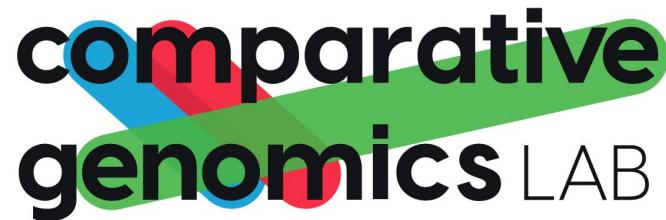


Biodiversity Bioinformatics

From large-scale phylogenomics to gene families and functions

25 Aug 2025: Orthology with OMA



ELIXIR Code of Conduct



Learning objectives

This course is centered around comparative genomics. After the course, you will be able to:

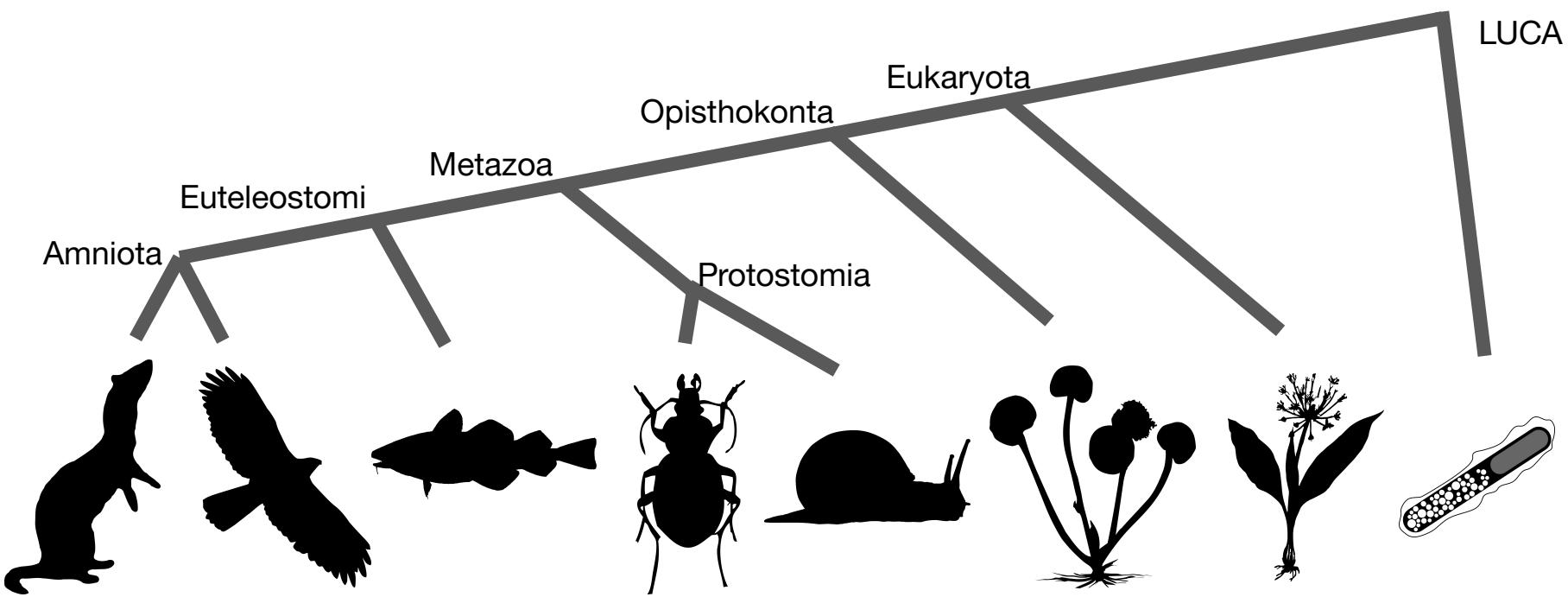
- Define orthology, paralogy and their subtypes
- Retrieve orthology information from the OMA database
- Map sequences quickly to their Hierarchical Orthologous Groups
- Perform genome annotation quality assessment with OMArk
- Infer orthologs on custom genomes using the FastOMA pipeline
- Construct and interpret phylogenetic species trees using OMA data

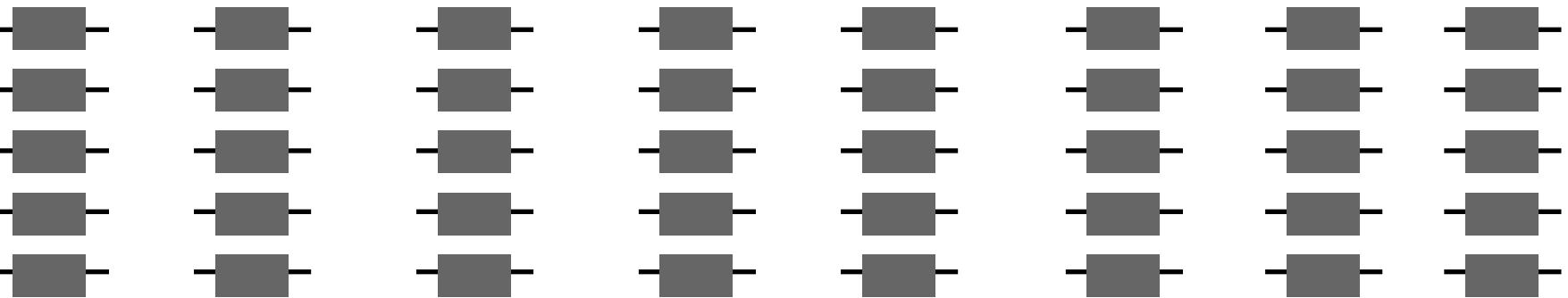
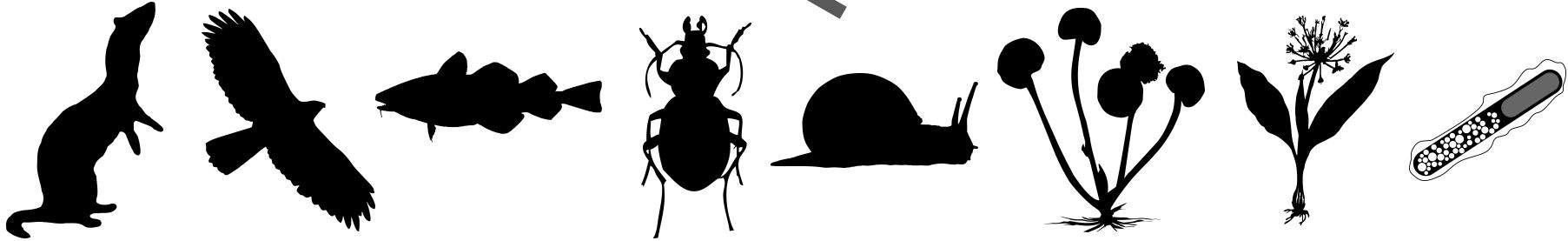
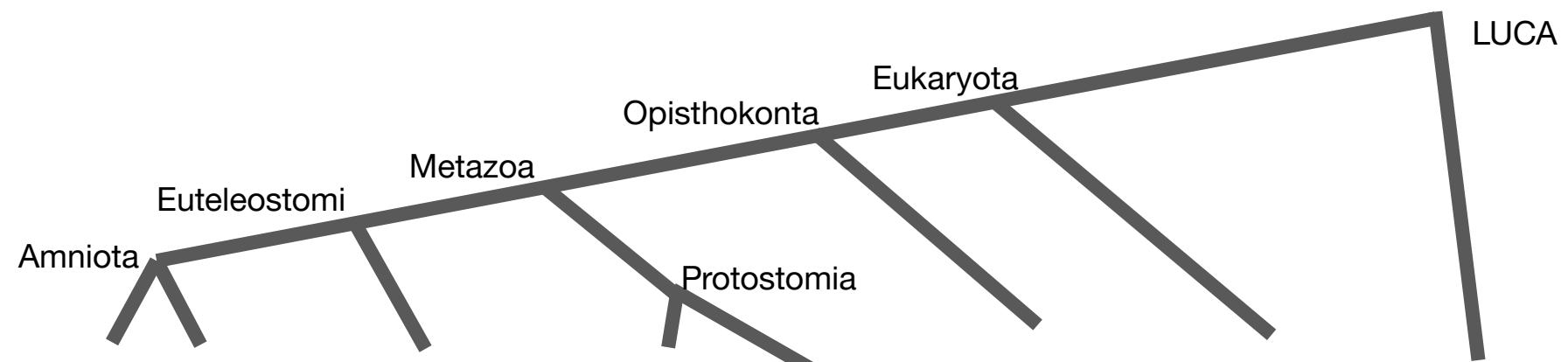
Schedule Aug 25 2025

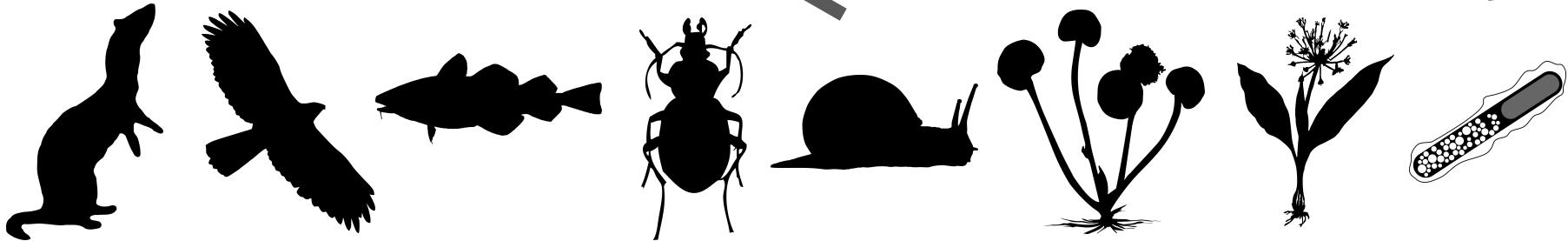
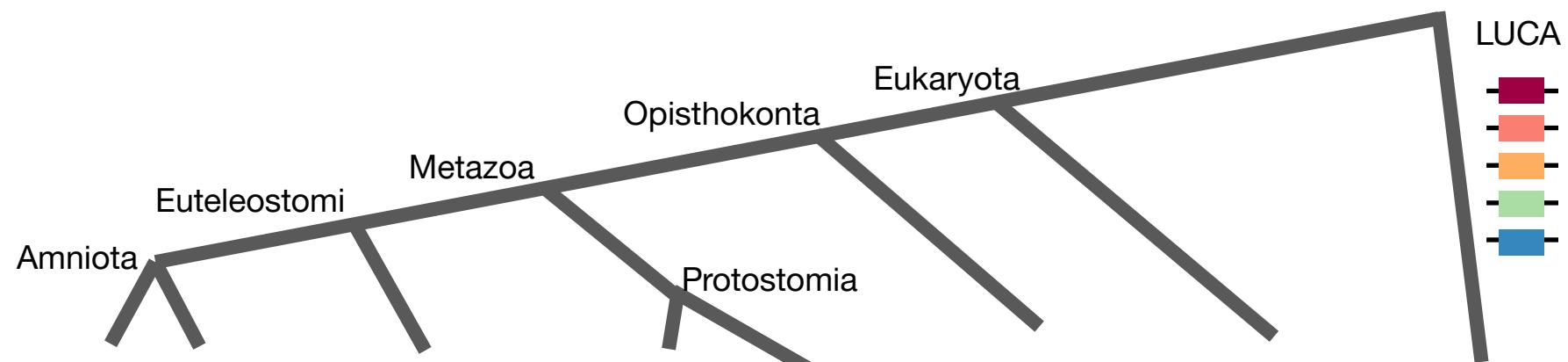
Time	Activity	In charge
09:00 - 09:15	Welcome, Introductions	Natasha Glover
09:15 - 09:45	Lecture: Overview, objectives, motivation, concept of orthologs, HOGs	Natasha Glover
09:45 - 10:25	Module 1: The OMA Browser	Natasha Glover
10:25 - 10:45	Coffee break	
11:25 - 11:35	Lecture: Fast placement into hierarchical orthologous groups with OMAMer	Nikolai Romashchenko
11:35 - 12:05	Module 2: OMAMer	Nikolai Romashchenko
12:15 - 13:30	Lunch	
13:30 - 13:40	Lecture: Protein-coding gene assessment with OMARk	Natasha Glover
13:40 - 14:10	Module 3: OMARk	Natasha Glover
14:10 - 14:15	Quick break	
14:15 - 14:25	Lecture: Orthology inference with FastOMA	Stefano Pascarelli
14:25 - 15:25	Module 4: FastOMA	Stefano Pascarelli
15:25 - 15:45	Coffee break	
15:45 - 16:00	Lecture: Gene trees and species trees	Christophe Dessimoz
16:00 - 16:45	Module 5: Building species trees	Christophe Dessimoz
16:45 - 17:00	Wrap up	Christophe Dessimoz

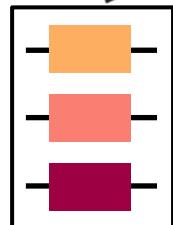
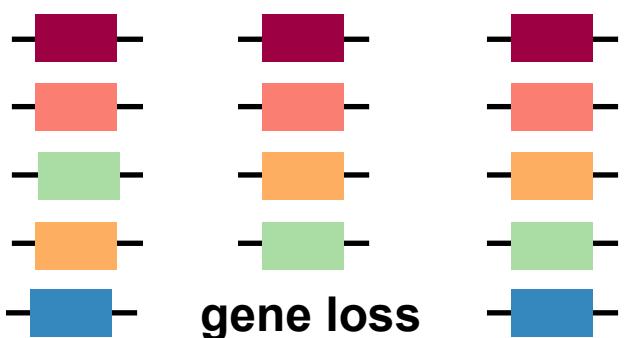
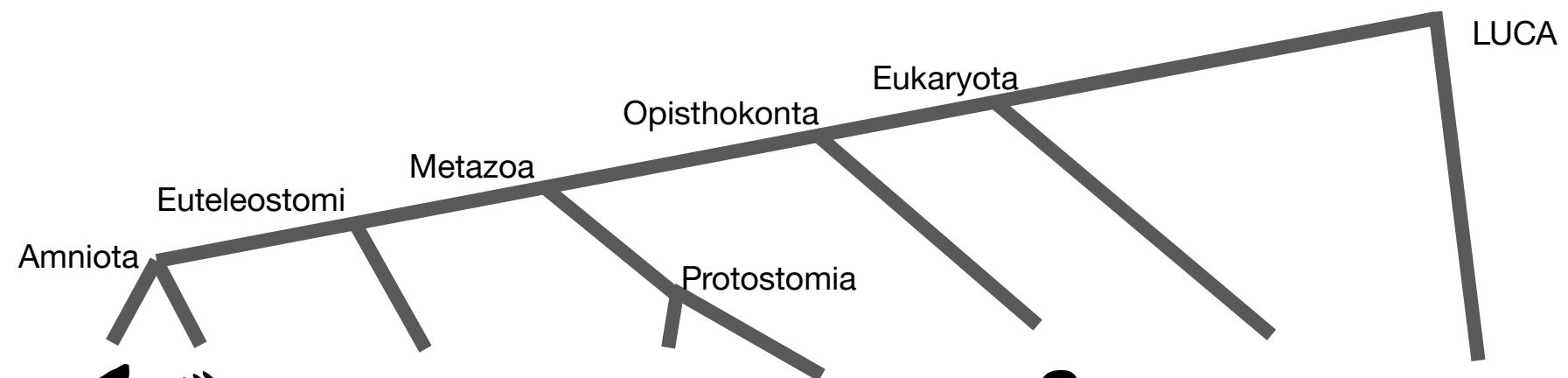
Module 1: Orthology and HOGs



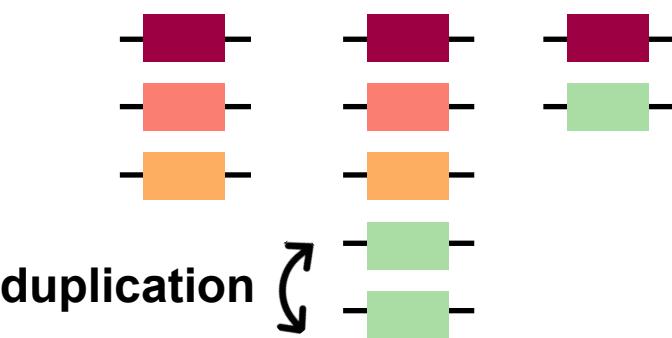








lineage-specific



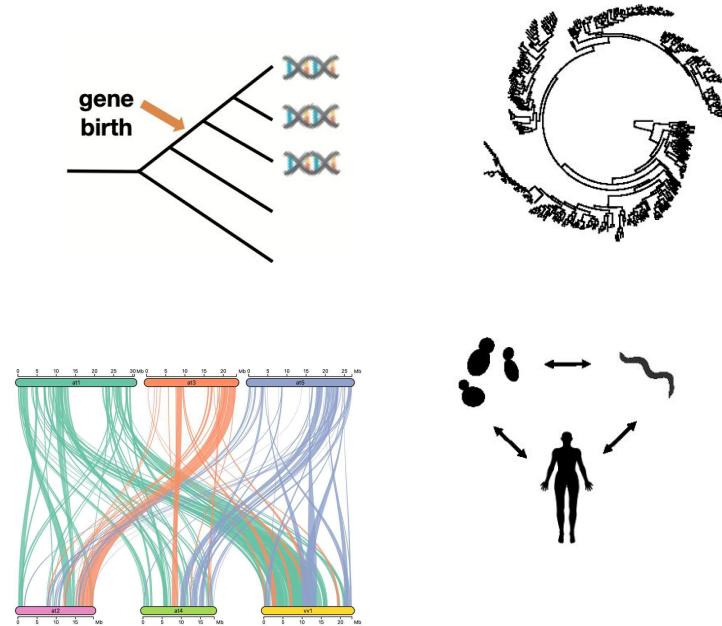
duplication



Orthology is fundamental to comparative genomics

Applications:

- Building phylogenetic trees
- Transferring gene function
- Finding lineage-specific genes
- Studying ancestral genomes
- Finding co-evolving genes
- Exploring synteny

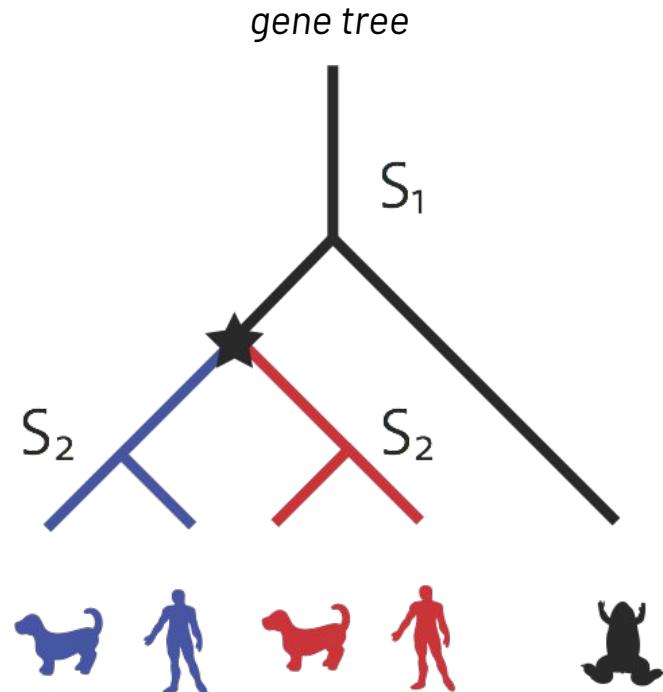


Orthology: What is it?

Homology

- The study of genetic material almost always starts with identifying, within or across species, **homologous regions**—regions of common ancestry.
- Homologs = **gene families**
- It is useful to distinguish between two classes of homologous genes.

