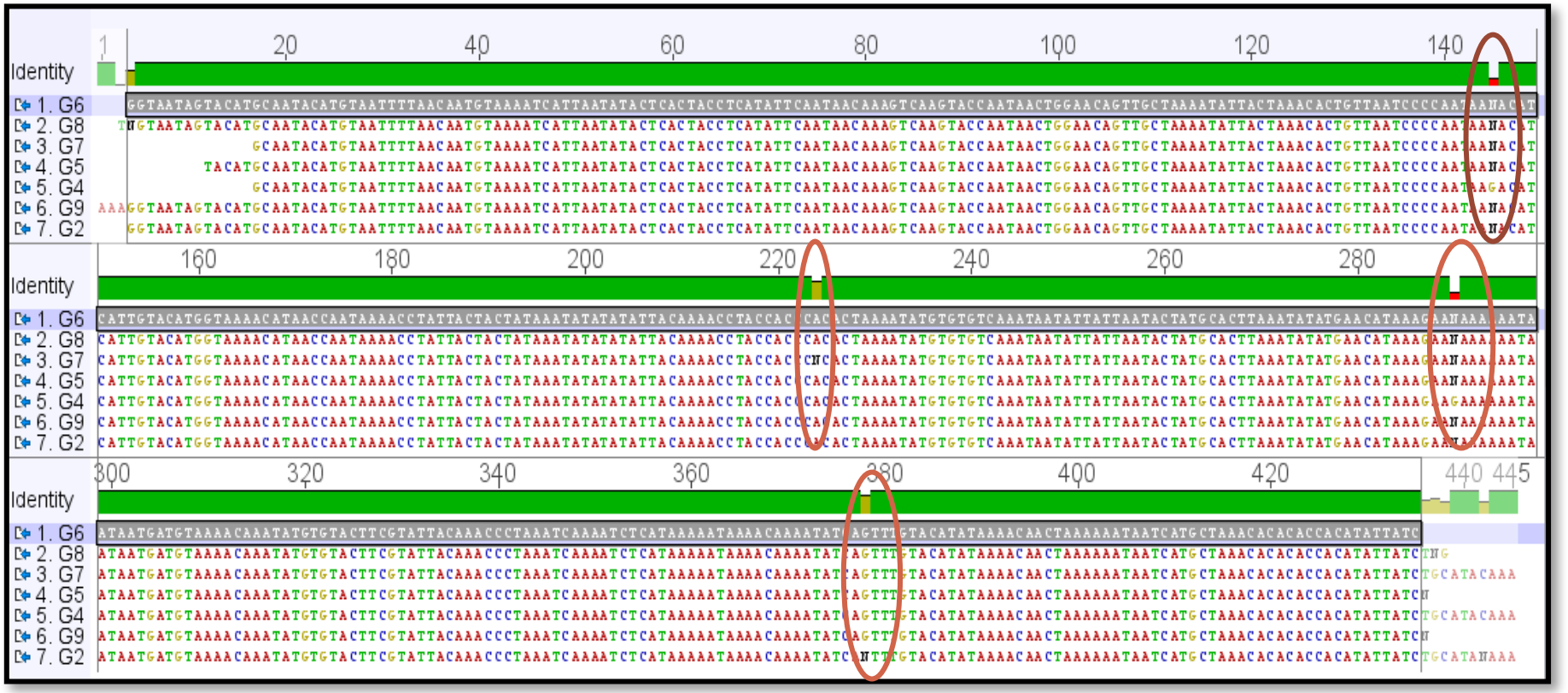
Phylogenetic relationships between group 7 and different members of the genus Leishmania based on the nucleotide sequences of the **Cyt b gene**.

