Diversity of Orientia tsutsugamushi strains in South East Asia (Thailand, Cambodia, Vietnam) based on the 56-kDa protein gene and multilocus typing

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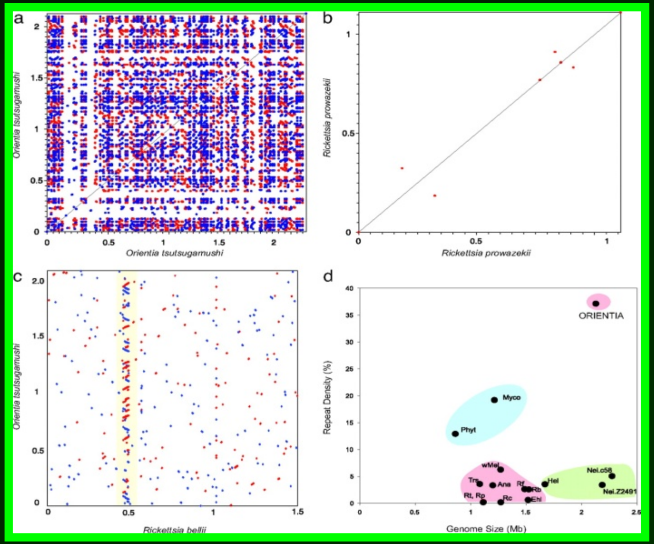
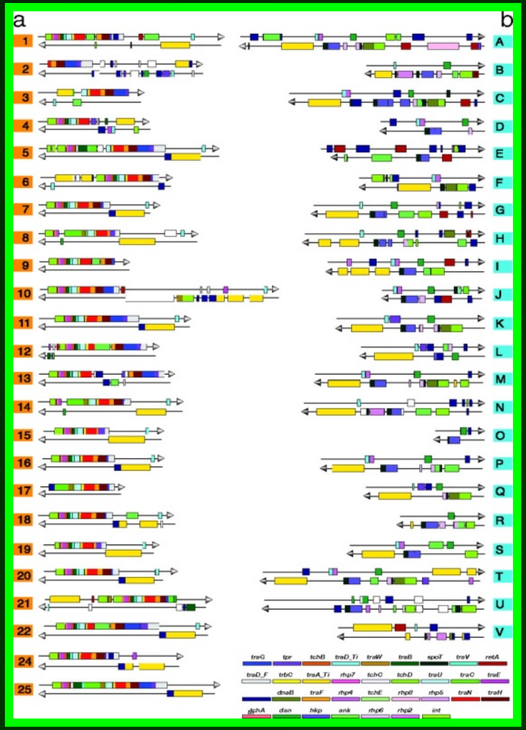
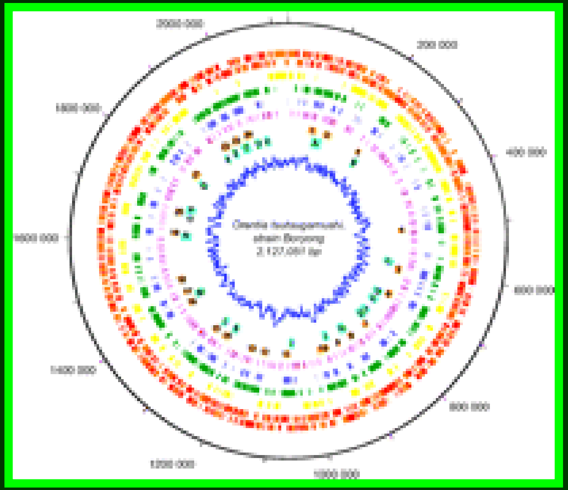
Orientia tsutsugamushi genome structure



