Identification of *Gracilaria* sp. Through Molecular Analysis

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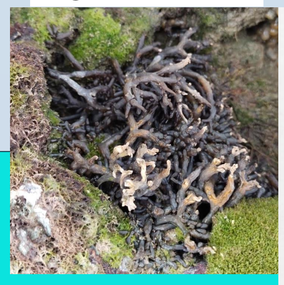
INTRODUCTION

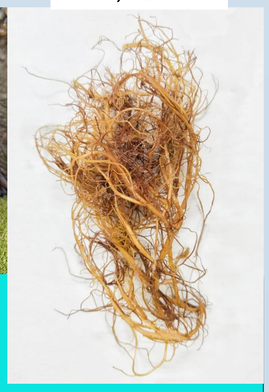
Red seaweed: *Gracilaria* sp.

- major contributors to Malaysia's algae production [1]
- highly commercial species – primarily produced for agar in the manufacturing industry [2]
- Annual production in Malaysia: approximately 200 metric tonnes [3]
- Global annual production over 30 million metric tonnes [4]

Port Dickson, Negeri Sembilan



Muar, Johor



Sample Source : Muar, Johor // Port Dickson, Negeri Sembilan

Problem Statement : Difficulty in assessing red seaweed species based solely on morphological data due to the limitation of distinct morphological differences [5]

Significance of study : To determine the distribution, taxonomy and the biodiversity of *Gracilaria* sp. using molecular analysis

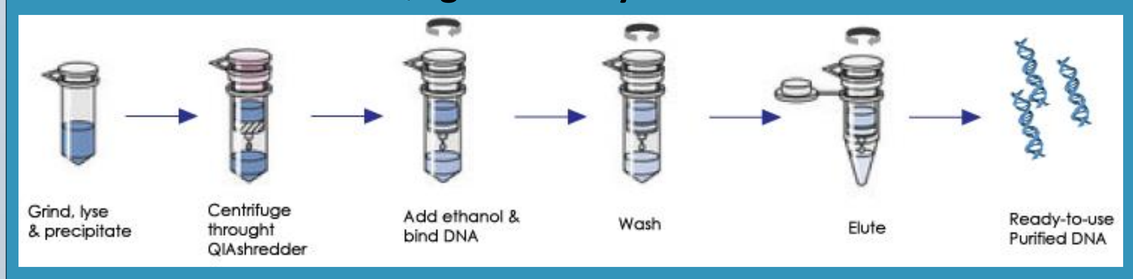
Objective : To identify *Gracilaria* sp. in south Peninsula Malaysia through PCR amplification and phylogenetic tree analysis

METHODOLOGY

1. **Sample collection – *Gracilaria* sp. samples were collected from Muar, Johor and Port Dickson, Negeri Sembilan respectively**

   Samples were washed with freshwater, rinsed with distilled water and left to dry

2. DNA extraction via Qiagen DNeasy® Plant Mini Kit



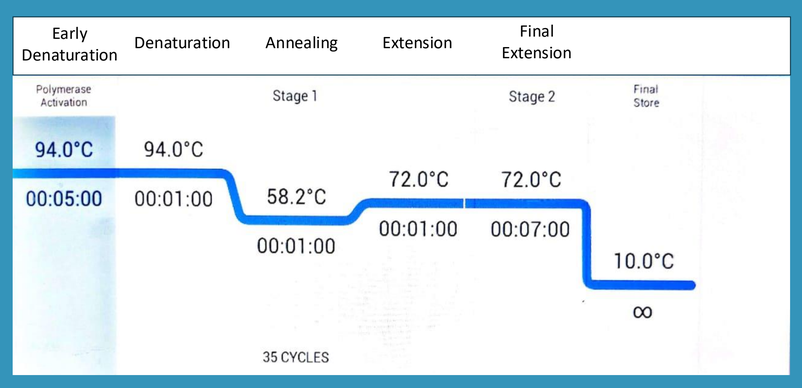
3. PCR amplification

Gene sequence used for PCR amplification: COX1 [6]

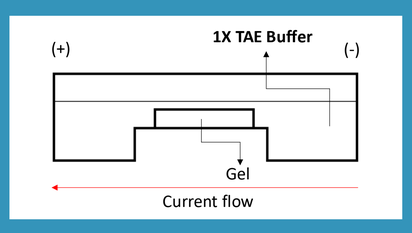
Primers	Oligonucleotide seq (5' – 3')
COX143F	TCA ACA AAT CAT AAA GAT ATT GGW ACT
COX11549R	AGG CAT TTC TTC AAA NGT ATG ATA

