

Using Meta-Transcriptomics and Ancient DNA to Reveal Microbial Diversity and Evolution

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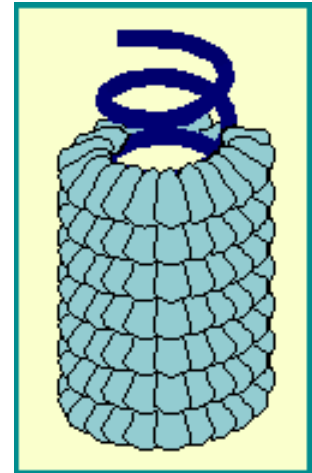


The Discovery of Viruses

The science of virology begin in earnest at the junction of the 19th and 20th centuries

Tobacco Mosaic Virus

- 1879: Adolf Mayer inoculated healthy plants with the juice of ground up leaves from plants that had tobacco mosaic disease.
- 1892: Dimitri Ivanofsky passed infected sap through unglazed porcelain filter and found it retained its infectious properties.
- 1898: Martinus Beijerinck showed that the diluted sap could regain its 'strength' after replication in living, growing plant tissue.



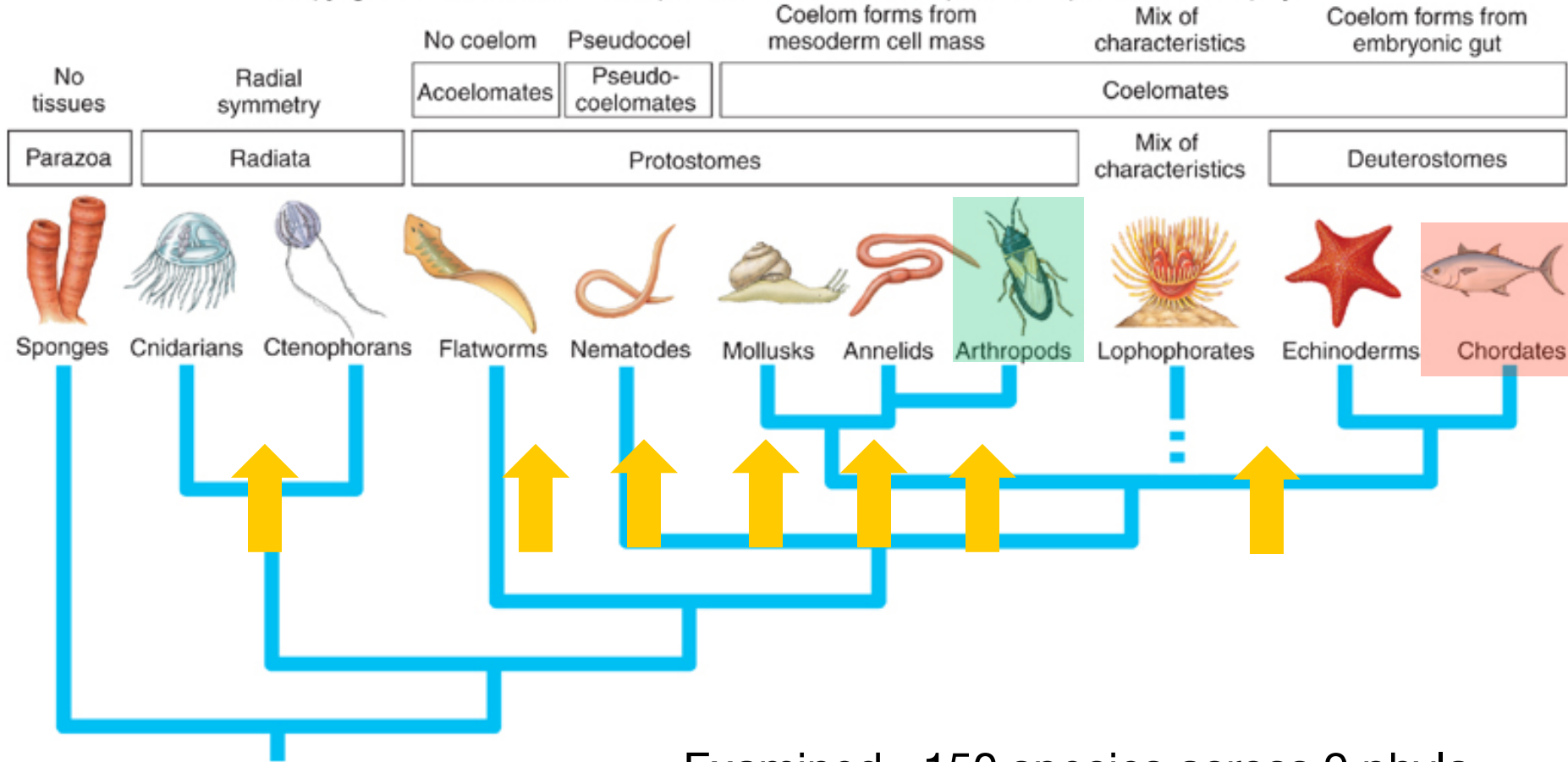
Tobacco Mosaic Virus (TMV)

More Viruses

- 1898 – 1915: Several 'filterable' agents isolated e.g. FMDV (1898), Myxoma (1898), Yellow Fever (1901), Rabies (1903), Polio (1909), Bacteriophage (1915).

