



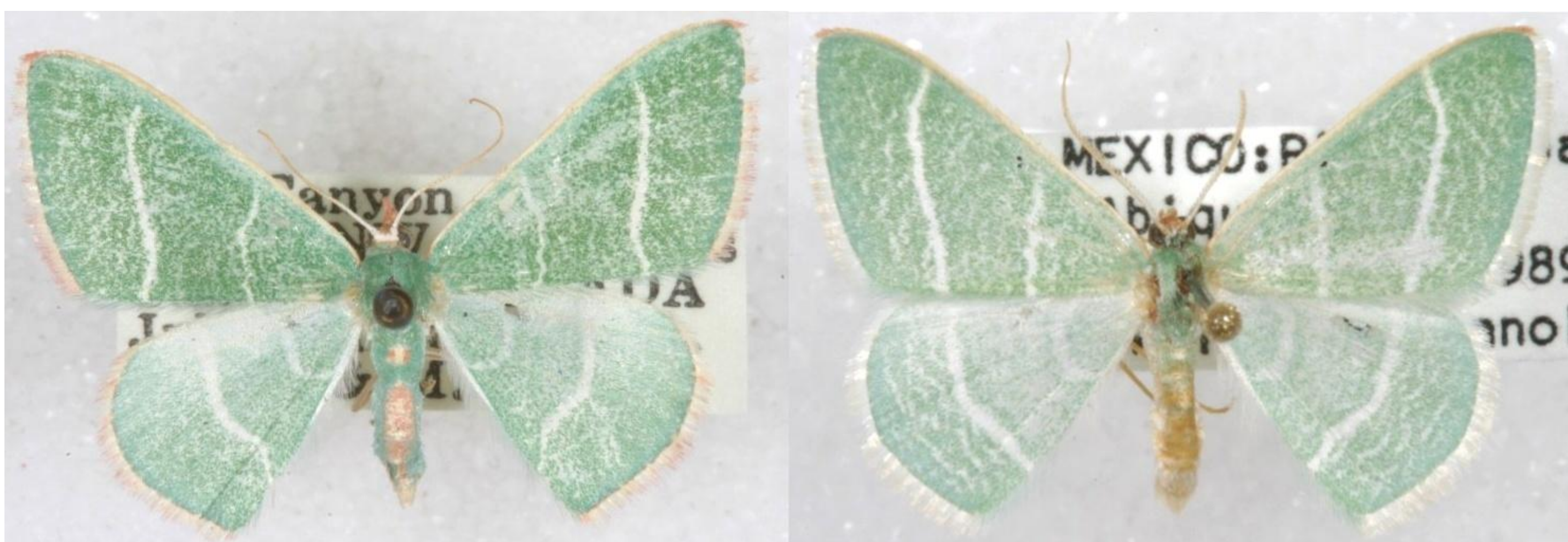
# Delimiting closely related *Nemoria* species (Lepidoptera: Geometridae) by molecular and morphological comparisons: *Nemoria intensaria* and *Nemoria caerulescens* are well separated by CO-I barcode sequences.

