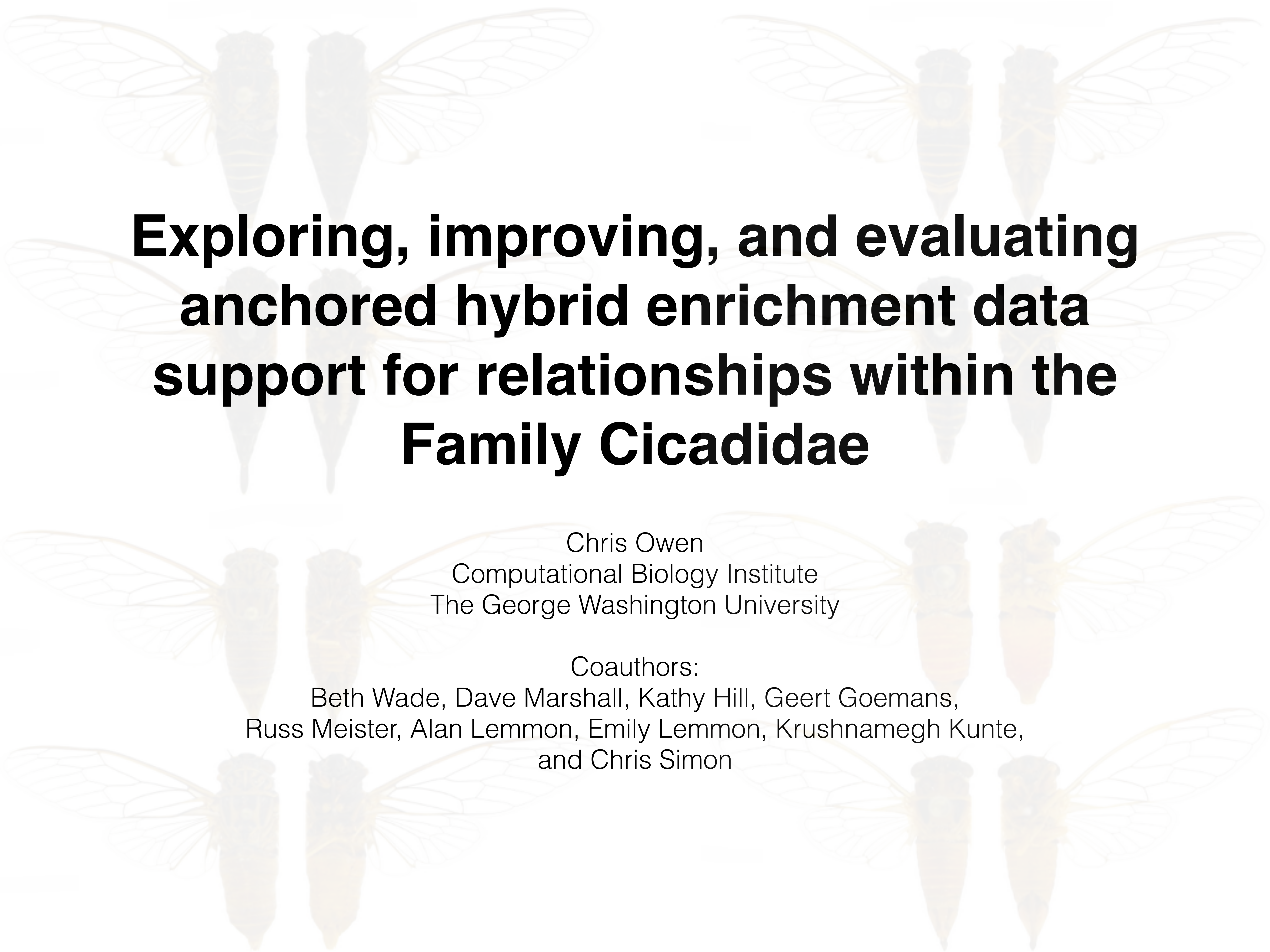

Exploring, improving, and evaluating anchored hybrid enrichment data support for relationships within the Family Cicadidae

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The George Washington University

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Russ Meister, Alan Lemmon, Emily Lemmon, Krushnamegh Kunte,
and Chris Simon

