

Arboreal Diversity in Sub-ecotypes 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-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 present⁷.
- Obtaining DNA from the cambial tissue found in bark eliminates the need to collect leaf tissue from high up in the canopy⁷.



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).

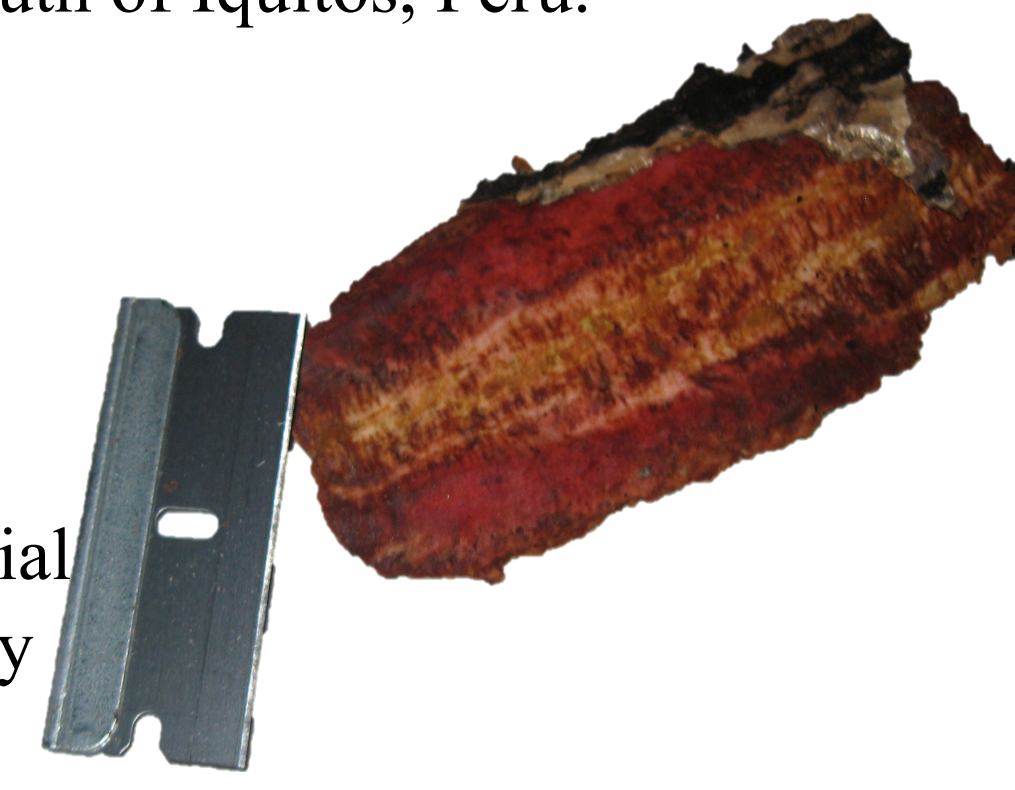


Figure 3. Bark sample, cambial side up before scraping.

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 protocols⁷.



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).

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

Sub-ecotype	Average DNA Yield (ng/ul)	Absorbance Average (260/280)	Amplification 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	KEW XF + MALPR12	19	0 %
ITS	17f + 25r	28	29 %
trnH-psbA	psbA3f + trnH	28	18 %