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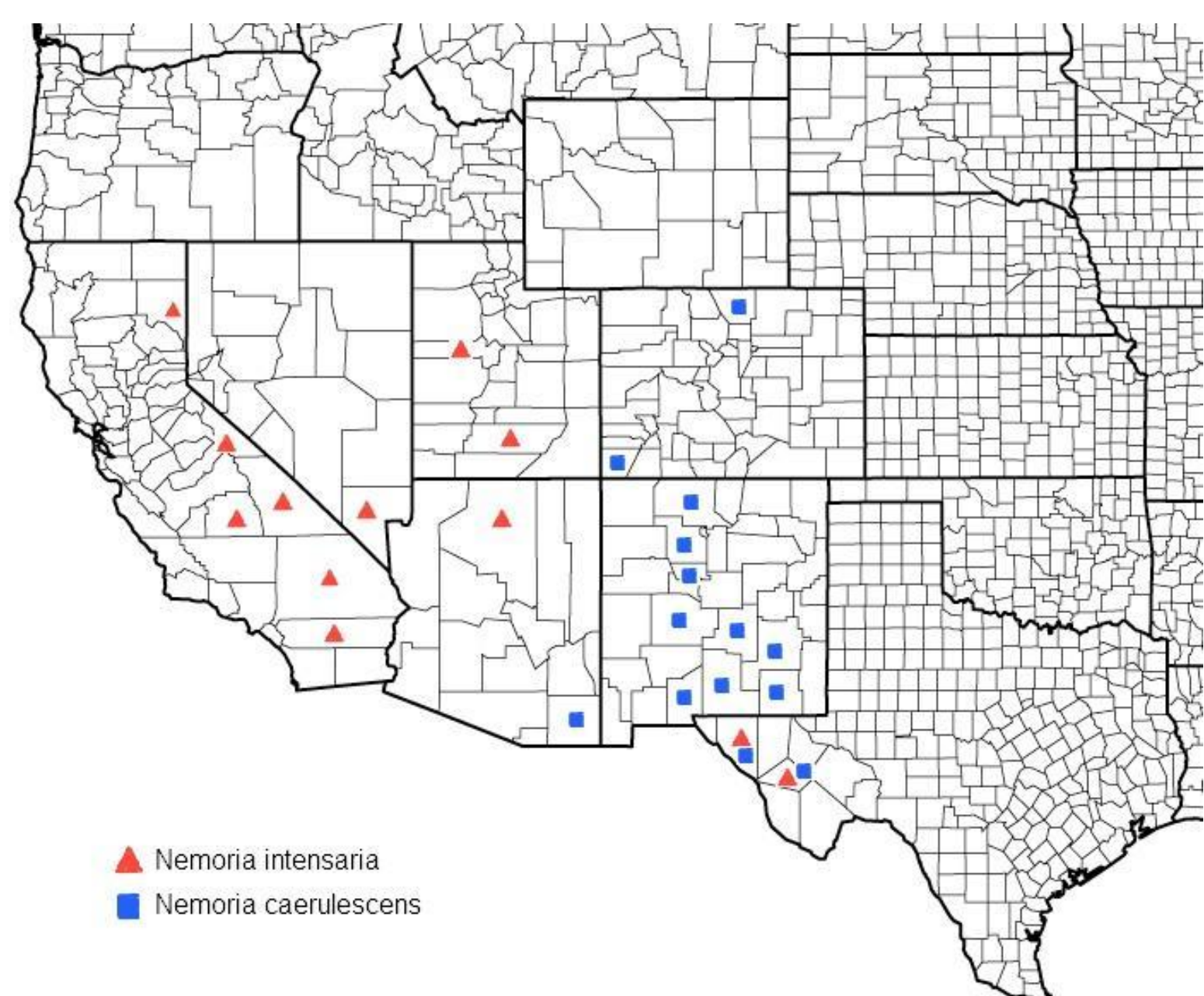
## Introduction

Among the Geometrid species of the genus *Nemoria*, two southwestern species have been poorly resolved in museum collections as a result of their strong outward resemblance, their largely indistinguishable genitalic structures and partially overlapping ranges. *Nemoria caerulescens* Prout and *Nemoria intensaria* (Pearsall) both display white striations on a grass green to faintly blue green background wing color. Both species lack a red terminal line on the wing margin, and both possess white abdominal spots. As an extension of field collecting expeditions in 2010 and 2013, a secondary school research team pursued CO-I sequencing of a Utah specimen of *Nemoria intensaria* to compare to published sequences of *Nemoria caerulescens* and other *Nemoria* species.

These two species have been found throughout several southwestern states. *N. intensaria* has been collected in southeastern California, southern Nevada, Utah, Arizona, and a small portion of western Texas. *N. caerulescens* has been collected in New Mexico, Colorado, Arizona, and the same region of Texas, where specimens of both species have been collected. Due to intraspecific variability, the similarities of *N. intensaria* and *N. caerulescens* were suggestive either of phenotypic variation of a single species in different locations, or alternatively two genetically distinct species with strong similarities in some morphological characters.



Above left, *Nemoria intensaria* adult male, Clark Co NV;  
Above right *Nemoria caerulescens* adult male, Rio Arriba Co., NM



At left, map of confirmed collection localities for *Nemoria intensaria* (red triangle) and *Nemoria caerulescens* (blue square); note presence of both in western Texas.

