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Proposals To Unify the Genera *Bartonella* and *Rochalimaea*, with Descriptions of *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov., and To Remove the Family *Bartonellaceae* from the Order *Rickettsiales*

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DNA hybridization data (hydroxyapatite method, 50 to 70°C) indicate that *Rickettsia prowazekii*, the type species of the type genus of the family *Rickettsiaceae*, is substantially less closely related to *Rochalimaea* species than was previously thought. The levels of relatedness of *Rickettsia prowazekii* to *Rochalimaea* species and to *Bartonella bacilliformis* under optimal conditions for DNA reassociation were 0 to 14%, with 25.5% or greater divergence in related sequences. When stringent reassociation criteria were used, the levels of relatedness were 0 to 2%. The genera *Bartonella* and *Rochalimaea* are currently classified in different families (the *Bartonellaceae* and the *Rickettsiaceae*) in the order *Rickettsiales*. On the basis of DNA relatedness data, previous 16S rRNA sequence data, guanine-plus-cytosine contents, and phenotypic characteristics, neither *Bartonella bacilliformis* nor *Rochalimaea* species are closely related to other organisms currently classified in the order *Rickettsiales*. In fact, the closest relative of these organisms is *Brucella abortus*. It is therefore proposed that the family *Bartonellaceae* should be removed from the order *Rickettsiales*. Previous 16S rRNA sequence data and DNA hybridization data revealed high levels of relatedness between *Bartonella bacilliformis* and the four *Rochalimaea* species, indicating that these species are members of a single genus. It is proposed that the genus *Rochalimaea* should be united with the genus *Bartonella* in the family *Bartonellaceae*. The name *Bartonella* is retained as the genus name since it has nomenclatural priority over the name *Rochalimaea*. This means that new combinations for the *Rochalimaea* species must be created. Proposals are therefore made for the creation of *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov.

All organisms considered to be rickettsiae are classified in the order *Rickettsiales*, which presently contains the three families *Rickettsiaceae*, *Bartonellaceae*, and *Anaplasmataceae* (54, 56). Rickettsiae were originally defined as parasites that could grow only in their hosts or in living tissues (30). Members of the genera *Bartonella* and *Grahamella* in the family *Bartonellaceae* (36) and the genus *Rochalimaea* in the family *Rickettsiaceae* (57), however, are cultivable on bacteriologic media.

Bartonella bacilliformis is the only *Bartonella* species that has been described previously (21). Forty or more species of the genus *Grahamella* have been described (21, 48), although only two species have been validly published and no cultures exist for these species (22). Until recently, *Rochalimaea quintana* and *Rochalimaea vinsonii* were the only *Rochalimaea* species that had been described (57). Two new species isolated from human clinical sources, *Rochalimaea henselae* and *Rochalimaea elizabethae*, were described recently (11, 31, 58).

Substantial efforts to determine the phylogenetic position of rickettsiae began with the 16S rRNA sequencing studies of Weisburg and his colleagues (49, 50, 52). In other recent studies workers described new *Rochalimaea* species and further determined the phylogenetic relationships of *Rochalimaea* and *Bartonella* species to each other and to members of the family *Rickettsiaceae* (6, 9, 26, 31, 33–35, 58). The data obtained in these studies do not support the present classification of the genera *Bartonella* and *Rochalimaea* in the order *Rickettsiales* or the classification of these taxa in different families. Furthermore, the data indicate that the five species currently in these genera should be in a single genus. In this paper we describe DNA hybridization data which reveal the levels of relatedness of *Rickettsia prowazekii* to members of the genera *Rochalimaea* and *Bartonella*. We also review the classification of these taxa, discuss the previous data which indicate that this classification should be changed, and make the following taxonomic proposals: (i) that the genus *Rochalimaea* should be transferred from the family *Rickettsiaceae* to the family *Bartonellaceae*; (ii) that the genus *Rochalimaea* should be united with the genus *Bartonella*; (iii) that *Rochalimaea* species should be transferred to the genus *Bartonella* as *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov.; and (iv) that the family *Bartonellaceae* should be removed from the order *Rickettsiales*.

MATERIALS AND METHODS

Bacterial strains. Type strains *Rochalimaea quintana* ATCC VR-358 and *Rochalimaea vinsonii* ATCC VR-152

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were obtained from the American Type Culture Collection and were provided by Ted Tzianabos, Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention. *Rochalimaea henselae* type strain G5436 (= Houston 1 = ATCC 49793), *Rochalimaea elizabethae* type strain F9251 (= B-91-002005 = ATCC 49927), *Bartonella bacilliformis* type strain KC 583 (= ATCC 35685), and *Brucella melitensis* type strain KC 1414 (= ATCC 23456) were obtained from the culture collection of the Special Bacteriology Laboratory, Emerging Bacterial and Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention. These organisms were cultivated on Trypticase soy agar supplemented with 5% defibrinated sheep blood or on heart infusion agar supplemented with 5% defibrinated rabbit blood at 37°C for 5 to 7 days. *Rickettsia prowazekii* Madrid E (= VR-233) from yolk sac passage 280 was cultivated in 6-day-old embryonated and antibiotic-free hen eggs (Truslow Farms, Chestertown, Md.); 8 days later, the rickettsiae were harvested and purified as described previously (59). *Rickettsia prowazekii* was further purified by using a variation of Renografin density gradient centrifugation (13, 18) in which the rickettsiae were suspended in 218 mM sucrose–3.76 mM KH_2PO_4–7.1 mM K_2HPO_4–5 mM glutamic acid (pH 7.0), layered onto 25% Renografin (E. R. Squibb, Inc., Princeton, N.J.), and sedimented by centrifugation at 30,240 × *g* for 60 min at 4°C in an SA-600 rotor (Du Pont Sorvall, Inc., Norwalk, Conn.).