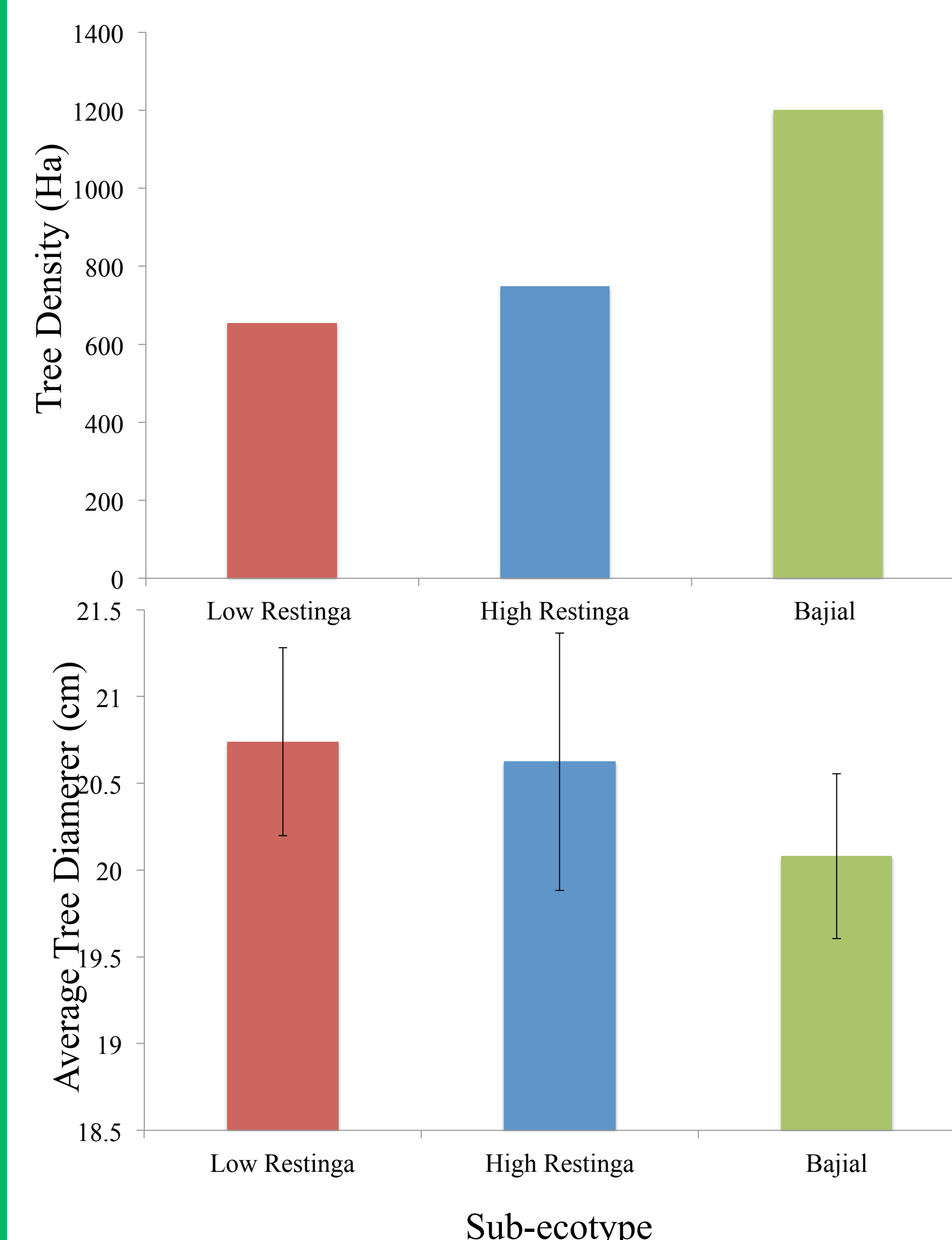


Figure 6. A) Density of trees per ha at the TRARC in the various sub-ecotypes of the Tahuayo River Amazon floodplain. LR (654), HR (748), and B (1200). B) The average tree diameter of each sub-ecotype studied. Error bars=SEM, ANOVA, F=0.42, p=0.655.

