TIPP: Taxon Identification using Phylogeny-Aware Profiles

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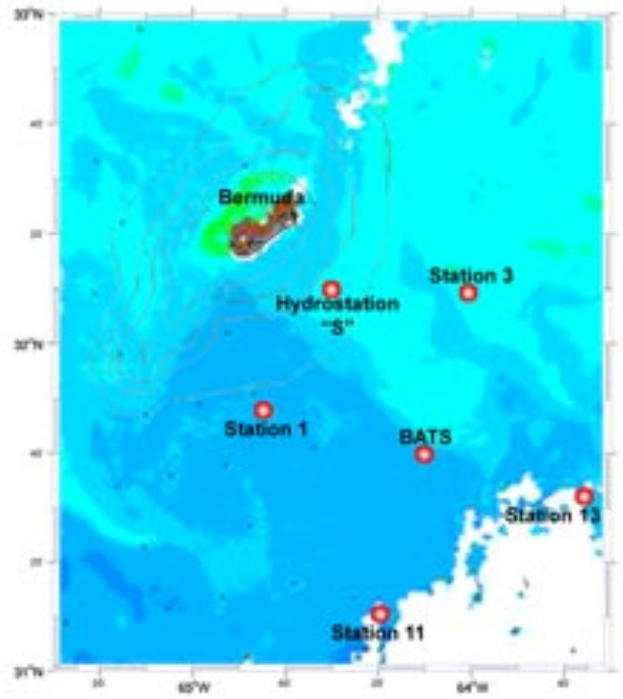
Metagenomic taxonomic identification and phylogenetic profiling



Metagenomics, Venter et al., Exploring the Sargasso Sea: Scientists Discover One Million New Genes in Ocean Microbes

Basic Questions

1. What is this fragment? (Classify each fragment as well as possible.)

2. What is the taxonomic distribution in the dataset? (Note: helpful to use marker genes.)

3. What are the organisms in this metagenomic sample doing together?

This talk

- SEPP (PSB 2012): SATé-enabled Phylogenetic Placement, and Ensembles of HMMs (eHMMs)
- Applications of the eHMM technique to metagenomic abundance classification (TIPP, Bioinformatics 2014)

Phylogenetic Placement

Input: Backbone alignment and tree on full-length sequences, and a set of homologous query sequences (e.g., reads in a metagenomic sample for the same gene)

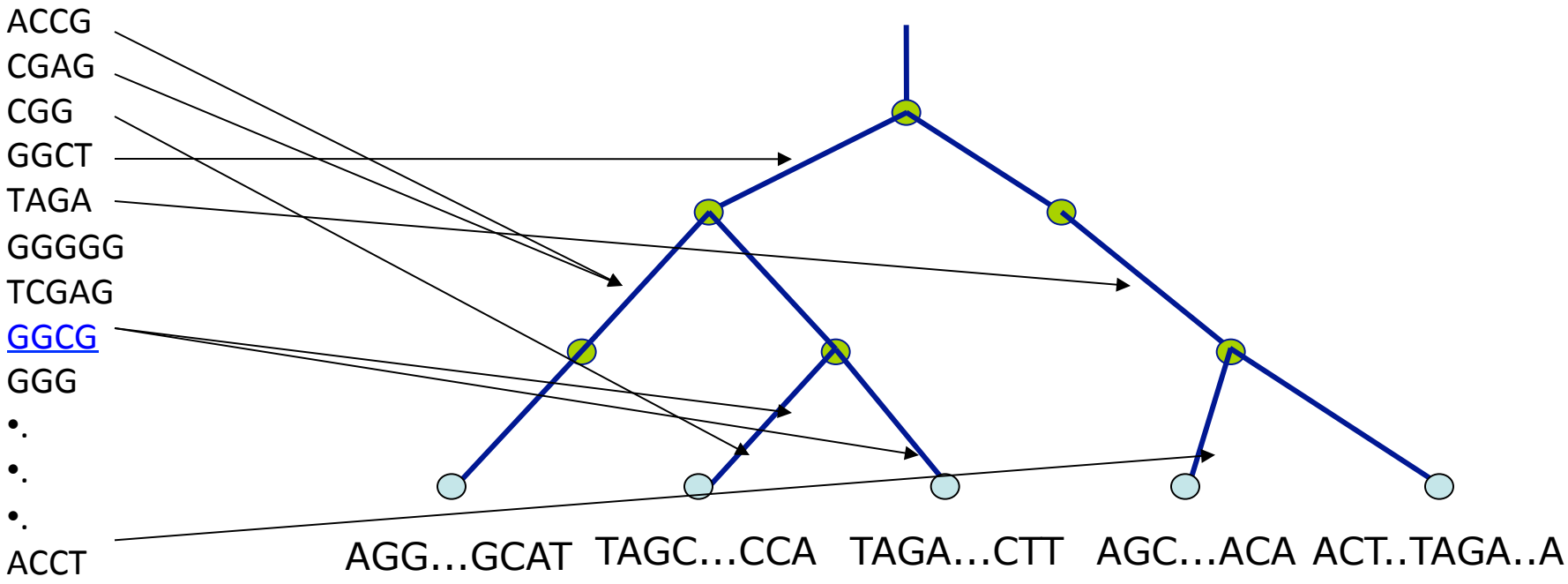
Output: Placement of query sequences on backbone tree

Phylogenetic placement can be used inside a pipeline, after determining the genes for each of the reads in the metagenomic sample.

