

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. ATGTCACGGTACAG

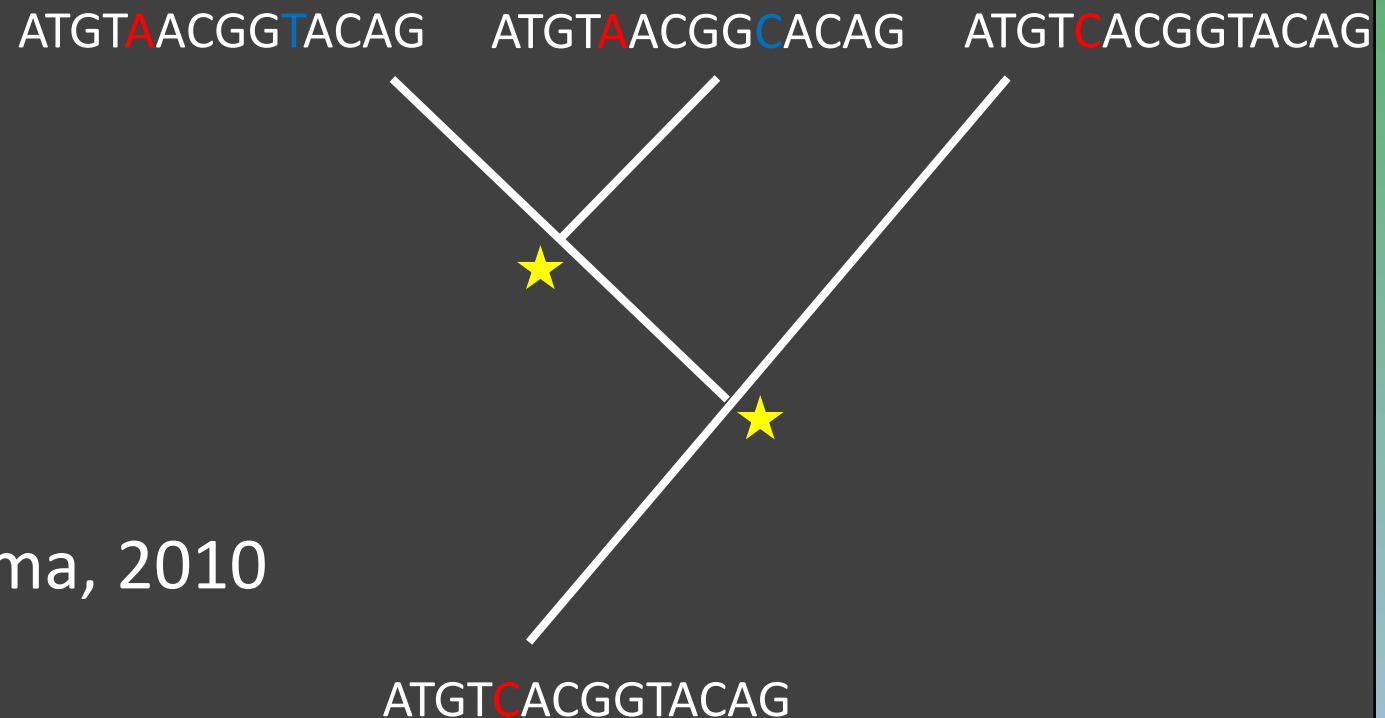
2. ATGTAACGGTACAG

3. ATGTAACGGCACAG

- 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

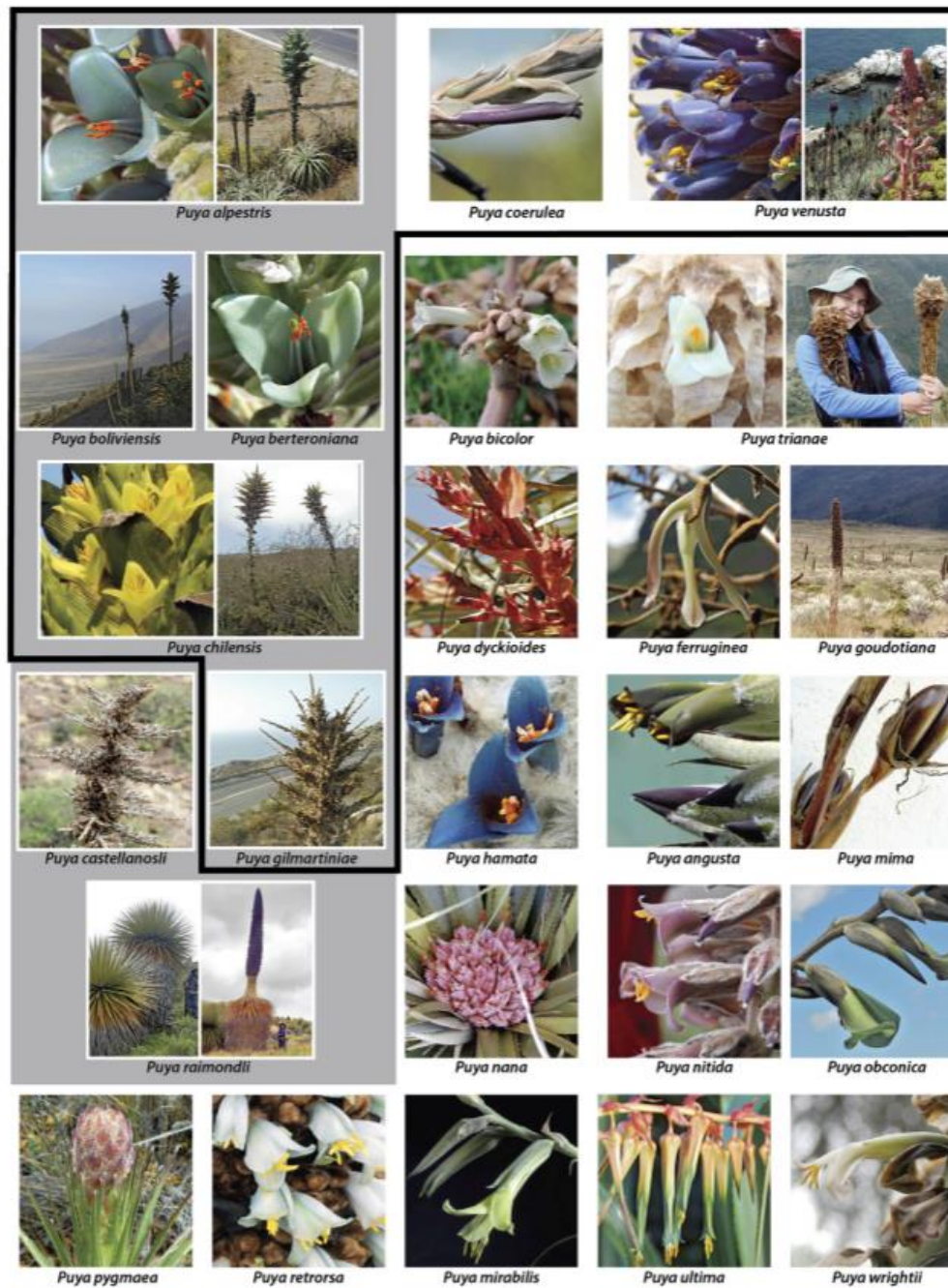


Fig. 1. Representative vegetative and floral diversity in both subgenera of *Puya* and in the Chilean species. Species in left gray box are members of subg. *Puya*. The remaining photos show species from subg. *Puyopsis*. Species united by the black line are Chilean *Puya* species.

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

Incongruence

