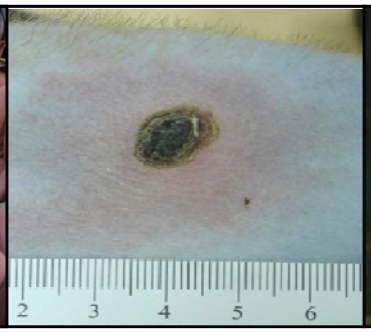
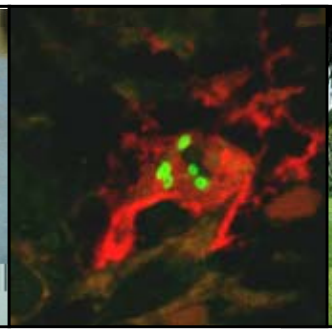
Unresolved Entomological issues related to Scrub Typhus

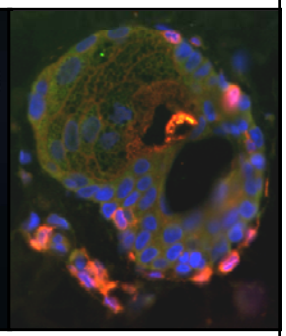
Daniel H. Paris

MORU, Bangkok, Thailand

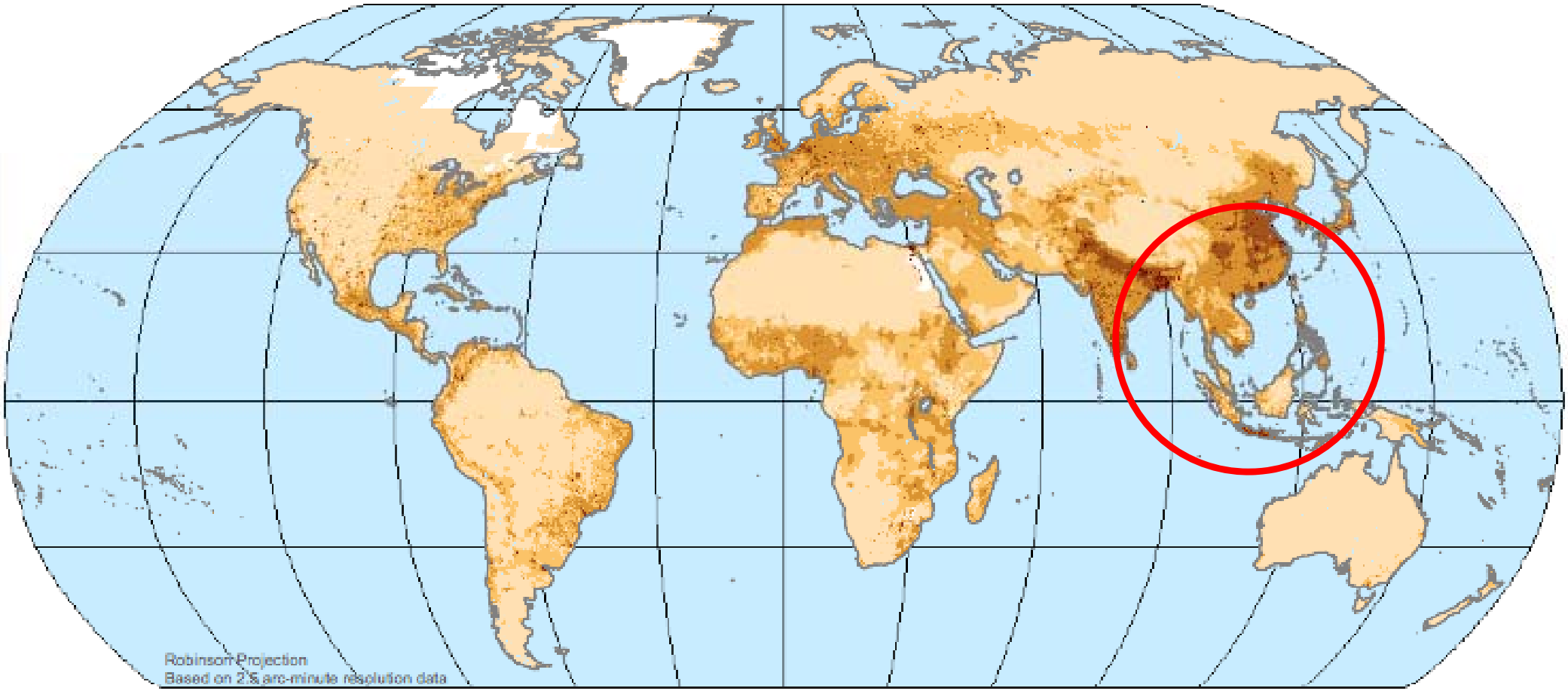
JITMM, November 2013



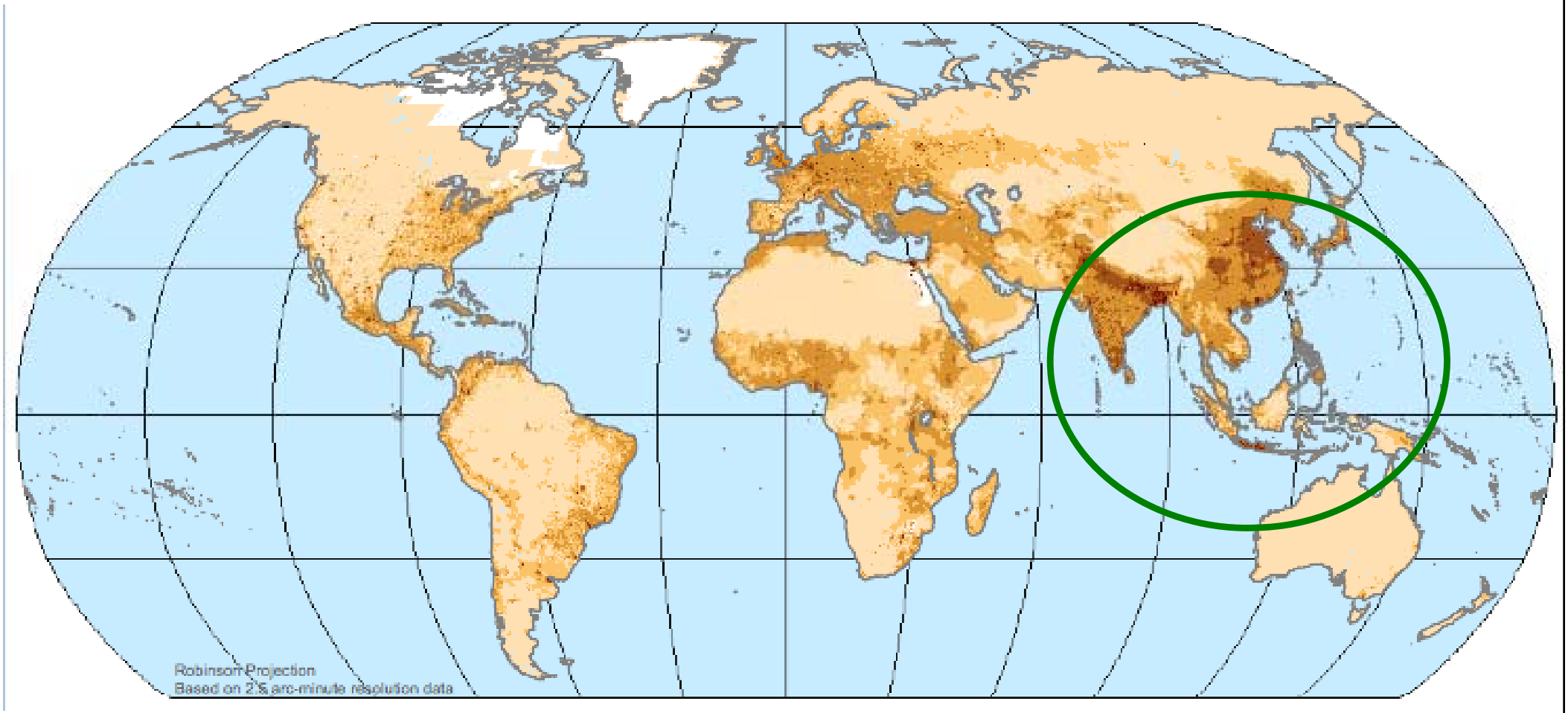



The importance of Asia …



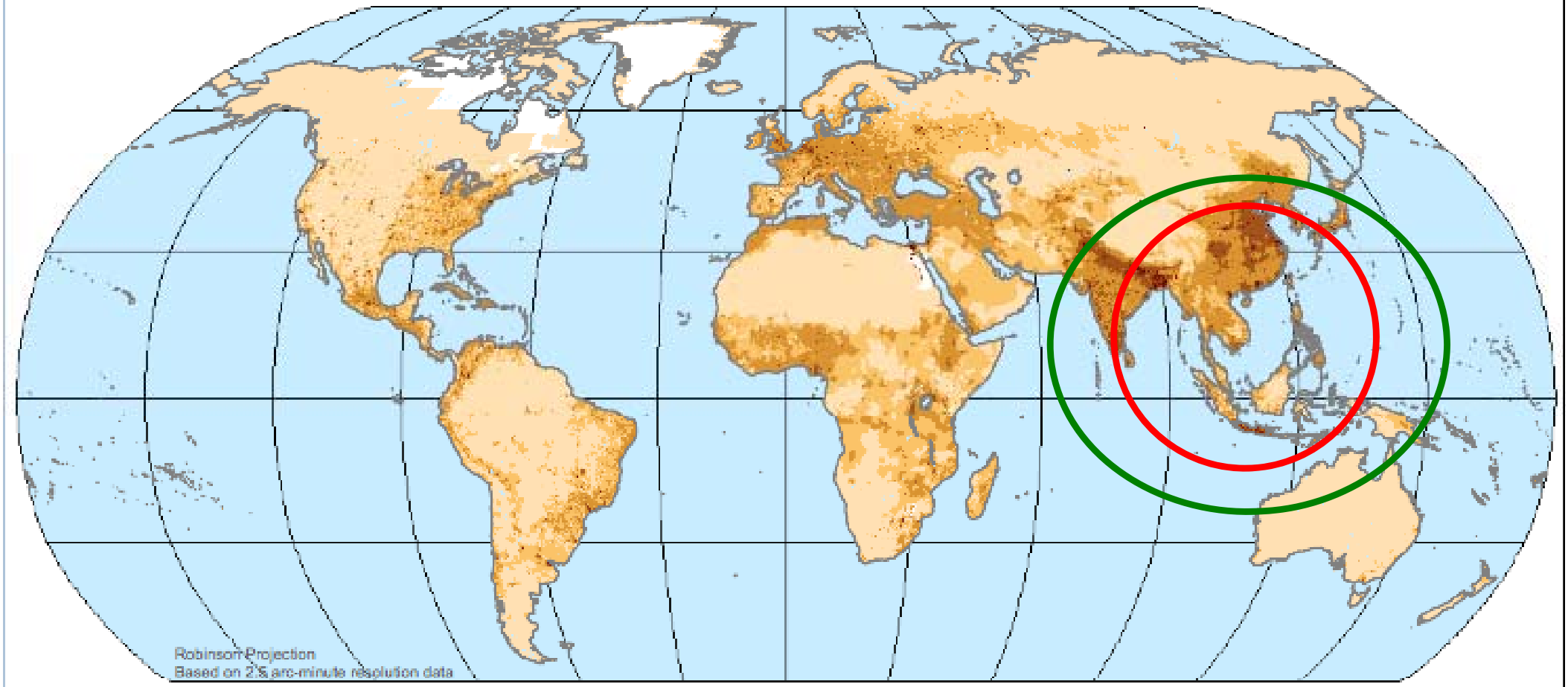
Half of the world's population within 2000 miles of Bangkok !!!

