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The *Bacteroidetes*, *Spirochaetes*, *Tenericutes* (*Mollicutes*), *Acidobacteria*, *Fibrobacteres*, *Fusobacteria*, *Dictyoglomi*, *Gemmatimonadetes*, *Lentisphaerae*, *Verrucomicrobia*, *Chlamydiae*, and *Planctomycetes*

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Phylum XXIV. Chlamydiae Garrity and Holt 2001

MATTHIAS HORN

Chla.my.di'ae. N.L. fem. pl. n. *Chlamydiales* type order of the phylum; N.L. fem. pl. n. *Chlamydiae* the phylum of the order *Chlamydiales*.

The phylum *Chlamydiae* is based on phylogenetic analysis of 16S rRNA sequences, clearly separating its members (showing ≥80% 16S rRNA sequence similarity with each other) from all other *Bacteria* by a long, deep branch in phylogenetic trees (Figure 154). All known members of this phylum are nonmotile, obligate intracellular bacteria and multiply in eukaryotic hosts (animals including humans, or protozoa). Unlike most other bacteria, they show a developmental cycle characterized by morphologically and physiologically distinct stages. All chlamydiae characterized so far possess small genomes (1–2.4 Mb); they are metabolically impaired and need to import essential building blocks like nucleotides, amino acids, and cofactors from their host cells. The intracellular life style of chlamydiae is thus thought to be an ancient trait of this phylum. Chlamydiae show a Gram-negative type cell wall, but they lack detectable amounts of peptidoglycan. They do not encode the cell division protein FtsZ, which is otherwise considered essential for bacteria. Chlamydiae possess several unique proteins found in no other microorganisms (n = 59) (Griffiths et al., 2006) and conserved insertions and deletions in widely distributed proteins (Griffiths and Gupta, 2007; Griffiths et al., 2005; Gupta and Griffiths, 2006).

Members of the phyla *Lentisphaera* and *Verrucomicrobia* seem to be the closest free-living relatives of *Chlamydiae* based on phylogenetic analysis of the 16S rRNA gene, concatenated protein datasets, and two unique inserts in LysRS and RpoB proteins (Griffiths and Gupta, 2007; Strous et al., 2006; Wagner and

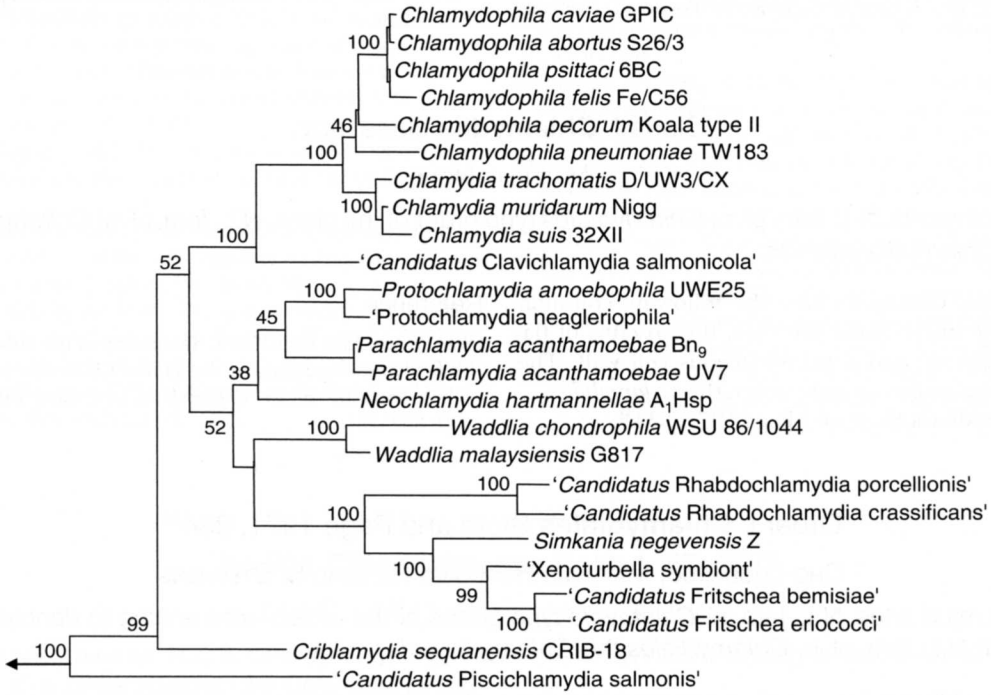

FIGURE 154. Phylogenetic tree showing the relationships of representative members of the *Chlamydiae*. The tree has been calculated with the ARB software, using a manually curated version of the SILVA database (SSU Ref, version 92) and the PhyML treeing algorithm implemented in ARB (Guindon et al., 2005; Ludwig et al., 2004; Pruesse et al., 2007). The HKY substitution model, empirical base frequency estimates, and a fixed transition/transversion ratio of 4.00 were used; non-parametric bootstrap analysis (100 resamplings) and a filter excluding highly variable positions were applied. Bar = 10% estimated evolutionary distance; arrow, to outgroup.

Horn, 2006). In addition, a phylogenetic relationship of the *Chlamydiae* with the phyla *Planctomycetes* and *Poribacteria* has been proposed (Cavalier-Smith, 2002; Wagner and Horn, 2006).

The phylum *Chlamydiae* currently comprises a single class, *Chlamydiia*, containing only one order, the *Chlamydiales*.

Type order: **Chlamydiales** Storz and Page 1971, 334[AL].

References

Cavalier-Smith, T. 2002. The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. Int. J. Syst. Evol. Microbiol. *52*: 297–354.

Garrity, G.M. and J.G. Holt. 2001. The Road Map to the *Manual*. *In* Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 1, The *Archaea* and the Deeply Branching and Phototrophic *Bacteria* (edited by Boone, Castenholz and Garrity). Springer, New York, pp. 119–166.

Griffiths, E., A.K. Petrich and R.S. Gupta. 2005. Conserved indels in essential proteins that are distinctive characteristics of *Chlamydiales* and provide novel means for their identification. Microbiology *151*: 2647–2657.

Griffiths, E., M.S. Ventresca and R.S. Gupta. 2006. BLAST screening of chlamydial genomes to identify signature proteins that are unique for the *Chlamydiales*, *Chlamydiaceae*, *Chlamydophila* and *Chlamydia* groups of species. BMC Genomics *7*: 14.

Griffiths, E. and R.S. Gupta. 2007. Phylogeny and shared conserved inserts in proteins provide evidence that *Verrucomicrobia* are the closest known free-living relatives of chlamydiae. Microbiology *153*: 2648–2654.

Guindon, S., F. Lethiec, P. Duroux and O. Gascuel. 2005. PHYML Online – a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res. *33*: W557–W559.

Gupta, R.S. and E. Griffiths. 2006. Chlamydiae-specific proteins and indels: novel tools for studies. Trends Microbiol. *14*: 527–535.

Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Forster, I. Brettske, S. Gerber, A.W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. Konig, T. Liss, R. Lussmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode and K.H. Schleifer. 2004. ARB: a software environment for sequence data. Nucleic Acids Res. *32*: 1363–1371.

Pruesse, E., C. Quast, K. Knittel, B. Fuchs, W. Ludwig, J. Peplies and F.O. Glöckner. 2007. SILVA: a comprehensive online resource for quality checked and aligned rRNA sequence data compatible with ARB. Nucleic Acids Res. *35*: 7188–7196.

Storz, J. and L.A. Page. 1971. Taxonomy of the chlamydiae: reasons for classifying organisms of the genus *Chlamydia*, family *Chlamydiaceae*, in a separate order *Chlamydiales* ord. nov. Int. J. Syst. Bacteriol. *21*: 332–334.

Strous, M., E. Pelletier, S. Mangenot, T. Rattei, A. Lehner, M.W. Taylor, M. Horn, H. Daims, D. Bartol-Mavel, P. Wincker, V. Barbe, N. Fonknechten, D. Vallenet, B. Segurens, C. Schenowitz-Truong, C. Medigue, A. Collingro, B. Snel, B.E. Dutilh, H.J. Op den Camp, C. van der Drift, I. Cirpus, K.T. van de Pas-Schoonen, H.R. Harhangi, L. van Niftrik, M. Schmid, J. Keltjens, J. van de Vossenberg, B. Kartal, H. Meier, D. Frishman, M.A. Huynen, H.W. Mewes, J. Weissenbach, M.S. Jetten, M. Wagner and D. Le Paslier. 2006. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature *440*: 790–794.

Wagner, M. and M. Horn. 2006. The *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae* and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr. Opin. Biotechnol. *17*: 241–249.

Class I. **Chlamydiia** class. nov.

Matthias Horn

Chla.my.di'ia. N.L. fem. pl. n. *Chlamydiales* type order of the class; N.L. fem. pl. n. *Chlamydiia* the *Chlamydiales* class.

Members of the class *Chlamydiia* have the following common features: an obligate intracellular life style, the absence of flagella and peptidoglycan, and a proteinaceous cell wall. The class *Chlamydiia* contains but a single order, the *Chlamydiales*.

Type order: **Chlamydiales** Storz and Page 1971, 334[AL].

Reference

Storz, J. and L.A. Page. 1971. Taxonomy of the chlamydiae: reasons for classifying organisms of the genus *Chlamydia*, family *Chlamydiaceae*, in a separate order *Chlamydiales* ord. nov. Int. J. Syst. Bacteriol. *21*: 332–334.

Order I. **Chlamydiales** Storz and Page 1971, 334[AL]

Cho-chou Kuo, Matthias Horn and Richard S. Stephens

Chla.my.di.a'les. N.L. fem. n. *Chlamydia* type genus of the order; *-ales* ending to denote an order; N.L. fem. pl. n. *Chlamydiales* the *Chlamydia* order.

Gram-stain-negative, obligately intracellular microorganisms that live in eukaryotes and may cause various diseases. The mode of multiplication within the cytoplasmic vacuoles is characterized by reorganization of small, rigid-walled, metabolically inactive infectious forms (elementary bodies) into large, flexible-walled, metabolically active forms (reticulate bodies) that are generally noninfectious and divide by binary fission. The developmental cycle is complete when the reticulate bodies reorganize into elementary bodies that survive extracellulary to infect new host cells upon their release from the host cell. The order *Chlamydiales* traditionally contained a single family, the *Chlamydiaceae*, which included the type genus *Chlamydia*. The recent discovery of novel chlamydia-related bacteria expanded the order *Chlamydiales* to include four additional families, having 80–90% 16S rRNA sequence similarity with the *Chlamydiaceae* (Everett et al., 1999; Rurangirwa et al., 1999; Thomas et al., 2006). Three

further families are added based on the descriptions of "*Candidatus* Piscichlamydia salmonis", "*Candidatus* Clavichlamydia salmonicola", and "*Candidatus* Rhabdochlamydia porcellionis" (Draghi et al., 2004; Karlsen et al., 2008; Kostanjsek et al., 2004), respectively, all of which show 80–90% 16S rRNA sequence similarity with all other *Chlamydiales* families (Figure 154).

For classification of members of the *Chlamydiales* at the family and genus level, the 16S rRNA sequence similarity thresholds proposed by Everett et al. (1999) are currently accepted. Members of a *Chlamydiales* family generally share ≥90% 16S rRNA similarity with each other; members of a *Chlamydiales* genus generally share ≥95% 16S rRNA sequence similarity, with the *Chlamydiaceae* being a notable exception. As the proposed separation of the *Chlamydiaceae* into the two genera *Chlamydia* and *Chlamydophila* (Everett et al., 1999) is not being used consistently in the scientific literature, it was recently proposed to abandon the genus name *Chlamydophila* and to transfer all *Chlamydophila* species to the genus *Chlamydia* (Bavoil and Wyrick, 2006; Kalayoglu and Byrne, 2006).

The order *Chlamydiales* consists currently of eight families, *Chlamydiaceae*, *Clavichlamydiaceae*, *Criblamydiaceae*, *Parachlamydiaceae*, *Piscichlamydiaceae*, *Rhabdochlamydiaceae*, *Simkaniaceae*, and *Waddliaceae*.

Further evidence for an even greater diversity of yet uncultured chlamydiae exists (Corsaro et al., 2003; Horn and Wagner, 2001; Ossewaarde and Meijer, 1999). Several oligonucleotide probes for the identification of chlamydiae by fluorescence *in situ* hybridization at different taxonomic levels are available (Loy et al., 2007; Poppert et al., 2002).

DNA G+C content (mol%): 35.8–41.3.

Type genus: **Chlamydia** Jones, Rake and Stearns 1945, 55[AL] emend. Everett, Bush and Andersen 1999, 430.

References

Bavoil, P.M. and P.B. Wyrick. 2006. Chlamydia: genomics and pathogenesis. Horizon Bioscience, Norwich, UK.

Corsaro, D., M. Valassina and D. Venditti. 2003. Increasing diversity within Chlamydiae. Crit. Rev. Microbiol. *29*: 37–78.

Draghi, A., 2nd, V.L. Popov, M.M. Kahl, J.B. Stanton, C.C. Brown, G.J. Tsongalis, A.B. West and S. Frasca, Jr. 2004. Characterization of "*Candidatus* piscichlamydia salmonis" (order *Chlamydiales*), a chlamydia-like bacterium associated with epitheliocystis in farmed Atlantic salmon (*Salmo salar*). J. Clin. Microbiol. *42*: 5286–5297.

Everett, K.D.E., R.M. Bush and A.A. Andersen. 1999. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol. *49*: 415–440.

Horn, M. and M. Wagner. 2001. Evidence for additional genus-level diversity of *Chlamydiales* in the environment. FEMS Microbiol. Lett. *204*: 71–74.

Jones, H., G. Rake and B. Stearns. 1945. Studies on lymphogranuloma venereum. III. The action of the sulfonamides on the agent of lymphogranuloma venereum. J. Infect. Dis. *76*: 55–69.

Kalayoglu, M.V. and G.I. Byrne. 2006. The genus *Chlamydia* - medical. *In* The Prokaryotes: a Handbook on the Biology of Bacteria, 3rd edn, vol. 7, *Proteobacteria*: Delta and Epsilon Subclasses. Deeply Rooting Bacteria (edited by Dworkin, Falkow, Rosenberg, Schleifer and Stackebrandt). Springer, New York, pp. 741–754.

Karlsen, M., A. Nylund, K. Watanabe, J.V. Helvik, S. Nylund and H. Plarre. 2008. Characterization of '*Candidatus* Clavochlamydia salmonicola': an intracellular bacterium infecting salmonid fish. Environ. Microbiol. *10*: 208–218.

Kostanjsek, R., J. Strus, D. Drobne and G. Avgustin. 2004. '*Candidatus* Rhabdochlamydia porcellionis', an intracellular bacterium from the hepatopancreas of the terrestrial isopod *Porcellio scaber* (Crustacea: Isopoda). Int. J. Syst. Evol. Microbiol. *54*: 543–549.

Loy, A., F. Maixner, M. Wagner and M. Horn. 2007. probeBase – an online resource for rRNA-targeted oligonucleotide probes: new features 2007. Nucleic Acids Res. *35*: D800–D804.

Ossewaarde, J.M. and A. Meijer. 1999. Molecular evidence for the existence of additional members of the order *Chlamydiales*. Microbiology *145*: 411–417.

Poppert, S., A. Essig, R. Marre, M. Wagner and M. Horn. 2002. Detection and differentiation of chlamydiae by fluorescence *in situ* hybridization. Appl. Environ. Microbiol. *68*: 4081–4089.

Rurangirwa, F.R., P.M. Dilbeck, T.B. Crawford, T.C. McGuire and T.F. McElwain. 1999. Analysis of the 16S rRNA gene of micro-organism WSU 86-1044 from an aborted bovine foetus reveals that it is a member of the order *Chlamydiales*: proposal of *Waddliaceae* fam. nov., *Waddlia chondrophila* gen. nov., sp. nov. Int. J. Syst. Bacteriol. *49*: 577–581.

Storz, J. and L.A. Page. 1971. Taxonomy of the chlamydiae: reasons for classifying organisms of the genus *Chlamydia*, family *Chlamydiaceae*, in a separate order *Chlamydiales* ord. nov. Int. J. Syst. Bacteriol. *21*: 332–334.

Thomas, V., N. Casson and G. Greub. 2006. *Criblamydia sequanensis*, a new intracellular *Chlamydiales* isolated from Seine river water using amoebal co-culture. Environ. Microbiol. *8*: 2125–2135.

Family I. **Chlamydiaceae** Rake 1957, 957[AL]

Cho-Chou Kuo and Richard S. Stephens

Chla.my.di.a.ce'ae. N.L. n. *Chlamydia* type genus of the family; *-aceae* ending to denote family; N.L. fem. pl. n. *Chlamydiaceae* the *Chlamydia* family.

In the previous edition of *Bergey's Manual of Systematic Bacteriology* (Volume 1, 1984), the family contained a single genus, *Chlamydia*. The family was emended to include an additional genus, *Chlamydophila*, by Everett et al. in 1999. The new genus and five species, *Chlamydophila abortus*, *Chlamydophila caviae*, *Chlamydophila felis*, *Chlamydophila pecorum*, *Chlamydophila pneumoniae*, and *Chlamydophila psittaci* were validly published, but the scientific community has not uniformly adopted the use of the new genus name. For this reason, in this edition of the *Manual* the original genus *Chlamydia* is retained as the sole genus in the family and the five additional species are classified in the genus.

DNA G+C content (mol%): 39–45.

Type genus: **Chlamydia** Jones, Rake and Stearns 1945, 55[AL].

Genus I. **Chlamydia** Jones, Rake and Stearns 1945, 55[AL]

CHO-CHOU KUO, RICHARD S. STEPHENS, PATRIK M. BAVOIL AND BERNHARD KALTENBOECK

Chla.my'di.a. Gr. fem. n. *chlamys, chlamydis* a cloak; N.L. fem. n. *Chlamydia* a cloak.

Coccoid, nonmotile, obligate intracellular organisms, 0.2–1.5 µm in diameter that parasitize and multiply in the cytoplasm of eukaryotic cells within membrane bound vacuoles, termed inclusions, by a unique developmental cycle. The cycle is characterized by physical changes in the outer membrane and nucleoid structure of metabolically inert elementary bodies that change into larger metabolically active reticulate bodies that divide by binary fission. The cycle is complete when reticulate bodies reorganize into intermediate bodies then to elementary bodies that exit the host cells and start a new generation of the developmental cycle. In a young inclusion, only reticulate bodies are present. However in a mature inclusion, all three forms (reticulate, intermediate, and elementary bodies) are present because the developmental cycle is not synchronized. The intracellular survival of the organism is facilitated by its ability to prevent fusion of lysosomes to chlamydia-containing inclusions by an unknown mechanism.

