Survey of *Diorhabda* spp. along Rio Grande

A unique opportunity to monitor population expansion and hybridization

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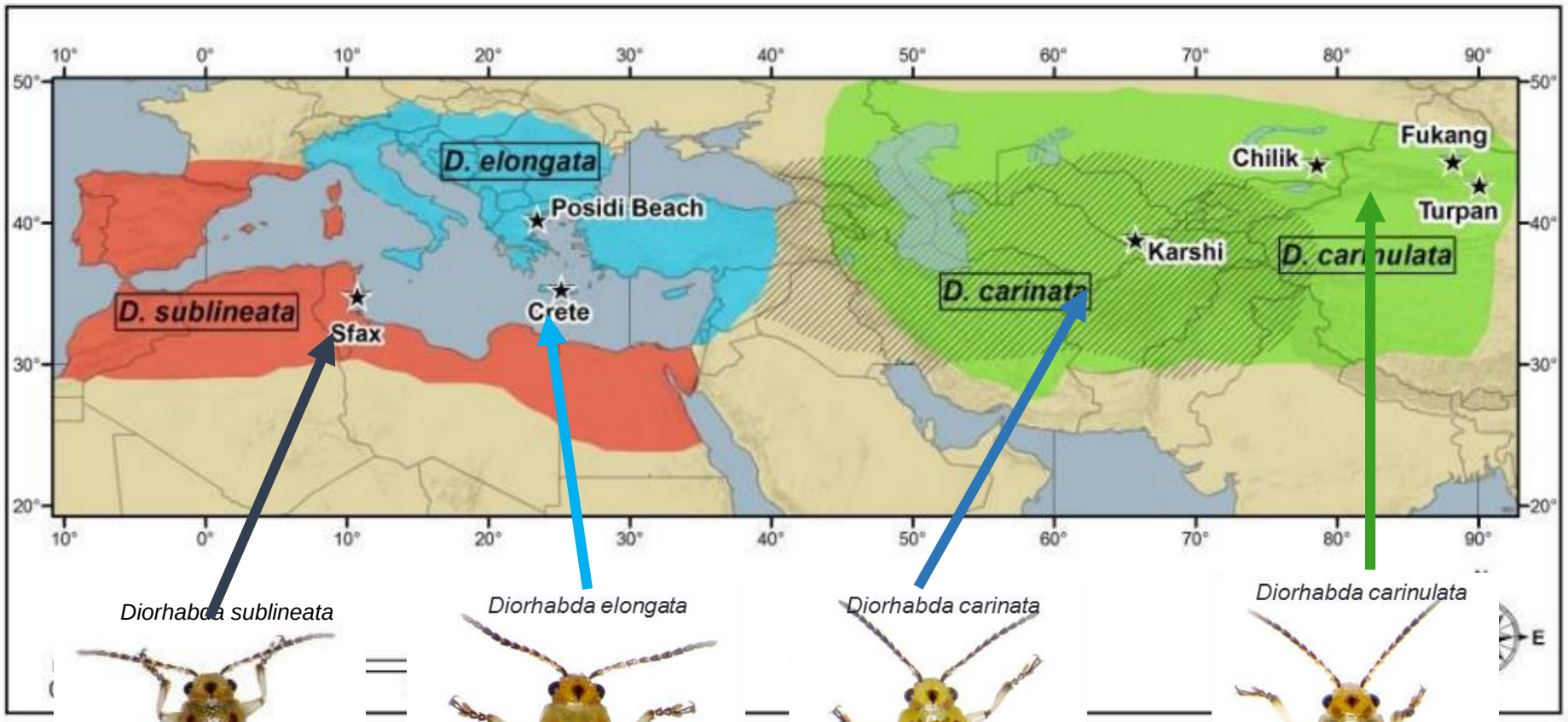
History of *Diorhabda* spp. in Western U.S.

- Original populations collected in Fukang, China and in Chilik, Kazakhstan
  - Lovell – WY
  - Delta – UT
  - Lovelock and Schurz – NV
  - Pueblo – CO
  - Owens Valley – CA
  - Temple - TX
- Upon failure of establishment of beetles in locations south of 37° N latitude other populations were collected in
  - Sfax, Tunisia
  - Crete, Greece
  - Karshi, Uzbekistan
  - Turpan, China

Diorhabda elongata

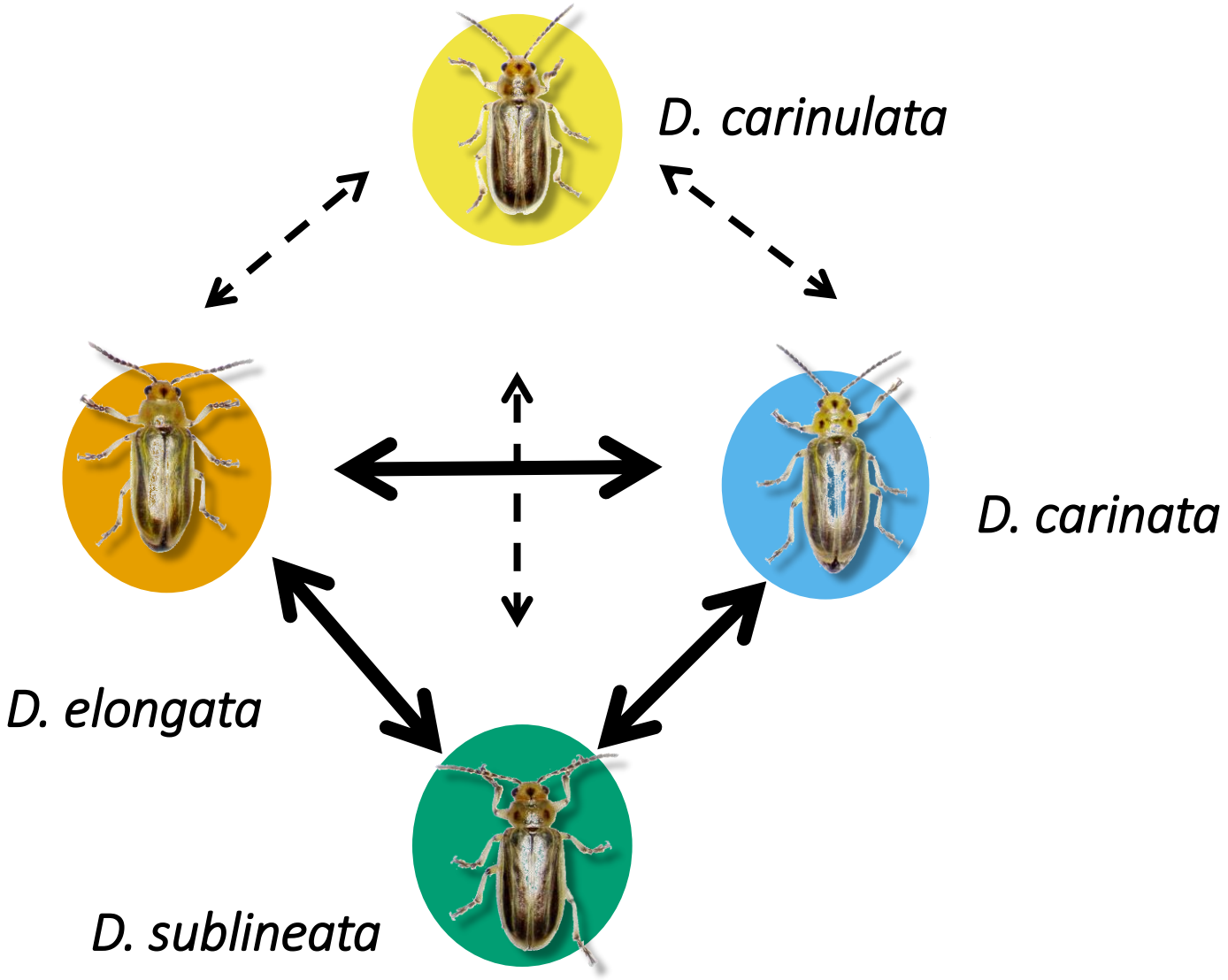
Diorhabda spp.



Tracy and Robbins, 2009

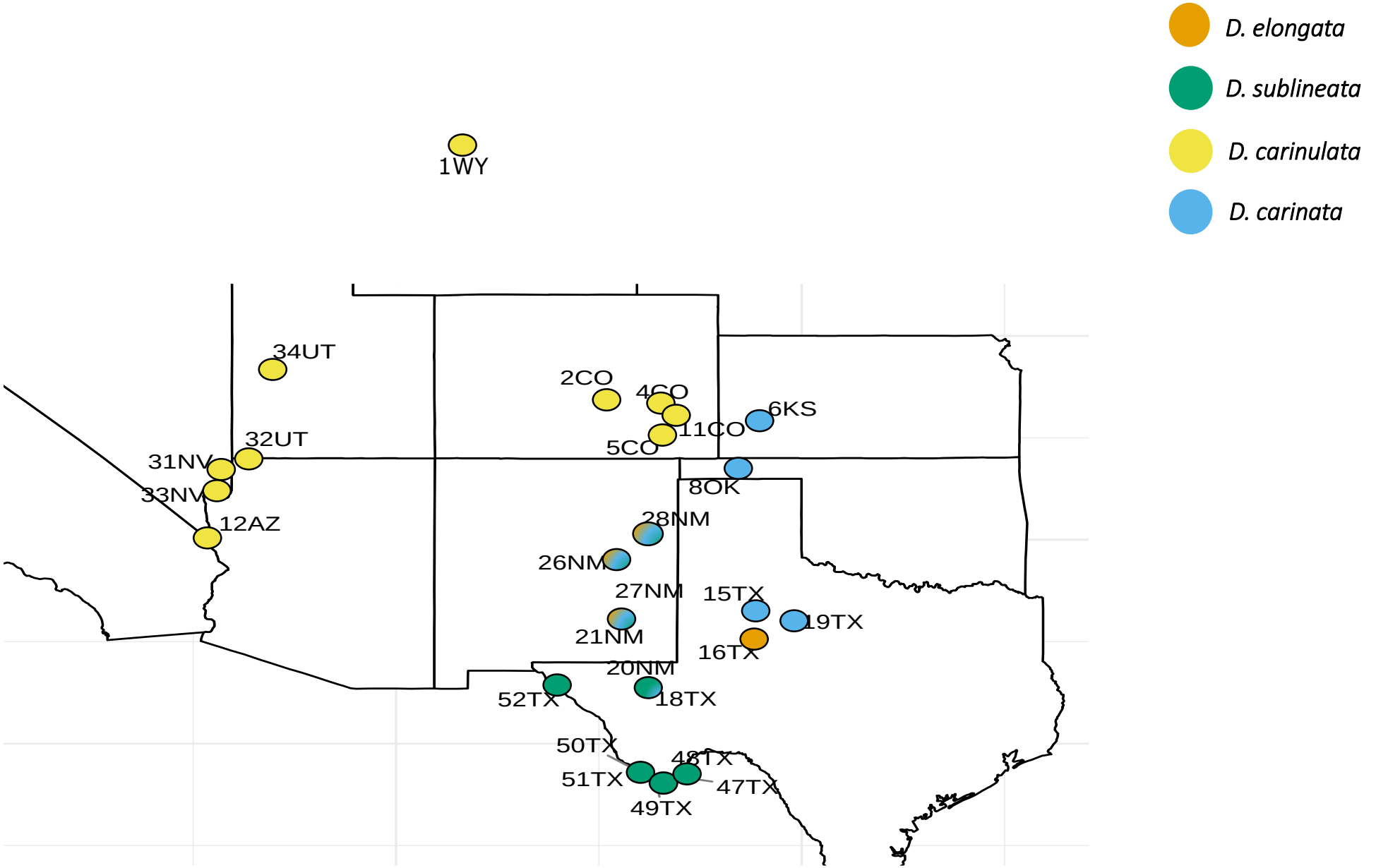
Bean, Kazmer, Gardner, Thompson, Reynolds, Keller, Gaskin (2013).