Orthologs vs. paralogs



Orthologs vs. paralogs

- **Orthologs:** pairs of genes that started diverging via evolutionary **speciation**
- **Paralogs:** pairs of genes that started diverging via gene **duplication**
- **Orthology:** A relation between pairs of genes that started diverging via evolutionary **speciation**
- **Paralogy:** A relation between pairs of genes that started diverging via gene **duplication**

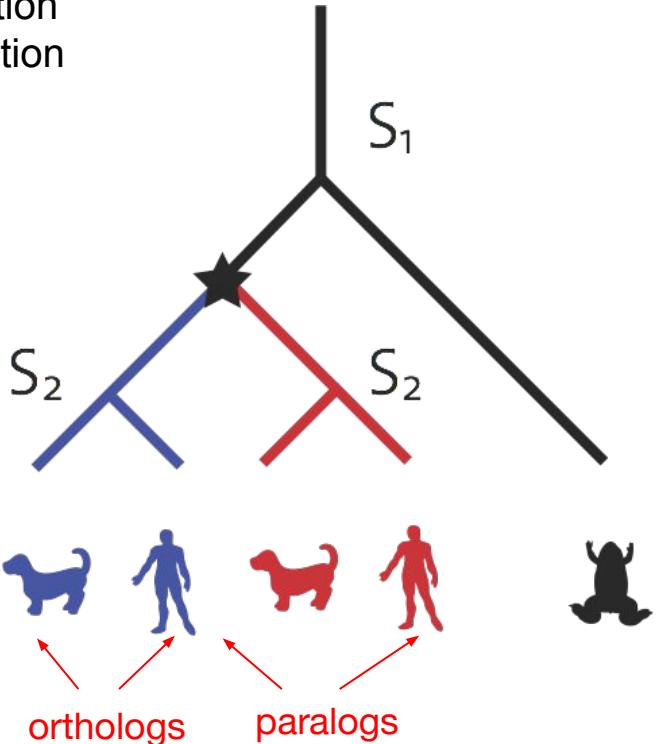
Ortho = exact
Para = beside/next to

Orthologs

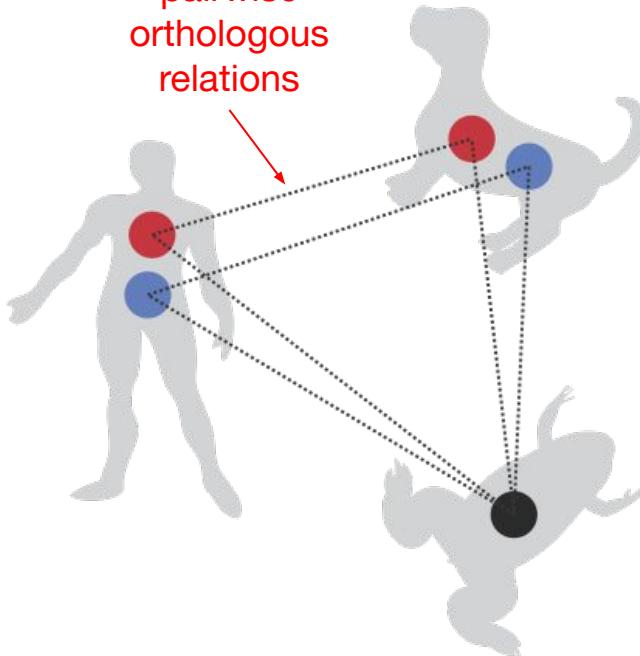
- Two genes in two species are **orthologous** if they derive from one gene in their last common ancestor
- Can be thought of as “corresponding genes” between species

S = speciation
★ = duplication

gene tree



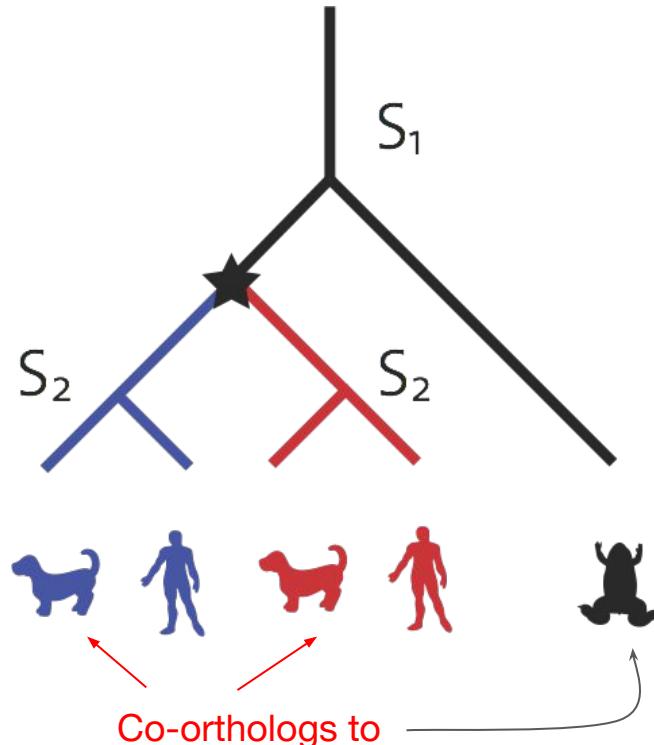
pairwise
orthologous
relations



Common misconceptions

S = speciation
★ = duplication

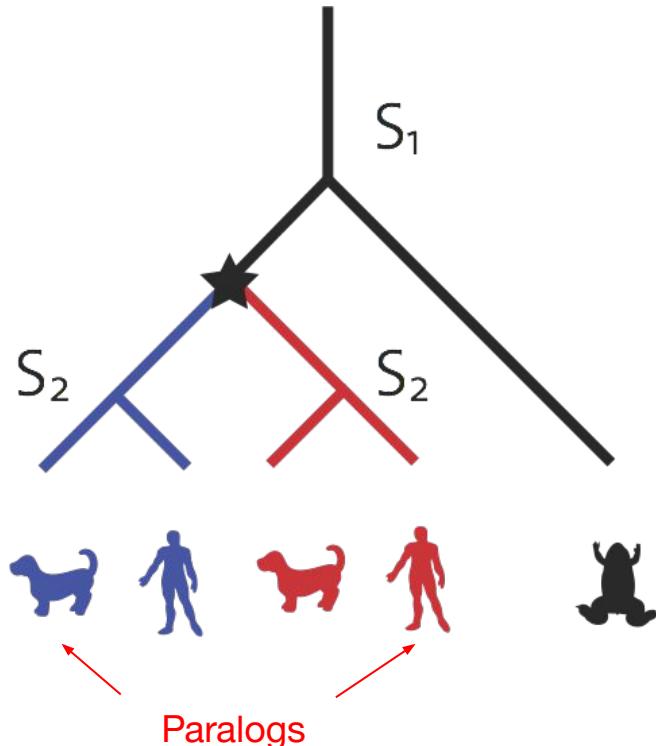
- Orthologs don't have to be one-to-one
- Orthology can also be a one-to-many, many-to-one, or many-to-many relationship
- Paralogs don't have to be in the same species

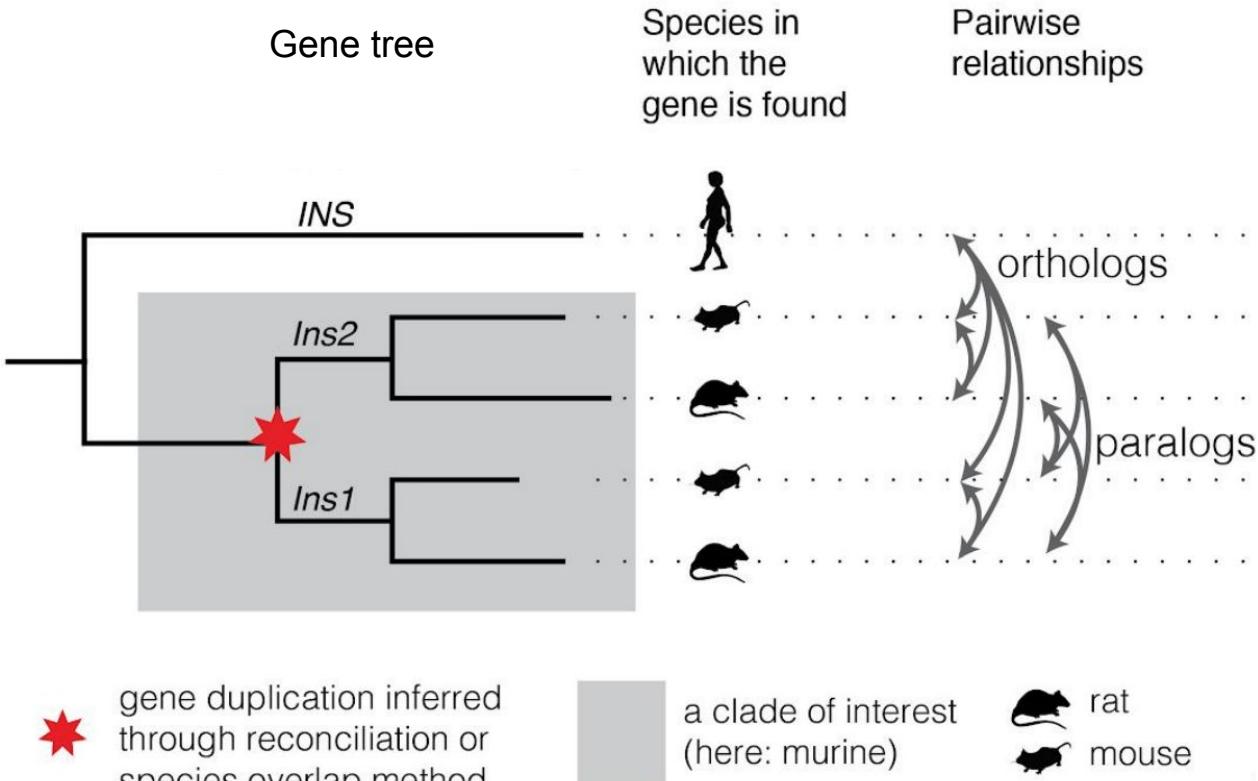


Common misconceptions

S = speciation
★ = duplication

- Orthologs don't have to be one-to-one
- Orthology can also be a one-to-many, many-to-one, or many-to-many relationship
- Paralogs don't have to be in the same species





Difficult to interpret pairwise relationships when referring to groups of genes in several species...

Orthologous Groups (i.e. OMA Groups/OGs)

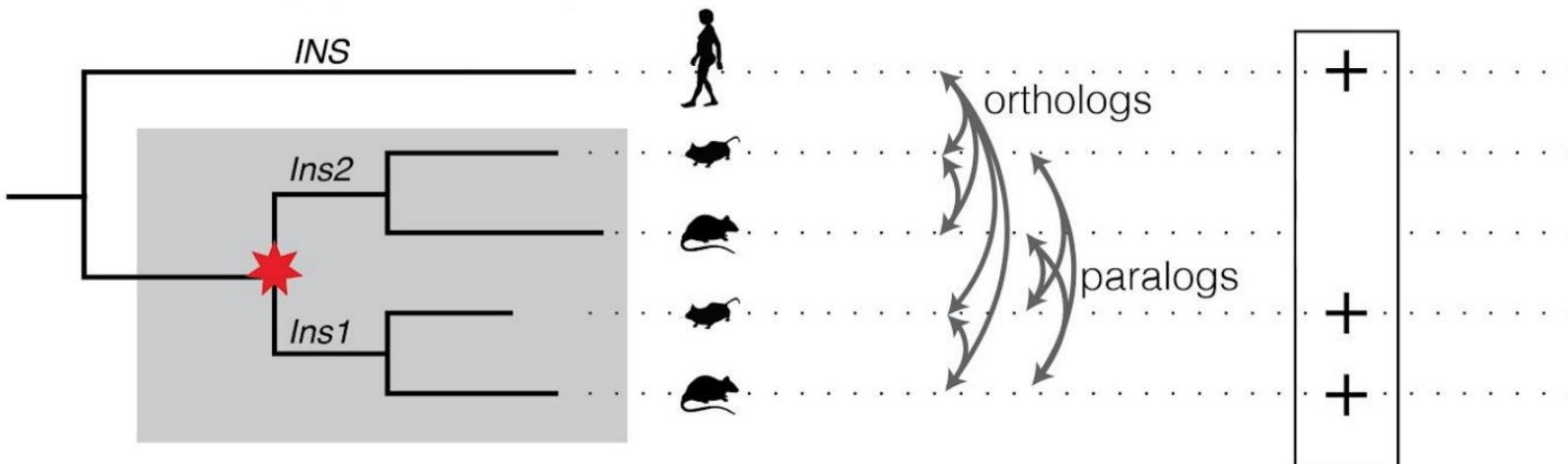
- Groups of genes which are all orthologous to each other
- Strict orthologous group
- Not necessarily 1:1 orthology, but each group contains at most one gene per species

Gene tree

Species in which the gene is found

Pairwise relationships

Example of strict orthologous group



gene duplication inferred through reconciliation or species overlap method



a clade of interest
(here: murine)



rat
mouse



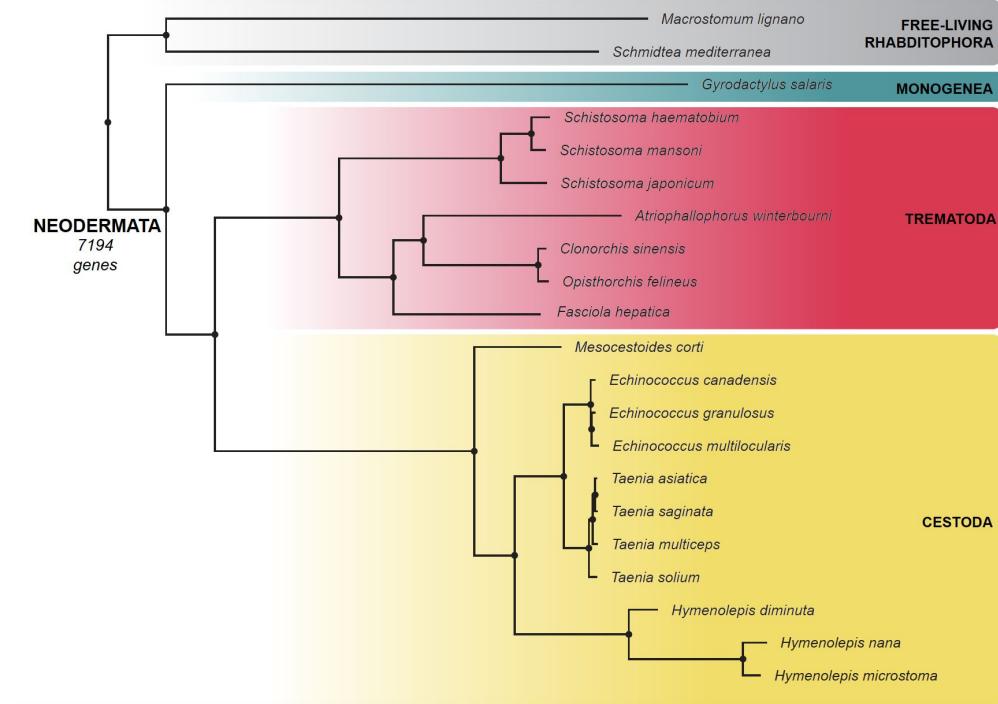
human



group membership

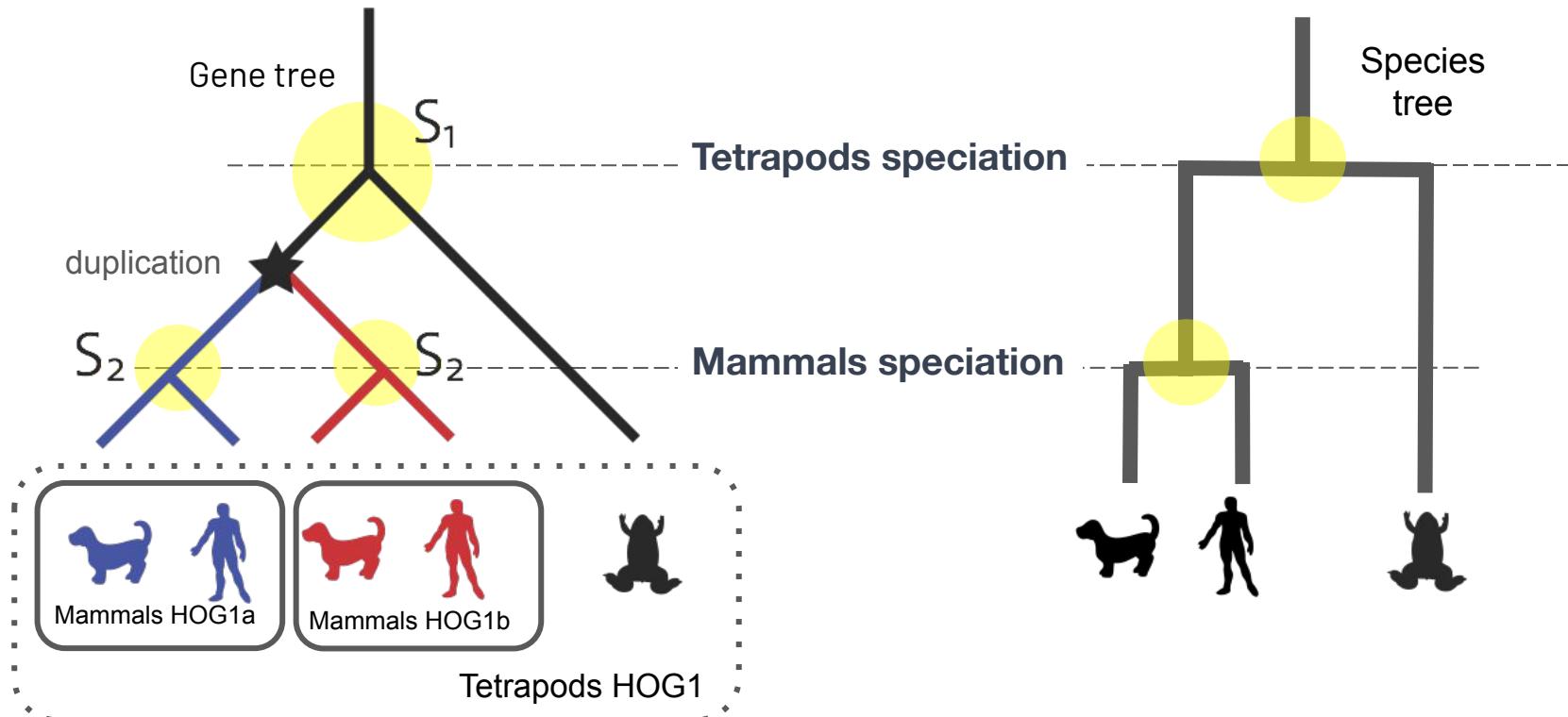
Making species trees

Create **species** trees
for a clade of interest
using the genes in the
**OMA Group (strict
Orthologous Group)**
at that taxonomic
level

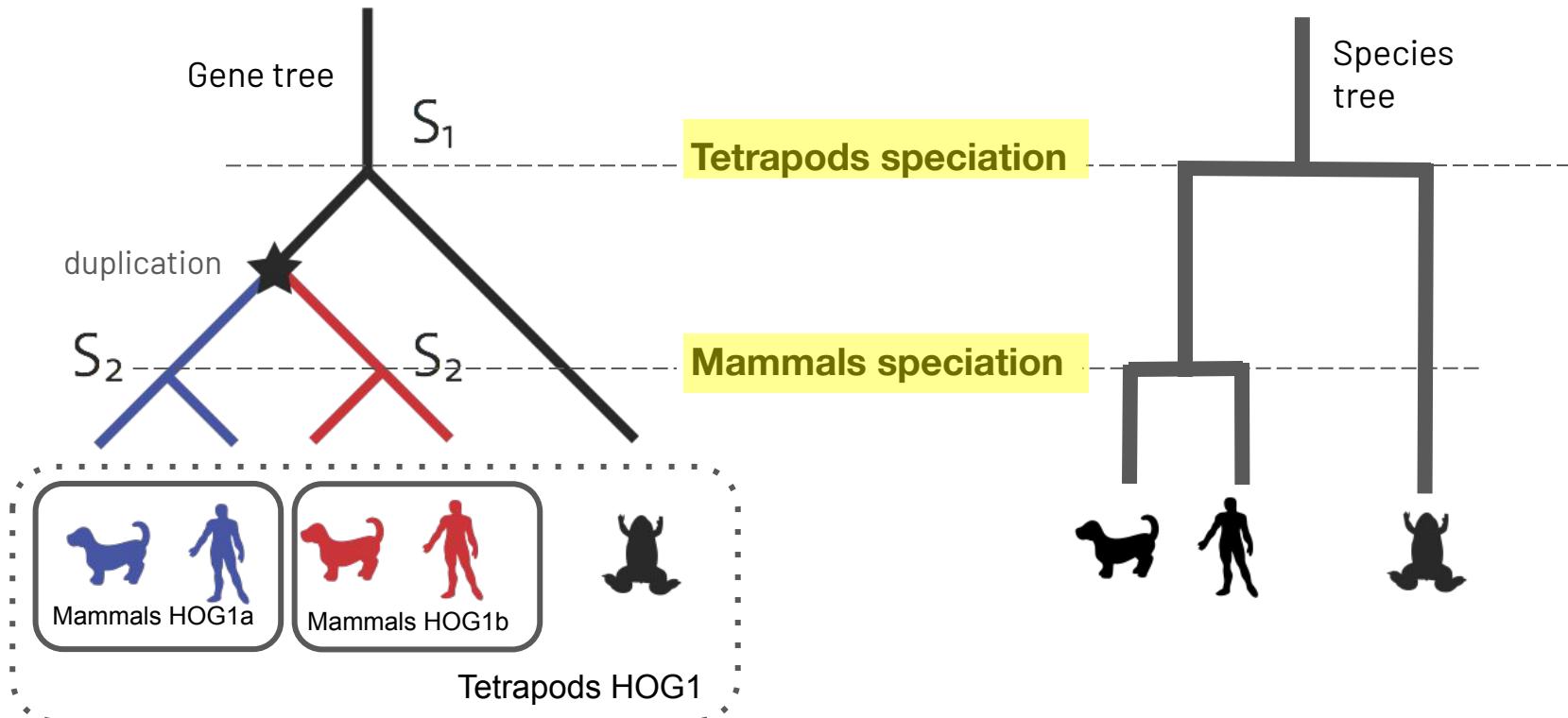


Hierarchical Orthologous Groups (HOGs)

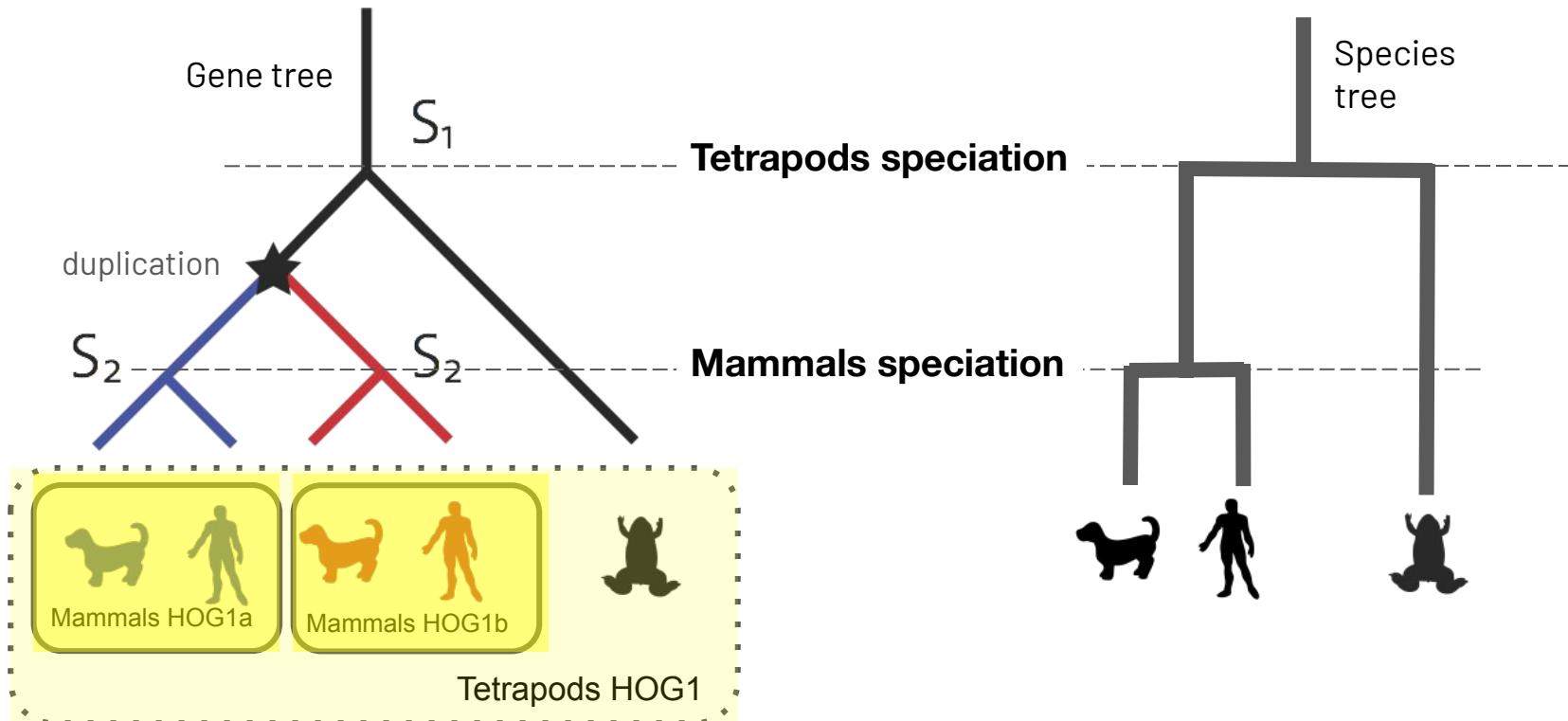
HOGs = Sets of genes that descended from a **common ancestral gene** in a given ancestral species



