

Mitochondrial molecular markers for US
lineages of *P. infestans*

Brian J. Knaus, Javier F. Tabima and Niklaus J. Grünwald

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1 The sample

The sample includes data which was opportunistically gathered from previous publications as well as data which is not yet available to the public (Judelson, unpublished).

Sample	Count	Reference
T30-4	1	[4]
PIC99189 & 90128	2	[8]
13_a2	1	[2]
Yoshida et al.	13	[9]
Martin et al.	3	[7]
Judelson	8	NA
Total	28	

- The sample ‘T30-4’ was the first sequenced genome and is considered the reference for nuclear work [4]. This genome was assembled prior to high-throughput sequencing (i.e., Illumina and 454 technologies). The data presented here are not the sequences used for the paper but are part of a project by The Broad to resequence this individual using Illumina and 454 technologies.
- Note that both the Yoshida and Martin papers included ancient DNA in their analyses [9, 7]. Here we have omitted those samples and focused on modern samples.
- For enigmatic reasons, not all of the samples from the Yoshida and Martin papers were actually available online. Therefore our numbers here do not match those presented in the papers.
- The Judelson data include a sample of US1 which was sampled at three different time points (us1_1, us1_2 and us1_3). We suspect that these were different samples and not necessarily the same clone. Therefore differences among these samples may either be due to biological or technical factors.
- The Judelson data includes a sample of US8 which has been characterized as having fungicide resistance [3]. This lineage was also sequenced by Martin et al. [7]. These are most likely different samples so differences among these samples may be interpreted as biological.
- The Yoshida data includes one sample of *P. mirabilis* (p7722), this should jump out in the analyses.
- For the mitochondrial data we used the type IIa form [1] because it was the longest sequence and we felt this would provide the best alignment.

31 We've used the term 'SNP' fairly loosely in this document. The term 'vari-
32 ant' may be more appropriate. Until fairly recently the software tools we've
33 been using could only handle SNPs. They now report short indels as well.
34 We've included both variant types here.

35 2 Variant discovery

36 Reads were mapped to the type IIa mitochondrial reference "AY898627.1".
37 Reads were mapped using bowtie2 [5]. Variants were called using SAMtools[6].

38 2.1 Variant filtering

39 As a quality control step, the variant files were filtered by quality, read depth
40 and mapping quality (Figures 1,2). For this we used an in-house R package
41 called `vcfR`. Here, sequencing depth is cumulative over all samples. Quality
42 here is for each variant over all samples and ranges from 1-999.

43 The genotype caller in Samtools assumes a diploid, bi-allelic model. Because
44 mitochondrial are assumed to be haploid we tried to filter out heterozygous
45 calls. Samples which included high quality heterozygote calls (p1362, p6096,
46 p10650, p12204, p10127) were mostly from the Yoshida et al. [9] paper and
47 were among the low sequencing depth samples they included. Because these
48 samples are not among the US lineages we're interested in, and because they
49 are apparently of low sequencing depth, we omitted them for now. However, the
50 sample nl07434 was among the high sequencing depth samples from this paper
51 and is perhaps noteworthy. T30-4 was called as a heterozygote for one variant
52 and is perplexing.

53 The variants remaining after filtering were visualized as a linear chromosome
54 in Figure 3.

```
## gt.m2sfs is commented out  
## Before filtering:  
## [1] 247  
## After filtering:  
## [1] 37
```

55 Filtering the variant panel based on quality (QUAL=999), cumulative se-
56 quencing depth (1st quartile >= DP >= 3rd quartile) and mapping quality (1st
57 quartile >= MQ >= 3rd quartile) resulted in 37 variants (Table 1). We have
58 identified a fraction of these as being diagnostic for a small group of samples
59 (Table 2).

60 3 Variant segregation

61 In order to visualize how variants segregated among the samples, a phylogeny
62 was inferred. We then used ancestral state reconstruction to map the characters

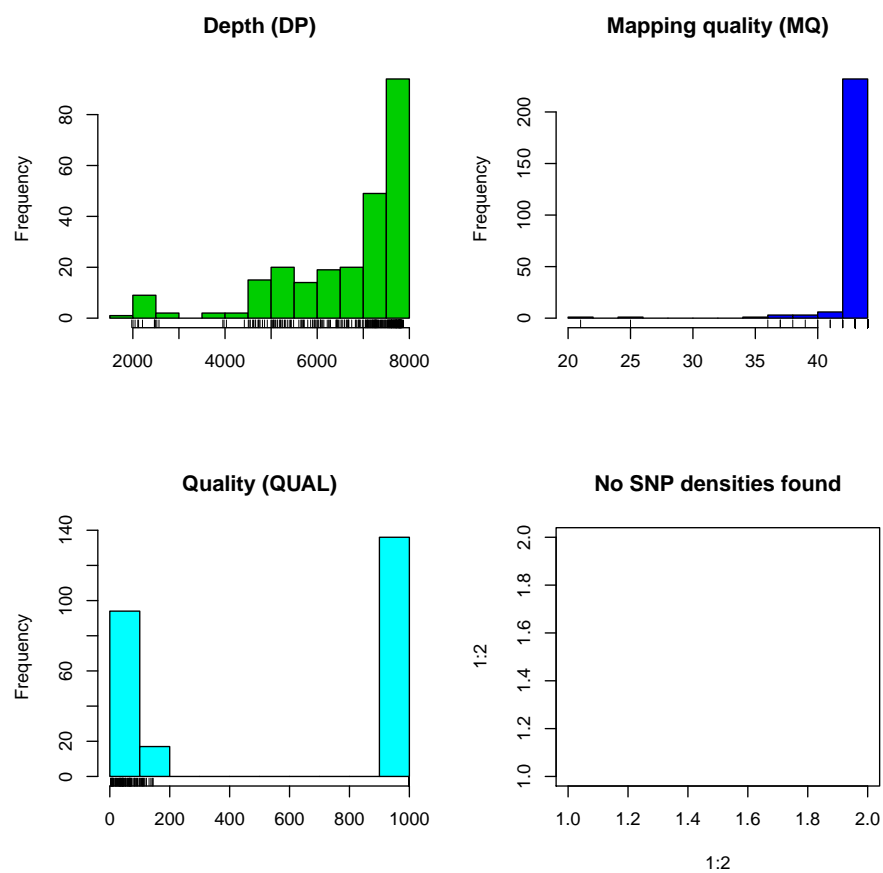


Figure 1: Quality control results for the mtDNA SNP calls before filtering.

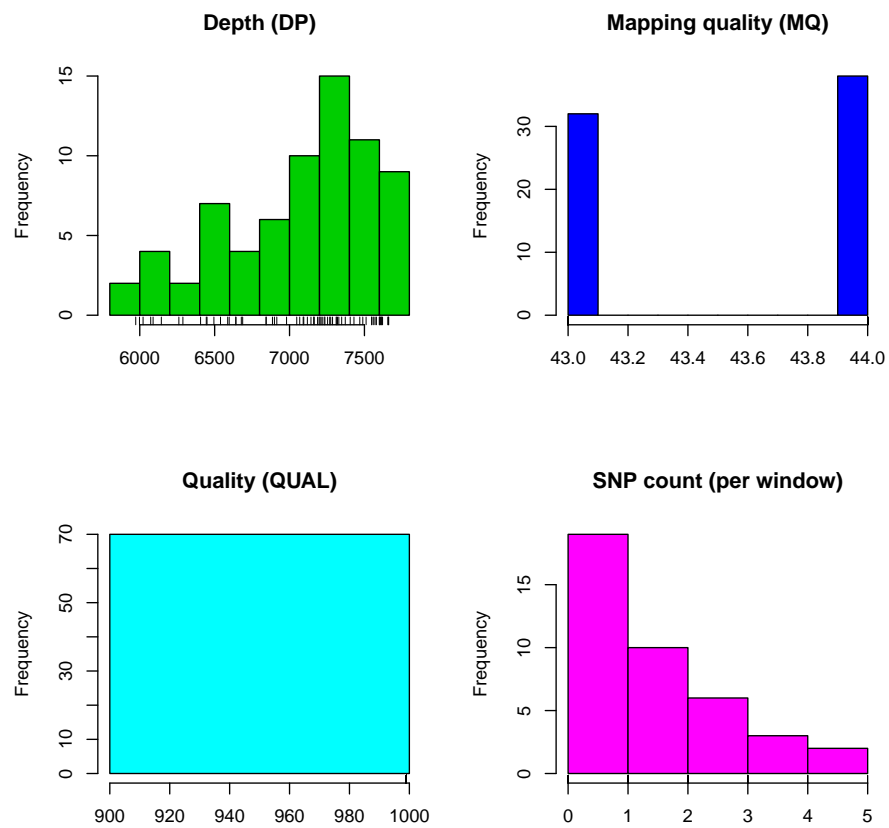


Figure 2: Quality control results for the mtDNA SNP calls after filtering and windowizing variants.

