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Spotted Fever Group *Rickettsia* sp. Closely Related to *R. japonica*, Thailand

To the Editor: In response to a recent report that suggested human infection with *Rickettsia japonica* in northeastern Thailand (*1*), we phylogenetically reexamined spotted fever group rickettsiae (SFGR) from Thailand. The organism had been isolated from a male *Haemaphysalis hystricis* tick found on Mt. Doi Suthep, Chiang Mai, northern Thailand, in December 2001. The strain was designated TCM1 and was not distinguishable from *R. japonica* by indirect immunoperoxidase stain using monoclonal antibody (*2*).

After propagating strain TCM1 in L-929 cell culture, we extracted DNA by using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). We subjected the DNA to sequencing that targeted a 491-bp fragment of rickettsial outer membrane protein A (*ompA*), a 394-bp fragment of the rickettsial genus–specific 17-kDa antigen gene, and a 1,250-bp fragment of citrate synthase gene (*gltA*). Direct sequencing of amplicons was performed as previously described. (*3*). Phylogenetic analyses based on o*mpA* indicated that strain TCM1 was closely related to and clustered within the same clade as *R. japonica* strain YH (98.4% identity) (Figure, panel A). Also, a 17-kDa antigen gene obtained from strain TCM1 showed 99.5% identity to the corresponding gene of *R. japonica* (Figure, panel B). Our phylogenetic analysis with *ompA* and 17-kDa antigen gene showed that strain TCM1 was closely related to *R. japonica* but distinguished from *Rickettsia* sp. PMK94 (which was closely related to *R. heilongjiangensis* from northeastern China) (*3*); another SFGR agent, *R. honei* from *Ixodes granulatus* ticks in Thailand (*4*), was apparently different from strain TCM1 (Figure). Phylogenetic analyses based on *gltA* (99.4%–99.6% identity) showed that strain TCM1 is also closely related to *R. japonica* and *Rickettsia* sp. strain PMK94 (data not shown). Thus, we describe the *R. japonica* group in Thailand. DNA sequences of strain TCM1 were determined and deposited in GenBank/EMBL/DDBJ under the following accession nos.: *ompA*, AB359459; 17-kDa antigen, AB359457; *gltA*, AB359458.

