EVOLUTION AND GENOMICS
Intensive and immersive training opportunities

2020 WORKSHOP ON POPULATION AND SPECIATION GENOMICS, CESKY KRUMLOV

The Multi-Species Coalescent (MSC) and its Application in Phylogenetics and Species Delimitation

L. Lacey Knowles

Dept. of Ecology and Evolutionary Biology
University of Michigan

Software: *Delineate*

Jeet Sukumaran
Dept. of Biology, Evolutionary Biology Program
San Diego State University

https://github.com/jeetsukumaran/delineate

Software: *Decrypt*

Arnaud Becheler
Dept. of Ecology and Evolutionary Biology
University of Michigan

https://becheler.github.io/pages/applications.html

Delimitation models that bring speciation to the multispecies coalescent

Inference of species boundaries (beyond the MSC)

Software: *Delineate* https://github.com/jeetsukumaran/delineate

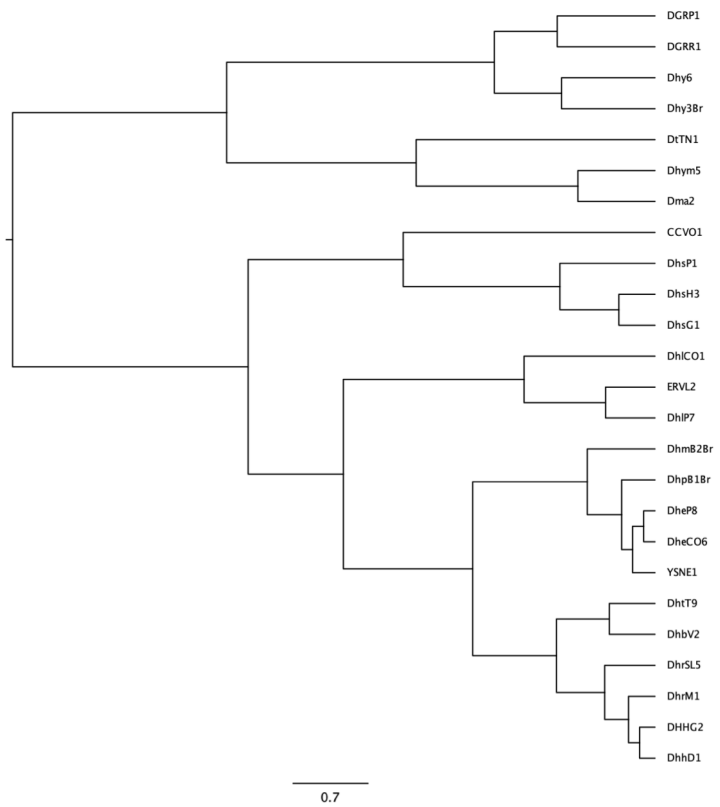
- Phylogenetic modeling approach that delineates species versus population lineages under a protracted speciation model

Software: *Decrypt* https://becheler.github.io/pages/applications.html

- Model of the geography of genetic divergence under a spatially explicit coalescent to evaluate competing hypotheses about cryptic diversity (inferred under the MSC)

Software: DELINEATE

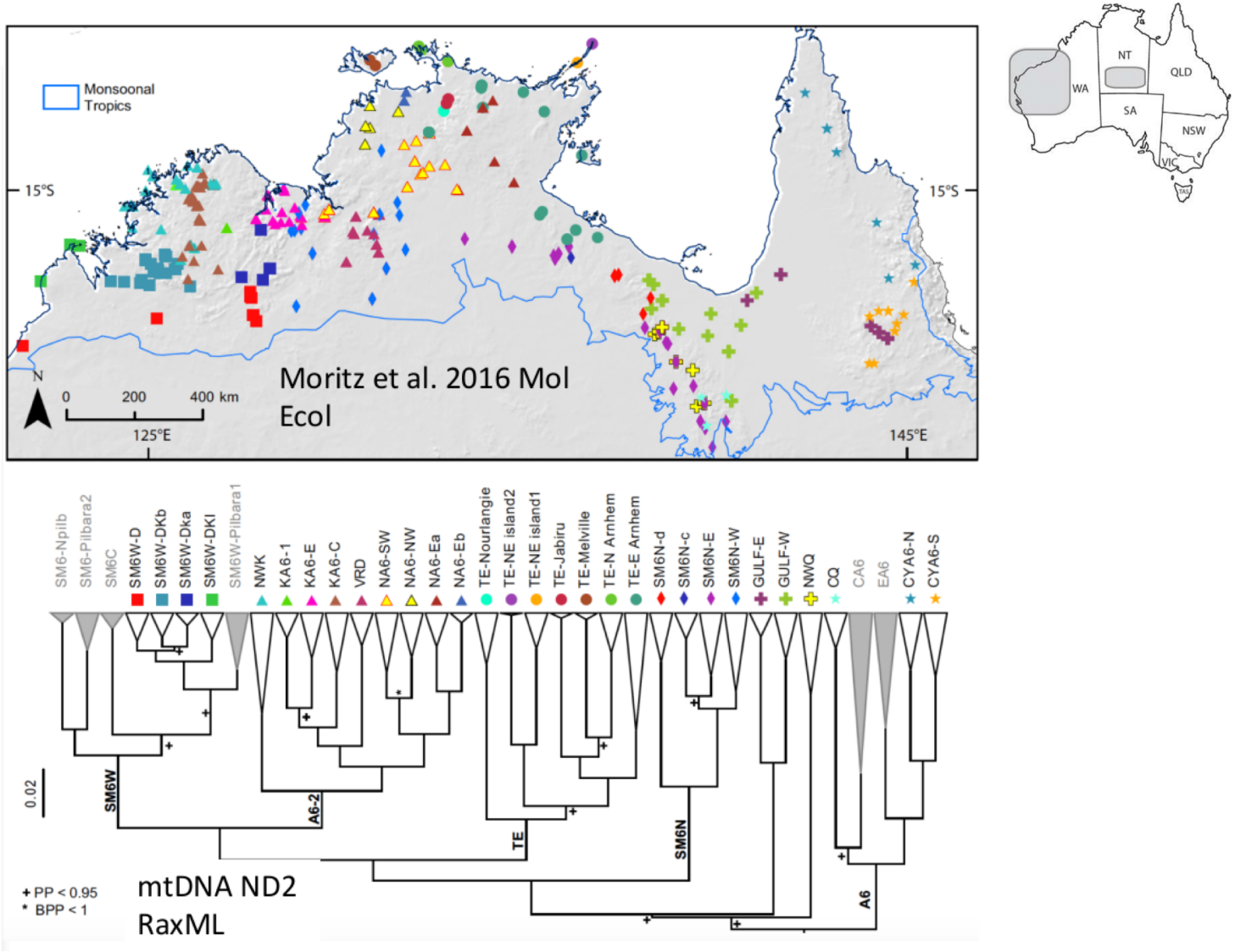
- approach integrates an explicit model of speciation into the “censored” or “multispecies” coalescent model to organize a set of population lineages sampled from one or more species into mutually-exclusive and jointly-comprehensive subsets, where each subset of population lineages represents a distinct species