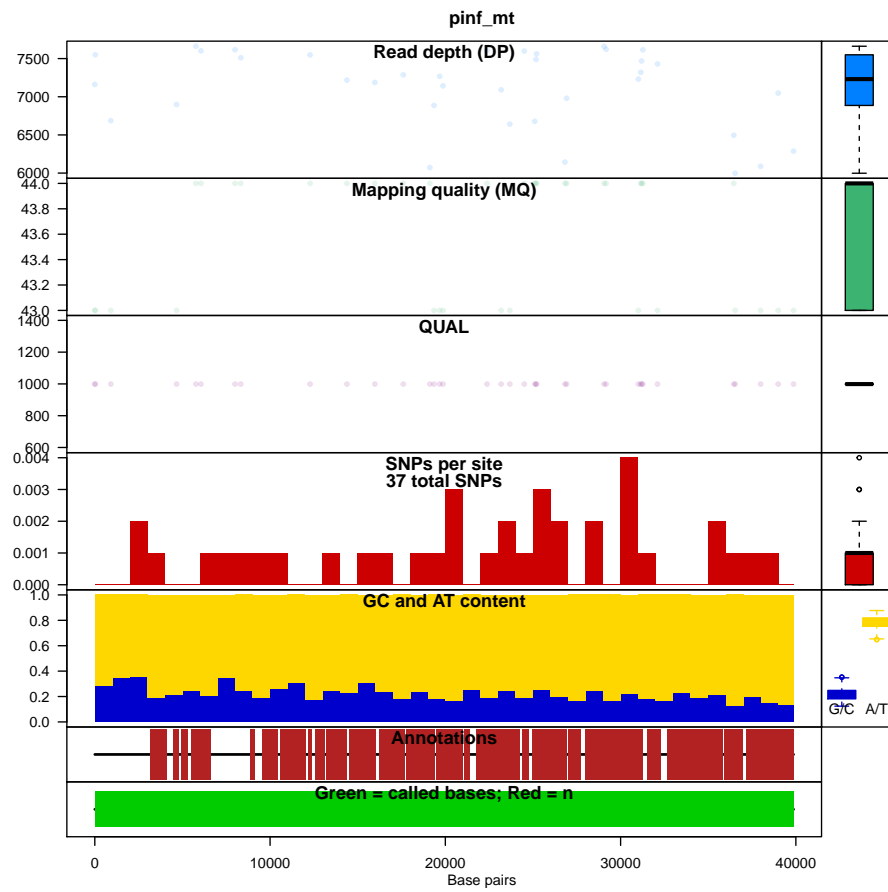


Figure 3: Whole mtDNA genome scan for the *P. infestans* samples.

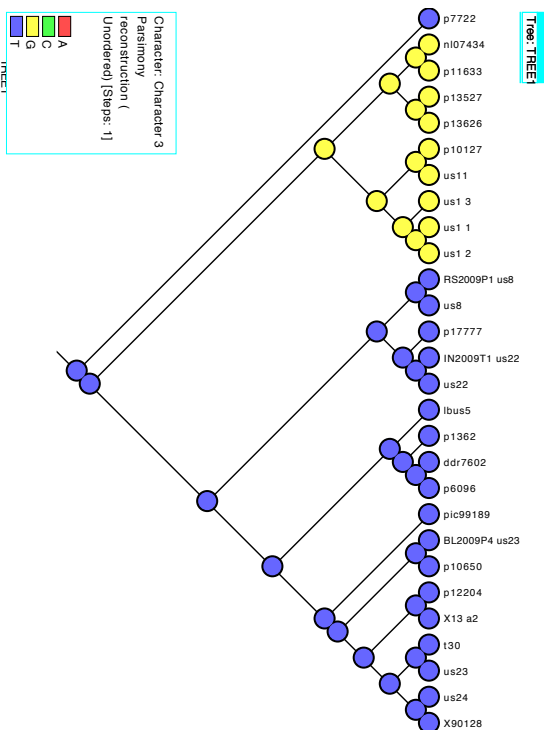
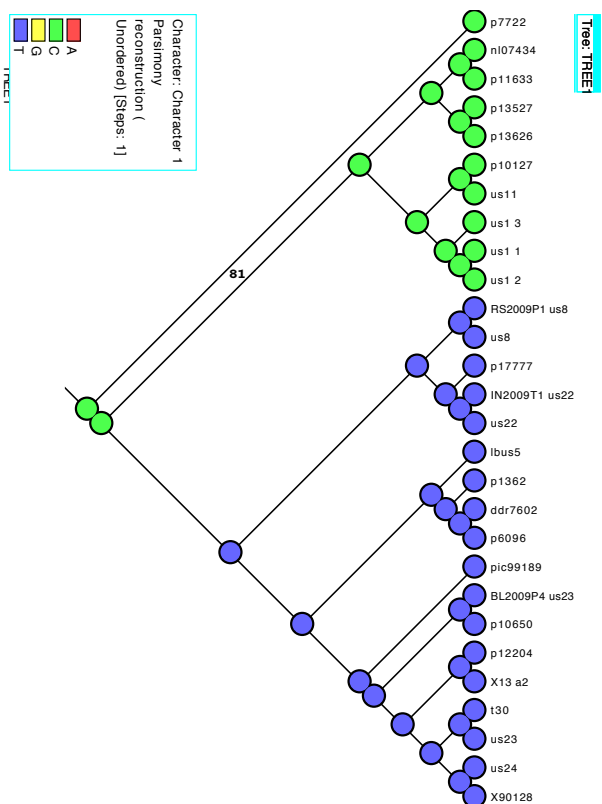
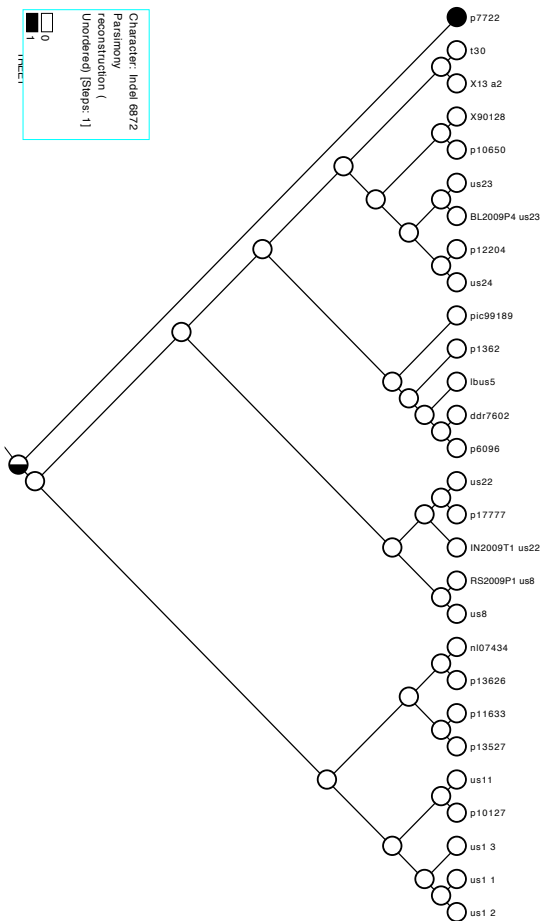
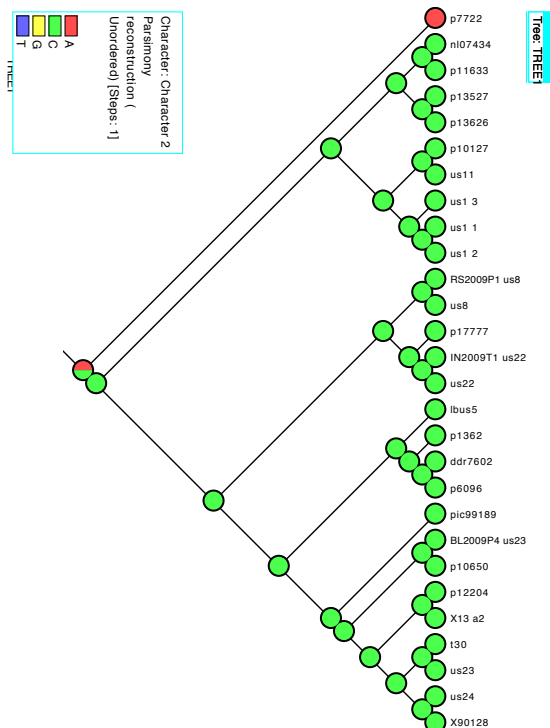
63 to the tree. At this time we're not trying to say anything bold about phylogeny
64 or character evolution. We're simply using these tools to visualize how the
65 variants segregate.

