



# Determining Correlation between Invasive Species Infested Trees and Lichen Growth in Garden City

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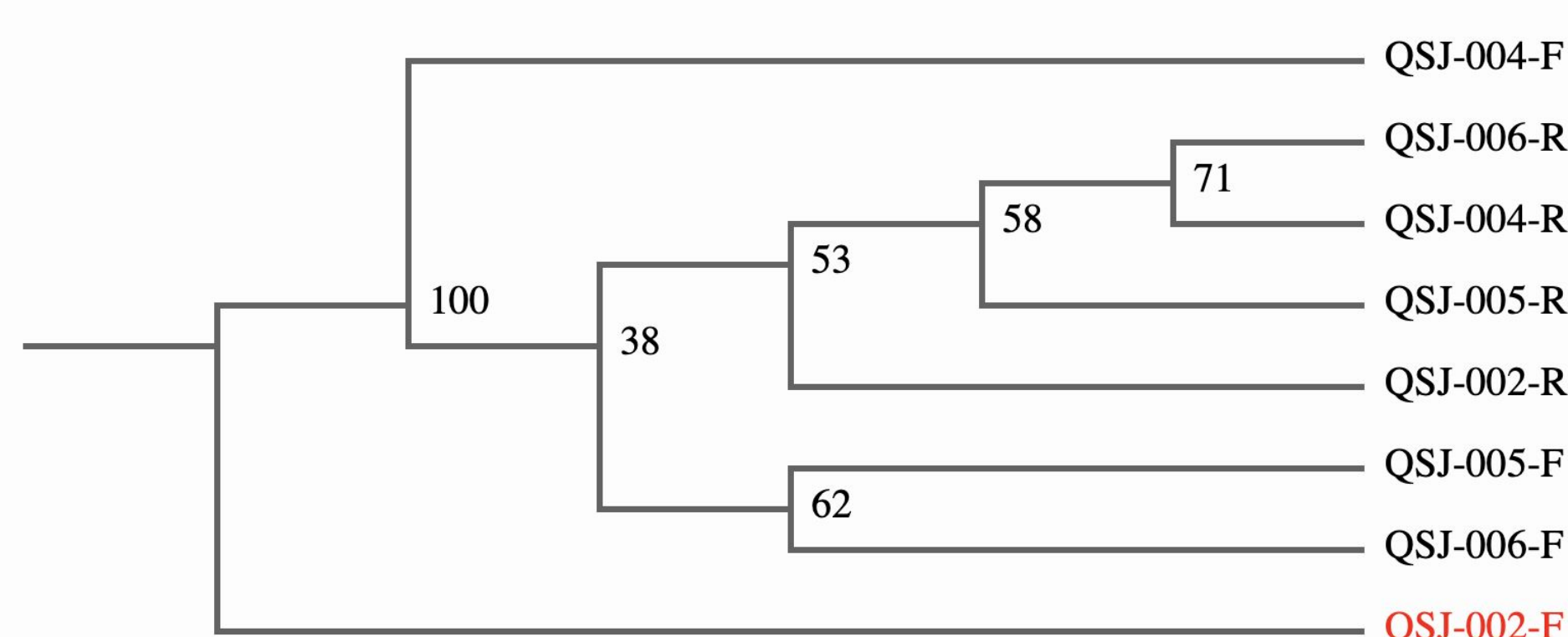
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## Abstract

**Emerald ash borers (EAB)** are an **invasive species** in the United States that specifically **target ash trees**. **Lichens**, plant-fungus symbiotic organisms, are often used as **health indicators for the trees they grow on**. We hypothesized that a specific species of lichen growth may correlate with EAB infestation. **DNA sequencing** of lichen and bark samples from infested ash trees was done to determine present lichen species. One sample was identified as *Gallowayella weberi* (*Xanthomendoza weberi*), a common type of lichen ubiquitous across much of the contiguous United States. Since trees in declining health tend to be suitable lichen substrates, this could suggest that lichen are useful indicators on ash trees for possible locations of future infestations.