Sampling RNA Viruses Across the Tree of Animal Life

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- Examined ~150 species across 9 phyla

RNA Virus Discovery

(‘Meta-transcriptomics’)

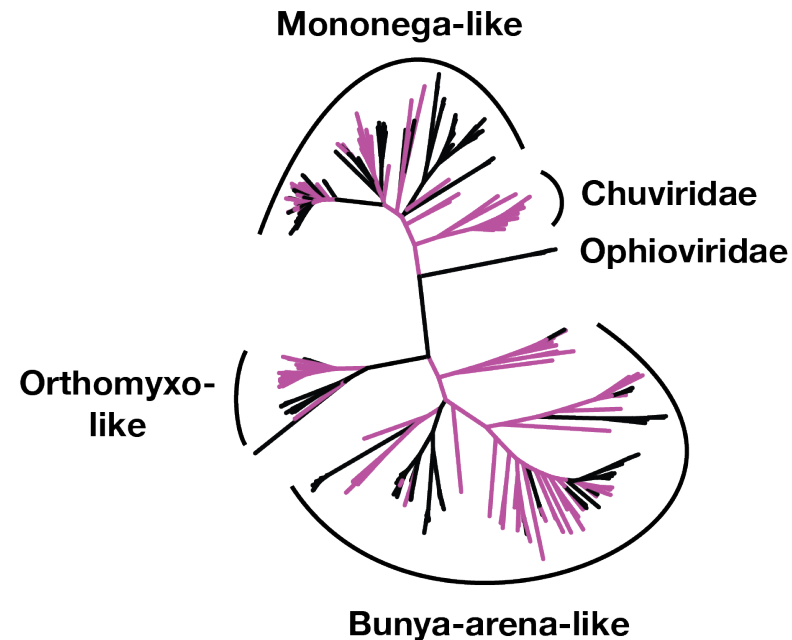
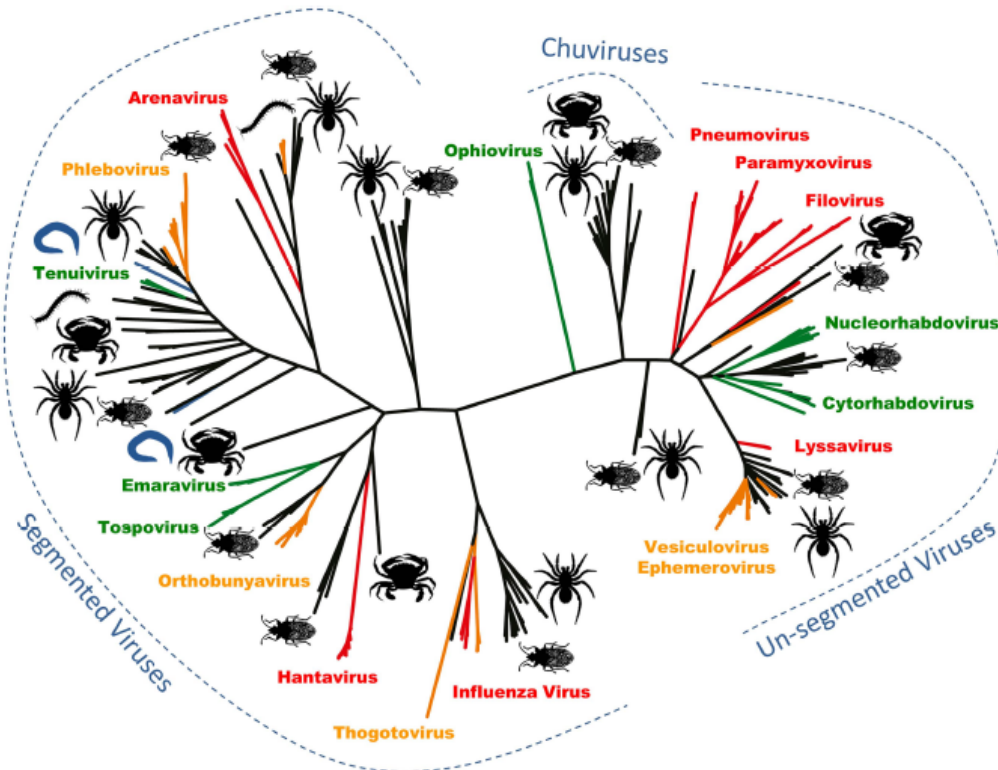
- Sample processing (homogenize whole animals or internal organs)
- cDNA library preparation
 - Extract total RNA
 - Remove host ribosomal RNA
 - Fragmentation, and cDNA library construction
- Next-generation sequencing (RNA-seq; Illumina Hi-Seq)
 - Huge data (72 libraries, >1 Tbp)
 - Rich information (hosts, viruses, bacteria, other parasites)
- Data analyses
 - *De novo* assembly using Trinity
 - Blastx (translated nucleotide against protein database)
 - Structural similarity searching
 - Mapping and quantification (bowtie2, trinity, etc.)
 - Phylogenetics (particularly RdRp)

Untapped Viral Biodiversity

- Negative-Sense RNA Viruses

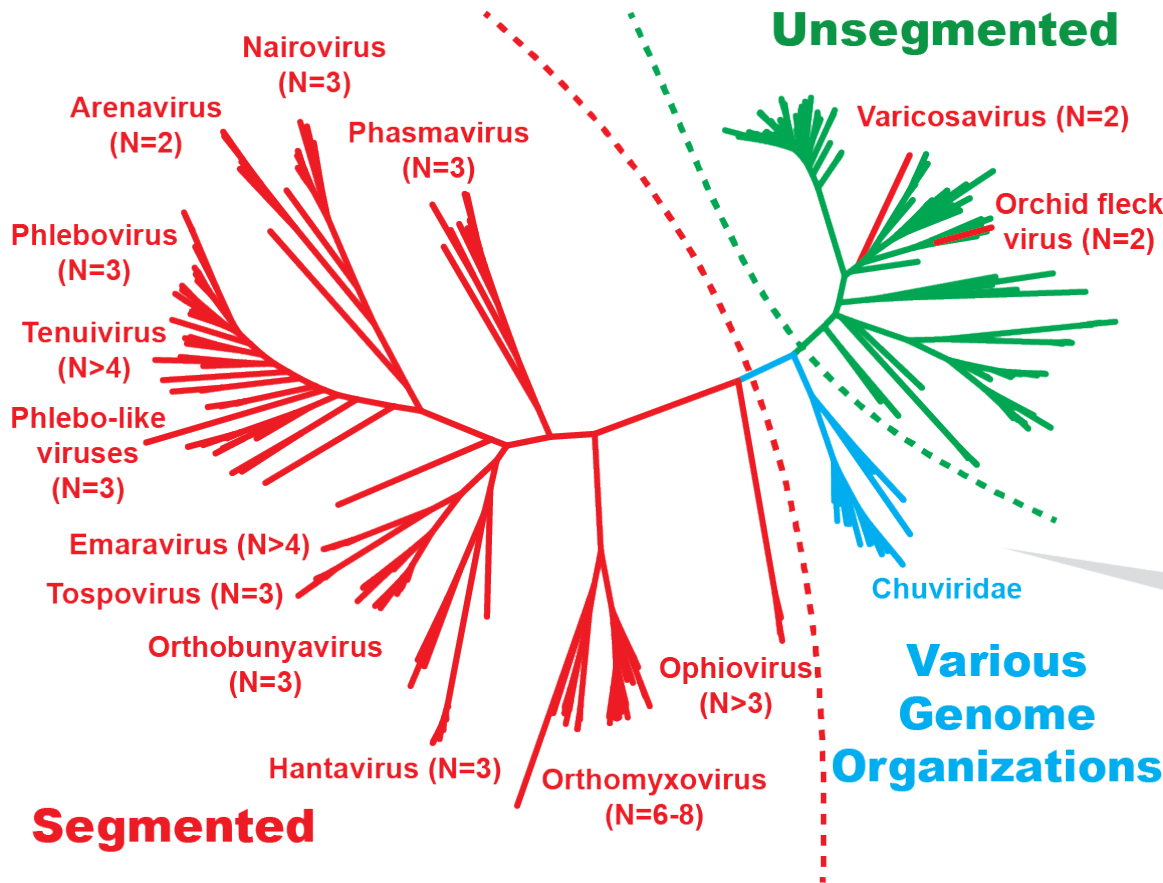
- Invertebrates carry an extraordinary diversity of RNA viruses that often fall basal to what is seen in vertebrates

