2024 Workshop on Phylogenomics

RAxML-NG Introduction and Laboratory

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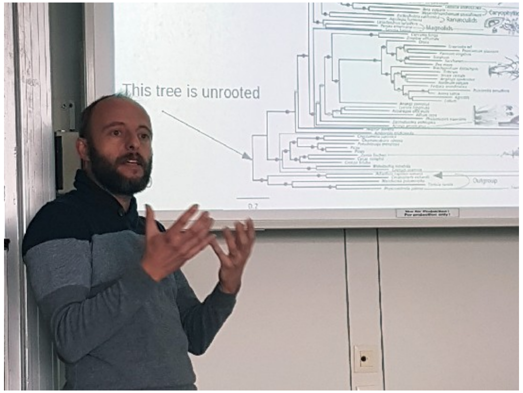
Český Krumlov – January 25, 2024

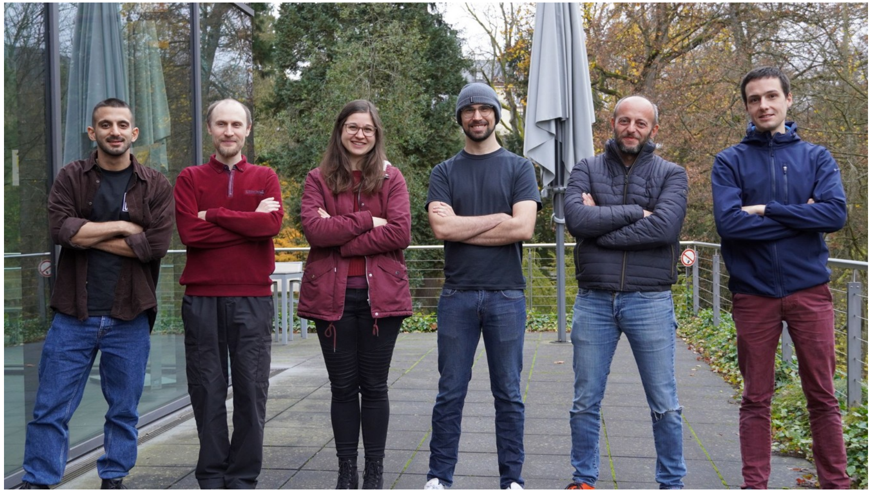
Bio

- 2007 Bachelor of Computer Science (Kyiv, Ukraine)
- 2007 – 2010 Software developer
- 2013 Master of Computer Science (KIT, Karlsruhe, Germany)
- 2013 – 2018 PhD student (Stamatakis lab)
- 2018 – ... Staff scientist (HITS, Heidelberg, Germany)
- 2023 – ... Sustainable Computing Advisor (part-time) → Sat. 2pm
- Maintainer and lead developer of RAxML-NG
- First name: Oleksiy (preferred), Alexey (before 2022)

Stamatakis lab(s)

- Prof. Alexandros Stamatakis
  - Author of the “original” RAxML
- “The Germans” → Heidelberg
  - https://cme.h-its.org/exelixis
  - 1 staff scientist, 1 PostDoc, 5 PhD students
- “The Cretans” → Heraklion
  - https://www.biocomp.gr/
  - 3 PostDocs, 2 PhD students





Outline

- RAxML-NG Intro
- Lab #1: Basics
- Results & Discussion

- RAxML-NG Parallelization
- Lab #2: Large datasets & Parallelization
- Results & Conclusions

RAxML-NG

- Maximum-likelihood (ML) phylogenetic inference tool
- Design goal #1: performance & scalability
  - B10K: 363 taxa x 63M sites (Stiller et al., under review)
- Design goal #2: ease-of-use
  - Defaults & auto-tuning whenever possible
- Design goal #3: efficiency
  - Avoid wasting time & resources

Evolution of RAxML(-NG)

2006 – 2018

RAxML

(Stamatakis 2006, 2014)

- Fast & feature-rich
- Some limitations
- Hard to maintain

2018 – 2024

RAxML-NG 1.x

(Kozlov 2019)

- More flexible
- Easier to use
- Easier to maintain

2024 –

RAxML-NG 2

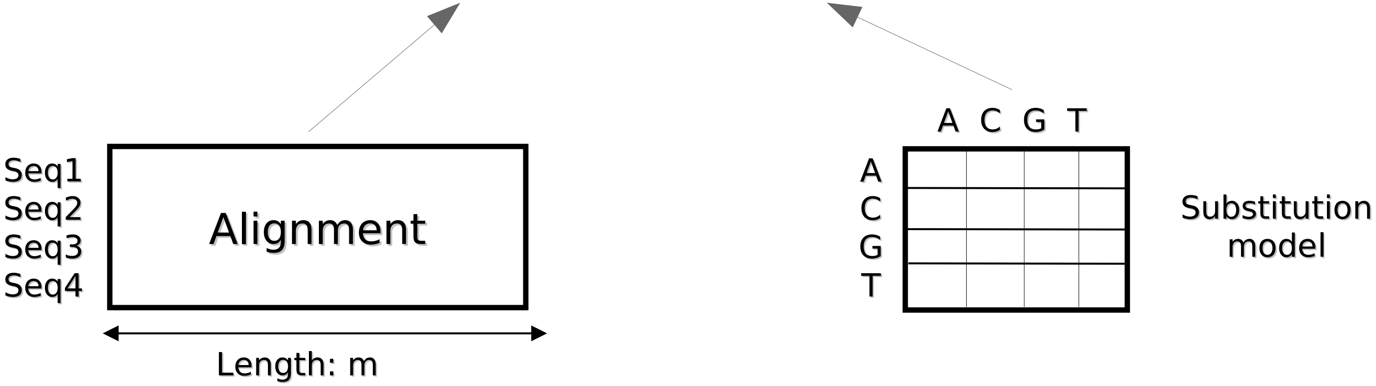
(in preparation)

- Adaptive tree search
- Fast support metrics

Quick start: ML tree search

- Default command: --search
  - 20 starting trees (10 random + 10 parsimony)
  - Pick the best-scoring one
  - Disclaimer: defaults can and will change!

```
$ raxml-ng --msa prim.phy --model GTR+G
```



Seq1
Seq2
Seq3
Seq4

Alignment

Length: m

A C G T

A
C
G
T

Substitution model

Evolutionary model: Global

```
$ raxml-ng --msa prim.phy --model GTR+G
```

- Substitution matrix
  - `GTR`, `LG`, `MULTI8_MK`, ... → also defines input data type
- Modifiers
  - Stationary frequencies: `+FO` (estimated), `+FC` (counted), `+FE` (equal)
  - Rate heterogeneity: `+G` (Gamma), `+R` (FreeRate)
  - Full list: https://github.com/amkozlov/raxml-ng/wiki/Input-data#single-model
- Fixed parameter values
  - `HKY{1.0/2.5}+G{0.7}`
  - `PROTGTR{rates.txt}`

Evolutionary model: Partitioned

```
$ raxml-ng --msa prim.phy --model prim.part
```

- Alignment columns divided into disjoint subsets:

```
$ cat prim.part

GTR+G+FO, NADH4=1-504
GTR+G+FO, tRNA=505-656
GTR+G+FO, NADH5=657-898
```

Same model, independent parameters!

```
$ cat prim2.part

GTR+G+FO, NADH4=1-504/3,2-504/3
JC+I, tRNA=505-656
GTR+R4+FC, NADH5=657-898
HKY, NADH4p3=3-504/3
```

- Branch length linkage modes:

```
--brlen linked
```

```
--brlen scaled
```

```
--brlen unlinked
```

Which model to use?

- Tools for statistical model selection
  - jModelTest, ModelFinder, SMS, ...
- We will use ModelTest-NG

(Darriba 2019)

```
modeltest-ng-static --help
```

Important options are:

-i ALIGNMENT

-d nt (DNA, default) or -d aa (proteins)

Common command line options

- Input or starting tree(s): **`--tree`**
  - Auto-generated: `--tree rand{5}` or `--tree pars{2},rand{2}`
  - User-specified: `--tree my.tre` or `--tree S1.raxml.bestTree`
- Output file prefix: **`--prefix`**
  - e.g. `S1` or `myoutput/S1` or `/home/user/S1`
  - `S1.raxml.bestTree, S1.raxml.log` etc
- Overwrite output files: **`--redo`**
- RNG seed: **`--seed`**
  - Deterministic search path → Reproducibility!

Tree with support values / “all-in-one”

- Command: `--all`
  - ML tree search
  - Bootstrapping (FBP) with convergence test (autoMRE)
  - Compute branch supports + map to ML tree

```
$ raxml-ng --all --msa prim.phy --model GTR+G
```

Warning: Can be extremely slow for large datasets!

