Challenge and novel aproaches for multiple sequence alignment and phylogenetic estimation

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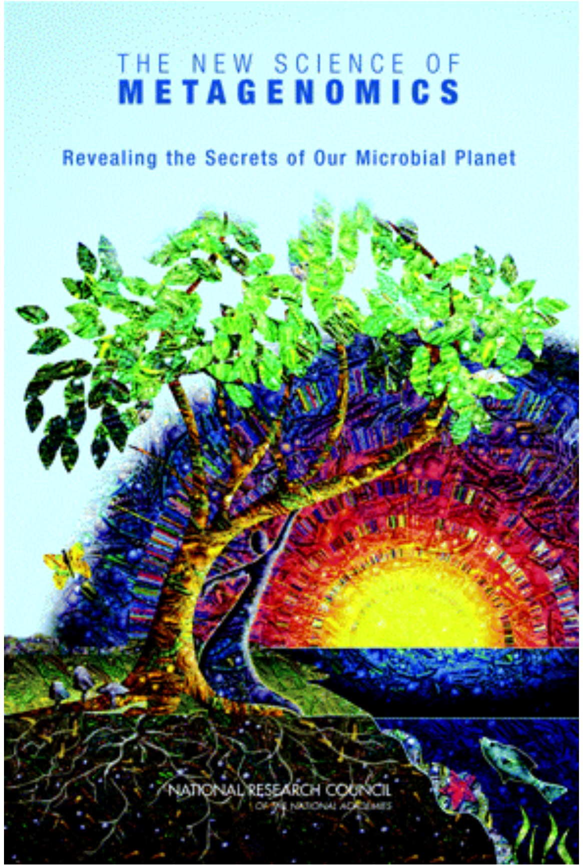
Computational Phylogenetics and Metagenomics

Courtesy of the Tree of Life project


THE NEW SCIENCE OF
METAGENOMICS
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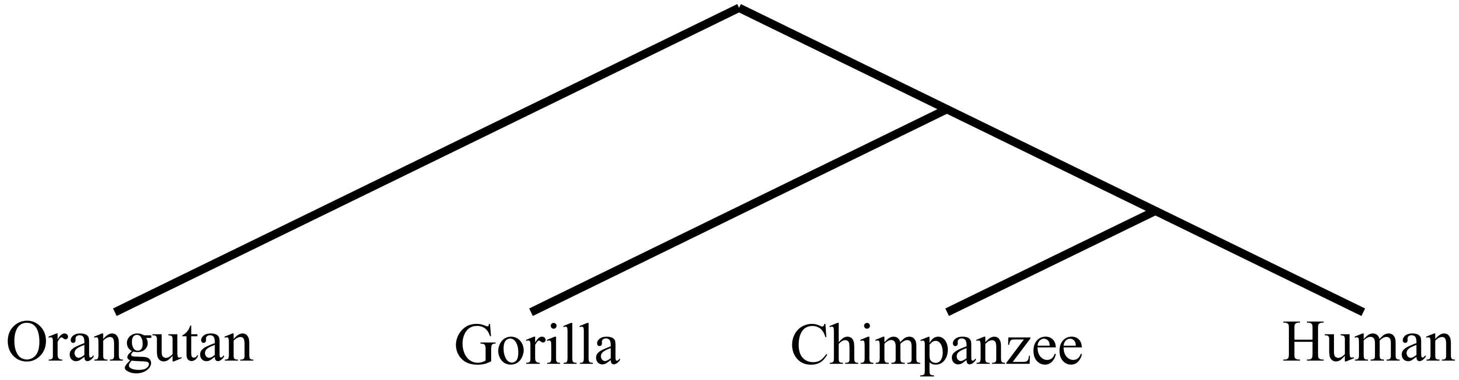
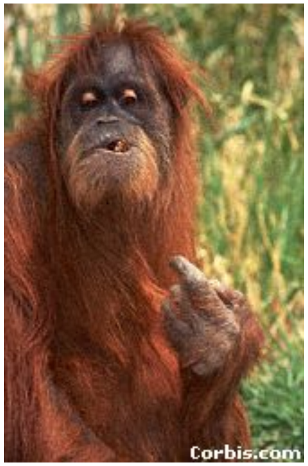
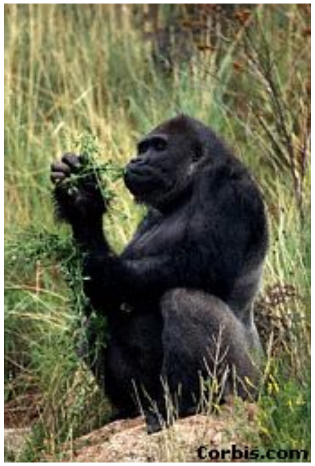
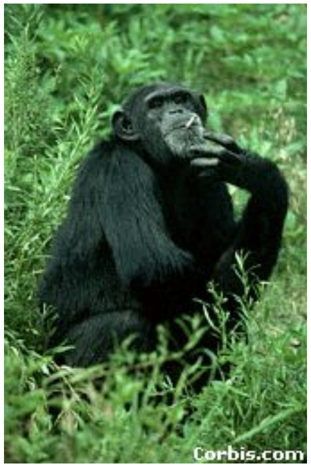
Phylogeny (evolutionary tree)









From the Tree of the Life Website,
University of Arizona

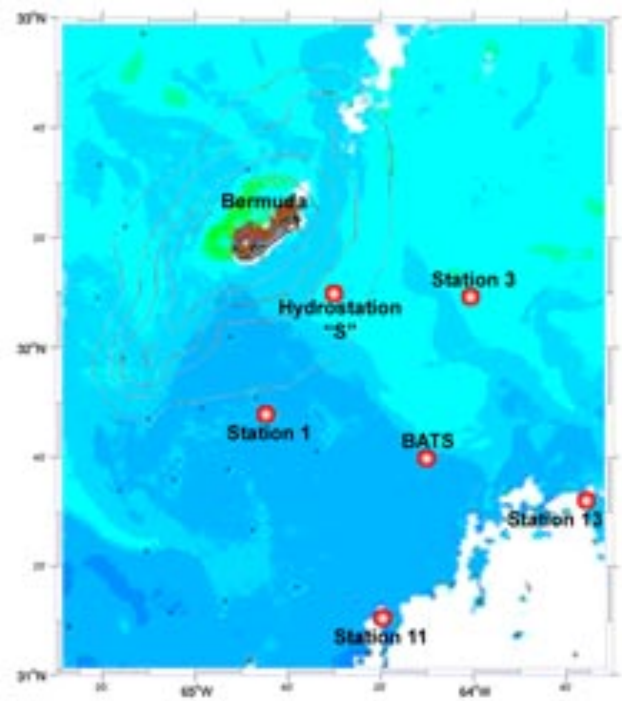
How did life evolve on earth?

Courtesy of the Tree of Life project

Metagenomics:

Venter et al., Exploring the Sargasso Sea:

Scientists Discover One Million New Genes in Ocean Microbes



Major Challenges

- **Phylogenetic analyses:** standard methods have *poor accuracy* on even moderately large datasets, and the most accurate methods are enormously *computationally intensive* (weeks or months, high memory requirements)
- **Metagenomic** analyses: methods for species classification of short reads have *poor sensitivity*. Efficient high throughput is necessary (millions of reads).

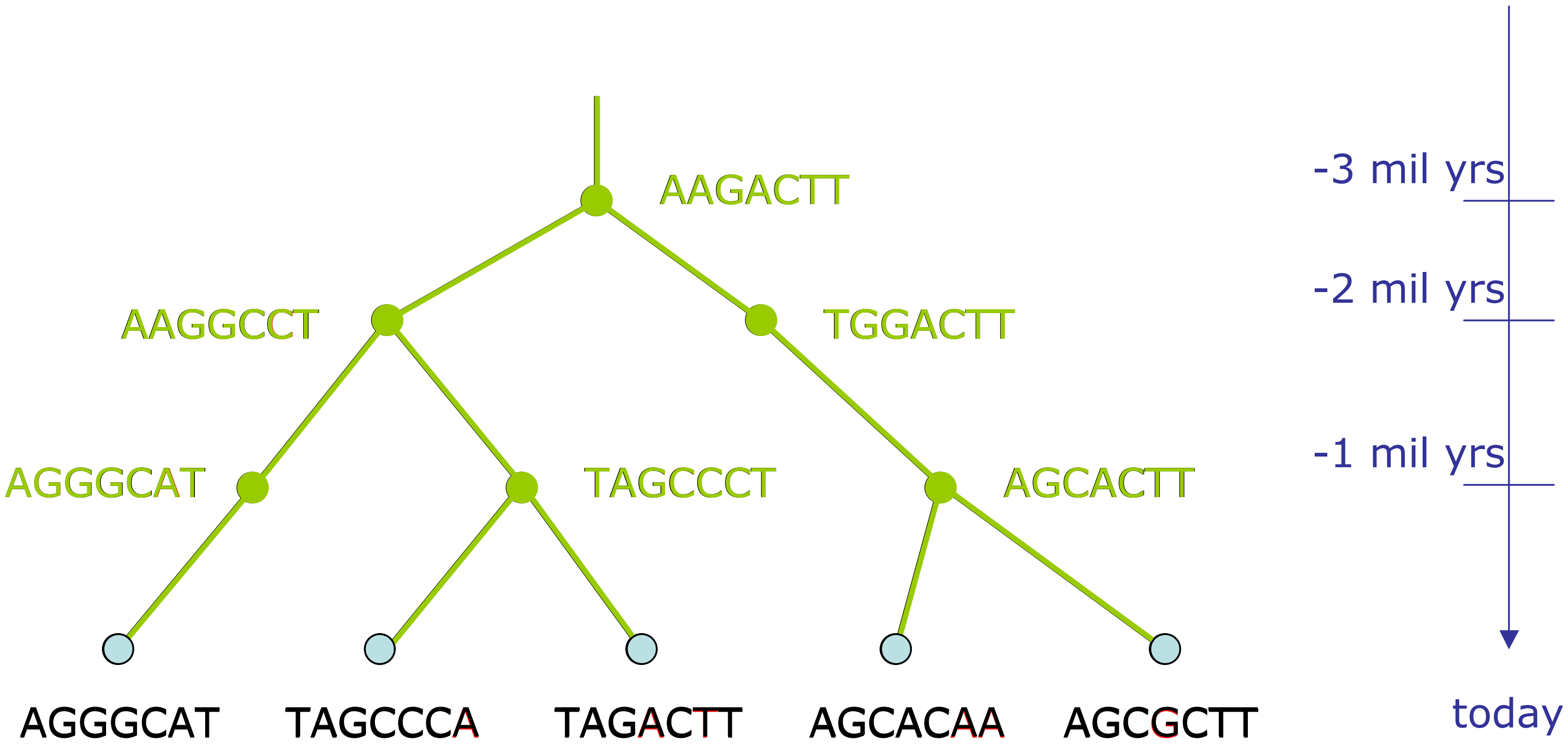
Phylogenetic “boosters” (meta-methods)

Goal: improve accuracy, speed, robustness, or theoretical guarantees of base methods

Examples:

- DCM-boosting for distance-based methods (1999)
- DCM-boosting for heuristics for NP-hard problems (1999)
- SATé-boosting for alignment methods (2009)
- SuperFine-boosting for supertree methods (2011)
- DACTAL-boosting: almost alignment-free phylogeny estimation methods (2011)
- SEPP-boosting for phylogenetic placement of short sequences (2012)
- TIPP-boosting for metagenomic taxon identification (2013)

DNA Sequence Evolution



AAGACTT

AAGGCCT

TGGACTT

AGGGCAT

TAGCCCT

AGCACTT

AGGGCAT

TAGCCCA

TAGACTT

AGCACAA

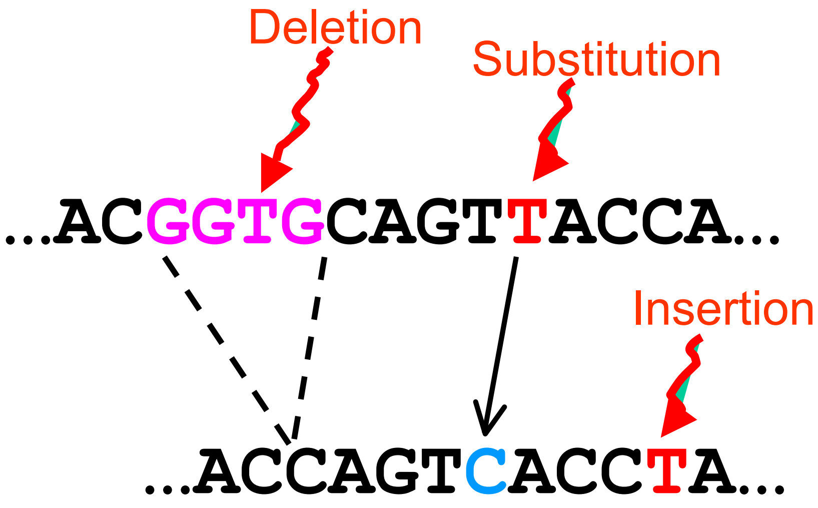
AGCGCTT

-3 mil yrs

-2 mil yrs

-1 mil yrs

today



Deletion

Substitution

...ACGGTGCAGTTACCA...

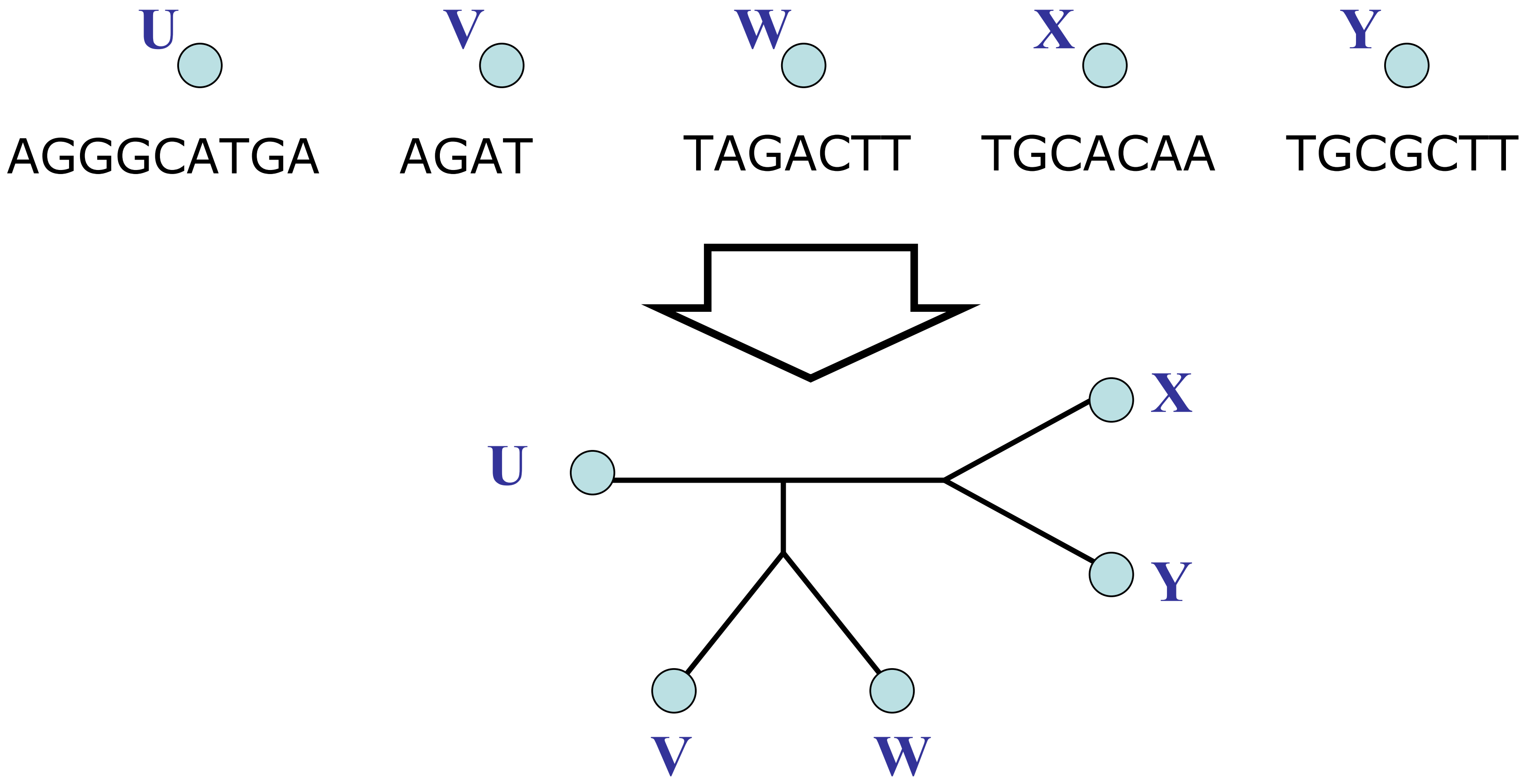
Insertion

...ACCAGTCACCTA...

```
...ACGGTGCAGTTACC-A...
...AC----CAGTCACCTA...
```

The true multiple alignment

- Reflects historical substitution, insertion, and deletion events
- Defined using transitive closure of pairwise alignments computed on edges of the true tree