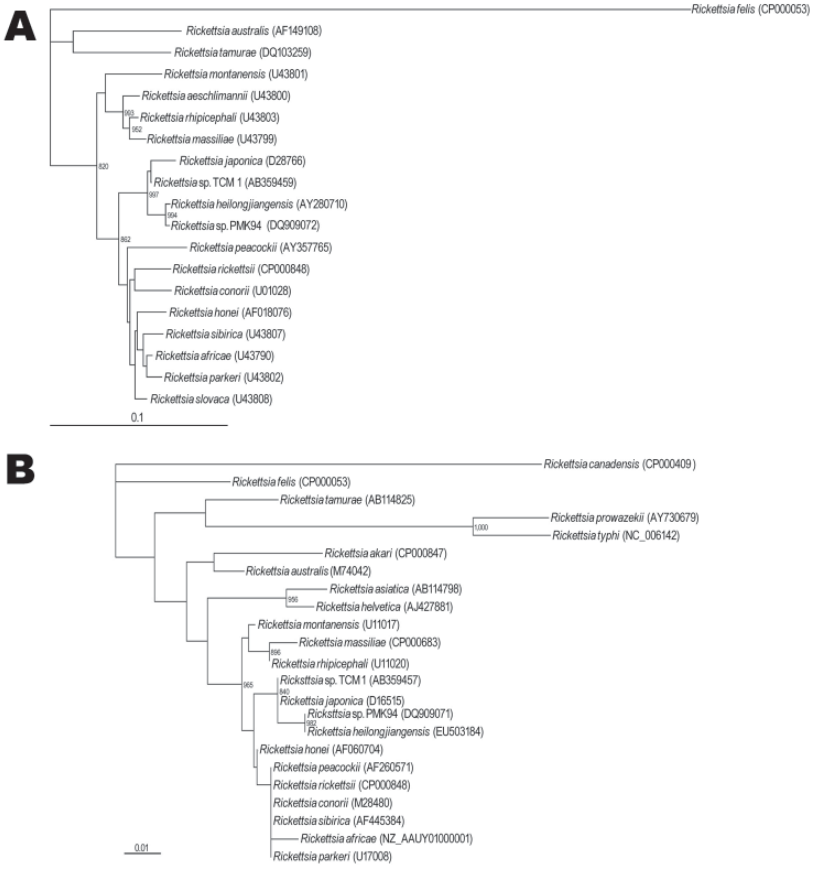

Figure. Phylogenetic analysis based on *ompA* gene (A) and rickettsial genus–specific 17-kDa antigen gene (B). Sequences were aligned by using the ClustalW software package (http://clustalw.ddbj.nig.ac.jp/top-j.html), and neighbor-joining phylogenetic tree construction and bootstrap analysis were conducted according to the Kimura 2-parameter method (www.ddbj.nig.ac.jp). Pairwise alignments were performed with an open-gap penalty of 10, a gap extension penalty of 0.5, and a gap distance of 8. Multiple alignments were also performed with the same values, and the phylogenetic branches were supported by bootstrap analysis with 1,000 replications (>800 were indicated). *Rickettsia felis* (CP000053) and *R. canadensis* (CP000409) were used as outgroups for *ompA* and 17-kDa antigen gene, respectively. The phylogenetic tree was constructed by using TreeView software version 1.5 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). Scale bars indicate nucleotide substitutions (%) per site.

R. japonica is the specific pathogen of Japanese spotted fever, which has been found mainly in southwestern Japan (*5*). The present strain, closely related to *R. japonica*, is likely to have been isolated from *H. hystricis* in Thailand because *R. japonica* frequently has been isolated, or detected by PCR, from the same tick species in Japan (*6*). Such tick species–specificity of SFGR should be considered when speculating on any geopathologic relationships of rickettsioses among different SFGR-endemic areas. Previous reports on spotted fever–positive results of human serosurveys (*7*,*8*) and on a clinical case (*9*) in northern Thailand may provide epidemiologic background. In Asia, multiple species of rickettsiea (e.g., *R. japonica*, *R. heilongjiangensis*, *R. honei*) are the causative agents of spotted fever rickettsioses, so the agent closely related to *R. japonica* could cause spotted fever in Thailand. Additionally, *R. japonica* has been found in Korea (*10*), and our current study indicates that *R. japonica* and its genetic variants are widely distributed in Far Eastern countries, including Japan (Grant-in-Aid for International Cooperative Research, unpub. data). Therefore, the epidemiology and genetic variation of SFGR throughout Asia should be examined by molecular studies.

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Segniliparus rugosus Infection, Australia

To the Editor: Recently, a female teenager with cystic fibrosis who resided in tropical north Queensland, Australia, was found to be infected with *Segniliparus rugosus*. She was homozygous for the deltaF508 mutation, had well-preserved lung function, and regularly played competitive sports. Unlike many cystic fibrosis patients, she did not have a history of chronic *Pseudomonas aeruginosa* infections, but *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* had been previously isolated from her sputum. In May 2007, she described reduced exercise tolerance and increased cough with excess sputum production. Lung function testing showed modest spirometric decline. A computed tomographic scan of the chest showed significant mucus plugging and bronchiectasis, uncommon without previous *P. aeruginosa* infection. Sputum was 3+ smear positive for acid-fast bacilli (AFB), and *S. rugosus* was isolated from liquid culture. Empiric antimicrobial drug therapy was changed to rifabutin and co-trimoxazole because these drugs have been effective in previous cases (*1*). Clinically, the patient showed response to the treatment. After 12 months of treatment, her sputum was still 3+ positive for AFB, and *S. rugosus* was again found in culture. She was referred to a pediatric teaching hospital in Brisbane with worsening respiratory symptoms precipitated by influenza B infection. Antimicrobial drug therapy with intravenous imipenem, oral moxifloxacin, and co-trimoxazole for 2 weeks resulted in clinical improvement but little reduction in smear positivity.

The initial AFB smear-positive sputum specimen underwent routine decontamination with sodium hydroxide and neutralization and was inoculated into radiometric 12B vials (Bec-