

Chloroplast phylogenomics in *Bartsia* L. (Orobanchaceae): a subgenomic approach using microfluidic PCR

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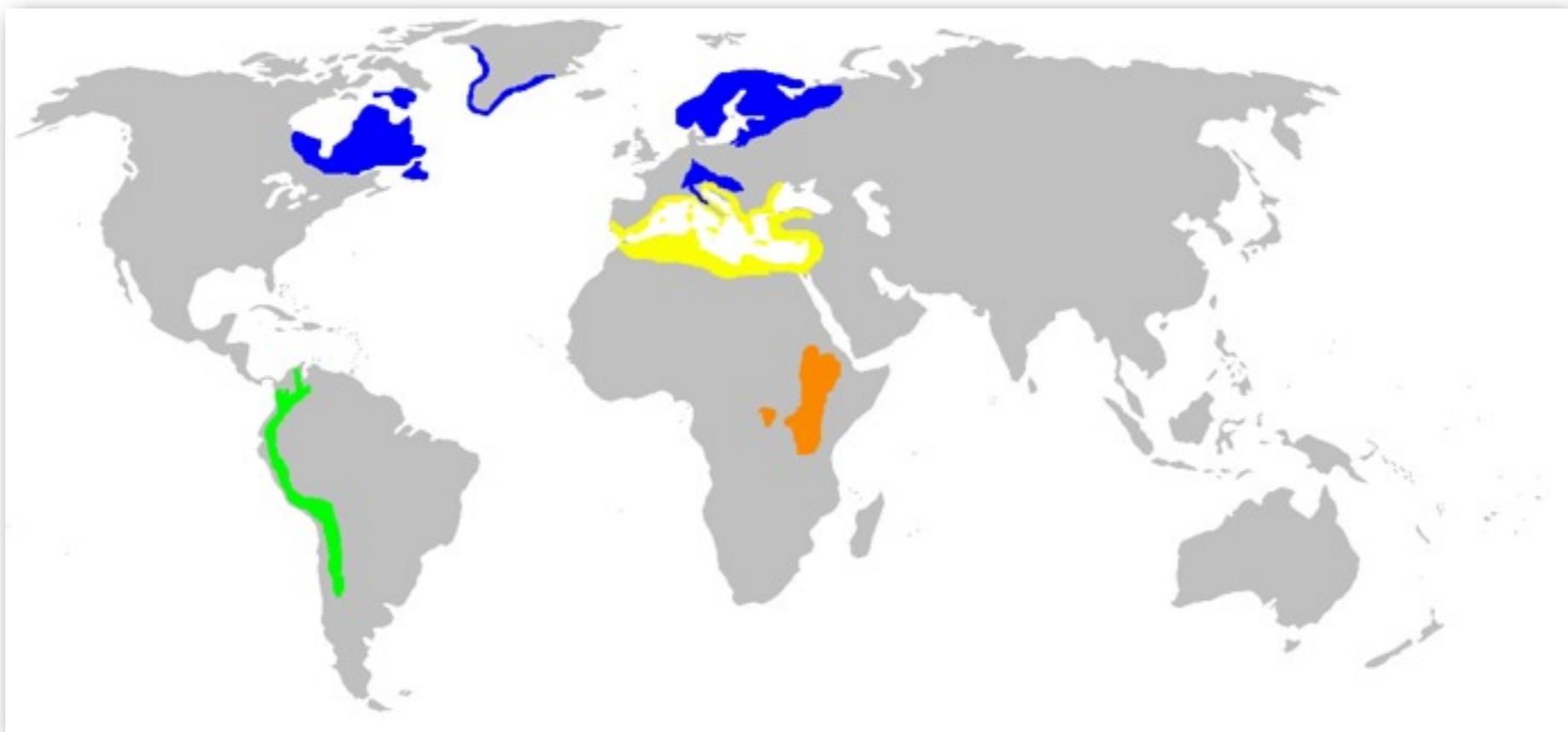
Bartsia L.

- Annual and perennial herbs
- Hemiparasite
- Grows in montane environments
- 49 species

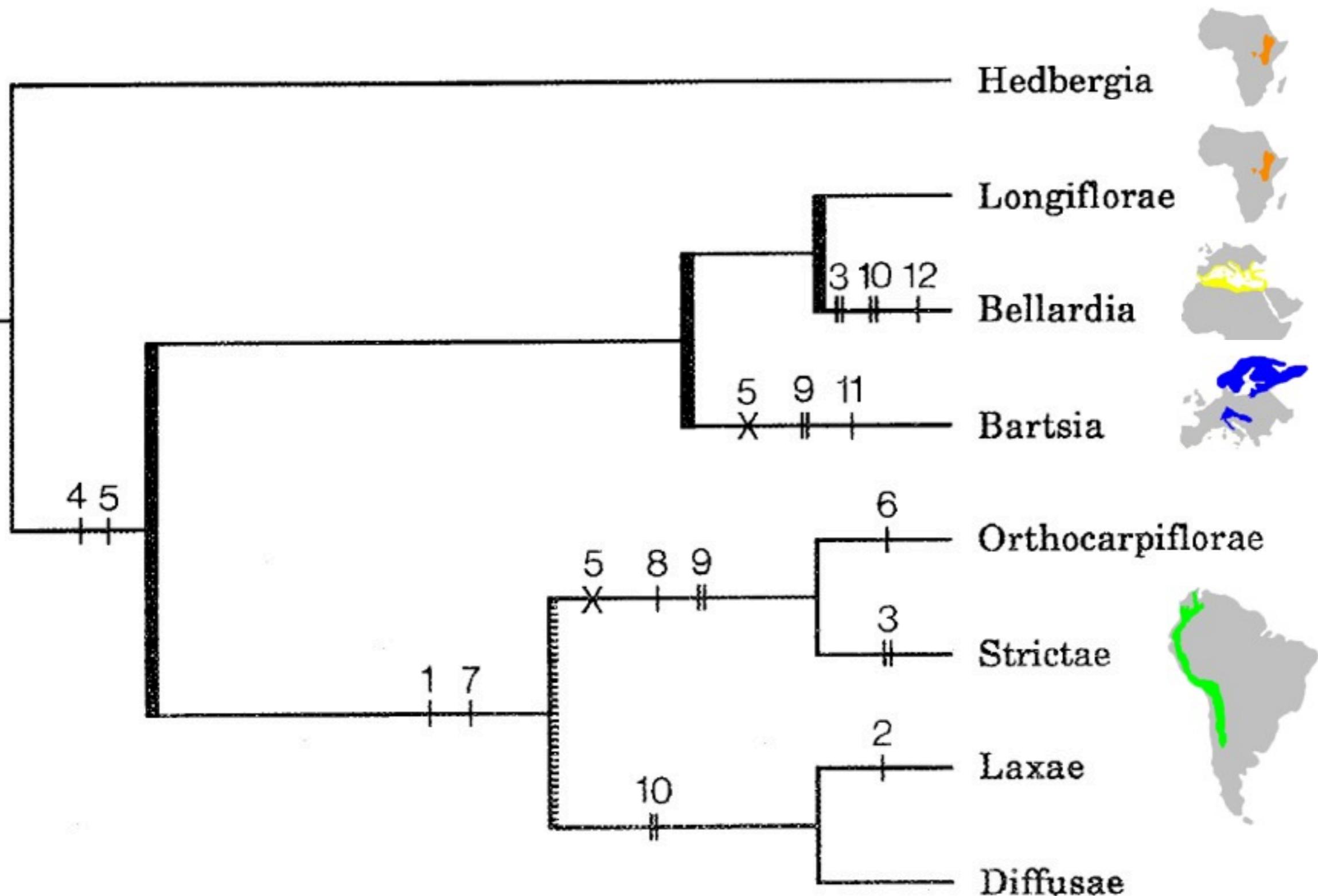


Bartsia L.

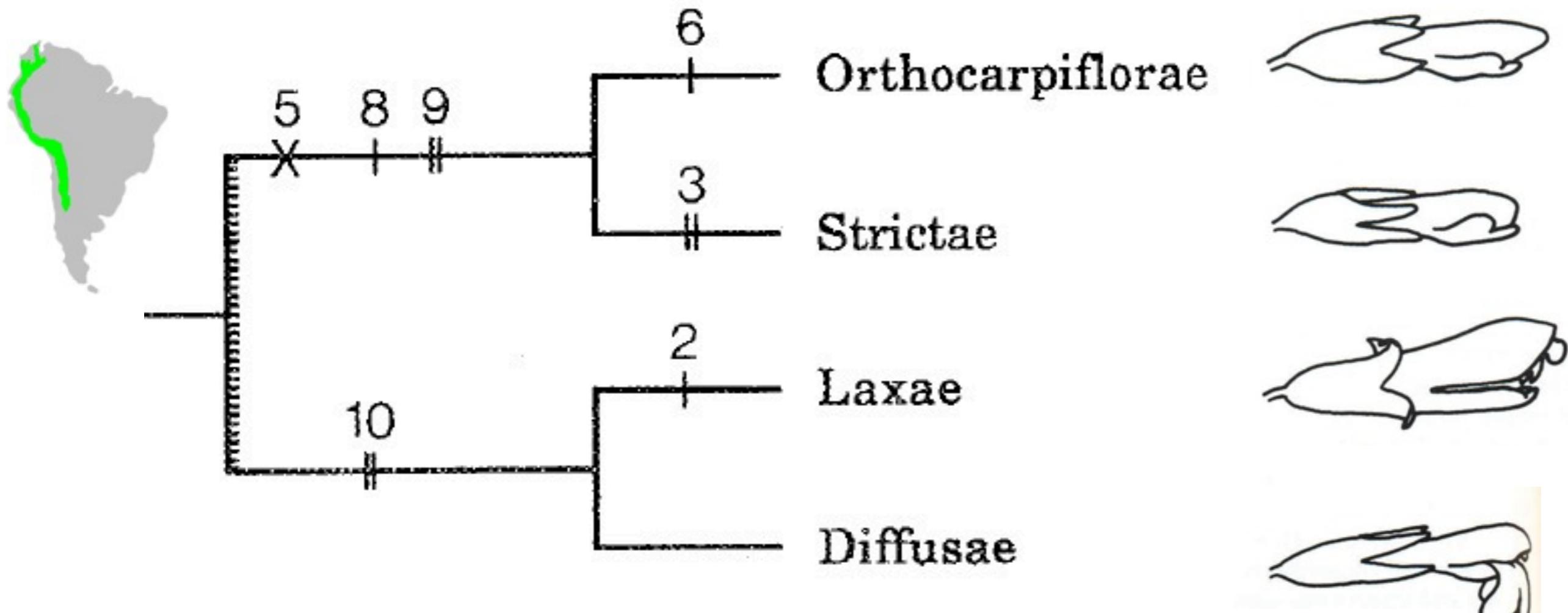
- 1 sp. in the Alps, Scandinavia,
northeastern North America
- 2 spp. in northeastern Africa
- 1 sp. in the Mediterranean but now has
naturalized to Chile, Australia, USA
- 45 spp. in the páramos of Andean South America