(standard FBP too expensive → will be adressed in RAxML-NG 2.x)

Tree likelihood evaluation

- Command: **`--evaluate`**
  - Optimize all branch lengths and free model parameters
  - On a given fixed tree topology

```
$ raxml-ng --evaluate --msa prim.phy --tree S1.raxml.bestTree --model GTR+G
```

- Command: **`--loglh`**
  - Compute and print tree log-likelihood
  - No optimization, no files created

```
$ raxml-ng --loglh --msa prim.phy --tree S1.raxml.bestTree --model S1.raxml.bestModel
```

Comparing tree topologies

- Command: **`--rfdist`**
  - Compute pairwise and average Robinson-Foulds (RF) distance
  - For a given set of trees

```
$ raxml-ng --rfdist --tree S1.raxml.mlTrees
```

- Command: **`--rf`**
  - Compute and print RF distance between two trees

```
$ raxml-ng --rf S1.raxml.bestTree,S1A.raxml.bestTree
```

Topological constraints

- Option: `--tree-constraint`
  - Fix some relationships (e.g., monophyletic families)
  - By providing a (multifurcating) constraint tree
  - Can be incomplete (= some taxa “free to move”)
- Enjoy responsibly!
  - Prior knoweldge vs. Bias
  - Speed vs. Bias

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Exercise 0: Getting ready

1. Check input datasets

```
$ cd /home/phylogenomics/workshop_materials/
$ git clone https://github.com/amkozlov/ng-tutorial
$ cd ng-tutorial
$ ls
```

2. Run raxml-ng without parameters to get help

```
$ raxml-ng
```

3. Check alignment for formatting errors → prim.phy

```
$ raxml-ng --check --msa prim.phy --model GTR+G
```

Exercises: Lab #1

1. Tree search
2. Bootstrapping / “all-in-one”
3. Tree likelihood evaluation
4. Partitioned models
5. Topological constraints
6. Model testing

https://github.com/amkozlov/ng-tutorial/blob/master/evomics2024.md

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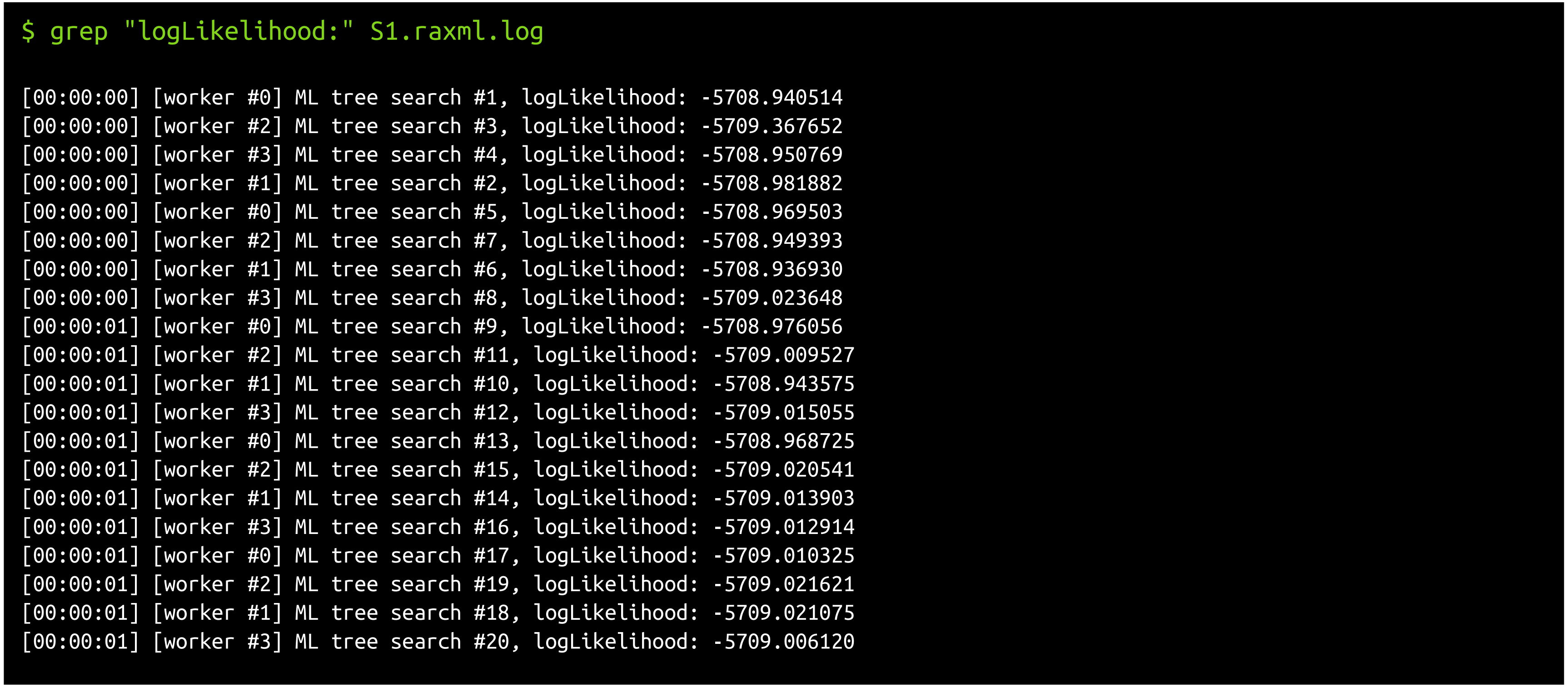
Exercise 1: Answers

2. ML tree likelihoods



```
$ grep "logLikelihood:" S1.raxml.log

[00:00:00] [worker #0] ML tree search #1, logLikelihood: -5708.940514
[00:00:00] [worker #2] ML tree search #3, logLikelihood: -5709.367652
[00:00:00] [worker #3] ML tree search #4, logLikelihood: -5708.950769
[00:00:00] [worker #1] ML tree search #2, logLikelihood: -5708.981882
[00:00:00] [worker #0] ML tree search #5, logLikelihood: -5708.969503
[00:00:00] [worker #2] ML tree search #7, logLikelihood: -5708.949393
[00:00:00] [worker #1] ML tree search #6, logLikelihood: -5708.936930
[00:00:00] [worker #3] ML tree search #8, logLikelihood: -5709.023648
[00:00:01] [worker #0] ML tree search #9, logLikelihood: -5708.976056
[00:00:01] [worker #2] ML tree search #11, logLikelihood: -5709.009527
[00:00:01] [worker #1] ML tree search #10, logLikelihood: -5708.943575
[00:00:01] [worker #3] ML tree search #12, logLikelihood: -5709.015055
[00:00:01] [worker #0] ML tree search #13, logLikelihood: -5708.968725
[00:00:01] [worker #2] ML tree search #15, logLikelihood: -5709.020541
[00:00:01] [worker #1] ML tree search #14, logLikelihood: -5709.013903
[00:00:01] [worker #3] ML tree search #16, logLikelihood: -5709.012914
[00:00:01] [worker #0] ML tree search #17, logLikelihood: -5709.010325
[00:00:01] [worker #2] ML tree search #19, logLikelihood: -5709.021621
[00:00:01] [worker #1] ML tree search #18, logLikelihood: -5709.021075
[00:00:01] [worker #3] ML tree search #20, logLikelihood: -5709.006120
```

Exercise 1: Answers (2)

3. Average topological (RF) distance

```
$ raxml-ng --rfdist --tree S1.raxml.mlTrees --prefix RF1

Reading input trees from file: S1.raxml.mlTrees
Loaded 20 trees with 12 taxa.

Average absolute RF distance in this tree set: 0.000000
Average relative RF distance in this tree set: 0.000000
Number of unique topologies in this tree set: 1
```

Absolute RF = # branches **not shared** by both trees

Relative RF = Absolute RF / max. possible RF

Exercise 1: Answers (3)

