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Orientia tsutsugamushi, agent of scrub typhus, displays a single metapopulation with maintenance of ancestral haplotypes throughout continental South East Asia

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ABSTRACT

Orientia tsutsugamushi is the causative agent of scrub typhus, a major cause of febrile illness in rural area of Asia–Pacific region. A multi-locus sequence typing (MLST) analysis was performed on strains isolated from human patients from 3 countries in Southeast Asia: Cambodia, Vietnam and Thailand. The phylogeny of the 56-kDa protein encoding gene was analyzed on the same strains and showed a structured topology with genetically distinct clusters. MLST analysis did not lead to the same conclusion. DNA polymorphism and phylogeny of individual gene loci indicated a significant level of recombination and genetic diversity whereas the ST distribution indicated the presence of isolated patches. No correlation was found with the geographic origin. This work suggests that weak divergence in core genome and ancestral haplotypes are maintained by permanent recombination in mites while the 56-kDa protein gene is diverging in higher speed due to selection by the mammalian immune system.

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- **Identification and analyze of sequence types (ST)**

Table 1
Sequence types (ST) of *O. tsutsugamushi* isolated from patient samples.

Strains	MLST loci						
	adk	lepB	lipA	lipB	secY	sodB	sucA
S005	1	1	1	1	1	1	1
S009	2	2	2	2	2	2	2
S072	3	3	2	3	2	3	3
S210	4	4	3	4	3	4	4
S257	5	5	4	5	2	5	5
S358	6	6	2	6	2	2	4
New	7	7	2	7	3	6	4
U0720315	13	6	18	7	17	12	4
U0809161	6	18	2	2	2	2	5
U0820321	6	21	2	2	2	2	5
U0902310	12	19	19	15	16	22	14
U1012321	16	20	17	4	2	24	4
U1014302	6	6	2	2	2	23	16
42BVKH	13	24	11	7	17	28	4
47BVKH	16	6	21	4	2	18	17
47QNg	18	2	2	4	18	26	4
49QNg	17	23	10	16	2	25	4
54BVKH	13	7	11	7	3	12	18
91BVKH	13	7	11	7	3	31	19
92BVKH	18	22	20	7	19	27	4
TH2011	6	15	22	10	20	12	4

Strains	MLST loci						
	adk	lepB	lipA	lipB	secY	sodB	sucA
TH2033	6	12	6	10	8	12	4
TH2037	11	13	7	7	3	12	10
TH2043	11	14	8	11	9	13	11
TH2059	19	13	6	17	5	29	20
TH2066	12	14	10	18	11	29	4
TH2082	11	15	9	11	10	13	11
TH2085	13	25	23	19	3	25	10
TH2190	12	14	10	12	11	14	5
TH2191	11	7	11	7	3	12	12
TH2208	11	12	12	11	10	15	11
TH2221	11	15	9	11	9	15	11
TH3007	11	13	7	7	2	16	12
TH3029	13	16	13	4	12	17	13
TH4005	14	14	11	13	13	18	14
TH4006	15	17	14	1	14	19	13
TH4007	13	14	15	4	3	20	15
TH4016	12	26	24	4	21	30	21
TH4024	12	6	16	14	15	21	12
Otb	9	8	5	1	4	8	7
Oti	8	9	6	8	5	7	6
Otgm	10	10	6	4	7	9	8
Otkp	8	11	6	9	6	11	4
Otkt	8	9	6	8	5	10	9
N* of Haplotypes	19	26	24	19	21	31	21

