**Supplementary Methods and Data**

**Extracting query segments from pair-wise MAF entries**

Post LCB identification, for each entry in the pair-wise MAF file, one FASTA entry contig is generated as below: sequence of contig is simply the query sequence of the MAF entry where gaps (character “-“) are removed and the contig name is constructed as below:

Start and end positions of the query are added to the query contig name separated by the ‘!’ character. These indices help the final backtracking algorithm. The generated FASTA file is used to align against the next subject genome FASTA file

**CoreDetector Backtracking Algorithm**

All *N - 1* generated pair-wise MAF files are processed recursively (starting from the last MAF file through to the first one). For each HSP entry of the last MAF file, a backtracking algorithm extracts each subject sequence part and finally builds an MSA entry containing *N* sequences. MSA entries are written as a final output MAF file or all concatenated into a final output FASTA file containing *N* contigs.

Given *N* genomes results in *N* *- 1* pairwise MAF file alignments, each pairwise alignment is accessed through an array defined as *MAF[1 .. N - 1]*. Foreach pairwise MAF alignment, let *msaK* be a variable list of *K* sequences that represent the multiple sequence alignment profile of the *K* sequences, and let the function *getHomolog (hsp, query, maf)* query part of the input *hsp* in the input pairwise alignment maf file to return the subject *hsp* equivalent.

The adjustment of alignment gaps occurs using an algorithm that consists of two processes. First process is *gap surveyor* that essentially prepares a map of gap positions and their numbers. The second process is *gap merger* that merges the gap survey of 2 sequences. The algorithm starts with preparing a gap survey for the query sequence as a Java Map object. When a subject profile is added to the end of alignment profile of *msaK*, the gaps survey is merged with each previous sequence’s gap survey profile in *msaK* and as a result of merging, all sequences in the profile and their gap surveys are updated.

**Complexity of back tracking algorithm**

Order of the algorithm is a function of number of genomes (N), level of divergence between genomes (D) and length of genomes (L). The first loop in the algorithm has an order equal to number of HSPs in the final pair-wise alignment, that itself is a function of N, L and D and can be shown with . Then the order of the algorithm is .

**Selecting homologous sequences**

The pairwise aligner is run in low and high sensitivity modes and the results of the two runs are merged into a single MAF file. In low sensitivity mode, more similar sequences are captured and in high sensitivity mode more divergent sequences are detected. Adjacent hits from the two runs are then merged into larger hits.

As pairwise alignments can report multiple hits (HSPs) due to paralogue sequences (duplication) or by chance, CoreDetector can use the chromosome number (if available) and relative position of an HSP in that chromosome to select the best hit that likely reflects the true orthologs. HSPs shorter than a user selected threshold (default 500bp) is excluded from the results to minimize the chance of a false positive orthologs.

**Experiment 1) Analysis of 27 fungal pathogen (Ptr) genomes**

**Table S1.*Pyrenophora tritici-repentis* (Ptr) isolate genomes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Assembly Accession | Strain | Sequencing techonolgy | Length | Assembly Level |
| GCA\_003171515.3 | M4 | PacBio | 40738834 | Chromosome |
| GCA\_003171545.1 | ARCrossB10v1 | Illumina | 33619417 | Scaffold |
| GCA\_003231325.2 | Ptr134 | Illumina | 40622625 | Chromosome |
| GCA\_003231345.1 | Ptr5213 | Illumina | 34183844 | Scaffold |
| GCA\_003231355.1 | Ptr11137 | Illumina | 33921061 | Scaffold |
| GCA\_003231365.1 | Ptr239 | Illumina | 34483177 | Scaffold |
| GCA\_003231415.2 | DW5 | PacBio | 40616955 | Chromosome |
| GCA\_003231425.2 | 86-124 | PacBio | 40927694 | Chromosome |
| GCA\_008692205.1 | V0001 | PacBio | 40136298 | Contig |
| GCA\_018492725.1 | AR CrossB10v2 | PacBio | 39876747 | Contig |
| GCA\_022544795.1 | Alg130 | Illumina | 34824145 | Scaffold |
| GCA\_022544805.1 | Alg215 | Illumina | 34570617 | Scaffold |
| GCA\_022578365.1 | T205 | Illumina | 34133756 | Scaffold |
| GCA\_022578395.1 | T199 | Illumina | 34281657 | Scaffold |
| GCA\_022788405.1 | EW4-4 | Illumina | 34368509 | Scaffold |
| GCA\_022788415.1 | SN001A | Illumina | 34152043 | Scaffold |
| GCA\_022788425.1 | SN002B | Illumina | 35159469 | Scaffold |
| GCA\_022788435.1 | EW7m1 | Illumina | 34228317 | Scaffold |
| GCA\_022788445.1 | SN001C | Illumina | 34292940 | Scaffold |
| GCA\_022788505.1 | EW306-2-1 | Illumina | 34540381 | Scaffold |
| GCA\_022788515.1 | CC142 | Illumina | 34345883 | Scaffold |
| GCA\_022813025.1 | Biotrigo9-1 | PacBio | 42003943 | Chromosome |
| GCA\_022813065.1 | L13-192 | PacBio | 36964309 | Chromosome |
| GCA\_022837075.1 | Ptr90-2 | Pacbio | 39319516 | Chromosome |
| GCA\_000149985.1 | Pt-1C-BFP | Sanger | 37840464 | Scaffold |
|  | \*DW7 | Illumina |  | Scaffold |
|  | \*SD20 | Illumina |  | Scaffold |

\*Assembly originally sourced from DOI: 10.1186/s12864-018-4680-3 published in 2018.

**Run CoreDetector:**

Command:

Result:

User time (seconds): 1218.40

System time (seconds): 66.87

Elapsed (wall clock) time (h:mm:ss or m:ss): 9:34.17

Maximum resident set size (kbytes): 10897008

Exit status: 0

Neighbor-joining:

(Alg215:0.00006,((Ptr239:0.00005,(Ptr11137:0.00007,(Ptr5213:0.00003,

Ptr134:0.00035):0.00003):0.00001):0.00001,(((DW7:0.00007,

DW5:0.00002):0.00008,V0001:0.00006):0.00001,((((((CC142:0.00002,

SN001C:0.00002):0.00003,EW4-4:0.00004):0.00002,EW7m1:0.00006):0.00001,

(EW306-2-1:0.00006,(SN002B:0.00004,SN001A:0.00007):0.00001):0.00001):0.00001,

(((ARCrossB10v2:0.00002,ARCrossB10:0.00003):0.00004,Pt-1C-BFP:0.00028):0.00001,

Ptr86-124:0.00007):0.00000):0.00001,(((SD20:0.00032,Ptr90-2:0.00033):0.00161,

((T199:0.00006,Alg130:0.00004):0.00000,T205:0.00005):0.00026):0.00009,

(L13-192 :0.00002,Biotrigo9-1:0.00002):0.00006):0.00000):0.00000):0.00001):0.00002,M4:0.00004);

UPMGA:

((((Alg130:0.00005,T199:0.00005):0.00000,T205:0.00005):0.00021,(((((((((SN002B:0.00005,EW306-2-1:0.00005):0.00002,SN001A:0.00007):0.00001,(((CC142:0.00002,SN001C:0.00002):0.00003,EW4-4:0.00005):0.00002,EW7m1:0.00006):0.00001):0.00001,(Ptr86-124:0.00007,(ARCrossB10v2:0.00003,ARCrossB10:0.00003):0.00005):0.00001):0.00000,((((Ptr239:0.00005,V0001:0.00005):0.00001,(M4:0.00005,Alg215:0.00005):0.00002):0.00001,Ptr5213:0.00007):0.00001,(Biotrigo9-1:0.00002,L13-192 :0.00002):0.00006):0.00001):0.00000,Ptr11137:0.00009):0.00003,(DW5:0.00005,DW7:0.00005):0.00007):0.00008,Pt-1C-BFP:0.00019):0.00006,Ptr134:0.00025):0.00001):0.00082,(SD20:0.00033,Ptr90-2:0.00033):0.00075);

**Run Mugsy:**

Command:

Results: Failed to run successfully.

**Run Parsnp**:

Command:

Result:

User time (seconds): 4731.08

System time (seconds): 386.56

Elapsed (wall clock) time (h:mm:ss or m:ss): 47:31.20

Maximum resident set size (kbytes): 57934588

Unrooted:

((Pt-1C-BFP:0.030111495,(ARCrossB10:0.000725282,ARCrossB10v2:0.000000005)1.000:0.009131949)1.000:0.009151922,((L13-192 :0.000839896,Biotrigo9-1:0.000000005)1.000:0.018349328,((((SD20:0.121634781,Ptr90-2:0.069672138)1.000:0.975283247,(T199:0.008920380,(T205:0.007747596,Alg130:0.009982138)1.000:0.002489951)1.000:0.050158285)1.000:0.009971009,(DW5:0.000009269,DW7:0.000188489)1.000:0.020201877)1.000:0.006079762,(((M4:0.009065674,Alg215:0.009688859)1.000:0.009751548,(Ptr239:0.010327575,V0001:0.013442618)1.000:0.004540371)1.000:0.003622329,(Ptr11137:0.016657137,(Ptr5213:0.014138296,Ptr134:0.022974970)1.000:0.006837888)1.000:0.003903821)0.810:0.002240127)1.000:0.005324087)0.990:0.003219396,(Ptr86-124:0.015861706,((SN001A:0.010563118,(EW306-2-1:0.014965848,SN002B:0.006463687)1.000:0.004768299)1.000:0.006384112,(EW7m1:0.014258299,(EW4-4:0.008241354,(SN001C:0.001795244,CC142:0.001581154)1.000:0.009669646)1.000:0.006727149)1.000:0.002565941)1.000:0.003233247)1.000:0.003207640);

Rooted:

((SD20:0.121634781,Ptr90-2:0.069672138)1.000:0.4587530685,((((((Pt-1C-BFP:0.030111495,(ARCrossB10:0.000725282,ARCrossB10v2:5e-09)1.000:0.009131949)1.000:0.009151922,(Ptr86-124:0.015861706,((SN001A:0.010563118,(EW306-2-1:0.014965848,SN002B:0.006463687)1.000:0.004768299)1.000:0.006384112,(EW7m1:0.014258299,(EW4-4:0.008241354,((SN001C:0):0.001795244,CC142:0.001581154)1.000:0.009669646)1.000:0.006727149)1.000:0.002565941)1.000:0.003233247)1.000:0.00320764)0.990:0.003219396,(L13-192:0.000839896,Biotrigo9-1:5e-09)1.000:0.018349328)1.000:0.005324087,(((M4:0.009065674,Alg215:0.009688859)1.000:0.009751548,(Ptr239:0.010327575,V0001:0.013442618)1.000:0.004540371)1.000:0.003622329,(Ptr11137:0.016657137,(Ptr5213:0.014138296,Ptr134:0.02297497)1.000:0.006837888)1.000:0.003903821)0.810:0.002240127)1.000:0.006079762,(DW5:9.269e-06,DW7:0.000188489)1.000:0.020201877)1.000:0.009971009,(T199:0.00892038,(T205:0.007747596,Alg130:0.009982138)1.000:0.002489951)1.000:0.050158285)1.000:0.5165301785);