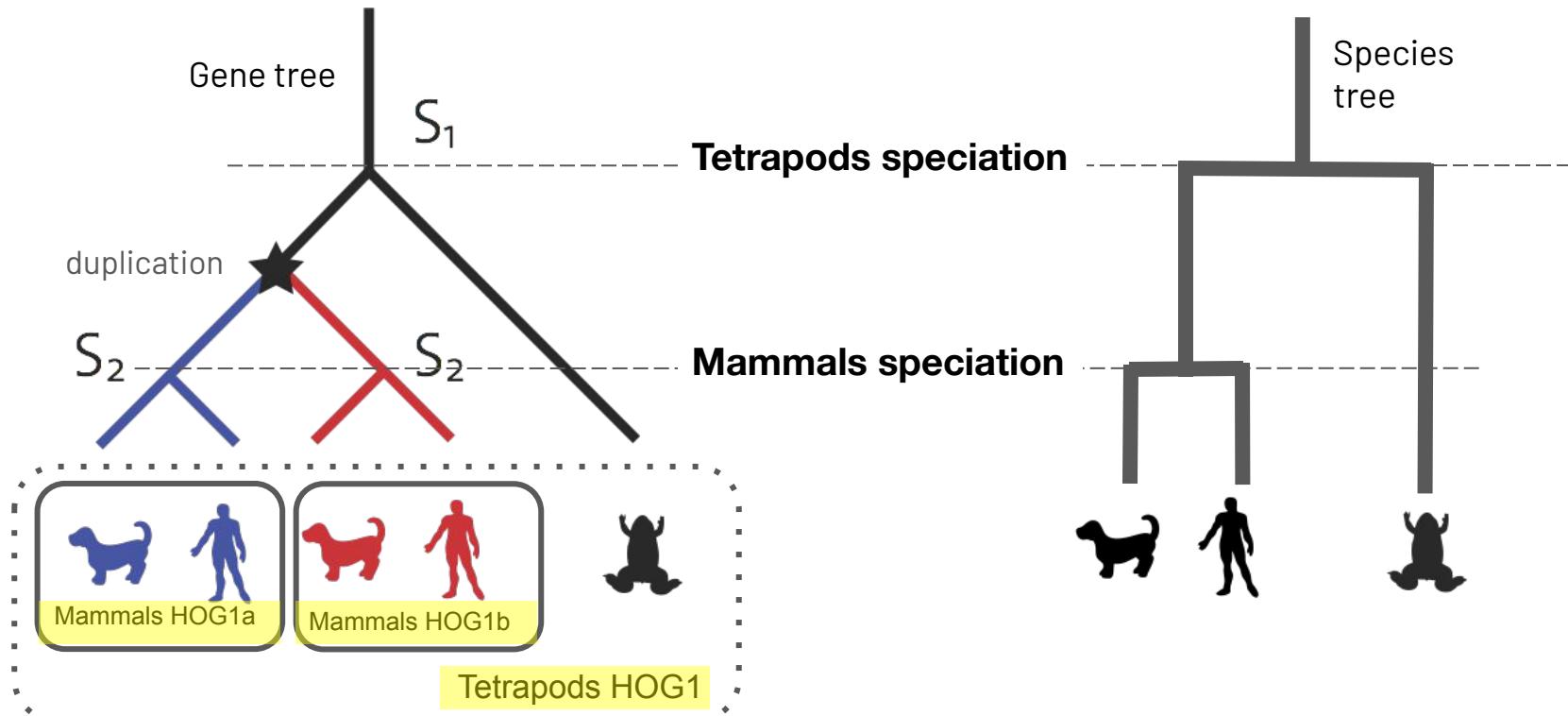
HOGs are defined with respect to specific clades



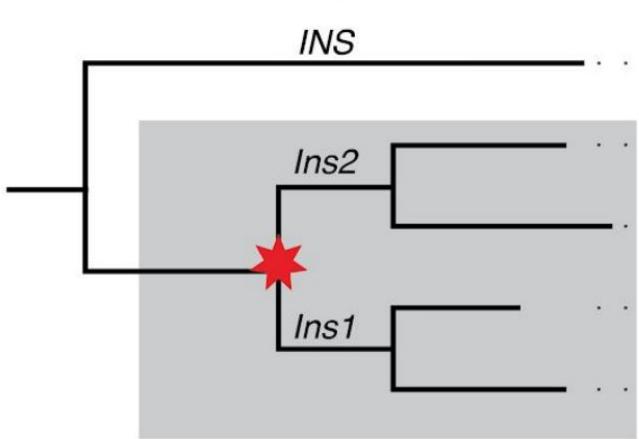
HOGs are **hierarchical** because the groups are defined with respect to deeper clades that **subsume** multiple groups defined on their descendants



HOGs are gene families; SubHOGs are nested subfamilies



Labelled gene trees
(marked internal nodes are
duplication nodes; others
are implicitly speciation nodes)



Species in
which the
gene is found

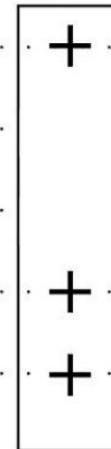


Pairwise
relationships

orthologs

paralogs

Example of
strict orthologous
group



Hierarchical
Orthologous
Groups



★ gene duplication inferred
through reconciliation or
species overlap method



a clade of interest
(here: murine)



rat



mouse



human



group membership

How to infer orthology?

Orthology Databases

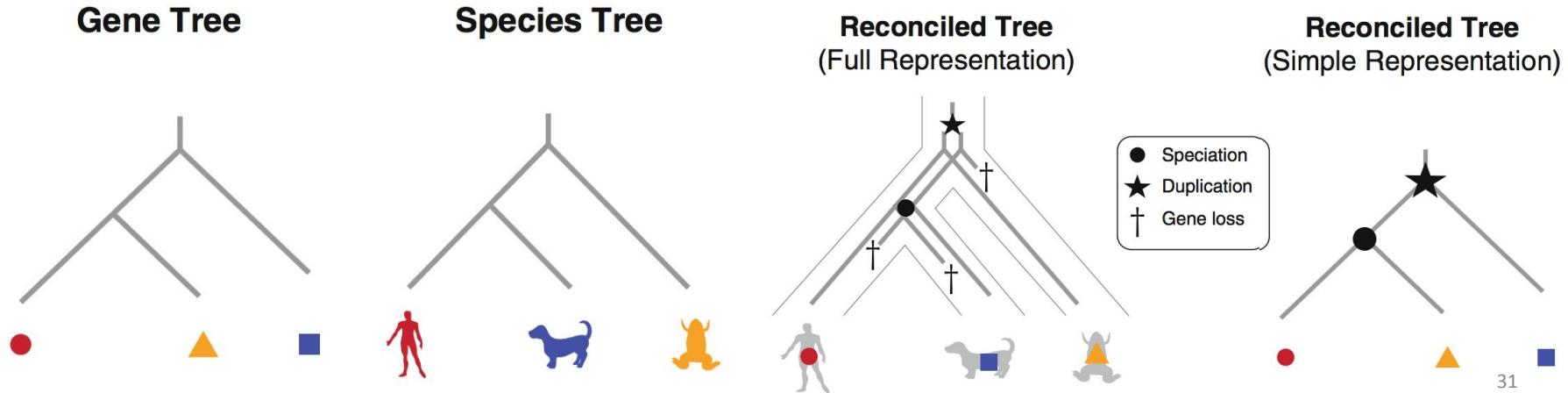
- OMA: <https://omabrowser.org/>
- OrthoDB: <https://www.orthodb.org/>
- EggNOG: <http://eggnogdb.embl.de>
- InParanoid/Hieranoid: <http://hieranoidb.sbc.su.se/>
- COG: <https://www.ncbi.nlm.nih.gov/COG/>
- PANTHER: <http://www.pantherdb.org/genes/>
- PhylomeDB: <http://phylomedb.org/>
- OrthoFinder: <https://github.com/davidemms/OrthoFinder>

Many different methods:
*Namely tree-based or
graph-based*

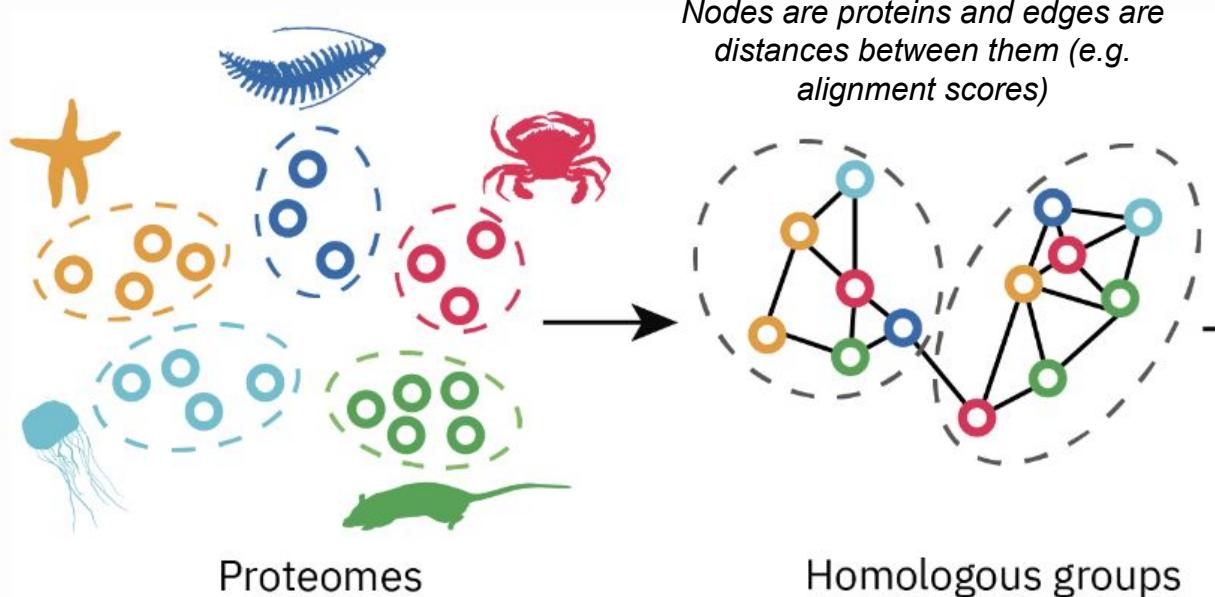
Tree-based methods

- Start with a group of homologous sequences
 - Reconstruct a gene tree
 - Infer the type of evolutionary event represented by each internal node of the tree

Gene tree/species tree reconciliation:



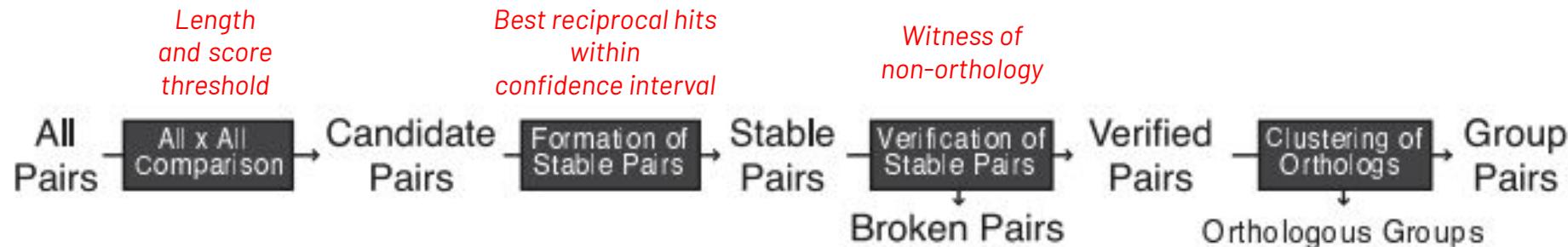
Graph-based methods



- Compare pairs of genes within and between species
- For pairs of genes between 2 species, orthologs tend to be the pairs of sequences that have diverged the least



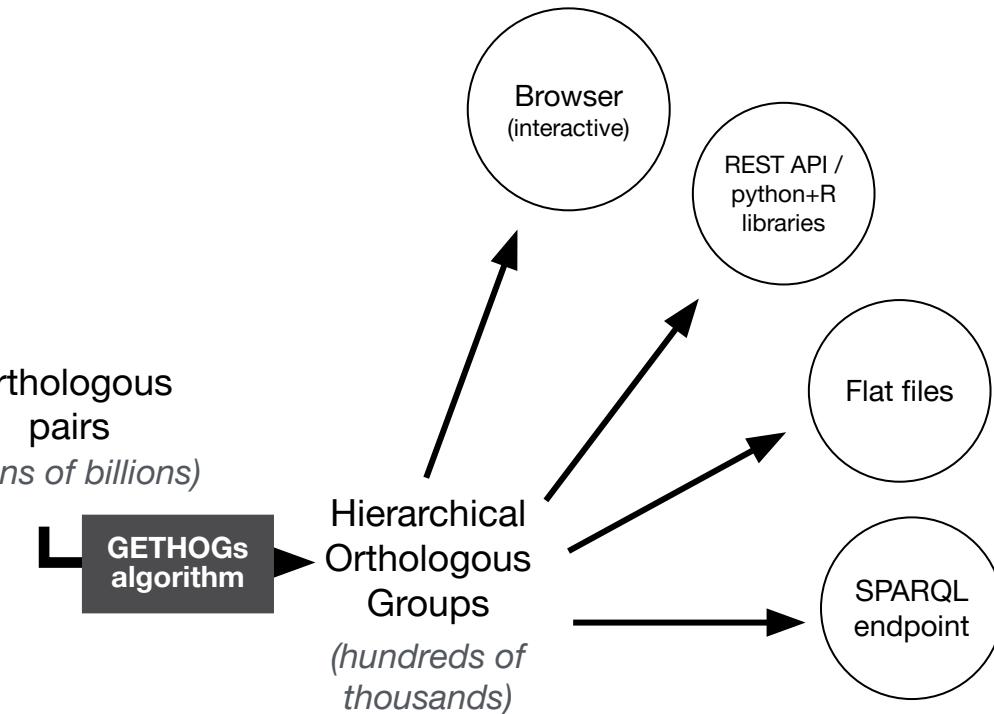
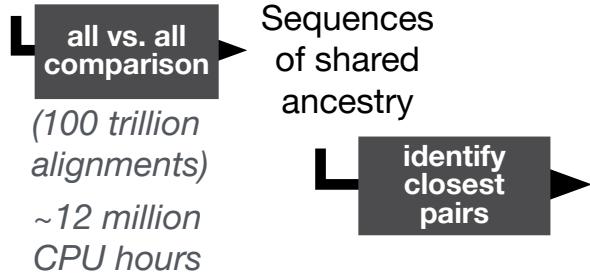
Pipeline:





orthologous
matrix

All protein
sequences from
>2800 full genomes
(22 million)

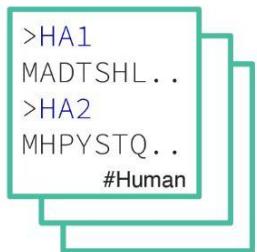


Dessimoz et al., RECOMB CG 2005
Dessimoz et al., Nucl Acids Res 2006
Roth et al., BMC Bioinformatics 2008
Altenhoff et al., Nucl Acids Res 2011
Altenhoff et al., PLoS ONE 2013
Altenhoff et al., NAR 2015
Train et al. Bioinformatics / ISMB 2017

Altenhoff et al. NAR 2018
Altenhoff et al. Genome Research 2019
Zahn-Zabal et al. F1000Res 2020
Glover F1000Res 2020
Altenhoff et al. NAR 2021
Altenhoff et al. NAR 2024

FastOMA, our new tool

Input Proteomes

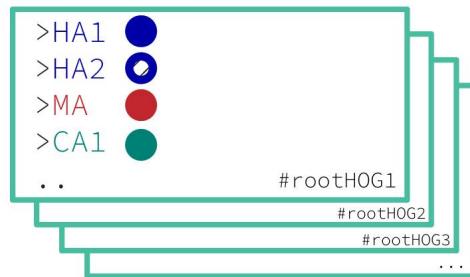


OMAmer

Mapping sequences
on OMA gene families
based on k-mers

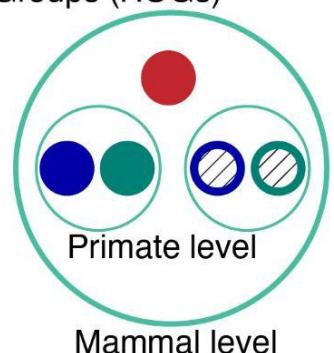


Root HOGs (Gene families)



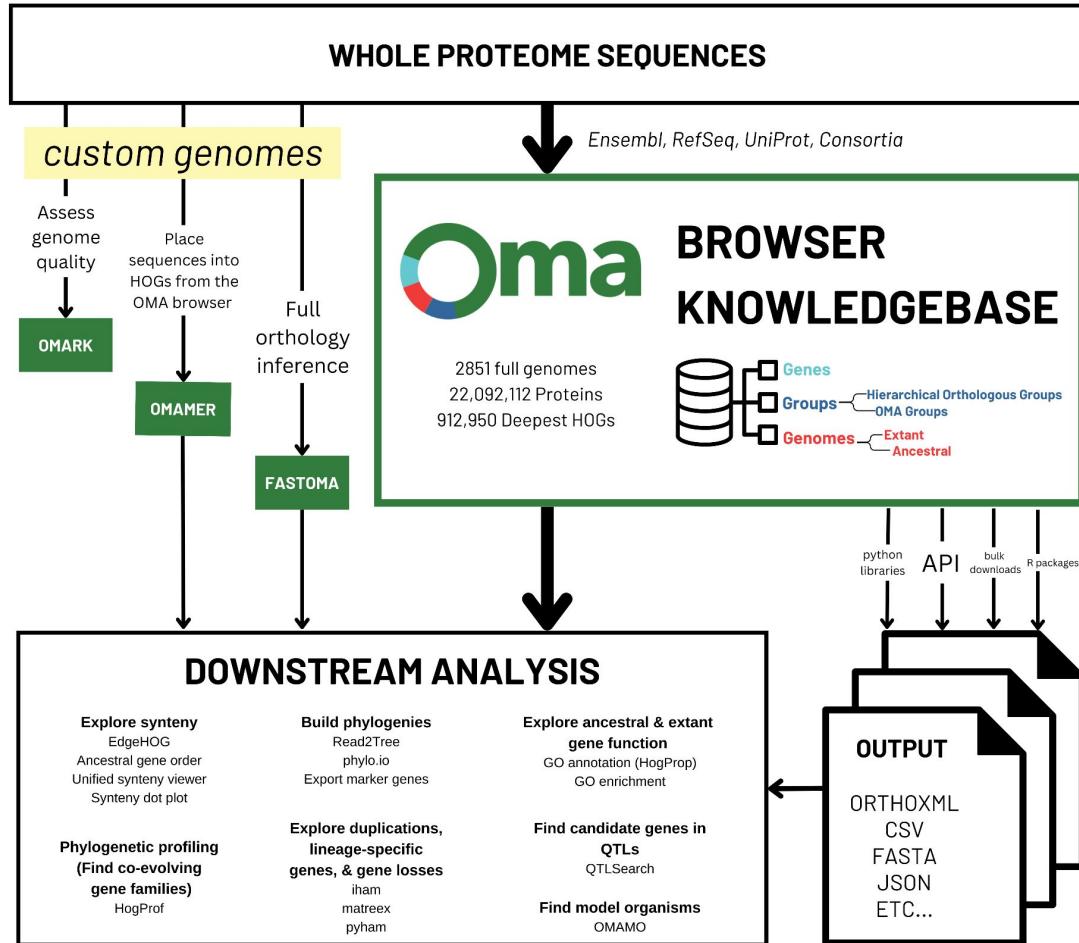
SubHOG/event
inference
(in parallel)

Hierarchical Orthologous Groups (HOGs)



The OMA Ecosystem

From public and private genomes, orthology inference, a database of over 2900 species, and a variety of downstream analyses for comparative genomics



The OMA browser

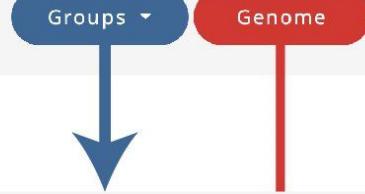
The screenshot shows the Oma browser interface. At the top left is the SIB logo and the Oma browser logo. At the top right are navigation links: Explore, Tools, Download, Help, and About. The main header features the Oma logo with the text "orthologous matrix" next to it. Below the header is a search bar with the query: "Blue-light photoreceptor" | proteinid:P53_RAT | species:"Drosophila melanogaster". To the right of the search bar is a circular progress or refresh icon. A callout box below the search bar contains the text: "Examples: Entry P53_RAT - 'EWGKQSF' in Tetraodon - Search for 'Blue-light photoreceptor' - 'Drosophila melanogaster' species". In the center, there is a "SCROLL TO DISCOVER MORE" button. At the bottom, a green footer bar displays statistics: 2,927 Full genomes, 24,790,217 Proteins, 1,428,654 OMA groups, 1,040,435 Deepest HOGs, and All.Jul2024 Release. The URL <https://omabrowser.org/> is highlighted in a white box on the left side of the footer.