AGGGCATGA AGAT TAGACTT TGCACAA TGCGCTT

Input: unaligned sequences

```
S1 = AGGCTATCACCTGACCTCCA
S2 = TAGCTATCACGACCGC
S3 = TAGCTGACCGC
S4 = TCACGACCGACA
```

Phase 1: Multiple Sequence Alignment

```
S1 = AGGCTATCACCTGACCTCCA
S2 = TAGCTATCACGACCGC
S3 = TAGCTGACCGC
S4 = TCACGACCGACA
```

→

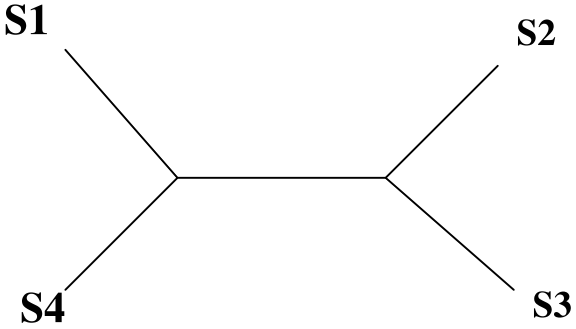
```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-CT-------GACCGC--
S4 = -------TCAC--GACCGACA
```

Phase 2: Construct tree

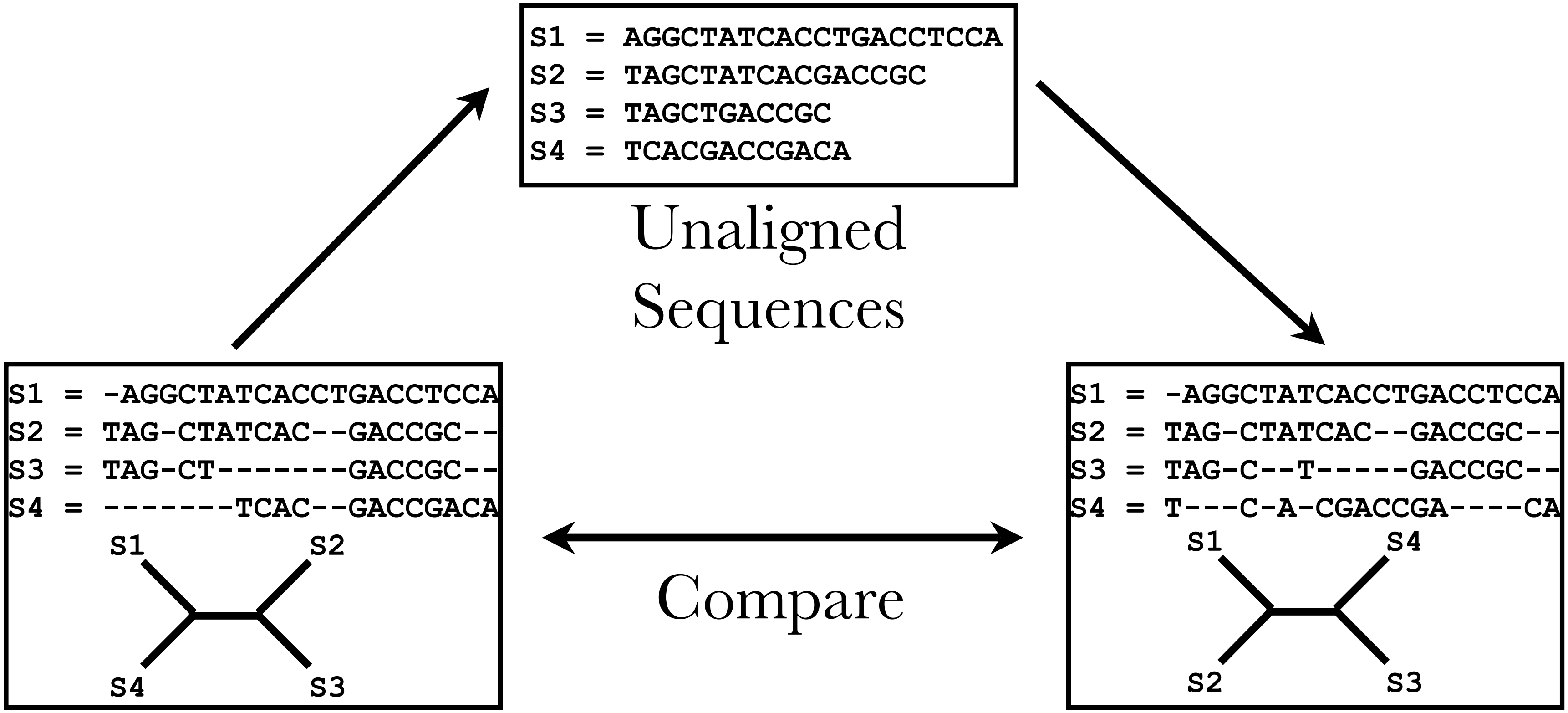
```
S1 = AGGCTATCACCTGACCTCCA
S2 = TAGCTATCACGACCGC
S3 = TAGCTGACCGC
S4 = TCACGACCGACA
```

→

```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-CT-------GACCGC--
S4 = -------TCAC--GACCGACA
```



Simulation Studies



```
S1 = AGGCTATCACCTGACCTCCA
S2 = TAGCTATCACGACCGC
S3 = TAGCTGACCGC
S4 = TCACGACCGACA
```

Unaligned Sequences

```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-CT-------GACCGC--
S4 = -------TCAC--GACCGACA
```

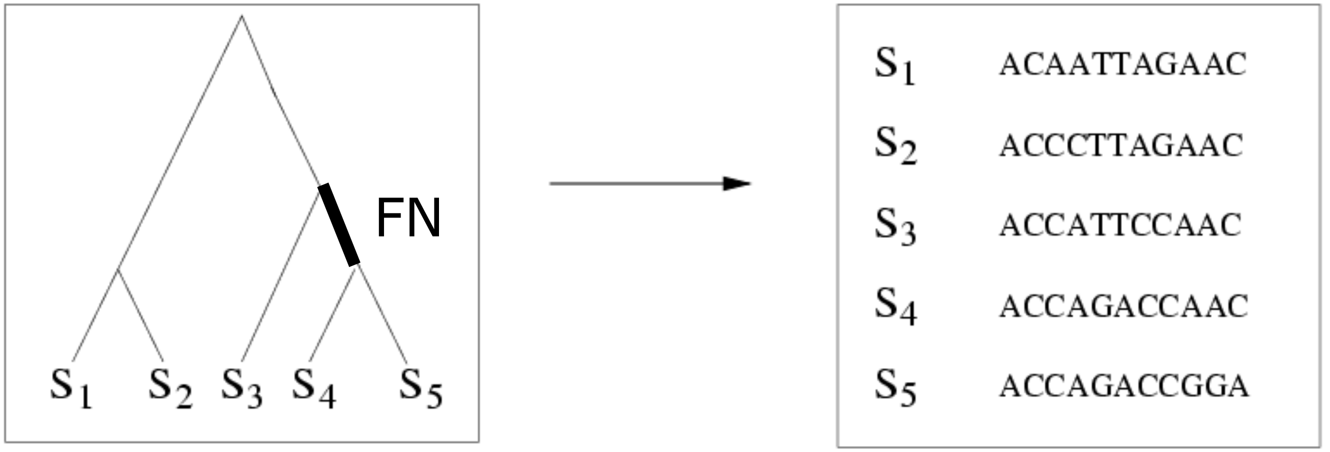
True tree and alignment

Compare

```
S1 = -AGGCTATCACCTGACCTCCA
S2 = TAG-CTATCAC--GACCGC--
S3 = TAG-C--T-----GACCGC--
S4 = T---C-A-CGACCGA----CA
```

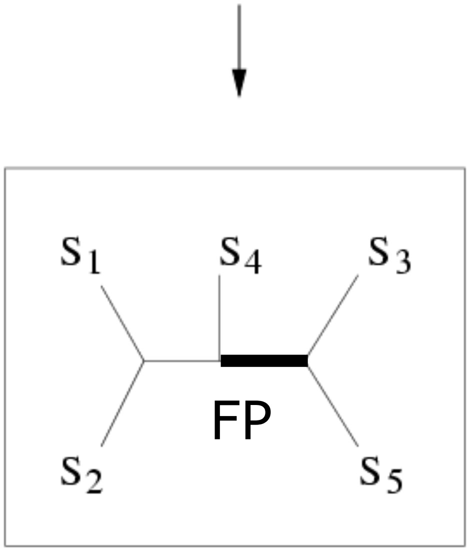
Estimated tree and alignment

Quantifying Error



TRUE TREE

DNA SEQUENCES

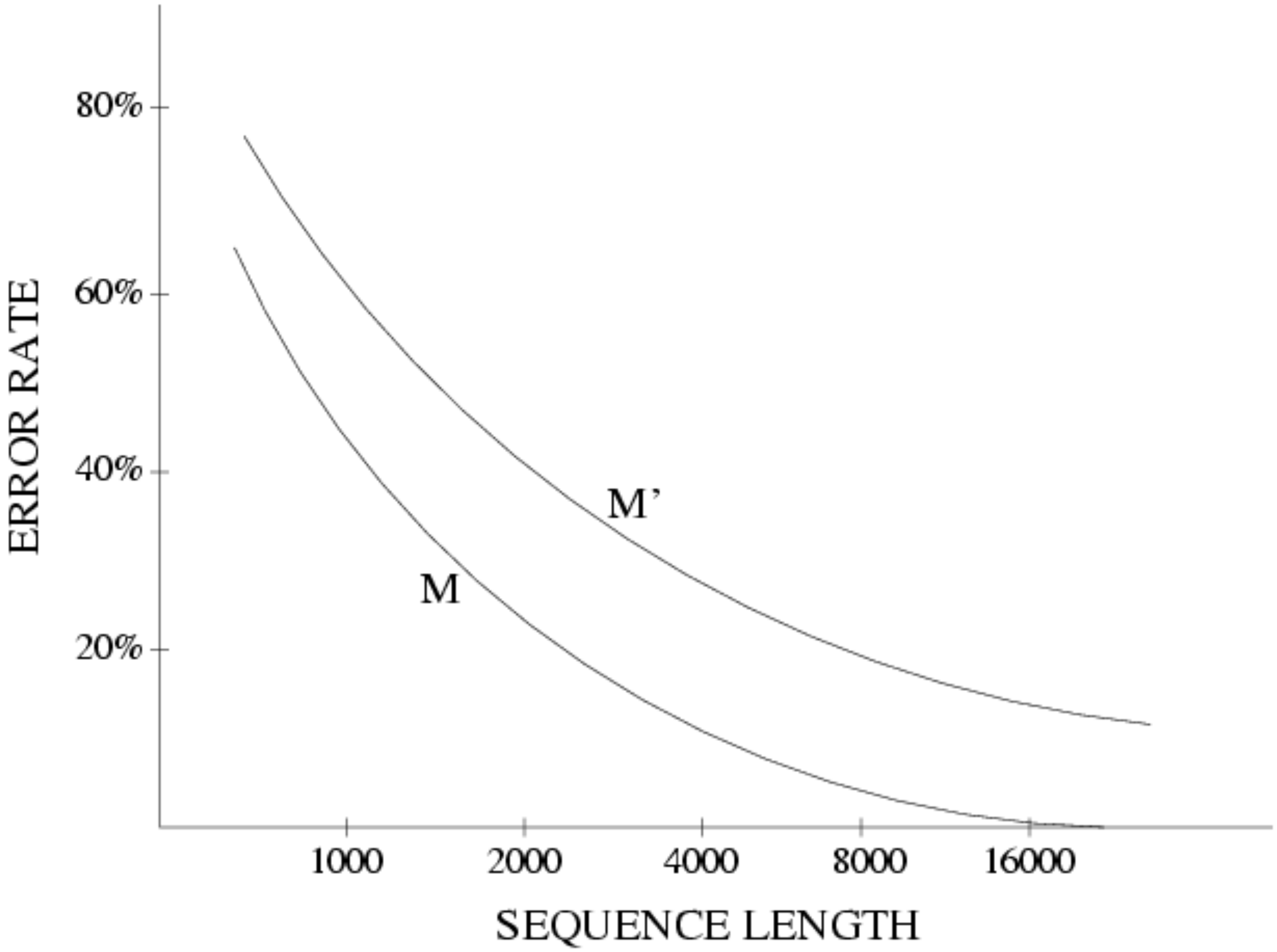


INFERRED TREE

FN: false negative
(missing edge)
FP: false positive
(incorrect edge)

50% error rate

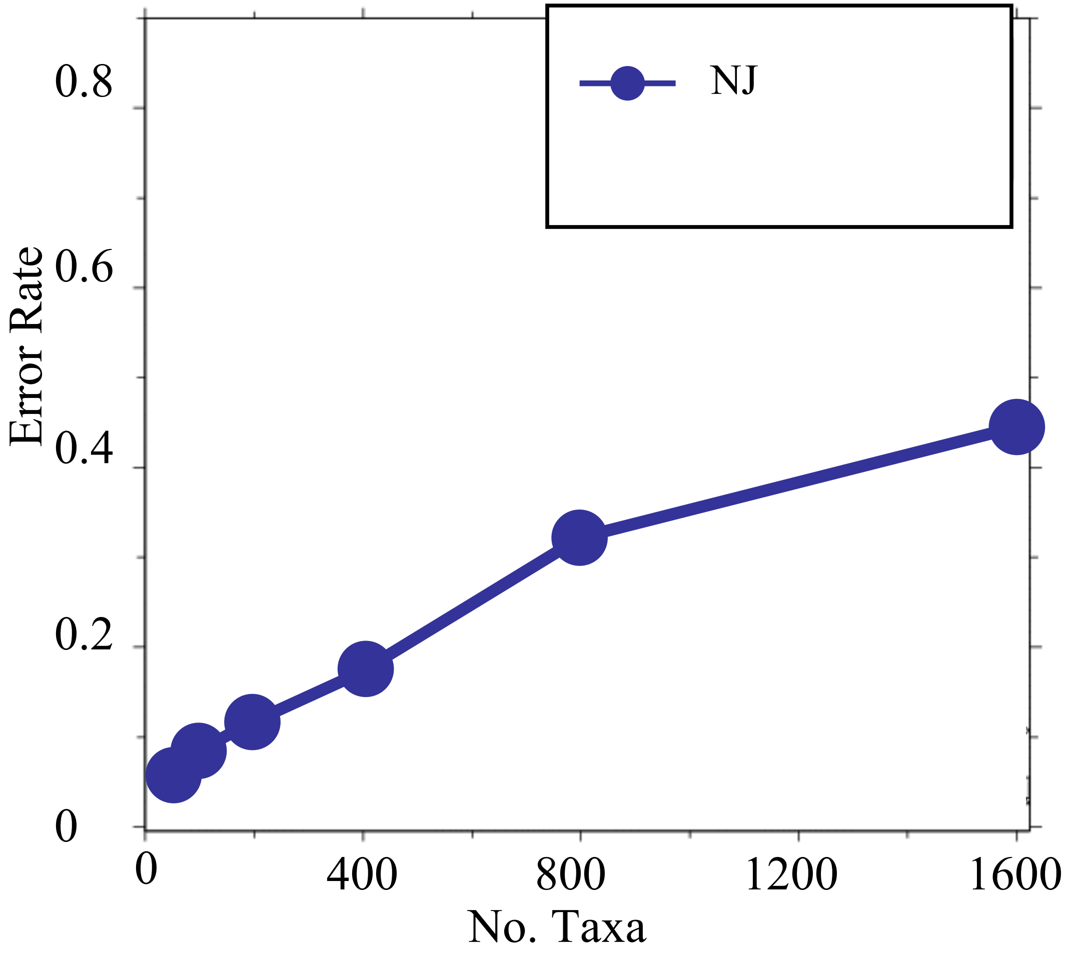
Statistical consistency and convergence rates