Marker-based Taxon Identification

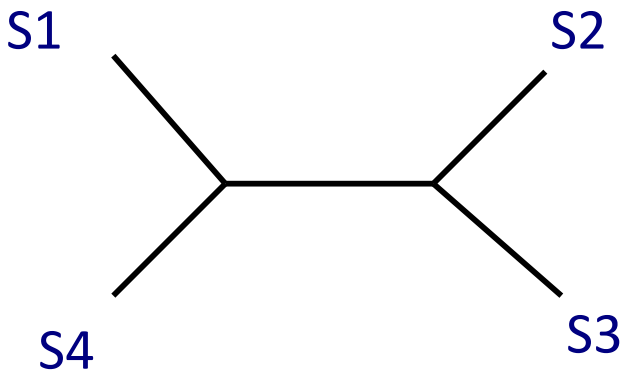
Fragmentary sequences from some gene

Full-length sequences for same gene, and an alignment and a tree



Align Sequence

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



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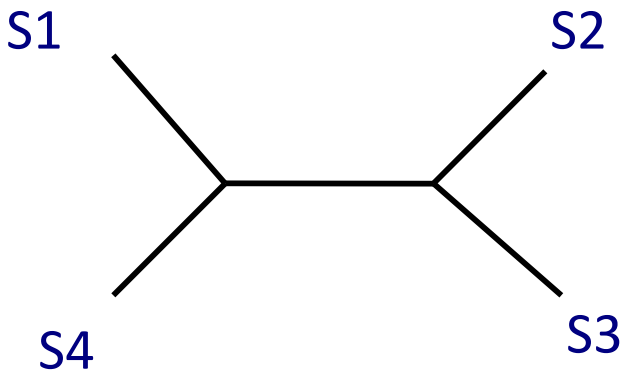

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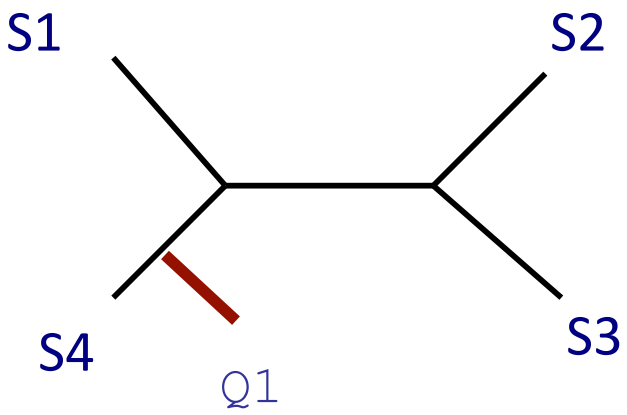
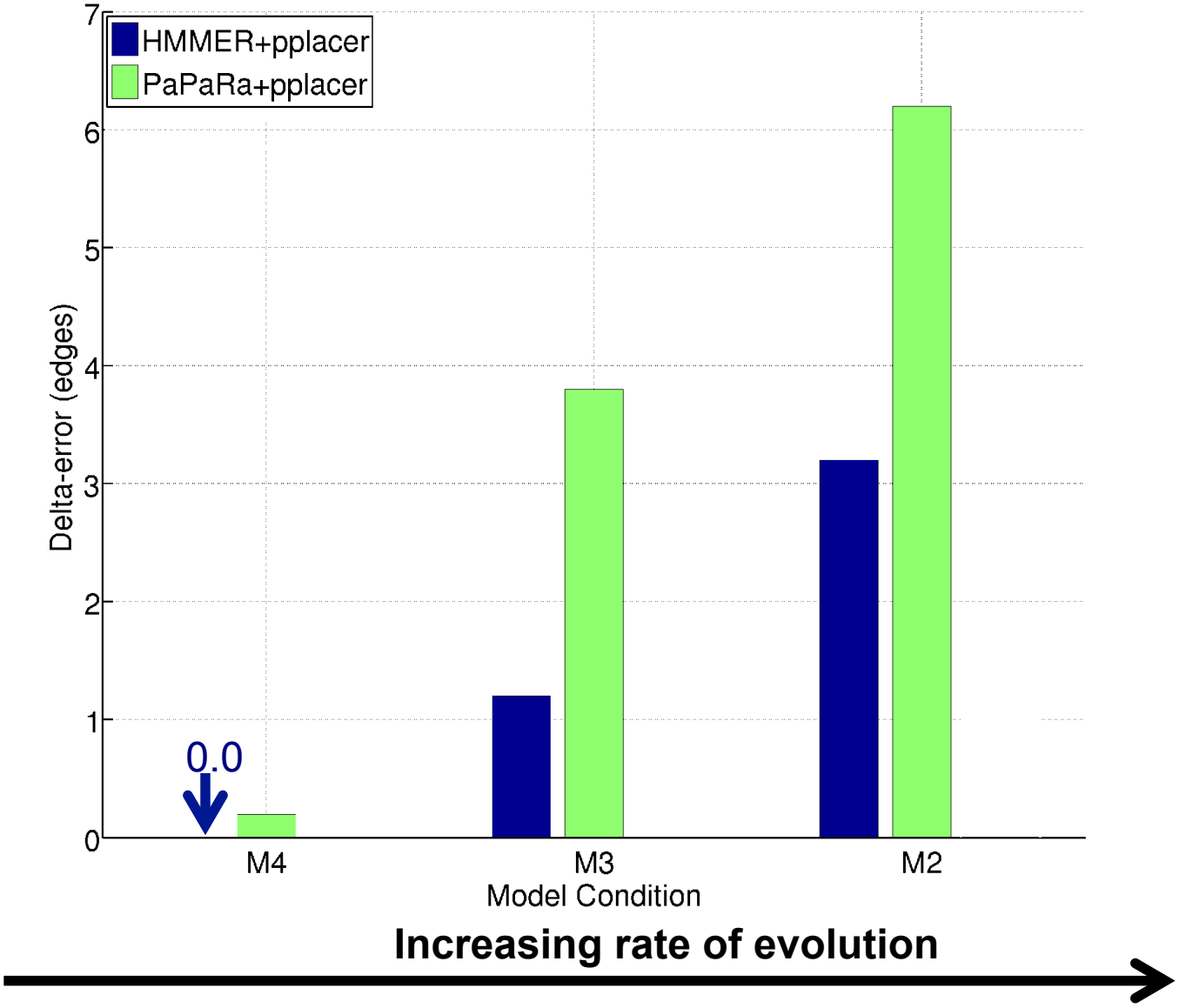


Phylogenetic Placement

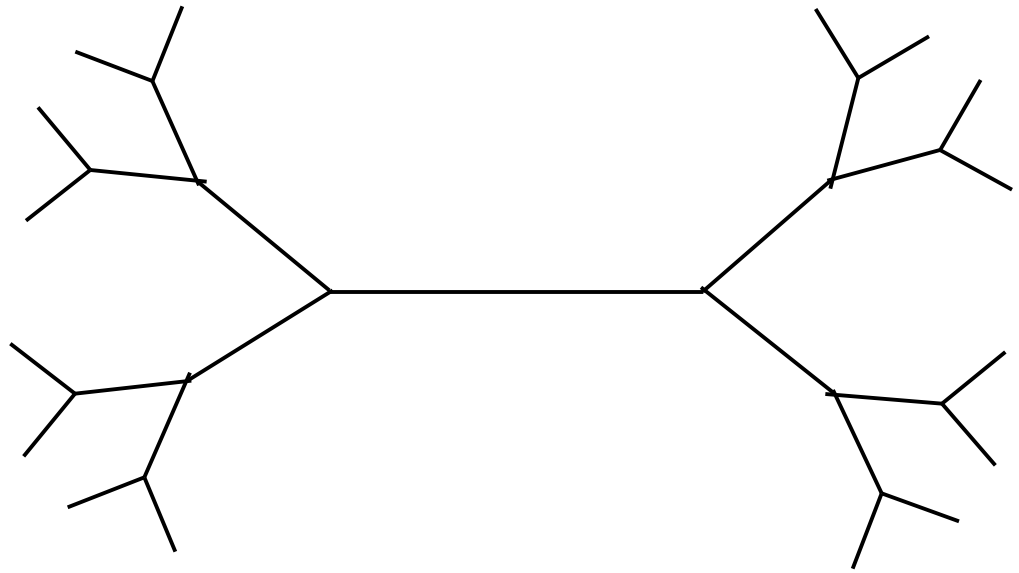
- Align each query sequence to backbone alignment
  - HMMALIGN (Eddy, Bioinformatics 1998)
  - PaPaRa (Berger and Stamatakis, Bioinformatics 2011)
- Place each query sequence into backbone tree
  - Pplacer (Matsen et al., BMC Bioinformatics, 2011)
  - EPA (Berger and Stamatakis, Systematic Biology 2011)

Note: pplacer and EPA use maximum likelihood

HMMER vs. PaPaRa Alignments



One Hidden Markov Model for the entire alignment?



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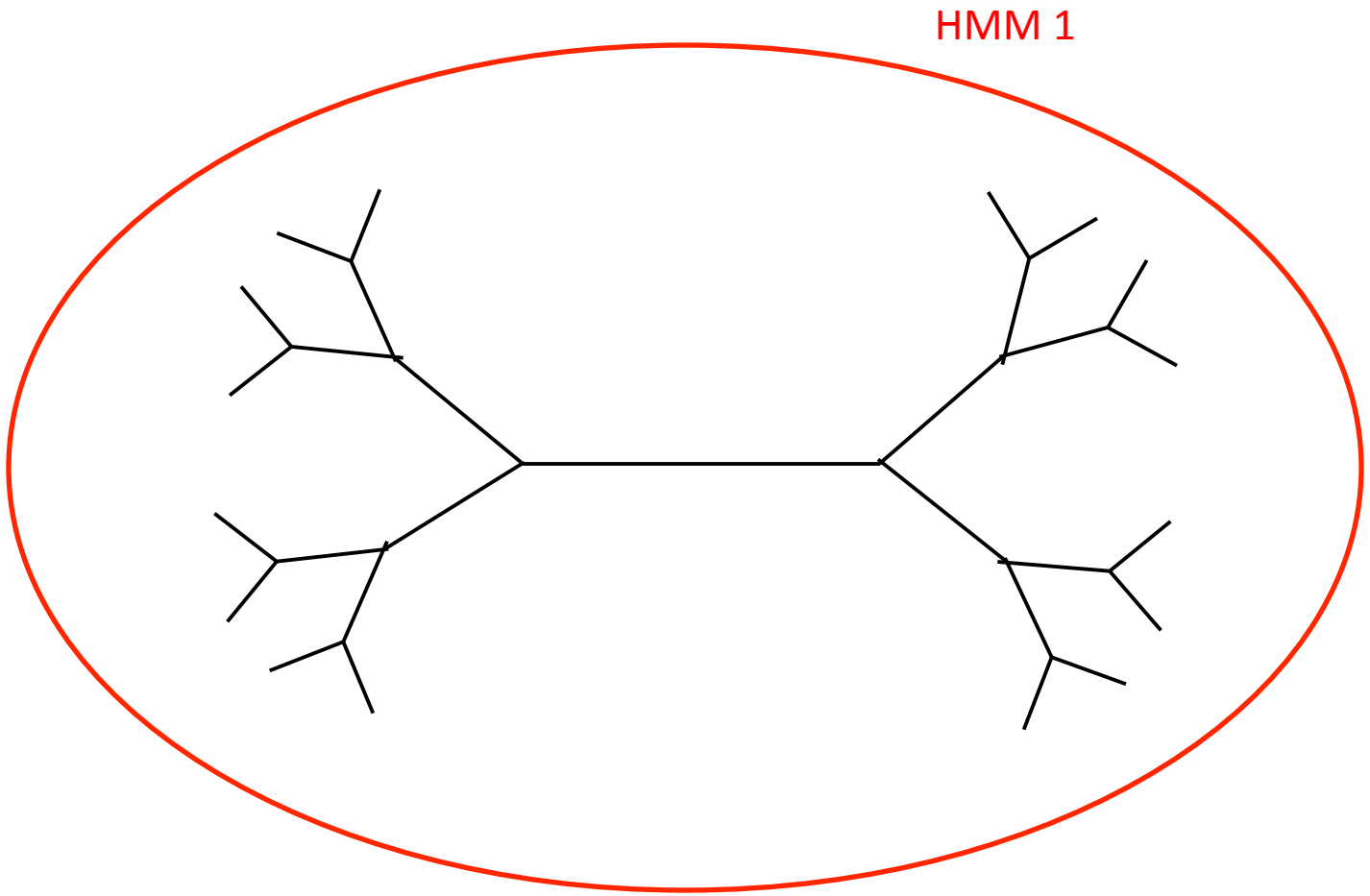
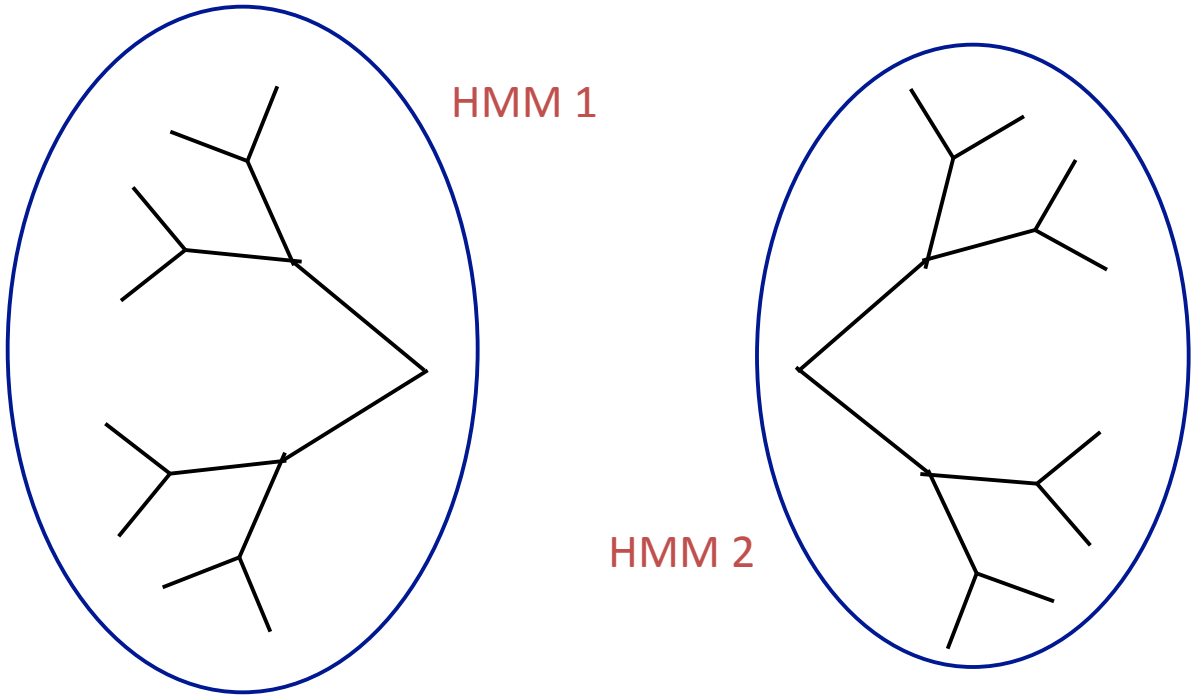
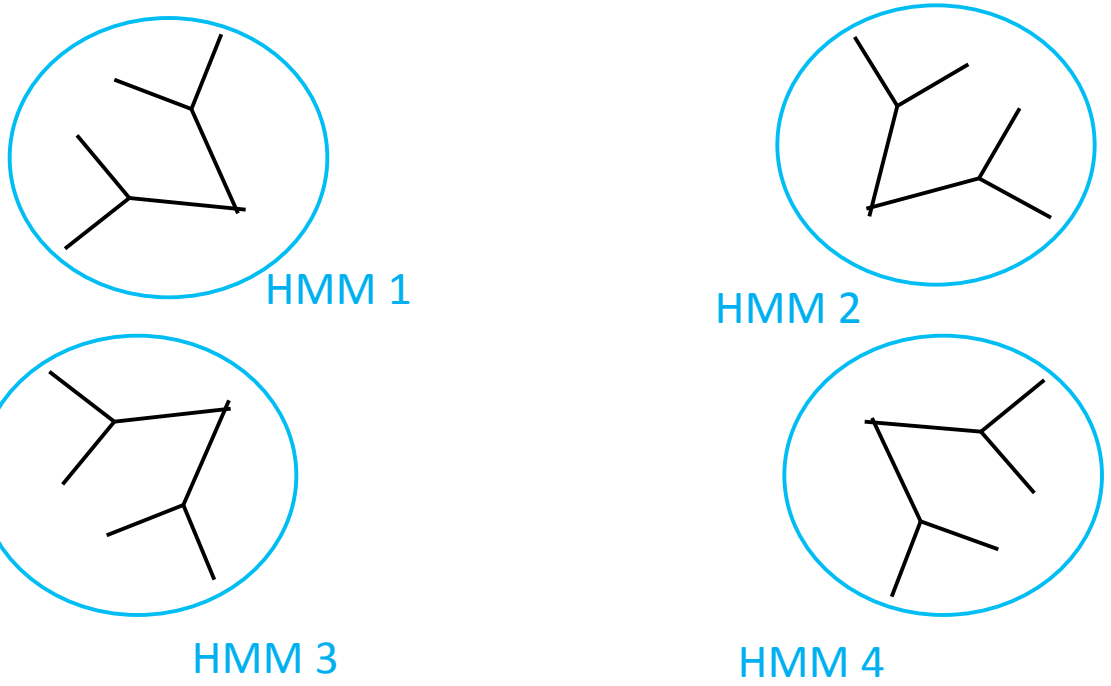


