Chlamydiae as Symbionts in Eukaryotes

Matthias Horn

Department of Microbial Ecology, University of Vienna, A-1090 Vienna, Austria; email: horn@microbial-ecology.net

Annu. Rev. Microbiol. 2008. 62:113–31

First published online as a Review in Advance on May 12, 2008

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi: 10.1146/annurev.micro.62.081307.162818

0066-4227/08/1013-0113$20.00

Key Words

chlamydia, symbiosis, amoeba, evolution

Abstract

Members of the phylum *Chlamydiae* are obligate intracellular bacteria that were discovered about a century ago. Although *Chlamydiae* are major pathogens of humans and animals, they were long recognized only as a phylogenetically well-separated, small group of closely related microorganisms. The diversity of chlamydiae, their host range, and their occurrence in the environment had been largely underestimated. Today, several chlamydia-like bacteria have been described as symbionts of free-living amoebae and other eukaryotic hosts. Some of these environmental chlamydiae might also be of medical relevance for humans. Their analysis has contributed to a broader understanding of chlamydial biology and to novel insights into the evolution of these unique microorganisms.

Contents

INTRODUCTION

It has to be tried to collect by the term 'Chlamydozoa' a group of peculiar microorganisms that do belong neither to the protozoa nor to the bacteria. They pass filters, cause inclusion bodies and will multiply as contagium only in egg culture.

Stanislaus von Prowazek, 1912 (58)

In Java, Indonesia, in 1907, the German radiologist Ludwig Halberstädter and the Austrian zoologist Stanislaus von Prowazek went on a research expedition to find the causative agent of syphilis. Among the discoveries they brought back from this trip was a conspicuous agent they considered responsible for trachoma, which was at that time a global disease (47). Within Giemsa-stained conjunctival epithelial cells of trachoma patients they had found irregularly blue-stained inclusions with small, dense particles, which they called "Chlamydozoa" (from the Greek word χλαμυσ, meaning mantle or cloak) (47). Originally considered neither protozoa nor bacteria and then regarded as viruses, in the 1960s they were recognized as bacteria (84). Later, these unique microorganisms were found to be among the most important bacterial pathogens of humankind.

Halberstädter's and Prowazek's Chlamydozoa are now called *Chlamydia trachomatis*, and it is the most prominent representative of a small group of closely related bacteria, the chlamydiae. Trachoma affects about 84 million people, of whom about 8 million are visually impaired as a consequence (103). *C. trachomatis* is also the most common cause of sexually transmitted diseases, with over 90 million new cases each year (102). The second prime human pathogen among the chlamydiae is *Chlamydophila pneumoniae*, a causative agent of pneumonia, which has also been associated with a number of chronic diseases such as atherosclerosis, asthma, and Alzheimer's disease (67). In addition, several other chlamydial species are primarily considered pathogens of animals, but some of them also show zoonotic potential (67).

Chlamydiae were long considered to comprise exclusively obligate intracellular bacterial pathogens that show a characteristic developmental cycle, including metabolically inert elementary bodies (EBs) and actively dividing reticulate bodies (RBs), which thrive within a host-derived vacuole termed inclusion (1). This phylogenetically well-isolated group of closely related bacteria constituted the single family *Chlamydiaceae* of the order *Chlamydiales*, which form a separate phylum in the domain *Bacteria*, the *Chlamydiae* (**Figure 1**). Our perception of chlamydial diversity changed substantially when in the 1990s novel chlamydia-like bacteria were discovered. This review summarizes our current knowledge about these novel chlamydiae (also called environmental chlamydiae).