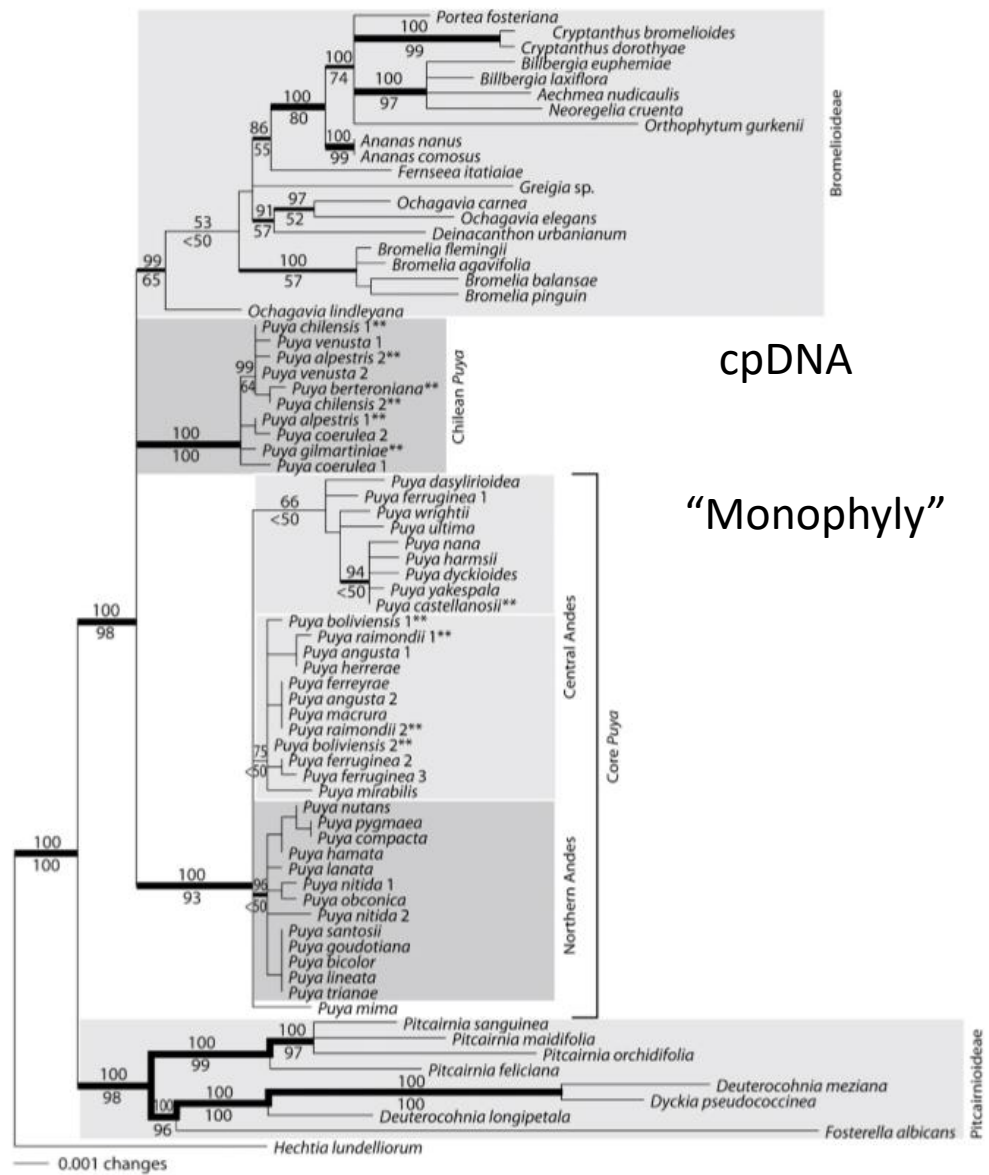


Fig. 3. Maximum likelihood cpDNA phylogeny for *Puya* and all outgroups based on *trnS-trnG*, *matK*, and *rps16*. *Hechtia* is used as the outgroup. Numbers above branches correspond to Bayesian MCMC inference posterior probabilities (PP) and numbers below correspond to maximum likelihood bootstrap (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 80), medium thick lines indicate medium support (either PP or MLB above 80), and thin lines indicate weak support (both PP and MLB below 80). If both PP and MLB are below 50, the clade is collapsed. Two asterisks (**): subgenus *Puya*.