Outline

1. Lessons learned from transcriptome phylogenomic datasets

2. Dealing with the unknown: what should the phylogeny look like and what are these sequences?

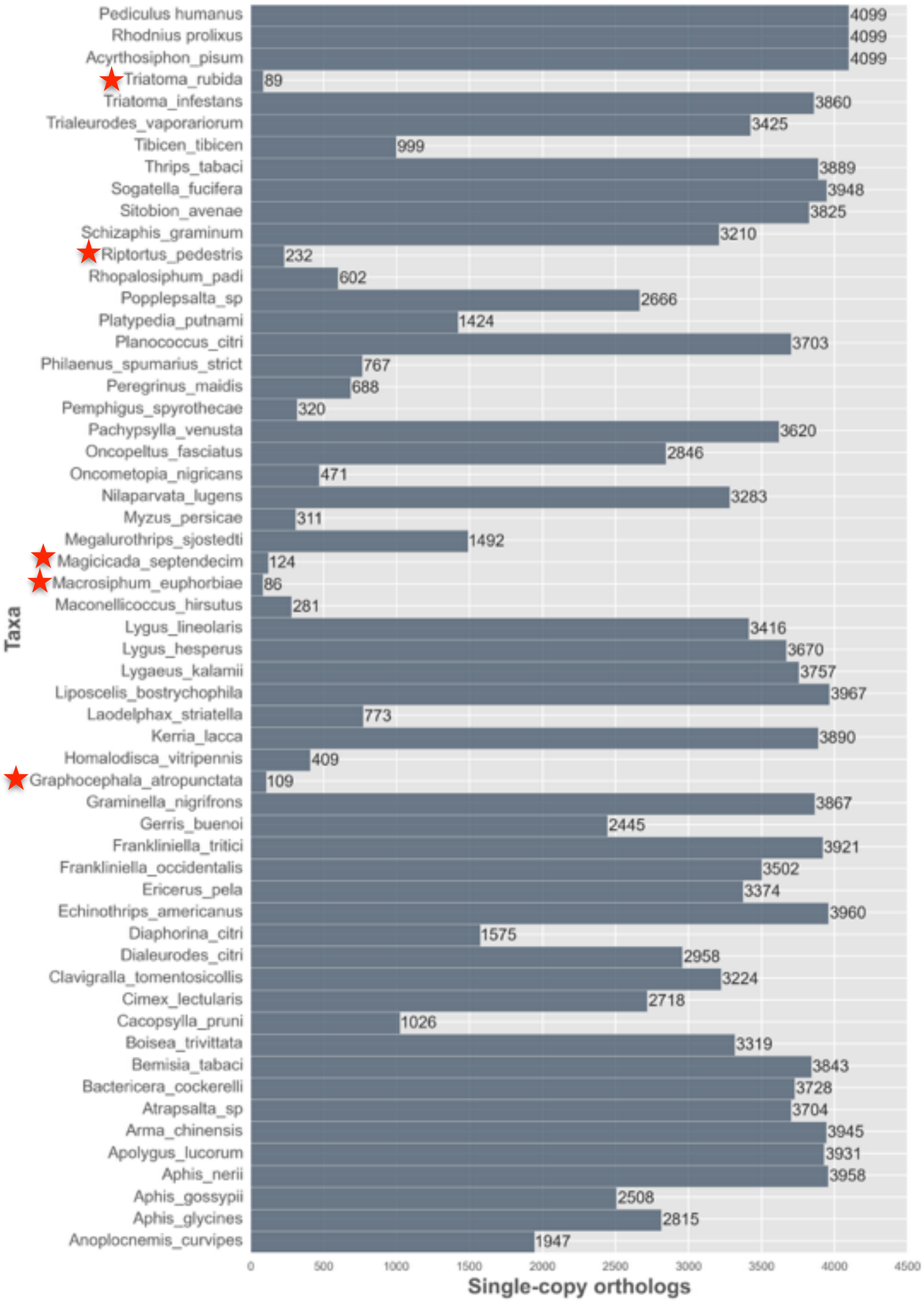
3. Model violation and gene filtering

4. Gene tree / species tree estimation

5. Exploring tree space

Lessons learned from Hemiptera transcriptome phylogenomic datasets

Taxon & Ortholog Sampling



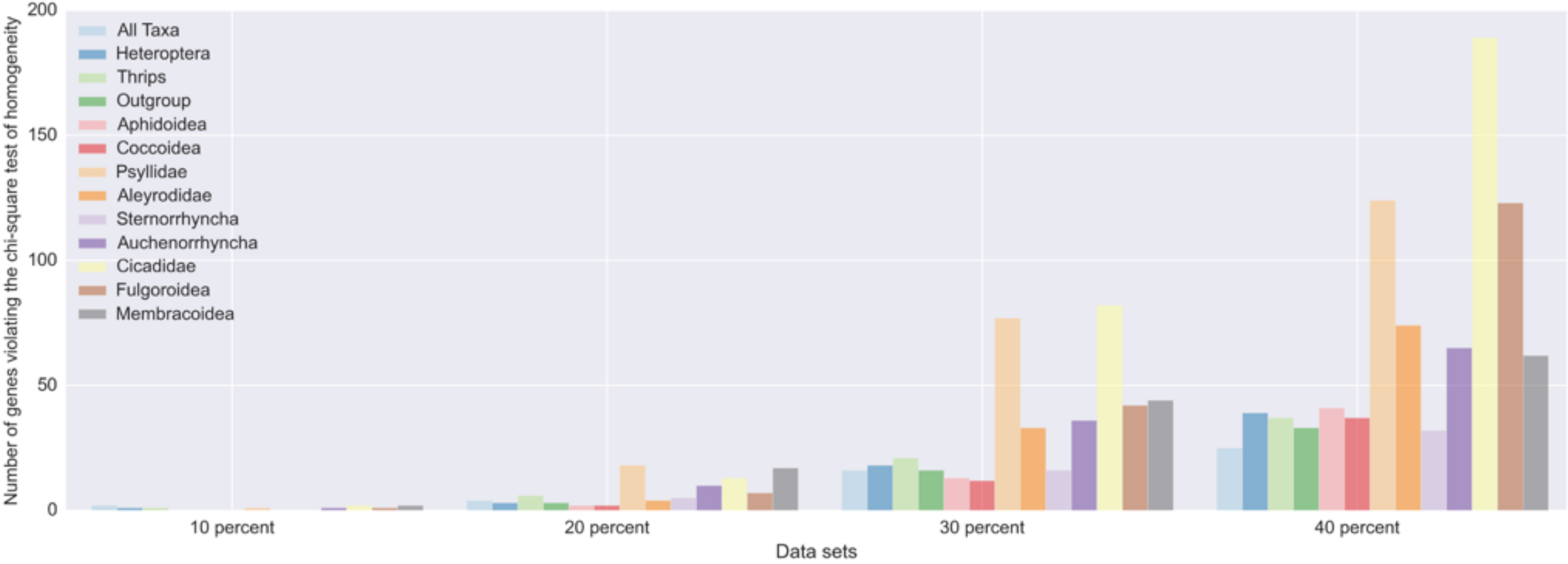
Outgroups
- Thysanoptera: 5 species
- Phthiraptera: 1 species
- Psocoptera: 1 species

Auchenorrhyncha
- Cicadoidea: 5 species
- Cercopoidea: 1 species
- Membracoidea: 4 species
- Fulgoroidea: 4 species

Heteroptera
- Pentatomoidea: 1 species
- Coreoidea: 4 species
- Lygaeoidea: 2 species
- Cimicoidea: 1 species
- Miroidea: 3 species
- Reduvioidea: 3 species
- Gerroidea: 1 species

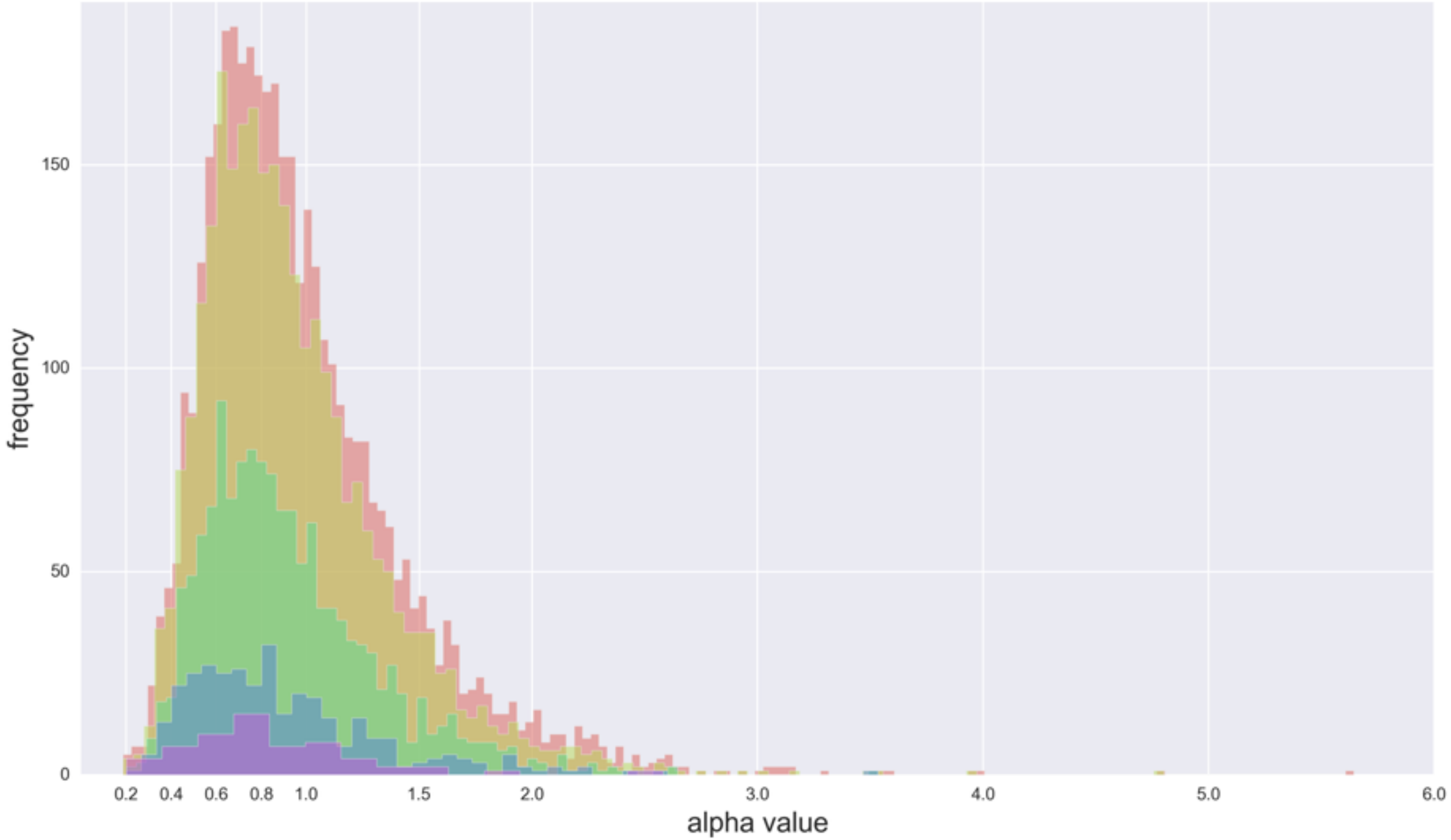
Sternorrhyncha
- Coccoidea: 4 species
- Aphidoidea: 10 species
- Psylloidea: 4 species
- Aleyrodidae: 3 species

Choosing the best genes: compositional bias



- Chi-square test among taxa and higher taxa ($p<0.5$)
- Suffers from type 2 error (Foster 2004)