Wuhan, China
October 2014

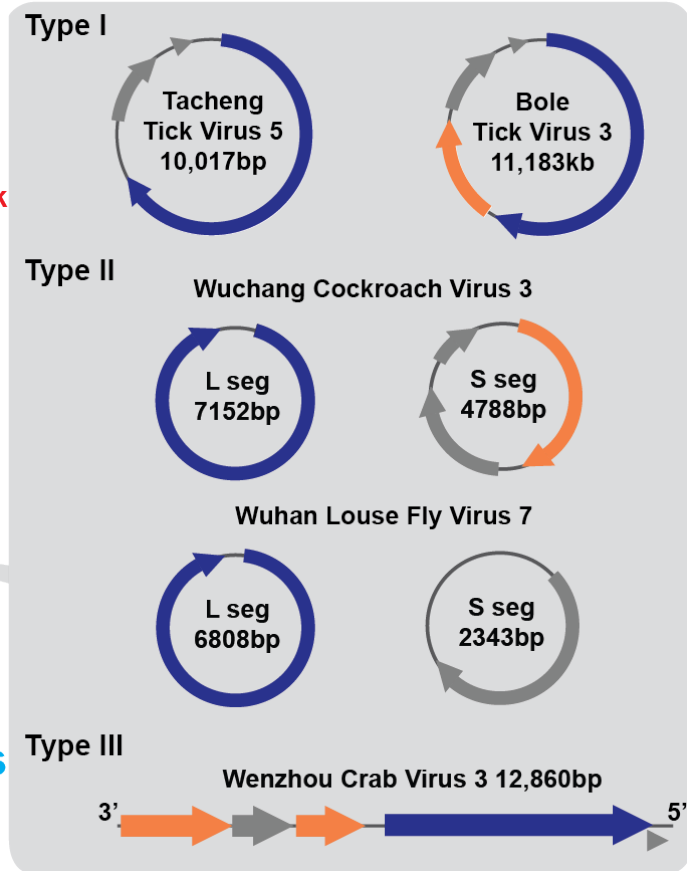


The Unusual “*Chuviridae*”