**Run Phylonium**:

Command:

Results:

User time (seconds): 42.87

System time (seconds): 38.95

Elapsed (wall clock) time (h:mm:ss or m:ss): 0:26.78

Maximum resident set size (kbytes): 3135796

Neighbor-joining:

(DW5:0.00004,(((Alg130:0.00013,(T205:0.00006,

T199:0.00008):0.00001):0.00014,(Ptr90-2:0.00051,

SD20:0.00041):0.00176):0.00012,((Biotrigo9-1:0.00006,L13-192 :0.00018):0.00006,

((((Pt-1C-BFP:0.00013,(ARCrossB10:0.00004,ARCrossB10v2:0.00004):0.00005):0.00002,

Ptr86-124:0.00012):0.00001,(((EW4-4:0.00010,(SN001C:0.00004,

CC142:0.00004):0.00007):0.00002,EW7m1:0.00012):0.00001,

((SN001A:0.00007,SN002B:0.00009):0.00002,EW306-2-1:0.00013):0.00002):0.00000):0.00001,

((((M4:0.00008,Alg215:0.00009):0.00003,V0001:0.00011):0.00001,

Ptr239:0.00011):0.00001,((Ptr134:0.00012,Ptr5213:0.00012):0.00000,

Ptr11137:0.00011):0.00000):0.00002):0.00002):0.00002):0.00008,DW7:0.00003);

UPGMA:

((((((((SN002B:0.00008,SN001A:0.00008):0.00003,EW306-2-1:0.00012):0.00002,

(((SN001C:0.00004,CC142:0.00004):0.00006,EW4-4:0.00010):0.00002,

EW7m1:0.00012):0.00001):0.00001,((Ptr86-124:0.00012,

Biotrigo9-1:0.00012):0.00001,(Pt-1C-BFP:0.00011,(ARCrossB10:0.00004,

ARCrossB10v2:0.00004):0.00007):0.00002):0.00001):0.00001,((DW7:0.00003,

DW5:0.00003):0.00011,(((Ptr134:0.00012,(Ptr11137:0.00011,

Ptr239:0.00011):0.00001):0.00001,Ptr5213:0.00012):0.00001,

((M4:0.00009,Alg215:0.00009):0.00002,V0001:0.00011):0.00002):0.00001):0.00001):0.00006,

L13-192 :0.00020):0.00007,(Alg130:0.00010,(T199:0.00007,

T205:0.00007):0.00004):0.00016):0.00099,(SD20:0.00046,

Ptr90-2:0.00046):0.00079);

**Table S2.** *The comparative matrix of fungal pathogen rooted phylogenetic tree metrics generated from CoreDetector, Parsnp, Phylonium and ProgressiveCactus results*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Distance Metric | Triplets | RFCluster0.5 | Matching Pair | Nodal Splitted | Matching Cluster | MAST | CopheneticL2 |
| CoreDetector vs Phylonium | 667 | 13 | 109 | 55.24 | 68 | 10 | 42.94 |
| CoreDetector vs Parsnp | 447 | **8** | 93 | 43.97 | 55 | **6** | 37.56 |
| Phylonium vs Parsnp | **370** | 11 | **64** | **36.08** | **38** | 9 | **20.02** |

**Experiment 2) Analysis of 12 Drosophila genomes**

**Table S3 Drosophila genus genomes**

|  |  |  |  |
| --- | --- | --- | --- |
| **Assembly Accession** | **Organism Name** | **Length** | **Assembly Level** |
| GCA\_000001215.4 | Drosophila melanogaster | 143706478 | Chromosome |
| GCA\_003285735.2 | Drosophila virilis | 189443829 | Contig |
| GCA\_003286085.2 | Drosophila persimilis | 195512972 | Contig |
| GCA\_003286155.2 | Drosophila erecta | 146538397 | Contig |
| GCA\_004382195.2 | Drosophila sechellia | 153084571 | Chromosome |
| GCA\_009870125.2 | Drosophila pseudoobscura | 163266851 | Chromosome |
| GCA\_016746365.2 | Drosophila yakuba | 147883098 | Chromosome |
| GCA\_016746395.2 | Drosophila simulans | 131663590 | Chromosome |
| GCA\_017639315.2 | Drosophila ananassae | 213817545 | Chromosome |
| GCA\_018153295.1 | Drosophila grimshawi | 191382978 | Contig |
| GCA\_018153725.1 | Drosophila mojavensis | 163170721 | Contig |
| GCA\_018902025.2 | Drosophila willistoni | 246985538 | Chromosome |

**Run CoreDetector:**

Command:

Rooted:

(((((((Drosophila\_simulans:0.008174107023423829,Drosophila\_sechellia:0.008174107023423829):0.009515109277212375,Drosophila\_melanogaster:0.017689216300636204):0.017472352808081557,(Drosophila\_erecta:0.030463867073209444,Drosophila\_yakuba:0.030463867073209444):0.0046977020355083166):0.07355794300192398,Drosophila\_ananassae:0.10871951211064174):0.011486292441234155,(Drosophila\_persimilis:0.016693666913404535,Drosophila\_pseudoobscura:0.016693666913404535):0.10351213763847136):0.02422227983882487,(Drosophila\_grimshawi:0.11904825027337734,(Drosophila\_mojavensis:0.10776819000195577,Drosophila\_virilis:0.10776819000195577):0.011280060271421574):0.02537983411732342):0.010896132196410502,Drosophila\_willistoni:0.15532421658711126);

Unrooted:

((Drosophila\_grimshawi:0.12083208972967743,(Drosophila\_mojavensis:0.11110534675310578,Drosophila\_virilis:0.10443103325080576):0.009496220815121498):0.031638164501544205,Drosophila\_willistoni:0.16768072935138384,((Drosophila\_persimilis:0.017718830324930455,Drosophila\_pseudoobscura:0.015668503501878615):0.10310866345208547,(Drosophila\_ananassae:0.1144907916158992,(((Drosophila\_simulans:0.007092298060915315,Drosophila\_sechellia:0.009255915985932343):0.0077790331576218456,Drosophila\_melanogaster:0.019425292420226732):0.01674803324390453,(Drosophila\_erecta:0.02994878679984983,Drosophila\_yakuba:0.030978947346569058):0.0060045067870934665):0.06804119678140327):0.015737286297791697):0.01903491015632574);

**Run Parsnp:**

Command:

Result:

Output error after 54 minutes as below:

|->[ERROR]: aligned regions cover less than 1% of reference genome, something is not right.. please adjust params and rerun. If problem persists, please contact developers (treangen@gmail.com)

Rooted: Run failed to complete

Unrooted: Run failed to complete

**Run Phylonium:**

Command:

Install mattools from https://github.com/EvolBioInf/mattools

Rooted:

(((((Drosophila\_melanogaster:0.00294,( Drosophila\_sechellia:0.00155, Drosophila\_simulans:0.00155):0.00139):0.00217,

(Drosophila\_yakuba:0.00459,ere:0.00459):0.00053):0.00967, Drosophila\_ananassae:0.01479):0.00799,

(Drosophila\_willistoni:0.01518,(( Drosophila\_virilis:0.01352, Drosophila\_mojavensis:0.01352):0.00126, Drosophila\_grimshawi:0.01478):0.00040):0.00759):0.00700,

(Drosophila\_pseudoobscura:0.00575, Drosophila\_persimilis:0.00575):0.02402);

Unrooted (NJ):

((Drosophila\_sechellia:0.00183,sim:0.00128):0.00131,(( Drosophila\_yakuba:0.00447, Drosophila\_erecta:0.00470):0.00105,

(Drosophila\_ananassae:0.01517,(( Drosophila\_pseudoobscura:0.00713, Drosophila\_persimilis:0.00437):0.03078,( Drosophila\_willistoni:0.00578,

((Drosophila\_virilis:0.01243, Drosophila\_mojavensis:0.01461):0.00179, Drosophila\_grimshawi:0.01425):0.00980):0.00487):0.00634):0.00932):0.00171, Drosophila\_melanogaster:0.00303);

Mario’s:

(((Drosophila\_melanogaster:0.003026,(Drosophila\_sechellia:0.001828,Drosophila\_simulans:1.2788e-03)100:1.3090e-03)100:0.001712,(Drosophila\_ananassae:0.015175,((Drosophila\_pseudoobscura:0.007133,Drosophila\_persimilis:4.3670e-03)100:0.030782,((Drosophila\_grimshawi:0.014247,(Drosophila\_virilis:0.012432,Drosophila\_mojavensis:1.4611e-02)100:1.7932e-03)100:0.009804,Drosophila\_willistoni:5.7760e-03)100:4.8668e-03)100:6.3374e-03)100:9.3183e-03)95:0.001054,Drosophila\_erecta:4.7021e-03,Drosophila\_yakuba:4.4731e-03);

**Run ProgressiveCactus:**

Commands:

1. Make sure you update TOIL directory to a directory that has enough space:
2. Run halStats to generate phylogenetics tree from hal file:
3. Convert hal to maf format:

Results:

Finished after 35 hours.

In-house program to extract core genome from the maf file,

Core extraction output:

records written:1269

Total Written Alignment Length(bp):88243

N50: 269

Unrooted:

(((Drosophila\_mojavensis:0.09867336734,Drosophila\_virilis:0.09867336734):0.01308244145,Drosophila\_grimshawi:0.1117558088):0.04693903063,((((Drosophila\_melanogaster:0.01826274433,(Drosophila\_sechellia:0.009326507309,Drosophila\_simulans:0.009326507309):0.008936237023):0.0186073202,(Drosophila\_erecta:0.03045180414,Drosophila\_yakuba:0.03045180414):0.006418260395):0.08406741592,Drosophila\_ananassae:0.1209374805):0.01109715615,(Drosophila\_pseudoobscura:0.007388763055,Drosophila\_persimilis:0.007388763055):0.1246458736):0.02666020281,Drosophila\_willistoni:0.1800255662);

Rooted:

((((Drosophila\_mojavensis:0.09867336734035617,Drosophila\_virilis:0.09867336734035617):0.013082441453579688,Drosophila\_grimshawi:0.11175580879393586):0.046939030625989045,((((Drosophila\_melanogaster:0.01826274433177766,(Drosophila\_sechellia:0.009326507308650894,Drosophila\_simulans:0.009326507308650894):0.008936237023126764):0.018607320204269154,(Drosophila\_erecta:0.03045180414066633,Drosophila\_yakuba:0.03045180414066633):0.00641826039538048):0.08406741592305332,Drosophila\_ananassae:0.12093748045910013):0.011097156153211968,(Drosophila\_pseudoobscura:0.007388763054697205,Drosophila\_persimilis:0.007388763054697205):0.12464587355761489):0.026660202807612804):0.010665363410652356,Drosophila\_willistoni:0.16936020283057726);

**Run SibeliaZ**

Using 32 cores and 256GB of RAM

Commands:

**Results:**

Finished after 5 hours and 30 minutes.

Analysis of maf file using in-house program to extract core:

**Output:**

2046 records written calculated 2046

564121 Total Written Alignment Length(bp)

N50:354 Min:59 Max:1114

Rooted:

((((((((Drosophila\_sechellia:0.003495490805469818,Drosophila\_simulans:0.003495490805469818):0.00367145780176285,Drosophila\_melanogaster:0.007166948607232668):0.007999975447184388,Drosophila\_erecta:0.015166924054417056):0.0011902187386093784,Drosophila\_yakuba:0.016357142793026434):0.031278309140855005,Drosophila\_ananassae:0.04763545193388144):0.01016604842224779,(Drosophila\_pseudoobscura:0.004386348717713648,Drosophila\_persimilis:0.004386348717713648):0.05341515163841558):0.01081730631900936,((Drosophila\_mojavensis:0.040377900205304504,Drosophila\_virilis:0.040377900205304504):0.003377393702676257,Drosophila\_grimshawi:0.04375529390798076):0.024863512767157828):0.0023791775128990528,Drosophila\_willistoni:0.07099798418803764);

Unrooted:

(((((((Drosophila\_sechellia:0.003495490805,Drosophila\_simulans:0.003495490805):0.003671457802,Drosophila\_melanogaster:0.007166948607):0.007999975447,Drosophila\_erecta:0.01516692405):0.001190218739,Drosophila\_yakuba:0.01635714279):0.03127830914,Drosophila\_ananassae:0.04763545193):0.01016604842,(Drosophila\_pseudoobscura:0.004386348718,Drosophila\_persimilis:0.004386348718):0.05341515164):0.01081730632,((Drosophila\_mojavensis:0.04037790021,Drosophila\_virilis:0.04037790021):0.003377393703,Drosophila\_grimshawi:0.04375529391):0.02486351277, Drosophila\_willistoni:0.0733771617);

Fly (Drosophila)species TimeTree ground truth rooted tree:

(((Drosophila\_mojavensis:27.02604000,Drosophila\_virilis:27.02604000)'16':10.95780500,Drosophila\_grimshawi:37.98384500)'19':0.00000000,(Drosophila\_willistoni:32.40000000,(((((Drosophila\_simulans:2.24134000,Drosophila\_sechellia:2.24134000)'15':2.38266000,Drosophila\_melanogaster:4.62400000)'13':6.94573000,(Drosophila\_erecta:8.00000000,Drosophila\_yakuba:8.00000000)'11':3.56973000)'9':19.13933500,Drosophila\_ananassae:30.70906500)'8':1.69093500,(Drosophila\_pseudoobscura:1.36028000,Drosophila\_persimilis:1.36028000)'7':31.03972000)'32':0.00000000)'31':5.58384500);

Fly (Drosophila)species TimeTree ground truth unrooted tree:

((Drosophila\_mojavensis:27.02604,Drosophila\_virilis:27.02604)'16':10.957805,Drosophila\_grimshawi:37.983845,(Drosophila\_willistoni:32.4,(((((Drosophila\_simulans:2.24134,Drosophila\_sechellia:2.24134)'15':2.38266,Drosophila\_melanogaster:4.624)'13':6.94573,(Drosophila\_erecta:8,Drosophila\_yakuba:8)'11':3.56973)'9':19.139335,Drosophila\_ananassae:30.709065)'8':1.690935,(Drosophila\_pseudoobscura:1.36028,Drosophila\_persimilis:1.36028)'7':31.03972)'32':0)'31':5.583845)'19';

**Table S4.** *The comparative phylogenetic tree distance metrics of fly unrooted ground truth tree compared to neighbor joining unrooted phylogenetic trees generated from the results obtained from CoreDetector, Phylonium, ProgressiveCactus and SibeliaZ.*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Distance Metric | Quartet | Path Difference | Matching Triplets | Matching Split | UMAST | GeoUnrooted | RF Weighted(0.5) | RF(0.5) |
| CoreDetector | 0.0 | 0.00 | 4 | 0 | 0 | 80.99 | 131.64 | 0 |
| Phylonium | 0.0 | 0.00 | 10 | 0 | 0 | 81.26 | 132.06 | 0 |
| ProgressiveCactus | 0.0 | 0.00 | 11 | 0 | 0 | 80.98 | 131.63 | 0 |
| SibeliaZ | 21.0 | 5.65 | 43 | 4 | 1 | 81.17 | 131.92 | 1 |

**Experiment 3) Analysis of 34 Rodent genomes**

**Table S5 Rodent order genomes**

|  |  |  |  |
| --- | --- | --- | --- |
| Assembly Accession | Organism Name | Length | Assembly Level |
| GCA\_004027535.1 | Acomys cahirinus | 2306070819 | Scaffold |
| GCA\_004027875.1 | Aplodontia rufa | 3005535537 | Scaffold |
| GCA\_001984765.1 | Castor canadensis | 2518306565 | Scaffold |
| GCA\_004027575.1 | Cricetomys gambianus | 2397721602 | Scaffold |
| GCA\_000223135.1 | Cricetulus griseus | 2399770464 | Scaffold |
| GCA\_000151885.2 | Dipodomys ordii | 2236368823 | Scaffold |
| GCA\_004024685.1 | Dipodomys stephensi | 2346418196 | Scaffold |
| GCA\_001685075.1 | Ellobius lutescens | 2353188398 | Scaffold |
| GCA\_001685095.1 | Ellobius talpinus | 2265966243 | Scaffold |
| GCA\_004027185.1 | Glis glis | 2462087207 | Scaffold |
| GCA\_004027655.1 | Graphiurus murinus | 2815014629 | Scaffold |
| GCA\_016881025.1 | Ictidomys tridecemlineatus | 2478949113 | Chromosome |
| GCA\_020740685.1 | Jaculus jaculus | 2863848715 | Chromosome |
| GCA\_001458135.2 | Marmota marmota marmota | 2506852125 | Scaffold |
| GCA\_002204375.1 | Meriones unguiculatus | 2523107715 | Scaffold |
| GCA\_017639785.1 | Mesocricetus auratus | 2457062007 | Scaffold |
| GCA\_000317375.1 | Microtus ochrogaster | 2287340943 | Chromosome |
| GCA\_900094665.2 | Mus caroli | 2553112587 | Chromosome |
| GCA\_000001635.9 | Mus musculus | 2728206152 | Chromosome |
| GCA\_900095145.2 | Mus pahari | 2475012951 | Chromosome |
| GCA\_921997135.2 | Mus spretus | 2546527799 | Chromosome |
| GCA\_004027005.1 | Muscardinus avellanarius | 2527147110 | Scaffold |
| GCA\_000622305.1 | Nannospalax galili | 3061408210 | Scaffold |
| GCA\_004026605.1 | Ondatra zibethicus | 2562752769 | Scaffold |
| GCA\_903995425.1 | Onychomys torridus | 2468394440 | Chromosome |
| \*GCA\_004027895.1 | Orientallactaga bullata | 3093575781 | Scaffold |
| GCA\_023159225.1 | Perognathus longimembris pacificus | 2212099196 | Chromosome |
| GCA\_003704035.3 | Peromyscus maniculatus bairdii | 2512423440 | Chromosome |
| GCA\_907164565.1 | Psammomys obesus | 2364980841 | Scaffold |
| GCA\_015227675.2 | Rattus norvegicus | 2647899415 | Chromosome |
| GCA\_004025045.1 | Sigmodon hispidus | 2730600022 | Scaffold |
| GCA\_002406435.1 | Spermophilus dauricus | 3106271744 | Scaffold |
| GCA\_004024805.1 | Xerus inauris | 2601418404 | Scaffold |
| GCA\_004024765.1 | Zapus hudsonius | 2611189839 | Scaffold |

* Note Orientallactaga bullata (Gobi jerboa)= Allactaga\_bullata

**Run CoreDetector:**

Command:

Result:

finished after 64 minutes

Unrooted:

((((Xerus\_inauris:0.04563286401452538,((Spermophilus\_dauricus:0.018822623629487945,Ictidomys\_tridecemlineatus:0.015342563771858164):0.0030441135878078868,Marmota\_marmota:0.01731352748306507):0.02310672928931263):0.014001804952393865,(Muscardinus\_avellanarius:0.000224,Aplodontia\_rufa:0.000138):0.06079758027426871):0.015571970975994567,(Graphiurus\_murinus:0.04740403384208957,Glis\_glis:0.0397043221387531):0.029560495502444592):0.01036716719290955,((Perognathus\_longimembris:0.06540744427342234,(Dipodomys\_stephensi:0.017788713927013162,Dipodomys\_ordii:0.017866076083536398):0.04272773994314567):0.048184481115534146,Castor\_canadensis:0.07564306413524288):0.003378315726325982,((Zapus\_hudsonius:0.07902915983217484,(Jaculus\_jaculus:0.04847551103115187,Allactaga\_bullata:0.041995506231676125):0.02176017166840645):0.030539844458416045,(Nannospalax\_galili:0.07384640883127151,(Cricetomys\_gambianus:0.0530893698911733,((((((Mus\_musculus:0.004059312793664881,Mus\_spretus:0.004998327191883716):0.005414772128704969,Mus\_caroli:0.010125807689125584):0.007389865443305492,Mus\_pahari:0.019185110841492624):0.016001054561249842,Rattus\_norvegicus:0.040766616796822405):0.01891898799787854,(Acomys\_cahirinus:0.053039103587052064,(Psammomys\_obesus:0.01375742246232382,Meriones\_unguiculatus:0.013577453060707272):0.03812677060768861):0.005156653413415844):0.004676846051713507,((Sigmodon\_hispidus:0.05315444587949699,(Peromyscus\_maniculatus:0.023467153844726737,Onychomys\_torridus:0.02580358099991778):0.016804906595060377):0.000987,((Ondatra\_zibethicus:0.020928173264636488,(Microtus\_ochrogaster:0.023443738663380894,(Ellobius\_talpinus:0.014078364479043787,Ellobius\_lutescens:0.017330011192865105):0.006148549827235294):0.0037865058392253133):0.0259636768031962,(Mesocricetus\_auratus:0.034212270400556744,Cricetulus\_griseus:0.028215748606156603):0.014531397366713491):0.000283):0.009843337668717136):0.001278660796591155):0.02917005812164214):0.011580704278110088):0.012446092891302989);