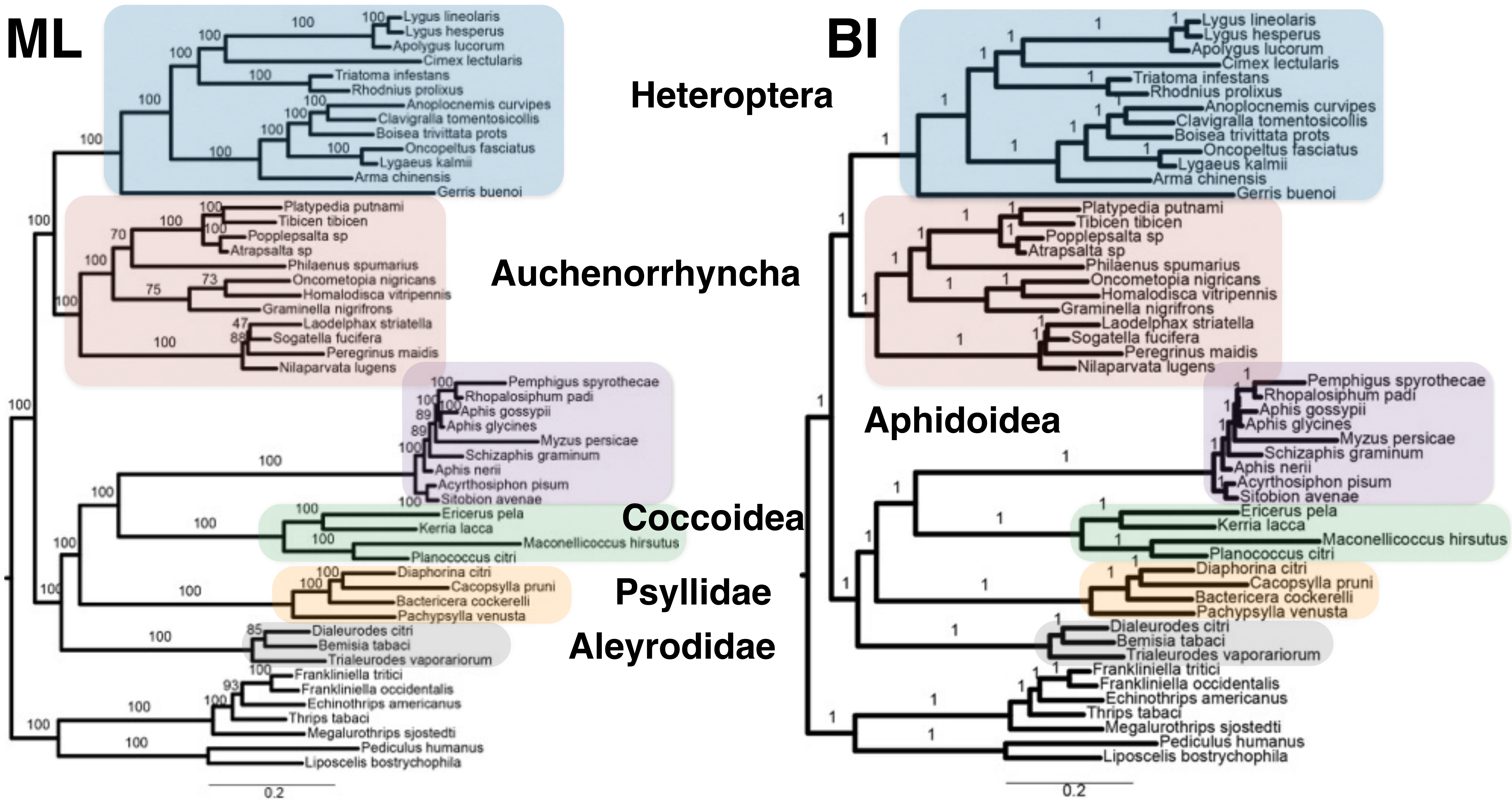
Choosing the best genes: among site rate variation



- removed genes alpha value < 1 (Yang 1994)

Hemiptera phylogeny: most curated dataset

Alignment: 463 genes; 106,740 AA; 52 taxa

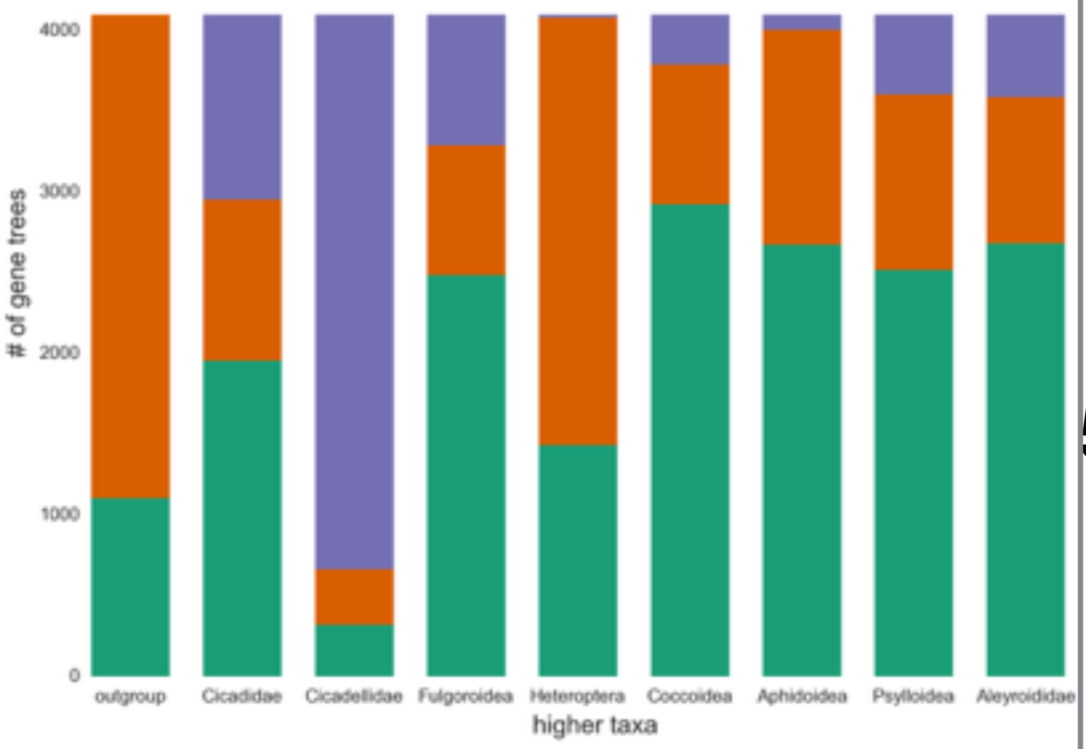


Comparison of monophyly among datasets

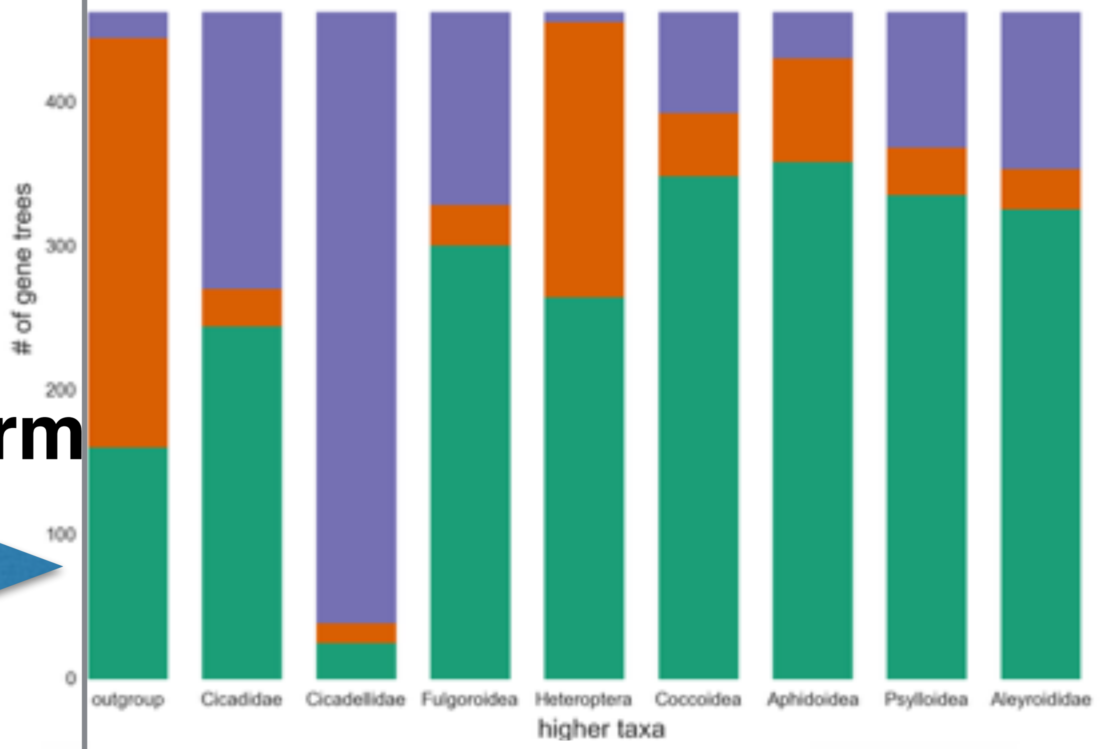
🟩 monophyletic 🟧 non-monophyletic 🟪 single sp.