Elementary bodies are 0.2–0.4 µm in diameter, have electron-dense DNA condensed with protein and a few ribosomes, and are surrounded by rigid trilaminar cell walls resistant to sonic lysis. Elementary bodies are infectious. **Reticulate bodies** are 0.6–1.5 µm in diameter, have less dense, fibrillar nuclear material, more ribosomes, and plastic trilaminar walls that are sensitive to sonic lysis. Reticulate bodies are not infectious. **Intermediate bodies** are organisms in transition from reticulate bodies to elementary bodies that are variable in size and have a small plaque of dense nuclear material in the center of the cytoplasm with a characteristic target-like appearance. Infectivity of intermediate bodies has not been demonstrated.

Cell walls of elementary and reticulate bodies exhibit regular hexagonal arrays of subunits on the inner surface and single patches of hexagonal ordered hemispheric projections on their outer surface that resemble in structure the type III secretion apparatus of Gram-stain-negative bacteria. Cell walls contain lipopolysaccharide and are analogous in structure and composition to walls of Gram-stain-negative bacteria, however, muramic acid is either absent or is present only in trace amounts in elementary bodies.

Chlamydiae cause a variety of diseases in humans, other mammals, and birds. Cultivation of *Chlamydia* in cell-free medium has not been achieved. They may be propagated in laboratory animals, the yolk sac of chicken embryos, or in cell culture. Chlamydiae require their hosts for low-molecular-weight synthetic intermediates that they use to synthesize DNA, RNA, proteins, and lipids. The genome size is among the smallest of prokaryotes, 1–1.3 × 10^6 bp.

DNA G+C content (mol%): 39–45.

Type species: **Chlamydia trachomatis** (Busacca 1935) Rake 1957, 958[AL] [*Rickettsia trachomae* (*sic*) Busacca 1935, 567].

TABLE 160. Characteristics of chlamydial elementary and reticulate bodies[a,b]

Characteristic	Elementary	Reticulate
General:		
Diameter (µm)	0.2–0.4	0.5–1.5
Density (g/cm^3)	1.21	1.18
Time of appearance in developmental cycle	Late	Early
Infectivity for new host cells	+	–
Intravenous lethality for mice	+	–
Immediate cytotoxicity in cell culture	+	–
Cell wall:		
Rigidity	Rigid	Flexible
Cross-linking of outer membrane	+	–
Susceptibility to:		
Mechanical stress	–	+
Osmotic stress	–	+
Lysis by trypsin	–	+
Trilamminar structure	+	+
Projections on surface	+	+
Hexagonal arrays on inner surface	+	+
Muramic acid	–	nd
Synthesis inhibited by penicillin	+	–
Genus-specific antigen	+	+
Hemagglutinin	+	–
Nucleic acids:		
DNA	Compact	Disperse
RNA/DNA ratio	1	3–4
Ribosomes	Scanty	Abundant
Metabolism:		
Net generation of ATP	–	–
ATP/ADP transport system	–	–
ATP-dependent liberation of CO_2 from glucose 6-P, pyruvate, glutamate, and aspartate	+[c]	+[c]
ATP-dependent host-free protein synthesis	–	+

[a]Symbols: +, >85% positive; –, 0–15% positive; nd, not determined.

[b]Adapted and modified from Table 9.13 in the chapter on the Genus *Chlamydia* in *Bergey's Manual of Systematic Bacteriology*, 1984, Vol. 1, p. 730.

[c]These experiments were conducted with mixtures of the two chlamydial cell types, so it is not certain whether only one or both types were responsible for the observed metabolic activity.

Further descriptive information

The voluminous *Chlamydia* literature has been exhaustively reviewed elsewhere. Some classical reviews, but mainly recent publications in books and articles of broad coverage on the subject treated in this chapter have been cited individually under Further Reading. Articles on the specific subjects discussed in this chapter are cited in the Bibliography Section in this book.

Cell morphology and developmental cycle. The structural forms of chlamydiae as well as their composition and biology are highly dependent on the developmental cycle. Therefore, the description of these characteristics can only be described in the context of the developmental cycle. The properties of the two principal cell forms are summarized in Table 160 and Figure 155. The developmental cycle may be divided into three phases: (1) attachment and entry of elementary bodies into host cells and their reorganization into reticulate bodies,

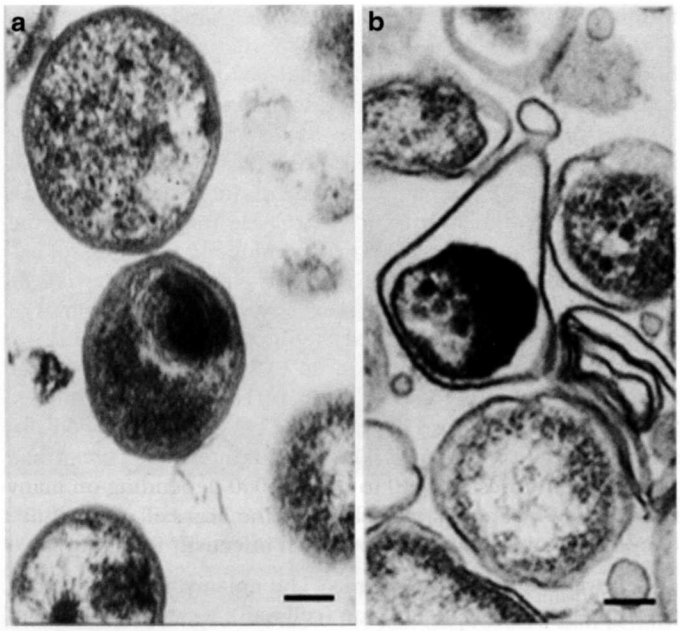

FIGURE 155. Elementary body form of chlamydiae is round, except for *Chlamydia pneumoniae* which is pear-shaped. (a) *Chlamydia trachomatis* serovar B, strain TW-5, (b). *Chlamydia pneumoniae* strain TW-183. The reticulate body is round for all species. Bars = 0.1 μm. (Reproduced with permission from Grayston et al., 1989. Int. J. Syst. Bacteriol. *39:* 88–90.)

(2) multiplication of reticulate bodies, and (3) conversion of a large fraction of the reticulate body population into a new generation of elementary bodies that are released from the host cells. The cycle is not synchronized, and each of the first two phases overlaps into the next succeeding one (Figure 156).

The initial stage of interaction between chlamydiae and host cells involves the electrostatic interaction of elementary bodies with host cells, which requires divalent cations (Kuo and Grayston, 1976). This interaction is enhanced by polycations and inhibited by polyanions (Kuo et al., 1973). The binding is further secured by other ligands, such as the hsp70 molecular chaperone (Raulston et al., 1993), the OmcB protein (Stephens et al., 2001; Ting et al., 1995), and the major outer-membrane protein (Su et al., 1990). Ligands, such as the high mannose oligosaccharide, may be involved (Kuo et al., 1996). These interactions lead to the entry of elementary bodies into host cells by microfilament independent and perhaps receptor-mediated endocytosis (Wyrick et al., 1989), although the mechanism remains uncharacterized. Candidate receptors, mannose receptor (Kuo et al., 2002), mannose 6-phosphate/insulin-like growth factor-2 receptor (Puolakkainen et al., 2005), and estrogen receptor (Davis et al., 2002), have been suggested, however, use of these receptors may vary in different cell types and species. Uptake of chlamydia appears to be an actin-mediated process that may be directed by *Chlamydia*-specific proteins (Clifton et al., 2005).

Ingestion of chlamydial elementary bodies requires expenditure of energy by the host but not by the parasite. Elementary bodies contain intrinsic ATPase activity. Disulfide bonds of the major outer-membrane protein of chlamydiae are reduced perhaps by the reducing agents of the host cell, such as glutathione,

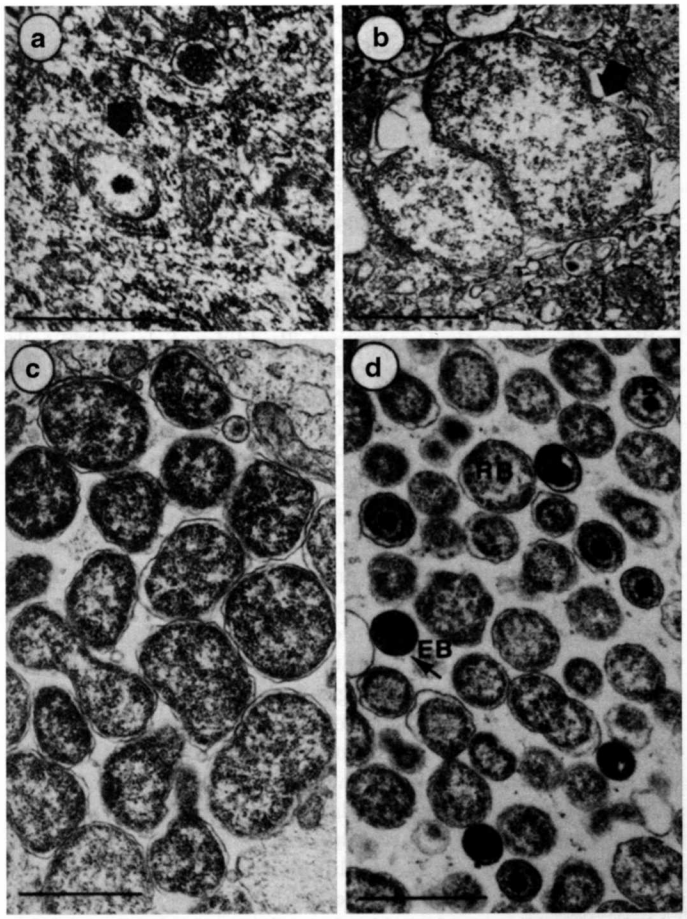

FIGURE 156. Transmission electron micrograph of thin sections of mouse fibroblasts (L cells) infected with *Chlamydia psittaci* strain meningopneumonitis. Bars = 1.0 μm. (a) At 2.5 h after infection. The arrow points to an elementary body that has just begun to differentiate into a reticulate body. (b) at 12 h after infection. The arrow points to a dividing reticulate body. (c) at 20 h after infection. The chlamydial population is almost entirely composed of dividing reticulate bodies. (d) at 30 h after infection. Some reticulate bodies are still dividing, but others have begun to reorganize into elementary bodies. EB, elementary body; RB, reticulate body; IB, intermediate body – a chlamydial cell intermediate in appearance between an elementary body and a reticulate body. (Reproduced with permission from Tribby et al., 1973. J. Infect. Dis. *127:* 155–163.)

to render the outer membrane permeable to host ATP which is then hydrolyzed by ATPase to generate energy for chlamydial metabolism immediately after entry into the host cells (Peeling et al., 1989). Ingested elementary bodies enter host cells inside phagosomes, which are cytoplasmic vacuoles surrounded by membranes derived from the plasma membranes of the host cell. Following ingestion of the phagocytized microorganisms and throughout the developmental cycle, some unidentified chlamydial activity(ies) prevents the fusion of lysosomes with phagosomes and destruction of the phagocytized organism. This fundamental property is seen only with live, but not with dead, elementary bodies. The *Chlamydia*-bearing phagosomes are transported rapidly (within minutes) to a perinuclear region within the host cell (Majeed and Kihlstrom, 1991). In a monkey model of subcutaneous autotransplantation of salpingeal

fimbrial tissues, this process happens within 10 min (Patton et al., 1998). Within the inclusion vacuole, the elementary body transforms quickly into the initial reticulate body which then grows and divides. The reticulate body is devoid of infectivity; its initially dense nucleoid is dispersed into more evenly distributed fibrillar DNA, ribosomes increase in number, and the cell wall becomes thinner, more flexible, and more fragile. Completion of the reorganization is signaled by division of the reticulate body, which is first seen 8–10 h after infection (Chi et al., 1987).

Multiplication of chlamydiae is by binary fission of reticulate bodies without apparent septation. Budding of daughter cells that are much smaller in size, contain no nucleoids, and are not infectious has been observed. Nuclear segregation is accomplished by separation of two low electron-dense zones filled with fine fibrillar material and indentation of walls at the plane of separation. From approximately 10–16 h after infection, a large fraction of the reticulate bodies is at some stage of division. The proportion of dividing forms decreases thereafter, but division occurs throughout the developmental cycle. Generally, within 24 h after infection, the inclusion contains only reticulate bodies (Figure 156), and the doubling time during exponential-phase growth is 2–3 h. Multiplication of reticulate bodies takes place within an expanding membrane-bound vacuole, or inclusion, that is an extension in time and space of the phagocytic vacuole in which the parent elementary body was brought into the host cell. Chlamydiae acquire endogenously synthesized macromolecules produced by the Golgi apparatus, including sphingolipids (Scidmore et al., 1996). Thus, the biogenesis of the inclusion membrane, including the acquisition of lipids, occurs by the intersection of the lipid distribution pathway. In addition, proteins produced by the reticulate body remodel the inclusion membrane (Rockey and Rosquist, 1994). By 12–15 h after infection, chlamydial inclusions are large enough to be seen by ordinary light microscopy of stained cells or by phase-contrast microscopy of live cells. Near the end of the developmental cycle, an inclusion may contain hundreds of chlamydial cells and fill nearly the entire cytoplasm of the host cell.

At 20–25 h after infection, host cell-associated infectivity begins to appear, and elementary bodies are seen again in electron micrographs (Figure 156). The beginning of the maturation process is signaled by the up-regulation of nuclear-binding protein genes that produce eukaryotic-like histone proteins that condense nuclear material into an electron-dense mass located at the center of the cytoplasm. These condense within reticulate bodies to form intermediate bodies (Hackstadt et al., 1991; Wagar and Stephens, 1988). Mature elementary bodies do not divide. Reticulate bodies continue to divide and reorganize into elementary bodies until the host cell can no longer support the multiplication of chlamydiae. Therefore, there is no clear-cut termination to the developmental cycle, although the end is usually considered to come 48–72 h after infection, at which time the maximum yields of infectious elementary bodies are obtained depending on the chlamydial strain. At this stage, all three developmental forms (reticulate, intermediate, and elementary bodies) coexist in an inclusion (Figure 156). The mechanism by which chlamydiae are released from host cells is poorly understood. Under cinematographic observation, the reticulate bodies are stationary and localized at the periphery of the inclusion, while the elementary bodies are in Brownian movement (Neeper et al., 1990). At the point of contact with the inclusion membrane the outer envelope of the reticulate body is rearranged into a rigid, well-developed trilaminar structure (Figure 157) (Peterson and de la Maza, 1988; Yang et al., 1994). Prior to the release of the inclusion contents, inclusions appear to rotate within the cell, extend and retract large blunt protrusions at the plasma membrane of the host cell which last 1–2 h, and eventually rupture to release the contents of the inclusion (Neeper et al., 1990). Two independent modes of cellular exit that occur with approximately equal frequency *in vitro* have been characterized. One is extrusion of the inclusion from the infected cell (Hybiske and Stephens, 2007; Neeper et al., 1990), and the second mechanism is lysis of the intracellular inclusion followed by lysis of the cell (Hybiske and Stephens, 2007). Terminal release of lysosomal enzymes may speed dissolution of moribund cells. The yield of infectious chlamydiae per host cell varies from 10 to nearly 1000 depending on many factors such as the chlamydial strain, the host cell, the culture conditions, and the method by which infectivity is measured.

Cell walls and fine structure. The chlamydial cell exhibits a double-layer outer membrane (cell wall), a cytoplasmic membrane that is separated from the outer membrane by a narrow periplasmic space (Figure 155), hexagonally arrayed subunits lining the inner surface, and spatially related patches of hexagonal projections on its outer surface (Figure 158) (Matsumoto, 1981, 1982). The hexagonally arranged projections are likely type III secretion system-like structures (Bavoil and Hsia, 1998; Hsia et al., 1997). It appears that these structures are also found in the reticulate body (Matsumoto, 1981). The morphology of chlamydial organisms is typically round except for the human biovar of *Chlamydia pneumoniae* which is pleiomorphic but typically pear-shaped and exhibits a large periplasmic space (Figure 155). *Chlamydia pneumoniae* with round morphology have been isolated, though these isolates also have a wider periplasmic space than the typical round elementary bodies

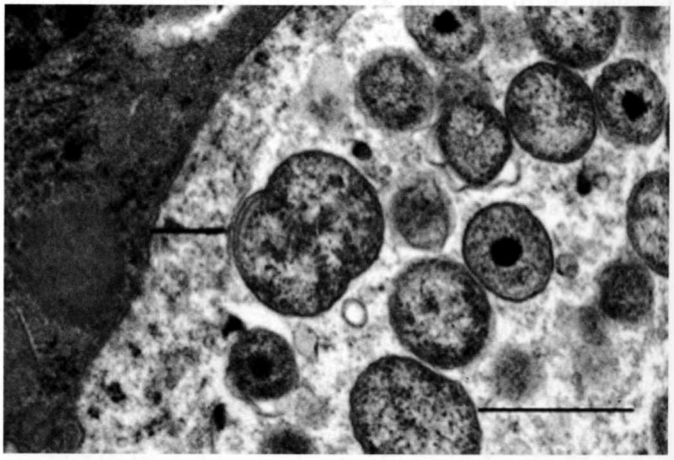

FIGURE 157. Transmission electron-micrograph of a portion of a *Chlamydia pneumoniae* inclusion in a ciliated bronchial epithelial cell in the mouse lungs demonstrating the outer envelope of reticulate body rearranged into a rigid well-developed trilaminar structure at the point of contact with the inclusion membrane (arrow). The reticulate body is undergoing division. Bar = 1.0 μm. (Reproduced with permission from Yang et al., 1994. J. Infect. Dis. *170:* 464–467.)

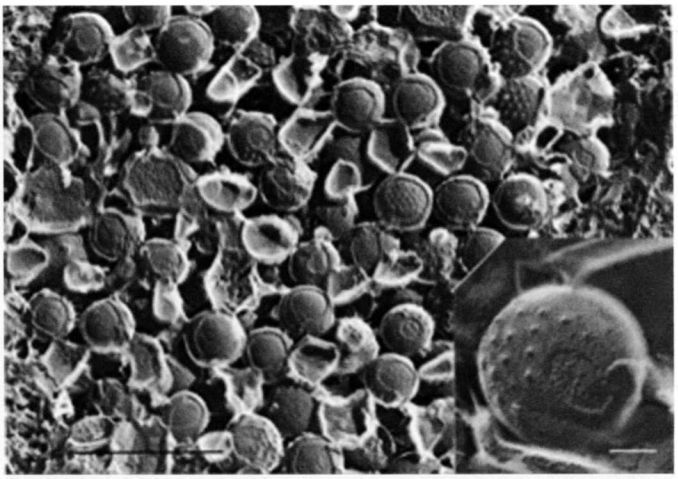

FIGURE 158. Hexagonally arrayed subunits lining the inner surface and spatially related patches of hexagonal projections on the outer surface revealed by freeze-etching of elementary bodies in *Chlamydia psittaci.* Bar = 1.0 μm. Inset: individual elementary body illustrating the arrangement of projections. Bar = 100 nm. (Reproduced with permission from Matsumoto, 1982. J. Bacteriol. *151:* 1040–1042.)

