

Title of proposed project: Phylogeography and lineage diversity of *Pitcairnia flammea* Lindl. complex (Bromeliaceae) adapted to Neotropical inselbergs.

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I) Financial report – Financial support IAPT 2014

The total amount of US\$ 1000.00 were expended as follow:

- 1) Field work to collect samples from Atlantic Rainforest populations = US\$ 900.00. This amount cover travelling costs (full, bus and airplane tickets), hotel and food during field work in Southeastern Brazil;
- 2) Silica gel and plastic pots were used to dry and store collected samples = US\$ 100.00. This amount cover the costs of materials to store the approximately 600 samples collected.

II) Scientific Report – Financial support IAPT 2014

Introduction

In Neotropics the isolation and ancient age of the rock outcrops is reflected in the high number of endemic species. Because of their fragmented nature, species adapted to rock outcrops are interesting models for studying the evolutionary consequences of limited gene flow among disjoint populations. *Pitcairnia flammea* (Bromeliaceae) (Figure 6) is adapted to rock outcrops (inselbergs), within the Atlantic rainforest. This wide-range species possess huge morphological variability with at least seven recognized varieties occurring in allopatry and/or sympatry.

Main Goals

1) to describe the lineage diversity and phylogeographic patterns of *P. flammea* complex; 2) to evaluate the current extent of genetic structure and the degree of isolation among disjoint populations in inselbergs; 3) to evaluate the effects of Pleistocene glaciation on the population demography (fragmentation/expansion events); 4) to aid comparisons of a continuous forest species (*Vriesea gigantea*) with those of *P. flammea* 'inselberg' taxa distributed in the same geographical range in Southeastern and southern Brazil.

Methodology

Samples from the entire species geographical range (ca. 1500 km) in the Atlantic Rainforest in Brazil were collected for DNA extraction (Table 1). Ten nuclear microsatellites (SSR) loci were used to assess population levels of genetic diversity and structure. Two plastidial regions were sequenced in order to make a combined use of biparental and uniparental inherited markers. Genetic diversity estimates, demographic analyses and the evolutionary relationships among haplotypes were

inferred according to methods appropriate and broadly used to describe phylogeographic patterns and to infer population fragmentation/expansion events.

Main results

We observed extremely low levels of INTRA-specific gene flow among inselbergs and a latitudinal gradient in genetic diversity from south to north of the species distribution (Figure 1). This pattern is in opposite to observed for dwelling-forest species (i.e. *Vriesea gigantea* – Palma-Silva et al 2009) in the same geographical region. *P. flammea* populations are genetically structured (Table 3; Figure 2; 3; 4), suggesting restricted pollen and seed dispersal, and consequently strong role of drift in evolutionary history of these naturally fragmented populations. M-ratios values (Table 2) suggested that bottlenecks have occurred in recent past for all populations studied. These results indicate the Neotropics has a mosaic of patterns pointing to highly complex processes responsible for its astonishing species diversity.

Table 1. Collected populations of *Pitcairnia flammea* in Brazil.

| Population | Species / Variety | City | Locality | Federal State |
|------------|---------------------------------------|-----------------------|--|---------------|
| MCPR | <i>P. flammea</i> var. <i>flocosa</i> | Campina Grande do Sul | Morro do Capivari | PR |
| ATISP | <i>P. flammea</i> var. <i>flocosa</i> | Atibaia | Pedra Grande | SP |
| BESP | <i>P. flammea</i> var. <i>flammea</i> | Bertioga | Estrada Mogi-Bertioga | SP |
| PISP | <i>P. flammea</i> var. <i>flammea</i> | Picinguaba | Praia da Fazenda | SP |
| MPPR | <i>P. flammea</i> var. <i>flocosa</i> | Guaratuba | Morro dos perdidos | PR |
| SSSP | <i>P. flammea</i> var. <i>flammea</i> | São Sebastião | Praia toque-toque | SP |
| MFSP | <i>P. flammea</i> var. <i>flocosa</i> | Altinópolis | Morro do Forno | SP |
| GRURJ | <i>P. flammea</i> var. <i>flammea</i> | Grajaú | Grajaú | RJ |
| CORJ | <i>P. flammea</i> var. <i>flammea</i> | Rio de Janeiro | Morro do Corcovado | RJ |
| JUFLA | <i>P. flammea</i> var. <i>flocosa</i> | Peruíbe | Estação ecológica da Jureia | SP |
| PARJ | <i>P. flammea</i> var. <i>flammea</i> | Paraty | Pedra 1 | RJ |
| IBIFLO | <i>P. flammea</i> var. <i>flocosa</i> | Ibitipoca | Cachoeira dos Macacos | MG |
| IBIFLA | <i>P. flammea</i> var. <i>flammea</i> | Ibitipoca | Cachoeira dos Macacos | MG |
| IBICEU | <i>P. curvidens</i> | Ibitipoca | Janela do ceu | MG |
| CAPMG | <i>P. flammea</i> var. <i>flammea</i> | Alto do Capará | Pedra Roxa | RJ |
| CARMG | <i>P. flammea</i> var. <i>flammea</i> | Catas Altas | RPPN Santurario do Caraça Reserva Ecologica Santa | MG |
| STES | <i>P. flammea</i> var. <i>flammea</i> | Santa Teresa | Lucia | ES |