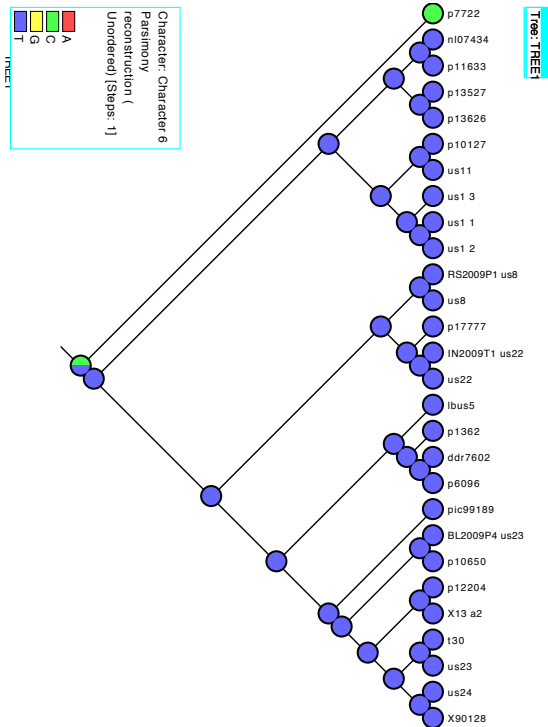
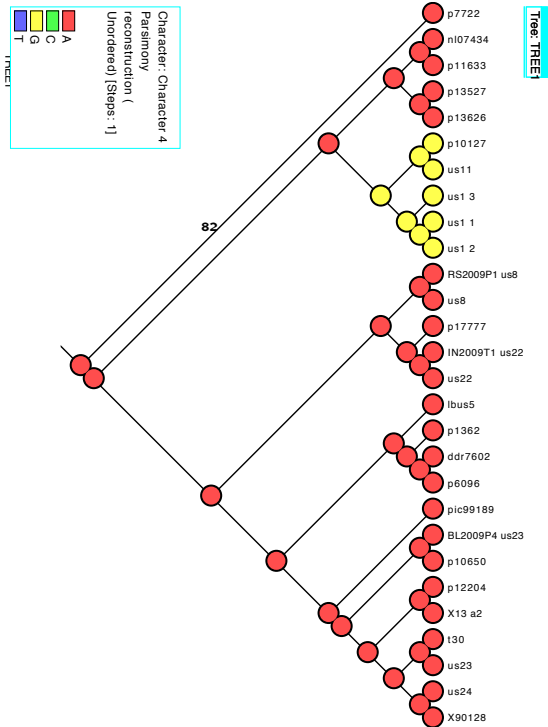
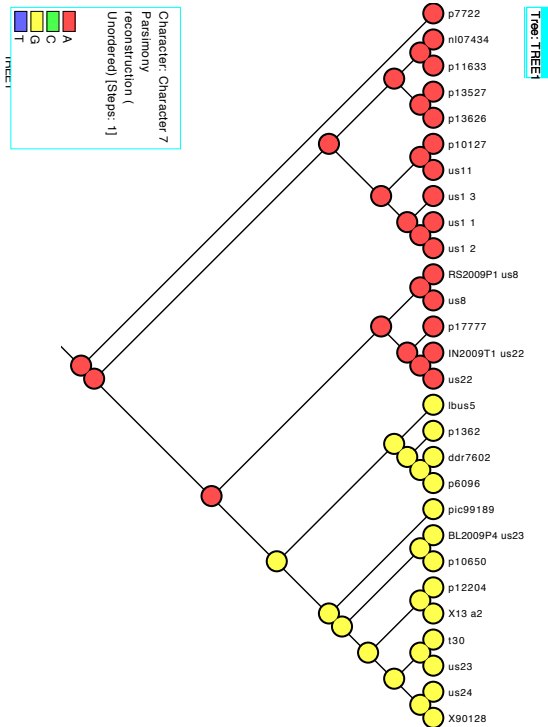
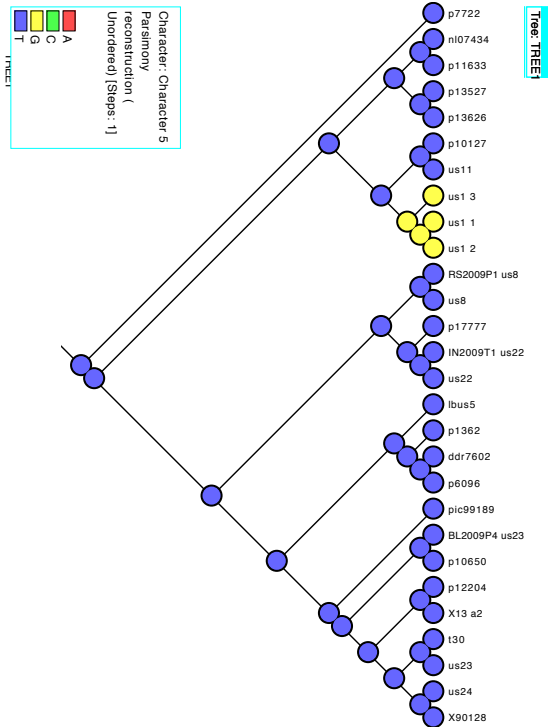
66 3.1 Phylogenetic reconstruction