DNA hybridization. The methods used to obtain purified DNA and to determine levels of DNA relatedness in free solutions by the hydroxyapatite method have been described previously (8); 60 μg of unlabeled DNA per ml instead of 150 μg of unlabeled DNA per ml was used in all hybridization reactions. This was because of the small genome size of these organisms and the scarcity of *Rickettsia prowazekii* DNA. The reaction kinetics were not significantly changed. DNAs from *Rickettsia prowazekii* and *Rochalimaea quintana* were labeled enzymatically in vitro with [^{32}P]dCTP by using a nick translation reagent kit (GIBCO BRL, Gaithersburg, Md.). To obtain optimal reassociation, reaction mixtures containing labeled *Rickettsia prowazekii* DNA were incubated at 50°C, and reaction mixtures containing labeled *Rochalimaea quintana* DNA were incubated at 55°C. This was because *Rickettsia prowazekii* has a lower guanine-plus-cytosine (G+C) content than *Rochalimaea quintana*. Stringent reassociation reaction mixtures were incubated at 65 and 70°C. The level of divergence in related sequences was estimated to be approximately 1% for each 1°C of decreased thermal stability in a heterologous reassociated DNA duplex compared with the thermal stability of the homologous reassociated DNA duplex. Levels of divergence were calculated to the nearest 0.5%.

16S rRNA sequence analysis. All of the 16S rRNA sequences used in this study were retrieved electronically from the GenBank data base (5) (Table 1). The sequences were aligned with the multisequence alignment program PILEUP, which is part of the GOG software package (14). The alignment was masked to remove variable regions where the nucleotide positions could not be reliably determined. This reduced the number of positions included in the analysis to 1,317. Phylogenetic relationships were inferred from the data by using version 3.4 of the PHYLIP software package (17). Evolutionary distance values determined by the method of Jukes and Cantor (19) were used to calculate the rRNA similarity values shown in Tables 2 and 3 and to construct the dendrogram in Fig. 1 by the neighbor-joining method (37). The reliability of the tree was analyzed by bootstrapping the data (16).

Nucleotide sequence accession numbers. The nucleotide sequence accession numbers for the organisms which we studied are shown in Table 1.

RESULTS

DNA hybridization data are shown in Table 4. Labeled DNA from *Rickettsia prowazekii* was 6 to 14% related to unlabeled DNAs from *Rochalimaea* species and *Bartonella bacilliformis*. The levels of divergence in related sequences were 26.5 to 27.5%. The levels of relatedness of *Rickettsia prowazekii* to *Rochalimaea* and *Bartonella* species were 0 to 1% in 65°C reactions and 1 to 2% in 70°C reactions. Labeled *Rochalimaea quintana* DNA exhibited 0% relatedness to *Rickettsia prowazekii* DNA in reactions performed at 55°C. The levels of relatedness of labeled *Rickettsia prowazekii* and *Rochalimaea quintana* DNAs to unlabeled DNA from *Brucella melitensis* were 0 and 1%, respectively.

The dendrogram in Fig. 1 was inferred by the neighbor-joining method, using the sequence from *Desulfovibrio desulfuricans* as the outgroup. The overall topology of the tree agrees with the results of previous phylogenetic studies based on 16S rRNA sequence data. The two *Chlamydia* species were not found to be closely related to eubacteria that cluster in the *Proteobacteria* branch (51, 61). Among the members of the *Proteobacteria* branch, organisms previously determined to be members of the α or τ subgroup were found to cluster together. The three species which form the group at the top of Fig. 1 have been identified as members of the τ subgroup (27, 50, 51, 61). The 34 representatives of the α subgroup were on a single branch that was divided into two major clusters. With the exception of the four *Rochalimaea* species and *Bartonella bacilliformis*, one cluster was composed of all of the species in the order *Rickettsiales* for which sequence data were available at the time that this study was performed. The eight *Ehrlichia* species were distributed into three well-delineated groups. *Ehrlichia risticii* and *Ehrlichia sennetsu* formed a deeply branching group (group I). Group II consisted of *Ehrlichia canis*, *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, and *Cowdria ruminatium*, and group III contained *Ehrlichia phagocytophila*, *Ehrlichia equi*, "*Ehrlichia platys*," and *Anaplasma marginale*. *Wolbachia pipientis* occupied an intermediate position in the evolution of these species. The three *Rickettsia* species formed a monophyletic group (group IV) which diverged prior to the 11 species contained in groups I through III.

A second cluster of the α subgroup of *Proteobacteria* contained all of the species that we propose should be removed from the order *Rickettsiales*, including the four *Rochalimaea* species and *Bartonella bacilliformis*. In this cluster, *Rochalimaea* species and *Bartonella bacilliformis* formed a coherent subcluster (group V) that was on a branch with *Brucella abortus* and *Agrobacterium tumefaciens*.

A bootstrap analysis of the data was performed to measure the reliability of the polyphyletic nature of the *Rickettsiales* shown in Fig. 1. A consensus tree derived from the 100 trees generated by this analysis had exactly the same structure as the tree shown in Fig. 1, with the single exception that the positions of *E. canis* and *E. chaffeensis* were interchanged. Groups I through V were absolutely stable, occurring as shown in Fig. 1 in 100% of the bootstrapping trials. The overall structure of the cluster consisting of the majority of the *Rickettsiales* was also very stable, with the taxonomic