Or 2 HMMs?



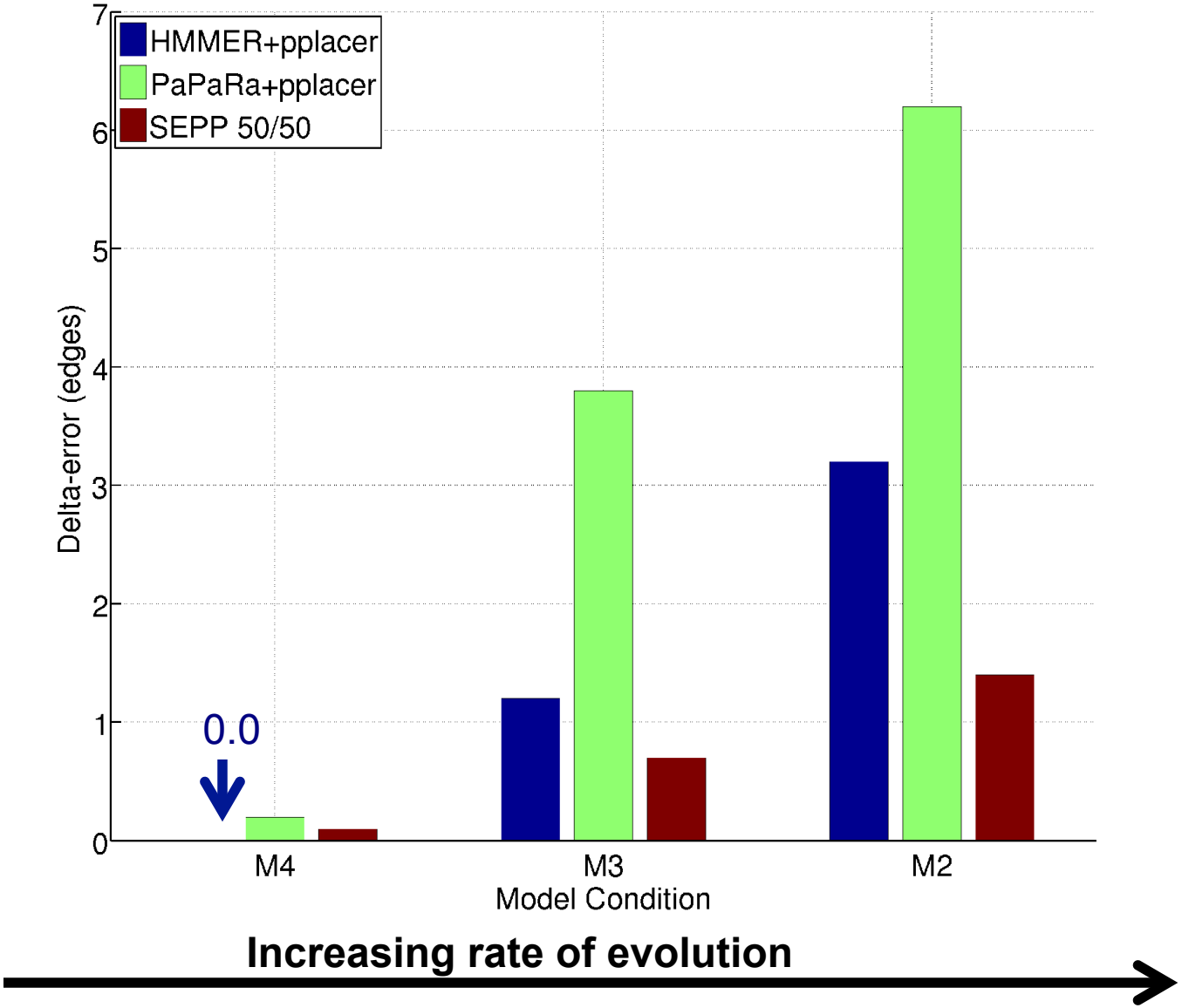
Or 4 HMMs?



