



Universidade de São Paulo
Instituto de Química



Análise filogenética para dados moleculares – aula 2

João Carlos Setubal

2020

Programas para inferência filogenética

- **Pacotes**
 - Oferecem vários diferentes programas
 - Diferentes métodos para o mesmo objetivo
 - Podem incluir programas auxiliares
- **Programas individuais**
 - São especializados num método

Pacotes

- PHYLIP
 - Joe Felsenstein
 - <http://evolution.genetics.washington.edu/phylip.html>
- PAUP
 - David Swofford
 - <http://paup.csit.fsu.edu/>
- MEGA
 - Sudhir Kumar, Koichiro Tamura & Masatoshi Nei
 - <http://www.megasoftware.net/>
 - Atualmente na versão X

Programas que implementam métodos não-probabilísticos

- **Distância**

- Pacotes mencionados no slide anterior

- Neighbor-joining

- UPGMA

- **Parcimônia**

- pacotes mencionados no slide anterior

Máxima verossimilhança

- RaXML
 - A. Stamatakis
 - <http://www.exelixis-lab.org/>
- phyML
 - O. Gascuel et al. Systematic Biology, 59(3):307-21, 2010
 - <http://www.atgc-montpellier.fr/phyml/>
- fastTree
 - Morgan N. Price in Adam Arkin's group
 - <http://www.microbesonline.org/fasttree/>
 - “FastTree can handle alignments with up to a million of sequences in a reasonable amount of time and memory”
- IQ-Tree
 - <http://www.iqtree.org/>
 - A fast and effective stochastic algorithm to infer phylogenetic trees by maximum likelihood. *IQ-TREE compares favorably to RAXML and PhyML* in terms of likelihoods with similar computing time ([Nguyen et al., 2015](#))

Um resultado de desempenho pontual

- Criação de uma árvore ML para 500 sequências de proteínas com aprox. 300 aa
- Computador desktop “normal” (4 GB de RAM)
- RAxML or PHYml levaram aprox. 10 horas
- Fasttree levou menos do que 1 hora

Inferência bayesiana

- MrBayes
- Ronquist and Huelsenbeck. Bioinformatics. 2003 19(12):1572-4.
- <http://mrbayes.sourceforge.net/>
- Mais lento comparado a RAxML e phyML
- Resultados não são conclusivamente melhores do que ML

O problema da caixa preta

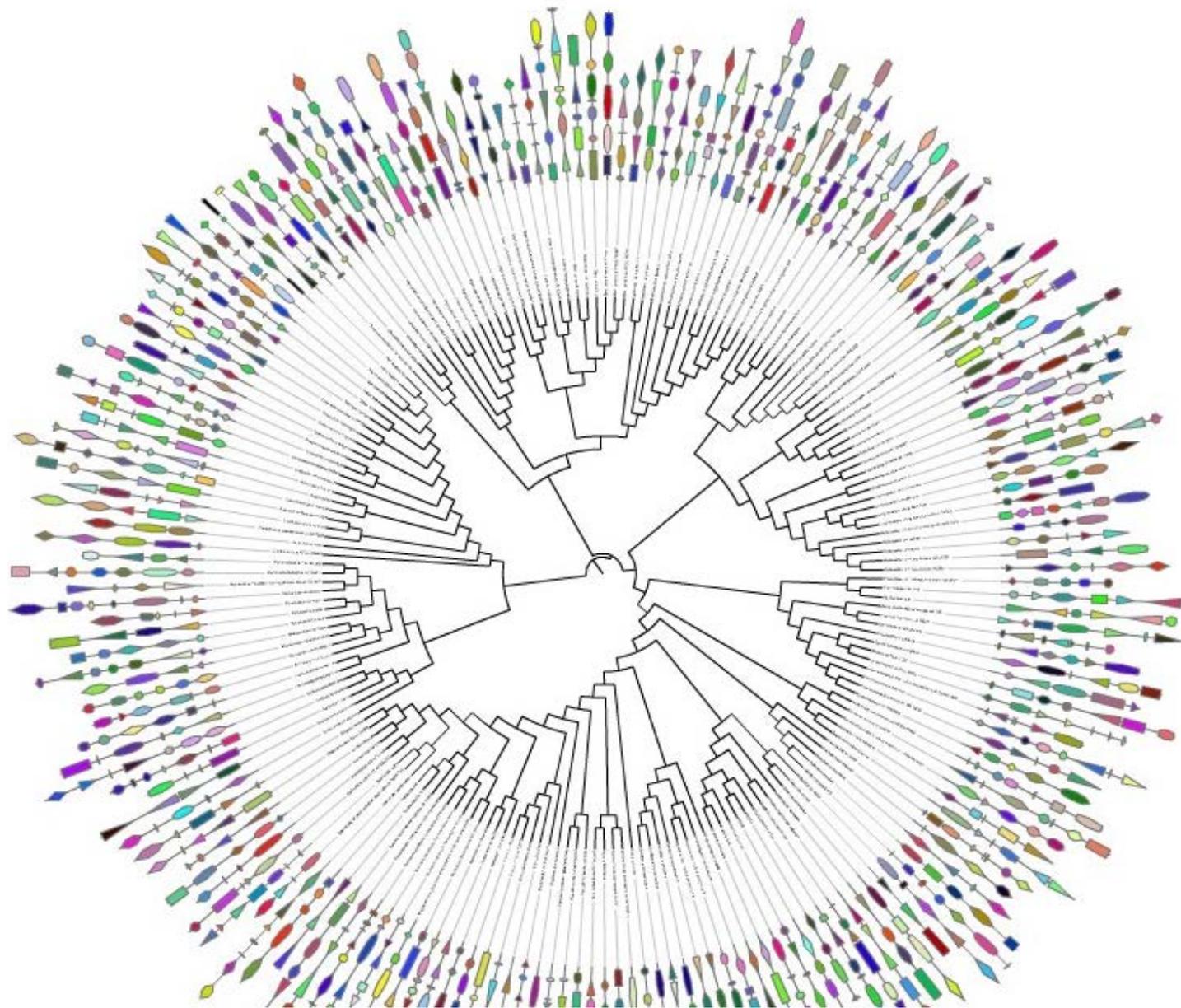
- Idealmente: todo usuário de um método e respectivo programa deveria entender os princípios do método
- No caso de métodos de filogenia
 - Estatística não trivial

Visualização de árvores: formatos

- **Newick, NEXUS**
- (((erHomoC:0.28006,erCaelC:0.22089):0.40998,(erHomoA:0.32304,(erpCaelC:0.58815,((erHomoB:0.5807,erCaelB:0.23569):0.03586,erCaelA:0.38272):0.06516):0.03492):0.14265):0.63594,(TRXHomo:0.65866,TRXSacch:0.38791):0.32147,TRXEcoli:0.57336);
- **<http://molecularevolution.org/resources/treeformats>**

Visualização de árvores

- Interactive Tree of Life <http://itol.embl.de>
- http://en.wikipedia.org/wiki/List_of_phylogenetic_tree_visualization_software