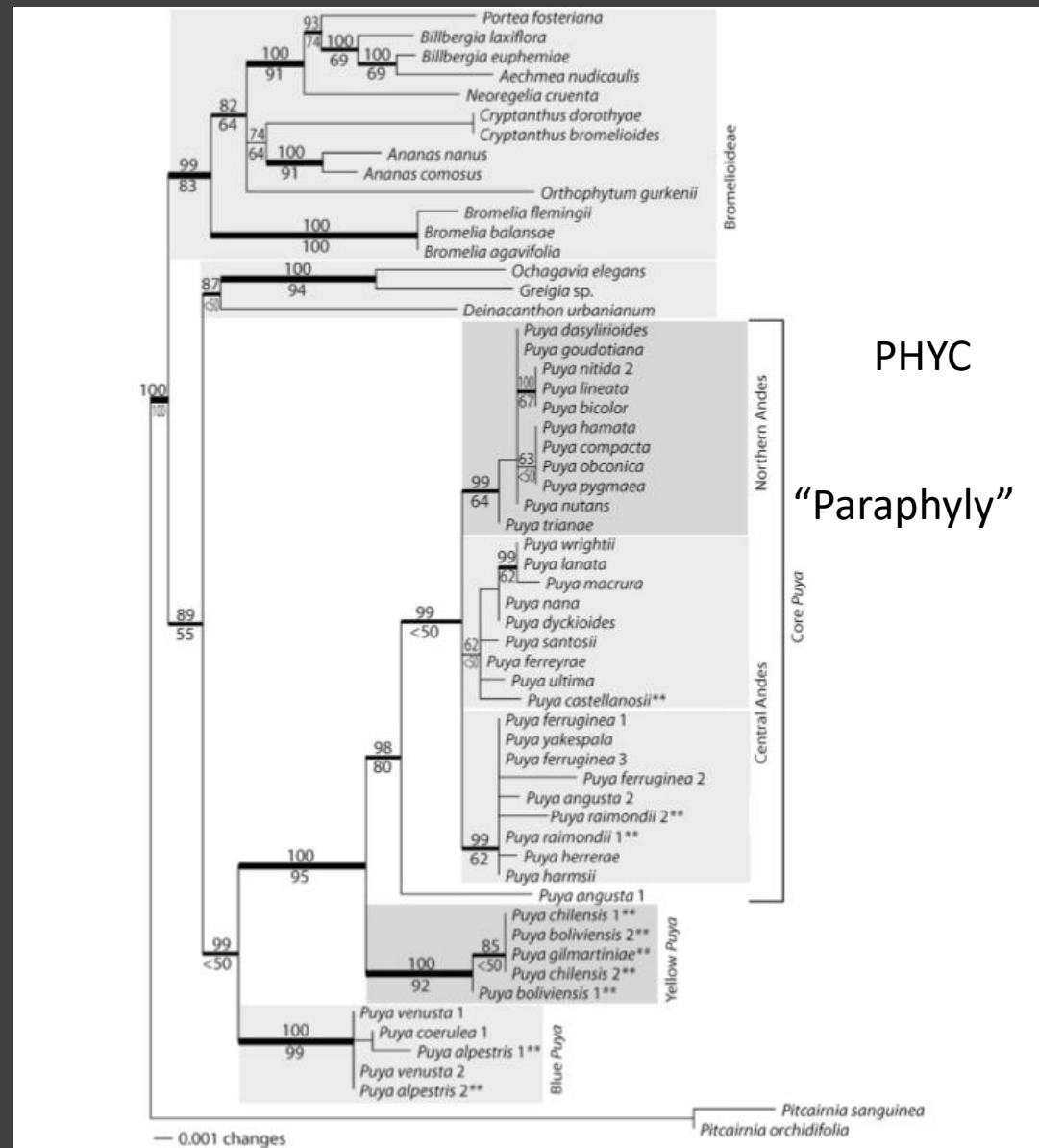


Fig. 4. Maximum likelihood nuclear DNA (PHYC) phylogeny for *Puya* and subfamily Bromelioideae with *Pitcairnia* spp. used as a monophyletic outgroup. Numbers above branches correspond to Bayesian inference posterior probabilities (PP), and numbers below correspond to maximum likelihood bootstrap (MLB) 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 80), medium thick lines indicate medium support (either PP or MLB above 80), and thin lines indicate weak support (both PP and MLB below 80). If both PP and MLB are below 50, the clade is collapsed. Two asterisks (**): subgenus *Puya*.