(Sukumaran, Holder, and Knowles, 2020)

Seemingly fractal nature of species diversity

With more geographic sampling, get more and more species inferred under the MSC

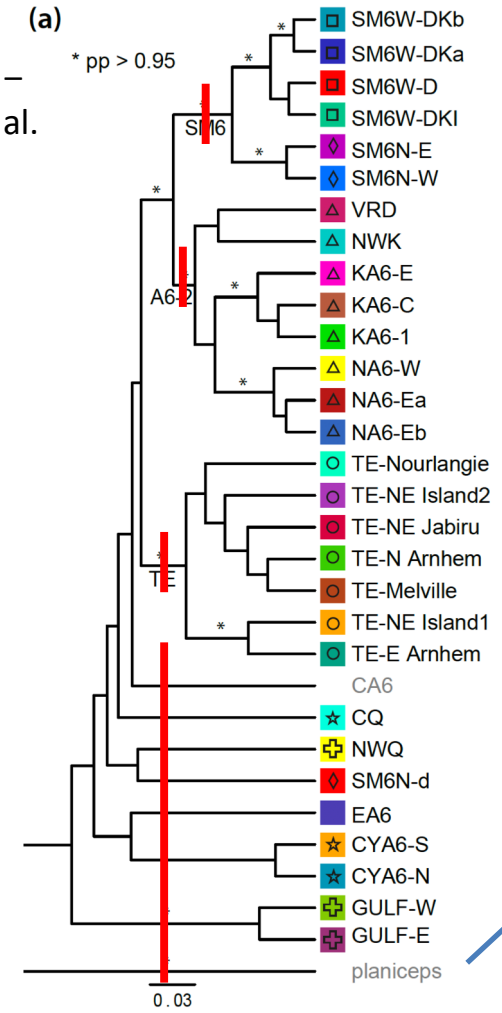


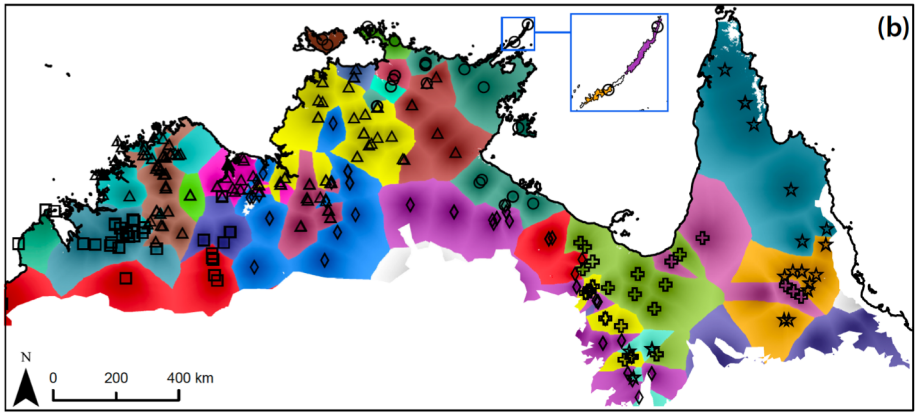
Seemingly fractal nature of species diversity

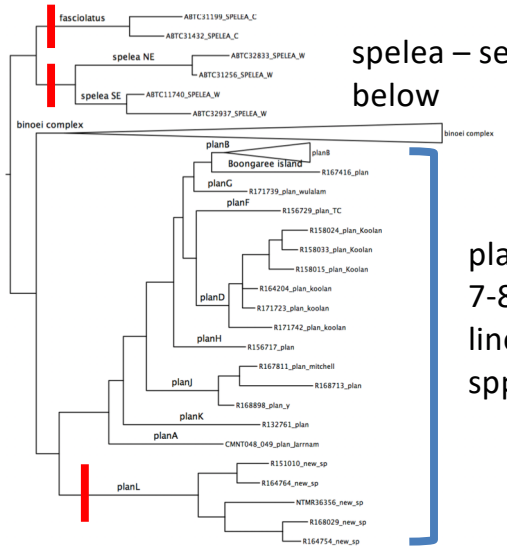
With more geographic sampling, get more and more species inferred under the MSC

Heteronotia binoei complex – species tree from Moritz et al. 2016 (7-8 loci) All lineages diagnosable using BPP.

146 samples
10 candidate species
30 lineages







spelea – see below

planiceps – 7-8 lineages, 2 spp

Software: DELINEATE

Data Requirements

DELINEATE requires two items of data:

- A *population tree*
- A *species assignment table*

File: Dynastes_calib_GeoPop.tre

File: constraints1.txt

Input Data: Population Tree

This is rooted ultrametric tree where each tip lineage represents a population or deme. This tree is typically obtained through a classical “censored” or “multispecies” coalescent analysis, such as results from BP&P (either mode A01 or A10) or StarBeast. The tree can be be specified either in NEXUS or Newick format.

Software: DELINEATE

Data Requirements

- A *population tree*
- A *species assignment table*

Input Data: Species Assignment Table

This is a tab-delimited plain text file with at least three columns:

- "lineage"
- "species"
- "status"

```
lineage species status
DGRP1   gr  1
DGRR1   gr  1
Dhy6    hy  1
  .     .     .
  .     .     .
  .     .     .
```

There may be more than these three columns, but these columns are mandatory (and all other columns will be ignored). The order of columns does not matter, as the DELINEATE programs will use the labels specified in the header row (see below) to identify the columns.

The first row is the header row: i.e., column labels. Subsequent rows will map each tip in the population tree (the "lineage" column) to a species label ("species") as well as an indication of whether this specis assignment is known or not ("status"). *Every* tip in the population tree must be represented by a row (and no more than one row) in this table. If species assignments are not known for some population lineages (as would be expected in species discovery type analyses), then the "species" field can be left blank or populated with an arbitrary value, such as a "?" or "NewSp.?" etc., but the "status" field should be set to "0". Species assignments that *are* known will have the status field set to "1".