EB: elementary body

RB: reticulate body

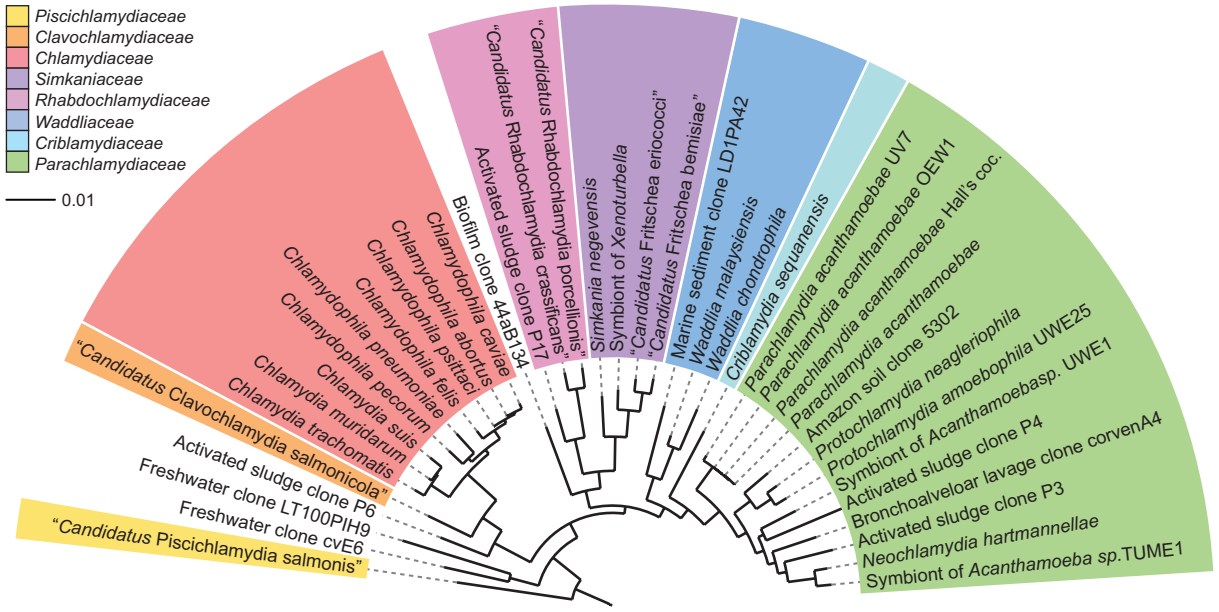


Figure 1

Phylogenetic 16S rRNA tree showing relationships among members of the phylum *Chlamydiae*. Representatives of all recognized families and a few clone sequences are shown. The tree was calculated with the ARB software, using a manually curated version of the SILVA database (16S/18S reference database, Version 92) and the PhyML treeing algorithm implemented in ARB, and visualized using the iTOL tool (43, 74, 77, 88). Bar, 10% estimated evolutionary distance.

DIVERSITY AND ECOLOGY OF CHLAMYDIA-LIKE BACTERIA

Ultrastructural evidence of the presence of unidentified bacteria resembling known chlamydiae was available in the 1980s (92). Yet it took another decade until the first chlamydia-like bacteria were identified on the molecular level, initially in laboratory cell culture (66) but mainly as symbionts of free-living amoebae (2, 5, 31, 54) [in this review the terms symbiosis and symbiont are used sensu deBary, i.e., to include mutualism, parasitism, and commensalism (19)]. These findings were the beginning of a series of reports describing a continuing increase of recognized diversity within the *Chlamydiae* (**Figure 2**) (16). Remarkably, these bacteria are not mere phylogenetic relatives of the *Chlamydiaceae*; they also show an obligate intracellular lifestyle and the unique chlamydial developmental cycle (**Figure 3**).

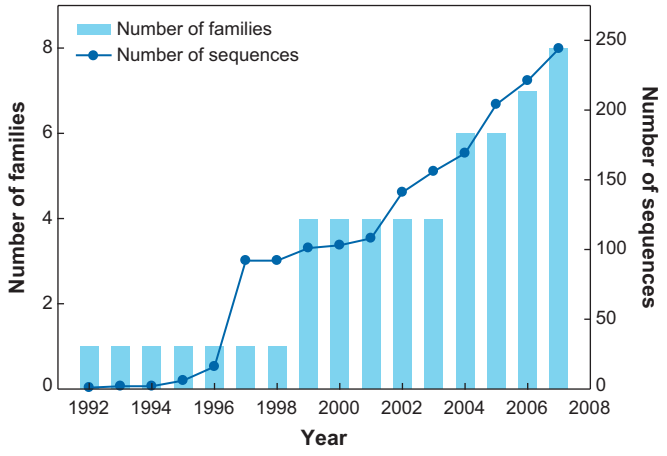


Figure 2

Increase in described diversity of the phylum *Chlamydiae* from 1992 to 2007. Numbers of 16S rRNA sequences deposited in public databases (>1000 nucleotides) and numbers of chlamydial families based on described chlamydiae [using the 90% 16S rRNA sequence similarity threshold suggested by Everett et al. (25) and Kuo et al. (73)] are shown.

