

# **A PANMICTIC AMAZONIAN WORLD?**

**Epiphytic bryophytes testify**

# Introduction

Understanding population connectivity is fundamental for biodiversity conservation and management

- *Different spatial scale*
- *Different temporal scale*

## Amazonian rainforest

- *One of the biggest biodiversity hotspot*
- *6 million square kilometers*
- *High  $\alpha$ -diversity but low  $\beta$ -diversity*
  - Lot of species broadly distributed
- *Pleistocene climate oscillations*
- *human-induced fragmentation*

# Introduction

Understanding population connectivity is fundamental for biodiversity conservation and management

- *Different spatial scale*
- *Different temporal scale*

Amazonian rainforest

- *One of the biggest biodiversity hotspot*
- *6 million square kilometers*
- *High  $\alpha$ -diversity but low  $\beta$ -diversity*
  - Lot of species broadly distributed
- *Pleistocene climate oscillations*
- *human-induced fragmentation*



# Introduction

## Epiphytic bryophytes

- *Spatially explicit habitats*
- Short life-cycles and poikilohydric condition
- *Factors influencing distribution are variable*
- Niche segregation
- Dispersal limitation
- Historical factors



## Mota & ter Steege (2015) : One panmictic metapopulation

- *Null model analyze*
- *Individuals randomly distributed across the Amazonian forest*
- *No ecological mechanism driving their distribution*
- *No trace of impact of global change or deforestation yet*  
→ need of genetic data

# Introduction

## Epiphytic bryophytes

- *Spatially explicit habitats*
- Short life-cycles and poikilohydric condition
- *Factors influencing distribution are variable*
- Niche segregation
- Dispersal limitation
- Historical factors



## Mota & ter Steege (2015) : One panmictic metapopulation

- *Null model analyze*
  - *Individuals randomly distributed across the Amazonian forest*
  - *No ecological mechanism driving their distribution*
  - *No trace of impact of global change or deforestation yet*
- need of genetic data!

