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Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*

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The genera *Anaplasma*, *Ehrlichia*, *Cowdria*, *Neorickettsia* and *Wolbachia* encompass a group of obligate intracellular bacteria that reside in vacuoles of eukaryotic cells and were previously placed in taxa based upon morphological, ecological, epidemiological and clinical characteristics. Recent genetic analyses of 16S rRNA genes, *groESL* and surface protein genes have indicated that the existing taxa designations are flawed. All 16S rRNA gene and *groESL* sequences deposited in GenBank prior to 2000 and selected sequences deposited thereafter were aligned and phylogenetic trees and bootstrap values were calculated using the neighbour-joining method and compared with trees generated with maximum-probability, maximum-likelihood, majority-rule consensus and parsimony methods. Supported by bootstrap probabilities of at least 54%, 16S rRNA gene comparisons consistently clustered to yield four distinct clades characterized roughly as *Anaplasma* (including the *Ehrlichia phagocytophila* group, *Ehrlichia platys* and *Ehrlichia bovis*) with a minimum of 96·1% similarity, *Ehrlichia* (including *Cowdria ruminantium*) with a minimum of 97·7% similarity, *Wolbachia* with a minimum of 95·6% similarity and *Neorickettsia* (including *Ehrlichia sennetsu* and *Ehrlichia risticii*) with a minimum of 94·9% similarity. Maximum similarity between clades ranged from 87·1 to 94·9%. Insufficient differences existed among *E. phagocytophila*, *Ehrlichia equi* and the human granulocytic ehrlichiosis (HGE) agent to support separate species designations, and this group was at least 98·2% similar to any *Anaplasma* species. These 16S rRNA gene analyses are strongly supported by similar *groESL* clades, as well as biological and antigenic characteristics. It is proposed that all members of the tribes *Ehrlichieae* and *Wolbachieae* be transferred to the family *Anaplasmataceae* and that the tribe structure of the family *Rickettsiaceae* be eliminated. The genus *Anaplasma* should be emended to include *Anaplasma* (*Ehrlichia*) *phagocytophila* comb. nov. (which also encompasses the former *E. equi* and the HGE agent), *Anaplasma* (*Ehrlichia*) *bovis* comb. nov. and *Anaplasma* (*Ehrlichia*) *platys* comb. nov., the genus *Ehrlichia* should be emended to include *Ehrlichia* (*Cowdria*) *ruminantium* comb. nov. and the genus *Neorickettsia* should be emended to include *Neorickettsia* (*Ehrlichia*) *risticii* comb. nov. and *Neorickettsia* (*Ehrlichia*) *sennetsu* comb. nov.

Keywords: *Anaplasmataceae*, *Ehrlichia*, *Anaplasma*, *Neorickettsia*, *Cowdria*

Abbreviation: HGE, human granulocytic ehrlichiosis.

Details of the similarity values used in construction of the trees are available in IJSEM Online at http://ijs.sgmjournals.org/

INTRODUCTION

Recent improvements in molecular technologies have significantly advanced our abilities to conduct genetic analyses and, for the first time, clearly indicated the proper phylogenetic positions of most of the fastidious bacterial species in the families *Rickettsiaceae*, *Bartonellaceae* and *Anaplasmataceae* in the order *Rickettsiales* (Woese *et al*., 1990; Weisburg *et al*., 1989; Brenner *et al*., 1993; Birtles *et al*., 1995). By 16S rRNA sequencing, Weisburg *et al*. (1989) demonstrated that *Coxiella burnetii* and *Wolbachia persica* belonged to the *γ-Proteobacteria*, while the remaining members of the order *Rickettsiales* that they examined (three species of *Rickettsia* and *Ehrlichia risticii*) formed a tight monophyletic cluster within the *α-Proteobacteria*. In fact, *Wolbachia persica* and related tick symbionts are most closely related to species of *Francisella* (Forsman *et al*., 1994; Noda *et al*., 1997; Niebylski *et al*., 1997). Subsequently, *Anaplasma marginale* and *Cowdria ruminantium* were also found to be closely related to *Rickettsia* and *Ehrlichia* (Weisburg *et al*., 1991; van Vliet *et al*., 1992; Dame *et al*., 1992). The second major reorganization of the order *Rickettsiales* came with the removal of the *Bartonellaceae* from the order and with the unification of the genera *Grahamella* and *Rochalimaea* in the genus *Bartonella* (Brenner *et al*., 1993; Birtles *et al*., 1995). Subsequently, additional species have been removed from the order *Rickettsiales* as their 16S rRNA sequences were determined. *Rickettsiella grylli* was found to be closely related to *Coxiella* and *Legionella* (Roux *et al*., 1997), while the genera *Haemobartonella* and *Eperythrozoon* were unified in the order *Mollicutes* (Neimark & Kocan, 1997; Rikihisa *et al*., 1997). *Wolbachia* was found to be polyphyletic, as *Wolbachia pipientis* belongs to the cluster of rickettsial species in the *α-Proteobacteria* (O'Neill *et al*., 1992) while *Wolbachia melophagi* is actually a species of *Bartonella* (R. J. Birtles and D. H. Molyneux, unpublished GenBank accession no. X89110).

We propose here a reorganization of the remaining members of the order *Rickettsiales* in the families *Rickettsiaceae* and *Anaplasmataceae*. We emend the order by elimination of the tribes *Rickettsieae*, *Ehrlichieae*, *Wolbachieae* and *Anaplasmataceae* because (i) many of the genera contained in each tribe have no phylogenetic affinities and have already been removed from the order and (ii), as described further below, the remaining species previously placed in the tribes *Ehrlichiaeae*, *Wolbachieae* and *Anaplasmataceae* have molecular and phenotypic affinities that are more appropriate to recognition at the family level. We propose that the family *Rickettsiaceae* be composed of the closely related genera *Rickettsia* and *Orientia*, which was recently split from *Rickettsia* (Tamura *et al*., 1995). All of the species in the family *Rickettsiaceae* are obligate intracellular bacteria that grow freely in the cytoplasm of their eukaryotic host cells.

We retain the family *Anaplasmataceae*, but broaden it to include all species of the *α-Proteobacteria* presently contained in the genera *Ehrlichia*, *Anaplasma*, *Cowdria*, *Wolbachia* and *Neorickettsia*, as described below. *Aegyptianella* is also retained provisionally in the *Anaplasmataceae*, but designated as *genus incertae sedis*, since its 16S rRNA and other gene sequences have not been determined but it has strong phenotypic similarities to the species of *Anaplasma*. All members of the family *Anaplasmataceae* are obligate intracellular bacteria that replicate while enclosed in a eukaryotic host cell membrane-derived vacuole (Rikihisa, 1991a). Except for the genus *Wolbachia*, each species can replicate in vertebrate hosts, usually within cells derived from mesodermal structures, in particular, mature and immature haematopoietic cells (Rikihisa, 1991a; Barbet, 1995; Logan *et al*., 1987). Moreover, for each species of these genera for which sufficient study has been accomplished, an invertebrate vector host has been identified, predominantly ticks or trematodes (Rikihisa, 1991a), except for *Wolbachia* species, which are highly promiscuous for diverse invertebrate hosts and are also found in a variety of helminths (Werren, 1997; Zhou *et al*., 1998).

The data generated by 16S rRNA gene sequencing studies support the prior classification of the species and genera in the newly constituted family *Anaplasmataceae* (Weisburg *et al*., 1989; van Vliet *et al*., 1992; Dame *et al*., 1992). Based upon 16S rRNA gene and *groESL* operon sequence results (Sumner *et al*., 1997; Zhang *et al*., 1997) and antigenic analyses (Zhang *et al*., 1997), the data suggest strongly that an accurate reorganization of these taxa would require the reorganization of most members of the existing genera *Anaplasma*, *Cowdria*, *Neorickettsia*, *Wolbachia* and *Ehrlichia* into four distinct genetic groups. Consistent with these genetic groups, which also have parallel differences in phenotype, we propose the following: (i) that the present genus *Anaplasma* be expanded to include *Ehrlichia phagocytophila*, *Ehrlichia bovis* and *Ehrlichia platys* and that *Anaplasma phagocytophila* comb. nov. will include the subjective synonyms *Ehrlichia equi* and *Ehrlichia* 'HGE agent'; (ii) that the species *Cowdria ruminantium* be placed in the genus *Ehrlichia as Ehrlichia ruminantium* comb. nov. with the existing species *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii* and *Ehrlichia muris*; (iii) that the genus *Neorickettsia* be expanded to include the species *Ehrlichia risticii* and *Ehrlichia sennetsu*; and (iv) that the species *Wolbachia pipientis* be provisionally retained as the sole member of the genus *Wolbachia*. Molecular and biological data supporting this taxonomic reorganization of species and genera in the family *Anaplasmataceae* are presented here.

METHODS

The literature on species in the family *Anaplasmataceae*, including analysis of nucleic acid sequences, antigenic properties, their ecology and geographical distribution and pathogenicity, was reviewed in order to determine the most

scientifically supported scheme for classification. Due to the subjective nature of the clinical and non-microbial phenotypic parameters used in previous taxonomic associations, accepted standards of phylogenetic analysis based upon identified gene nucleic acid sequences or protein amino acid sequences of ehrlichiae have been given greater weight in the final determination of the positions of proposed taxa. Sequence analyses were conducted by obtaining all 16S rRNA gene and *groESL* sequences deposited in GenBank that could be retrieved with a key word search for *Ehrlichia*, *Anaplasma*, *Cowdria*, *Wolbachia* or *Neorickettsia* (Tables 1 and 2). Because of a paucity of sequences available for *Anaplasma* species and the absence of sequence data for *Ehrlichia ovina*, additional 16S rRNA gene sequences were determined by participating authors, submitted for inclusion in GenBank and included in the final analyses. The methods and details of these sequences will be presented elsewhere. The 16S rRNA gene sequences of *Escherichia coli*, *Rickettsia* species, *Chlamydia trachomatis* and a variety of other bacteria with arthropod associations were included for comparison. Sequences were aligned using CLUSTAL X version 1.8 (Thompson *et al.*, 1997) and then corrected by hand to preserve codon alignment and conserved protein motifs, where relevant. Sites containing gaps or having ambiguous alignment were removed prior to phylogenetic analysis.

Phylogenetic trees were inferred from nucleotide sequences using PAUP* (Swofford, 2000). Trees were constructed using the maximum-parsimony, minimum-evolution and maximum-likelihood criteria as implemented in PAUP*. The most parsimonious tree was sought using a heuristic search procedure with 100 random addition sequence replicates and tree bisection–reconnection branch swapping. For distance-based methods, the HKY85 two-parameter model of sequence evolution was applied, with empirical estimation of transition/transversion ratio and base frequency. The minimum-evolution tree was used as the starting tree for maximum-likelihood analyses. Internal node support was verified using the bootstrap method (Felsenstein, 1985) with 1000 replicates.