4*. fusob.phy

```
$ raxml-ng --msa fusob.phy --model GTR+G --prefix S2 --tree pars{5},rand{5}
```

```
$ grep "logLikelihood:" S2.raxml.log

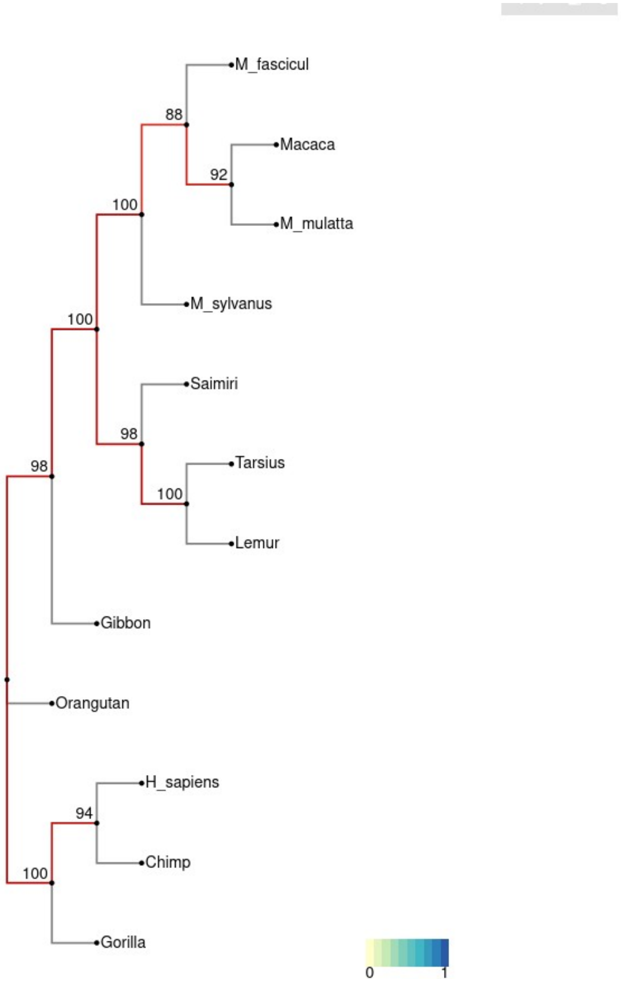
[00:00:02] [worker #2] ML tree search #3, logLikelihood: -9974.673091
[00:00:02] [worker #0] ML tree search #1, logLikelihood: -9974.665558
[00:00:02] [worker #1] ML tree search #2, logLikelihood: -9980.906638
[00:00:03] [worker #3] ML tree search #4, logLikelihood: -9974.663320
[00:00:04] [worker #2] ML tree search #7, logLikelihood: -9980.906494
[00:00:04] [worker #1] ML tree search #6, logLikelihood: -9974.670122
[00:00:04] [worker #3] ML tree search #8, logLikelihood: -9974.669716
[00:00:05] [worker #0] ML tree search #5, logLikelihood: -9974.671526
[00:00:06] [worker #1] ML tree search #10, logLikelihood: -9974.666947
[00:00:07] [worker #0] ML tree search #9, logLikelihood: -9981.909653
```

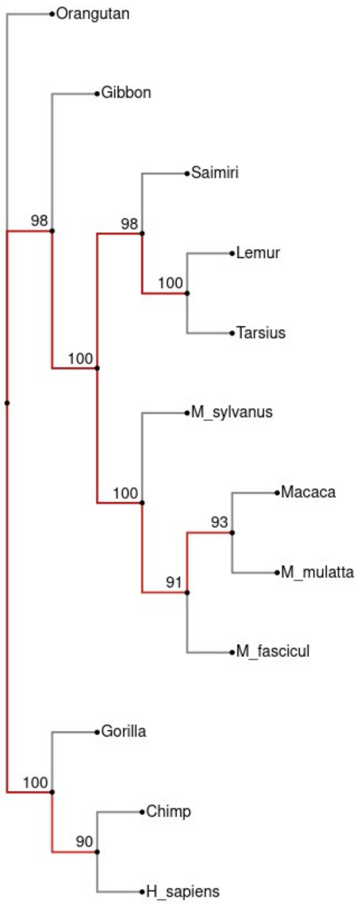
```
$ raxml-ng --rfdist --tree S2.raxml.mlTrees --prefix RF2


Average absolute RF distance in this tree set: 5.244444
Average relative RF distance in this tree set: 0.074921
Number of unique topologies in this tree set: 3
```

Exercise 2: Answers

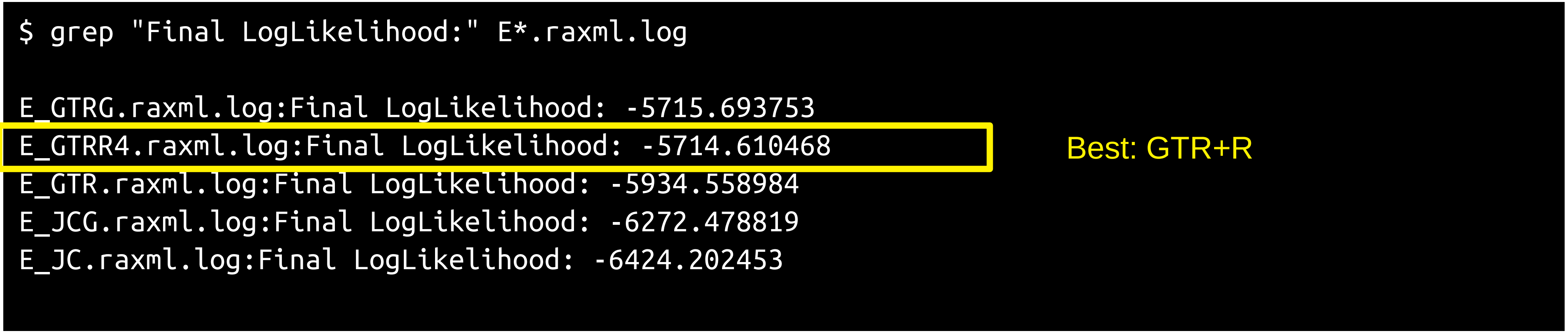
50 reps





100 reps

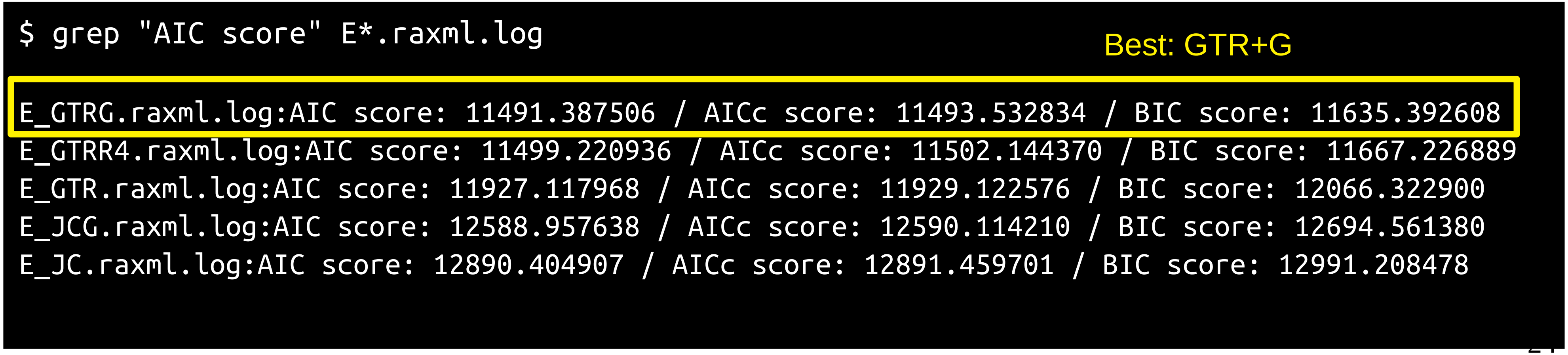
Exercise 3: Answers



```
$ grep "Final LogLikelihood:" E*.raxml.log

E_GTRG.raxml.log:Final LogLikelihood: -5715.693753
E_GTRR4.raxml.log:Final LogLikelihood: -5714.610468
E_GTR.raxml.log:Final LogLikelihood: -5934.558984
E_JCG.raxml.log:Final LogLikelihood: -6272.478819
E_JC.raxml.log:Final LogLikelihood: -6424.202453
```

Best: GTR+R



```
$ grep "AIC score" E*.raxml.log

E_GTRG.raxml.log:AIC score: 11491.387506 / AICc score: 11493.532834 / BIC score: 11635.392608
E_GTRR4.raxml.log:AIC score: 11499.220936 / AICc score: 11502.144370 / BIC score: 11667.226889
E_GTR.raxml.log:AIC score: 11927.117968 / AICc score: 11929.122576 / BIC score: 12066.322900
E_JCG.raxml.log:AIC score: 12588.957638 / AICc score: 12590.114210 / BIC score: 12694.561380
E_JC.raxml.log:AIC score: 12890.404907 / AICc score: 12891.459701 / BIC score: 12991.208478
```