TABLE 1. List of organisms included in the phylogenetic analysis

Species	Strain[a]	Accession no.	Reference(s)[b]
Anaplasmataceae			
Anaplasma marginale		M60313	49
Bartonellaceae			
Bartonella bacilliformis	ATCC 35685^T[c]	M65249	11, 26
Rickettsiaceae (*Rickettsieae*)			
Coxiella burnetii	Q117	M21291	50
Rickettsia prowazekii	Brein1^T	M21789	50
Rickettsia rickettsii	ATCC VR-891^T	M21293	50
Rickettsia typhi	Wilmington^T	M20499	50
Rochalimaea elizabethae	ATCC 49927^T	L01260	11
Rochalimaea henselae	ATCC 49882^T	M73229	31
Rochalimaea quintana	ATCC VR-358^T	M73228	31
Rochalimaea vinsonii	ATCC VR-152^T	L01259	11
Rickettsiaceae (*Ehrlichieae*)			
Cowdria ruminantium	Crystal Springs	X61659	12
Ehrlichia canis	Oklahoma	M73221	2
Ehrlichia chaffeensis		M73222	2
Ehrlichia equi		M73223	2
Ehrlichia ewingii	Stillwater^T	M73227	3
Ehrlichia phagocytophila	Old Sourhope	M73220	2
'Ehrlichia platys''		M82801	3
Ehrlichia risticii	ATCC VR-986^T	M21290	50
Ehrlichia sennetsu	Miyayama	M73219	2
Rickettsiaceae (*Wolbachieae*)			
Wolbachia persica	ATCC VR-331^T	M21292	50
Wolbachia pipientis		X61768	27
Chlamydiaceae			
Chlamydia psittaci	6BC^T	M13769	51
Chlamydia trachomatis	434	M59178	
Other α subgroup *Proteobacteria*			
Afipia felis	ATCC 53690^T	M65248	26
Agrobacterium tumefaciens	DMS 30105	M11223	62
Azospirillum lipoferum	ATCC 29707^T	M59061	
Brucella abortus	11-19	X13695	15
Erythrobacter longus	OCH 101^T	M96744	
Hyphomicrobium vulgare	MC-750	X53182	
Methylobacterium extorquens	NCIMB 9399^T	M95656	7
Methylobacterium organophilum	XX^T	M29028	42
Pseudomonas diminuta	ATCC 11568^T	M59064	
Rhodopseudomonas acidophila	7050^T	M34128	
Rhodopseudomonas globiformis	ATCC 35887^T	M59066	
Rhodopseudomonas palustris	GH	M59068	
Rhodospirillum fulvum	ATCC 15798^T	M59065	
Rhodospirillum molischianum	ATCC 14031^T	M59067	
Rhodospirillum salexigens	DSM 2132^T	M59070	
Subgroup γ *Proteobacteria*			
Legionella pneumophila	ATCC 33152^T	M59157	
Subgroup γ *Proteobacteria*			
Desulfovibrio desulfuricans	ATCC 27774	M34113	29

[a] For each strain for which a strain designation is not given, the template DNA used for sequencing was obtained by enzymatic amplification of 16S rRNA genes from Renografin-purified cells, clinical specimens, or tissue obtained from insect hosts.
[b] For each strain for which a reference is not given, the sequence was submitted to GenBank as "unpublished."
[c] T = type strain.

units occurring to the right of the interior nodes present at levels of 95% or greater. The values for the interior nodes in the other α subgroup of *Proteobacteria* cluster were generally lower; however, the group consisting of *Agrobacterium tumefaciens*, *Brucella abortus*, *Bartonella bacilliformis*, and the *Rochalimaea* species occurred in 100% of the trials.

Table 2 shows the 16S rRNA similarity values for *Agrobacterium tumefaciens*, *Brucella abortus*, and members of the *Rickettsiales*. The sequences of *Coxiella burnetii* and *Wolbachia persica* did not exhibit high levels of homology to any of the α subgroup of *Proteobacteria* sequences, which is consistent with the position of these organisms outside this subgroup. The sequence of *W. pipientis* exhibited the highest levels of homology (approximately 89.5%) to the sequences of species belonging to groups II and III. The sequence of *Bartonella bacilliformis* was most similar to the sequences of the *Rochalimaea* species, with levels of similarity ranging from 98.7 to 98.8%. These values were only slightly less than the values of 99.1 to 99.8% obtained in comparisons among the four *Rochalimaea* species. The sequences of *Bartonella bacilliformis* and the *Rochalimaea* species were substantially more similar to the sequences of *Brucella abortus* and *Agrobacterium tumefaciens* than to the sequences of members of the *Rickettsiales*.

TABLE 2. 16S rRNA similarity matrix

Species	% Similarity to[a]: *Wolbachia persica*	*Ehrlichia risticii*	*Ehrlichia sennetsu*	*Ehrlichia canis*	*Ehrlichia chaffeensis*	*Ehrlichia ewingii*	*Cowdria ruminantium*	*Ehrlichia equi*	*Ehrlichia phagocytophila*	*"Ehrlichia platys"*	*Anaplasma marginale*	*Wolbachia pipientis*	*Rickettsia prowazekii*	*Rickettsia typhi*	*Rickettsia rickettsii*	*Rochalimaea henselae*	*Rochalimaea vinsonii*	*Rochalimaea elizabethae*	*Rochalimaea quintana*	*Bartonella bacilliformis*	*Brucella abortus*	*Agrobacterium tumefaciens*	*Coxiella burnetii*
Ehrlichia risticii	79.2																						
Ehrlichia sennetsu	79.0	**99.4**[b]																					
Ehrlichia canis	80.6	85.0	85.0																				
Ehrlichia chaffeensis	80.9	85.3	85.2	**98.8**																			
Ehrlichia ewingii	81.0	85.0	85.0	**99.1**	**99.3**																		
Cowdria ruminantium	81.5	85.3	85.2	**98.1**	**98.5**	**98.5**																	
Ehrlichia equi	81.4	86.2	86.0	93.6	94.0	93.7	93.7																
Ehrlichia phagocytophila	81.5	86.3	86.0	93.7	94.0	93.8	93.8	**99.9**															
"Ehrlichia platys"	81.5	86.0	85.7	93.0	93.6	93.2	93.2	**99.2**	**99.2**														
Anaplasma marginale	81.3	86.1	85.8	94.1	94.3	94.0	93.9	**98.0**	**98.1**	**97.5**													
Wolbachia pipientis	80.5	85.7	85.5	89.2	89.5	89.4	89.5	89.5	89.6	89.2	89.2												
Rickettsia prowazekii	81.4	84.1	83.8	84.8	84.8	84.7	85.5	85.7	85.7	85.0	85.8	85.7											
Rickettsia typhi	81.0	83.8	83.3	85.1	85.0	85.1	85.8	85.7	85.8	84.9	86.0	85.7	**99.7**										
Rickettsia rickettsii	81.3	84.1	83.7	85.3	85.3	85.2	85.9	85.8	85.9	85.1	86.0	85.9	**99.4**	**99.3**									
Rochalimaea henselae	83.1	82.4	82.3	84.2	84.5	84.5	84.5	85.5	85.6	85.5	85.0	84.3	86.2	86.5	86.5								
Rochalimaea vinsonii	83.1	82.5	82.4	84.2	84.5	84.5	84.6	85.4	85.5	85.4	84.9	84.5	86.4	86.7	86.8	**99.5**							
Rochalimaea elizabethae	83.1	82.5	82.3	84.3	84.5	84.6	84.6	85.5	85.5	85.5	85.0	84.5	86.6	86.9	87.0	**99.4**	**99.7**						
Rochalimaea quintana	83.2	82.4	82.2	84.2	84.5	84.5	84.5	85.5	85.6	85.5	85.0	84.4	86.2	86.5	86.5	**99.2**	**99.1**	**99.2**					
Bartonella bacilliformis	83.2	82.4	82.2	84.3	84.5	84.6	84.5	85.7	85.8	85.8	85.3	84.3	85.9	86.0	86.2	**98.8**	**98.8**	**98.7**	**98.7**				
Brucella abortus	82.3	83.3	83.2	83.5	83.7	83.7	83.9	84.7	84.7	84.7	84.3	84.0	85.7	85.9	85.9	95.7	96.3	96.2	95.7	95.4			
Agrobacterium tumefaciens	83.8	83.4	83.0	84.1	84.1	84.3	84.5	85.4	85.5	85.5	85.2	84.5	85.6	86.2	85.9	94.7	95.1	95.0	94.4	94.3	94.5		
Coxiella burnetii	85.9	81.7	81.5	82.3	82.5	82.5	82.6	82.9	83.0	82.8	83.2	82.4	83.1	83.2	83.4	83.8	84.1	84.1	83.8	83.8	83.9	84.6	
Legionella pneumophila	85.9	80.7	80.5	81.5	81.7	81.4	82.0	82.2	82.2	82.2	82.4	81.5	82.4	82.2	82.3	82.7	82.8	82.9	83.0	83.1	82.9	83.5	89.0