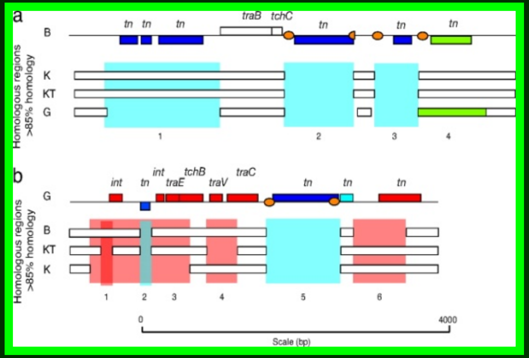




ca. 2 Mb

High repeat number

Low coding percentage



Gene duplication

High pseudogene contents

High number of mobile elements

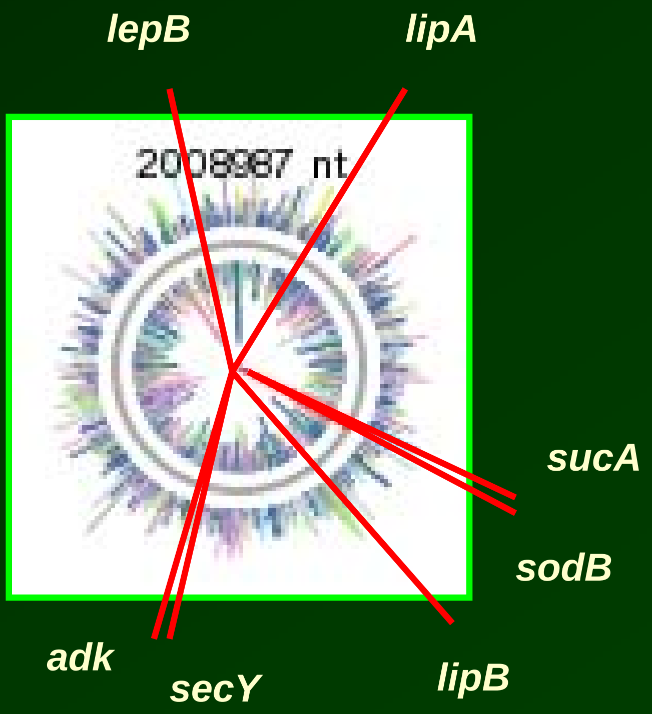
Highly repeated tra sequences ‘type IV secretory pathway’

Limited core genome

MLST target loci

7 target genes

adk	**adenosine kinase**
lepB	**GTP-binding protein**
lipA	**lipoyl synthase**
lipB	**lipoyltransferase**
secY	**preprotein translocase**
sodB	**Superoxide dismutase**
sucA	**2-oxoglutarate dehydrogenase**



Validated on other Rickettsiales (*Ehrlichia ruminantium*)
Part of the core genome

Strains

Region-wide analysis

MLST : Cambodia (Duong et al., 2010 – this presentation)

Thailand : under way
Vietnam : under way

56-kDa protein gene

Cambodia - Thailand - Vietnam

Combination of several works

Patients diagnosed with scrub typhus
Strains pathogenics for humans
2 reference strains from Korea and Japan

Goal: have an idea of the distribution, diversity and dynamics at the scale of the South East Asia region