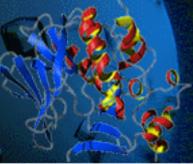


All-in-one: <http://phylogeny.fr>



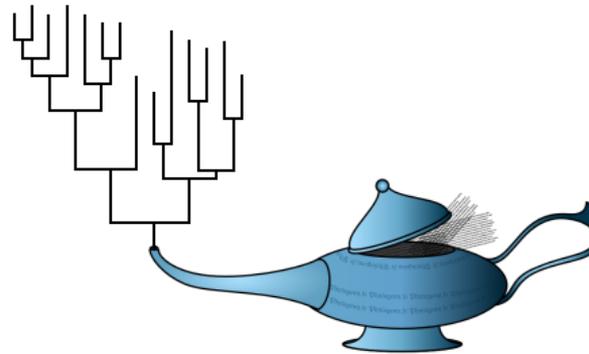
```
TTTCTTAAATGCAATTAATGCGATGATATATCTTTGGATATATTTCAAAGAT  
AAATGAAATTTAGTCTATCTTTTTCACATTCACATTCGATTCGATTCGATTCGAC  
AAGAAACAACATGGATATCATACTACTGGTATGCGGCATATAAGTGGCCCAT  
TTTCTTAAATGCAATTAATGCGATGATATATCTTTGGATATATTTCAAAGAT  
AAATGAAATTTAGTCTATCTTTTTCACATTCACATTCGATTCGATTCGATTCGAC  
AAGAAACAACATGGATATCATACTACTGGTATGCGGCATATAAGTGGCCCAT  
AAATGCGATTTATTAAGAATTTACACCCAAAGATTTGTTACCGACATATTT  
TTTGGTAAAGAAATTTGAAAGATTTAGTCTATCTTTTTCACATTCACATTCGAT  
TCGATTCGATTTTACGTCATTTTAGTGAATTTATAAATTAATGATTAATGTT  
TTTCTTAAATGCAATTAATGCGATGATATATCTTTGGATATATTTCAAAGAT  
AAATGAAATTTAGTCTATCTTTTTCACATTCACATTCGATTCGATTCGATTCGAC  
AAGAAACAACATGGATATCATACTACTGGTATGCGGCATATAAGTGGCCCAT  
AAATGCGATTTATTAAGAATTTACACCCAAAGATTTGTTACCGACATATTT  
TTTGGTAAAGAAATTTGAAAGATTTAGTCTATCTTTTTCACATTCACATTCGAT  
TCGATTCGATTTTACGTCATTTTAGTGAATTTATAAATTAATGATTAATGTT  
AAATGCGATTTATTAAGAATTTACACCCAAAGATTTGTTACCGACATATTT  
TTTGGTAAAGAAATTTGAAAGATTTAGTCTATCTTTTTCACATTCACATTCGAT  
TCGATTCGATTTTACGTCATTTTAGTGAATTTATAAATTAATGATTAATGTT
```

Information
Génomique et
Structurale



Home	Phylogeny Analysis	Blast Explorer	Online Programs	Your Workspace	Documentation	Downloads	Contacts
------	--------------------	----------------	-----------------	----------------	---------------	-----------	----------

Phylogeny.fr Robust Phylogenetic Analysis For The Non-Specialist



Phylogeny.fr is a free, simple to use web service dedicated to reconstructing and analysing phylogenetic relationships between molecular sequences.

Phylogeny.fr runs and connects various bioinformatics programs to reconstruct a **robust phylogenetic tree from a set of sequences**.

If you use this site, please cite:

[Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.-F., Guindon S., Lefort V., Lescot M., Claverie J.-M., Gascuel O. *Phylogeny.fr: robust phylogenetic analysis for the non-specialist* Nucleic Acids Research. 2008 Jul 1; 36 \(Web Server Issue\):W465-9. Epub 2008 Apr 19. \(PubMed\)](#)

Phylogeny.fr (2)

● Phylogeny analysis

"One Click"

Paste your set of sequences and let the software make decisions on your behalf (Each step is optimized for your data).

"Advanced"

Manually set parameters for the various steps.

"A la Carte"

Create your own phylogeny workflow using more programs available.

● Explore your sequence neighbors

Paste your single sequence, run Blast and explore its homologous sequences.

● Online phylogeny programs

Direct access to the individual tools available on this server.

Multiple Alignment:

[MUSCLE](#)
[T-Coffee / 3D-Coffee](#)
[ClustalW](#)
[ProbCons](#)

Phylogeny:

[PhyML](#)
[TNT](#)
[BioNJ](#)
[MrBayes](#)

Tree viewers:

[TreeDyn](#)
[Drawgram](#)
[Drawtree](#)
[ATV](#)

Utilities:

[Gblocks](#)
[Jalview](#)
[Readseq](#)
[Format converter](#)

This project is funded by the Réseau National des Génopoles (RNG).

This project is managed in a GForge project, which aims to help collaboration and development management (using Subversion).

 [RSS Feed](#)  [Mailing-list](#)  [Mentions légales](#)



Building your tree locally: SeaView

SeaView

Version 4.3.5

NEW: seaview computes and draws parsimony, distance and PhyML phylogenetic trees.

NEW: seaview prints trees and outputs them in scalable vector graphics (SVG) format.

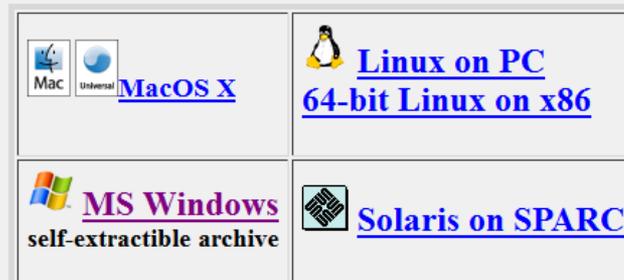
NEW: seaview drives the Gblocks program to select blocks of conserved sites.

SeaView is a multiplatform, graphical user interface for multiple sequence alignment and molecular phylogeny.

- SeaView reads and writes various file formats ([NEXUS](#), MSF, CLUSTAL, FASTA, PHYLIP, [MASE](#), Newick) of DNA and protein sequences and of phylogenetic trees.
- SeaView drives programs [muscle](#) or [clustalw](#) for multiple sequence alignment, and also allows to use any external alignment algorithm able to read and write FASTA-formatted files.
- Seaview drives the [Gblocks](#) program to select blocks of evolutionarily conserved sites.
- SeaView computes phylogenetic trees by
 - parsimony, using PHYLIP's [dnapars/protpars](#) algorithm,
 - distance, with [NJ](#) or [BioNJ](#) algorithms on a variety of evolutionary distances,
 - maximum likelihood, driving program [PhyML](#) 3.0.
- SeaView prints and draws phylogenetic trees on screen, SVG, PDF or PostScript files.
- SeaView allows to download sequences from EMBL/GenBank/UniProt using the Internet.