Hybridization and admixture in biocontrol

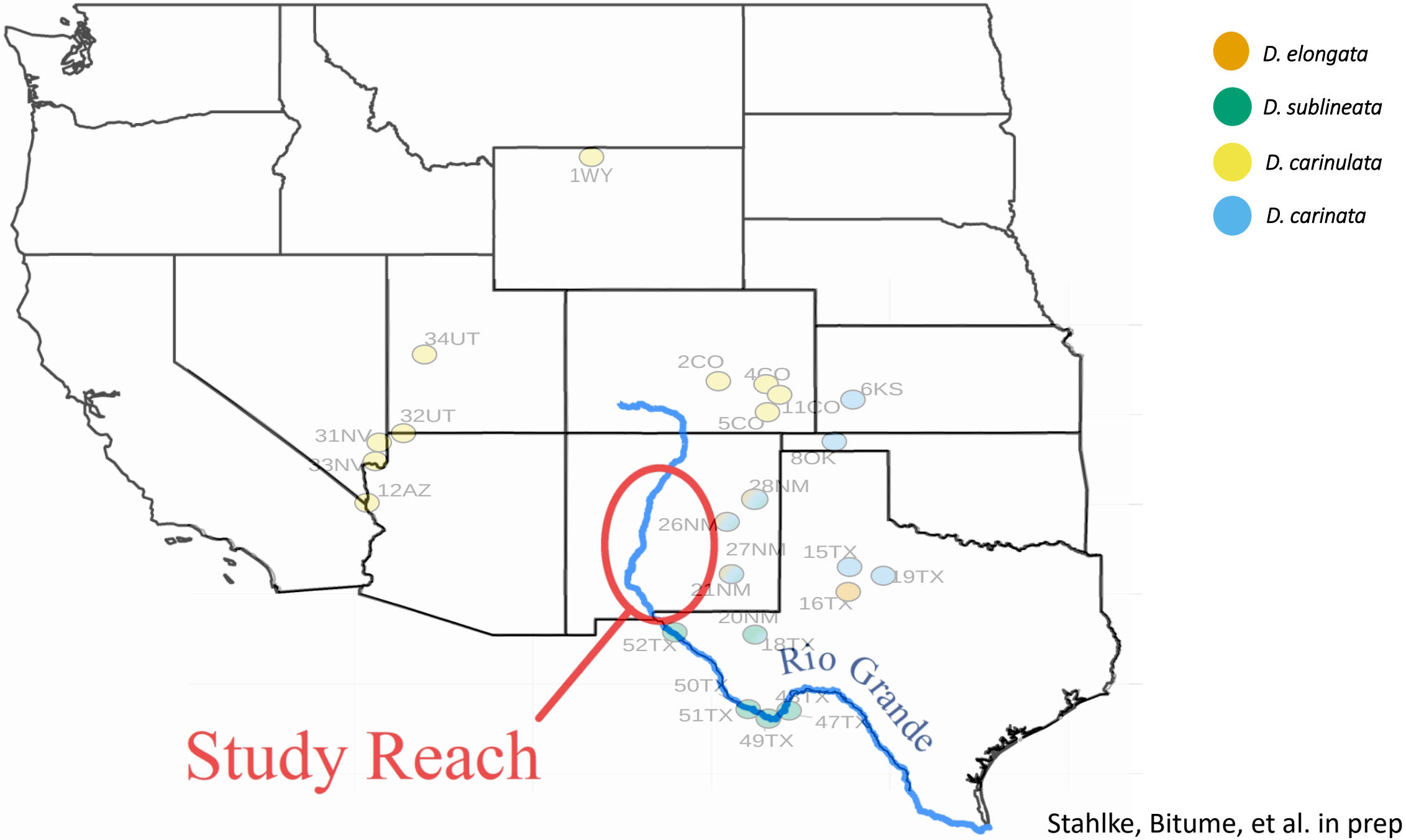


- **Relief from inbreeding** load due to culturing
- **Novel combinations** may allow adaptation to novel environment
  - Increase in efficacy or risk
- **Incompatibilities** may decrease fitness

Bean, Kazmer, Gardner, Thompson, Reynolds, Keller, Gaskin (2013).



Stahlke, Bitume, et al. in prep



Stahlke, Bitume, et al. in prep

Identification methods

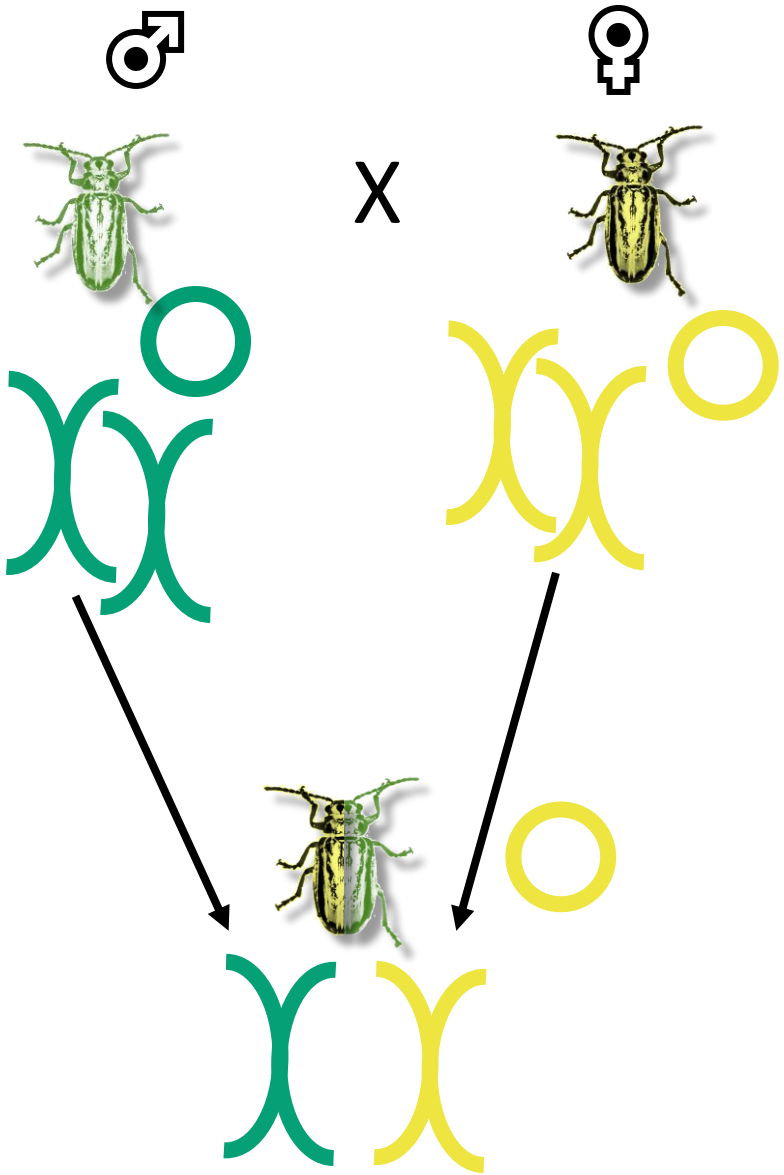
Mitochondrial CO1 - Sanger Sequencing

- Focuses only on one gene
- Gives information of the maternal heritage
- Does NOT reveal hybridization status
- ~$17/ sample