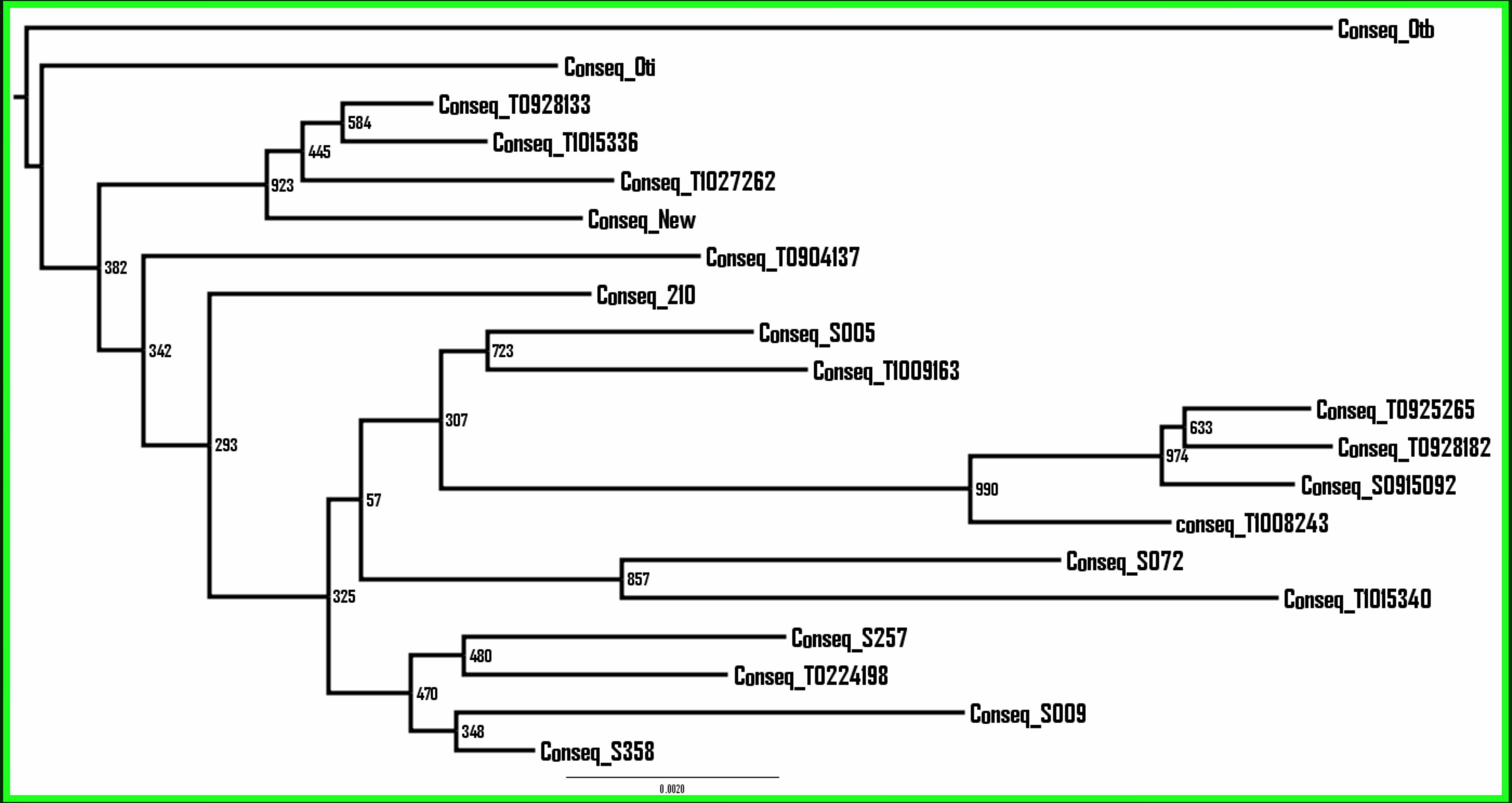
MLST

	adk	*lepB*	*lipA*	*lipB*	*secY*	*sodB*	*sucA*
Strain 005	1	1	1	1	1	1	1
Strain 009	2	2	2	2	2	2	2
Strain 072	3	3	3	3	2	3	3
Strain 210	4	4	4	4	3	4	4
Strain 257	5	5	5	5	2	5	5
Strain 358	6	6	2	6	2	2	4
Strain New	7	7	2	7	3	6	4
Strain Otb	8	8	6	1	4	7	6
Strain Oti	9	9	7	8	5	8	7
Strain S0915092	10	10	8	4	6	9	8
Strain T0224198	6	6	9	9	2	10	4
Strain T0904137	6	11	10	10	7	11	9
Strain T0925265	11	12	11	11	6	12	4
Strain T0928133	12	7	2	7	8	13	4
Strain T0928182	10	12	8	11	9	14	8
Strain T1008243	1	12	8	11	6	13	10
Strain T1009163	1	13	4	12	1	15	4
Strain T1015336	12	7	2	7	3	16	8
Strain T1015340	13	14	12	7	2	14	11
Strain T1027262	12	15	13	7	3	13	4
N Haplotypes	13	15	13	12	9	16	11

One patient – One strain – One ST
Clonal structure

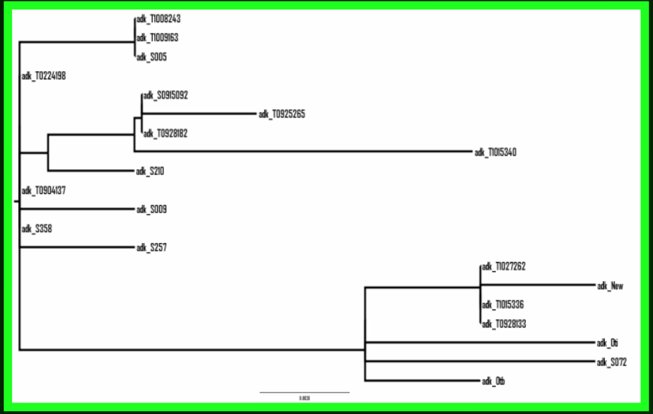
Specific habitat (chigger mites)
Divergent evolutionary paths (literature)
Positive selection