Hypothesis by Jabaily & Sytsma, 2010 Adapted by me

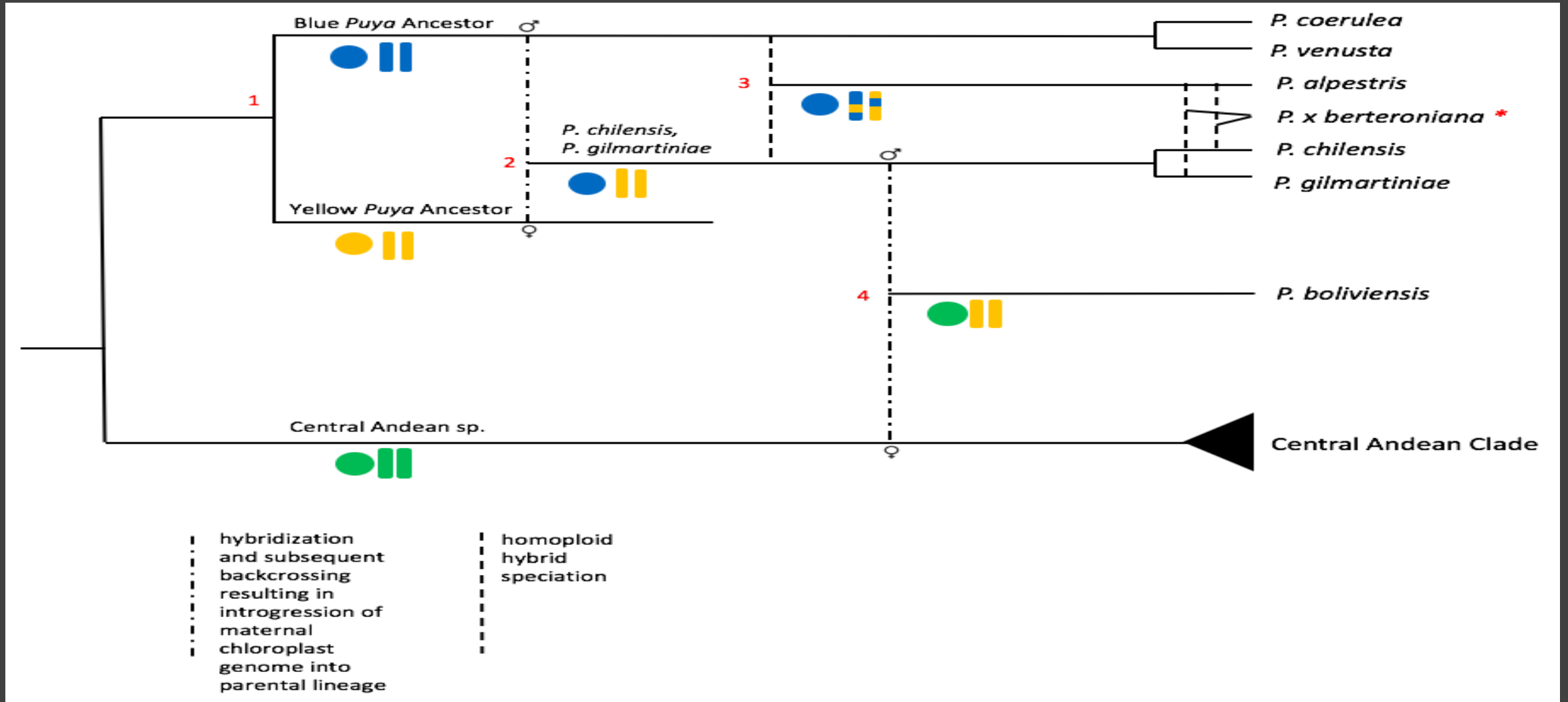
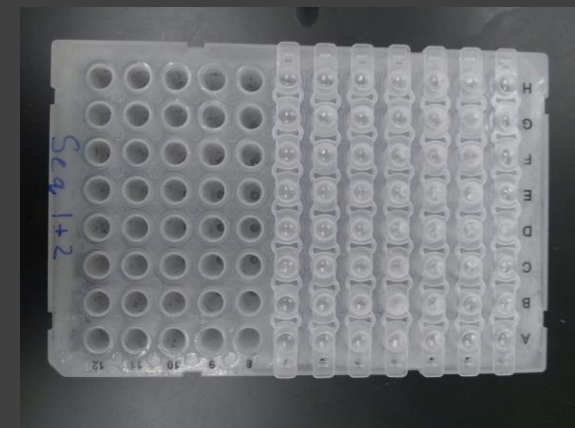
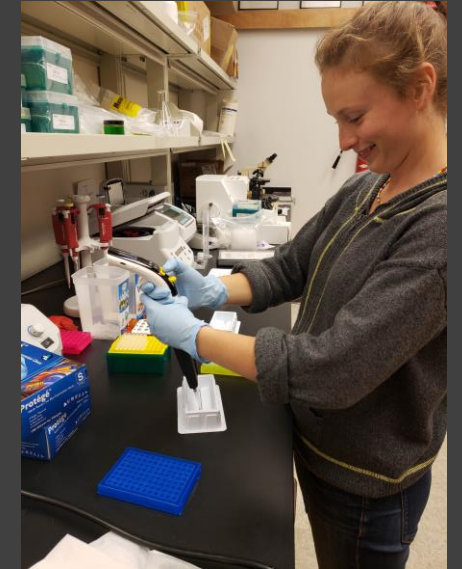
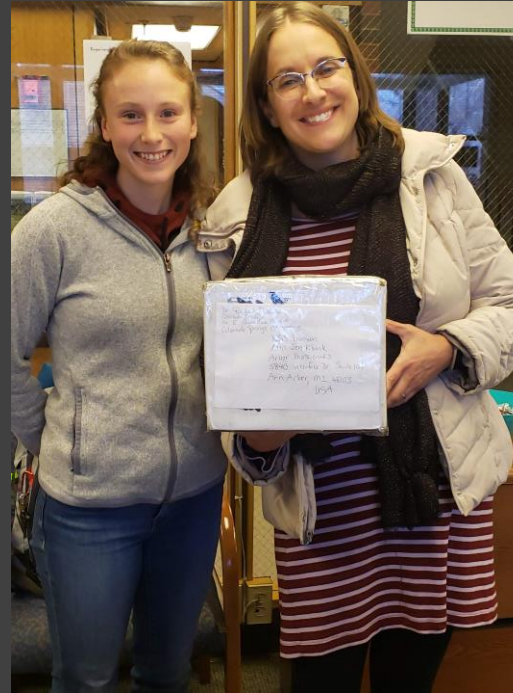