Software: DELINEATE Running a Species Delimitation Analysis

To do a quick check of your files from your terminal:

```
(base) m-c02wt02ehh24:~ knowlesl$ delineate-check
```

delineate-check -c Downloads/constraints1.txt -t Downloads/Dynastes_calib_GeoPop.tre -f newick

Software: DELINEATE Running a Species Delimitation Analysis

Basic Run

Given a population lineage tree file, "population-tree.nex", and a species assignment table file "species-mappings.tsv", then the following command will run a DELINEATE analysis on the data::

```
delineate-estimate partitions --tree-file population-tree.nex --config-file data1.tsv"
```

or, using the short-form options:

```
delineate-estimate partitions -t population-tree.nex --t data1.tsv"
```

This command has the following components:

- `delineate-estimate` : This is the name of the program to be run.
- `estimate` : This is the command or operation that the program will be running.
- `--tree-file population-tree.nex` or `-t population-tree.nex` : The `--tree-file` flag, or its short-form synonym, `-t` , specifies that the next element will be the path to tree file with data on the population lineage tree. In this example, the file is located in the current working directory, i.e., *population-tree.nex*. If it was in another directory, then the path could be */home/bilbo/projects /orc-species-delimitation/data1/population-tree.nex*, for example.
- `--config-file data1.tsv` or `-c data1.tsv` The `--config-file` flag, or its short-form synonym, `-c` , specifies that the next element will be the path to species assignment configuration file. In this example, the file is located in the current working directory, i.e., *delineate-species.tsv*. Again, if it was in another directory, then the path could be */home/bilbo /projects/orc-species-delimitation/data1/data1.tsv*, for example.

(base) m-c02wt02ehh24:~ knowlesl$ delineate-estimate partitions -c Downloads/constraints1.txt -t Downloads/Dynastes_calib_GeoPop.tre -f newick

Software: DELINEATE Running a Species Delimitation Analysis

Basic Run Output

Executing this command will run the DELINEATE analysis and will produce the following output files:

- *"data1.delimitation-results.json"* File: constraints1.delimitation-results.json
- *"data1.delimitation-results.trees"* File: constraints1.delimitation-results.trees

More generally, unless the `-o` or `--output-prefix` flag (see below) is used to explicity specify an alternate output prefix for all results generated, the files will take on a prefix given by the file stemname of the configuration file, *"data1"* in the this example.

Software: DELINEATE Running a Species Delimitation Analysis

Basic Run Output

Executing this command will run the DELINEATE analysis and will produce the following output files:

- *"data1.delimitation-results.json"* File: constraints1.delimitation-results.json
- *"data1.delimitation-results.trees"*

The first file, with the general name of *"<output-prefix>.delimitation-results.json"*, is the primary results file. As can be inferred from its extension, it is a JSON format text file, and it consists of a single a dictionary. The dictionary provides information on the estimated speciation completion rate as well as the probabilities of all the possible partitions of the population lineage leafset into species sets, given the species assignment constraints, ranked by the probability of each partition.

Software: DELINEATE Running a Species Delimitation Analysis

Basic Run Output

Executing this command will run the DELINEATE analysis and will produce the following output files:

- *"data1.delimitation-results.json"*
- *"data1.delimitation-results.trees"*

File: constraints1.delimitation-results.trees

The first file, with the general name of *"<output-prefix>.delimitation-results.json"*, is the primary results file. As can be inferred from its extension, it is a JSON format text file, and it consists of a single a dictionary. The dictionary provides information on the estimated speciation completion rate as well as the probabilities of all the possible partitions of the population lineage leafset into species sets, given the species assignment constraints, ranked by the probability of each partition.

Software: DELINEATE Running a Species Delimitation Analysis

Basic Run Output

Executing this command will run the DELINEATE analysis and will produce the following output files:

- "*data1.delimitation-results.json*"
- "*data1.delimitation-results.trees*"

File: constraints1.delimitation-results.trees

The second file, with the general name of "*<output-prefix>.delimitation-results.trees*", provides supporting results. Basically this is a collection of trees, with one tree for each partition considered. The topology of the trees are identical, corresponding to the topology of the input tree (i.e., the population lineage tree), as are the tip labels. However, the tips have associated with them some extra metadata that will be available for viewing in a program like FigTree. Most important of this is `species`, i.e., the label corresponding to the identity of the species assignment in that partition. In the case of species assignments that are constrained (i.e., status indicated by "1"), these will be identical to the assignment and invariant across all partitions, of course. However, in the case of population lineages of *unknown* species affinities (i.e., status indicated by "0"), this may be an existing species label (if the population lineage was assigned to an existing species in the partition under consideration) or a new, arbitrary species label (if the population lineage was assigned to a new distinct species in the partition under consideration). In addition, in FigTree you can also choose to have the branches colored by "status", and this will highlight population lineages of (*a priori*) known vs unknown species affinities, and thus quickly identify the assigned species identities of the lineages of interest.

Software: DELINEATE Running a Species Delimitation Analysis

Basic Run Output

Executing this command will run the DELINEATE analysis and will produce the following output files:

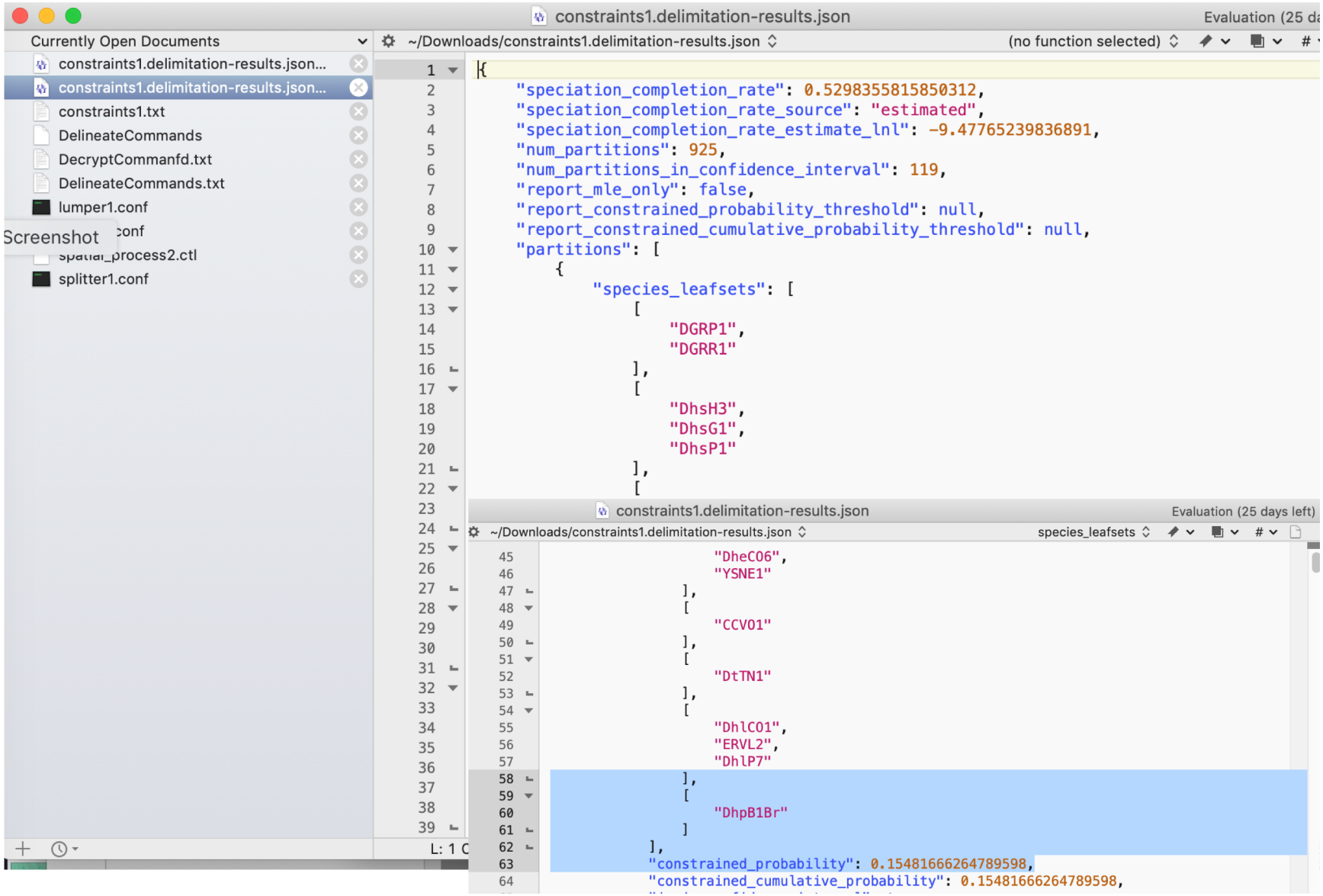
- "*data1.delimitation-results.json*"
- "*data1.delimitation-results.trees*"

File: constraints1.delimitation-results.trees

The second file, with the general name of "*<output-prefix>.delimitation-results.trees*", provides supporting results. Basically this is a collection of trees, with one tree for each partition considered. The topology of the trees are identical, corresponding to the topology of the input tree (i.e., the population lineage tree), as are the tip labels. However, the tips have associated with them some extra metadata that will be available for viewing in a program like FigTree. Most important of this is `species`, i.e., the label corresponding to the identity of the species assignment in that partition. In the case of species assignments that are constrained (i.e., status indicated by "1"), these will be identical to the assignment and invariant across all partitions, of course. However, in the case of population lineages of *unknown* species affinities (i.e., status indicated by "0"), this may be an existing species label (if the population lineage was assigned to an existing species in the partition under consideration) or a new, arbitrary species label (if the population lineage was assigned to a new distinct species in the partition under consideration). In addition, in FigTree you can also choose to have the branches colored by "status", and this will highlight population lineages of (*a priori*) known vs unknown species affinities, and thus quickly identify the assigned species identities of the lineages of interest.

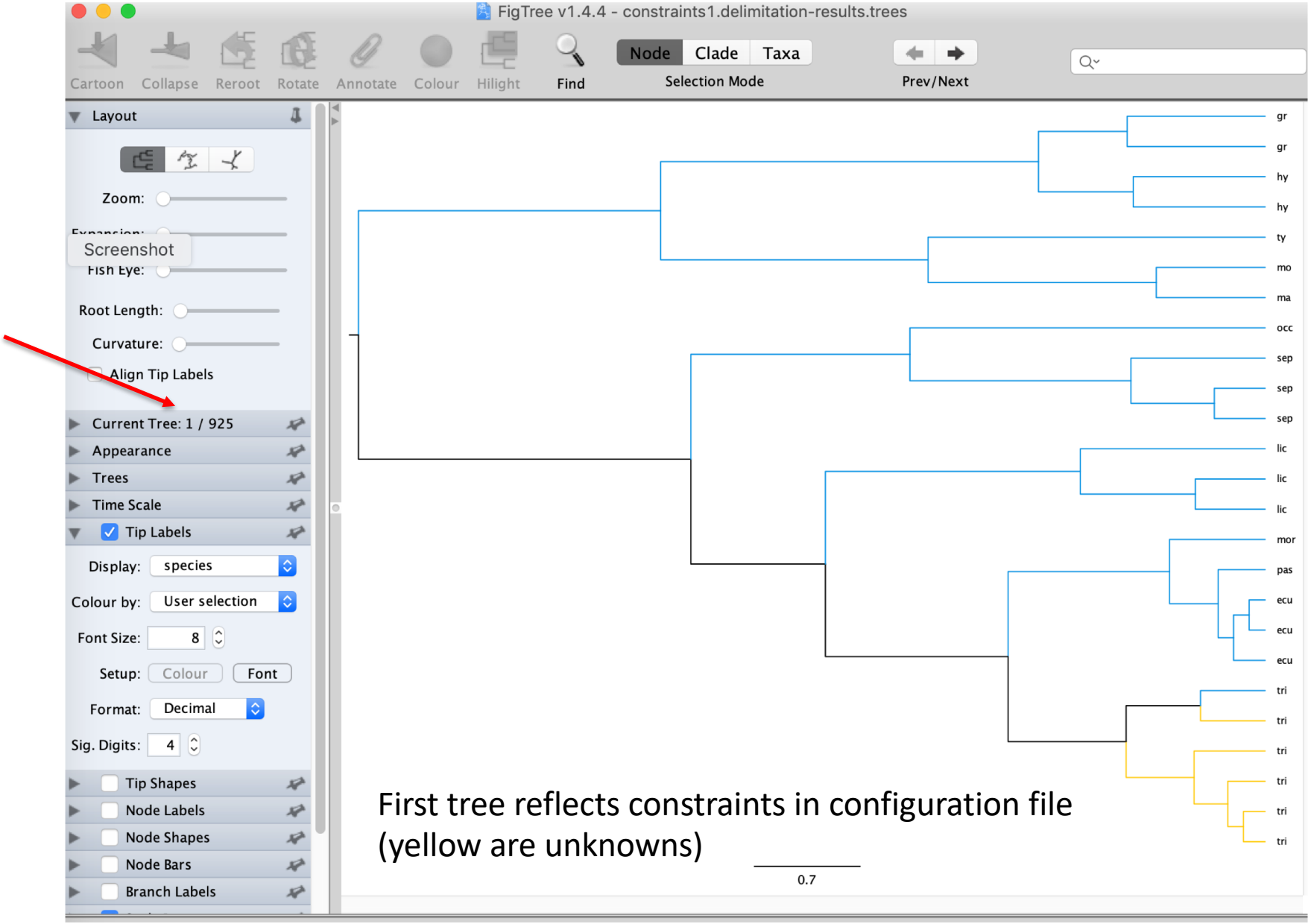
Software: DELINEATE Running a Species Delimitation Analysis