The OMA Database: 3 kinds of pages

genes

Gene LEIIN03631 (A4I3J2)

E Leishmania infantum is on Chromosome 28.



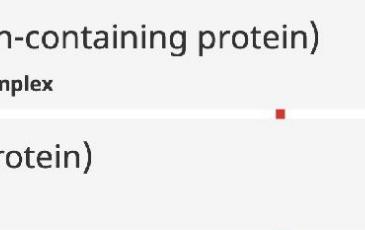
groups

HOG:0495624.7c with 2 members (DYW_deaminase domain-containing protein)

Eukaryota / Kinetoplastida / Trypanosomatidae / Leishmaniinae / Leishmania / Leishmania donovani species complex

OMA GROUP 402875 with 9 members. (Uncharacterized protein)

Fingerprint: MRFARFE



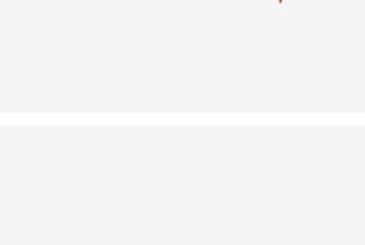
genomes

LEIIN - Leishmania infantum

Proteome version: 03-JAN-2001 (Rel. 66, Last updated, Version 1) with 8031 proteins.

Ancestral genome of Leishmaniinae

with 6 descendant species and 7893 ancestral genes (HOGs).



Hierarchical Orthologous Groups (HOGs)

HOG:D0606964 with 42 members (Ig-like domain-containing protein)

Primates / Lower Level ▾

Completeness score: 0.75 ⓘ

Ancestral Genome

Hierarchical group HOG:0606964 open at level of Primates

OPTIONS ▾

Graphical viewer

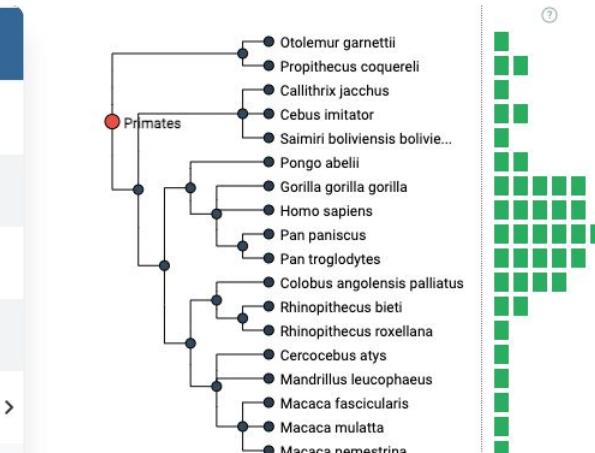
Members

Ancestral GO

Alignment

Ancestral synteny

Similar HOGs



?

- A HOG is a gene family
- A collection of orthologs and paralogs which descended from a common ancestral gene

HOGs

HOG:D0606964 with 42 members (Ig-like domain-containing protein)

Completeness score: 0.75 ⓘ Ancestral Genome

Primates / Lower Level ▾

[OrthoXML](#) / [Phyloxml species tree \(Primates\)](#) / [Sequences \(fasta\)](#)

Search

Graphical viewer

Members

Ancestral GO

Alignment

Ancestral synteny

Similar HOGs >

Protein ID	Cross reference	Domain Architectures	Taxon
CERAT42572	★ A0A2K5KV26	[blue bar]	Cercocebus atys
MACFA00770	ENSMFAG00000035597.1	[blue bar]	Macaca fascicularis
MACMU01493	★ A0A5F8APY1	[blue bar]	Macaca mulatta
MACNE44496	★ A0A2K6B118	[blue bar]	Macaca nemestrina
MANLE27898	★ A0A2K6A627	[blue bar] -	Mandrillus leucophaeus
COLAP38015	★ A0A2K5HL19	[blue bar]	Colobus angolensis palliatus
COLAP38016	★ A0A2K5INJ2	- [blue bar]	Colobus angolensis palliatus
COLAP38023	★ A0A2K5HLG0	[blue bar] -	Colobus angolensis palliatus
COLAP38026	★ A0A2K5JAM3	[blue bar] -	Colobus angolensis palliatus
RHIBE28045	★ A0A2K6M7G8	- [blue bar]	Rhinopithecus bieti

OMA ID

Ancestral genomes

The collection of HOGs at a given taxonomic level

Ancestral genome of Primates

with 24 descendant species and 38457 ancestral genes (HOGs).

Remove HOGs with completeness score below

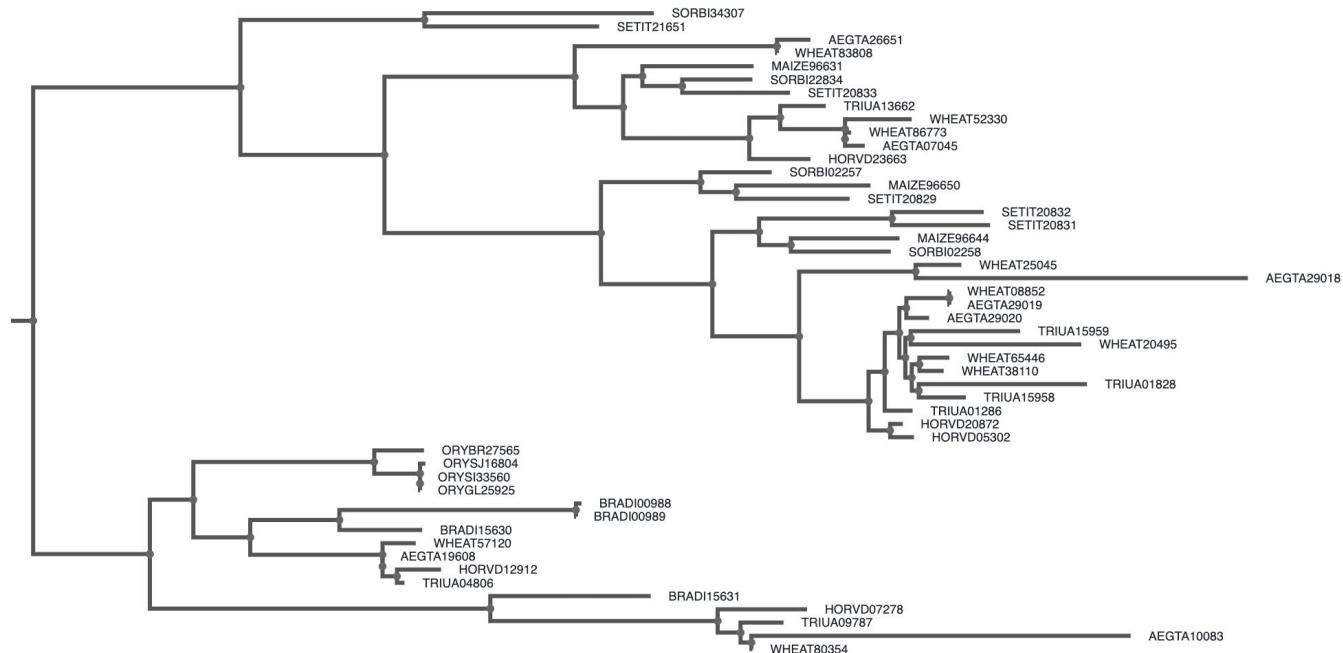
Search grid icon download icon

HOG ID	Root HOG ID	Completeness	Nr genes in HOG	Description
HOG:D0912633.1a	HOG:D0912633	1.00	24	autophagy related 16 like
HOG:D0912535.3b.8a.7b.4b	HOG:D0912535	1.00	24	leukocyte cell derived chemotaxin
HOG:D0911480.5b.3b	HOG:D0911480	1.00	24	prostaglandin G/H synthase
HOG:D0911480.5a.2b	HOG:D0911480	1.00	24	prostaglandin G/H synthase
HOG:D0911074.2b.12b	HOG:D0911074	1.00	25	thioredoxin domain containing
HOG:D0911067.13d.9b	HOG:D0911067	1.00	24	paraoxonase
HOG:D0909668	HOG:D0909668	1.00	25	alkB homolog
HOG:D0909574.1b.7a.4b	HOG:D0909574	1.00	24	kinase regulatory subunit
HOG:D0909570.1a.6g	HOG:D0909570	1.00	24	rna helicase
HOG:D0909570.1a.6d.20a.23a.11b	HOG:D0909570	1.00	24	rna helicase
HOG:D0908691.1b.1b.2a.1b	HOG:D0908691	1.00	24	5'-nucleotidase

OMA's Downstream analyses

Make gene trees*

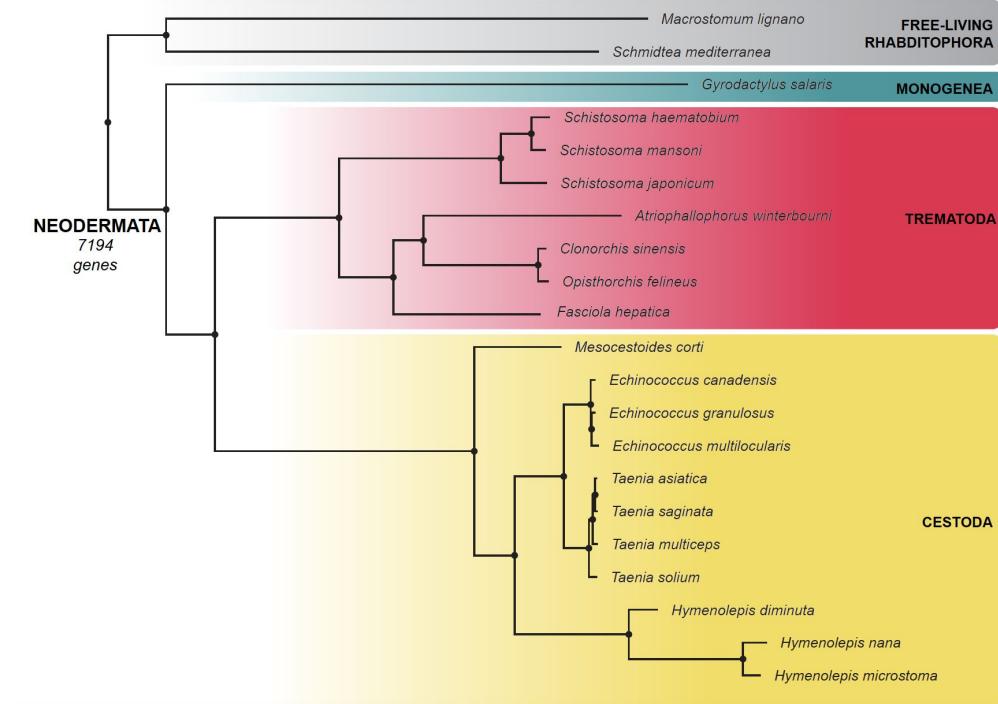
Create **gene** trees
for a clade of
interest using the
genes in the **HOG**
at that taxonomic
level



*Uses external software outside
of the OMA Ecosystem

Making species trees*

Create **species** trees
for a clade of interest
using the genes in the
**OMA Group (strict
Orthologous Group)**
at that taxonomic
level



Infer gene function and perform GO enrichment analysis

Gene Ontology Function Projection

Email Email
We will send an email to this address once the predictions are ready.

Name of Dataset Name of Dataset

Sequence File (fasta format)* Choose file | No file chosen

Captcha* I'm not a robot 
reCAPTCHA
Privacy - Terms

Submit

Transfer Gene Ontology annotations from genes in the same HOG from well-studied model species to other species

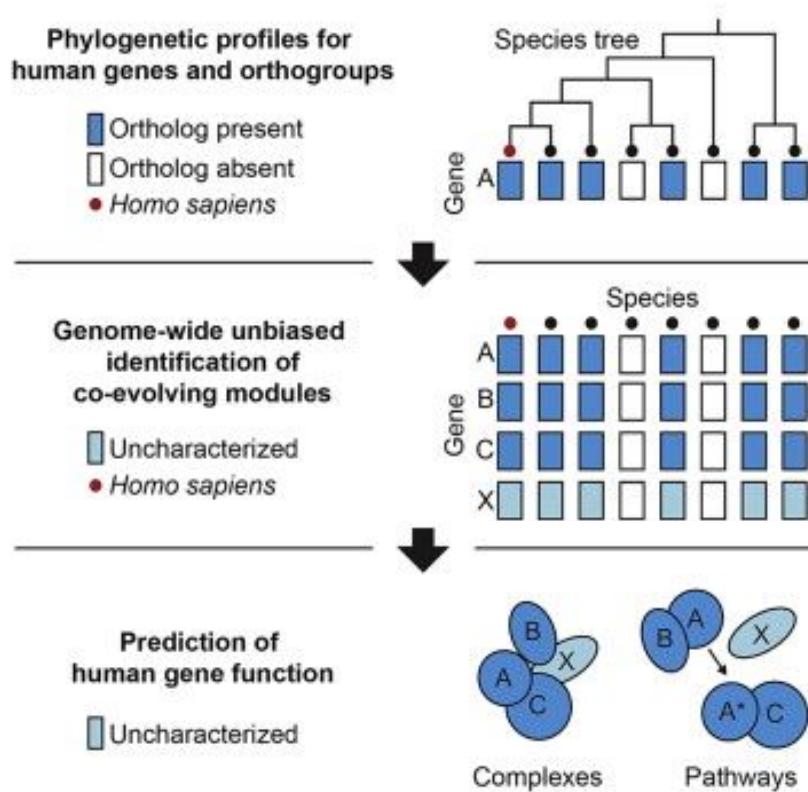
GO ENRICHMENT

You can download the detailed result for this GO enrichment analysis by clicking [here](#)

GO_ID	GO_Name	study_count	pop_count	Study_Entries	study_n	p_uncorrected	p_bonferroni	p_fdr
GO:0033041	sweet taste receptor activity	6	32	DROME01345, DROME0317, DROME04384, DROME04694, DROME05340, DROME05633	6	1.428750553e-16	0	0
GO:0001582	detection of chemical stimulus involved in sensory perception of sweet taste	6	34	DROME01345, DROME0317, DROME04384, DROME04694, DROME05340, DROME05633	6	2.120447249e-16	0	0

Perform a GO enrichment analysis to find overrepresented functions in a set of extant or ancestral genes

Phylogenetic profiling



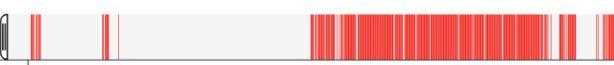
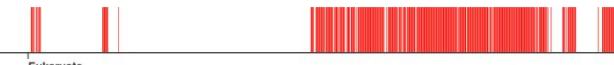
Phylogenetic profiling

HOG:D0679560 with 323 members (intraflagellar transport protein 57 homolog) Completeness score: 0.44 ⓘ Ancestral Genome

Eukaryota / Lower Level ▾

This HOG has 49 similar HOGs:

Co-evolving HOGs

HOG ID	Jaccard similarity ⓘ	Reset	Description
HOG:D0680756	0.8515625		intraflagellar transport
HOG:D0680535	0.8515625		intraflagellar transport
HOG:D0680689	0.84375		intraflagellar transport

Graphical viewer

Members

Ancestral GO

Alignment

Ancestral synteny

Similar HOGs

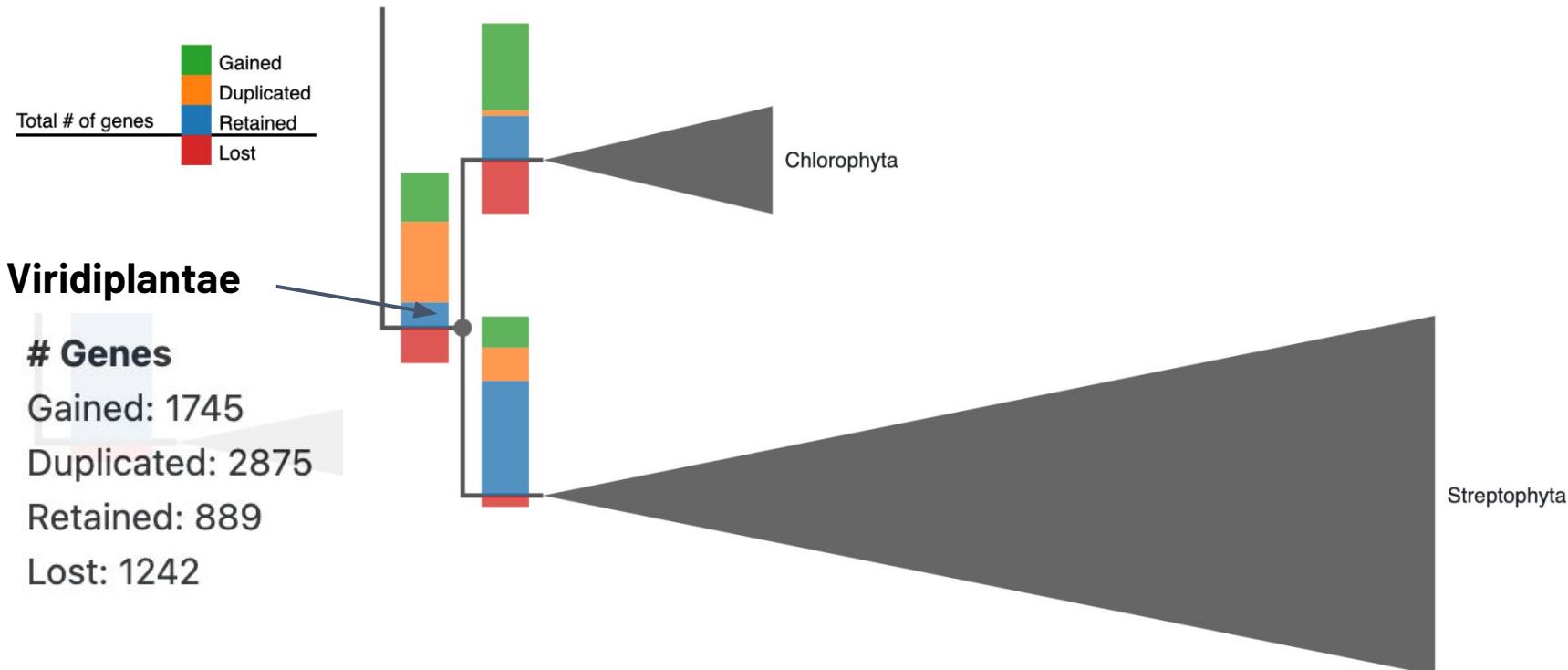
BASED ON:

Similar domains

Search

Related functions

Tracking the evolutionary history of a gene family



Gene content of ancestral genomes

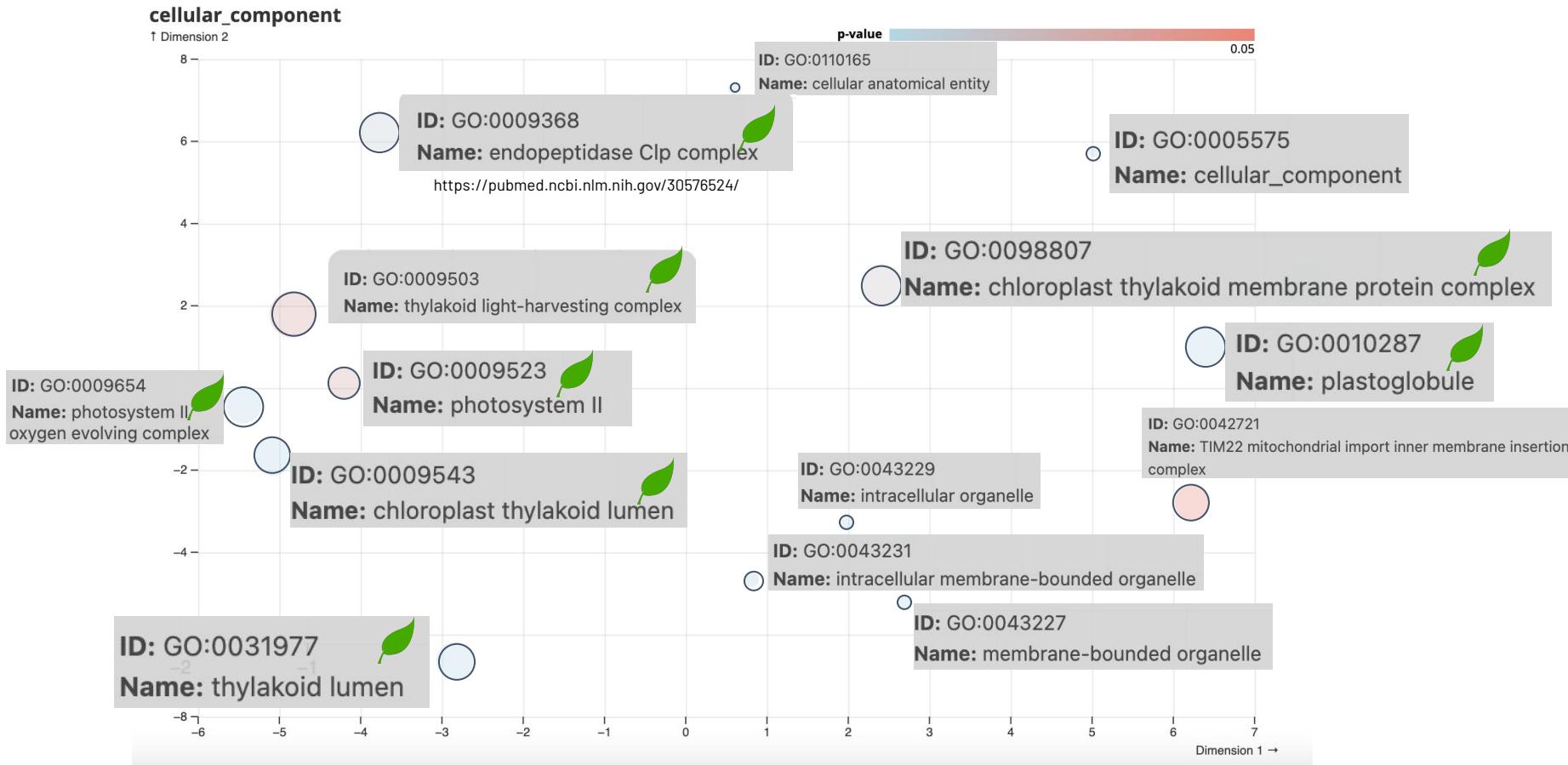
Ancestral genome of Viridiplantae

with 85 descendant species and 42339 ancestral genes (HOGs).