Screen shots of the main [alignment](#) and [tree](#) windows. On-line [help](#) document. Old [seaview version 3.2](#)

Download SeaView



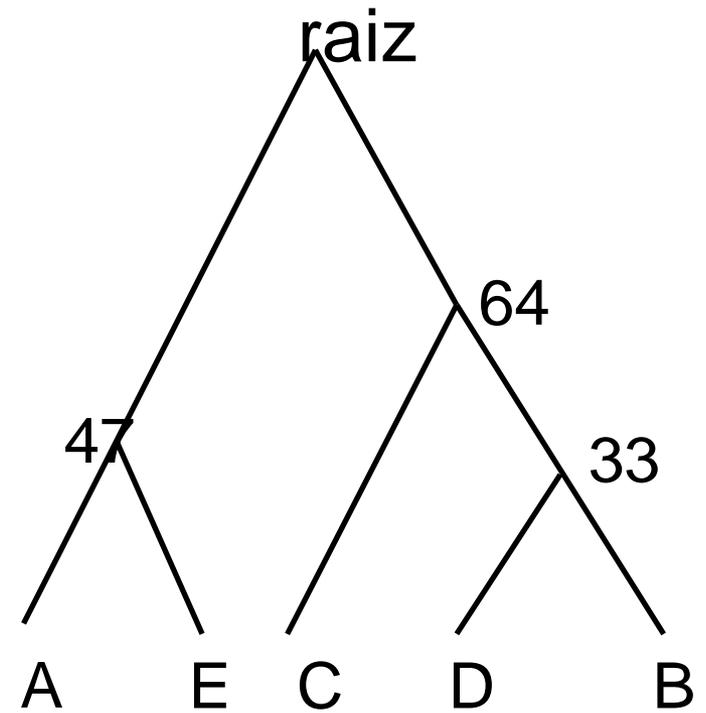
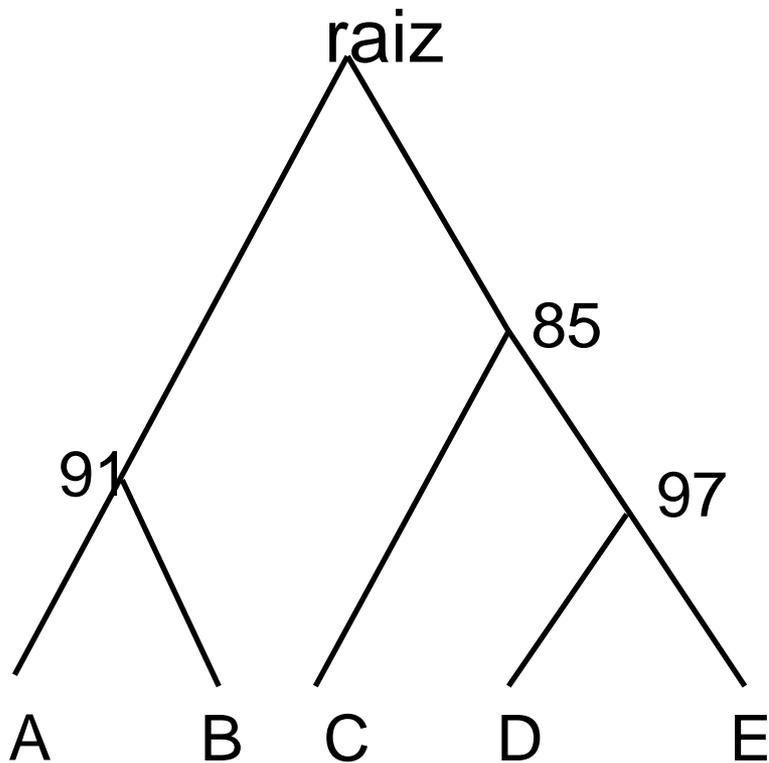
Interpretação

- Árvores são apenas hipóteses
- GIGO: garbage in, garbage out
- Os métodos em geral (menos distância) fornecem uma árvore com **nota (score)**
 - Parcimônia: **número mínimo de mutações**
 - ML: **valor da verossimilhança logarítmica**
 - Bayesiano: **probabilidade posterior**
- A árvore de melhor nota pode não ser a árvore “verdadeira”
- Para avaliar a qualidade da árvore
 - Confiabilidade de sua topologia

Confiabilidade da topologia

- Valores de **bootstrap**
- Colunas do AM são amostradas aleatoriamente em várias corridas (**replicatas**; geralmente entre 100 e 1000)
- Árvores resultantes são comparadas entre si
- Concordâncias nos clados são calculadas, resultando em número de vezes (ou %) que clados se repetem nas replicatas
- Valores bons são considerados aqueles maiores do que 0.7 (70%)
- Custosos para calcular
- PhyML fornece valores aproximados de bootstrap (ALRT) muito mais rapidamente

Exemplo de árvores com bootstrap



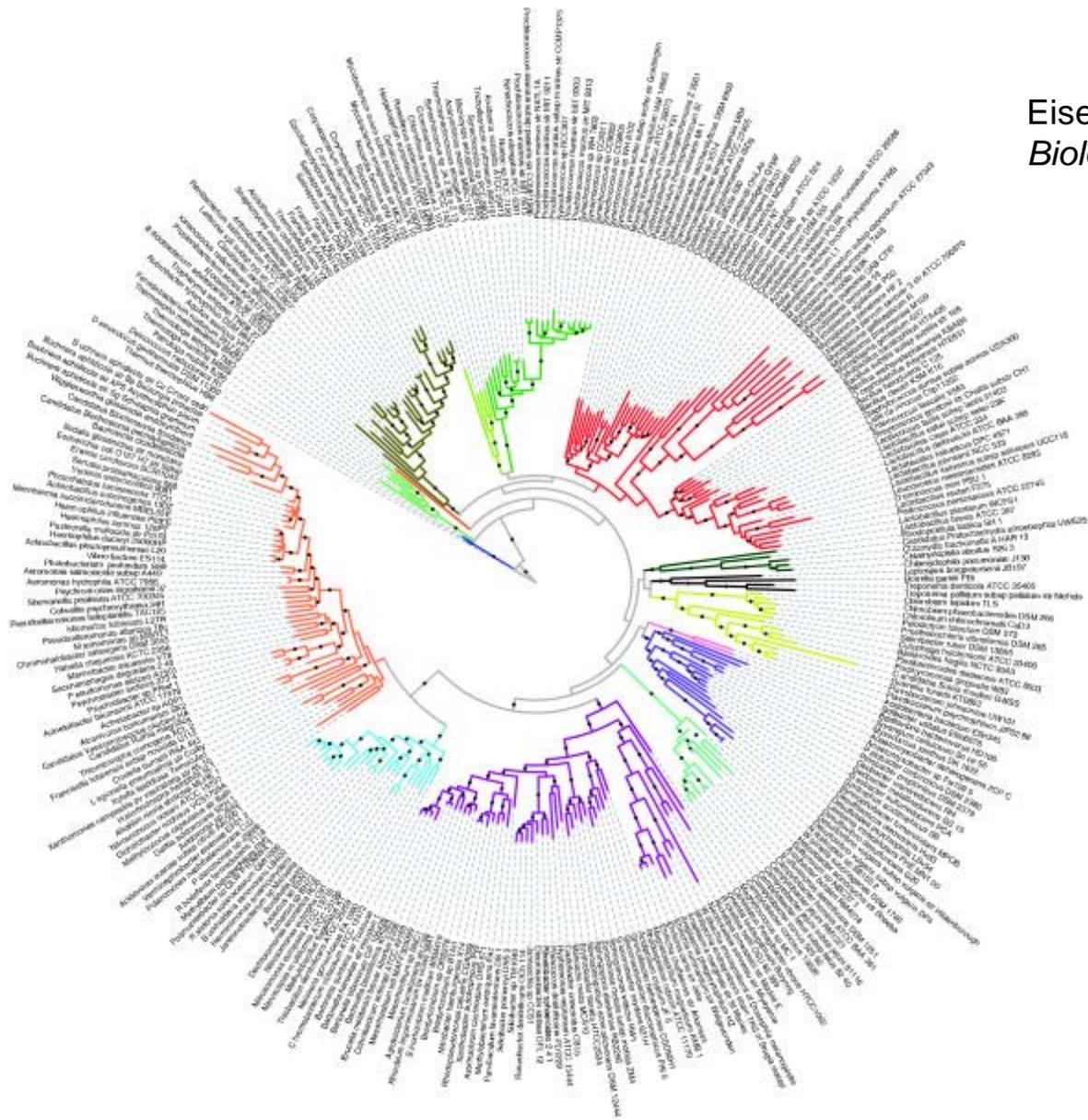
Como lidar com todas essas incertezas?