Best: GTR+G

Exercise 4: Answers

```
$ grep "Final LogLikelihood:" {S,P}1.raxml.log

S1.raxml.log:Final LogLikelihood: -5708.926872
P1.raxml.log:Final LogLikelihood: -5673.806570
```

```
$ raxml-ng --rf S1.raxml.bestTree,P1.raxml.bestTree

Average absolute RF distance in this tree set: 0.000000
Average relative RF distance in this tree set: 0.000000
Number of unique topologies in this tree set: 1
```

Exercise 5: Answers

```
$ grep "Final LogLikelihood:" C*.raxml.log

C0.raxml.log:Final LogLikelihood: -5708.923405   no constraint
C1.raxml.log:Final LogLikelihood: -5708.926239   constraint #1
C2.raxml.log:Final LogLikelihood: -5779.491583   constraint #2
```

```
$ grep "Elapsed time:" C*.raxml.log

C0.raxml.log:Elapsed time: 23.629 seconds        no constraint
C1.raxml.log:Elapsed time: 17.553 seconds        constraint #1
C2.raxml.log:Elapsed time: 17.987 seconds        constraint #2
```

Exercise 6: Answers

```
$ modeltest-ng -i prot21.fa -d aa

Partition 1/1:
                         Model         Score        Weight
----------------------------------------------------------
       BIC               LG+G4     6005.4554        0.5062
       AIC             LG+I+G4     5893.6825        0.7923
      AICc               LG+G4     5941.3599        0.5402
```

```
$ raxml-ng --msa prot21.fa --model LG+G4 --prefix S6

Final LogLikelihood: -2872.979205
```

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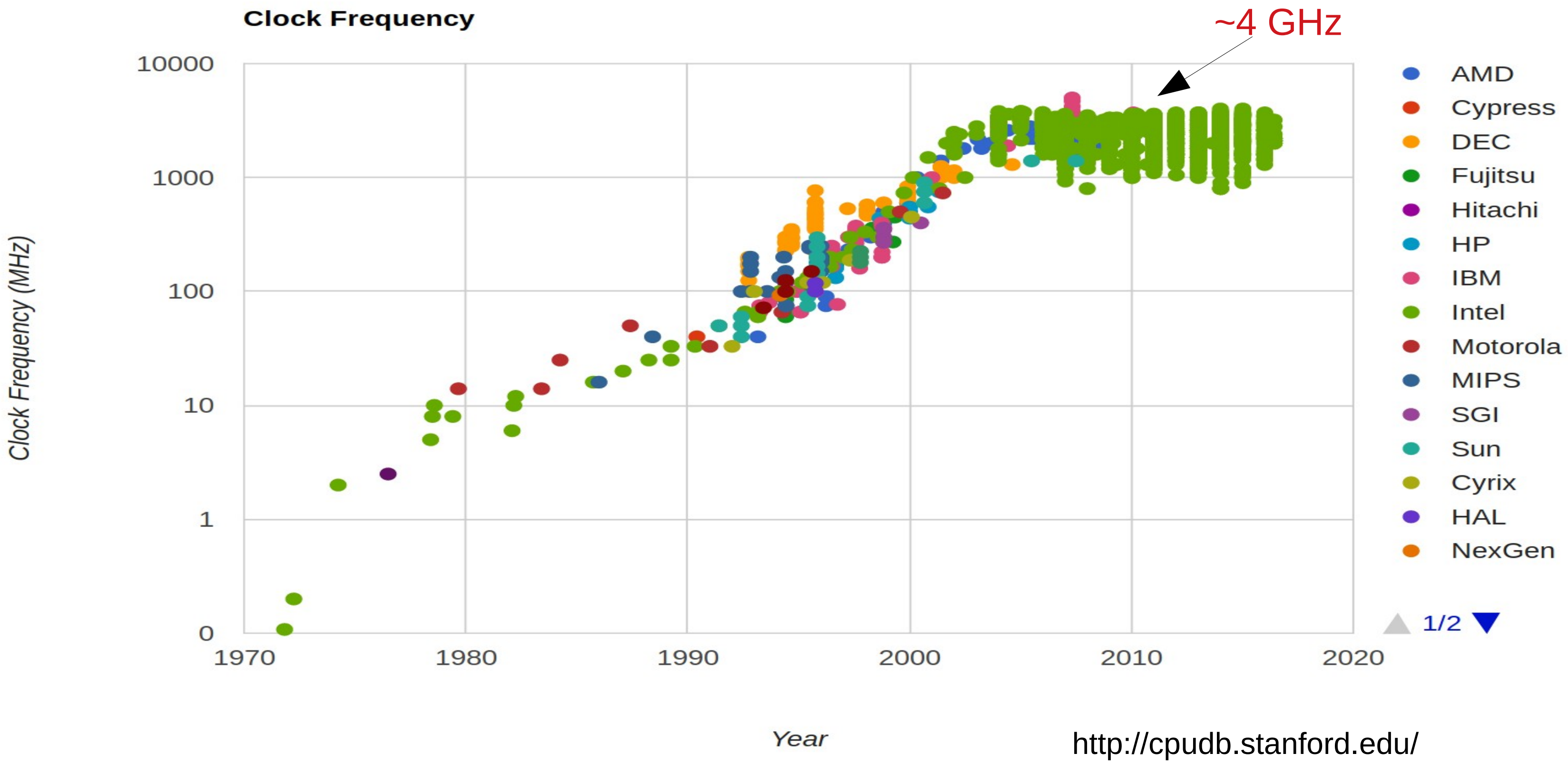
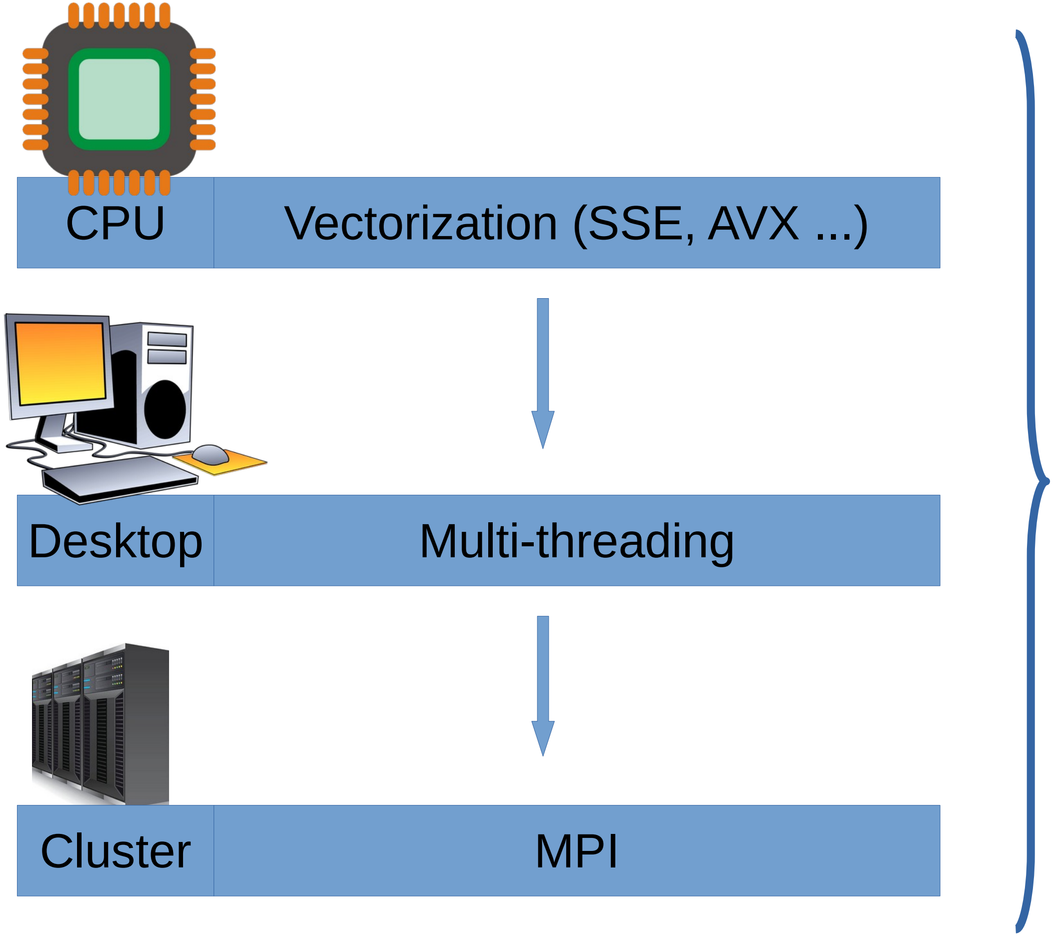
Why is parallelization so important?



http://cpudb.stanford.edu/

Parallelization: hardware



CPU | Vectorization (SSE, AVX ...)