(Miyashita et al., 1997). One half of the protein content of the outer membrane is in the form of one protein with a molecular weight of approximately 40,000 (Caldwell et al., 1981) and having species and type-specific immunoreactivity (Kuo and Chi, 1987).

Nutrition and growth. Chlamydiae competitively acquire biosynthetic intermediates from the metabolic pools of their hosts, a process augmented by treatment of the host cell with cycloheximide or emetine (Alexander, 1968; Plaunt and Hatch, 1988; Reed et al., 1981). Chlamydiae have free access to some constituents of these pools but not to exogenous substrates for ATP synthesis. Therefore, it is essential that chlamydiae transport and use exogenous ATP for their own biosynthetic needs. Nutritional requirements of chlamydiae cannot be strictly defined because cell-free growth has not been achieved. Therefore, determinations of the nutritional requirements of chlamydiae have been performed in embryonated eggs or in cultured cells. The observed requirements for amino acids determined in cell culture (Allan and Pearce, 1983a, b; Kuo and Grayston, 1990a) indicate that the number of required amino acids varies among the species, the biovars within the species, and the cell types (HeLa or McCoy) used for determination (Table 161).

Metabolism. Investigations of the metabolism of host-free chlamydiae must be interpreted with two cautions in mind: (1) that the metabolic activities detected using host-free preparations are not due to contamination with host enzymes; and (2) that the correct cell type, i.e., the reticulate body or elementary body, is examined. Metabolic activity is traditionally analyzed at 20 h post-infection when the metabolic activity of chlamydiae is at its peak and the organisms are all in the reticulate body form, or at 48 h post-infection when there are mixed populations of developmental forms and the majority are elementary bodies. It has long been apparent that the reticulate body is the metabolically active cell type, while the elementary body is metabolically inactive. In recent years, the cloning of mutant cells deficient in specific enzymes of energy and nucleotide metabolic pathways has provided additional means for analyzing chlamydial metabolism (Iliffe-Lee and McClarty, 1999; Tjaden et al., 1999).

When separated from host cells, chlamydiae have few metabolic activities. They do not catabolize glucose, but they produce carbon dioxide from glucose 6-phosphate, glutamate, pyruvate, and aspartate in the presence of ATP, NADP, and other cofactors (Iliffe-Lee and McClarty, 1999). However, chlamydiae do not metabolize glutamate beyond succinate, and glutamate does not serve as an energy source.

Cytochromes, flavins, and reactions that result in a net gain of ATP have not been demonstrated. Because chlamydiae have appeared in the past to be unable to synthesize their own high energy compounds, they have been described as "energy parasites" (Hatch et al., 1982). Genomic analysis has, however, revealed that *Chlamydia* species encode enzymes of energy producing pathways and substrate-level phosphorylation, indicating that the energy burden of the chlamydiae is at least partially fulfilled by the *Chlamydia* themselves (Iliffe-Lee and McClarty, 1999).

Chlamydiae synthesize protein, lipid, and glycogen. Glycogen is produced and deposited within cytoplasmic inclusions of *Chlamydia trachomatis, Chlamydia muridarum,* and *Chlamydia suis,* but not in amounts detectable by iodine staining in inclusions of other chlamydial species (Gordon and Quan, 1965). Folic acid and its derivatives are synthesized by most strains of *Chlamydia trachomatis, Chlamydia muridarum,* and *Chlamydia suis* but not by other chlamydial species (Lin and Moulder, 1966). Chlamydiae also synthesize lipids such as phosphatidyl glycerol and branched-chain fatty acids that are characteristic of prokaryotes and are not found in their host cells (Reed et al., 1981).

Chlamydiae are auxotrophic for ATP, GTP, and UTP. CTP is obtained from the host cell or synthesized from UTP (Tipples and McClarty, 1993). Chlamydiae contain two structural genes encoding nucleotide transport proteins (Npt), $Npt1_{Ct}$ and $Npt2_{Ct}$, respectively, for the uptake of ribonucleoside triphosphate and transport of energy. $Npt1_{Ct}$ mediates uptake of ATP by chlamydiae and $Npt2_{Ct}$ mediates the exchange of ADP for ATP after ATP is catabolized to ADP by chlamydiae (Tjaden et al., 1999). Paradoxically, chlamydiae have the functional capacity to produce their own ATP and reducing power because they contain the required functional enzymes. These enzymes are pyruvate kinase, phosphoglycerate kinase, and glyceraldehde-3-phophate dehydrogenase of glycolysis and glucose-6-phosphate dehydrogenase of the pentose phosphate pathway. The existence of these enzymes has been demonstrated by complementation in *Eschericia coli* mutants deficient in these specific genes (Iliffe-Lee and McClarty, 1999).

Genetics. The DNA G+C content ranges from 39 to 45 mol% (T_m) (Table 162). The percentage DNA homology between the species and between the biovars within the species is presented in Table 162 (Cox et al., 1988; Fukushi and Hirai, 1992; Gerloff et al., 1970; Kingsbury and Weiss, 1968). Genome sequences for ten strains representing five chlamydial species have been reported with many more in progress. *Chlamydia* species have small genomes relative to most free-living organisms. The genome size varies from 1.03×10^6 bp for *Chlamydia trachomatis* to 1.23×10^6 bp for *Chlamydia pneumoniae* (Table 163). One of the remarkable outcomes from comparing genomes is how similar they are despite being separated

TABLE 161. Amino acid requirements of *Chlamydia* species determined in cell culture[a,b]

Amino acid	*C. trachomatis* Ocular	*C. trachomatis* Genital	*C. trachomatis* LGV	*C. abortus*	*C. caviae*	*C. felis*	*C. pneumoniae* (human)	Meningopneumonitis
Arginine	–	–	–	–	–	–	(d)	–
Cysteine	–	–	–	–	–	–	+	–
Glutamine	d	d	+	+	–	+	d	–
Histidine	+	+[c]	+	–	–	–	+	–
Isoleucine	–	–	–	–	–	–	(d)	–
Leucine	d	d	+	+	+	+	(+)	+
Lysine	–	–	–	–	–	–	–	–
Methionine	–	d	+	–	–	–	+	–
Phenylalanine	+	d	+	+	+	–	+	+
Threonine	–	–	–	–	–	–	(d)	–
Tryptophan	+	–	–	–	–	–	+	d
Tyrosine	–	–	–	+	–	–	+	–
Valine	+	+	+	+	+	+	(+)	+

[a]Symbols: +, >85% positive; d, different strains give different reactions (16–84% positive); –, 0–15% positive; ( ) indicates values from 90% amino acid depletion when 100% depletion of amino acid resulted in cell detachment.

[b]Determined in McCoy cells (*Chlamydia pneumoniae* in HeLa cells).

[c]All serovars except H.

TABLE 162. DNA G+C content and DNA relatedness based on DNA–DNA hybridization within and between species of the genus *Chlamydia*[a,b]

DNA from species	G+C (mol %)	Percent relatedness to DNA from: *C. trachomatis*	*C. psittaci*	*C. pneumoniae*
C. trachomatis:				
Trachoma	42–45	96–100	≤5	≤5
Genital	42–45	96–100	≤5	≤5
LGV	42–45	95–97	12	na
C. muridarum	42–44	63	na	na
C. psittaci	39–42	≤5	100	≤5
Meningopneumonitis[c]	39–43	11–12	93–96	na
C. pneumoniae	40	≤5–6	≤5–7	94–100
C. pecorum	39–42	1–10	na	na
C. abortus	na	6	85	na
C. caviae	na	≤5	32	≤5
C. felis	na	11	37	6–8

[a]Strains tested: trachoma and genital (serovars A–E); *Chlamydia psittaci* (6BC, parakeet, ornithosis, chicken); *Chlamydia pneumoniae* (TW183, AR39, AR458, LR65); Data for *Chlamydia suis* are not available.

[b]na, Data not available.

[c]Ferret, unclassified.

by a 100 million years of evolution. Gene content identity of any chlamydial genome to that for the prototypical *Chlamydia trachomatis* (D/UW-3/Cx) is greater than 90%. Given the similarity of the intracellular environment (niche) and the small genome size for all *Chlamydia* species, it could be expected that they would share a largely identical complement of genes. However, even more remarkable is the high level of conservation of the order of their genes, called genome synteny. Gene order is one of the least-conserved characteristics of bacteria as synteny is the first genomic property to be lost during evolution. This has not been the case for the *Chlamydiaceae*. For example, alignment of the genomes of *Chlamydia trachomatis* and *Chlamydia muridarum* results in precise conservation of gene order throughout the entire genome despite the fact that they are separated from a common ancestor over 50 million years ago as evidenced by sharing only 56% nucleotide identity between the genomes. Measurement of synteny between all available genomes reveals analogous outcomes (Table 163). The conclusion is that species of *Chlamydia* have been separated for a long geological time as reflected, for example, in 16S rRNA phylogenetic analyzes; nevertheless, they have changed very incrementally in the framework of biological evolution.

Mutants. Targeted mutant strains have not been generated due to the lack of a genetic tool for *Chlamydiaceae*. A nitrosoguanidine-generated, temperature-sensitive attenuated mutant of *Chlamydia abortus* has been isolated and is used as a live vaccine against abortion of ewes in Europe (Rodolakis, 1983).

Plasmids. Most *Chlamydia* species have a conserved 7500 bp plasmid (Joseph et al., 1986; Palmer and Falkow, 1986) that is not required for growth *in vitro* (Peterson et al., 1990). The plasmid has been detected in nearly all isolates of *Chlamydia trachomatis*, *Chlamydia psittaci* and in one strain of *Chlamydia pneumoniae* (Thomas et al., 1997). There are four plasmid copies per elementary body chromosome for *Chlamydia trachomatis* (Pickett et al., 2005). Clinical isolates representing the

TABLE 163. Characteristics differentiating species of the genus *Chlamydia*[a]

Characteristic	*C. trachomatis* genital biovar	*C. trachomatis* LGV biovar	*C. abortus*	*C. caviae*	*C. felis*	*C. muridarum*	*C. pecorum*	*C. pneumoniae*	*C. psittaci*	*C. suis*
Natural host	Human	Human	Ruminants, pig	Guinea pig	Cat	Rodent	Ruminants, pig, koala	Human, horse, koala	Bird	Pig
Site of infection	Eye, genital	Genital lymph node	Genital, respiratory	Eye, genital	Eye, respiratory	Respiratory?	Intestinal, urogenital, respiratory	Respiratory	Intestinal, respiratory	Genital, respiratory
16S rRNA difference (%)[b]	0	0	4.8	4.7	4.8	1.6	4.8	6.1	4.8	2.5
Genome size (bp)	1,042,519	1,038,841	1,144,377	1,173,390	nd	1,069,412	nd	1,230,230	nd	nd
Gene content identity[a]	100	nd	92.4	92.5	nd	96	nd	91.2	nd	nd
Genome synteny	100	nd	87.6	85.4	nd	87.4	nd	89.8	nd	nd
Plasmid	+	+	–	+	+	+	+	–	+	+
Bacteriophage	–	–	+	+	–	–	+	"+ (rare)"	+	–
pmp gene paralogs	9	9	18	17	nd	9	nd	21	nd	nd

[a]Symbols: +, >85% positive; –, 0–15% positive; nd, not determined.

[b]Compared to *Chlamydia trachomatis* (D/UW-3/Cx).

lymphogranuloma venereum (LGV) biovar and the genital biovar that lack the common plasmid have been characterized (Peterson et al., 1990; Stothard et al., 1998). This suggests that the plasmid is not essential for growth and persistence *in vivo* (i.e., pathogenesis). Plasmids from each species encode eight analogous proteins whose functions in the biology of *Chlamydia* are unknown, further mystifying the role of plasmid in chlamydial biology.

Phages. Several phages that infect *Chlamydia* species termed chlamydiaphages have been isolated and characterized. The first chlamydiaphage, named Chp1, was isolated from a *Chlamydia psittaci* outbreak in a duck farm in England in 1982 (Richmond et al., 1982; Storey et al., 1989a; Storey et al., 1989b). Chp1 forms large characteristic paracrystalline virion arrays that allow its identification by transmission electron microscopy. Several other chlamydiaphages were subsequently isolated from *Chlamydia caviae* (PhiCPG1) (Hsia et al., 2000), *Chlamydia pneumoniae* (PhiAR39) (Read et al., 2000), *Chlamydia abortus* (Chp2) (Liu et al., 2000), and *Chlamydia pecorum* (Chp3) (Garner et al., 2004). However, phages have not been isolated from *Chlamydia trachomatis* or *Chlamydia muridarum*. All chlamydiaphages isolated to date are members of the microviridae and are genetically and structurally related to phages of *Spiroplasma melliferum* and *Bdellovibrio bacteriovorus* and more distantly to the coliphage phiX174. The phage host range is specified by a peptidic loop of the major capsid protein, VP1 (Read et al., 2000), and sequence variation within the loop is consistent with the observed overlapping host ranges of the phages. For instance, PhiCPG1 and PhiAR39, which have identical VP1 sequences, are both able to infect *Chlamydia caviae* and *Chlamydia pneumoniae*. However, Chp2, whose VP1 sequence differs, may also infect *Chlamydia caviae* and other *Chlamydia* species (Everson et al., 2002), suggesting that distinct receptors to multiple phages exist at the surface of chlamydiae. Receptors for these phages have not been identified. The phage infectious cycle was characterized by transmission electron microscopy for phage PhiCPG1 infection of *Chlamydia caviae* (Hsia et al., 2000). Briefly, PhiCP1 virions gain access to intracellular chlamydiae by first attaching to and cointernalizing with extracellular elementary bodies. Once inside, the nascent inclusion virions infect the newly formed reticulate body. Abundant phage progeny are generated by rolling circle replication of the single-stranded DNA genome and packaging into phage procapsids. Phage-infected reticulate bodies appear as aberrantly enlarged cells similar to *in vitro* persistent forms of chlamydiae. Infectious phage progeny are released by phage-specified lysis of the inclusion membrane approximately 24 h post phage infection.

Antigenic structure. All chlamydiae share a similar antigenic framework consisting of common and specific antigenic determinants (Table 164). Monoclonal antibodies to the most important chlamydial antigens are commercially available. The *Chlamydiaceae* are characterized by a common lipopolysaccharide (LPS) antigen. This antigen is heat-stable and is the antigen for complement fixation tests. The common antigenic determinant is a LPS core terminating in a 2-keto-3-deoxyoctanoic acid (Brade et al., 1986). Antibodies to LPS react strongly to antigen present within inclusions and in the reticulate body membranes, however, reactivity to elementary bodies by immunofluorescence is weak. All chlamydiae encode a major 60,000 molecular weight heat-shock protein (HSP-60) family member

TABLE 164. Structure and function of the major immunoreactive antigens identified in *Chlamydia*[a]

Molecular weight	Name	Function
≥ 98,000	Pmp	Unknown. Six Pmp subtypes: A, B, C/D, E/F, G/I, and H
75,000	HSP	Chaperone HSP-70 family
60,000	OmcB	Structural, cysteine-rich OMP
60,000	HSP	Chaperone HSP-60 family
40,000	OmpA (MOMP)	Outer-membrane porin. Contains serological determinants of species-, subspecies-, and serovar-specificity
40,000	PorB	Outer-membrane porin
12,500	OmcA	Structural lipoprotein, cysteine-rich OMP
~12,000	LPS	Endotoxin, genus antigen

[a]Abbreviations: Pmp, polymorphic membrane protein; HSP, heat shock protein; Crp, cysteine-rich protein (cross linking of disulfide bonding of cysteine-rich proteins give rigidity to the outer membrane of the EB); Omc, outer membrane complex protein; MOMP, major outer membrane protein; LPS, lipopolysaccharide.

(Bavoil et al., 1990) that is associated with seroreactivity in individuals at risk for pathogenic sequelae of infection (Wagar et al., 1990). This should not be confused with another 60,000 molecular weight protein (OmcB) that is cysteine-rich and present in elementary-body outer membranes but not reticulate-body outer membranes (Batteiger et al., 1985; Hatch et al., 1986). This protein is a potent immunogen and useful marker for serological studies of chlamydial infection.

A major antigen is the major outer-membrane protein (OmpA or MOMP) that is the quantitatively predominant protein in *Chlamydia trachomatis* (Caldwell et al., 1981) and other *Chlamydia* species (Campbell et al., 1990). This protein is an outer membrane porin (Bavoil et al., 1984) and is antigenically complex (Stephens et al., 1982). For *Chlamydia trachomatis* and *Chlamydia suis*, four variable sequence (VS) loops are exposed on the surface and accessible to binding by antibodies (Stephens et al., 1988). *Chlamydia trachomatis* serovar-specific antigens have been mapped and are associated with vs1 and vs2; more broadly reactive determinants, including a species-specific antigen, are localized with vs4. For *Chlamydia trachomatis* (Fan and Stephens, 1997) and *Chlamydia pneumoniae* (Wolf et al., 2001), some of the OmpA antigenic determinants are conformation dependent. Sequence based antigenic variations of this protein singularly account for the antigenic specificities originally characterized by the micro-immunofluorescence test in which 15 prototype serovars were defined (Figure 159) (Wang and Grayston, 1970). Serovars A through K, including Ba, are associated with ocular and urogenital tract infection and serovars L_1, L_2, and L_3 are serovars of the LGV biovar. Antigenic variation is thought to derive from selection by the host immune response to infection (Stephens, 1989). While the other *Chlamydia* species genomes each have *ompA*, only *Chlamydia trachomatis*, *Chlamydia pecorum*, and *Chlamydia suis* have alleles of *ompA* that significantly differ among isolates. A new porin, PorB, was discovered following genome sequencing (Kubo and Stephens, 2000). Like OmpA, PorB is surface-exposed but, unlike OmpA, PorB is not quantitatively predominant and does not display significant sequence variation among isolates within a species.