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



SEPP and eHMMs

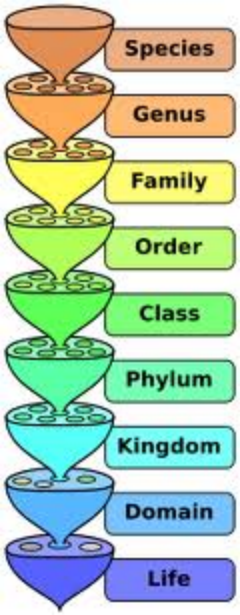
An ensemble of HMMs provides a better model of a multiple sequence alignment than a single HMM, and is better able to

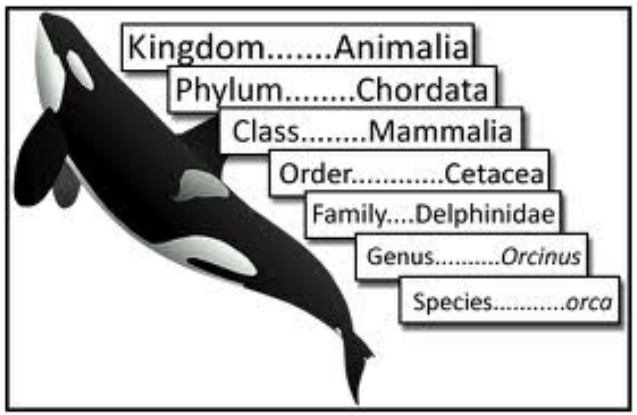
- detect homology between full length sequences and fragmentary sequences
- add fragmentary sequences into an existing alignment

especially when there are many indels and/or substitutions.

Metagenomic Taxon Identification

Objective: classify short reads in a metagenomic sample





Abundance Profiling

Objective: Distribution of the species (or genera, or families, etc.) within the sample.

For example: The distribution of the sample at the species-level is:

50%	species A
20%	species B
15%	species C
14%	species D
1%	species E

TIPP (https://github.com/smirarab/sepp)

TIPP (Nguyen, Mirarb, Liu, Pop, and Warnow, Bioinformatics 2014), marker-based method that only characterizes those reads that map to the Metaphyler's marker genes

TIPP pipeline

1. Uses BLAST to assign reads to marker genes
2. Computes UPP/PASTA reference alignments
3. Uses reference taxonomies, refined to binary trees using reference alignment
4. Modifies SEPP by considering statistical uncertainty in the extended alignment and placement within the tree

TIPP Design (Step 4)

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    - Taxonomically characterize each read at this MRCA.

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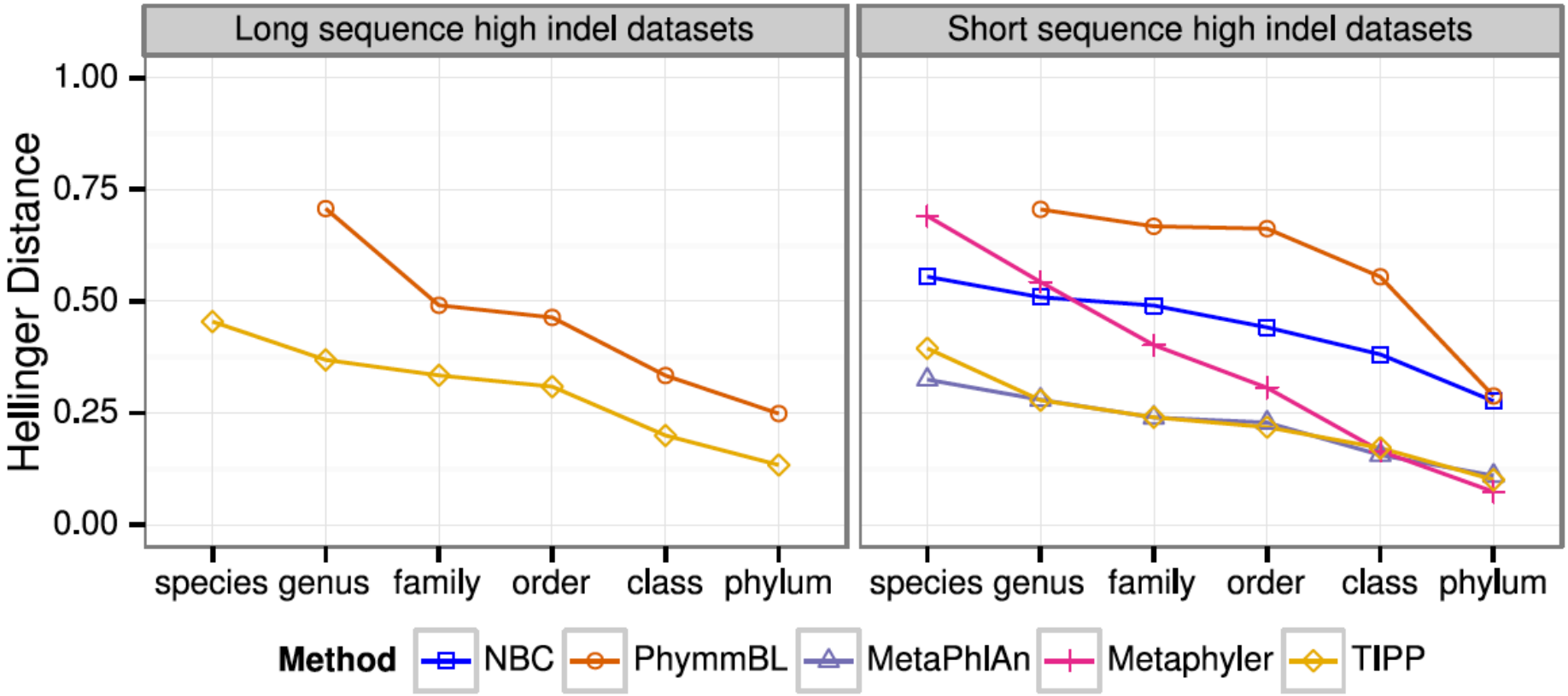
We compared TIPP to

- PhymmBL (Brady & Salzberg, Nature Methods 2009)
- NBC (Rosen, Reichenberger, and Rosenfeld, Bioinformatics 2011)
- MetaPhyler (Liu et al., BMC Genomics 2011), from the Pop lab at the University of Maryland
- MetaPhlAn (Segata et al., Nature Methods 2012), from the Huttenhower Lab at Harvard
- mOTU (Bork et al., Nature Methods 2013)

MetaPhyler, MetaPhlAn, and mOTU are marker-based techniques (but use different marker genes).