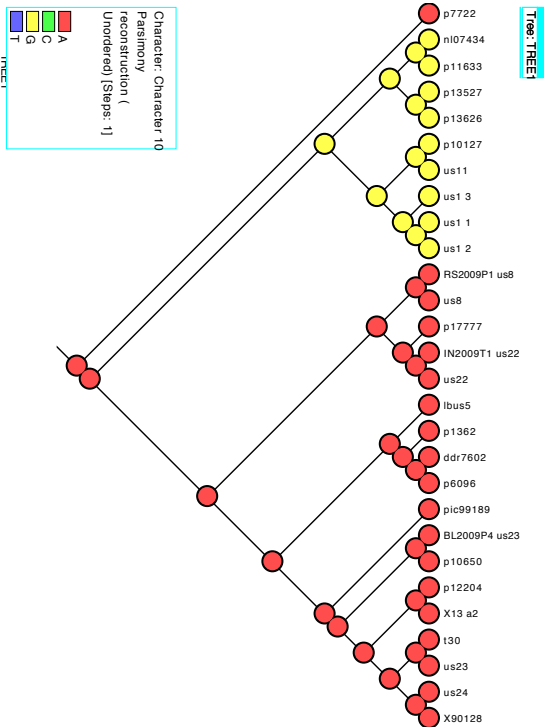
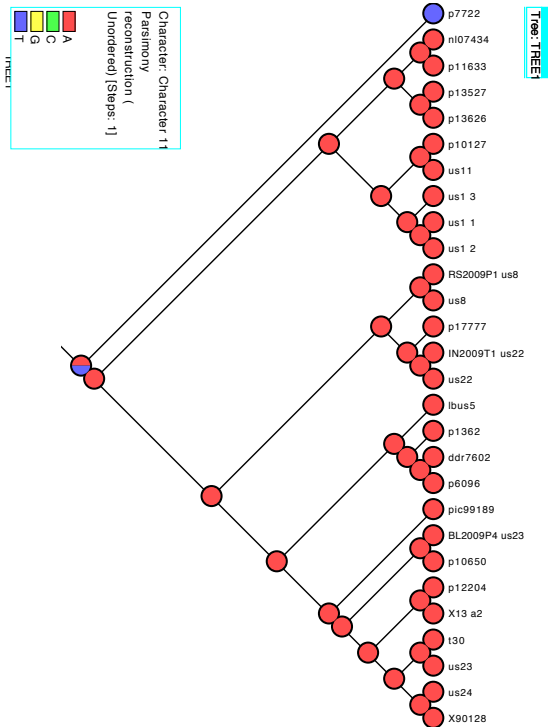
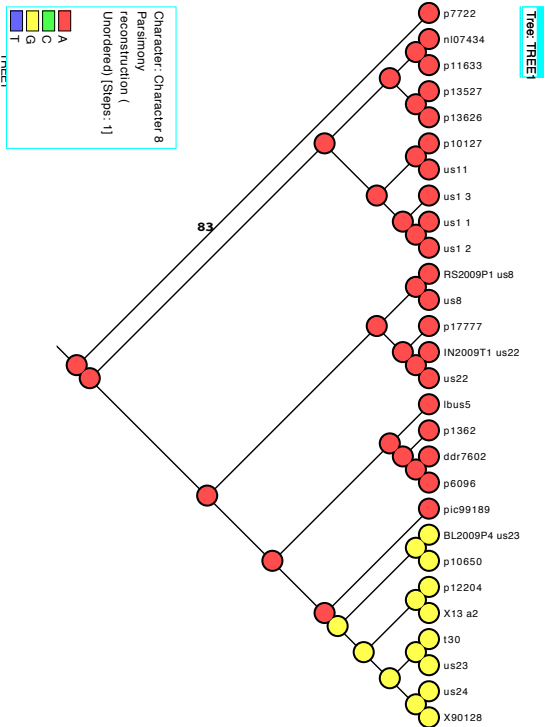
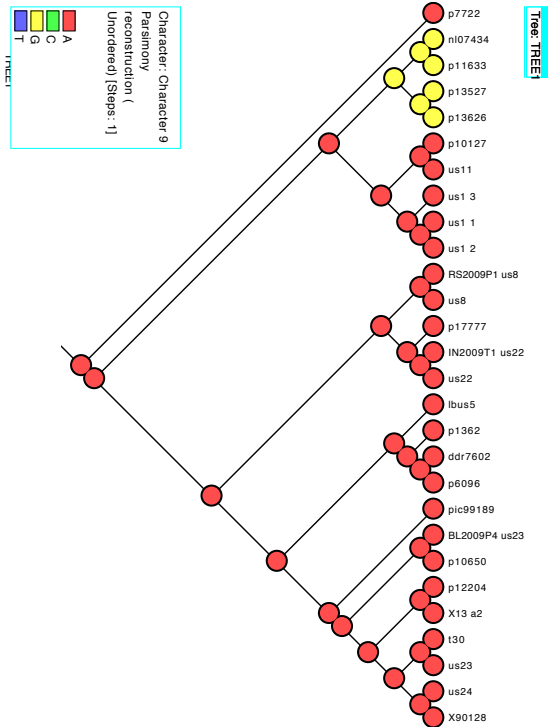
67 Using the whole genome alignment (28 sequences, 39,870 nucleotides) we per-
68 formed a whole-genome phylogeny using maximum likelihood (RAxML) and
69 Bayesian inference (BEAST). We used RAxML using no partitions, 1000 boot-
70 strap replicates, a GTR+I+G model of nucleotide evolution to obtain a biparti-
71 tioned tree with the bootstrap values mapped to the branches. For BEAST, we
72 specified p7722 (*P.mirabilis*) as the outgroup. We used a HKY+G+I model of nu-
73 cleotide substitutions, a strict molecular clock, a constant population size prior,
74 UPGMA starting tree and 10 million Markov chains. The best tree is shown in
75 Figure 4.

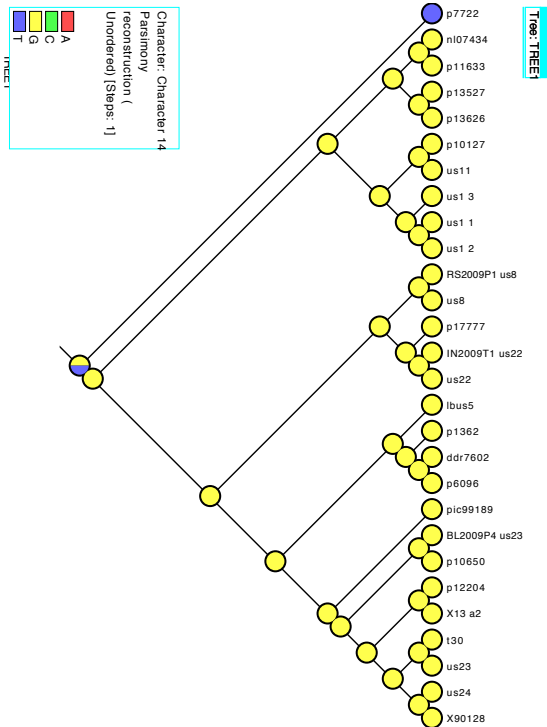
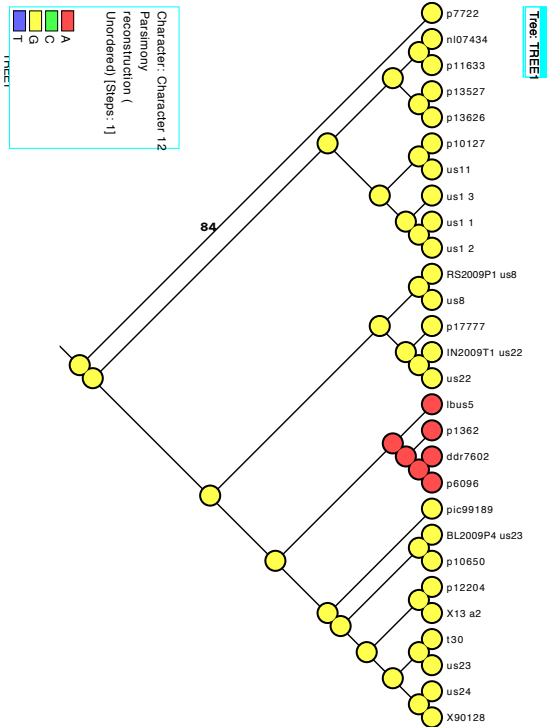
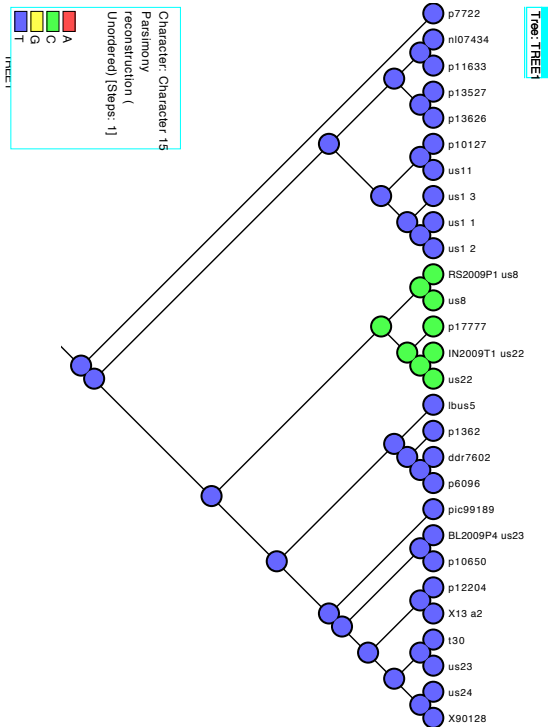
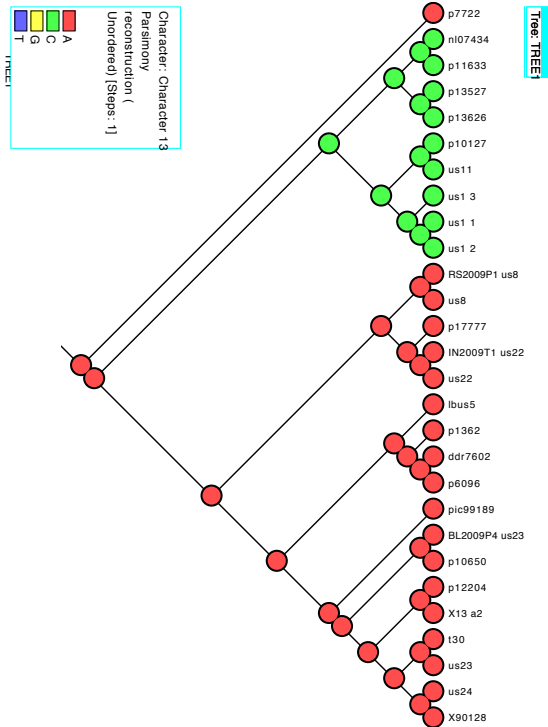
76 3.2 Mapping the SNP's in the BEAST tree