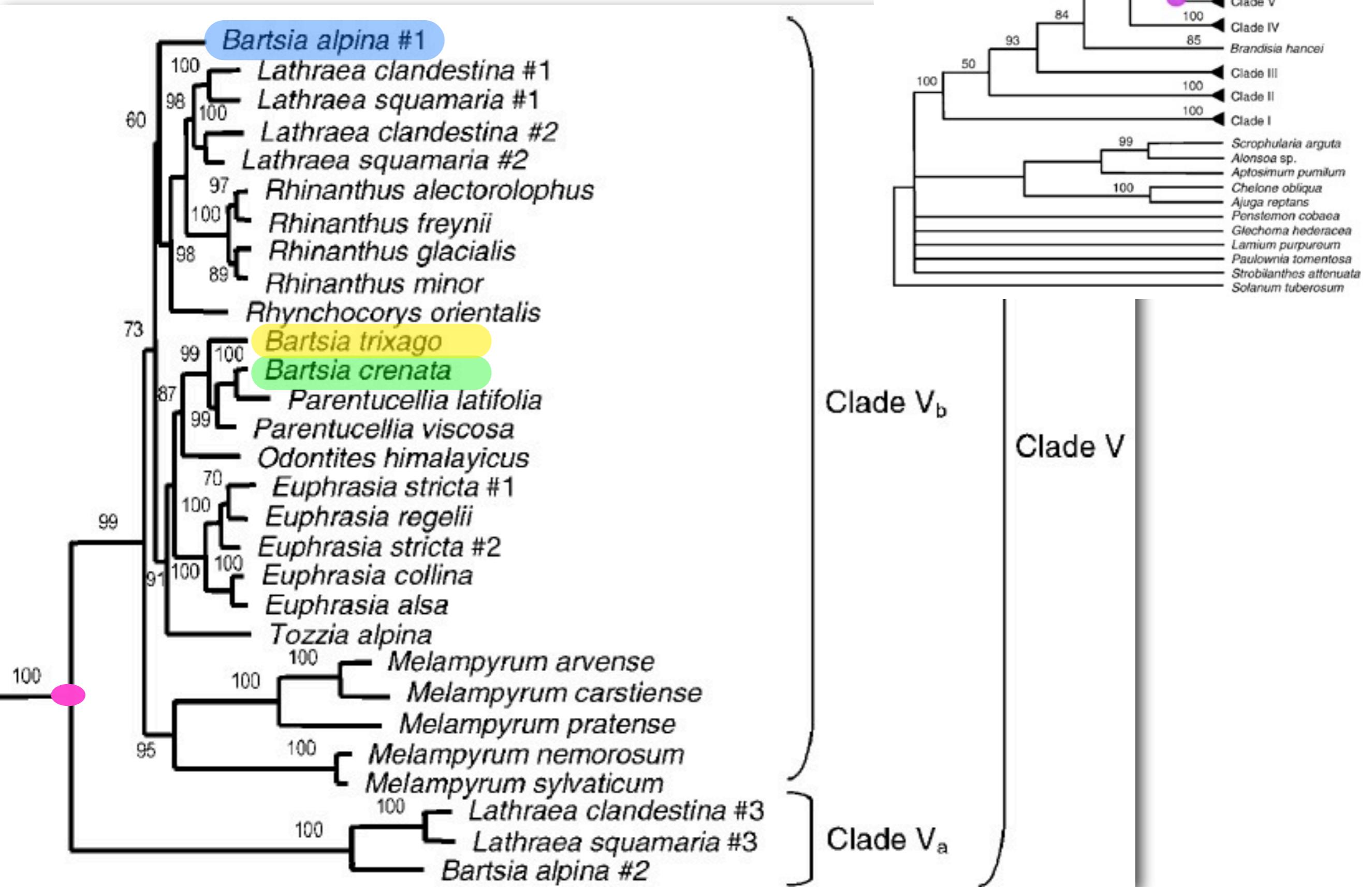


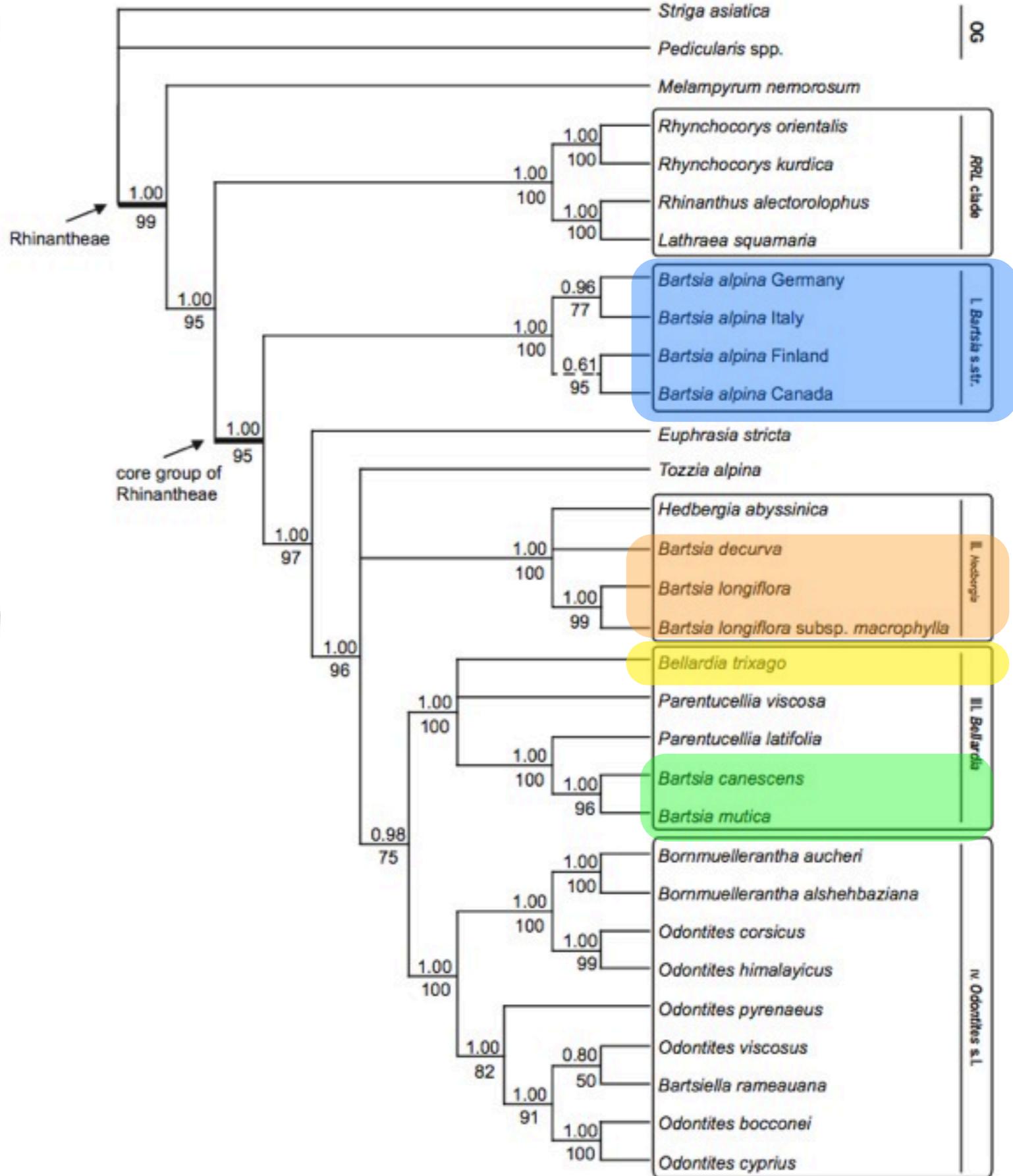
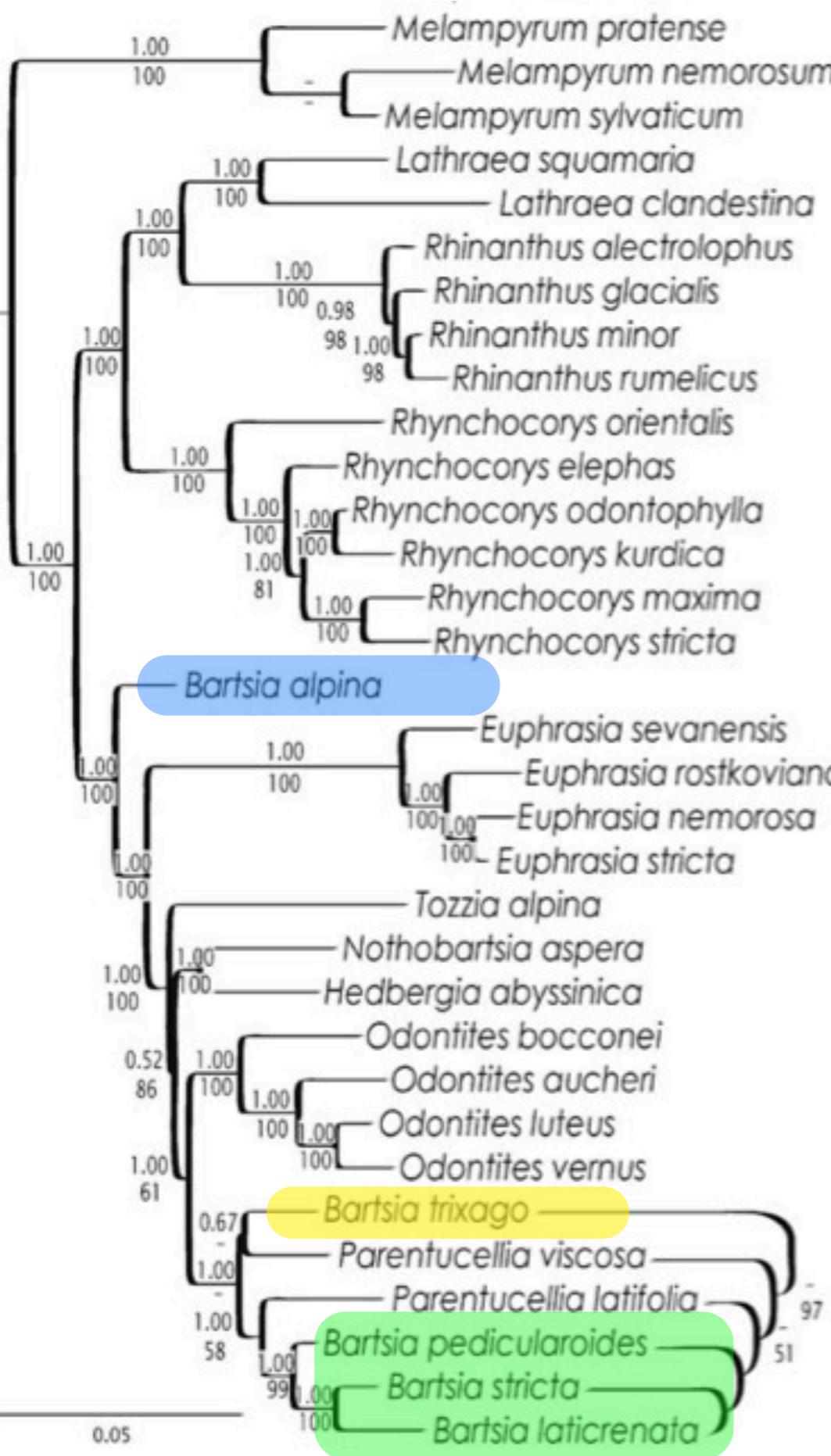
Back in 1990...