Rooted:

((((((((((Mus\_musculus:0.004528819992774298,Mus\_spretus:0.004528819992774298):0.005505879912528128,Mus\_caroli:0.010034699905302426):0.008267771420973595,Mus\_pahari:0.01830247132627602):0.01901666999192793,Rattus\_norvegicus:0.03731914131820395):0.01852020539912596,(Acomys\_cahirinus:0.05241665597812811,(Psammomys\_obesus:0.013667437761515546,Meriones\_unguiculatus:0.013667437761515546):0.03874921821661256):0.003422690739201796):0.003152944405884664,((Sigmodon\_hispidus:0.05074318485867878,(((Peromyscus\_maniculatus:0.02463536742232226,Onychomys\_torridus:0.02463536742232226):0.01960852327016275,(Mesocricetus\_auratus:0.031214009503356673,Cricetulus\_griseus:0.031214009503356673):0.013029881189128335):0.0031896668388563323,(Ondatra\_zibethicus:0.02349682747467054,(Microtus\_ochrogaster:0.022648238163285317,(Ellobius\_talpinus:0.015704187835954446,Ellobius\_lutescens:0.015704187835954446):0.006944050327330871):0.000849):0.0239367300566708):0.0033096273273374374):0.005502989612303867,Cricetomys\_gambianus:0.056246174470982645):0.002746116652231928):0.02246175440689447,Nannospalax\_galili:0.08145404553010904):0.016352119494199374,((((Xerus\_inauris:0.04397401293802554,((Spermophilus\_dauricus:0.017082593700673054,Ictidomys\_tridecemlineatus:0.017082593700673054):0.0016375236850999508,Marmota\_marmota:0.018720117385773005):0.025253895552252534):0.015204671057490009,(Muscardinus\_avellanarius:0.000181,Aplodontia\_rufa:0.000181):0.05899809318374064):0.0142916908856121,(Graphiurus\_murinus:0.043554177990421335,Glis\_glis:0.043554177990421335):0.029916196890706313):0.0069893701426651245,Castor\_canadensis:0.08045974502379277):0.017346420000515644):0.0021328261499582724,(Zapus\_hudsonius:0.07301242006599765,(Jaculus\_jaculus:0.045235508631414,Allactaga\_bullata:0.045235508631414):0.027776911434583648):0.026926571108269043):0.00904671338979246,(Perognathus\_longimembris:0.06298128961092139,(Dipodomys\_stephensi:0.01782739500527478,Dipodomys\_ordii:0.01782739500527478):0.045153894605646616):0.04600441495313776);

**Run Parsnp (Harvest):**

Resources: We allocated a machine with 16 OCPU and 256GB RAM.

Command:

Result:

Exited with error:

Segmentation fault (core dumped)

**Run Mugsy:**

Resources: We allocated a machine with 16 OCPU and 256GB RAM.

Command:

Result:

After 6 days exited with error:.ERROR: malloc failed, there is not enough memory

**Run ProgressiveCactus:**

Resources: We allocated a machine with 32 OCPU and 512GB RAM.

Commands:

Make sure you update TOIL directory to a directory that has enough space:

Result:

Progress 20% after 4 weeks.

**Run Skmer:**

Command:

Using 32 Cores and 128GB of RAM

Result:

Finished in 5 minutes.

Unrooted tree:

(((((((Allactaga\_bullata:0.04958322,Jaculus\_jaculus:0.05197459):0.01794275,Zapus\_hudsonius:0.06932182):0.0219338,((((Aplodontia\_rufa:0,Muscardinus\_avellanarius:0):0.07355467,(((Ictidomys\_tridecemlineatus:0.01455103,Spermophilus\_dauricus:0.01265189):0.00573622,Marmota\_marmota:0.01802827):0.0356563,Xerus\_inauris:0.05217473):0.01470148):0.01130398,(Glis\_glis:0.04880233,Graphiurus\_murinus:0.05129643):0.02670274):0.00839197,(Castor\_canadensis:0.08671625,((Dipodomys\_ordii:0.0126652,Dipodomys\_stephensi:0.0110145):0.04794225,Perognathus\_longimembris:0.06089611):0.03075502):0.00253732):0.00910904):0.00709203,Nannospalax\_galili:0.08208703):0.01345912,(Cricetomys\_gambianus:0.07259805,(((Cricetulus\_griseus:0.04699794,Mesocricetus\_auratus:0.05191294):0.01650423,((Onychomys\_torridus:0.03885649,Peromyscus\_maniculatus:0.0369781):0.0242405,(((Ellobius\_lutescens:0.02352138,Ellobius\_talpinus:0.01961805):0.01288021,Microtus\_ochrogaster:0.03605388):0.00455279,Ondatra\_zibethicus:0.03635395):0.03100664):1.677e-05):0.00155957,Sigmodon\_hispidus:0.07262961):0.00650883):0.00089139):0.00335233,(((Mus\_caroli:0.02069438,(Mus\_spretus:0.01079456,Mus\_musculus:0.01034558):0.01062353):0.01566396,Mus\_pahari:0.03739409):0.0222235,Rattus\_norvegicus:0.0620213):0.01471257):0.00565823,(Meriones\_unguiculatus:0.02080334,Psammomys\_obesus:0.02184482):0.04918628,Acomys\_cahirinus:0.06697);

