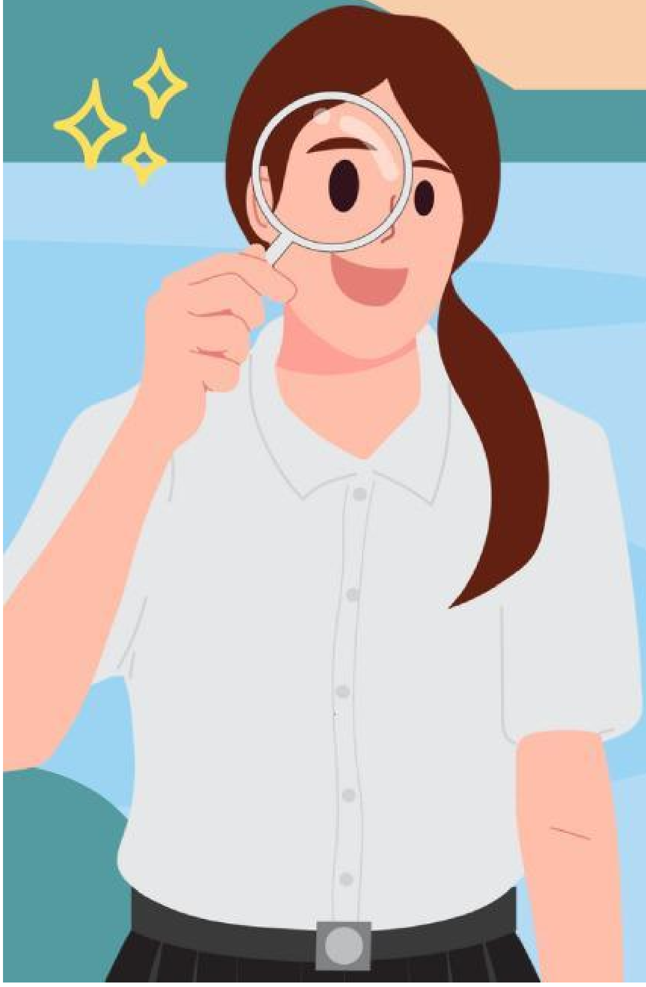
STUDENT WORKSHEET

Understanding the Diversity and Evolutionary Relationships in Nepenthaceae

Name:

Class:

Group:



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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.

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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

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

a. Biodiversity

https://youtu.be/pSUfsWzWbeg?si=Z1lbPBYR-K8rsBID

b. Phylogenetic

https://youtu.be/5x7k-Nyqbb8?si=CzWyg3wVMBIddk5-

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INVESTIGATION

Tropical rainforests are home to a variety of unique plants, one of which is the pitcher plant. This carnivorous plant belongs to the Nepenthaceae family, which is the largest group of pitcher plants with around 120 known species.

The characteristic of this plant is the presence of a cup-shaped pouch that functions to trap and digest insects with the help of digestive enzymes. Despite having similar functions, many species in this family have high morphological similarities, especially in the early growth phase. This makes the identification process based on physical characteristics quite difficult. To overcome these limitations, scientists use DNA analysis. By comparing DNA sequences from various pitcher plant species.

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

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INVESTIGATION

Sample A:
rbcL: OP534746
ITS1: OQ123732
ITS2: OQ123720

Sample B:
rbcL: OP534748
ITS1: OQ123725
ITS2: OQ123722

Sample C:
rbcL: OP534747
ITS1: OQ123732
ITS2: OQ123721

As a scientist involved in research on pitcher plants, do you think the phylogenetic tree created can explain the kinship relationships between pitcher plant species? Explain your answer by including reasons supported by available data or information.

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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)

```
>AGTGTTTTTTTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATAC
GAAACGAAAG
ATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTTCCGCCTGAAGAAG
CAGGGGCAGC
GGTAGCTGCCGAATCTTCCACTGGTACATGGACAACCGTGTGGACTGATGGACTTACCAG
CCTTGATCGT
TACAAAGGGCGATGCTACAACATCGAGCCCGTTCTTGGCGAAACAGATCAATATATCTGT
TATGTAGCTT
ACCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACCAACATGTTTACGTCCATTGTAGGAAA
TGTATTTGG
ATTCAAAGCCCTGCGTGCTCTACGCCTGGAAGATCTGCGAATCCCTACTGCTTATATTAAA
ACTTTCCAA
GGTCCGCCTCATGGGATCCAAGTTGAGAGAGATAAATTGAACAAATATGGTCGTCCTCTG
CTGGGATGTA
CTATTAAACCTAAATTGGGTTTATCCGCTAAAAACTACGG
```