An entire multigene family of antigens [polymorphic membrane proteins (Pmps)] was also discovered from genome

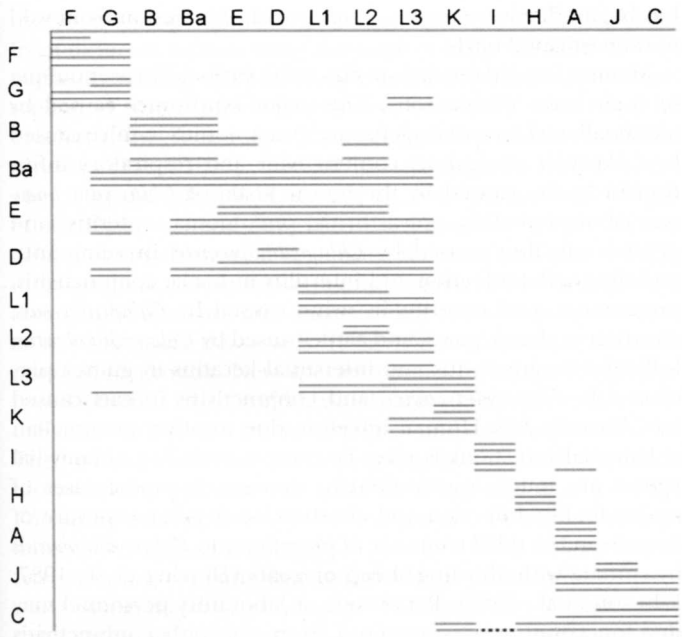

FIGURE 159. Microimmunofluorescence pattern of *Chlamydia trachomatis* antisera prepared in mice. A difference of one line expresses a twofold difference from the homologous titer which is shown with five lines. (Reproduced with permission from Wang et al., 1973. Infect. Immun. 7: 356–360; adapted from the 1984 edition of the *Bergey's Manual of Systematic Bacteriology*, vol. 1; Chapter Genus I *Chlamydia*, Figure 9.14.)

sequencing to include nine representatives in *Chlamydia trachomatis* (Stephens et al., 1998) and 21 representatives in *Chlamydia pneumoniae* (Kalman et al., 1999) and intermediate numbers of paralogs for other species. The protein sequences are very different, but they are unambiguously related (Grimwood et al., 2001). The functions of these proteins are unknown, but some have been shown to be surface-exposed outer-membrane proteins, and their expression is dependent upon phase variation.

Antibiotic sensitivity. The susceptibility of chlamydiae to antibiotics has been tested in yolk sac of chicken embryos (Gordon et al., 1957; Molder et al., 1955) or cell culture (Kuo and Grayston, 1988; Kuo et al., 1977b). The growth of chlamydiae in culture is inhibited by the tetracyclines, macrolides, azalides, chloramphenicol, rifampin, and fluoroquinolones. Chlamydial multiplication is not blocked by aminoglycosides, bacitracin, or vancomycin. *Chlamydia trachomatis*, *Chlamydia muridarum*, and *Chlamydia suis* are sensitive to sulfonamides, whereas other species, except the 6BC strain of *Chlamydia psittaci*, are resistant. Beta-lactam antibiotics (penicillin), which inhibit the synthesis of peptidoglycan, interrupt the developmental cycle by preventing the maturation of reticulate bodies into elementary bodies. However, if these antibiotics are removed, development proceeds normally. The inhibitory effect of penicillin on chlamydiae which contain no or only trace amounts of muramic acid has been attributed to the presence of penicillin-binding proteins in their outer membrane (Barbour et al., 1982). D-Cycloserine acts similarly to penicillin, but *Chlamydia trachomatis* is much more susceptible to growth inhibition by this antimicrobial than is *Chlamydia psittaci*. Antagonism is not observed among sulfa drugs, tetracycline, erythromycin, chloramphenicol, penicillin, and ciprofloxacine. An additive inhibition is observed between tetracycline and penicillin, tetracycline and erythromycin, tetracycline and chloramphenicol, erythromycin and penicillin, erythromycin and chloramphenicol (How et al., 1985) and azalides and rifampin derivatives (Kuo, C.C., unpublished data). Synergistic activity is observed between trimethoprim and sulfamethoxazole (How et al., 1985). The drugs of choice for treatment of chlamydial infections are tetracyclines, macrolide, and azalides. Long-acting antibiotics (doxycycline) and those antibiotics that achieve high intracellular concentrations (azithromycin) are preferred.

Chlamydial strains resistant to sulfonamides, penicillin, chlorotetracycline, and rifampin have been produced by *in vitro* passage in the presence of these drugs. However, stable drug-resistant mutants of *Chlamydia trachomatis* or *Chlamydia pneumoniae* have not been isolated. *Chlamydia suis* in American pigs is resistant to tetracycline by virtue of the integration in its genome of a Tet(R) transposon-like genetic element from another bacterium (Dugan et al., 2004).

Pathogenesis. Chlamydiae are among the most widely distributed pathogens of humans and animals and they produce variable clinical syndromes. Humans are the natural hosts of *Chlamydia trachomatis* (Grayston and Wang, 1975) and the human biovar of *Chlamydia pneumoniae* (Kuo et al., 1986). *Chlamydia trachomatis* causes ocular infection, which may lead to blindness (trachoma), a disease still found in tropical and subtropical endemic areas (Grayston et al., 1985), and it causes genitourinary tract infections, which may cause tubal factor infertility (fallopian tube obstruction), which is a serious complication of chronic salpingitis (Grayston and Wang, 1975) (Table 165). The human biovar of *Chlamydia pneumoniae* is a common respiratory pathogen with manifestations of pharyngitis, bronchitis, and pneumonia (Grayston et al., 1989) and is associated with atherosclerosis and coronary heart disease (Kuo et al., 1993) (Table 165). The organism has been isolated from the atherosclerotic lesions of the artery. A unique characteristic of human infection with these chlamydial agents is their ability to establish persistent or chronic infection, as exemplified by the difficulty in reisolating the organism from lesion sites, such as from the occluded fallopian tube in *Chlamydia trachomatis*

TABLE 165. Diseases caused by human chlamydial agents

Species	Biovar	Disease or syndrome
Chlamydia trachomatis	Ocular[a]	Inclusion conjunctivitis of newborns, children, and adults; trachoma and blindness
	Genital	Infantile pneumonia; urethritis in men; epididymitis; urethral syndrome in women; cervicitis; endometritis; salpingitis, tubal factor infertility; proctitis; perihepatitis and peritonitis (Fitz–Hugh–Curtis syndrome)?; bartholinitis?; endocarditis?
	LGV	Lymphogranuloma venereum
Chlamydia pneumoniae	Human	Pharyngitis; bronchitis; pneumonia; sinusitis?; otitis media?; atherosclerosis (a co-risk factor with hyperlipidemia for atherosclerosis)?

[a]Trachoma biovar in old nomenclature

salpingitis (Campbell et al., 1993) and from the lungs and atherosclerotic arteries in *Chlamydia pneumoniae* infections (Kuo et al., 1993). However, the presence of organisms (chlamydial DNA, RNA, and antigens) can still be detected by PCR, RT-PCR, or immunohistochemistry. This observation has been attributed to the arrest of the developmental cycle at the reticulate body stage by depletion of intracellular tryptophan or other required amino acids (Allan and Pearce, 1983b, a). Tryptophan may become limiting as a result of gamma-interferon produced in the context of the host's innate immune response (Beatty et al., 1994). Gamma-interferon induces indoleamine 2,3 dioxygenase (IDO) that catabolizes oxidative decyclization of tryptophan (Kane et al., 1999).

Chlamydia psittaci causes systemic infection in birds and the infection is often asymptomatic (Table 166). *Chlamydia psittaci* is highly infectious to humans. Humans may contract *Chlamydia psittaci* infection by the airborne route, either by direct contact with infected birds or indirectly by inhalation of dust contaminated with excreta of infected birds. The major manifestation of *Chlamydia psittaci* infection in humans is atypical pneumonia, and the infection in humans is often systemic. Psittacosis is a term used to describe the infections acquired from a psittacine bird, and ornithosis is the infection contracted from birds other than psittacine. *Chlamydia psittaci* is widespread in wild and domestic birds including parrots, parakeets, pigeons, ducks, and turkeys. Outbreaks of *Chlamydia psittaci* infection may occur in poultry, and transmissions of infection to poultry farmers occur sporadically. Quarantine of trapped wild birds in combination with preventive antibiotic (tetracycline) treatment has been effective in controlling spread of infection from wild to domesticated birds.

Mammalian chlamydial species cause various diseases unique to their hosts (Table 166). The major syndromes caused by mammalian chlamydial agents are: pneumonitis in mice caused by *Chlamydia muridarum*; conjunctivitis and respiratory infection in koalas caused by the biovar koala of *Chlamydia pneumoniae*; encephalitis, polyarthritis, pneumonia, enteritis, and genital infection caused by *Chlamydia pecorum* in ruminants, and urogenital infection and infertility in koala; conjunctivitis, pneumonia, and enteritis in swine caused by *Chlamydia suis*; abortion in sheep, goats, and cattle caused by *Chlamydia abortus*; follicular conjunctivitis and interstitial keratitis in guinea pigs caused by *Chlamydia caviae*; and conjunctivitis in cats caused by *Chlamydia felis*. Human infection due to other mammalian chlamydial infections is rare, because mammalian chlamydial agents are much less virulent to humans. Sporadic cases of severe flu-like infection and abortion occur after exposure of farmers in the third trimester of pregnancy to *Chlamydia abortus* by contact with aborting sheep or goats (Herring et al., 1987; Johnson et al., 1985). Pet owners or laboratory personnel may develop conjunctivitis acquired from cats with conjunctivitis due to *Chlamydia felis* (Schachter et al., 1969).

Phylogenetic relationships based on 16S rRNA. Several investigators have evaluated the relationships among many of the *Chlamydia* and *Chlamydia*-related organisms by tree-methodologies using 16S rRNA (Corsaro et al., 2002; Everett et al., 1999; Petersson et al., 1997; Pudjiatmoko et al., 1997; Weisburg et al., 1986). Complete 16S rRNA sequences of most of

TABLE 166. Disease caused by avian and mammalian chlamydial agents

Species	Host	Disease
Chlamydia abortus	Sheep, goats, cattle, swine	Abortion, vaginitis, endometritis, seminal vesiculitis, mastitis; often latent infection
Chlamydia caviae	Guinea pigs	Follicular conjunctivitis, interstitial keratitis
Chlamydia felis	Cats	Conjunctivitis with or without rhinitis
Chlamydia muridarum	Mice, hamsters	Pneumonitis; often latent ileitis
Chlamydia pecorum	Sheep, cattle, swine, koala	Encephalomyelitis, polyarthritis, pneumonia, enteritis, vaginitis, endometritis in sheep and cattle; polyarthritis, serocitis, enteritis, pneumonia in swine; keratoconjunctitis, vaginitis, ovarian cyst, infertility in koala; often latent infection
Chlamydia pneumoniae:		
Biovar koala	Koala	Rhinitis, pneumonia
Biovar equine	Horse	Rhinitis[a]
Chlamydia psittaci	Birds	Systemic, often latent respiratory and enteric infection
Chlamydia suis	Swine	Conjunctivitis, pneumonia, enteritis, polyarthritis
Chlamydia abortus	Sheep, goats, cattle, swine	Abortion, vaginitis, endometritis, seminal vesiculitis, mastitis; often latent infection
Chlamydia caviae	Guinea pigs	Follicular conjunctivitis, interstitial keratitis
Chlamydia felis	Cats	Conjunctivitis with or without rhinitis
Chlamydia muridarum	Mice, hamsters	Pneumonitis; often latent ileitis
Chlamydia pecorum	Sheep, cattle, swine, koala	Encephalomyelitis, polyarthritis, pneumonia, enteritis, vaginitis, endometritis in sheep and cattle; polyarthritis, serocitis, enteritis, pneumonia in swine; keratoconjunctitis, vaginitis, ovarian cyst, infertility in koala; often latent infection
Chlamydia pneumoniae:		
Biovar koala	Koala	Rhinitis, pneumonia
Biovar equine	Horse	Rhinitis[a]
Chlamydia psittaci	Birds	Systemic, often latent respiratory and enteric infection
Chlamydia suis	Swine	Conjunctivitis, pneumonia, enteritis, polyarthritis

[a]Only one isolate from a horse with serous nasal discharge has been reported.

the prototypical strains are available, and the resulting trees from different reports using the same or different methods are markedly similar. All *Chlamydia*-related organisms are deeply separated from other bacteria and form their own bacterial division as they lack a significant relationship to other bacterial divisions (Pace, 1997). Distantly related organisms such as the *Piscichlamydia* (Draghi et al., 2004), *Protochlamydia* (Collingro et al., 2005a, b), and *Parachlamydia* (Amann et al., 1997) are distinct from chlamydial organisms that establish the strongly monophyletic *Chlamydiaceae* family (Figure 160). Three groups diverged from a common ancestor that contain species related to the *Chlamydia trachomatis*, *Chlamydia psittaci*, and *Chlamydia pneumoniae* groups. New species names have also been proposed (Everett et al., 1999), which are often representative of the relationship with the natural host and are adopted herein. The *Chlamydia trachomatis* grouping contains the murine biovar mouse pneumonitis (MoPn) (Nigg and Eaton, 1944) *Chlamydia muridarum* and related organisms isolated from pigs named *Chlamydia suis* (Hoelzle et al., 2000). The *Chlamydia psittaci* grouping contains strains from birds (*Chlamydia psittaci*), cats (*Chlamydia felis*), guinea pigs (*Chlamydia caviae*), and ruminants and pigs (*Chlamydia abortus*). The third group contains *Chlamydia pneumoniae* (Grayston et al., 1989), originally isolated from humans, and *Chlamydia pecorum* isolated from cattle (Fukushi and Hirai, 1992).

Analyzes of a variety of other single components such as protein and 23S rRNA sequences support these relationships. The application of simple DNA amplification procedures for the detection of novel 16S or 23S sequences related to *Chlamydia* has resulted in the detection of *Chlamydia*-like organisms

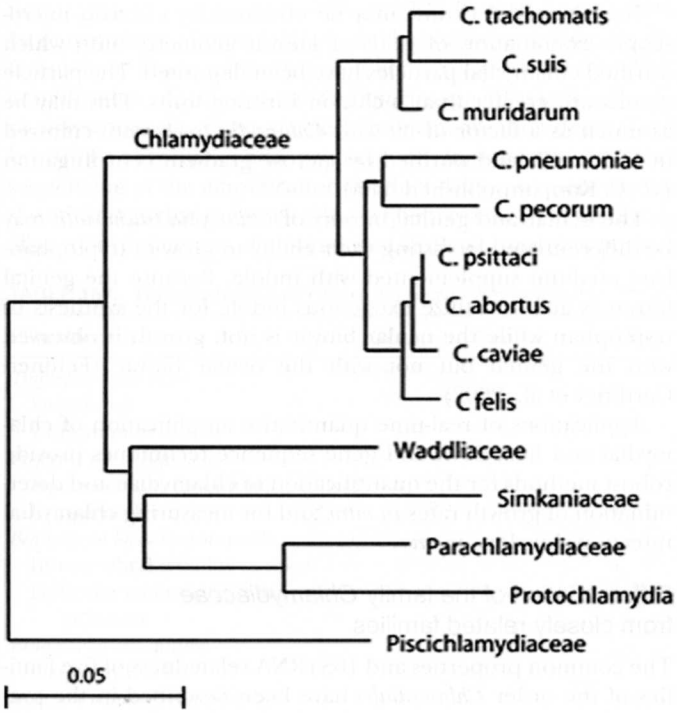

FIGURE 160. 16S rRNA gene phylogeny of *Chlamydiaceae* and *Chlamydiaceae*-related organisms using neighbor joining with Jukes–Cantor distance corrections. Bar = 0.05 substitutions/site.

from many animals, fish, reptiles, and environmental samples. These activities will likely continue, but many do not result in full-length gene sequences and are not supported by microbiological isolation.

Enrichment and isolation procedures

Laboratory handling of *Chlamydia*-containing specimens and growth of chlamydiae should be conducted by employing biosafety level 2 (BSL-2) or higher containment facilities and practice (Chosewood and Wilson 2007). Strains from all chlamydial species grow in the yolk sacs of chicken embryos. All chlamydial strains multiply in a variety of cell cultures with the assistance of infection-promoting procedures such as pretreatment of host cells with polycations (Kuo et al., 1972b), centrifugation of the inoculum onto host cell monolayers (Gordon et al., 1969), and inhibition of the synthesis of host macromolecules with cycloheximide (Ripa and Mardh, 1977). The efficiency of growth in cell culture varies among the species and the biovars within the species. For example, *Chlamydia psittaci* grows more efficiently than other mammalian chlamydial species in both chicken embryos and cell cultures, while the LGV biovar multiplies more readily than the trachoma and genital biovars of *Chlamydia trachomatis* in both systems. Chlamydiae also infect and are lethal to mice following intracerebral inoculation. This test of virulence varies among chlamydial strains and is similar to their behavior in chicken embryos and cell cultures. *Chlamydia psittaci* strains are particularly virulent to humans. The risk is so great that attempts to isolate *Chlamydia psittaci* from birds should be made only by experienced workers in specially equipped laboratories.

Collection and processing of specimens. Collection of an appropriate specimen for isolation of chlamydiae depends on the disease, the host, and the chlamydial agent. Clinical samples, especially swab samples from the bodily openings and intestinal tissues, are contaminated with other bacteria and yeasts, therefore antibacterial and antifungal agents that are not inhibitory to chlamydiae are added to the transport medium. Frequently used isolation mixtures contain gentamicin, or streptomycin and vancomycin, and amphotericin B. Clinical samples are usually suspended in phosphate buffer containing sucrose and glutamate or cell culture medium, supplemented with suitable antimicrobial agents. A suitable medium contains 0.2 M sucrose in phosphate buffer and 10 μg/ml gentamicin and 3.75 μg/ml amphotericin B. Intracellular chlamydiae are released by shaking with glass beads or by sonication. Specimens may be kept at 4°C if inoculated within 24 h. If they must be held for longer times, they should be frozen at −75°C or lower.

Isolation in cell culture. The cell lines most commonly used for isolation of chlamydiae are McCoy cells (a heterodiploid mouse line) (Gordon et al., 1969) and HeLa 229 cells (derived from a human cervical carcinoma) (Kuo et al., 1977a; Kuo et al., 1972b). Other cell lines are used for certain species. For example, HL (human line) (Kuo and Grayston, 1990b) and Hep-2 (human nasopharyngeal carcinoma) (Roblin et al., 1992) cells for *Chlamydia pneumoniae* and L-929 (mouse fibroblast) or BGMK (buffalo green monkey kidney) cells for *Chlamydia psittaci*, *Chlamydia pecorum*, *Chlamydia felis*, and *Chlamydia suis*. Enhancement of infection is achieved by pretreating cell monolayers with 20 μg/ml di-ethyl-amino-ethyl (DEAE)-dextran,

centrifuging the inoculum onto the monolayer at 1000–3000× g for 1 h at 20–35°C, and incubating the inoculated cells with medium containing 0.5–1.0 μg/ml cycloheximide. After 2 or 3 d of incubation at 35 or 37°C, the presence of chlamydial inclusions is determined by fluorescent antibody stain of the cell monolayer.