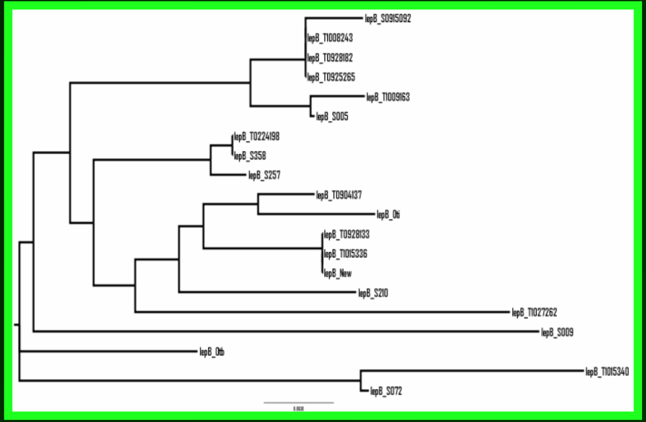
Concatenated sequences

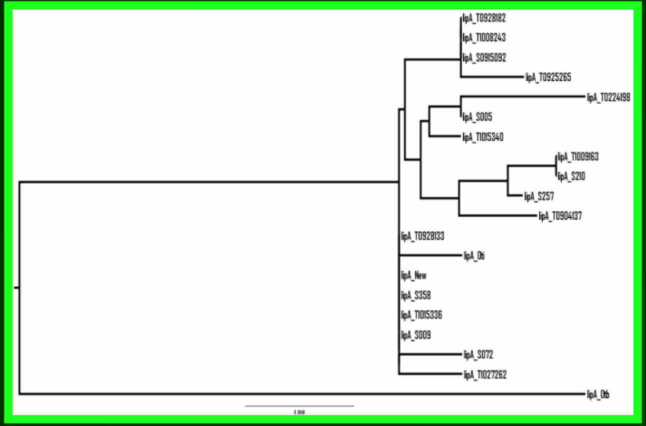


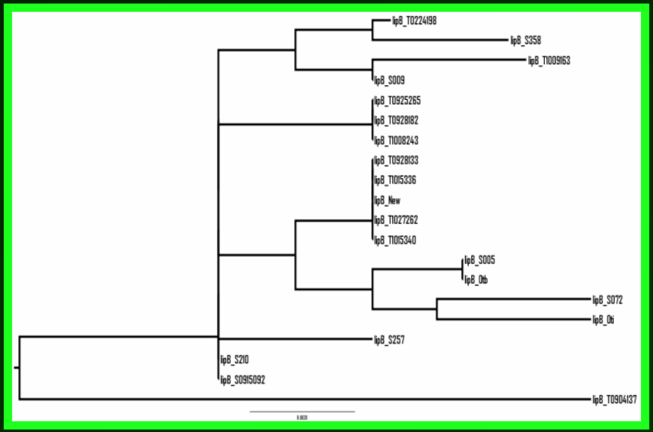
Cambodia vs. Korea/Japan
Weak bootstraps
Not clonal