Open in Text editor: constraints1.delimitation-results.trees



Software: DELINEATE Running a Species Delimitation Analysis

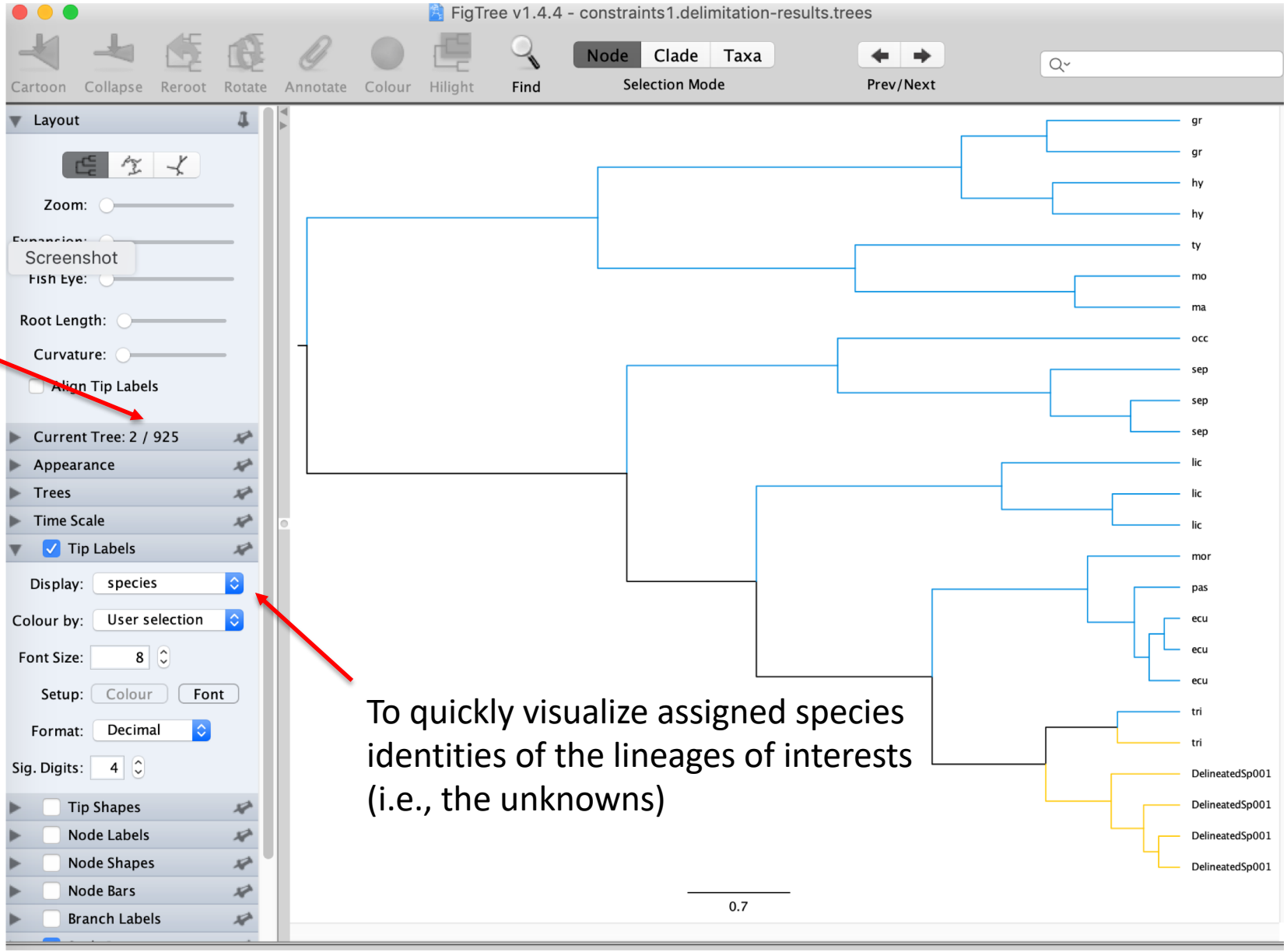
Open in FigTree; File: constraints1.delimitation-results.trees



First tree reflects constraints in configuration file (yellow are unknowns)