The importance of Asia …



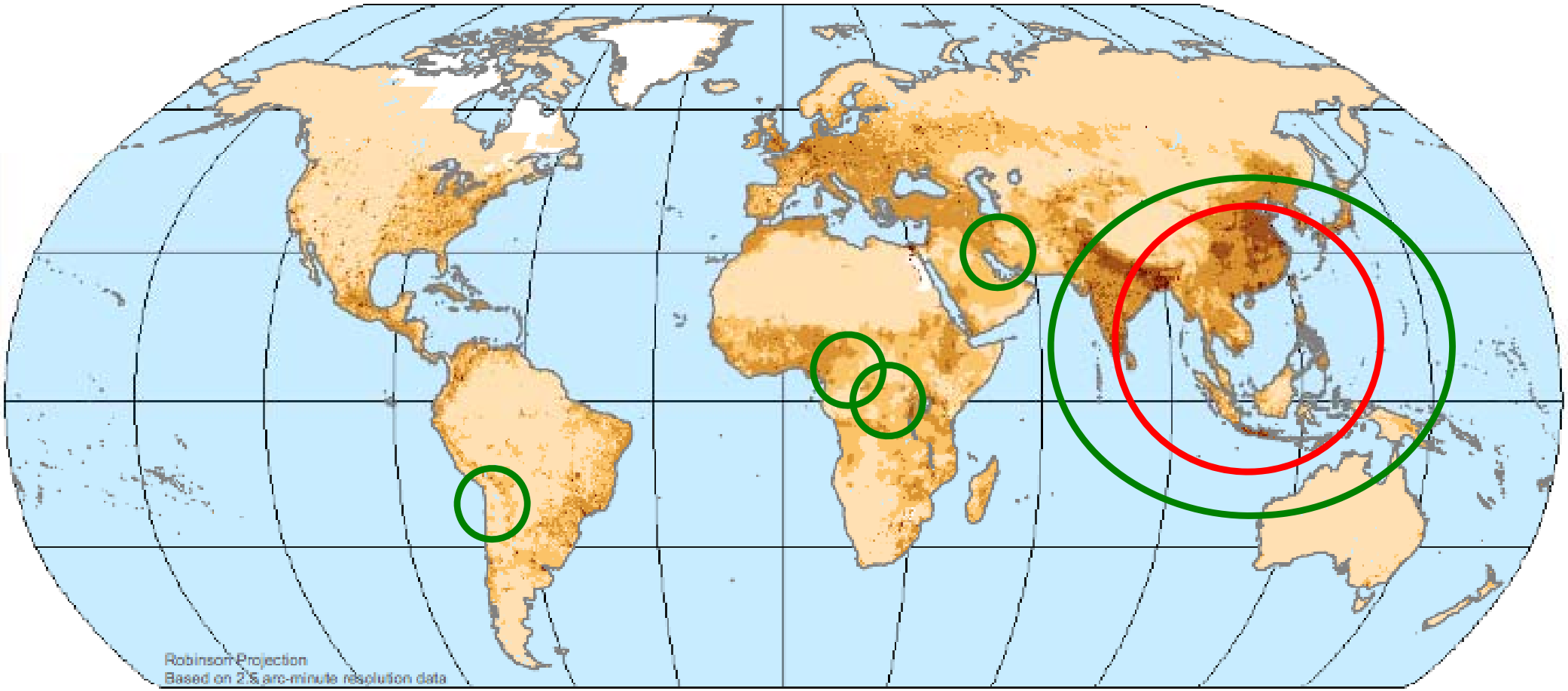
>90 % of reported scrub typhus cases within 3000 miles of Bangkok !!!

The importance of Asia …



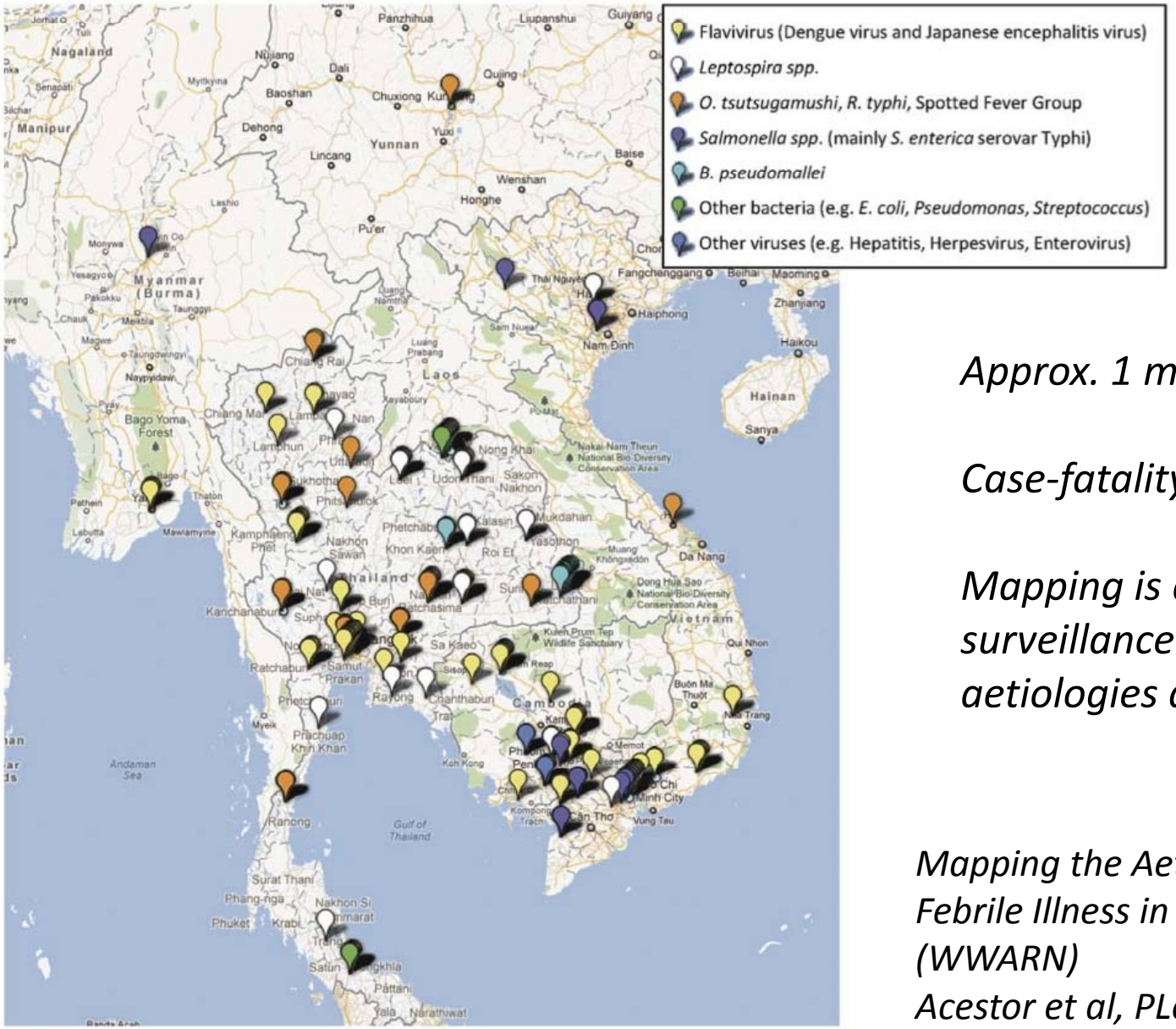
… What about the rest of the world … ??!!