RESULTS AND DISCUSSION

Multiple analyses and alignments of the 16S rRNA gene sequences of these organisms have revealed four distinct clusters, regardless of method. This phenomenon was also confirmed by comparing the nucleotide sequences of the *groESL* operon for organisms where those sequences have been described (van Vliet *et al.*, 1992; Dame *et al.*, 1992; Rikihisa *et al.*, 1997; Zhang *et al.*, 1997; Roux & Raoult, 1995, 1999; Drancourt & Raoult, 1994; Anderson *et al.*, 1991; Chen *et al.*, 1994a; Wen *et al.*, 1995a, b; Sumner *et al.*, 1997). In the genetic analyses, full-length sequences were not available for many 16S rRNA gene entries. Therefore, analysis was performed using the largest fragment that was available for most taxa. Thus, a 1292 nt fragment (after gap-stripping) including 87 taxa was used to validate subsequent comparisons using a smaller fragment so that the remaining taxa could also be assessed. This smaller fragment included the first 455 nt of the larger fragment, representing 138 taxa. Four groups were consistently identified (Fig. 1; details of the similarity values are available as Additional Table 1 in IJSEM Online at http://ijs.sgmjournals.org/cgi/content/full/51/6/2145/DC1) in both the large and small fragment comparisons, with 16S rRNA gene sequence similarities between 82·2 and 100%, but generally greater than 91·0% (mean 90·9%). These analyses also revealed the genus *Rickettsia* to be at least 80·2% but not more than 86·1% similar to any member in the genus *Ehrlichia*, *Neorickettsia helminthoeca*, *A. marginale*, *C. ruminantium* and *W. pipientis*. In the dendrograms, *E. phagocytophila*, *E. equi*, the human granulocytic ehrlichiosis (HGE) agent, *E. platys* and *A. marginale* (*E. phagocytophila* group) clustered to obtain at least 96·1% similarity, but were at most 94·9% similar to the next closest grouping, which included *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and *C. ruminantium* (*E. canis* group). Likewise, members of the *E. canis* group clustered to obtain at least 97·7% similarity. In contrast, the group defined by *E. sennetsu* (including *E. sennetsu*, *E. risticii*, *Neorickettsia helminthoeca* and the SF agent) was less than 88·3% similar to any member of the *E. canis* or *E. phagocytophila* groups or to *W. pipientis*. *W. pipientis* is an obligate intracellular bacterium that is transmitted vertically (maternally) in arthropod and helminth hosts. This species seems to occupy an intermediate phylogenetic position, between 82·3 and 90·0% similar to each of the other three genetic clusters. The legitimacy of this grouping analysis was confirmed, as very similar results were obtained with nucleotide sequence alignments of *groESL* (Fig. 2; details of the similarity values are available as Additional Table 2 in IJSEM Online at http://ijs.sgmjournals.org/cgi/content/full/51/6/2145/DC2) and comprehensive analyses of the outer-membrane protein genes that are shared among the *E. phagocytophila* and *E. canis* groups and with members of the genus *Wolbachia* but not among *E. sennetsu*, *E. risticii* or *N. helminthoeca* (Sumner *et al.*, 1997; Zhang *et al.*, 1997; Yu *et al.*, 1999a; Ohashi *et al.*, 1998a, b; Murphy *et al.*, 1998; Dawson *et al.*, 1996a; Lally *et al.*, 1995).

With the 16S rRNA gene and *groESL* alignments used as an initial starting template for a genetically based taxonomic classification system, further evidence of validity was sought by evaluation of other objective phenotypic characteristics, especially analyses of the amino acid or nucleotide sequences of outer-membrane protein genes, antigenic analyses, biological characteristics including infected host cell type, potential vectors, mammalian hosts with and without clinically evident signs of infection and clinical signs in infected hosts. Progressively less weight was attributed to these characteristics as objectivity decreased.

The *E. phagocytophila/Anaplasma* group

Within the *E. phagocytophila*/*Anaplasma* group cluster, three organisms share at least 99·1% nucleotide

Table 1. 16S rRNA sequences used in the phylogenetic analyses and associated information

Accession no.	Location	Source	Designation	Prior taxonomic classification
AF283007	Japan	Bovine	Japan	*Anaplasma centrale*
AF318944	South Africa	Ovine	NA	*Anaplasma centrale*
AF309866	Virginia, USA	Bovine	Virginia	*Anaplasma marginale*
AF309867	Florida, USA	Bovine	Florida	*Anaplasma marginale*
AF309868	Idaho, USA	Bovine	South Idaho	*Anaplasma marginale*
AF309869	Israel	Bovine	Israel	*Anaplasma marginale*
AF311303	Virginia, USA	Bovine	Virginia	*Anaplasma marginale*
M60313	NA	Bovine	NA	*Anaplasma marginale*
AF309865	NA	Ovine	South Africa	*Anaplasma ovis*
AF318945	NA	Ovine	NA	*Anaplasma ovis*
NKIT36586	South Africa	Ovine	Sheep 3573/7	*Anaplasma ovis*
AB001521	Africa	*Ornithodoros moubata* tick	Symbiote A	Argasid tick 'symbiote A'
AB001522	Africa	*Ornithodoros moubata* tick	Symbiote B	Argasid tick 'symbiote B'
AE001345	NA	Human	D/UW-3/CX	*Chlamydia trachomatis*
AF069758	South Africa	Ruminant	Mara 87/7	*Cowdria ruminantium*
U03776	South Africa	Ruminant	Omatjenne	*Cowdria ruminantium*
U03777	South Africa	Ruminant	Ball3	*Cowdria ruminantium*
X61659	Zimbabwe	Ruminant	Crystal Springs	*Cowdria ruminantium*
X62432	Senegal	Ruminant	Senegal	*Cowdria ruminantium*
D84559	NA	*Rhipicephalus sanguineus* tick	NA	*Coxiella* sp.
U03775	South Africa	Bovine	NA	*Ehrlichia bovis*
AF162860	Guangzhou, China	Dog	Gzh982	*Ehrlichia canis*
M73221	Oklahoma, USA	Dog	Oklahoma^T	*Ehrlichia canis*
M73226	Florida, USA	Dog	Florida	*Ehrlichia canis*
U26740	Israel	Dog	611	*Ehrlichia canis*
AF147752	China	*Amblyomma testudinarium* tick	NA	*Ehrlichia chaffeensis*
M73222	Arkansas, USA	Human	Arkansas^T	*Ehrlichia chaffeensis*
U23503	Arkansas, USA	Human	91HE17	*Ehrlichia chaffeensis*
U60476	Oklahoma, USA	Human	Sapulpa	*Ehrlichia chaffeensis*
U86664	Florida, USA	Human	Jax	*Ehrlichia chaffeensis*
U86665	Florida/Georgia, USA	Human	St Vincent	*Ehrlichia chaffeensis*
AF036645	California, USA	Horse	Alice	*Ehrlichia equi*
AF036646	California, USA	*Ixodes pacificus* tick/horse	Atempo	*Ehrlichia equi*
AF036647	California, USA	Horse	Meretricious	*Ehrlichia equi*
AF172164	California, USA	Horse	CASOLJ	*Ehrlichia equi*
AF172165	California, USA	Horse	CAMEBS	*Ehrlichia equi*
AF172166	California, USA	Horse	CASITL	*Ehrlichia equi*
AF172167	California, USA	Horse	CAMAWI	*Ehrlichia equi*
M73223	North America	Horse	NA	*Ehrlichia equi*
M73227	Oklahoma, USA	Dog	Stillwater^T	*Ehrlichia ewingii*
U96436	North Carolina/Virginia, USA	Dog	95E9-TS	*Ehrlichia ewingii*
AB013008	Japan	*Apodemus speciosus*	I268	*Ehrlichia muris*
AB013009	Japan	*Haemaphysalis flava* tick	NA1	*Ehrlichia muris*
U15527	Japan	*Eothenomys kageus*	AS145^T	*Ehrlichia muris*
AF318946	Turkey	Ovine	NA	*Ehrlichia ovina*
M73220	Scotland, UK	Sheep	Old Sourhope	*Ehrlichia phagocytophila*
M73224	Scotland, UK	Goat	Feral goat	*Ehrlichia phagocytophila*
AF156784	Guangzhou, China	Dog	Gzh981	*Ehrlichia platys*
M82801	North America	Dog	NA	*Ehrlichia platys*
AF036648	Oregon, USA	Horse	Buck	*Ehrlichia risticii*
AF036649	Oregon, USA	Horse	Bunn	*Ehrlichia risticii*
AF036650	Oregon, USA	Horse	Danny	*Ehrlichia risticii*
AF036651	California, USA	*Juga* spp. (snail)	None	*Ehrlichia risticii*
AF036652	California, USA	*Juga* spp. (snail)	DrPepper	*Ehrlichia risticii*
AF036653	Pennsylvania, USA	Horse	Eclipse	*Ehrlichia risticii*
AF036654	California, USA	*Juga* spp. (snail)	Juga/snail	*Ehrlichia risticii*
AF036655	Oregon, USA	*Juga* spp. (snail)	Stagnicola	*Ehrlichia risticii*
AF036656	Michigan, USA	Horse	MostlyMemories	*Ehrlichia risticii*
AF036657	California, USA	*Juga* spp. (snail)	MsAnnie	*Ehrlichia risticii*
AF036658	Oregon, USA	*Juga* spp. (snail)	Tate	*Ehrlichia risticii*
AF036659	Oregon, USA	*Juga* spp. (snail)	Thorenberg	*Ehrlichia risticii*
AF037210	California, USA	*Juga* spp. (snail)	SHSN-1	*Ehrlichia risticii*
AF037211	California, USA	*Juga* spp. (snail)	SHSN-2	*Ehrlichia risticii*
AF170727	California, USA	Coyote	CATE	*Ehrlichia risticii*
AF170729	California, USA	Coyote	CAPL	*Ehrlichia risticii*
M21290	Maryland, USA	Horse	Illinois^T	*Ehrlichia risticii*
M73219	Japan	Human	Miyayama^T	*Ehrlichia sennetsu*
M73225	NA	Human	11908	*Ehrlichia sennetsu*
AF012528	France	*Ixodes ricinus* tick	EHR62	*Ehrlichia* sp.
AF057707	Switzerland	Horse	NA	*Ehrlichia* sp.
AF069062	California, USA	*Haliotis cracherodii* (abalone)	WSA	*Ehrlichia* sp.
AF084907	Switzerland	*Ixodes ricinus* tick	NA	*Ehrlichia* sp.
AF104680	Netherlands	*Ixodes ricinus* tick	Schotti variant	*Ehrlichia* sp.
AF136712	Germany	*Ixodes ricinus* tick	Frankonia 2	*Ehrlichia* sp.
AF136713	Germany	*Ixodes ricinus* tick	Frankonia 1	*Ehrlichia* sp.