- Chuviruses can be unsegmented, bi-segmented and perhaps circular RNA molecules



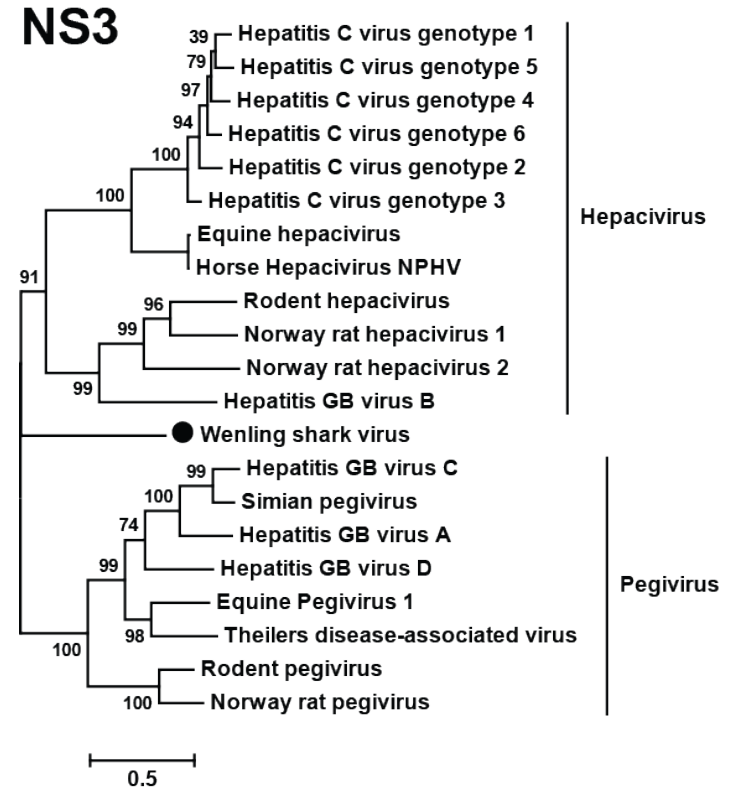
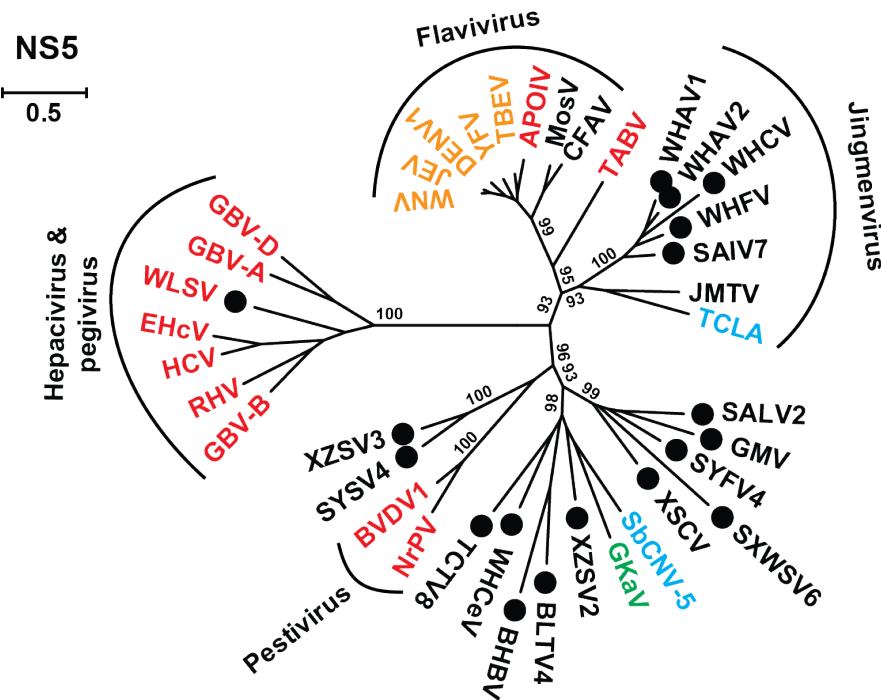
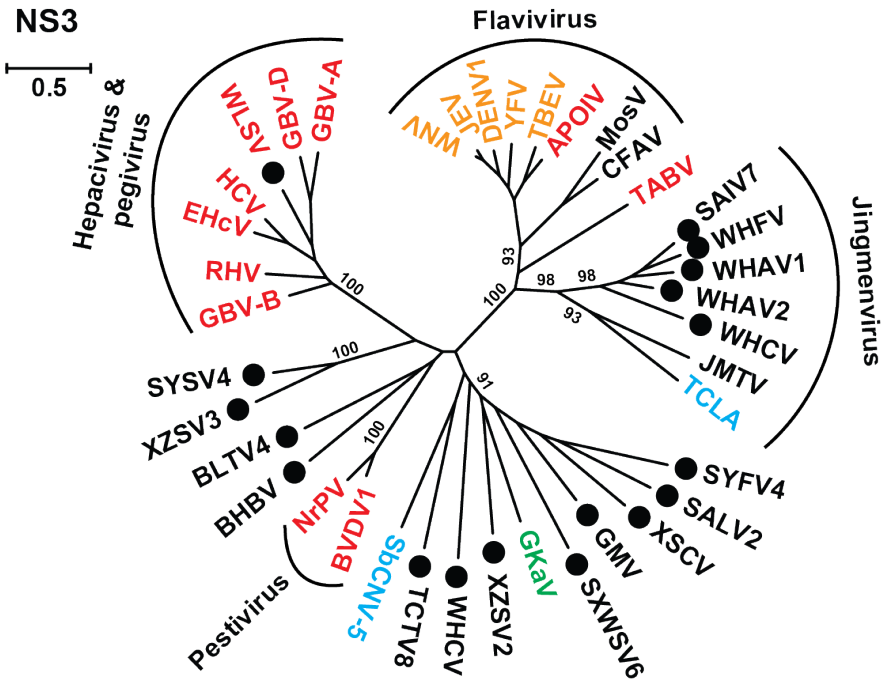
Circular RNA genomes



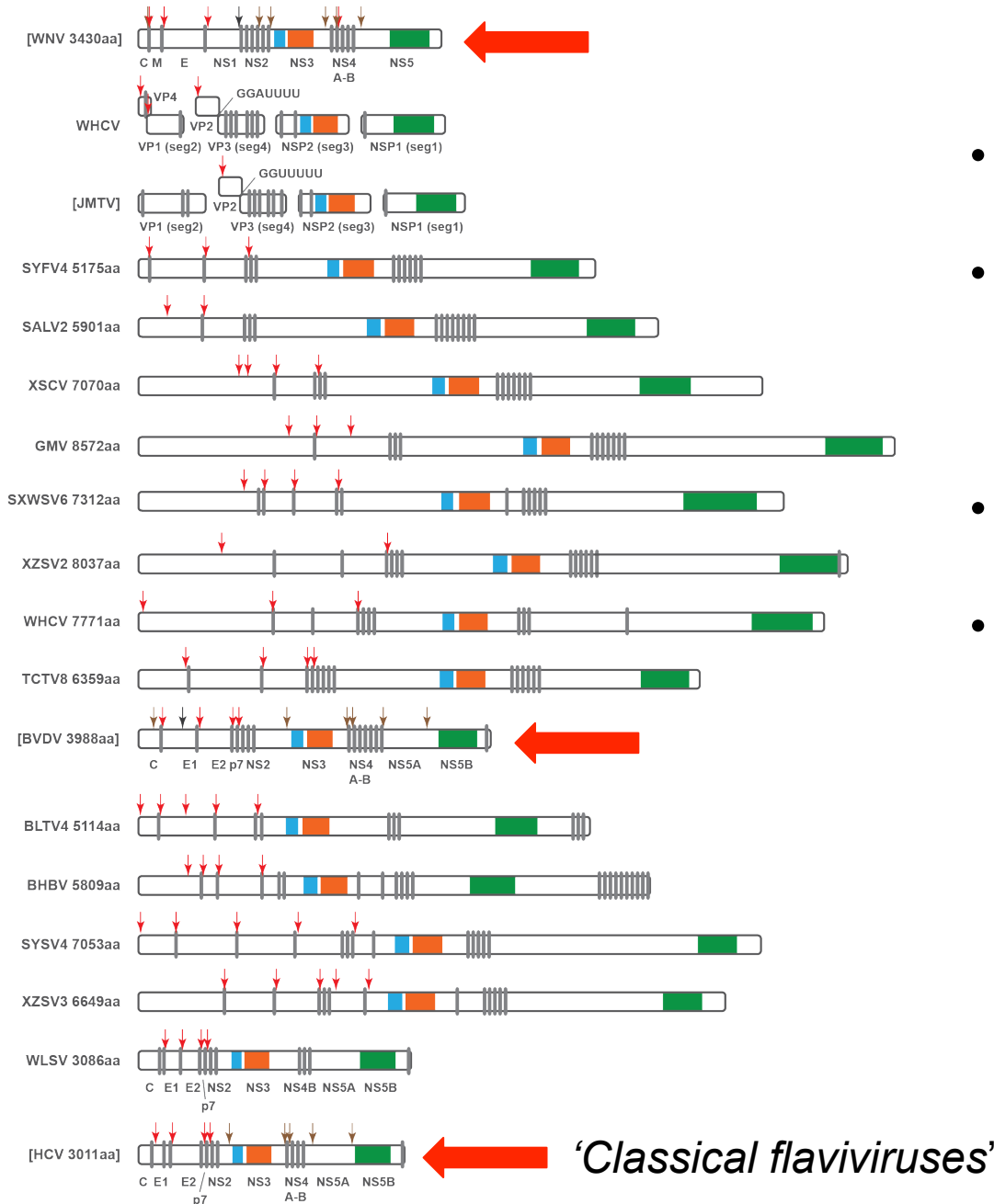
- Genome segmentation and size are relatively fluid characteristics (although all RNA virus genomes are still <32 kb)