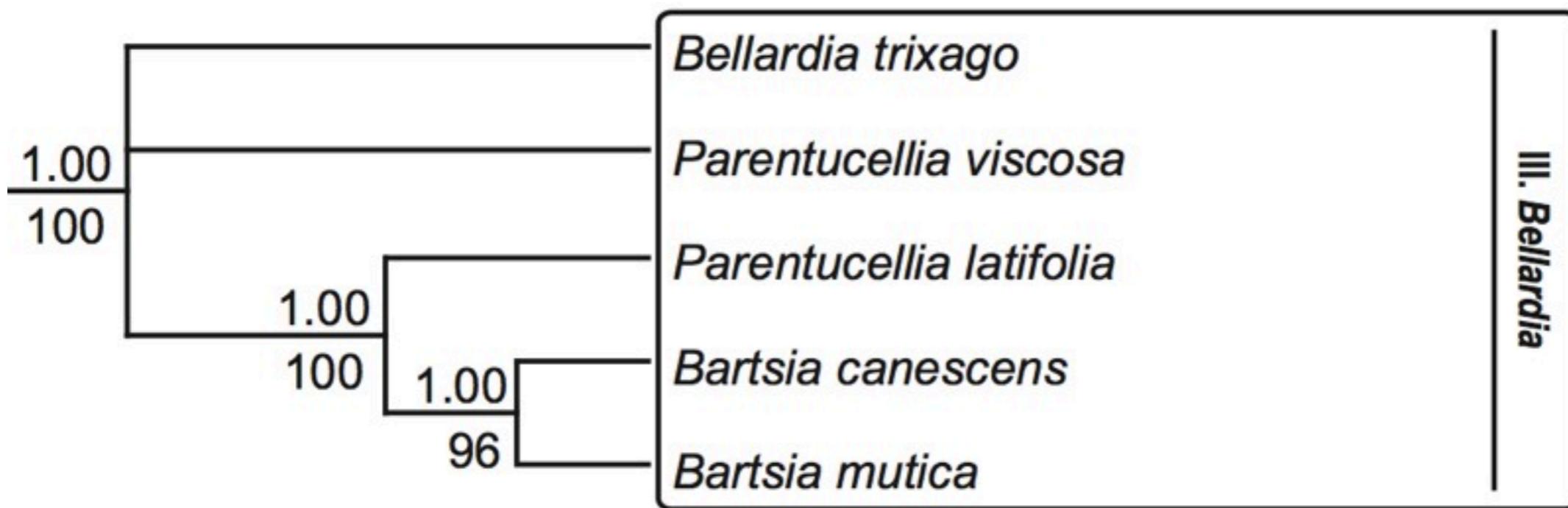


Back in 1990...



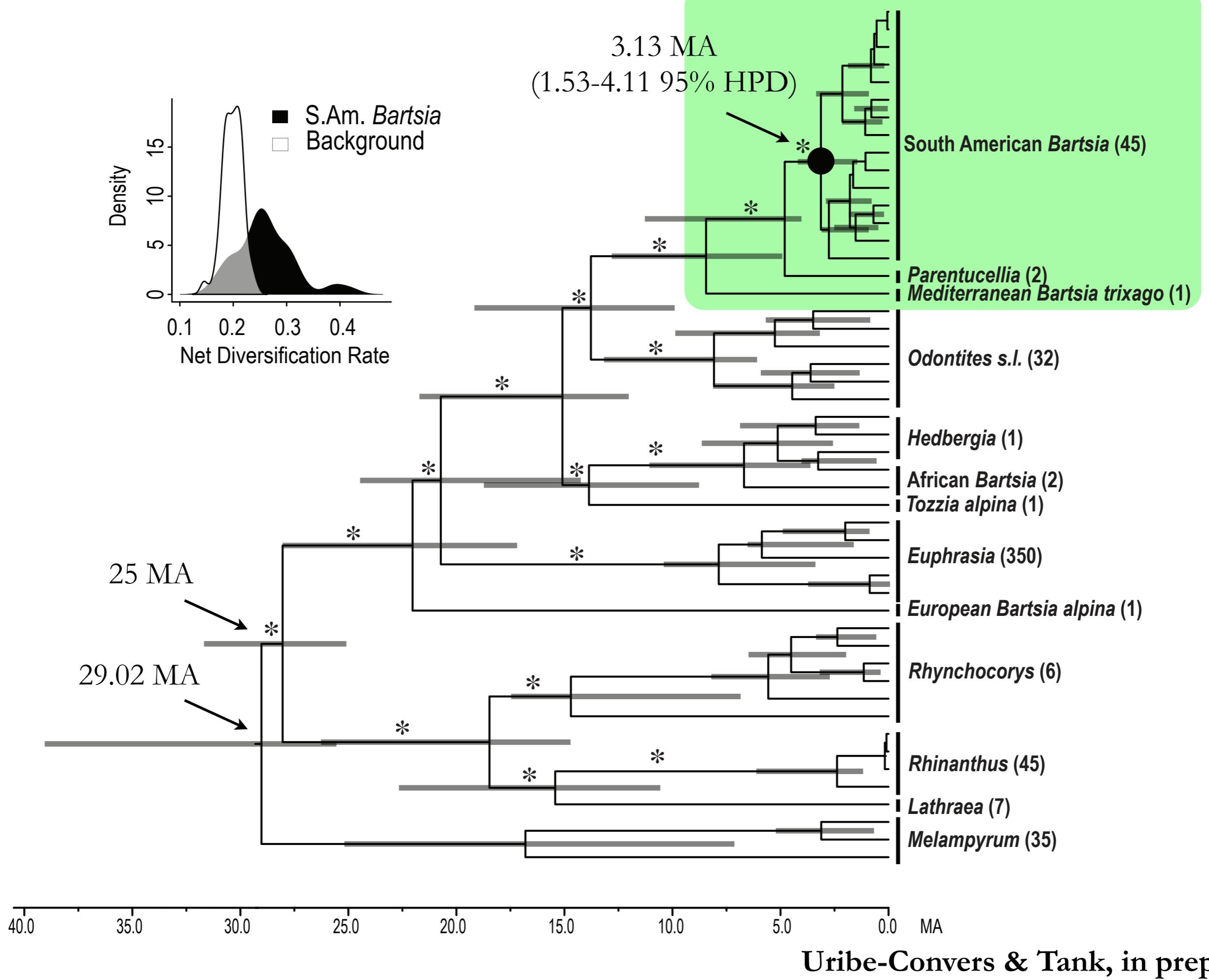




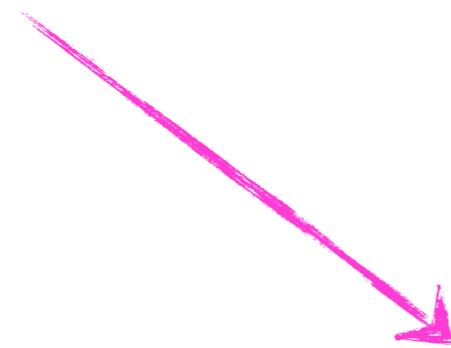


Bellardia All.

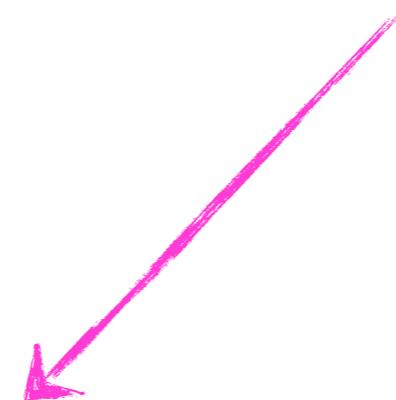




Low genetic divergence?



Next-generation sequencing!



But which method?