Distance Matrix (Substitutions per site)

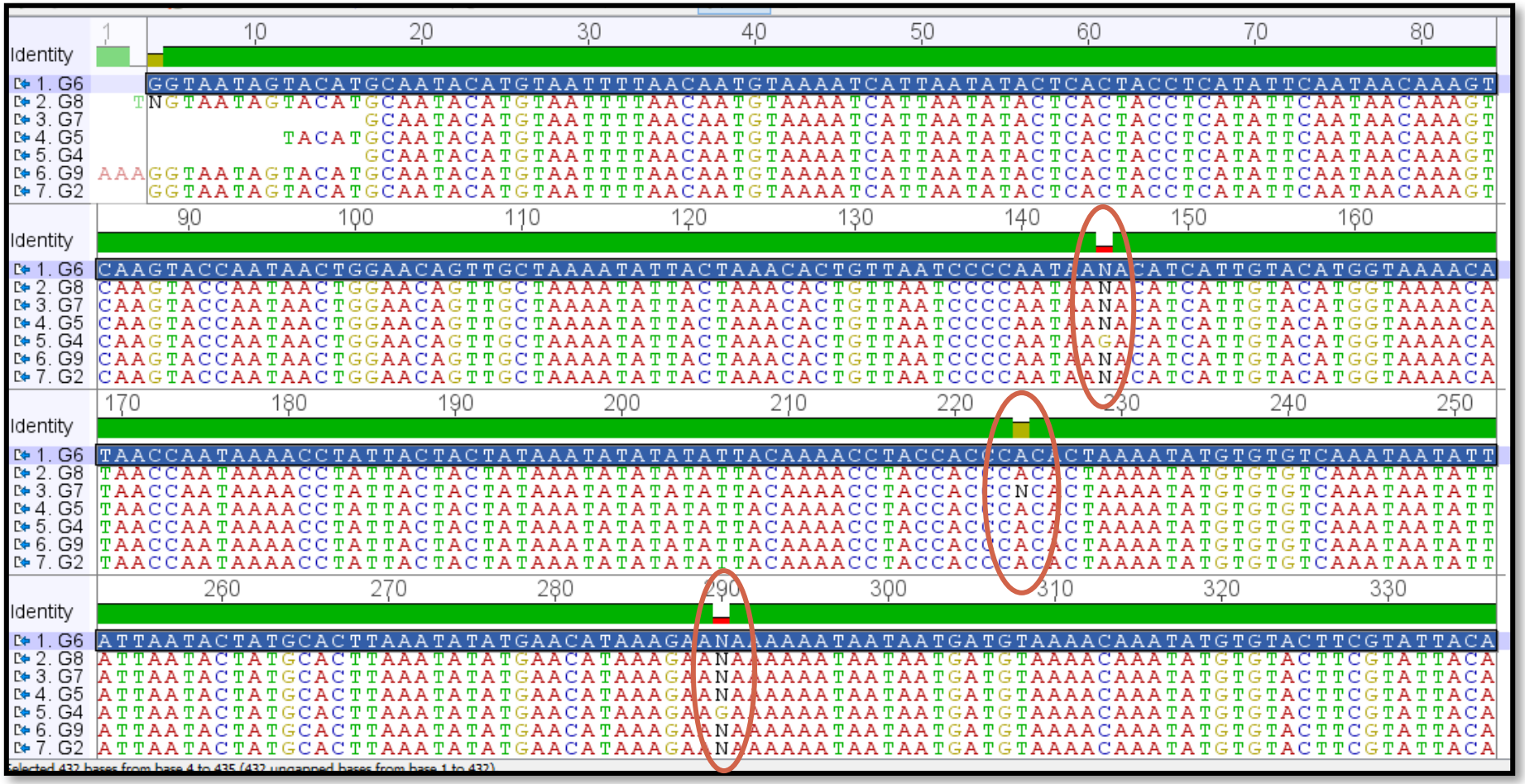
	G7	Leishmania tarentolae	Leishmania braziliensis	Leishmania tropica	L. donovani	L. infantum	L. killicki	L. major	L. mexicana	L. panamensis
G7	-	0.59725	0.41722	0.49258	0.57161	0.49258	0.63725	0.49258	0.51986	0.41722
Leishmania tarentolae	0.59725	-	0.52372	0.50603	0.52623	0.52623	0.23262	0.52555	0.60478	0.53934
Leishmania braziliensis	0.41722	0.52372	-	0.22846	0.22433	0.22023	0.66641	0.22599	0.25111	0.018491
Leishmania tropica	0.49258	0.50603	0.22846	-	0.066817	0.060191	0.67346	0.039051	0.11057	0.22640
L. donovani	0.57161	0.52623	0.22433	0.066817	-	0.0061131	0.67346	0.081943	0.11782	0.22228
L. infantum	0.49258	0.52623	0.22023	0.060191	0.0061131	-	0.67346	0.075183	0.11419	0.21818
L. killicki	0.63725	0.23262	0.66641	0.67346	0.67346	0.67346	-	0.38312	0.38312	0.33899
L. major	0.49258	0.52555	0.22599	0.039051	0.081943	0.075183	0.38312	-	0.12331	0.22228
L. mexicana	0.51986	0.60478	0.25111	0.11057	0.11782	0.11419	0.38312	0.12331	-	0.24245
L. panamensis	0.41722	0.53934	0.018491	0.22640	0.22228	0.21818	0.33899	0.22228	0.24245	-