Desktop | Multi-threading

Cluster | MPI

raxmlHPC

raxmlHPC-SSE

raxmlHPC-AVX

raxmlHPC-PTHREADS

raxmlHPC-PTHREADS-SSE

raxmlHPC-HYBRID

raxmlHPC-MPI

raxmlHPC-MPI-AVX2

raxml-ng

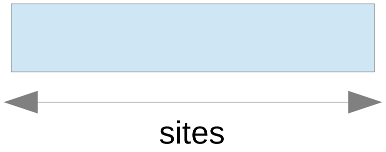
raxml-ng-mpi

RAxML-NG parallelization setup

- Vectorization → fully automatic 😁
- Multi-threading → usually works fine™
  - Automatic CPU detection & thread tuning 😑
  - Core oversubscription warning
- MPI/hybrid → more tricky
  - Read your cluster manual 😠
  - Ask your sysadmin/technician
  - Benchmark!

Parallelization: software

MSA



4 searches

e.g. from 4 starting trees

4 threads

T1, T2, T3, T4

1 – 4 workers

W1, W2, W3, W4

Fine-grained

Search 1	T1	T2	T3	T4	W1
Search 2	T1	T2	T3	T4	W1
Search 3	T1	T2	T3	T4	W1
Search 4	T1	T2	T3	T4	W1

Coarse-grained

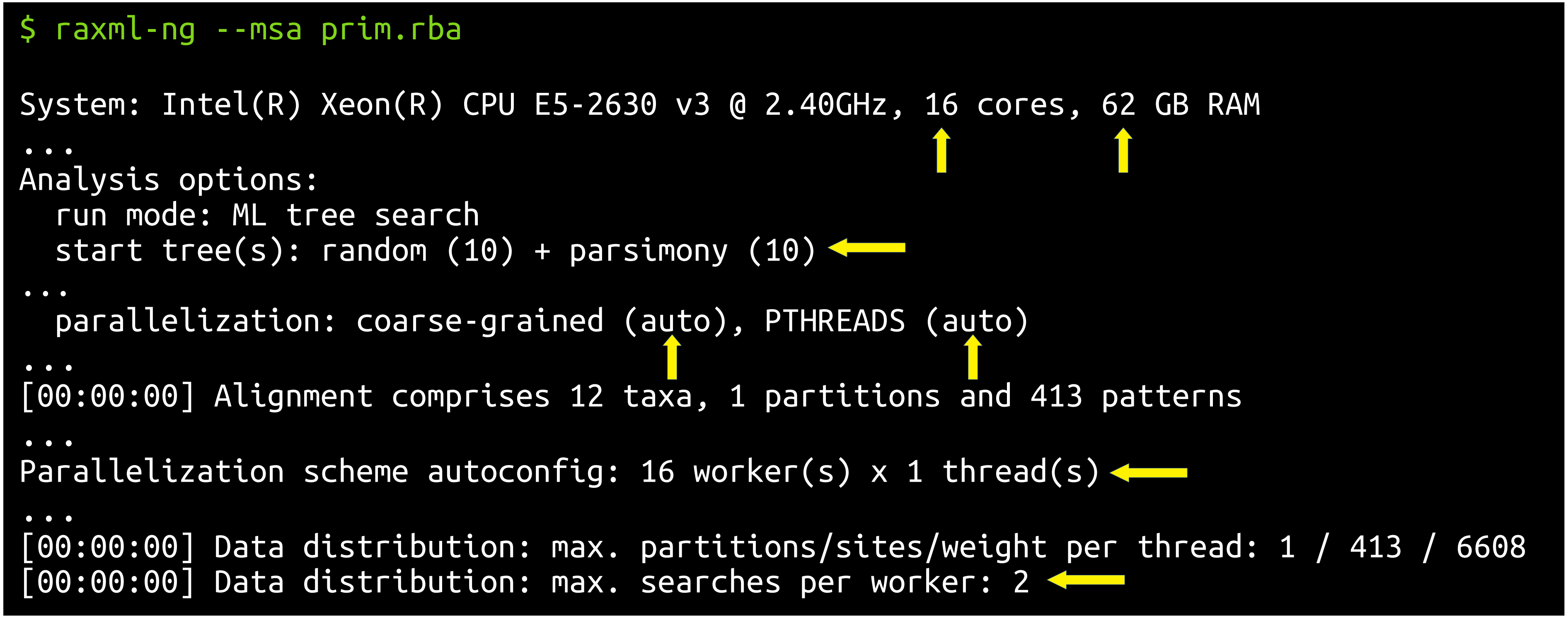
Search 1	T1	W1
Search 2	T2	W2
Search 3	T3	W3
Search 4	T4	W4

Mixed/hybrid

Search 1	T1	T2	W1
Search 2	T1	T2	W1
Search 3	T3	T4	W2
Search 4	T3	T4	W2

Since v1.0: Full native support and automatic configuration!

Parallelization: auto-tuning



```
$ raxml-ng --msa prim.rba

System: Intel(R) Xeon(R) CPU E5-2630 v3 @ 2.40GHz, 16 cores, 62 GB RAM
...
Analysis options:
  run mode: ML tree search
  start tree(s): random (10) + parsimony (10)
...
  parallelization: coarse-grained (auto), PTHREADS (auto)
...
[00:00:00] Alignment comprises 12 taxa, 1 partitions and 413 patterns
...
Parallelization scheme autoconfig: 16 worker(s) x 1 thread(s)
...
[00:00:00] Data distribution: max. partitions/sites/weight per thread: 1 / 413 / 6608
[00:00:00] Data distribution: max. searches per worker: 2
```

Parallelization: manual tuning

- Automatic with upper limits

```
$ raxml-ng --msa prim.rba --threads auto{16} --workers auto{2}
```

- Manual

```
$ raxml-ng --msa prim.rba --threads 16 --workers 2
```

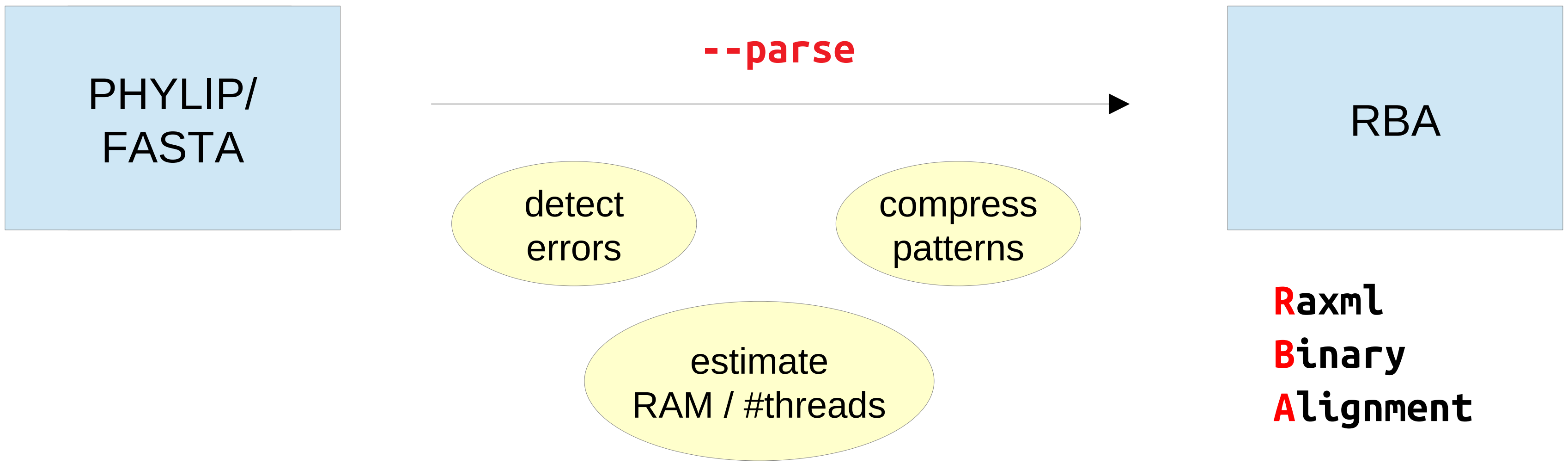
- Also works with MPI

```
$ mpirun -n 4 raxml-ng-mpi --msa prim.rba --threads 16 --workers 8
```

4 ranks * 16 threads = 64 = 8 workers * 8 threads

Alignment pre-processing

```
$ raxml-ng --parse --msa prim.phy --model GTR+G --prefix prim
```