RADseq - Whole genome – reduced representation

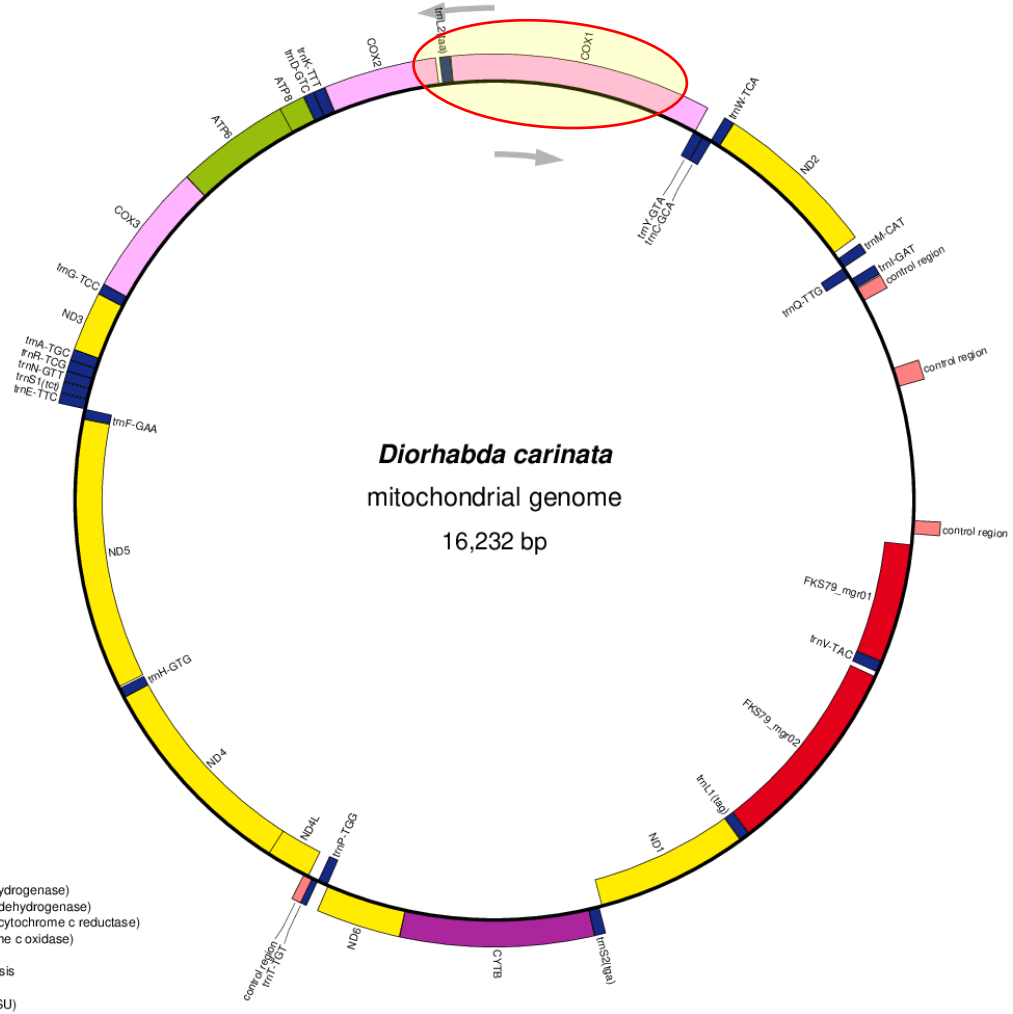
- 1,000s of loci in the whole genome
- Reveals hybridization status
- Can provide both neutral and adaptive markers
- ~$8 → ? / sample

Nuclear DNA - from both parents
Mitochondrial DNA - from the mother

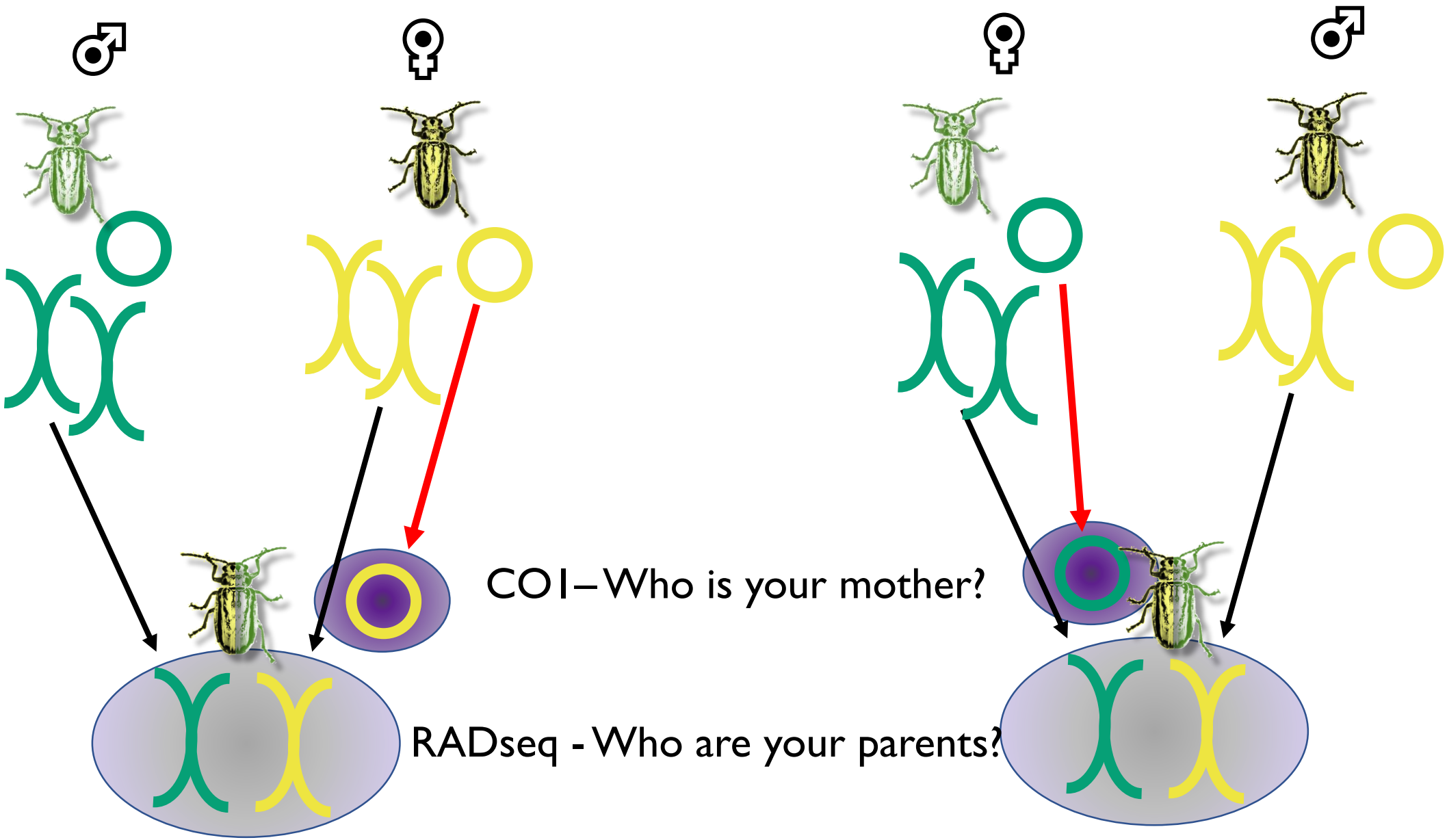


mitochondrial DNA
(0.004% of total)