Isolation in chicken embryos. Six-to-seven-day-old fertile hen eggs from a flock maintained on antibiotic-free feed are candled for viability, inoculated into their yolk sacs and incubated at 35–37°C for 14 d (T'ang et al., 1957). On the third day of incubation, eggs containing dead embryos are discarded. The eggs are candled every day thereafter. All dead embryos are examined for the presence of chlamydiae by staining impress smears of the yolk sac with Gimenez or modified Macchiavello. Negative primary passage may be passed again blind. Criteria for positive isolation are the presence of elementary bodies and serially transmissible embryo mortality in the absence of contaminating bacteria or viruses. For isolation of human strains of chlamydiae, the yolk sac is less sensitive than cell culture.

Isolation in mice. Most chlamydial strains tested were found to infect mice after intranasal instillation. BALB/c or A/J inbred mice are preferred for isolation. Under light metofane or isoflurane anesthesia, 20–40 μl of the inoculum is deposited on the nostrils of 3 week-old mice that readily inhale the inoculum due to anesthesia-induced hyperventilation. This method has been used for isolation of chlamydiae from avian hosts and of virulent strains from other hosts, but is less sensitive than cell culture for isolation of low-virulence strains. Within one week after inoculation, mice develop typical lung lesions consisting of grayish foci of consolidation, sometimes involving a major portion of the lung. For verification of the infection, mice are sacrificed after 1 week, and impression smears from lesions are stained with Gimenez or FITC-labeled monoclonal antibody against chlamydial LPS, or lung tissue is examined by PCR. Standard bacteriological culture is used to confirm the absence of contaminating bacteria, and cell culture is used for exclusion of viral infections. In negative isolation attempts, subculture in BGMK cells is preferred over blind passages.

Maintenance procedures

Chlamydiae are maintained and propagated in chicken embryos and cell cultures by the procedure just described. Chlamydiae are best preserved by freezing at −75°C or lower in buffers containing sucrose and glutamic acid, such as SPG (sucrose, 75 g; KH_2PO_4, 0.52 g; Na_2HPO_4, 1.22 g; glutamic acid, 0.72 g; H_2O to 1 l; pH 7.4–7.6).

Chlamydiae can be propagated to a high titer by serial passages in chicken embryo yolk sac cultures or cell monolayer cultures in a large flask. For example, it is possible to achieve 100% infection of HeLa 229 cells by serial passage without the assistance of centrifugation or inhibitors of host-cell macromolecular synthesis with *Chlamydia trachomatis*, *Chlamydia psittaci*, and *Chlamydia muridarum* (Kuo et al., 1977a) and of HL (human line) (Kuo and Grayston, 1990b) and Hep-2 (Roblin et al., 1992) cells with *Chlamydia pneumoniae*. After amplification of chlamydial titer to grow to 100% infectivity in stationary cell cultures, the LGV biovar of *Chlamydia trachomatis* and *Chlamydia psittaci* can be propagated in a suspension culture of L-cells. Alternatively, BGMK monolayer cells can be used for effective propagation of chlamydiae, and elementary bodies released into the culture medium can be concentrated into stocks of high purity and infectivity (Li et al., 2005).

Procedures for testing special characters

Several methods are used for quantification of infectivity titer and enumeration of bacterial particles. For example, *Chlamydia psittaci*, the LGV biovar *Chlamydia trachomatis*, and *Chlamydia muridarum* readily form plaques on monolayers of L-cells (a heterodiploid mouse line) after extended incubation. Ocular and genital biovar strains of *Chlamydia trachomatis* can also be coaxed to form plaques (Matsumoto et al., 1998). The infectivity titer is expressed as plaque forming units. A simple method is to determine the inclusion-forming units by infecting a monolayer by centrifugation and culturing infected cells with medium containing inhibitors of host macromolecular synthesis, incubating for 48–72 h, and staining with Giemsa, iodine, or fluorescent antibodies and counting the inclusions. The number of chlamydial inclusions in each of a number of microscopic fields is counted. The inclusion counts are standardized, and the titer is expressed in term of inclusion forming units per milliliter (Furness et al., 1960). The burst size is defined in term of the number of infectious elementary bodies produced from a single inclusion. for example, the burst size of *Chlamydia pneumoniae* in HL cells is 73 with the assistance of centrifugation and inhibition of host cell macromolecular synthesis by cycloheximide. The m.o.i. is the number of infectious organisms inoculated per cell in a monolayer culture. Chlamydial infectivity in cell culture, chicken embryos, and mice may be expressed in term of the 50% infectious dose or the 50% lethal dose. Infectivity for chicken embryos inoculated via the yolk sac may also be estimated from the nearly linear relation between the logarithm of the inoculum dilution and the survival time of the inoculated embryos.

Total bacterial counts may be obtained by electron microscopic examination of grids of known geometry onto which purified chlamydial particles have been deposited. The particle counts are greater than inclusion forming units. This may be as much as a factor of 30 with *Chlamydia trachomatis* cultured in HeLa cells and purified by sucrose gradient centrifugation (C.- C. Kuo, unpublished data).

The ocular and genital biovars of *Chlamydia trachomatis* may be differentiated by testing their ability to grow in tryptophan-free medium supplemented with indole. Because the genital biovar is able to utilize exogenous indole for the synthesis of tryptophan while the ocular biovar is not, growth is observed with the genital but not with the ocular biovar (Fehlner-Gardiner et al., 2002).

Applications of real-time quantitative amplification of chlamydial and host DNA and gene sequence techniques provide robust methods for the quantification of chlamydiae and determination of growth rates *in vitro*, and for measuring chlamydial infectious burdens *in vivo*.

Differentiation of the family *Chlamydiaceae* from closely related families

The common properties and 16S rRNA relatedness of five families of the order *Chlamydiales* have been described in the preceding section under the Order *Chlamydiales*. Therefore, only some other biological and pathogenic characteristics useful for differentiation of the family *Chlamydiaceae* from the families *Parachlamydiaceae*, *Piscichlamydiaceae*, *Waddliaceae*, and *Simkaniaceae* are presented below.

The natural hosts for *Parachlamydiae* are free-living amoebae, although they have been recovered from soil (Collingro et al., 2005a, b); for *Piscichlamydia*, Atlantic salmon (Draghi et al., 2004); for *Waddlia*, bovines (Dilbeck et al., 1990); and for *Simkania*, soil (Kahane et al., 1995). Isolations of *Simkaniaceae* from humans with respiratory infection have been reported. However, the pathogenesis of *Parachlamydia* in humans has not been described. These intracellular microorganisms form inclusions and exhibit similar developmental cycles. However, the developmental forms vary in morphology. Specifically, *Piscichlamydia* forms variable elongated reticulate bodies with distinctive head and tail cells and the intermediate form which does not evolve to the elementary form (Draghi et al., 2004). Antigenically, cross-reactivity to chlamydial LPS has been demonstrated with *Piscichlamydia*, but not with *Parachlamydia*.

Differentiation of species, biovars, and serovars of the genus *Chlamydia*

Nine species of Chlamydia are currently recognized including *Chlamydia trachomatis*, *Chlamydia muridarum*, *Chlamydia pecorum*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Chlamydia suis*, *Chlamydia abortus*, *Chlamydia caviae*, and *Chlamydia felis*. *Chlamydia trachomatis* is further subdivided into three biovars (Table 167) and *Chlamydia pneumoniae* into three biovars (Table 168). In the following sections, if the chlamydial property being described is not common to all members of the genus, the species or biovars to which the description applies will be stated.

Characteristics useful for differentiation of the species of *Chlamydia* are listed in Tables 161, 162, and 163. Nucleic acid-based techniques have emerged as technically easier for use in species differentiation than direct determination of biological differences. Initially, whole-genome hybridization and restriction fragment length polymorphism (RFLP) methods required large quantities of pure DNA of all chlamydial species to be differentiated. These methods have been largely replaced by PCR amplification of genes containing species-specific nucleotide signature sequences. These species signatures are then determined by RFLP, oligonucleotide probing, or, increasingly, by DNA sequencing of the amplification product. Direct sequencing has the advantage that the nucleotide sequences can be immediately aligned to sequences deposited in GenBank, and comparison with amplification products from standards of the chlamydial species is unnecessary. The availability in the near future of genome sequences of all chlamydial species, even for multiple strains of *Chlamydia trachomatis* and *Chlamydia pneumoniae*, allows the use of any gene with species signatures. Nevertheless, it will be preferable to rely for routine differentiation on the *ompA* and rRNA genes. The *ompA* gene contains highly polymorphic serovar determinants interspersed among conserved domains that allow genus-specific priming and species as well as serovar differentiation (Kaltenboeck et al., 1993, 1997). The 16S and 23S rRNA genes (but not the intergenic rRNA gene spacer sequence) are less polymorphic but allow design of primers with higher GC content than *ompA* primers, and thus more robust and sensitive PCR methods for direct amplification and species differentiation in disease specimens (DeGraves et al., 2003).

TABLE 167. Differentiation of *Chlamydia trachomatis* biovars[a]

Characteristic	Ocular	Genital	LGV
Tryptophan synthase:			
Functional	–	+	+
Utilization of indole	–	+	+
Preferred site of infection:			
Squamocolumnar epithelial cells	+	+	–
Lymph nodes	–	–	+
Behavior in laboratory animals:			
Intracerebral lethality in mice	–	–	+
Follicular conjunctivitis in primates	+	+	–
Behavior in cell culture:			
Plaque in L cells	–	–	+
Infection markedly enhanced by:			
Centrifugation onto cell monolayer	+	+	–
Treatment of host cells with diethyl- aminoethyl-dextran	+	+	–

[a]Symbols: +, >85% positive; –, 0–15% positive.

TABLE 168. Differentiation of *Chlamydia pneumoniae* biovars

Human	Koala	Equine
Pharyngitis	Rhinitis	Serous nasal discharge
Bronchitis	Pneumonia	
Pneumonia		
One strain	na[a]	Only one isolate[b]
No plasmid in EB	na	Plasmid in EB
EB pear-shape[c]	na	EB oval
Large periplasmic space	na	Tight periplasmic space

[a]na, No information is available.

[b]No second isolate to confirm.

[c]Isolates with oval elementary bodies have been reported for the human biovar. However, unlike the elementary body of other chlamydial species which demonstrates a tight periplasmic space, these isolates still manifest a discernible periplasmic space, although less wide than that described originally for pear-shaped elementary bodies of *Chlamydia pneumoniae*.

Differentiation of the three biovars of *Chlamydia trachomatis* is listed in Table 167. Differentiation of the three biovars of *Chlamydia pneumoniae* is provided in Table 168.

Only a single serovar or serotype has been identified by the micro-immunofluorescence test in all chlamydial species, except for *Chlamydia trachomatis* which has 15 serovars, and for *Chlamydia psittaci* which has 6 serovars. Cross-reaction patterns of the micro-immunofluorescence antibody reaction in *Chlamydia trachomatis* are depicted in Figure 159 (Wang et al., 1973). No cross-reactivities are observed when *Chlamydia psittaci* serovar-specific monoclonal antibodies are used in the micro-immunofluorescence test for differentiation of *Chlamydia psittaci* serovars (Andersen, 1991).

Taxonomic considerations

The *Chlamydiales* represent a large clade of the bacteria that were pathogens of single eukaryotic cells nearly one billion years ago (Greub and Raoult, 2003; Stephens, 2002). Since that time, they have become obligate intracellular pathogens that have had very limited opportunity to acquire genes from other organisms. The ability to sample the microbial gene pool has played a decisive role in the adaptation to changing environments, evolution, and speciation of bacteria. From the perspective of chlamydiae, the intracellular vacuole is a largely invariant environment whether it is within a single-celled organism, or within a cell of a bird, koala, or human. During this billion years of history, all bacteria acquired and collected mutations in their 16S rRNA genes while rapidly evolving and adapting to novel environmental niches (i.e., speciation). The

chlamydiae, too, collected 16S rRNA mutations, however, chlamydiae lacked the opportunity or the necessity to evolve in significantly new directions. While there has been considerable gene loss, even the amount of intragenomic recombination has been limited as evidenced by the high level of genome synteny among *Chlamydia* species.

From 1971, chlamydiae have been classified within one order (*Chlamydiales*) and one family (*Chlamydiaceae*) and with one genus (*Chlamydia*) (Molder et al., 1984). With the advent of PCR, *Chlamydia*-like DNA has been found in many types of animals and environmental samples; the extent of diversity of *Chlamydia* began to emerge, and this continues today, unfortunately without microbiological isolation. During this period, there was a growing need for more accommodating and accurate polyphasic taxonomic characterization, and this proceeded through a deliberate process highlighted by the detailed and comprehensive characterization of *Chlamydia pneumoniae* (Grayston et al., 1989) and *Chlamydia pecorum* (Fukushi and Hirai, 1992). In 1999, the taxonomy of *Chlamydia* was thrown in disarray and created confusion in the field (Schachter et al., 2001) when Everett et al. (1999) proposed a new second genus for the order *Chlamydiales* and family *Chlamydiaceae* in 1999. The genus proposal was based upon an arbitrary difference of greater than 4% in 16S rRNA gene sequences. This resulted in the separation of *Chlamydia pneumoniae*, *Chlamydia pecorum*, and *Chlamydia psittaci* into a new genus *Chlamydophila* leaving the *Chlamydia trachomatis* biovars in the original *Chlamydia* genus. This separation was inconsistent with the 4% genus threshold, since the 16S rRNA genes of several species within the proposed *Chlamydophila* genus differ by 4% or more.

One of the goals of taxonomy is to maintain stability in the nomenclature and to avoid unnecessary confusion according to Principle 1, Subprinciples 1 and 2 of the International Code of Nomenclature of Bacteria (Sneath, 1992). Indeed, for bacterial pathogens, renaming them even though it may be justified scientifically, may not be advisable. For example, the Judicial Commission of the International Committee on Nomenclature of Bacteria rejected the proposal for renaming the pathogen, *Yersinia pestis*, as a subspecies *Yersinia pseudotuberculosis* thereby giving rise to the name *Yersinia pseudotuberculosis* subspecies *pestis* even though this was justified based on DNA–DNA hybridization (Becovier et al., 1980; Judicial Commission, 1985). Because of the ruling of the Judicial Commission, the validated name *Yersinia pseudotuberculosis* subsp. *pestis* was rejected and the name *Yersinia pestis* was conserved on the approved list, due to the inherent confusion its renaming would cause to the field as well as the potential hazard it posed as a *nomen periculosum* for the general public welfare (Judicial Commission, 1985; Williams, 1984).

These principles can be applied to the pathogens of the family *Chlamydiaceae*. Therefore, for this edition of *Bergey's Manual of Systematic Bacteriology*, the sole genus name *Chlamydia* is being retained for all of the species of the family *Chlamydiaceae*, all of which are human or animal pathogens.

Until now there has been a single *Chlamydia trachomatis* biovar designation called the trachoma biovar that included strains responsible for natural ocular infection and trachoma and strains that naturally caused urogenital infection and disease. Although the trachoma-causing strains were typically represented by serovars A-C and the urogenital strains by serovars D-K, no biological markers were available to differentiate strains that caused these different diseases. It now appears that trachoma-causing strains lack a functional tryptophan synthase, whereas urogenital strains contain a functional enzyme (Fehlner-Gardiner et al., 2002). The enzymic function can easily be tested *in vitro* or by gene sequence techniques for non-isolated samples. It is proposed that trachoma strains lacking tryptophan synthase function compose the "trachoma" biovar and those from the urogenital tract that have a functional enzyme compose a new "genital" biovar. Thus, *Chlamydia trachomatis* consists of three biovars including ocular, genital, and LGV.

Further reading

Allegra, L and F. Blasi (editors). 1999. *Chlamydia pneumoniae*. Springer-Verlag Italiana, Milano.

Barron A.L. (Editor). 1988. Microbiology of *Chlamydia*. CRC Press, Boca Raton.

Bavoil, P. and P. Wyrick (editors). 2006. *Chlamydia*: Genomics, Pathogenesis and Implications for Control. Horizon Press, Norwich, UK.

Campbell, L.A. and C.-C. Kuo. 2004. *Chlamydia pneumoniae* - an infectious risk factor for atherosclerosis? Nature Rev. Microbiol. *2*: 23–32.

Campbell, L.A., C.-C. Kuo and C. Gaydos. 2006. Chlamydial Infections. *In* Detrick, B., R. G. Hamilton and J. D. Folds. (editors) Manual of Molecular and Clinical Laboratory Immunology. 7th edn. American Society for Microbiology Press, Washington, D.C., pp. 518–525.

Campbell, L.A., J.M. Marrazzo, W.E. Stamm and C.-C. Kuo. 2001. Chapter 27. Chlamydiae. *In* Laboratory Diagnosis of Bacterial Infections (edited by Cimolai). Marcel Dekker, New York, pp. 795–821.

Gordon, F.B. (editor). 1962. The Biology of the Trachoma Agent. New York Academy of Science, New York.

Hanna, L, J. Schachter, C.R. Dawson and P. Thygeson (editors). 1967. Trachoma and Allied Diseases. Am. J. Ophthal. *63*: Part II.

Kuo, C.-C., L.A. Jackson, L.A. Campbell and J.T. Grayston. 1995. *Chlamydia pneumoniae* (TWAR). Clin. Microbiol. Rev. *8*: 451–461.

Molder, J.W. (editor). 1989. Intracellular Parasitism. CRC Press, Boca Raton.

Nichols, R.L. (editor) 1971. Trachoma and Related Diseases Caused by Chlamydial Agents. Excerpta Medica, Amsterdam.

Stephens, R.S. (editor). 1999. *Chlamydia*: *Intracellular Biology, Pathogenesis*, and *Immunity*. American Society for Microbiology Press, Washington, D.C.

Storz, J. 1971. *Chlamydia* and *Chlamydia*-induced Diseases. Charles C. Thomas, Springfield, IL.

Storz, J. and B. Kaltenboeck, 1993. Disease diversity of Chlamydial Infections. *In* Rickettsial and Chlamydial Diseases of Domestic Animals (edited by Woldehiwet and Ristic). Pergamon Press, Oxford, pp. 363–392.