Biodiversity of the “Flavi-Like” Viruses

- RNA-seq analysis of pools of arthropod and vertebrate samples
- Discovered one novel hepacivirus, 12 diverse flavi-like viruses, and 5 novel segmented Jingmenviruses



Genomic Diversity of the “Flavi-Like” Viruses



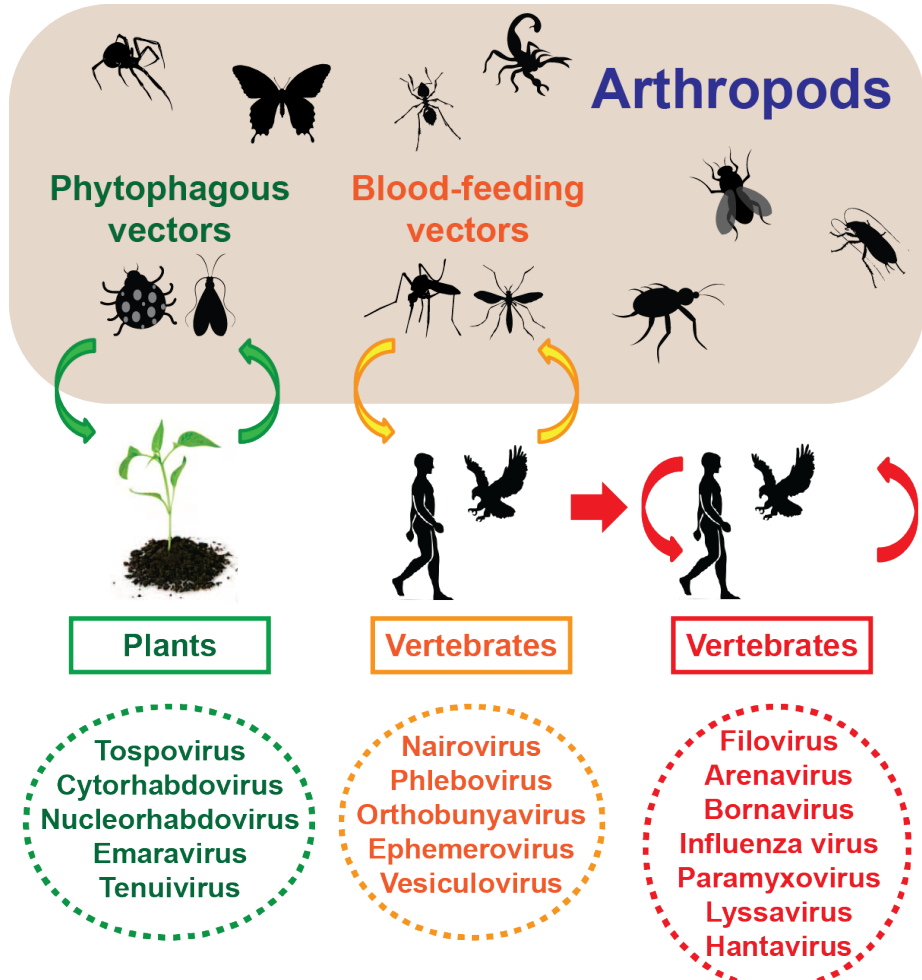
- Huge diversity of genome structures and sizes
- Length range from 9-26 kb: shortest in the hepaciviruses/pegiviruses and in vertebrate viruses
- Both segmented and unsegmented genomes
- Segmented genomes derived from unsegmented ones

Arthropods are a bridge between the vertebrate, invertebrate and plant worlds

Table 1. Distribution and Age of Endogenous Viral Elements Derived from RNA and DNA Viruses Found in Animal Genomes

Viral Family and Type	Host Range	Number of Elements	Estimated Minimum Age	Reference
<i>Bomaviridae</i> (ssRNA ⁻) ^a	Mammals ^b	67	93 MYA	Belyi et al. (2010b), Horie et al. (2010), Katzourakis and Gifford (2010)
<i>Filoviridae</i> (ssRNA ⁻) ^a	Mammals	25	30 MYA ^c	Belyi et al. (2010b), Katzourakis and Gifford (2010), Taylor et al. (2010)
<i>Bunyaviridae</i> (ssRNA ⁻) ^a	Insects	40	Unknown	Katzourakis and Gifford (2010)
<i>Rhabdoviridae</i> (ssRNA ⁻) ^a	Insects	143	Unknown	Katzourakis and Gifford (2010)
<i>Orthomyxoviridae</i> (ssRNA ⁻)	Insects	1	Unknown	Katzourakis and Gifford (2010)
<i>Reoviridae</i> (dsRNA)	Insects	1	Unknown	Katzourakis and Gifford (2010)
<i>Flaviviridae</i> (ssRNA ⁺)	Insects	5	Unknown	Crochu et al. (2004), Katzourakis and Gifford (2010)

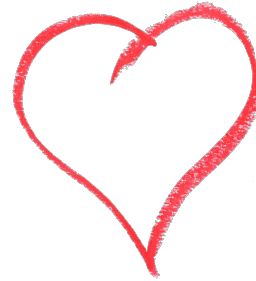
Still scratching the surface of the virosphere



Example of negative-sense RNA viruses

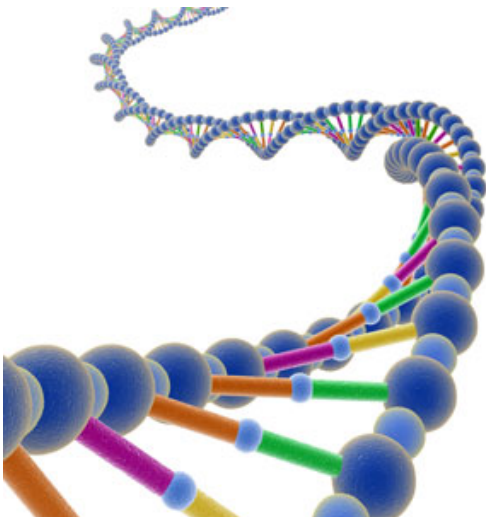
Using Ancient Pathogens to Understand Microbial Evolution

“Ancient DNA” Opens Up New Ways to Study Disease Pandemics



www.stinkymeat.net

Modern DNA



Ancient and degraded DNA





Three Pandemic Waves of Plague

1. Justinian's Plague (540-590 AD)
2. Black Death-18th Century plagues
3. 19th Century-modern plagues

Brisbane, 1901

Plague bacterium
(*Yersinia pestis*)

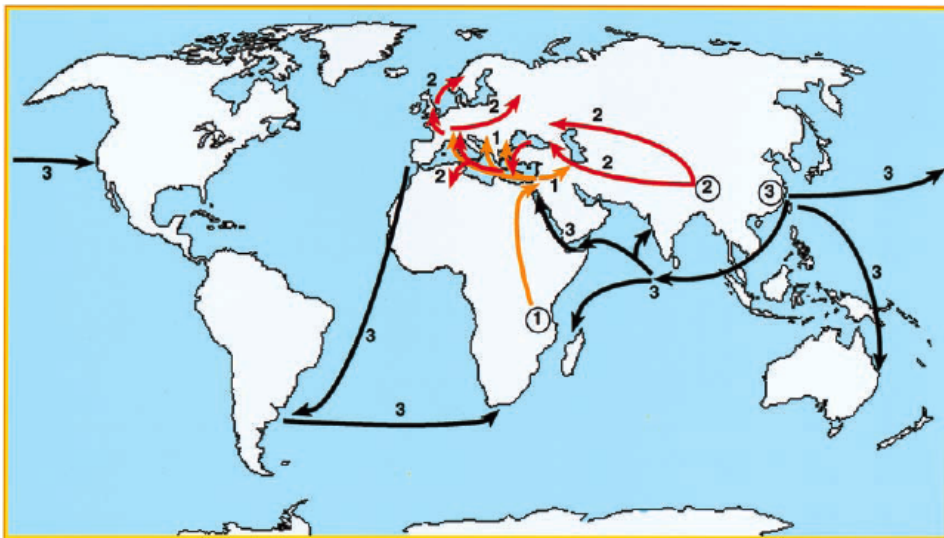


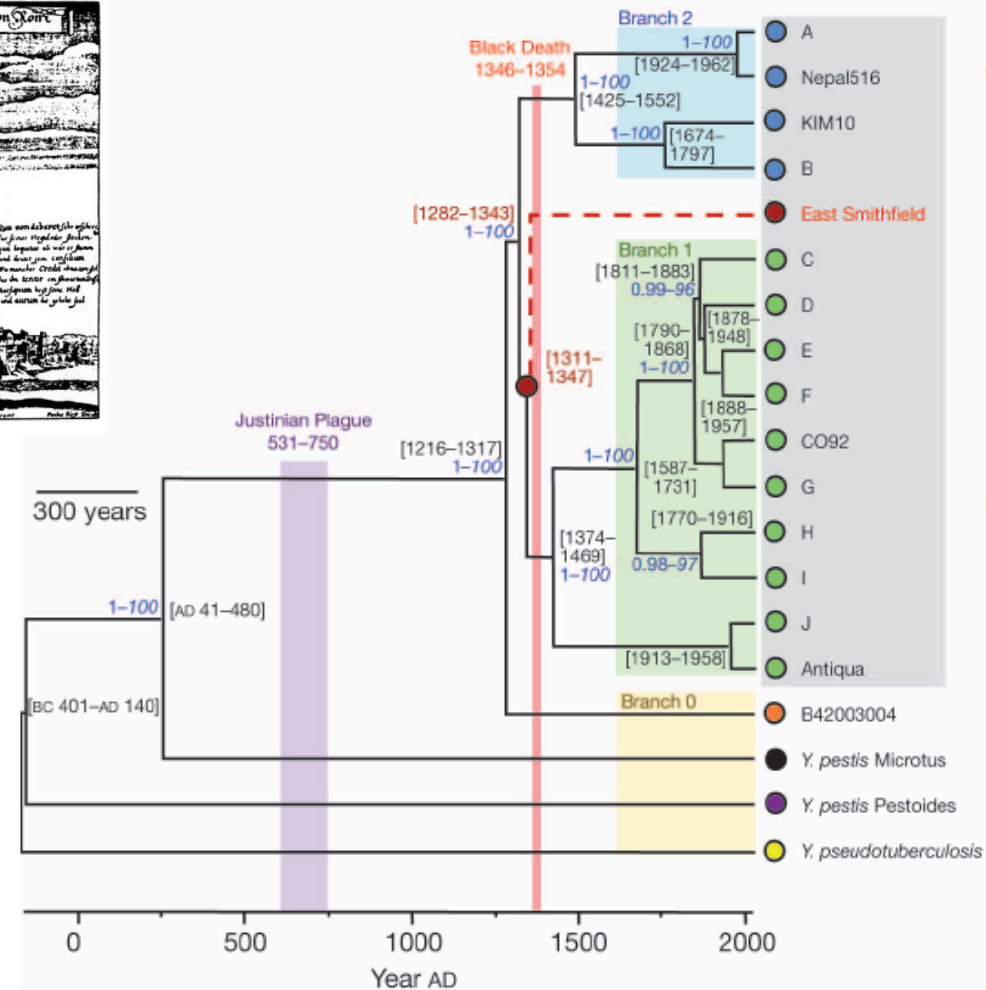
Fig. 1. Routes followed by the three plague pandemic waves, labeled 1, 2, and 3. Circled numbers indicate the regions thought to be the origins of these pandemic waves.

Black Death Genomics



Hendrik Poinar
(McMaster University)

'Targeted Enrichment coupled with
Next-Generation Sequencing (TENGS)'



East Smithfield, London



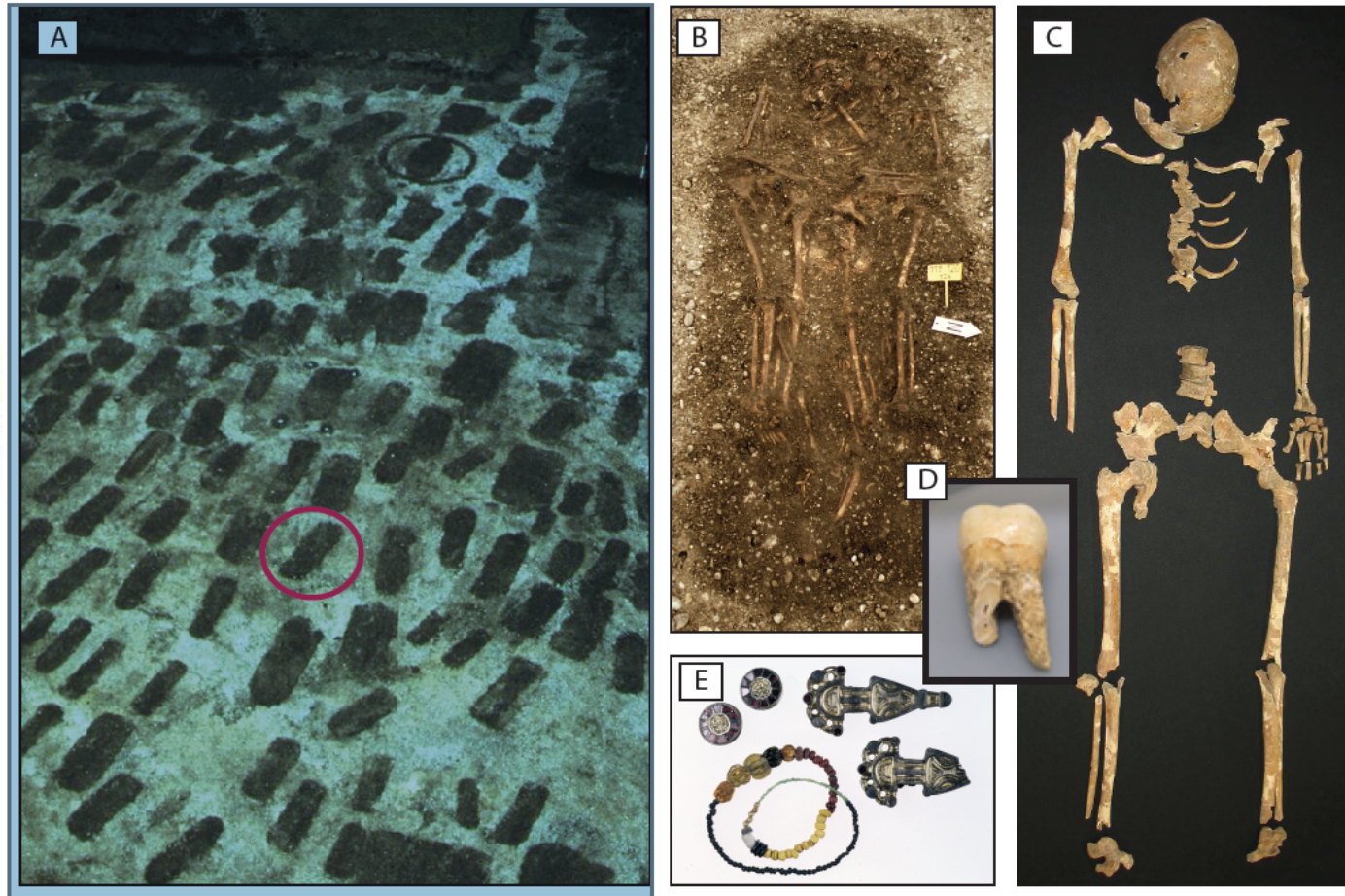
The East Smithfield strain (1348 AD) falls
basal to all modern human *Y. pestis* strains

Byzantine Genomics (Justinian's Plague)

- Early Medieval graveyard; Aschheim-Bajuwarenring, Bavaria, Germany (438 individuals)
- Extracted DNA from 5 human teeth of two individuals (A120, A76)
- Radiocarbon dating of both individuals (A120 to 533 +/- 98 cal. CE and A76 to 504 +/- 61 cal. CE) place it within the time frame of Justinian's Plague