Table 1 (*cont.*)

Accession no.	Location	Source	Designation	Prior taxonomic classification
AF136714	Germany	*Ixodes ricinus* tick	Baden	*Ehrlichia* sp.
AF170728	California, USA	Coyote	CASC	*Ehrlichia* sp.
AF241532	California, USA	Llama	NA	*Ehrlichia* sp.
U02521	Wisconsin, USA	Human	NA	*Ehrlichia* sp.
U10873	Sweden	Dog	Rosa	*Ehrlichia* sp.
U27101	Oklahoma, USA	*Odocoileus virginianus* (white-tailed deer)	OK3	*Ehrlichia* sp.
U27102	Oklahoma, USA	*Odocoileus virginianus* (white-tailed deer)	OK1	*Ehrlichia* sp.
U27103	Georgia, USA	*Odocoileus virginianus* (white-tailed deer)	GA2	*Ehrlichia* sp.
U27104	Georgia, USA	*Odocoileus virginianus* (white-tailed deer)	GA4	*Ehrlichia* sp.
U34280	Japan	*Stellantchasmus falcatus* (fluke)	SF agent	*Ehrlichia* sp.
U52514	Missouri, USA	*Amblyomma americanum* tick	NA	*Ehrlichia* sp.
U54805	South Africa	Sheep	Germishuys	*Ehrlichia* sp.
U54806	South Africa	Bovine	Omatjenne	*Ehrlichia* sp.
U72878	Minnesota, USA	*Peromyscus leucopus* (white-footed mouse)	PL1559	*Ehrlichia* sp.
U72879	Minnesota, USA	*Peromyscus leucopus* (white-footed mouse)	PL505	*Ehrlichia* sp.
U77389	Switzerland	Horse	Swiss horse 1	*Ehrlichia* sp.
AF093788	California, USA	Human	CAHU-HGE1	*Ehrlichia* sp. HGE agent
AF093789	California, USA	Human	CAHU-HGE2	*Ehrlichia* sp. HGE agent
AF189153	Minnesota, USA	*Peromyscus leucopus* (white-footed mouse)	PL59	*Ehrlichia* sp. HGE agent
AJ242785	Sweden	*Ixodes ricinus* tick	NA	*Ehrlichia* sp. type Ia
AJ242783	Sweden	*Ixodes ricinus* tick	NA	*Ehrlichia* sp. type Ib
AJ242784	Sweden	*Ixodes ricinus* tick	NA	*Ehrlichia* sp. type IIb
U88565	NA	University of Illinois, Urbana, USA	Illinois	*Eperythrozoon suis*
AF016546	NA	*Bos taurus*	NA	*Eperythrozoon wenyonii*
J01859	NA	NA	NA	*Escherichia coli*
U95297	NA	*Cat*	NA	*Haemobartonella felis*
U82963	Japan	*Apodemus argentus*	Shizuoka	*Haemobartonella muris*
AB001519	NA	*Haemaphysalis longicornis* tick	NA	Ixodid tick 'symbiote A'
U12457	NA	*Nanophyetus salmincola* in dog	NA	*Neorickettsia helminthoeca*
D38622	Japan	Human	Gilliam	*Orientia tsutsugamushi*
L36217	NA	Human	R strain	*Rickettsia rickettsii*
D84558	NA	*Ixodes scapularis* tick	NA	*Rickettsia* sp.
U12463	North Carolina, USA	Human	Wilmington^T	*Rickettsia typhi*
X89110	NA	*Melophagus ovinus*	MO6	*Wolbachia melophagi*
M21292	NA	NA	NA	*Wolbachia persica*
AF179630	NA	*Folsomia candida*	NA	*Wolbachia pipientis*
U23709	NA	*Culex pipiens*	NA	*Wolbachia pipientis*
X61768	Champaign, IL, USA	*Culex pipiens*	NA	*Wolbachia pipientis*
AB025965	NA	*Callosobruchus chinensis*	jC strain	*Wolbachia* sp.
AF035160	Guangzhou, China	*Sitophilus oryzae*	Ch	*Wolbachia* sp.
AF220604	Korea	*Thecodiplosis japonensis*	NA	*Wolbachia* sp.
AJ010275	NA	*Brugia malayi*	NA	*Wolbachia* sp.
AJ010276	NA	*Onchocerca ochengi*	NA	*Wolbachia* sp.
AJ012646	NA	*Brugia pahangi*	NA	*Wolbachia* sp.
L02882	NA	*Muscidifurax uniraptor*	NA	*Wolbachia* sp.
L02883	Spain	*Trichogramma cordubensis*	Spain	*Wolbachia* sp.
L02884	Texas, USA	*Trichogramma deion*	Texas	*Wolbachia* sp.
L02887	NA	*Trichogramma deion*	Bautista Canyon	*Wolbachia* sp.
L02888	South Dakota, USA	*Trichogramma deion*	South Dakota	*Wolbachia* sp.
U17059	NA	*Drosophila sechellia*	NA	*Wolbachia* sp.
U17060	NA	*Drosophila mauritiana*	NA	*Wolbachia* sp.
U80584	NA	*Phlebotomus papatasi*	NA	*Wolbachia* sp.
U83090	Urbana, IL, USA	*Gryllus pennsylvanicus*	NA	*Wolbachia* sp.
U83091	NA	*Gryllus assimilis*	NA	*Wolbachia* sp.
U83092	Gainesville, FL, USA	*Gryllus rubens*	NA	*Wolbachia* sp.
U83093	Gainesville, FL, USA	*Gryllus ovisopis*	NA	*Wolbachia* sp.
U83094	Austin, TX, USA	*Gryllus integer*	NA	*Wolbachia* sp.
U83095	Davis, CA, USA	*Gryllus integer*	NA	*Wolbachia* sp.
U83096	Humboldt Co., NV, USA	*Gryllus integer*	NA	*Wolbachia* sp.
U83097	Wayne Co., UT, USA	*Gryllus integer*	NA	*Wolbachia* sp.
U83098	Urbana, IL, USA	*Diabrotica virgifera virgifera*	NA	*Wolbachia* sp.
Z49261	NA	*Dirofilaria immitis*	NA	*Wolbachia* sp.
AF069068	NA	*Litomosoides sigmodontis*	NA	*Wolbachia*-like endobacterium

NA, Not available or none assigned.

sequence similarity in their 16S rRNA genes and have identical GroEL amino acid sequences (van Vliet *et al.*, 1992; Sumner *et al.*, 1997; Zhang *et al.*, 1997; Roux & Raoult, 1999; Drancourt & Raoult, 1994; Anderson *et al.*, 1991; Chen *et al.*, 1994a; Wen *et al.*, 1995a, b; Dawson *et al.*, 1996a). Each of *E. phagocytophila*, *E. equi* and the HGE agent is also closely related on the basis of antigenic analyses by indirect fluorescent antibody tests (Dumler *et al.*, 1995). Protein immunoblots and cloned recombinant proteins indicate the presence of several outer-membrane protein antigens in each of these species, including an immunodominant antigen of variable molecular size (mean 44 kDa) (Dumler *et al.*, 1995; Asanovich *et al.*, 1997; Zhi *et al.*,

Table 2. *groESL* operon sequences used in the phylogenetic analyses and associated information

Accession no.	Location	Source	Designation	Prior taxonomic classification
AF165812	North America	Bovine	NA	*Anaplasma marginale*
M98257	South America	Human	ATCC 35685	*Bartonella bacilliformis*
AF008210	NA	*Schizaphis graminum*	NA	*Buchnera aphidicola*
AE001285	NA	Human	D/UW-3/CX	*Chlamydia trachomatis*
U13638	South Africa	Bovine	Welgevonden^T	*Cowdria ruminantium*
U96731	Florida, USA	Dog	Florida	*Ehrlichia canis*
L10917	Arkansas, USA	Human	Arkansas^T	*Ehrlichia chaffeensis*
AF172158	California, USA	Horse	CASOLJ	*Ehrlichia equi*
AF172160	California, USA	Horse	CAMAWI	*Ehrlichia equi*
AF172162	California, USA	Horse	CASITL	*Ehrlichia equi*
U96727	California, USA	Horse	California horse	*Ehrlichia equi*
U96729	Scotland, UK	Goat	Feral goat	*Ehrlichia phagocytophila*
U96730	Scotland, UK	Sheep	Old Sourhope	*Ehrlichia phagocytophila*
U96735	Switzerland	Horse	Swiss horse	*Ehrlichia phagocytophila*
U24396	NA	Horse	90-12	*Ehrlichia risticii*
U96732	Maryland, USA	Horse	Illinois^T	*Ehrlichia risticii*
U88092	Japan	Human	Japan	*Ehrlichia sennetsu*
AF033101	Slovenia	Human	NA	*Ehrlichia* sp. HGE agent
AF172159	California, USA	Human	CAHU-HGE2	*Ehrlichia* sp. HGE agent
AF172163	California, USA	Human	CAHU-HGE1	*Ehrlichia* sp. HGE agent
U96728	New York, USA	Human	HGE agent	*Ehrlichia* sp. HGE agent
X07850	NA	NA	NA	*Escherichia coli*
U64996	NA	Human	MS11-A	*Neisseria gonorrhoeae*
M31887	NA	Human	Karp	*Orientia tsutsugamushi*
AJ235272	NA	Human	Madrid E	*Rickettsia prowazekii*
U96733	Montana, USA	NA	R	*Rickettsia rickettsii*
AF075440	North Carolina, USA	Human	Wilmington^T	*Rickettsia typhi*
AB002286	NA	*Teleogryllus taiwanemma*	Group B	*Wolbachia* sp.

NA, Not available.