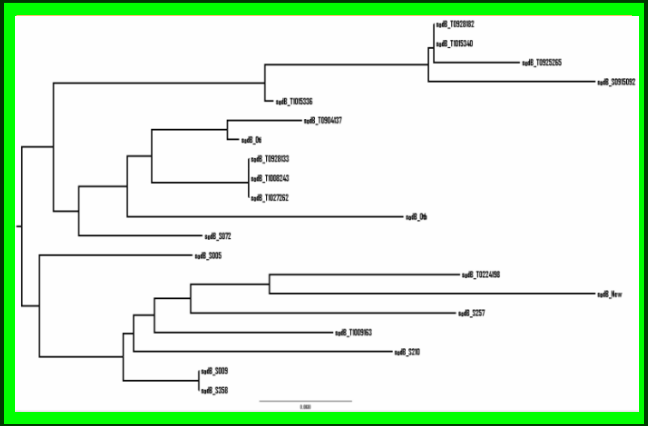
Individual gene trees

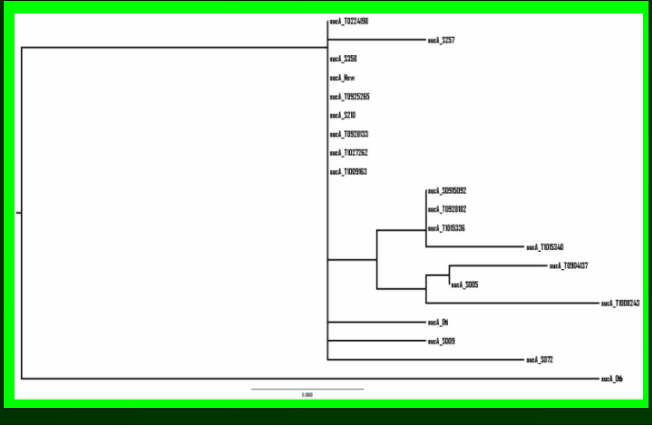






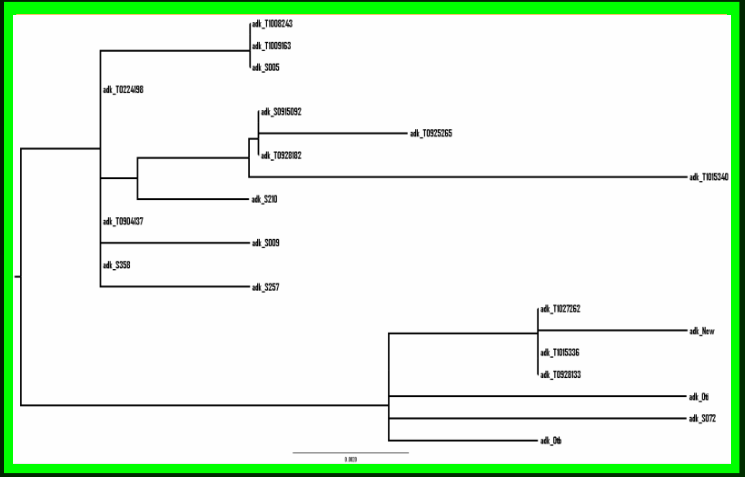


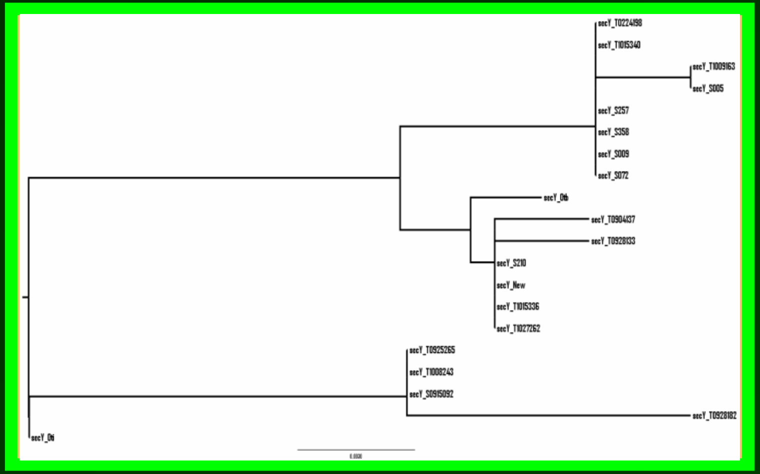


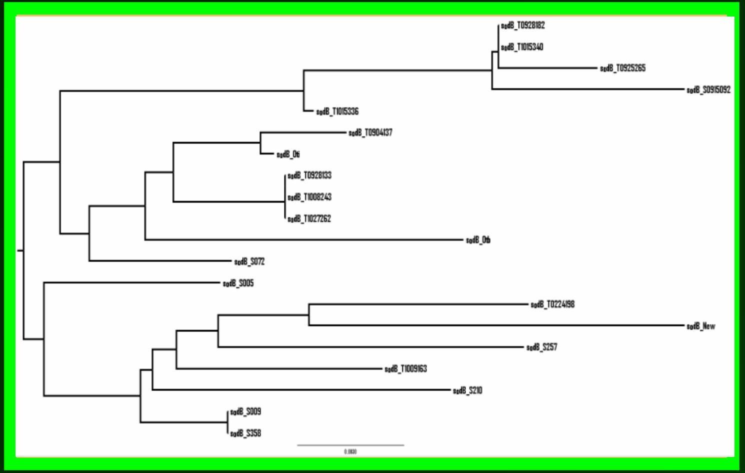


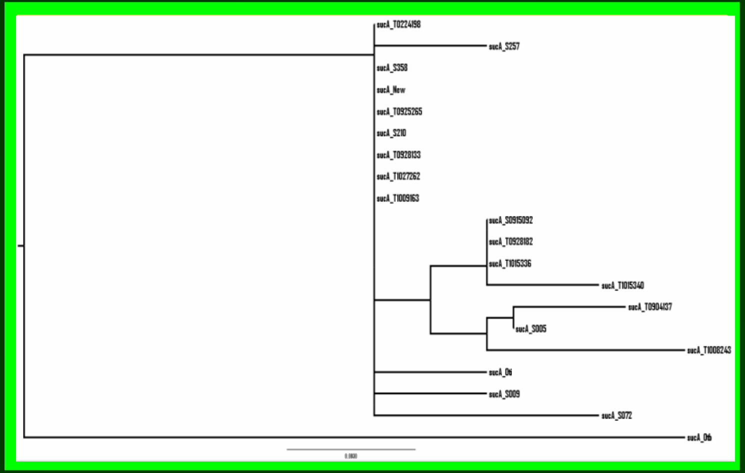
Differing topologies
Different evolutionary paths
Otb and Oti often separate
Not always / not at the same level

Differing topologies









Genes expected to be linked
Differing evolutionary paths

Neutrality

	Hp	S	h	h (s)	Pa	Na	Ns	Tajima's D	D*	F*
adk	13	18	19	12	6	10	9	-1.18764 NS	-2.05288 NS	-2.09066 NS
lepB	15	34	36	12	22	11	25	-0.51375 NS	-0.36526 NS	-0.47702 NS
lipA	13	24	26	21	3	9	17	-2.05011*	-3.02265**	-3.18223**
lipB	12	14	14	9	5	8	6	-1.39525 NS	-1.75136 NS	-1.91243 NS
secY	9	17	18	5	12	3	14	0.07808 NS	-0.19261NS	-0.13160 NS
sodB	16	27	27	12	15	9	18	-0.55983 NS	-0.81597 NS	-0.86129 NS
sucA	11	19	19	17	2	10	9	-2.11308*	-3.16817 NS	-3.32210 NS