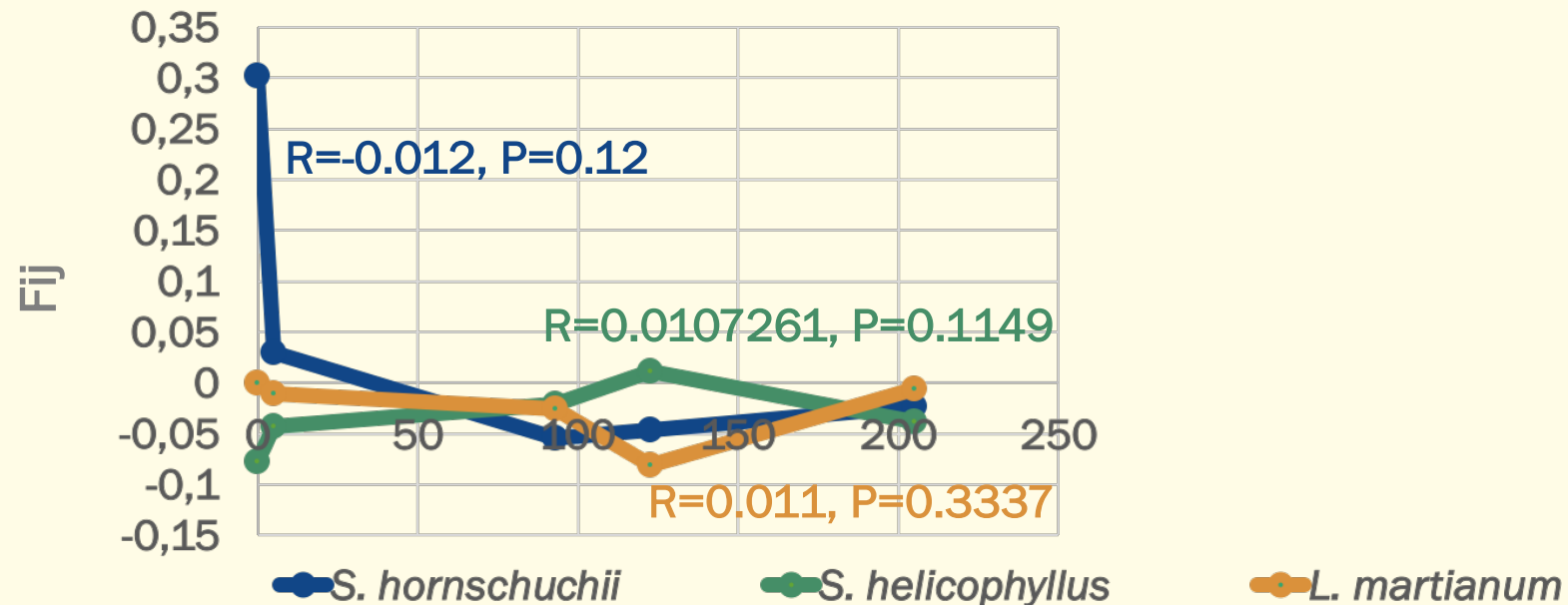
# Objectives

Amazonian epiphytic bryophytes = one panmictic population ?

- *Do Amazonian epiphytes exhibit a spatial genetic structure (SGS) at regional (< 500 km) scales ?*

→ Mantel test : regression between  $F_{ij}$  and distances among individuals

→ Is the p-value of the slope significant (<0.05) ?



# Objectives

Amazonian epiphytic bryophytes = one panmictic population ?

- *Do Amazonian epiphytes exhibit a spatial genetic structure (SGS) at regional (< 500 km) scales ?*

→ Mantel test : regression between  $F_{ij}$  and distances among individuals

→ Is the p-value of the slope significant?

*If there is a significant SGS,*

- At which spatial scale does it occur?
- Is niche conservatism (isolation-by-ecology) rather than dispersal limitation (isolation-by-distance) the main driver of distribution across Amazonia ?
  - Partial Mantel test : comparison of the variance explained by those factors
  - Regression between  $F_{ij}$  and geographic distances among individuals
  - Regression between  $F_{ij}$  and ecological distances among individuals

*Ecological distances : 0 = same forest type; 1 = different forest types*

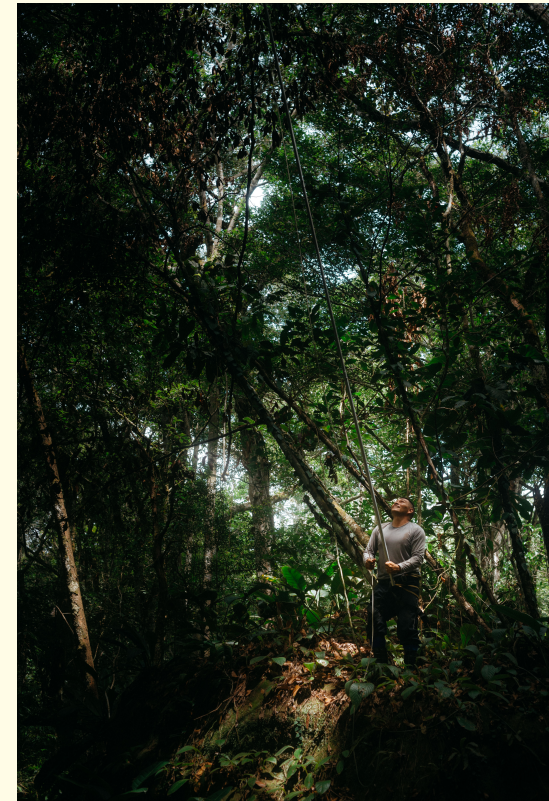
# Material and methods

## Sampling

- *14 species of Amazonian epiphytic bryophytes*
- *> 500 individuals (15-50 per species)*
- *2 forest types : lowland and white sand forest*
- *50,000 km<sup>2</sup> area*

## Lab technique

- *Sanger sequencing at traditional cpDNA and nDNA loci → rake  
→ Next Generation sequencing*
- *Genotyping By Sequencing (GBS)*
- *Elshire et al. 2011 (except reverse PCR primer : Sonah et al. 2013)*
- *Libraries sequenced on Illumina NextSeq 500*
- *Data demultiplexed and loci built de novo on Stacks*