The importance of Asia …



… potential global tropical / subtropical distribution … ?!

How important is scrub typhus?



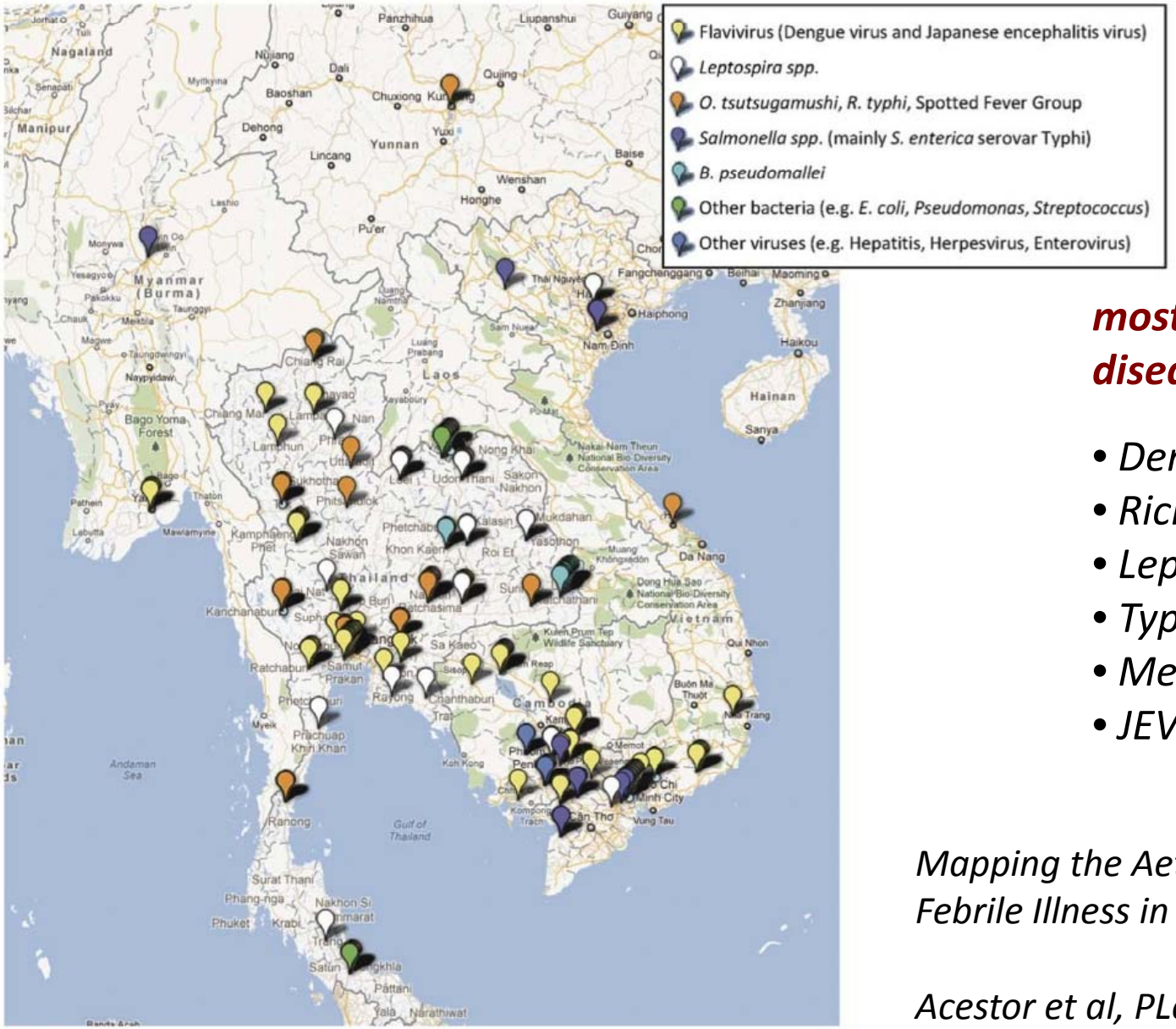
Approx. 1 mio cases estimated

Case-fatality rate 3-7%

Mapping is an excellent surveillance tool to identify aetiologies and localise 'gaps'

Mapping the Aetiology of Non-Malarial Febrile Illness in Southeast Asia 2012 (WWARN)
Acestor et al, PLoS ONE, 2012

How important is scrub typhus?



most frequent reported diseases/pathogens:

- *Dengue*
- *Rickettsial infections*
- *Leptospirosis*
- *Typhoid*
- *Melioidosis*
- *JEV*

Mapping the Aetiology of Non-Malarial Febrile Illness in Southeast Asia 2012

Acestor et al, PLoS ONE, 2012

Typhus in SEA

Thailand
Malaysia
Laos
Vietnam
Cambodia
Myanmar
Bangladesh
India
… ?! etc.