## Introduction

- Emerald Ash Borer (EAB) are an invasive species that target ash trees
  - Bore through bark and lay eggs which consume phloem and cambium of tree
  - Identifiable by D-shaped exit holes
- EAB infestation has killed tens of millions of ash trees throughout the US and Canada
  - Older-growth ash trees in Garden City are being cut down to curb the spread of infestation
- Lichen = mycobiont (fungi) + photobiont (algae or cyanobacteria)
  - Does not negatively impact substrate
  - Often used as marker of thriving environment
- Previous studies have suggested relationship between lichen growth and diseased trees
  - Decreased foliage → increased sunlight → increased lichen growth
- We hypothesize there may be a potential correlation between infested trees and the type of lichen growth



## Materials and Methods

### Sample Collection

- Collection location: Terrace Park (40.73°06'67", -73°67'00")
- Utilized direct hand collection method
  - Tweezers, gloves, plastic bag
- 4-6 cm in length
- Sampled 3 EAB infested ash trees marked to be cut down
- Collected late March
- Stored in freezer until isolation

### Bioinformatics

- DNA Sequences manually trimmed
- Samples identified on NCBI BLAST
- MUSCLE and Phylogenetic diagram constructed on DNA Subway

### Isolating the DNA

- Utilized Silica DNA extraction method
  - Using tweezers, 1 cm pieces of samples were deposited into Eppendorf tubes
  - Lysis solution and pestle used to mechanically break membranes and release DNA
  - Supernatant was separated into another tube and silica resin was pipetted in
    - Silica resin's polarity allowed it to bind to DNA
  - Silica pellet was washed with wash buffer 2 times
  - dH<sub>2</sub>O mixed with pellet to dissolve
- Fungi (lichen specific) ITS-1 primer amplified using CSHL protocol
- Gel electrophoresis performed to confirm success of PCR
- Samples sent to Cyverse to obtain DNA sequences

