

# STUDENT WORKSHEET

## Understanding the Diversity and Evolutionary Relationships in Nepenthaceae

Name:

Class:

Group:



# LEARNING OBJECTIVES



**After participating in this activity, students are expected to be able to:**

- **Explain the evolutionary relationships among living organisms based on DNA sequence similarities from three examples provided in the journal accurately.**
- **Classify living organisms according to the similarity of their DNA sequences presented in the journal, appropriately based on their level of relatedness.**
- **Analyze a phylogenetic tree using DNA data and logically conclude the evolutionary relationships among the organisms.**

# INTRODUCTION



## Question

1. Do you know about "Molecular Evolutionary Genetic Analysis (MEGA)" ?
2. Do you know about "BLAST" ?
3. What do you know about biodiversity?
4. What are some examples of biodiversity that you know?
5. Do you know about phylogenetic?
6. Do you know about Intergenic Spacer (ITS)?

## Expected Answer



# Now, let's watch a video about biodiversity and phylogenetics!

## a. Biodiversity

<https://youtu.be/rclOz8Fsbmg?feature=shared>

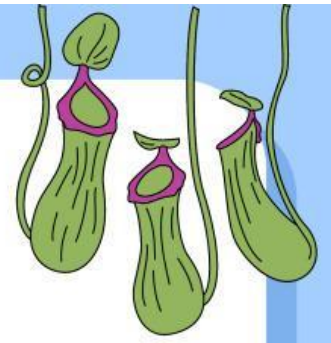


## b. Phylogenetic

<https://youtu.be/5x7k-Nyqbb8?si=CzWyg3wVMBlddk5->



# INVESTIGATION



Among the pitcher plants, the Nepenthaceae family is the largest, with 120 species that produce specialized cup-shaped pitcher that attract small and kill them through digestive enzymes. These plants look very similar to each other, especially when they're young so it's difficult to tell them apart just by physical appearance. The use of DNA samples from various types of Nepenthaceae allows for a more accurate analysis of the relationship between species, thus assisting in the preparation of a more precise taxonomic classification.

Modified from article: Genes. (2023). DNA Barcoding, Phylogenetic Analysis and Secondary Structure Predictions of *Nepenthes ampullaria*, *Nepenthes gracilis* and *Nepenthes rafflesiana*.

Article Access:

## Sample A (*Nepenthes ampullaria*)

### rbcl: OP534746

```
GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCTTGGCAG
CATTCCGAG
TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCCGCGGTAGCTGCCGAAT
CTTCTACTGGTAC
ATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGC
TACCACATCGAG
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACCTTTTT
GAAGAAGGTT
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGTG
CTCTACGCTT
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACCTTCCAAGGACCGCCTCACGGTA
TCCAAGTTGAG
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGAAATTG
GGGTTATCCG
CTAAGAAGTACG
```



# INVESTIGATION



## ITS1: OQ123732

TTGTCGAAATTGCTGAGCAGAGCGACCCGCGGAACTGTTGAAATAAATCGTCGGGGG  
CACGGCGCCCTCG  
ACTGCCGGCCGAGCCCGCACCTTACGGCCTGCTCCAGGAGCGGCGGTGGGGGTGCC  
GGGCCGGGCTTGCC  
ACGAGAACGAACCCCGGCGCGGATCGCGCCAAGGAAGAGTAACATAGCGCGCGTTG  
CCTTGCCGCCGGAT  
GCCCGGCGG

## ITS2: OQ123720

GGAGCTCGGCAACTGCGCCGCGACGTTCCGGTGGTCGGCAAGGCGAAGGCCAAGGTT  
GCATCGCGTCGCGC  
CCAGCTCGTCACCCGTGAAGCTCACACGACCCCGCTGCGCCGTCCGTACGGCACCAAC  
GCAGCGACCCCA  
GGTCAGGCGAGGCTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGGAAC  
TTACAAGGATTCC  
CCTAGTAACGGCGAGCGAACC GGGAAGTGCC