## Methods

DNA extraction from *Nemoria intensaria* midlegs tissue was conducted according to standard protocols using Qiagen DNEasy tissue kit. PCR was completed using Illustra PuReTaq Ready-To-Go PCR beads. Partial sequences were amplified from mitochondrial CO-I regions using two primer pairs (see below). PCR was carried out on an Eppendorf Mastercycler thermocycler. PCR products were observed on 1% agarose gels in Tris-borate-EDTA TBE buffer for verification of amplification of the correct fragment size and sequencing reactions were performed by Genewiz (South Plainfield NJ) after clean-up with Exo-SAP-IT. Sequences were aligned, merged, and analyzed using Geneious version 7.03 software (Biomatters Ltd., Auckland New Zealand). DNA sequences for other *Nemoria* species were obtained from published data on NCBI Genbank after the work of Canfield et al (2008) (1)

Primer Name	Primer Sequence	Nucleotide Position*	Length of Fragment
Ron (forward)	5'-GGATCACCTGATAGCATTCCC-3'	1730	
Nancy (reverse)	5'-CCCGGTAAAATATAAACCTTC-3'	2217	487
TN2126 (forward)	5'-TTGAYCCTGCAGTGGWGGAGA-3'	2126	
Hobbes (reverse)	5'-AAATGTTGNGGAAAAATGTTA-3'	2757	631

\* Nucleotide position referenced to CO-I sequence of *Drosophila yakuba*

## Results

### Distance Matrix – CO-I Sequences

	<i>N. intensaria</i>	<i>N. leptalea</i>	<i>N. caerulescens</i>	<i>N. festaria</i>
<i>N. intensaria</i>		96.9%	96.3%	96.8%
<i>N. leptalea</i>	96.9%		96.1%	96.6%
<i>N. caerulescens</i>	96.3%	96.1%		99.7%
<i>N. festaria</i>	96.8%	96.6%	99.7%	