Impossible to reject the null hypothesis of neutrality
Negative selection of core function genes (not neutral) : No
Positive selection of strains (not neutral) : No
Blurred figures : Not clonal but kind of "amphimictic"

DNA polymorphism

	Hp	**S**	η	**Pa**	η**(s)**	θ	π
Adk	13	18	19	6	12	5,35	0.00947
lepB	15	34	36	22	12	10.15	0.02191
lipA	13	24	26	3	21	7.33	0.00806
lipB	12	14	14	5	9	9.95	0.00718
secY	9	17	18	12	5	5.07	0.01257
sodB	16	27	27	15	12	7.52	0.01825
sucA	11	19	19	2	17	5.36	0.00619

Recombination and linkage disequilibrium

	Rm	ZnS	B	Q	PW	F	BP
adk	0	0.0863	0,0000	0,0000	136	6**	3
lepB	8	0.1195	0.1290	0.2188	496	33*/**	1
lipA	1	0.3997	0.4899	0.5000	231	0	0
lipB	1	0.1307	0.2308	0.2857	91	0	0
secY	0	0,2660	0.2667	0.4375	120	24*/**	16
sodB	7	0.0906	0.0385	0.0741	351	16*/**	9
sucA	1	0.2257	0.2222	0.2632	171	0	0

Evidence for recombination

Contradictory trends ?

STs	DNA polymorphism
Clonal populations	Recombination
Isolated patches	Exchange of genetic material
Divergent evolutionary paths	Co-infection (intracellular)

Contradiction does not exist in nature

Only things we do not understand yet

Chigger mite: the true host

- Vertical transmission
- Symbiont-like association
- No host to host movement

Small mammals: the maintenance host

- Multiple infections
- Recombination process
- Genetic diversity

Humans: the accidental host

- Rare and unique genetic events
- Looks clonal but is not

Different works same results

Mahidol Oxford Tropical Medicine Research Unit

Sonthayanon et al. 2010. High rates of homologous recombination in the mite endosymbiont and opportunistic human pathogen *Orientia tsutsugamushi*. PLoS Negl Trop Dis 4, e752.

Pasteur Institute in Cambodia – Cirad –University Montpellier 2

Duong et al., 2010. Diversity of *Orientia tsutsugamushi* clinical isolates in Cambodia reveals active selection and recombination process. Infect Genet Evol. doi:10.1016/j.meegid.2010.08.015

Same approach
Different strains
Different countries
Different loci

SAME RESULTS
SAME CONCLUSIONS

56-kDa type specific antigen (TSA)

High genomic plasticity
High level of duplication and rearrangements
Numerous repeats transposons and conjugative elements
Horizontal transfer
MLST analysis: extensive recombination

56-kDa protein gene

- **Unique to *O. tsutsugamushi***
- **Outer membrane surface**
- **Induces neutralizing antibody responses**
- **Involved in cell invasion (specific binding to fibronectin)**
- **Interacts directly with the mammalian host**
- **Subjected to host-driven adaptive selection**
- **9 significant clusters**