Phylogenetic relationships of various members of the genus Leishmania based on the nucleotide sequences of the **Cyt b gene**.



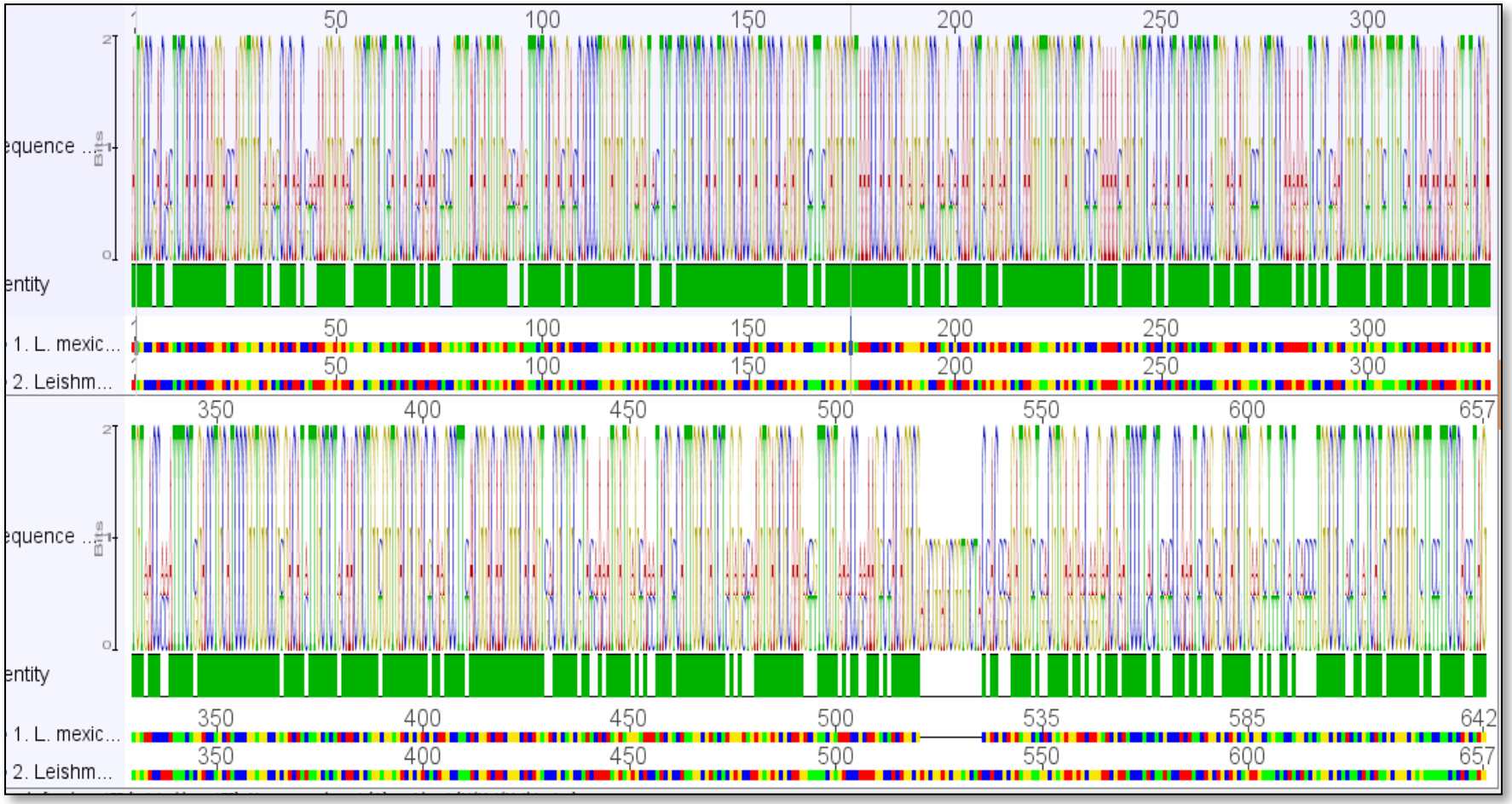
Alignment of the **Cyt b gene** of all tested Leishmania groups analysed in this course



