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Arboral Diversity in Sub-ecotypes of the Peruvian Amazon Blackwater Rainforest: Barcoding from Cambial Tissue

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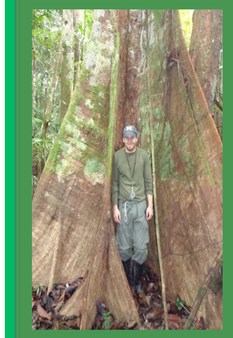

Arboral Diversity in Sub-ecotypes of the Peruvian Amazon Blackwater Rainforest: Barcoding from Cambial Tissue

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Objectives:

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

Background Information:

- The Lowland Amazon Igapo forest floodplain, identified by the black water that floods it, is home to four distinct sub-ecotypes 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 [1,3].
- 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[7].
- Obtaining DNA from the cambial tissue found in bark elimin-ates the need to collect leaf tissue from high up in the canopy [7].

Figure 1. Igapo forest floodplain during the wet season.

Methods:

Study Site: Tamshiyacu 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)

DNA Extraction and Amplification:

1) Isolating cambial tissue: The cambial tissue from 77 samples was isolated by scraping the samples' underside after removal of wood (Fig 3).

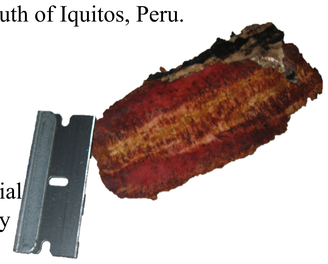
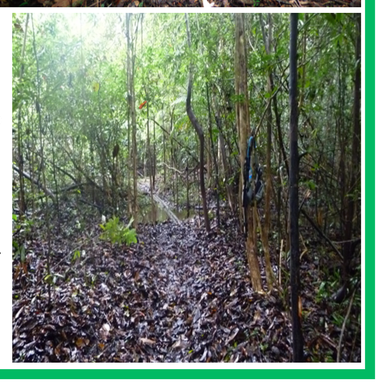


Figure 3. Bark sample, cambial side up before scraping.

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 [7].

Figure 4. Three sub-ecotypes 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).



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 (matK), 17f + 25r (ITS), and psbA3f + trnH (psbA3-trnH) (Table 2).

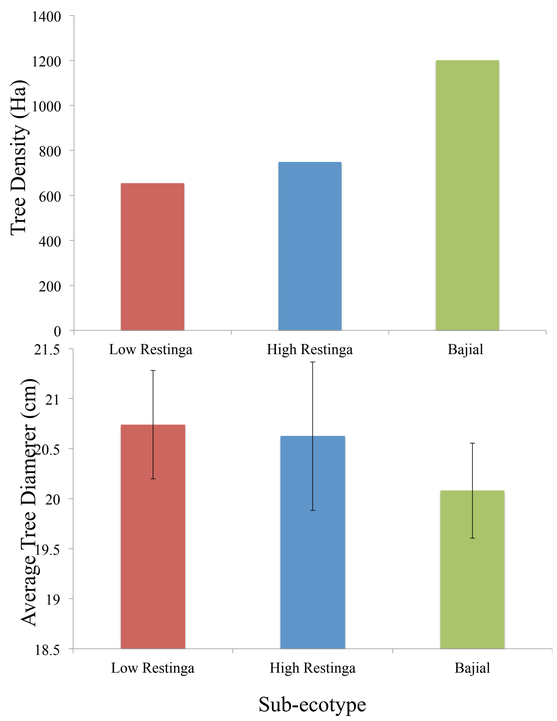


Figure 6. A) Density of trees per ha at the TRARC in the various sub-ecotypes of the Tahuayo River Amazon floodplain. LR (654), HR (748) , and B (1200). B) The average tree diameter of each sub-ecotype studied. Error bars=SEM, ANOVA, F=0.42, p=0.655.

Table 1. Average DNA concentration, purity, and amplification success of each sub-ecotype examined.

Sub-ecotype	Average DNA Yield (ng/ul)	Absorb-ance Average (260/280)	Amplifica-tion Success (%)
High Restinga	49.73	1.53	36
Low Restinga	43.68	1.40	0.76
Bajial	18.67	1.41	0.8

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

Gene	Primer	# Samples Attempted	Percent Success
rbcLa	1f + r724	28	21 %
rbcLa	SI_For + SI_Rev	15	0 %
matK	KIM3f + KIM1r	28	21 %
matK	1329f + 320r	32	22 %
matK	KEWXF + MALPR12	19	0 %
ITS	17f + 25r	28	29 %
trnH-psbA	psbA3f + trnH	28	18 %

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.