### Sample B (*Nepenthes gracilis*)

## rbcl: OP534748

GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCTTGGCAG  
CATTCCGAG  
TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCCGCGGTAGCTGCCGAAT  
CTTCTACTGGTAC  
ATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGC  
TACCACATCGAG  
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACCTTTTT  
GAAGAAGGTT  
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGTG  
CTCTACGCTT  
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACCTTCCAAGGACCGCCTCACGGTA  
TCCAAGTTGAG  
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGAAATTG  
GGGTTATCCG  
CTAAGAACTACGGTCGAGC



# INVESTIGATION



## ITS1: OQ123725

GTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCTCTAG  
GCTGAGG  
GCACGTCTGCCTGGGCGTCACGCATAGCGTCGCCCCGACCTAGCCGCCGCGTCTCTGCATGC  
TAGGTTG  
GGGCGGAGAGTGGCCTCCCGTGCGACGTTGTGCGGTTGGCCTAAAATCGGAGCTCGGCGACT  
GCGCCACG  
ACGTCCGGTGGTCGACAAGGCGATGGCCAAGGTTGCATGGCGTGGCGCTCGGCATGTCGCCC  
GTGAAGCT  
CGCACGAACCCGCCGTGCCGTCCGCACGGCACCAACGCAGCGACCCC

## ITS2: OQ123722

AACCGGAGCTCGGCGACTGCGCCGCGACGTCCGGTGGTTCGGCAAGGCGAAGGCCAAGGTTG  
CATCGCGTC  
GCGCCCGGCACGTCGCCCGTGAAGCTCGCACGACCCCGCCGCGCGGTCCGTACGGCACCAA  
CGCAGCGAC  
CCCAGGTCGGGCGGGGCTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGGAACT  
TACAAGGA  
TTCCCCTAGTAACGGCGAGCGAACC GGGAAGTGCCCAGCT

## Sample C (*Nepenthes rafflesiana*)

## rbcl: OP534747

GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCTTGGCAG  
CATTCCGAG  
TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCGCGGTAGCTGCCGAAT  
CTTCTACTGGTAC  
ATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGC  
TACCACATCGAG  
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACCTTTTT  
GAAGAAGGTT  
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGTG  
CTCTACGCTT  
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACCTTCCAAGGACCGCCTCACGGTA  
TCCAAGTTGAG  
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGAAATTG  
GGGTTATCCG  
CTAAGAACTACG

# INVESTIGATION

## ITS1: OQ123732

GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCTTGGCAG  
CATTCCGAG  
TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCCGCGGTAGCTGCCGAAT  
CTTCTACTGGTAC  
ATGGACAACCTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGACGATGC  
TACCACATCGAG  
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACCTTTTT  
GAAGAAGGTT  
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTGCGTG  
CTCTACGCTT  
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACCTTCCAAGGACCGCCTCACGGTA  
TCCAAGTTGAG  
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGAAATTG  
GGGTTATCCG  
CTAAGAACTACG

## ITS2: OQ123721

GGAGCTCGGCGACTGCGCCGCGACGTCCGGTGGTCGGCAAGGCGAAGGCCAAGGTT  
GCATCGCGTCGCGC  
CCGGCACGTGCCCCGTGAAGCTCGCACGACCCCGCCGCGCGGTCCGTACGGCACCAA  
CGCAGCGACCCCA  
GGTCGGGCGGGGCTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGGAAC  
TTACAAGGATTCC  
CCTAGTAACGGCGAGCGAACC GG

**Based on the existing sequence data, how to make a phylogenetic tree to show the relationship between species? Using BLASTN and MEGA for identifying homologous sequences, performing multiple sequence alignment, and generating a phylogenetic tree that illustrates the evolutionary relationships among the species!**



# STEPS TO USE BLASTN (via NCBI Website)



1

Open the NCBI website

Access:

2

On the homepage, click the "Nucleotide" tab  
(located below the search box)

3

Enter the FASTA code provided in the sample section, using the FASTA format  
starting with the ">" symbol

**Enter Query Sequence:**

*Nepenthes alata* (MW601008.1)

```
>AGTGTTCCTTCAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATAC
GAAACGAAAG
ATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAAGAAG
CAGGGGCAGC
GGTAGCTGCCGAATCTTCCACTGGTACATGGACAACCGTGTGGACTGATGGACTTACCAG
CCTTGATCGT
TACAAAGGGCGATGCTACAACATCGAGCCCGTTCTTGGCGAAACAGATCAATATATCTGT
TATGTAGCTT
ACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACCAACATGTTTACGTCCATTGTAGGAAA
TGTATTTGG
ATTCAAAGCCCTGCGTGCTCTACGCCTGGAAGATCTGCGAATCCCTACTGCTTATATTA
ACTTTCCAA
GGTCCGCCTCATGGGATCCAAGTTGAGAGAGATAAATTGAACAAATATGGTCGTCCTCTG
CTGGGATGTA
CTATTAAACCTAAATTGGGTTTATCCGCTAAAACTACGG
```

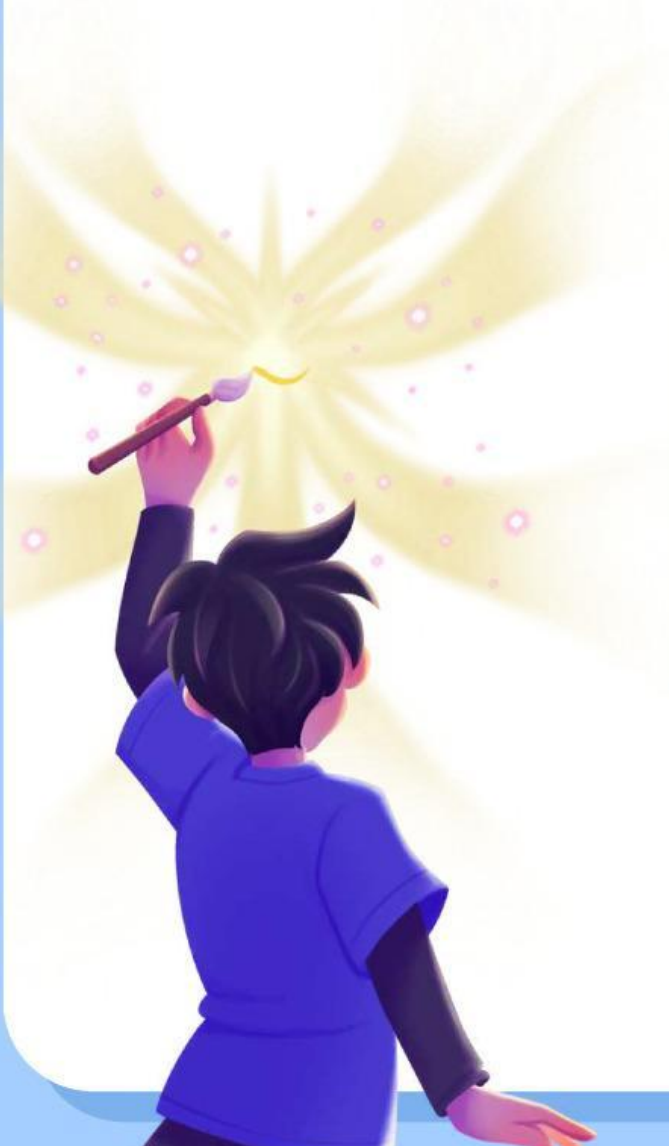
*Nepenthes mirabilis* (PQ550816.1)

```
>GTTGGATTAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCTGAGTATCAAC
CCCAGGACA
CTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCATCAGAAGAAGCAG
GGGCTGCAGT
AGCTGCCGAATCTTCTACTGGTACATGGACAACCTGTATGGACCGACGGACTTACCAGTCT
TGATCGTTAC
AAAGGACGATGCTACCACATCGATCCCGTTCCTGGAGAAGACAATCAATATATTTGTTATG
TAGCTTACC
CTTTAGACCTTTTTGAAGAGGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTA
TTTGGGTT
CAAAGCCTTGCGTGCTCTACGTTTGAGGATTGCGAATCCCTATTGCTTATATAAAAACT
TTCCAAGGA
CCACCTCACGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGCCGTCCTTATTG
GGATGCACCA
TTAAACCGAAATTGGGGTTATCTGCTAAAACTATGGTCGAGCAGTTTA
```

**Enter Subject Sequence:**

```
>GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCT
TGGCAGCATTCCGAG
TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCCGCGGTAGCTGC
CGAATCTTCTACTGGTAC
ATGGACAACCTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGAC
GATGCTACCACATCGAG
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACC
TTTTGAAGAAGGTT
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTG
CGTGCTCTACGCTT
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACCTTCCAAGGACCGCCTCA
CGGTATCCAAGTTGAG
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGA
AATTGGGGTTATCCG
CTAAGAACTACG
>GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCT
TGGCAGCATTCCGAG
TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCCGCGGTAGCTGC
CGAATCTTCTACTGGTAC
ATGGACAACCTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGAC
GATGCTACCACATCGAG
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACC
TTTTGAAGAAGGTT
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTG
CGTGCTCTACGCTT
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACCTTCCAAGGACCGCCTCA
CGGTATCCAAGTTGAG
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGA
AATTGGGGTTATCCG
CTAAGAACTACGGTCGAGC
>GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCT
TGGCAGCATTCCGAG
TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCCGCGGTAGCTGC
CGAATCTTCTACTGGTAC
ATGGACAACCTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGAC
GATGCTACCACATCGAG
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACC
TTTTGAAGAAGGTT
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTG
CGTGCTCTACGCTT
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACCTTCCAAGGACCGCCTCA
CGGTATCCAAGTTGAG
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGA
AATTGGGGTTATCCG
CTAAGAACTACG
```

- 4 Go to the BLASTN page  
Access:
- 5 In the "Enter Query Sequence" section, paste the available DNA sample
- 6 Click "Align two or more sequences"
- 7 Paste both sequences in the provided fields
- 8 Scroll down and click the "BLAST" button to run the alignment
- 9 Wait a few seconds until the results appear. You will see a list of homologous DNA sequences

A child with dark hair, wearing a blue long-sleeved shirt, is seen from behind, holding a paintbrush and painting a large, glowing yellow starburst shape on a white background. The starburst has many small pink dots around it.

**Ready to apply  
what you've  
learned? Let's  
construct a  
phylogenetic tree!**

# STEPS TO USE MEGA



- 1 Launch the MEGA application
- 2 Go to Align > Edit/Build Alignment > Create a New Alignment.
- 3 Select DNA as the sequence type
- 4 Click Edit > Insert Sequences from File.
- 5 Import your .fasta or .meg file containing the DNA sequences of interest (e.g., rbcL, ITS1, ITS2 from *Nepenthes* species and outgroup if applicable).
- 6 In the alignment window, click Alignment > Align by ClustalW to align all sequences
- 7 Save the aligned data: File > Save Session > Save as .meg format.
- 8 Return to the MEGA main window
- 9 Go to Phylogeny > Construct/Test Neighbor-Joining Tree
- 10 Open the aligned .meg file
- 11 In the options window, check Bootstrap method (e.g., 1000 replicates) to test tree reliability.
- 12 Click Compute to generate the tree
- 13 A phylogenetic tree will be displayed
- 14 Save the tree as an image or export it as a Newick format for further analysis

# EVALUATION



## Question

- 
1. Do you know about "Molecular Evolutionary Genetic Analysis (MEGA)" ?
  2. Do you know about "BLAST" ?
  3. What do you know about biodiversity?
  4. What are some examples of biodiversity that you know?
  5. Do you know about phylogenetic?
  6. Do you know about Intergenic Spacer (ITS)?

**Please answer the question below again**

