Redefining the Virosphere: Metagenomics & Disease Emergence

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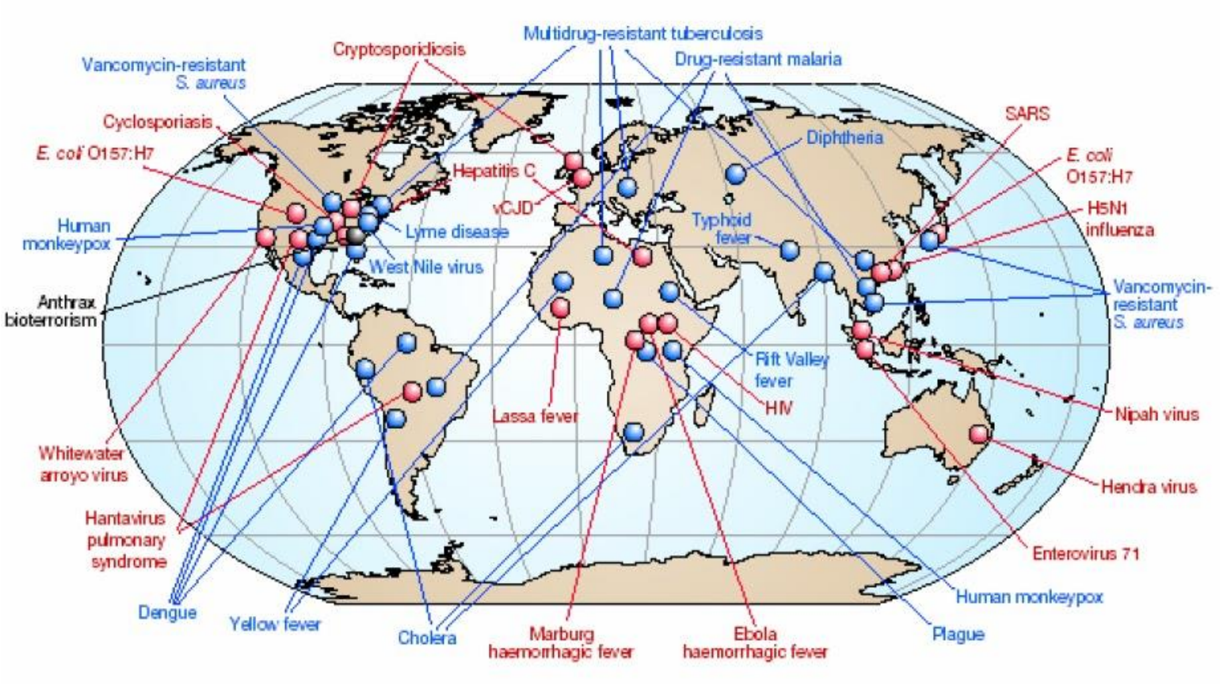
Understanding Disease Emergence

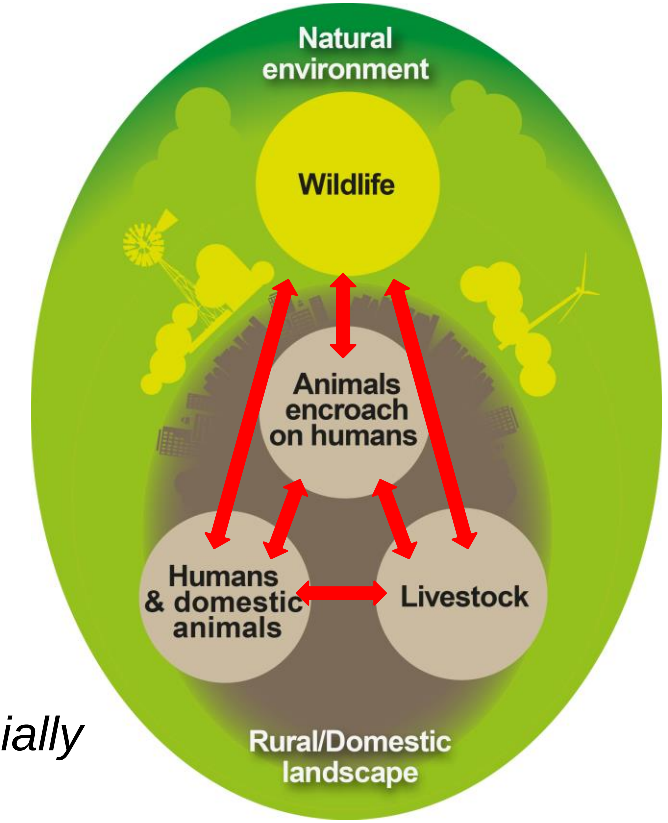
Infectious disease emergence is often due to host-jumping of pathogens

Questions

- How do viruses adapt to and spread in new hosts?
- How large is the virosphere and how is it structured?
- What determines virus diversity and abundance?

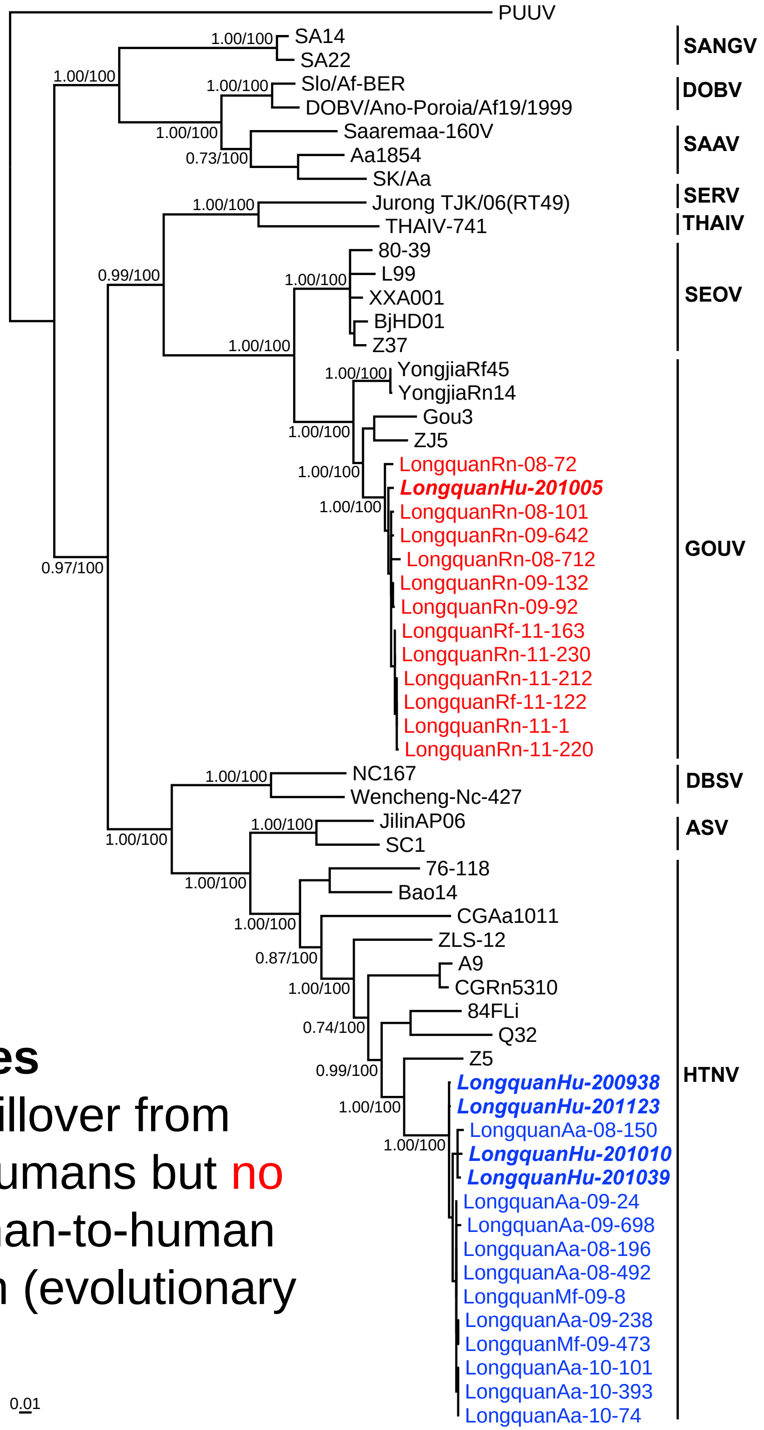


'Fault lines' in Emergence



We characterize virus biodiversity and evolution, especially in locations where humans and animals interact

Tracking Viral Emergence

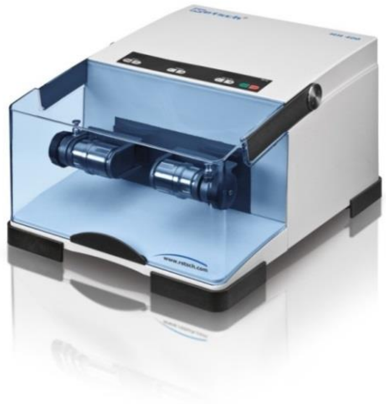


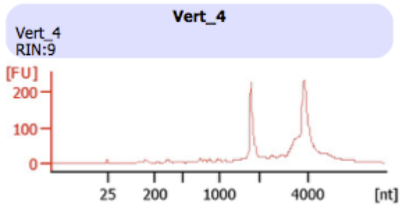
Hantaviruses

Frequent spillover from rodents to humans but no onward human-to-human transmission (evolutionary dead-end)

Meta-Transcriptomics (Bulk RNA-Seq)

- **Sample processing**
  - Homogenize samples from representative tissue
  - Extract total RNA
  - Pool samples
- **Library preparation**
  - Deplete ribosomal RNA (rRNA)
  - Prepare cDNA library
- **High-throughput sequencing on Illumina platform**
  - Target ~50 millions reads per library
  - Sequencing all ‘non-ribosomal’ RNA in sample
  - Confirmation by PCR and Sanger sequencing





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Transcriptome Data Analysis Pipeline

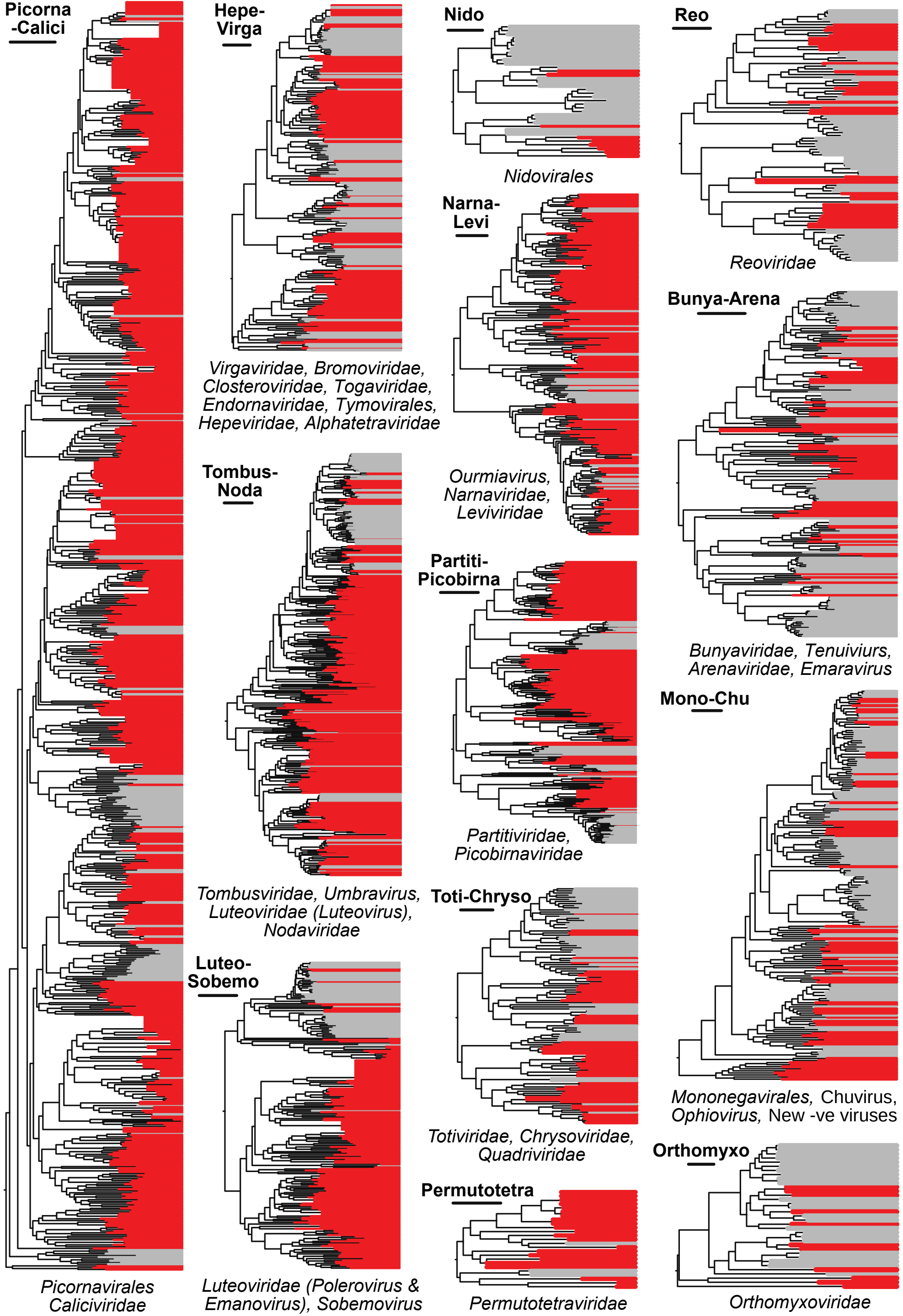
- **Remove host data by mapping against reference (optional)**
- ***De novo* assemble transcriptome**
  - Trinity or any decent assembler
  - Estimate abundance/frequency of transcripts
- **Compare contigs against sequence databases using BLAST**
  - Blastn: simple and fast for known pathogens
  - Blastx: slower but better for novel pathogens
  - CD search: find gene homologs
- **Refine assembly and annotate hits**
  - Phylogenetic analysis (using the RdRp as a marker)
- These data provide an unbiased view of the virome/pathogen discovery

Mang Shi, J-S Eden

Sample quality is critical, including rapid storage at -80ºc.

redefining the virosphere

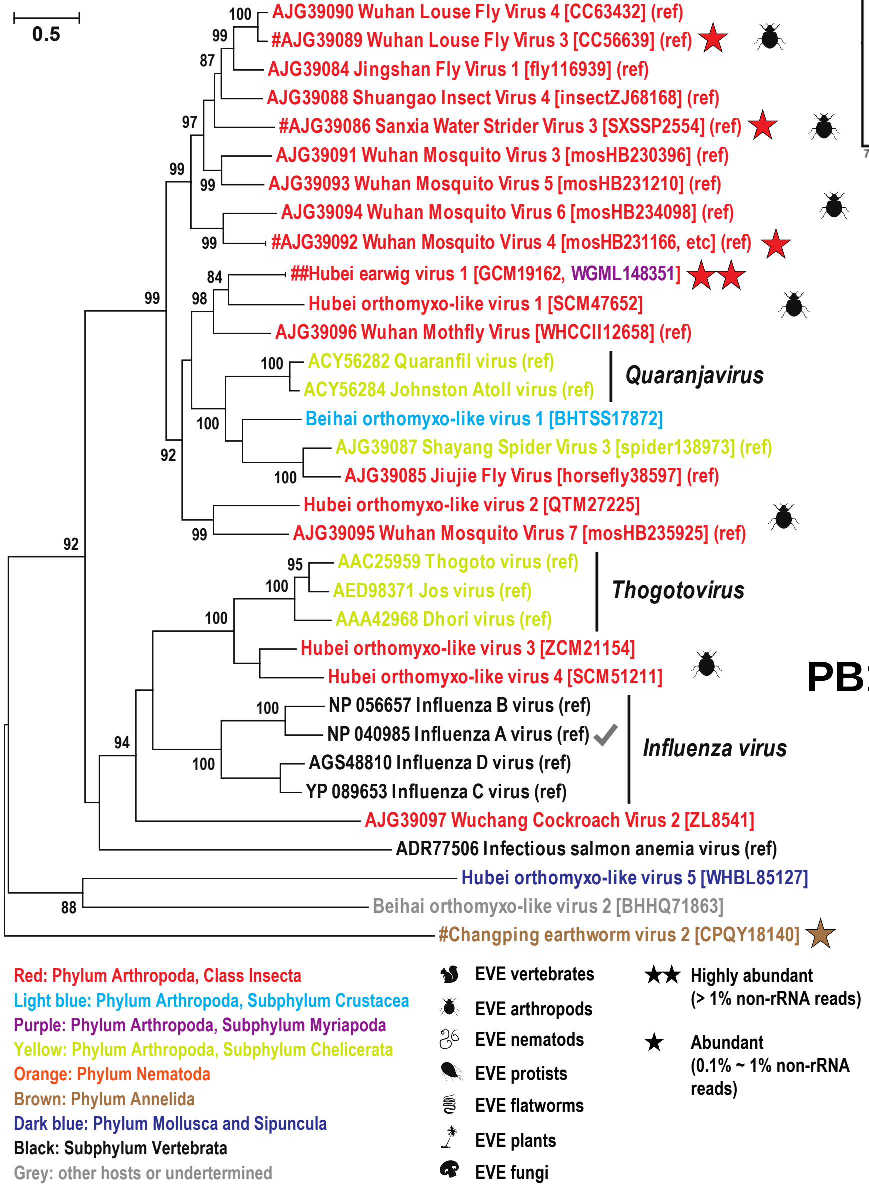
Extraordinary RNA Virus Diversity in Invertebrates



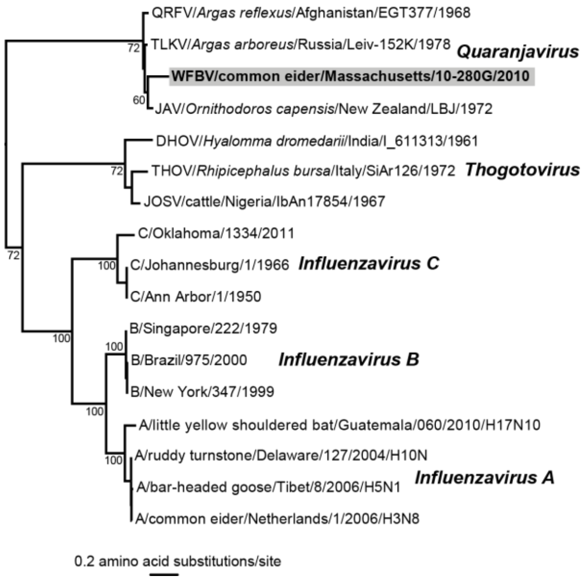
- Collaboration with the Chinese CDC, Beijing
- Examined >220 species across 9 invertebrate phyla
- Huge data (>100 libraries, >3 Tbp)
- Overall ~1500 newly discovered viruses in diverse invertebrate hosts (new genera, families, orders)
- Invertebrate viruses often ancestral to vertebrate viruses
- Fills in ‘gaps’ in phylogenetic diversity – taxonomic implications
- Combination of virus-host co-divergence and cross-species virus transmission

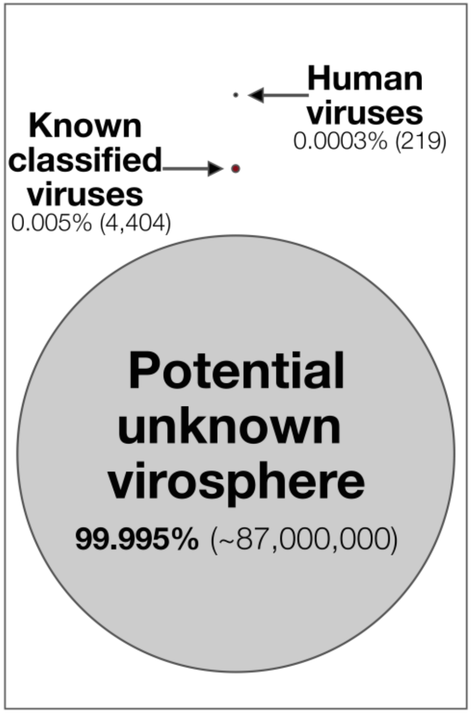
- **Red = newly described here**
  Grey = known viruses

"Orthomyxo-Like" Virus Phylogenetic Diversity



PB1 Phylogeny





Human viruses
0.0003% (219)

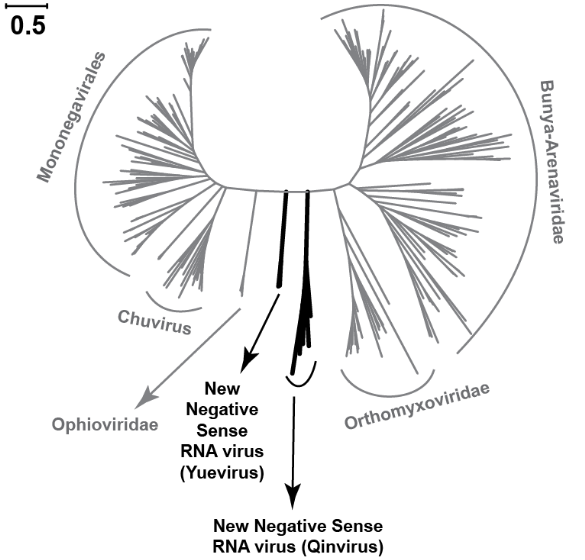
Known classified viruses
0.005% (4,404)

Potential unknown virosphere
99.995% (~87,000,000)

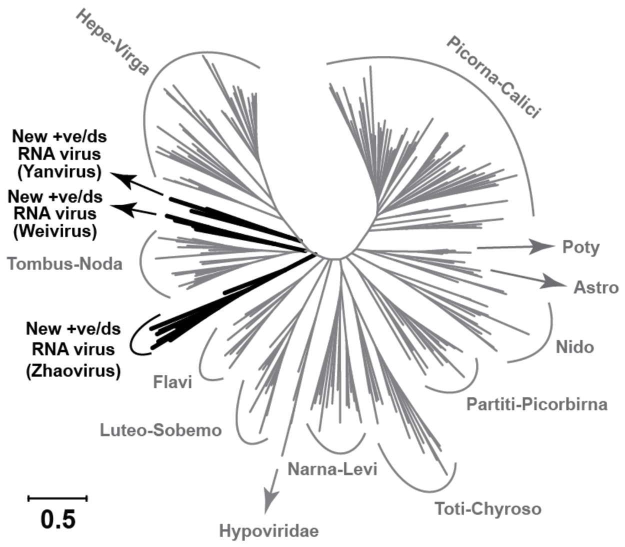
Still scratching the surface of virus biodiversity: may be ~100 million viruses in eukaryotes

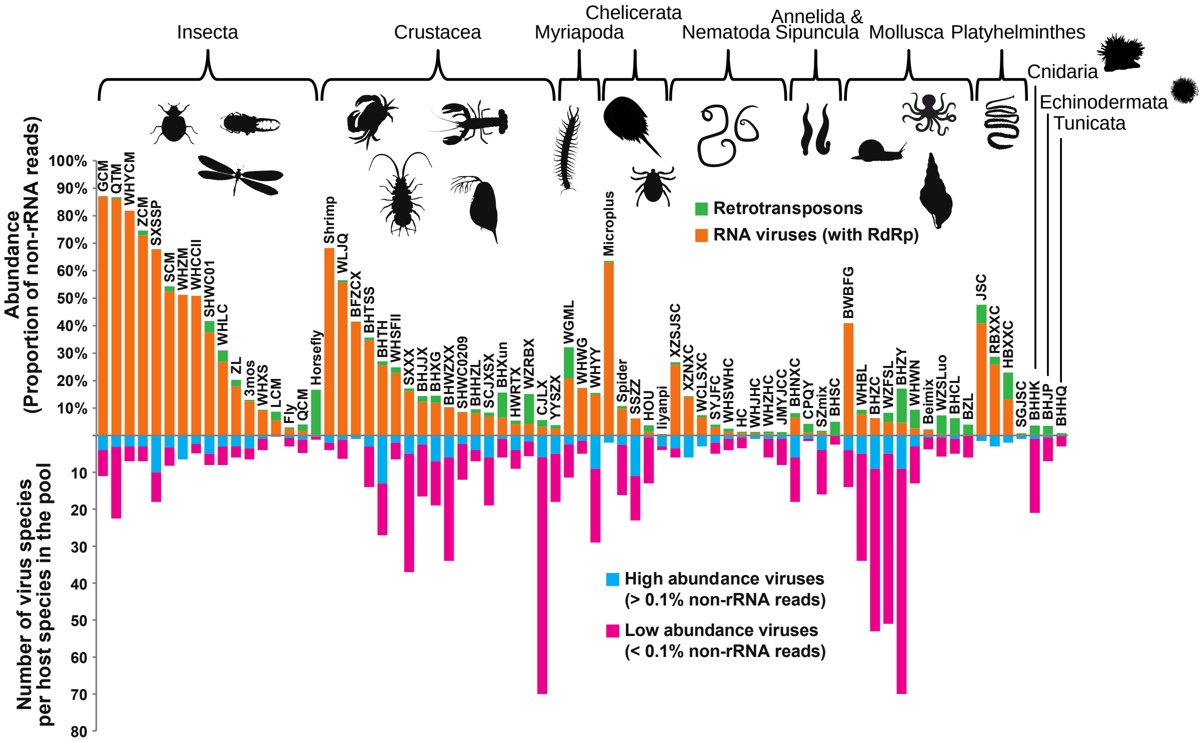
Diverse, Abundant Invertebrate RNA Viruses

Negative-sense RNA viruses



Positive-sense or Double Strand RNA viruses

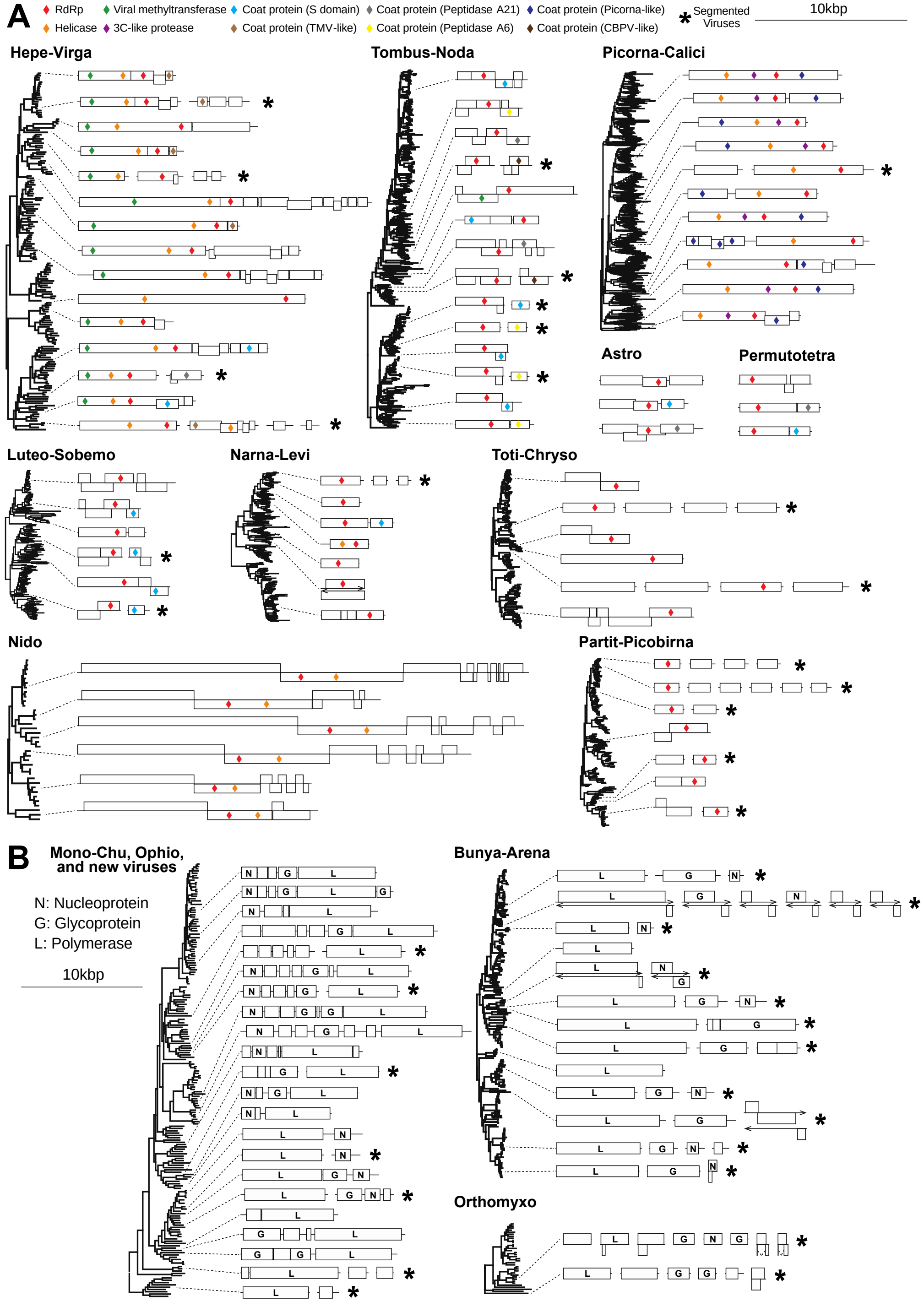




Total RNA with host rRNA excluded

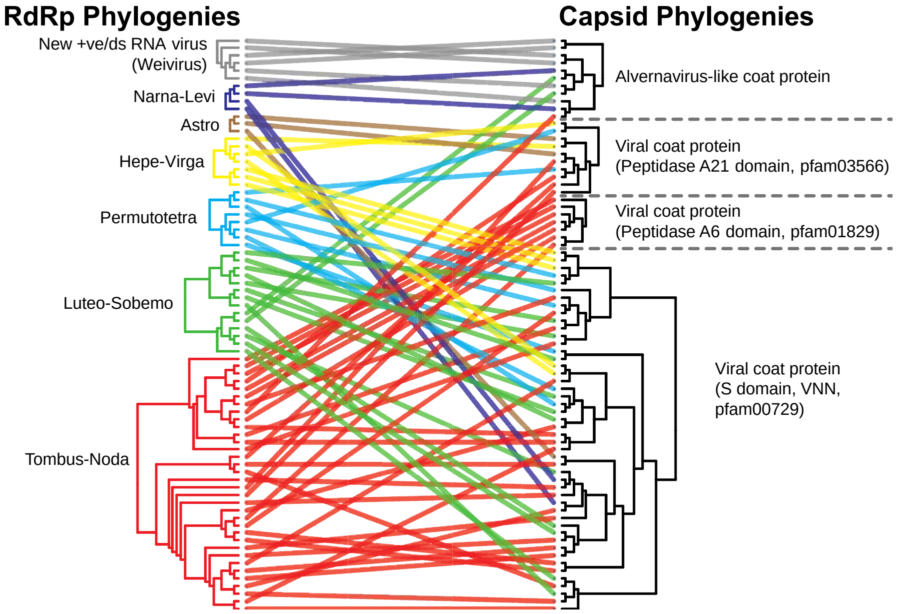
- Abundance provides clues to host type
- Some invertebrate viruses appear to be highly abundant (although this needs to be confirmed)

Complex Genome Organizations and Evolution



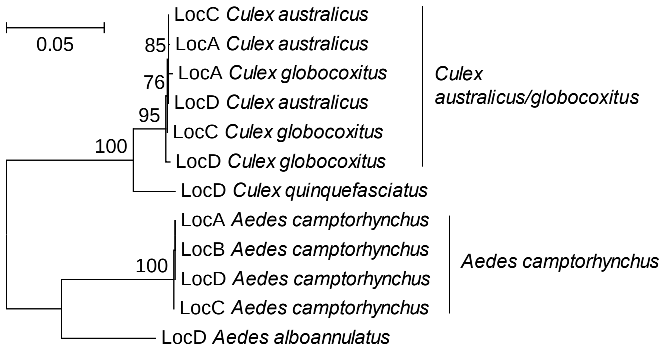
Remarkable Genome Fluidity

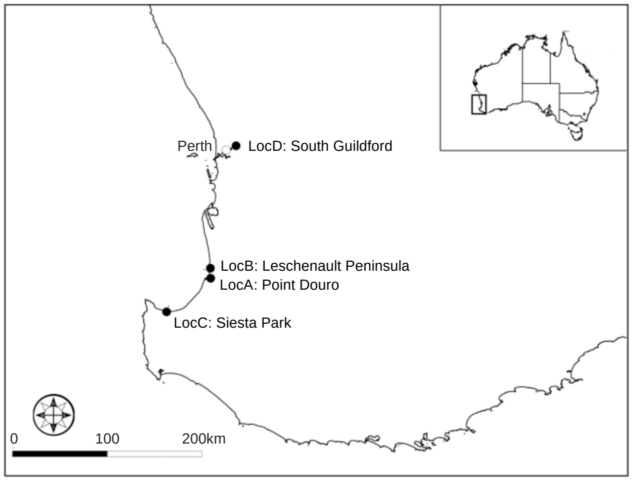
- Frequent recombination, lateral gene transfer (inc. with hosts), genome reorganization, change in segment numbers, gene gain and gene loss

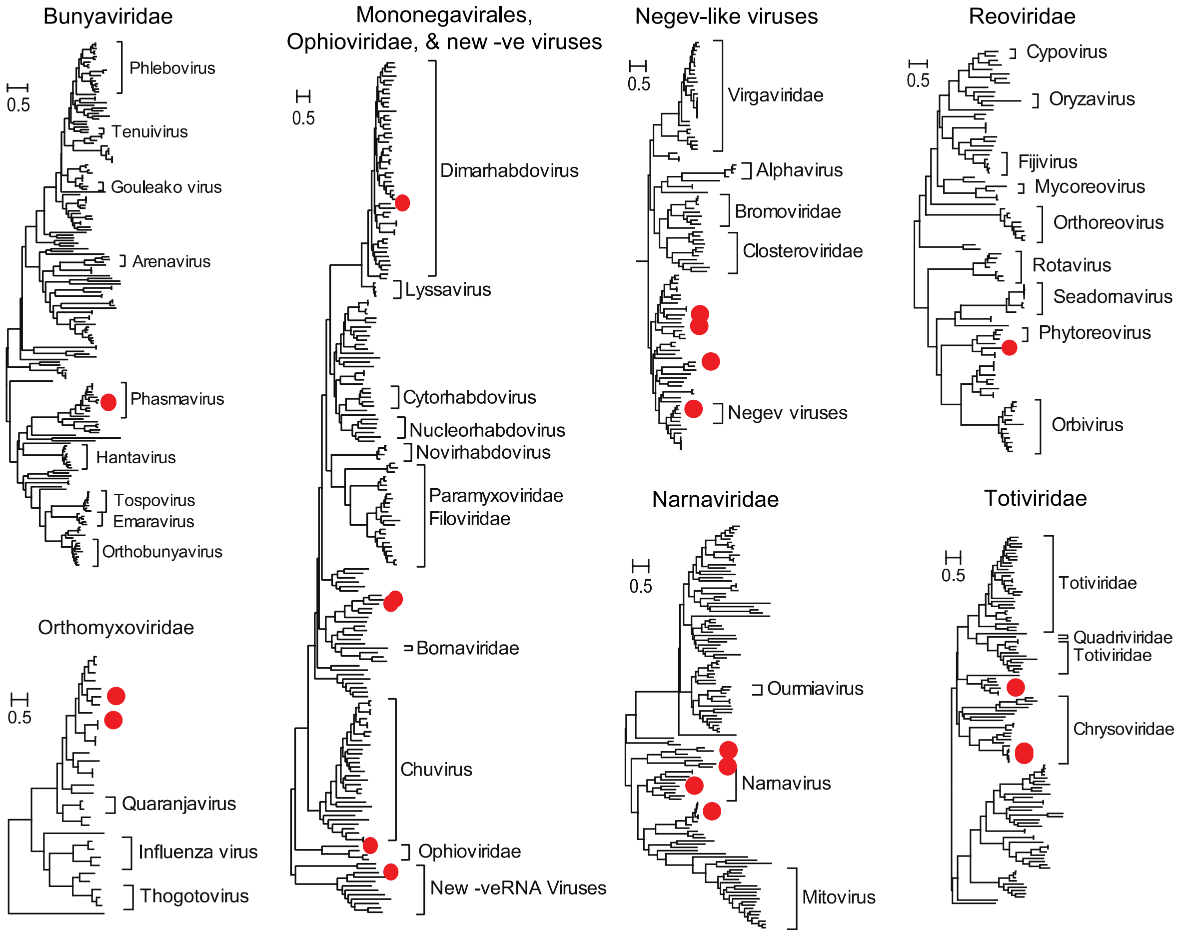


Recombination of functional modules

Novel Mosquito Viruses in Australia

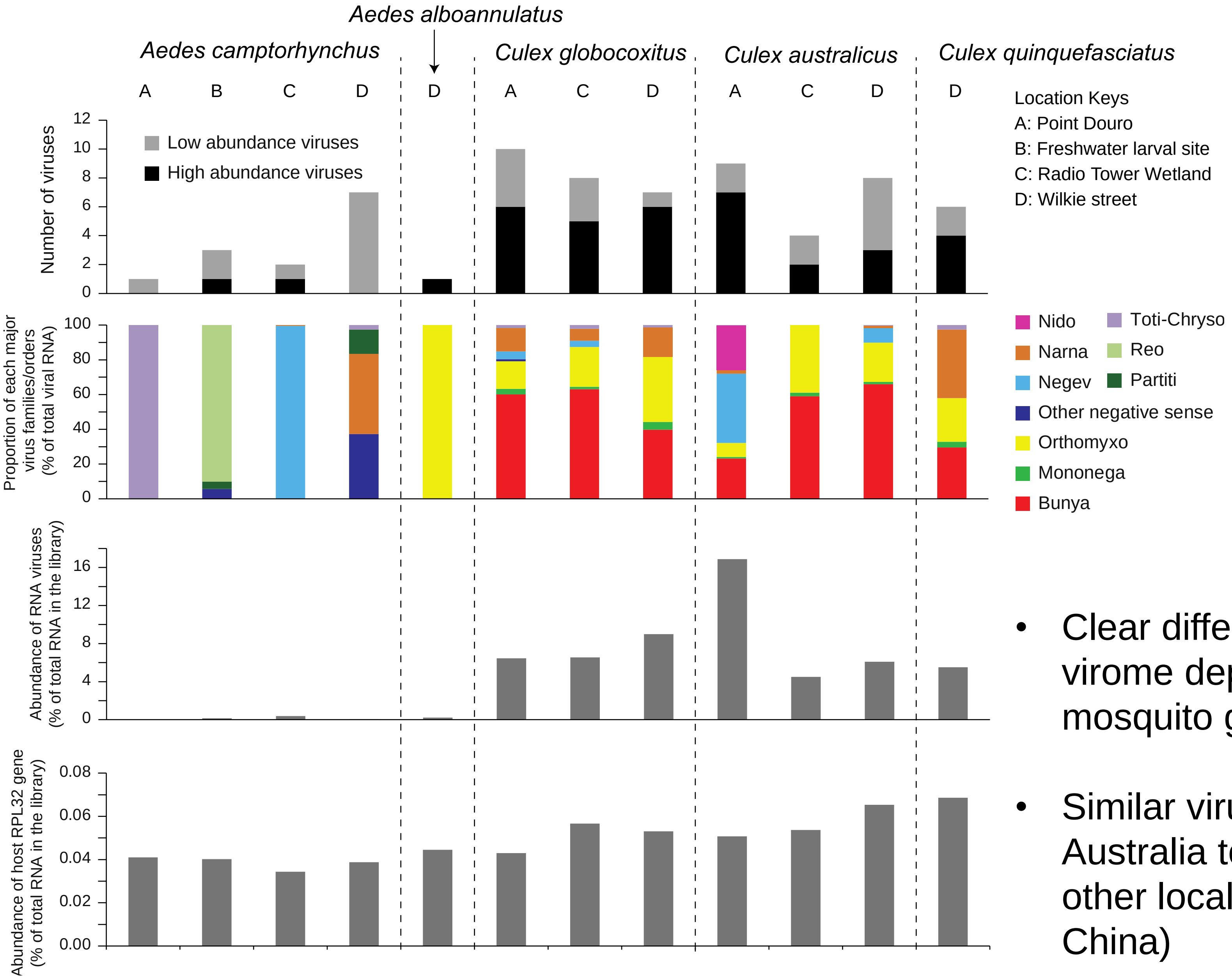






23 RNA viruses identified, 19 novel

Virome Division by Mosquito Genus



- Clear difference in RNA virome depending on mosquito genus
- Similar viruses found in Australia to those from other localities (e.g. China)

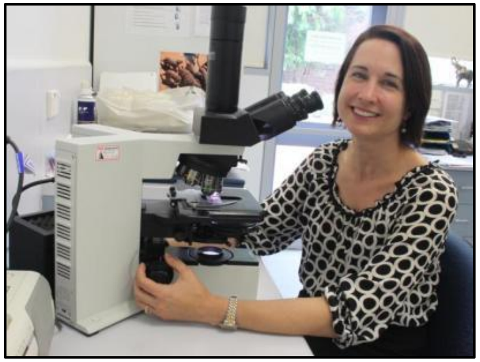
meta-transcriptomics for diagnostics

Neglected Wildlife Diseases of Unknown Aetiology

SYNDROME	SPECIES		SYMPTOMS
Tammar sudden death	Swamp wallabies		Acute mass mortality
Clenched claw	Rainbow lorikeets		Non-suppurative encephalitis, necrotic enteritis
● Wobbly possum	Brushtail possums		Non-suppurative encephalitis
Bird haemorrhagic X	Pelicans, ibis & other water birds		Mortality, non-suppurative myocarditis with necrosis, fibrinoid vasculopathy, fibrinous coelomitis, multifocal haemorrhages
Black and white bird	Magpies, ravens, currawongs		Non-suppurative encephalitis, myocarditis, enteritis
● Ringtail X	Ringtail possums		Acute mortality, necrotising enteritis & hepatitis with some splenic lympholysis (concurrent with rabbit calicivirus mortalities)

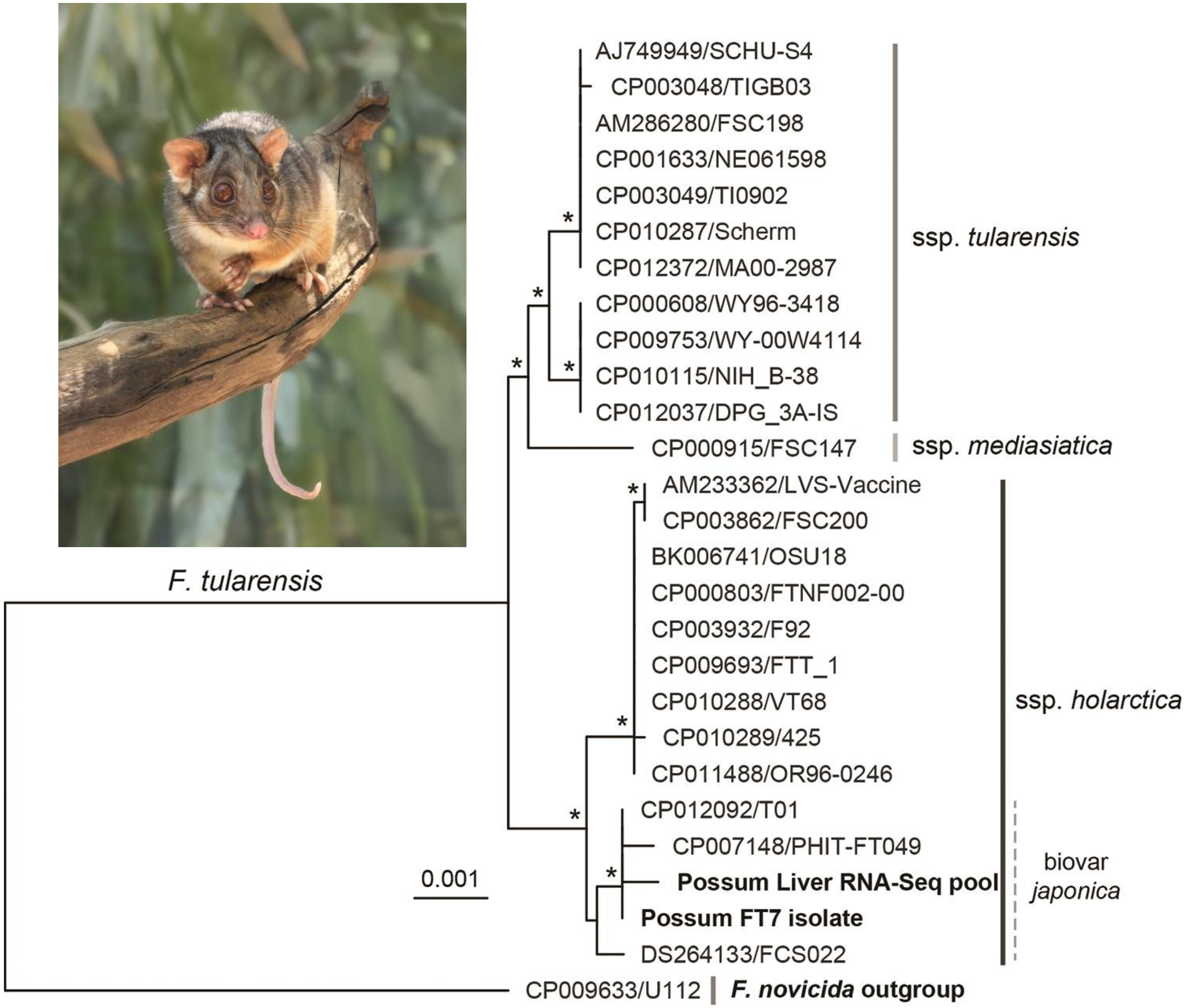
Dr. Karrie Rose

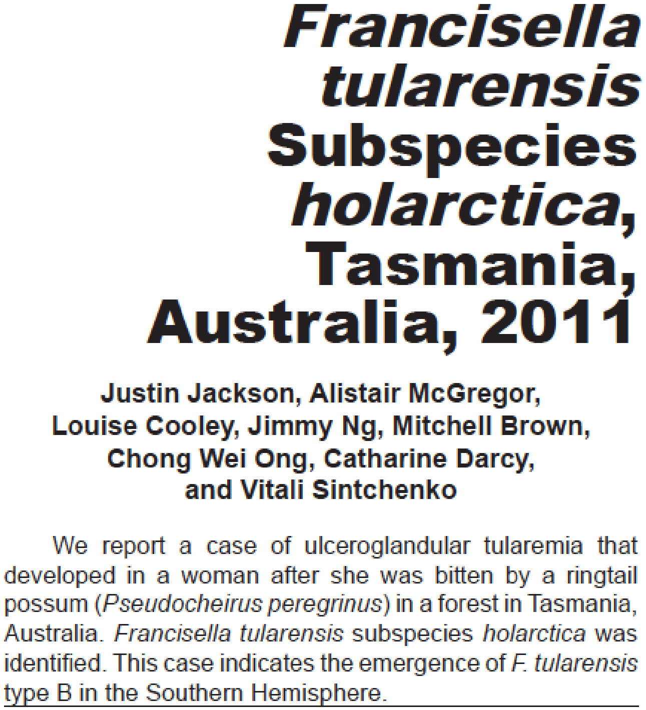
TARONGA
CONSERVATION SOCIETY AUSTRALIA.
Australian Registry
of Wildlife Health



Tularaemia in Australia

- RNA-Seq of 8 possums from the Sydney north shore – identified the presence of *Francisella tularensis* holarctica.
- Confirmed by culture and the official CDC test (PCR & DFA). Also confirmed at AAHL/CSIRO. Complete genome (DNA) sequence also obtained.





Francisella tularensis Subspecies *holarctica*, Tasmania, Australia, 2011

Justin Jackson, Alistair McGregor, Louise Cooley, Jimmy Ng, Mitchell Brown, Chong Wei Ong, Catharine Darcy, and Vitali Sintchenko

We report a case of ulceroglandular tularemia that developed in a woman after she was bitten by a ringtail possum (*Pseudocheirus peregrinus*) in a forest in Tasmania, Australia. *Francisella tularensis* subspecies *holarctica* was identified. This case indicates the emergence of *F. tularensis* type B in the Southern Hemisphere.

EID **18**, 1484-1486 (2012)

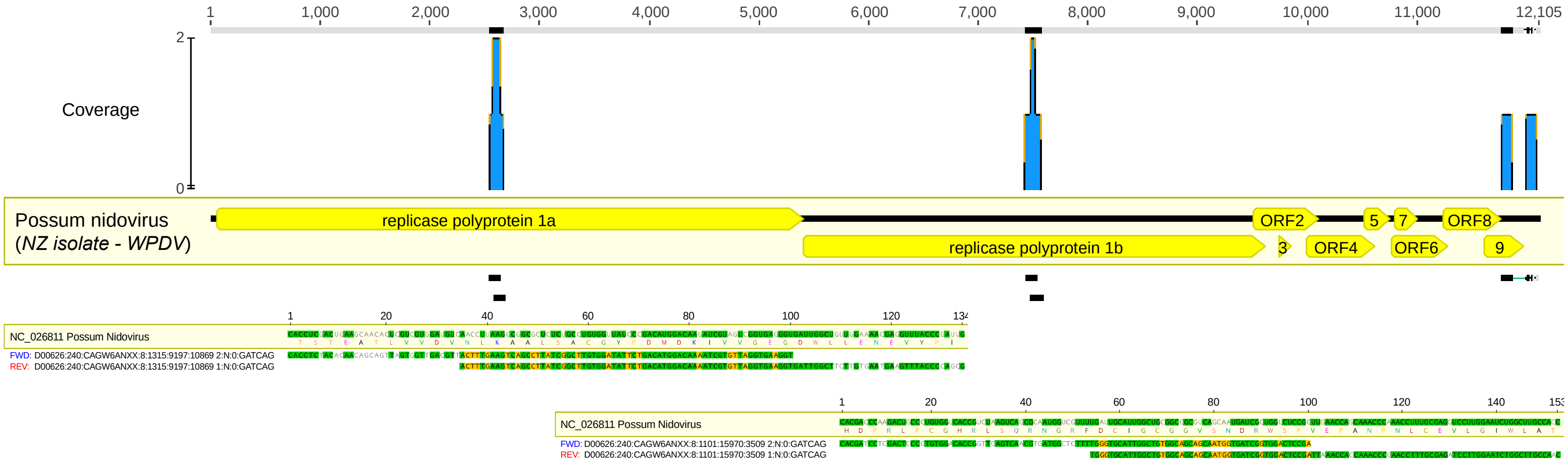
Wobbly Possum Disease

- Non-suppurative encephalitis in brushtail possums
- Only described in New Zealand with a novel nidovirus the likely cause
- A condition clinically similar to wobbly possum disease, has been reported from brushtail possums in eastern Australia and Tasmania
- Case fatality rate is greater than 95%
- Brain tissue examined from 7 animals

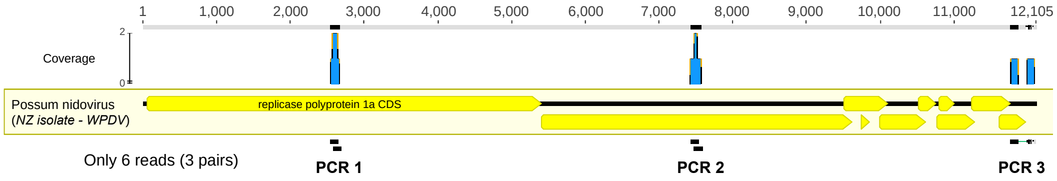
Source: www.youtube.com/watch?v=twFbAvNwcZ8

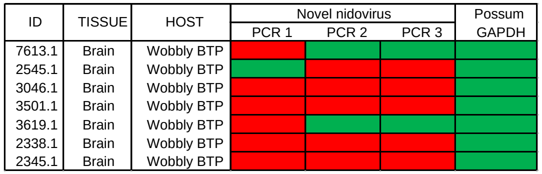
Wobbly Possum Disease

Australian Possum Nidovirus?



- Very low abundance: 6 reads from 50 million
- Viral titers in brain tissue averaged only ~10^4 copies/pg RNA in experimental infections with NZ isolates
- ~80% nucleotide identity to NZ isolate
- Previous screens for NZ possum nidovirus in Australia were negative



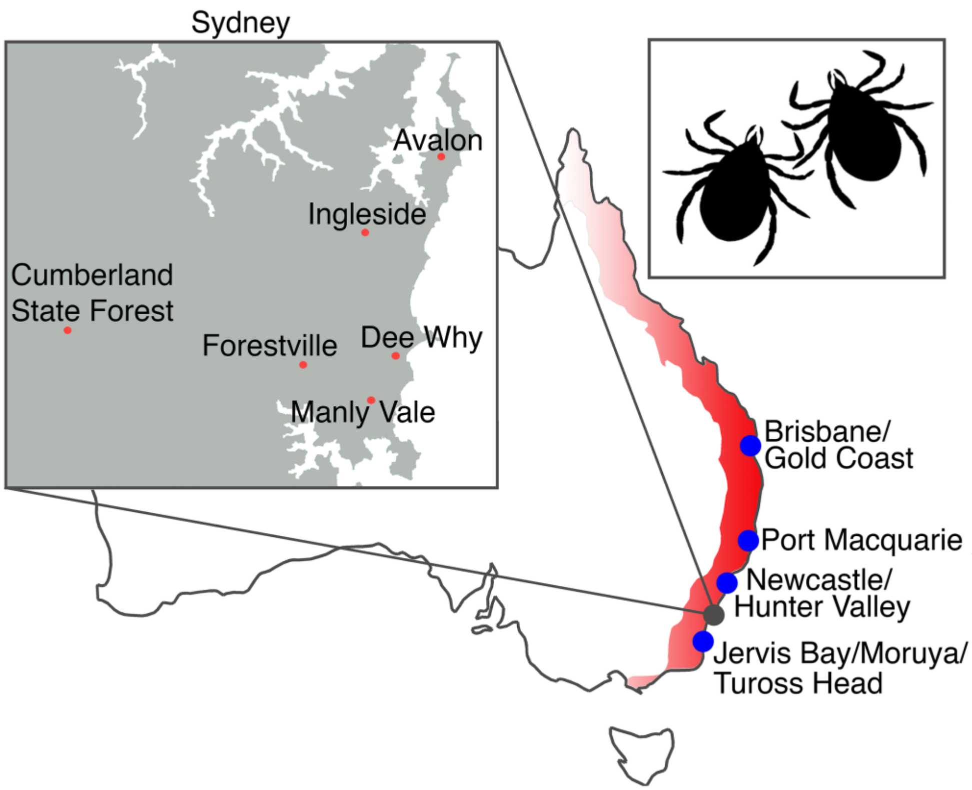


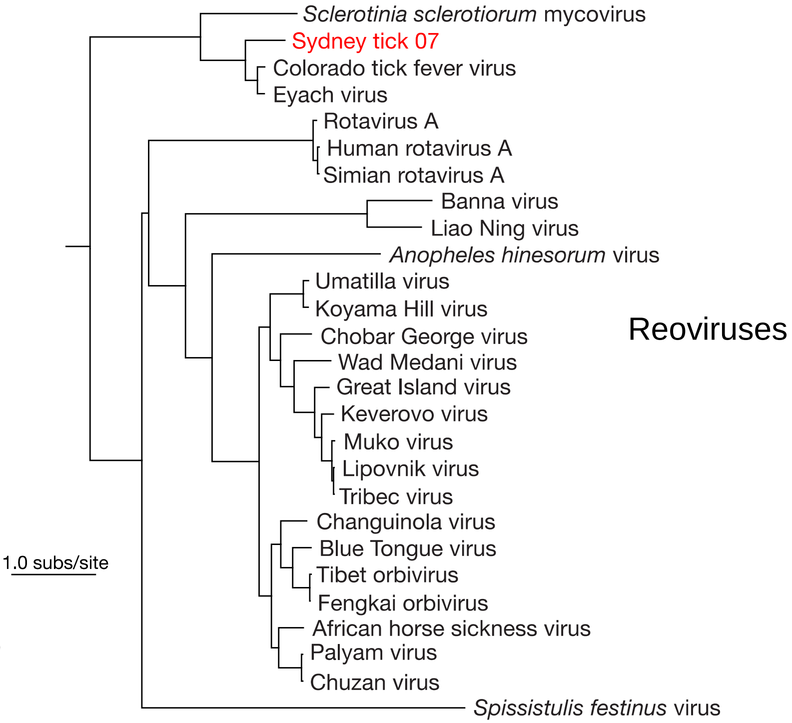
ID	TISSUE	HOST	Novel nidovirus			Possum
			PCR 1	PCR 2	PCR 3	GAPDH
7613.1	Brain	Wobbly BTP	🟥	🟩	🟩	🟩
2545.1	Brain	Wobbly BTP	🟩	🟥	🟥	🟩
3046.1	Brain	Wobbly BTP	🟥	🟥	🟥	🟩
3501.1	Brain	Wobbly BTP	🟥	🟥	🟥	🟩
3619.1	Brain	Wobbly BTP	🟥	🟩	🟩	🟩
2338.1	Brain	Wobbly BTP	🟥	🟥	🟥	🟩
2345.1	Brain	Wobbly BTP	🟥	🟥	🟥	🟩

‘Lyme-Like’ Tick-Borne Disease in Australia

Highly controversial disease syndrome along the Australian east coast associated with tick bites and with some clinical similarity to Lyme disease

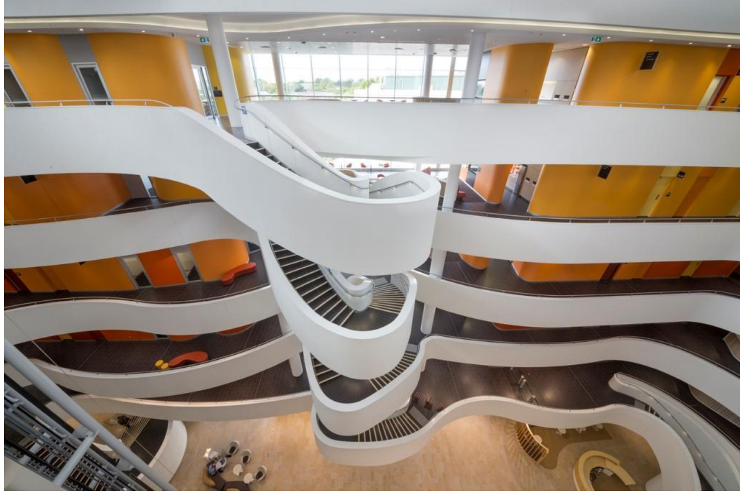
Ixodes holocyclus ticks on bandicoots





- **No evidence** of *Borrelia burgdorferi* in any Australian tick or human sample
- *Borrelia afzelli*, an agent of Lyme disease, found in the skin sample of a patient infected overseas; *Rickettsia australis* also identified in Australian samples
- An abundance of viruses in ticks from Australian wildlife

Thanks To:



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Redefining the Virosphere & Pathogen Discovery

- Ci-Xiu Li, Jun-Hua Tian, Wen Wang, Wen-Ping Guo, Xian-Dan Lin,Yong-Zhen Zhang (China CDC, Beijing)
- Mang Shi, John-Sebastian Eden, Jemma Geoghegan (University of Sydney)

Arthropod RNA Viruses in Australia

- Allison Imrie (University of Western Australia)
- Peter Neville (WA Department of Health)

Tularaemia In Australia

- Karrie Rose (Australian Registry of Wildlife Health)
- John-Sebastian Eden, Jimmy Ng, Vitali Sintchenko (University of Sydney)

Tick-Borne Disease in Australia

- Erin Harvey (University of Sydney)
- Bernie Hudson, Alice Kizny Gordon (Royal North Shore Hospital, Sydney)