Tabela 2. Genetic diversity in populations of *Pitcairnia flammea*. Allelic richness: Rs; number of alleles: A; variance in allele size: Var; expected and observed heterozygosities: He e Ho, respectively; inbreeding coefficient: FIS; values of M-ratio statistics.

| Popultions | Rs | A | Var | He | Ho | FIS | M-ratio |
|------------|------|-----|-------|-------|-------|----------|---------|
| MCPR | 5,43 | 6,5 | 8,89 | 0,626 | 0,441 | 0.296*** | 0,305 |
| MPPR | 3,96 | 4,8 | 37,55 | 0,458 | 0,458 | 0.260*** | 0,419 |
| ATISP | 4,17 | 5,1 | 9,82 | 0,586 | 0,488 | 0.174*** | 0,265 |
| MFSP | 4,91 | 5,7 | 11,25 | 0,512 | 0,401 | 0.220*** | 0,336 |
| BESP | 2,45 | 2,8 | 2,74 | 0,295 | 0,201 | 0.326*** | 0,291 |
| PGSP | 3,64 | 3 | 4,19 | 0,379 | 0,280 | 0.227*** | 0,275 |
| PISP | 2,85 | 3,2 | 3,62 | 0,406 | 0,352 | 0.137 | 0,298 |
| SSSP | 3,06 | 3 | 7,16 | 0,367 | 0,210 | 0.432*** | 0,236 |
| GRURJ | 2,78 | 2,3 | 9,04 | 0,403 | 0,406 | 0.002 | 0,264 |
| CORJ | 3,39 | 3,3 | 7,45 | 0,407 | 0,268 | 0.358*** | 0,268 |

A population is considered to have undergone a bottleneck if its M-value falls below a threshold of 0.680, following the procedure described by Garza & Williamson (2001).

Tabela 3. Analysis of molecular variance (AMOVA) for chloroplast DNA (cpDNA) data

| Source of variation | d.f | Sum of squares | Variance components | Percentage of variation |
|----------------------------|------------|-----------------------|----------------------------|--------------------------------|
| Among populations | 4 | 120.941 | 1.63178 Va | 97.66 |
| Within populations | 94 | 3.675 | 0.03910 Vb | 2.34 |
| Total | 98 | 124.616 | 1.67088 | |

