**BLAST analysis instructions:**

1. Go to <https://blast.ncbi.nlm.nih.gov/Blast.cgi>

2. Click on Nucleotide BLAST

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Description automatically generated

3. Open up the .fasta file with our bat fly sequences in it. (Phylogeny\_seq\_alignment\_07282023).

4. Copy the sequence but do not include the name.

A computer screen with a white and blue text box

Description automatically generated

5. Paste sequence into Enter Query Sequence box.

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6. Make sure Highly similar sequences (megablast) is selected in program Selection. You can also click the show results in a new window box next to the BLAST button to have results open in separate tab.

7. Click BLAST

A computer screen with text boxes

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8. You will receive a list of sequences that share similarities with your submitted sequence

9. Look at the top sequence and do these 3 things.

1. The species is the same as the species you reported

a. ie. Cyclopodia dubia = Cyclopodia dupia in our samples

2. Look at the Per. Ident. Number

b. report this number in the Excel Sequence\_metadata file under & Identity

3. Report the Accession number for the top sequence in the Sequence\_metadata file.

10. Repeat for all sequences

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