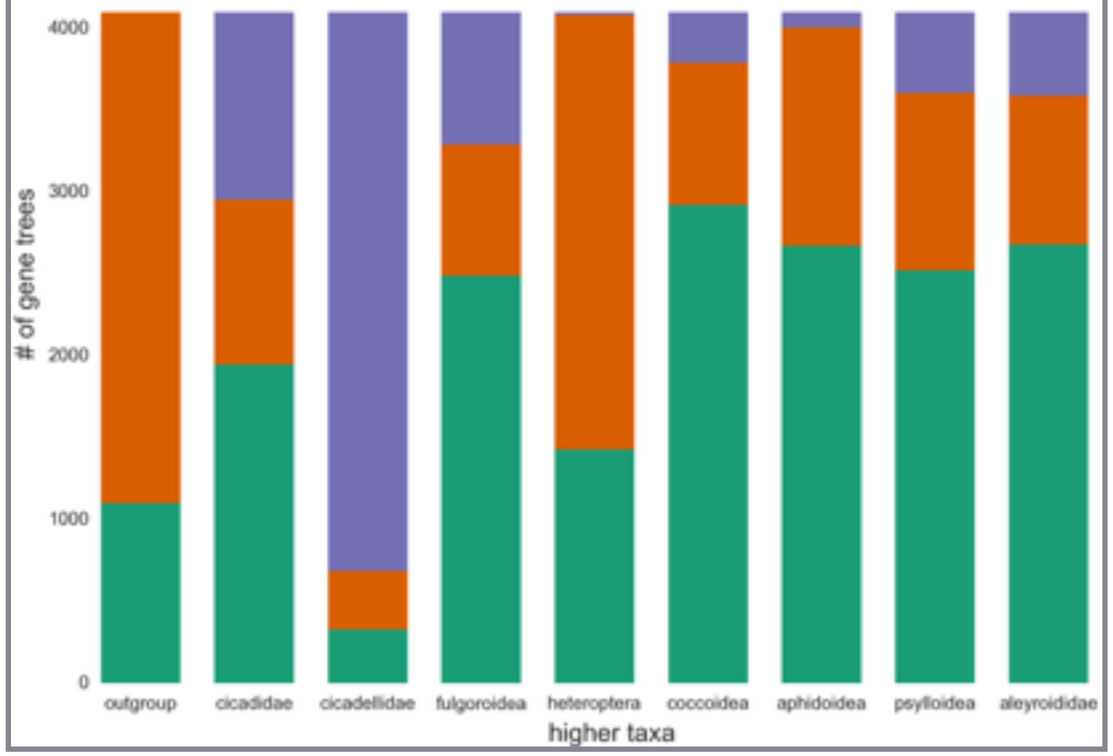
All genes

Curated dataset

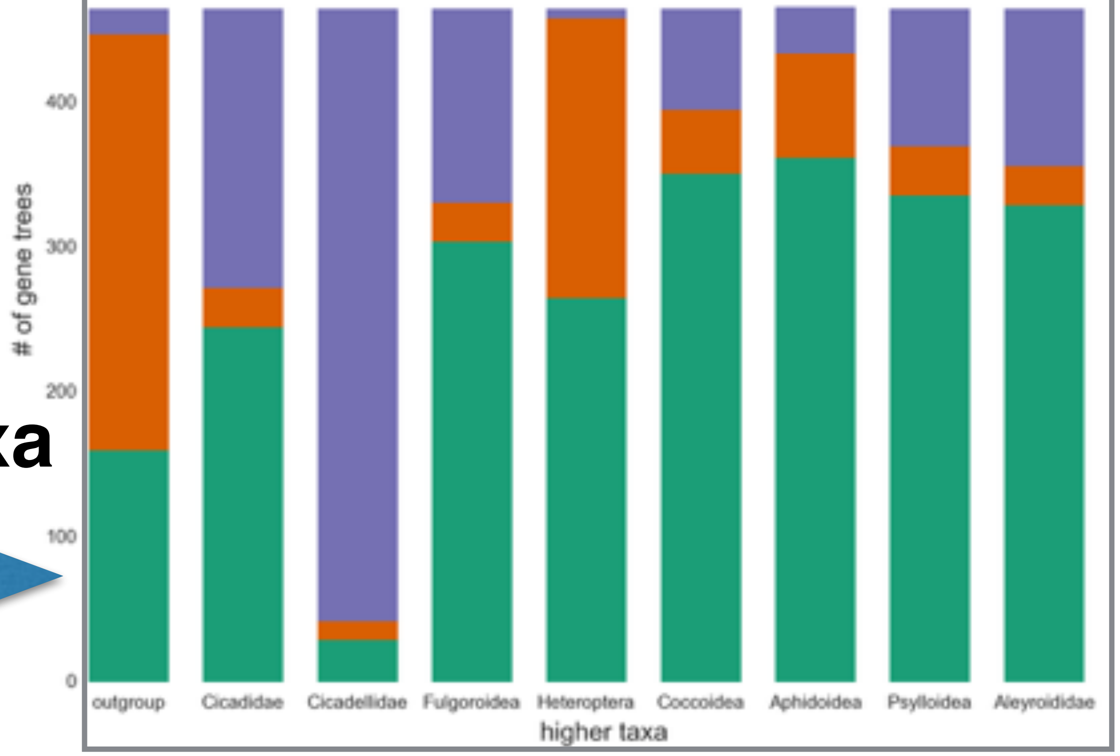


5 taxa rm





All taxa

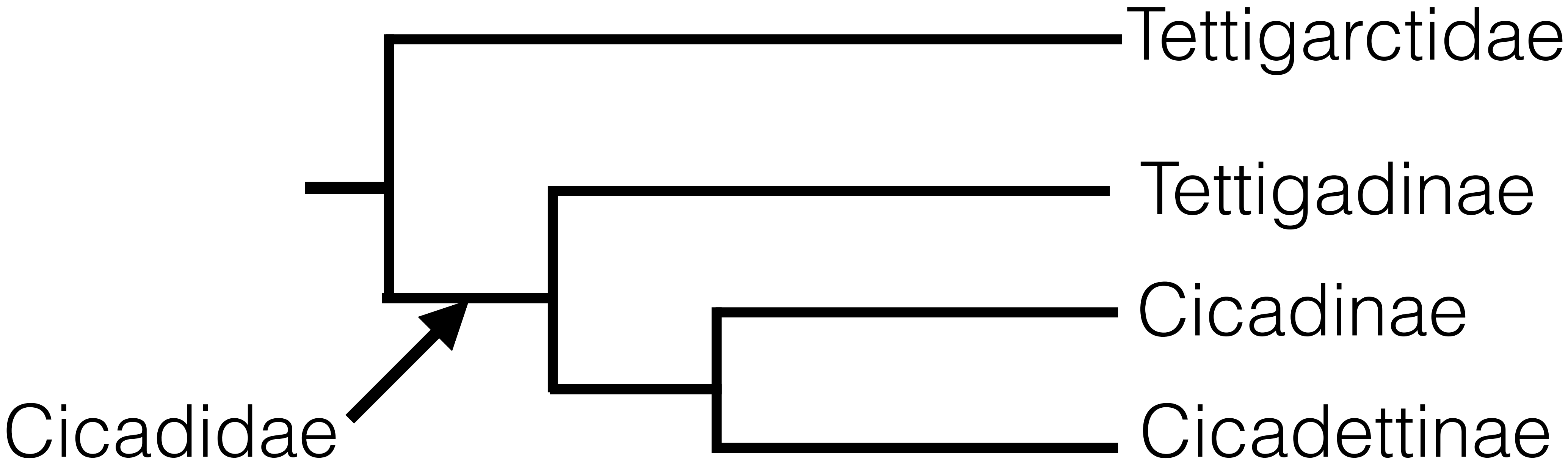


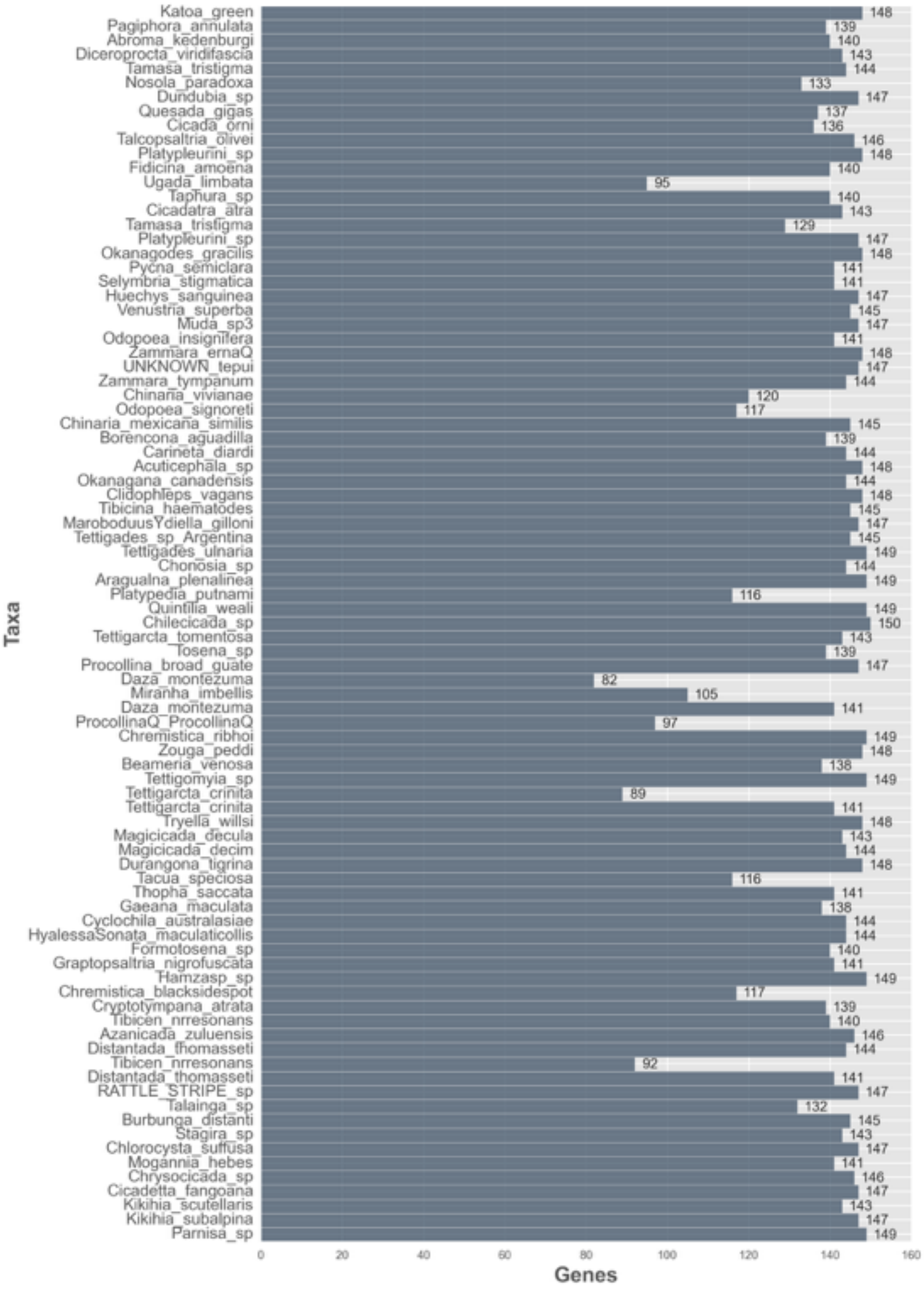
Cicadidae hybrid capture phylogenomics

Dealing with the unknown: what should the phylogeny look like?

- No molecular hypotheses of subfamily and tribal relationships

- Moulds (2005) proposed subfamily relationships and Australian tribal relationships (117 morphological characters)





Gene matrix occupancy

- 150 loci; 87 taxa; ~50k bp
- 2 taxa < 90 loci
- 2 taxa 90 < loci < 100
- 1 taxon with 150 loci

Dealing with the unknown: what are these loci?

Pipeline

1. Blast cds transcripts to loci or map transcriptome short reads to loci using bowtie2
2. Use MACSE (Ranwez et al. 2011) for first alignment