- Aprenda mais sobre evolução e inferência filogenética
- Se a filogenia é crucial para seus resultados
 - Use mais de um método!

Supermatrizes

- Método bom para obter árvores robustas de espécies quando genomas completos ou quase completos estão disponíveis
- Determinar famílias de proteínas para os genomas de interesse
- Determinar quais famílias tem exatamente um representante de cada genoma
- AM para cada família
- Concatenar todos os AMs (“a supermatriz”)
- Construir árvore com base no AM concatenado
- Foi usando esse método que foi construída a árvore da vida do próximo slide

No próximo slide há um outro exemplo de uso da técnica de supermatrizes, neste caso para construir uma árvore apenas de bactérias



- | | | | |
|-------------------------|-----------------------------|------------------|-----------------------|
| ■ Gammaproteobacteria | ■ Acidobacteria | ■ Cyanobacteria | ■ Deinococcus/Thermus |
| ■ Betaproteobacteria | ■ Bacteroidetes/Chlorobi | ■ Chloroflexi | |
| ■ Alphaproteobacteria | ■ Spirochaetes | ■ Actinobacteria | |
| ■ Epsilonproteobacteria | ■ Chlamydiae/Planctomycetes | ■ Aquificae | |
| ■ Deltaproteobacteria | ■ Firmicutes | ■ Thermotogae | |

Problemas de supermatrizes

- Diferentes genes tem diferentes taxas de evolução
- Long branch attraction
 - Ramos longos (muitas mutações) tendem a ficar artificialmente próximos uns dos outros (e próximos da raiz)
 - Topologia errada
- HGT insuspeita sempre vai introduzir erros

Próximo slide: exemplo recente de árvore de bactérias e arqueias

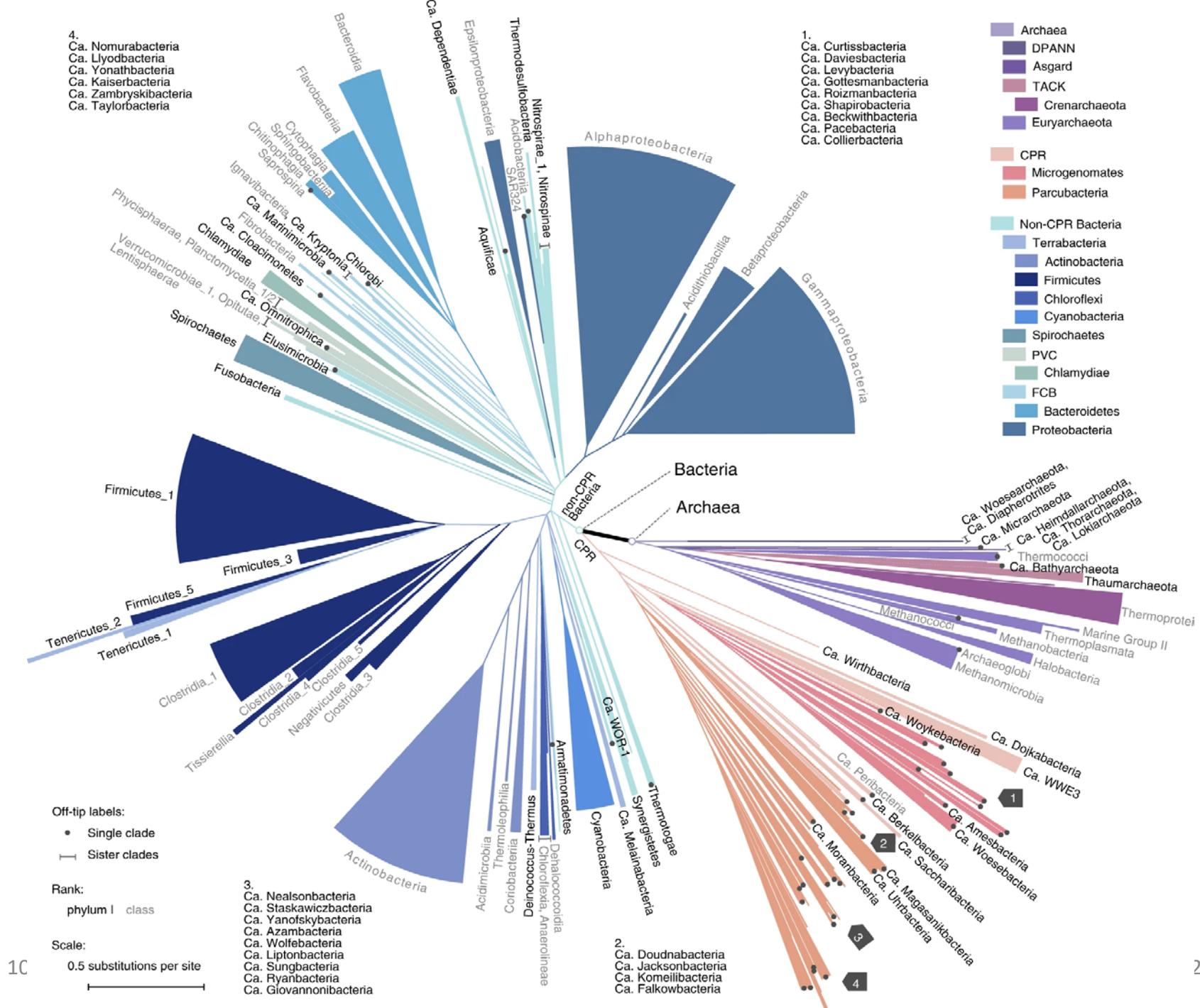
Exercício: compare essa árvore com a árvore de Wu e Eisen do slide anterior