Completeness Score =
Number of species in the HOG/
number species in the clade

Remove HOGs with completeness score below 0.3						Search	grid icon	download icon	
Genome information						Description			
Ancestral genes									
	HOG ID	Root HOG ID	Evolutionary event	Completeness	Nr genes in HOG				
	HOG:D0271083	HOG:D0271083	gained	1.00	454	EGF-like calcium-binding domain-containing protein			
	HOG:D0271236	HOG:D0271236	gained	0.988	302	Hyaluronan/mRNA-binding protein domain-containing protein			
	HOG:D0271378	HOG:D0271378	gained	0.976	235	t-SNARE coiled-coil homology domain-containing protein			
	HOG:D0271254	HOG:D0271254	gained	0.976	104	ATP-dependent Clp protease proteolytic subunit			
	HOG:D0271213	HOG:D0271213	gained	0.976	145	Chlorophyll a-b binding protein chloroplastic			
	HOG:D0271177	HOG:D0271177	gained	0.976	276	phosphoglycerate mutase-like protein			

Ancestral GO enrichment of genes gained at the **Viridiplantae** level



Resources and References

- Orthology: definitions, inferences and impact on species phylogeny inference (Fernández, Gabaldon & Dessimoz)
<https://hal.science/hal-02535414v1>
- Inferring orthology and paralogy (Altenhoff, Glover & Dessimoz, 2019)
https://link.springer.com/protocol/10.1007/978-1-4939-9074-0_5
- Quest for Orthologs (consortium): <https://questfororthologs.org/>

Module 1 exercise: OMA Browser



OMA Academy

Welcome to the OMA Academy! Here, you will find online exercises which will aid you in becoming more familiar with orthology, phylogenies, and comparative genomics.

Background

OMA ("Orthologous MAtrix") is a method and database for the inference of orthologs among complete genomes. It can be found at omabrowser.org. Many of the exercises use the OMA browser as a starting point. The OMA pipeline can also run on custom genomic/transcriptomic data using the OMA stand-alone software, and it is even possible to combine precomputed data with custom data by exporting parts of the OMA database.

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7. [IBE Workshop - OMAnnotator: Consensus annotation using evolutionary data](#)



Gartner: 60% of cloud workloads will be built us



CDEs

Resources ▾

Solutions ▾

Platform ▾

Note: preferably use Chrome
(other browsers may have
restrictions on clipboard access
that affect copy-pasting)

Always ready-to-code.

The screenshot shows a GitHub repository page. At the top, there is a navigation bar with icons for back, forward, and search. The URL in the address bar is `github.com/DessimozLab/SIBBiodiversityBioinformatics2025`, with the last part highlighted by a red circle. Below the address bar, there are two tabs: `README` and `License`. On the right side of the header, there are edit and more options icons.

We will be mainly working on an [GitPod](#), an online integrated development environment (IDE) that allows users to write, edit, and run code directly in a web browser. GitPod is cloud-based, meaning that all software, code, and files needed for the course are stored and processed on remote servers; you will not need to install or configure anything locally.

You can access the GitPod here:

<https://gitpod.io/#https://github.com/DessimozLab/SIBBiodiversityBioinformatics2025>

Participants need to sign up for a GitPod account via Github and/or LinkedIn to access 10 hours per month for free, which is enough time to complete the exercises. After logging in, create a new workspace by choosing SIBBiodiversityGenomics2025, Browser Editor, and Large configuration (8 cores, 16 GB RAM, 50 GB storage).

New Workspace

Create a new workspace in the **Yannis' Org** organization.



github.com/DessimozLab/SIBBiodiversi...

Context URL



VS Code · 1.81.1

Editor · Browser



Large

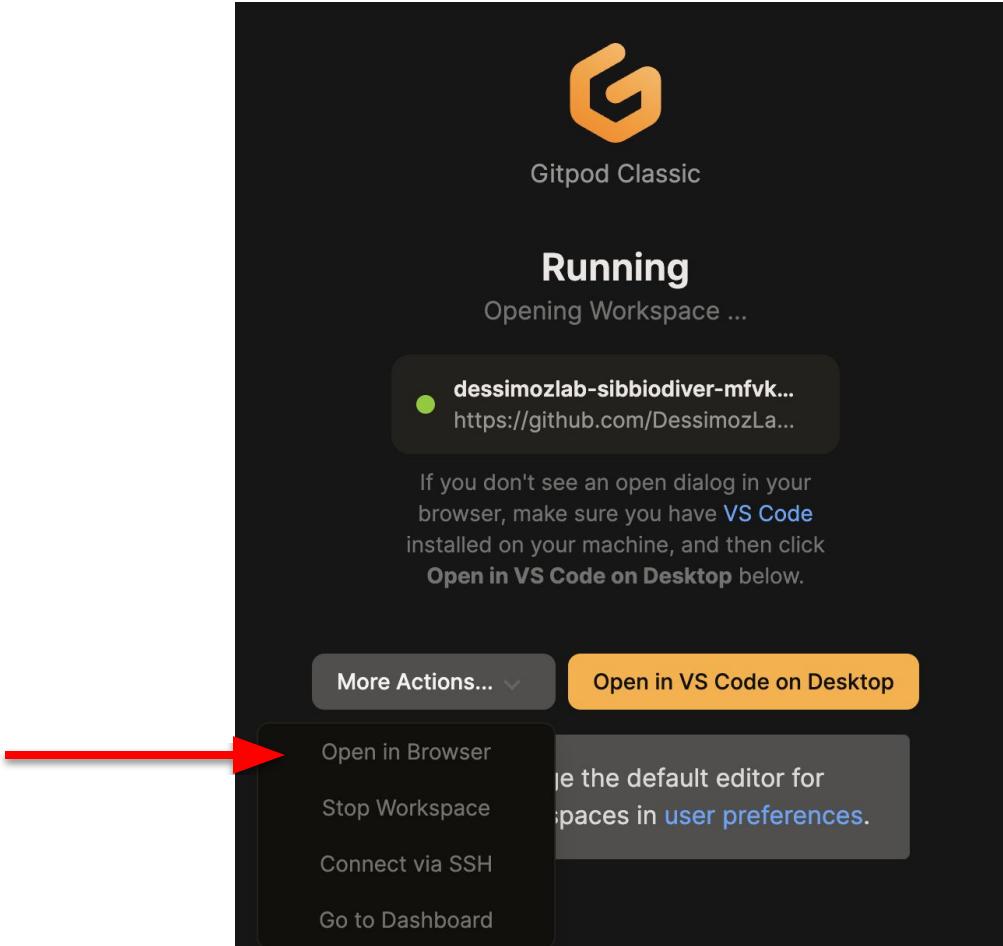
Class · Up to 8 cores, 16GB RAM, 50GB storage



Continue

Autostart with these options for this repository.

Don't worry, you can reset this anytime in your [preferences](#).



After the installation, check if everything works

```
(omacademy) gitpod /workspace $ omamer -h
usage: omamer [-h] [--version] {mkdb,search,info} ...

OMAMer - tree-driven and alignment-free protein assignment to sub-families.

optional arguments:
  -h, --help      show this help message and exit
  --version, -v  Show version and exit.

Commands:
```

omamer -h

```
(omacademy) gitpod /workspace/SIBBiodiversityBioinformatics2024/Module3_FastOMA (main) $ nextflow -h
Usage: nextflow [options] COMMAND [arg...]

Options:
  -C
    Use the specified configuration file(s) overriding any defaults
  -D
    Set JVM properties
  -bg
    Execute nextflow in background
  -c, -config
    Add the specified file to configuration set
  -config-ignore-includes
    Disable the parsing of config includes
  -h
    Print this help
  -log
    Set nextflow log file path
  -q, -quiet
    Do not print information messages
```

nextflow -h

iqtree -h

Timeouts

Workspaces will stop after a period of inactivity without any user input.

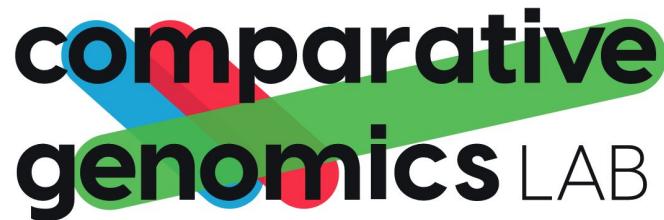
Default Workspace Timeout

1h

Save

Use minutes or hours, like 30m or 2h

Module 2: OMAmer for sequence placement into HOGs



Why sequence placement?

	Speed	Input	Proteomes
Orthology database	Instantaneous	Known identifiers	Only in the database
Placement into HOGs	Few minutes	Few sequences or whole proteome	Any proteome
Orthology inference	Hours	Whole proteomes	Any proteomes (one or several)

Why sequence placement?

	Speed	Input	Proteomes
Orthology database	Instantaneous	Known identifiers	Only in the database
Full orthology inference	Hours	Whole proteomes	Any proteomes (one or several)
Placement into HOGs	Few minutes	Few sequences or whole proteome	Any proteome

What is OM Amer?

- Fast sequence placement into existing HOGs from the OMA Browsers
- More accurate than closest sequence matching for subfamily placement!

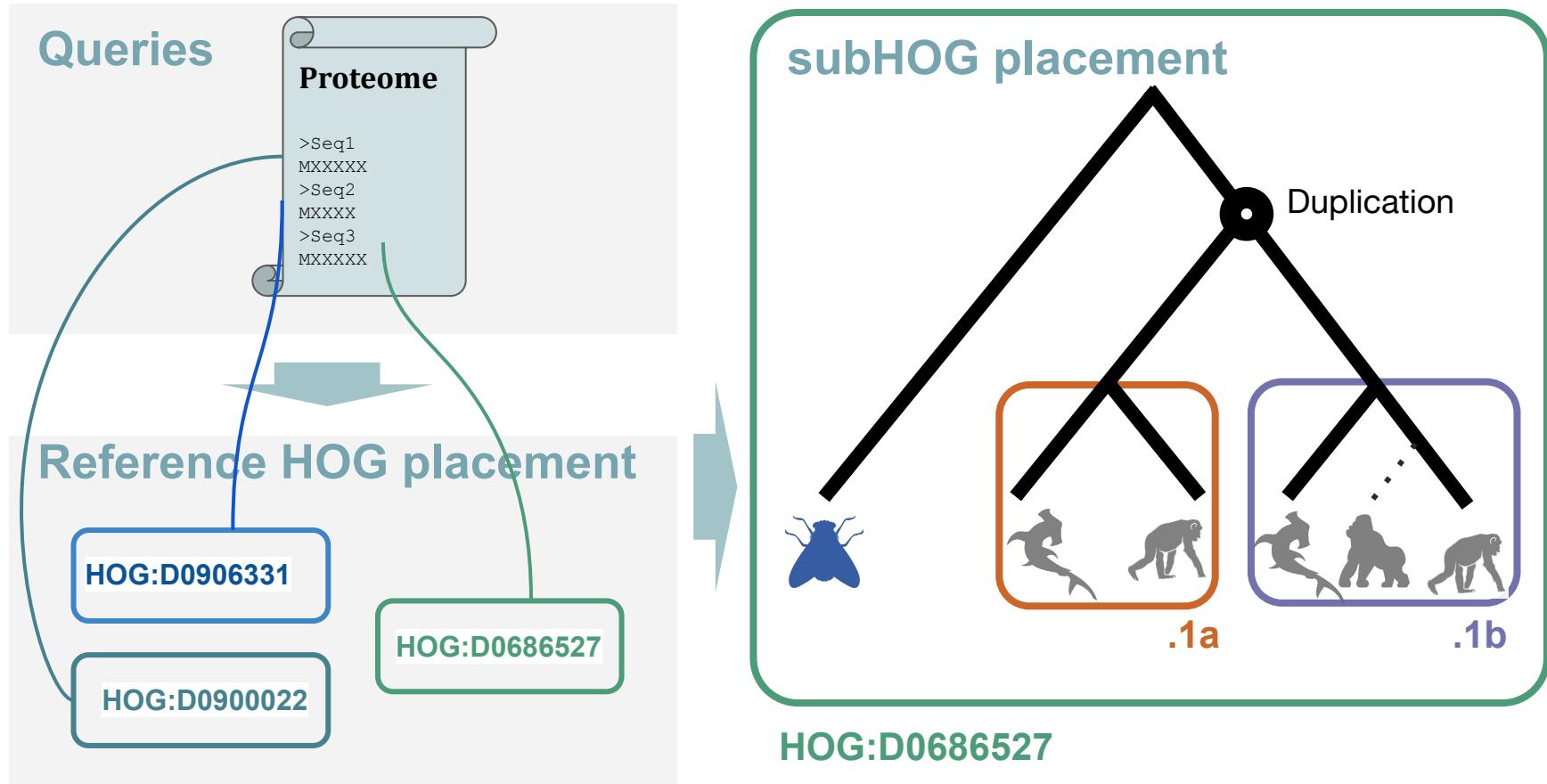
OM Amer: tree-driven and alignment-free protein assignment to subfamilies outperforms closest sequence approaches

Victor Rossier  ^{1,2,3}, Alex Warwick Vesztrocy  ^{1,2,3}, Marc Robinson-Rechavi  ^{3,4,*} and Christophe Dessimoz  ^{1,2,3,5,6,*}



<https://github.com/DessimozLab/omamer>

OMAmer placement - principle



k-mer based placement

- ❖ **k-mers** : words of k characters in a sequence

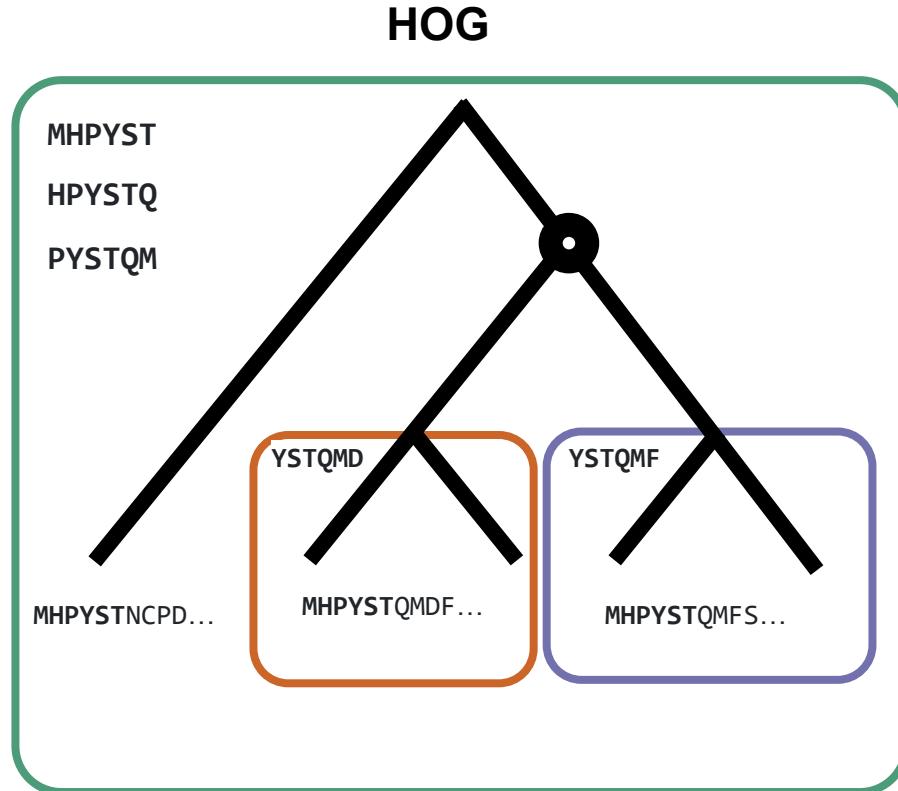
Query sequence

MHPYSTQMFS LQITVMEDSQ SDMSIELPLS

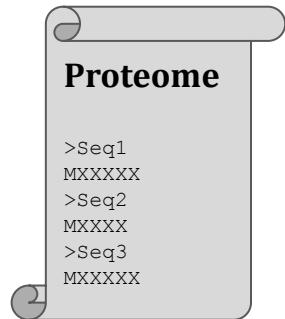
MHPYST
HPYSTQ
PYSTQM

...
...
...

MSIELP
SIELPL
IELPLS



How to use OMAMer



OMAMer database
HDF5 format

Built with HOGs from the OMA Browser

```
omamer search --query query.fa --db db.h5 --output results.txt
```

Query sequences
FASTA format

From any species

Seq1 HOG:D0578800.1c.1d
Seq2 HOG:D0571029
Seq3 HOG:D0606120.3n

OMAMer output
Tab separated format

All HOG placements

Interpreting the output

qseqid	hogid	hoglevel	family_p	family_count	family_normcount
Seq1	HOG:D0630083.1g.9c.31d	Theria	858.4562422	133	0.970765763
Seq2	HOG:D0630583	Gnathostomata	1002.895597	122	1

subfamily_score	subfamily_count	qseqlen	subfamily_medianseqlen	qseq_overlap
0.9298	53	143	143	1
1	122	128	155	1

Interpreting the output

qseqid	hogid	family_p	family_normcount	subfamily_score	qseq_overlap
Seq1	HOG:D0630083.1g.9c.31d	858.4562422	0.970765763	0.929822828	1
Seq2	HOG:D0630583	1002.895597	1	0.99999372	1

- **qseqid:** Query identifier

Interpreting the output

qseqid	hogid	family_p	family_normcount	subfamily_score	qseq_overlap
Seq1	HOG:D0630083.1g.9c.31d	858.4562422	0.970765763	0.929822828	1
Seq2	HOG:D0630583	1002.895597	1	0.99999372	1

- qseqid: Query identifier
- **hogid : HOG ID where the query is placed**

Interpreting the output

qseqid	hogid	family_p	family_normcount	subfamily_score	qseq_overlap
Seq1	HOG:D0630083.1g.9c.31d	858.4562422	0.970765763	0.929822828	1
Seq2	HOG:D0630583	1002.895597	1	0.99999372	1

- qseqid: Query identifier
- hogid : HOG ID where the query is placed
- **family_p** : p-value for query matching this family/rootHOG - expressed in neg log units (*higher is better*)

Significance threshold:
 $-\log(\text{family_p}) \geq \alpha$
 $\alpha = -\log(10^{-6})$

Interpreting the output

qseqid	hogid	family_p	family_normcount	subfamily_score	qseq_overlap
Seq1	HOG:D0630083.1g.9c.31d	858.4562422	0.970765763	0.929822828	1
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qseqid	hogid	family_p	family_normcount	subfamily_score	qseq_overlap
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- **subfamily_score:** subfamily OMAMer score (similarity between query and subHOG)

Significance threshold:
 $-\log(\text{family}_p) \geq \alpha$
 $\alpha = -\log(10^{-6})$

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qseqid	hogid	family_p	family_normcount	subfamily_score	qseq_overlap
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- qseqid: Query identifier
- hogid : HOG ID where the query is placed
- family_p : p-value for query matching this family/rootHOG - expressed in neg log units (*higher is better*)
- family_normcount : count of query k-mers in common with family/rootHOG (normalized)
- subfamily_score: subfamily OMAMer score (similarity between query and subHOG)
- **qseq_overlap** : query k-mers (%) overlapping with k-mers of reference root-HOGs

Significance threshold:
 $-\log(\text{family}_p) \geq \alpha$
 $\alpha = -\log(10^{-6})$

To remember

- Placement into HOGs allows us to find gene families for **species not in the database**
- Can be used on **any number of sequences** - from one to whole proteomes
- Precise to the subfamily level, but not a definitive proof of orthology
- Allows us to still **take advantage of OMA Browser** wealth of data and features

Module 2 exercise: OMAMer

- Go to the corresponding directory on gitpod:
/workspace/SIBBiodiversityBioinformatics202
5/Module2_OMAMer/working_dir



OMA Academy

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Background

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Schedule Aug 25 2025

Time	Activity	In charge
09:00 - 09:15	Welcome, Introductions	Natasha Glover
09:15 - 09:45	Lecture: Overview, objectives, motivation, concept of orthologs, HOGs	Natasha Glover
09:45 - 10:25	Module 1: The OMA Browser	Natasha Glover
10:25 - 10:45	Coffee break	
11:25 - 11:35	Lecture: Fast placement into hierarchical orthologous groups with OMAMer	Nikolai Romashchenko
11:35 - 12:05	Module 2: OMAMer	Nikolai Romashchenko
12:15 - 13:30	Lunch	
13:30 - 13:40	Lecture: Protein-coding gene assessment with OMArk	Natasha Glover
13:40 - 14:10	Module 3: OMArk	Natasha Glover
14:10 - 14:15	Quick break	
14:15 - 14:25	Lecture: Orthology inference with FastOMA	Stefano Pascarelli
14:25 - 15:25	Module 4: FastOMA	Stefano Pascarelli
15:25 - 15:45	Coffee break	
15:45 - 16:00	Lecture: Gene trees and species trees	Christophe Dessimoz
16:00 - 16:45	Module 5: Building species trees	Christophe Dessimoz
16:45 - 17:00	Wrap up	Christophe Dessimoz

```
source /workspace/conda/bin/activate  
conda activate omacademy
```

```
nextflow FastOMA/FastOMA_light.nf --input_folder in_folder --output_folder out_folder  
-resume
```

```
cd /workspace/SIBBiodiversityBioinformatics2023/Module3_FastOMA/expected_output/
```

Typo alert! (Module 3.1):

In this exercise, we will run FastOMA standalone to infer the orthology information for five yeast species. We already provided the proteomes of five species in the GitPod environment, located at

/workspace/SIBBiodiversityBioinformatics2023/Module3_FastOMA/working_dir/in_folder/proteome.