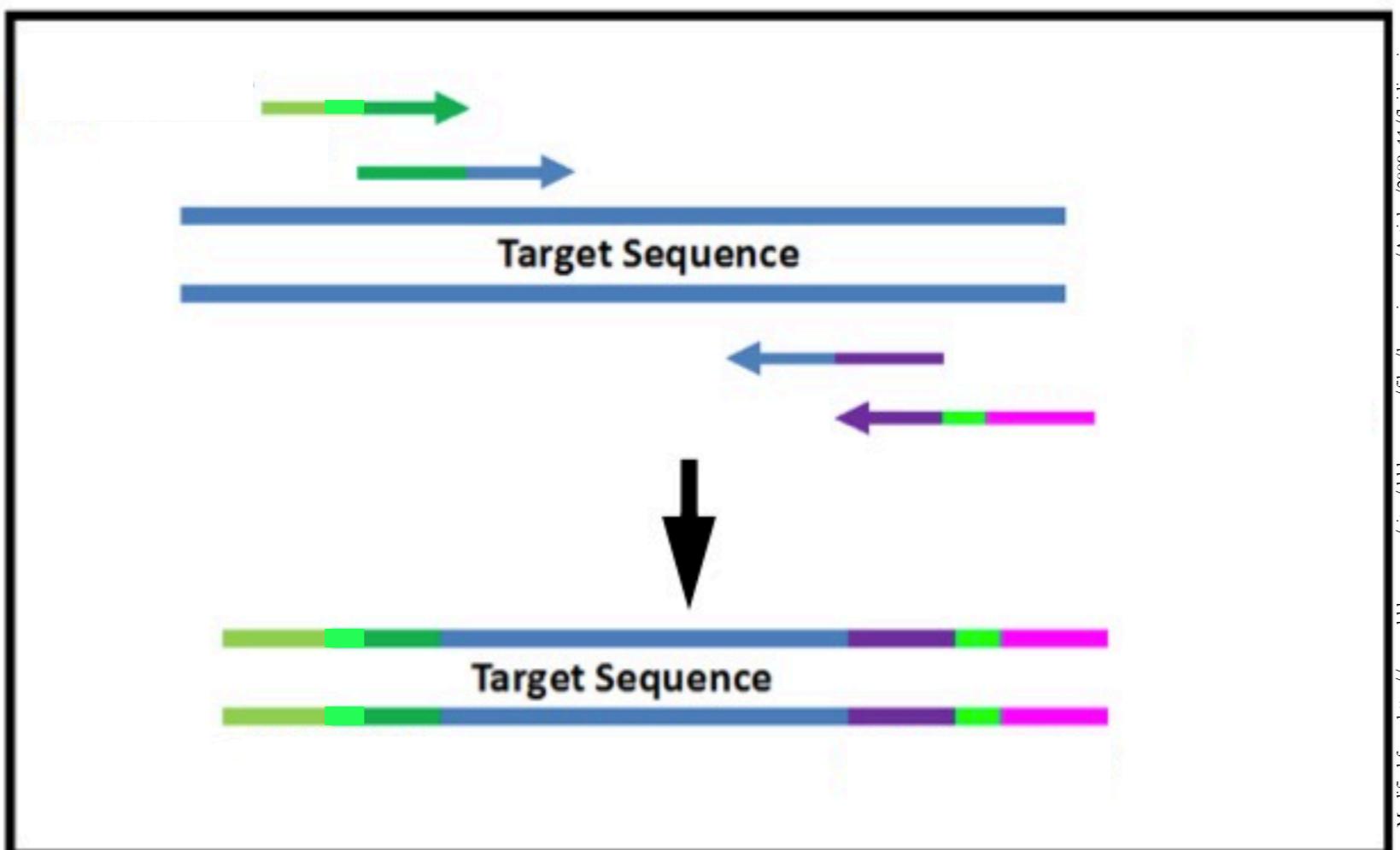
Microfluidic PCR

- Using Fluidigm Access Array
- 48 x 48 (2304 PCRs)
- Ready for next-gen sequencing



Microfluidic PCR

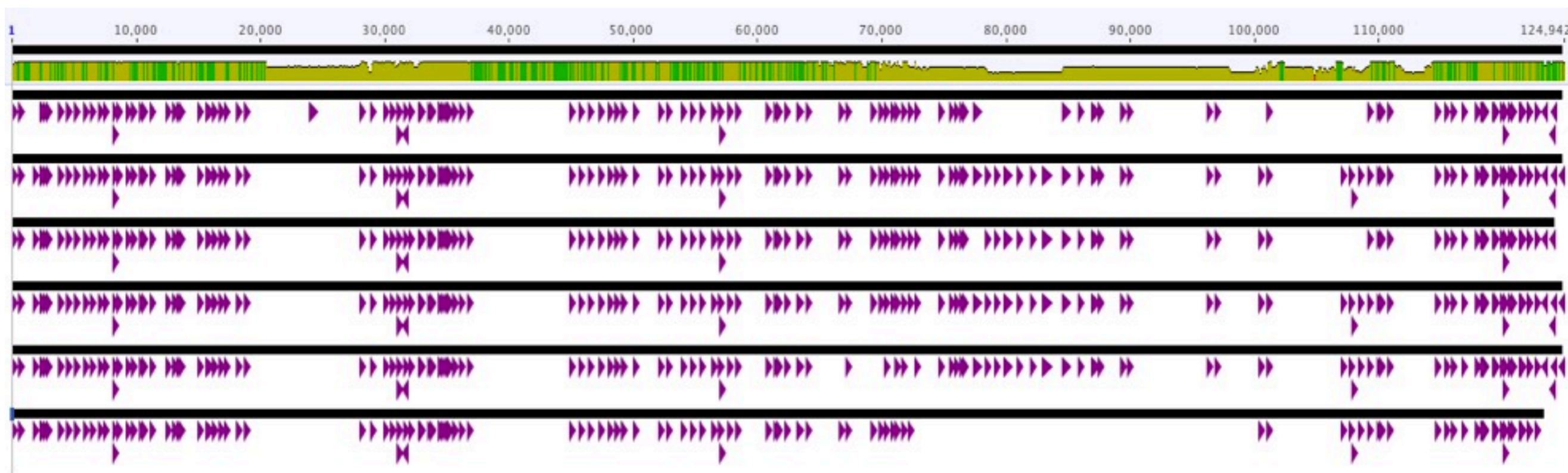
- 4 primer reaction
- Barcodes and adapters are incorporated in the reaction
- No need for library preparation!



Primer: forward & reverse
 Conserved sequence
 Barcodes
 Next-Gen adaptor

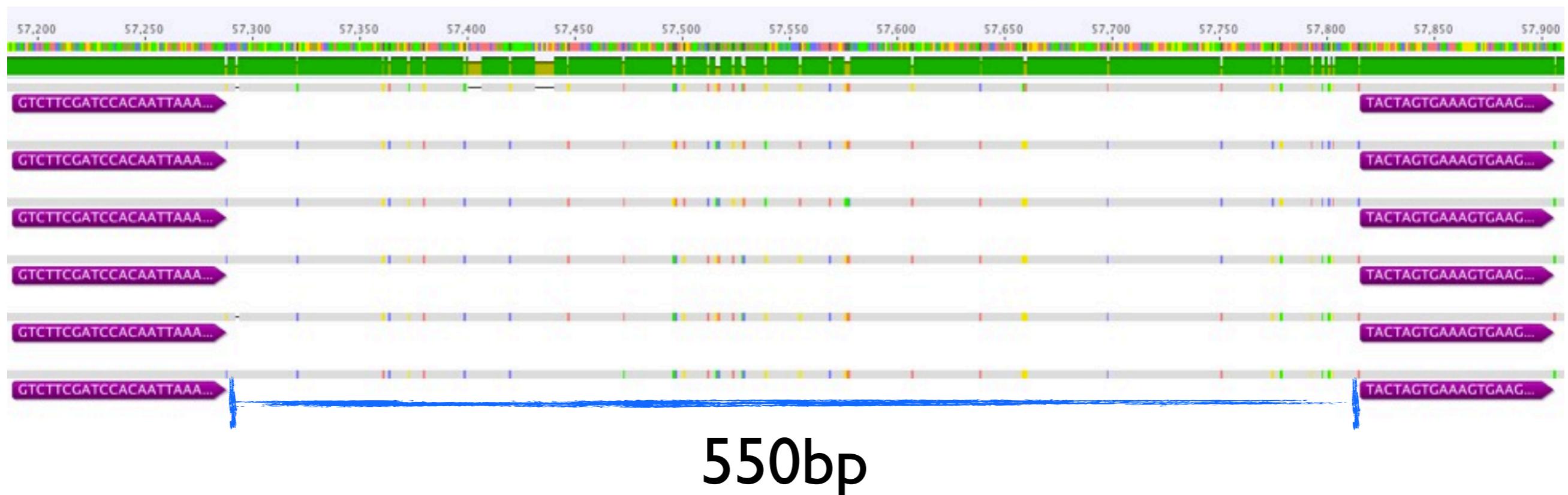
Primer design

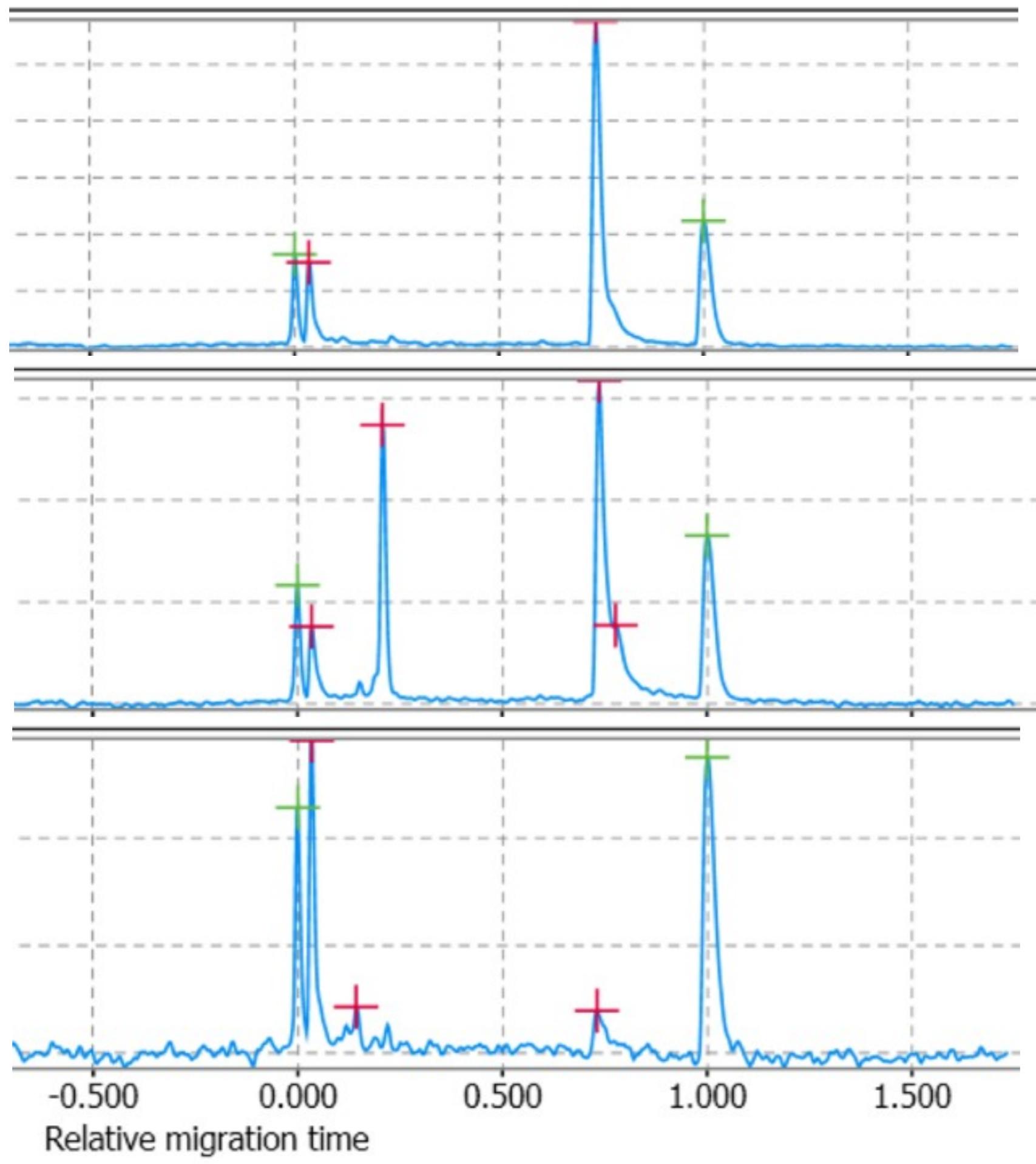
- Six complete plastomes (via long PCR)
- Designed 74 primer pairs
- Most variable regions in the chloroplast