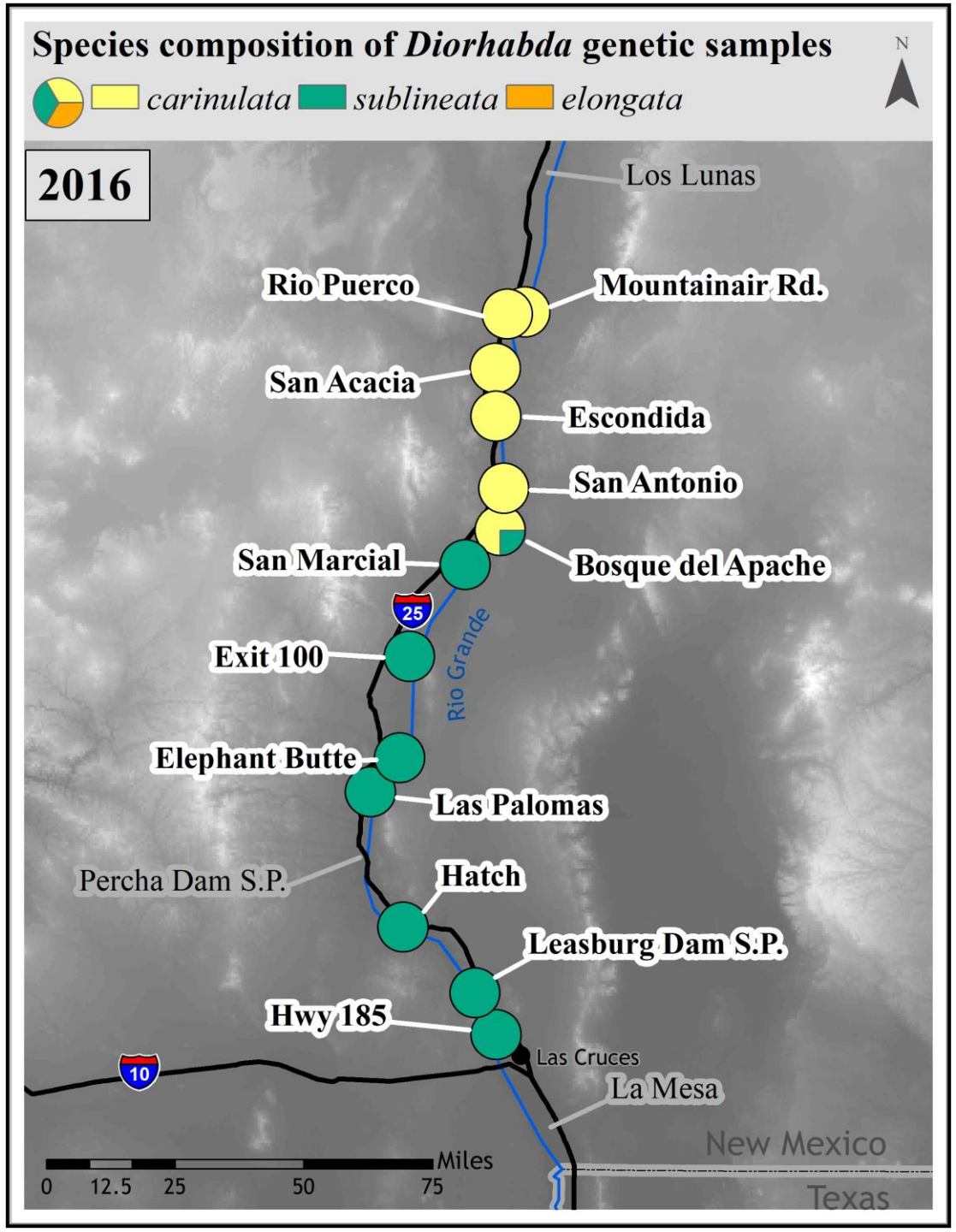
Nuclear DNA



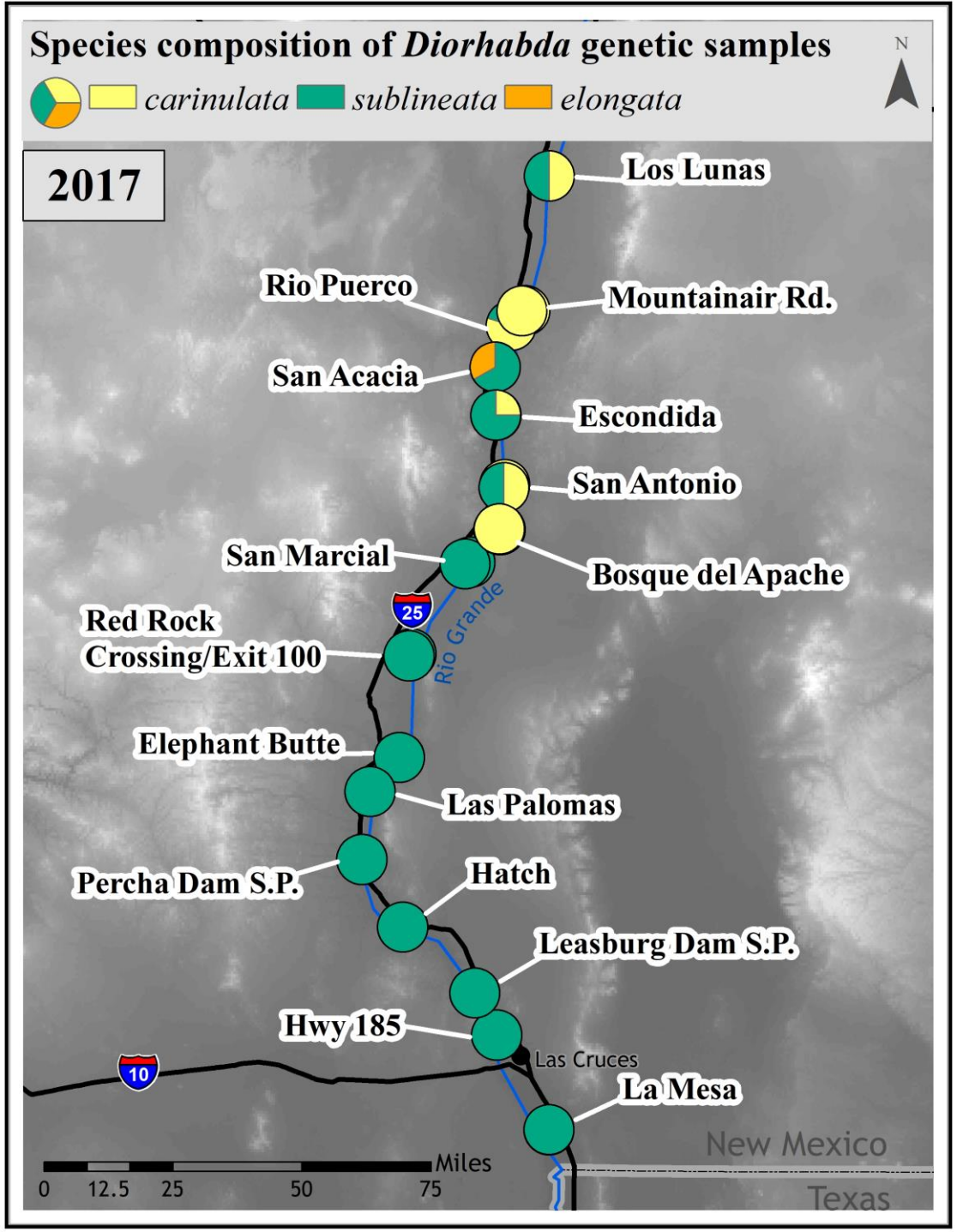
Stahlke AR, Ozsoy AZ, Bean DW, Hohenlohe PA. 2019. Mitochondrial genome sequences of *Diorhabda carinata* and *Diorhabda carinulata*, two beetle species introduced to North America for biological control. Microbiology Resource Announcements 8:e00690-19.


♂
♀
♀
♂
CO1–Who is your mother?
RADseq - Who are your parents?

CO1


Species composition of *Diorhabda* genetic samples
carinulata
sublineata
elongata
N
2016
Los Lunas
Rio Puerco
Mountainair Rd.
San Acacia
Escondida
San Antonio
San Marcial
Bosque del Apache
25
Exit 100
Rio Grande
Elephant Butte
Las Palomas
Percha Dam S.P.
Hatch
Leasburg Dam S.P.
Hwy 185
10
Las Cruces
La Mesa
New Mexico
Texas
Miles
0
12.5
25
50
75

CO1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N
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San Antonio
San Marcial
Bosque del Apache
25
Red Rock
Crossing/Exit 100
Rio Grande
Elephant Butte
Las Palomas
Percha Dam S.P.
Hatch
Leasburg Dam S.P.
Hwy 185
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New Mexico
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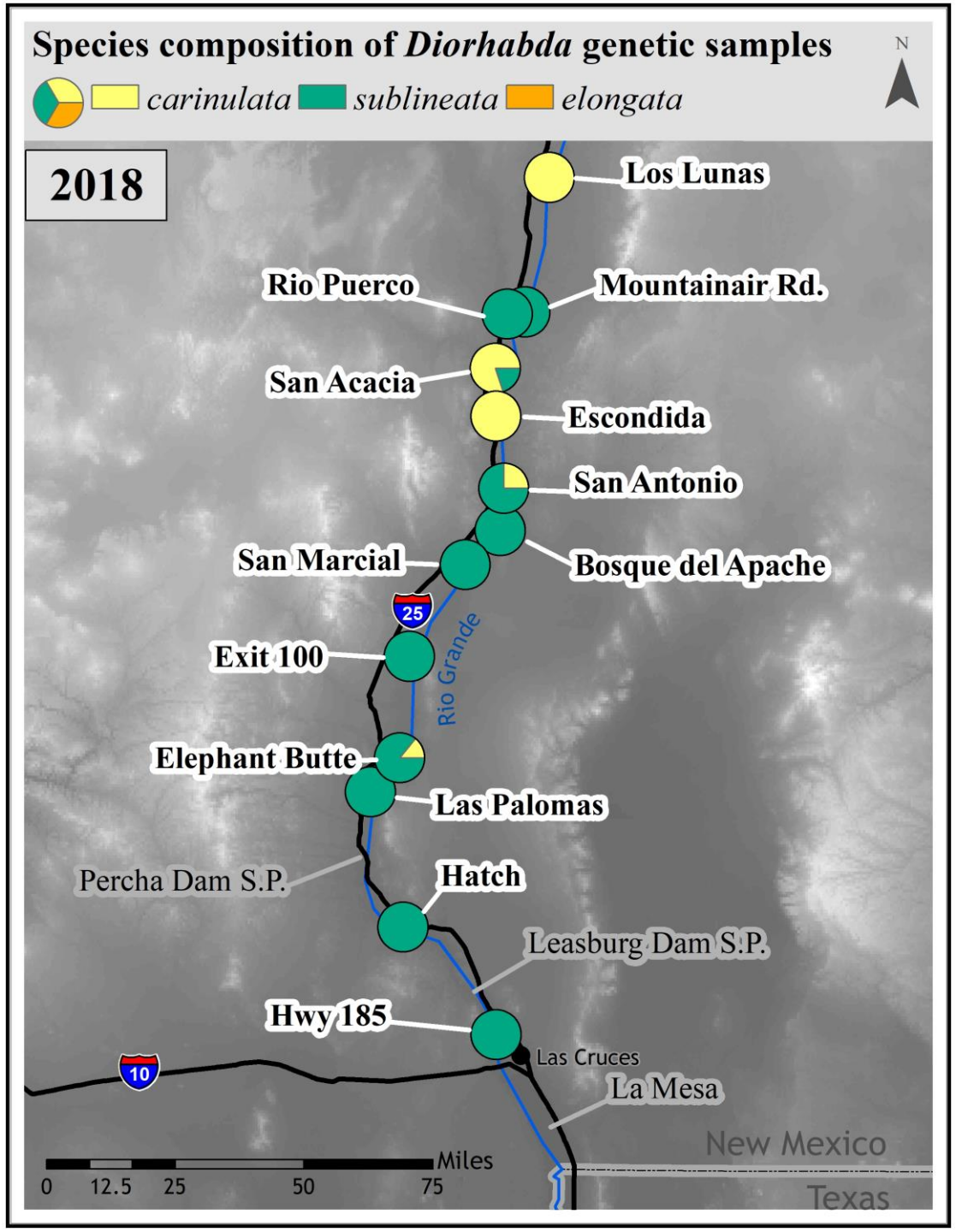
CO1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elongata
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Escondida
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San Marcial
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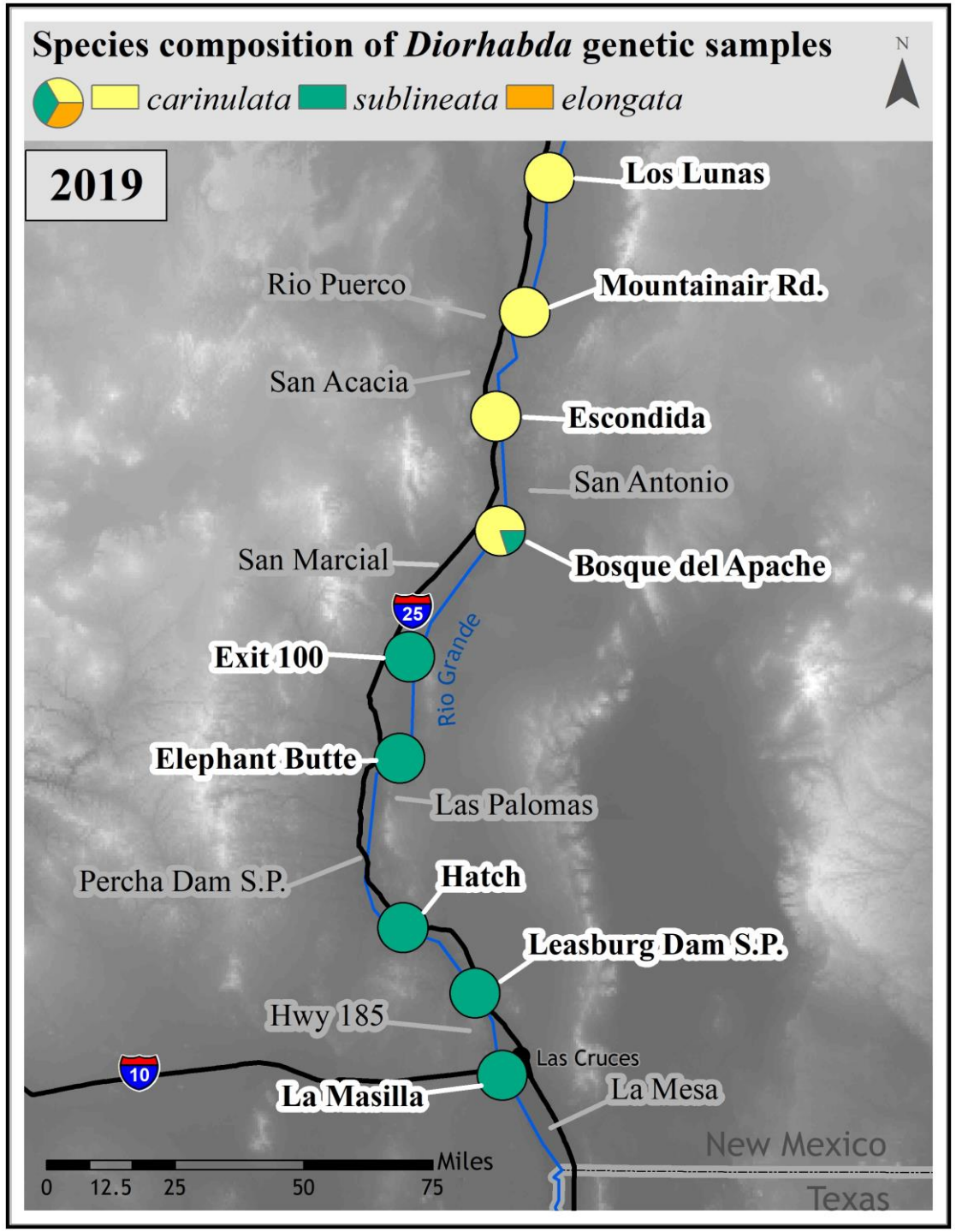
25
50
75