[a] Levels of similarity corrected for multiple base changes by the method of Jukes and Cantor (19).
[b] Values in boldface type are similarity values for members of the *Rickettsiales* grouped on the basis of levels of rRNA similarity.

The coherence among the members of the five groups defined above and the distinctiveness of these groups are evident from the intra- and intergroup similarity values shown in Table 3. Within each of the five groups, the average similarity values are high, ranging from 98.7 to 99.5%. When comparisons are made across groups, the average similarity values are approximately 10% lower, except for groups II and III, whose members exhibit an average level of similarity of 93.7%.

TABLE 3. Levels of 16S rRNA similarity among groups of the *Rickettsiales*

Group	% Similarity to: Group I[a]	Group II[b]	Group III[c]	Group IV[d]	Group V[e]
I	99.4				
II	85.1	98.7			
III	86.0	93.7	98.7		
IV	83.8	85.2	85.6	99.5	
V	82.4	84.5	85.4	86.5	99.1

[a] *E. risticii* and *E. sennetsu*.
[b] *E. canis*, *E. chaffeensis*, *E. ewingii*, and *Cowdria ruminantium*.
[c] *E. equi*, *E. phagocytophila*, *"E. platys,"* and *Anaplasma marginale*.
[d] *Rickettsia prowazekii*, *Rickettsia typhi*, and *Rickettsia rickettsii*.
[e] *Rochalimaea henselae*, *Rochalimaea vinsonii*, *Rochalimaea elizabethae*, *Rochalimaea quintana*, and *Bartonella bacilliformis*.

DISCUSSION

The order *Rickettsiales* has been a repository for small, gram-negative, usually intracellular, parasitic or mutualistic bacteria, almost all of which are unable to grow on bacteriologic media. These organisms have included rickettsiae, parasites of protozoa, endosymbionts of insects, and, until 1971, chlamydiae (30, 41).

As defined by Weiss and Moulder (56), the members of the *Rickettsiales* are usually rod-shaped or coccoid, often pleomorphic, parasitic or mutualistic, gram-negative, nonmotile bacteria with typical bacterial cell walls. They multiply by binary fission and are cultivable only in their host cells or in living tissues. Except for binary fission, there are notable exceptions to all of these characteristics. The parasitic forms are often associated with arthropods that act as vectors or primary hosts.

Specialists have long recognized the difficulties in accurately classifying the *Rickettsiales*, as well as its families, genera, and species (28, 43, 47, 53a, 54, 60). Since most rickettsiae could not be grown in vitro, their classification was based heavily on their hosts, their intracellular morphol-