Good target for investigating population structure

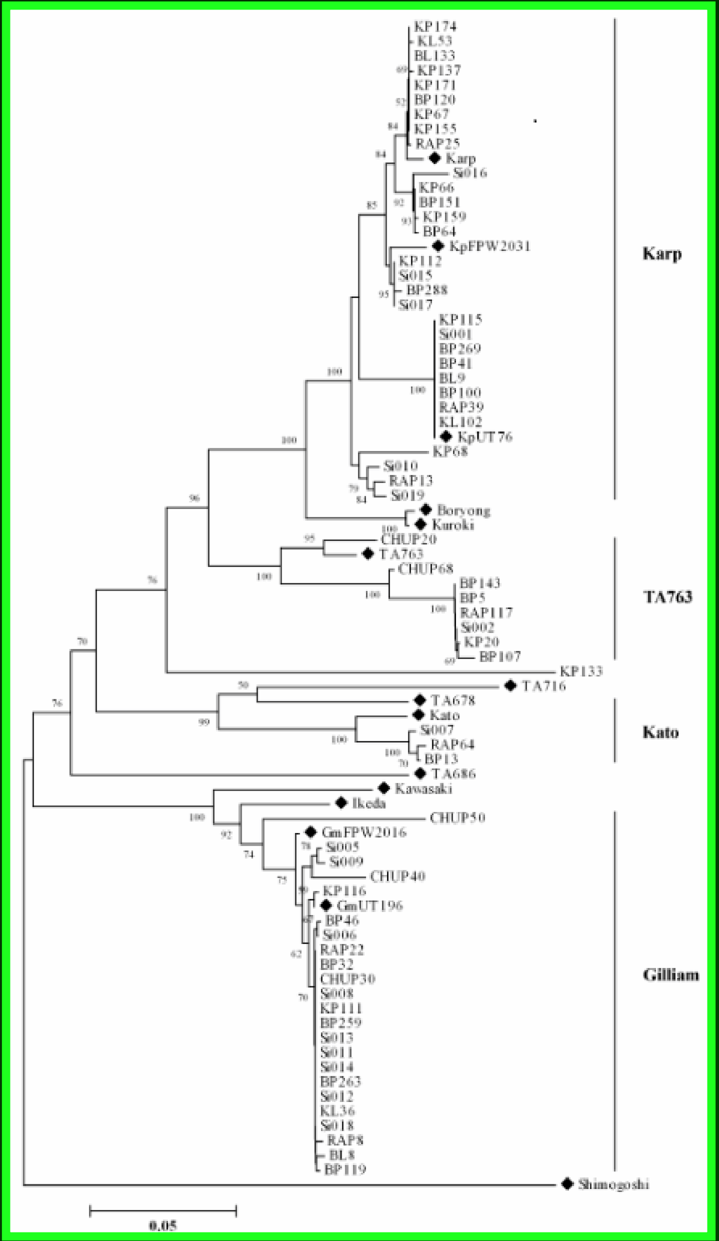
56-kDa TSA diversity in Thailand

Variable domain I (VD I) to domain IV (VD IV)
PCR amplification and sequencing

Country-wide sampling scheme (63 strains)
8 genotypes reported previously
Only 4 detected here

Karp: 46%
Gilliam: 34.9%
TA763: 12.7%
Kato: 4.8%

Karp genotype confirmed as the most common
Gilliam and TA763 also commonly found (contrast)
TA716 was not found
Difference with previous reports
Spatial and temporal variations ?



56-kDa TSA diversity in Cambodia and Vietnam

Full length sequence
PCR amplification and sequencing

Vietnam
3 groups

Karp (77%)
TA763 (15.5%)
JG-v (7.5%)