Click to go back, hold to see history

.gitpod.yml	Changed path in the github file to reflect changing name of repo	yesterday
LICENSE	Initial commit	5 days ago
README.md	Update README.md	now

README.md



SIB Biodiversity Bioinformatics 2023

Teachers

- Natasha Glover
- Yannis Nevers
- Sina Majidian
- Christophe Dessimoz

FastOMA (temp)

FastOMA command line

```
cd /workspace/SIBBiodiversityBioinformatics2023/Module3_FastOMA/working_dir/  
nextflow FastOMA_light.nf --input_folder in_folder --output_folder out_folder
```



expected output structure for test data

Then, following files and folders should appear in the folder `out_folder` which was the argument.

```
$ls out_folder  
hogmap OrthologousGroupsFasta OrthologousGroups.tsv output_hog.orthoxml
```

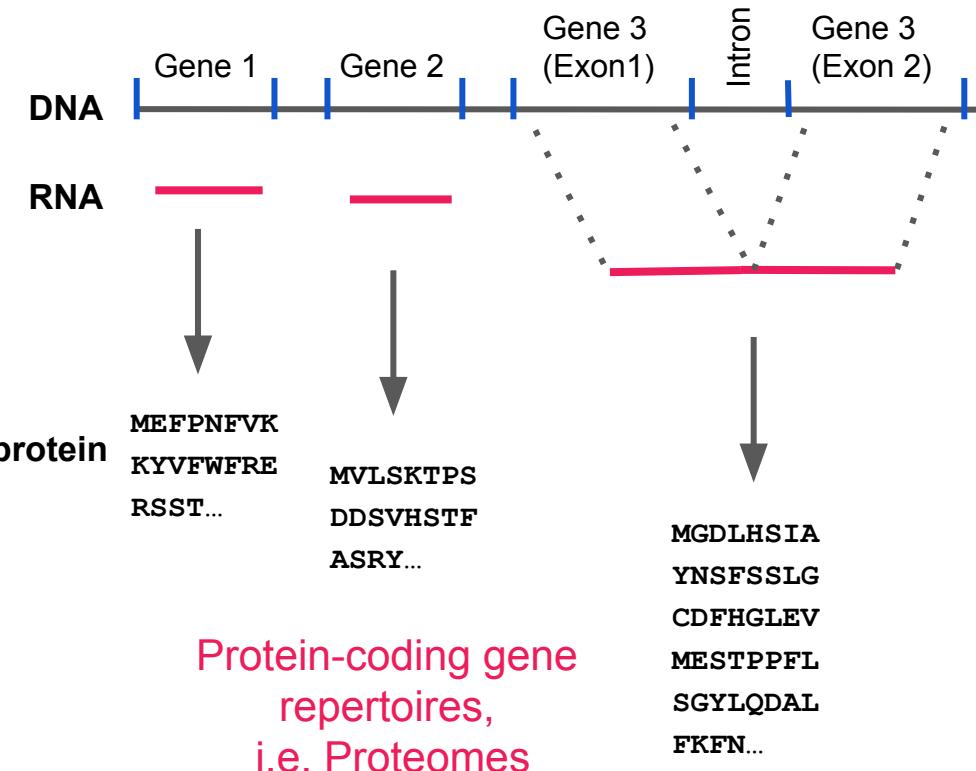


Module 3: OMArk

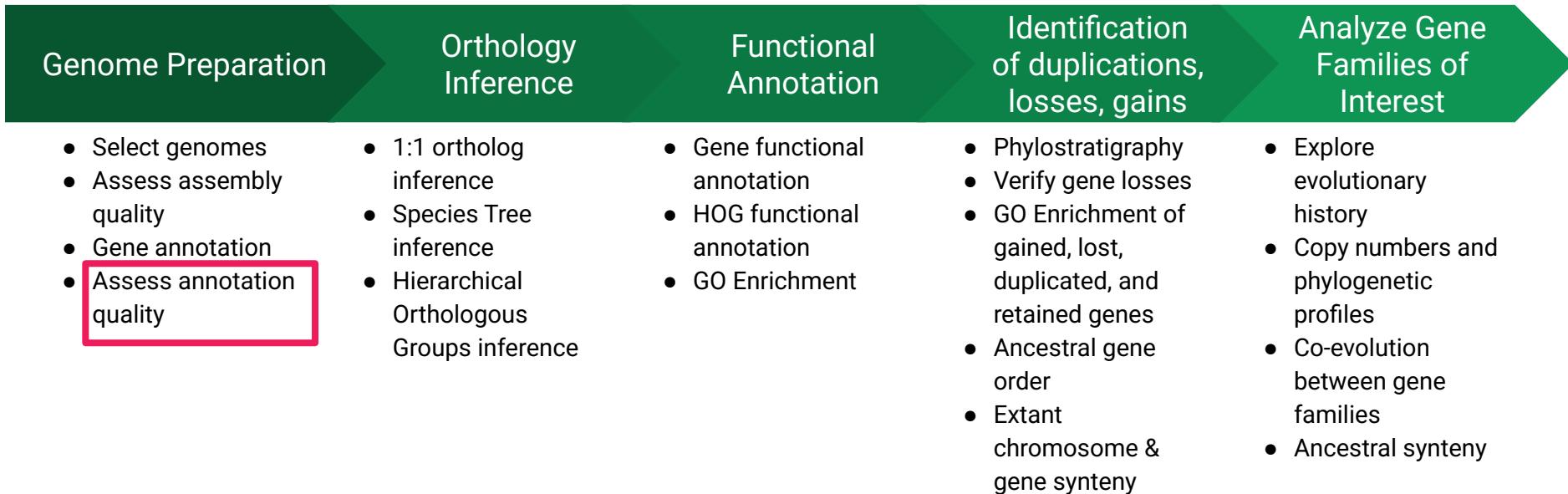
Structural gene annotation

Identifying the physical locations of genes and their components:

- coding regions
- non-coding regions
- start and stop codons
- regulatory elements
- untranslated regions (UTRs)



Comparative Genomics Pipeline



Protein-coding gene repertoire quality

- **Coding-gene repertoire:** set of coding-genes annotated on a given genome sequence
- Available on databases as **proteomes**

Subject to quality issues

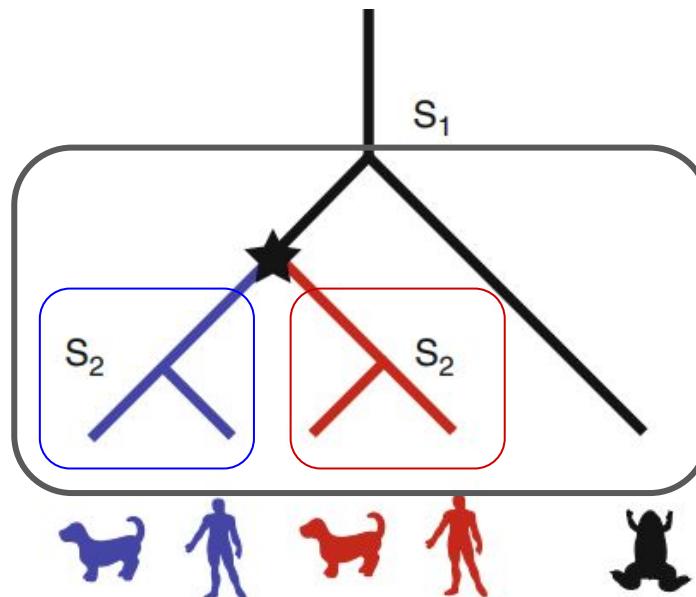


- Missing genes
- Fragmented genes
- Inclusion of non-coding regions
- Contamination

Lack of tool to detect all these issues!

HOGs defined at a taxon represent ancestral genes

- Duplication event in mammals gave rise to 2 subfamilies
- 2 ancestral genes at mammals level
- 1 ancestral gene at Tetrapod level



All HOGs defined at a taxon = **ancestral gene repertoire**

OMArk workflow overview

Inputs



OMAmer
database
(Defined at
LUCA)

Proteome

```
>Seq1  
XXXXXX  
>Seq2  
XXXXXX  
>Seq3  
XXXXXX
```

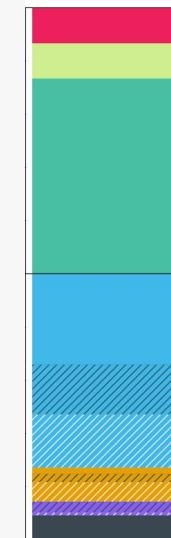
Gene Family Placement



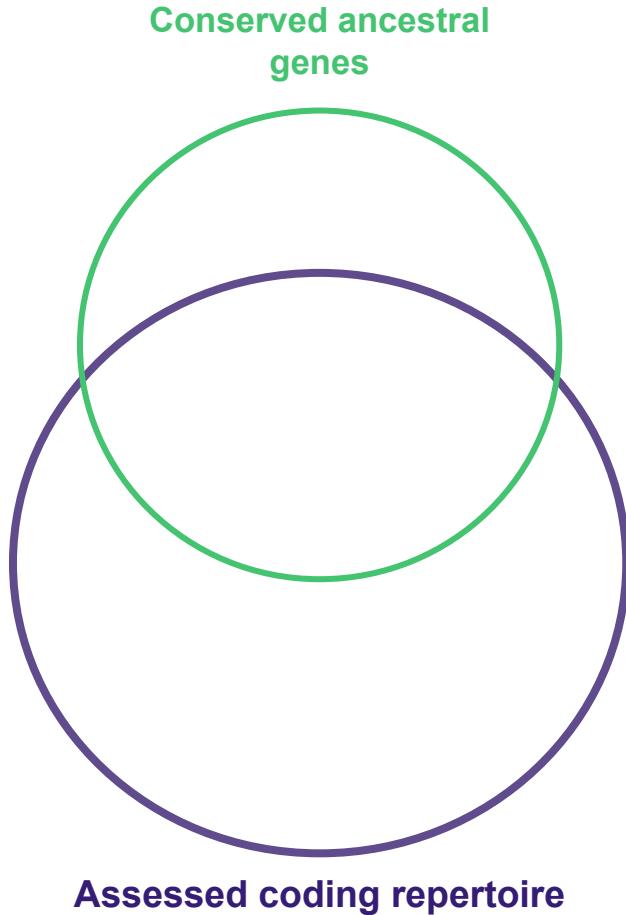
Fast k-mer based
mapping of proteins to
gene families (HOGs)

Seq1	HOG:B0578800.1c.1d
Seq2	HOG:B0571029
Seq3	HOG:B0606120.3n

Annotation Quality Assessment



QA summary



Ancestral lineage:

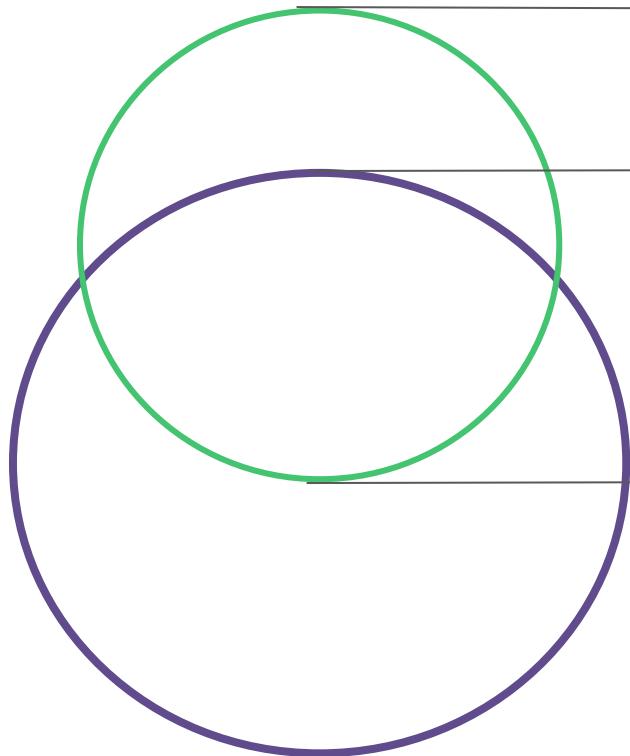
- Latest ancestor clade in OMA with 5+ representatives
- Dynamically selected from taxid or from the placements

Conserved ancestral genes:

- Gene families defined at the ancestral lineage level (ancestral gene repertoire)
- Present in at least 80% species

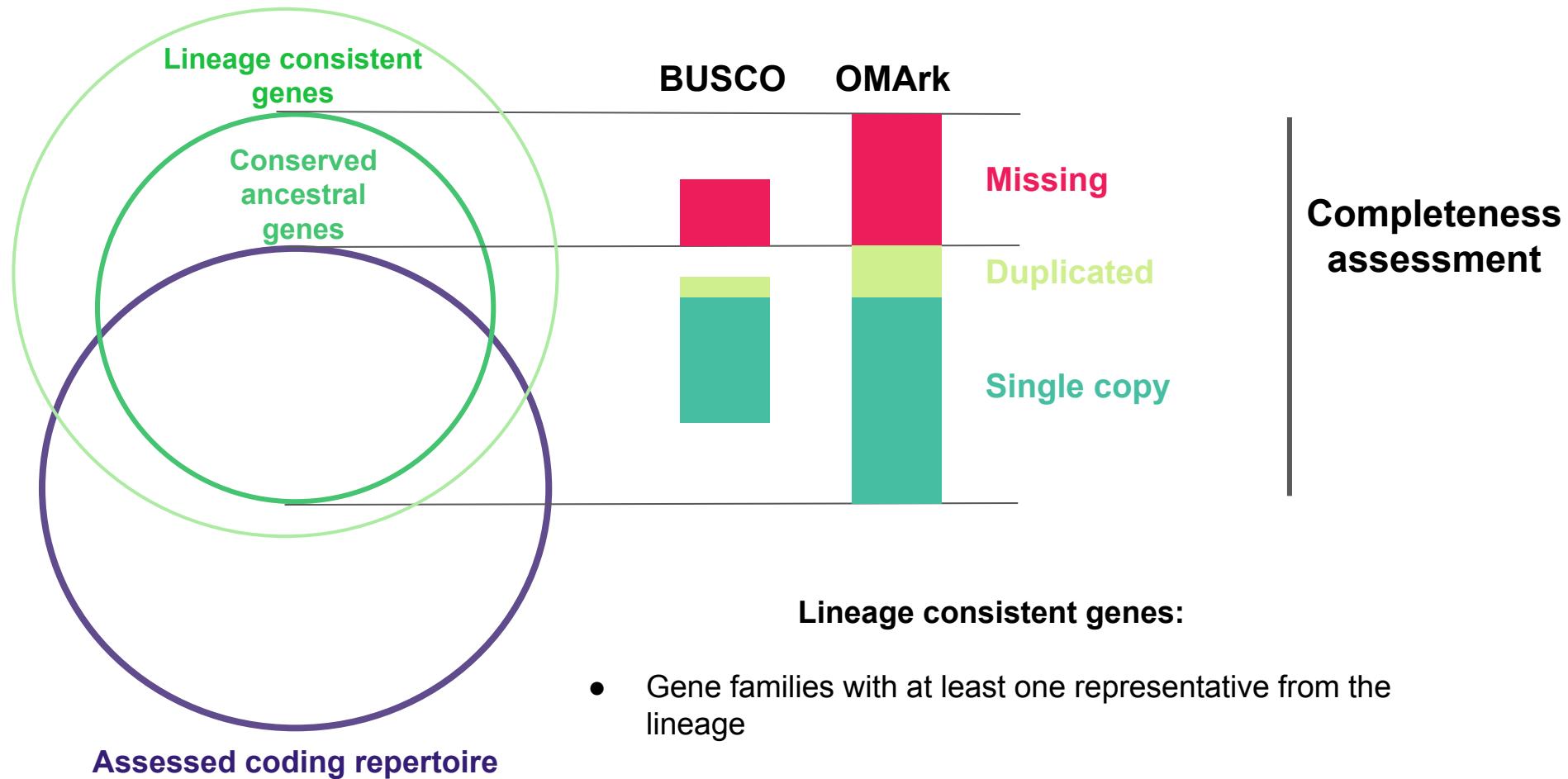
Conserved ancestral
genes

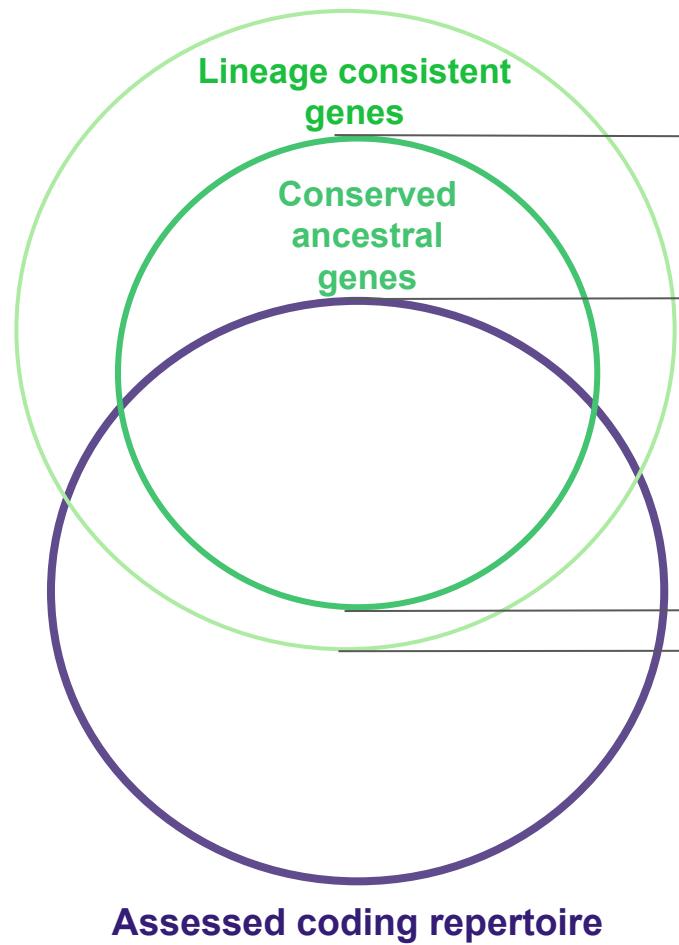
BUSCO OMArk



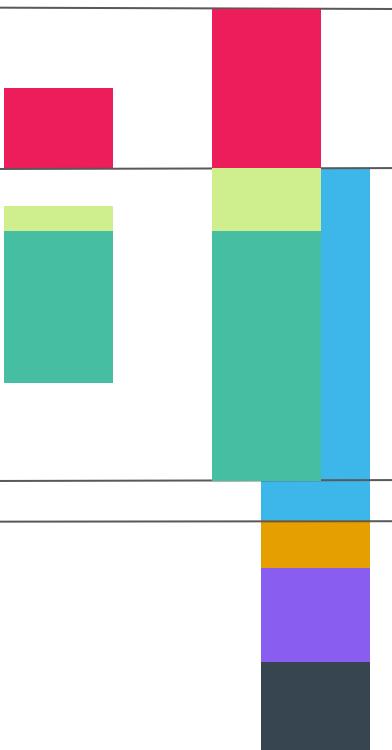
Missing
Duplicated
Single copy

Completeness
assessment





BUSCO OMark



Consistent

Contamination

Inconsistent

Unknown

Consistency Assessment
(whole repertoire)

