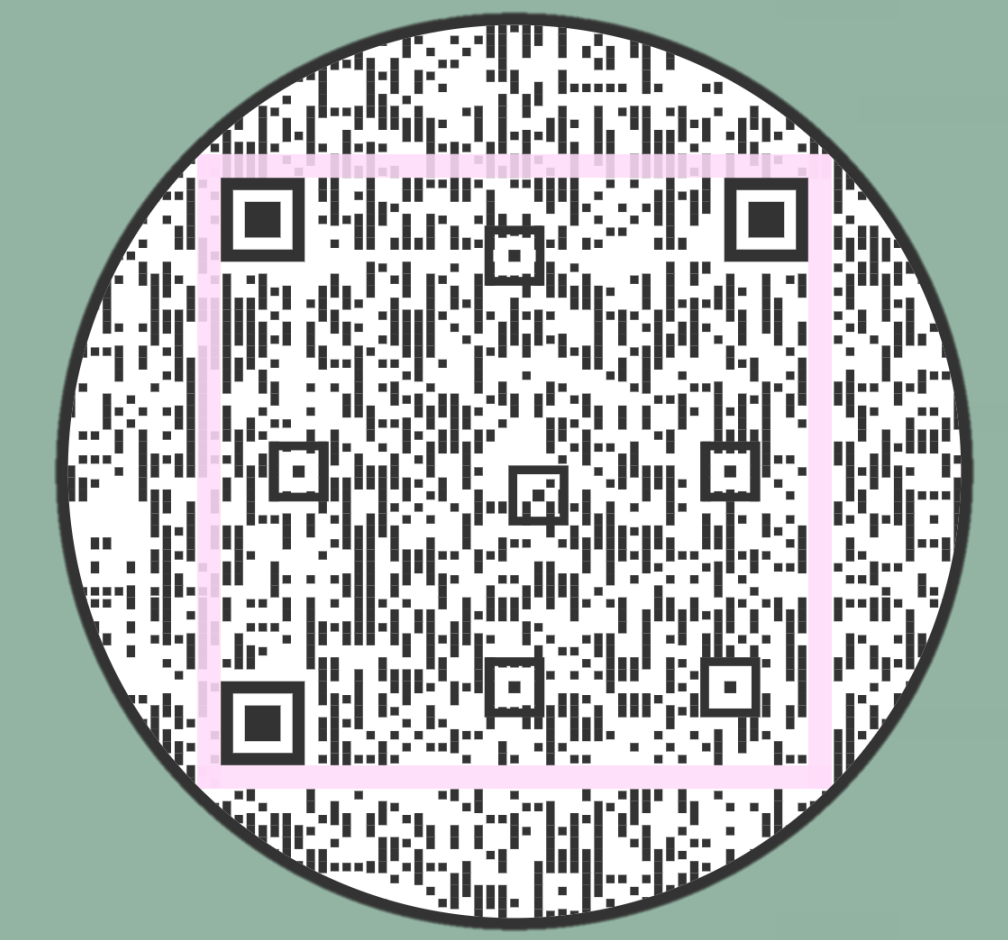


Phylogenetic Analysis of Threatened *Cypripedium* Populations in NH and VT

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New Hampshire Academy of Science