Article | [Open Access](#) | Published: 02 December 2019

Phylogenomics of 10,575 genomes reveals evolutionary proximity between domains Bacteria and Archaea

Qiyun Zhu, Uyen Mai, [...] Rob Knight 

Nature Communications **10**, Article number: 5477 (2019) | [Cite this article](#)



Transferência Horizontal de Genes

- Material genético é passado de uma célula (doadora) para outra (receptora)
- O doador pode ser completamente diferente do receptor
- Exemplo: humanos e bactérias

Exemplo de HGT



Fungos e queijos

- Fabricação de queijos depende da ação de fungos
- Roquefort
 - *Penicillium roqueforti*
- Camembert
 - *P. camemberti*
- Esses fungos vem sendo selecionados e cultivados há séculos

Resultado recente

- Ao comparar diferentes espécies de fungos usados em queijos, descobriu-se
 - *Multiple Recent Horizontal Gene Transfers between Distant Penicillium Species, Flanked by Specific Retrotransposons*

Adaptive Horizontal Gene Transfers between Multiple Cheese-Associated Fungi

Jeanne Ropars,^{1,2,8} Ricardo C. Rodríguez de la Vega,^{1,2,8} Manuela López-Villavicencio,³ Jérôme Gouzy,^{4,5} Erika Sallet,^{4,5} Émilie Dumas,^{1,2} Sandrine Lacoste,³ Robert Debuchy,^{6,7} Joëlle Dupont,³ Antoine Branca,^{1,2,9,*} and Tatiana Giraud^{1,2,9,*}

¹Ecologie, Systématique et Evolution, UMR8079, Univ. Paris-Sud, 91405 Orsay, France

²Ecologie, Systématique et Evolution, UMR8079, CNRS, 91405 Orsay, France

³Institut de Systématique, Evolution, Biodiversité, UMR 7205 CNRS-MNHN-UPMC-EPHE, Muséum national d'Histoire naturelle, Sorbonne Université, CP39, 57 Rue Cuvier, 75231 Paris Cedex 05, France

⁴Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, INRA, Castanet-Tolosan 31326, France

⁵Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR2594, CNRS, Castanet-Tolosan 31326, France

⁶Institut de Génétique et Microbiologie, UMR8621, Univ. Paris-Sud, 91405 Orsay, France

⁷Institut de Génétique et Microbiologie, UMR8621, CNRS, 91405 Orsay, France

⁸Co-first author

⁹Co-senior author

*Correspondence: antoine.branca@u-psud.fr (A.B.), tatiana.giraud@u-psud.fr (T.G.)

<http://dx.doi.org/10.1016/j.cub.2015.08.025>

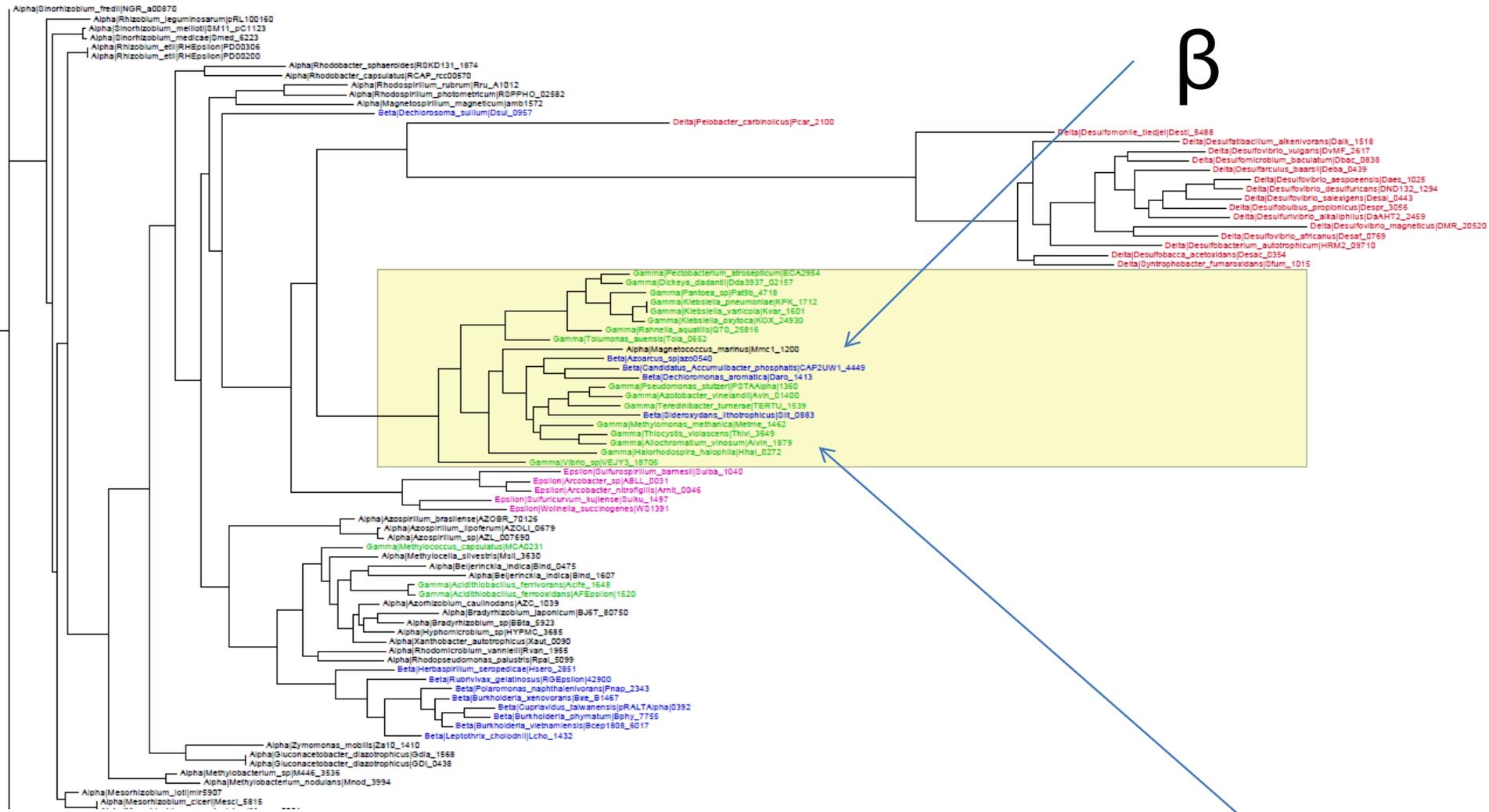
This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Transferência Horizontal de Genes

- Atrapalha a construção de árvores de espécies
- Como detectar?
- THG antiga
- THG recente

THG antiga

- Incongruência de árvores
 - Quando a árvore de um gene difere da árvore (robusta) de espécies