PHYLIP/
FASTA

--parse

RBA

detect
errors

compress
patterns

estimate
RAM / #threads

Raxml
Binary
Alignment

Bootstrapping revisited

1. Infer bootstrap trees

```
$ raxml-ng --bootstrap --msa prim.phy --model GTR+G --prefix BS1 --bs-trees 50 --seed $RANDOM
```

2. Check convergence

```
$ raxml-ng --bsconverge --bs-trees BS1.raxml.bootstraps --prefix BC
```

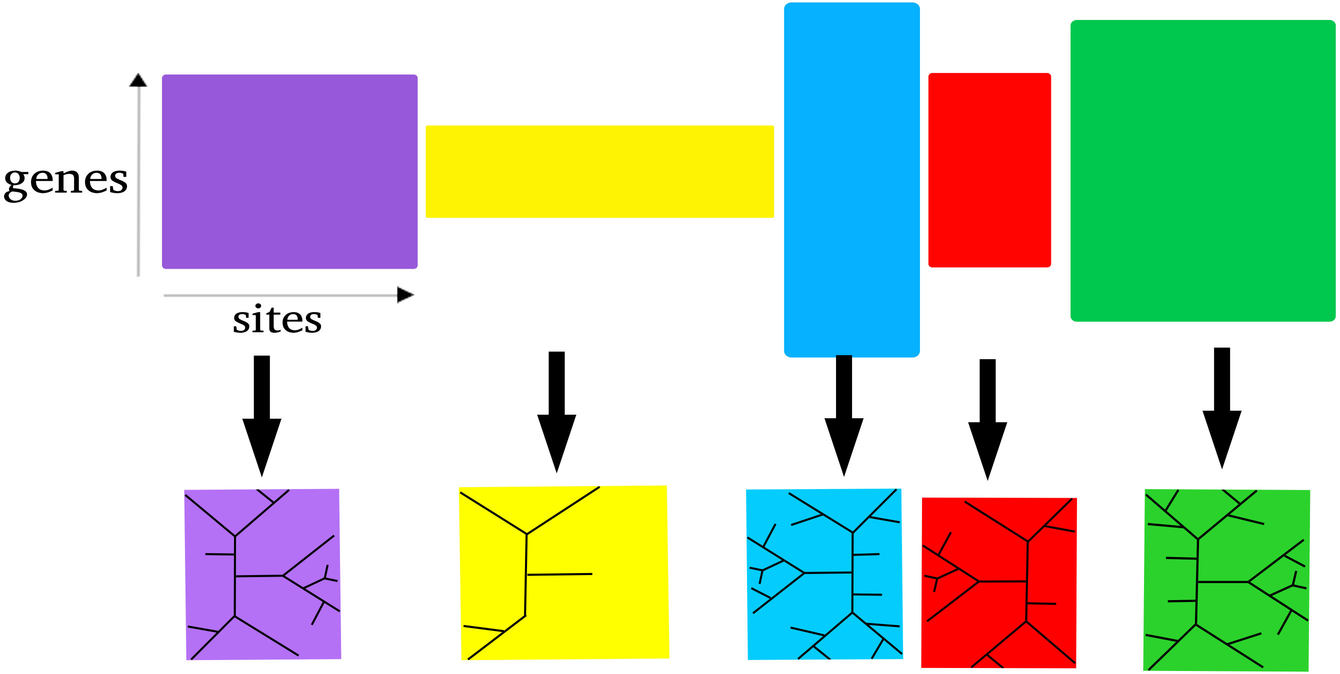
3. Map bootstrap support values to the best ML tree

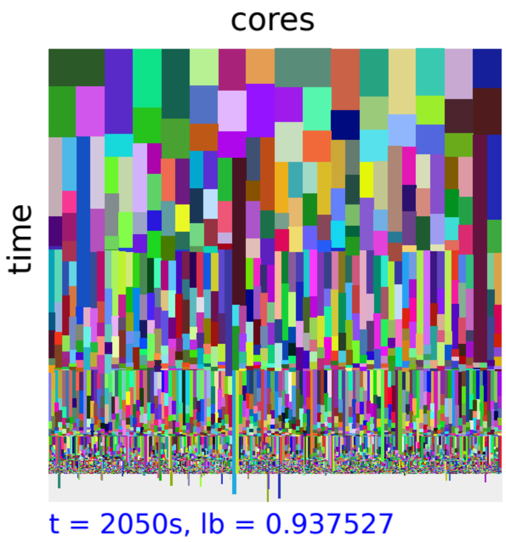
```
$ raxml-ng --support --tree S1.raxml.bestTree --bs-trees BS1.raxml.bootstraps --prefix B2
```

ParGenes

- Infer thousands of (gene) trees in parallel
  - Load balancing + Checkpointing
  - Model testing (ModelTest-NG) + species tree reconstruction (ASTRAL)

(Morel 2018)





ParGenes command line - example

Do not run this one!

Folder containing
the alignments
-a msa_dir

Folder for
result files
-o output_dir

Number of
cores
-c 256

```
$ python pargenes.py -a msa_dir -o output_dir -m -s 10 -p 20 -b 100 -p 256
```

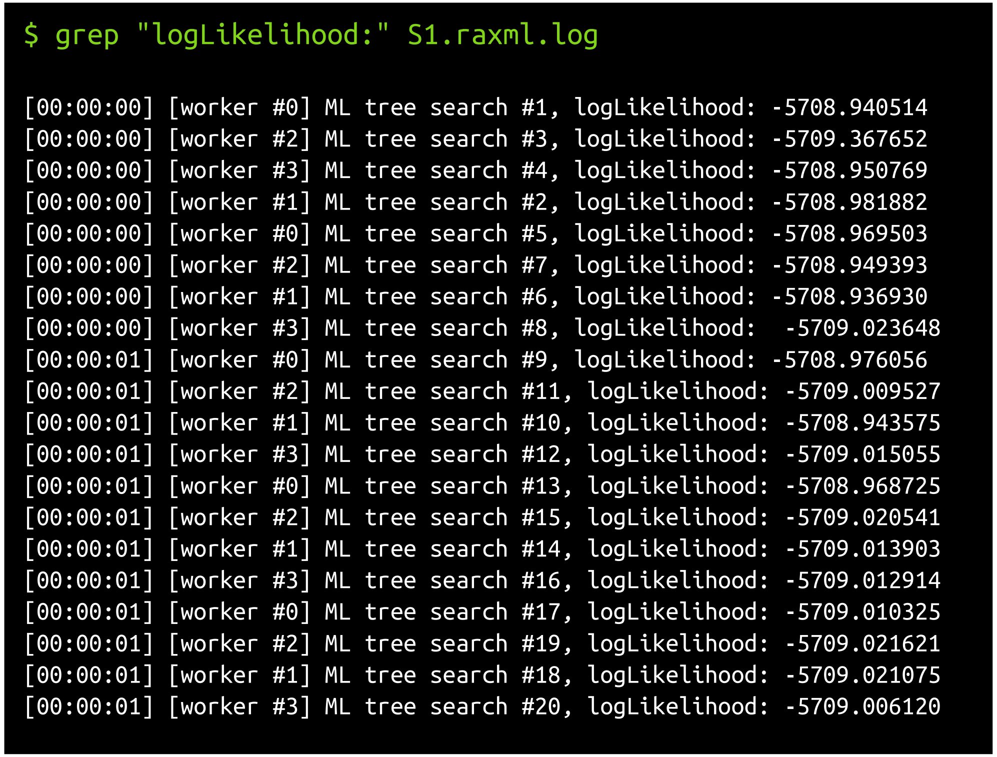
Apply model
selection?
-m

Number of
starting trees
-p 20 -s 10

Number of BS
replicates
-b 100

More examples: /home/phylogenomics/software/.source/ParGenes/examples/

“From easy to hopeless”