Primer design criteria

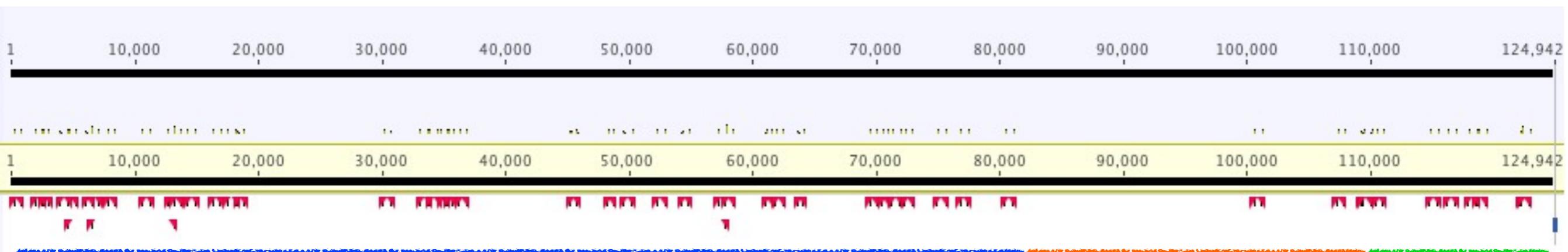
- Variable regions between 400-800bp
 - Conserved flanking regions
 - Every primer had the same annealing temperature (60°C)





Primer design

- 53 primer pairs were successfully validated
- 72% success rate
- The 48 most informative ones were chosen
 - average variability 2.7% (0.8%-7.5%)

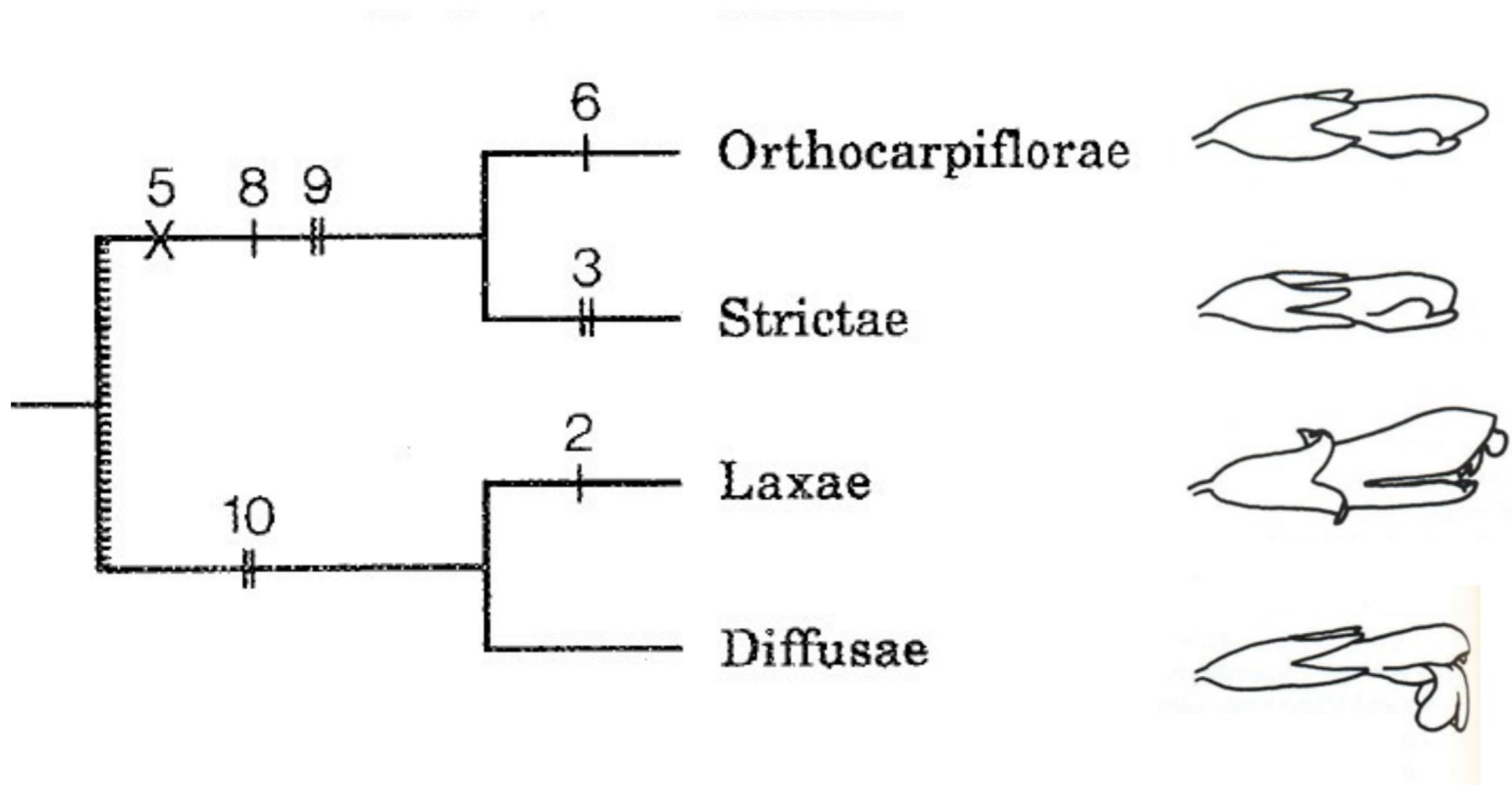


LSC
Large Single Copy

IRB
Inverted Repeat

SSC
Small Single Copy

Are Molau's morphological sections monophyletic?



Sampling

-192 accessions

-42 (94%) species



- Amplification of 4 chips in a Fluidigm Access Array System
- Sequencing Illumina MiSeq (1 million 250 paired -end)
- Cleaned adaptors and primers
- For every region in each sample, the most frequent read chosen as the correct one
- Forward and reverse reads were concatenated (500bp)

No. of Samples	No. of Regions	Total bp
156	48	
176	39-48	24,378
	Matrix coverage	0.995147

-Regions were independently aligned with MUSCLE
(v. 3.28. Edgar, 2004)



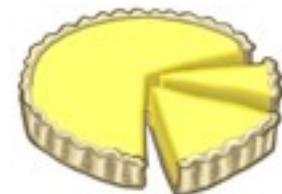
-Each alignment was checked by eye in Geneious
(v. 6.1. Biomatters)



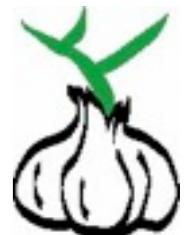
-The 48 alignments were concatenated in Phyutility
(v. 2.2. Smith & Dunn, 2008)



-Data partition and model selection in PartitionFinder
(v. 1.1. Lanfear et al., 2012)