"Dengue-like illness"

"Typhus-like illness"

- *burden 15-25% ST, 10-15% MT*
- *estimated 1 mio cases per year*
- *seroprevalence 30-50%*

Fringe habitats - rural

Markets - urban

***ST and MT** (and SFG) combined = leading cause of treatable undifferentiated febrile illnesses*

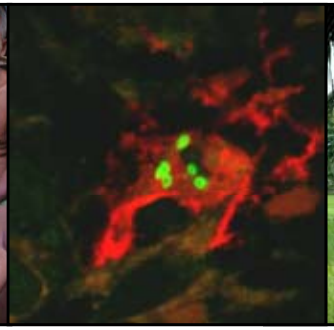
"Scrub typhus is probably one of the most underdiagnosed and underreported febrile illnesses requiring hospitalization in the region"

World Health Organization, 1999

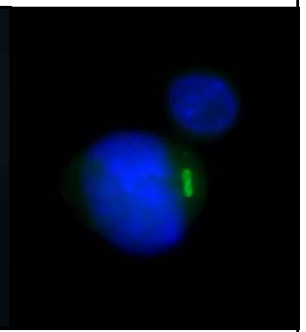
Unresolved issues

- *Ecology and Epidemiology*
- ***Entomology***
- *Diagnosis and Diagnostics*
- *Scrub Typhus Pathogenesis / Immunity*
- *Developing a Vaccine against Orientia*

Concerted efforts of multiple specialities …





Entomology – open questions

- *Are Leptotrombidia the only species transmitting Orientia?*
- *What are the risk factors for getting bitten?*
- *What are the true hosts of Orientia?*
- *How do Orientia modulate the sex ratio of infected mites?*
- *What selective processes influence Orientia evolution?*
- *What do multiple strain infections (mites/humans) tell us?*
- *Mite bite site processes? What is the role of mite saliva?*

Are Leptotrombidia the only species transmitting Orientia?

The established vectors are all species of the genus Leptotrombidium

L. deliense, L. scutellare, L. imphalum, L. arenicola, L. umbricola
L. akamushi, L. deliense, L. pallidum …
(Thailand, Malaysia, Japan, Korea, India)

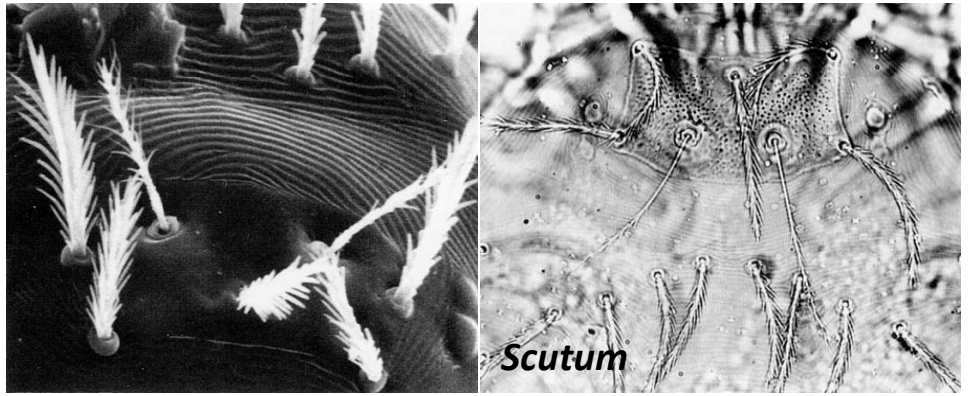
http://livingwithinsects.wordpress.com/

Reports of non-Leptotrombidium mites assoc. w/ Orientia

- ***Blankaartia*** sp. (Thailand)
- ***Eutrombicula*** sp. (Thailand)
- ***Schoengastia*** sp. (Thailand)
- ***Gahrliepia*** sp. (Japan, India)
- ***Neotrombicula*** sp. (Korea)
- ***Cheladonta*** sp. (Korea)

Challenge

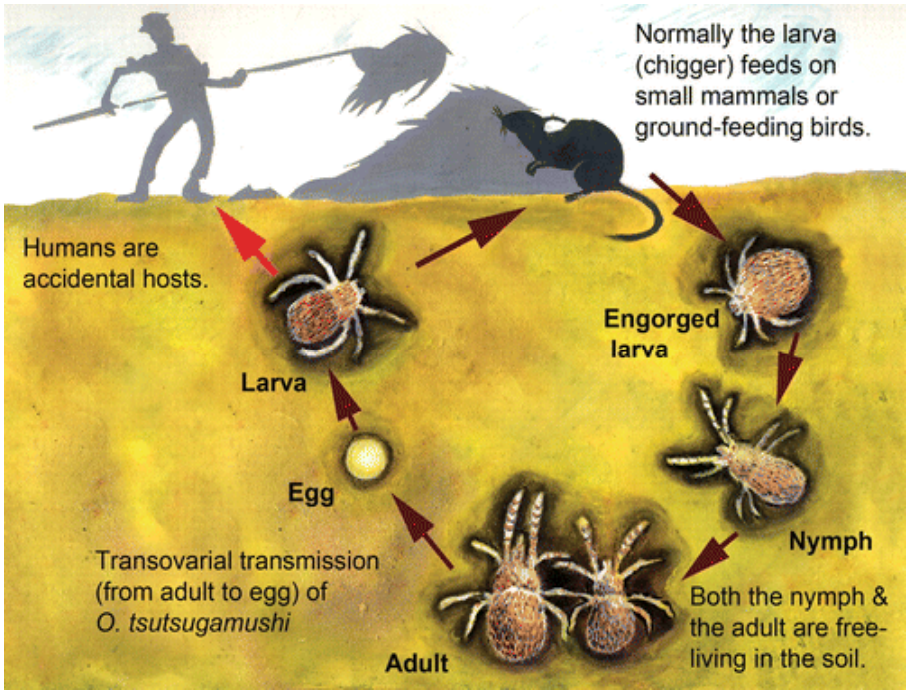
- *Morpholgy* (scutum, hairs etc.)
- *Genetic characterisation* (COX genes etc.)
- *other …*



What are the true hosts of Orientia?