- **Identification and analyze of sequence types (ST)**

- **Phylogenetic analysis of the 56-kDa TSA**
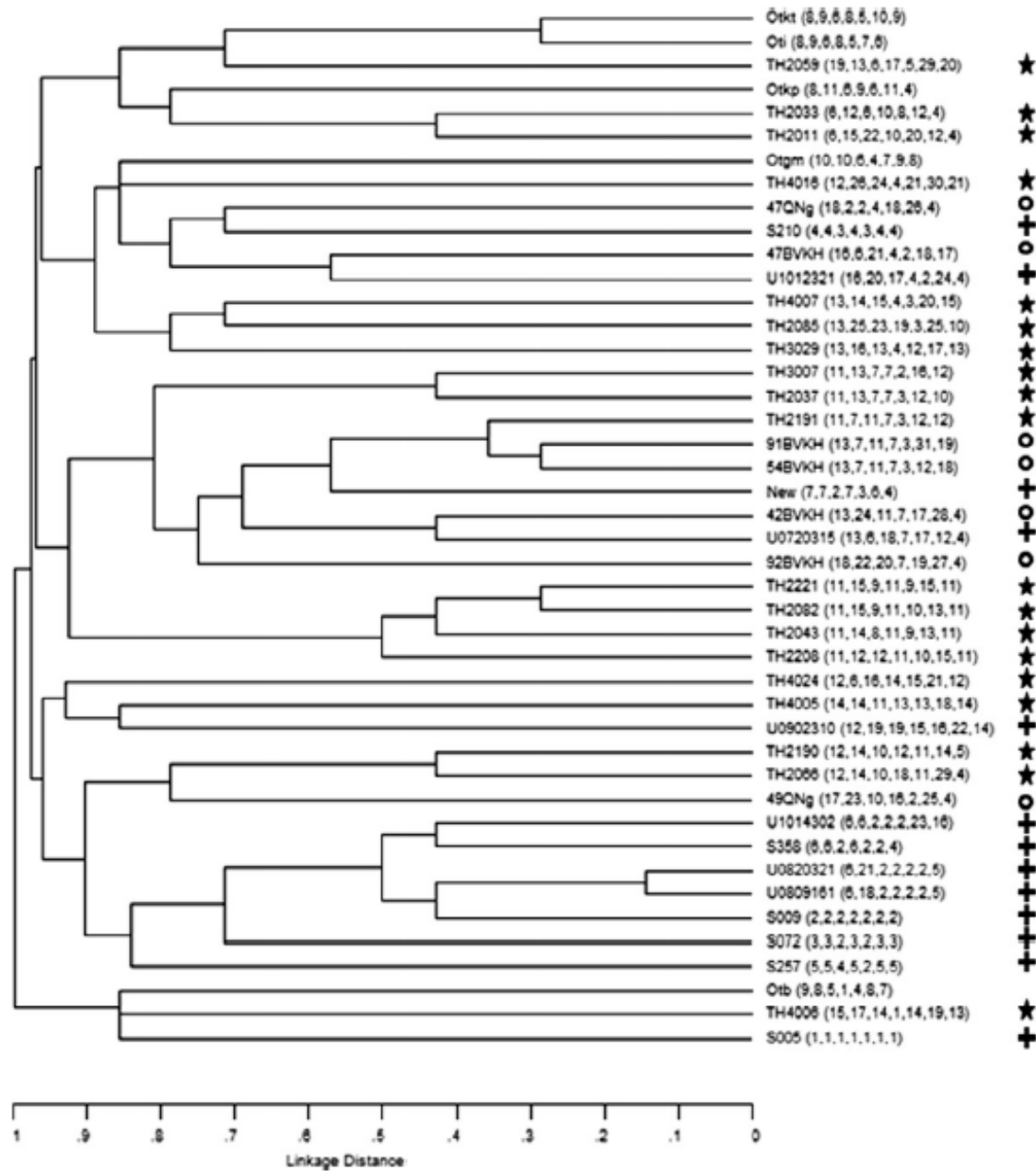