Software: DELINEATE Running a Species Delimitation Analysis

Open in FigTree; File: constraints1.delimitation-results.trees



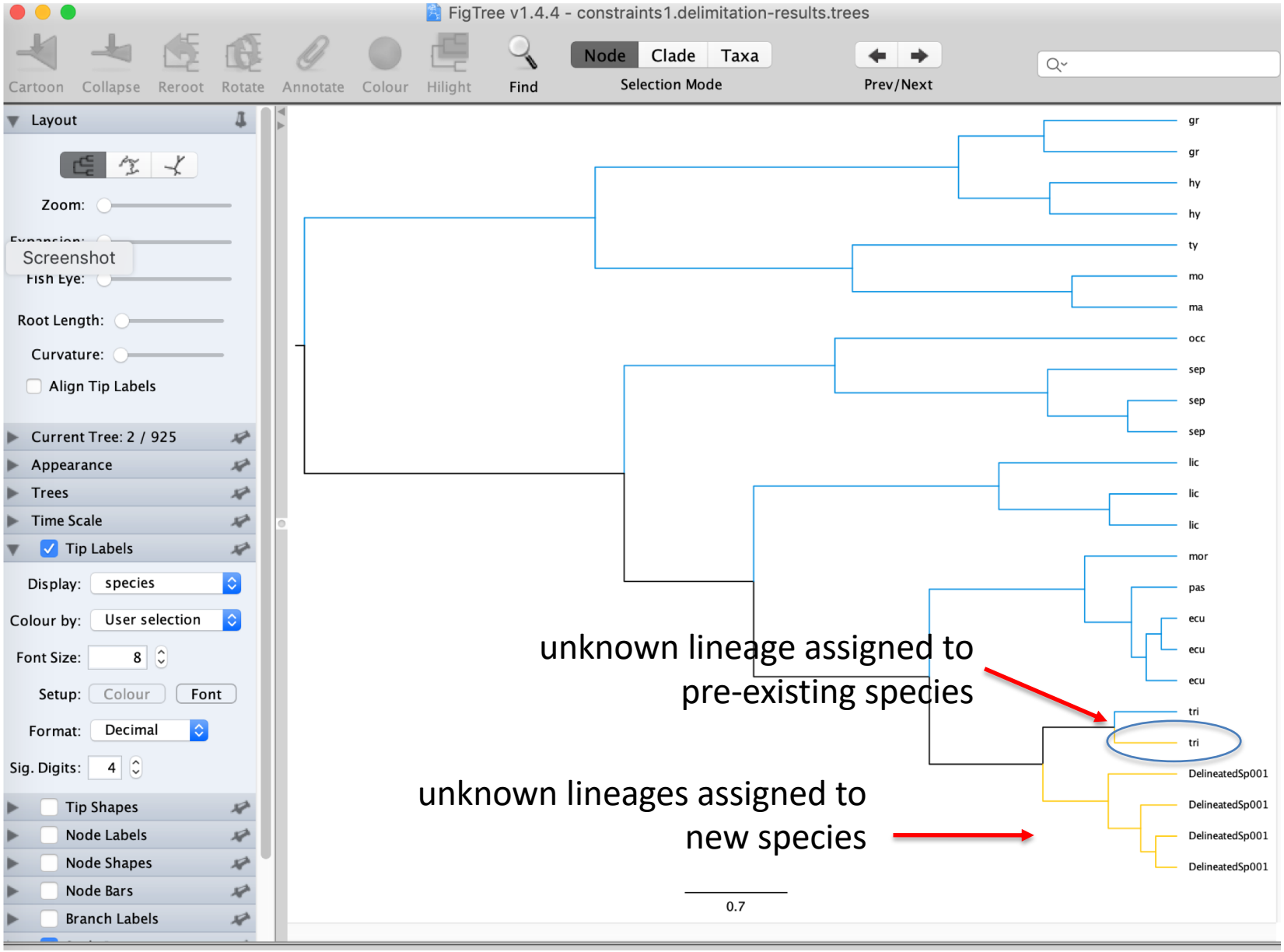
Screenshot

MLE

To quickly visualize assigned species identities of the lineages of interests (i.e., the unknowns)

Software: DELINEATE Running a Species Delimitation Analysis

Open in FigTree; File: constraints1.delimitation-results.trees



Software: DELINEATE

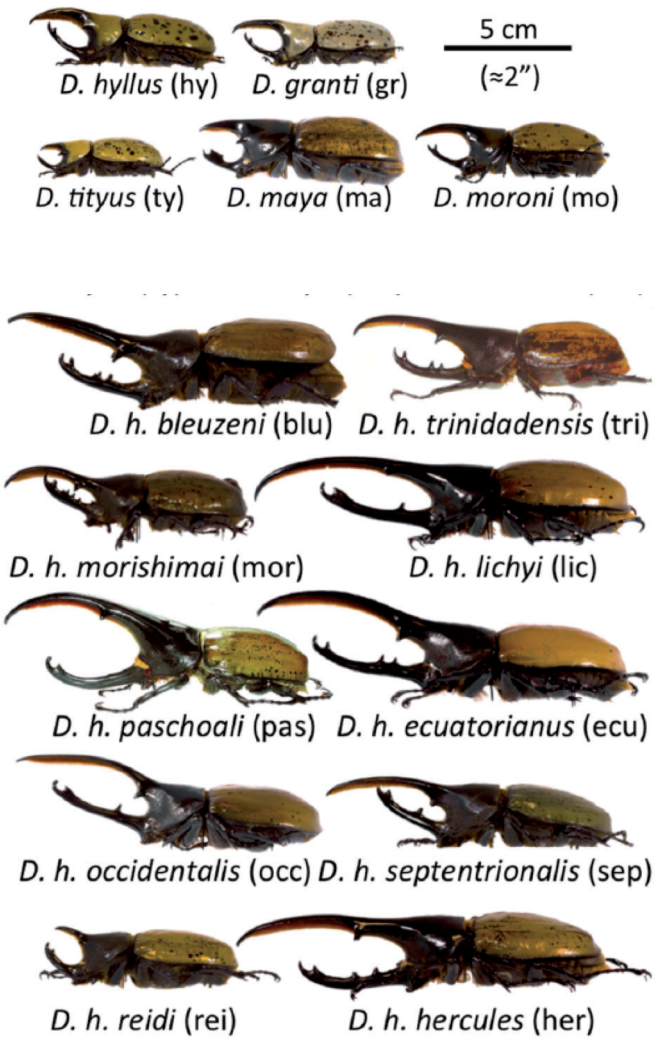
Data Requirements

- A *population tree*
- A *species assignment table*

Original Alpha Taxonomy

lineage	species	status
DGRP1	gr	1
DGRR1	gr	1
Dhy6	hy	1
Dhy3Br	hy	1
DtTN1	ty	1
Dhym5	mo	1
Dma2	ma	1
CCV01	occ	1
DhsP1	sep	1
DhsH3	sep	1
DhsG1	sep	1
DhlC01	lic	1
ERVL2	lic	1
DhlP7	lic	1
DhmB2Br	mor	0
DhpB1Br	pas	0
DheP8	ecu	0
DheC06	ecu	0
YSNE1	ecu	0
DhtT9	tri	0
DhbV2	blu	0
DhrSL5	rei	0
DhrM1	rei	0
DHHG2	her	0
DhhD1	her	0

File: constraints1.txt



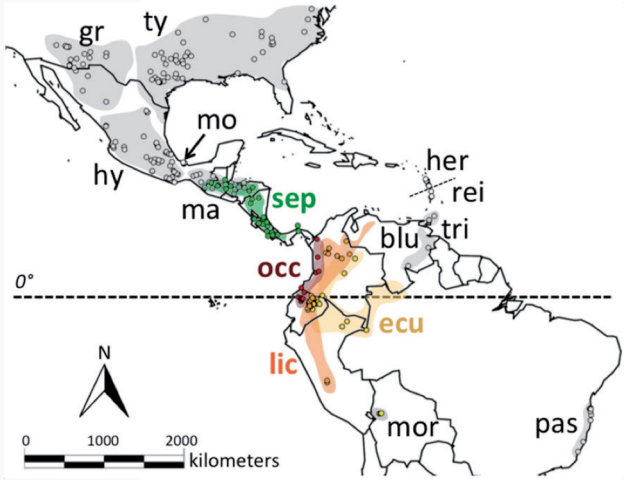
Software: DELINEATE

Hypotheses based on different species criteria:

Original Alpha Taxonomy

lineage	species	status
DGRP1	gr	1
DGRR1	gr	1
Dhy6	hy	1
Dhy3Br	hy	1
DtTN1	ty	1
Dhym5	mo	1
Dma2	ma	1
CCV01	occ	1
DhsP1	sep	1
DhsH3	sep	1
DhsG1	sep	1
DhlC01	lic	1
ERVL2	lic	1
DhlP7	lic	1
DhmB2Br	mor	0
DhpB1Br	pas	0
DheP8	ecu	0
DheC06	ecu	0
YSNE1	ecu	0
DhtT9	tri	0
DhbV2	blu	0
DhrSL5	rei	0
DhrM1	rei	0
DHHG2	her	0
DhhD1	her	0

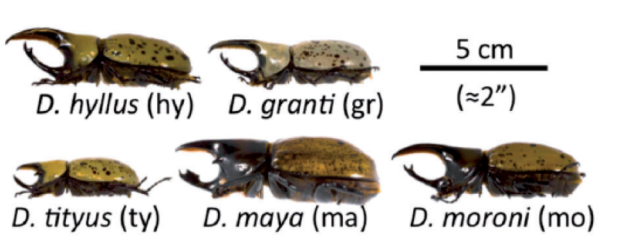
- Taxonomic practices vs diversity differences



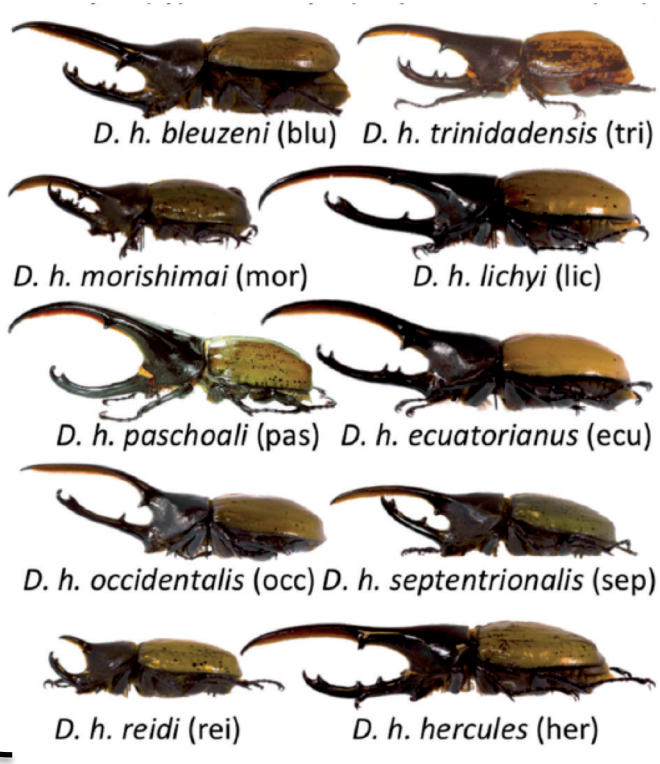
Data Requirements

- A *population tree*
- A *species assignment table*

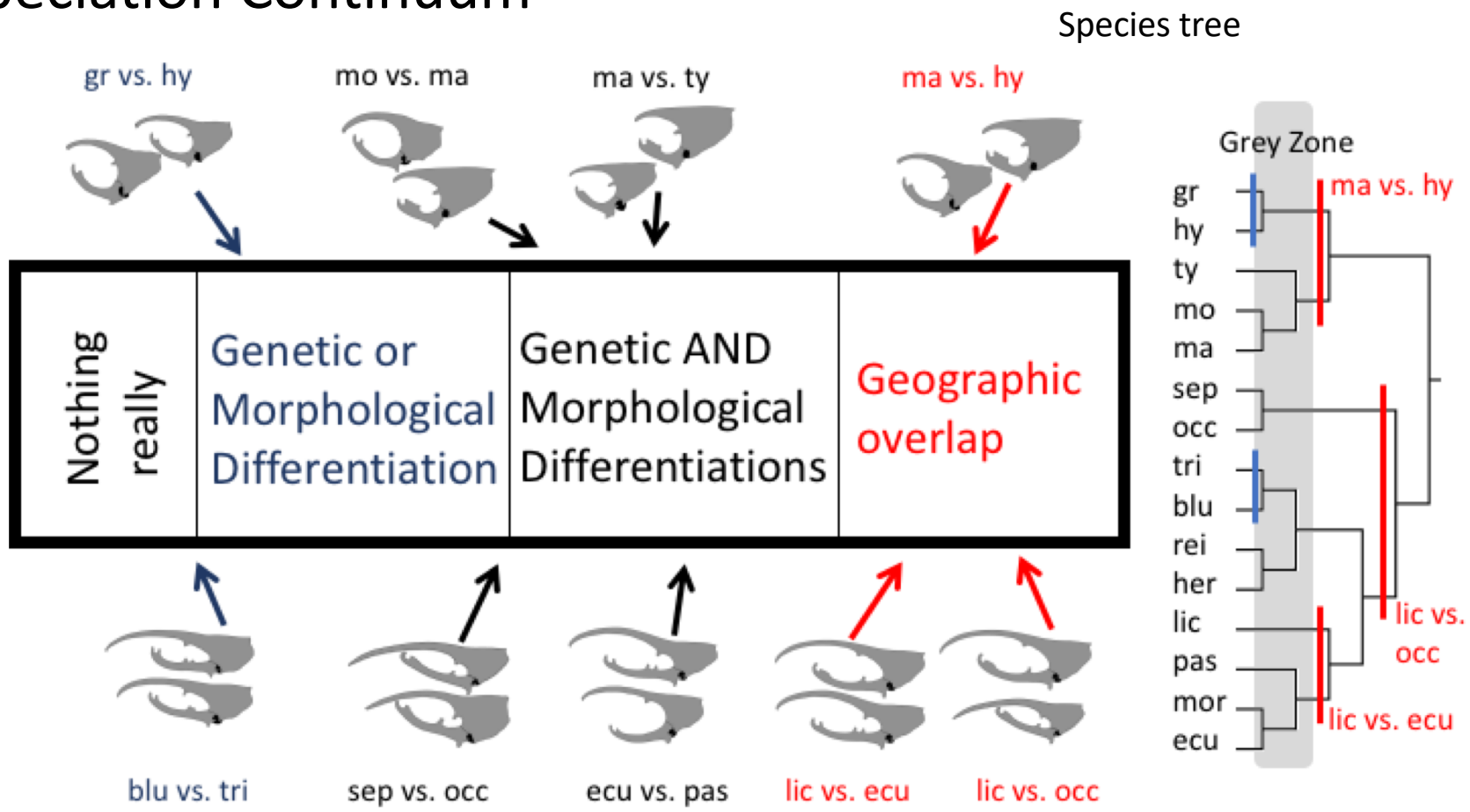
5 species



1 species



Speciation Continuum



Could test different hypotheses about species boundaries based on different species criteria