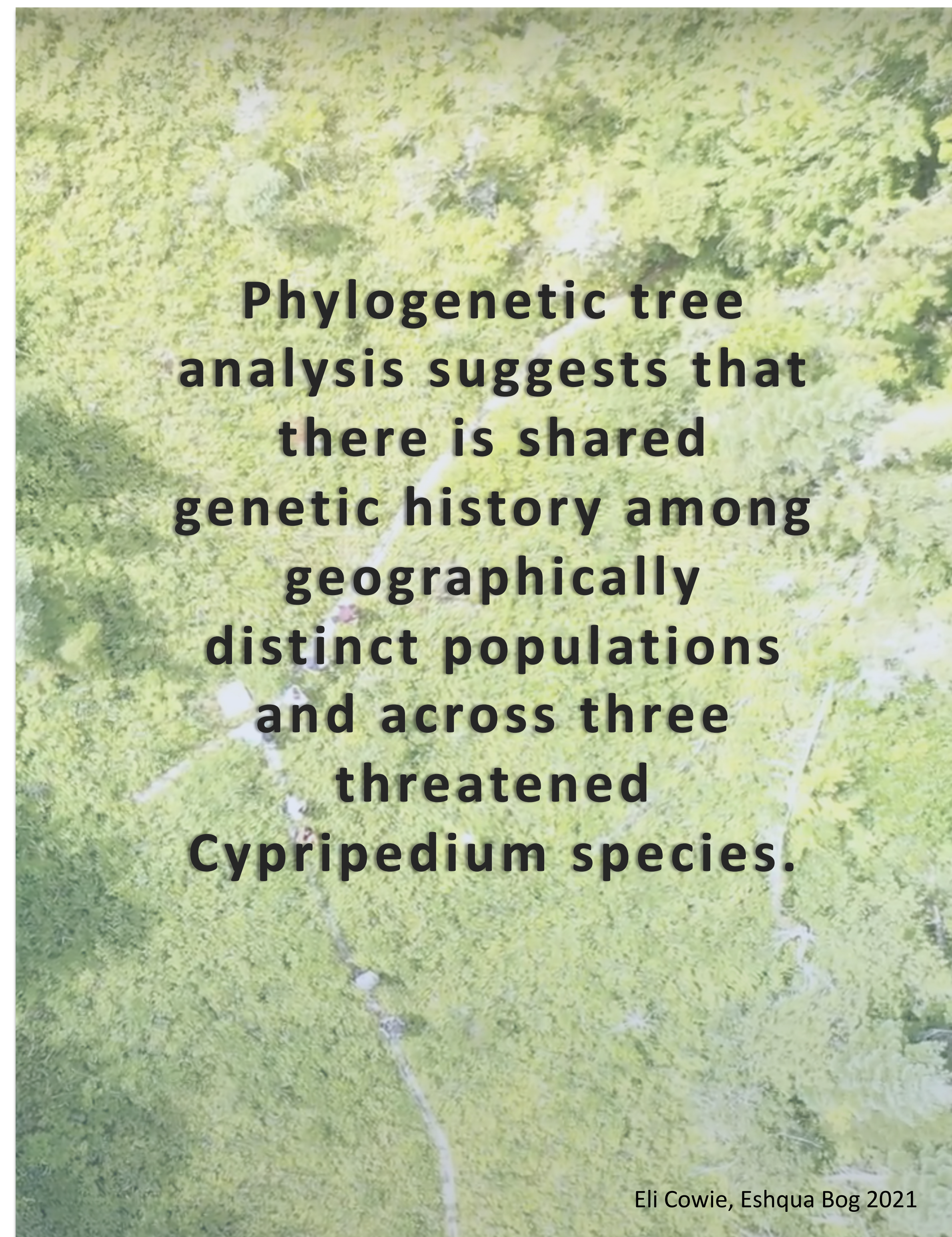


Introduction

Cypripedium reginae, or the Showy Lady's Slipper, are native to North America and are classified as endangered in New Hampshire, due to habitat loss and a lack of genetic diversity. Genetic diversity confers upon a population a higher tolerance of environmental changes, creating a more sustainable population. We can test for genetic diversity by amplifying regions of the genome identified by microsatellite primers using polymerase chain reaction (PCR) and sequencing the DNA. Microsatellites are sequences of up to a few hundred nucleotides with repeats that mutate more quickly than coding regions of the genome, allowing for detection of changes among individual plants. The genome of *Cyp. reginae* has not yet been sequenced, so 20 primer sets previously established for looking at microsatellites in a similar species (*Cyp. tibeticum*), were used. These microsatellite primers: 172, 209, 164, 880, 289, 182, 233, and 130 were used to amplify specific areas of the DNA in the 58 plant samples tested.



Figure 1. *Cyp. reginae*



Phylogenetic tree analysis suggests that there is shared genetic history among geographically distinct populations and across three threatened *Cypripedium* species.