List of species of the genus *Chlamydia*

1. **Chlamydia trachomatis** (Busacca 1935) Rake 1957, 958[AL]
[*Rickettsia trachomae* (*sic*) Busacca 1935, 567]

tra.cho′ma.tis. Gr. n. *trachusma-atos* roughness; N.L. n. *trachoma, -atis* the disease trachoma; N.L. gen. n. *trachomatis* of trachoma.

The characteristics are as described for the genus and as listed in Tables 161, 162, 163, and 167. The inclusion morphology is depicted in Figure 161.

All *Chlamydia trachomatis* isolates have come from humans. *Chlamydia trachomatis* isolates are classified into three biovars, ocular, genital, and lymphogranuloma venereum (LGV) on the basis of tissue tropism, pathogenesis in humans and laboratory animals, and their biological characteristics in cell cultures (Kuo et al., 1972a). The molecular basis of the separation of ocular from genital biovar is the ability to utilize exogenous indole for tryptophan biosynthesis by the genital, but not by the ocular biovar. The LGV biovar also utilizes indole for tryptophan biosynthesis. Three biovars are genetically (Table 162) and serologically (Figure 159) closely related. Serologically distinguishable from other chlamydial species by the species-specific monoclonal antibody in the micro-immunofluorescence test. Isolates are serotyped by the same test, however, a broad-reactivity among BED serovars and LGV are noted. Strains of ocular biovars parasitize mucous membranes of the conjunctiva, while strains of the genital biovar parasitize the squamous and columnar epithelium of the urogenital mucosa. The LGV biovar has tropism for lymphoid organs and is more invasive than the other two biovars. This behavior parallels its behavior in animals and cultured cells.

DNA G+C content (mol%): 43–44.2 (T_m).

Type strain: A/Har-13 (Trachoma type A strain HAR-13), ATCC VR-571-B.

Sequence accession no. (16S rRNA gene): D89067, E17344, NR 025888.

Further information concerning the three biovars of *Chlamydia trachomatis* is given below:

(i) Biovar ocular. Chlamydiae of this biovar exclusively parasitize the conjunctival mucosal cells. Transmission is by discharge from the inflamed eyes commonly in family or school. The primary infection is in the form of follicular conjunctivitis, which may heal spontaneously. However, in endemic areas, repeated infections sustain the chronicity of infection and cause scarring of the conjunctiva and cornea. The end stage of the disease is blindness. Table 165 lists the diseases caused by the ocular biovar of *Chlamydia trachomatis*. Serovars of the ocular biovar are A, B, Ba, and C.

DNA G+C content (mol%) of five strains: 44.0 (T_m).

Type strain: PK-2, serovar C, ATCC VR-576.

Sequence accession no. (16S rRNA gene) for strain C/TW-3/OT: D85720.

(ii) Biovar genital. Strains of this biovar are sexually transmitted. The primary site of infection is the mucosal membranes of the urogenital tract. Diseases caused by the genital biovar are listed in Table 165. The infection may be auto-transferred to the conjunctiva or to contacts to cause acute follicular conjunctivitis. Chronic infection due to the genital biovar of *Chlamydia trachomatis* is a common cause of infertility in women. Serovars of genital isolates are D through K. No difference in the virulence among the serovars has been observed.

DNA G+C content (mol%) of two strains is: 44.2 (T_m).

Type strain: UW-3/Cx, serovar D, ATCC VR-885.

Sequence accession no. (16S rRNA gene) for strain D/UW-3Cx: D85721.

(iii) Biovar lymphogranuloma venereum (LGV). Strains of this biovar are sexually transmitted and cause systemic infection with the major manifestation of lymphadenopathy or swelling of lymph nodes draining the extragenitalia. Three serovars are recognized which are L_1, L_2, and L_3.

DNA G+C content (mol%): 43.0 (T_m).

Type strain: 434, serovar L_2, ATCC VR-902B.

Sequence accession no. (16S and 23S rRNA genes): U68443.

2. **Chlamydia abortus** comb. nov. Everett, Bush and Andersen 1999 (*Chlamydophila abortus* Everett, Bush and Andersen 1999, 434)

a.bor'tus. L. n. *abortus, -us* an abortion, a miscarriage; L. gen. n. *abortus* of an abortion, a miscarriage.

The characteristics are as described for the genus and as listed in Tables 162, 163, and 166. This species was separated from *Chlamydia psittaci* with the establishment of five new

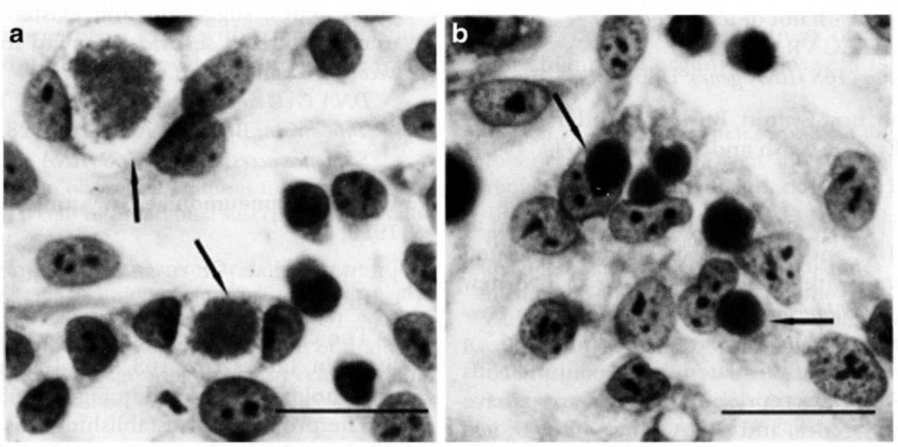

FIGURE 161. Two different morphologies of chlamydial inclusions. Infected host cell monolayers were fixed with methanol and stained with May-Greenwald Giemsa. Bars = 20 μm. Arrows point to a typical inclusion. (a) Vacuolar, glycogen-positive (*Chlamydia trachomatis, Chlamydia muridarum* and *Chlamydia suis*). (b) Solid, glycogen-negative (*Chlamydia psittaci, Chlamydia pneumoniae, Chlamydia pecorum, Chlamydia abortus, Chlamydia caviae* and *Chlamydia felis*). (Courtesy of author, C.-C. Kuo.)

species in addition to *Chlamydia trachomatis, Chlamydia pecorum, Chlamydia pneumoniae,* and *Chlamydia psittaci.*

Chlamydia abortus is endemic in ruminants, and it is a frequent cause of late-term abortion in flocks of sheep or goats (enzootic abortion of ewes, EAE), but less frequently in cattle. It has also been associated with seminal vesiculitis in bulls and with bovine infertility. *Chlamydia abortus* has also infrequently been identified as the cause of abortion in other mammals such as horse and pig, and experimentally causes abortion in laboratory animals such as rabbits, guinea pigs, and mice. Infection of pregnant women with *Chlamydia abortus* is typically contracted during contact with small ruminants and can cause flu-like symptoms of systemic infection followed by abortion (Herring et al., 1987; Johnson et al., 1985). *Chlamydia abortus* isolates worldwide represent a single serovar and have virtually identical *ompA* and rRNA genes.

DNA G+C content (mol%): not determined.

Type strain: B577, Ovine Chlamydial Abortion strain B-577, ATCC VR-656.

Sequence accession no. (16S rRNA gene): AB001783, D85709.

3. **Chlamydia caviae** comb. nov. Everett, Bush and Andersen 1999 (*Chlamydophila caviae* Everett, Bush and Andersen 1999, 434.)

ca.vi'ae. N.L. gen. n. *caviae* of *Cavia,* of the guinea pig (*Cavia cobaya*) because the type strain was isolated from *Cavia cobaya.*

The characteristics are as described for the genus and as listed in Tables 162, 163, and 166. This species was separated from *Chlamydia psittaci* with the establishment of five new species in addition to *Chlamydia trachomatis, Chlamydia pecorum, Chlamydia pneumoniae,* and *Chlamydia psittaci.*

Chlamydia caviae is a chlamydial pathogen that has exclusively been isolated from guinea pigs. The *ompA* sequences of five *Chlamydia caviae* isolates are identical. The primary isolation site is the conjunctiva (guinea pig inclusion conjunctivitis, GPIC), but inoculation of the guinea pig genital tract results in disease and infertility that are remarkably similar to those caused by human genital infection with *Chlamydia trachomatis.*

DNA G+C content (mol%): not determined.

Type strain: GPIC, ATCC VR 813.

Sequence accession no. (16S rRNA gene): D85708.

4. **Chlamydia felis** comb. nov. Everett, Bush and Andersen 1999 (*Chlamydophila felis* Everett, Bush and Andersen 1999, 434.)

fe'lis. L. n. *felis, -is* a cat; L. gen. n. *felis* of a cat.

The characteristics are as described for the genus and as listed in Table 162, 163, and 166. This species was separated from *Chlamydia psittaci* with the establishment of five new species in addition to *Chlamydia trachomatis, Chlamydia pecorum, Chlamydia pneumoniae,* and *Chlamydia psittaci.*

Chlamydia felis is frequently isolated from conjunctivitis and rhinitis in cats. All strains represent a single serovar, have highly conserved *ompA* genes, and rRNA genes differ by less than 0.6%. Human conjunctivitis caused by infection with *Chlamydia felis* contracted from pet cats appears to be common (Schachter et al., 1969).

DNA G+C content (mol%): not determined.

Type strain: FP Baker, ATCC VR-120.

Sequence accession no. (16S rRNA gene): D85701.

5. **Chlamydia muridarum** Everett, Bush and Andersen 1999, 431[VP]

mu.ri.da'rum. N.L. pl. gen. n. *muridarum* of the *Muridae* (i.e., the mouse/hamster family).

The characteristics are as described for the genus and as listed in Tables 162, 163, and 166. This species was separated from *Chlamydia trachomatis* with the establishment of five new species in addition to *Chlamydia trachomatis, Chlamydia pecorum, Chlamydia pneumoniae,* and *Chlamydia psittaci.*

Two virtually identical *Chlamydia muridarum* strains are currently known, isolated from the lungs of clinically healthy mice (strain MoPn Nigg II) and from the intestinal tract of a hamster with proliferative ileitis (strain SFPD). Strain MoPn has been found to be highly virulent for mice and can cause severe interstitial pneumonia. It is also frequently used in mouse models of chlamydial genital tract infection and infertility.

DNA G+C content (mol%): 42–44 (T_m).

Type strain: Nigg II, ATCC VR123.

Sequence accession no. (16S rRNA gene): D85718.

6. **Chlamydia pecorum** Fukushi and Hirai 1992, 307[VP]

pe.co'rum. L. gen. pl. n. *pecorum* of flocks of sheep or herds of cattle.

The characteristics are as described for the genus and as listed in Tables 162, 163, and 166. This was the fourth species established for the genus, after *Chlamydia trachomatis, Chlamydia psittaci,* and *Chlamydia pneumoniae.*

Chlamydia pecorum has been isolated from ruminants, swine, and koalas, and is frequently found in low numbers on the mucosal membranes of healthy host animals. *Chlamydia pecorum* causes severe diseases in these animals such as sporadic bovine encephalomyelitis (SBE) in feedlot cattle, and enteritis, pneumonia, polyarthritis, and urogenital infection in cattle and swine. In koalas, *Chlamydia pecorum* causes keratoconjunctivitis, vaginitis, cystitis, and infertility, and is strongly associated with the decline of the koala population in combination with stress resulting from destruction of the koala habitat. *Chlamydia pecorum* isolates show polymorphic *ompA* genes, suggesting the existence of numerous as yet uncharacterized serovars. Human infection with *Chlamydia pecorum* has not been observed.

DNA G+C content (mol%): 39.3 (T_m).

Type strain: Bo/E58, ATCC VR-628.

Sequence accession no. (16S rRNA gene): D88371.

7. **Chlamydia pneumoniae** Grayston, Kuo, Campbell and Wang 1989, 88[VP]

pneu.mo'ni.ae. Gr. n. *pneumonia* pneumonia, inflammation of the lungs; N.L. gen. n. *pneumoniae* of pneumonia.

The characteristics are as described for the genus and as listed in Tables 162, 163, 165, 166, and 168. The inclusion morphology is depicted in Figure 155.

The proposal for establishing this species, which was the third species in the genus, was based on the ultrastructural morphology of the elementary bodies that is typically pear-shaped (Figure 155), presence of a species-specific antigen identifiable with a species-specific monoclonal antibody, and its clear separation from the two other species by the DNA–DNA homology (Table 162). The species includes three

biovars: human, koala, and horse (Table 168). Since 1983, no other isolate has been obtained to confirm the original isolate from horse (Spencer and Johnson, 1983; Willis et al., 1990). The inclusions contain no glycogen, hence stain negative with iodine. Diseases caused by *Chlamydia pneumoniae* in humans are listed in Table 165 and diseases in animals in Table 166.

DNA G+C content (mol%): 40 (T_m).

Type strain: TW-183, ATCC VR-2282.

Sequence accession no. (16S rRNA gene): L06108, NR 026527, Z49873.

8. **Chlamydia psittaci** (Lillie 1930) Page 1968, 60[AL] (*Rickettsia psittaci* Lillie 1930, 778)

psit'ta.ci. L. n. *psittacus* a parrot; L. gen. n. *psittaci* of a parrot.

The characteristics are as described for the genus and as listed in Tables 162 and 163. This was the type species and strain of the first two species, *Chlamydia trachomatis* and *Chlamydia psittaci*, established for the genus.

Chlamydia psittaci is comprised of six serovars, A–F, of avian chlamydial isolates (Andersen, 1991, 1997) that cause latent and clinically overt infections of the intestinal and respiratory tracts in many species of birds. Upon exposure of birds to crowding and stress, these infections often become severe, with diarrhea, air sacculitis, pneumonia, and fatal systemic spread with pericarditis and splenomegaly. Human *Chlamydia psittaci*-induced disease, typically contracted by inhalation of dried bird feces, is called psittacosis or ornithosis if contracted from psittacine or from non-psittacine birds, respectively. Psittacosis is characterized by early flu-like symptoms followed by severe, sometimes fatal atypical interstitial pneumonia that requires intensive antibiotic and symptomatic therapy and resolves only slowly after several weeks. *Chlamydia psittaci* isolates should be handled only in a biosafety level 3 laboratory.

DNA G+C content (mol%): 41.3 (T_m).

Type strain: 6BC, Psittacosis strain 6 BC, ATCC VR-125.

Sequence accession no. (16S rRNA gene): AB001778, U68447.

9. **Chlamydia suis** Everett, Bush and Andersen 1999, 431[VP]

su'is. L. n. *sus suis* a swine; L. gen. n. *suis* of a swine.

The characteristics are as described for the genus and as listed in Tables 163 and 166. This species was separated from *Chlamydia trachomatis* with the establishment of five new species in addition to *Chlamydia trachomatis*, *Chlamydia pecorum*, *Chlamydia pneumoniae*, and *Chlamydia psittaci*.

The S45 type strain of *Chlamydia suis* was isolated by Koelbl in the 1960s in Austria from a pig with inapparent intestinal infection (Koelbl, 1969; Koelbl et al., 1970). Since 1995, high incidences and tetracycline-resistant strains of *Chlamydia suis* have been found in enteric and conjunctival porcine specimens, suggesting that *Chlamydia suis* infections are endemic in swine (Dugan et al., 2004). *Chlamydia suis* strains show marked *ompA* gene polymorphism, particularly of the variable domains, but less than 1.1% 16S rRNA gene diversity. This suggests the existence of numerous serovars, similar to *Chlamydia trachomatis*. Human infection with *Chlamydia suis* has not been observed.

DNA G+C content (mol%): not determined.

Type strain: S45, ATCC VR-1474.

Sequence accession no. (16S rRNA gene): U73110.

Other organisms

Chlamydia-like organisms have been described in reptiles (turtle, tortoise, and boar) (Homer et al., 1994; Vanrompay et al., 1994), amphibians (frog) (Bodetti et al., 2002), bivalves (mussel and clam) (Cajaraville and Angulo, 1991; Harshbarger and Chang, 1977; Page and Cutlip, 1982), and crab (Sparks et al., 1985). These *Chlamydia*-like organisms have ultra-structural morphology and developmental forms resembling those of chlamydiae in that the organisms grow within a cytoplasmic vacuole (inclusion) and undergo a developmental cycle from the reticulate body-like form to an elementary body-like form. The relatedness of these organisms to other *Chlamydia* species has been demonstrated by electron microscopy, PCR analysis of OMP and rRNA genes, and immunoreactivity with *Chlamydia*-specific antibodies. Isolation of the organisms by inoculation of chicken eggs has been reported from Moorish tortoise and green sea turtle (Homer et al., 1994; Vanrompay et al., 1994). However, taxonomic relatedness of these microorganisms to other chlamydial organisms has yet to be determined.

References

Alexander, J.J. 1968. Separation of protein synthesis in meningopneumonitisgent from that in L cells by differential susceptibility to cycloheximide. J. Bacteriol. *95*: 327–332.

Allan, I. and J.H. Pearce. 1983a. Differential amino acid utilization by *Chlamydia psittaci* (strain guinea pig inclusion conjunctivitis) and its regulatory effect on chlamydial growth. J. Gen. Microbiol. *129*: 1991–2000.

Allan, I. and J.H. Pearce. 1983b. Amino acid requirements of strains of *Chlamydia trachomatis* and *C. psittaci* growing in McCoy cells: relationship with clinical syndrome and host origin. J. Gen. Microbiol. *129*: 2001–2007.

Amann, R., N. Springer, W. Schonhuber, W. Ludwig, E.N. Schmid, K.D. Muller and R. Michel. 1997. Obligate intracellular bacterial parasites of acanthamoebae related to *Chlamydia* spp. Appl. Environ. Microbiol. *63*: 115–121.

Andersen, A.A. 1991. Serotyping of *Chlamydia psittaci* isolates using serovar-specific monoclonal antibodies with the microimmunofluorescence test. J. Clin. Microbiol. *29*: 707–711.

Andersen, A.A. 1997. Two new serovars of *Chlamydia psittaci* from North American birds. J. Vet. Diagn. Invest. *9*: 159–164.

Barbour, A.G., K. Amano, T. Hackstadt, L. Perry and H.D. Caldwell. 1982. *Chlamydia trachomatis* has penicillin-binding proteins but not detectable muramic acid. J. Bacteriol. *151*: 420–428.

Batteiger, B.E., W.J.t. Newhall and R.B. Jones. 1985. Differences in outer membrane proteins of the lymphogranuloma venereum and trachoma biovars of *Chlamydia trachomatis*. Infect. Immun. *50*: 488–494.

Bavoil, P., A. Ohlin and J. Schachter. 1984. Role of disulfide bonding in outer membrane structure and permeability in *Chlamydia trachomatis*. Infect. Immun. *44*: 479–485.