## Results

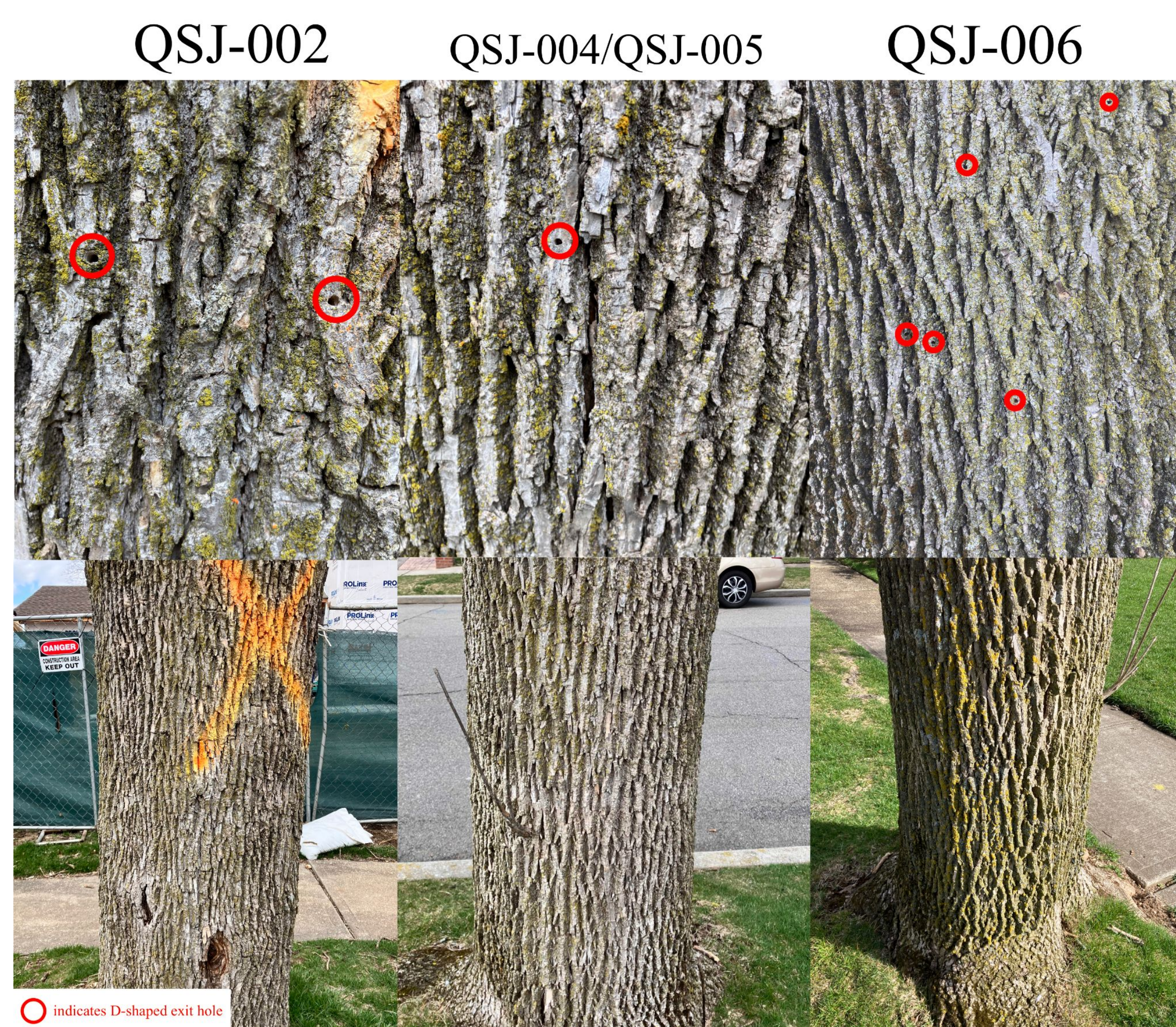


Figure 1 (above): EAB infested ash trees on Terrace Park where samples were collected 3/28/23. Trees are marked to be cut down due to infestation.

Figure 3 (left): Phylogenetic tree shows low genetic relations between samples.

Figure 4 (right): Comparison of samples using MUSCLE diagram shows poor quality sequencing (frequent gray areas) except for QSJ-005.