CO1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sublineata
elongata
N
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25
Exit 100
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Percha Dam S.P.
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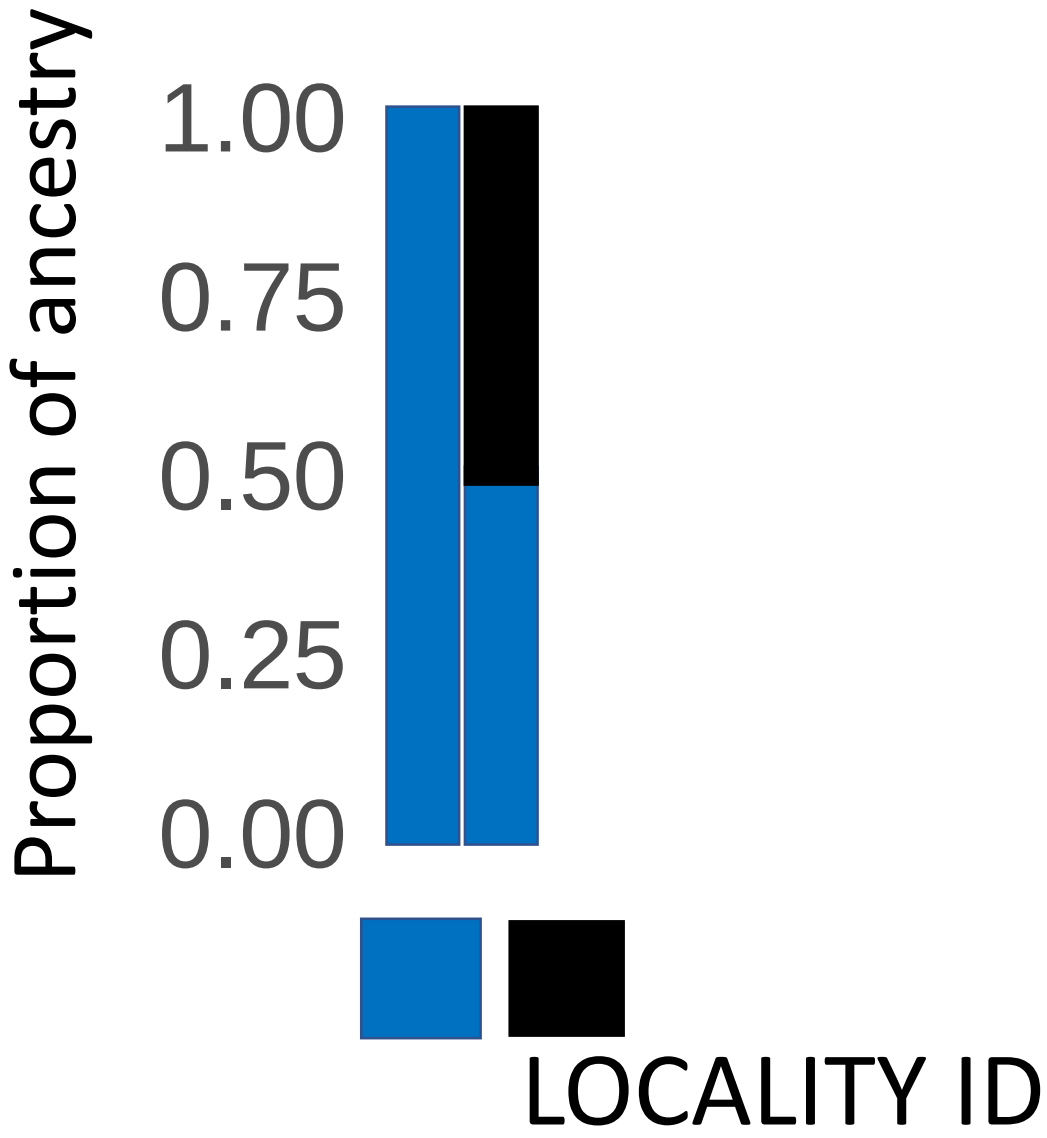
Hwy 185
Las Cruces
10
La Masilla
La Mesa
New Mexico
Texas
Miles
0
12.5
25
50
75

Who is where?

STRUCTURE, a Bayesian clustering algorithm that calculates the posterior probability of genetic data fitting K clusters given a model of allele frequency distributions

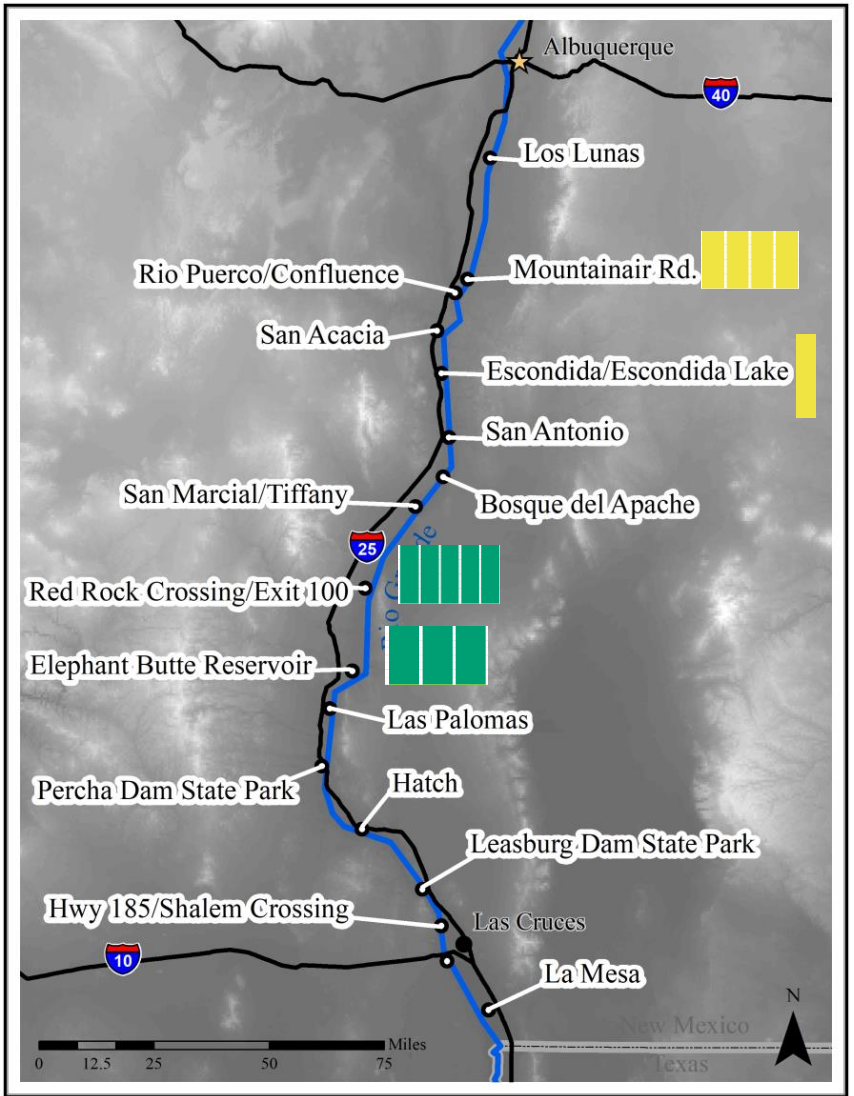
Probability(X|K)