- Different mite species within genus have different infection rates
- Transmission of *Orientia*? Role of rodents? Humans?

- ***Transmission rates***
  - Rodent carriage of *Orientia* (range 14% to 33%)
  - Rodent-mite transmission (range from ***<1%*** to max. 9%)
  - Establishment of infection or vertical transmission rates post rodent feeding in nature remains unknown
  - Co-feeding transmission rates?



- ***Transmission experiments***
  - Vertical transmission of mites infected via feeding on mice is low (<1 in 10,000)
  - Basically the rodent seems to be a dead-end host as well?
  - ***"chicken and egg"*** question … ?!

Takahasi 1990
Tanskul 1998
Lerdthusnee 2003
Jeong 2007

What is the basis of "(re-)emergence"?

- *De novo* emergence vs. Re-emergence?
- Endemic disease – increased awareness?
- Other factors for maintenance of Orientia in nature? (other than vertical transmission that can explain antigenic diversity)
- Are there *Orientia* species other than *O. tsutsugamushi*?
- Actual geographic distribution?

Highly efficient vertical transmission

- Over 5 generations infection rates almost 100%
- Progeny is female in 95-100%, normal ratio is 2:1 (f:m)
- Variation per mite species
  lower female rates in F1-F3 generations

Entity termed "mite islands"

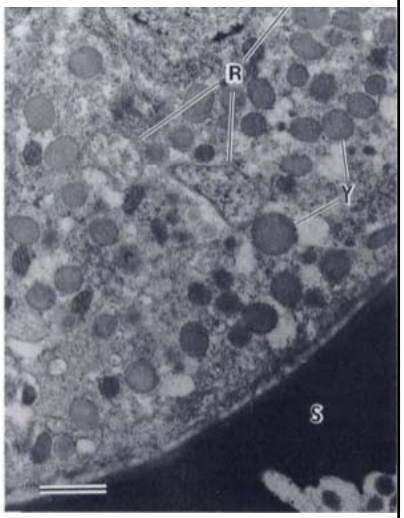
- Defined areas with high presence of infected chiggers
- Clonal characteristics of *Orientia*
- Chigger 're-attach' to another rodent
  if incomplete feed, co-feeding issues

How do Orientia modulate the sex ratio?

Why are not all mites infected in nature?

- Data shows around 1-3% are infected (Japan, SEA)
  - Population-survival advantage
    (Fewer eggs, longer development time)
  - Uninfected larvae acquire *Orientia* very rarely via rodent feeding
    (infection rates <1%, vertical transmission almost absent)
  - Proportion of transmission not vertically transmitted
    (lose ability of female infected offspring)

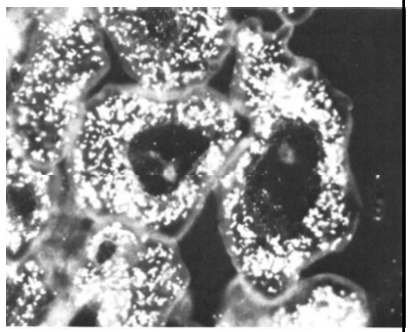
Spermatophore
bar 5 um



Oocytes
bar 1 um

Association with oocytes / ovaries and not male spermatophores

- Cytoplasmic inheritance factor (?)
- Parthenogenesis (?)



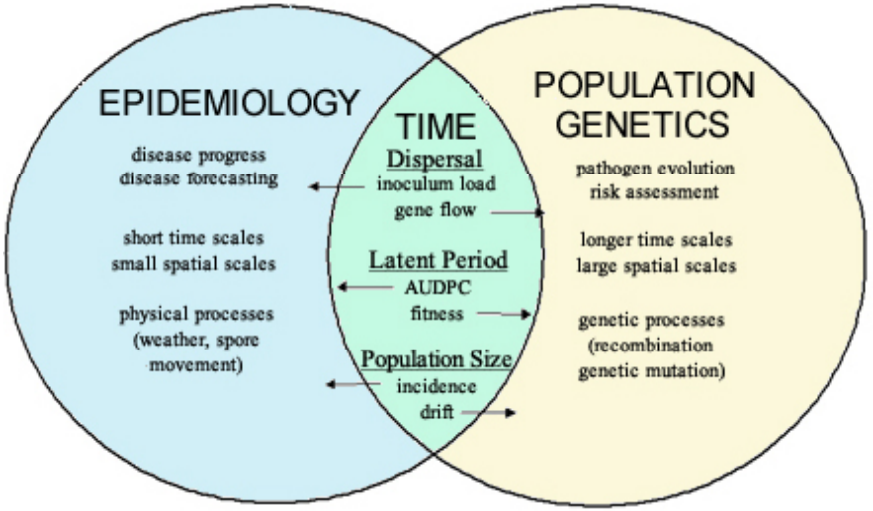
Ovary
Oocytes

Walker JS, 1975 / Rapmund G, 1972, 1984 / Phasomkusolsil 2009

What selective processes influence Orientia evolution?

Some considerations

- Clonal organization of *Orientia*
- DNA polymorphism, genomic plasticity
- Small core genome, highly streamlined
- Gene transfer tools available
- Rodents less important than assumed
- *Role of co-feeding?*



- Which *Orientia* undergo transstadial and transovarial transmission?
- What are the characteristics of these endosymbionts?
  = usually mutually beneficial interactions
- *Infected mites - longer development times and decreased fecundity*
  = more time for reproduction?

What tools to use?!

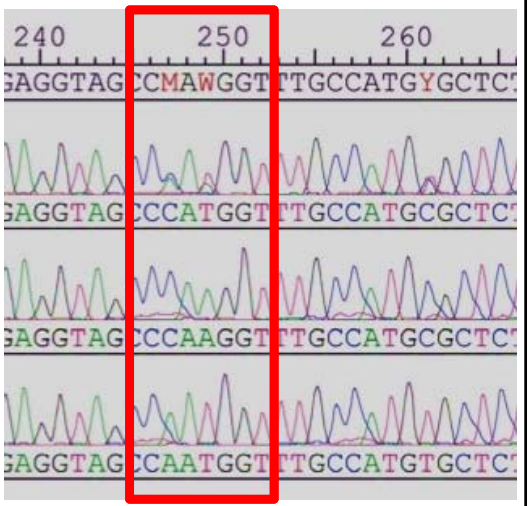
- *56kDa*: HV regions / immunogenic protein / selective pressure
- *MLST*: one mite – one strain – one ST (seq. type)
- *Whole genome sequencing*: population genetics

Cho 2007
Nakayama 2010
Sonthayanon 2010
Duong 2010
Takhampunya 2013

What do multiple strain infections tell us?

Evidence for multiple infections

- In humans: Thailand 25% with multiple infections
- In rodents: multiple genotypes described
- In mites: laboratory-reared and naturally-infected mites



Heterogeneity / strain diversity

- Human pathogenic
- Human non-pathogenic = strains causing sub-clinical disease (*unknown*)
- Strains causing varying levels of disease severity
  - virulence factors (??) affected by background immunity

Questions – Issues

- *Is recombination (and other mechanisms for increasing genetic diversity) occurring in rodents and mites ?*
- *If these 'maintenance hosts' are producing recombinant forms what are the underlying mechanisms?*

Shirai 1982
Sonthayanon 2010
Duong 2010
Takhampunya 2013

Mite Bite Site

Why is the mite bite and resulting eschar painless?

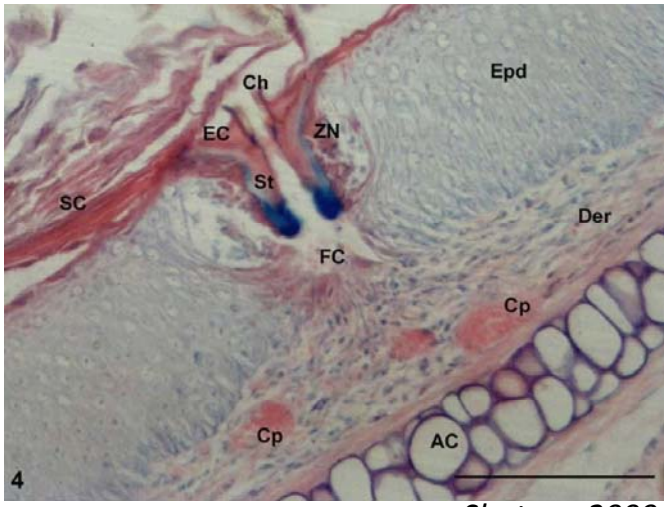
Stylostome formation and different depths?
- Affects dissemination pathways?

Early host defenses?
- Neutrophils, monocytes influx

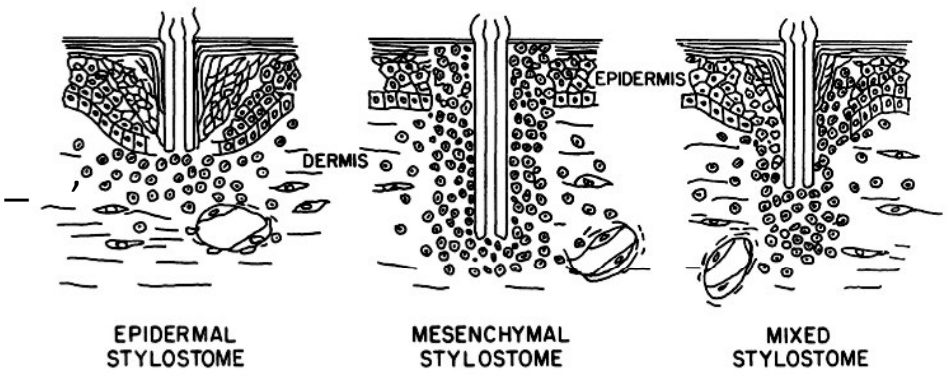
Immuno modulation – saliva/pathogen?
- IL-10, TGFβ, ag-presentation etc.

Local replication vs. early dissemination
- Haematogenic / lymphatic / 'monocyte-shuttles'
- Role of bacterial virulence?

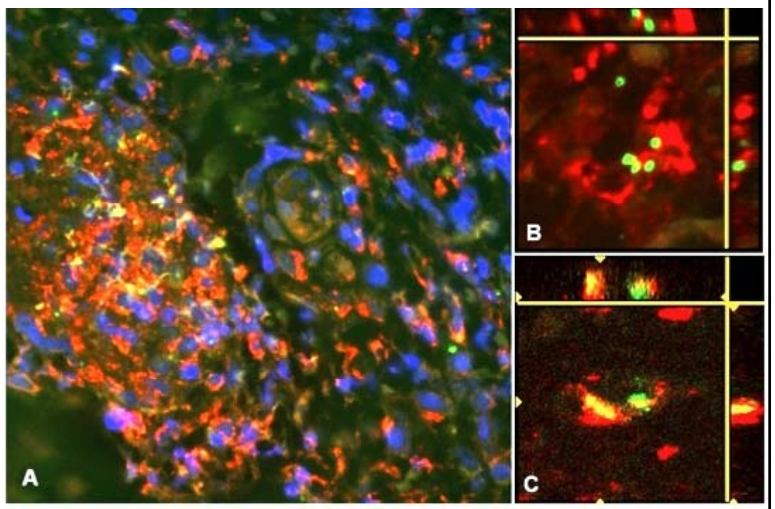
Inoculation bacterial load?