Part I: “Fast-Converging Methods”

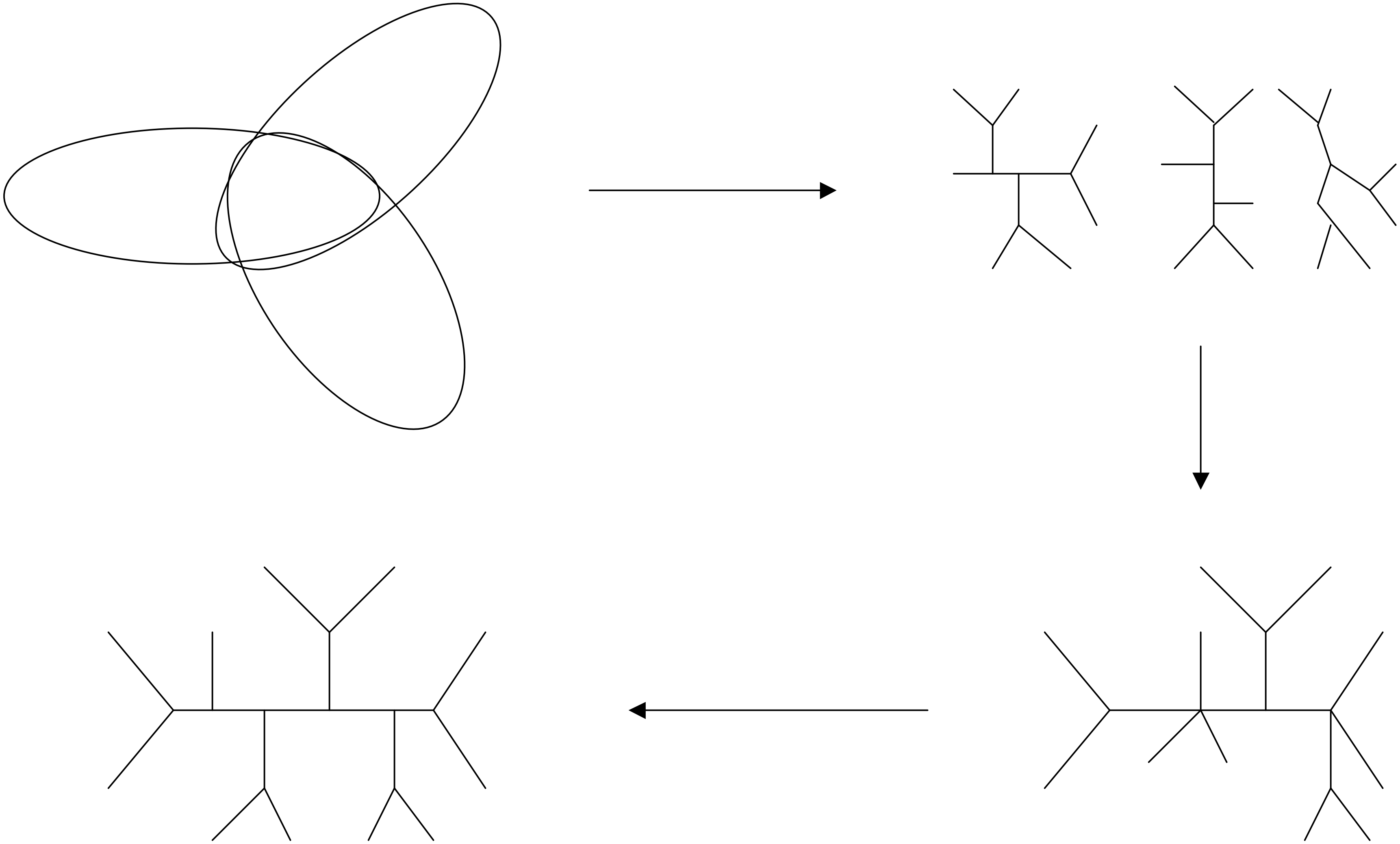
- Basic question: how much data does a phylogeny estimation method need to produce the true tree with high probability?

Neighbor joining has poor performance on large diameter trees *[Nakhleh et al. ISMB 2001]*



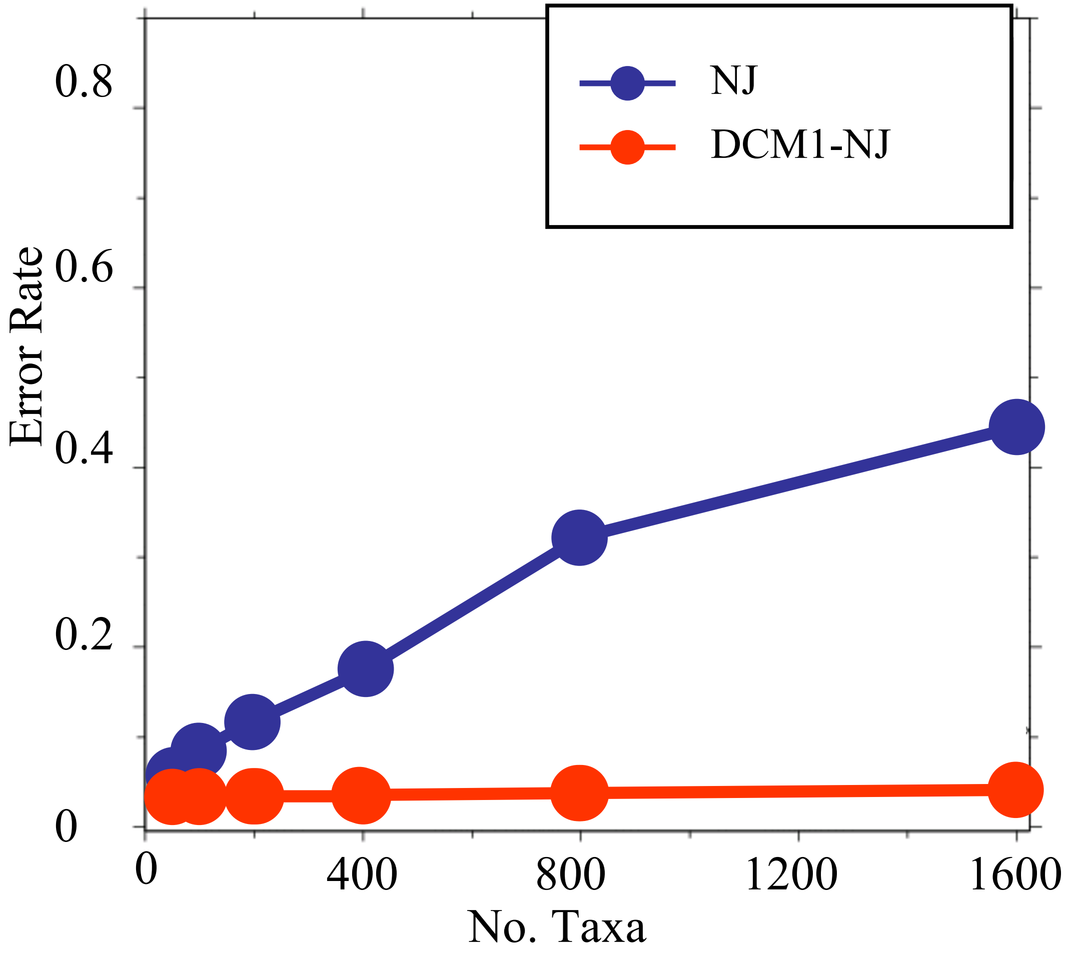
Theorem (Atteson): Exponential sequence length requirement for Neighbor Joining!

Disk-Covering Methods (DCMs)
(starting in 1998)



DCM1-boosting distance-based methods

[Nakhleh et al. ISMB 2001]



DCM1-boosting makes distance-based methods more accurate

Theoretical guarantees that DCM1-NJ converges to the true tree from polynomial length sequences

Part II: SATé

Simultaneous Alignment and Tree Estimation

Liu, Nelesen, Raghavan, Linder, and Warnow, *Science*, 19 June 2009, pp. 1561-1564.

Liu et al., Systematic Biology 2012

Public software distribution (open source) through the Mark Holder’s group at the University of Kansas

Two-phase estimation

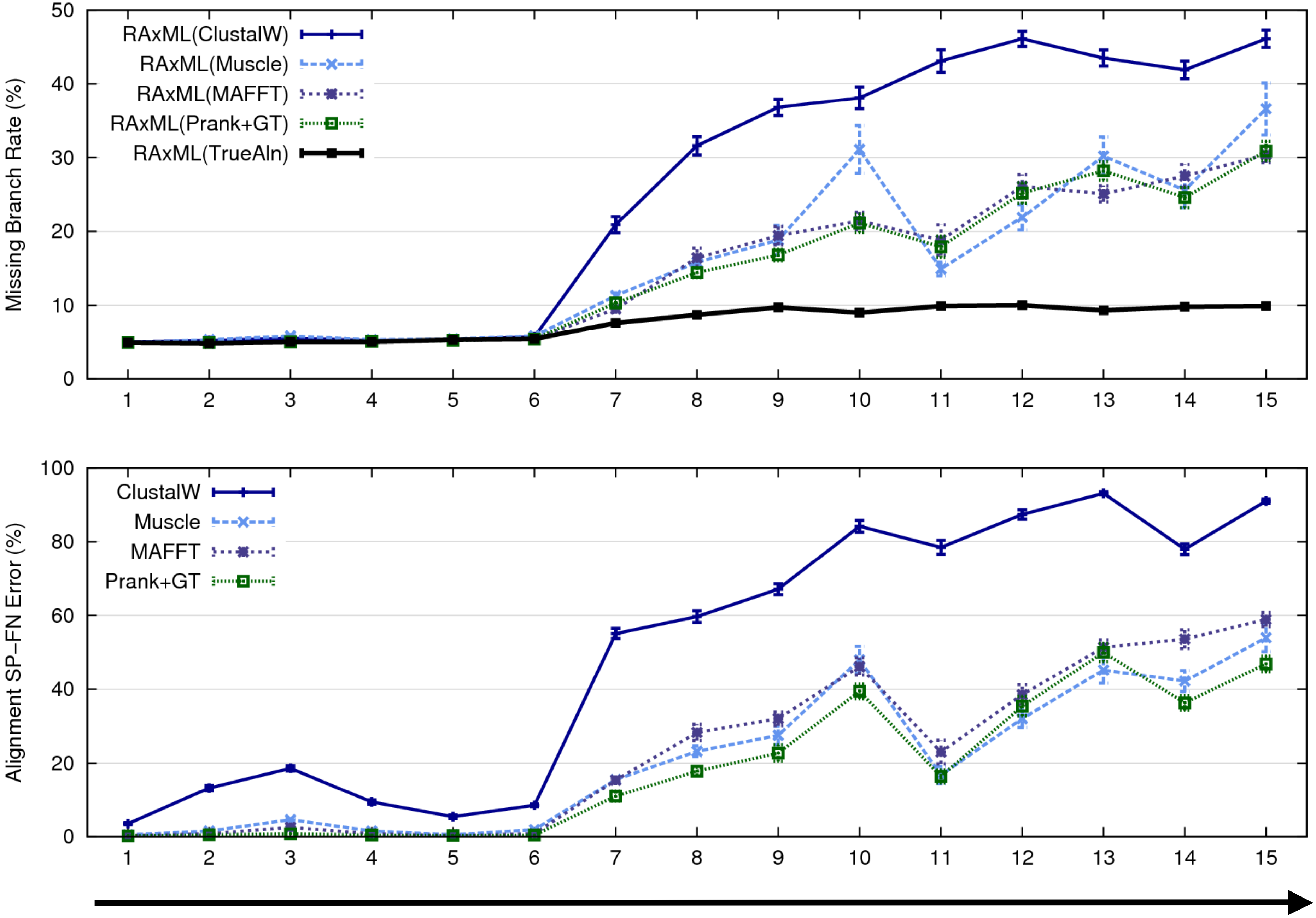
Alignment methods

- Clustal
- POY (and POY*)
- Probcons (and Probtree)
- Probalign
- MAFFT
- Muscle
- Di-align
- T-Coffee
- Prank (PNAS 2005, Science 2008)
- Opal (ISMB and Bioinf. 2007)
- *FSA (PLoS Comp. Bio. 2009)*
- *Infernal (Bioinf. 2009)*
- Etc.

Phylogeny methods

- Bayesian MCMC
- Maximum parsimony
- Maximum likelihood
- Neighbor joining
- FastME
- UPGMA
- Quartet puzzling
- Etc.

***RAxML**: heuristic for large-scale ML optimization*



1000 taxon models, ordered by difficulty (Liu et al., 2009)

Problems

- Large datasets with high rates of evolution are hard to align accurately, and phylogeny estimation methods produce poor trees when alignments are poor.
- Many phylogeny estimation methods have poor accuracy on large datasets (even if given correct alignments)
- *Potentially useful genes are often discarded* if they are difficult to align.