Fig. 1. UPGMA tree of O. tsutsugamushi strains ST.
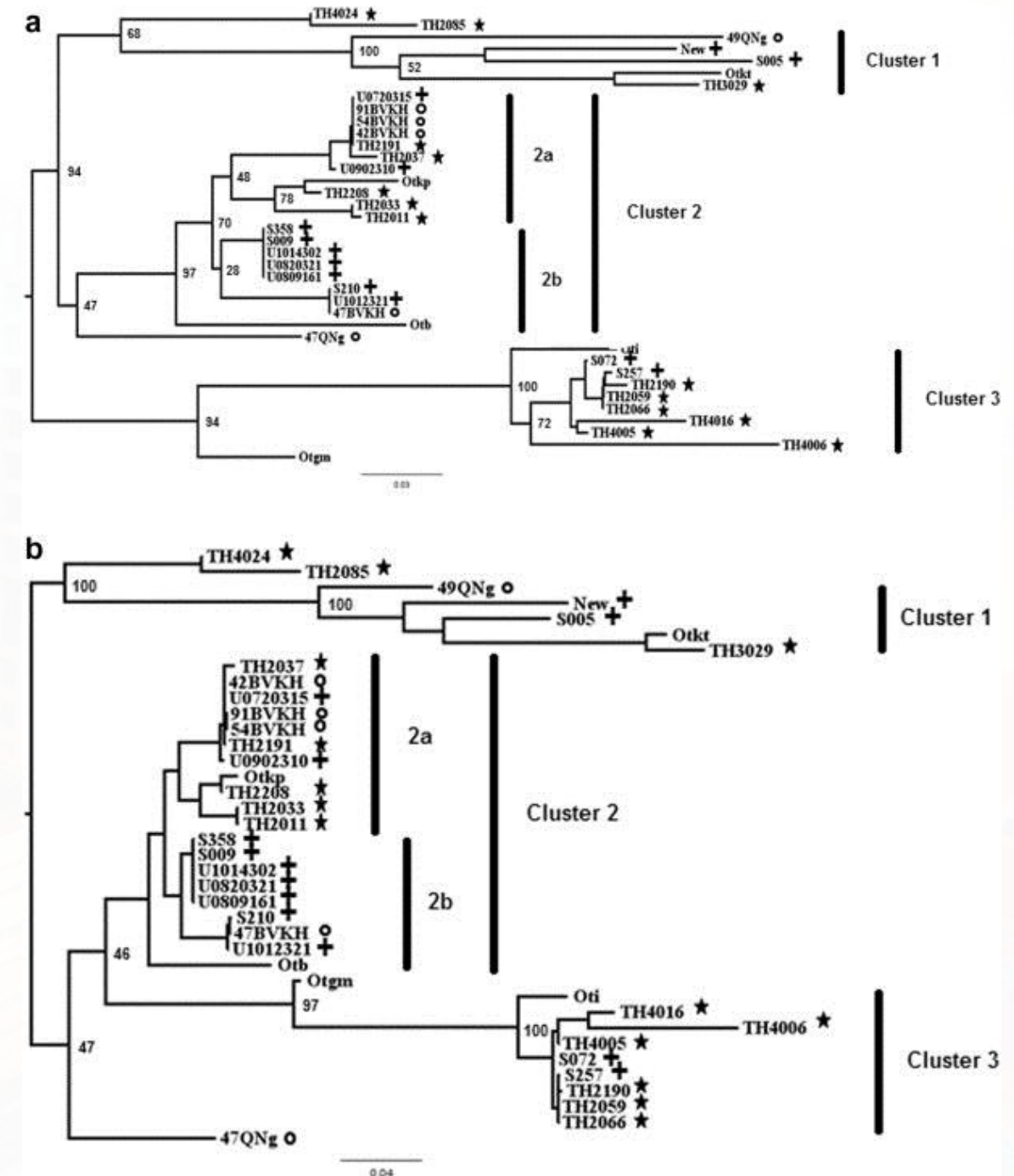
Fig. 2. Phylogenetic trees of 56-kDa protein gene from VD I to VD IV region.

