Molecular phylogenetics and molecular dating of Chilean Puya (Bromeliaceae)

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Molecular Phylogenetics

- Differences in DNA sequence serve as proxies for evolution
  1. ATGT\textcolor{red}{C}ACGG\textcolor{green}{T}ACAG
  2. ATG\textcolor{red}{T}A\textcolor{green}{A}CGG\textcolor{red}{T}ACAG
  3. ATG\textcolor{red}{T}A\textcolor{green}{A}CGG\textcolor{red}{G}ACAG

- Phylogenies allow for:
  - Molecular Dating
  - Biogeographical Analysis
  - Character Mapping

- Incongruences: Jabaily & Sytsma, 2010
Background of *Puya*
Background of *Puya*
Background of Chilean Puya

Jabaily & Sytsma, 2010, Figure 1

- Black outline: Chilean Puya
- “Blue” Puya
- “Yellow” Puya
Incongruence

Fig. 3. Minimum likelihood cpDNA phylogeny for Puyo and all outgroups based on an raxml-ng, aucML, and est ML analysis. Hechita is used as the outgroup. Numbers above branches correspond to Bayesian MCMC posterior probability (PP) and numbers below correspond to maximum likelihood bootstrap (MLB) values calculated using RAXML. Support values are shown for only clades of interest. Line thickness corresponds to relative support: thickest lines indicate strong support (both PP and MLB above 90%), medium thick lines indicate medium support (either PP or MLB above 60%), and thin lines indicate weak support (both PP and MLB below 50%). If both PP and MLB are below 50%, the clade is collapsed. Two asterisks (**), subgenus Puyaeae.

“I Monophyly”

Fig. 4. Maximum likelihood nuclear DNA (MFL) phylogeny for Puyo and all outgroups. Hechita, Meziluma, and Puyaeae with Pitcairnella spp. used as a monophyletic outgroup. Numbers above branches correspond to Bayesian inference posterior probabilities (PP), and numbers below correspond to maximum likelihood bootstrap (MLB) values calculated using RAxML. Support values are shown for only clades of interest. Line thickness corresponds to relative support: thickest lines indicate strong support (both PP and MLB above 90%), medium thick lines indicate medium support (either PP or MLB above 60%), and thin lines indicate weak support (both PP and MLB below 50%). If both PP and MLB are below 50%, the clade is collapsed. Two asterisks (**), subgenus Puyaeae.

“Paraphyly”
Hypothesis by Jabaily & Sytsma, 2010 Adapted by me

Figure 1

Hybridization and subsequent backcrossing resulting in introgression of maternal chloroplast genome into parental lineage.

Central Andean Clade

P. coerulea
P. venusta
P. alpestris
P. × berteroniana *
P. chilensis
P. gilmartiniae
P. boliviensis

Central Andean sp.
Methods – Wet Lab

- DNA samples from Jabaily & Sytsma, 2010
- Re-extraction from silica-dried tissue
  - DNeasy Plant Mini Kit from Qiagen
- PCR amplification of nuclear locus g3pdh
  - Primers from Sass & Specht, 2010 – phylogeny of Aechmea
  - Thermal Cycling settings from Aguirre-Santoro, 2016 – phylogeny of Ronnbergia Alliance
- PCR product cleaned
  - HighPrep PCR Beads from MagBio Genomics
- Sequencing
  - Macrogen in Seoul
- Troubleshooting (ETS)
Troubleshooting ETS locus
Methods - Geneious

- Contiged forward and reverse sequences
- Ends trimmed
- Ambiguity checked manually
- Gaps stripped
- No indels because g3pdh is a coding region
- Alignment length of 1,187 bp
- Maximum Likelihood
  - RAxML v.8 in Geneious using GTR model
- Bayesian Inference
  - MrBayes v.3.2.6 in Genius using GTR model
Methods – Molecular Dating

• BEAST v. 1.10.4
• Givnish et al., 2011
  • Molecular dated phylogeny of Bromeliaceae including 8 *Puya* species
• 3 secondary calibration points
## Results

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Results – ML Phylogeny

- **Paraphyletic Blue *Puya***
  - Low support: bootstrap not shown

- **Yellow *Puya* embedded in Core *Puya***
  - ED clade – in red
  - Fairly well supported – bootstrap 80

- **Bromelia embedded in *Puya***
  - Fairly low support – bootstrap 68

- **Little structure in Core *Puya***
  - As expected – little genetic variation in this genus

Figure 2. Maximum likelihood phylogeny with bootstrap support
Results

• Paraphyletic Blue *Puya*
  • Low support – not shown
  • Monophyletic Blue *Puya* hypothesis rejected

• Yellow *Puya* embedded in Core *Puya*

• ED clade falls to the base of Core *Puya* excluding *P. mima*

• *Bromelia* embedded in *Puya*
  • Low support - 50

• A little more structure in Core *Puya*

Figure 3. Bayesian Inference phylogeny
Results

- *Puya* and *Bromelia* split 11.4 Ma
- *P. venusta* and *P. coerulea* diverge first (10.04 Ma) and *P. alpestris* diverges next (8.05 Ma)
- Core *Puya* (excluding *P. mima*) diverges 5.99 Ma
- Structure in *P. yakespala* clade
  - Surprisingly, *P. chilensis* diverges first

Figure 4. Molecular dated phylogeny with Bayesian Inference
Discussion

PHYC

• Monophyletic Blue *Puya*
• Yellow *Puya* sister to Core *Puya*
  • A basal position but closer to Core *Puya* than is Blue *Puya*

cpDNA

• Monophyletic Chilean *Puya*

g3pdh

• Paraphyletic Blue *Puya*
  • Ancestral Blue *Puya* was two lineages, not one
• Yellow *Puya* embedded in Core *Puya*
  • Confirm the close ties between Yellow *Puya* and Core *Puya*
Elevational Disjunct Clade (ED clade)

- *P. chilensis*, *P. gilmartiniae*, *P. raimondii*, and *P. yakespala*

- All lack the common *Puya* blue/purple pigment

- *P. boliviensis*, the third member of Yellow *Puya*, also has close ties to Core *Puya*

- Ancestral Yellow *Puya* hypothesis
Updated Hypothesis

Figure 8

hybridization and subsequent backcrossing resulting in introgression of maternal chloroplast genome into parental lineage
Molecular Dating and Biogeography

- Chile is home to the oldest lineages of Bromeliioideae as well as Puyoideae and is a hotspot of diversity today
- Split between Puyoideae and Bromeliioideae at 11.4 Ma
  - Beginning of seasonality in what is now Chile 12-15 Ma
  - Aridification of what is now Atacama Desert in northern Chile Pliocene to Miocene
Conclusion

Key Findings

• Paraphyletic Blue Puya
• ED clade and the ancestral Yellow *Puya* hypothesis
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