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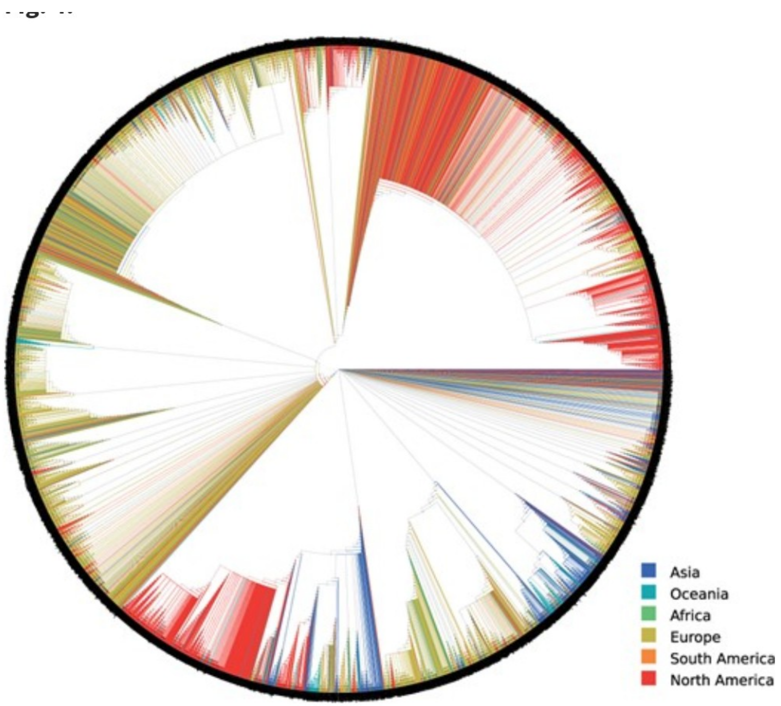
[00:00:01] [worker #3] ML tree search #16, logLikelihood: -5709.012914
[00:00:01] [worker #0] ML tree search #17, logLikelihood: -5709.010325
[00:00:01] [worker #2] ML tree search #19, logLikelihood: -5709.021621
[00:00:01] [worker #1] ML tree search #18, logLikelihood: -5709.021075
[00:00:01] [worker #3] ML tree search #20, logLikelihood: -5709.006120
```



(Morel 2020)

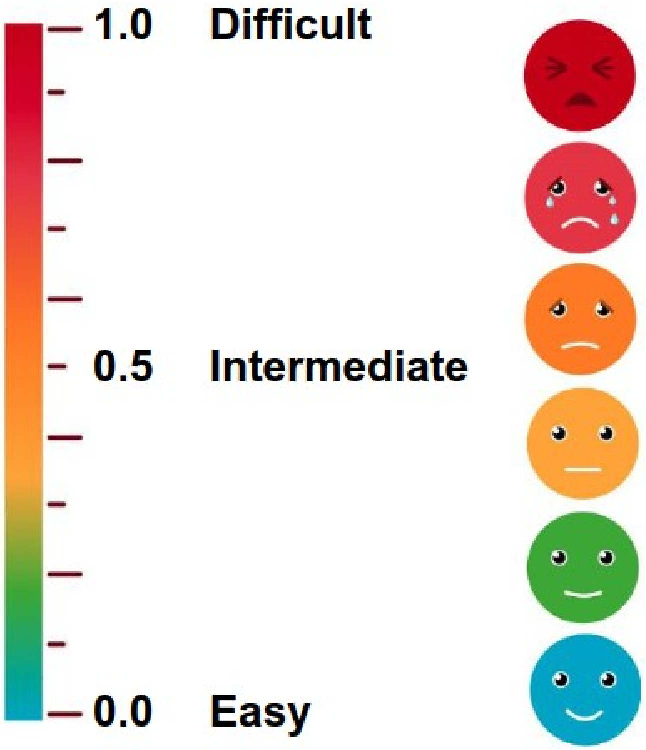
Few taxa, clear signal

10k … 100k taxa, little variation

Prediction of phylogenetic difficulty

Input MSA → **Pythia** →

(Haag 2022)

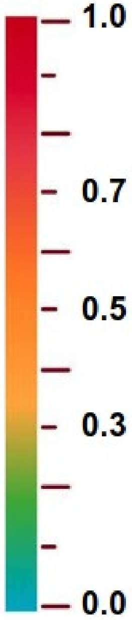


(Adapted from Togkousidis 2023b)

Julia Haag

Are we searching too hard?

- RAxML-NG vs. IQ-TREE 2 vs. FastTree (Hoehler, 2022 preprint)



On **difficult** MSAs, all tools perform **similarly** but **only** in terms of **likelihood** score

---

On **intermediate** MSAs, **RAxML-NG** and **IQ-TREE 2** infer significantly **better trees**

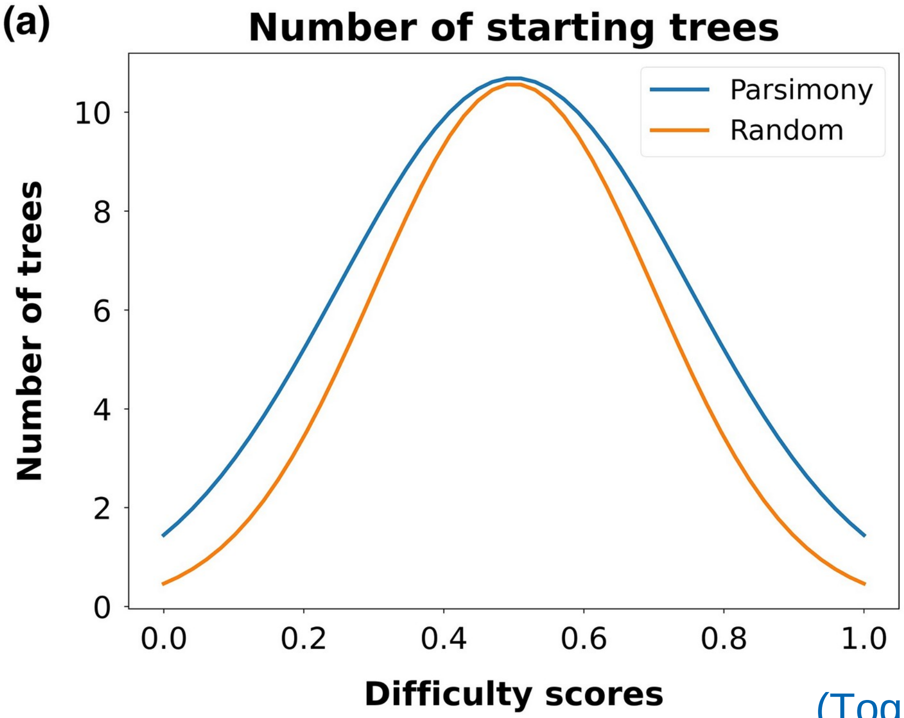
On **easy** MSAs, all tools perform **similarly** in terms of **likelihood** score and **topological accuracy**

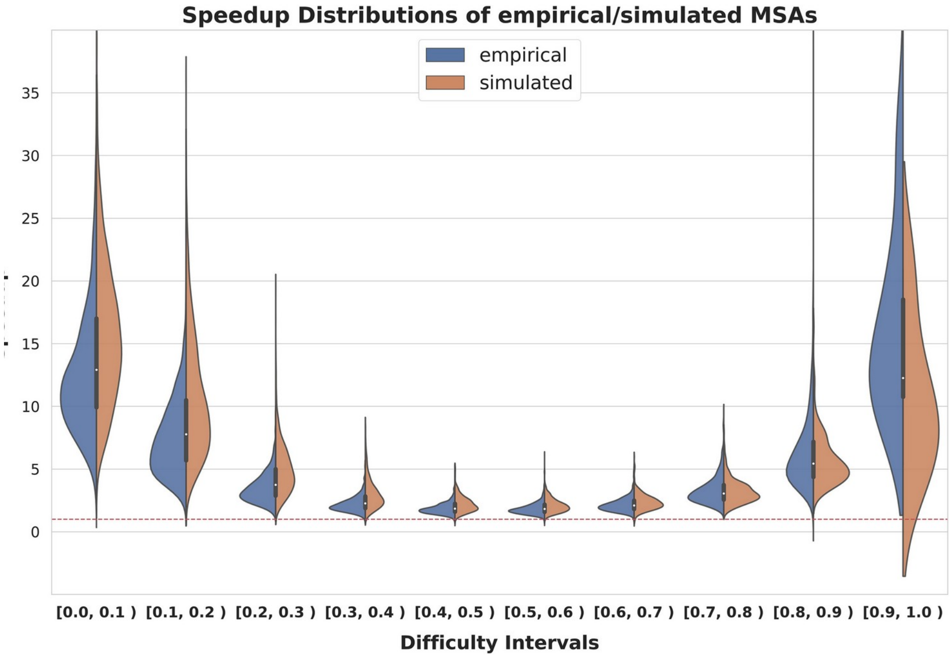
(Adapted from Togkousidis 2023b)

Adaptive RAxML-NG

- Use predicted MSA difficulty to adust
- number of starting trees + search heuristic (SPR radius, NNIs)

Anastasis Togkousidis





(Togkousidis 2023a)

Outline

- RAxML-NG Intro
- Lab #1: Basics
- Results & Discussion

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- RAxML-NG Parallelization
- Lab #2: Large datasets & Parallelization
- Results & Conclusions

Knowing your system

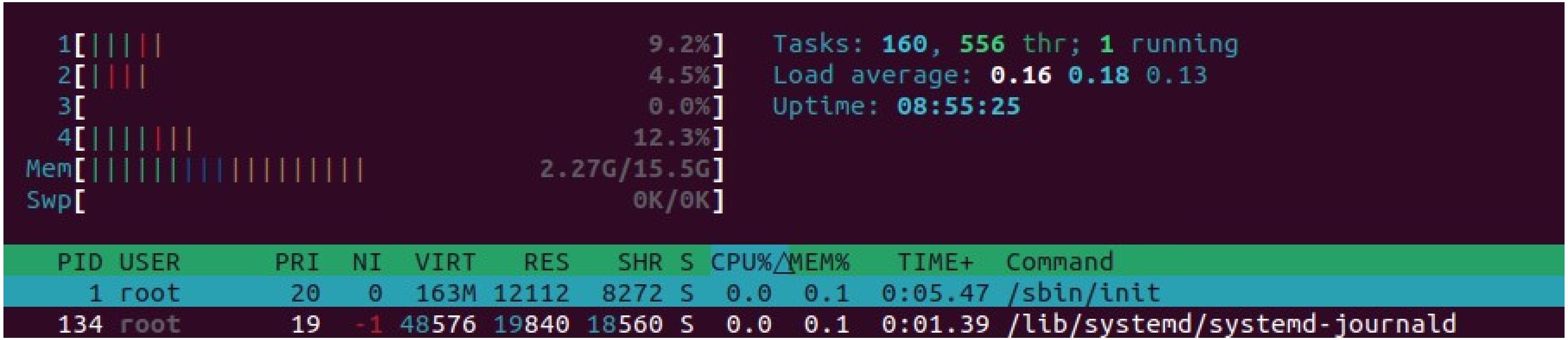
```
$ lscpu