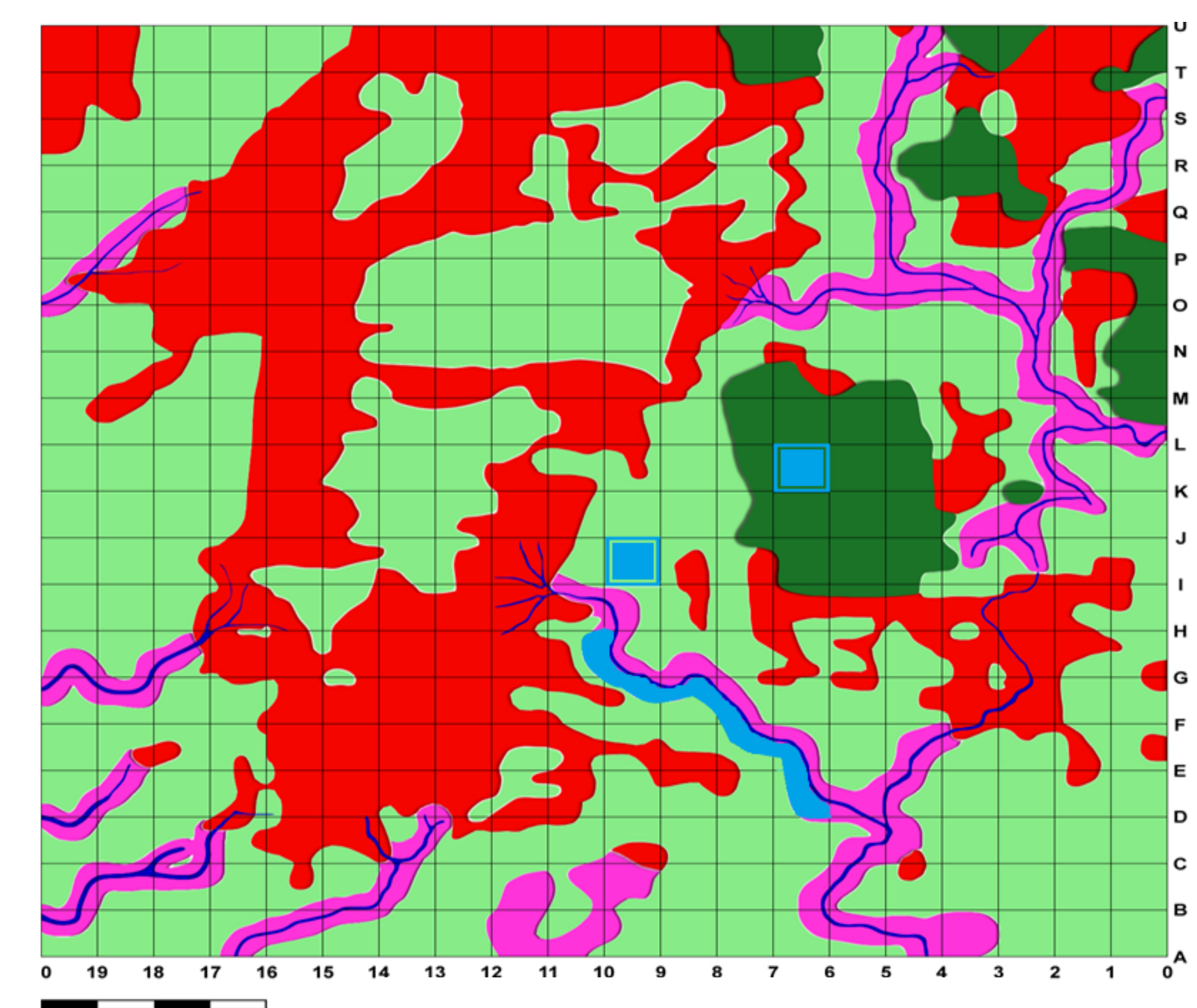


Figure 2. The TRARC gridded research site southeast of the Tahuayo River divided into 100-hectare regions: Low Restinga (light green), High Restinga (dark green), Bajial (pink), and Palm Swamp (red). Three, 1 hectare (ha) forest plots (light blue) were surveyed in our preliminary study. Palm swamp was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