Bavoil, P., R.S. Stephens and S. Falkow. 1990. A soluble 60 kiloDalton antigen of *Chlamydia* spp. is a homologue of *Escherichia coli* GroEL. Mol. Microbiol. *4*: 461–469.

Bavoil, P.M. and R.C. Hsia. 1998. Type III secretion in *Chlamydia*: a case of deja vu? Mol. Microbiol. *28*: 860–862.

Beatty, W.L., T.A. Belanger, A.A. Desai, R.P. Morrison and G.I. Byrne. 1994. Tryptophan depletion as a mechanism of gamma interferon-mediated chlamydial persistence. Infect. Immun. *62*: 3705–3711.

Becovier, H., H.H. Mollaret, J.M. Alonso, J. Brault, G.R. Fanning, A.G. Steigerwalt and D.J. Brenner. 1980. Intra- and interspecies relatedness of *Yersinia pestis* by DNA hybridization and its relationship to *Yersinia pseudotuberculosis*. Curr. Microbiol. *4*: 225–229.

Bodetti, T.J., E. Jacobson, C. Wan, L. Hafner, A. Pospischil, K. Rose and P. Timms. 2002. Molecular evidence to support the expansion of the hostrange of *Chlamydophila pneumoniae* to include reptiles as well as humans, horses, koalas and amphibians. Syst. Appl. Microbiol. *25*: 146–152.

Brade, L., S. Schramek, U. Schade and H. Brade. 1986. Chemical, biological, and immunochemical properties of the *Chlamydia psittaci* lipopolysaccharide. Infect. Immun. *54*: 568–574.

Busacca, A. 1935. Un germe caractères de rickettsies (*Rickettsia trachomæ*) dans tissues trachomateux. Arch. Ophthalmol. *52*: 567–572.

Cajaraville, M.P. and E. Angulo. 1991. *Chlamydia*-like organisms in digestive and duct cells of mussels from the Basque coast. J. Invertebr. Pathol. *58*: 381–386.

Caldwell, H.D., J. Kromhout and J. Schachter. 1981. Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. Infect. Immun. *31*: 1161–1176.

Campbell, L.A., C.C. Kuo, S.P. Wang and J.T. Grayston. 1990. Serological response to *Chlamydia pneumoniae* infection. J. Clin. Microbiol. *28*: 1261–1264.

Campbell, L.A., D.L. Patton, D.E. Moore, A.L. Cappuccio, B.A. Mueller and S.P. Wang. 1993. Detection of *Chlamydia trachomatis* deoxyribonucleic acid in women with tubal infertility. Fertil Steril. *59*: 45–50.

Chi, E.Y., C.C. Kuo and J.T. Grayston. 1987. Unique ultrastructure in the elementary body of *Chlamydia* sp. strain TWAR. J. Bacteriol. *169*: 3757–3763.

Chosewood, L.C. and D.E. Wilson 2007. Biosafety in Microbiological and Biomedical Laboratories, 5th edn, U.S. Department of Health and Human Services, Public Health Service Centers for Disease Control and Prevention and National Institutes of Health. U.S. Government Printing Office, Washington, D.C.

Clifton, D.R., C.A. Dooley, S.S. Grieshaber, R.A. Carabeo, K.A. Fields and T. Hackstadt. 2005. Tyrosine phosphorylation of the chlamydial effector protein Tarp is species specific and not required for recruitment of actin. Infect. Immun. *73*: 3860–3868.

Collingro, A., S. Poppert, E. Heinz, S. Schmitz-Esser, A. Essig, M. Schweikert, M. Wagner and M. Horn. 2005a. Recovery of an environmental *Chlamydia* strain from activated sludge by co-cultivation with *Acanthamoeba* sp. Microbiology *151*: 301–309.

Collingro, A., E.R. Toenshoff, M.W. Taylor, T.R. Fritsche, M. Wagner and M. Horn. 2005b. '*Candidatus* Protochlamydia amoebophila', an endosymbiont of *Acanthamoeba* spp. Int. J. Syst. Evol. Microbiol. *55*: 1863–1866.

Corsaro, D., D. Venditti and M. Valassina. 2002. New parachlamydial 16S rDNA phylotypes detected in human clinical samples. Res. Microbiol. *153*: 563–567.

Cox, R.L., C.-C. Kuo, J.T. Grayston and L.A. Campbell. 1988. Deoxyribonucleic acid relatedness of *Chlamydia* sp. strain TWAR to *Chlamydia trachomatis* and *Chlamydia* psitaci. Int. J. Syst. Bacteriol. *38*: 265–267.

Davis, C.H., J.E. Raulston and P.B. Wyrick. 2002. Protein disulfide isomerase, a component of the estrogen receptor complex, is associated with *Chlamydia trachomatis* serovar E attached to human endometrial epithelial cells. Infect. Immun. *70*: 3413–3418.

DeGraves, F.J., D. Gao and B. Kaltenboeck. 2003. High-sensitivity quantitative PCR platform. BioTechniques *34*: 106–110, 112–105.

Dilbeck, P.M., J.F. Evermann, T.B. Crawford, A.C. Ward, C.W. Leathers, C.J. Holland, C.A. Mebus, L.L. Logan, F.R. Rurangirwa and T.C. McGuire. 1990. Isolation of a previously undescribed rickettsia from an aborted bovine fetus. J. Clin. Microbiol. *28*: 814–816.

Draghi, A., 2nd, V.L. Popov, M.M. Kahl, J.B. Stanton, C.C. Brown, G.J. Tsongalis, A.B. West and S. Frasca, Jr. 2004. Characterization of "*Candidatus* Piscichlamydia salmonis" (order *Chlamydiales*), a chlamydia-like bacterium associated with epitheliocystis in farmed Atlantic salmon (*Salmo salar*). J. Clin. Microbiol. *42*: 5286–5297.

Dugan, J., D.D. Rockey, L. Jones and A.A. Andersen. 2004. Tetracycline resistance in *Chlamydia suis* mediated by genomic islands inserted into the chlamydial *inv*-like gene. Antimicrob. Agents Chemother. *48*: 3989–3995.

Everett, K.D.E., R.M. Bush and A.A. Andersen. 1999. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol. *49*: 415–440.

Everson, J.S., S.A. Garner, B. Fane, B.L. Liu, P.R. Lambden and I.N. Clarke. 2002. Biological properties and cell tropism of Chp2, a bacteriophage of the obligate intracellular bacterium *Chlamydophila abortus*. J. Bacteriol. *184*: 2748–2754.

Fan, J. and R.S. Stephens. 1997. Antigen conformation dependence of *Chlamydia trachomatis* infectivity neutralization. J. Infect. Dis. *176*: 713–721.

Fehlner-Gardiner, C., C. Roshick, J.H. Carlson, S. Hughes, R.J. Belland, H.D. Caldwell and G. McClarty. 2002. Molecular basis defining human *Chlamydia trachomatis* tissue tropism. A possible role for tryptophan synthase. J. Biol. Chem. *277*: 26893–26903.

Fukushi, H. and K. Hirai. 1992. Proposal of *Chlamydia pecorum* sp. nov. for *Chlamydia* strains derived from ruminants. Int. J. Syst. Bacteriol. *42*: 306–308.

Furness, G., D.M. Graham and P. Reeve. 1960. The titration of trachoma and inclusion blennorrhoea viruses in cell cultures. J. Gen. Microbiol. *23*: 613–619.

Garner, S.A., J.S. Everson, P.R. Lambden, B.A. Fane and I.N. Clarke. 2004. Isolation, molecular characterisation and genome sequence of a bacteriophage (Chp3) from *Chlamydophila pecorum*. Virus Genes *28*: 207–214.

Gerloff, R.K., D.B. Ritter and R.O. Watson. 1970. Studies on thermal denaturation of DNA from various chlamydiae. J. Infect. Dis. *121*: 65–69.

Gordon, F.B., V.W. Andrew and J.C. Wagner. 1957. Development of resistance to penicillin and to chlortetracycline in psittacosis virus. Virology *4*: 156–171.

Gordon, F.B. and A.L. Quan. 1965. Occurence of glycogen in inclusions of the psittacosis-lymphogranuloma venereum-trachoma agents. J. Infect. Dis. *115*: 186–196.

Gordon, F.B., I.A. Harper, A.L. Quan, J.D. Treharne, R.S. Dwyer and J.A. Garland. 1969. Detection of *Chlamydia* (Bedsonia) in certain infections of man. I. Laboratory procedures: comparison of yolk sac and cell culture for detection and isolation. J. Infect. Dis. *120*: 451–462.

Grayston, J.T. and S. Wang. 1975. New knowledge of chlamydiae and the diseases they cause. J. Infect. Dis. *132*: 87–105.

Grayston, J.T., S.P. Wang, L.J. Yeh and C.C. Kuo. 1985. Importance of reinfection in the pathogenesis of trachoma. Rev. Infect. Dis. *7*: 717–725.

Grayston, J.T., C.C. Kuo, L.A. Campbell and S.P. Wang. 1989. *Chlamydia pneumoniae* sp. nov. for *Chlamydia* sp. strain twar. Int. J. Syst. Bacteriol. *39*: 88–90.

Greub, G. and D. Raoult. 2003. History of the ADP/ATP-translocase-encoding gene, a parasitism gene transferred from a *Chlamydiales* ancestor to plants 1 billion years ago. Appl. Environ. Microbiol. *69*: 5530–5535.

Grimwood, J., L. Olinger and R.S. Stephens. 2001. Expression of *Chlamydia pneumoniae* polymorphic membrane protein family genes. Infect. Immun. *69*: 2383–2389.

Hackstadt, T., W. Baehr and Y. Ying. 1991. *Chlamydia trachomatis* developmentally regulated protein is homologous to eukaryotic histone H1. Proc. Natl. Acad. Sci. U. S. A. *88*: 3937–3941.

Harshbarger, J.C. and S.C. Chang. 1977. *Chlamydiae* (with phages), mycoplasmas, and richettsiae in Chesapeake Bay bivalves. Science *196*: 666–668.

Hatch, T.P., E. Al-Hossainy and J.A. Silverman. 1982. Adenine nucleotide and lysine transport in *Chlamydia psittaci*. J. Bacteriol. *150*: 662–670.

Hatch, T.P., M. Miceli and J.E. Sublett. 1986. Synthesis of disulfide-bonded outer membrane proteins during the developmental cycle of *Chlamydia psittaci* and *Chlamydia trachomatis*. J. Bacteriol. *165*: 379–385.

Herring, A.J., I.E. Anderson, M. McClenaghan, N.F. Inglis, H. Williams, B.A. Matheson, C.P. West, M. Rodger and P.P. Brettle. 1987. Restriction endonuclease analysis of DNA from two isolates of *Chlamydia psittaci* obtained from human abortions. Br. Med. J. (Clin. Res. Ed.) *295*: 1239.

Hoelzle, L.E., G. Steinhausen and M.M. Wittenbrink. 2000. PCR-based detection of chlamydial infection in swine and subsequent PCR-coupled genotyping of chlamydial *ompl*-gene amplicons by DNA-hybridization, RFLP-analysis, and nucleotide sequence analysis. Epidemiol. Infect. *125*: 427–439.

Homer, B.L., E.R. Jacobson, J. Schumacher and G. Scherba. 1994. Chlamydiosis in mariculture-reared green sea turtles (*Chelonia mydas*). Vet. Pathol. *31*: 1–7.

How, S.J., D. Hobson, C.A. Hart and R.E. Webster. 1985. An *in-vitro* investigation of synergy and antagonism between antimicrobials against *Chlamydia trachomatis*. J. Antimicrob. Chemother. *15*: 533–538.

Hsia, R.C., Y. Pannekoek, E. Ingerowski and P.M. Bavoil. 1997. Type III secretion genes identify a putative virulence locus of *Chlamydia*. Mol. Microbiol. *25*: 351–359.

Hsia, R.C., L.M. Ting and P.M. Bavoil. 2000. Microvirus of chlamydia psittaci strain guinea pig inclusion conjunctivitis: isolation and molecular characterization. Microbiology *146*: 1651–1660.

Hybiske, K. and R.S. Stephens. 2007. Mechanisms of host cell exit by the intracellular bacterium *Chlamydia*. Proc. Natl. Acad. Sci. U. S. A. *104*: 11430–11435.

Iliffe-Lee, E.R. and G. McClarty. 1999. Glucose metabolism in *Chlamydia trachomatis*: the 'energy parasite' hypothesis revisited. Mol. Microbiol. *33*: 177–187.

Johnson, F.W., B.A. Matheson, H. Williams, A.G. Laing, V. Jandial, R. Davidson-Lamb, G.J. Halliday, D. Hobson, S.Y. Wong, K.M. Hadley and et al. 1985. Abortion due to infection with *Chlamydia psittaci* in a sheep farmer's wife. Br. Med. J. (Clin. Res. Ed.) *290*: 592–594.

Jones, H., G. Rake and B. Stearns. 1945. Studies on lymphogranuloma venereum. III. The action of the sulfonamides on the agent of lymphogranuloma venereum. J. Infect. Dis. *76*: 55–69.

Joseph, T., F.E. Nano, C.F. Garon and H.D. Caldwell. 1986. Molecular characterization of *Chlamydia trachomatis* and *Chlamydia psittaci* plasmids. Infect. Immun. *51*: 699–703.

Judicial Commission. 1985. Opinion 60. Rejection of the name *Yersinia pseudotuberculosis* subsp. *pestis* (van Loghem) Bechovier et al. 1991 and conservation of the name *Yersinia pestis* (Lehman and Neumann) van Loghem 1944 for the plague bacillus. Int. J. Syst. Bacteriol. *35*: 540–541.

Kahane, S., E. Metzer and M.G. Friedman. 1995. Evidence that the novel microorganism 'Z' may belong to a new genus in the family *Chlamydiaceae*. FEMS Microbiol. Lett. *126*: 203–207.

Kalman, S., W. Mitchell, R. Marathe, C. Lammel, J. Fan, R.W. Hyman, L. Olinger, J. Grimwood, R.W. Davis and R.S. Stephens. 1999. Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*. Nat. Genet. *21*: 385–389.

Kaltenboeck, B., K.G. Kousoulas and J. Storz. 1993. Structures of and allelic diversity and relationships among the major outer membrane protein (*ompA*) genes of the four chlamydial species. J. Bacteriol. *175*: 487–502.

Kaltenboeck, B., N. Schmeer and R. Schneider. 1997. Evidence for numerous *ompl* alleles of porcine *Chlamydia trachomatis* and novel chlamydial species obtained by PCR. J. Clin. Microbiol. *35*: 1835–1841.

Kane, C.D., R.M. Vena, S.P. Ouellette and G.I. Byrne. 1999. Intracellular tryptophan pool sizes may account for differences in gamma interferon-mediated inhibition and persistence of chlamydial growth in polarized and nonpolarized cells. Infect. Immun. *67*: 1666–1671.

Kingsbury, D.T. and E. Weiss. 1968. Lack of deoxyribonucleic acid homology between species of the genus *Chlamydia*. J. Bacteriol. *96*: 1421–1423.

Koelbl, O. 1969. Untersuchungen uebe das Vorkommen von Miyagawanellen beim Schwein. Wien Tierarztl Mschr *56*: 332–335.

Koelbl, O., H. Burtscher and J Hebensreit. 1970. Polyarthritis bei Schlachtschweinen. Mikrobiologische, histologische und fleischhygienische Aspekte. Wien Tierarztl. Mschr *57*: 355–361.

Kubo, A. and R.S. Stephens. 2000. Characterization and functional analysis of PorB, a *Chlamydia* porin and neutralizing target. Mol. Microbiol. *38*: 772–780.

Kuo, C.-C., S.-P. Wang and J.T. Grayston. 1977a. Growth of trachoma organisms in HeLa 229 cell culture. *In* Nongonococal Urethritis and Related Infection (edited by Hobson and Holmes). American Society for Microbiology, Washington, D.C., pp. 328–336.

Kuo, C.-C., H.-H. Chen, S.-P. Wang and J.T. Grayston. 1986. Identification of a new group of *Chlamydia psittaci* strains called TWAR. J. Clin. Microbiol. *24*: 1034–1037.

Kuo, C., S. Wang and J.T. Grayston. 1972a. Differentiation of TRIC and LGV organisms based on enhancement of infectivity by DEAE-dextran in cell culture. J. Infect. Dis. *125*: 313–317.

Kuo, C., S. Wang, B.B. Wentworth and J.T. Grayston. 1972b. Primary isolation of TRIC organisms in HeLa 229 cells treated with DEAE-dextran. J. Infect. Dis. *125*: 665–668.

Kuo, C., N. Takahashi, A.F. Swanson, Y. Ozeki and S. Hakomori. 1996. An N-linked high-mannose type oligosaccharide, expressed at the major outer membrane protein of *Chlamydia trachomatis*, mediates attachment and infectivity of the microorganism to HeLa cells. J. Clin. Invest *98*: 2813–2818.

Kuo, C.C., S.P. Wang and J.T. Grayston. 1973. Effect of polycations, polyanions and neuraminidase on the infectivity of trachoma-inclusin conjunctivitis and lymphogranuloma venereum organisms HeLa cells: sialic acid residues as possible receptors for trachoma-inclusion conjunction. Infect. Immun. *8*: 74–79.

Kuo, C.C. and T. Grayston. 1976. Interaction of *Chlamydia trachomatis* organisms and HeLa 229 cells. Infect. Immun. *13*: 1103–1109.

Kuo, C.C., S.P. Wang and J.T. Grayston. 1977b. Antimicrobial activity of several antibiotics and a sulfonamide against *Chlamydia trachomatis* organisms in cell culture. Antimicrob. Agents Chemother. *12*: 80–83.

Kuo, C.C. and E.Y. Chi. 1987. Ultrastructural study of *Chlamydia trachomatis* surface antigens by immunogold staining with monoclonal antibodies. Infect. Immun. *55*: 1324–1328.

Kuo, C.C. and J.T. Grayston. 1988. *In vitro* drug susceptibility of *Chlamydia* sp. strain TWAR. Antimicrob. Agents Chemother. *32*: 257–258.

Kuo, C.C. and J.T. Grayston. 1990a. Amino acid requirements for growth of *Chlamydia pneumoniae* in cell cultures: growth enhancement by lysine or methionine depletion. J. Clin. Microbiol. *28*: 1098–1100.

Kuo, C.C. and J.T. Grayston. 1990b. A sensitive cell line, HL cells, for isolation and propagation of *Chlamydia pneumoniae* strain TWAR. J. Infect. Dis. *162*: 755–758.