CPU(s):                 4   logical
Thread(s) per core:     2   hyperthreading ON
Model name:             AMD EPYC 7571
```

```
$ raxml-ng -v

System: AMD EPYC 7571, 2 cores, 15 GB RAM
                           physical
```

```
$ htop
```



```
  1[|||||                                          9.2%]   Tasks: 160, 556 thr; 1 running
  2[||||                                           4.5%]   Load average: 0.16 0.18 0.13
  3[                                               0.0%]   Uptime: 08:55:25
  4[|||||||                                       12.3%]
Mem[||||||||||||||||||                      2.27G/15.5G]
Swp[                                             0K/0K]

  PID USER       PRI  NI  VIRT   RES   SHR S CPU%△MEM%   TIME+  Command
    1 root        20   0  163M 12112  8272 S  0.0  0.1  0:05.47 /sbin/init
  134 root        19  -1 48576 19840 18560 S  0.0  0.1  0:01.39 /lib/systemd/systemd-journald
```

Exercises: Lab #2

7. Alignment compression
8. Automatic & manual parallelization
9. Bootstrapping revisited
10. Adaptive search
11. ParGenes

https://github.com/amkozlov/ng-tutorial/blob/master/evomics2024.md

Outline

- RAxML-NG Intro
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- RAxML-NG Parallelization
- Lab #2: Large datasets & Parallelization
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Exercise 7: Answers

```
$ raxml-ng --parse --msa fusob.phy --model GTR+G --prefix fusob
```

```
Partition 0: noname
Model: GTR+FO+G4m
Alignment sites / patterns: 1602 / 635
Gaps: 10.13 %
Invariant sites: 9.61 %


NOTE: Binary MSA file created: fusob.raxml.rba

* Estimated memory requirements                : 6 MB

* Recommended number of threads / MPI processes: 3
```

Exercise 8: Answers

```
raxml-ng -search -msa fusob.raxml.rba -tree rand{10} -seed 1 -prefix TXWX
raxml-ng -search -msa fusob.raxml.rba -tree rand{10} -seed 1 -workers 1 -prefix TXW1
raxml-ng -search -msa fusob.raxml.rba -tree rand{10} -seed 1 -workers 2 -prefix TXW2
raxml-ng -search -msa fusob.raxml.rba -tree rand{10} -seed 1 -threads 2 -workers 2 -prefix T2W2
```

```
$ grep "Elapsed time:" T?W?.raxml.log

T2W2.raxml.log:Elapsed time: 11.995 seconds
TXW1.raxml.log:Elapsed time: 9.752 seconds
TXW2.raxml.log:Elapsed time: 8.551 seconds
TXWX.raxml.log:Elapsed time: 10.398 seconds
```

~18 % faster

Exercise 9: Answers

```
$ raxml-ng --bootstrap --msa prim.phy --model GTR+G --prefix BS1 --seed $RANDOM --bs-trees 50
$ raxml-ng --bootstrap --msa prim.phy --model GTR+G --prefix BS2 --seed $RANDOM --bs-trees 50
```

```
$ cat BS*.raxml.bootstraps > bstrees.txt
```

```
$ raxml-ng --support -tree S1.raxml.bestTree -bs-trees bstrees.txt -prefix B2
```

```
$ raxml-ng --support -tree S1.raxml.bestTree -bs-trees bstrees.txt -prefix B3 -bs-metric TBE
```

Exercise 10: Answers

```
Analysis options:
  run mode: ML tree search



Starting ML tree search
with 20 distinct starting trees

Final LogLikelihood: -5673.760245

Elapsed time: 8.618 seconds
```

```
Analysis options:
  run mode: Adaptive ML tree search

[00:00:00] Predicted difficulty: 0.00

Starting ML tree search
with 2 distinct starting trees

Final LogLikelihood: -5673.844106

Elapsed time: 1.635 seconds
```

```
$ raxml-ng-adaptive --rf P1.raxml.bestTree,P1A.raxml.bestTree

Average absolute RF distance in this tree set: 0.000000
Average relative RF distance in this tree set: 0.000000
Number of unique topologies in this tree set: 1
```

Answers

- Full tutorial script with all answers & results:
  - https://github.com/amkozlov/ng-tutorial/wiki/evomics2024

Software availability

- Web server
  - CIPRES: http://www.phylo.org/ → registration required
- Graphical interface
  - raxmlGUI: https://github.com/AntonelliLab/raxmlGUI
  - Mesquite: http://www.mesquiteproject.org/
- Stand-alone command line (Linux/macOS)
  - GitHub: https://github.com/amkozlov/raxml-ng
  - Conda: https://anaconda.org/bioconda/raxml-ng

Where to get help?

- Documentation
  - https://github.com/amkozlov/raxml-ng/wiki
- Tutorial
  - https://github.com/amkozlov/raxml-ng/wiki/Tutorial
- User support group
  - https://groups.google.com/forum/#!forum/raxml

Which tool to use as of 2024 ?

- FastTree
  - “Hopeless” alignments (100k+, few genes)
- RAxML, IQ-Tree, PhyML
  - Models/features not available in RAxML-NG
- ParGenes
  - Lots of gene trees, coalescent methods
- RAxML-NG
  - All other cases :)

Děkuji

Questions?

References

- Darriba et al. (2019) **ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models.** *Molecular Biology and Evolution.* https://doi.org/10.1093/molbev/msz189
- Haag et al. (2022) **From Easy to Hopeless—Predicting the Difficulty of Phylogenetic Analyses.** *Molecular Biology and Evolution.* https://doi.org/10.1093/molbev/msac254
- Hoehler et al. (2022) **A representative Performance Assessment of Maximum Likelihood based Phylogenetic Inference Tools.** BioRxiv. https://doi.org/10.1101/2022.10.31.514545
- Kozlov et al. (2019) **RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference**. *Bioinformatics.* https://doi.org/10.1093/bioinformatics/btz305
- Morel, Kozlov, Stamatakis (2018) **ParGenes: a tool for massively parallel model selection and phylogenetic tree inference on thousands of genes.** *Bioinformatics.* https://doi.org/10.1093/bioinformatics/bty839
- Morel et al. (2020) **Phylogenetic Analysis of SARS-CoV-2 Data Is Difficult**. *Molecular Biology and Evolution* https://doi.org/10.1093/molbev/msaa314
- Stamatakis (2006) **RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models**. *Bioinformatics*, https://doi.org/10.1093/bioinformatics/btl446
- Stamatakis (2014) **RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies.** *Bioinformatics*, https://doi.org/10.1093/bioinformatics/btu033
- Togkousidis (2023a) **Adaptive RAxML-NG: Accelerating Phylogenetic Inference under Maximum Likelihood using Dataset Difficulty.** *In Molecular Biology and Evolution.* https://doi.org/10.1093/molbev/msad227
- Togkousidis (2023b) **Adaptive RAxML-NG.** *ERGA BioGenome Analysis and Applications Seminar*, https://cme.h-its.org/exelixis/pubs/Anastasis_adaptive_raxmlng_erga_nov_2023.pdf