Eli Cowie, Eshqua Bog 2021

Results

M172 and M209 Artificial DNA Sequences

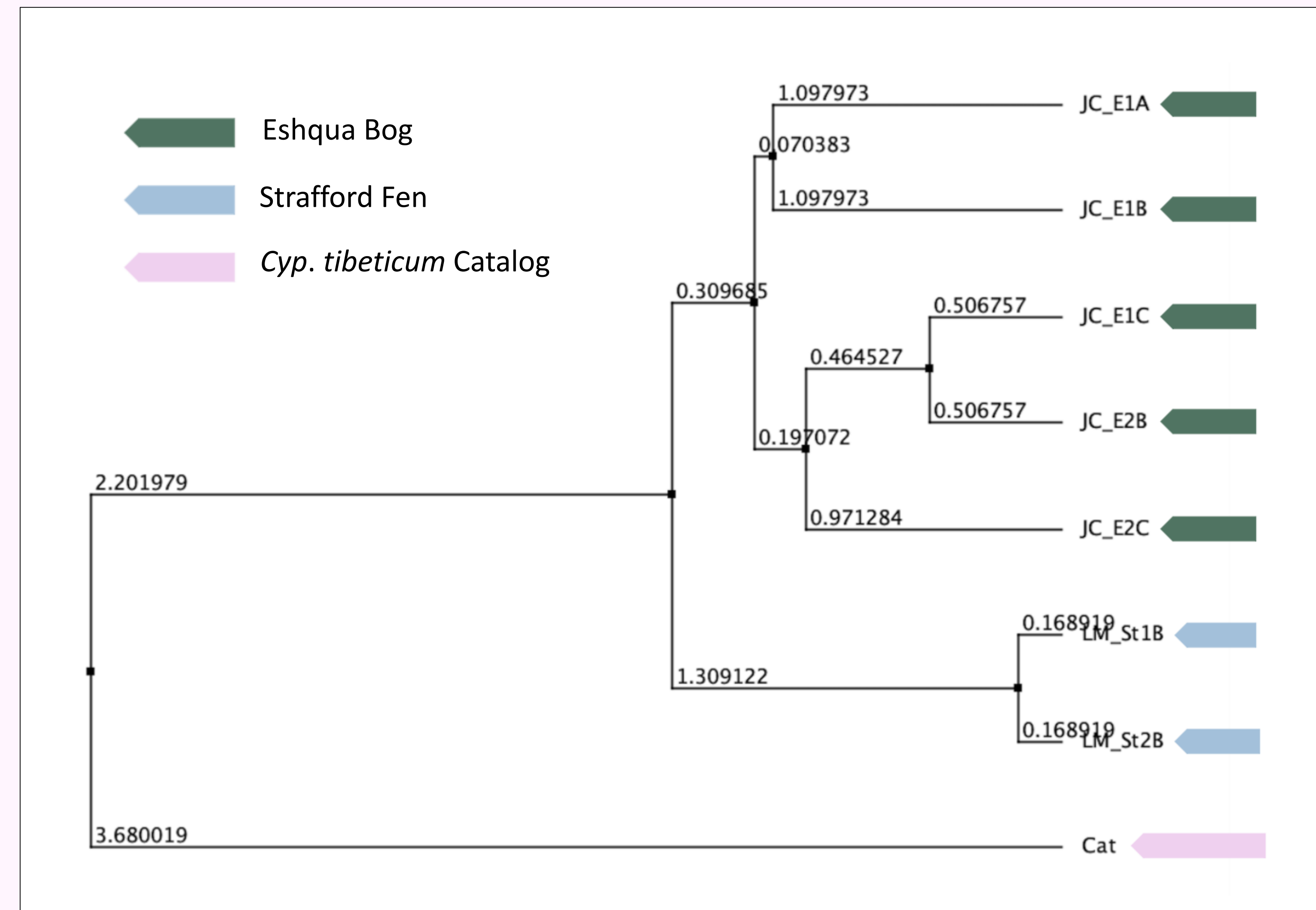
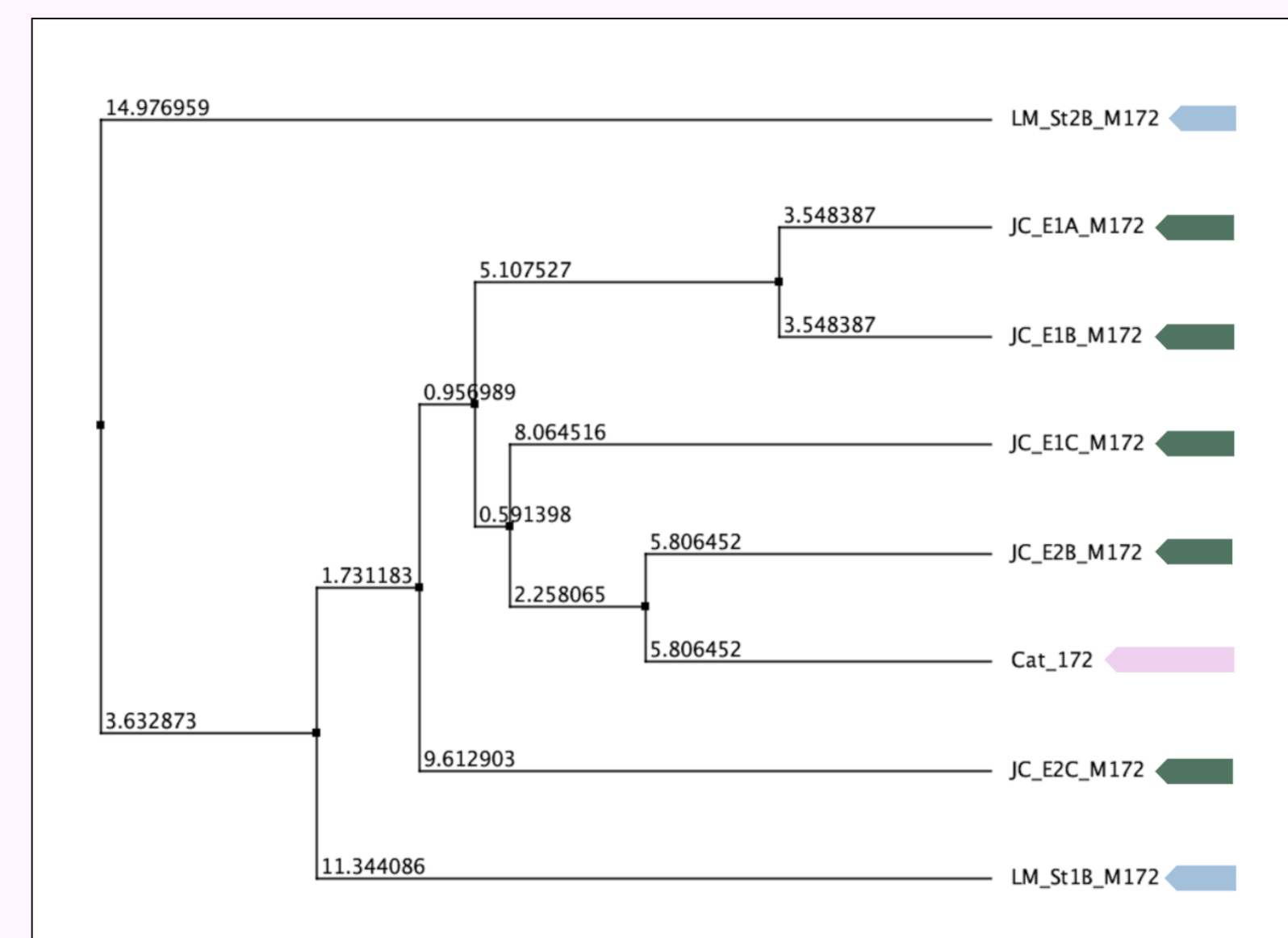