These issues seriously impact large-scale phylogeny estimation (and Tree of Life projects)

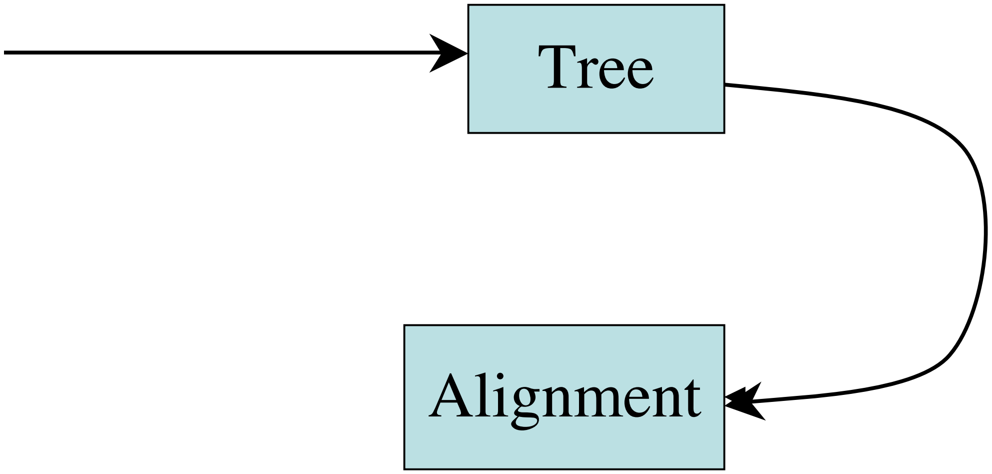
SATé Algorithm

Obtain initial alignment and estimated ML tree

Tree

SATé Algorithm

Obtain initial alignment
and estimated ML tree

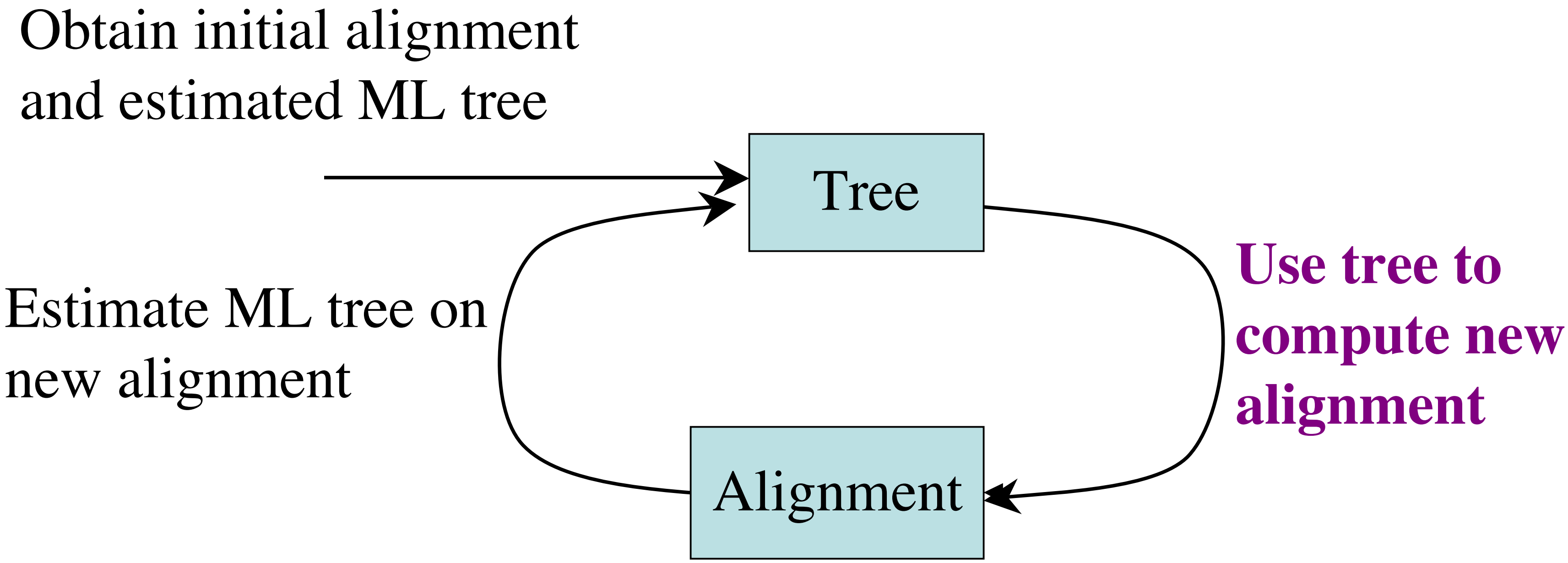


Tree

Use tree to
compute new
alignment

Alignment

SATé Algorithm



Obtain initial alignment and estimated ML tree

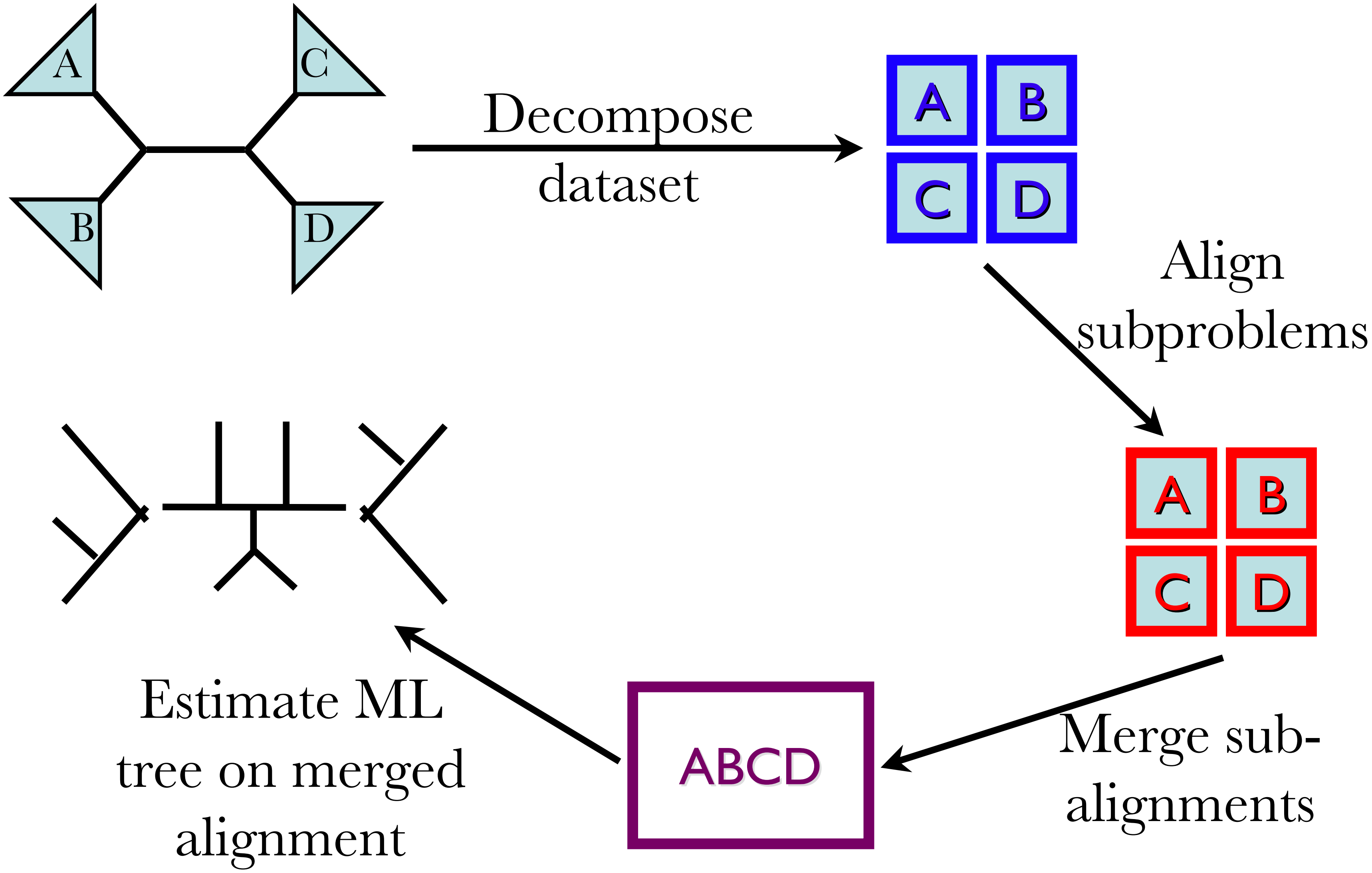
Tree

Use tree to compute new alignment

Alignment

Estimate ML tree on new alignment

Re-aligning on a tree

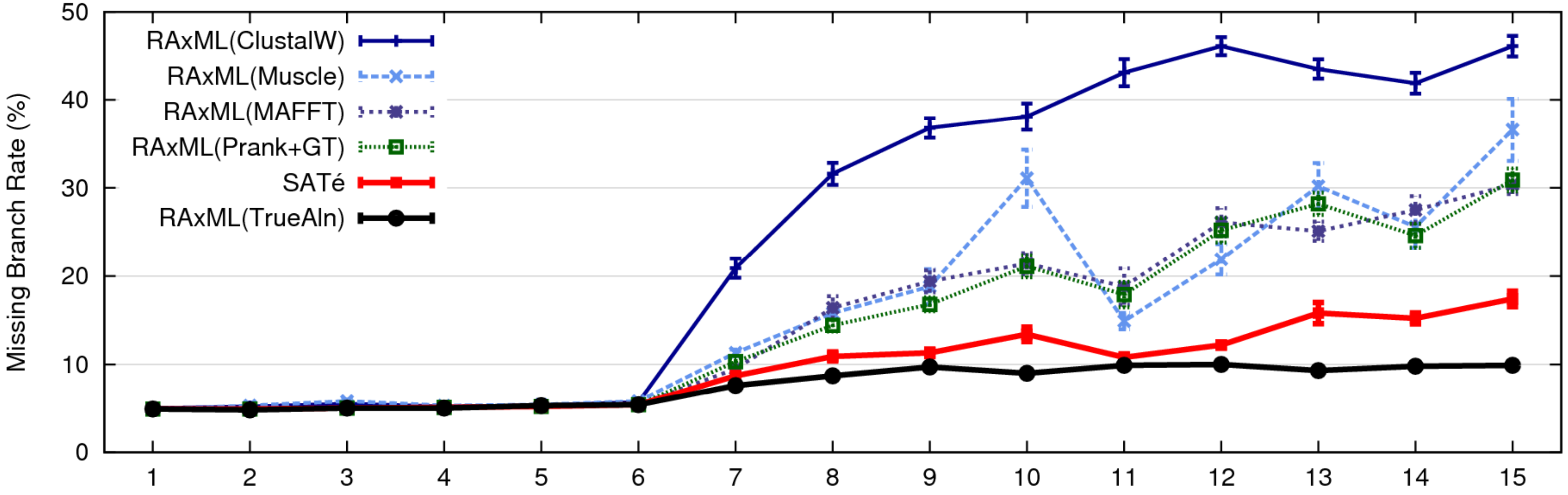


Decompose dataset

Align subproblems

Merge sub-alignments

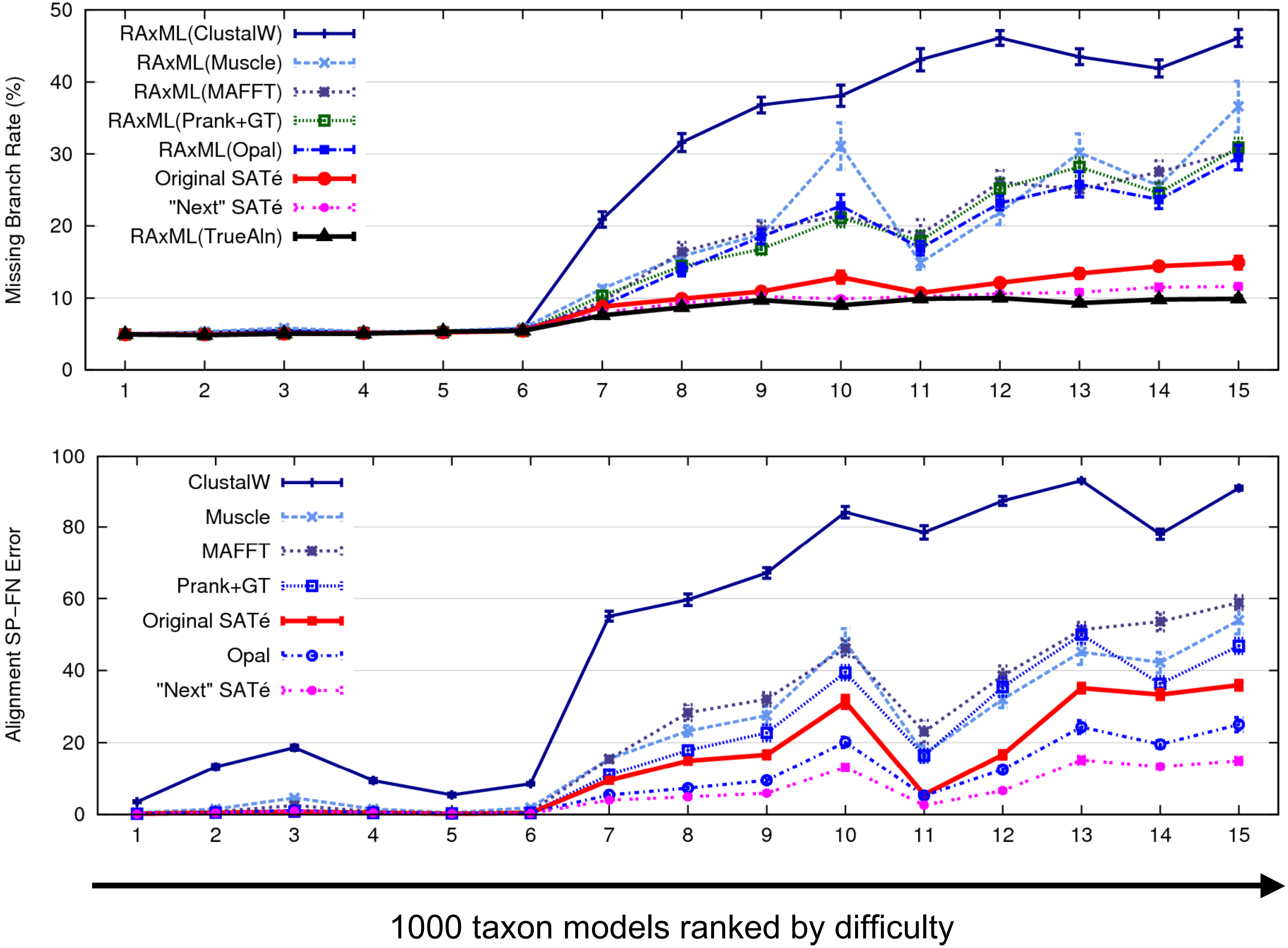
Estimate ML tree on merged alignment



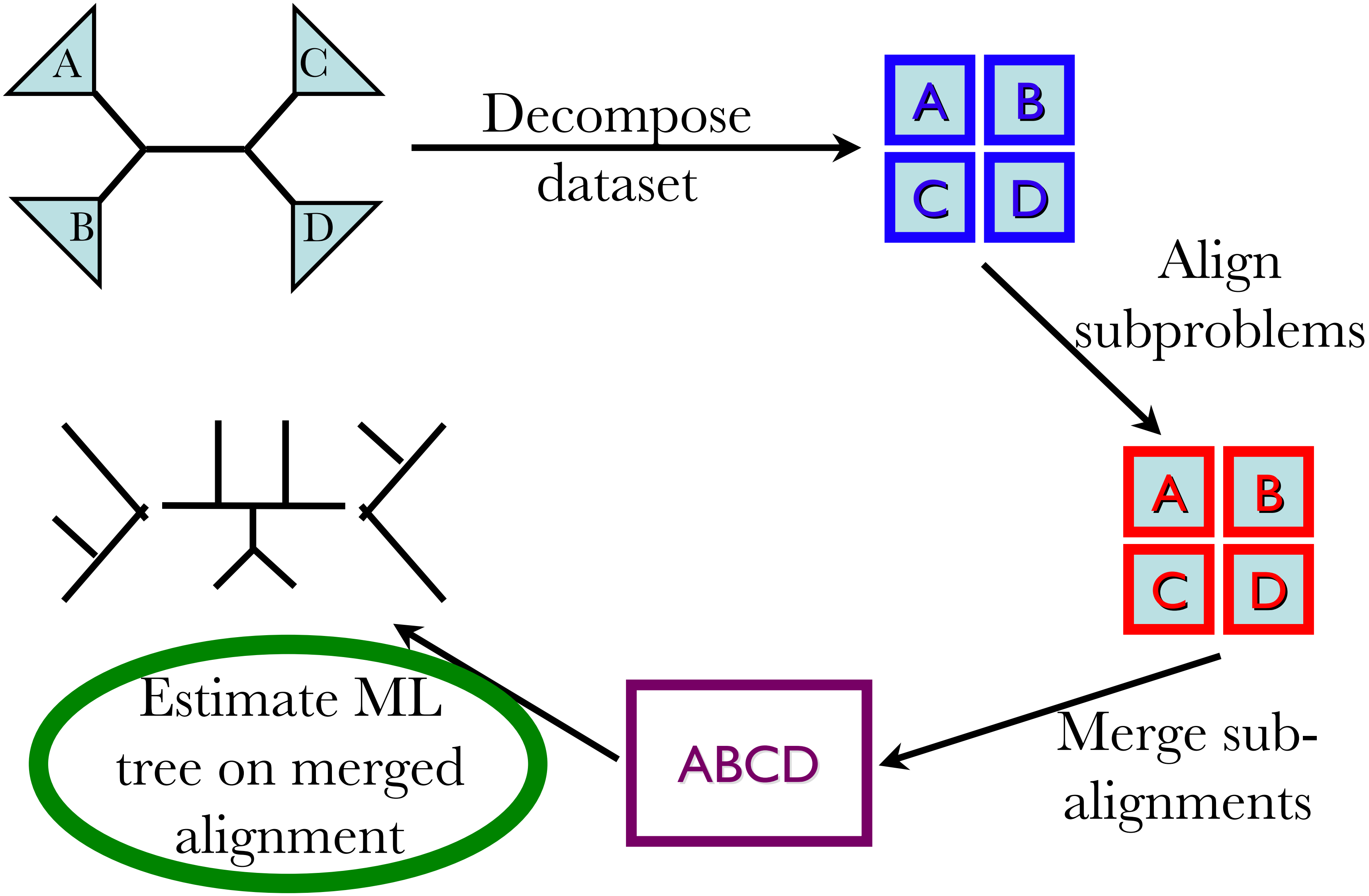
1000 taxon models, ordered by difficulty