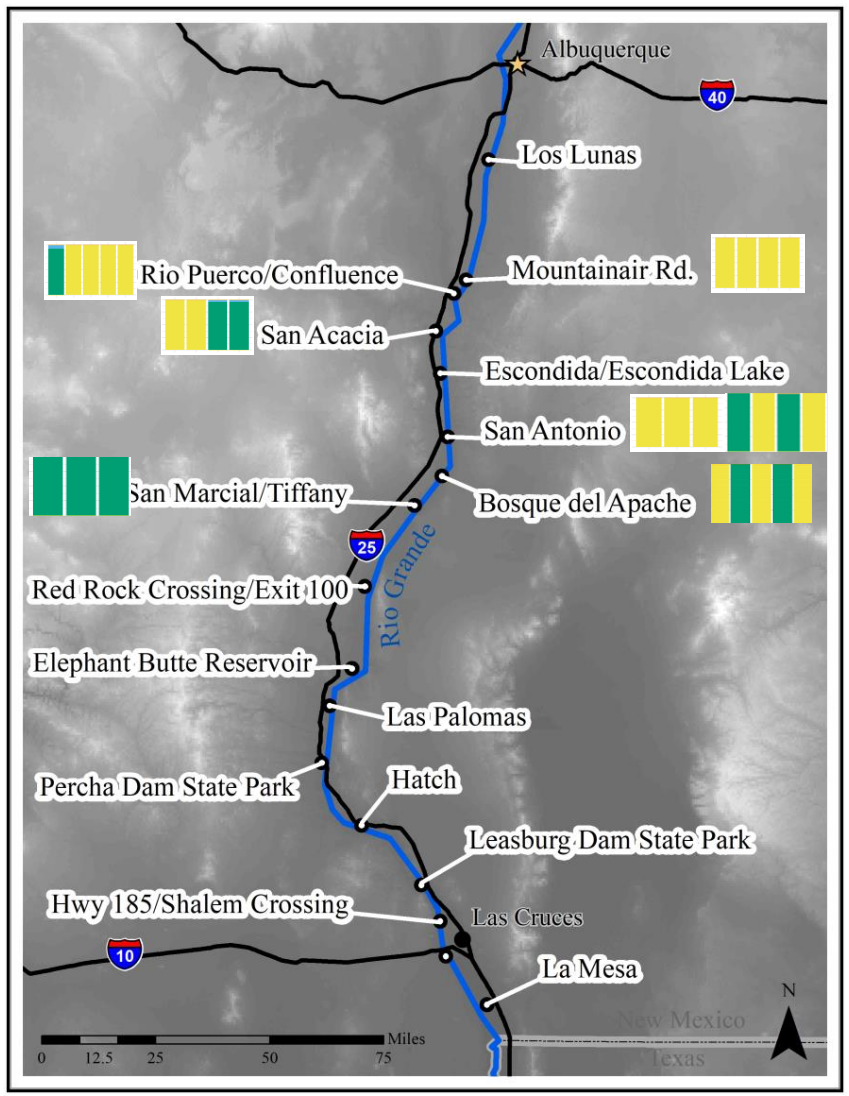
2017

D. elongata
D. sublineata
D. carinulata
D. carinata

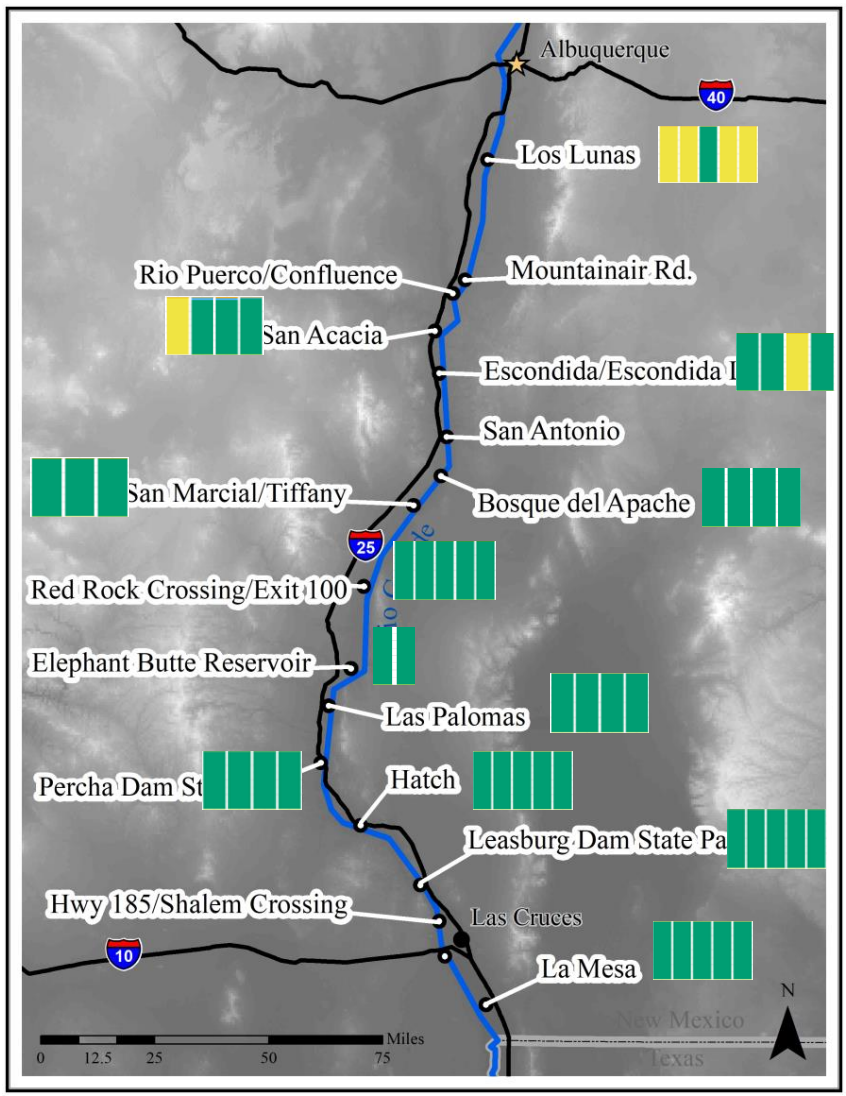
May



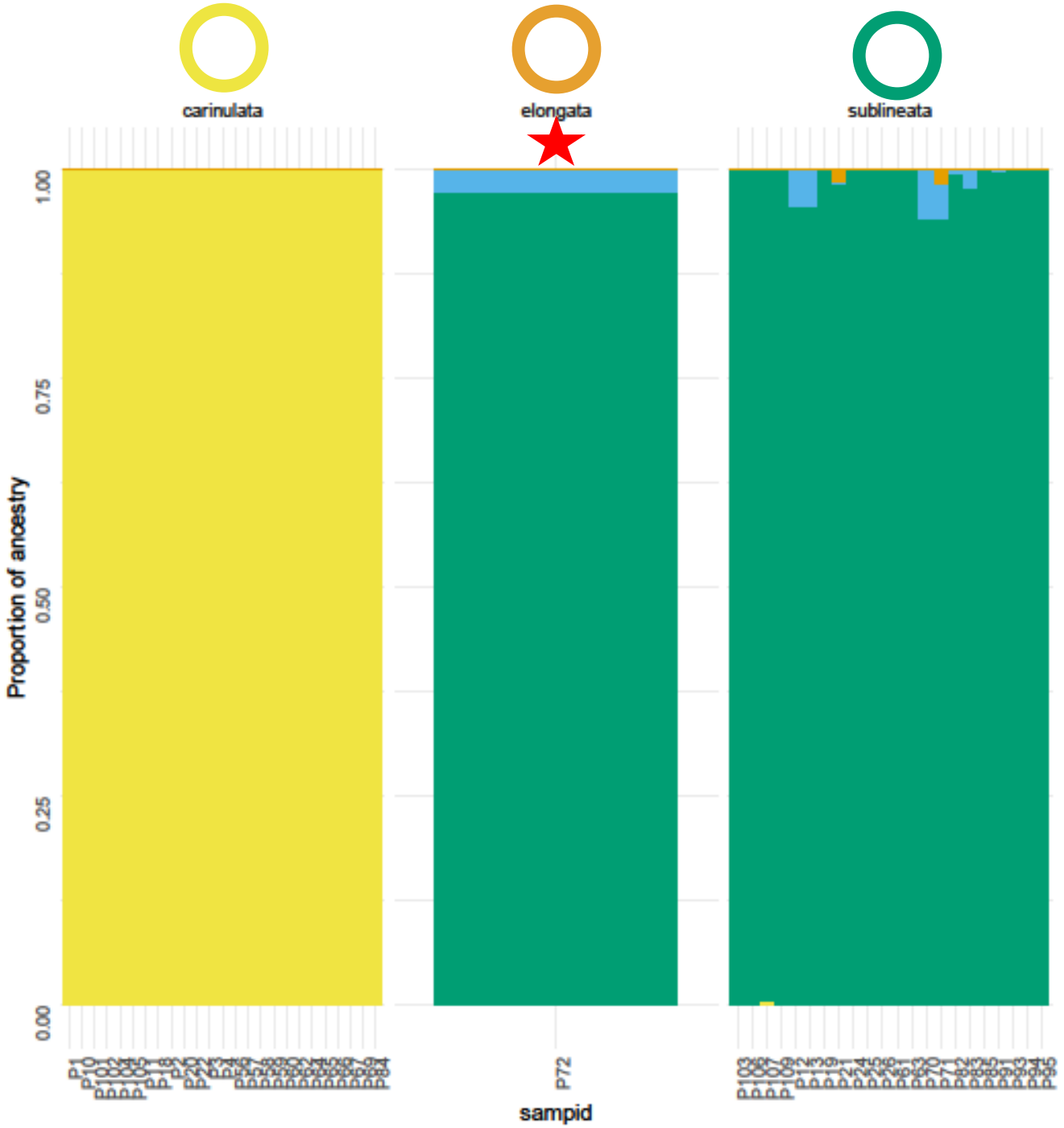
July



September



2017 CO1 ~ RADseq



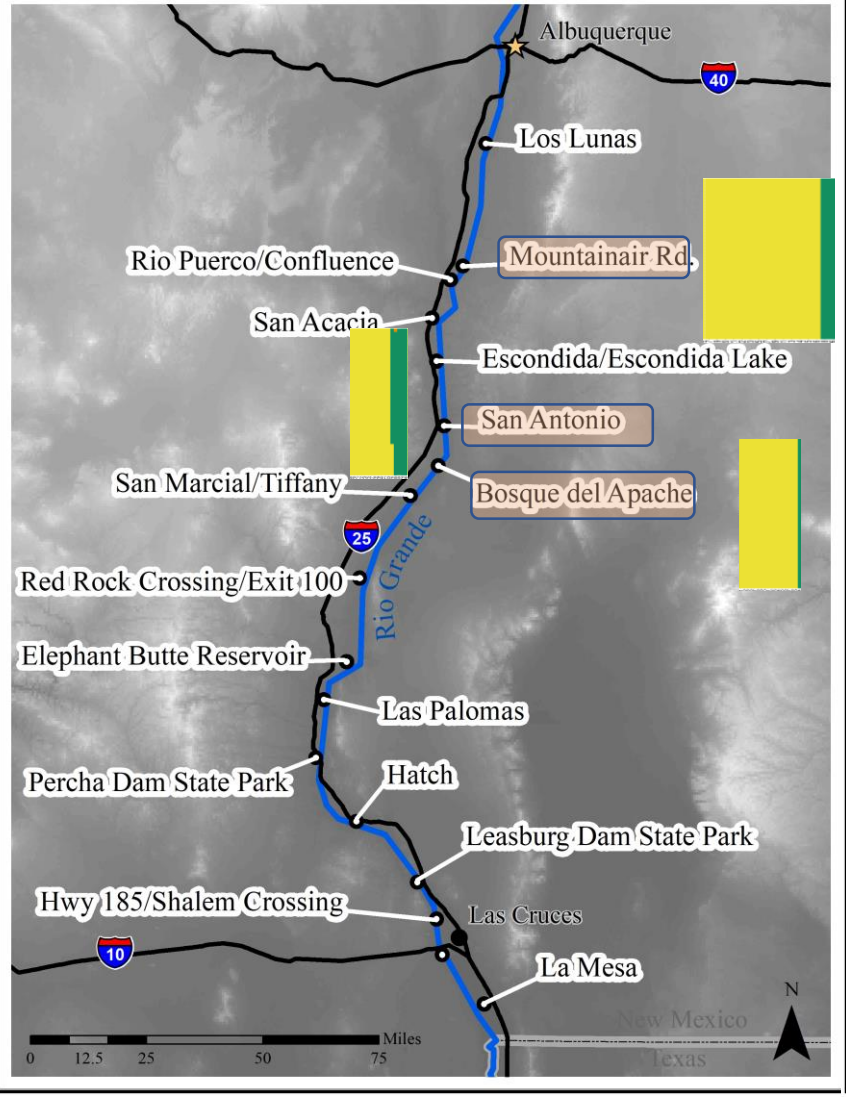
Inferred ancestry

- D. elongata
- D. carinata
- D. sublineata
- D. carinulata

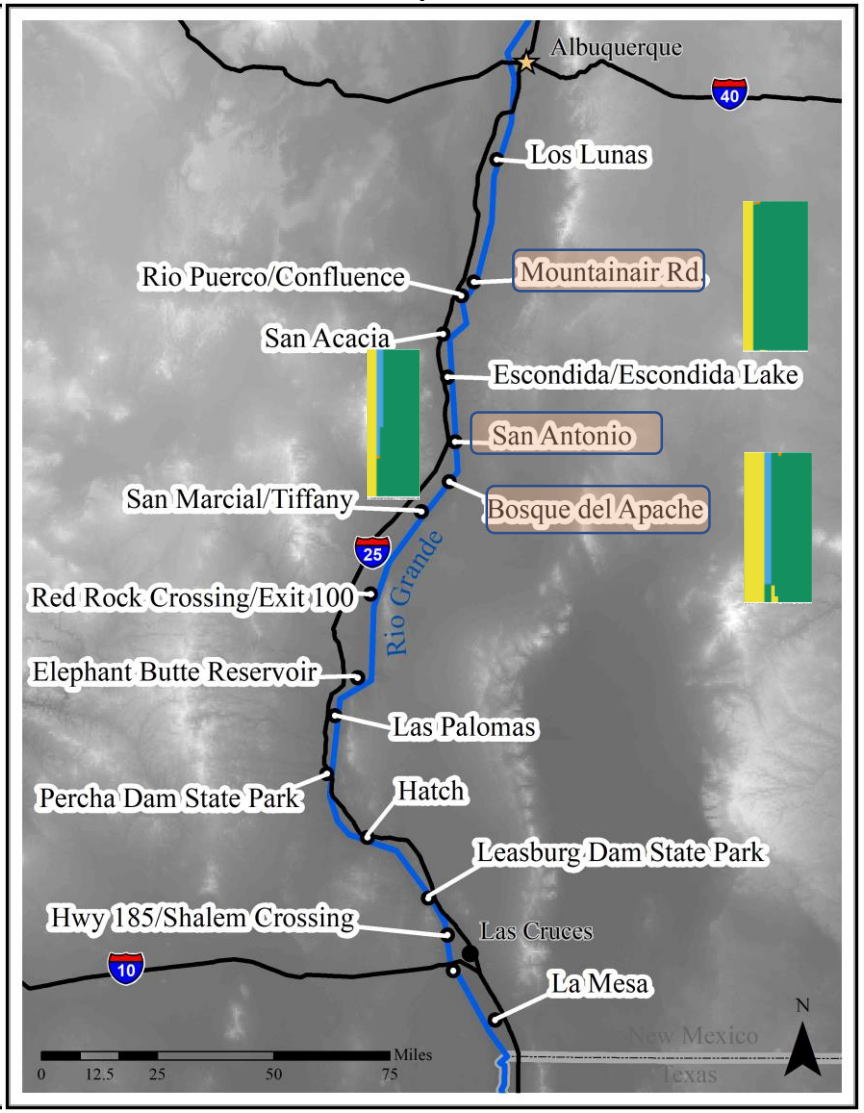
2018

D. elongata
D. sublineata
D. carinulata
D. carinata

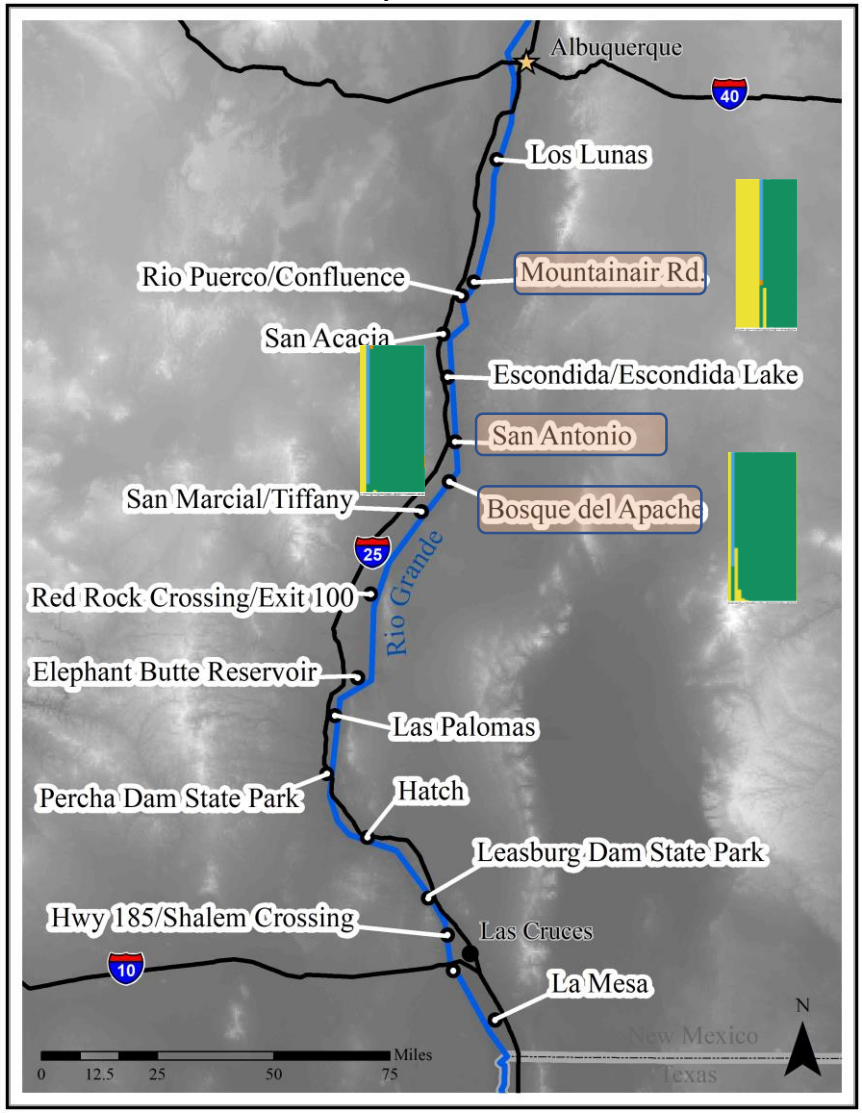