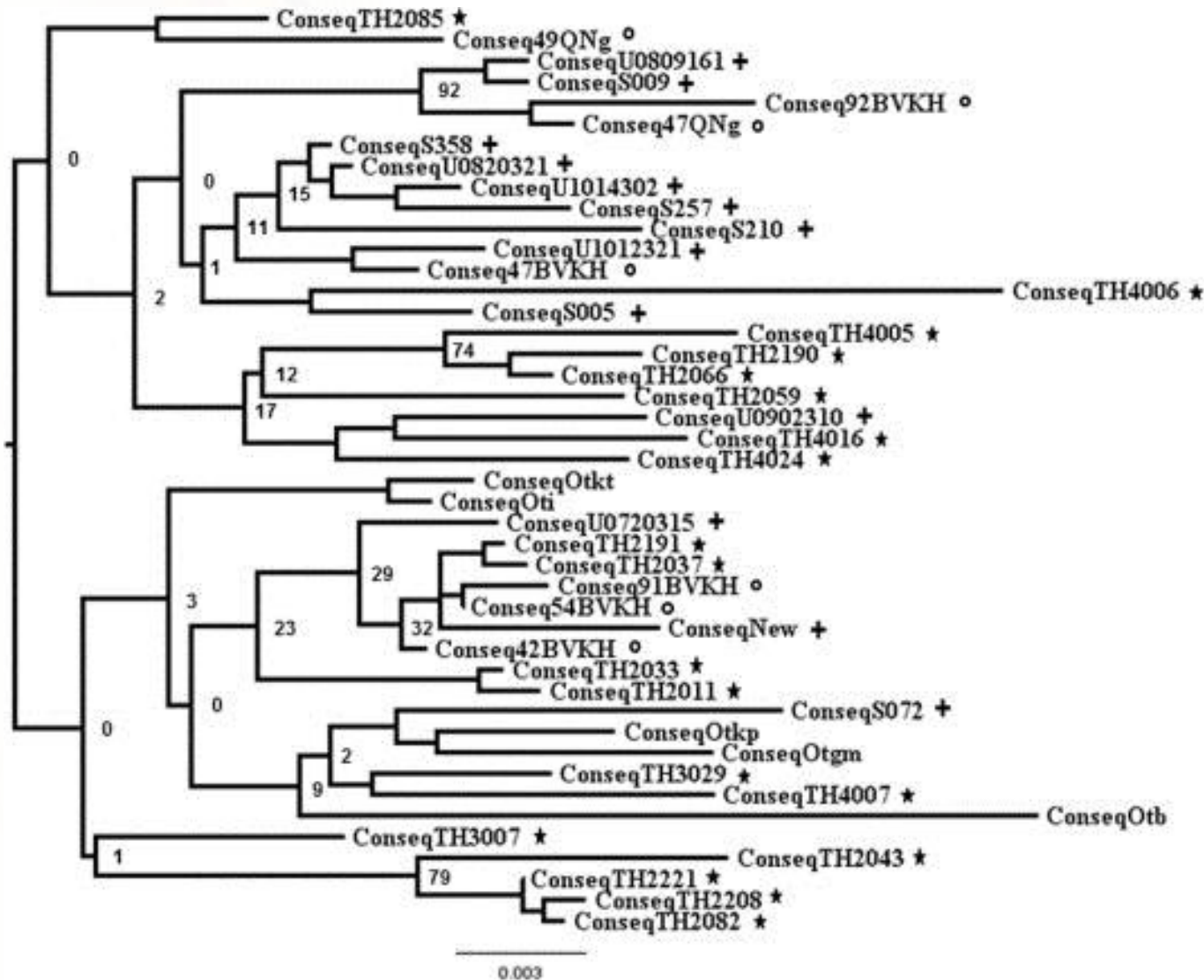- **Phylogenic analysis of sequenced MLST loci**