1997). The gene encoding this 44 kDa immunodominant protein is one of a multigene family comprising multiple distinct genes (Murphy *et al*., 1998; Zhi *et al*., 1998; IJdo *et al*., 1998) that also encode proteins with significant amino acid similarity to (i) the 36 kDa antigen called major surface protein 2 (MSP2) and the precursor of the 31 kDa antigen of *A. marginale* called MSP4 (Murphy *et al*., 1998; Zhi *et al*., 1998; IJdo *et al*., 1998), (ii) the *C. ruminantium* 28 kDa major antigenic protein 1 (MAP1) (Jongejan & Thielemans, 1989; Jongejan *et al*., 1993; Ohashi *et al*., 1998b; Yu *et al*., 1999a), (iii) the *E. chaffeensis* and *E. canis* P28 and P30 protein families (Yu *et al*., 1999a; Ohashi *et al*., 1998a, b; Reddy *et al*., 1998) and (iv) *Wolbachia* spp. outer-surface protein precursors (Yu *et al*., 1999a; Ohashi *et al*., 1998b). This complex of outer-membrane proteins is encoded in the HGE agent, *A. marginale*, *E. chaffeensis*, *E. canis*, *E. muris*, *C. ruminantium* and potentially in other *Ehrlichia* species by polymorphic multigene families that are suspected to contribute to immune evasion or persistence in reservoir hosts (Reddy *et al*., 1998; Alleman *et al*., 1997; French *et al*., 1998; Reddy & Streck, 1999).

A gene encoding a protein antigen of approximately 150–160 kDa that has repeated ankyrin motifs on the amino terminus, *ankA*, has been cloned from the HGE agent (Storey *et al*., 1998; Caturegli *et al*., 2000). The function of this protein is unknown and it is a unique but relatively minor antigen among the HGE agent, *E. equi* and *E. phagocytophila*. Comparison of the nucleotide sequence of a 444 bp region of the ankyrin repeat region from five Wisconsin strains and one New York strain designated as HGE agent by 16S rRNA gene sequence revealed 100 % similarity, whereas the sequence of the MRK strain of *E. equi* is 99·6 % similar to that of the HGE agent. Similarly, the sequence of *ankA* of the HGE agent is between 95·5 and 96·8 % similar to those of Swedish and Spanish strains of *E. phagocytophila* from cattle and goats, respectively (Caturegli *et al*., 2000). These data are confirmed by full gene sequences of a larger number of *E. phagocytophila*-group organisms from various geographical regions (Massung *et al*., 2000).

Biologically, *A. marginale*, *E. phagocytophila*, *E. equi*, *E. platys*, *E. bovis* and the HGE agent are most often detected in cells in the peripheral blood that are derived

from bone marrow precursors. *E. phagocytophila*, *E. equi* and the HGE agent are capable of growth *in vitro* in undifferentiated HL-60 promyelocytic cells, HL-60 cells differentiated into neutrophil-like cells and potentially in precursors of the myelomonocytic lineage, as well as in embryonic *Ixodes scapularis* tick cell lines (Goodman *et al*., 1996; Heimer *et al*., 1997; Klein *et al*., 1997; Feng, 1997; Munderloh *et al*., 1996a). The HGE agent and *E. equi* do not propagate in HL-60 cells differentiated into mature macrophages. This situation resembles that *in vivo*, since each of these species is detected most often in neutrophils or band neutrophils in the blood of infected animals and humans. *A. marginale* infects predominantly erythrocytes *in vivo* and a suitable equivalent mammalian cell line for propagation has not been identified. *A. marginale* can be grown in embryonic tick cells *in vitro* and short-term propagation in erythrocyte culture and endothelial/erythrocyte co-cultures has also been achieved (Munderloh *et al*., 1996b; Kessler *et al*., 1979; Waghela *et al*., 1997). *E. platys* infects canine platelets *in vivo* and *E. bovis* infects bovine monocytes; neither has been cultivated *in vitro*. Although the host cell ligand is not known for *E. platys* or *E. bovis*, the HGE agent, a member of the *E. phagocytophila* group, adheres to platelet glycoprotein selectin ligand-1 (PGSL-1; Herron *et al*., 2000), a sialic acid-bearing surface protein molecule that shares many chemical characteristics, such as sensitivity to neuraminidase and chymotrypsin, with the erythrocyte ligand of *A. marginale* (McGarey & Allred, 1994).

Ticks transmit all of these species, but transovarial transmission in ticks does not occur for those investigated. *E. phagocytophila*, *E. equi* and the HGE agent are each transmitted by members of the *Ixodes persulcatus* complex, whereas *A. marginale* is transmitted by *Dermacentor* spp. ticks in temperate regions of North America and by *Boophilus* spp. or other genera in other geographical regions (Kuttler, 1984; Eriks *et al*., 1993; Kocan *et al*., 1992; Telford *et al*., 1996; Richter *et al*., 1996; Walls *et al*., 1997; *Gordon et al*., 1932; MacLeod & Gordon, 1933). Except for *E. platys* and *E. bovis*, the life cycles of these agents are partially known. *A. marginale* and the closely related *Anaplasma centrale* and *Anaplasma ovis* are usually maintained by persistent subclinical infection of ruminants, including wild ruminants such as deer (Kuttler, 1984; Eriks *et al*., 1993). A role exists for transmission by male ticks among multiple animals in a single herd and mechanical transmission via biting flies provides a potential alternative transmission vehicle (Kocan *et al*., 1992). The HGE agent is maintained, at least in part, by infection of small mammal species such as the white-footed mouse, *Peromyscus leucopus*, or the dusky-footed wood rat, *Neotoma fuscipes*, in which occasional persistent infections may be detected (Telford *et al*., 1996; Walls *et al*., 1997; Nicholson *et al*., 1999). *E. phagocytophila* may establish persistent infections in ruminants under natural and experimental circumstances (Gordon *et al*., 1932; MacLeod & Gordon, 1933; Hudson, 1950; Foggie, 1951; Foster & Cameron, 1970; McDiarmid, 1965) and mounting evidence suggests that both *E. equi* and the HGE agent establish subclinical persistent infections in domestic and wild ruminants, including deer (Foley *et al*., 1998; Belongia *et al*., 1997; Walls *et al*., 1998; Magnarelli *et al*., 1999). The HGE agent produces disease typical of *E. equi* infection in horses and induces protective immunity to challenge with *E. equi* (Madigan *et al*., 1995; Barlough *et al*., 1995). Likewise, *E. equi*-like bacteria have caused infection in humans that is indistinguishable from HGE (Foley *et al*., 1999). Clinical manifestations, even in typical mammalian hosts, are highly variable for each of *E. phagocytophila*, *E. equi* and the HGE agent; clinical features therefore provide a lower degree of certainty about classification, since these are likely to be at least in part host-dependent (Gordon *et al*., 1932; MacLeod & Gordon, 1933; Hudson, 1950; Foggie, 1951; Madigan, 1993; Reubel *et al*., 1998b; Bakken *et al*., 1994, 1996, 1998; Aguero-Rosenfeld *et al*., 1996).

These common features are expected of organisms with a high degree of relatedness and indicate that these bacteria should be unified within a single genus. Moreover, the data indicate that sufficient similarity exists among *E. phagocytophila*, *E. equi* and the HGE agent for them to be classified as a single species. *A. marginale* is sufficiently divergent to be considered a separate species, but the 16S rRNA gene sequences of strains of *A. marginale*, *A. ovis* and *A. centrale*, excepting a Japanese strain, are nearly identical (minimum 99·1 % similarity), suggesting the possibility that these also represent variants of a single species, as denoted initially by Theiler (1911). The existence of a strain of *A. centrale* that has 1·8 % nucleotide difference from other phenotypically characterized strains of *A. centrale* indicates the polygenic nature of this designation and casts some doubt upon the classical morphological taxonomic methods for this species and genus. Overall, a close grouping of erythrocytic anaplasmas is supported by other genetic, phenotypic and antigenic characteristics that also indicate a close grouping with *A. marginale* (McGuire *et al*., 1984; Palmer *et al*., 1988, 1998; Visser *et al*., 1992). In fact, all species of *Anaplasma* are known to share antigens that reside on 19, 36 and 105 kDa proteins, data that strengthen the close relationship based upon host cell type and morphological characteristics (Palmer *et al*., 1988; Visser *et al*., 1992).

A large genetic distance (minimum 74·3 % similarity) in *groESL* sequences was noted between the *E. phagocytophila* group members and *A. marginale*, which is in part explained by the paucity of *groESL* sequences examined. All members of the *E. phagocytophila* group were at least 98·8 % similar and no other sequence representatives (*E. platys*, *E. bovis* etc.) of the *Anaplasma*/*E. phagocytophila* group were available. Similarly large genetic distances (minimum 86·31 % similarity) were observed for *groESL* sequences between *E. canis* and *C. ruminantium*, which

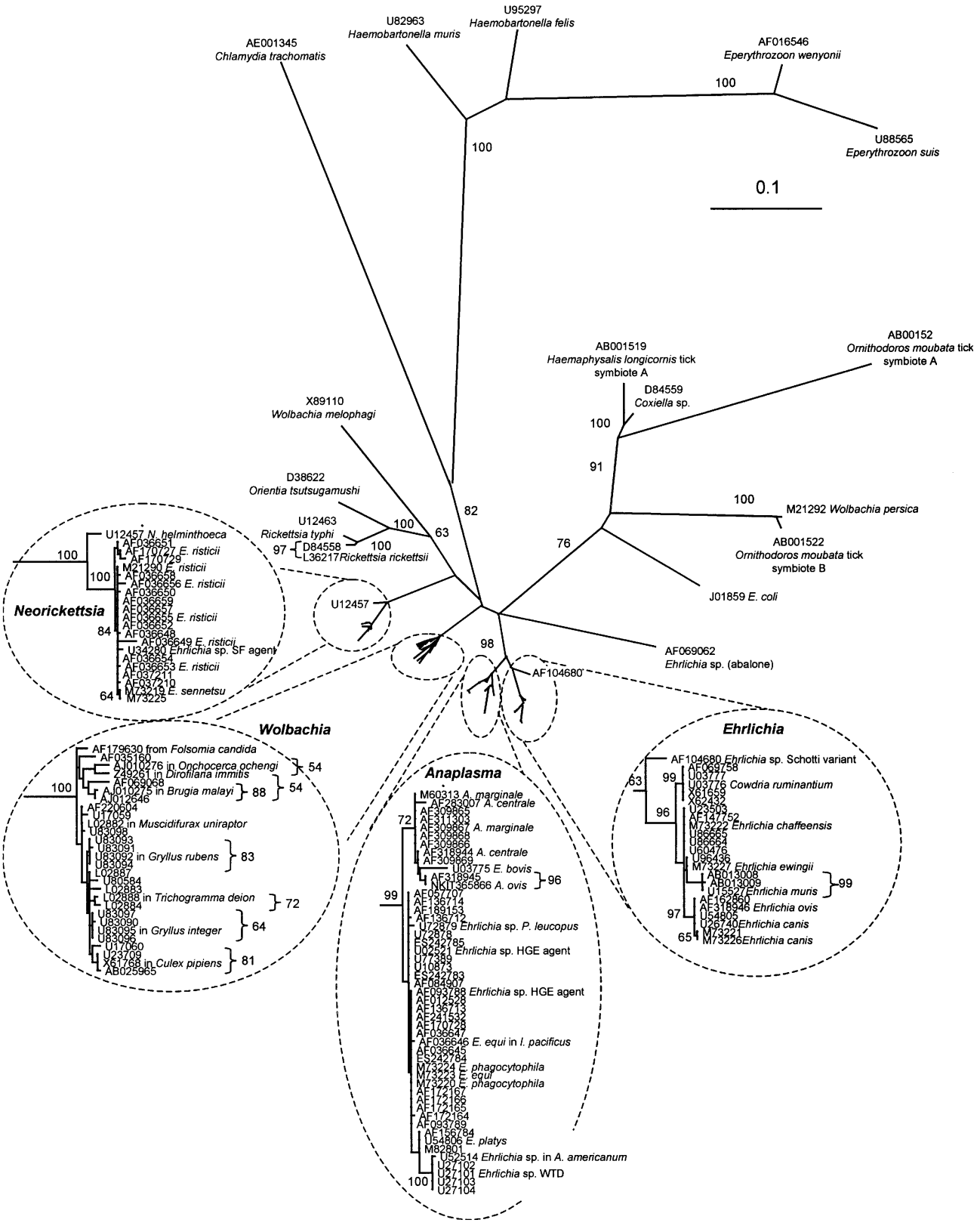

Fig. 1. For legend see facing page.