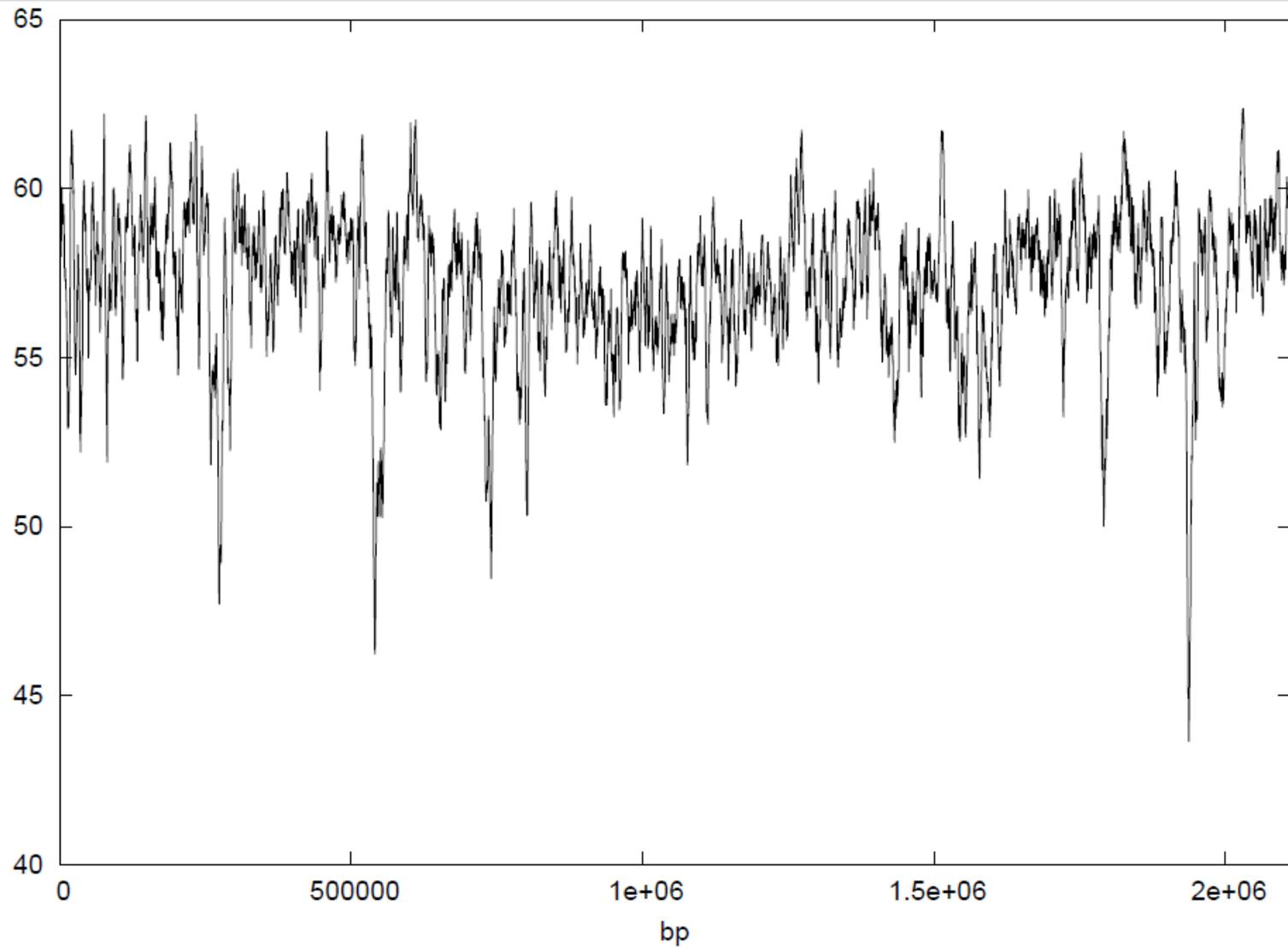


β

γ gamma

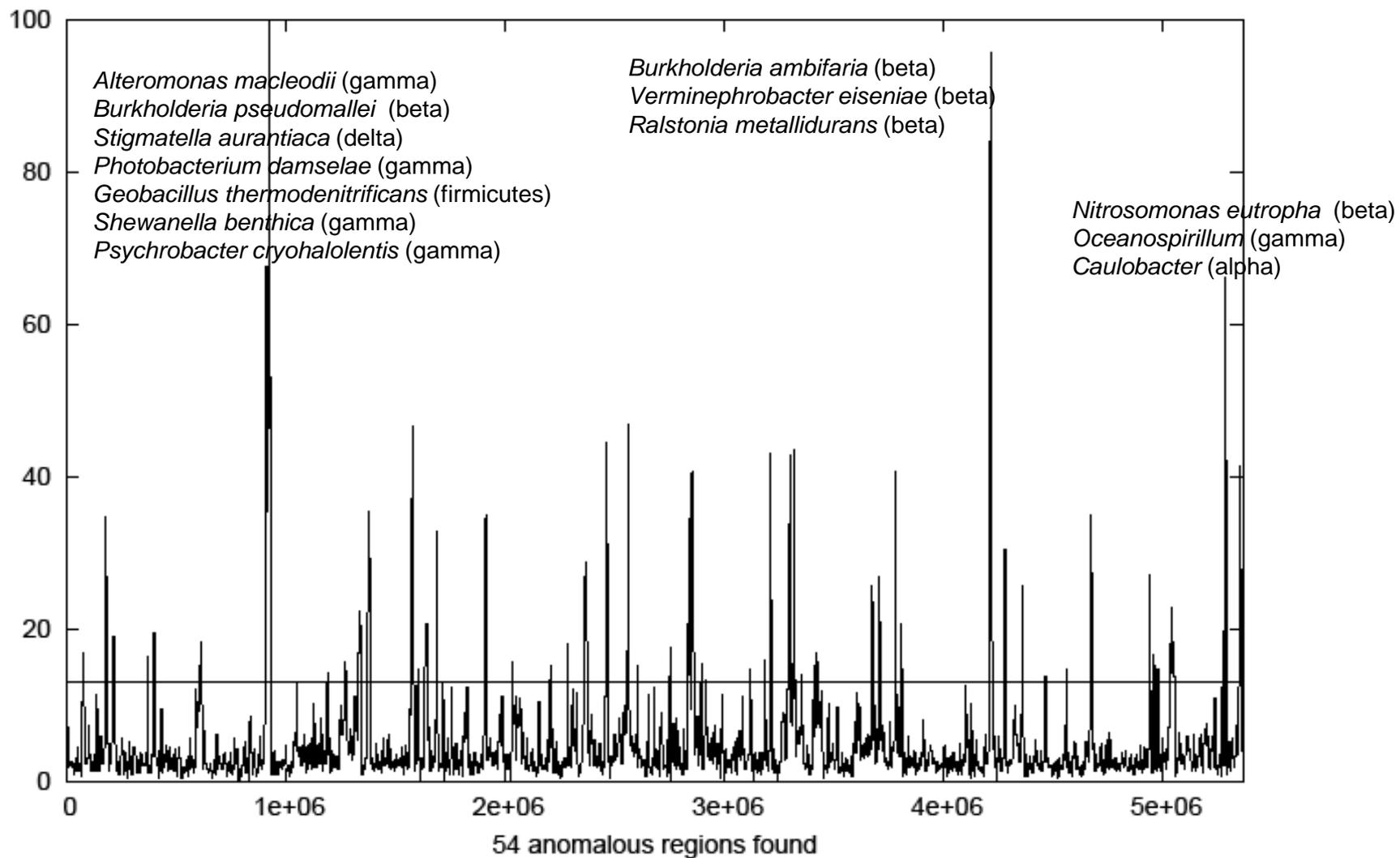
THG recente

- Incongruência de árvores
- Outros métodos
 - Desvios na composição (%GC, dinucleotídeos, uso de codons) da sequência
 - Ilhas genômicas