Ground truth rooted tree from TimeTree database:

(((Muscardinus\_avellanarius:35.20500000,(Graphiurus\_murinus:24.31800000,Glis\_glis:24.31800000)'16':10.88700000)'19':22.40060000,(Aplodontia\_rufa:49.95000000,((Marmota\_marmota:11.90000000,(Ictidomys\_tridecemlineatus:9.44000000,Spermophilus\_dauricus:9.44000000)'15':2.46000000)'13':20.11000000,Xerus\_inauris:32.01000000)'11':17.94000000)'9':7.65560000)'8':11.93440000,(((((((Peromyscus\_maniculatus:11.69300000,Onychomys\_torridus:11.69300000)'7':6.80700000,((((Microtus\_ochrogaster:9.10000000,(Ellobius\_talpinus:8.12500000,Ellobius\_lutescens:8.12500000)'32':0.97500000)'31':2.35200000,Ondatra\_zibethicus:11.45200000)'29':5.61900000,(Mesocricetus\_auratus:12.14093500,Cricetulus\_griseus:12.14093500)'28':4.93006500)'27':0.07700000,Sigmodon\_hispidus:17.14800000)'40':1.35200000)'38':7.55000000,((Acomys\_cahirinus:17.83302000,(Psammomys\_obesus:9.81600000,Meriones\_unguiculatus:9.81600000)'26':8.01702000)'24':5.99216500,(Rattus\_norvegicus:10.25000000,(((Mus\_musculus:2.30000000,Mus\_spretus:2.30000000)'6':2.05971000,Mus\_caroli:4.35971000)'48':2.16029000,Mus\_pahari:6.52000000)'53':3.73000000)'51':13.57518500)'47':2.22481500)'46':0.00000000,Cricetomys\_gambianus:26.05000000)'45':17.06196000,Nannospalax\_galili:43.11196000)'43':8.78804000,((Allactaga\_bullata:20.99000000,Jaculus\_jaculus:20.99000000)'5':12.21000000,Zapus\_hudsonius:33.20000000)'4':18.70000000)'73':17.18200000,(Castor\_canadensis:63.61000000,(Perognathus\_longimembris:30.60000000,(Dipodomys\_stephensi:9.51654500,Dipodomys\_ordii:9.51654500)'72':21.08345500)'79':33.01000000)'77':5.47200000)'71':0.45800000);

Ground truth unrooted tree from TimeTree database:

((Muscardinus\_avellanarius:35.205,(Graphiurus\_murinus:24.318,Glis\_glis:24.318)'16':10.887)'19':22.4006,(Aplodontia\_rufa:49.95,((Marmota\_marmota:11.9,(Ictidomys\_tridecemlineatus:9.44,Spermophilus\_dauricus:9.44)'15':2.46)'13':20.11,Xerus\_inauris:32.01)'11':17.94)'9':7.6556,(((((((Peromyscus\_maniculatus:11.693,Onychomys\_torridus:11.693)'7':6.807,((((Microtus\_ochrogaster:9.1,(Ellobius\_talpinus:8.125,Ellobius\_lutescens:8.125)'32':0.975)'31':2.352,Ondatra\_zibethicus:11.452)'29':5.619,(Mesocricetus\_auratus:12.140935,Cricetulus\_griseus:12.140935)'28':4.930065)'27':0.077,Sigmodon\_hispidus:17.148)'40':1.352)'38':7.55,((Acomys\_cahirinus:17.83302,(Psammomys\_obesus:9.816,Meriones\_unguiculatus:9.816)'26':8.01702)'24':5.992165,(Rattus\_norvegicus:10.25,(((Mus\_musculus:2.3,Mus\_spretus:2.3)'6':2.05971,Mus\_caroli:4.35971)'48':2.16029,Mus\_pahari:6.52)'53':3.73)'51':13.575185)'47':2.224815)'46':0,Cricetomys\_gambianus:26.05)'45':17.06196,Nannospalax\_galili:43.11196)'43':8.78804,((Allactaga\_bullata:20.99,Jaculus\_jaculus:20.99)'5':12.21,Zapus\_hudsonius:33.2)'4':18.7)'73':17.182,(Castor\_canadensis:63.61,(Perognathus\_longimembris:30.6,(Dipodomys\_stephensi:9.516545,Dipodomys\_ordii:9.516545)'72':21.083455)'79':33.01)'77':5.472)'71':12.3924)'8';

**Table S6.** *Comparative phylogenetic tree distance metrics of unrooted rodent ground truth tree compared to unrooted trees generated from CoreDetector, Phylonium and ProgressiveCactus and SibeliaZ results.*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Distance Metric | Quartet | Path Difference | Matching Triplets | Matching Split | Umast | GeoUnrooted | RF Weighted(0.5) | RF(0.5) |
| CoreDetector | 672 | 18.43 | 496 | 12 | 2 | 150.17 | 456.10 | 3 |
| Skmer | 2240 | 26.38 | 669 | 20 | 4 | 150.15 | 456.03 | 5 |