OMArk Results: Graph summary



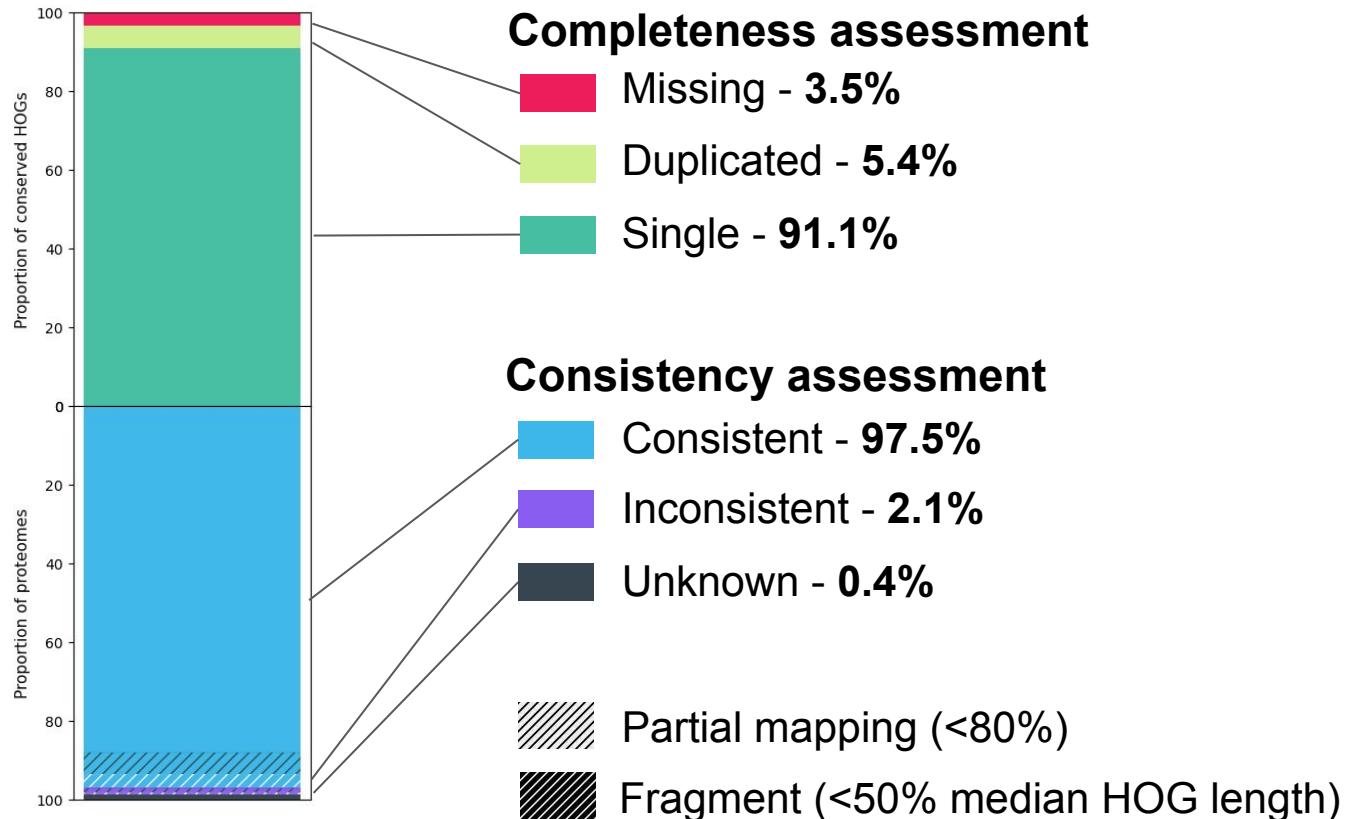
Source: wikipedia

Zebrafish
Danio rerio

Otophysi clade

16,336 conserved
HOGs

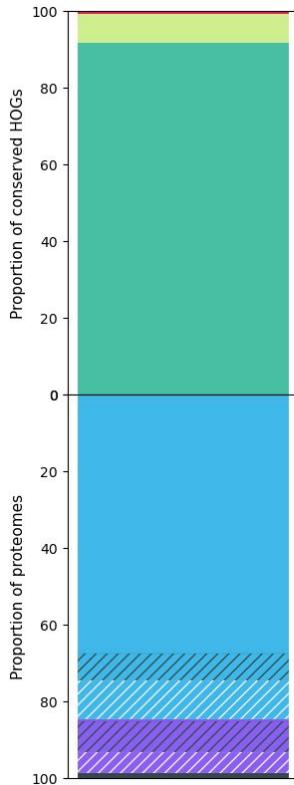
25,706 genes



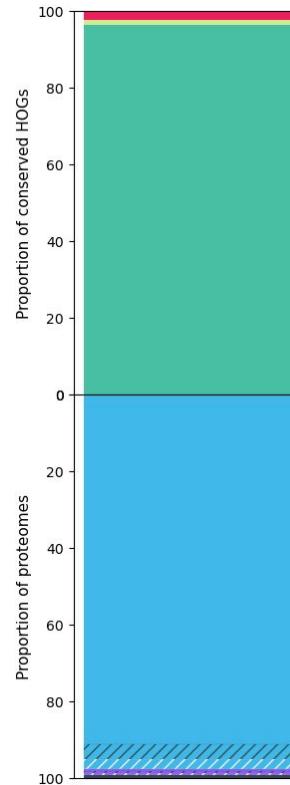
Use-case: Annotation comparison



Ensembl Metazoa 48
July 2020



Ensembl Metazoa 54
July 2024



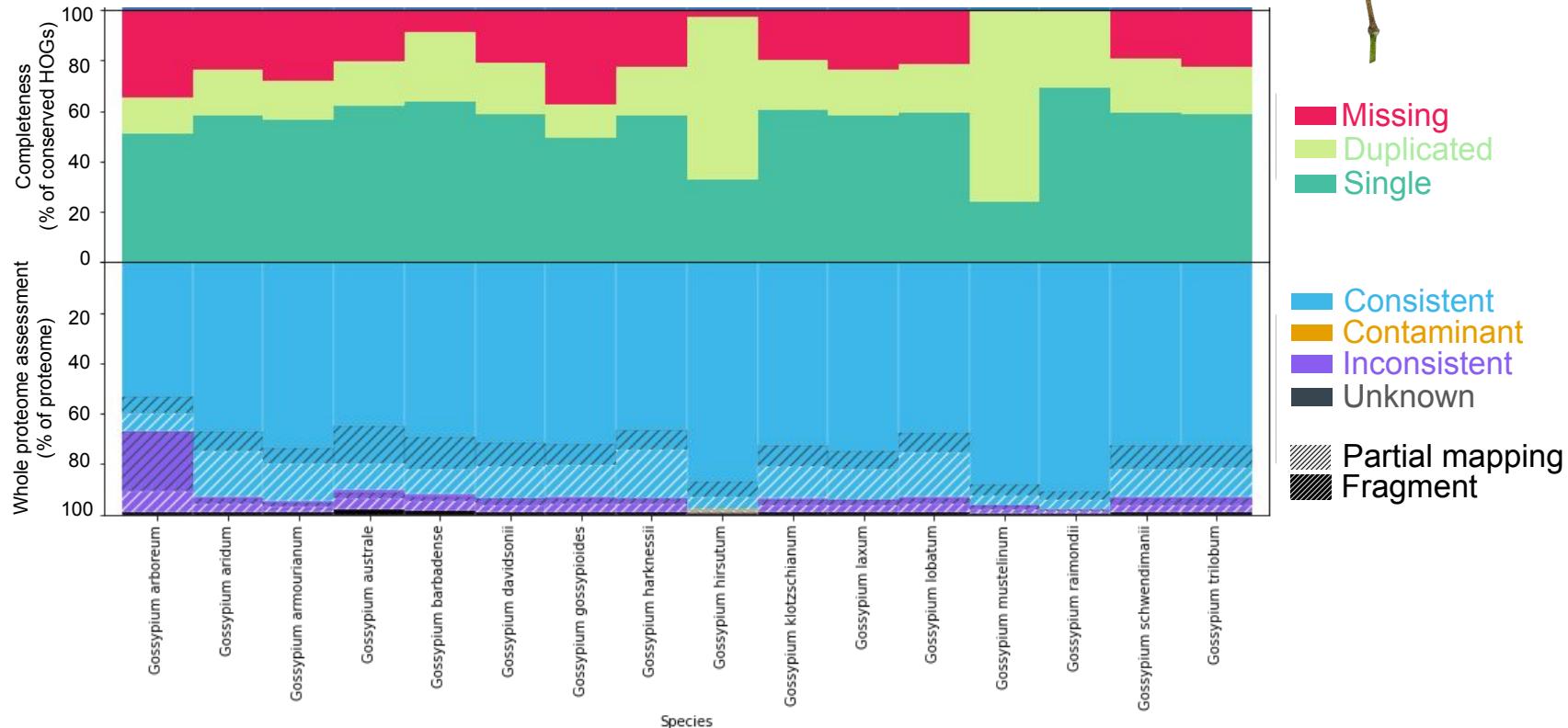
- Missing
- Duplicated
- Single

- Consistent
- Contaminant
- Inconsistent
- Unknown

- Partial mapping
- Fragment

Use-case: proteome selection

Comparison of *Gossypium* (Cotton-shrub) proteomes



OMArk browser

<https://omark.omabrowser.org/>

 Enter species name Submit Genome Help Sign In Sign Up Change datasets

OMArk is a software to assess the quality of gene repertoire annotated from a genomic sequence - also called proteome. It relies on comparisons to the predicted ancestral gene repertoire of the target species and to the extant gene repertoire of close species to:

- Estimate the completeness of the gene repertoire by comparison to conserved orthologous groups.
- Estimate the proportion of accurate and erroneous gene models in the proteome.
- Detect possible contamination from other species in the proteome.

The software is available as a command-line tool on [GitHub](#) or can be executed from this webserver.

Submit genomes



Euarchontoglires

Proteome size

% of conserved HOGs

% of proteome

Select Taxon ▾

PNG SVG

Protein Number

Missing Duplicated Single

Consistent Contamination Inconsistent Partial mapping Fragment Unknown

Module 3 exercise: OMArk

- Go to the corresponding directory on gitpod:
/workspace/SIBBiodiversityBioinformatics202
5/Module3_OMArk/working_dir



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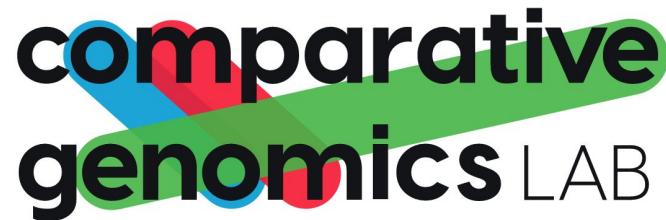
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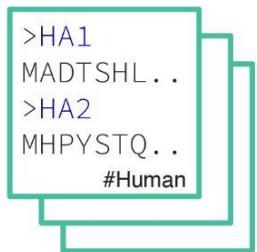
1. [Exploring Orthology with the OMA Browser](#)
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3. [OMArk](#)
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Module 4: FastOMA



FastOMA, our new tool

Input Proteomes

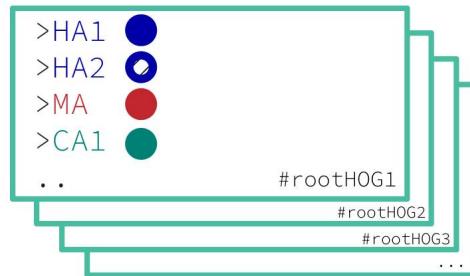


OMAmer

Mapping sequences
on OMA gene families
based on k-mers

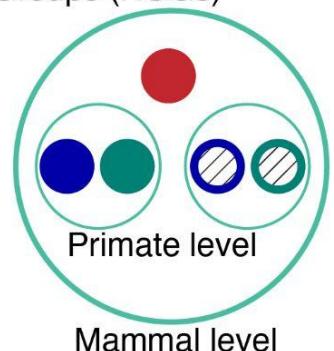


Root HOGs (Gene families)



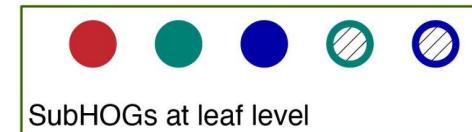
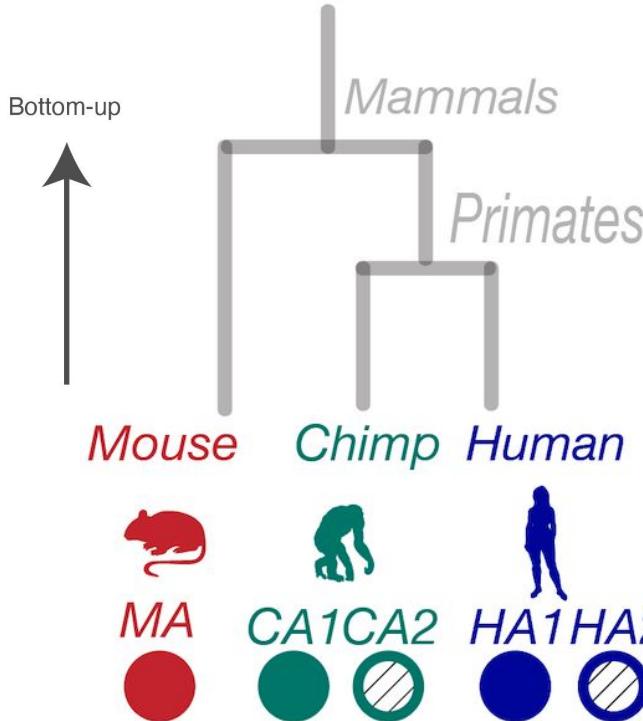
SubHOG/event
inference
(in parallel)

Hierarchical Orthologous Groups (HOGs)

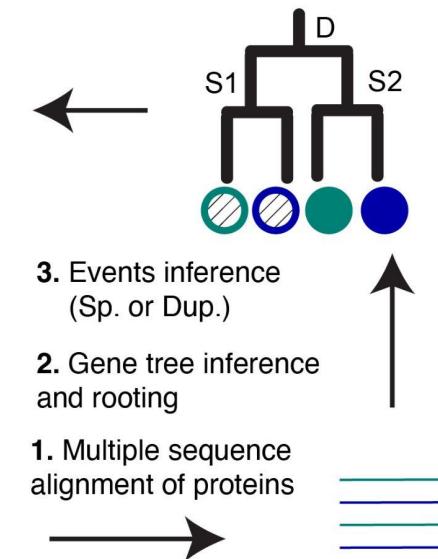
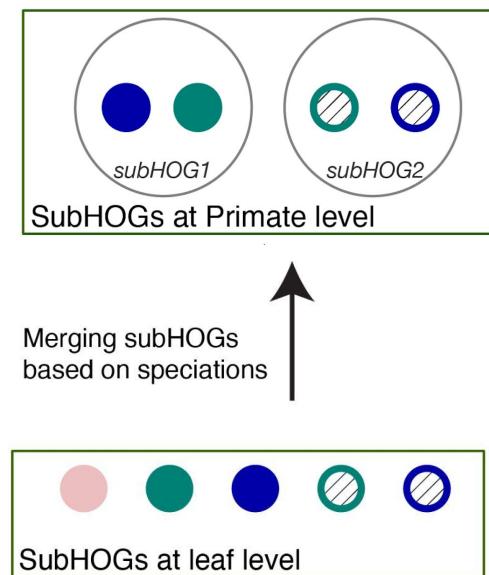
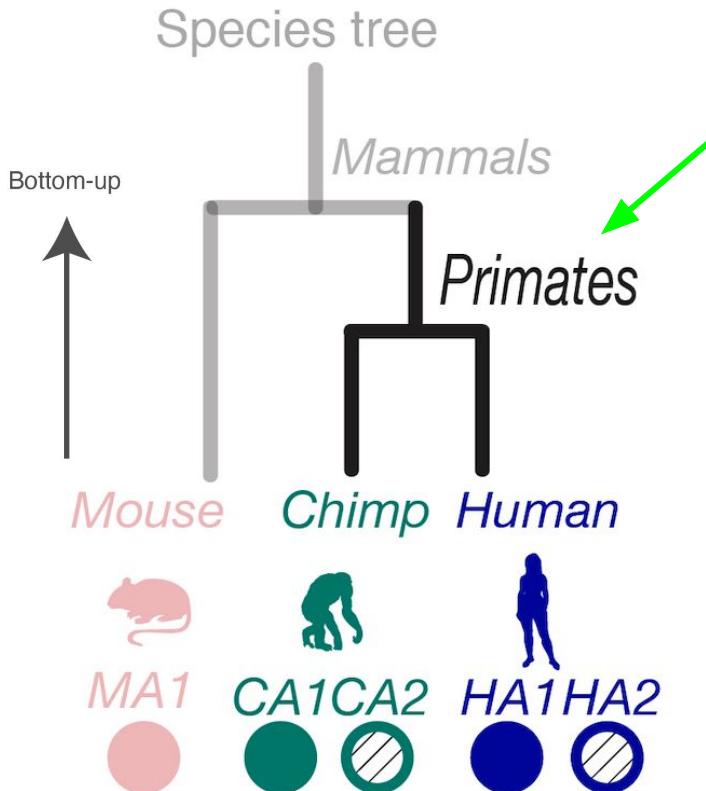


HOG inference

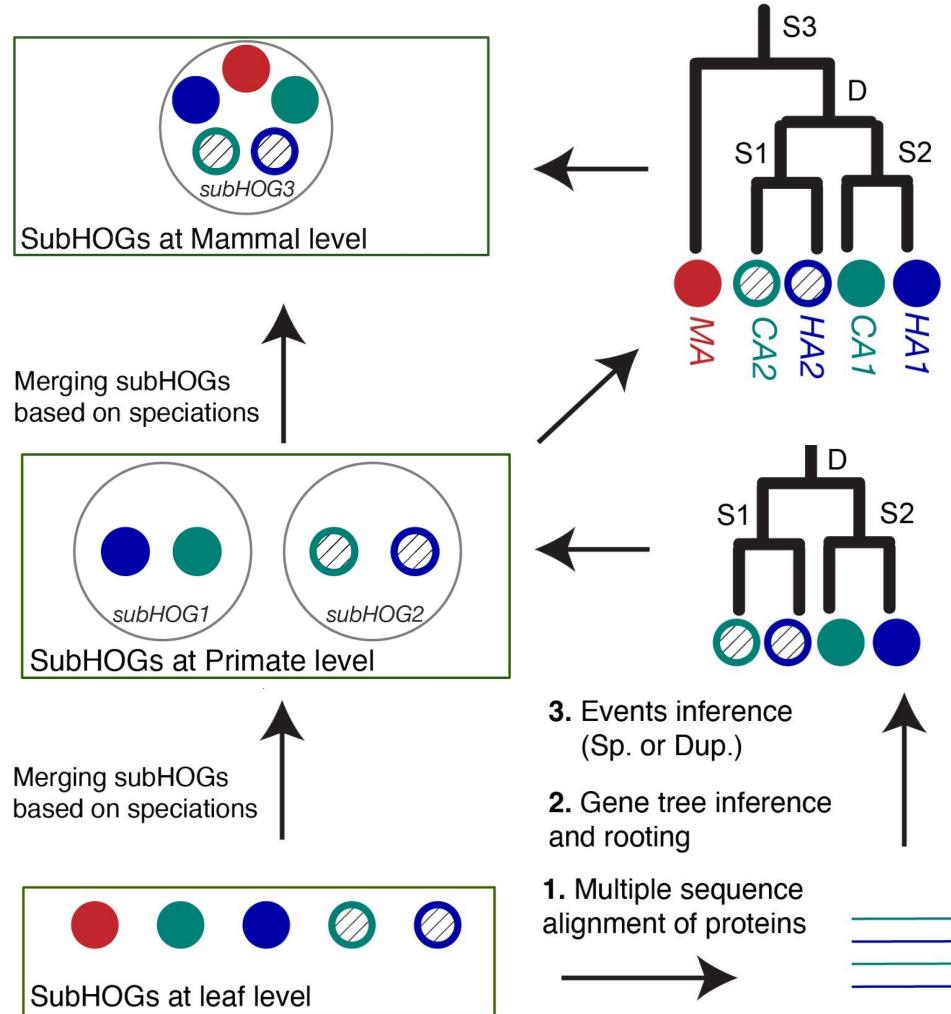
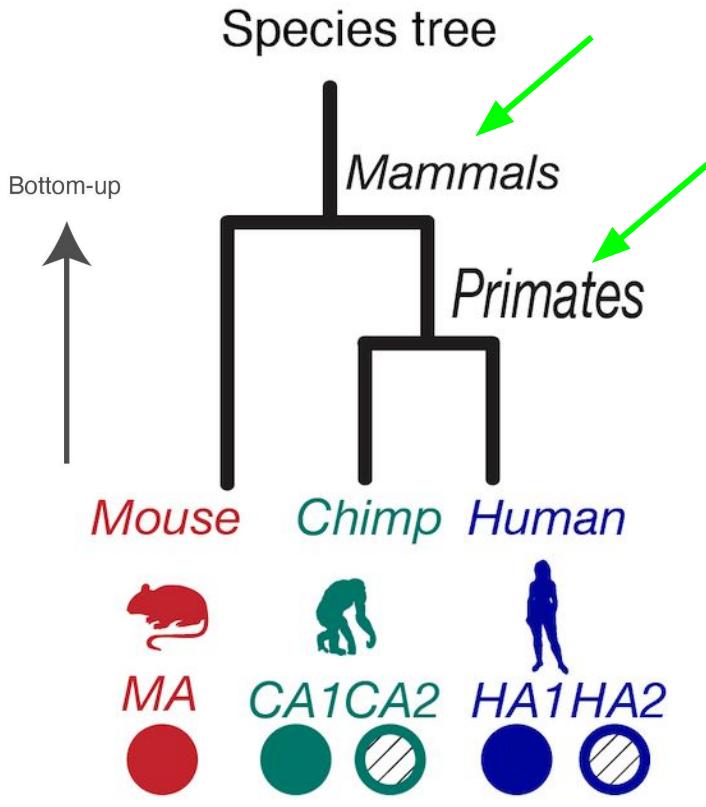
Species tree
Bottom-up



HOG inference

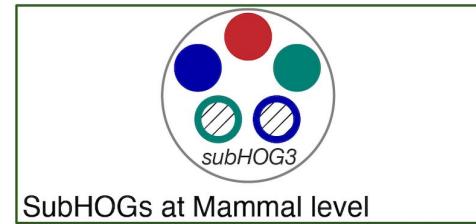
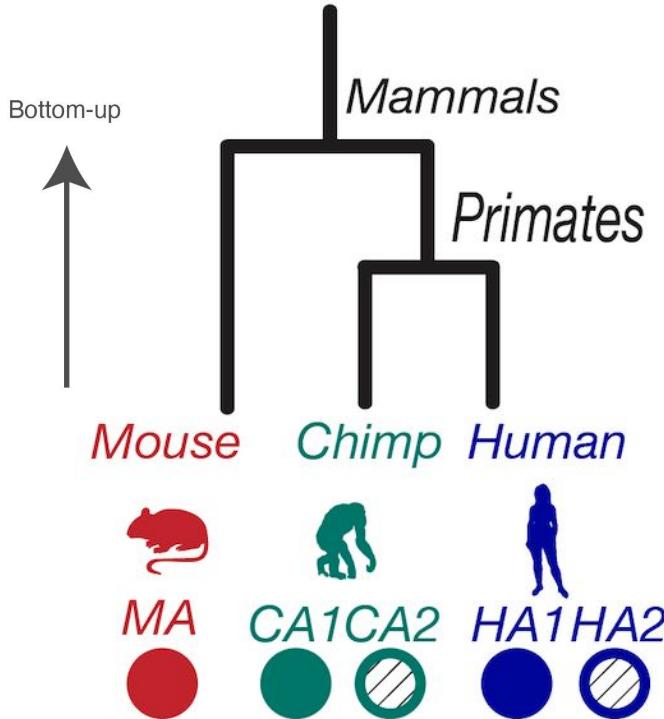


HOG inference



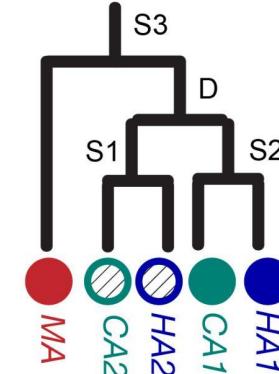
HOG inference

Species tree

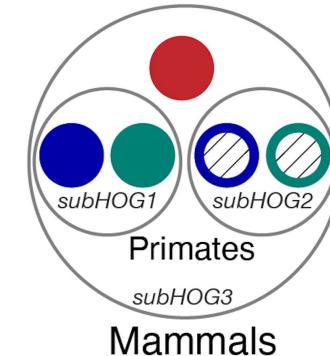


HOG in orthoXML

```
<orthoGroup3 Mammals>
  MA
  <paraGroup>
    <orthoGroup1 Primate>
      HA1
      CA1
    </orthoGroup1>
    <orthoGroup2 Primate>
      HA2
      CA2
    </orthoGroup2>
  </paraGroup>
</orthoGroup3>
```



Nested structure of HOG

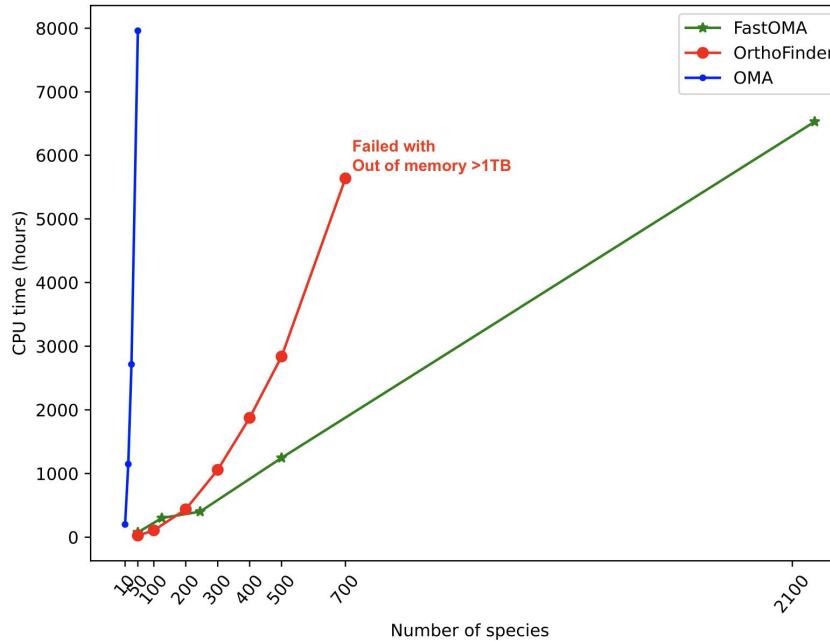


Orthology inference for Eukaryote dataset

- 2180 eukaryotic species
- Uniprot reference proteomes
- in a single day using 300 CPUs



[github.com/DessimozLab/
FastOMA](https://github.com/DessimozLab/FastOMA)



Module 3.3

- 4. How many Root HOGs are in the HOG file?