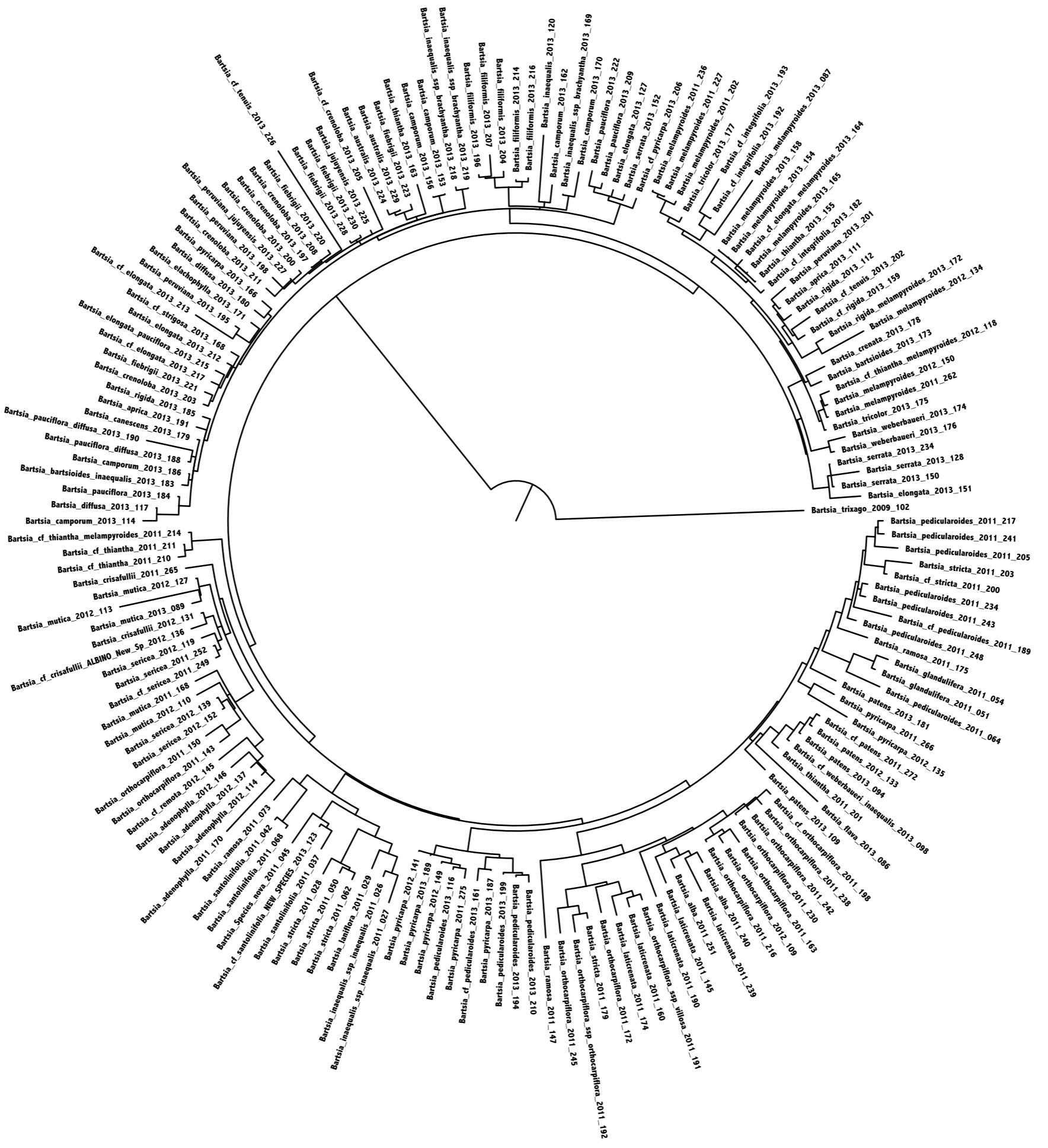


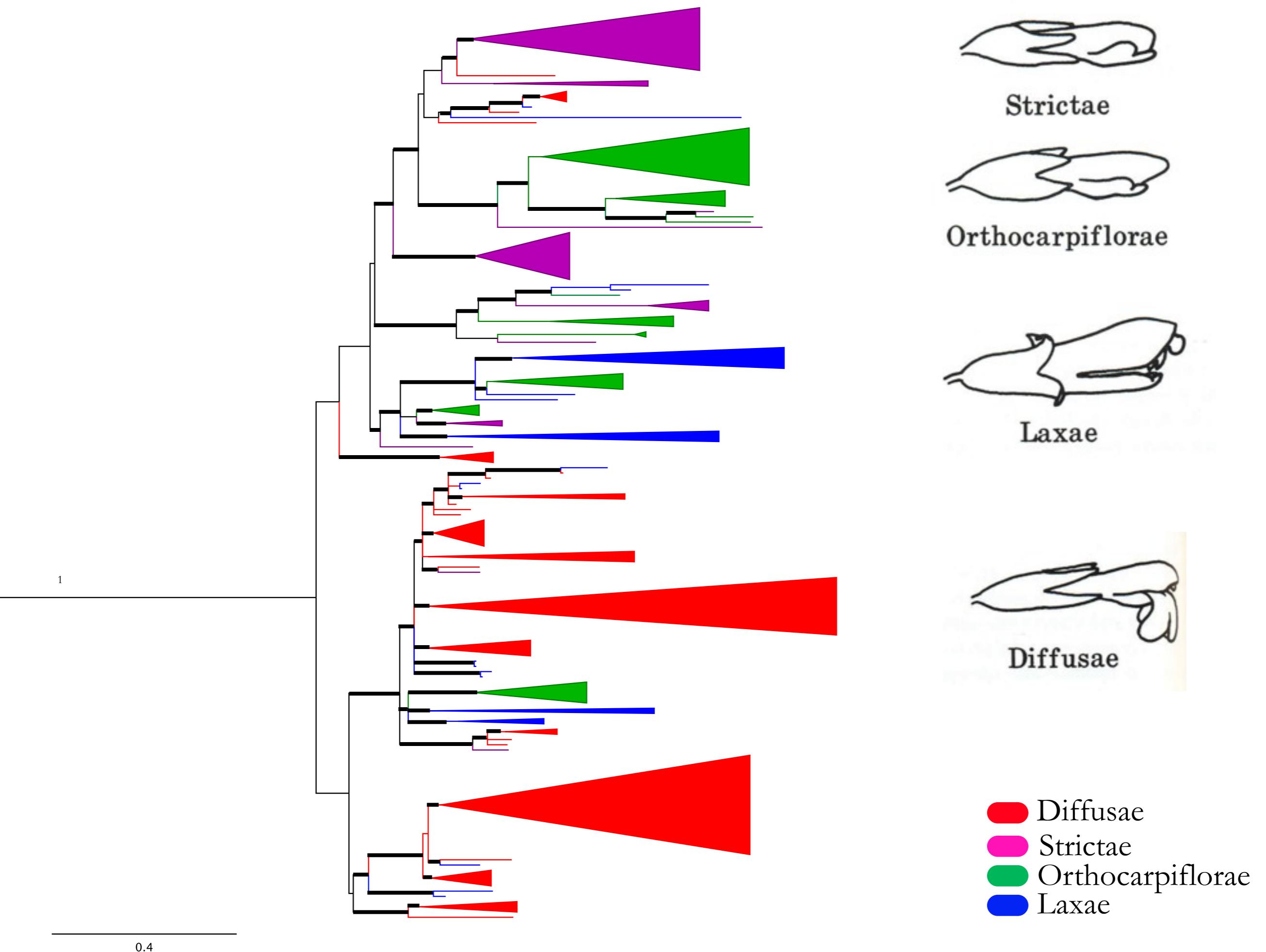
-Maximum likelihood analyses in GARLI
(v. 2.0. Zwickl, 2006)
-11 partitions, 5 models

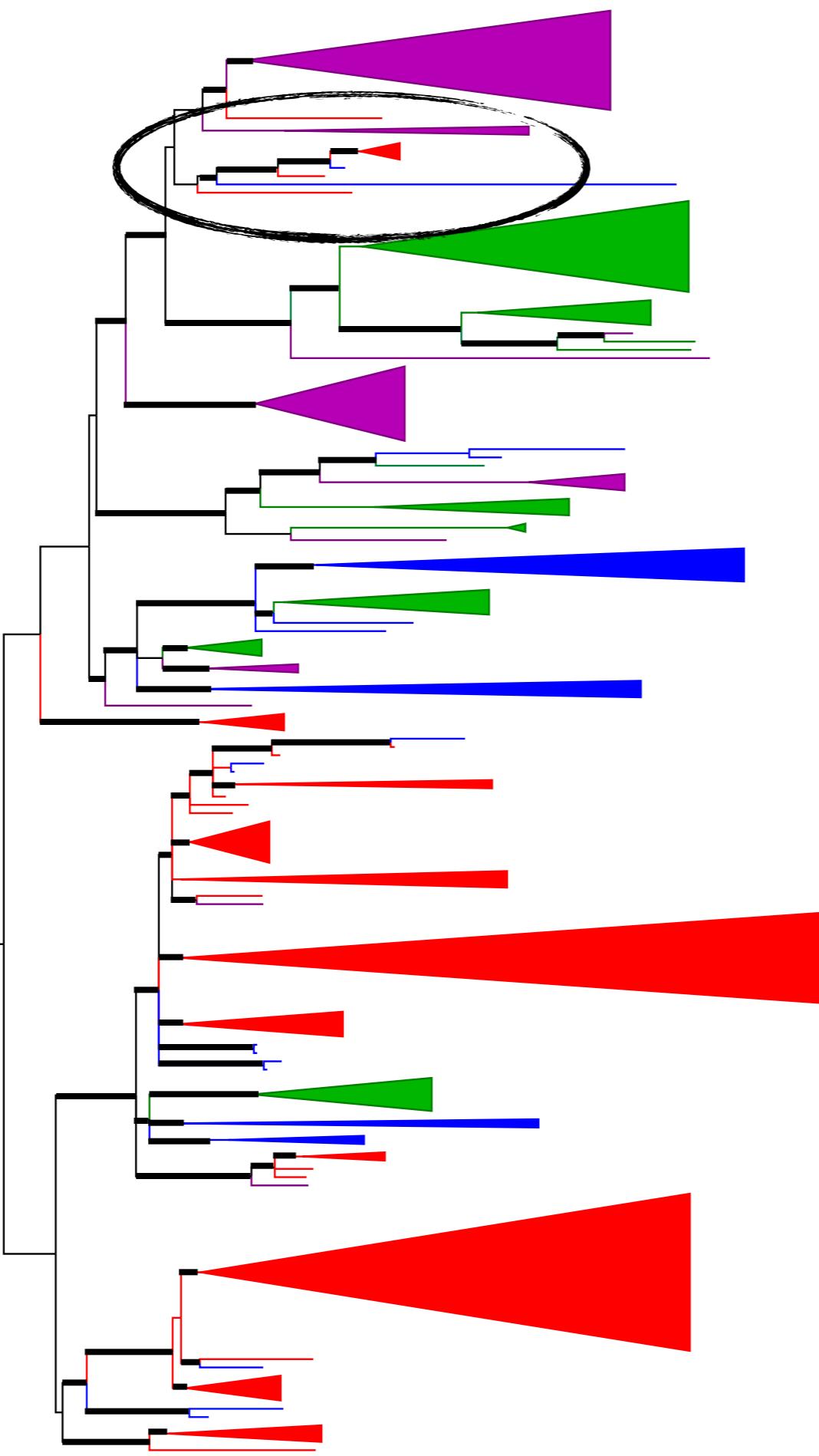


-Bayesian inference analyses in MrBayes
(v. 3.2. Ronquist & Huelsenbeck, 2003)
-12 partitions, 5 models