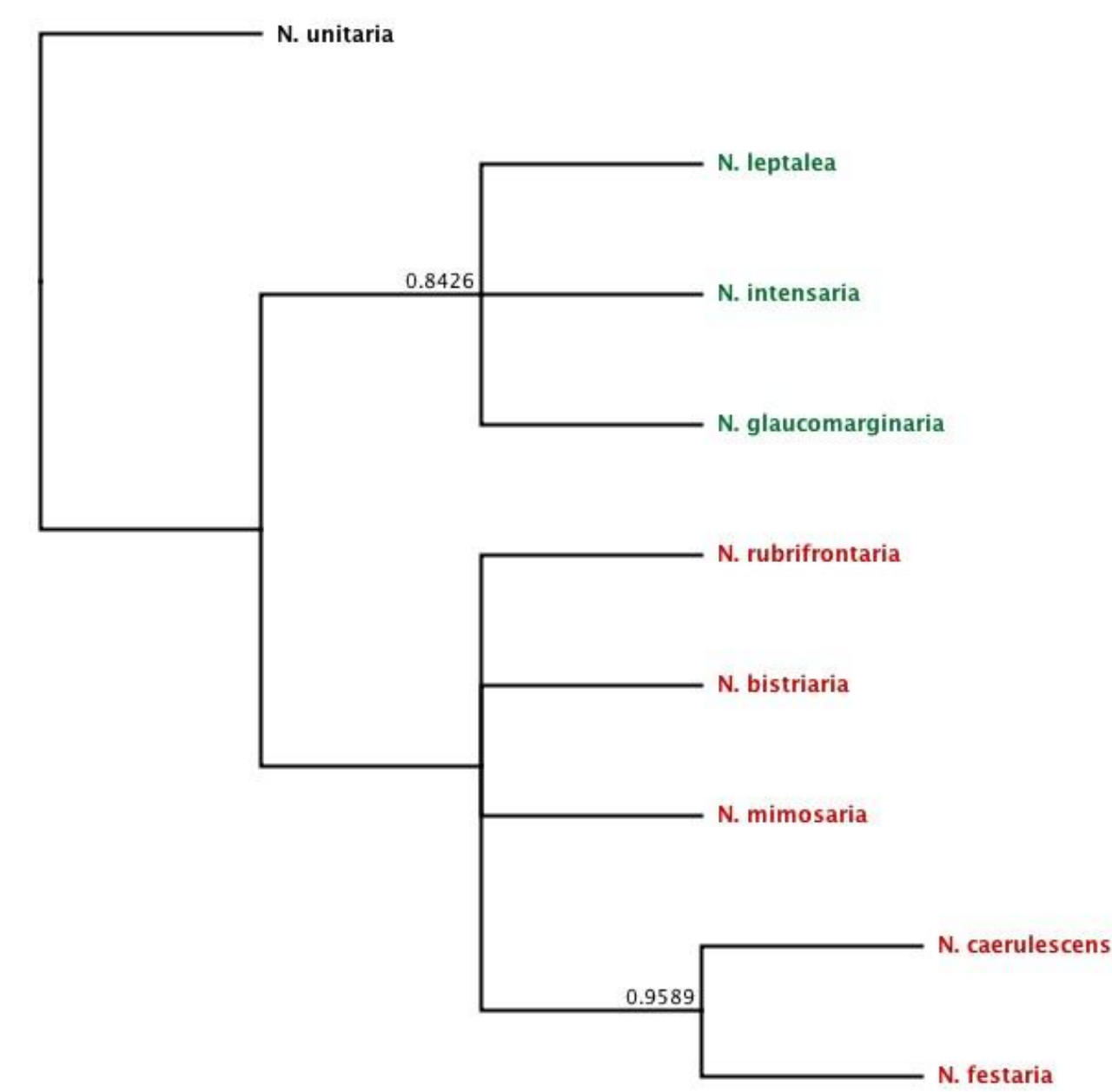
### Nucleotide Variation Table

32 sites where *N. intensaria* does not match with *N. caerulescens*

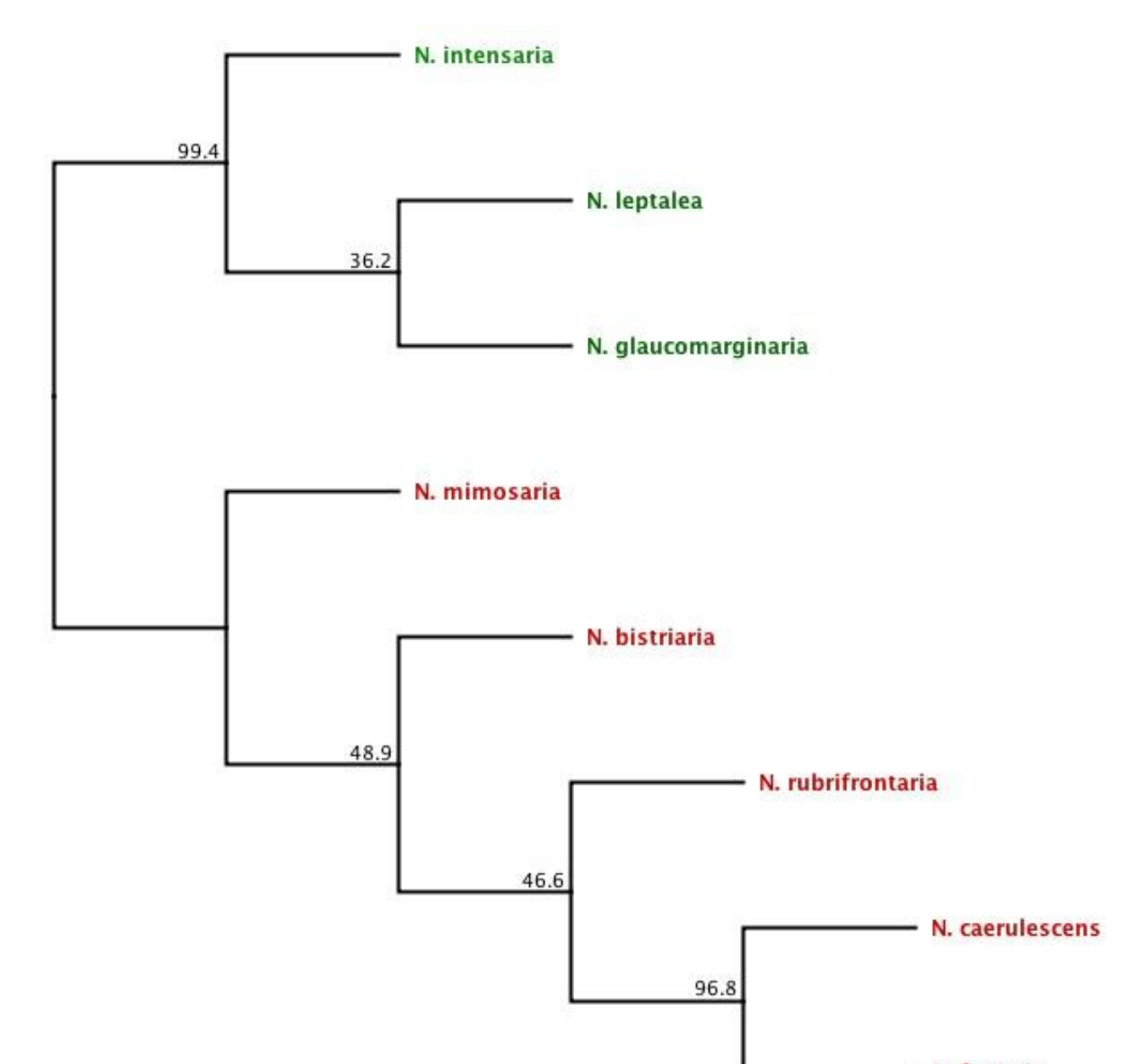
Nemoria Species	Nucleotide Position															
	15	18	27	61	78	87	114	144	159	213	292	299	360	375	378	525
<i>N. leptalea</i>	T	T	C	T	A	T	C	A	A	A	T	T	C	A	T	A
<i>N. intensaria</i>	C	T	T	G	A	T	C	C	A	A	C	T	T	A	C	A
<i>N. rubrifrontaria</i>	C	C	C	T	A	C	T	T	T	A	T	A	C	A	T	A
<i>N. caerulescens</i>	T	C	C	T	G	C	T	T	T	G	T	A	C	G	T	T
<i>N. festaria</i>	T	C	C	T	G	C	T	T	T	A	T	A	C	A	T	T