24 hour SATé analysis, on desktop machines

(Similar improvements for biological datasets)


Missing Branch Rate (%)
50
40
30
20
10
0
RAxML(ClustalW)
RAxML(Muscle)
RAxML(MAFFT)
RAxML(Prank+GT)
RAxML(Opal)
Original SATé
"Next" SATé
RAxML(TrueAln)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Alignment SP–FN Error
100
80
60
40
20
0
ClustalW
Muscle
MAFFT
Prank+GT
Original SATé
Opal
"Next" SATé
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1000 taxon models ranked by difficulty

Limitations



Decompose dataset

Align subproblems

Merge sub-alignments

Estimate ML tree on merged alignment

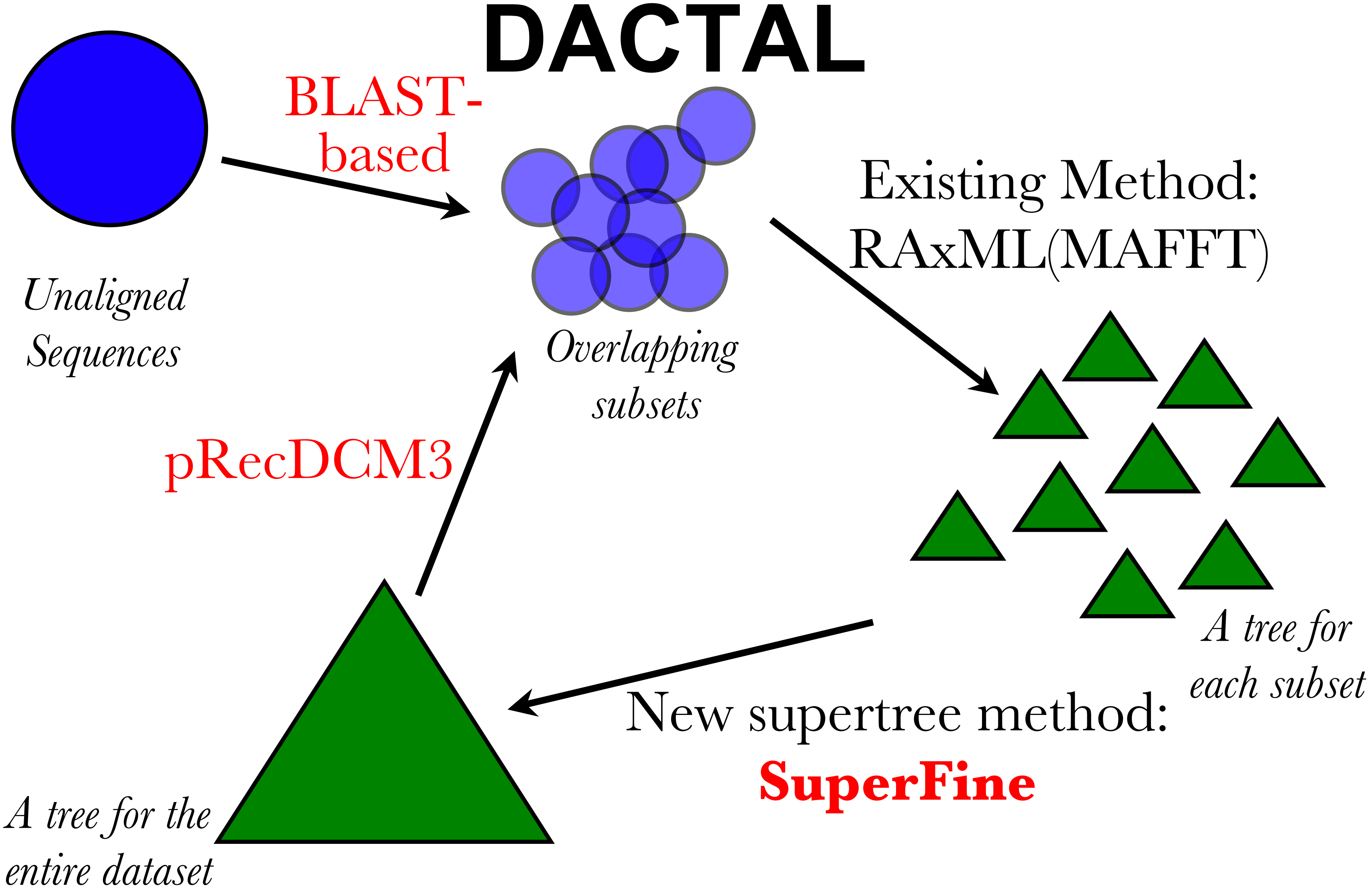
Part III: DACTAL

(Divide-And-Conquer Trees (Almost) without alignments)

- Input: set S of unaligned sequences
- Output: tree on S (but no alignment)

Nelesen, Liu, Wang, Linder, and Warnow, ISMB 2012 and Bioinformatics 2012

DACTAL



Unaligned Sequences

BLAST-based

Overlapping subsets

Existing Method: RAxML(MAFFT)

A tree for each subset

New supertree method: **SuperFine**

A tree for the entire dataset

pRecDCM3

Average of 3 Largest CRW Datasets

CRW: Comparative RNA database,

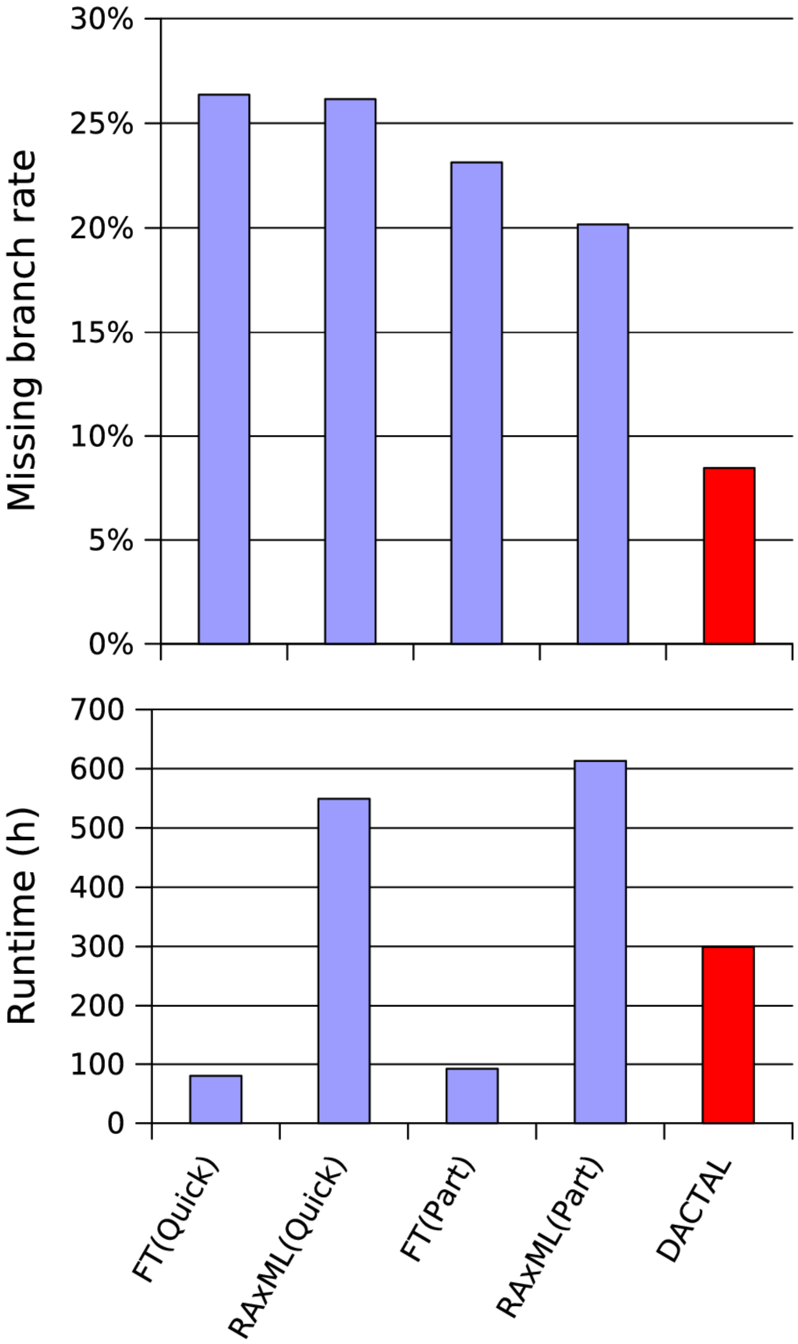
Three 16S datasets with **6,323** to **27,643** sequences

Reference alignments based on secondary structure

Reference trees are 75% RAxML bootstrap trees

DACTAL (shown in red) run for 5 iterations starting from FT(Part)

FastTree (FT) and RAxML are ML methods



Part III: SEPP

- SEPP: SATé-enabled Phylogenetic Placement, by Mirarab, Nguyen, and Warnow
- Pacific Symposium on Biocomputing, 2012 (special session on the Human Microbiome)

Phylogenetic Placement

Input: Backbone alignment and tree on full-length sequences, and a set of query sequences (short fragments)

Output: Placement of query sequences on backbone tree

Phylogenetic placement can be used for taxon identification, but it has general applications for phylogenetic analyses of NGS data.

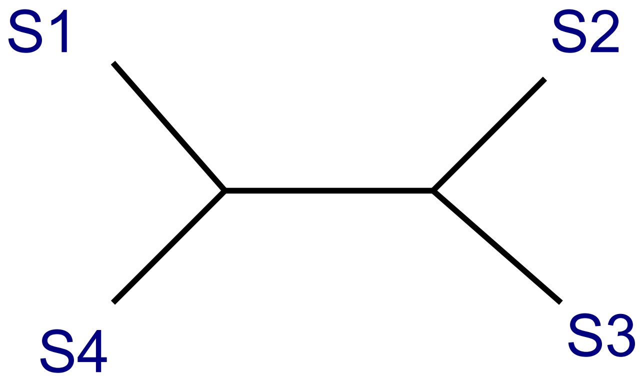
Phylogenetic Placement

- Align each query sequence to backbone alignment

- Place each query sequence into backbone tree, using extended alignment

Align Sequence

```
S1   = -AGGCTATCACCTGACCTCCA-AA
S2   = TAG-CTATCAC--GACCGC--GCA
S3   = TAG-CT-------GACCGC--GCT
S4   = TAC----TCAC--GACCGACAGCT
Q1   = TAAAAC
```



Align Sequence

```
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S2  = TAG-CTATCAC--GACCGC--GCA
S3  = TAG-CT-------GACCGC--GCT
S4  = TAC----TCAC--GACCGACAGCT
Q1  = -------T-A--AAAC--------
```
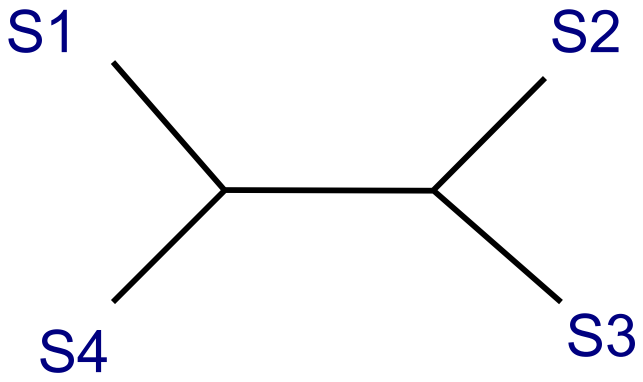
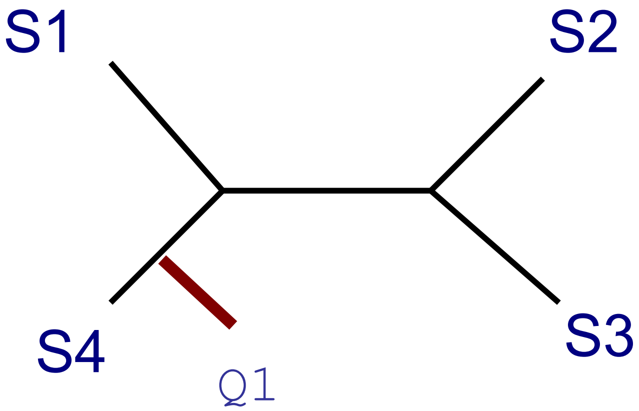



Place Sequence

```
S1  = -AGGCTATCACCTGACCTCCA-AA
S2  = TAG-CTATCAC--GACCGC--GCA
S3  = TAG-CT-------GACCGC--GCT
S4  = TAC----TCAC--GACCGACAGCT
Q1  = -------T-A--AAAC--------
```