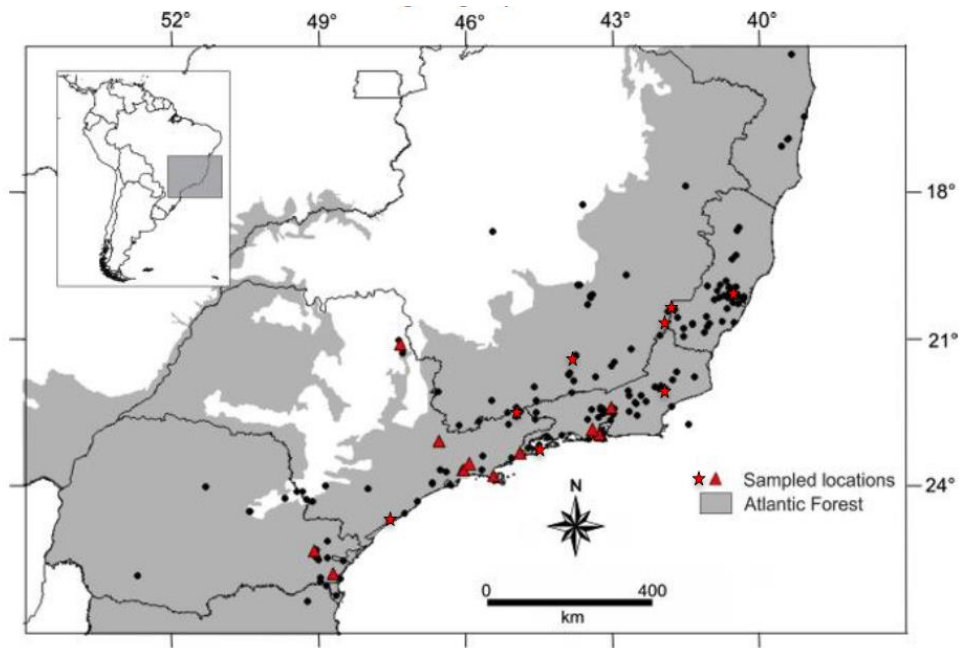
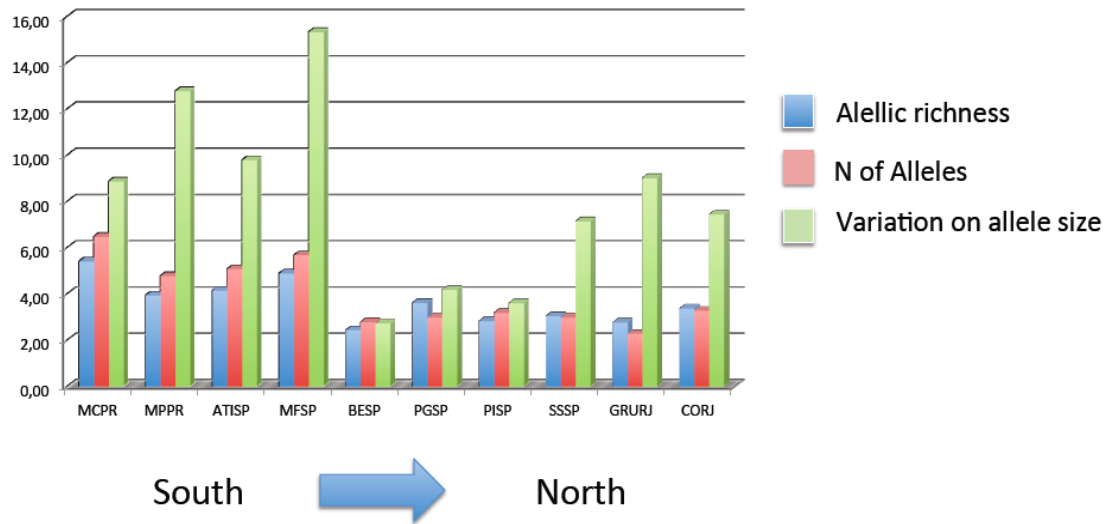


Figure 1. Map showing populations of *Pitcarnia flammea* collected and analyzed in this project.



Latitudinal gradient

Figure 2. Latitudinal gradient South-North in genetic diversity based on: Allelic richness, Number of alleles, and variation in allele size.

14 allopatric populations – nuclear SSR

Structure $K = 13$

$$G'_{ST} = 0.76$$

Strong geographic structure

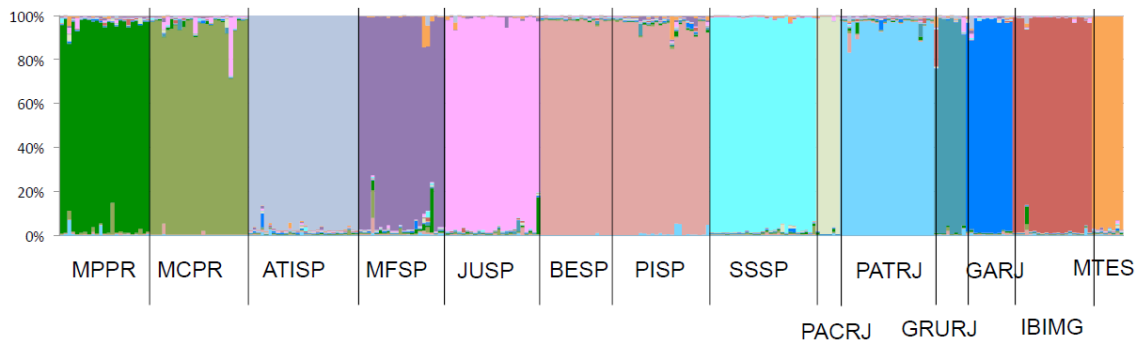


Figure 3. Results of STRUCURE 2.3.4 analysis showing from $K=9$ in *Pitcairnia flammae*. Clusters of populations are represented by colors.