Kuo, C.C., A. Shor, L.A. Campbell, H. Fukushi, D.L. Patton and J.T. Grayston. 1993. Demonstration of *Chlamydia pneumoniae* in atherosclerotic lesions of coronary arteries. J. Infect. Dis. *167*: 841–849.

Kuo, C.C., M. Puolakkainen, T.M. Lin, M. Witte and L.A. Campbell. 2002. Mannose-receptor positive and negative mouse macrophages differ in their susceptibility to infection by *Chlamydia* species. Microb. Pathog. *32*: 43–48.

Li, D., A. Vaglenov, T. Kim, C. Wang, D. Gao and B. Kaltenboeck. 2005. High-yield culture and purification of *Chlamydiaceae* bacteria. J. Microbiol. Methods *61*: 17–24.

Lillie, R.D. 1930. Psittacosis-rickettsia-like inclusions in man and in experimental animals. Pub. Health Rep *45*: 773–778.

Lin, H.S. and J.W. Moulder. 1966. Patterns of response to sulfadiazine, D-cycloserine and D-alanine in members of the psittacosis group. J. Infect. Dis. *116*: 372–376.

Liu, B.L., J.S. Everson, B. Fane, P. Giannikopoulou, E. Vretou, P.R. Lambden and I.N. Clarke. 2000. Molecular characterization of a bacteriophage (Chp2) from *Chlamydia psittaci*. J. Virol. *74*: 3464–3469.

Majeed, M. and E. Kihlstrom. 1991. Mobilization of F-actin and clathrin during redistribution of *Chlamydia trachomatis* to an intracellular site in eucaryotic cells. Infect. Immun. *59*: 4465–4472.

Matsumoto, A. 1981. Electron microscopic observations of surface projections and related intracellular structures of *Chlamydia* organisms. J. Electron Microsc. (Tokyo) *30*: 315–320.

Matsumoto, A. 1982. Surface projections of *Chlamydia psittaci* elementary bodies as revealed by freeze-deep-etching. J. Bacteriol. *151*: 1040–1042.

Matsumoto, A., H. Izutsu, N. Miyashita and M. Ohuchi. 1998. Plaque formation by and plaque cloning of *Chlamydia trachomatis* biovar trachoma. J. Clin. Microbiol. *36*: 3013–3019.

Miyashita, N., A. Matsumoto, R. Soejima, T. Kishimoto, M. Nakajima, Y. Niki and T. Matsushima. 1997. Morphological analysis of *Chlamydia pneumoniae*. J. Jpn. Chemother. *45*: 255–264.

Molder, J.W., B.R.S. McCormack, F.M. Gogolak, M.M. Zebovitz and M.K. Itatani. 1955. Production and properties of a penicillin-resistant strain of feline pneumonitis virus. J. Infect. Dis. *96*: 57–74.

Molder, J.W., T.P. Hatch, C.-C. Kuo, J. Schachter and J. Storz. 1984. Genus *Chlamydia*. *In* Bergey's Manual of Systematic Bacteriology, vol. 1 (edited by Krieg). Williams & Wilkins, Baltimore, pp. 729–739.

Neeper, I.D., D.L. Patton and C.C. Kuo. 1990. Cinematographic observations of growth cycles of *Chlamydia trachomatis* in primary cultures of human amniotic cells. Infect. Immun. *58*: 2042–2047.

Nigg, C. and M.D. Eaton. 1944. Isolation from normal mice of a pneumotropic virus which forms elementary bodies. J. Exp. Med. *79*: 497–510.

Pace, N.R. 1997. A molecular view of microbial diversity and the biosphere. Science *276*: 734–740.

Page, L.A. 1968. Proposal for the recognition of two species in the genus *Chlamydia* Jones, Rake, and Stearns 1945. Int. J. Syst. Bacteriol. *18*: 51–66.

Page, L.A. and R.C. Cutlip. 1982. Morphological and immunological confirmation of the presence of *Chlamydiae* in the gut tissues of the Chesapeake Bay clam, *Mercenaria mercenaria*. Curr. Microbiol. *7*: 297–300.

Palmer, L. and S. Falkow. 1986. A common plasmid of *Chlamydia trachomatis*. Plasmid *16*: 52–62.

Patton, D.L., P.K. Cummings, C. Sweeney, T. Yvonne and C. C. Kuo. 1998. *In vivo* uptake of *Chlamydia trachomatis* by fallopian tube epithelial cells is rapid. *In* Chlamydial Infections. Proceedings of the Ninth International Symposium on Human Chlamydial Infection (edited by Stephens, Byrne, Christiansen, Clarke, Grayston, D. Rank, Ridgway, Saikku, Schachter and Stamm), San Francisco, pp. 87–90.

Peeling, R.W., J. Peeling and R.C. Brunham. 1989. High-resolution 31P nuclear magnetic resonance study of *Chlamydia trachomatis*: induction of ATPase activity in elementary bodies. Infect. Immun. *57*: 3338–3344.

Peterson, E.M. and L.M. de la Maza. 1988. *Chlamydia* parasitism: ultrastructural characterization of the interaction between the chlamydial cell envelope and the host cell. J. Bacteriol. *170*: 1389–1392.

Peterson, E.M., B.A. Markoff, J. Schachter and L.M. de la Maza. 1990. The 7.5-kb plasmid present in *Chlamydia trachomatis* is not essential for the growth of this microorganism. Plasmid *23*: 144–148.

Petersson, B., A. Andersson, T. Leitiner, O. Olsvik, M. Uhlen, C. Storey and C. Black. 1997. Evolutionary relationships among members of the genus *Chlamydia* based on 16S rDNA analysis. J. Bacteriol. *179*: 4195–4205.

Pickett, M.A., J.S. Everson, P.J. Pead and I.N. Clarke. 2005. The plasmids of *Chlamydia trachomatis* and *Chlamydophila pneumoniae* (N16): accurate determination of copy number and the paradoxical effect of plasmid-curing agents. Microbiology *151*: 893–903.

Plaunt, M.R. and T.P. Hatch. 1988. Protein synthesis early in the developmental cycle of *Chlamydia psittaci*. Infect. Immun. *56*: 3021–3025.

Pudjiatmoko, H. Fukushi, Y. Ochiai, T. Yamaguchi and K. Hirai. 1997. Phylogenetic analysis of the genus *Chlamydia* based on 16S rRNA gene sequences. Int. J. Syst. Bacteriol. *47*: 425–431.

Puolakkainen, M., C.C. Kuo and L.A. Campbell. 2005. *Chlamydia pneumoniae* uses the mannose 6-phosphate/insulin-like growth factor 2 receptor for infection of endothelial cells. Infect. Immun. *73*: 4620–4625.

Rake, E.G. 1957. Family II. *Chlamydiaceae* fam. nov. *In* Bergey's Manual of Determinative Bacteriology, 7th edn (edited by Breed, Murray and Smith). Williams & Wilkins, Baltimore, pp. 957–968.

Raulston, J.E., C.H. Davis, D.H. Schmiel, M.W. Morgan and P.B. Wyrick. 1993. Molecular characterization and outer membrane association of a *Chlamydia trachomatis* protein related to the Hsp70 family of proteins. J. Biol. Chem. *268*: 23139–23147.

Read, T.D., C.M. Fraser, R.C. Hsia and P.M. Bavoil. 2000. Comparative analysis of *Chlamydia* bacteriophages reveals variation localized to a putative receptor binding domain. Microb. Comp. Genomics *5*: 223–231.

Reed, S.I., L.E. Anderson and H.M. Jenkin. 1981. Use of cycloheximide to study independent lipid metabolism of *Chlamydia trachomatis* cultivated in mouse L cells grown in serum-free medium. Infect. Immun. *31*: 668–673.

Richmond, S.J., P. Stirling and C.R. Ashley. 1982. Virus infecting the reticulate bodies of an avian strain of *Chlamydia psittaci*. FEMS Microbiol. Lett. *14*: 31–36.

Ripa, K.R. and P. A. Mardh. 1977. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. J. Clin. Microbiol. *6*: 328–331.

Roblin, P.M., W. Dumornay and M.R. Hammerschlag. 1992. Use of HEp-2 cells for improved isolation and passage of *Chlamydia pneumoniae*. J. Clin. Microbiol. *30*: 1968–1971.

Rockey, D.D. and J.L. Rosquist. 1994. Protein antigens of *Chlamydia psittaci* present in infected cells but not detected in the infectious elementary body. Infect. Immun. *62*: 106–112.

Rodolakis, A. 1983. *In vitro* and *in vivo* properties of chemically induced temperature-sensitive mutants of *Chlamydia psittaci* var. ovis: screening in a murine model. Infect. Immun. *42*: 525–530.

Schachter, J., H.B. Ostler and K.F. Meyer. 1969. Human infection with the agent of feline pneumonitis. Lancet *1*: 1063–1065.

Schachter, J., R.S. Stephens, P. Timms, C. Kuo, P.M. Bavoil, S. Birkelund, J. Boman, H. Caldwell, L.A. Campbell, M. Chernesky, G. Christiansen, I.N. Clarke, C. Gaydos, J.T. Grayston, T. Hackstadt, R. Hsia, B. Kaltenboeck, M. Leinonnen, D. Ojcius, G. McClarty, J. Orfila, R. Peeling, M. Puolakkainen, T.C. Quinn, R.G. Rank, J. Raulston, G.L. Ridgeway, P. Saikku, W.E. Stamm, D. Taylor-Robinson, S.P. Wang and P.B. Wyrick. 2001. Radical changes to chlamydial taxonomy are not necessary just yet. Int. J. Syst. Evol. Microbiol. *51*: 249; author reply 251–253.

Scidmore, M.A., E.R. Fischer and T. Hackstadt. 1996. Sphingolipids and glycoproteins are differentially trafficked to the *Chlamydia trachomatis* inclusion. J. Cell Biol. *134*: 363–374.

Sneath, P.H.A. 1992. International Code of Nomenclature of Bacteria (1990 Revision). American Society for Microbiology, Washington, D.C.

Sparks, A.K., J.F. Morado and J.W. Hawkes. 1985. A systemic microbial disease in the Dungeness crab, *Cancer magister*, caused by a *Chlamydia*-like organism. J. Invertebr. Pathol. *45*: 204–217.

Spencer, W.N. and F.W. Johnson. 1983. Simple transport medium for the isolation of *Chlamydia psittaci* from clinical material. Vet. Rec. *113*: 535–536.

Stephens, R.S., M.R. Tam, C.C. Kuo and R.C. Nowinski. 1982. Monoclonal antibodies to *Chlamydia trachomatis*: antibody specificities and antigen characterization. J. Immunol. *128*: 1083–1089.

Stephens, R.S., E.A. Wagar and G.K. Schoolnik. 1988. High-resolution mapping of serovar-specific and common antigenic determinants of the major outer membrane protein of *Chlamydia trachomatis*. J. Exp Med. *167*: 817–831.

Stephens, R.S. 1989. Antigenic variation of *Chlamydia trachomatis*. *In* Intracellular Parasitism (edited by Molder). CRC Press, Boca Raton, FL, pp. 51–62.

Stephens, R.S., S. Kalman, C. Lammel, J. Fan, R. Marathe, L. Aravind, W. Mitchell, L. Olinger, R.L. Tatusov, Q. Zhao, E.V. Koonin and R.W. Davis. 1998. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science *282*: 754–759.

Stephens, R.S., K. Koshiyama, E. Lewis and A. Kubo. 2001. Heparin-binding outer membrane protein of chlamydiae. Mol. Microbiol. *40*: 691–699.

Stephens, R.S. 2002. *Chlamydiae* and evolution: a billion years and counting. *In* Chlamydial Infections. Proceedings of the Tenth International Symposium on Human Chlamydial Infections (edited by Schachter, Christiansen, Clarke et al.), Basim Yeri, Turkey, pp. 3–12.

Storey, C.C., M. Lusher and S.J. Richmond. 1989a. Analysis of the complete nucleotide sequence of Chp1, a phage which infects avian *Chlamydia psittaci*. J. Gen. Virol. *70*: 3381–3390.

Storey, C.C., M. Lusher, S.J. Richmond and J. Bacon. 1989b. Further characterization of a bacteriophage recovered from an avian strain of *Chlamydia psittaci*. J. Gen. Virol. *70*: 1321–1327.

Stothard, D.R., J.A. Williams, B. Van Der Pol and R.B. Jones. 1998. Identification of a *Chlamydia trachomatis* serovar E urogenital isolate which lacks the cryptic plasmid. Infect. Immun. *66*: 6010–6013.

Su, H., N.G. Watkins, Y.X. Zhang and H.D. Caldwell. 1990. *Chlamydia trachomatis*-host cell interactions: role of the chlamydial major outer membrane protein as an adhesin. Infect. Immun. *58*: 1017–1025.

T'ang, F., J. Chang, Y. Huang and K. Wang. 1957. Studies on the etiology of trachoma with special reference to isolation of the virus in chick embryo. Chin. Med. J. *75*: 429–447.

Thomas, N.S., M. Lusher, C.C. Storey and I.N. Clarke. 1997. Plasmid diversity in *Chlamydia*. Microbiology *143*: 1847–1854.

Ting, L.M., R.C. Hsia, C.G. Haidaris and P.M. Bavoil. 1995. Interaction of outer envelope proteins of *Chlamydia psittaci* GPIC with the HeLa cell surface. Infect. Immun. *63*: 3600–3608.

Tipples, G. and G. McClarty. 1993. The obligate intracellular bacterium *Chlamydia trachomatis* is auxotrophic for three of the four ribonucleoside triphosphates. Mol. Microbiol. *8*: 1105–1114.

Tjaden, J., H.H. Winkler, C. Schwoppe, M. Van Der Laan, T. Mohlmann and H.E. Neuhaus. 1999. Two nucleotide transport proteins in *Chlamydia trachomatis*, one for net nucleoside triphosphate uptake and the other for transport of energy. J. Bacteriol. *181*: 1196–1202.

Tribby, II, R.R. Friis and J.W. Moulder. 1973. Effect of chloramphenicol, rifampicin, and nalidixic acid on *Chlamydia psittaci* growing in L cells. J. Infect. Dis. *127*: 155–163.

Vanrompay, D., W. De Meurichy, R. Ducatelle and F. Haesebrouck. 1994. Pneumonia in Moorish tortoises (*Testudo graeca*) associated with avian serovar A *Chlamydia psittaci*. Vet. Rec. *135*: 284–285.

Wagar, E.A. and R.S. Stephens. 1988. Developmental-form-specific DNA-binding proteins in *Chlamydia* spp. Infect. Immun. *56*: 1678–1684.

Wagar, E.A., J. Schachter, P. Bavoil and R.S. Stephens. 1990. Differential human serologic response to two 60,000 molecular weight *Chlamydia trachomatis* antigens. J. Infect. Dis. *162*: 922–927.

Wang, S.P. and J.T. Grayston. 1970. Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test. Am. J. Ophthalmol. *70*: 367–374.

Wang, S.P., C.C. Kuo and J.T. Grayston. 1973. A simplified method for immunological typing of trachoma-inclusion conjunctivitis-lymphogranuloma venereum organisms. Infect. Immun. *7*: 356–360.

Weisburg, W.G., T.P. Hatch and C.R. Woese. 1986. Eubacterial origin of *Chlamydiae*. J. Bacteriol. *167*: 570–574.

Williams, J.E. 1984. Proposal to reject the new combination *Yersinia pseudotuberculosis* subsp. *pestis* for violation of the First Principle of the International Code of Nomenclature of Bacteria. Int. J. Syst. Bacteriol. *34*: 268–269.

Willis, J.M., G. Watson, M. Lusher, T.S. Mair, D. Wood and S.J. Richmond. 1990. Characterization of *Chlamydia psittaci* isolated from a horse. Vet. Microbiol. *24*: 11–19.

Wolf, K., E. Fischer, D. Mead, G. Zhong, R. Peeling, B. Whitmire and H.D. Caldwell. 2001. *Chlamydia pneumoniae* major outer membrane protein is a surface-exposed antigen that elicits antibodies primarily directed against conformation-dependent determinants. Infect. Immun. *69*: 3082–3091.

Wyrick, P.B., J. Choong, C.H. Davis, S.T. Knight, M.O. Royal, A.S. Maslow and C.R. Bagnell. 1989. Entry of genital *Chlamydia trachomatis* into polarized human epithelial cells. Infect. Immun. *57*: 2378–2389.

Yang, Z.P., P.K. Cummings, D.L. Patton and C.C. Kuo. 1994. Ultrastructural lung pathology of experimental *Chlamydia pneumoniae* pneumonitis in mice. J. Infect. Dis. *170*: 464–467.

Family II. "*Candidatus* Clavichlamydiaceae"

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Cla.vi.chla.my.di.a.ce'ae. N.L. fem. n. *Clavichlamydia* type genus of the family; *-aceae* ending to denote a family, N.L. fem. pl. n. *Clavichlamydiaceae* the *Clavichlamydia* family.

The family "*Candidatus* Clavichlamydiaceae" is currently solely based on 16S rRNA sequences moderately related to members of the *Chlamydiaceae* (around 90% sequence similarity) (Figure 154). The family "*Candidatus* Clavichlamydiaceae" is a sister family of the *Chlamydiaceae* and contains a single genus, "*Candidatus* Clavichlamydia".

Type genus: **"*Candidatus* Clavichlamydia"** corrig. Karlsen, Nylund, Watanabe, Helvik, Nylund and Plarre 2008.

Genus I. "*Candidatus* Clavichlamydia" corrig. Karlsen, Nylund, Watanabe, Helvik, Nylund and Plarre 2008

MATTHIAS HORN

Cla.vi.chla.my'di.a. L. n. *clava* a knotty branch, rough stick, cudgel, club; N.L. fem. n. *Chlamydia* taxonomic name of a bacterial genus; N.L. fem. n. *Clavichlamydia* a club *Chlamydia* (the morphology of the bacteria includes the characteristic head-and-tail cells that have been described earlier from salmonid fish suffering from epitheliocystis).

Pleomorphic or elongated, nonmotile, obligate intracellular bacteria, up to 2 µm in length. Cells show a **developmental cycle** with morphologically distinct stages and thrive **inside a host vacuole.** Organisms were found within gill lesions of fish and are differentiated from all other chlamydiae by the morphology of the proposed **elementary bodies, which show a characteristic head-and-tail form** (Figure 162). Members have not yet been obtained in cell culture.

Type species: **"*Candidatus* Clavichlamydia salmonicola"** corrig. Karlsen, Nylund, Watanabe, Helvik, Nylund and Plarre 2008.

Further descriptive information

Three distinct morphological forms, representing different developmental stages, have been observed inside the host-derived vacuole that surrounded the bacteria. The proposed reticulate bodies are large pleomorphic cells (up to 2 µm) with