3. Use Muscle for refinement (-*refine*) to clean it up

Results

147 loci are a combination of coding and non-coding

3 loci are non-coding UTR’s

Sequence partitioning, modeling, and phylogeny estimation

1. Each gene partitioned by coding PartitionFinder and non-coding

2. Models estimated in PartitionFinder

3. Gblocks / no Gblocks

4. Phylogenies:

    - Partitioned gene trees: Garli

    - Concatenated matrices (including k-means cluster modeling (Frandsen et al. 2015)): RAxML

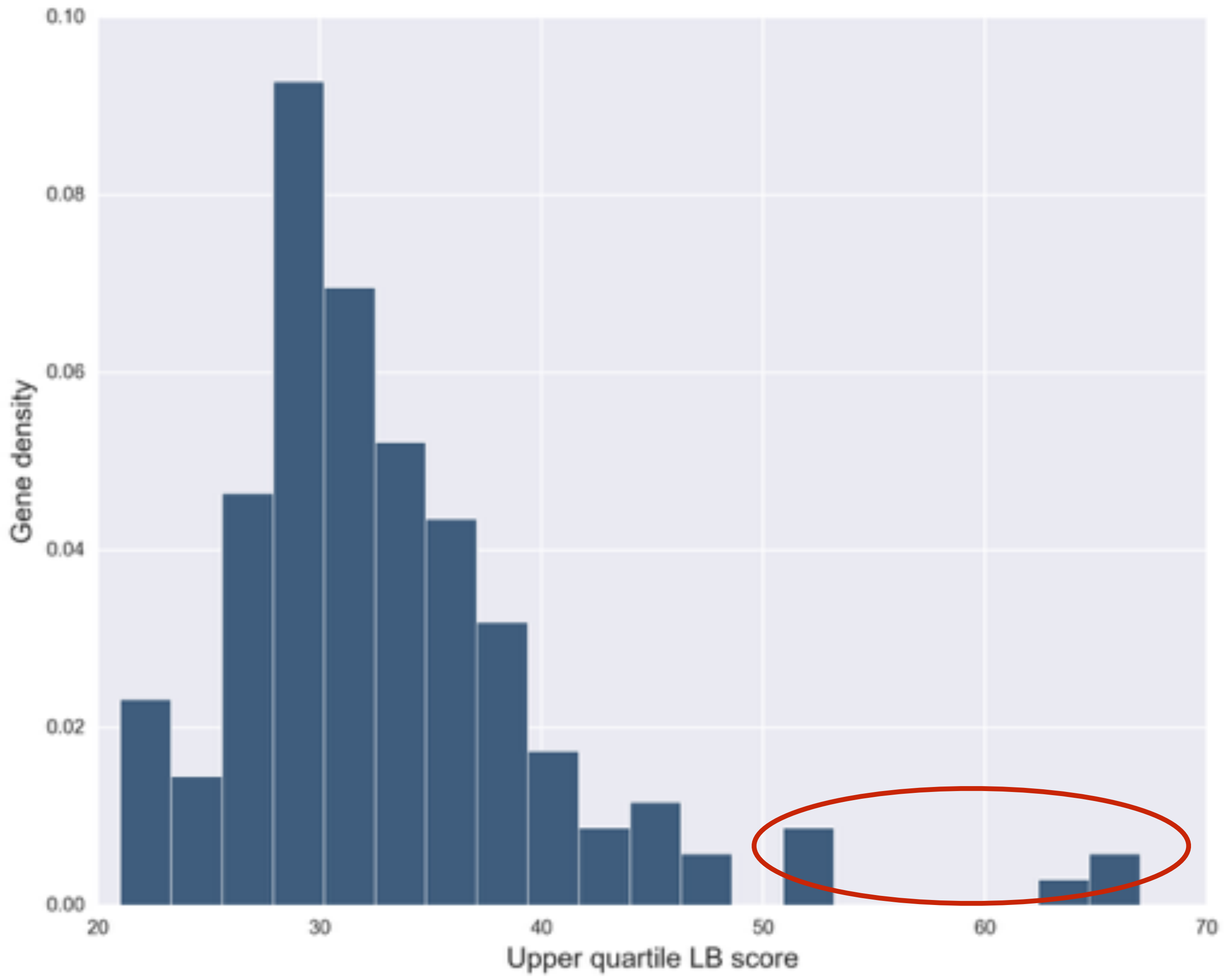
    - Concatenated partitioned matrices: RAxML

    - Gene tree / species tree methods: Astral

Gene filtering: base composition

- Chi-square test in BaCoCa (Kuck and Struck 2014)
- Cicadidae hybrid capture dataset: No significant differences at $p = 0.05$ -> $p = 0.20$

Gene tree filtering: removing loci with long branches



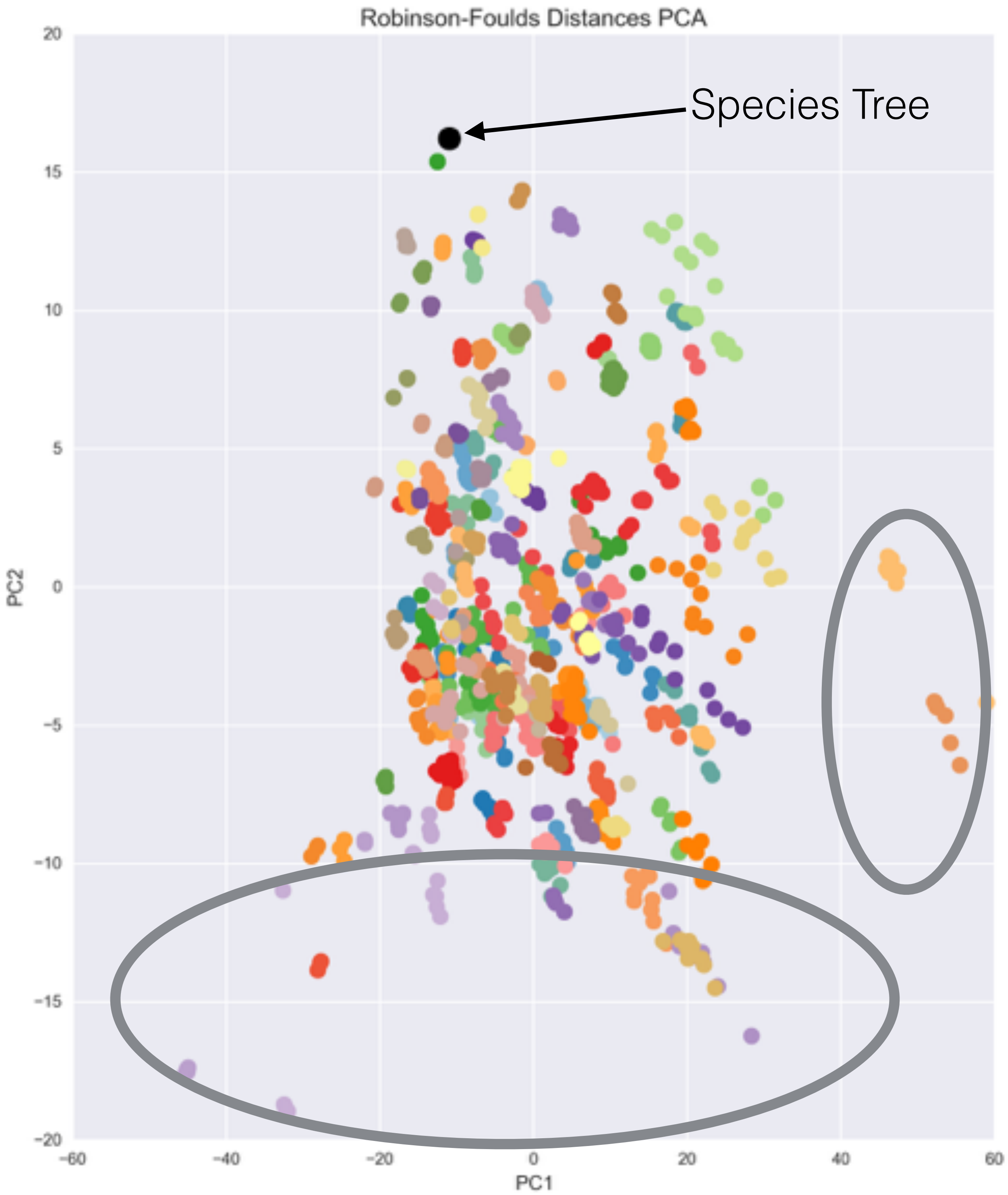
Long Branch Score

$$LB_i = \left(\frac{\overline{PD_i}}{\overline{PD_a}} - 1\right) * 100$$

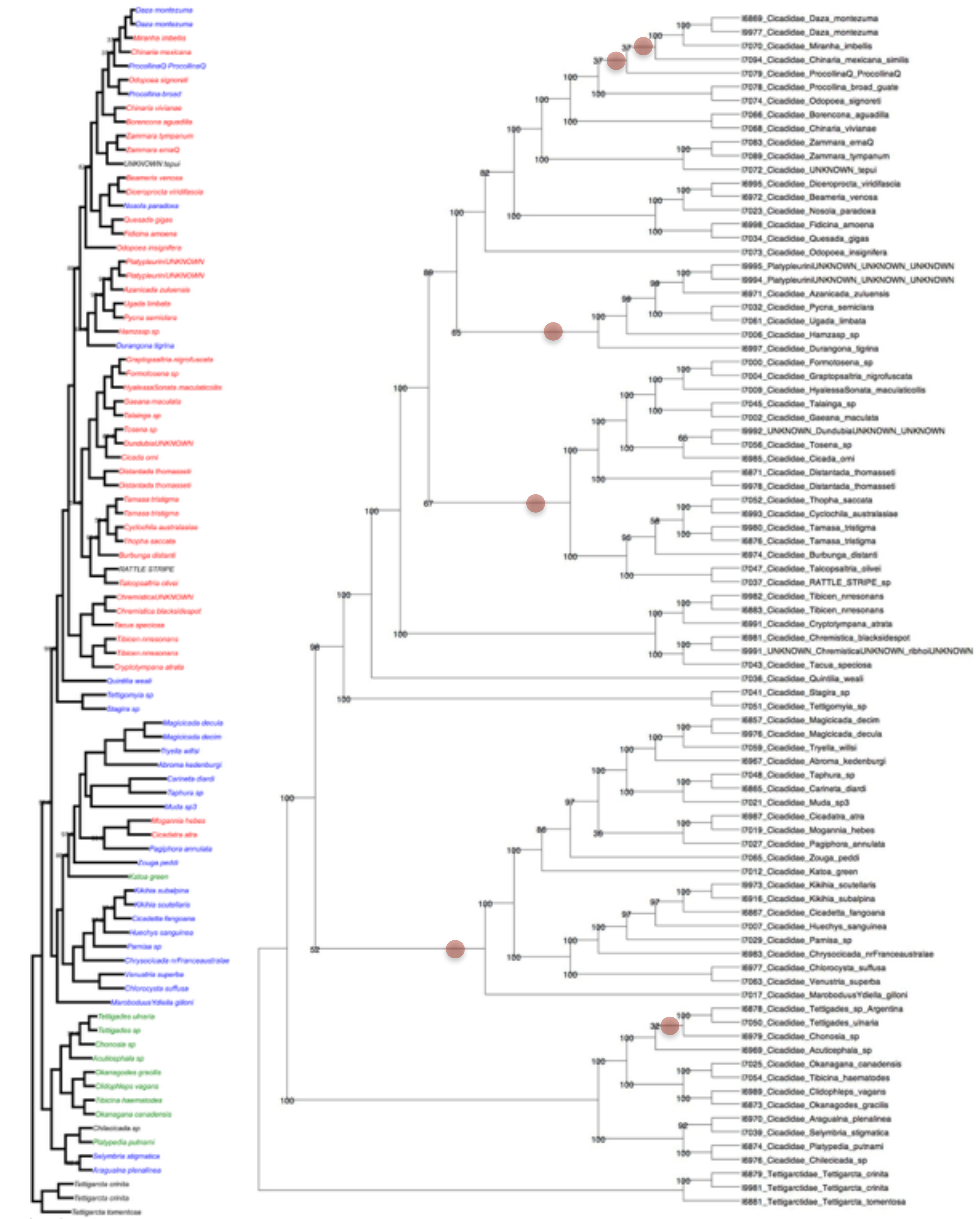
- mean pairwise patristic distance (PD) of a taxon to all other taxa in the tree relative to the avg. pairwise PD over all taxa

- LB score upper quartile of each partition in TreSpEx (Struck 2014)
- unrooted gene trees
- Brinkman & Philippe 2008; Bergsten 2005

Gene filtering: Robinson-Foulds distances



- Weighted Robinson-Foulds distances
    - shared bipartitions and sum of square differences in branch lengths
- 10 best trees from each gene tree search
- Species tree from RAxML partitioned concatenated search
- Removed 12 genes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Daza montezuma
Miranha imbellis
Chinaria mexicana
ProcollinaQ ProcollinaQ
Odopoea signoreti
Procollina broad
Chinaria vivianae
Borencona aguadilla
Zammara tympanum
Zammara ernaQ
UNKNOWN tepui
Beameria venosa
Diceroprocta viridifascia
Nosola paradoxa
Quesada gigas
Fidicina amoena
Odopoea insignifera
PlatypleuriniUNKNOWN
PlatypleuriniUNKNOWN
Azanicada zuluensis
Ugada limbata
Pycna semiclara
Hamzasp sp
Durangona tigrina
Graptopsaltria nigrofuscata
Formotosena sp
HyalessaSonata maculaticollis
Gaeana maculata
Talainga sp
Tosena sp
DundubiaUNKNOWN
Cicada orni
Distantada thomasseti
Distantada thomasseti
Tamasa tristigma
Tamasa tristigma
Cyclochila australasiae
Thopha saccata
Burbunga distanti
RATTLE STRIPE
Talcopsaltria olivei
ChremisticaUNKNOWN
Chremistica blacksidespot
Tacua speciosa
Tibicen nmesonans
Tibicen nmesonans
Cryptotympana atrata
Quintilia weali
Tettigomyia sp
Stagira sp
Magicicada decula
Magicicada decim
Tryella willsi
Abroma kedenburgi
Carineta diardi
Taphura sp
Muda sp3
Mogannia hebes
Cicadatra atra
Pagiphora annulata
Zouga peddi
Katoa green
Kikihia subalpina
Kikihia scutellaris
Cicadetta fangoana
Huechys sanguinea
Pamisa sp
Chrysocicada nrFranceaustralae
Venustria superba
Chlorocysta suffusa
MaroboduusYdiella gilloni
Tettigades ulnaria
Tettigades sp
Chonosia sp
Acuticephala sp
Okanagodes gracilis
Clidophleps vagans
Tibicina haematodes
Okanagana canadensis
Chilecicada sp
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I6881_Tettigarctidae_Tettigarcta_tomentosa

Concatenated Tree

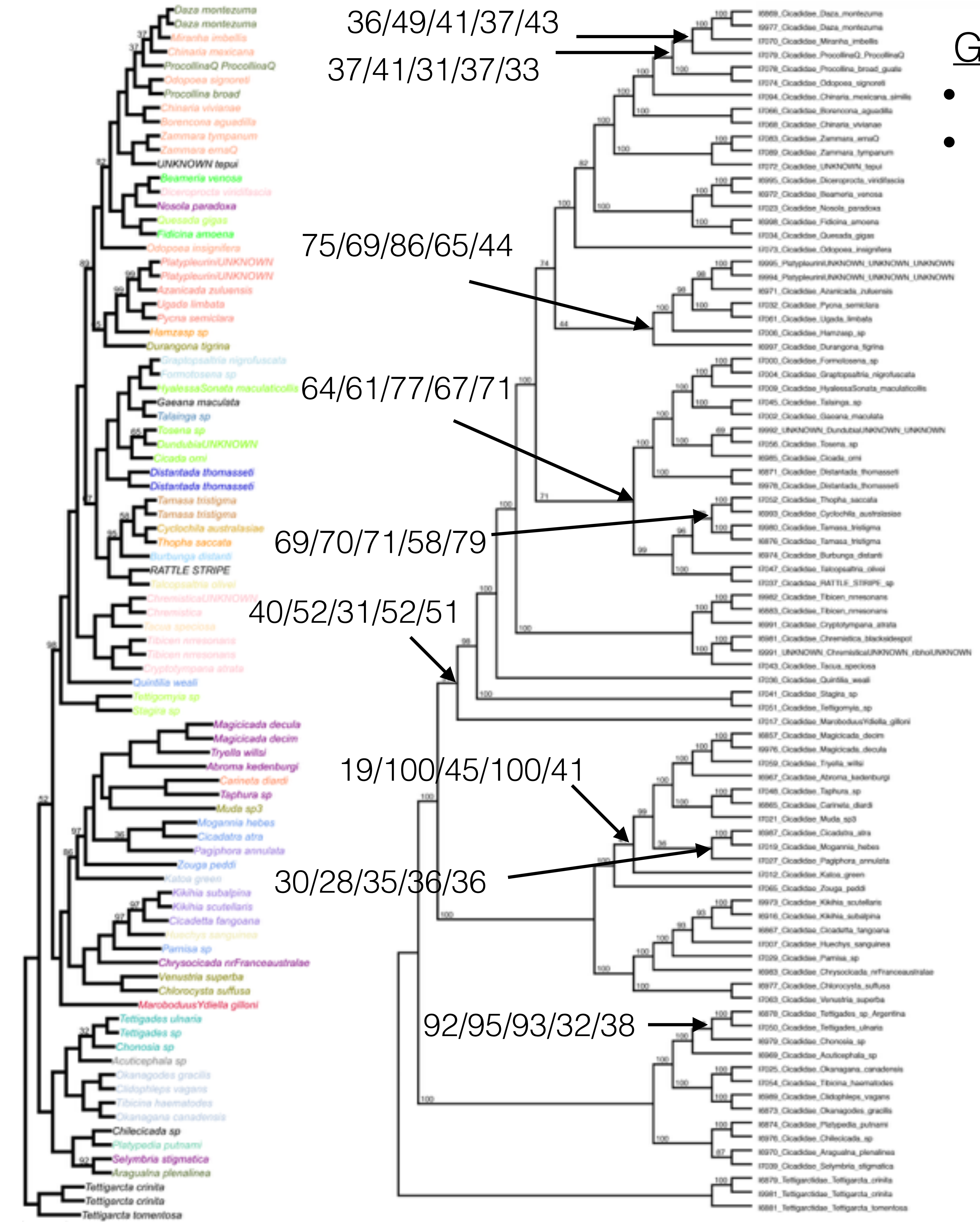
- 6 branches not supported by bootstrap support
- 2 unsupported branches are sister to larger clades
- RAxML gene partitioned

🟥 Cicadinae

🟦 Cicadettinae

🟩 Tettigadinae

● BS < 67



Gene filtering and alternative models

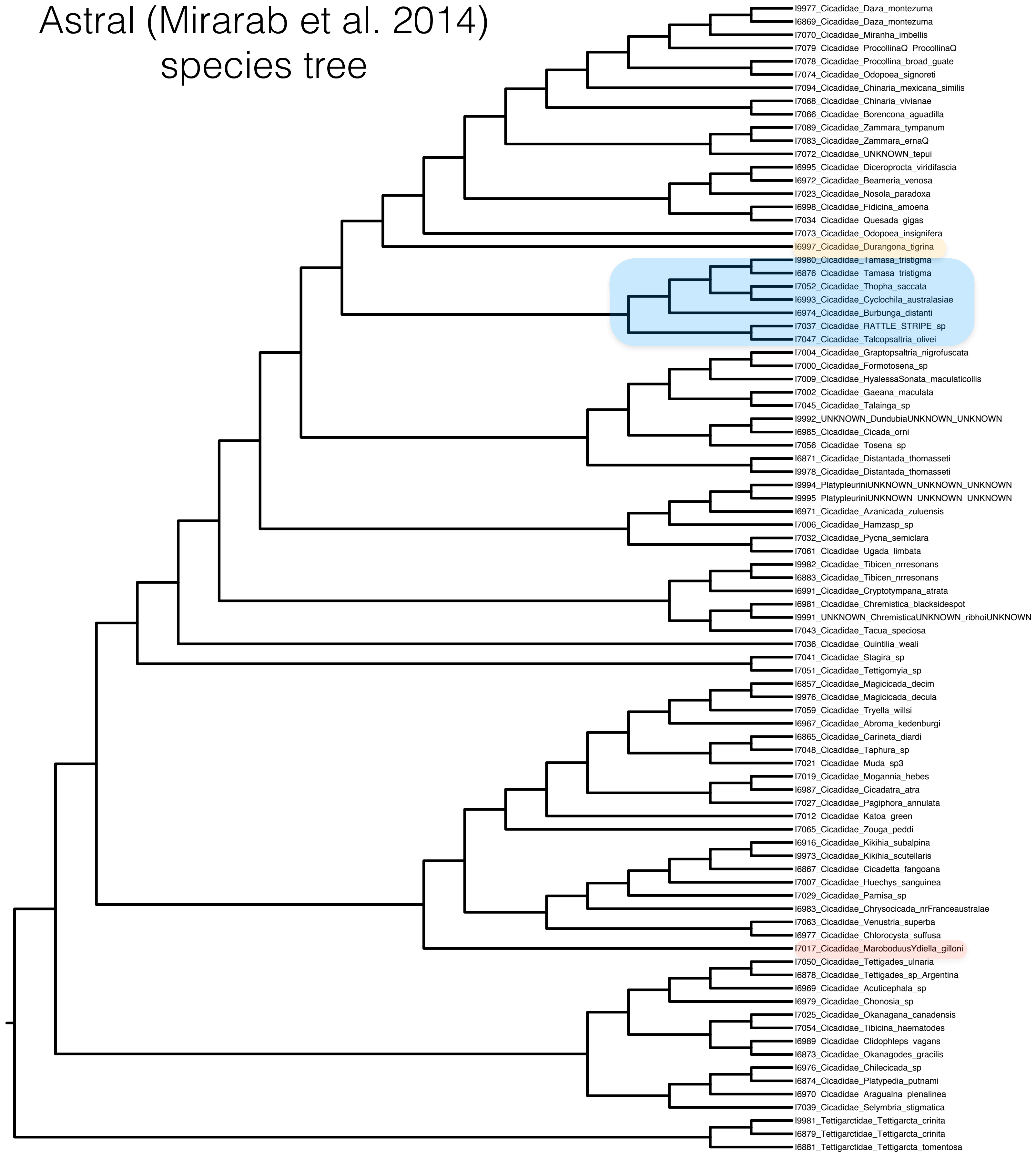
- all produce the same concat tree
- six unsupported branches still unsupported to varying degrees in addition to other branches

Bootstrap support values =
no filtering, one model /
Gblocks, partitioned/
Gblocks, K-means/
RF 12 loci rm, gene partitioned/
RF 12 loci rm, k-means partitioned

unique colors = tribes

Gene tree / species tree analysis

Astral (Mirarab et al. 2014) species tree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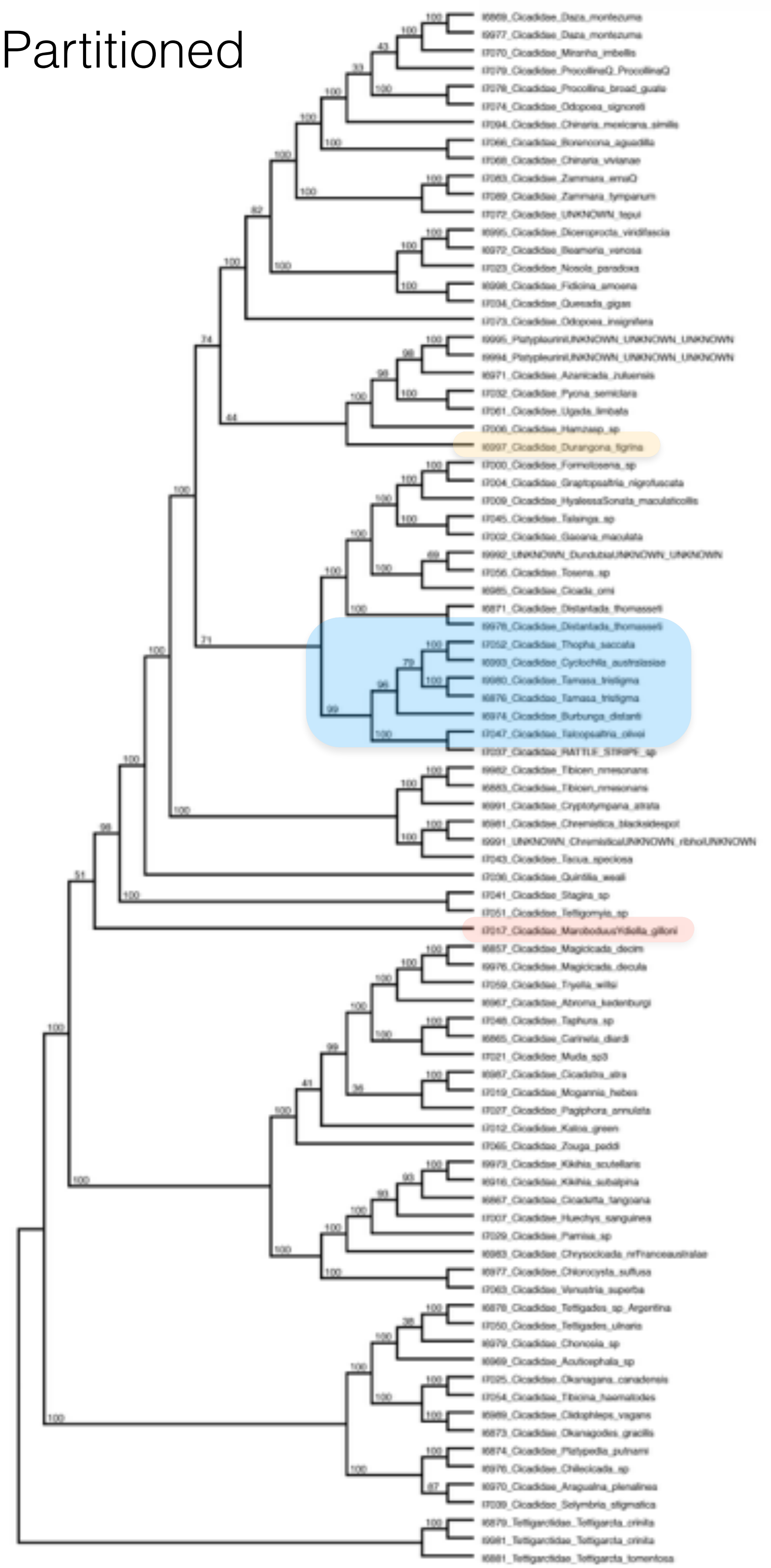
I6881_Tettigarctidae_Tettigarcta_tomentosa

RAxML Partitioned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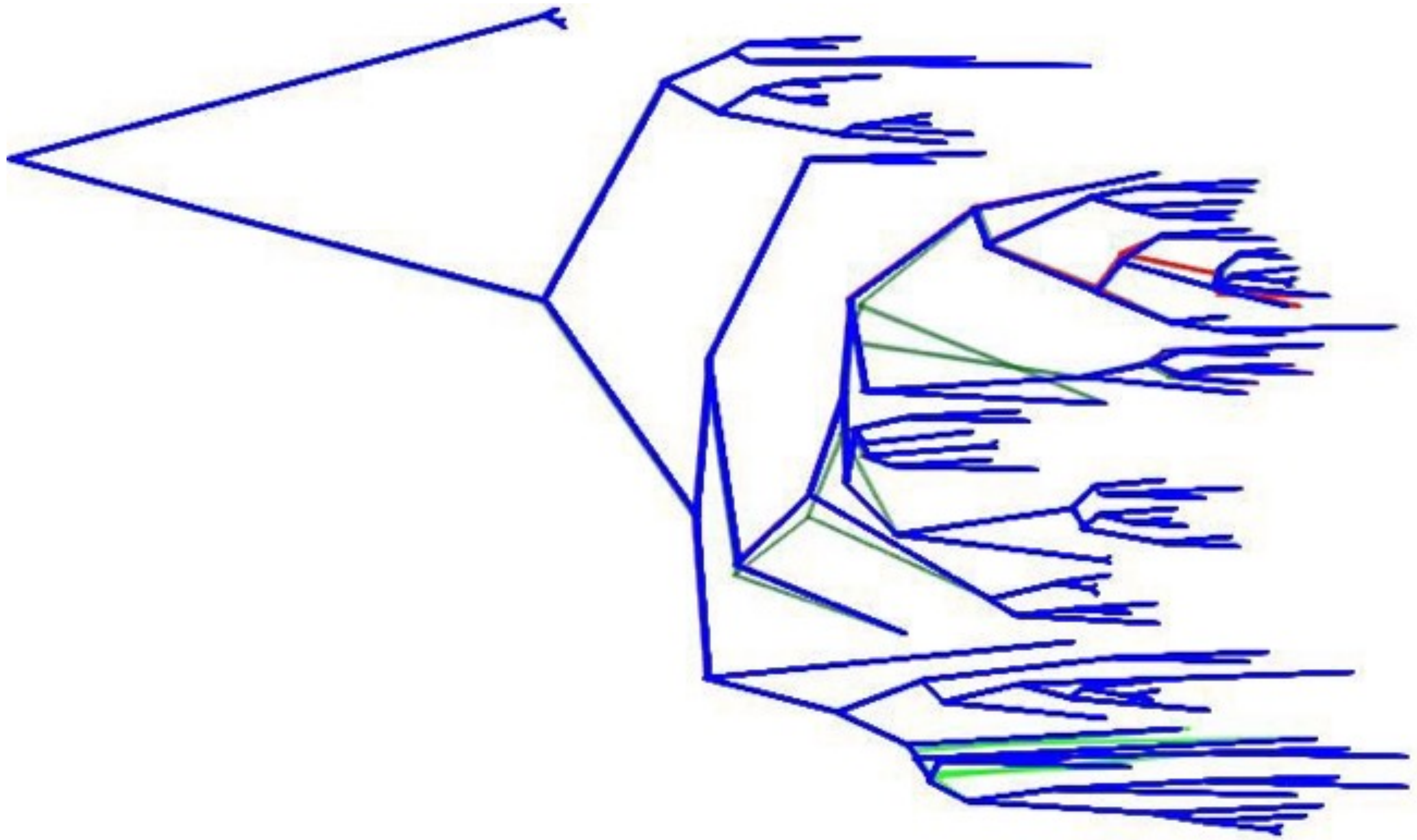
I7021_Cicadidae_Muda_sp3
I6987_Cicadidae_Cicadatra_atra
I7019_Cicadidae_Mogannia_hebes
I7027_Cicadidae_Pagiphora_annulata
I7012_Cicadidae_Katoa_green
I7065_Cicadidae_Zouga_peddi
I9973_Cicadidae_Kikihia_scutellaris
I6916_Cicadidae_Kikihia_subalpina
I6867_Cicadidae_Cicadetta_fangoana
I7007_Cicadidae_Huechys_sanguinea
I7029_Cicadidae_Parnisa_sp
I6983_Cicadidae_Chrysocicada_nrFranceaustralae
I6977_Cicadidae_Chlorocysta_suffusa
I7063_Cicadidae_Venustria_superba
I6878_Cicadidae_Tettigades_sp_Argentina
I7050_Cicadidae_Tettigades_ulnaria
I6979_Cicadidae_Chonosia_sp
I6969_Cicadidae_Acuticephala_sp
I7025_Cicadidae_Okanagana_canadensis
I7054_Cicadidae_Tibicina_haematodes
I6989_Cicadidae_Clidophleps_vagans
I6873_Cicadidae_Okanagodes_gracilis
I6874_Cicadidae_Platypedia_putnami
I6976_Cicadidae_Chilecicada_sp
I6970_Cicadidae_Aragualna_plenalinea
I7039_Cicadidae_Selymbria_stigmatica
I6879_Tettigarctidae_Tettigarcta_crinita
I9981_Tettigarctidae_Tettigarcta_crinita
I6881_Tettigarctidae_Tettigarcta_tomentosa

Exploring tree space: highly divergent starting trees



- 100 random starting trees sharing no bipartitions from ML tree
- RAxML gene partitioned analyses

Conclusions

1. Most of the tree is supported, but some rogue taxa and clades

2. Higher taxonomy needs to be reexamined in light of well-supported relationships

3. Additional sensitivity analyses needed

Questions?