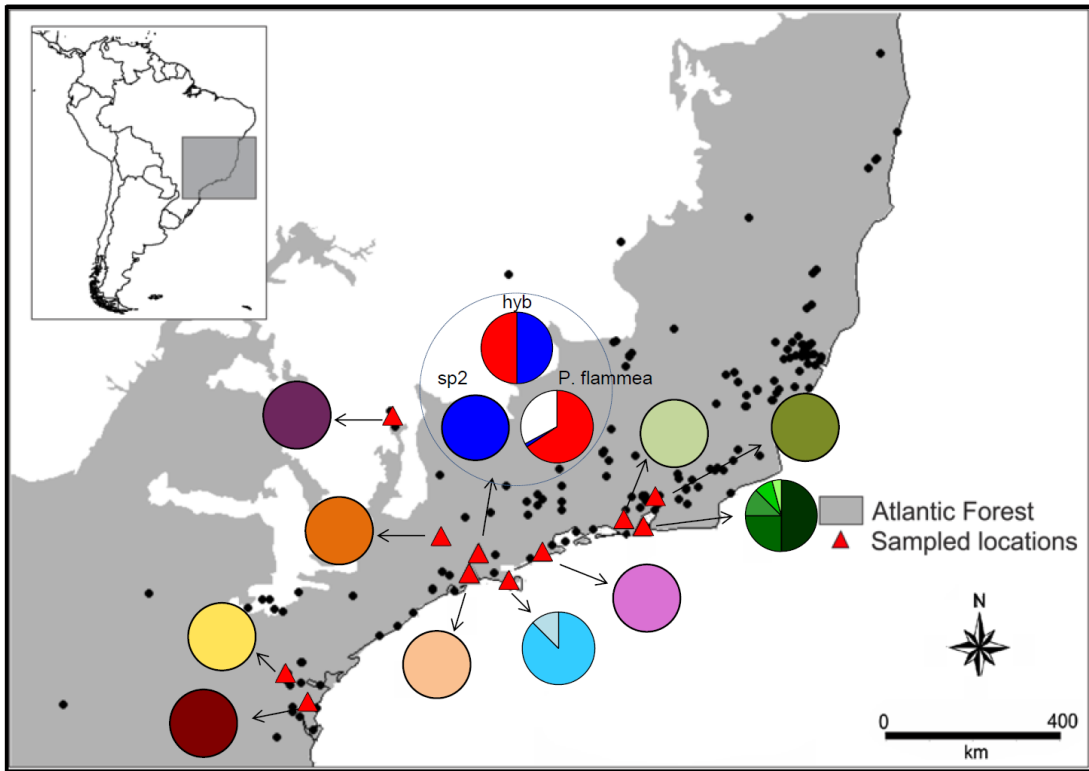


Figure 4. Map showing haplotypes from *Pitcairnia flammea* populations.

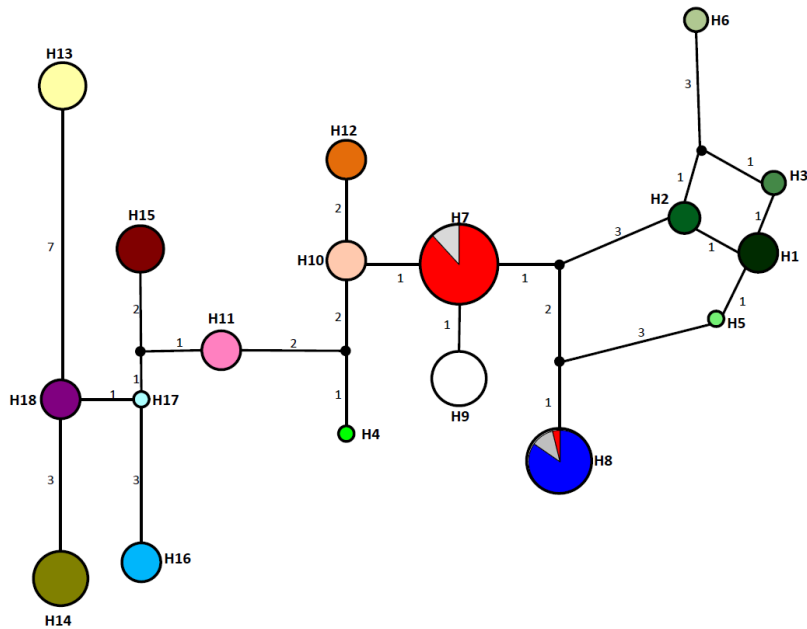


Figure 5. Network analyses of plastidial genome. The color represent the haplotypes.

Figure 6. *Pitcairnia flammea*