Huang & Knowles (2016) *Syst. Biol.*

Software: DELINEATE

Data Requirements

- A *population tree*
- A *species assignment table*

Hypotheses based on different species criteria:

Original Alpha Taxonomy

lineage	species	status
DGRP1	gr	1
DGRR1	gr	1
Dhy6	hy	1
Dhy3Br	hy	1
DtTN1	ty	1
Dhym5	mo	1
Dma2	ma	1
CCVO1	occ	1
DhsP1	sep	1
DhsH3	sep	1
DhsG1	sep	1
DhlCO1	lic	1
ERVL2	lic	1
DhlP7	lic	1
DhmB2Br	mor	0
DhpB1Br	pas	0
DheP8	ecu	0
DheCO6	ecu	0
YSNE1	ecu	0
DhtT9	tri	0
DhbV2	blu	0
DhrSL5	rei	0
DhrM1	rei	0
DHHG2	her	0
DhhD1	her	0

"Lumper"Taxonomy

lineage	species	status
DGRP1	gr grhy	1
DGRR1	gr grhy	1
Dhy6	hy grhy	1
Dhy3Br	hy grhy	1
DtTN1	ty motyma	1
Dhym5	mo motyma	1
Dma2	ma motyma	1
CCVO1	occ sepocc	1
DhsP1	sep sepocc	1
DhsH3	sep sepocc	1
DhsG1	sep seppcc	1
DhlCO1	lic lic	1
ERVL2	lic lic	1
DhlP7	lic lic	1
DhmB2Br	mor mor	0
DhpB1Br	pas pas	0
DheP8	ecu ecu	0
DheCO6	ecu ecu	0
YSNE1	ecu ecu	0
DhtT9	tri tri	0
DhbV2	blu blu	0
DhrSL5	rei rei	0
DhrM1	rei rei	0
DHHG2	her her	0
DhhD1	her her	0

"Splitter"Taxonomy

lineage	species	status
DGRP1	gr1	1
DGRR1	gr2	1
Dhy6	hy1	1
Dhy3Br	hy2	1
DtTN1	ty	1
Dhym5	mo	1
Dma2	ma	1
CCVO1	occ	1
DhsP1	sep	1
DhsH3	sep	1
DhsG1	sep	1
DhlCO1	lic	1
ERVL2	lic	1
DhlP7	lic	1
DhmB2Br	mor	0
DhpB1Br	pas	0
DheP8	ecu	0
DheCO6	ecu	0
YSNE1	ecu	0
DhtT9	tri	0
DhbV2	blu	0
DhrSL5	rei	0
DhrM1	rei	0
DHHG2	her	0
DhhD1	her	0

Software: DELINEATE

Data Requirements

- A *population tree*
- A *species assignment table*

- Number of inferred species naturally increases as the known species assignments are made under more of a splitter, rather than a lumper, than perspective.
- However, naturally as well, the relationship is not a simple numerical one but is rather dependent on the branch lengths leading to the assigned species subtrees.

Regime	Constrained	Inferred	New
lumper2	3	1	0
lumper1	4	1	0
original	8	2	2
splitter1	12	2	2
splitter2	14	10	10

lineage	species	status
DGRP1	gr	1
DGRR1	gr	1
Dhy6	hy	1
Dhy3Br	hy	1
DtTN1	ty	1
Dhym5	mo	1
Dma2	ma	1
CCV01	occ	1
DhsP1	sep	1
DhsH3	sep	1
DhsG1	sep	1
DhlC01	lic	1
ERVL2	lic	1
DhlP7	lic	1
DhmB2Br	mor	0
DhpB1Br	pas	0
DheP8	ecu	0
DheC06	ecu	0
YSNE1	ecu	0
DhtT9	tri	0
DhbV2	blu	0
DhrSL5	rei	0
DhrM1	rei	0
DHHG2	her	0
DhhD1	her	0

A new era of species delimitation models that brings speciation models to the multispecies coalescent

- Erroneous species boundaries are inferred from current model-based genetic approaches based on MSC

  Delimitation under the MSC:

  - genetic structure = species

- Relying on heuristics to interpret results from current genetic methods (e.g., bpp) is not the answer

- Future of genetic-based species delimitation is with speciation-based delimitation models in which species criteria is explicitly incorporated into test of species status of unknowns (e.g., “splitter” vs “lumper”)

Incorporating the Speciation Process into Species Delimitation

Jeet Sukumaran[a,c,1,2], Mark T. Holder[b,1], and L. Lacey Knowles[a]

[a]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor MI, USA 41809-1079; [b]Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence KS, USA 66045; [c]Department of Biology, San Diego State University, San Diego CA, USA 92182-4614

This manuscript was compiled on October 10, 2019

Processes contribute to the generation of this structure apart from speciation, including population isolation or divergence. This results in “population” units being conflated with “species” units when these approaches are applied without corroborating data. This confusion between populations and species has become more and more problematical recently, ironically due to the increasing resolution and scope of the data

Our work pioneers a new age of species delimitation approaches that deal with the problem directly by modeling the issue instead of ignoring it! That is, by actually incorporating an explicit model of the speciation process — in particular, an extended or protracted speciation process — into species delimitation, we are able to discriminate between species and population (or other) boundaries in genomic data.

knowlesl@umich.edu

QUESTIONS?

Jeet Sukumaran
San Diego State Univ.

Mark Holder
Univ. of Kansas

support NSF & the UM

Software: *Decrypt* https://becheler.github.io/pages/applications.html

- Model of the geography of genetic divergence under a spatially explicit coalescent to evaluate competing hypotheses about cryptic diversity (inferred under the MSC)