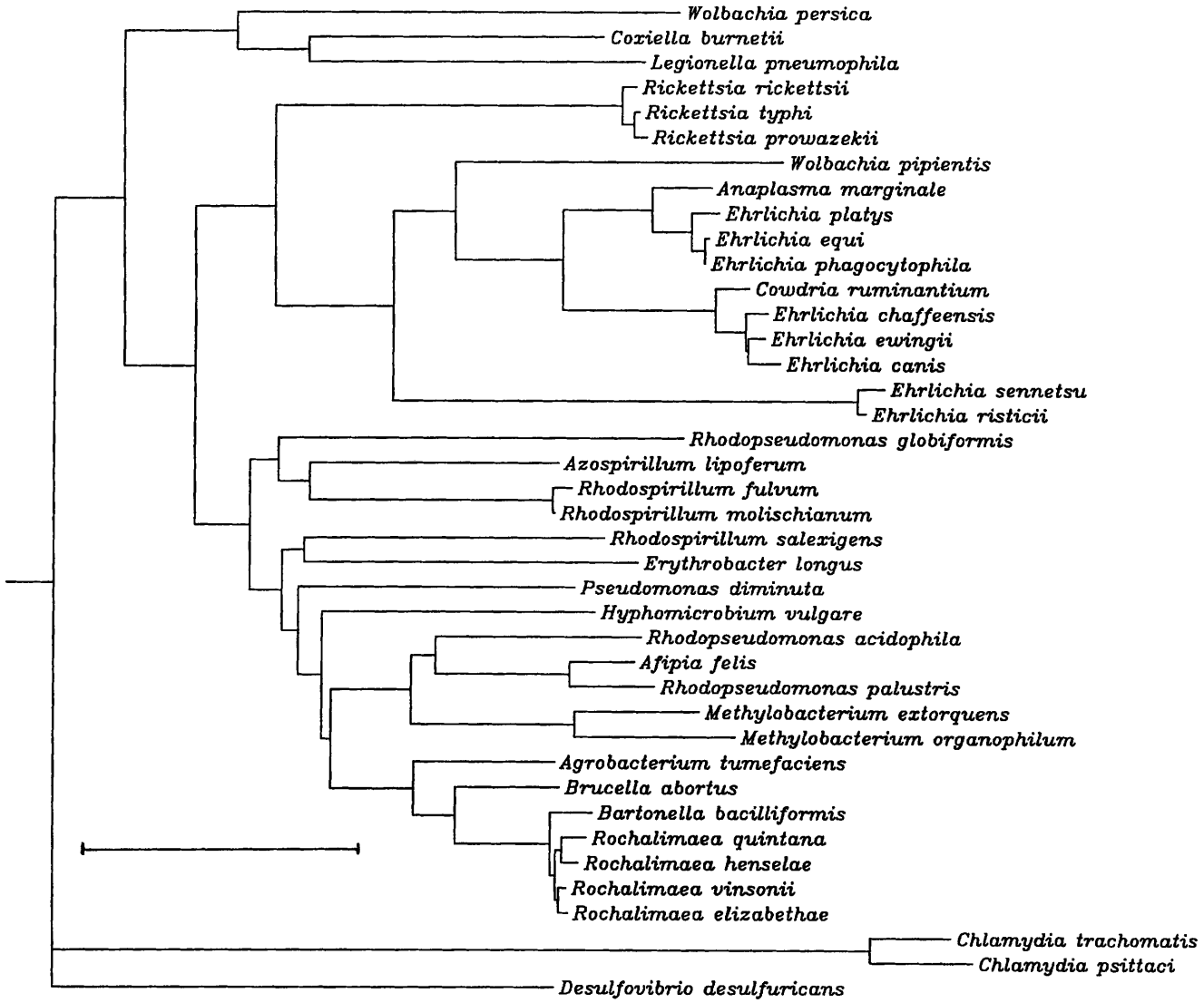

FIG. 1. Phylogenetic tree for members of the order *Rickettsiales*, members of the order *Chlamydiales*, and other species representing the α and τ subgroups of the class *Proteobacteria*. The sequence of *D. desulfuricans* (σ subgroup of the *Proteobacteria*) was used as an outgroup. The tree was reconstructed as described in Materials and Methods. The scale bar (lower left) represents a 5% difference in nucleotide sequences, as determined by taking the sum of all branch lengths that connect two species. GenBank accession numbers and references for the sequences included are given in Table 1.

ogy and locations, their arthropod vectors, their antigenic differences, and a small number of other phenotypic characteristics (43). Another barrier to accurate classification was the fact that the important human and animal pathogens were studied in much more detail than were agents that had little or no effect on their hosts (54).

Rickettsiologists realized that in-depth taxonomic studies were not possible until molecular methods could be used to determine genetic relatedness among rickettsial species and the relatedness of rickettsiae to other bacteria. Tyeryar et al. (43) stated the need "to establish an objective index of genetic relatedness among rickettsiae" and took the first step in doing so by determining G+C contents in rickettsial DNAs. In 1982, Weiss (53) wrote, "What we hope to learn in the next decade is the identity of the next-of-kin of rickettsiae by analysis of ribosomal RNA nucleotide sequences or amino acid sequences in ribosomal proteins. This information will greatly enhance our understanding of the place of rickettsiae in the microbiological world." Ormsbee (28), after reviewing G+C content and genome size data, concluded that the major rickettsial groups exhibited substantial divergence. He believed that most of the characteristics common to these major groups were the result of convergent evolution in response to the common pressures of a parasitic or commensal life style. Wisseman (60) came to a similar conclusion, stating that the "organisms so conveniently considered collectively in the past as 'rickettsial agents of human disease' . . . show enormous diversity of properties, actions and host cell associations and . . . may actually be of diverse origins showing both convergent and divergent evolution—e.g., *Rochalimaea*, *Rickettsia*, and *Coxiella*."

Most recently, Kreier and his colleagues (21) concluded that, "*Bartonella* and *Grahamella* of the family *Bartonellaceae* are typical bacteria with cell walls morphologically

TABLE 4. Levels of DNA relatedness of *Rickettsia prowazekii* to *Rochalimaea* species and to *Bartonella bacilliformis*

Source of unlabeled DNA	*Rickettsia prowazekii*				% Relatedness to *Rochalimaea quintana* at 55°C
	% Relatedness at 50°C	% Divergence	% Relatedness at 65°C	% Relatedness at 70°C	
Rickettsia prowazekii Madrid E	100	0.0	100	100	0
Rochalimaea quintana ATCC VR-358^T	9	27.5	0	2	
Rochalimaea vinsonii ATCC VR-152^T	10	26.5	1	2	
Rochalimaea henselae ATCC 49882^T	14	27.0	1	2	
Rochalimaea elizabethae ATCC 49927^T	6	26.5	1	1	
Bartonella bacilliformis ATCC 35685^T	13	26.5	1	1	
Brucella melitensis ATCC 23456^T	0		0	1	1

comparable to the cell walls of other gram-negative bacteria. Despite the fact that in vivo they often grow intracellularly, they can be cultured without difficulty on culture media. . . . *Bartonella* and *Grahamella* are thus small, facultatively intracellular gram-negative bacteria. It is apparent that these organisms do not have the attributes classically ascribed to the rickettsiae." Certainly these conclusions are equally applicable to the genus *Rochalimaea*.