Variação do %GC no cromossomo principal de *Brucella ovis* ATCC25840

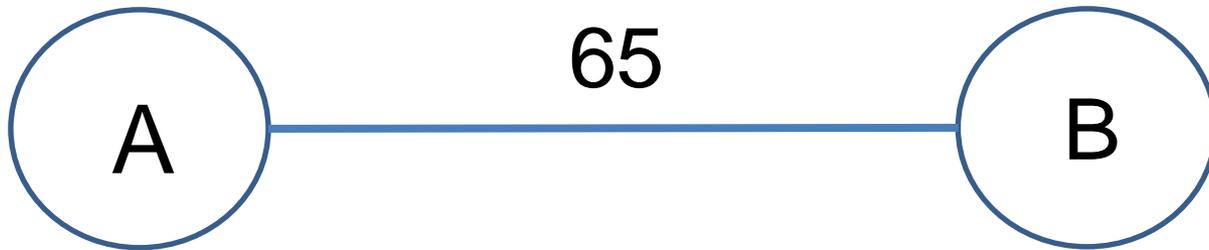
Azotobacter vinelandii anomalous regions (Alien_hunter threshold: 13.020)



Redes filogenômicas

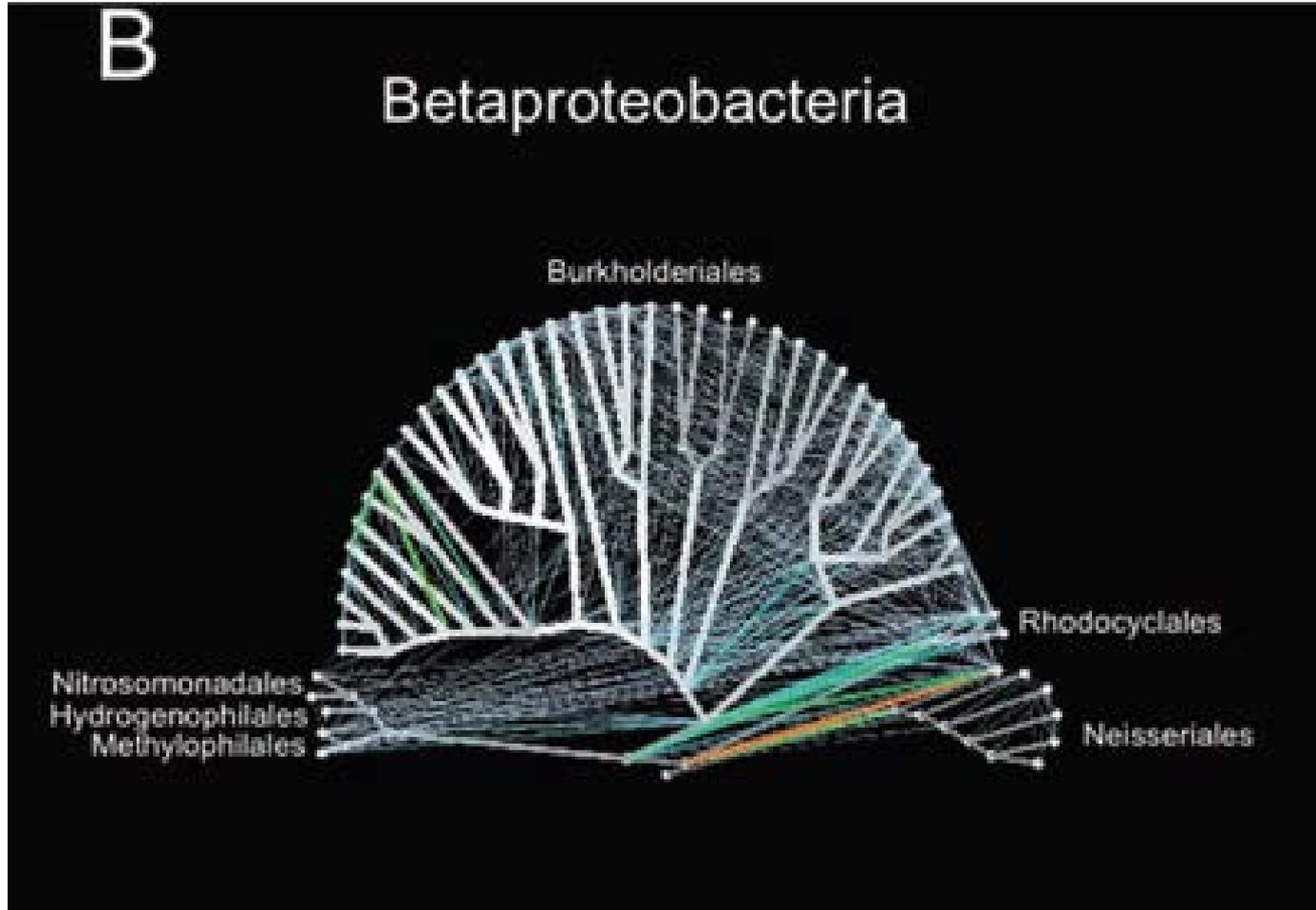
- Redes que mostram compartilhamento de genes

Genoma A compartilha 65 genes com genoma B



A superposição de uma **árvore de espécies** numa tal rede mostra possíveis eventos de **transferência horizontal**