Shatrov, 2009



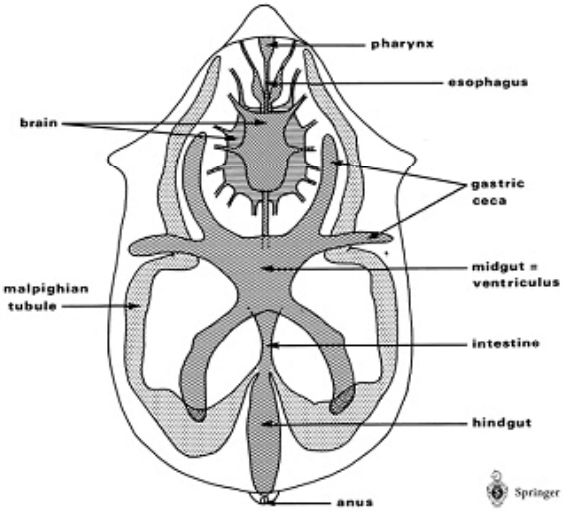
Hase, 1978



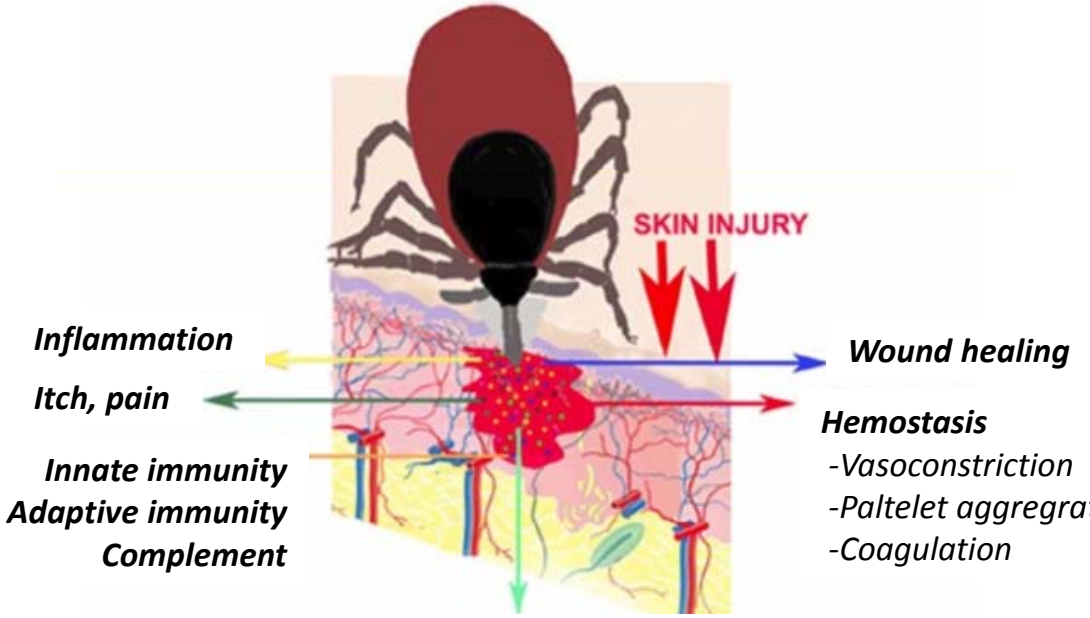
Paris, 2012

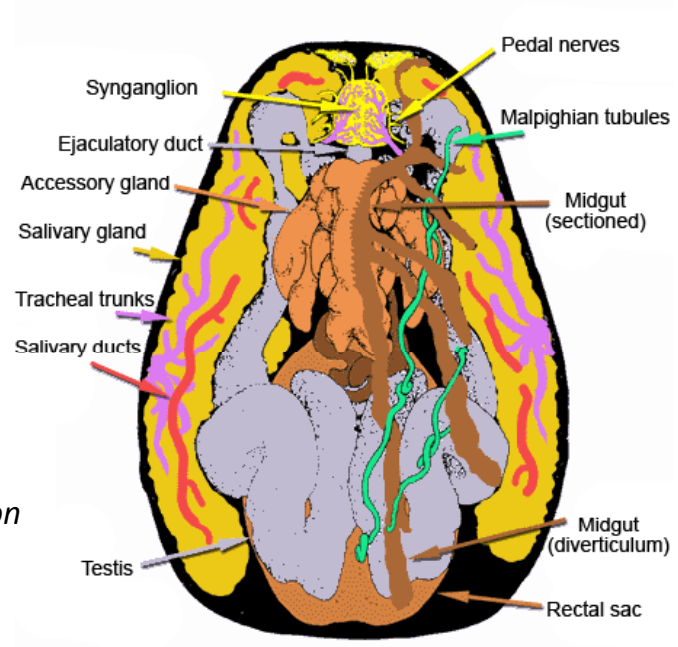
What is the role of mite saliva?

- ***Totally unexplored … !***
- Parallels to tick saliva?
  - No blood feeding, lymphatic fluids
  - Inflammation, analgesia, immunomodulation, complement activation etc.
  - Hemostasis, vasoconstriction …



Melhorn 2001





Tick viscera

Thank you !

MORU

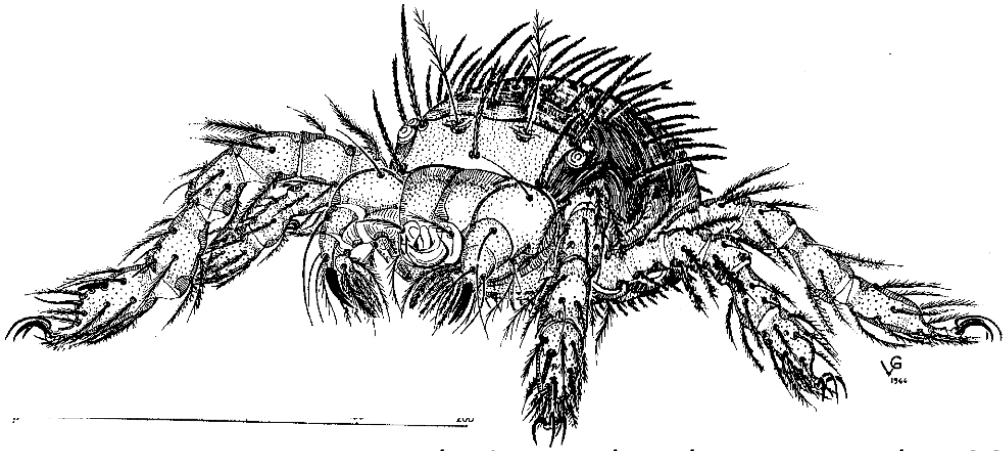
Mahidol University

Oxford University

AFRIMS

NMRC

… and many more …



Red mites and Typhus, R. J. Audy, 1965

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