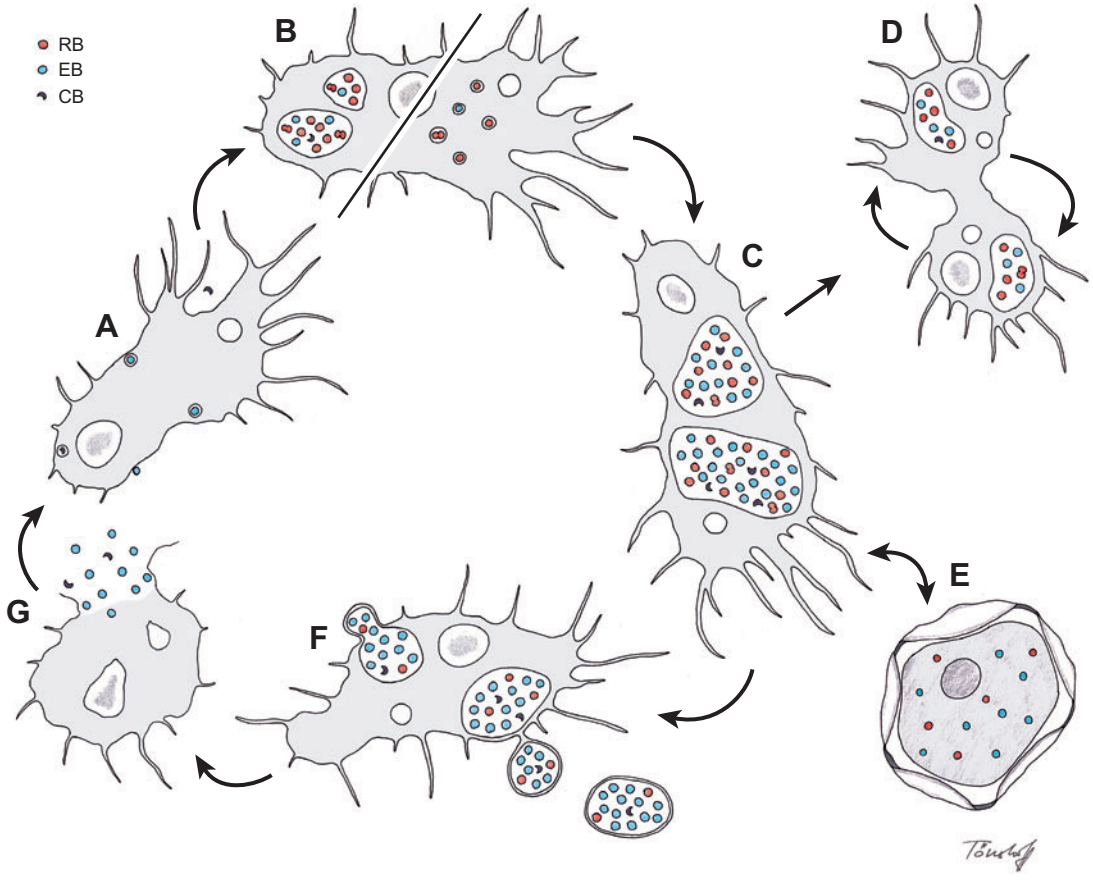


Figure 3

The developmental cycle of chlamydia-like bacteria in free-living amoebae. Elementary bodies (EBs) are shown in blue; reticulate bodies (RBs) are in red, the proposed crescent bodies (CBs) of *Parachlamydia acanthamoebae* are shown in dark purple. The occurrence of *P. acanthamoebae*, *Simkania negevensis*, and *Waddlia chondrophila* in larger inclusions compared with *Protochlamydia amoebophila* generally found in single-cell inclusion is indicated in step B; see text for further details. Figure by E. Toenshoff.

Symbionts of Free-Living Amoebae

Free-living amoebae are ubiquitous protozoa. They are the main predators controlling bacterial populations in soil, but they are also present in marine and freshwater habitats and the air (91). Frequently, they are found in human-made environments such as public water supplies, air-conditioning units, and sewage treatment plants. As a result, they can also be isolated from the human body, e.g., nasal cavities (69). Consistent with our common exposure to these amoebae, close to 90% of healthy individuals show specific immunoglobulin G reactivity with free-living amoebae of the genus *Acanthamoeba* (10). Ubiquitous *Acanthamoeba* species frequently contain bacterial endosymbionts, and in about 5% of all *Acanthamoeba* isolates studied by Fritsche et al. (30, 31) these symbionts were novel chlamydia-like bacteria. To date, only a few of these chlamydial symbionts of amoebae have been characterized to some extent.