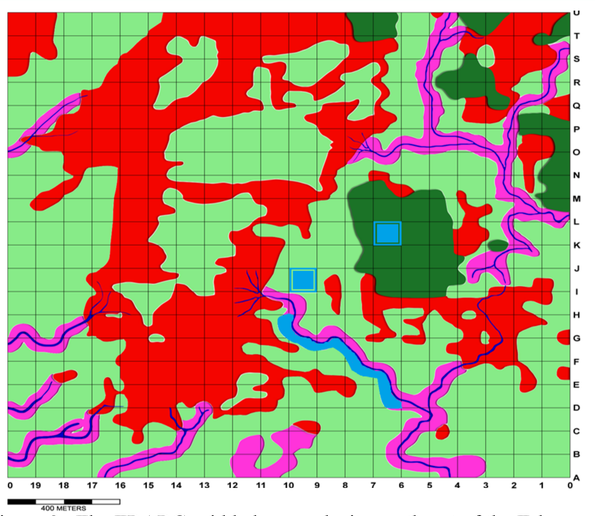


Figure 2. The TRARC gridded research site southeast of the Tahuayo River divided into 100-hectare regions: Low Restinga (light green), High Restinga (dark green), Bajial (pink), and Palm Swamp (red) . Three, 1 hectare (ha) forest plots (light blue) were surveyed in our preliminary study. Palm swamp was not studied at this time.

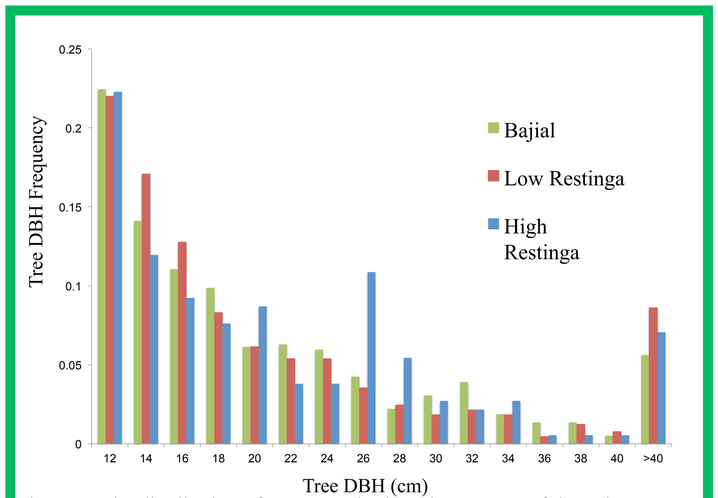


Figure 5. The distribution of tree DBH in the sub-ecotypes of the Tahuayo River Igapo Forest Floodplain. DBH was grouped in 2 cm increments for all sub-ecotypes and followed a logarithmic curve (n=1200 for LR, n=748 for HR, and n=654 for B). Chi-Square, X^2=47.490,df=30,p=0.022

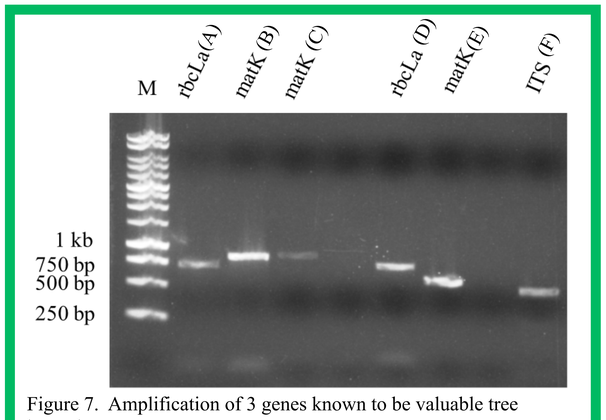


Figure 7. Amplification of 3 genes known to be valuable tree barcodes.
From left to right: (A) 1f + r724, (B) KIM3f + KIM1R, (C) 1329 + 320, (D) SI_For + SI_Rev, (E) KEW XF + MALPR12, (F) 17F + 25r. Barcoding genes included the conservative coding region rbcL, the protein coding gene matK, the noncoding spacing region trnH-psbA, and the nuclear ribosomal ITS[5]

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[10]
2) Lyophilization of plant tissue prior to DNA extraction[8]
3) Addition of NaCl and Bovine Serum Albumin (BSA) to extraction buffer to remove polyphenolic impurities[9]
4) CTAB DNA extraction method to increase DNA yield[10]

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