Nephentes mirabilis (PQ550816.1)

```
>GTTGGATTTAAAGCTGGTGTTAAAGATTACAAATTGACTTATTATACTCCTGAGTATCAAC
CCCAGGACA
CTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCATCAGAAGAAGCAG
GGGCTGCAGT
AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTATGGACCGACGGACTTACCAGTCT
TGATCGTTAC
AAAGGACGATGCTACCACATCGATCCCGTTCCTGGAGAAGACAATCAATATATTTGTTATG
TAGCTTACC
CTTTAGACCTTTTTGAAGAGGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTA
TTTGGGTT
CAAAGCCTTGCGTGCTCTACGTTTGGAGGATTTGCGAATCCCTATTGCTTATATAAAAACT
TTCCAAGGA
CCACCTCACGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGCCGTCCCTTATTG
GGATGCACCA
TTAAACCGAAATTGGGGTTATCTGCTAAAAACTATGGTCGAGCAGTTTA
```

LIVEWORKSHEETS

Enter Subject Sequence:

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TCACTCCTCAACCTGGAGTTCCGCCGGAAGAAGCAGGGGCCGCGGTAGCTGC
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GATGCTACCACATCGAG
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACC
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ATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAAAGGAC
GATGCTACCACATCGAG
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACC
TTTTTGAAGAAGGTT
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCCTG
CGTGCTCTACGCTT
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACTTTCCAAGGACCGCCTCA
CGGTATCCAAGTTGAG
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AATTGGGGTTATCCG
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>GATTACAAATTGACTTATTATACTCCTGACTATGAAACCCTAGATACTGATATCT
TGGCAGCATTCCGAG
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GATGCTACCACATCGAG
CCTGTTGCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACC
TTTTTGAAGAAGGTT
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CGTGCTCTACGCTT
GGAGGATTTGCGAATCCCTCCTGCTTATTCGAAAACTTTCCAAGGACCGCCTCA
CGGTATCCAAGTTGAG
AGAGATAAATTGAACAAATATGGTCGTCCACTATTGGGATGTACTATTAAACCGA
AATTGGGGTTATCCG
CTAAGAACTACG

4 Go to the BLASTN page
Access:

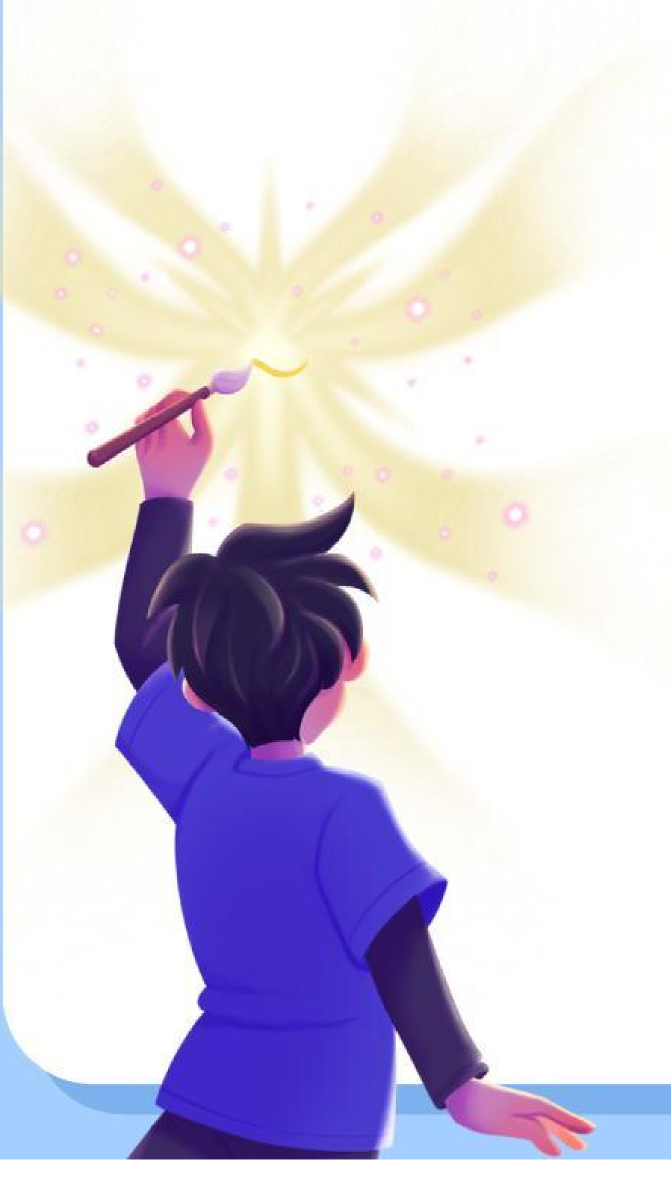
5 In the "Enter Query Sequence" section, paste the available DNA sample

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



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

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EVALUATION

Question

1. **Do you know about "Molecular Evolutionary Genetic Analysis (MEGA)" ?**
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6. **Do you know about Intergenic Spacer (ITS)?**

Please answer the question below again

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