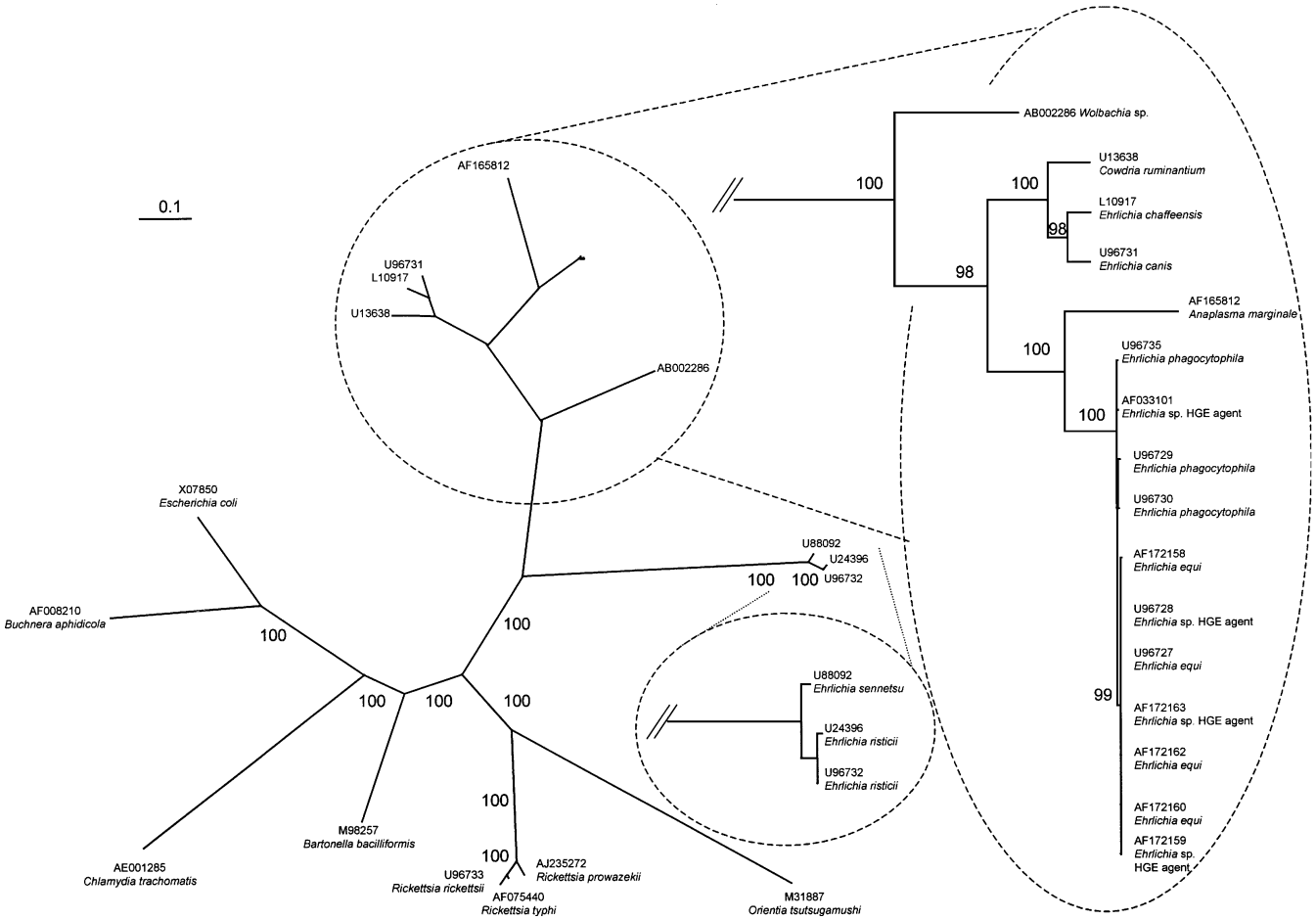

Fig. 2. Phylogenetic tree inferred from *groESL* gene sequences of *Ehrlichia*, *Anaplasma*, *Neorickettsia* and *Wolbachia* species, including 1077 sites after removal of sites containing a gap in any sequence. The sequence from *Chlamydia trachomatis* (accession no. AE001285) was used as an outgroup. Numbers above internal nodes indicate the percentage of 1000 bootstrap replicates that supported the branch. All bootstrap values are included for clades that were consistently observed using the phylogenetic methods applied (maximum parsimony, minimum evolution, maximum likelihood and majority-rule bootstrap analysis of neighbour-joining trees). The maximum-likelihood tree is shown. Bar, estimated number of substitutions per site; scale for the figure and insets are the same.

also appear to be clearly related on the basis of 16S rRNA gene sequences and phenotypic findings. Overall, the *groESL* sequences support the divisions as indicated by 16S rRNA gene sequences and provide evidence of polymorphisms that may be random or may represent subtleties of evolutionary selection. Thus, despite these ambiguous differences, insufficient genetic distance and biological differences exist among the *Anaplasma* species, the *E. phagocytophila* group, *E. bovis* and the *E. platys* clade to designate them into separate genera. This is supported further by the lack of bootstrap support for the clear separation of the two major arms of this clade and by the inconsistent presence of *E. bovis* in either the *Anaplasma* or *E. phagocytophila* clades in the various phylogenetic analyses. Additional sequence analyses of conserved and semi-conserved genes (e.g. *gltA*), whole genome analysis, as well as analysis of additional strains may further identify taxonomic divisions or support the current analyses of 16S rRNA and *groESL* genes.

Little is known about the antigenic characteristics of

Fig. 1. Phylogenetic tree inferred from small subunit (16S) rRNA gene sequences of *Ehrlichia*, *Anaplasma*, *Neorickettsia* and *Wolbachia* species, including 455 sites after removal of sites containing a gap in any sequence. The sequence from *Chlamydia trachomatis* (accession no. AE001345) was used as an outgroup. Numbers above internal nodes indicate the percentage of 1000 bootstrap replicates that supported the branch. All bootstrap values are included for clades that were consistently observed using the phylogenetic methods applied (maximum parsimony, minimum evolution, maximum likelihood and majority-rule bootstrap analysis of neighbour-joining trees). The maximum-likelihood tree is shown. Bars, estimated number of substitutions per site; the scale for the figure and insets are the same.

either *E. platys* or *E. bovis*; their taxonomic positions must therefore be assigned on the basis of what is known about their genetic characteristics (Anderson *et al.*, 1992). For some previously described agents, such as '*Cytoecetes microti*' (Tyzzer, 1938), no isolates or genetic information are available for analysis and their relationships to other named species cannot be assessed objectively. Of interest is the identification of several 16S rRNA gene sequences from the blood of white-tailed deer (*Odocoileus virginianus*) from Oklahoma and Georgia in the USA (Dawson *et al.*, 1996c), from an *Amblyomma americanum* tick in Missouri (USA) and from the blood of sheep in South Africa (Allsopp *et al.*, 1997), each of which is most similar to *E. platys*. A definitive bacterial morphology has never been identified for any of these sequences; their taxonomic positions can therefore only be judged on the basis of the 16S rRNA gene sequences.

The *E. canis*/*Cowdria* group

The second genetic cluster includes *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and *C. ruminantium*, all of which are at least 97·7% similar in 16S rRNA gene sequences (van Vliet *et al.*, 1992; Dame *et al.*, 1992; Rikihisa *et al.*, 1997; Zhang *et al.*, 1997; Roux & Raoult, 1995, 1999; Drancourt & Raoult, 1994; Anderson *et al.*, 1991; Wen *et al.*, 1995a, b; Shibata *et al.*, 2000). *E. canis*, *E. chaffeensis* and *E. muris* are detected mostly in macrophages and monocytes *in vivo* and can be propagated *in vitro*, most effectively in macrophage cell lines (Dawson *et al.*, 1991a, b; Barnewell & Rikihisa, 1994; Heimer *et al.*, 1998). *C. ruminantium* is most often found in endothelial cells, neutrophils or macrophages *in vivo* and can also be propagated in cell lines derived from both endothelial cells and macrophages (Cowdry, 1926; Logan *et al.*, 1987; Bezuidenhout *et al.*, 1985; Sahu, 1986; Prozesky & Du Plessis, 1987). *E. ewingii* is the exception in that it is detected most frequently in peripheral blood neutrophils and it has not been grown in long-term culture (Ewing *et al.*, 1971). *E. canis* is best recognized as a pathogen of canids (Huxsoll, 1976; Woody & Hoskins, 1991), but can infect humans and may infect felines (Perez *et al.*, 1996; Bouloy *et al.*, 1994), whereas *E. chaffeensis* causes symptomatic infection in humans and subclinical persistent infections in deer and canids (Fishbein *et al.*, 1994; Ewing *et al.*, 1995, Lockhart *et al.*, 1997; Dawson *et al.*, 1996b; Dawson & Ewing, 1992). *E. ewingii* causes low-grade infections of canids that are sometimes characterized by lameness due to polyarthritis (Ewing *et al.*, 1971) and has recently been implicated as a human pathogen (Buller *et al.*, 1999). *C. ruminantium* is best known as the cause of heartwater in African and Caribbean ruminants (Cowdry, 1926; Uilenberg, 1983; Camus *et al.*, 1993). Each of these species is known to be transmitted and maintained in a tick vector reservoir, including *Amblyomma* spp. for *C. ruminantium* (Bezuidenhout, 1987), *Amblyomma americanum* for *E. chaffeensis* and *E. ewingii* (Ewing *et al.*, 1995; Anziani *et al.*, 1990) and *Rhipicephalus sanguineus* for *E. canis* (Groves *et al.*, 1975). Transovarial transmission is ineffective for *E. canis* and *C. ruminantium*, the only species studied sufficiently (Bezuidenhout, 1987; Groves *et al.*, 1975).