Strictae



Orthocarpiflorae

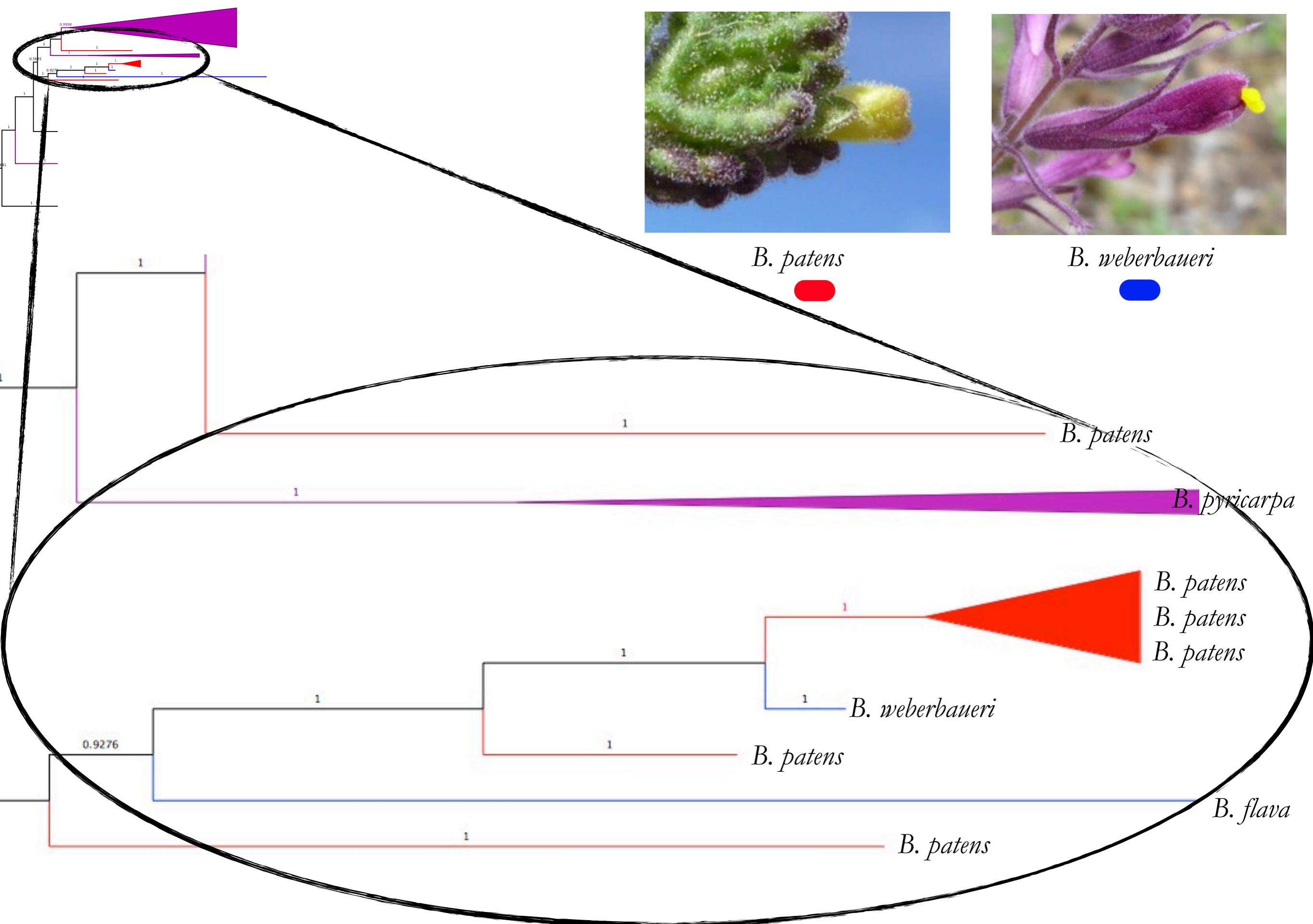


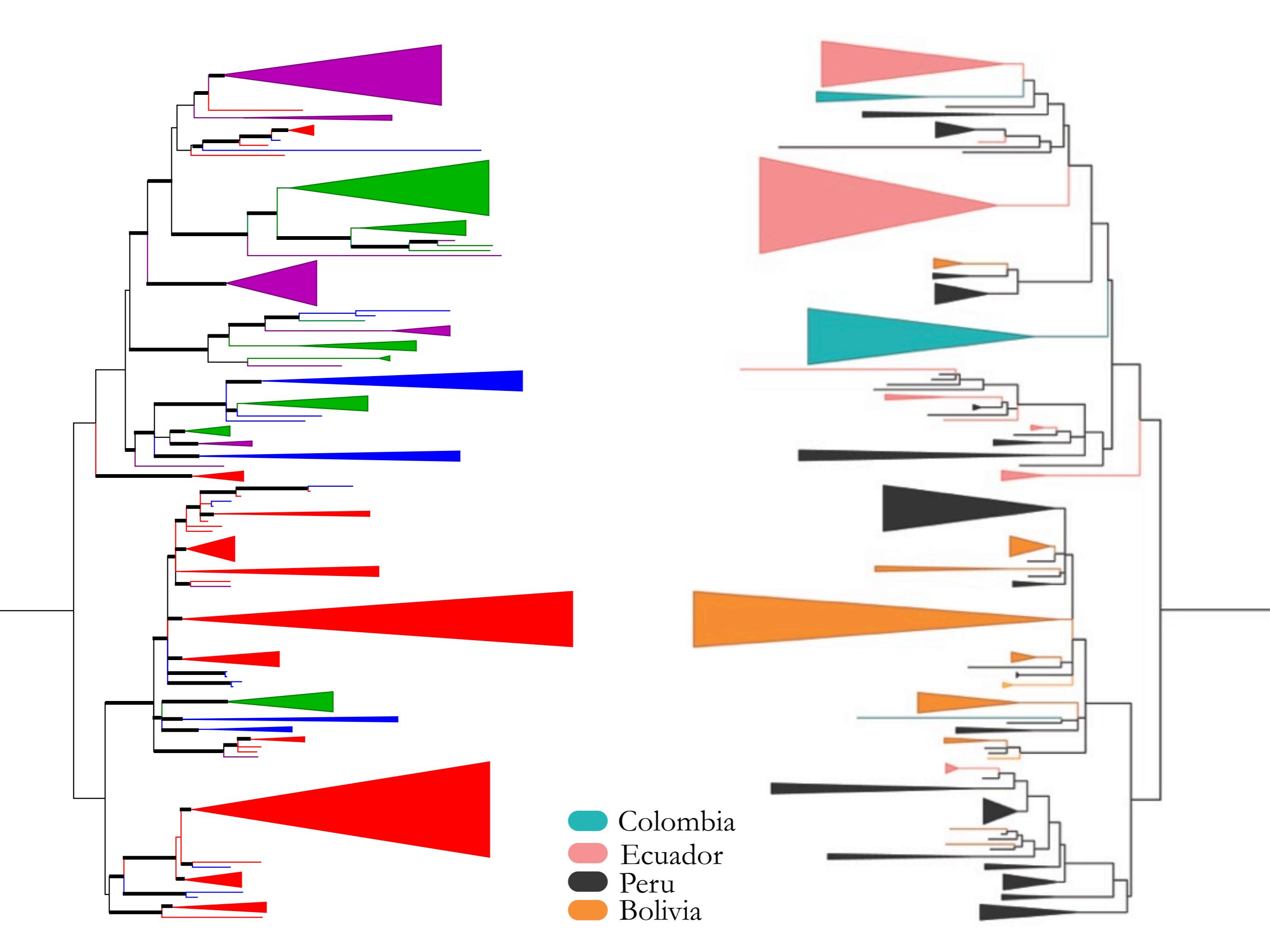
Laxae

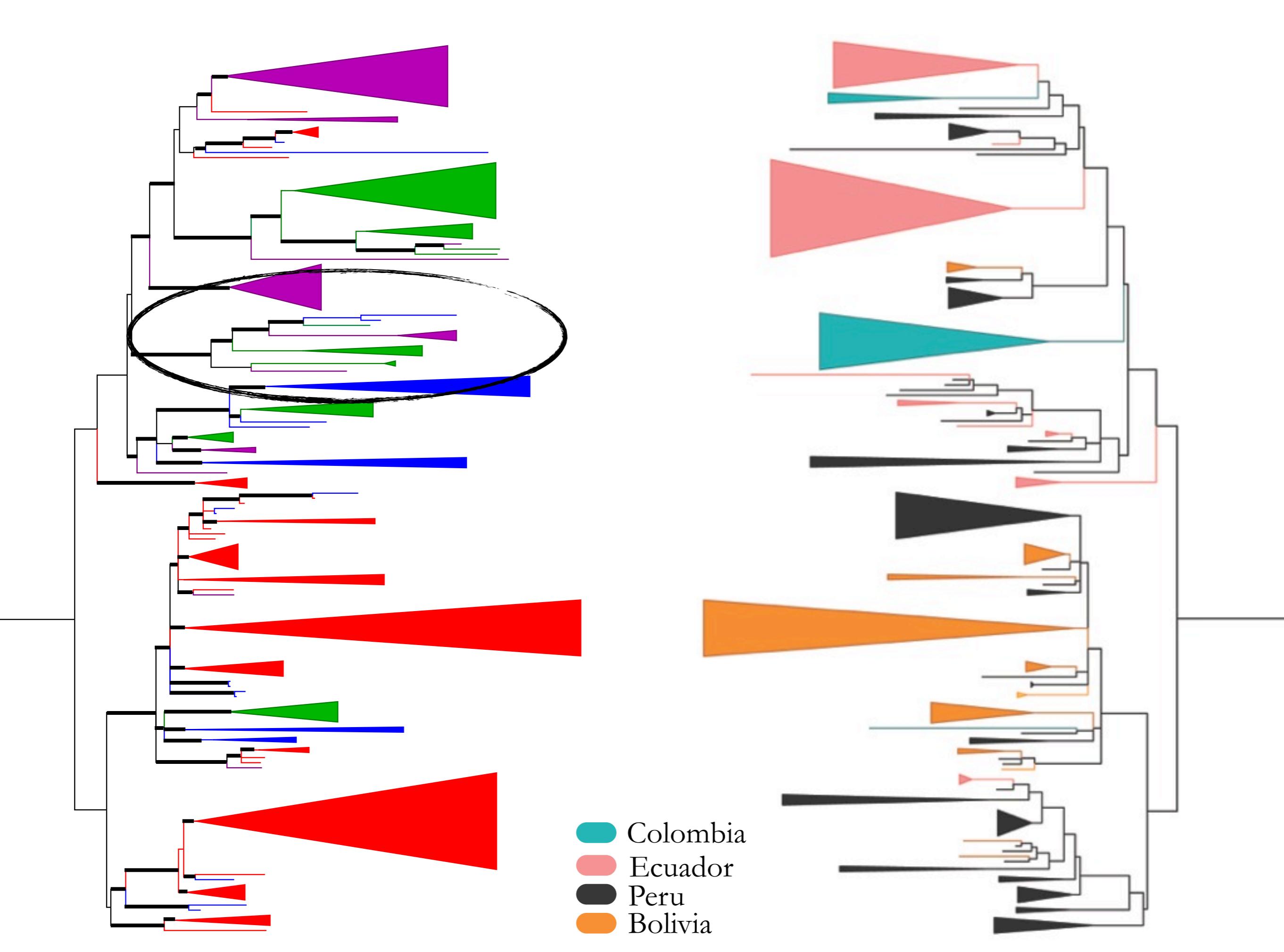


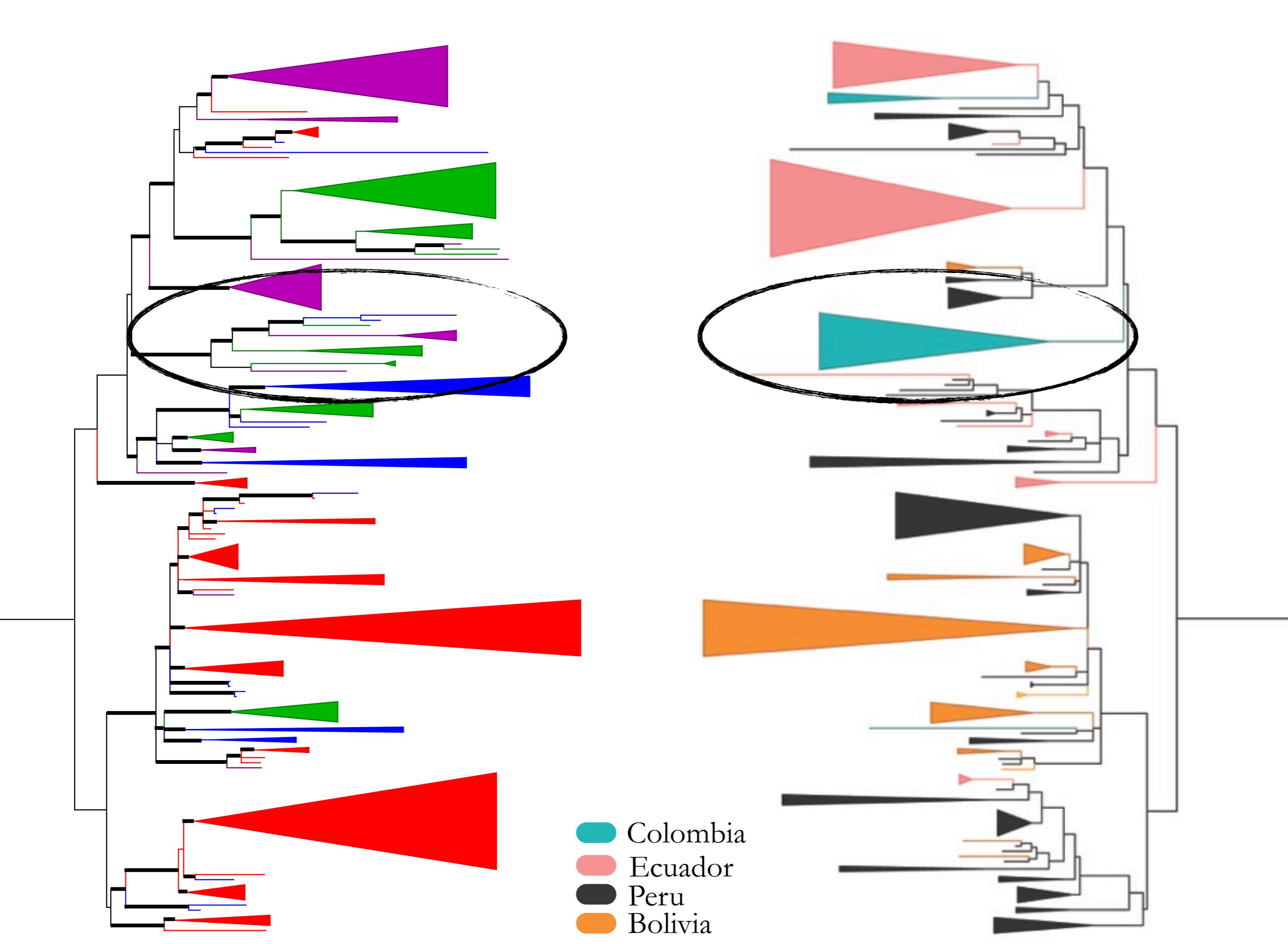
Diffusae

- Diffusae
- Strictae
- Orthocarpiflorae
- Laxae









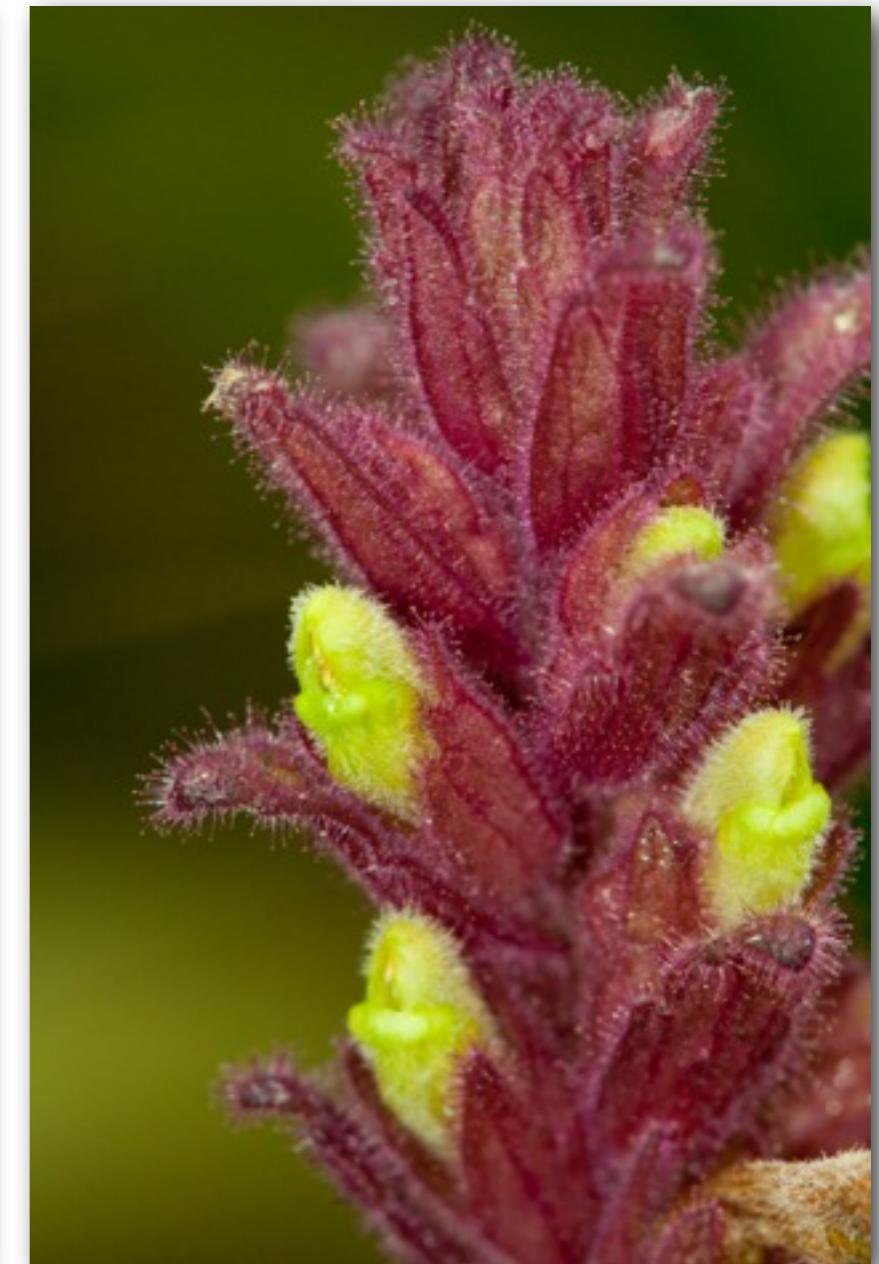
Conclusions

- Microfluidic PCR is a cost effective method
 - each chip is \$600 ($48 \times 48 = 2304$ PCRs!)
 - possibility to multiplex in each well
 - multiple chips per lane
- Subgenomics is a good approach for species level phylogenetics