Uma rede filogenômica

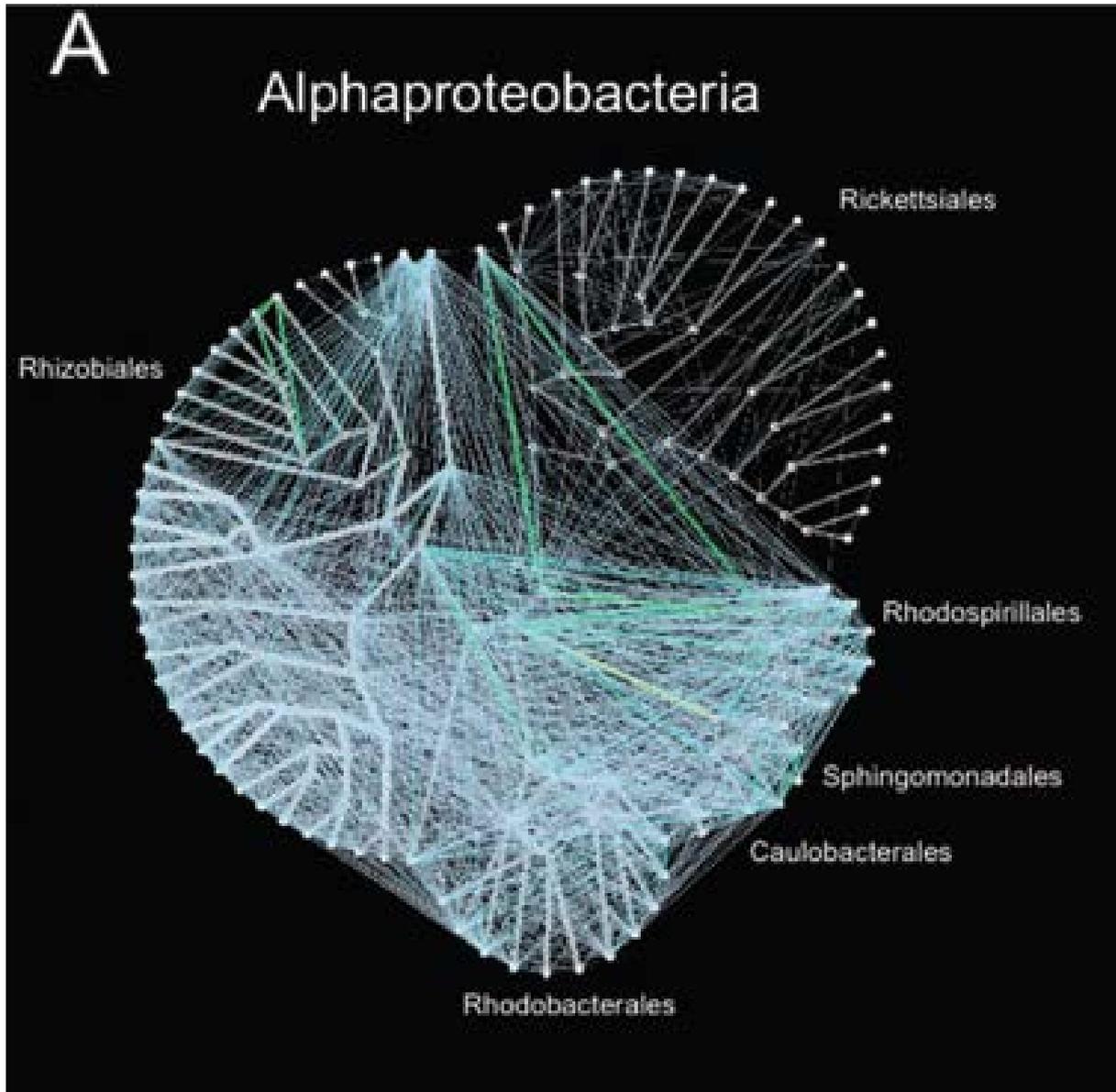


Kloesges et al, *Molecular Biology and Evolution*, 2011

10/22/2020

J. C. Setubal

39



Substituições sinônimas e não-sinônimas

- Código genético é degenerado
- Glicina: GGA, GGC, GGG, GGU
- Mutação na terceira base **não altera** o aminoácido
 - Sinônima (silenciosa)
- Mutação na primeira base altera o aminoácido
 - Não-sinônima

Razão Ka/Ks

- **Ka/Ks ou dN/dS**
- Razão entre o número de subs. não-sinônimas (Ka) e o número de subs. sinônimas (Ks)
- Usado para inferir a direção e magnitude de seleção natural agindo em genes codificadores de proteínas
- $Ka/Ks > 1$: seleção positiva ou Darwiniana
- $Ka/Ks < 1$: seleção purificadora ou estabilizadora
- $Ka/Ks = 1$: não há seleção (neutra)

Para calcular Ka/Ks

- Hurst, L. (2002). "The Ka/Ks ratio: diagnosing the form of sequence evolution". *Trends in Genetics* **18**: 486–489
- <http://services.cbu.uib.no/tools/kaks>

Para saber mais

- Yang e Rannala. Molecular phylogenetics: principles and practice. *Nature Reviews Genetics*, 13:303-314, 2012
- ***Bioinformatics***. Baxevanis and Ouellette (Eds.) Wiley-Interscience, 2005 (3rd edition), **ch. 14**
- D. Mount. ***Bioinformatics***. CSHL Press, 2004 (2nd edition), **ch. 7**
- ***The phylogenetic handbook***. Lemey, Salemi and Vandamme (Eds.) Cambridge University Press, 2009 (2nd edition)

THE TOP 100 PAPERS

Nature explores the most-cited research of all time.

BY RICHARD VAN NOORDEN,
BRENDAN MAHER AND REGINA NUZZO

The discovery of high-temperature superconductors, the determination of DNA's double-helix structure, the first observations that the expansion of the Universe is accelerating — all of these breakthroughs won Nobel prizes and international acclaim. Yet none of the papers that announced them comes anywhere close to ranking among the 100 most highly cited papers of all time.

of carbon nanotubes (number 36) are indeed classic discoveries. But the vast majority describe experimental methods or software that have become essential in their fields.

The most cited work in history, for example, is a 1951 paper² describing an assay to determine the amount of protein in a solution. It has now gathered more than 305,000 citations — a recognition that always puzzled its lead author, the late US biochemist Oliver Lowry. “Although J. Cell. Biol. is not a

to other scientists what kind of work we are doing”. Another common practice is to ensure that truly foundational discoveries — Einstein's special theory of relativity for instance — get fewer citations than they might deserve: they are so important that they quickly enter the textbooks or are inserted into the main text of papers as terms so familiar that they do not need a citation.

Citation counts are riddled with confounding factors. The volume of citations has increased, for example — yet older papers had more time to accrue citations. Engineers tend to cite one another's work more frequently than, say, physicists. And not all fields have the same number of publications. Molecular biologists therefore recoil from metrics as crude as simply counting citations when they want to measure a paper's value: instead, they prefer to compare counts for papers of similar age, and in comparable fields.

Nor is Thomson Reuters' list the only citation system available. Google Scholar has its own top-100 list for *Nature*. It includes many more citations because the search engine pulls references from a much greater (and poorly characterized) literature base, including books from a large range of publishers. In that list, available at www.nature.com/top100, ecology papers have more prominence. Google Scholar's list also features books, which Thomson Reuters did not analyse. But among the top papers, many of the same titles show

up. Yet even with all the caveats, the Thomson Reuters hall of fame still has value. If not for anything else, it serves as a reminder of the nature of scientific knowledge. To make exciting discoveries, researchers rely on relatively unsung papers that describe experimental methods, data analysis software.

Here *Nature* tours some of the key papers that tens of thousands of citations have brought to the top of science's Kilimanjaro — but rarely thrust into the limelight.

Nature, 30/10/2014

Papers de bioinformática

- 10) clustalW: 40289
- 12) blast1: 38380
- 14) blast2: 36410
- 20) NJ: 30176
- 28) clustalX: 24098
- 41) bootstrap: 21373
- 45) MEGA: 18286
- 76) modelTest: 14099
- 100) MrBayes: 12209