Polyclonal antibodies to these organisms have a high degree of cross-reactivity by immunofluorescence, a result consistent with a close genetic relationship. Low-level antigenic cross-reactivity is also recognized between *C. ruminantium* and *E. phagocytophila* and between *E. phagocytophila*, *E. equi*, the HGE agent and *E. chaffeensis*, *E. canis* or *E. ewingii* (Dumler *et al.*, 1995; Dawson *et al.*, 1991a; Jongejan *et al.*, 1989; Buller *et al.*, 1999; Brouqui *et al.*, 1992; Rikihisa *et al.*, 1992). The antigens of these organisms have been studied in some detail by Western blotting, which reveals the presence of cross-reactive immunodominant antigens of similar molecular size but with a degree of diversity when detected with monoclonal antibodies (Dumler *et al.*, 1995; Asanovich *et al.*, 1997; Zhi *et al.*, 1997; Visser *et al.*, 1992; Palmer *et al.*, 1985, 1998; Brouqui *et al.*, 1992, 1994; Rikihisa *et al.*, 1992, 1994; Yu *et al.*, 1993; Chen *et al.*, 1994b, 1996; Kim & Rikihisa, 1998; Ravyn *et al.*, 1999; Adams *et al.*, 1986; Vidotto *et al.*, 1994; Alleman & Barbet, 1996; Barbet *et al.*, 1994; Rossouw *et al.*, 1990; Mahan *et al.*, 1993, 1994; Bowie *et al.*, 1999; Kelly *et al.*, 1994). A group of antigens that range between 27 and 32 kDa is common among these organisms and is shared between these different species when analysed by immunoblotting methods (Rikihisa, 1991a; Rikihisa *et al.*, 1992, 1994; Iqbal *et al.*, 1994; Wen *et al.*, 1995a; Ohashi *et al.*, 1998a, b; Jongejan *et al.*, 1993). Monoclonal antibodies reactive with proteins in this molecular size range that are raised against one isolate do not always react with other isolates (Chen *et al.*, 1996, 1997). These proteins are encoded by polymorphic genes and are called MAP1 in *C. ruminantium*, MAP1 homologue, p28 and p30 in *E. canis* and Omp1 or p28 in *E. chaffeensis*, but have yet to be described in *E. ewingii*; a homologous gene has been identified in other *Ehrlichia* species (Ohashi *et al.*, 1998a, b; Reddy *et al.*, 1998; Yu *et al.*, 1999b; McBride *et al.*, 1999; van Vliet *et al.*, 1994). In fact, a high degree of amino acid similarity exists between these proteins and the MSP4 of *A. marginale*, further clarifying the basis for prior evidence of serological cross-reactions obtained by immunofluorescence studies (Ohashi *et al.*, 1998a, b; Yu *et al.*, 1999b; McBride *et al.*, 1999; van Vliet *et al.*, 1994).

The data on the tick-transmitted ehrlichiae in the *Anaplasma*/*E. phagocytophila* and *E. canis*/*Cowdria* groups argue convincingly for the unification of these species within either one or two separate genera. However, the large degree of internal genetic similarity (Fig. 1), the extent of shared amino acid sequences in major outer-membrane proteins, the similarity in host cells and similarity in serological cross-reactions argue for consolidation of the species of the *E. phagocytophila* complex in a genus that contains only *A. marginale*, *E. platys* and *E. bovis*. Moreover, the

repeated genetic clustering of members of the *E. canis*/*Cowdria* group to the exclusion of members of the *E. phagocytophila*/*Anaplasma* group suggests that the establishment of two separate genera for these groups is the best way to emphasize the degree of biological difference between these clades. However, should a large number of apparently ancestral types to both these groups be found, like the 'Schotti variant' (Fig. 1), future consolidation of these two closely related groups may be warranted.

The *E. sennetsu*/*Neorickettsia* group

The third and most divergent genetic cluster of the ehrlichiae includes *E. sennetsu*, *E. risticii* (van Vliet *et al*., 1992; Dame *et al*., 1992; Rikihisa *et al*., 1997; Zhang *et al*., 1997; Roux & Raoult, 1995, 1999; Drancourt & Raoult, 1994; Anderson *et al*., 1991; Chen *et al*., 1994a; Wen *et al*., 1995a, b), *N. helminthoeca* and an ehrlichia-like bacterium present in the metacercarial stage of the fluke *Stellantchasmus falcatus* (SF), all of which exhibit between 94·9 and 100·0 % similarity in 16S rRNA gene sequences (Wen *et al*., 1996; Barlough *et al*., 1998; Pretzman *et al*., 1995; Chaichanasiriwithaya *et al*., 1994). However, individual isolates of *E. risticii* may diverge in 16S rRNA gene sequence by as many as 15 nucleotides (Wen *et al*., 1995b; Barlough *et al*., 1998). These data underscore the phylogenetic heterogeneity of this clade. In spite of these observations, fluorescent antibody and protein immunoblot studies show a high degree of antigenic similarity among *E. sennetsu*, *E. risticii*, *N. helminthoeca* and the SF agent, but not to other species of *Ehrlichia* (Rikihisa, 1991b; Rikihisa *et al*., 1988; Dumler *et al*., 1995; Wen *et al*., 1996; Holland *et al*., 1985a, b; Ristic *et al*., 1986; Shankarappa *et al*., 1992). Each of these species infects predominantly mononuclear phagocytes *in vivo* and can be propagated *in vitro* most efficiently in cell lines derived from macrophages (Zhang *et al*., 1997; Wen *et al*., 1996; Shankarappa *et al*., 1992; Rikihisa *et al*., 1991, 1995). Ticks have never been implicated in transmission of these agents, whereas transmission via infected metacercariae or cercariae of flukes that infest either snails, fish or aquatic insects has been shown for *N. helminthoeca* and *E. risticii* and is strongly suspected for *E. sennetsu* (Rikihisa, 1991a; Barlough *et al*., 1998; Madigan *et al*., 2000). While no naturally existing mammalian infection with the SF agent has been recognized, its presence in flukes and pathogenicity in mice is consistent with the above observations in other *E. sennetsu*-group organisms (Rikihisa, 1991a; Wen *et al*., 1996; Fukuda & Yamamoto, 1981).

E. sennetsu is best known as the agent of sennetsu fever, a mononucleosis-like illness described only in Japan and Malaysia (Misao & Kobayashi, 1955; Rapmund, 1984). Early epidemiological studies suggested that individuals who consumed uncooked fish from certain areas of Japan were at risk (Rikihisa, 1991a; Tachibana *et al*., 1976). Although not proven, this epidemiology has long suggested the possibility of enteral ingestion of fish contaminated with ehrlichia-infected flukes as the mechanism for transmission. *E. sennetsu* causes a fatal infection in mice and produces no clinical signs in horses, but protects horses against challenge by *E. risticii* (Tachibana & Kobayashi, 1975; Rikihisa *et al*., 1988). *E. risticii* causes Potomac horse fever, also known as equine monocytic ehrlichiosis or 'Shasta River crud' (Holland *et al*., 1985a; Rikihisa & Perry, 1985; Madigan *et al*., 1997). Presumably, the agent is either ingested when horses feed upon snail-ridden grasses or by ingestion of infected metacercaria-containing aquatic insects (Reubel *et al*., 1998a; Barlough *et al*., 1998; Madigan *et al*., 2000). The presentation is that of a febrile illness with profuse watery diarrhoea. *N. helminthoeca* is acquired by ingestion of fluke-infested fish by dogs and causes a febrile infection called salmon poisoning disease (Rikihisa, 1991a).

The degree of 16S rRNA gene sequence similarity of the *E. sennetsu* group to those in the *E. phagocytophila* and *E. canis* groups is not more than exists between the *E. sennetsu* group and *Rickettsia* species (Wen *et al*., 1995a, b). Although minor serological cross-reactivity has been described in some studies (Holland *et al*., 1985a; Ristic *et al*., 1981), no firm similarities in outer-membrane protein amino acid sequences have been established and there appear to be no haematophagous arthropod vectors such as ticks involved in the life cycle. However, the common infected host cells are similar to those of other *Ehrlichia* species, although the clinical manifestations of enteric involvement are more pronounced. The significant genetic, antigenic and ecological traits of the species of the *E. sennetsu* group suggest that it is a distinct clade deserving of designation as a separate genus.

Wolbachia species

The sole remaining named species of the genus *Wolbachia* is *W. pipientis*, an obligate intracellular bacterium that resides within cytoplasmic vacuoles, predominantly in the ovaries of many species of arthropods and increasingly identified in helminths (Werren, 1997; Popov *et al*., 1998; O'Neill *et al*., 1992; Dobson *et al*., 1992; Bandi *et al*., 1998). Analysis of *ftsZ* gene amplicons of arthropod and filarial wolbachiae indicates the existence of at least two distinct host-associated clades (Bandi *et al*., 1998; Vandekerckhove *et al*., 1999). However, by 16S rRNA gene sequence analysis, *W. pipientis* and the *Wolbachia* spp. occupy a position intermediate between the two tick-transmitted groups (*E. canis*/*C. ruminantium* and *E. phagocytophila*/*Anaplasma*) and the helminth-borne *E. sennetsu*/*Neorickettsia* group (Roux & Raoult, 1995; Wen *et al*., 1995b; O'Neill *et al*., 1992). Deduced amino acid sequences of *Wolbachia* spp. outer-membrane protein genes exhibit similarity to those of the major outer-membrane proteins of *A. marginale*, the *E. phagocytophila* complex, *E. chaffeensis*, *E. canis* and

C. ruminantium, thus corroborating the phylogenetic position of *W. pipientis* (Yu *et al.*, 1999a; Ohashi *et al.*, 1998b; Zhou *et al.*, 1998). However, *W. pipientis* is not recognized as a vertebrate pathogen, since mammalian infection has never been documented.

Although there are significant morphological, genetic and amino acid sequence similarities between *W. pipientis* and the other *Ehrlichia*/*Cowdria*/*Anaplasma* groups, the significant degree of differences in 16S rRNA and *groESL* gene sequences, the lack of a significant vertebrate host phase, the promiscuous invertebrate host associations and its highly efficient transovarial transmission adequately differentiate *W. pipientis* and related organisms from species found in the genera *Ehrlichia*, *Cowdria*, *Anaplasma* and *Neorickettsia*.