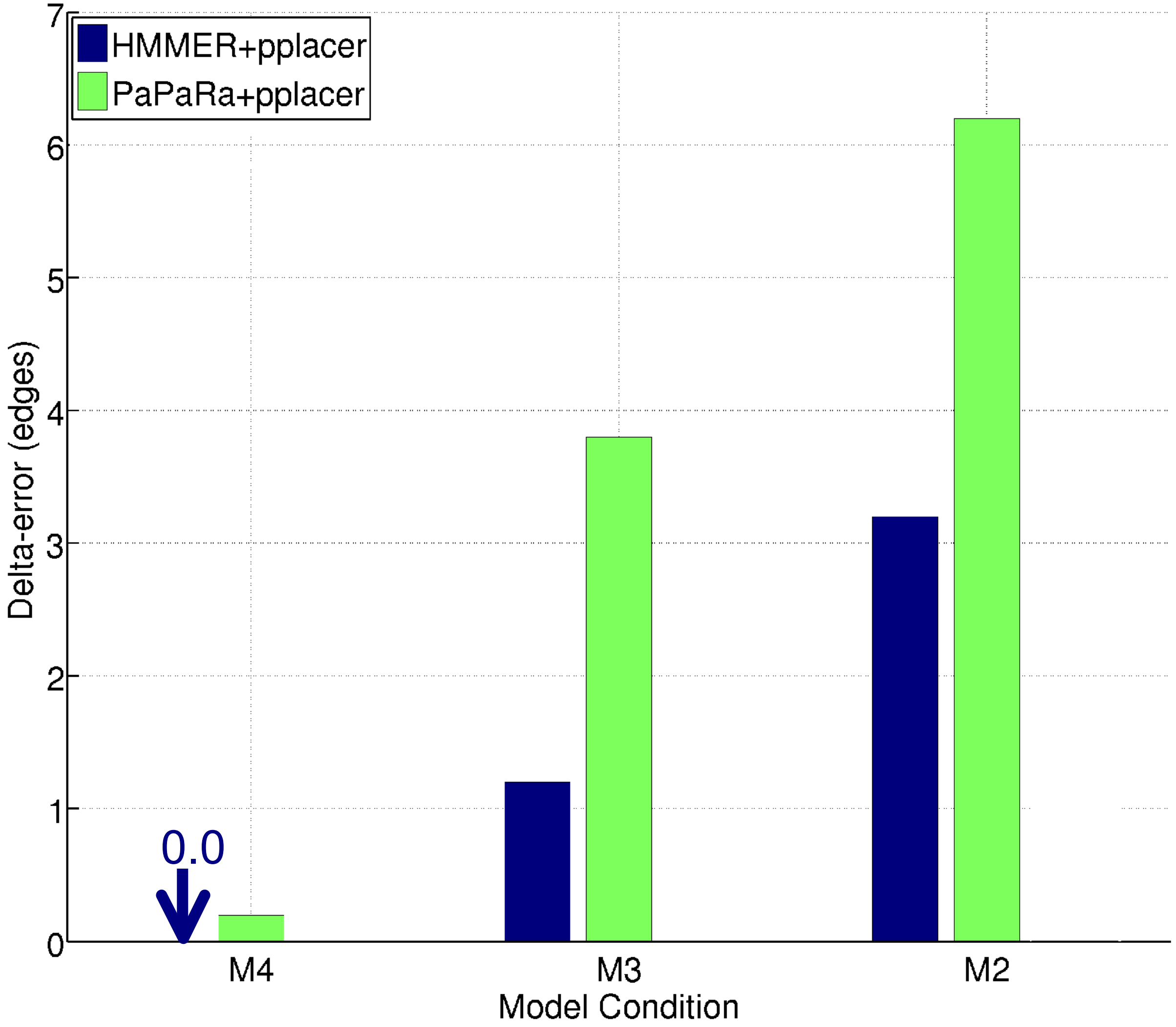


Phylogenetic Placement

- Align each query sequence to backbone alignment
  - HMMALIGN (Eddy, Bioinformatics 1998)
  - PaPaRa (Berger and Stamatakis, Bioinformatics 2011)
- Place each query sequence into backbone tree
  - Pplacer (Matsen et al., BMC Bioinformatics, 2011)
  - EPA (Berger and Stamatakis, Systematic Biology 2011)

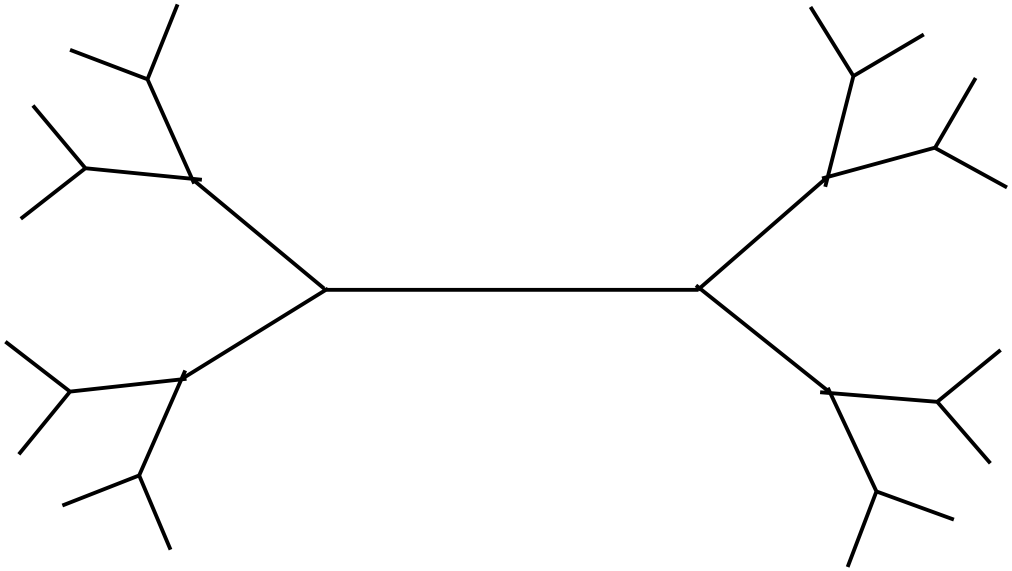
Note: pplacer and EPA use maximum likelihood