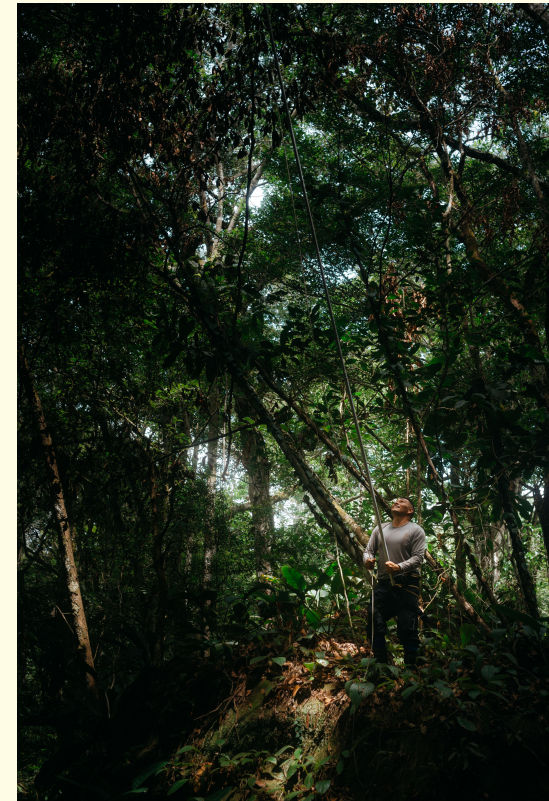
# Material and methods

## Sampling

- *14 species of Amazonian epiphytic bryophytes*
- *> 500 individuals (15-50 per species)*
- *2 forest types : lowland and white sand forest*
- *50,000 km<sup>2</sup> area*

## Lab technique

- *Sanger sequencing at traditional cpDNA and nDNA loci → rake*  
→ Next Generation sequencing
- *Genotyping By Sequencing (GBS)*
- *Elshire et al. 2011 (except reverse PCR primer : Sonah et al. 2013)*
- *Libraries sequenced on Illumina NextSeq 500*
- *Data demultiplexed and loci built de novo on Stacks*



# First results on Stacks

## Parameters

- Min depth of coverage to create a stacks : 3
- Max distance allowed between stacks : 2
- Max distance allowed to align secondary reads : 4
- Max number of stacks allowed per *de novo locus* : 3
- % of the population having the SNP needed to retain the SNP : 50

⇒ small number of SNPs polymorphic among individuals!

Species	Number of SNPs
<i>Archilejeunea parviflora</i>	204
<i>Bazzania hookeri</i>	193
<i>Leucobryum martianum</i>	180
<i>Syrrhopodon helicophyllus</i>	243
<i>Syrrhopodon hornschuchii</i>	159
<i>Thysananthus amazonicus</i>	221
<i>Octoblepharum albidum</i>	275
<i>Octoblepharum pulvinatum</i>	232
<i>Syrrhopodon annotinus</i>	163
<i>Syrrhopodon simmondsii</i>	144
<i>Calymperes lonchophyllum</i>	79

# Improvements

Why small number of SNPs polymorphic?

- *Stacks ? Haploid data? Parameters choice? → Try with iPyrad*
- *Lab? Adapter concentration? Poor quality sequences → Trim the sequences*

Reliability of the SNPs

- *Enough intra-individual variation to asses population genetic questions?*
- *Structure or phylogeny to see if they can distinguish 2 species?*

Check for contaminations

- *Blast?*

Presence of mixed specimen

- *Especially in challenging genera*
- *Barcoding? But no known loci for these species*
- *Structure to identify different clusters within « one species »*

# Improvements

Why small number of SNPs polymorphic?

- *Stacks ? Haploid data? Parameters choice? → Try with iPyrad*
- *Lab? Adapter concentration? Poor quality sequences → Trim the sequences*

Reliability of the SNPs

- *Enough intra-individual variation to asses population genetic questions?*
- *Structure or phylogeny to see if they can distinguish 2 species?*

Check for contaminations

- *Blast?*

Presence of mixed specimen

- *Especially in challenging genera*
- *Barcoding? But no known specific loci for these species*
- *Structure to identify different clusters within « one species »*



THANK YOU FOR YOUR ATTENTION!