Historical precedents

The historical precedent for naming species in the entire group of ehrlichiae is *A. marginale*, which was first described and named by Theiler (1910). The organism currently denoted *C. ruminantium* was described initially by Cowdry (1925) and given the genus designation *Cowdria* by Moshkovski (1947). Gordon first clearly differentiated tick-borne fever from louping ill in goats in 1932 and suggested that the disease was caused by a rickettsia, an assertion that was affirmed in 1940 by Foggie (Gordon *et al.*, 1932; Foggie, 1951). The genus designation *Ehrlichia* was first coined in 1945 to honour Paul Ehrlich (Moshkovski, 1945; Silverstein, 1998), 2 years before the designation of *C. ruminantium*; however, the type species, *E. canis*, was first described as *Rickettsia canis* by Donatien & Lestoquard (1935) and, in 1936, the same authors described *E. bovis* as *Rickettsia bovis* (Donatien & Lestoquard, 1936). Hertig first described rickettsia-like organisms in insects in 1936 and these were placed in the genus *Wolbachia* in honour of S. Burt Wolbach, who demonstrated the presence of rickettsiae in pathological lesions in the vasculotropic rickettsioses (Hertig, 1936). The designation '*Cytoecetes microti*' was created to describe a microorganism with morphological features similar to the organism now called *E. phagocytophila* (Tyzzer, 1938); however, original materials and isolates no longer exist for verification of its identity (Ristic & Huxsoll, 1984). Subsequently, other designations were made into the genus *Ehrlichia* (in North America) or '*Cytoecetes*' (in Europe and Asia) for organisms that were recognized to be pathogenic for mammals (Ristic & Huxsoll, 1984; Moshkovski, 1945). *N. helminthoeca* was described in 1953, *E. sennetsu* in 1954, *E. equi* in 1969, *E. platys* in 1982, *E. risticii* in 1984, *E. chaffeensis* in 1991, *E. ewingii* in 1993 and *E. muris* in 1995 (Wen *et al.*, 1995a; Anderson *et al.*, 1992; Misao & Kobayashi, 1955; Philip *et al.*, 1953; Stannard *et al.*, 1969; Gribble, 1969).

Convincing phylogenetic data now show that a series of significant flaws exists in the taxonomic structure of the families *Anaplasmataceae* and *Rickettsiaceae* in the order *Rickettsiales*. Similar phylogenetic studies led to a significant taxonomic modification of the former genera *Rochalimaea* and *Grahamella* (Brenner *et al.*, 1993; Birtles *et al.*, 1995). It is now clear that a distinction between some members of the families *Rickettsiaceae* and *Anaplasmataceae* is not supported. Moreover, some members of the family *Anaplasmataceae*, the genera *Eperythrozoon* and *Haemobartonella*, are clearly not related to the genus *Anaplasma* and should be removed and reassigned within the family *Mycoplasmataceae* (Rikihisa *et al.*, 1997). While no classification system fits all criteria perfectly, genetic data have become the objective standards and, when evaluated carefully, often closely predict similar biological and clinical behaviours. Thus, the data compiled here indicate that a sufficient genotypic and phenotypic relationship exists among the genera *Anaplasma*, *Cowdria*, *Wolbachia* and *Ehrlichia*, excluding *N. helminthoeca*, *E. sennetsu* and *E. risticii*, to merit unification into two separate genera. Since the validly published names *Anaplasma* and *A. marginale* and *Ehrlichia* and *E. canis* predate *Cowdria* and *Wolbachia*, *Anaplasma* should be retained for the unified genus that encompasses the existing *Anaplasma* species, the *E. phagocytophila* group, *E. bovis* and *E. platys*, while the genus *Ehrlichia* should be retained and used to describe members of the *Ehrlichia canis* group, including *C. ruminantium*. This change further necessitates accommodation of the members of the *E. sennetsu* group within a single genus, *Neorickettsia*. Thus, a revised classification may be formulated that differentiates organisms in the order *Rickettsiale*s into two families, *Rickettsiaceae*, which contains the rickettsiae (*Rickettsia*, *Orientia*) that occupy an intracytoplasmic compartment, and *Anaplasmataceae*, which contains the ehrlichiae (*Neorickettsia*, *Wolbachia*, *Ehrlichia*, *Anaplasma*) that occupy an intravacuolar compartment within infected host cells. Consequently, new combinations for the multiple genera and species that are involved must also be created.

Emended description of *Rickettsiales* (Gieszczykiewicz 1939) Weiss and Moulder 1984

It is proposed that the tribes *Rickettsieae*, *Ehrlichieae* and *Wolbachieae* should be abolished. Furthermore, all species formerly within the tribes *Ehrlichieae* and *Wolbachieae* are transferred into the family *Anaplasmataceae*.

Emended description of *Rickettsiaceae* (Pinkerton 1936) Weiss and Moulder 1984

It is proposed that the genera *Ehrlichia*, *Cowdria*, *Neorickettsia* and *Wolbachia* be transferred from the family *Rickettsiaceae* to the family *Anaplasmataceae*, a change that results in the elimination of all tribes within the family *Rickettsiaceae*. It is also proposed that the genera *Haemobartonella* and *Eperythrozoon*

should be transferred from the family *Anaplasmataceae* to the order *Mycoplasmatales* and that *Coxiella*, *Rickettsiella*, *Francisella* (*Wolbachia*) *persica* and *Wolbachia melophagi* (Weisburg *et al*., 1989; Roux *et al*., 1997) should be removed from the family *Rickettsiaceae*. This proposal also requires emendation of the description of the family *Rickettsiaceae* to specify that organisms infect host cells within the cytoplasm or nucleus and are not bounded by a vacuole. The family *Rickettsiaceae* includes only the genera *Rickettsia* and *Orientia*.

Emended description of *Anaplasmataceae* (Philip 1957) Ristic and Kreier 1984

It is proposed that the family *Anaplasmataceae* be emended to include species in the genera *Wolbachia*, *Ehrlichia*, *Cowdria* and *Neorickettsia* and to retain species in the genera *Anaplasma* and *Aegyptianella*. This requires emendation of the description of the *Anaplasmataceae* to specify infection within a cytoplasmic vacuole of host cells that include erythrocytes, reticuloendothelial cells, bone marrow-derived phagocytic cells, endothelial cells and cells of insect, helminth and arthropod reproductive tissues. *Aegyptianella* is retained as *genus incertae sedis*.

Emended description of *Anaplasma* (Theiler 1910) Ristic and Kreier 1984

It is proposed that members of the *E. phagocytophila* group, including *E. phagocytophila*, *E. equi*, the HGE agent, as well as *E. bovis* and *E. platys*, should be united with the genus *Anaplasma*. This change requires emendation of the description of the genus *Anaplasma* (Ristic & Kreier, 1984) by integrating it with some descriptions of the genera *Ehrlichia* and new data for *Anaplasma* and *Ehrlichia*, as follows.

Gram-negative, small, often pleomorphic, coccoid to ellipsoidal organisms that reside within cytoplasmic vacuoles, either singly and more often in compact inclusions (morulae) present in mature or immature haematopoietic cells, particularly myeloid cells and neutrophils and including erythrocytes, in peripheral blood or in tissues, usually mononuclear phagocyte organs (spleen, liver, bone marrow) of mammalian hosts. By ultrastructure, two morphological forms are observed, including larger reticulate cells and smaller forms with condensed protoplasm called dense-core forms (Popov *et al*., 1998). Vectors, where known, are ticks. Organisms grow in tick vectors. Non-motile. Not cultivable in cell-free media or chicken embryos. Some species are cultivable in neutrophils, myelomonocytic cell lines, promyelocytic cell lines, erythrocytes and tick cell lines. Aetiological agents of diseases of dogs and other canids, humans and ruminants such as cattle, goats, sheep and llamas. Variably pathogenic or non-pathogenic infections in some ruminants such as cattle, goats, sheep and deer, horses and rodents. The estimated G+C content of the DNA varies between approximately 30 and 56 mol%. The type species is *Anaplasma marginale* (Theiler, 1910).

Emended description of *Ehrlichia* (Moshkovski 1945) Ristic and Huxsoll 1984

Gram-negative, small, often pleomorphic, coccoid to ellipsoidal organisms that reside within cytoplasmic vacuoles, either singly and more often in compact inclusions (morulae) present in mature or immature haematopoietic cells, especially mononuclear phagocytes such as monocytes and macrophages and for some species in myeloid cells such as neutrophils, in peripheral blood or in tissues, usually mononuclear phagocyte organs (spleen, liver, bone marrow, lymph node) of mammalian hosts. By ultrastructure, two morphological forms are observed, including larger reticulate cells and smaller forms with condensed protoplasm (dense-core forms) (Popov *et al*., 1998). Vectors, where known, are ticks. Organisms grow in tick vectors. Non-motile. Not cultivable in cell-free media or chicken embryos. Some species cultivable in blood monocytes, monocytic or macrophage cells lines, myelomonocytic cell lines, endothelial cell lines and tick cell lines. Aetiological agents of diseases of dogs and other canids, rodents and humans. Variably pathogenic or non-pathogenic infections in some ruminants such as deer and some rodents. The G+C content of the DNA varies between approximately 30 and 56 mol%. The type species is *Ehrlichia canis* (Donatien and Lestoquard 1935) Moshkovski 1945.

Emended description of *Neorickettsia* (Philip, Hadlow and Hughes 1953)

It is proposed that some descriptions of the genus *Ehrlichia* be united with the genus *Neorickettsia* (Pretzman *et al*., 1995). This requires emendation of the description of the genus *Neorickettsia* by integration with some descriptions of the genus *Ehrlichia* and new data, as follows.

Small, coccoid, often pleomorphic, intracytoplasmic bacteria that occur primarily in vacuoles of monocytes in the blood and macrophages of lymphoid or other tissues of dogs, horses and humans. Certain tissues of mature fluke vectors, all other fluke stages, eggs, rediae, cercariae and metacercariae have been proven infectious by injection into susceptible vertebrate hosts, as have mature stages of aquatic insects, which confirms that the infectious cycle includes transovarial and *trans*-stadial transmission in the vectors (Reubel *et al*., 1998a; Barlough *et al*., 1998). Gram-negative. Non-motile. Not cultivable in cell-free media or in chicken embryos. Sensitive to tetracycline antibiotics. The G+C content of the DNA is not known. The type species is *Neorickettsia helminthoeca* (Philip *et al*., 1953).

Description of *Anaplasma phagocytophila* comb. nov.