Parachlamydia acanthamoebae Bn_9 was discovered in an *Acanthamoeba* isolate recovered from a human nasal swab of a healthy volunteer (2, 25, 81). *Neochlamydia hartmannellae* A_1Hsp

was detected in a *Hartmannella vermiformis* isolate from a water conduit system of a dental care unit (54), *Protochlamydia amoebophila* UWE25 was originally found in an *Acanthamoeba* isolate from a soil sample (13, 31), and *Protochlamydia naegleriophila* was identified in a *Naegleria* sp. (9). All these bacteria thrive as symbionts in amoebae, and thus it might be tempting to simply lump them together as chlamydia-like symbionts. It is, however, important to recognize that they are not closely related but that they are separated by roughly 300 million years of evolution [if a divergence rate of 1%–2% per 50 million years for the 16S ribosomal RNA gene is assumed (85)]. In fact, these chlamydia-like symbionts represent three distinct genera within the family *Parachlamydiaceae* (**Figure 1**) (25) and show remarkable differences with respect to their biology and the interaction with their host cells (see below).

Cocultivation with amoebae was used successfully to recover novel chlamydiae from complex environmental samples, although in this case the natural host remains unknown (12, 99). For example, the *P. acanthamoebae* strains UV7 and Seine and *Criblamydia sequanensis* (**Figure 1**) were identified by this method from activated sludge and water samples of the Seine river, respectively (12, 99).

Simkania negevensis was discovered as contaminant in a human cell culture (66). Similar to *C. sequanensis*, its original host is thus unknown, but it grows well in *Acanthamoeba* spp. and other amoebae (59, 82). Given the ubiquity of free-living amoebae, it is tempting to speculate that they serve as natural hosts for *S. negevensis* in the environment.

Other Nonhuman Hosts

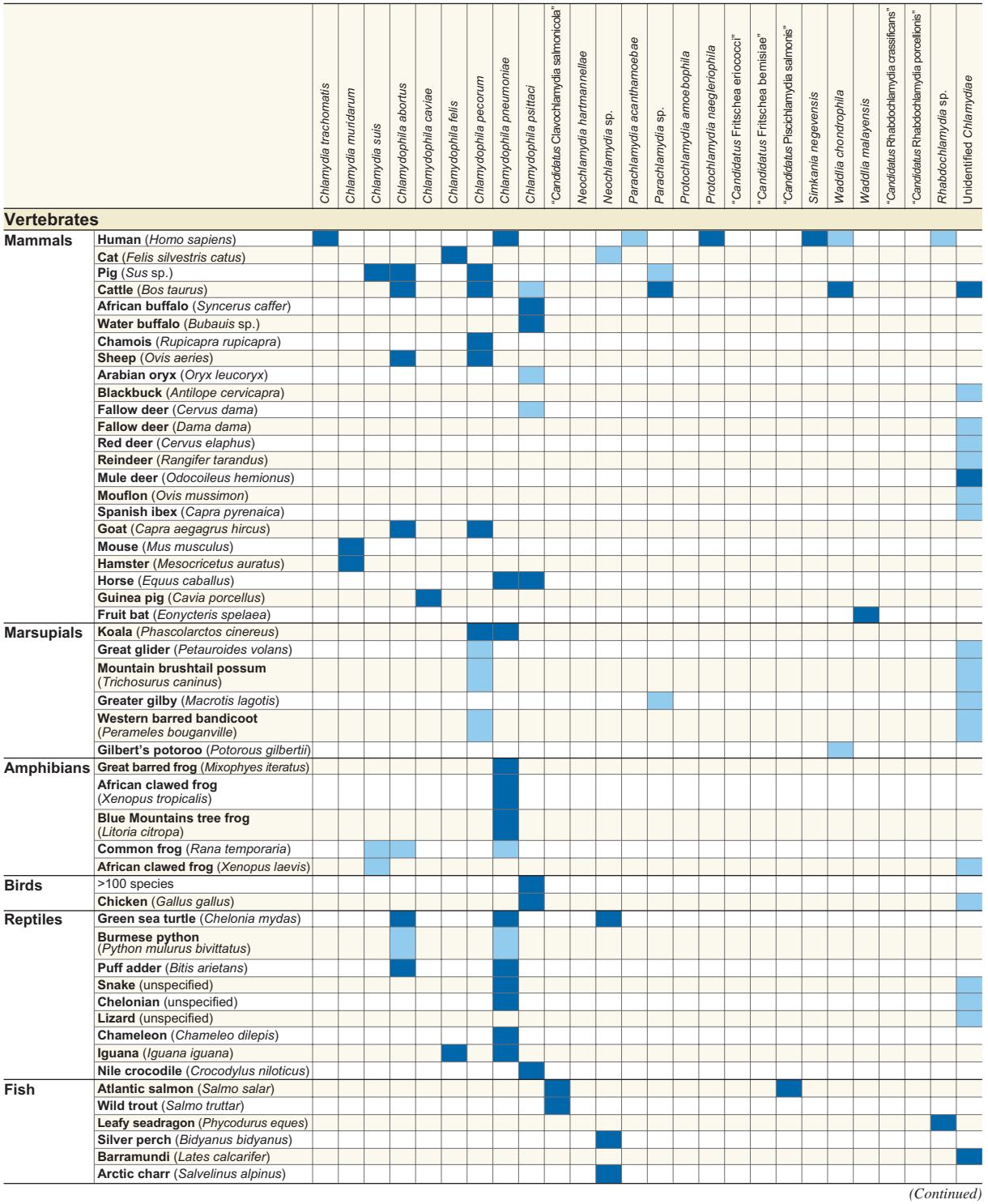
In addition to chlamydial symbionts of amoebae, a number of other chlamydia-like bacteria were identified in phylogenetically diverse animal hosts. *Waddlia chondrophila* was recovered from an aborted bovine fetus (94). *Waddlia malaysiensis* was detected in urine of fruit bats (11). Representatives of two novel *Chlamydiales* families, the *Piscichlamydiaceae* and the *Clavochlamydiaceae*, were identified in gill tissues of fish suffering from epitheliocystis (22, 68). *Rhabdochlamydia porcellionis* and *R. crassificans* infect terrestrial isopods and cockroaches, respectively (15, 71). *Fritschea bemisiae* and *F. eriococci* were described in insects (98), and a yet unnamed chlamydial symbiont was detected in the enigmatic worms *Xenoturbella bocki* and *X. westbladi* (57). Taken together, the chlamydiae identified so far show a broad host range across the animal kingdom (**Table 1**).