HMMER vs. PaPaRa

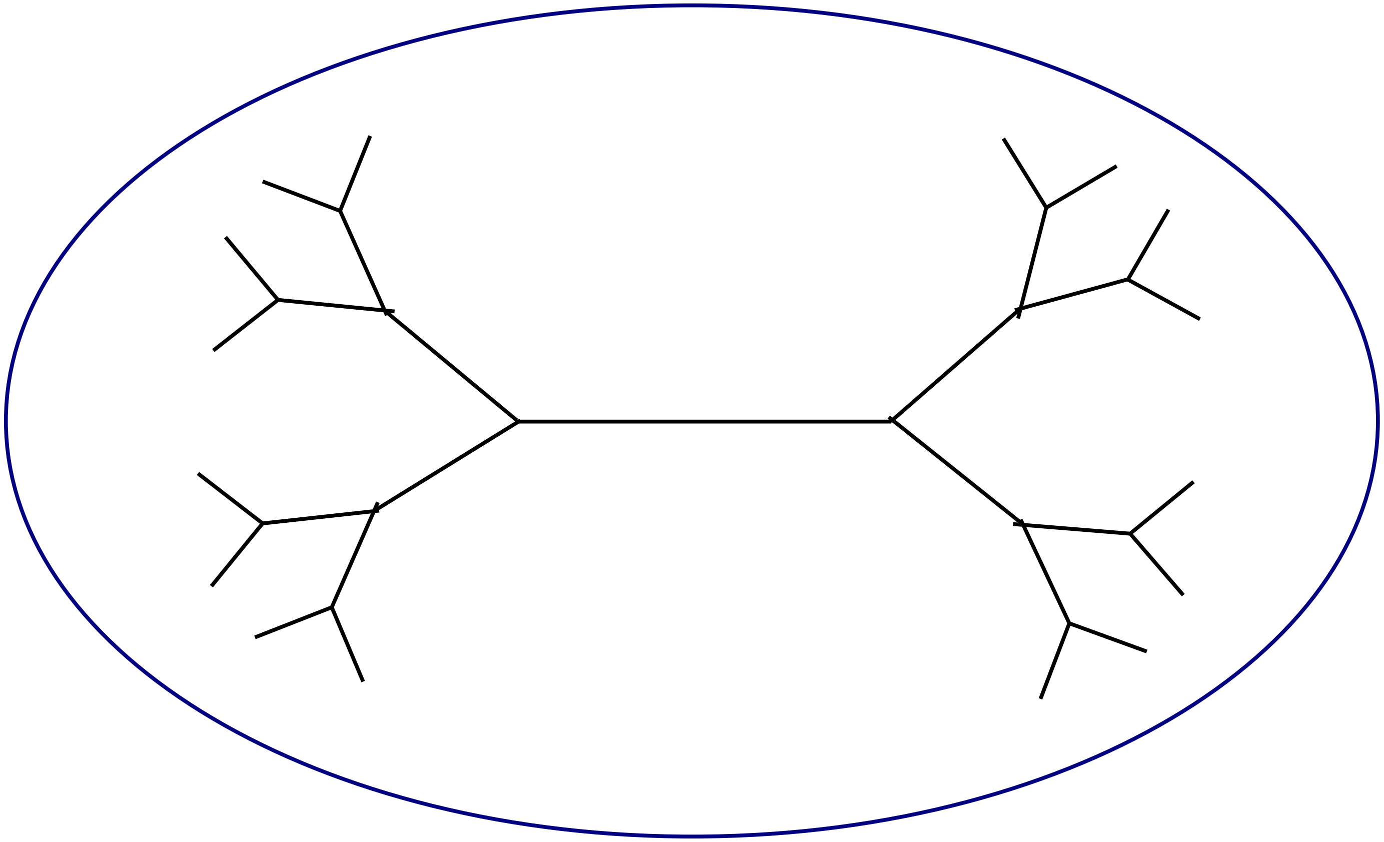


Increasing rate of evolution

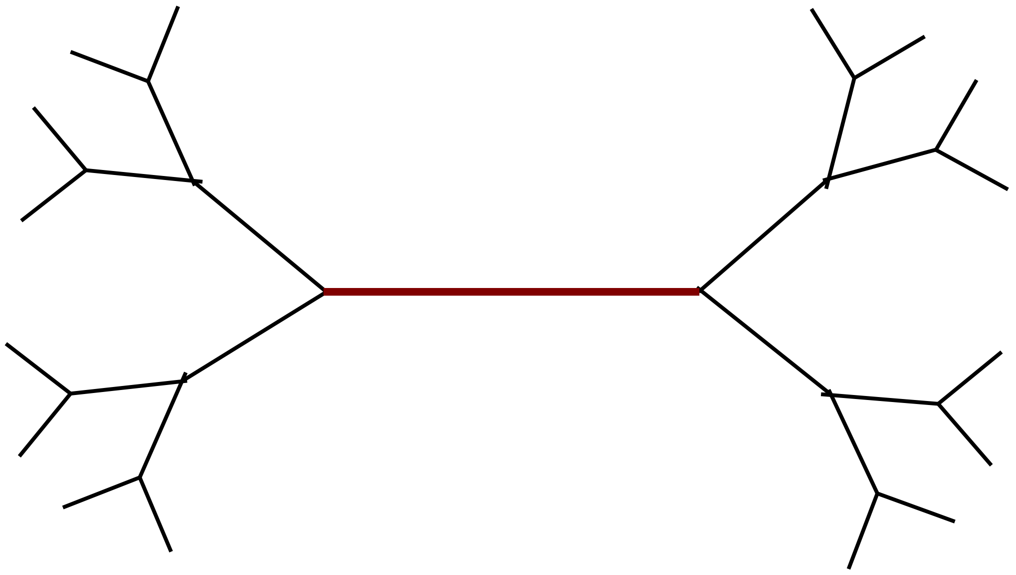
Insights from SATé



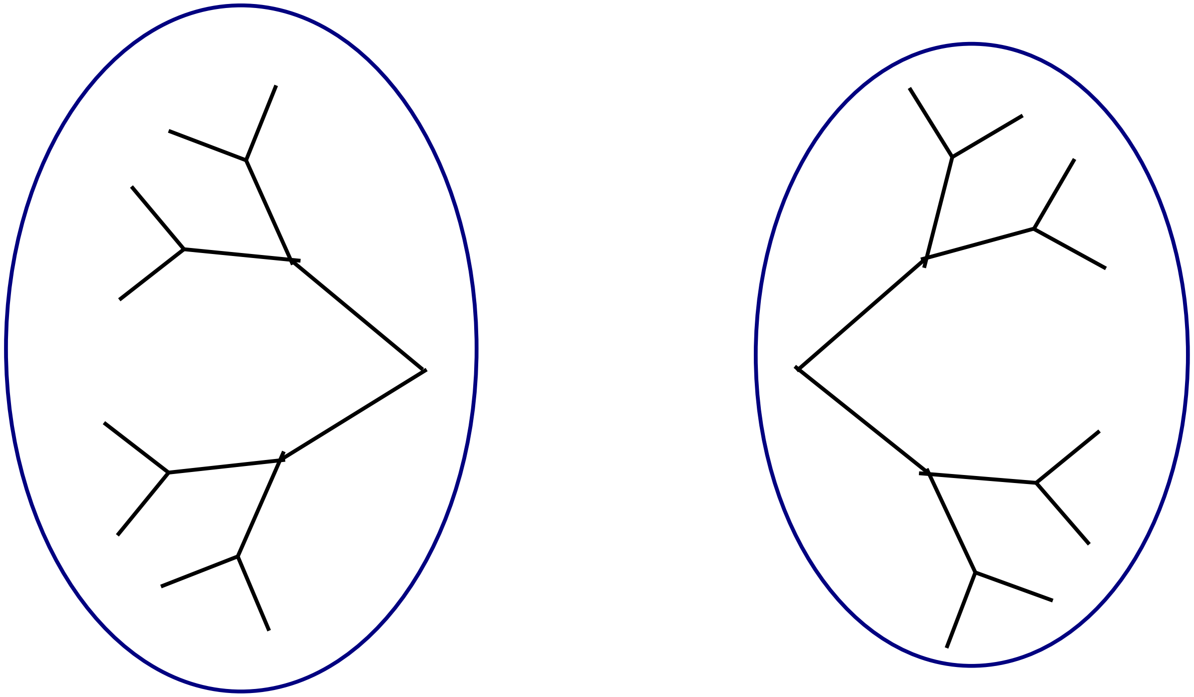
Insights from SATé



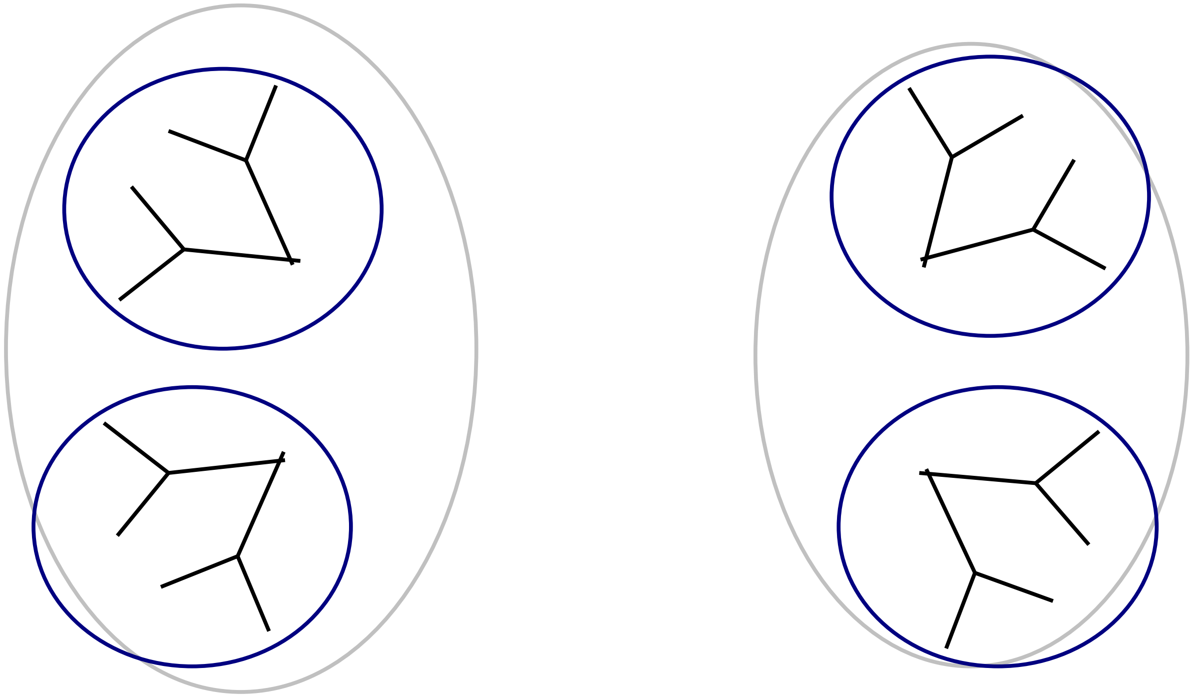
Insights from SATé



Insights from SATé



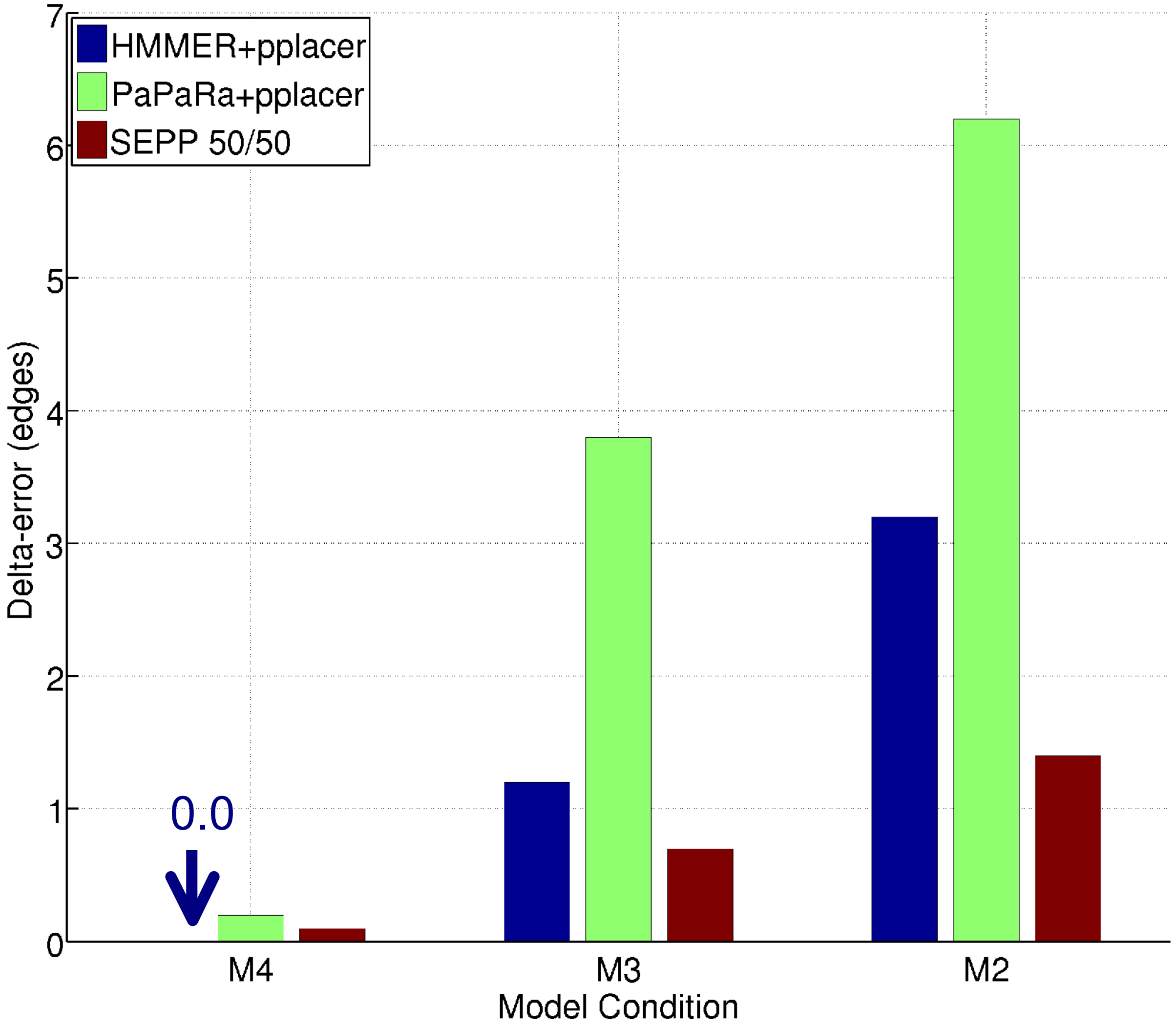
Insights from SATé



SEPP Parameter Exploration

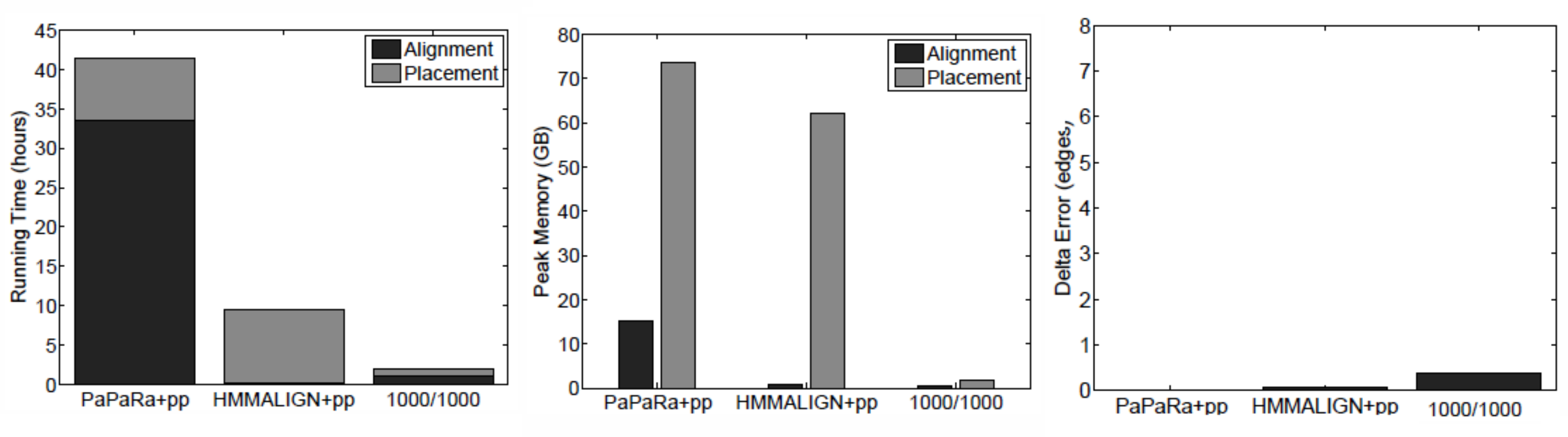
- Alignment subset size and placement subset size impact the accuracy, running time, and memory of SEPP
- 10% rule (subset sizes 10% of backbone) had best overall performance

SEPP (10%-rule) on simulated data



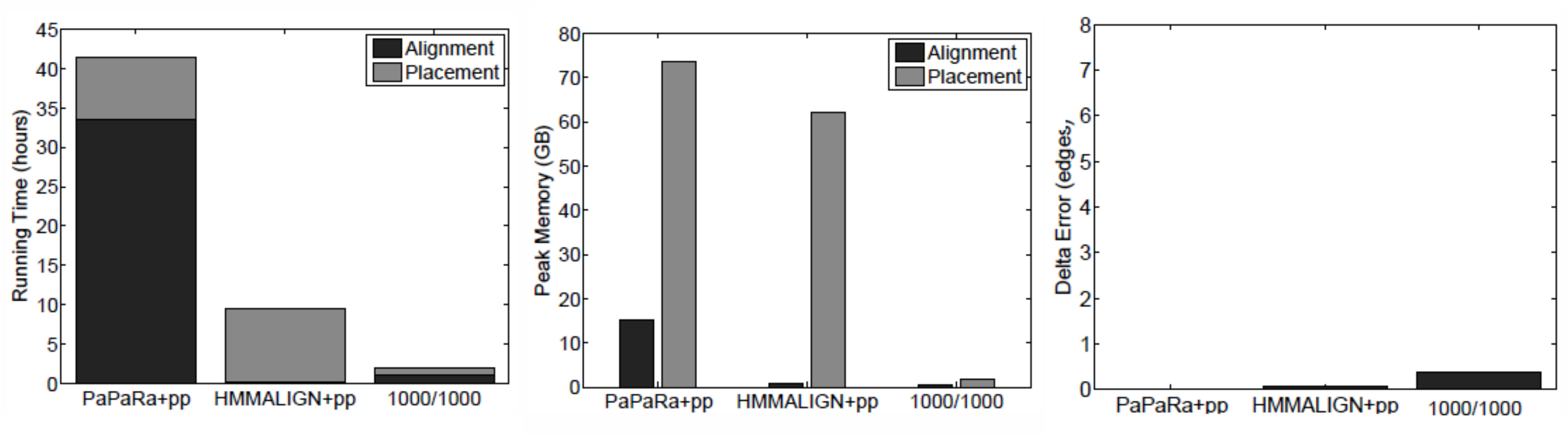
Increasing rate of evolution

SEPP (10%) on Biological Data



16S.B.ALL dataset, 13k curated backbone tree, 13k total fragments

SEPP (10%) on Biological Data



16S.B.ALL dataset, 13k curated backbone tree, 13k total fragments

For 1 million fragments:

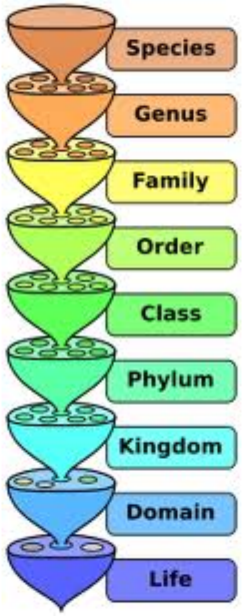
PaPaRa+pplacer: ~133 days

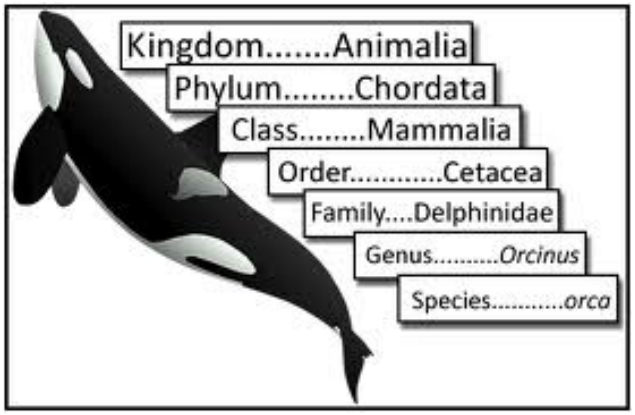
HMMALIGN+pplacer: ~30 days

SEPP 1000/1000: ~6 days

Part IV: Taxon Identification

Objective: classify short reads in a metagenomic sample





Metagenomic data analysis

NGS data produce fragmentary sequence data

Metagenomic analyses include unknown species

Taxon identification: given short sequences, identify the species for each fragment

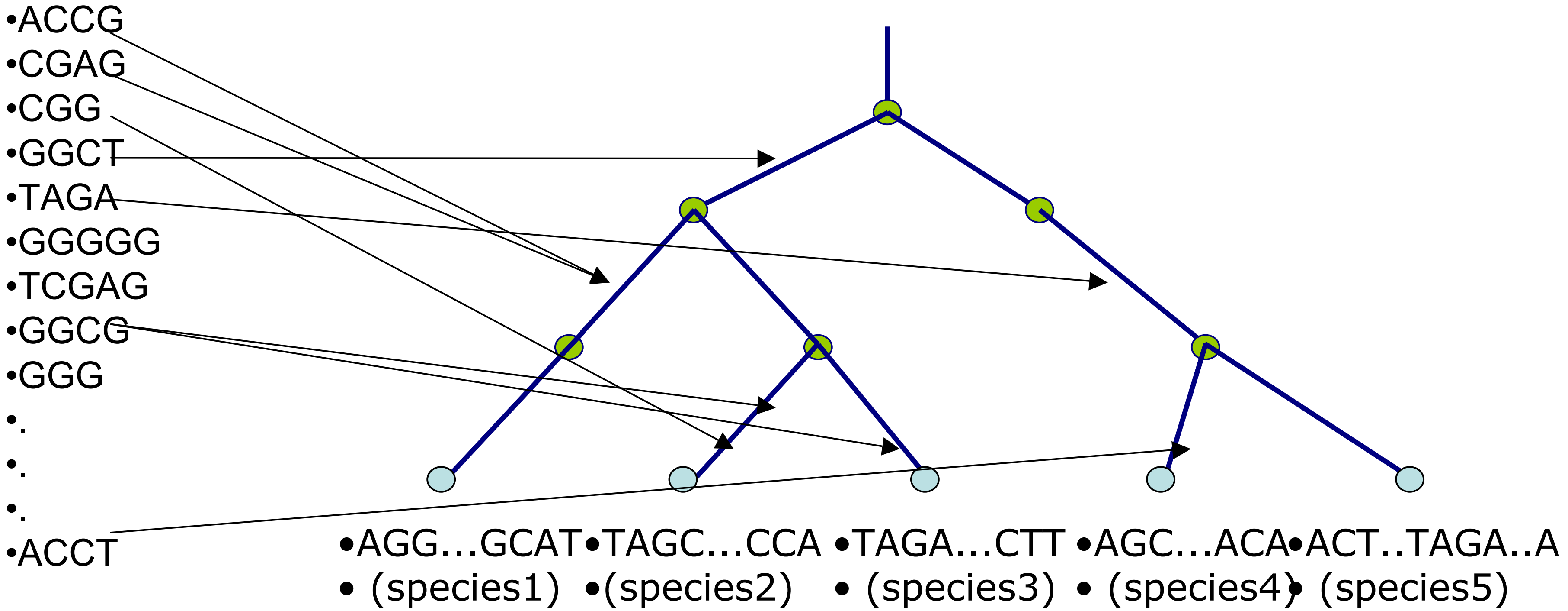
Applications: Human Microbiome

Issues: accuracy and speed

TIPP: Taxon Identification by Phylogenetic Placement

- Fragmentary Unknown Reads:
  - (60-200 bp long)

- Known Full length Sequences,
- and an alignment and a tree
  - (500-10,000 bp long)



- ACCG
- CGAG
- CGG
- GGCT
- TAGA
- GGGGG
- TCGAG
- GGCG
- GGG
- .
- .
- .
- ACCT

•AGG...GCAT	•TAGC...CCA	•TAGA...CTT	•AGC...ACA	•ACT..TAGA..A
• (species1)	•(species2)	• (species3)	• (species4)	• (species5)

TIPP: Taxon Identification using Phylogenetic Placement - Version 1

Given a set Q of query sequences for some gene, a taxonomy T, and a set of full-length sequences for the gene,

- Compute reference alignment and tree on the full-length sequences, using SATé
- Use SEPP to place each query sequence into the taxonomy (alignment subsets computed on the reference alignment/tree, then inserted into taxonomy T)

TIPP version 2- considering uncertainty

TIPP version 1 too aggressive (over-classification)