 Hint

 Answer



Each line in the output file denotes a gene family. After running, check the end of the file rootHOGs.tsv. Note that the indexing starts from 0.



There are 6793 rootHOG (gene families) in this file.

- 5. Consider the gene “60S ribosomal protein L15-A” in *Schizosaccharomyces pombe* with protein ID: RL15A_SCHPO. How many proteins are in the gene family (for these 5 species of interest)?

Module 4 exercise: FastOMA



OMA Academy

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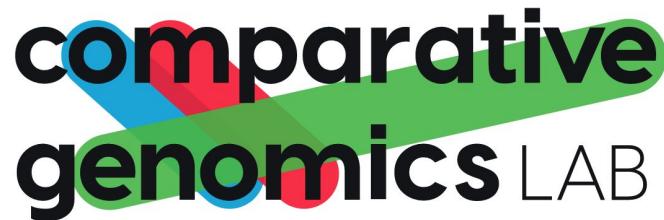
Background

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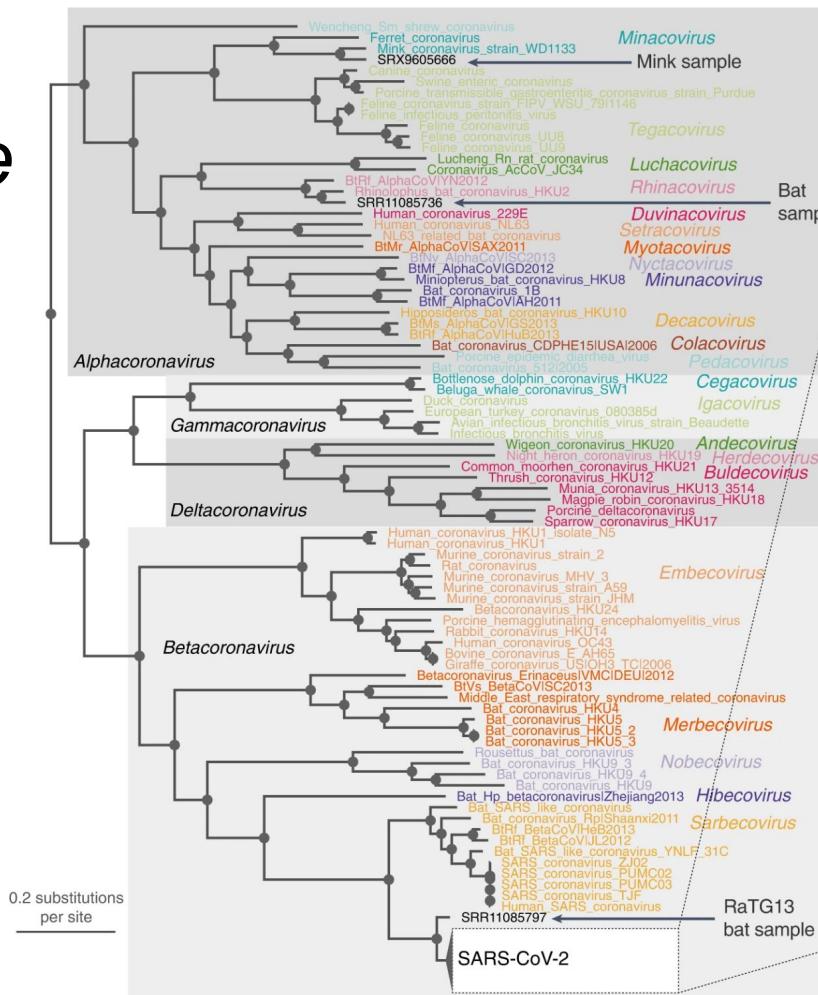
Module 5: Building species trees



A tree of Coronaviridae viruses

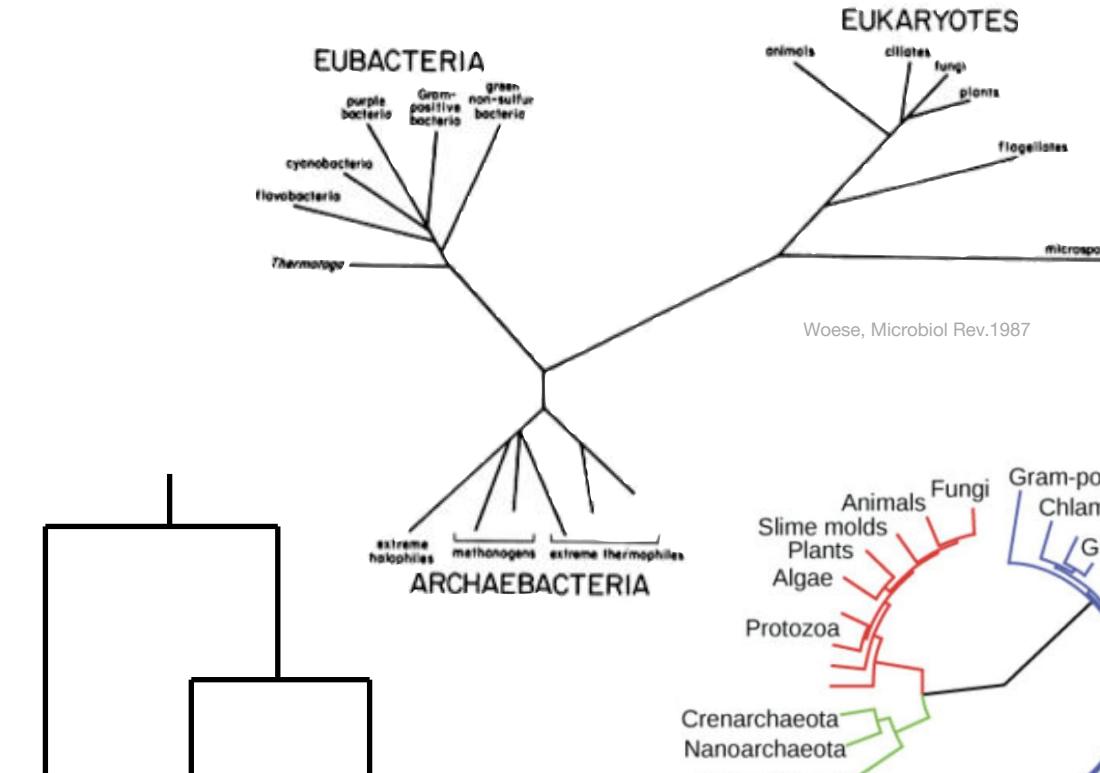
Concepts:

- Topology
- Branches lengths
- Internal nodes
- Leaves
- Root
- Clades / lineages

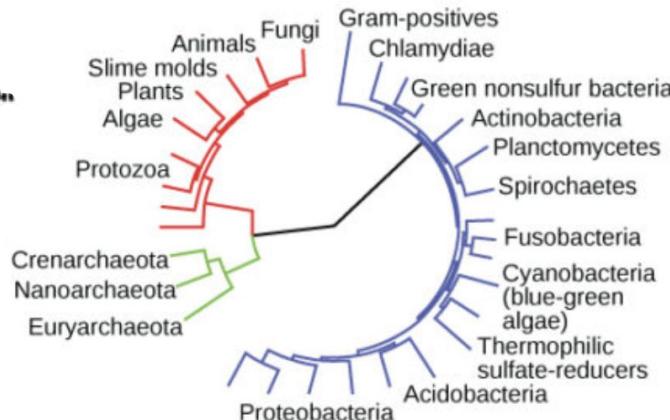


phylo.io

Other tree representations

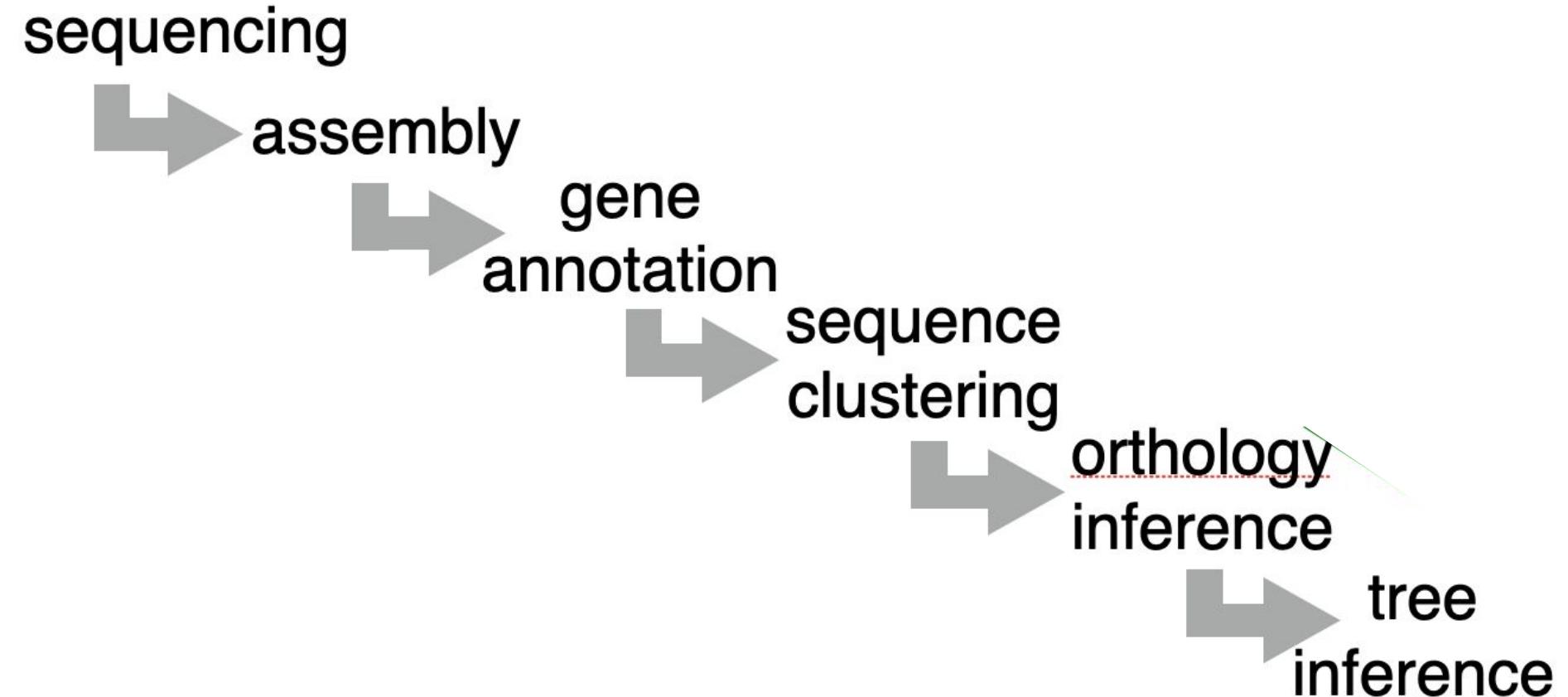


Gorilla Chimp Human



Concepts:

- Rooted vs. unrooted
- Scaled vs. unscaled



HOG:C0620879.1a with 61 members (insulin)

Vertebrata / Gnathostomata / Euteleostomi / Sarcopterygii / Tetrapoda / Amniota / Mammalia / Lower Level ▾



Graphical viewer

Members

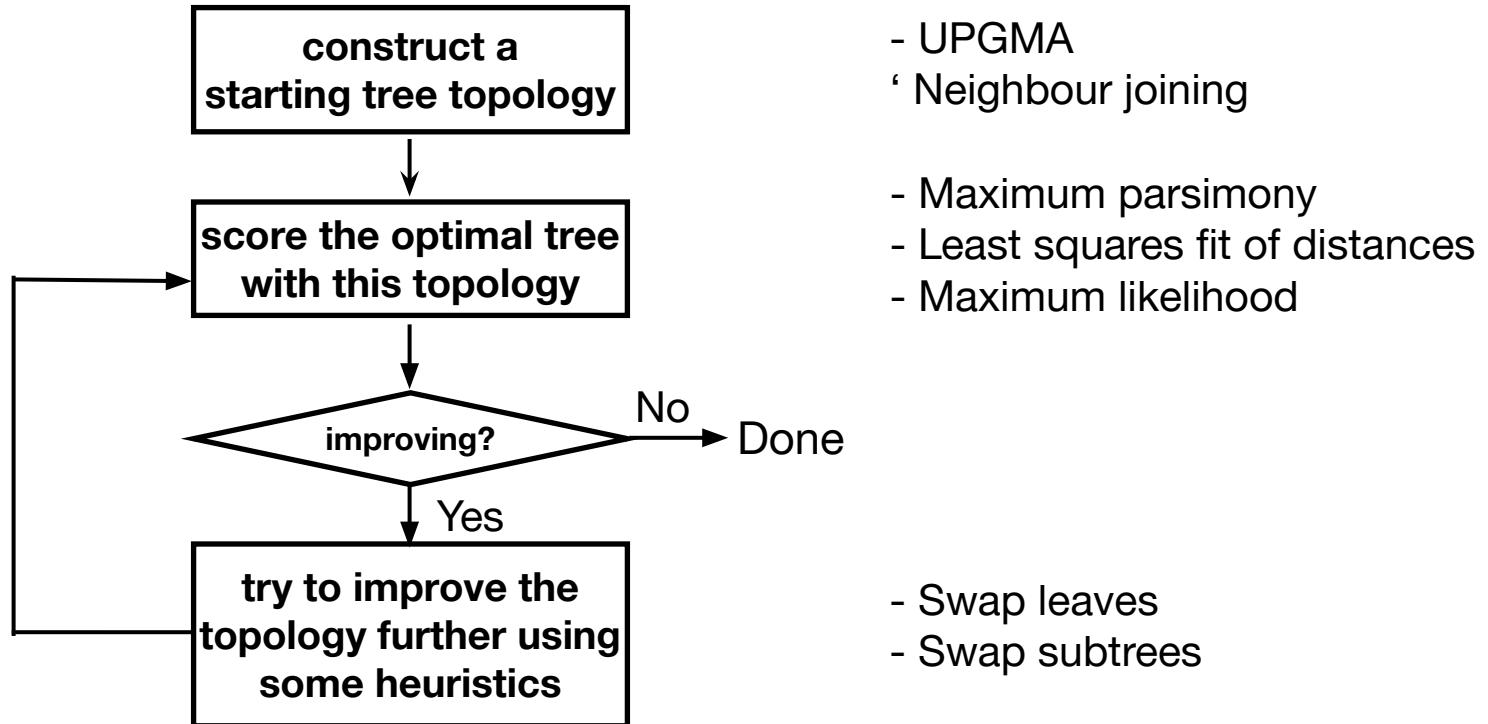
Alignment

Ancestral synteny

Similar HOGs

Label	2 . 4 . 6 . 8 . 10 . 12 . 14 . 16 . 18 . 20 . 22 . 24 . 26 . 28 . 30 . 32 . 34 . 36 . 38 . 40 . 42 . 44 . 46 . 48 . 50 . 52 . 54 . 56 . 58 . 60
CHIGR1/53/	- - - - - M A L W M K L L P L L A L L A L W E P N P A Q A F Y N Q H L C G S H L V E A L Y L V C G E R G F F Y T
MOUSE26691	- - - - - M A L L V H F L P L L A L L A L W E P K P T Q A F V K Q H L C G P H L V E A L Y L V C G E R G F F Y T
MOUSE57227	- - - - - M A L W M R F L P L L A L L A L F L W E S H P T Q A F V K Q H L C G S H L V E A L Y L V C G E R G F F Y T
RATNO17730	M P S C G H C S N M A L W I R F L P L L A L L I L W E P R P A Q A F V K Q H L C G S H L V E A L Y L V C G E R G F F Y T
RATNO18008	- - - - - M A L W M R F L P L L A L L A L V L W E P K P A Q A F V K Q H L C G P H L V E A L Y L V C G E R G F F Y T
NANGA19616	- - - - - M A L W M R F L L P L L A L L A F W G P N P G Q A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
ICTTR07348	- - - - - M A L W T R L L P L L A L L A L L W G P D P A Q A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
CERAT20372	- - - - - M A L W M R F L L P L L A L L A L W G P D P V P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
CHLSB00063	- - - - - M A L W M R F L L P L L A L L A L W G P D P V P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
MACFA08228	- - - - - M A L W M R F L L P L L A L L A L W G P D P P A P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
MACMU09924	- - - - - M A L W M R F L L P L L A L L A L W G P D P P A P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
MACNE29303	- - - - - M A L W M R F L L P L L A L L A L W G P D P P A P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
MANLE26401	- - - - - M A L W M R F L L P L L A L L A L W G P D P V P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
PAPAN06025	- - - - - M A L W M R F L L P L L A L L A L W G P D P V P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
COLAP22086	- - - - - M A L W M R F L L P L L A L L A L W G P D P V P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
RHIBE04930	- - - - - M A L W M R F L L P L L A L L A L W G P D P V P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
RHIRO07577	- - - - - M A L W M R F L L P L L A L L A C G P D P V P A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
GORG003436	- - - - - M A L W M R F L L P L L A L L A L W G P D P A A A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
HUMAN03911	- - - - - M A L W M R F L L P L L A L L A L W G P D P A A A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
PANPA05915	- - - - - M A L W M R F L L P L L A L L A L W G P D P A S A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
PANTR02017	- - - - - M A L W M R F L L P L L V L L A L W G P D P A S A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T
PONAB02480	- - - - - M A L W M R F L L P L L A L L A L W G P D P A - A F V V N Q H L C G S H L V E A L Y L V C G E R G F F Y T

General Scheme to Build Phylogenetic Trees

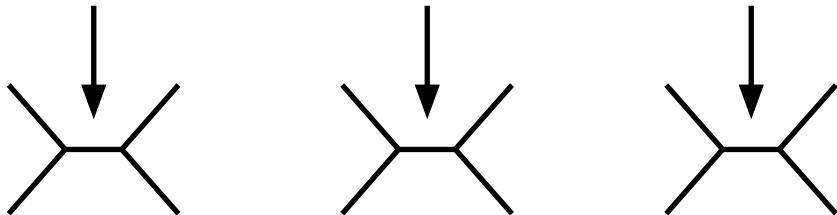
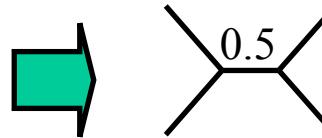


Measuring confidence with the Bootstrap

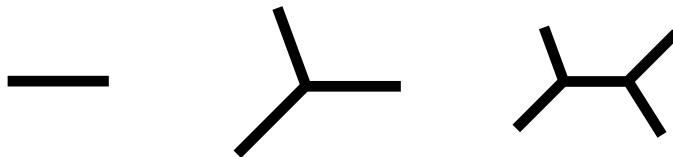
How would our tree inference change if we repeated the experiment with other data that came about through the same process?

Use “pseudo-replicates”: resample alignment columns (with replacement), same length.

Data	Replicate 1	Replicate 2
A: ALTF ^C G	A: LCGC ^A L	A: ATF ^A L ^F
B: NL ^T FCG	B: LCGC ^N L	B: NT ^F NLF
C: ALSFRG	C: LRGRAL	C: ASF ^A L ^F
D: NLSFRG	D: LRGRNL	D: NSF ^N LF



**How many branches are there
in an unrooted bifurcating
tree of n taxa?**



2

3

4

1

3

5

How many topologies?

**Number of
“taxa”**

3

**Number of binary trees
Unrooted Rooted**

1

Module 5 exercise: Building species tree



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Recap

