To determine the phylogenetic position of two new rickettsial strains isolated from ticks in China, 16S ribosomal DNA, gltA, and ompA (apart from the tandem repeat units) genes were amplified by PCR and sequenced. The phylogenetic relationships between these strains and other rickettsiae were inferred from the comparison of sequences of the three genes by the parsimony, neighbor-joining, and maximum-likelihood methods. The results demonstrated that the 054 strain, a rickettsia pathogenic in humans, and the HL-93 strain were related and clustered together with Rickettsia japonica. Significant statistical bootstrap values (100 and 92%) supported the nodes in this cluster. Based on previous genotypic and antigenic data and the phylogenetic analysis presented here, the 054 and HL-93 strains should be considered as new species, and we formally propose that they be named “Rickettsia heilongjiangii” and “Rickettsia hulinii,” respectively.
sequence Navigator and AutoAssembler software. Each base position was established at least three times in both the forward and reverse directions. Sequences of the 16S rDNA, gltA, and ompA genes were aligned by using the multisequence alignment program CLUSTAL, within the BISANCE environment (7). Phylogenetic relationships between strains 054 and HL-93 and other SFG rickettsiae were inferred by using version 3.4 of the PHYLIP software package (10). The distance matrices generated by DNADIST were determined under the assumptions of Kimura (13) and were used to infer dendrograms by the neighbor-joining method (25). Two other dendrograms were constructed by data processing with the maximum-likelihood and parsimony programs in PHYLIP. A bootstrap analysis based on 100 randomly generated trees by using SEQBOOT and CONSENSE in PHYLIP was performed to estimate the node reliability of the trees obtained by the three phylogenetic methods (4).

The primers fD1 and rP2 amplified 1,463- and 1,461-bp fragments of the 16S rDNA gene from strains 054 and HL-93, respectively. The gltA gene was amplified in two fragments. Overall, sequences of 1,238 and 1,237 bp were obtained from the 054 and HL-93 strains, respectively. PCR amplification and sequencing of the ompA gene excluded the central tandemly repeat region. The 5′-end fragment of the gene was 611 bp for both the 054 and HL-93 strains. The 3′ part of the gene was amplified in four fragments. Overall, sequences of 3,187 and 3,181 bp were obtained from strains 054 and HL-93, respectively. The sequenced fragments of the rOmpA gene were analyzed from base positions 91 to 680 and 3608 to 6789 with respect to the sequence published for *R. rickettsii* (2) and were therefore submitted to GenBank as two separate fragments. Strains 054 and HL-93 clustered together with *R. japonica* into a well-defined, strongly supported monophyletic group within the SFG rickettsiae, with bootstrap values of 91 and 100% for the node where strains and HL-93 branched together in the gltA and ompA trees, respectively, and values of 92 and 100% for the node where both strains clustered with *R. japonica*. Similar phylogenetic organizations were inferred from the analysis of all three genes by using the three phylogenetic analysis methods (Fig. 2).

Historically, the classification and identification of rickettsiae have been based on differences in their epidemiology, serology, and intracelllar growth characteristics. Because different species may have common ecological features (e.g., geographic distribution, arthropod vectors, etc.) and due to serological cross-reactivity, it is difficult to distinguish between rickettsiae. Moreover, DNA-DNA hybridization, a criterion used for the definition of species in the family *Enterobacteriaceae*, is not applicable to SFG rickettsiae (8). Other recognized tools for the taxonomic classification of bacteria, such as the study of protein profiles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, monoclonal antibodies, Western blotting, and hybridization of labeled cloned DNA probes (1, 3, 17, 21, 31, 32, 34), are not widely applicable and lack interlaboratory reproducibility. Therefore, for over 10 years, the reference method for the identification of members of SFG rickettsiae has been the indirect microimmunofluorescence serologic typing test with mouse sera (18). Recently, phylogenetic analyses and the determination of taxonomic relationships based on comparisons of sequences of a combination of various genes have greatly facilitated the study of rickettsial taxonomy. The 16S rDNA (23, 26, 28), gltA (24), and ompA (11, 22) genes appear to be the most useful sequences. In our study, the 054 and HL-93 strains demonstrated ompA sequence homologies of 98.1% with each other and 96.6 and 95.6% with *R. japonica*, respectively, which are higher than...
ettsia are the first showing isolates clustering with grouped into a well-supported cluster in the phylogenetic trees from our study show that strains 054 and HL-93 are distinct (94 and 90%, respectively). Although closely related, the data GenBank accession numbers are AF172942 and AF178037 for " and " heilongjiangii Rickettsia cies, and we formally propose that these be named "alternative genomic tools. However, based on data from pre-

Japan and in China. The current rickettsial taxonomy is based This suggests that an SFG rickettsia may have evolved sepa-

China, where the 054 and HL-93 strains were isolated, is lo-

main islands. The island is located between 132° to 135° East and 33° to 35° North. To the north is the Seto Inland Sea, while and 33° to 35° North. To the north is the Seto Inland Sea, while

Tick-associated rickettsiae are characterized by limited, spe-

program Abstr. EUWOG-ASR Joint Meeting, p. 12, 1999). (5, 6, 15, 29, 30, 35–41; L. Chen, J. Z. Zhang, and D. Z. Bi, in concordance with previous phenotypic and genotypic studies

REFERENCES


FIG. 2. Dendrogram representing phylogenetic relationships between Rickettsia species inferred from the comparison of gltA (left) and ompA (right) sequences by the neighbor-joining method. The bootstrap values are indicated at the nodes.