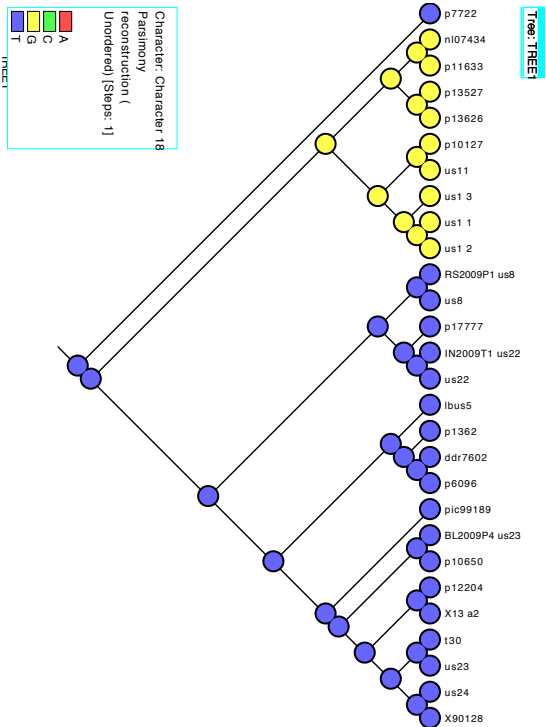
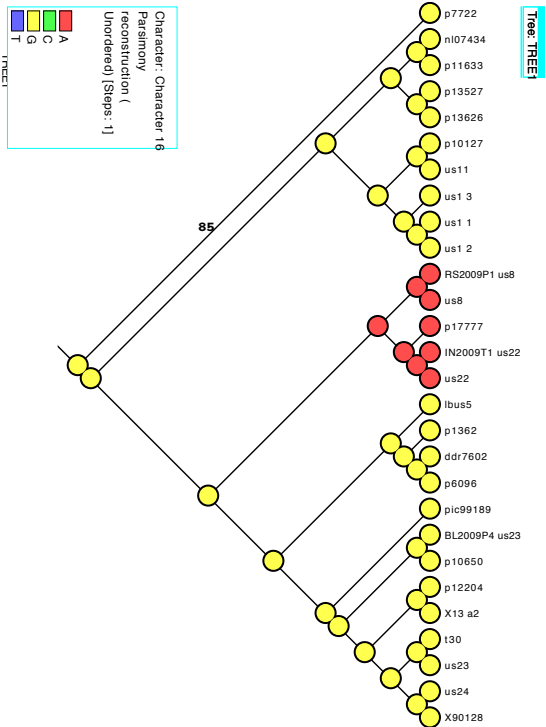
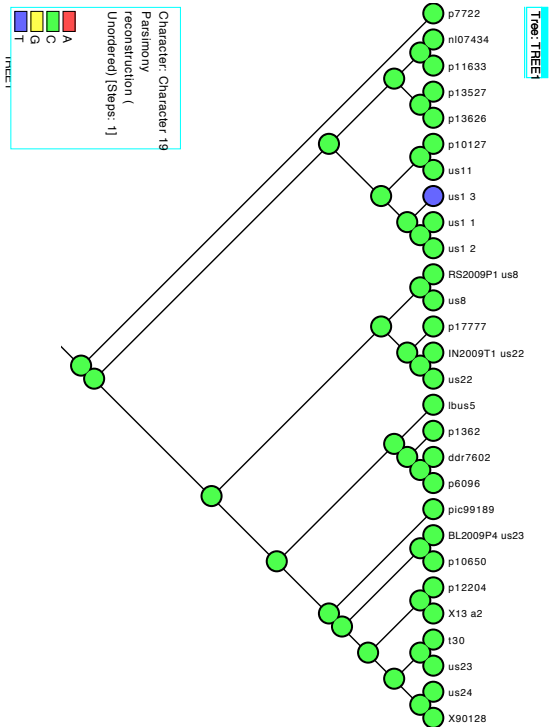
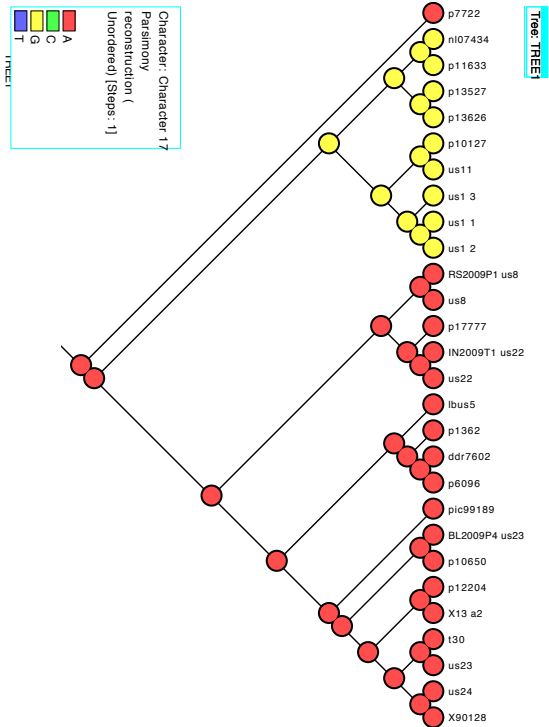
77 To map the variants found in the mtDNA genome to the coalescent tree, we
78 used Mesquite. We did a removal of invariable regions and ancestral state
79 reconstruction for all 37 SNPs using a parsimony reconstruction state (Figure
80 ??).

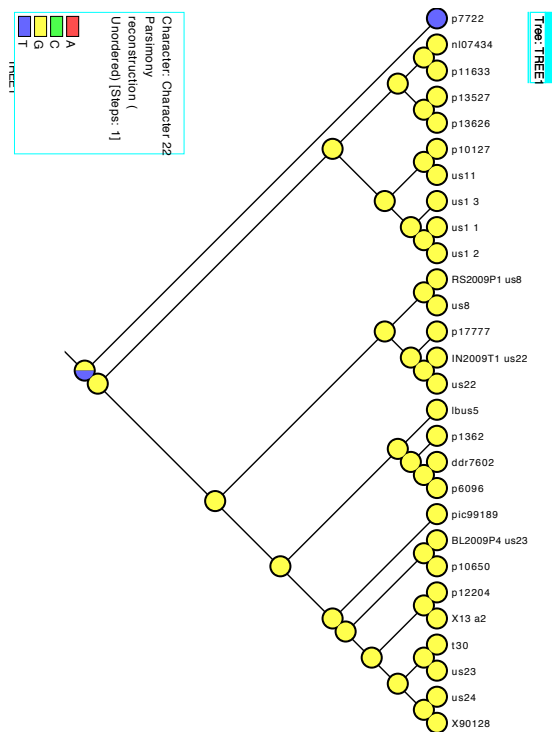
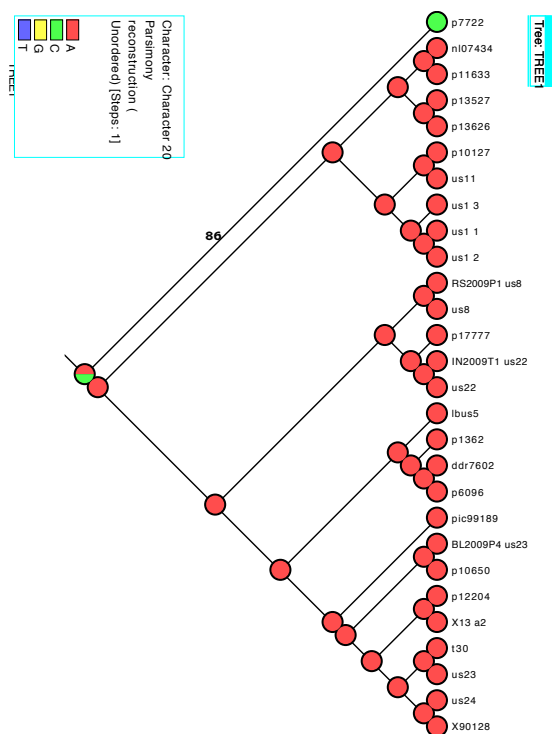
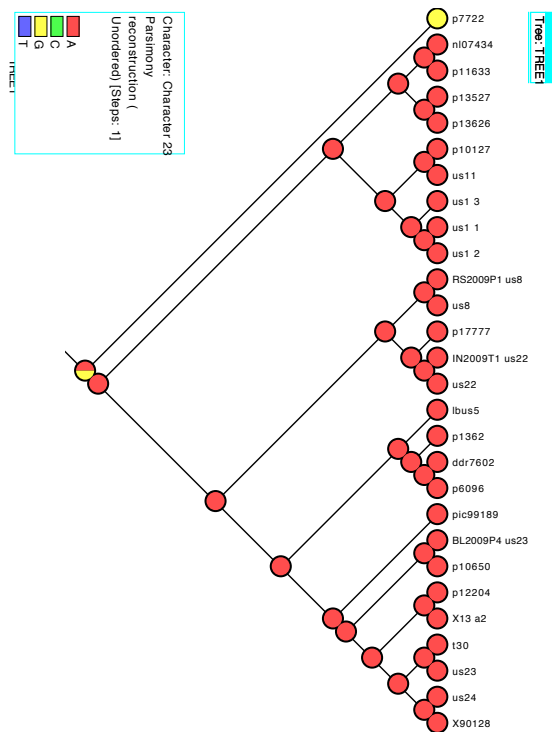
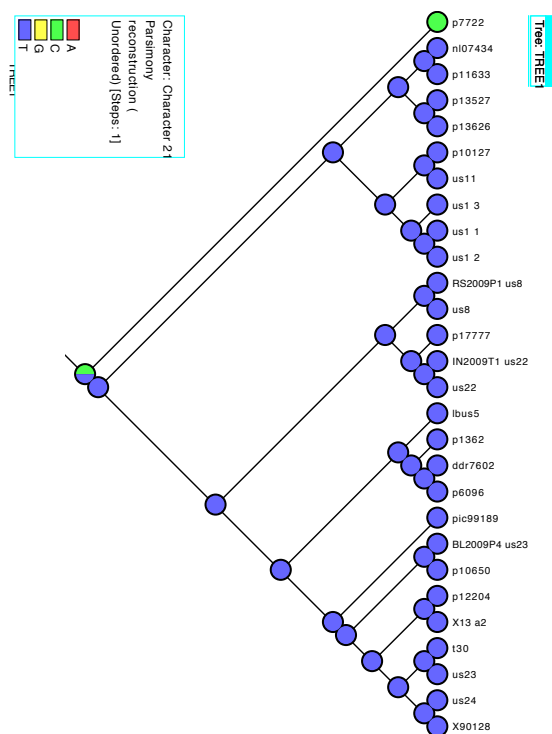


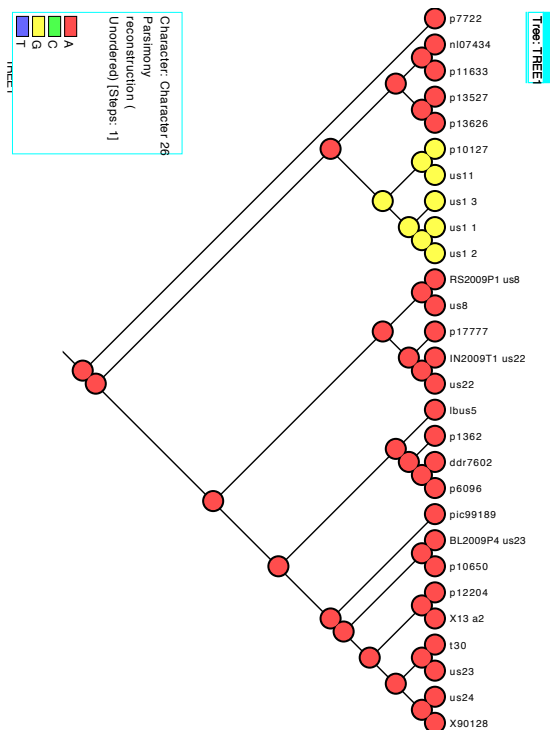
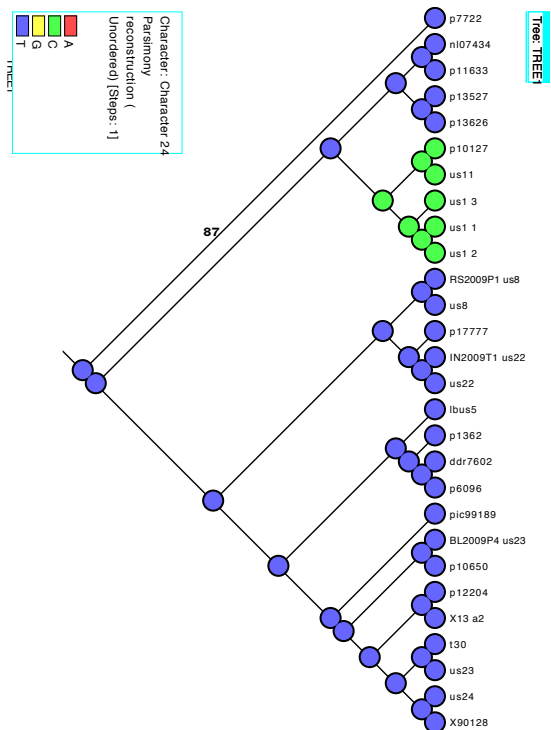
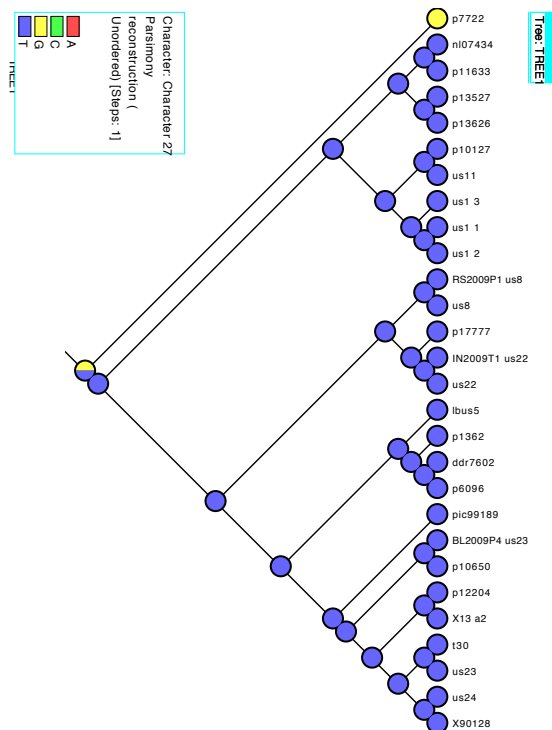
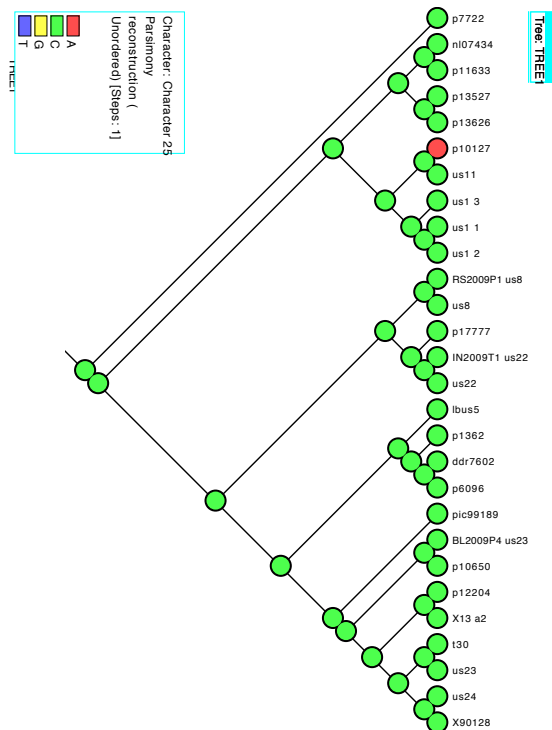