The most recent description of *E. phagocytophila* is that of Ristic & Huxsoll (1984). It is proposed that the species *E. equi* (Ristic & Huxsoll, 1984; Stannard *et al.*, 1969) and the unnamed HGE agent (Chen *et al.*, 1994a; Bakken *et al.*, 1994) be united within the single species designation *E. phagocytophila* and transferred into the genus *Anaplasma*. This requires emendation of the species description for *E. phagocytophila* by integrating portions of the description of the species *E. equi* (Ristic & Huxsoll, 1984) and new data for *E. equi* and the HGE agent as follows.

Gram-negative, coccoid to ellipsoidal, often pleomorphic, intracytoplasmic bacteria that infect cells of mammalian bone marrow derivation, predominantly cells in the myeloid lineage. Two ultrastructural morphologies are observed, including a larger reticulate form and a smaller dense-core form that contains condensed protoplasm. Tick vectors include species of the *Ixodes persulcatus* complex (Telford *et al.*, 1996; Richter *et al.*, 1996; MacLeod & Gordon, 1933; Foggie, 1951). In mammalian cells, morulae are usually 1·5–2·5 μm in diameter, but may be as large as 6 μm (Popov *et al.*, 1998). Individual bacterial cells are of two types, dense-core and reticulate, both present in the same vacuole; both may undergo equal or unequal binary fission. Individual cells may be as large as 2 μm in diameter. Empty vesicles may be present in the vacuolar space, but fibrillar matrix is lacking. Abundant cytoplasmic membrane may be present, forming protrusions into the periplasmic space or invaginations into the bacterial protoplasm. Mitochondria do not contact with or cluster around morulae. Causative agent of tick-borne fever of ruminants (Gordon *et al.*, 1932; Hudson, 1950; Foggie, 1951). Equine granulocytic ehrlichiosis (Madigan, 1993; Stannard *et al.*, 1969; Gribble, 1969), a type of canine granulocytic ehrlichiosis that lacks lameness as a significant sign (Greig *et al.*, 1996; Pusterla *et al.*, 1997) and human granulocytic ehrlichiosis (Chen *et al.*, 1994a; Goodman *et al.*, 1996; Bakken *et al.*, 1994) are caused by variants of *A. phagocytophila*, previously known as *E. equi* and the HGE agent, respectively. Tick-borne fever is chiefly reported as a febrile disease of goats, sheep and cattle in the UK, The Netherlands, Scandinavia, Spain, France, Germany and Switzerland. Clinical signs vary from no detectable illness to severe febrile disease associated with opportunistic infections, haemorrhage and abortions. Equine granulocytic ehrlichiosis and a form of canine granulocytic ehrlichiosis have been described broadly across the USA, Canada, Brazil, Venezuela and Northern Europe. Equine and canine diseases are characterized by fever, depression, anorexia, leukopaenia and thrombocytopaenia; equine infection also frequently results in limb oedema and ataxia and may lead to opportunistic infections. Human granulocytic ehrlichiosis has also been described in many of the same geographical areas of California, Wisconsin, Minnesota and the New England states in the USA and in Slovenia, Norway, Switzerland and Sweden in Europe; serological evidence of human infection in the absence of overt human disease has been described in the USA, UK, Switzerland, Norway, Sweden, Denmark, Germany and Bulgaria. Human disease is characterized by fever, headache, myalgia and malaise and by the presence of leukopaenia, thrombocytopaenia and evidence of hepatic injury (Bakken *et al.*, 1996; Aguero-Rosenfeld *et al.*, 1996). The case fatality rate in humans is less than 1%, but is associated with severe opportunistic infections (Walker & Dumler, 1997). Although *A. phagocytophila* has a broad geographical distribution and all isolates appear to have significant serological cross-reactivity, a minor degree of variation in the nucleotide sequence of up to 5 bp (> 99·5% identity) in the 16S rRNA gene and ⩾ 99·0% identity in *groESL* is detected. The organism shares significant antigens with *E. canis*, *E. chaffeensis* and *E.* (*Cowdria*) *ruminantium* comb. nov. The major constitutively produced protein antigens are encoded by a multigene family, vary between 42 and 49 kDa in molecular size and are expressed on the outer membrane (Murphy *et al.*, 1998; Dumler *et al.*, 1995; Asanovich *et al.*, 1997; Zhi *et al.*, 1997, 1998). The amino acid sequences of the major outer-membrane proteins are similar to those of *A. marginale*, *E.* (*Cowdria*) *ruminantium*, *E. canis*, *E. chaffeensis* and *Wolbachia* species. The genome size is approximately 1500 kbp (Rydkina *et al.*, 1999). The G+C content of the DNA estimated from sequenced genes is 41 mol%. The 16S rRNA gene sequence of the *A. phagocytophila* type strain Webster^T is the same as deposited in GenBank under the accession number U02521.

Description of *Anaplasma bovis* comb. nov.

The most recent description of *Ehrlichia bovis* is that of Scott (1994). In addition, *A. bovis* is a Gram-negative, coccoid to coccobacillary and often pleomorphic obligate intravacuolar bacterium that infects cattle and perhaps other mammals. Mononuclear cells are most often infected but are infrequently identified in peripheral blood. African tick vectors include *Hyalomma excavatum*, *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and possibly *Amblyomma cajennense* in Brazil. Serological cross-reactions with *E. ruminantium* have been reported (Du Plessis *et al.*, 1987). The 16S rRNA gene sequence of *A. bovis* is deposited in GenBank under the accession number U03775.

Description of *Ehrlichia ruminantium* comb. nov.

The most recent description of *Cowdria ruminantium* is that of van Vliet *et al.* (1992). In addition, *E. ruminantium* is a Gram-negative, coccoid to ellipsoidal, often pleomorphic, intracytoplasmic bacteria that infects cattle, sheep, goats and occasionally murine endothelial cells as well as cells of bone marrow derivation, predominantly cells in the myeloid and monocytic lineages. Various species of wild African ruminants are reservoir hosts (Neitz, 1933, 1935; Peter

et al., 1998, 1999). Tick vectors include at least 10 species of the genus *Amblyomma*. The major constitutionally produced protein antigens (MAP1) are encoded by a multigene family, vary between 31 and 32 kDa in molecular size and are expressed on the outer membrane. The amino acid sequences of this major outer-membrane protein are similar to those of *A. marginale*, *A. phagocytophila*, *E. chaffeensis*, *E. canis* and *W. pipientis*. The 16S rRNA gene sequence of the type strain, Welgevonden^T, is the same as that for the Crystal Springs strain deposited in GenBank under accession no. X61659 (M. T. Allsopp, personal communication).

Emended description of *Ehrlichia canis*

The most recent description of *E. canis* is that of Ristic & Huxsoll (1984). In addition, cells are Gram-negative, coccoid to ellipsoidal, often pleomorphic, intracytoplasmic bacteria that infect canid and perhaps human cells of bone marrow derivation (Perez *et al*., 1996), predominantly cells in the monocytic lineage. The predominant tick vector is *Rhipicephalus sanguineus*. The major constitutionally produced protein antigen varies between 28 and 32 kDa in molecular size and is expressed on the outer membrane (Yu *et al*., 1999a; Ohashi *et al*., 1998b; Reddy *et al*., 1998; McBride *et al*., 1999). The amino acid sequences of the major outer-membrane proteins are similar to those of *E. chaffeensis*, *E. ruminantium*, *A. marginale*, *A. phagocytophila* and *W. pipientis*. The 16S rRNA gene sequence of the type strain Oklahoma^T is deposited in GenBank under accession no. M73221.

Emended description of *Ehrlichia chaffeensis*

With the following additions, the description is the same as that given previously (Anderson *et al*., 1991). The tick vector is *Amblyomma americanum*. The type strain is strain Arkansas^T. The major constitutionally produced protein antigens are encoded by a multigene family, vary between 28 and 32 kDa in molecular size and are expressed on the outer membrane (Yu *et al*., 1999a; Ohashi *et al*., 1998a; Reddy *et al*., 1998). The amino acid sequences of the major outer-membrane proteins are similar to those of *E. canis*, *E. ruminantium*, *A. phagocytophila*, *A. marginale* and *Wolbachia* species. The genome size is approximately 1250 kbp (Rydkina *et al*., 1999). The 16S rRNA gene sequence of the type strain, Arkansas^T, is deposited in GenBank under accession no. M73222.

Emended description of *Ehrlichia ewingii*

With the following additions, the description is the same as that given previously (Anderson *et al*., 1992). The tick vector is *Amblyomma americanum*. The type strain is strain Stillwater^T. Aetiological agent of canine and human disease (Ewing *et al*., 1971; Buller *et al*., 1999). The 16S rRNA gene sequence is deposited in GenBank under accession no. M73227.

Emended description of *Ehrlichia muris*

With the following additions, the description is the same as that given previously (Wen *et al*., 1995a). *Haemaphysalis flava* ticks may be naturally infected, but a role as a vector has not been established (Kawahara *et al*., 1999). The 16S rRNA gene sequence of the type strain, AS145^T, is deposited in GenBank under accession no. U15527.

Description of *Anaplasma platys* comb. nov.

With the following additions, the description is the same as that given previously for *E. platys* (Ristic & Huxsoll, 1984; Anderson *et al*., 1992). A tick vector is suspected, but has not been established. The 16S rRNA gene sequence is deposited in GenBank under accession no. M82801.

Description of *Neorickettsia sennetsu* comb. nov.

With the following additions, the description is the same as that given previously for *E. sennetsu* (Ristic & Huxsoll, 1984). The organism shares antigens with *N.* (*Ehrlichia*) *risticii*. Not pathogenic for the horse but, after infection, horses are protected from infection with *N. risticii*. The mode of transmission is not known, although a fish parasite is suspected. Mice are highly susceptible to infection. The genome size is approximately 880 kbp (Rydkina *et al*., 1999). The type strain is Miyayama^T, for which the 16S rRNA gene sequence is deposited in GenBank under accession no. M73225 (Anderson *et al*., 1991).

Description of *Neorickettsia risticii* comb. nov.

With the following additions, the description is the same as that given previously for *E. risticii* (Holland *et al*., 1985b). Causative agent of Potomac horse fever, also called equine monocytic ehrlichiosis. Transmitted by the ingestion of fresh-water snail species or insects infested with infected trematodes or metacercariae (Reubel *et al*., 1998a; Madigan *et al*., 2000). Shares antigens with *N. sennetsu*, *N. helminthoeca* and the SF agent bacterium. The organism is found to infect peripheral blood monocytes, intestinal epithelial cells and equine mast cells. The 16S rRNA gene may vary by up to 15 bases in nucleic acid sequences. The approximate genome size is 880 kbp (Rydkina *et al*., 1999). The type strain is Illinois^T, for which the 16S rRNA gene sequence is deposited in GenBank under accession no. M21290.

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