Arboreal Diversity in Sub-ecotones of the Peruvian Amazon
Blackwater Rainforest: Barcoding from Cambial Tissue

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Objectives:
1) Establish an arboreal study of lowland forest floodplain dynamics across distinct sub-ecotopes in relation to abiotic and biotic parameters.
2) Record size, distribution, and density of the trees within each sub-ecotope
3) Establish a protocol for the extraction and amplification of cambial tree DNA from bark samples using traditional plant barcoding genes

Methods:

Study Site: Tarmishayuc Nature Preserve in the lowland Amazonian forest floodplain near the banks of the Tahuayo River (Fig 2), a tributary of the Amazon River upstream and south of Iquitos, Peru.

Tree Survey: Trees ≥ 10 cm, were tagged and measured at breast height (DBH) and cambial tree DNA from bark samples using traditional plant barcoding genes

1) Isolating cambial tissue: The cambial tissue from 77 samples was isolated by scraping the samples' underside after removal of wood (Fig 3).
2) DNA extraction and amplification: tissue was homogenized using liquid nitrogen and a mortar & pestle, extracted using a Qiagen DNEasy kit, and amplified using established PCR protocols.

Results:

- Bajial, the sub-ecotype with the highest flood level, had the highest density of trees (1200/ha) and the lowest avg. tree diameter (20.1 cm) (Fig 6).
  - The rarely flooded HR had the second highest density of trees (748/ha) and contained the second highest average tree diameter (20.6), behind the LR (Fig 6).
  - The Bajial yielded the lowest average DNA concentration and nucleic acid purity. It also had the lowest proportion of amplification success (Table 1).
  - The HR yielded the highest average DNA concentration and nucleic acid purity. It had the highest proportion of amplification success.
  - The 5 most effective primers were 1f+r724 (rbcLa), KIM3f+KIM1r (matK), 1329f+320r (ITS), and psbA3f+trnH (psbA-trnH) (Table 2).

Background Information:
- The Lowland Amazon Igapo forest floodplain, identified by the black water that floods it, is home to four distinct sub-ecotopes characterized by differences in annual flood levels: High Restinga (HR), Low Restinga (LR), Bajial (B) and Palm Swamp (PS) (Fig 4).
- Previous studies have put forth estimates of >11,000 tree species in the Amazon basin, with density estimates of 500-700 trees per square hectare.
- With up to 2200 mm of precipitation annually, the Igapo floodplain is completely flooded for 2-4 months of the year (Fig 1).
- Simplified species delimitation using DNA barcoding could be useful for baseline estimates of biodiversity and subsequently for making informed decisions regarding conservation management.
- Even without taxonomic species determination, DNA barcoding could provide a reasonable estimate of the species diversity present.
- Obtaining DNA from the cambial tissue found in bark eliminates the need to collect leaf tissue from high up in the canopy.

Figure 1. Igapo forest floodplain during the wet season.

Figure 2. The TRARC gridted research site southeast of the Tahuayo River

Figure 3. Bark sample, cambial side

Figure 4. Three sub-ecotones of the Tahuayo River Igapo forest floodplain organized by increasing annual flooding level: High Restinga (top left), Low Restinga (top right) and Bajial (bottom right).

Figure 5. The distribution of tree DBH in the sub-ecotones of the Tahuayo River Igapo Forest Floodplain. DBH was grouped in 2 cm increments for all sub-ecotopes and followed a logarithmic curve (n=1200 for LR, n=748 for HR, and n=654 for B). Chi-Square, X^2=47.490, df=30, p=0.022

Figure 6. A) Density of trees per ha at the TRARC in the various sub-ecotones of the Tahuayo River Amazon floodplain. LR (654), HR (748), and B (1200). B) The average tree diameter of each sub-ecotype studied. Error bars = SEM, ANOVA, F=0.42, p=0.655

Figure 7. Amplification of 3 genes known to be valuable tree barcodes.

Discussion and Future Work:
- While DNA extraction from bark was successful, amplification of target genes was less reliable. Potential modifications to protocol include:
  1) Improved on-site extraction methodology and immediately preserving bark samples in silica gel
  2) Lympholization of plant tissue prior to DNA extraction
  3) Addition of NaCl and Bovine SerumAlbumin (BSA) to extraction buffer to remove polyphenolic impurities
  4) CTAB DNA extraction method to increase DNA yield

Conclusions:
- DNA extraction from cambial tissue of bark samples is possible, albeit inconsistent in our preliminary study.
- Differences in sub-ecotype amplification success could be due to flooding that creates anoxic conditions that lead to death of cambial tissue.

Table 2. The genes and primers amplified in each successful experiment.

<table>
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<tr>
<th>Gene</th>
<th>Primer</th>
<th># Samples Attempted</th>
<th>Percent Success</th>
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<tbody>
<tr>
<td>rbcLa</td>
<td>1f+r724</td>
<td>28</td>
<td>21%</td>
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<tr>
<td>matK</td>
<td>SI For + SI Rev</td>
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<tr>
<td>matK</td>
<td>1329f+320</td>
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<tr>
<td>ITS</td>
<td>KEWXF + MALPR12</td>
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<tr>
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<td>28</td>
<td>18%</td>
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References: