Whole Genome Alignment

Phylogenomics June 2021

Start a job

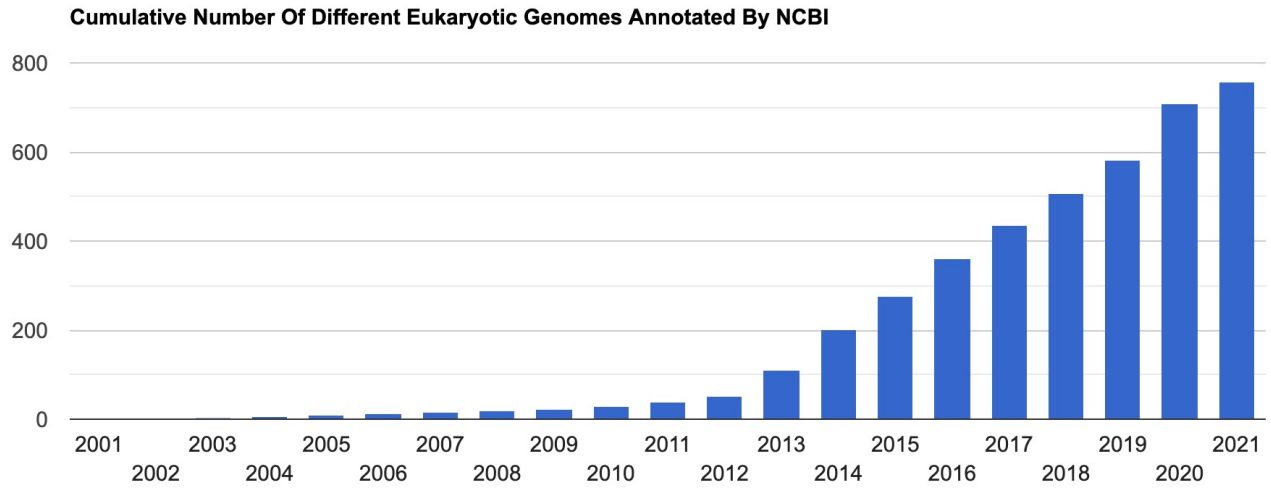
- Go to https://github.com/ForBioPhylogenomics/tutorials/blob/main/whole_genome_alignment/README.md
- Do this part:

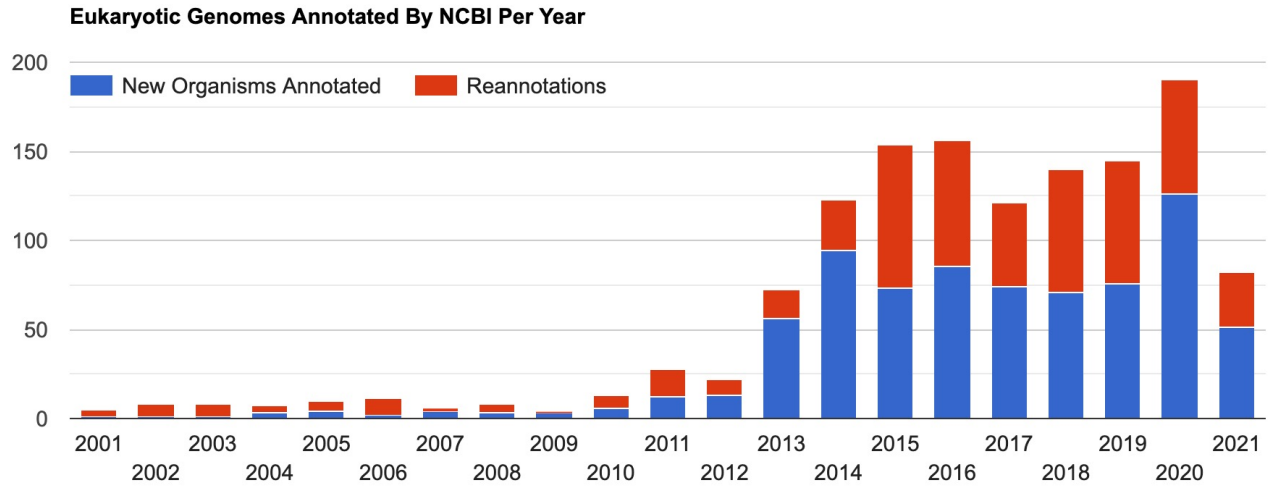
```
cd /cluster/projects/nn9458k/phylogenomics/
mkdir $YOURNAME
cd $YOURNAME
mkdir -p cichlids
cd cichlids
cp ../..//week2/data/cichlids/scripts/* .
```

- Submit this job: `sbatch run_cactus_chr5.sh`
- Two people can also submit this:

```
sbatch --reservation=nn9458k run_cactus_all.sh
```

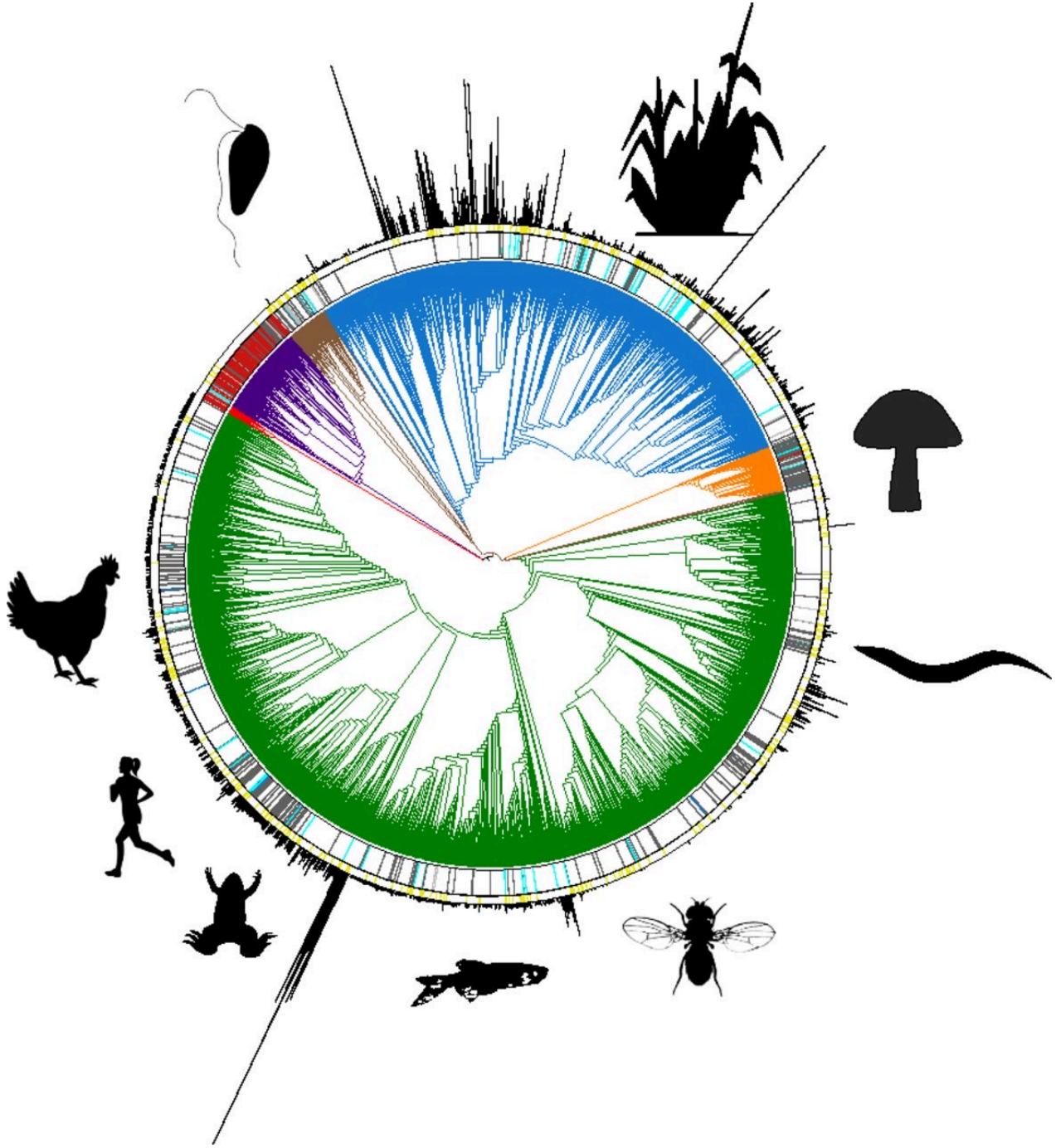
Number of annoted eukaryotic genomes increasing





https://www.ncbi.nlm.nih.gov/genome/annotation_euk/

Status of sequencing of life



Lewin et al 2018

Earth BioGenome Project and others

PERSPECTIVE

Earth BioGenome Project: Sequencing life for the future of life

Harris A. Lewin[a,b,c,d,1], Gene E. Robinson[e], W. John Kress[f], William J. Baker[g], Jonathan Coddington[f], Keith A. Crandall[h], Richard Durbin[i,j], Scott V. Edwards[k,l], Félix Forest[g], M. Thomas P. Gilbert[m,n], Melissa M. Goldstein[o], Igor V. Grigoriev[p,q], Kevin J. Hackett[r], David Haussler[s,t], Erich D. Jarvis[u], Warren E. Johnson[v], Aristides Patrinos[w], Stephen Richards[x], Juan Carlos Castilla-Rubio[y,z], Marie-Anne van Sluys[aa,bb], Pamela S. Soltis[cc], Xun Xu[dd], Huanming Yang[ee], and Guojie Zhang[dd,ff,gg]

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PERSPECTIVE

Analysis

A comparative genomics multitool for scientific discovery and conservation

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Accepted: 27 July 2020
Published online: 11 November 2020
Open access
Check for updates

Zoonomia Consortium*

The Zoonomia Project is investigating the genomics of shared and specialized traits in eutherian mammals. Here we provide genome assemblies for 131 species, of which all but 9 are previously uncharacterized, and describe a whole-genome alignment of 240 species of considerable phylogenetic diversity, comprising

Article

Dense sampling of bird diversity increases power of comparative genomics

https://doi.org/10.1038/s41586-020-2873-9
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Open access
Check for updates

A list of authors and affiliations appears at the end of the paper.

Whole-genome sequencing projects are increasingly populating the tree of life and characterizing biodiversity[1–4]. Sparse taxon sampling has previously been proposed to confound phylogenetic inference[5], and captures only a fraction of the genomic diversity. Here we report a substantial step towards the dense representation of avian phylogenetic and molecular diversity, by analysing 363 genomes from 92.4% of bird families—including 267 newly sequenced genomes produced for phase II of the Bird

Article

Towards complete and error-free genome assemblies of all vertebrate species

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Published online: 28 April 2021
Open access
Check for updates

A list of authors and their affiliations appears at the end of the paper.

High-quality and complete reference genome assemblies are fundamental for the application of genomics to biology, disease, and biodiversity conservation. However, such assemblies are available for only a few non-microbial species[1–4]. To address this issue, the international Genome 10K (G10K) consortium[5,6] has worked over a five-year period to evaluate and develop cost-effective methods for assembling highly accurate and nearly complete reference genomes. Here we present lessons learned from

The dataset

ID	Species	Common name	Genus
neobri	*Neolamprologus brichardi*	lyretail cichlid	*Neolamprologus*
neomar	*Neolamprologus marunguensis*	?	*Neolamprologus*
neogra	*Neolamprologus gracilis*	?	*Neolamprologus*
neooli	*Neolamprologus olivaceous*	?	*Neolamprologus*
neopul	*Neolamprologus pulcher*	daffodil cichlid	*Neolamprologus*
orenil	*Oreochromis niloticus*	Nile tilapia	*Oreochromis*

Progressive Cactus

- We need:
  - Softmasked genome assemblies
  - Guide tree

Article

Progressive Cactus is a multiple-genome aligner for the thousand-genome era

https://doi.org/10.1038/s41586-020-2871-y

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Published online: 11 November 2020

Open access

Check for updates

Joel Armstrong[1], Glenn Hickey[1], Mark Diekhans[1], Ian T. Fiddes[1], Adam M. Novak[1], Alden Deran[1], Qi Fang[2,3], Duo Xie[2,4], Shaohong Feng[2,5], Josefin Stiller[3], Diane Genereux[6], Jeremy Johnson[6], Voichita Dana Marinescu[7], Jessica Alföldi[6], Robert S. Harris[8], Kerstin Lindblad-Toh[6,7], David Haussler[1,9], Elinor Karlsson[6,10,11], Erich D. Jarvis[9,12], Guojie Zhang[3,5,13,14]✉ & Benedict Paten[1]✉

New genome assemblies have been arriving at a rapidly increasing pace, thanks to decreases in sequencing costs and improvements in third-generation sequencing

Soft masking

- Masking can be hard or soft:
  - Hard: replace repeats with Ns
  - Soft: lower case instead of capital

```
Original: AGGTCACTACTACTACTGACTTAC
Softmask: AGGTCactactactactGACTTAC
Hardmask: AGGTCNNNNNNNNNNNNGACTTAC
```

- With hardmasking we lose information
- Want softmasking of repeats

- Alignments will not start inside masked regions, avoids trying to align one repeat copy to all others

How to mask

- RepeatModeler/RepeatMasker:
  - Use RepeatModeler to generate a *de novo* repeat library for your species
  - Use RepeatMasker to mask repeats based on repeat library or existing database
  - Pros: Characterizes/annotates most/many repeats (STRs, TEs, etc)
  - Cons: Take quite some time (a day or so for a medium sized genome (1 Gbp))
- Red:
  - Use Red to characterize and mask all repetitive sequence in a genome
  - Pros: Quick, and only characterises actual repetitive sequence (ignores TEs only occurring once or a few times)
  - Cons: Doesn’t annotate the repeats (you don’t know what it is)

Exercises

- Go to https://github.com/ForBioPhylogenomics/tutorials/blob/main/whole_genome_alignment/README.md and do the tutorial up until "Creating a guide tree with Mash"

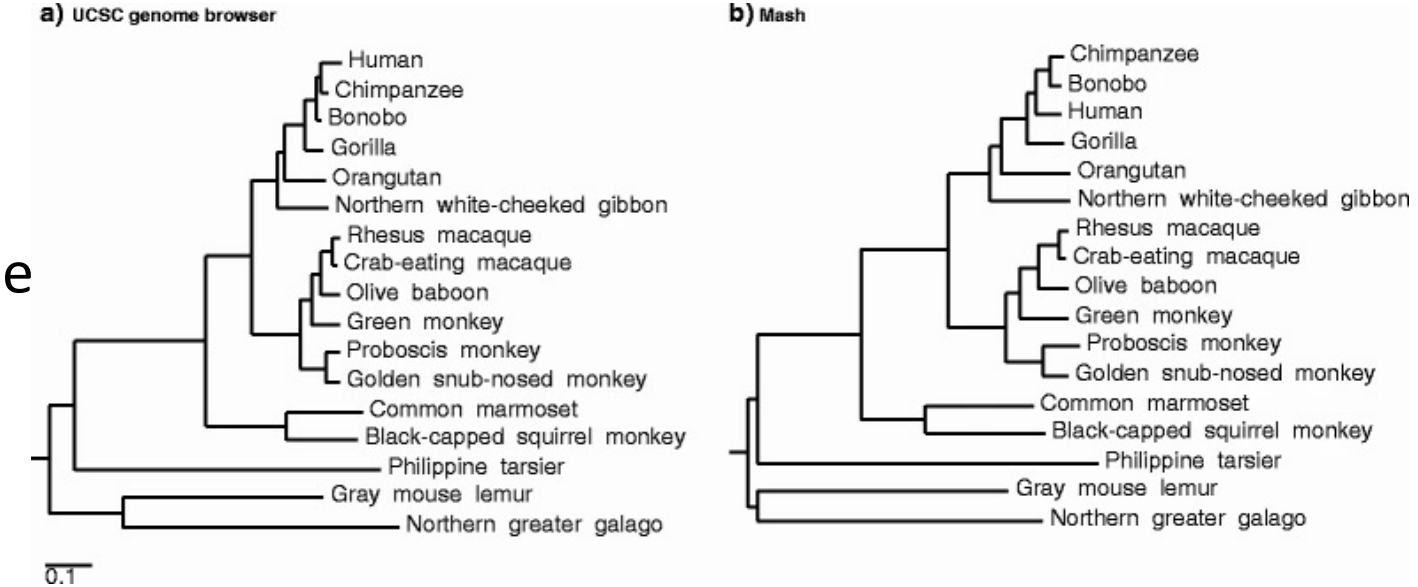
Results from masking

```
-rw-rw-r-- 1 olekto nn9458k   43497196 Jun  9 21:40 neobri.repeats.bed
-rw-rw-r-- 1 olekto nn9458k  275320088 Jun  9 21:40 neobri.repeats.fasta
-rw-rw-r-- 1 olekto nn9458k  865018698 Jun  9 21:40 neobri.softmasked.fa
-rw-rw-r-- 1 olekto nn9458k   45092622 Jun  9 22:16 neogra.repeats.bed
-rw-rw-r-- 1 olekto nn9458k  262196845 Jun  9 22:16 neogra.repeats.fasta
-rw-rw-r-- 1 olekto nn9458k  672846647 Jun  9 22:14 neogra.softmasked.fa
-rw-rw-r-- 1 olekto nn9458k   46494946 Jun  9 22:00 neomar.repeats.bed
-rw-rw-r-- 1 olekto nn9458k  269948250 Jun  9 22:01 neomar.repeats.fasta
-rw-rw-r-- 1 olekto nn9458k  683501275 Jun  9 21:59 neomar.softmasked.fa
-rw-rw-r-- 1 olekto nn9458k   46074867 Jun  9 22:02 neooli.repeats.bed
-rw-rw-r-- 1 olekto nn9458k  267874589 Jun  9 22:03 neooli.repeats.fasta
-rw-rw-r-- 1 olekto nn9458k  681893391 Jun  9 22:01 neooli.softmasked.fa
-rw-rw-r-- 1 olekto nn9458k   45076389 Jun  9 22:03 neopul.repeats.bed
-rw-rw-r-- 1 olekto nn9458k  262152533 Jun  9 22:03 neopul.repeats.fasta
-rw-rw-r-- 1 olekto nn9458k  674817636 Jun  9 22:02 neopul.softmasked.fa
-rw-rw-r-- 1 olekto nn9458k   30337323 Jun  9 21:52 orenil.repeats.bed
-rw-rw-r-- 1 olekto nn9458k  347698289 Jun  9 21:53 orenil.repeats.fasta
-rw-rw-r-- 1 olekto nn9458k 1025835685 Jun  9 21:52 orenil.softmasked.fa
```

Questions?

Obtaining a guide tree

- Often an complicated process
  - Find paralogs in some way
  - Align them to each other
  - Run some tree inference tool on the alignment
- Mash: fast genome distance estimation
  - Reduces sequences to sketches that are a thousandth as large
  - Can be used to quickly find distances between genomes



Ondov et al 2016

Distance matrix

- Mash triangle gives a distance matrix
- RapidNJ can generate a neighbour-joining tree based on a distance matrix

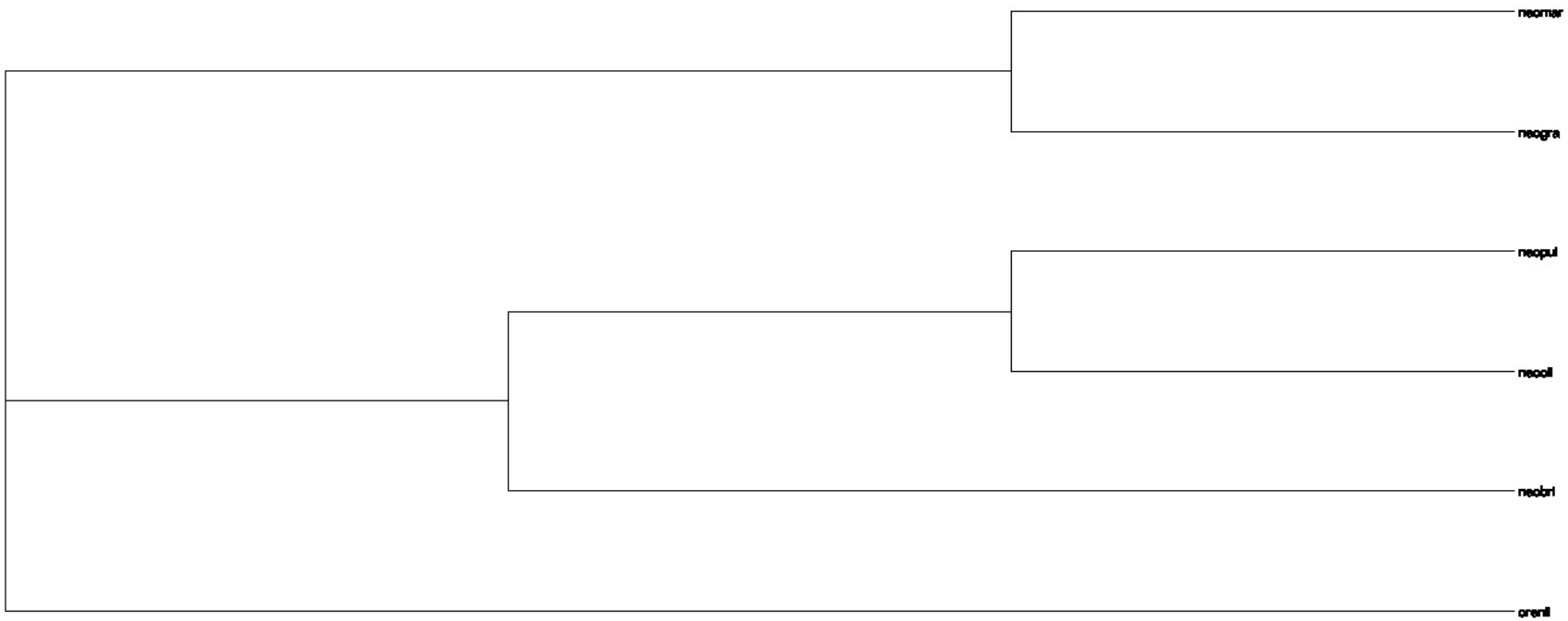
```
       6
neobri
neogra 0.00981378
neomar 0.00985499   0.00888862
neooli 0.00904645   0.00948708   0.0090861
neopul 0.00924552   0.00924552   0.00932572   0.00659353
orenil 0.0519203    0.0509537    0.0515304    0.0509537    0.0511448
```

Exercises

- Go to https://github.com/ForBioPhylogenomics/tutorials/blob/main/whole_genome_alignment/README.md and continue the tutorial up until "Running Cactus"

The guide tree

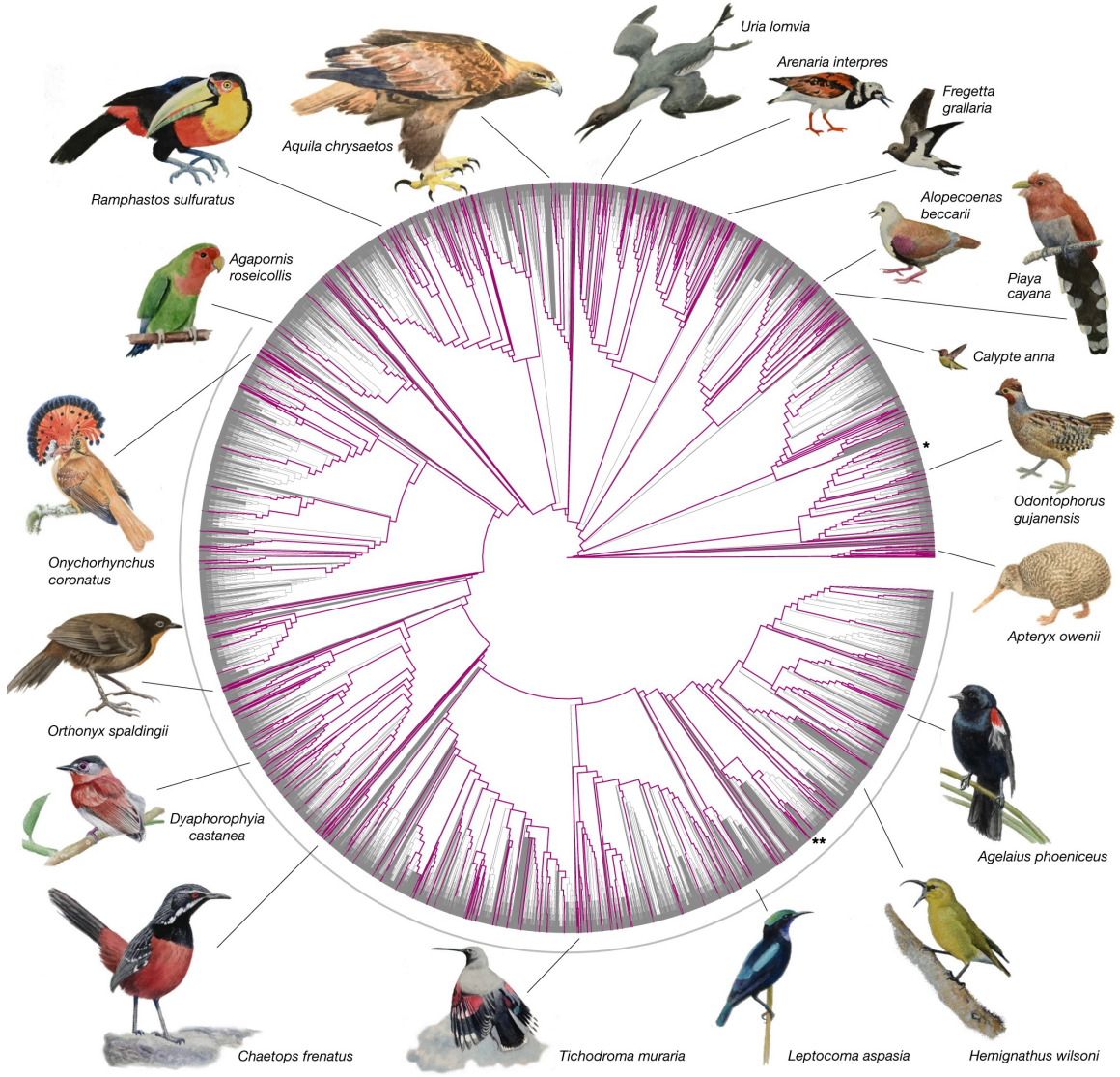
```
((neomar:0.0045736,neogra:0.004315):0.00021445,
((neopul:0.0033453,neooli:0.0032482):0.00094829,
neobri:0.0049009):0.00032849,orenil:0.046583);
```



Questions?

Bird 10k project

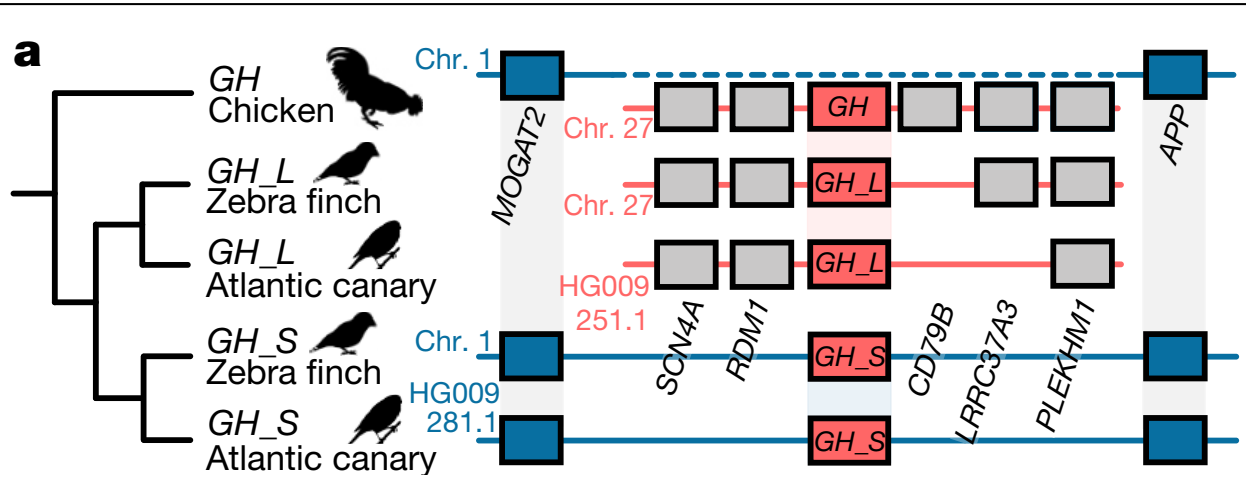
- 363 species (of 10,135 total), covering 92.4 % of families
- 267 new species
- Purple branches are with genomes



Feng et al 2020

Cactus alignment informs synteny

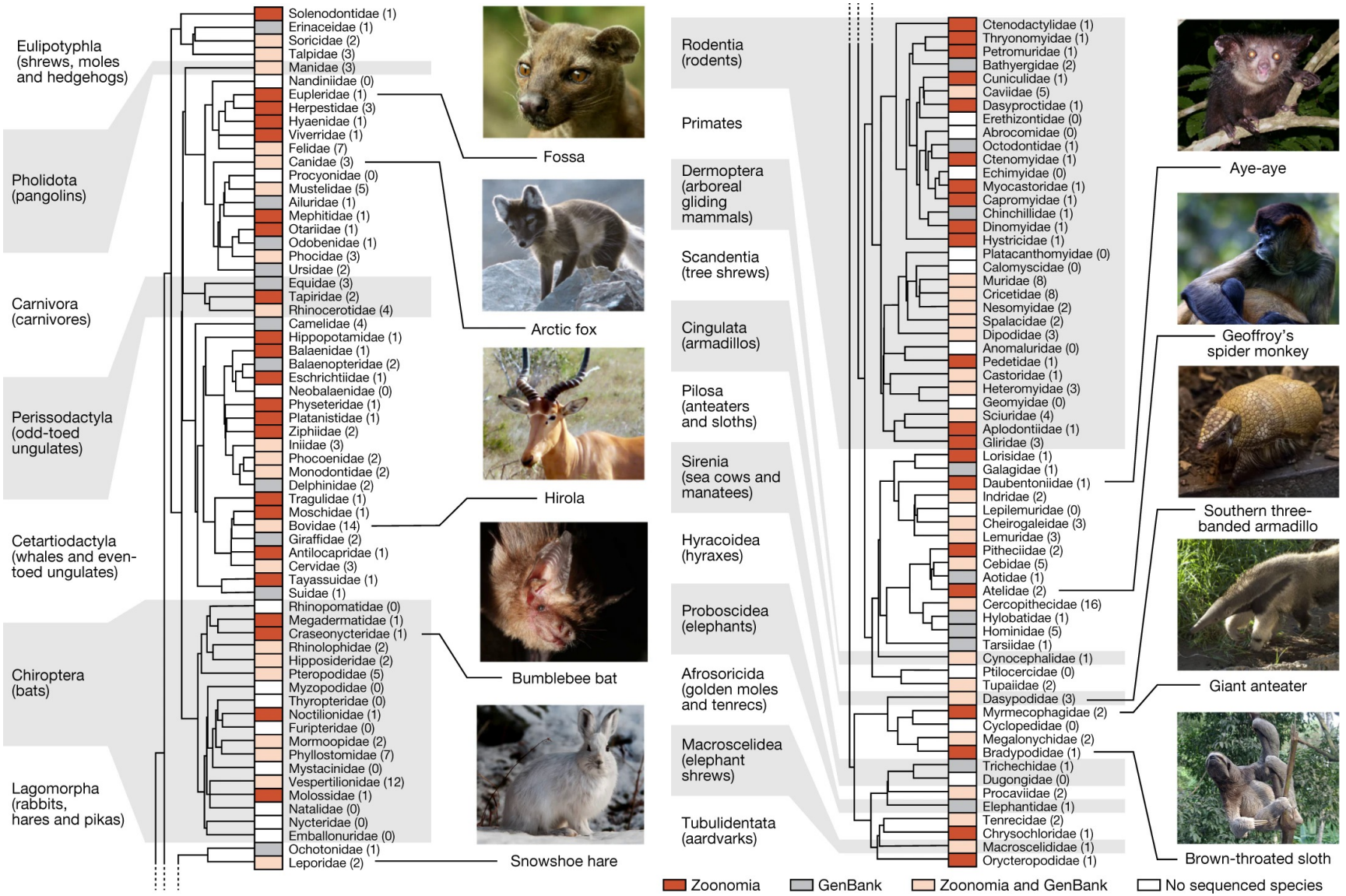
- *GH* one copy in chicken, two in Passeriformes
- Based on synteny, *GH_L* is ancestral, and not *GH_S* even though it is highly similar.



Feng et al 2020

Zoonomia

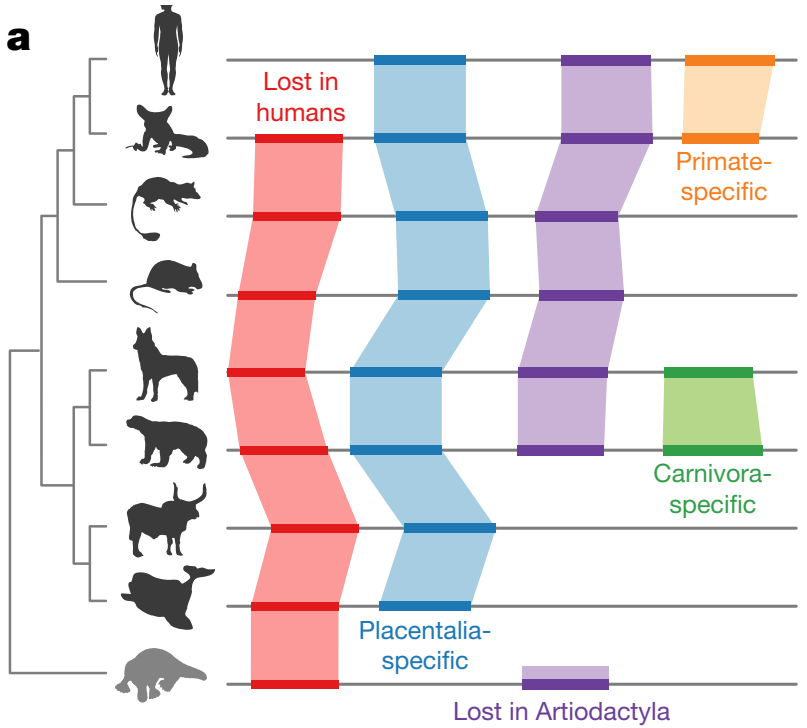
- 240 mammalian species, covering 83 % of families
- 131 new species



Zoonomia Consortium et al 2020

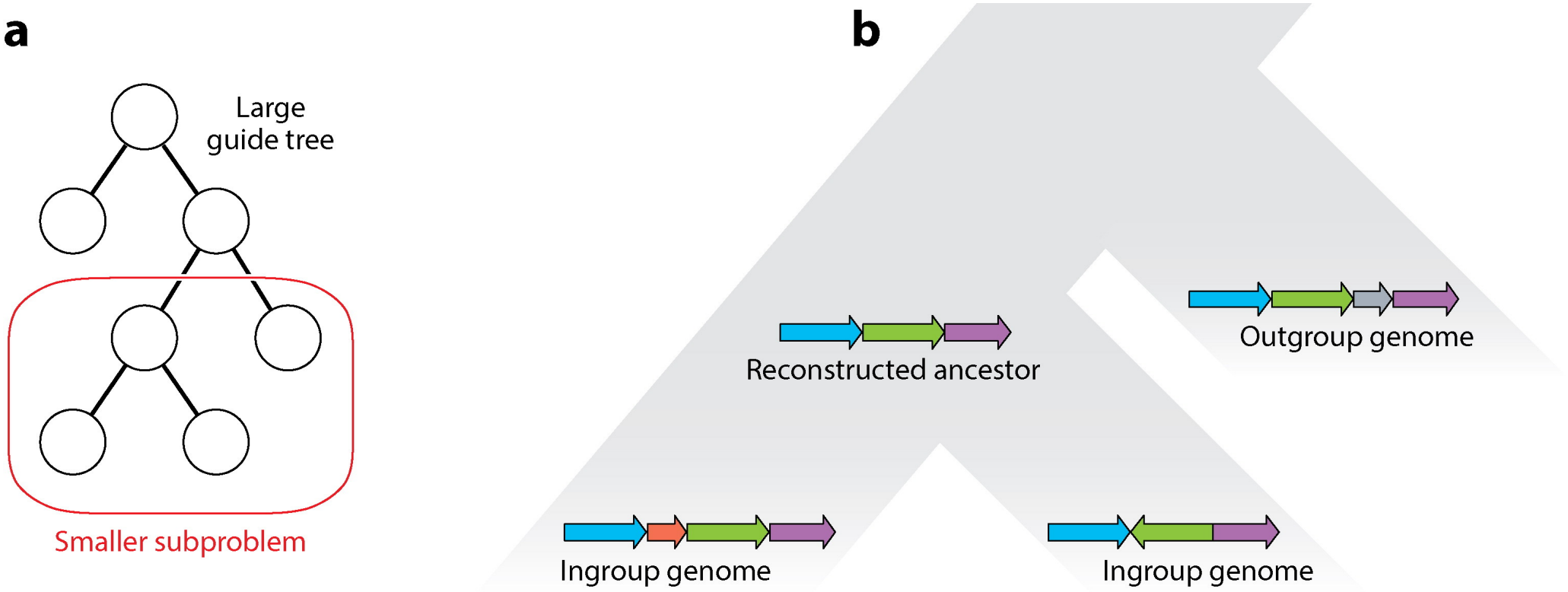
Uses of the alignment

- Alignment has been used to compare the structure of ACE2 (receptor for SARS-CoV-2) and identified 47 mammals with high likelihood of being virus reserviors (Damas et al 2020; PNAS)
- Reference free alignment detects sequence absent from human and other lineages



Zoonomia Consortium et al 2020

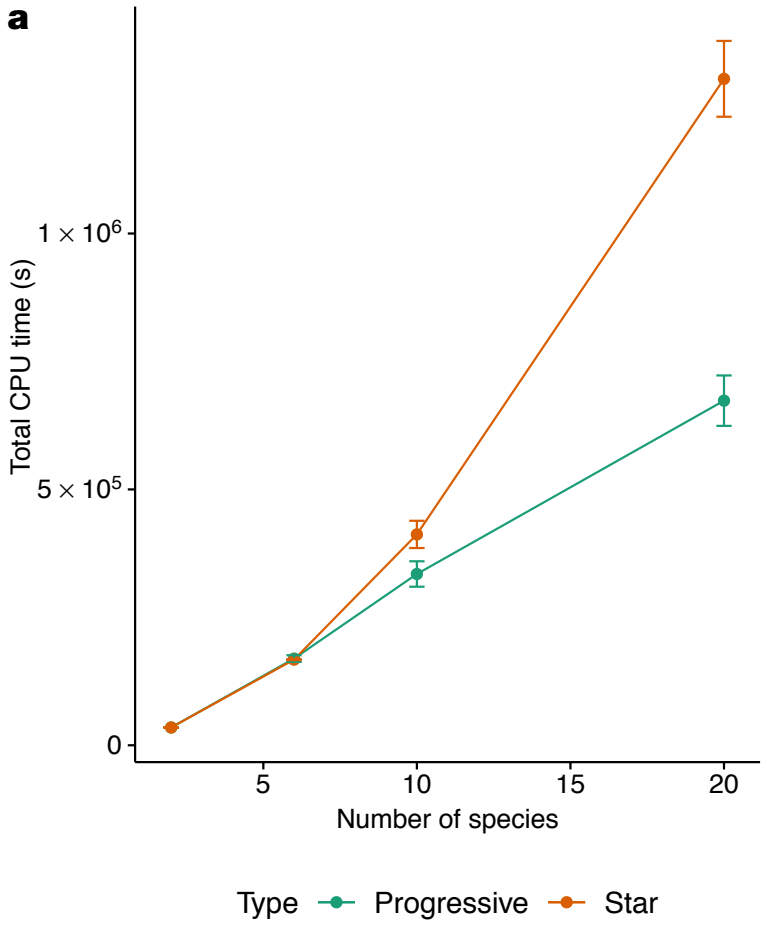
Progressive Cactus



Armstrong J, et al. 2019.
Annu. Rev. Anim. Biosci. 7:41–64

Progressive vs star

- Progessive uses less time than star
- Without progressive alignment in some way, aligning many genomes would take too much time/effort/computing power



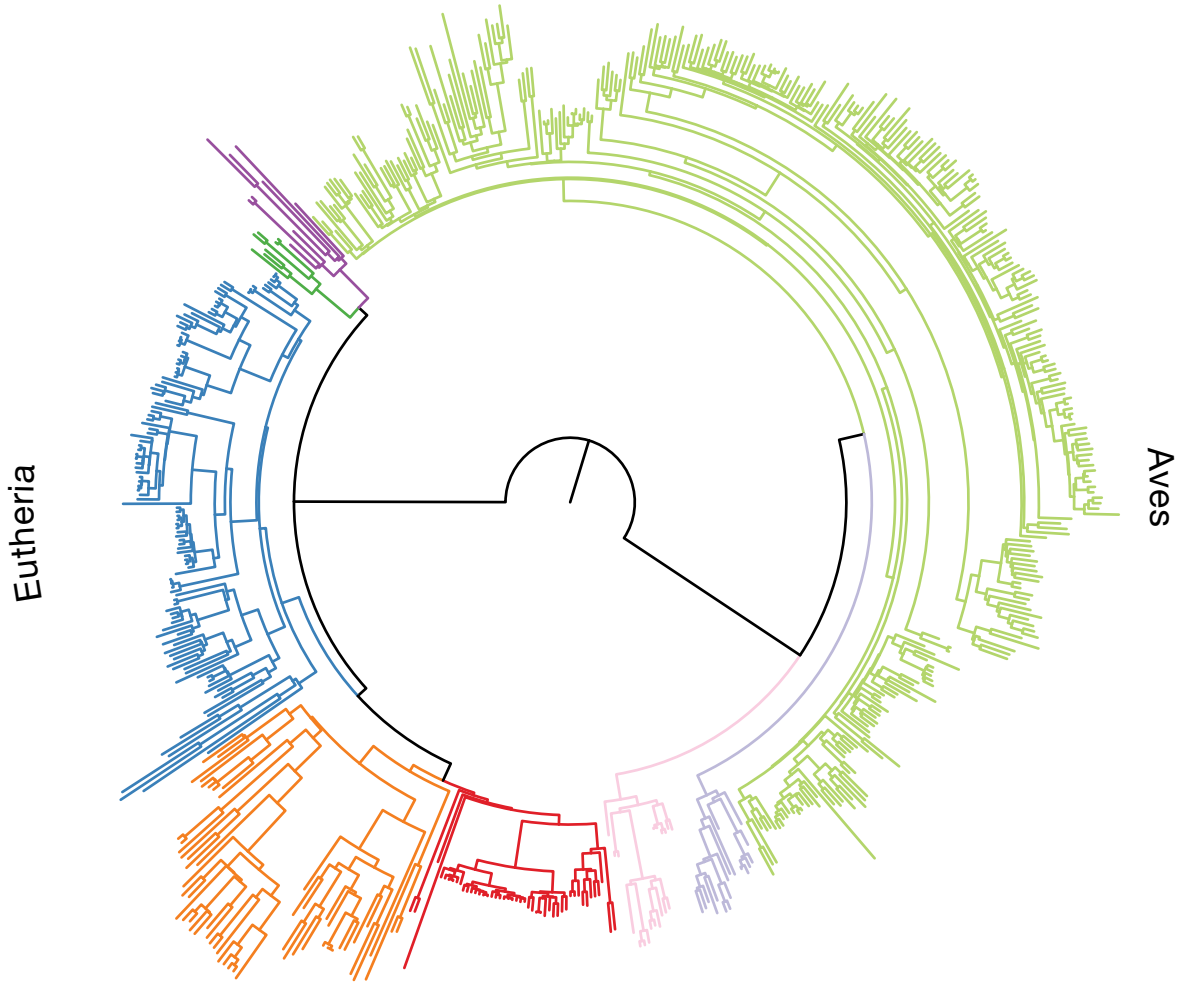
Armstrong et al 2020

605-way alignment

a

Alignment	No. of genomes	Total bases	Instance-hours	Core-hours	Common ancestor size
Zoonomia	242	669 billion	68,166	1.9 million	1.73 Gb
B10K	363	400 billion	5,302	0.2 million	1.13 Gb
Combined	605	1.07 trillion	73,692	2.1 million	181 Mb

The increase in computational work for the mammal alignment compared with the bird alignment is largely caused by the increase in the pairwise alignment phase runtime because it scales quadratically with the size of the genomes being aligned.



Armstrong et al 2020

Exercises

- Go to https://github.com/ForBioPhylogenomics/tutorials/blob/main/whole_genome_alignment/README.md finish the tutorial

Questions?