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

Simon Uribe-Convers and David C. Tank

University of Idaho

@uribe_convers  www.simonuribe.com
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.

Bellardia trixago
Bellardia viscosa
Bellardia latifolia
Bellardia canescens
Bellardia mutica

Modified from Scheunert et al., 2012
3.13 MA
(1.53-4.11 95% HPD)

South American Bartsia (45)

S.Am. Bartsia

Density

0 5 10 15
0.1 0.2 0.3 0.4
Net Diversification Rate

Odontites s.l. (32)

Euphrasia (350)

African Bartsia (2)

Tozzia alpina (1)

Rhinanthus (45)

Melampyrum (35)

Parentucellia (2)

Mediterranean Bartsia trixago (1)

Uribe-Convers & Tank, in prep.

29.02 MA

25 MA

20.0

15.0

10.0

5.0

0.0 MA
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)

550bp
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?

Molau, 1990
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)

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>No. of Regions</th>
<th>Total bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>156</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>176</td>
<td>39-48</td>
<td>24,378</td>
</tr>
</tbody>
</table>

Matrix coverage 0.995147
- Regions were independently aligned with MUSCLE (v. 3.2. 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
Colombia
Ecuador
Peru
Bolivia
Conclusions

-Microfluidic PCR is a cost effective method
  -each chip is $600 (48 x 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

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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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Tank Lab
FIGHTING POLYTOMY SINCE 2008

www.simonuribe.com
@uribe_convers