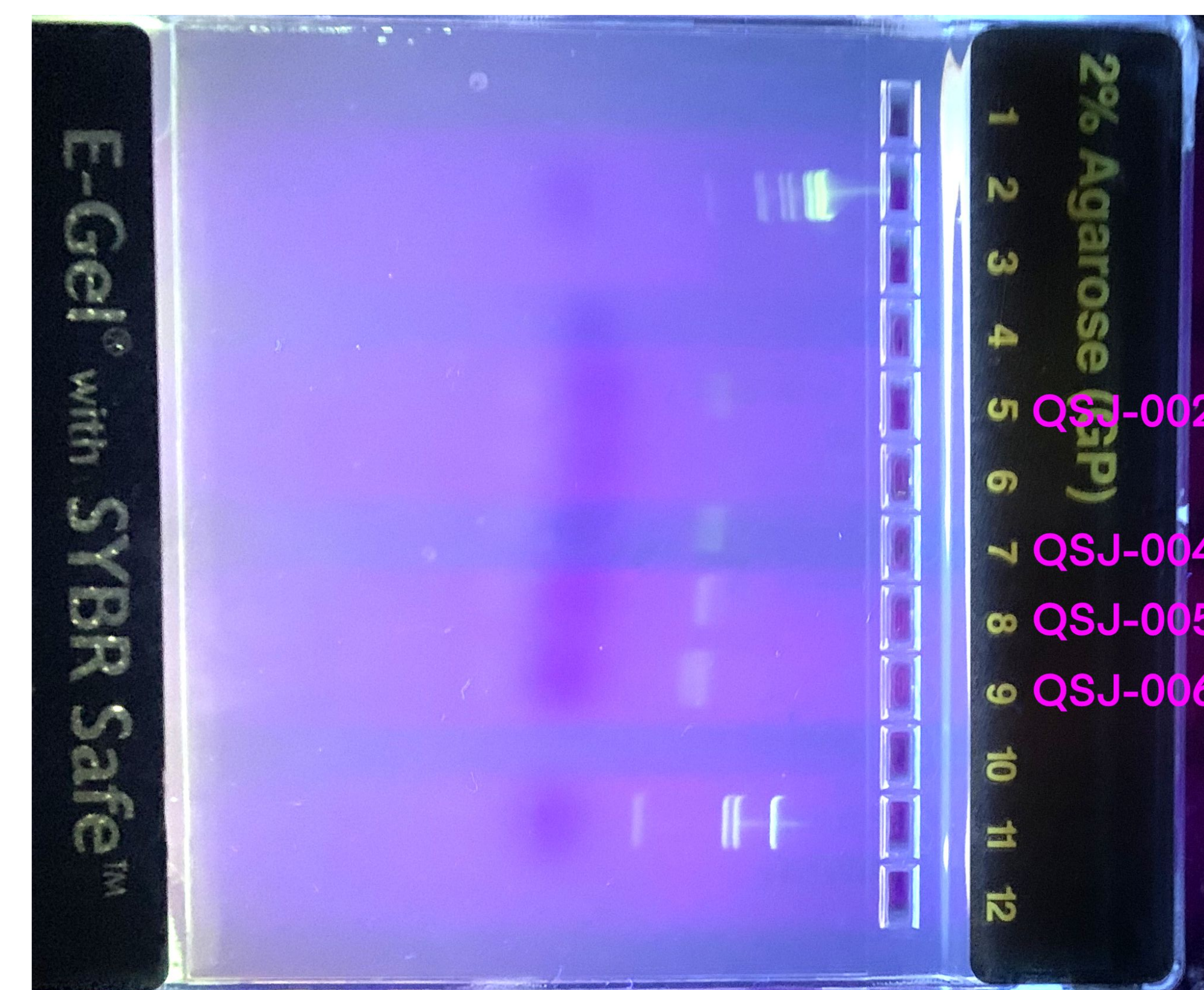
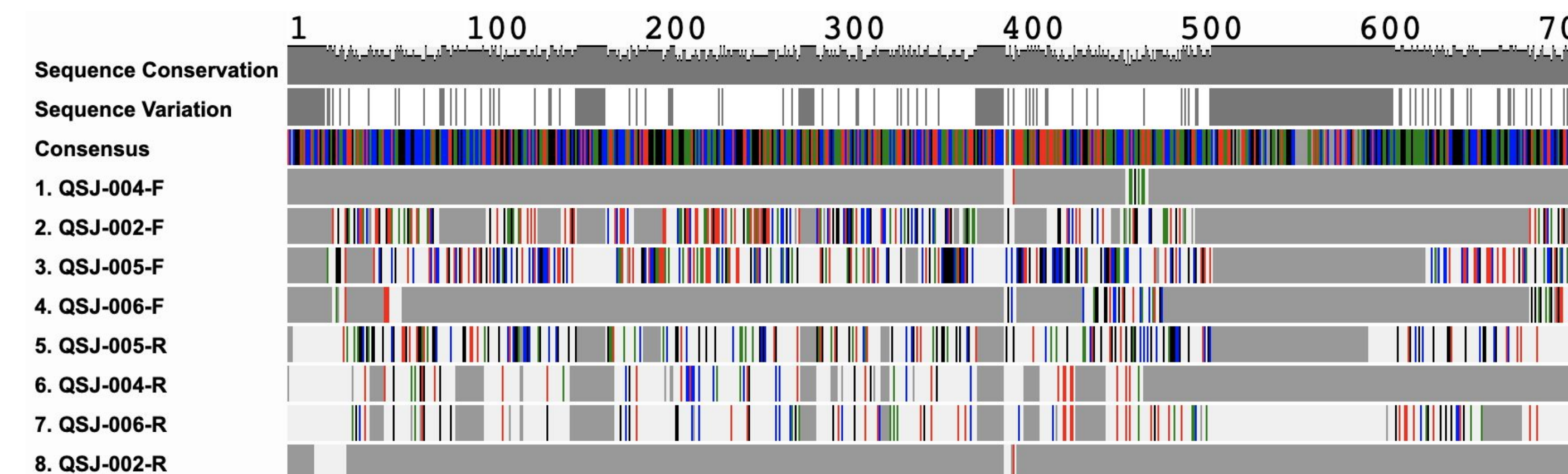


Figure 2 (above): PCR products were identified using Gel Electrophoresis. Lanes 4-9 contain samples; odd wells contain tree bark, even wells contain lichen. QSJ-002, QSJ-004, QSJ-005, and QSJ-006 show positive results. QSJ-002, QSJ-004, and QSJ-006 suggest double bands (see discussion).



## Discussion

- Sample QSJ-005 was identified as *Gallowayella weberi*, suggesting there may be a correlation between EAB infestation and *Gallowayella weberi*
- 3 out of 4 sequenced samples had too low quality to be identified on BLAST
  - QSJ-002, QSJ-004, and QSJ-006 in gel show double bands → suggests multiple fungi
  - Multiple species of fungi → background noise in sequencing, no dominant fluorescence signal detected → low-quality sequence/ unable to identify
- QSJ-005 (bark) likely yielded lichen due to trace bits of lichen left on bark amplified by ITS fungi primer; succeeded over intended lichen samples because less fungi present
- Future Directions
  - Collect lichens from non-infested ash trees to compare lichen species
  - Correlate severity of infestation with lichen amount
    - Decreased foliage in infested trees may lead to increased lichen growth
  - Bark DNA samples still remain → use insect specific primer to confirm EAB presence

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