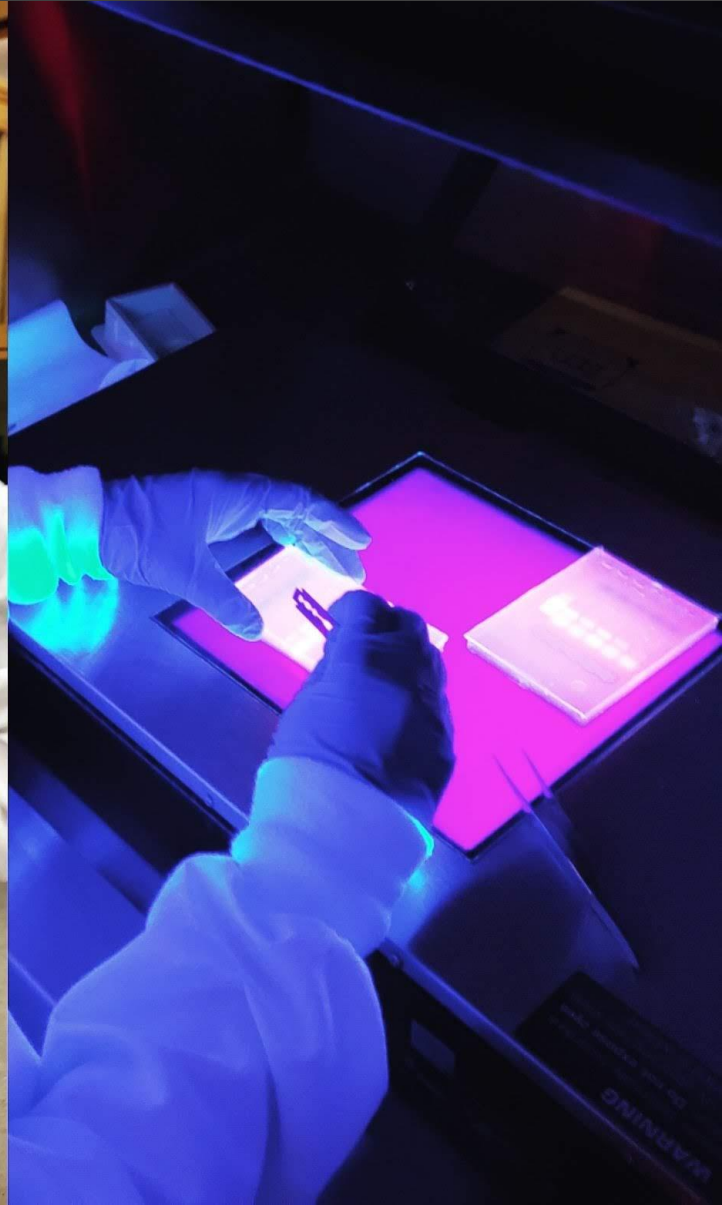
Figure 1

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



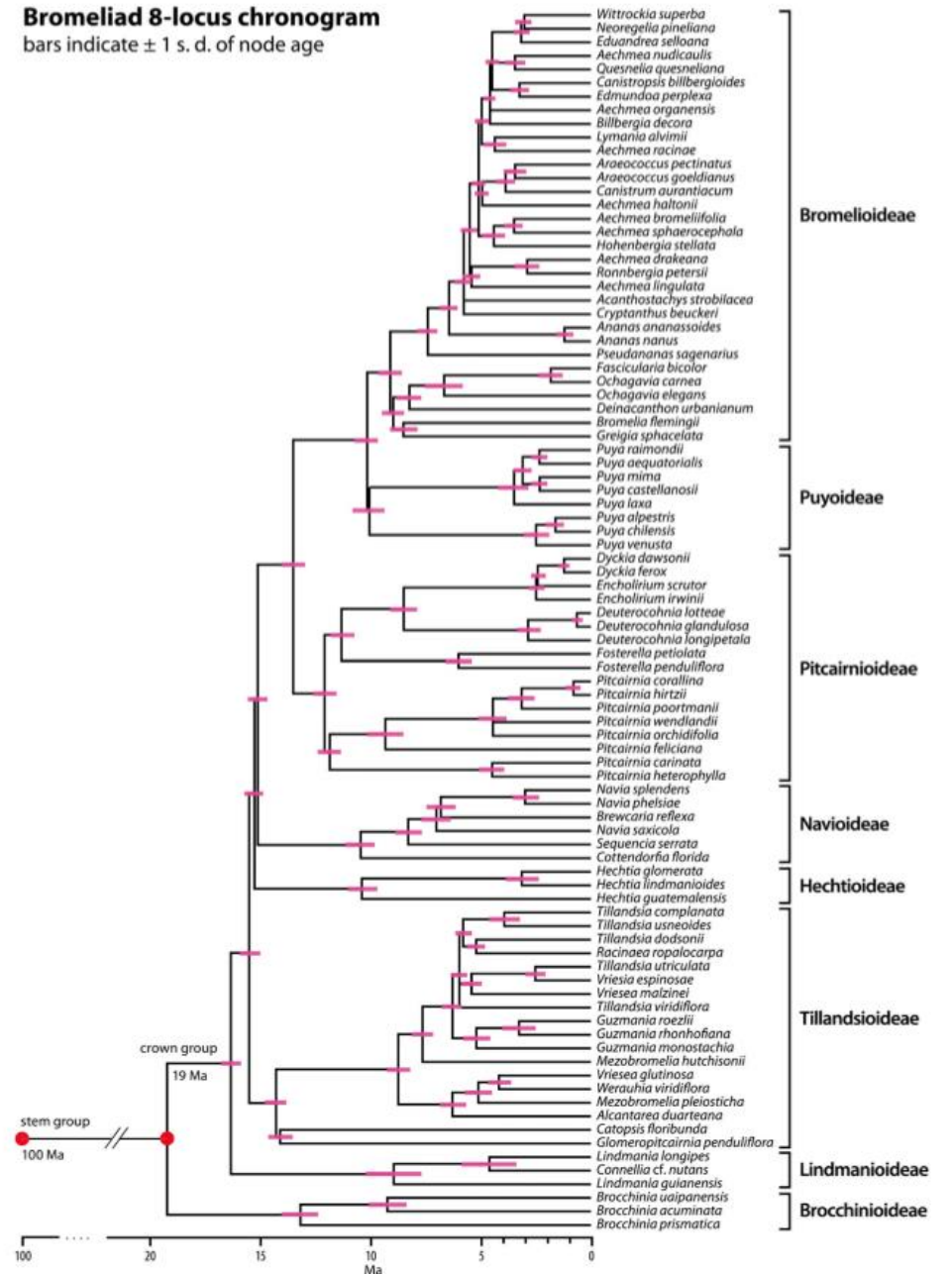
Methods – Molecular Dating

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

Fig. 7. Cross-verified penalized-likelihood (PL) chronogram for bromeliad evolution based on the maximum-likelihood phylogeny, using the crown and stem ages derived from the across-monocots PL analysis (see Fig. 6). Each magenta bar indicates ± 1 SD around the estimated age of the corresponding node based on bootstrap resamplings.