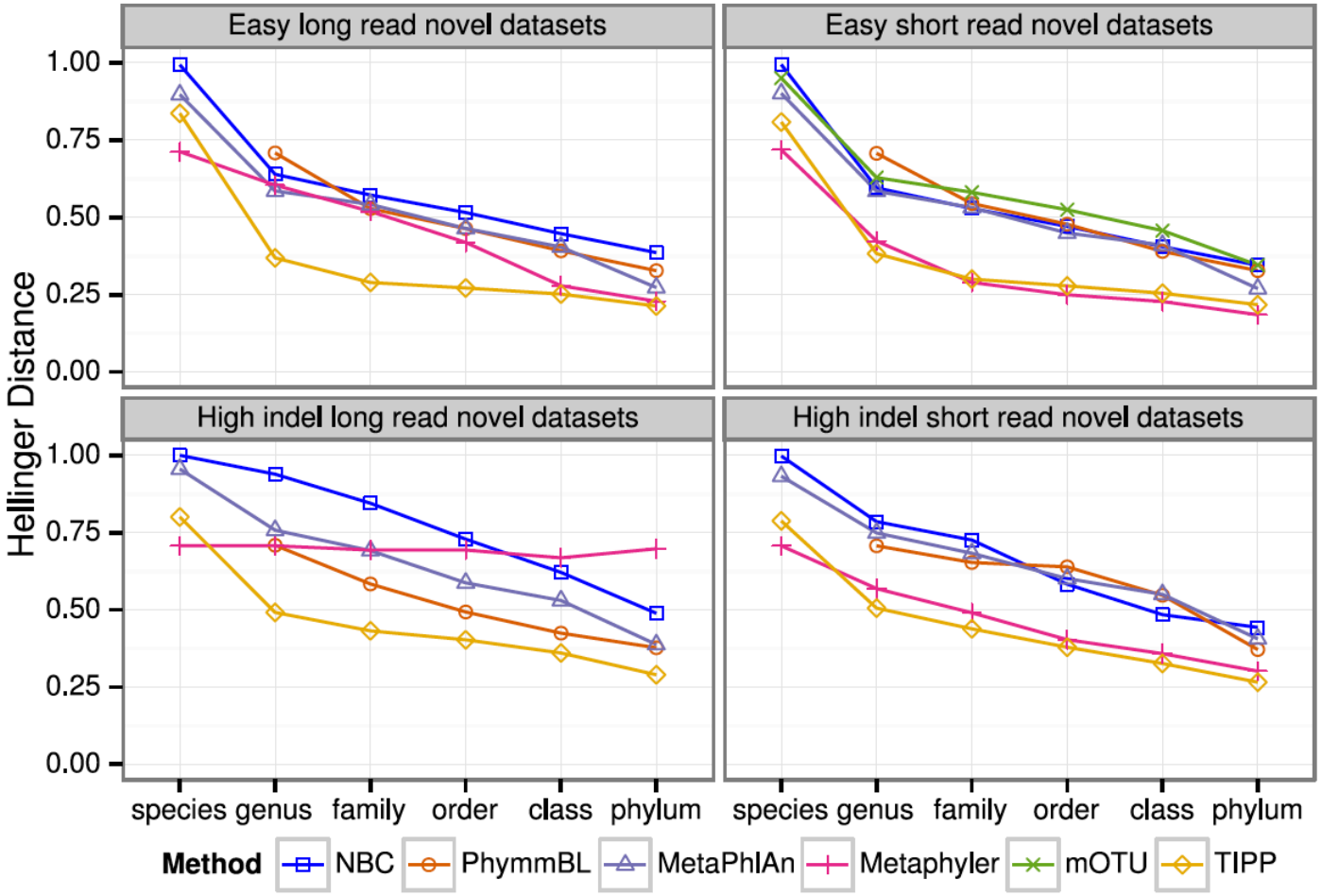
Marker gene are single-copy, universal, and resistant to horizontal transmission.

High indel datasets containing known genomes



Note: NBC, MetaPhlAn, and MetaPhyler cannot classify any sequences from at least one of the high indel long sequence datasets, and mOTU terminates with an error message on all the high indel datasets.

“Novel” genome datasets



Note: mOTU terminates with an error message on the long fragment datasets and high indel datasets.

TIPP vs. other abundance profilers

- TIPP is highly accurate, even in the presence of high indel rates and novel genomes, and for both short and long reads.

- All other methods have some vulnerability (e.g., mOTU is only accurate for short reads and is impacted by high indel rates).

- Improved accuracy is due to the use of eHMMs; single HMMs do not provide the same advantages, especially in the presence of high indel rates.

Still to do

- Evaluate TIPP in comparison to newer methods (e.g., Kraken)
- Evaluating TIPP with respect to taxonomic identification and identification of novel taxa.
- Update TIPP's design!