PCR Mixture:
DNA template
dNTP
$MgCl_2$
PCR Buffer
Forward primer
Reverse primer
Taq polymerase
Nuclease free water

PCR Programme Settings



4. Gel Electrophoresis (1.5% agarose + 100mL 1X TAE Buffer)

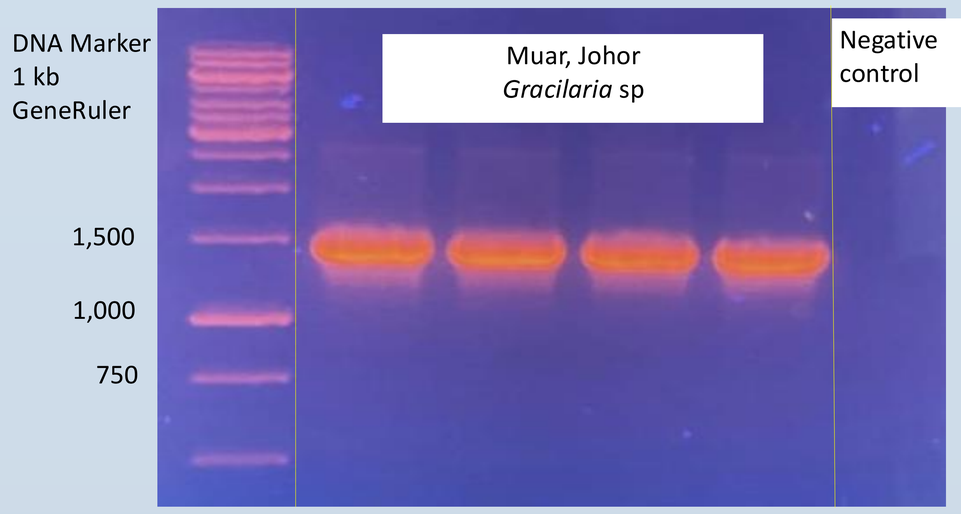


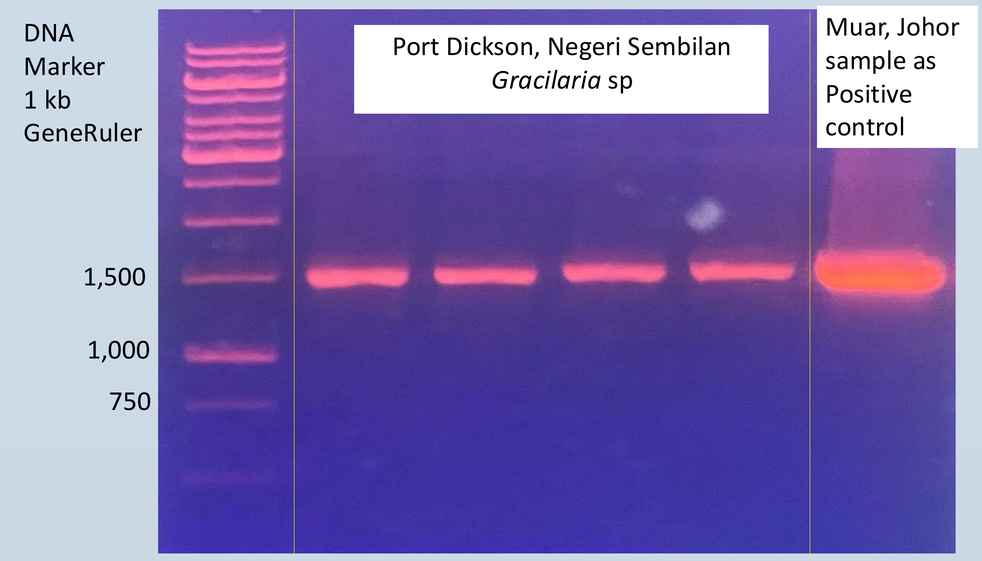
Voltage	90V
Power	120W
Time	60 min

5. DNA sequencing and Phylogenetic Analysis

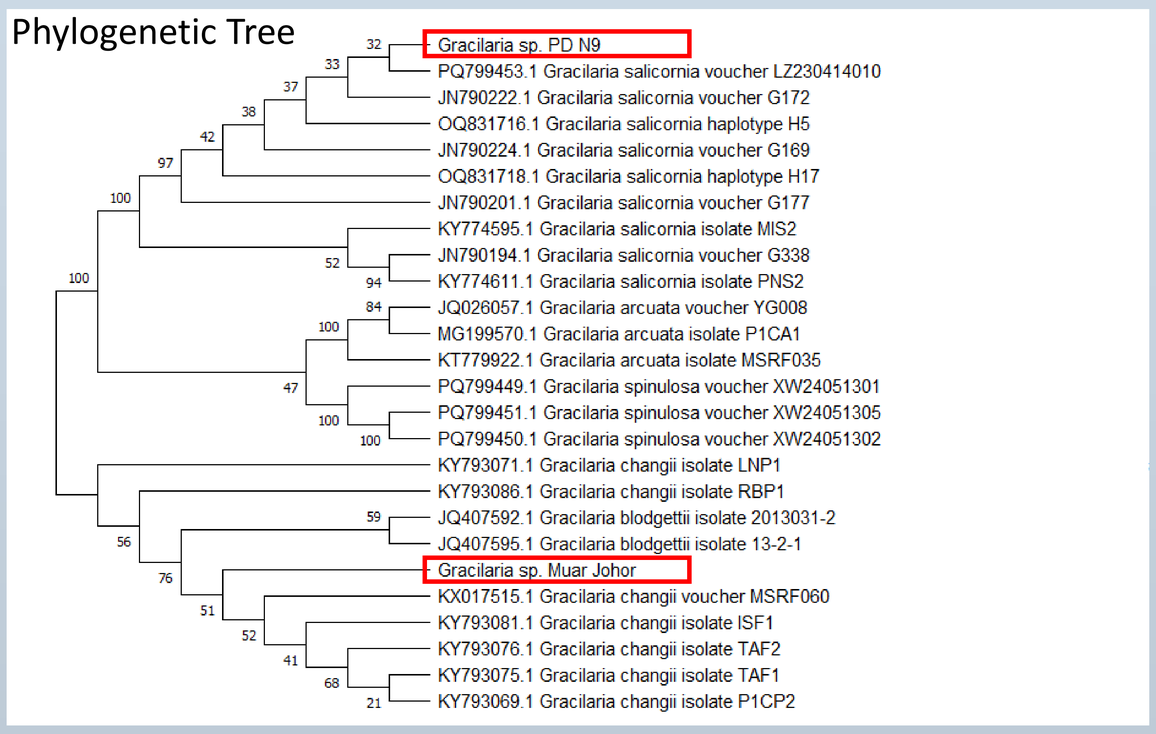
- PCR products were sequenced using Sanger's method
- DNA sequences were compared with available sequences through BLASTn and aligned using Bioedit
- Phylogenetic tree constructed using MEGA 11 software

RESULTS & DISCUSSION





The PCR product were observed at ~1,400 bp, respectively



Phylogenetic tree shows that the ***Gracilaria* sp. from Muar, Johor is closely associated with *Gracilaria blodgetti* and *Gracilaria Changii***, whereas the ***Gracilaria* sp. from Port Dickson is highly associated with *Gracilaria salicornia***. BLASTn indicated percentage identical for *Gracilaria changii* and *Gracilaria salicornia* to be 95-96% and 94-99%, respectively.

The *Gracilaria* sp. from Port Dickson, Negeri Sembilan matches the description of *Gracilaria salicornia* (Lim & Phang, 2004) in terms of distribution and morphology [7]. *Gracilaria changii* was discovered in Muar, Johor in 2023 [8]. Prior to this, *Gracilaria changii* was already distributed across the state of Johor in the coastal area of Mersing, Kukup, and Sungai Pulai [7]. This indicates that *Gracilaria changii* is a common species of red seaweed in Johor and is locally distributed across the state.

The **COX1 gene was used for its suitability in identifying the seaweed species up to genus and species level due to the differences in COI-amino acid sequences**. This method is straightforward, having an accuracy of more than 96.4%, is low cost and accessible for molecular analysis and species identification [9].

CONCLUSION

- Through molecular analysis, the species of red seaweed in Muar, Johor is *Gracilaria changii*; the species of red seaweed in Port Dickson, Negeri Sembilan is *Gracilaria salicornia*
- The COX1 gene is reliable in molecular analysis for species identification with high accuracy and accessibility.

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IDRC · CRDI

References