Bromeliad 8-locus chronogram

bars indicate ± 1 s. d. of node age



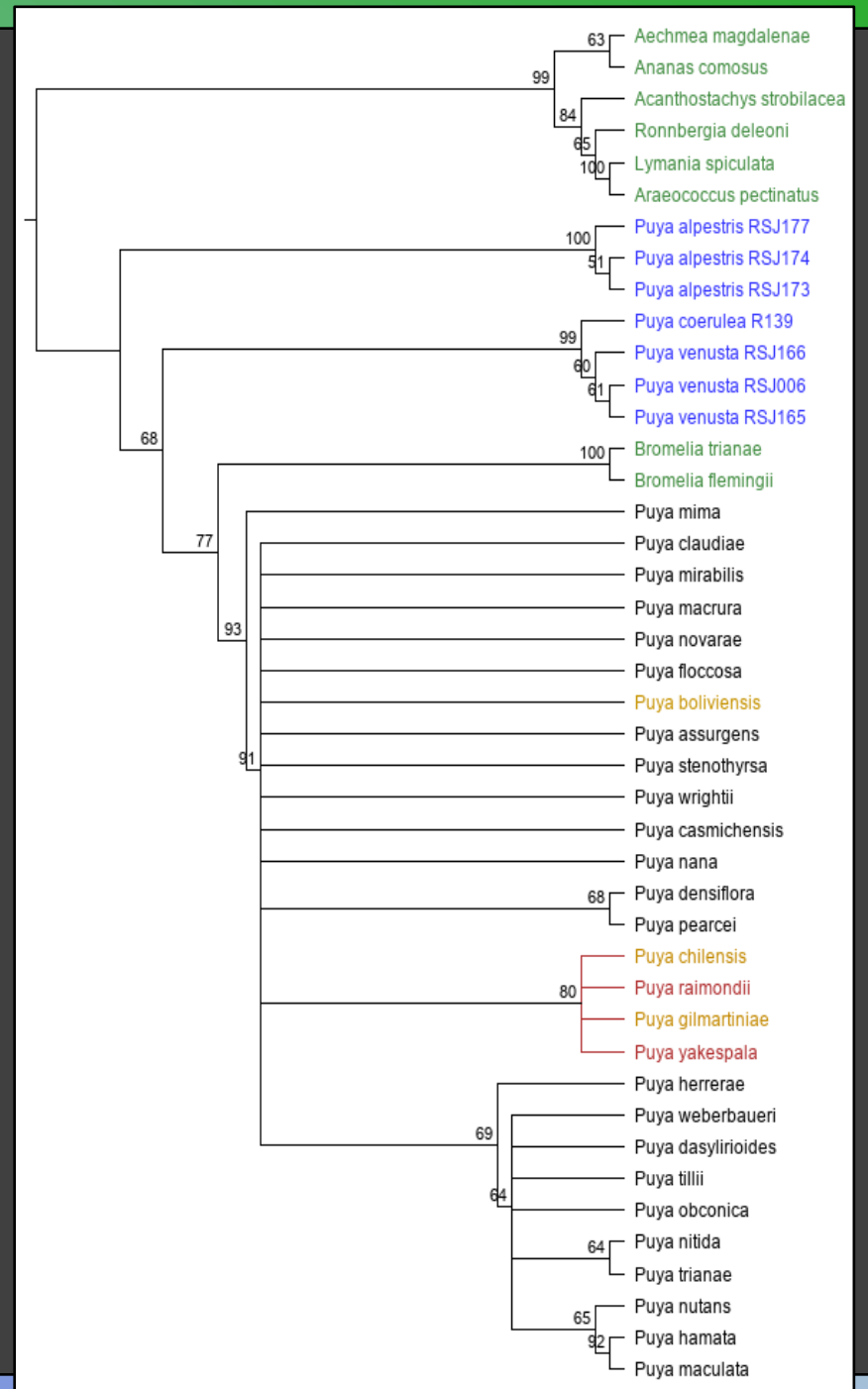
Results

Dataset	Total Characters	Variable and Parsimony Uninformative	Parsimony Informative Characters	Percent Parsimony Informative
g3pdh	1178	105	113	9.59%
PHYC	1048	178	97	9.3%
<i>matK</i>	819	77	32	3.9%
<i>trnS-trnG</i>	1048	104	49	5%
<i>rps16</i>	827	108	43	5.2%

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

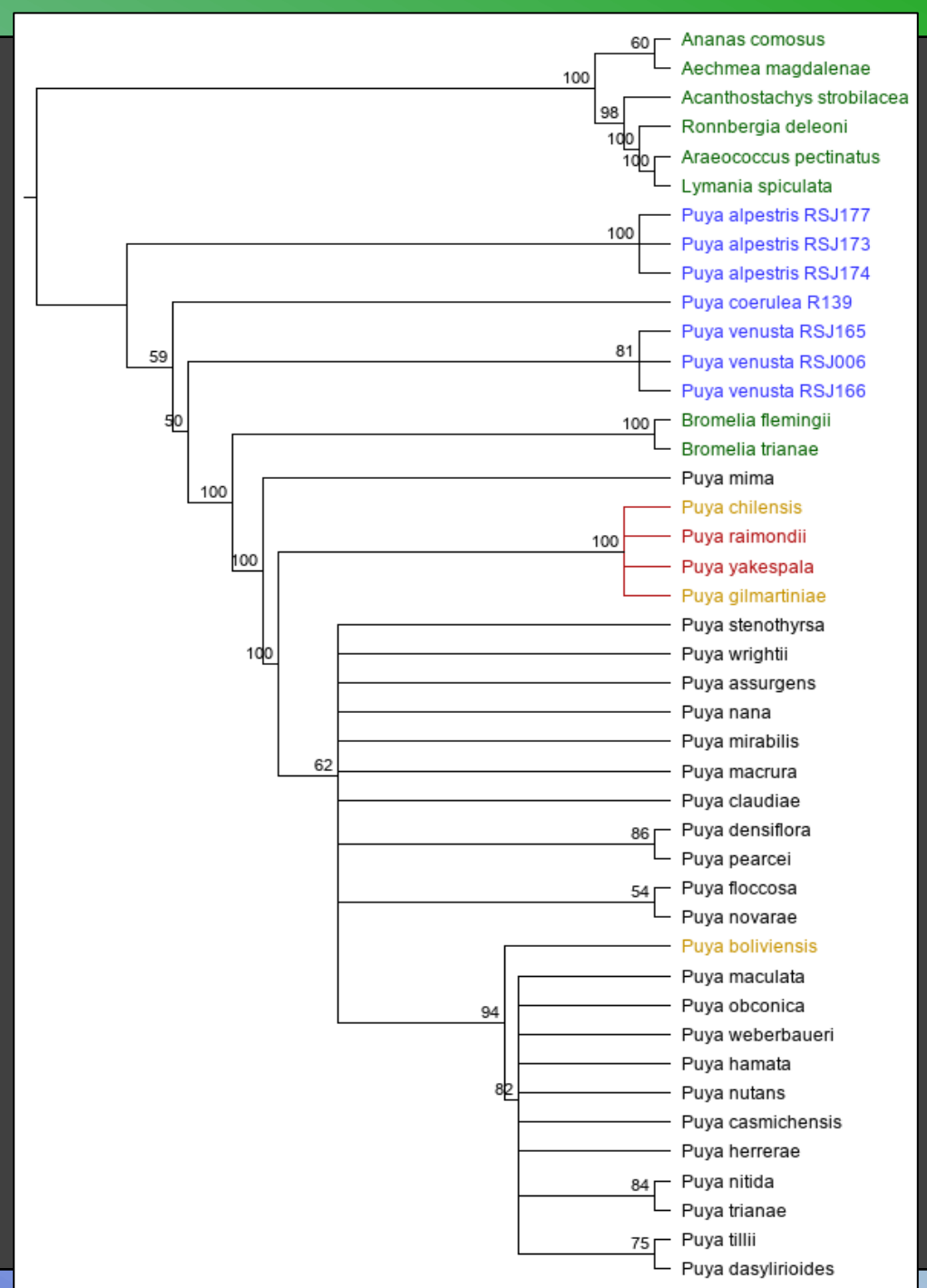
Figure 2. Maximum likelihood phylogeny with bootstrap support