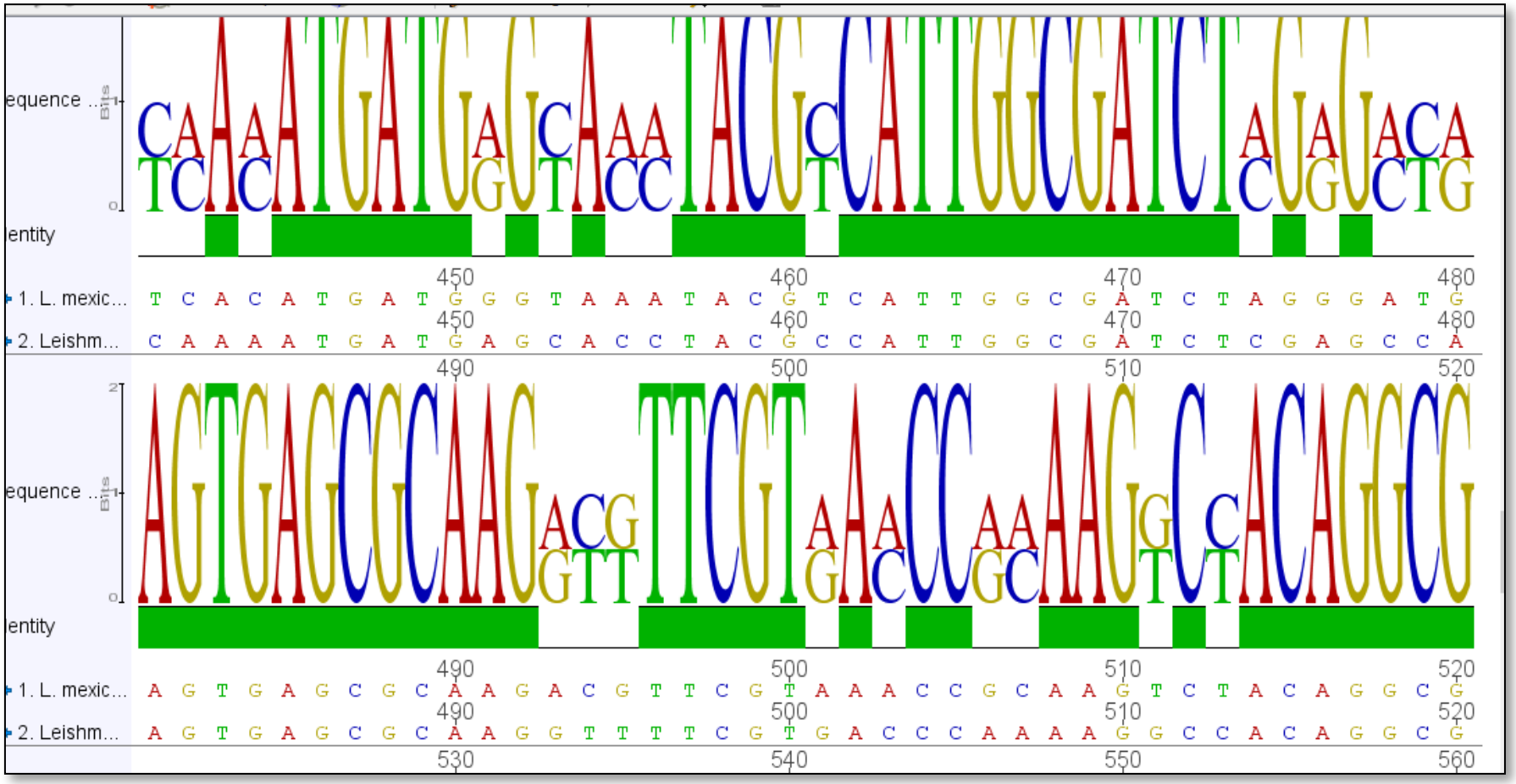
Alignment of the **Cyt b gene** of all tested Leishmania groups analysed in this course