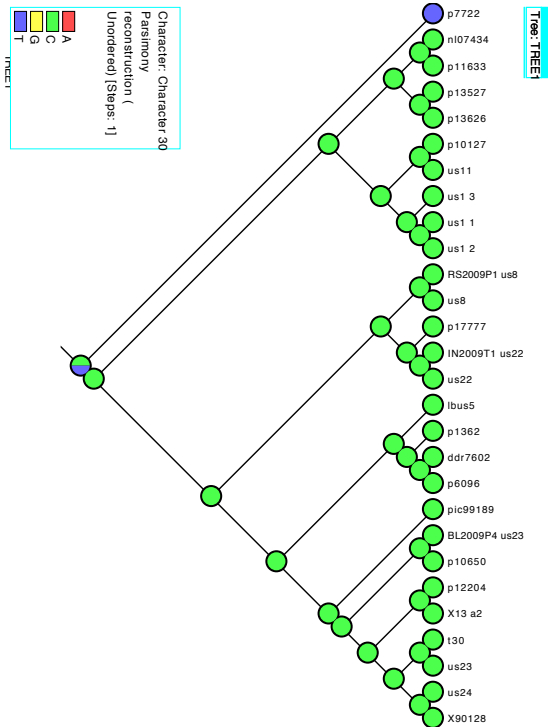
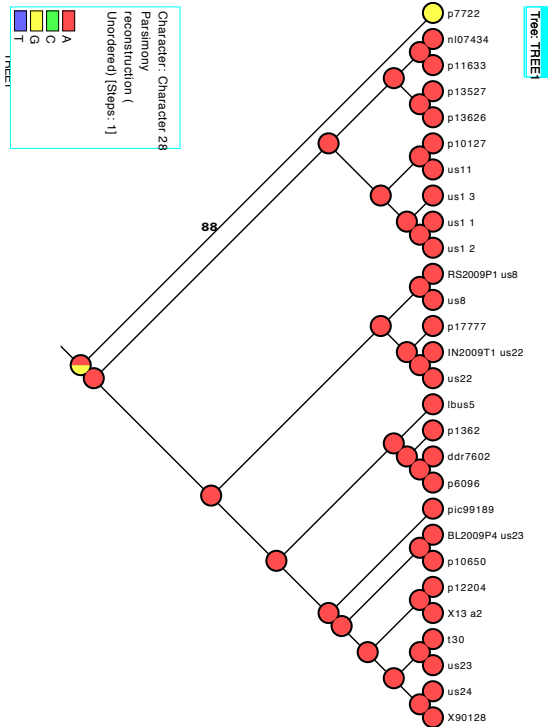
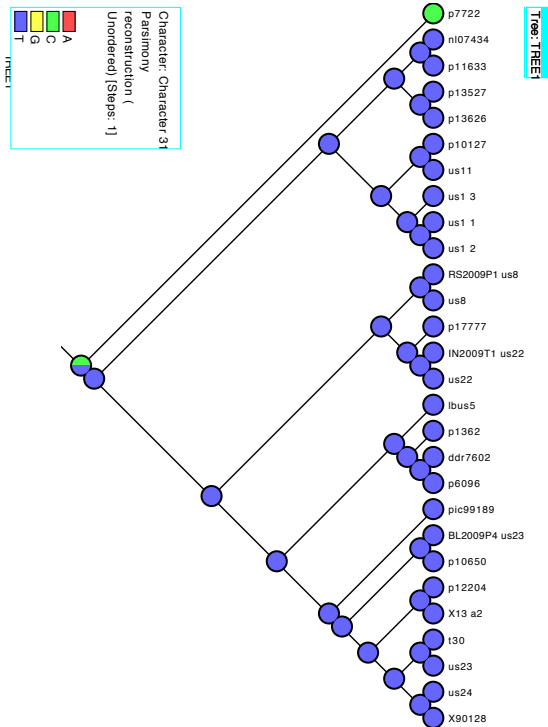
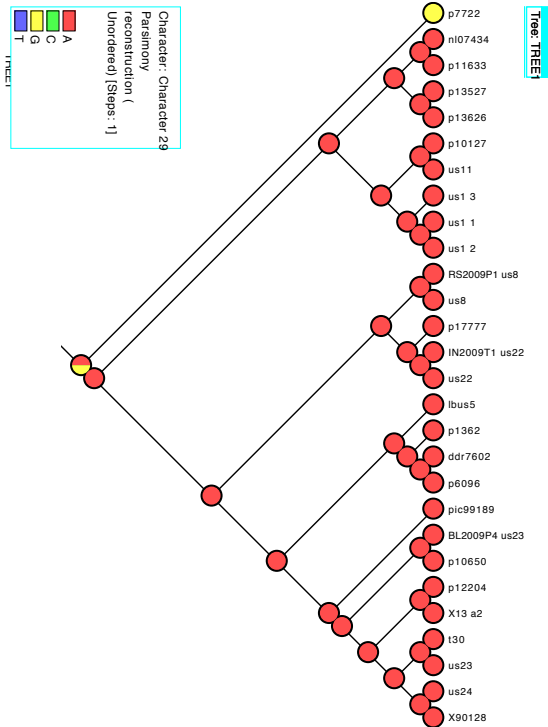




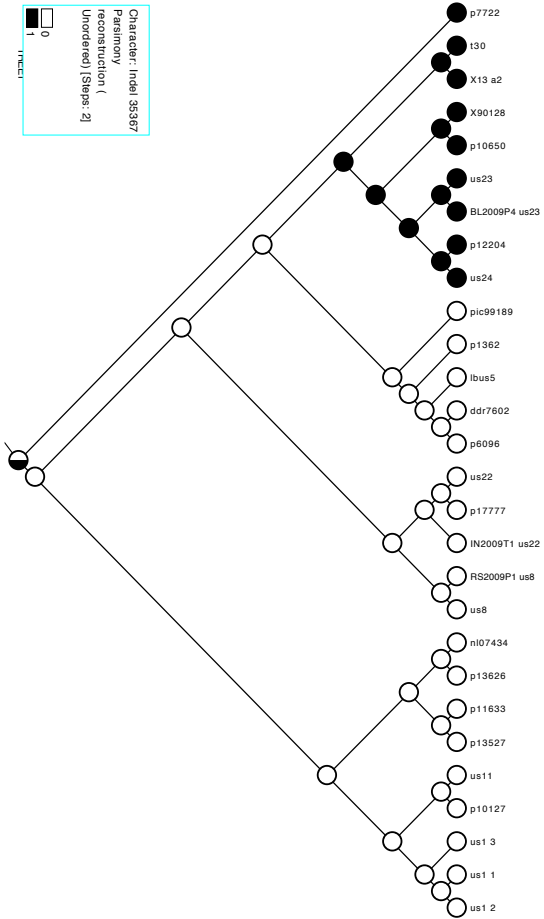




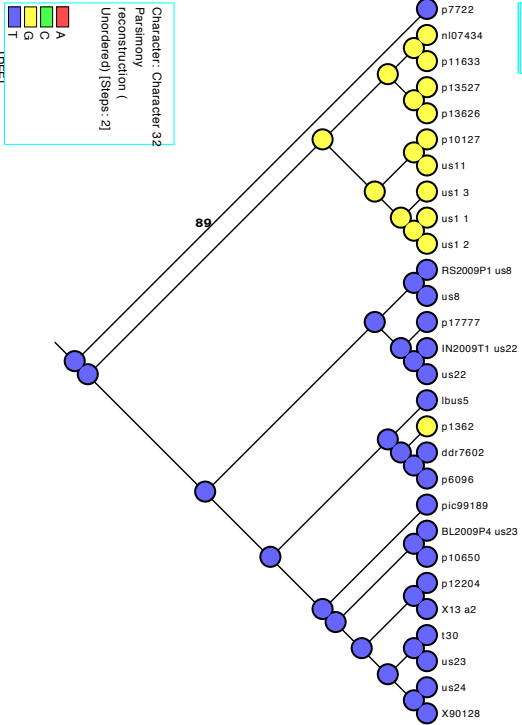




Character: Indel 35367
Parimony
reconstruction (Unordered) [Steps: 2]
0
1
Tree: TREE1