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*

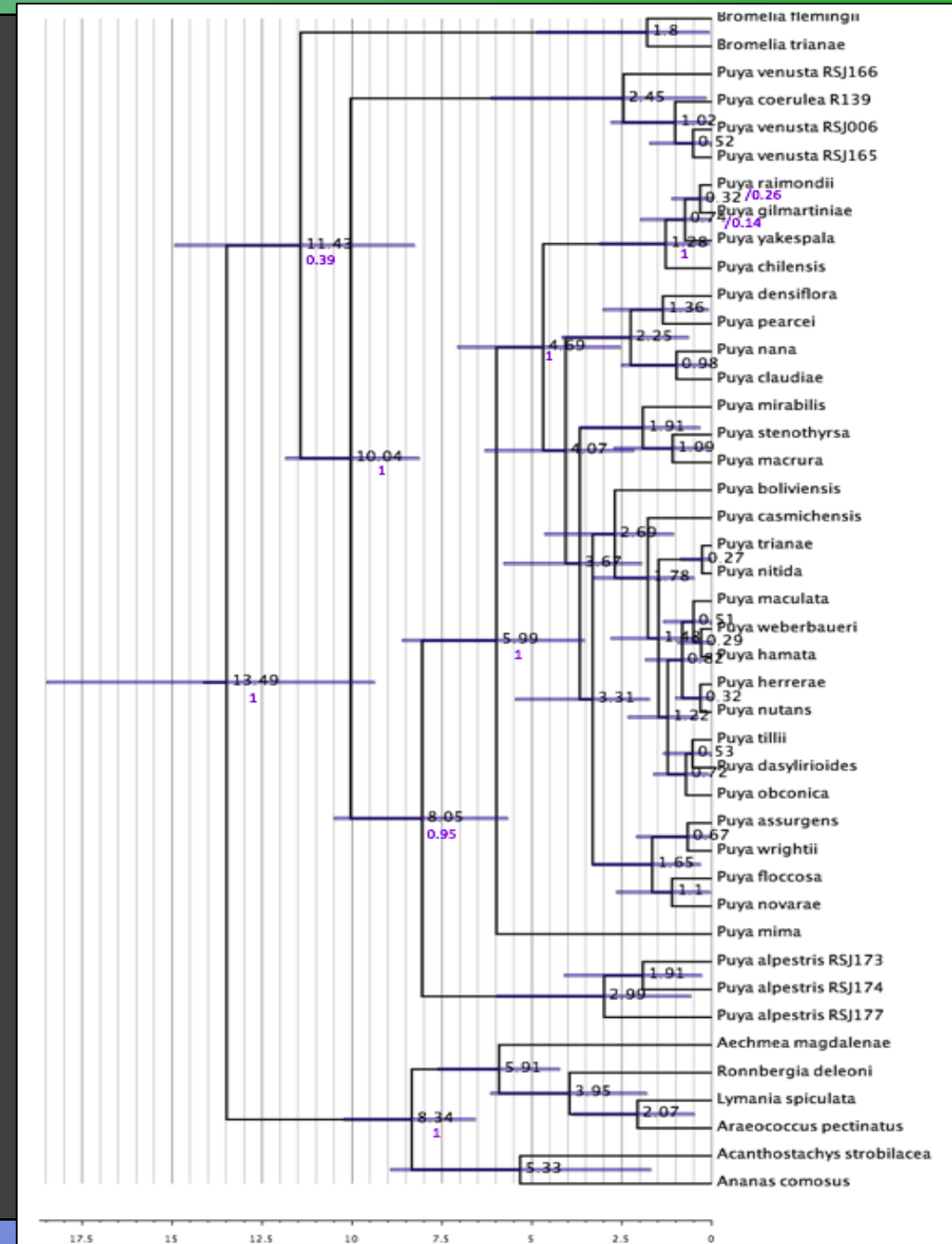
Figure 3. Bayesian Inference phylogeny



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 5.99Ma
- 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*

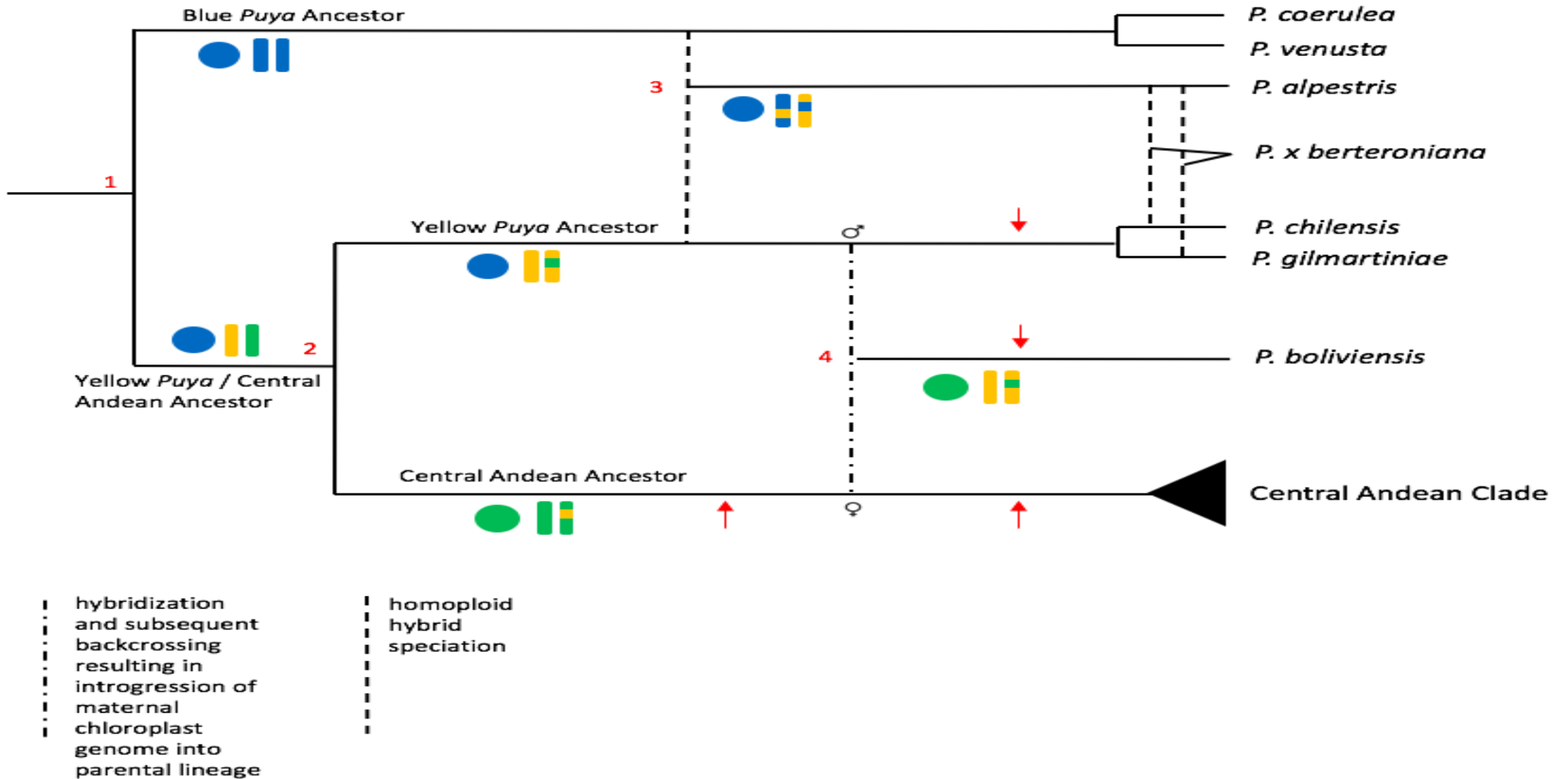
Elevational Disjunct Clade (ED clade)