May



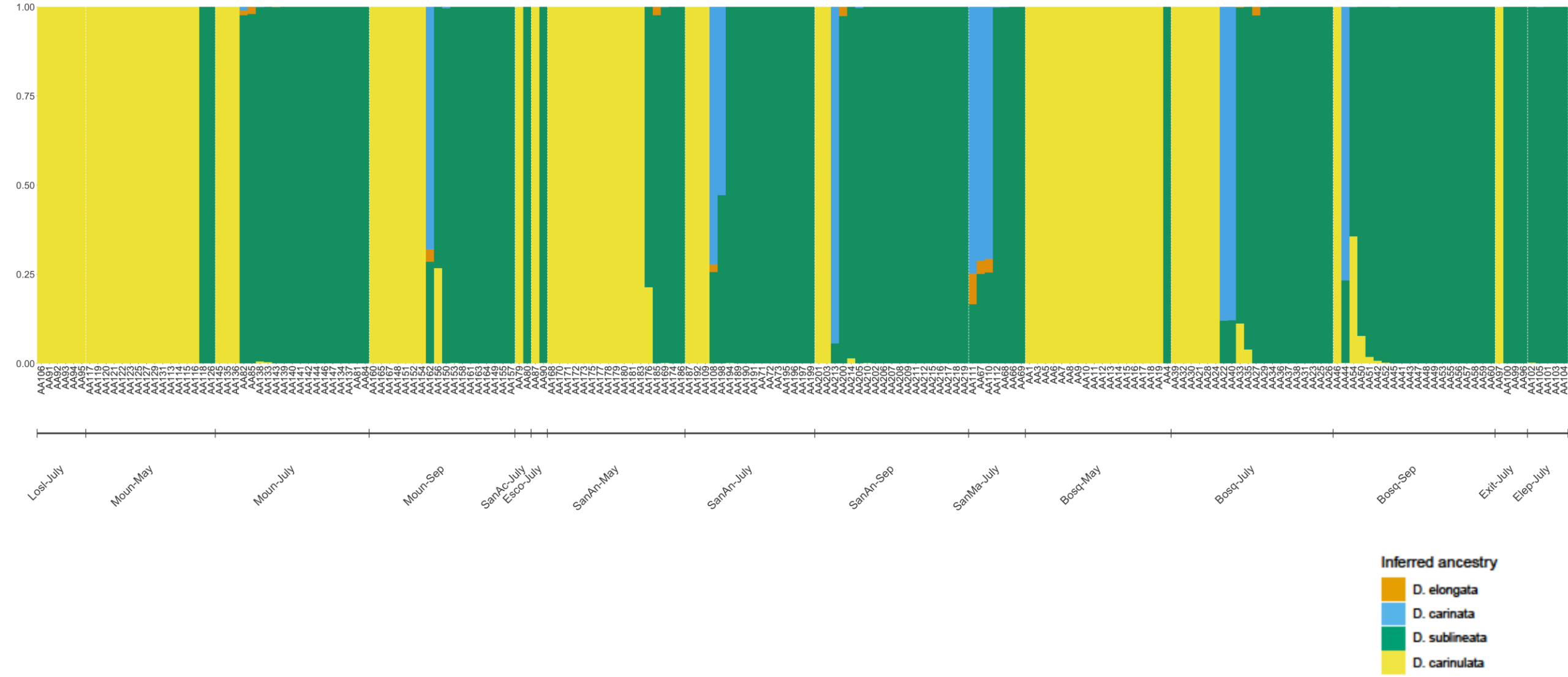
July



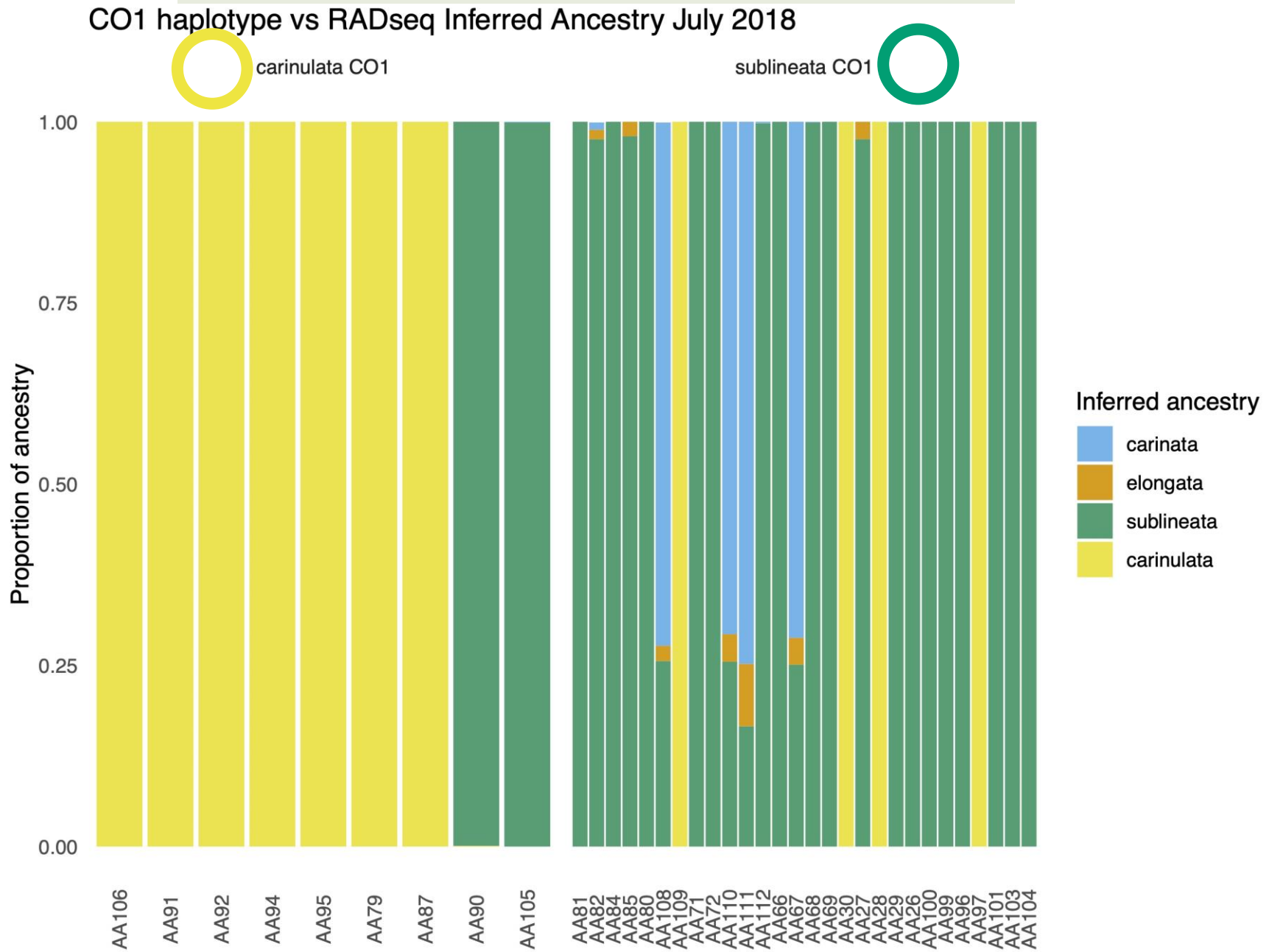
September

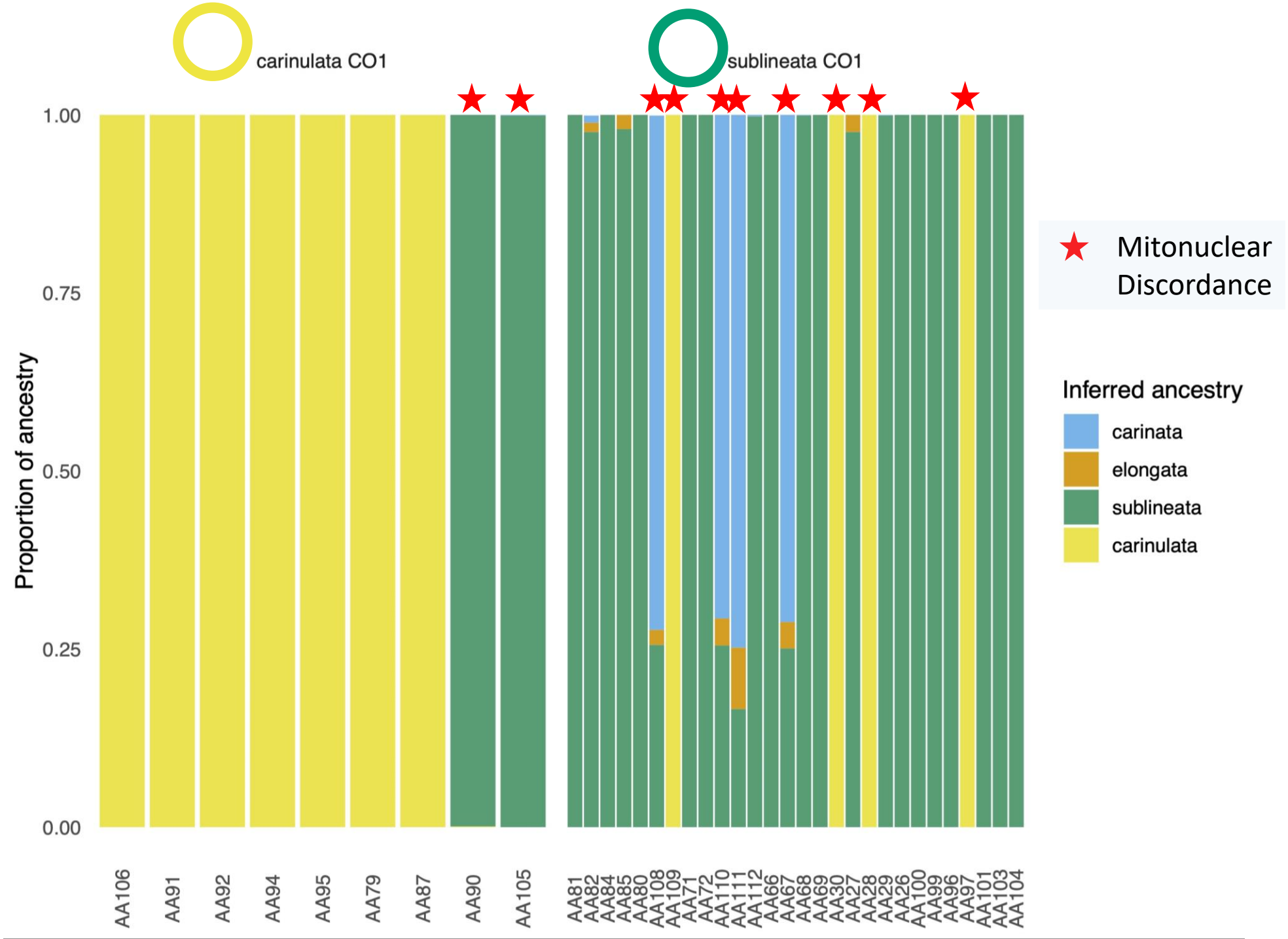


Over time-2018



2018 CO1 ≠ RADseq




carinulata CO1
sublineata CO1
Proportion of ancestry
1.00
0.75
0.50
0.25
0.00
AA106
AA91
AA92
AA94
AA95
AA79
AA87
AA90
AA105
AA81
AA82
AA84
AA85
AA80
AA108
AA109
AA71
AA72
AA110
AA111
AA112
AA66
AA67
AA68
AA69
AA30
AA27
AA28
AA29
AA26
AA100
AA99
AA96
AA97
AA101
AA103
AA104
Mitonuclear Discordance
Inferred ancestry
carinata
elongata
sublineata
carinulata

Take away

- *D. carinulata* seem to have stalled around Bosque del Apache
- *D. sublineata* have expanded their range to the north
- *D. carinulata* present early in the season then *D. sublineata* take over
- They are hybridizing!
  - Evidence of *D. sublineata* x *D. carinata* (x elongata)
  - But also *D. sublineata* x *D. carinulata*

- Discordance

Acknowledgements

- CMU
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