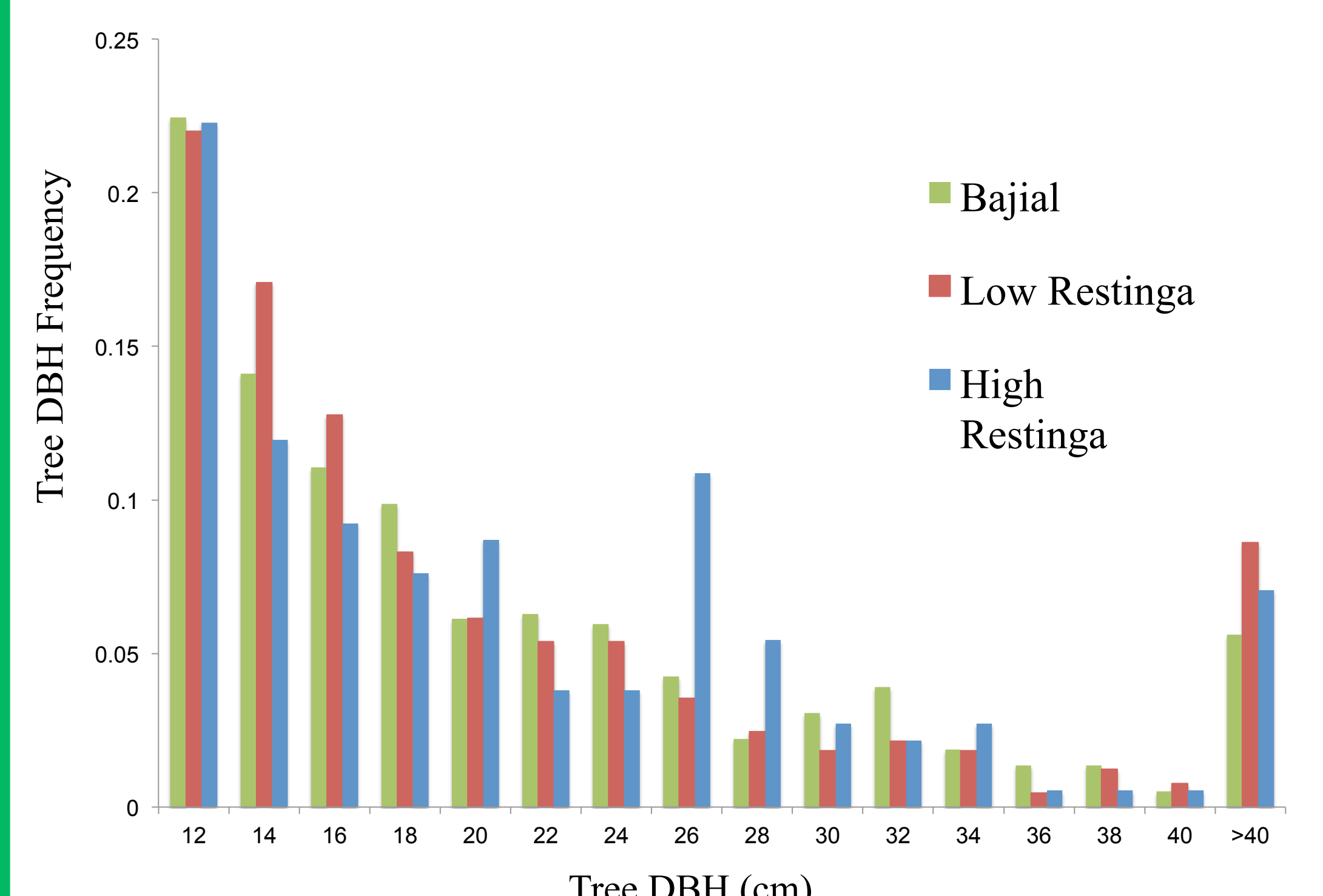


Figure 5. The distribution of tree DBH in the sub-ecotypes of the Tahuayo River Igapo Forest Floodplain. DBH was grouped in 2 cm increments for all sub-ecotypes and followed a logarithmic curve (n=1200 for LR, n=748 for HR, and n=654 for B). Chi-Square, $X^2=47.490, df=30, p=0.022$