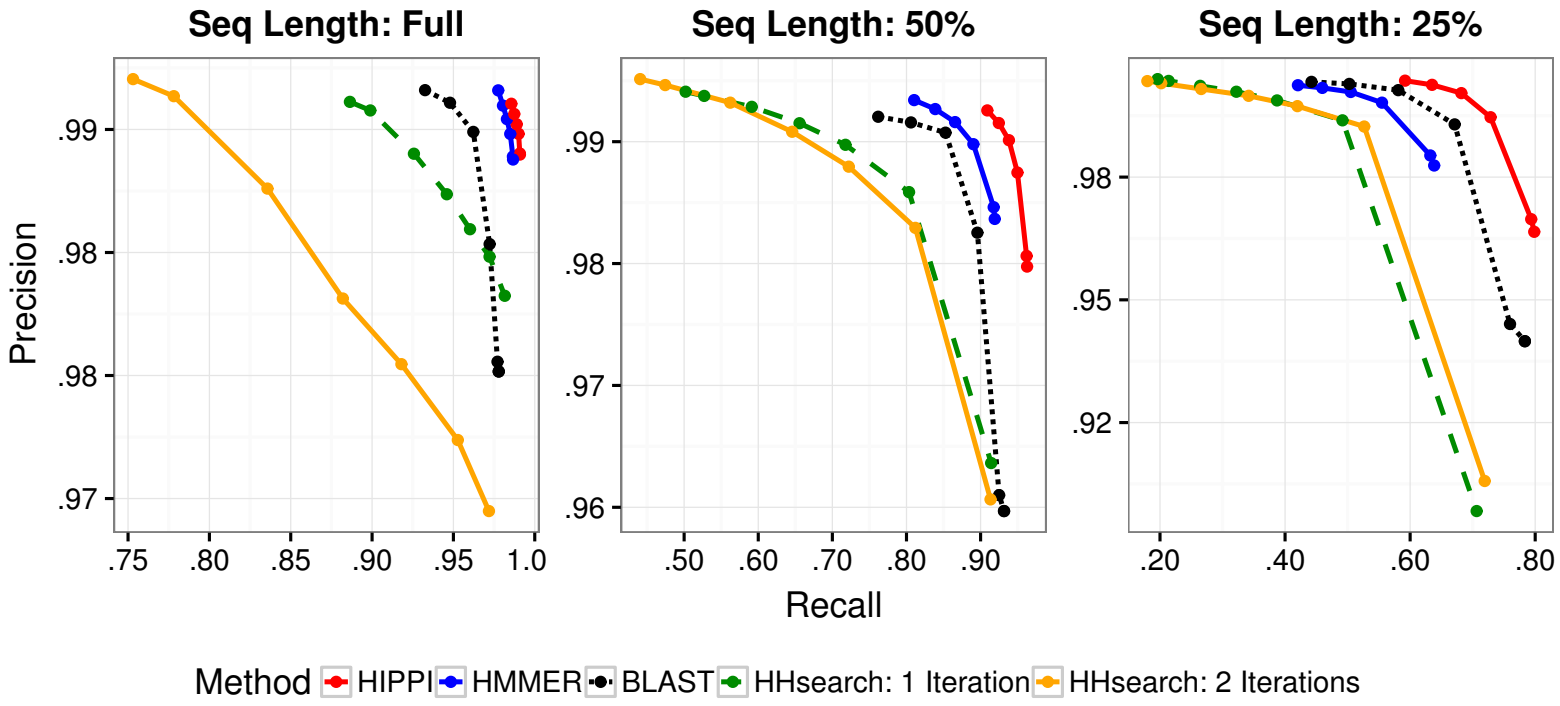
HIPPI

- HIerarchical Profile HMMs for Protein family Identification
- Nguyen, Nute, Mirarab, and Warnow, RECOMB-CG and BMC-Genomics 2016
- Uses an ensemble of HMMs to classify protein sequences
- Tested on HMMER

Protein Family Assignment

- Input: new AA sequence (might be fragmentary) and database of protein families (e.g., PFAM)
- Output: assignment (if justified) of the sequence to an existing family in the database

TIPPI: Replacing BLAST by HIPPI within TIPP



To appear, Nguyen et al., BMC Genomics

Our Publications using eHMMs

- S. Mirarab, N. Nguyen, and T. Warnow. "SEPP: SATé-Enabled Phylogenetic Placement." Proceedings of the 2012 Pacific Symposium on Biocomputing (PSB 2012) 17:247-258.
- N. Nguyen, S. Mirarab, B. Liu, M. Pop, and T. Warnow "TIPP:Taxonomic Identification and Phylogenetic Profiling." Bioinformatics (2014) 30(24): 3548-3555.
- N. Nguyen, S. Mirarab, K. Kumar, and T. Warnow, "Ultra-large alignments using phylogeny aware profiles". Proceedings RECOMB 2015 and Genome Biology (2015) 16:124
- N. Nguyen, M. Nute, S. Mirarab, and T. Warnow, HIPPI: Highly accurate protein family classification with ensembles of HMMs. BMC Genomics (2016): 17 (Suppl 10):765

All codes are available in open source form at
https://github.com/smirarab/sepp

Summary

- Using an ensemble of HMMs tends to improve accuracy, for a cost of running time. Applications so far to taxonomic placement (SEPP), multiple sequence alignment (UPP), protein family classification (HIPPI). Improvements are mostly noticeable for large diverse datasets.
- Phylogenetically-based construction of the ensemble helps accuracy (note: the decompositions we produce are not clade-based), but the design and use of these ensembles is still in its infancy. (Many relatively similar approaches have been used by others, including FlowerPower by Sjolander)
- The basic idea can be used with any kind of probabilistic model, doesn't have to be restricted to profile HMMs.
- Basic question: why does it help?

Acknowledgments



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Mihai Pop, University of Maryland

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