Cambodia

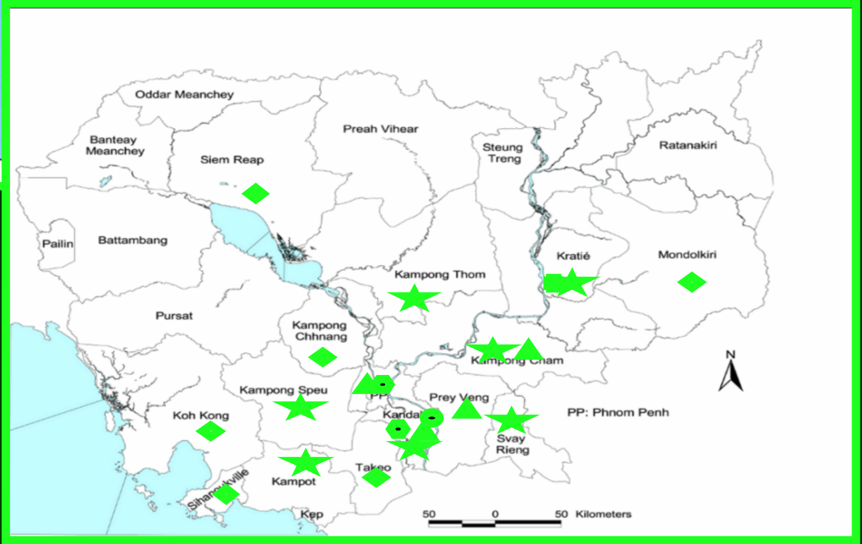
12 provinces
28 strains

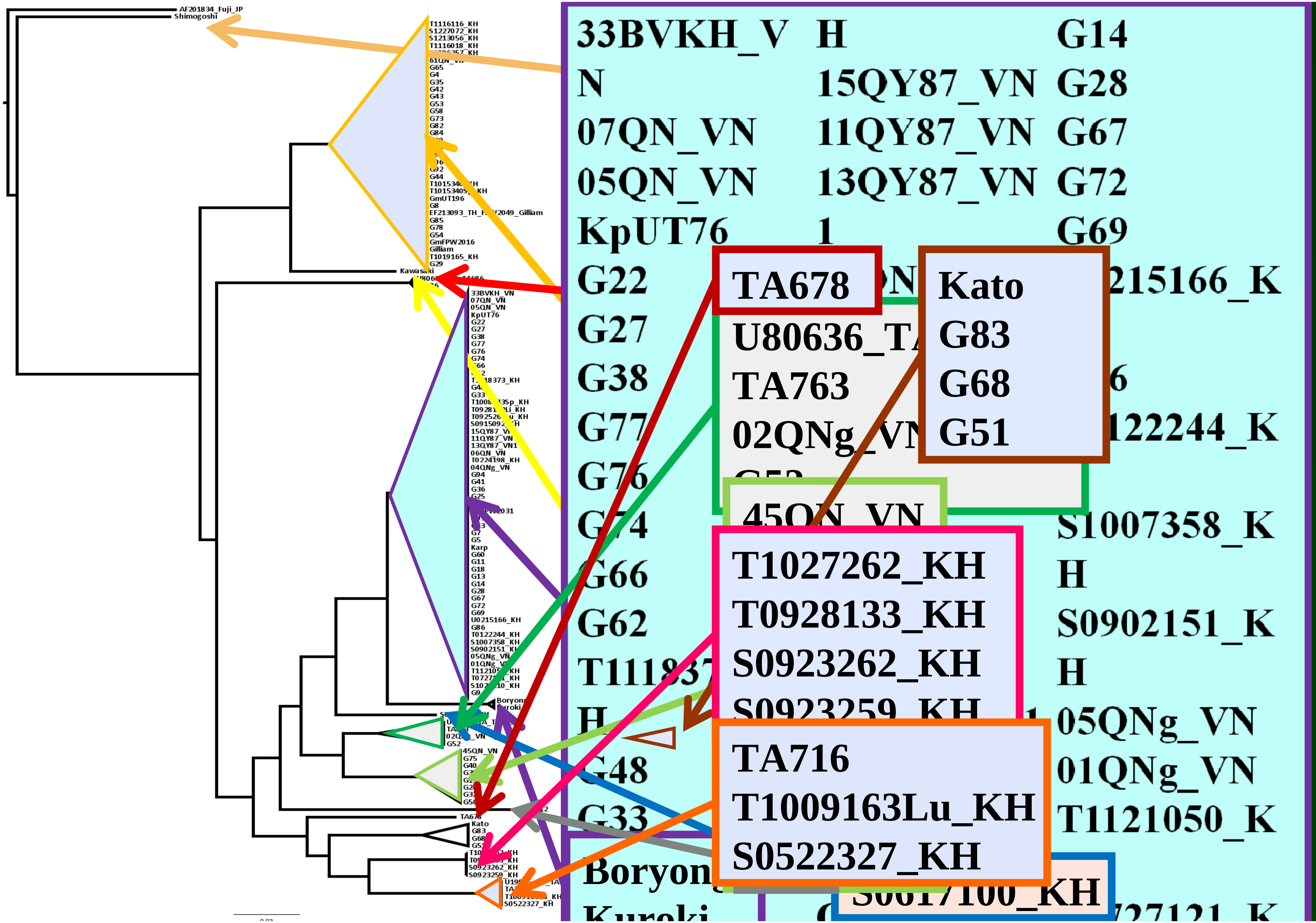
Vietnam

3 provinces
12 strains

Cambodia
5 major groups

Karp (43.5%),
JGv (25%)
Kato/TA716 (21.5%)
TA763 (3.5%)
Gilliam (3.5%)





33BVKH_V
N
07QN_VN
05QN_VN
KpUT76
G22
G27
G38
G77
G76
G74
G66
G62
T111837
H
G48
G33

H
15QY87_VN
11QY87_VN
13QY87_VN
1

G14
G28
G67
G72
G69
215166_K
6
122244_K
S1007358_K
H
S0902151_K
H
05QNg_VN
01QNg_VN
T1121050_K
727121_K

TA678

U80636_TA
TA763
02QNg_VN

Kato
G83
G68
G51

45QN_VN

T1027262_KH
T0928133_KH
S0923262_KH
S0923259_KH

TA716
T1009163Lu_KH
S0522327_KH

S0617100_KH

Boryong
Kuroki

Conclusion

No geographic clustering

- **56-kDa**
- **MLST**

Distribution depends on small mammals distribution
Distribution is impacted by human activities

- **Deforestation, land use**
- **Urban growth**
- **Global changes**

Distribution most likely to evolve in space and time
Under diagnosed in Cambodia and Vietnam
Clinical samples are not representative (rare and unique events)
Focus on mites and rodents for actual distribution

Priority probably not on epidemiology
Priority probably on awareness and efficient early diagnostic and treatment

Partnership

I.P. Cambodia
V. Duong
K.Blasdell
P. Buchy

Mahidol- Siriraj
P. Wongprompitak
W. Anukool
Y. Suputtamongkol
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P. Ekpo

CNRS / UM2
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I.P. Nha Trang
Trinh Thi Xuan Mai
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Calmette Hospital
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Kantha Bopha Hospital
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Thank you for your attention