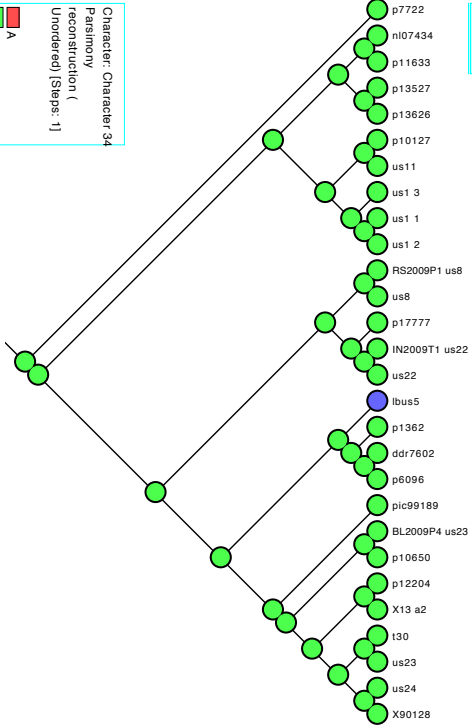


Tree: TREE1

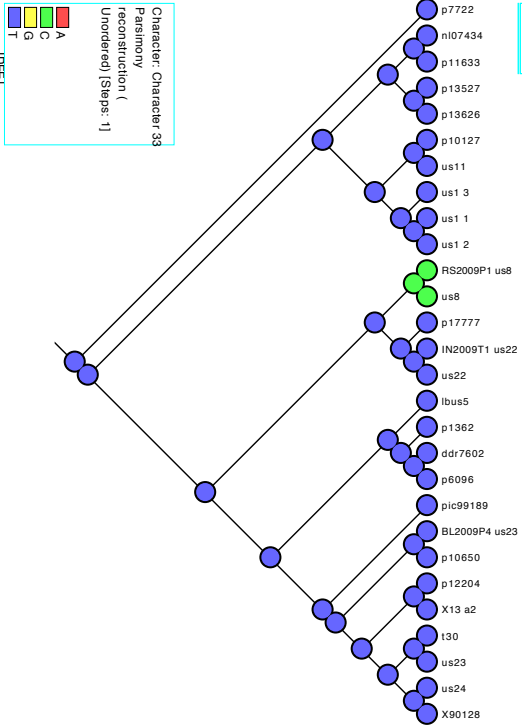


Character: Character 32
Parimony
reconstruction (Unordered) [Steps: 2]
A
C
G
T
Tree: TREE1

Character: Character 34
Parimony
reconstruction (Unordered) [Steps: 1]
A
C
G
T
Tree: TREE1



Tree: TREE1



Character: Character 33
Parimony
reconstruction (Unordered) [Steps: 1]
A
C
G
T
Tree: TREE1

Character: Character 35
Parimony
reconstruction (Unordered) [Steps: 1]

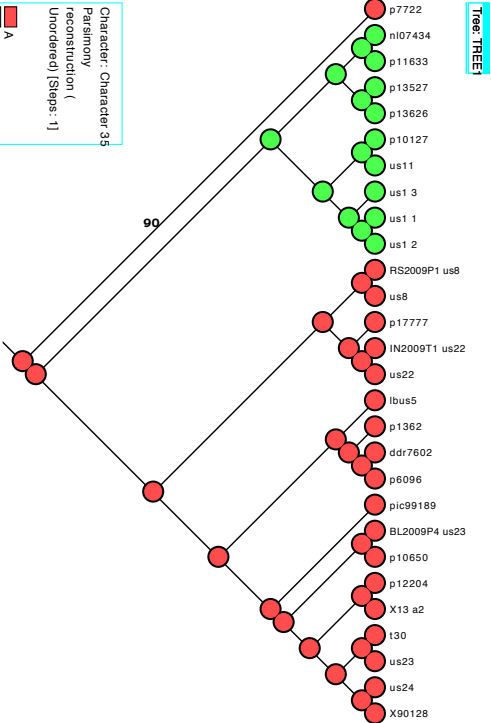
A

C

G

T

Tree



91 4 Session information

```
sessionInfo()

## R version 3.0.2 (2013-09-25)
## Platform: x86_64-apple-darwin10.8.0 (64-bit)
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
## [1] xtable_1.7-1 vcfR_0.1      knitr_1.5
##
## loaded via a namespace (and not attached):
## [1] ape_3.0-11      evaluate_0.5.1  formatR_0.10    grid_3.0.2
## [5] lattice_0.20-23 nlme_3.1-111    stringr_0.6.2    tools_3.0.2
```

92 References

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96 *tophthora infestans*. *Current genetics*, 49(1):39–46, 2006.
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98 Louise R Cooke, Graham J Etherington, Kenneth L Deahl, Rhys A Farrer,
99 Eleanor M Gilroy, Erica M Goss, et al. Genome analyses of an aggressive
100 and invasive lineage of the irish potato famine pathogen. *PLoS pathogens*,
101 8(10):e1002940, 2012.
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104 united states. *Plant Disease*, 97(7):873–881, 2013.
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106 Handsaker, Liliana M Cano, Manfred Grabherr, Chinnappa D Kodira, Syl-
107 vain Raffaele, Trudy Torto-Alalibo, et al. Genome sequence and analy-
108 sis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature*,
109 461(7262):393–398, 2009.
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113 Gabor Marth, Goncalo Abecasis, Richard Durbin, et al. The sequence align-
114 ment/map format and SAMtools. *Bioinformatics*, 25(16):2078–2079, 2009.
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118 evolution in historic samples of the Irish potato famine pathogen. *Nature*
119 *communications*, 4, 2013.
- 120 [8] Sylvain Raffaele, Joe Win, Liliana M Cano, and Sophien Kamoun. Analy-
121 ses of genome architecture and gene expression reveal novel candidate vir-
122 ulence factors in the secretome of *Phytophthora infestans*. *BMC genomics*,
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125 Bagdevi Mishra, Rahul Sharma, Chirsta Lanz, Frank N Martin, Sophien
126 Kamoun, Johannes Krause, et al. Correction: The rise and fall of the *Phy-*
127 *tophthora infestans* lineage that triggered the Irish potato famine. *eLife*, 2,
128 2013.

	CHROM	POS	REF	ALT	Tree Number
6	Supercontig_1.1	2701	C	T	1
7	Supercontig_1.1	2728	C	A	2
12	Supercontig_1.1	3519	G	T	3
36	Supercontig_1.1	6872	accc	acc	4
42	Supercontig_1.1	7857	A	G	5
44	Supercontig_1.1	8118	T	G	6
59	Supercontig_1.1	9857	T	C	7
62	Supercontig_1.1	10146	A	G	8
85	Supercontig_1.1	13684	A	G	9
106	Supercontig_1.1	15563	G	A	10
114	Supercontig_1.1	16986	G	A	11
119	Supercontig_1.1	18440	A	T	12
123	Supercontig_1.1	19793	G	A	13
124	Supercontig_1.1	20005	C	A	14
125	Supercontig_1.1	20290	G	T	15
128	Supercontig_1.1	20464	T	C	16
141	Supercontig_1.1	22711	G	A	17
147	Supercontig_1.1	23431	G	A	18
150	Supercontig_1.1	23872	G	T	19
154	Supercontig_1.1	24611	C	T	20
159	Supercontig_1.1	25142	A	C	21
160	Supercontig_1.1	25204	T	C	22
161	Supercontig_1.1	25237	G	T	23
169	Supercontig_1.1	26684	A	G	24
171	Supercontig_1.1	26767	T	C	25
186	Supercontig_1.1	28680	C	A	26
187	Supercontig_1.1	28783	A	G	27
192	Supercontig_1.1	30427	T	G	28
193	Supercontig_1.1	30552	A	G	29
194	Supercontig_1.1	30591	A	G	30
195	Supercontig_1.1	30660	C	T	31
201	Supercontig_1.1	31403	T	C	32
221	Supercontig_1.1	35296	G	T	33
222	Supercontig_1.1	35367	taaaaaaaaaaa	taaaaaaaaaaa	34
233	Supercontig_1.1	36663	T	C	35
239	Supercontig_1.1	37562	C	T	36
242	Supercontig_1.1	38345	C	A	37

Table 1: Variants remaining after filtering.

Table 2: Diagnostic SNP positions for the mtDNA genome after filtering.

Position	SNP	Diagnostic for
7857	A/G	p10127, us11 and us1
8118	T/G	us1
20464	T/C	p17777, us22 and us8
22711	G/A	p17777, us22 and us8
26767	A/G	p10127, us11 and us1
28783	A/G	p10127, us11 and us1
36663	T/C	us8