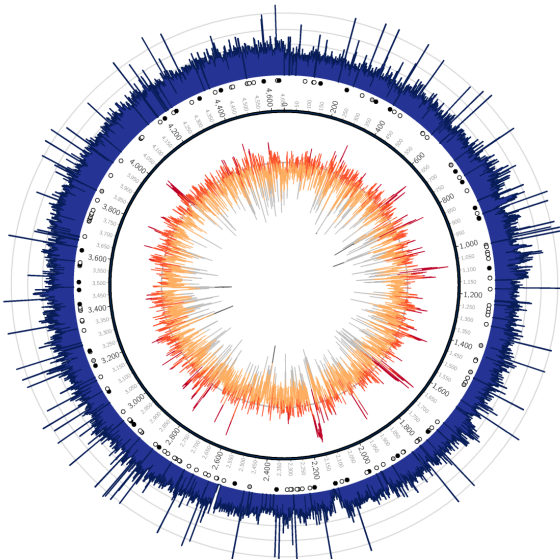


Justinian the Great

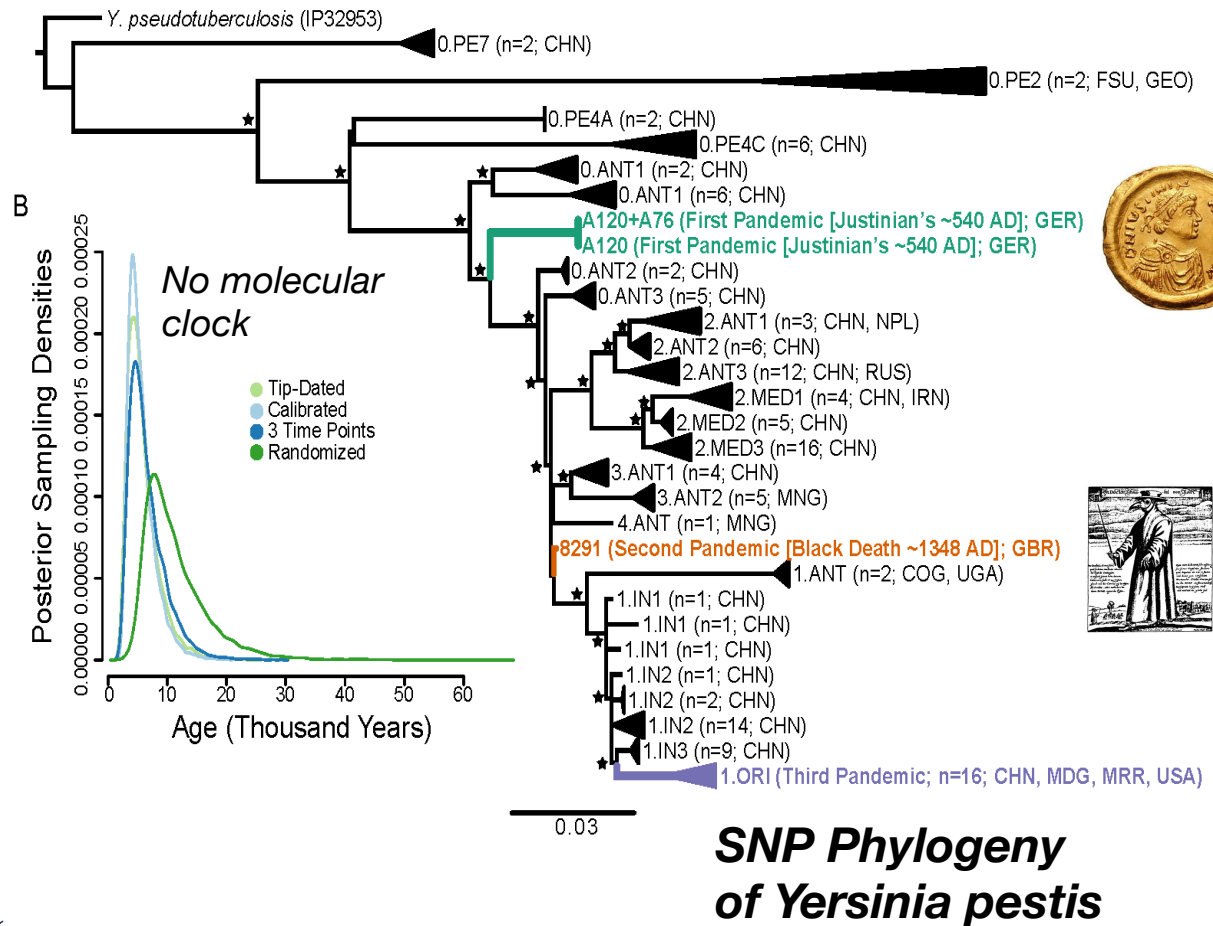


Evolutionary History of the Plague

- Ancient bacterial genome (~1500 yBP; 800 years older than the Black Death strain)
- Average of 7.6X coverage (blue)
- GC content (orange)



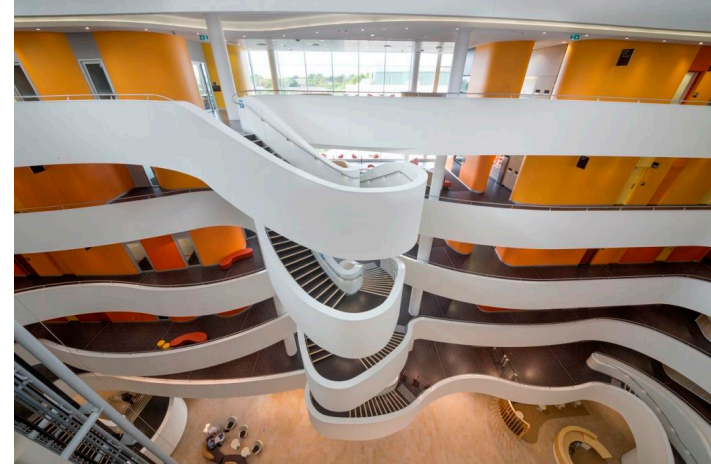
A120+A76_chromosome



- The 3 plague pandemics – Justinian's Plague, Black Death, 3rd (modern) pandemic strains – are *independent* emergence events from rodents
- What caused the extinction of the Justinian strain?
- Phylogeography suggests Asian not African origin
- No molecular clock in these data

Thanks To:

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RNA Virus Biodiversity

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Ancient Plague

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