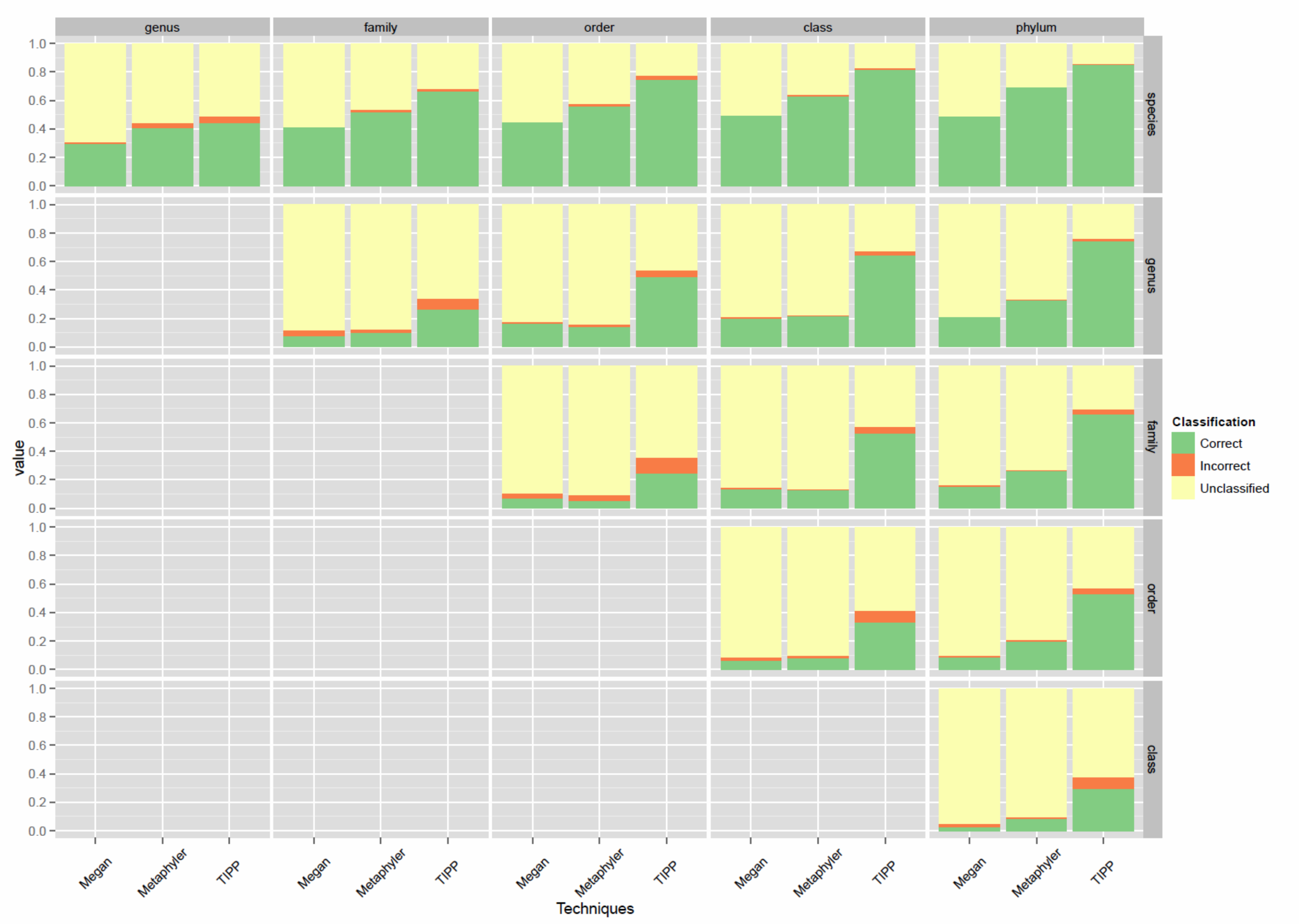
TIPP version 2 dramatically reduces false positive rate with small reduction in true positive rate, by considering uncertainty, using statistical techniques:

- For each reference alignment/tree pair, compute **many** extended alignments (using statistical support computed using HMMER to cover x% of the probability).

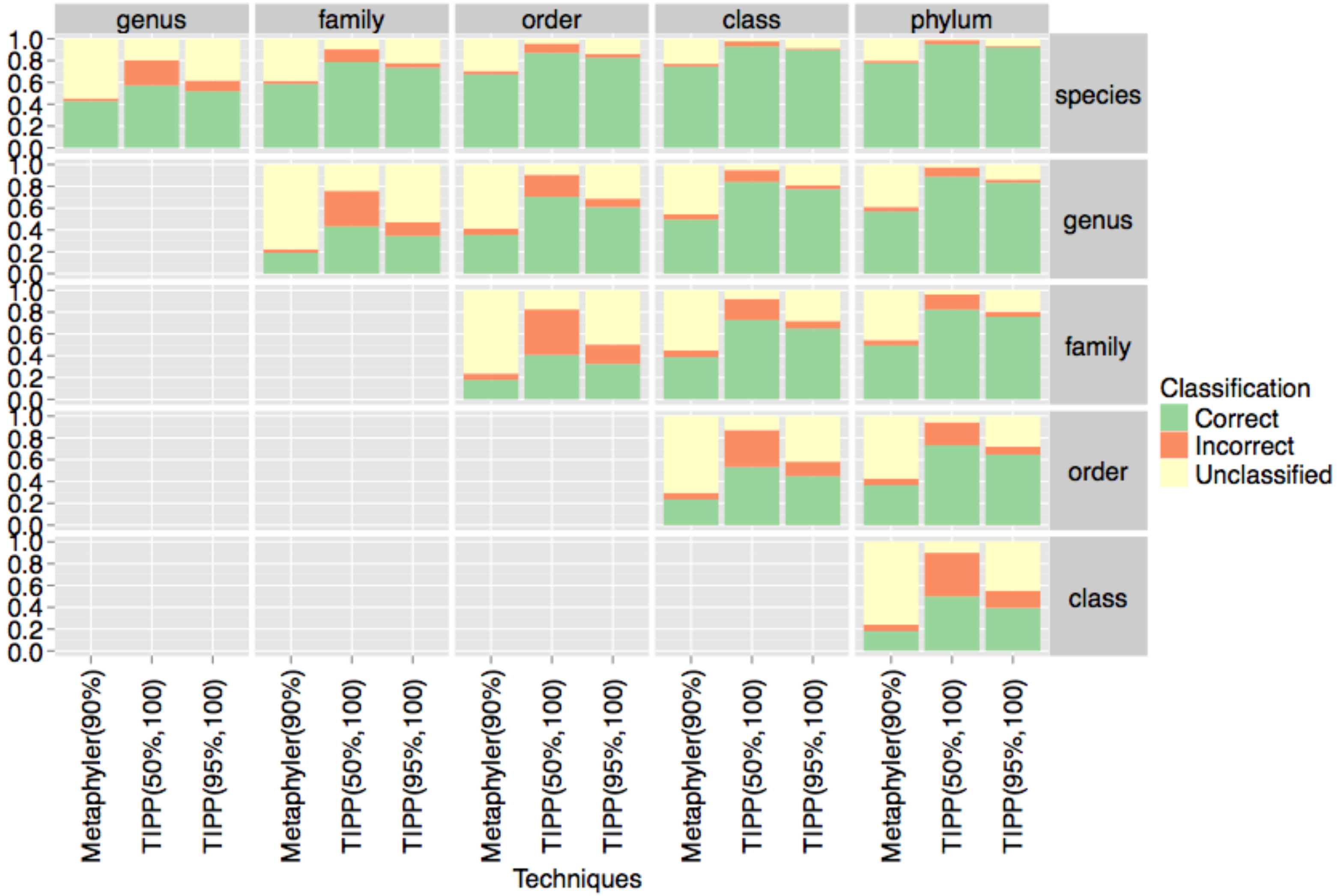
- For each extended alignment, use pplacer statistical support values to place fragment into taxonomy, so that the clade below the placement contains x% of the probability.

Classify each fragment at the **LCA** of all placements obtained for the fragment

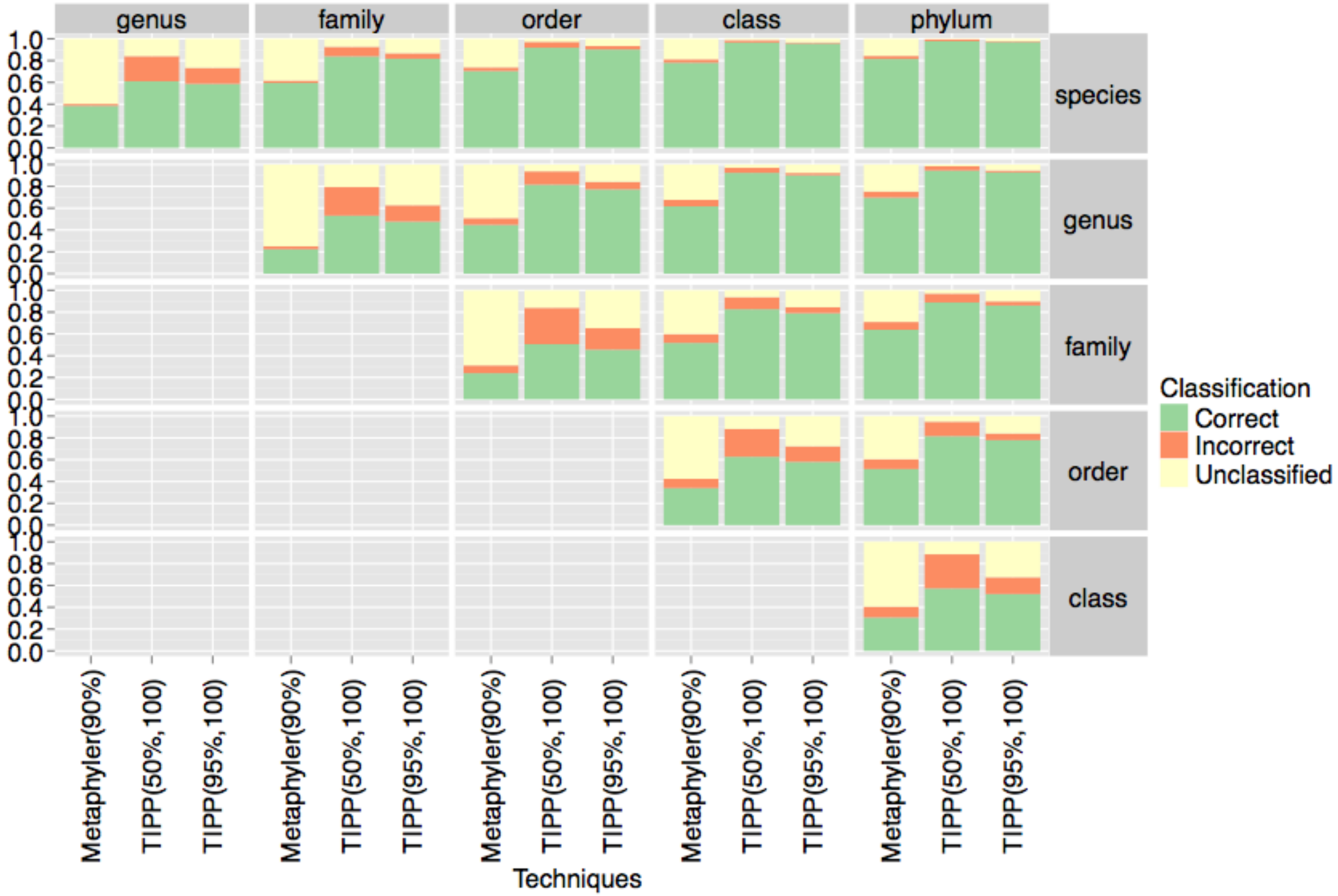
60bp error-free reads on rpsB marker gene



Results on 30 marker genes, leave-one-out experiment with Illumina errors



Results on 30 marker genes, leave-one-out experiment with 454 errors



Five “Boosters”

•DCM: distance-based tree estimation

•SATé: co-estimation of alignments and trees

•DACTAL: large trees without full alignments

•SEPP: phylogenetic placement of short reads

•TIPP: taxon identification of fragmentary data

Algorithmic strategies: divide-and-conquer and iteration to improve the accuracy and scalability of a *base method*

General Observations - Part I

- Relative performance of methods can change dramatically with dataset size
- Statistical inference methods often do not scale well

Observations - Part II

- Meta-methods can improve accuracy and even speed
- Hidden Markov Models (HMMs) can be improved by making a set of HMMs instead of a single HMM
- Algorithmic parameters let you explore sensitivity/specificity
- Parallelism is easily exploited

Overall message

- When data are difficult to analyze, develop better methods - don’t throw out the data.
- BIGDATA problems in biology are an opportunity for computer scientists to have a big impact!

Acknowledgments

- Guggenheim Foundation Fellowship, Microsoft Research New England, National Science Foundation: Assembling the Tree of Life (ATOL), ITR, and IGERT grants, and David Bruton Jr. Professorship

- Collaborators:
  - DCM-NJ: Bernard Moret and Katherine St. John
  - SATé: Kevin Liu, Serita Nelesen, Sindhu Raghavan, and Randy Linder (and also Mark Holder at Kansas for public distribution)
  - DACTAL: Serita Nelesen, Kevin Liu, Li-San Wang, and Randy Linder
  - TIPP: Siavash Mirarab, Nam Nguyen, Mihai Pop, and Bo Liu

Current Research Projects

Method development:

- Large-scale multiple sequence alignment and phylogeny estimation
- Metagenomic taxon identification
- Phylogenetic placement of NGS data (short reads or fragmentary sequences)
- Comparative genomics
- Estimating species trees from gene trees
- Supertree methods
- Phylogenetic estimation under statistical models

Dataset analyses (multi-institutional collaborations):

- Avian Phylogeny (and brain evolution)
- Human Microbiome
- Thousand Transcriptome (1KP) Project
- Conifer evolution

Steps in a phylogenetic analysis

- Gather data

- Estimate sequence alignment (NP-hard)
- Estimate phylogeny (NP-hard statistical estimation)

- Evaluate uncertainty in analysis (creates huge datasets)
- Visualize tree and alignment (unsolved)
- Perform post-tree analyses

But finding the “best tree” is … unlikely!

# of Taxa	# of Unrooted Trees
4	3
5	15
6	105
7	945
8	10395
9	135135
10	2027025
20	2.2×10^{20}
100	4.5×10^{190}
1000	2.7×10^{2900}

Observations

- DACTAL gives more accurate trees than all other methods on the largest datasets.
- DACTAL is much faster than SATé, and can analyze datasets that SATé cannot.
- DACTAL is robust to starting trees and other algorithmic parameters.

Metagenomic data analysis

NGS data produce fragmentary sequence data

Metagenomic analyses include unknown species

Taxon identification: given short sequences, identify the species, genus, etc., for each fragment

Applications: Human Microbiome

Issues: accuracy and speed

Not just data analysis

- Science is more complex than our mathematical models.
- Better analyses are needed in order to refine the models, and data are essential to accurate modelling.
- Hence, a *cycle* of mathematical modelling, statistical inference, methods for hard optimization problems, software development, extensive testing, …

Phylogenetic “Boosters”

- SATé: co-estimation of alignments and trees
- SEPP/TIPP: phylogenetic analysis of fragmentary data

Algorithmic strategies: divide-and-conquer and iteration to improve the accuracy and scalability of a *base method*

Major Challenges

- Many phylogenetic datasets contain hundreds to thousands of species, some with thousands of genes.
- Future datasets will be substantially larger (e.g., iPlant plans to construct a tree on 500,000 plant species)
- *Current methods have poor accuracy or cannot run on large datasets.*

Some “large dataset” problems (and algorithms)

- Absolute Fast Converging Methods (SODA 2001, TCS 1999, RSA 1999, ICALP 1997)
- SATé (Co-estimation of alignments and trees), Science 2009
- DACTAL (almost alignment-free estimation of trees), ISMB 2012)
- TIPP (Taxon identification of short reads for metagenomic analysis), in preparation

Today’s Talk

- SATé: Simultaneous Alignment and Tree Estimation (Liu et al., Science 2009, and Liu et al. Systematic Biology, 2011)
- SEPP: SATé-enabled Phylogenetic Placement (Mirarab, Nguyen and Warnow, Pacific Symposium on Biocomputing 2012)
- TIPP: Taxon Identification using Phylogenetic Placement (Nguyen, Mirarab, and Warnow, in preparation, collaboration with Mihai Pop and Bo Liu)

OBSERVATIONS

- MEGAN is very conservative
- MetaPhyler makes more correct predictions than MEGAN
- Other methods (Liu et al, BMC Bioinformatics 2011) not as sensitive (on these 31 marker genes) as MetaPhyler

Thus, the best taxon identification methods have **high precision** (make accurate predictions), but **low sensitivity** (i.e., they **fail to classify** a large portion of reads) even at higher taxonomy levels.

Summary

- SATé gives better alignments and trees than standard alignment estimation methods
- SEPP can enable alignment of short (fragmentary) sequences into alignments of full-length sequences, and phylogenetic placement into gene trees or taxonomies
- TIPP enables taxon identification of short reads -- not limited to 31 marker genes, and no training is needed.