 - Same regions of the genome for every sample
 - Less missing data



Conclusions

- First split in the S. Am. clade shows two major clades
Diffusae - Strictae, Orthocarpiflorae, Laxae
- Taxonomic incongruences



Future directions

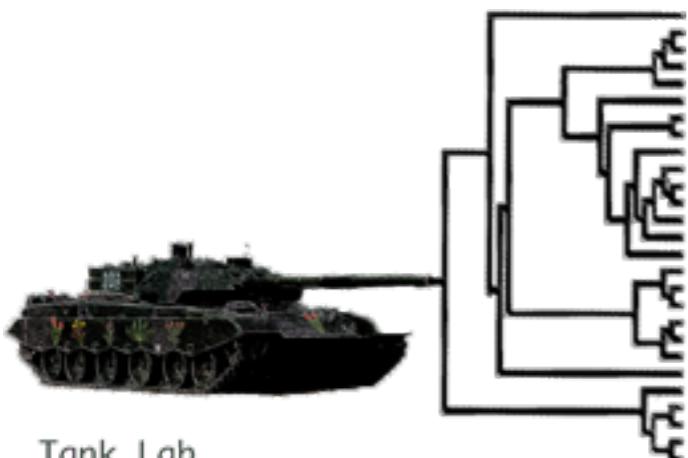
- Eight more microfluidic chips will be sequenced for cpDNA
- 48 single copy nuclear regions
 - PPR and COSII
- Multi-locus dataset for hundreds of samples
- Coalescent based analyses
 - Species tree estimation
 - Delimitation of species boundaries



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