Beginning in 1973, G+C contents were reported for species belonging to the genera *Rickettsia*, *Rochalimaea*, and *Bartonella* (1, 9, 11, 23–25, 38, 43, 44, 55). As Table 5 shows, the G+C contents of *Rickettsia* species are 28.5 to 33.3 mol%. These G+C contents are significantly lower than the values of 38.5 to 41.1 mol% reported for *Rochalimaea* species and the values of 39.0 to 40.0 mol% reported for *Bartonella bacilliformis*.

A summary of the results of previous DNA hybridization studies performed to determine the interrelatedness of *Rochalimaea*, *Bartonella*, and *Rickettsia* species is shown in Table 6. Myers et al. (25) and Myers and Wisseman (23, 24) reported that four strains of *Rochalimaea quintana* were more than 90% related and two strains of *Rochalimaea vinsonii* were 100% related and that the levels of relatedness between *Rochalimaea quintana* and *Rochalimaea vinsonii* strains were 31 to 42%. Welch et al. (58) and Daly et al. (11), using a somewhat more sensitive method, showed that the levels of relatedness between *Rochalimaea quintana* and *Rochalimaea vinsonii* strains were 55 to 62% and the levels of relatedness among the four species in the genus *Rochalimaea* ranged from 49 to 71%. Brenner et al. (9) reported a level of relatedness between two *Bartonella bacilliformis* strains of 97%, and Welch et al. (58) and Daly et al. (11) reported levels of relatedness between the four *Rochalimaea* species and *Bartonella bacilliformis* of 32 to 47% (average, 42%).

These data indicate that the four *Rochalimaea* species and *Bartonella bacilliformis* are valid genomospecies and that the genus *Rochalimaea*, containing four closely related species, is easily defendable. The data also strongly suggest that the members of the genera *Bartonella* and *Rochalimaea* are members of a single genus or two very closely related genera that must be in the same family.

Myers et al. (25) and Myers and Wisseman (23, 24) determined levels of DNA relatedness among *Rickettsia* species and between *Rickettsia* and *Rochalimaea* species. They found levels of relatedness between *Rickettsia prowazekii* and *Rochalimaea quintana* of 31 to 33% and levels of relatedness between *Rickettsia typhi* and *Rochalimaea quintana* of 27 to 31%. The levels of relatedness among strains of *Rickettsia prowazekii* were 93 to 100%, and the levels of relatedness among strains of *Rickettsia typhi* were 70 to 78%, indicating that these organisms are members of single species. The levels of interspecies relatedness when *Rickettsia typhi*, *Rickettsia canada*, and *Rickettsia rickettsii* strains were compared usually were 36 to 53%. Thus, these *Rickettsia* species form a good genus, but they are almost as closely related to members of the genus *Rochalimaea* as they are to each other. Although difficult to reconcile with the substantial G+C content differences observed between members of the genera *Rochalimaea* and *Rickettsia*, the DNA hybridization data of Myers et al. (25) and Myers and Wisseman (23, 24) support the present classification of the genus *Rochalimaea* (and therefore the genus *Bartonella*) in the family *Rickettsiaceae*.

Our DNA hybridization data (Table 4) indicate that *Rickettsia prowazekii* is substantially less closely related to the genera *Rochalimaea* and *Bartonella* (levels of relatedness, 6 to 14%) than previously reported (23–25). The related sequences have diverged extensively, as judged from the divergence values of 26.5 to 27.5%. This seems to indicate that the related sequences are extremely unstable and may result at least partially from the fact that the G+C content of 28.5 to 30.3 mol% reported for *Rickettsia prowazekii* is very low (Table 5). This possibility appears to be supported by the fact that levels of relatedness of only 0 to 2% were observed in reactions performed at 55°C with labeled DNA from *Rochalimaea quintana* (Table 4), whose G+C content is 38.5 to 40.3 mol% (Table 5). Our data correlate with the significant divergence of the genus *Rickettsia* from the genera *Rochalimaea* and *Bartonella* that is inferred from differences in the G+C contents of these organisms and from their relatively low levels of rRNA sequence homology, as discussed below.

16S rRNA sequencing of rickettsiae was first done in 1985. Summaries of sequence similarity data are shown in Table 1 and Fig. 1. Weisburg et al. (52) sequenced 16S rRNA from

TABLE 5. G+C content of *Rickettsia*, *Rochalimaea*, and *Bartonella* DNAs

Species	G+C content, mol%[a]
Rickettsia canada	29.3–30.3 (43), 29.2 (24)
Rickettsia prowazekii	29.0–30.3 (43), 28.5–29.7 (23), 29.0 (24)
Rickettsia typhi	29.0–30.8 (43)
Rickettsia akari	32.2–33.2 (43)
Rickettsia conorii	32.9–33.3 (43)
Rickettsia japonica	31.2 (44)
Rickettsia rhipicephali	32.2 (1)
Rickettsia rickettsii	32.0–33.2 (43), 32.6 (24)
Rickettsia sibirica	32.5 (38)
Bartonella bacilliformis	39.0–40.0 (9)
Rochalimaea elizabethae	41.0 (11)
Rochalimaea henselae	41.0 (11)
Rochalimaea quintana	40.3 (11), 38.5–38.8 (43), 39.0 (55), 39.3–39.8 (25), 39.3–39.8 (23)
Rochalimaea vinsonii	41.1 (11), 39.0 (55), 38.9–39.2 (25)

[a] The numbers in parentheses are the references from which the data were obtained.

TABLE 6. DNA relatedness data for *Rickettsia*, *Rochalimaea*, and *Bartonella* species

Species	% Relatedness to:															
	Rochalimaea elizabethae[a]	*Rochalimaea henselae*		*Rochalimae*[a] *quintana*			*Rochalimae*[a] *vinsonii*		*Bartonella bacilliformis*[e]	*Rickettsia canada*	*Rickettsia prowazekii*		*Rickettsia typhi*		*Rickettsia rickettsii*[f]
		Welch et al.[a]	Daly et al.[b]	Welch et al.[a]	Myers et al.[c]	Myers et al.[d]	Welch et al.[a]	Myers et al.[c]			Myers et al.[d]	Myers et al.[f]	Myers et al.[d]	Myers et al.[f]	
Rochalimaea elizabethae			49												
Rochalimaea henselae	67	100, 100, 100, 99, 98, 92, 92[g]	100, 100, 99, 92[g]	71, 69, 68, 68, 67, 67, 66, 62, 62[g]			64, 63, 58[g]								
Rochalimaea quintana	59	66, 56[g]		100	97, 94, 92[g]	94	55	42, 39, 34, 31,[g]			33, 31[g]		31, 27[g]		
Rochalimaea vinsonii	66	55		62	42, 39, 34, 31[g]			100							
Bartonella bacilliformis	32	43	47	45					97						
Rickettsia canada												37		41, 38[g]	47, 43[g]
Rickettsia prowazekii										37	100, 100, 97, 94, 93, 93[g]		78, 73, 70[g]		53, 47[g]
Rickettsia typhi						31, 27[g]				52, 47[g]	78, 73, 72, 72, 70[g]	100, 97[g]			27, 20[g]
Rickettsia rickettsii										39, 37[g]		47, 38[g]		42, 36[g]	

[a] Data from reference 58.
[b] Data from reference 11.
[c] Data from reference 25.
[d] Data from reference 23.
[e] Data from reference 9.
[f] Data from reference 24.
[g] More than one relatedness percentage under a species name indicates that multiple strains or multiple methods were used to obtain data.

Rochalimaea quintana and found that this organism belongs in the α-2 subgroup of the *Proteobacteria* and that its 16S rRNA sequence is most homologous (92.5%) with that of *Agrobacterium tumefaciens*. Weisburg and his collaborators (50) subsequently sequenced the 16S rRNAs of six additional species belonging to the *Rickettsiaceae*. *Rickettsia prowazekii* and *Rickettsia typhi* 16S rRNAs were 99.5% homologous and were 98.2 and 98.5% homologous, respectively, to *Rickettsia rickettsii* 16S rRNA. The *Rickettsia* species were in the α subgroup of the *Proteobacteria*, but not in the α-2 subgroup with *Rochalimaea quintana*. In fact, *E. risticii* was more closely related to *Rickettsia prowazekii* than was *Rochalimaea quintana*. *Coxiella burnetii* and *W. persica* were in the τ subgroup, with members of the genus *Coxiella* being specifically, although not extremely closely, related to *Legionella pneumophila*. These data leave no doubt that there is substantial heterogeneity within the family *Rickettsiaceae* and the tribe *Rickettsieae*, which contains the genera *Rickettsia*, *Rochalimaea*, and *Coxiella*.

The polyphyletic nature of the order *Rickettsiales* and the family *Rickettsiaceae* was confirmed, and additional taxonomic problems were identified as more rickettsial 16S rRNAs were sequenced. The genus *Ehrlichia* was shown to contain three very different groups (2, 3). *E. ewingii*, *E. chaffeensis*, and *E. canis* form one group whose members are 98% interrelated. This group is about 92% related to a second group consisting of *E. equi*, *E. phagocytophila*, and "*E. platys*" and about 84% related to the third group, which

contains *E. risticii* and *E. sennetsu*. *Cowdria ruminatium* was about 97% homologous to *E. chaffeensis* and *E. canis* (45) and was more closely related to members of the genus *Anaplasma* in the family *Anaplasmataceae* than to species belonging to the *Rickettsiaceae*, the family in which it is currently classified (12).

Another example is *Anaplasma marginale*, which exhibited higher levels of homology to members of the *Rickettsiaceae* (*Rickettsia* species and *E. risticii*) than were observed with members of genera in the *Rickettsiaceae* (*Rochalimaea* and *Rickettsia* species) (49). 16S rRNA sequences are now available for all four *Rochalimaea* species and for *Bartonella bacilliformis* (6, 11, 26, 31, 33–35, 52). It should be noted that the *Bartonella bacilliformis* sequence reported by O'Connor et al. (26) was incorrect and has been corrected (11) and that the results of workers in three laboratories who used the same strain of *Bartonella bacilliformis* differ by as much as 2% (6, 11, 34). Sequence comparisons indicate that the levels of homology among *Rochalimaea* species are 99.1 to 99.7% and the levels of homology between *Rochalimaea* species and *Bartonella bacilliformis* are 97.9 to 98.8% (not including data from the incorrect sequence generated by O'Connor et al. [26]). The 16S rRNA sequence data complement the DNA hybridization data that support the hypothesis that there should be a single genus for *Rochalimaea* and *Bartonella* species.

A constant and convincing fact from all of the sequence studies described above is that there has been substantial evolutionary divergence of *Rochalimaea* and *Bartonella* species from all "true" members of the *Rickettsiales*. In fact, *Rochalimaea* and *Bartonella* species are more closely related to a number of other bacteria, including *Brucella abortus* and *Agrobacterium tumefaciens*, than to any rickettsial species.

The available phylogenetic data quite convincingly identify a number of serious taxonomic problems in the *Rickettsiales*. It is likely that additional taxonomic inconsistencies will be revealed when 16S rRNA sequence data and DNA hybridization data are available for all genera and species. Changes in the classification of the genera *Rochalimaea* and *Bartonella* are now warranted on the basis of phylogenetic data. Although the order *Rickettsiales* is not precisely defined, it is clear that neither the genus *Rochalimaea* nor the genus *Bartonella* belongs in this order. Thus, the family *Bartonellaceae* and the genera *Bartonella* and *Rochalimaea* should be removed from the order *Rickettsiales*. Members of the genus *Rochalimaea* are most closely related to members of the genus *Bartonella* and therefore should be transferred from the family *Rickettsiaceae* to the family *Bartonellaceae*. While it can be argued that the genera *Rochalimaea* and *Bartonella* should remain separate genera, we believe that, in accord with the recommendations of Wayne et al. (46), the members of these taxa are sufficiently related genotypically and phenotypically to merit being in a single genus. Since the names *Bartonella* and *Bartonella bacilliformis* predate the name *Rochalimaea* and the name of its oldest species, *Rochalimaea quintana*, the name *Bartonella* should be retained for the unified genus. This means that new combinations for the four *Rochalimaea* species must be created. Formal taxonomic proposals are made below.