Fig. 3. Maximun-likelihood tree under the GTR+G model of concatenated MLST loci sequences.

- **DNA polymorphism and selective pressure**

Table 2
DNA polymorphism and neutrality tests of MLST loci of *O. tsutsugamushi* isolated from patient samples.

Locus	Hp	S	η	$\eta(S)$	Pa	θ	π	Na	Ns	K_a	K_s	K_a/K_s	Tajima's D	D*	F*
Adk	19	28	31	18	10	7.12644	0.00956	17	10	0.00606	0.02414	0.251	-1.59931^{NS}	-3.41374^{**}	-3.30358^{**}
lepB	26	37	41	13	24	9.42529	0.02139	11	30	0.00363	0.09110	0.040	-0.32835^{NS}	-1.24701^{NS}	-1.09754^{NS}
lipA	24	37	41	26	11	9.42529	0.00895	11	23	0.00463	0.02333	0.199	-2.12057^{*}	-3.79773^{**}	-3.81262^{**}
lipB	19	22	22	9	13	5.05747	0.00897	11	11	0.00537	0.02153	0.250	-1.33524^{NS}	-1.26122^{NS}	-1.52422^{NS}
secY	21	27	29	12	15	6.66667	0.01074	7	19	0.00175	0.03959	0.044	-1.02707^{NS}	-1.88993^{NS}	-1.88800^{NS}
sodB	31	38	40	15	23	9.19541	0.01723	12	23	0.00757	0.05727	0.132	-1.04306^{NS}	-1.52156^{NS}	-1.60871^{NS}
sucA	21	25	26	16	9	5.97701	0.00574	14	12	0.00233	0.01887	0.124	-2.07465^{*}	-3.13552^{*}	-3.28528^{**}

Hp: number of haplotypes; S: polymorphic sites; η: total number of mutations; $\eta(s)$: number of singletons; Pa: parsimony informative sites; θ: Watterson's mutation parameter (per sequence calculated from); π: nucleotide diversity; Na: number of non-synonymous substitutions; Ns: number of synonymous substitutions; k_a: rate of non-synonymous substitutions; k_s: rate of synonymous substitutions; *Significant at $p < 0.05$; **Significant at $p < 0.02$; NS: not significant. Tajima's D: Tajima's D test for neutrality; D* and F*, Fu and Li's D* and F* test for neutrality.

- **Age of most recent common ancestor**

Table 3
Estimated age of the most recent common ancestor for the concatenated MLST loci.

Model	Age mean value [a]	ESS [b]	95% HPD limits [c]	Clock rate (mean)
Relax, constant, HKY	140.964	364.755	25.529–235.776	1.57×10^{-4}
Strict, constant, HKY	316.741	584.904	34.679–793.026	9.19×10^{-5}
Relax, BSP, GTR	361.57	1189.612	25.722–1098.090	8.01×10^{-5}
Relax, BSP, GTR+g4	306.726	114.373	20.788–1131.545	1.12×10^{-4}
Relax, BSP, HKY+g4	508.695	94.806	30.779–2459.947	9.68×10^{-5}
Strict, constant, GTR+g4	145.853	953.683	24.815–271.296	1.98×10^{-4}
Strict, constant, HKY+g4	360.987	543.354	27.523–493.609	1.74×10^{-4}

[a] Value is given in years.
[b] ESS = effective sample size.
[c] HPD: higher posterior density.

Table 4
Estimated age of the most recent ancient common ancestor for the 56-kDa protein gene.

Model	Age mean value [a]	ESS [b]	95% HPD limits [c]	Clock rate (mean)
Relax, constant, HKY	10698.736	435.787	147.918–33810.385	1.40×10^{-4}
Strict, constant, HKY	24426.885	587.934	618.437–72325.069	3.99×10^{-5}
Relax, constant, GTR	27286.289	287.357	230.835–82040.054	6.88×10^{-5}
Strict, constant, GTR	21380.724	643.776	480.960–75770.973	4.03×10^{-5}
Strict, constant, GTR + g4	24156.207	845.462	778.238–83851.991	4.05×10^{-5}
Strict, constant, HKY + g4	37619.574	505.351	773.025–88060.418	4.14×10^{-5}

[a] Value is given in years.
[b] ESS = effective sample size.
[c] HPD: higher posterior density.