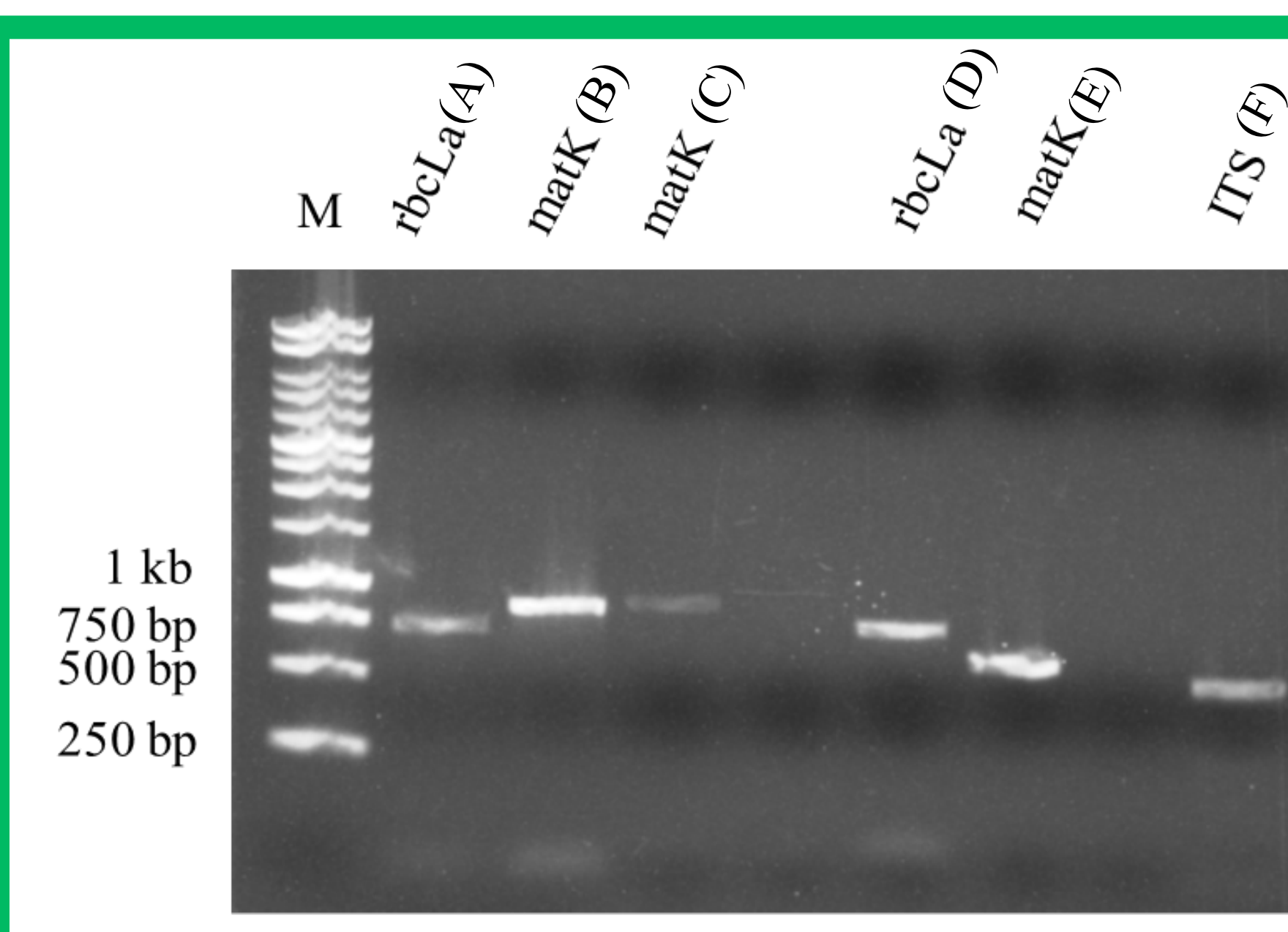


Figure 7. Amplification of 3 genes known to be valuable tree barcodes. From left to right: (A) 1f + r724, (B) KIM3f + KIM1R, (C) 1329 + 320, (D) SI_For + SI_Rev, (E) KEW XF + MALPR12, (F) 17F + 25r. Barcoding genes included the conservative coding region rbcL, the protein coding gene matK, the noncoding spacing region trnH-psbA, and the nuclear ribosomal ITS⁵

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.

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 gel¹⁰
- 2) Lyophilization of plant tissue prior to DNA extraction⁸
- 3) Addition of NaCl and Bovine Serum Albumin (BSA) to extraction buffer to remove polyphenolic impurities⁹
- 4) CTAB DNA extraction method to increase DNA yield¹⁰

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