Emendation of the order *Rickettsiales* (Gieszczykiewicz 1939) Weiss and Moulder 1984. It is proposed that the genus *Rochalimaea* and the family *Bartonellaceae*, containing the genera *Bartonella* and *Grahamella*, should be removed from the order *Rickettsiales*. This requires emending the description of the *Rickettsiales* (56) to exclude motile organisms and organisms that multiply on bacteriologic media. It also reduces the number of families to two, the *Rickettsiaceae* and the *Anaplasmataceae*.

Emendation of the family *Rickettsiaceae* (Pinkerton 1936) Weiss and Moulder 1984. It is proposed that the genus *Rochalimaea* should be removed from the family *Rickettsiaceae*. This requires emending the description of the *Rickettsiaceae* to exclude organisms that are cultivable in cell-free media.

Emendation of the tribe *Rickettsieae* (Philip 1953) Weiss and Moulder 1984. It is proposed that the genus *Rochalimaea* should be removed from the tribe *Rickettsieae*. This requires emending the description of the *Rickettsieae* (56) by reducing the number of genera to two, the genera *Rickettsia* and *Coxiella*.

Emendation of the family *Bartonellaceae* (Gieszczykiewicz 1939) Ristic and Kreier 1984. It is proposed that the genus *Rochalimaea* should be transferred to the family *Bartonellaceae*. This requires emending the description of the *Bartonellaceae* (36) by integrating portions of the description of the genus *Rochalimaea* (56) and data for *Rochalimaea henselae* and *Rochalimaea elizabethae*, as follows.

Parasites of erythrocytes of humans and other vertebrates and of human cutaneous and osseous tissue. Cells are rod shaped, cocci, or ring or disk shaped, often beaded or filamentous. Greatest cell diameter is less than 3 μm. Gram negative; aerobic; not acid fast. Strains do not utilize carbohydrates. One species has unipolar flagella. Cultivable on bacteriologic media. Arthropod transmission has been established for many species. Human pathogens include etiologic agents of trench fever, bartonellosis, bacillary angiomatosis (10, 39, 40), peliosis hepatis, septicemia, and possibly cat scratch disease (32). Animal species, which have been found in erythrocytes of rodents and other vertebrates, are nonpathogenic (48). The type genus is *Bartonella* Strong, Tyzzer, and Sellards 1915.

Emendation of the genus *Bartonella* (Strong, Tyzzer, and Sellards 1915) Ristic and Kreier 1984. It is proposed that the genus *Rochalimaea* should be united with the genus *Bartonella*. This requires emending the description of the genus *Bartonella* (36) by integrating portions of the description of the genus *Rochalimaea* (57) and data for *Rochalimaea henselae* and *Rochalimaea elizabethae*, as follows.

Gram-negative, oxidase-negative, fastidious, aerobic rods. One species is motile by means of polar flagella. Best growth is obtained on media containing 5% rabbit or sheep blood in the presence of 5% CO_2. The optimal incubation temperature varies from 25°C (*Bartonella bacilliformis*) to 35 to 37°C. Carbohydrates are not utilized. All but one species are pathogenic for humans. Arthropod vectors have been demonstrated for *Bartonella bacilliformis*, for *Bartonella quintana*, and probably for *Bartonella vinsonii* (4). One or more species cause bartonellosis, trench fever, bacillary angiomatosis, peliosis hepatis, septicemia, and perhaps cat scratch disease. The G+C contents of the DNAs are 38.5 to 41 mol%. The type species is *Bartonella bacilliformis* (Strong, Tyzzer, Brues, Sellards, and Gastiaburú 1913) Strong, Tyzzer, and Sellards 1915.

Description of *Bartonella quintana* comb. nov. The most recent description of *Rochalimaea quintana* is that of Weiss and Moulder (57). In addition, *Bartonella quintana* is oxidase positive as determined by the standard oxidase test or weakly oxidase positive as determined by the Kovacs modification of the standard test (11). *Bartonella quintana* is pathogenic for humans. It causes trench fever, is an etiologic agent of bacillary angiomatosis (20), and has been isolated

from patients with human septicemia (58). The 16S rRNA gene nucleotide sequence of the type strain, ATCC VR-358, has been deposited in the GenBank data base under accession number M73228 (31). A corrected sequence has been submitted to the GenBank, EMBL, and DDBJ data bases (accession number L01259) (11).

Description of *Bartonella vinsonii* comb. nov. The most recent description of *Rochalimaea vinsonii* is that by Weiss and Moulder (57). In addition, *Bartonella vinsonii* is oxidase negative in the standard oxidase test or weakly oxidase positive in the Kovacs modification of the standard test (11). The 16S rRNA gene nucleotide sequence of the type strain, ATCC VR-152, is deposited in the GenBank data base under accession number M73230 (31).

Description of *Bartonella henselae* comb. nov. With the following additions, the description of *Bartonella henselae* is the same as the description given previously (31) for *Rochalimaea henselae*. *Bartonella henselae* is pathogenic for humans. It is a major etiologic agent of bacillary angiomatosis (33, 35) and of hepatic and splenic peliosis (58). It is a causative agent of septicemia in immunocompromised persons as well as in otherwise healthy persons (58) and may be a major cause of cat scratch disease (32). No arthropod vector has been identified. The type strain is Houston 1 (= G5436 = ATCC 49882); it has a G+C content of 41 mol% (11), and its 16S rRNA gene nucleotide sequence has been deposited in the GenBank data base under accession number M73229 (31).

Description of *Bartonella elizabethae* comb. nov. The description of *Bartonella elizabethae* is the same as the description given by Daly et al. for *Rochalimaea elizabethae* (11). No arthropod vector has been identified. The 16S rRNA gene nucleotide sequence of the type strain, ATCC 49927 (= F9251 = B91-002005), has been deposited in the GenBank, EMBL, and DDBJ data bases under accession number L01260 (11).

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