Figure 2. Phylogenetic tree created using artificial sequences; M172 and M209 sequences were concatenated by individual

M172



M209

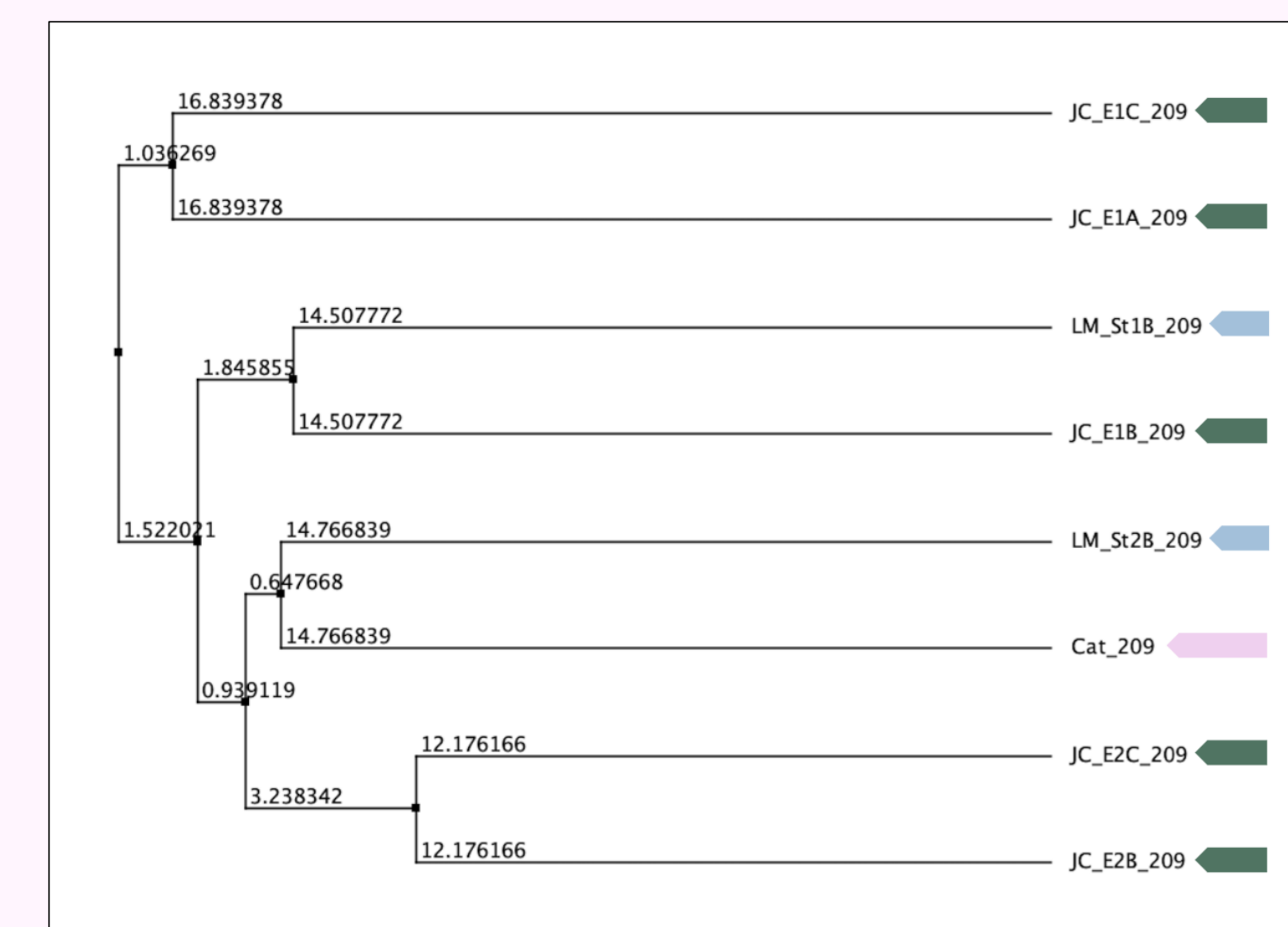


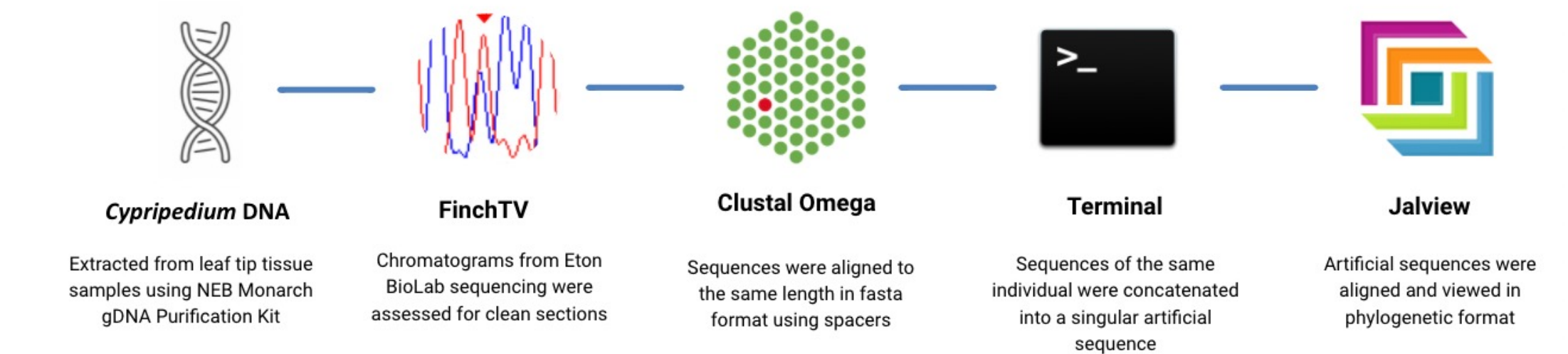
Figure 3. Phylogenetic tree created using individuals from two *Cyp. reginae* populations (shown in blue and green,) and catalog (shown in pink). Trees are separated by primer set

Methods

M112
M130
M136
M139
M142
M150
M164
M172
M182
M209

58 Individuals
20 Primer Sets
15 Clumps
5 Populations
3 Species

M233
M289
M294
M370
M372
M401
M576
M681
M880
M886



Discussion

❖ Bioinformatic challenges

- Complete data archive
- Multi-year collaboration
- Reproducible results

❖ Future directions

- Fill in sample matrix
- Quantify information gain

Acknowledgements

Li, Jing et al. "Development of microsatellite markers for *Cypripedium tibeticum* (Orchidaceae) and their applicability to two related species." *Applications in plant sciences* vol. 5,12 apps.1700084. 21 Dec. 2017, doi:10.3732/apps.1700084

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