Nemoria Species	Nucleotide Position															
	534	597	612	684	687	750	759	795	801	822	828	843	849	870	888	978
<i>N. leptalea</i>	T	C	T	T	T	G	A	T	T	T	C	T	G	A	T	T
<i>N. intensaria</i>	T	C	C	T	T	G	A	C	T	G	C	T	A	G	C	C
<i>N. rubrifrontaria</i>	A	T	T	T	A	A	A	C	T	T	T	C	A	A	T	T
<i>N. caerulescens</i>	A	T	T	G	A	A	G	T	C	T	T	C	G	A	T	T
<i>N. festaria</i>	A	T	T	T	A	A	G	T	C	T	T	C	G	A	T	T

Below, Bayesian inference tree, 1,000,000 generations, *N. unitaria* as outgroup.



Below, Maximum Likelihood tree made with PhyML plugin in Geneious, 10,000 bootstraps



## Conclusions

While outwardly very similar, *Nemoria caerulescens* and *Nemoria intensaria* appear to be well resolved by CO-I sequences according to these preliminary findings. Current studies on immature stages and host plants seem to support this species distinction as well. Two other interesting observations arise from the broader phylogenetic analysis of the species group reviewed: first, *Nemoria intensaria* appears to be more closely related to the two California species *N. leptalea* and *N. glaucomarginaria* rather than the *Nemoria caerulescens* which it closely resembles. Second, *Nemoria caerulescens* differs by very few nucleotides in the CO-I region analyzed from *Nemoria festaria*, a species from which it is easily distinguished on the basis of wing markings, abdominal markings, and male genitalic structures.

## Acknowledgments

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## Bibliography

- Canfield, M.J., Greene, E.; Moreau, C.S.; Chen, N.; Pierce, N.E. (2008). "Exploring phenotypic plasticity and biogeography in emerald moths: A phylogeny of the genus *Nemoria* (Lepidoptera: Geometridae)". *Molecular Phylogenetics and Evolution* 49 (2): 477-87.