LKPD

STUDENT WORKSHEET

Biodiversity and Evolutionary Relationships of Medicinal Plants in the Tianshan Wilderness, China

Group: _____

Grade: _____

Member Of The Group:

1.
2.
3.
4.
5.
6.



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LEARNING OBJECTIVES

- Through learning activities, students can identify levels of biodiversity correctly.
- Through learning activities, students are able to formulate efforts to preserve biodiversity correctly.
- Through discussion activities, students can group living things and understand evolutionary relationships by interpreting the correct phylogenetic tree.

A. INTRODUCTION

Answer the questions below!

Question	Answer
What do you know about MEGA (Molecular Evolutionary Genetics Analysis)?	
What do you know about biodiversity?	
Can knowing about genetic diversity help conservation efforts?	
What is meant by a phylogenetic tree?	
What are the stages in creating a phylogenetic tree?	
Why is it important to understand the genetic relationships between medicinal plants? Give your reasons!	
What do you know about Internal Transcribed Spacer (ITS)?	

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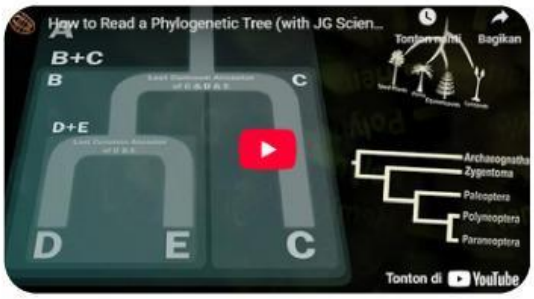
Next, watch the following video on the definition of biodiversity and how to read a phylogenetic tree.

1. Definition of Biodiversity



https://youtu.be/rclOz8Fsbmg?si=ri0Md46x0GAmS2dm

2. How to Read a Phylogenetic Tree



https://youtu.be/SFzk2fB9-mo?si=rhxeoP2hsFOoSaZk

After watching the two videos above, give your opinion below!

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B. INVESTIGATION

The Tianshan Wild Fruit Forest in Xinjiang, China, is one of the world's centers of medicinal plant biodiversity. However, this region faces serious threats from human activities, such as illegal mining, overgrazing, and unsustainable exploitation of medicinal plants. Many endemic medicinal plant species in this region remain incompletely documented, either morphologically or genetically. Identification based on physical characteristics often leads to errors due to the large number of similar-looking species. Therefore, researchers have begun using DNA barcoding and phylogenetic tree analysis to determine the relationships among medicinal plants.

In a recent study by Jiamahate et al. (2025), 101 medicinal plant species were analyzed using the ITS, matK, and rbcL genes. The results helped identify 23 species that should be prioritized for conservation due to their high genetic and ecological value. These include *Arnebia euchroma, Ligularia heterophylla, Rosa laxa, Sparganium stoloniferum, Amaranthus retroflexus, and Parnassia palustris.*

Source: Jiamahate, A., Bozorov, T. A., Wang, J., Zhang, J., Zhang, H., Wang, X., Yang, H., & Zhang, D. (2025). Insights from DNA Barcodes-Based Phylogenetic Analysis of Medicinal Plants and Estimation of Their Conservation Status: A Case Study in the Tianshan Wild Forest, China. *Plants*, 14(99), 1-20.

1. Case Analysis

A conservation team has been assigned by the government to help develop a conservation strategy for medicinal plants in the Tianshan Forest. If you were part of that conservation team, your task would be to determine the genetic relationship between the medicinal plant species mentioned in the article excerpt above. To do this, you would use DNA sequence data from each species and analyze it using bioinformatics software called MEGA (Molecular Evolutionary Genetics Analysis). The results of this analysis will be presented in the form of a phylogenetic tree, which illustrates the evolutionary relationships between species and helps determine conservation priorities based on their genetic relationships.

Based on the available sequence data, the relationships between the medicinal plants mentioned above can be demonstrated by constructing a phylogenetic tree. How do you construct a phylogenetic tree of medicinal plants using available sequence data?

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The sequence data for each species is as follows:

(https://pastebin.com/j9iE2RCQ)

SPECIES 1

Arnebia euchromal (Target):

GTGACACGTTCACTAAAAA
AAAATCCTTTTGTAGCCAAT
CATTTATTAAAAAAAATTGAA
AAGCTTAATA

SPECIES 2

Ligularia heterophylla:

TCAATAAACTCTTAGAATTTT
GCTATTAACTATTTAATTTAA
ATTCTTGGCTATTCATATTC
GCTATTAT

SPECIES 3

Rosa laxa

ATGGTTCTTCAAGGATCCTT
TTATACAGTATGTTAGATAT
CAAGGAAAATCGATTCTGG
CATCAAAAGAT

SPECIES 4

Sparganium stoloniferum

ATGAAACAATTACAAGGAT
ATTTAGAAAAAGCTAGATCT
CGGAAACAACACTTCCTAT
ATCCGCTTCTTT

SPECIES 5

Amaranthus retroflexus

GTGTTAGATATATTAATACC
TTACCCTGCCCATCTAGAA
ATCTTGGTTCAAACTCTTCG
CTATTGGTTGA

SPECIES 6

Parnassia palustris

ATGGAAGAGTTTCAAGGAT
ATTTAGAATTAGATCCCTCG
CAGCAGCATGACTTCCTAT
ATCCACTTATTT

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2. Creating a Phylogenetic Tree Using MEGA

1) Download and Install the MEGA Software
(https://www.megasoftware.net/)

2) Prepare DNA Sequence Data

- Sequence data for each species used can be seen above or if you have not prepared sequence data, you can search for and download it via NCB. (https://www.ncbi.nlm.nih.gov/) with FASTA file format

3) Open MEGA and Create an Alignment File

- Open MEGA
- Select Align > Edit/Build Alignment > Create New Alignment > DNA
- Click Edit > Insert Sequence From File, then insert the FASTA file containing the six sequences
- Save the alignment file (format: .meg)

4) Apply Multiple Sequence Alignment

- Select Alignment > Align by ClustalW or MUSCLE
- After the process is complete, save the alignment file again.

5) Create a Phylogenetic Tree

- Return to the MEGA home page
- Select Phylogeny > Construct/Test Neighbor-Joining Tree (or another method such as Maximum Likelihood)
- Select the created .meg alignment file and click OK
- Set parameters if necessary, then click Compute

6) Visualize and Save Phylogenetic Tree

- The phylogenetic tree will appear in a new window.
- Use the features in the toolbar to adjust the tree's appearance (e.g., radial, rectangular).
- Click File > Export Image to save the resulting tree in image format (.png/.jpg).

"After completing the above activity, now you can construct of a phylogenetic tree by your own version!"

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COME ON, ANSWER THE FOLLOWING QUESTIONS!

Once you've successfully created a phylogenetic tree from the available DNA sequence data, it's time to analyze and draw conclusions based on the results. Answer the following analysis questions to connect this bioinformatics activity to the issue of medicinal plant conservation:

Question	Answer
How do the kinship patterns between medicinal plant species appear in the phylogenetic tree you constructed?	
Which species is most closely related to Arnebia euchroma?	
Based on kinship relationships, which species should be prioritized for conservation? Why?	
If you use more than one gene such as ITS and rbcL, is there a difference in the relationship patterns between species of the two genes?	
What can you conclude about the accuracy or strengths/weaknesses of each gene in determining evolutionary relationships?	

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Answer the questions below!

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GREAT JOB!

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