Identity
1. Leishmania braziliensis
2. Leishmania tropica

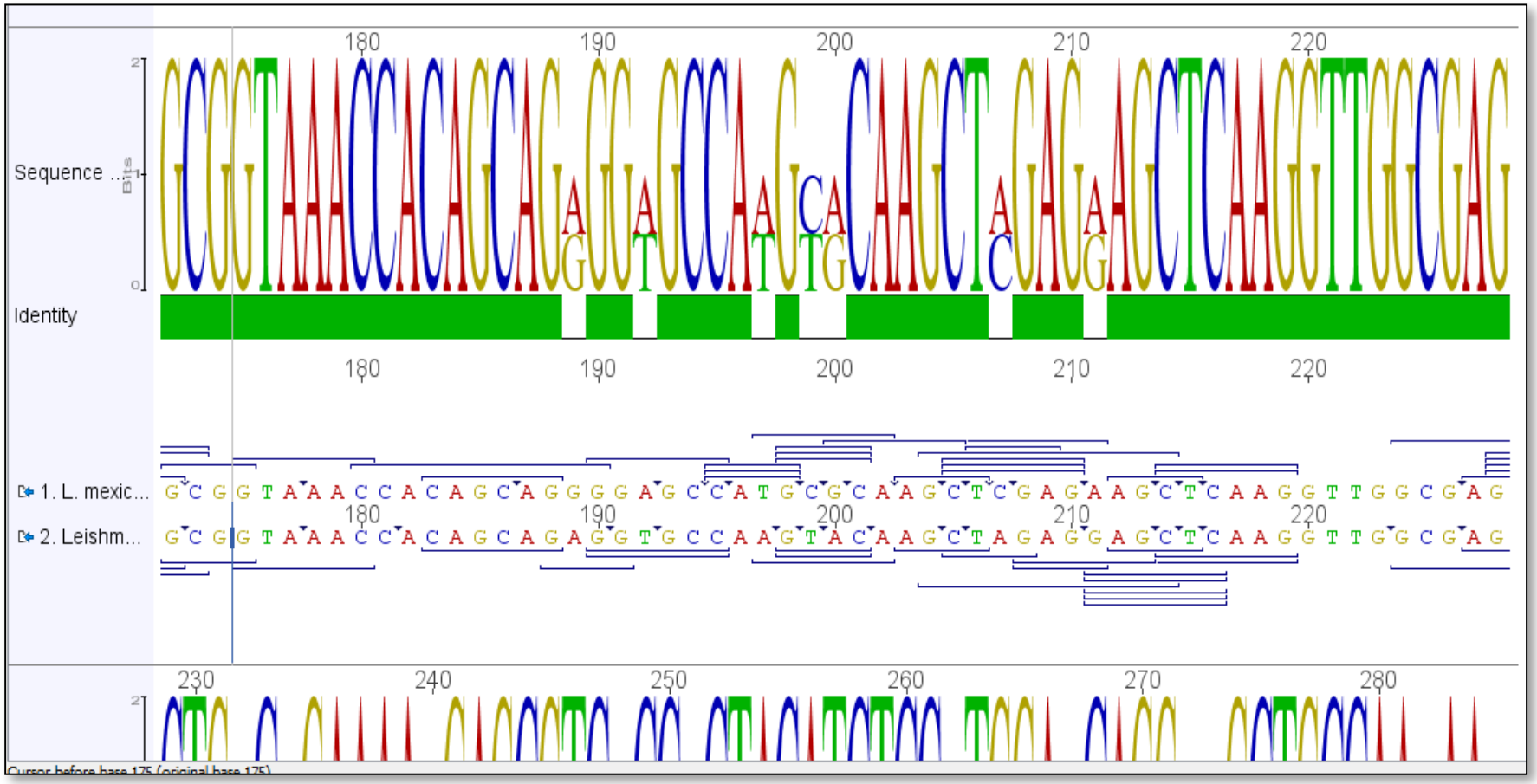
L. Mexicana vs *L. braziliensis*



L. Mexicana vs *L. braziliensis*



Restriction Enzyme sites




THANK YOU