- *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



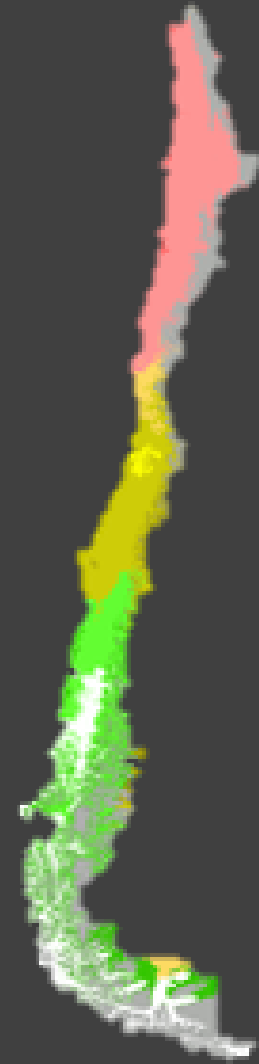
Updated Hypothesis

Figure 8



Molecular Dating and Biogeography

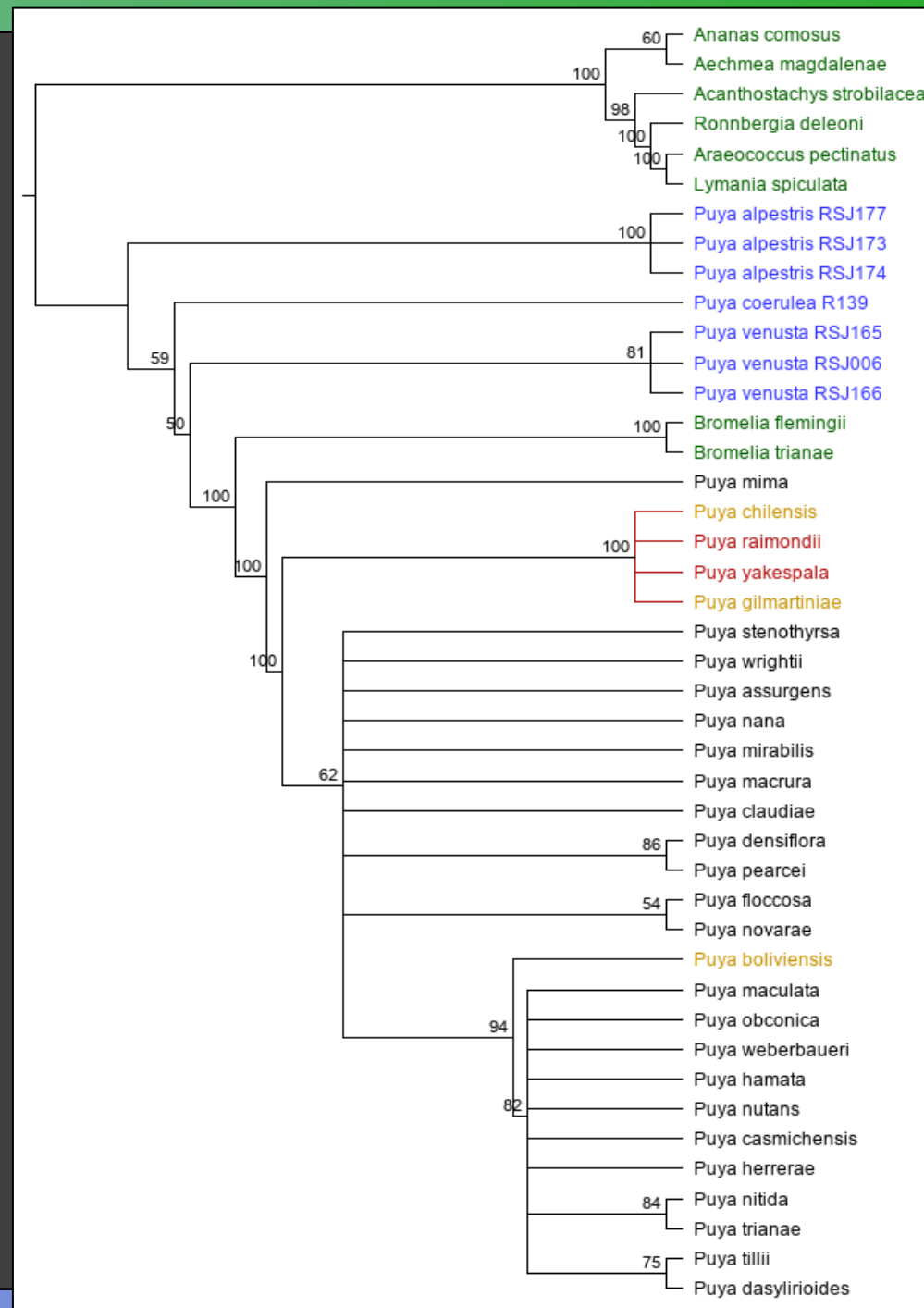
- Chile is home to the oldest lineages of Bromelioideae as well as Puyoideae and is a hotspot of diversity today
- Split between Puyoideae and Bromelioideae at 11.4 Ma
 - Beginning of seasonality in what is now Chile 12-15 Ma
 - Aridification of what is now Atacama Desert in northern Chile Pliocene to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



Conclusion

Key Findings

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



Acknowledgements

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