Free-living amoebae seem to play a special role in the ecology of chlamydiae, as many of them, including *Chlamydophila pneumoniae* (23), survive and/or multiply in acanthamoebae (2, 31, 54, 59, 83). Amoebae, particularly *Acanthamoeba* species, might thus serve as major reservoirs and vehicles of dispersal for chlamydiae. Compared with other protozoa and most other amoebae, *Acanthamoeba* species, however, are easy to isolate, to adapt to, and to maintain in axenic culture in the laboratory. Our current view of *Acanthamoeba* species as major hosts for chlamydia-like bacteria thus might be biased toward these amoebae. In fact, as amoebae are a polyphyletic, largely unrelated assemblage of protists, it would not be surprising if other protozoa are hosts for chlamydial symbionts.

The Tip of the Iceberg

As illustrated above, known diversity of *Chlamydiae* has increased from one to currently eight families during the past decade [if the 90% 16S rRNA sequence similarity threshold suggested by Everett et al. (25) and Kuo et al. (73) is applied] (**Figures 1** and **2**). Eleven of 13 genera in these families are, however, represented currently by only one (7 genera) or two (4 genera) species or isolates, indicating that the actual diversity within the recognized chlamydial families is even larger and yet undiscovered. Indeed, molecular evidence suggests that the recognized diversity of the chlamydiae is just the tip of the iceberg (16–18, 53, 86). A large number of phylogenetically diverse

Table 1 Host range of the phylum *Chlamydiae*[a]



(Continued)

Table 1 (*Continued*)

		Chlamydia trachomatis	*Chlamydia muridarum*	*Chlamydia suis*	*Chlamydophila abortus*	*Chlamydophila caviae*	*Chlamydophila felis*	*Chlamydophila pecorum*	*Chlamydophila pneumoniae*	*Chlamydophila psittaci*	"*Candidatus* Clavochlamydia salmonicola"	*Neochlamydia hartmannellae*	*Neochlamydia* sp.	*Parachlamydia acanthamoebae*	*Parachlamydia* sp.	*Protochlamydia amoebophila*	*Protochlamydia naegleriophila*	"*Candidatus* Fritschea eriococci"	"*Candidatus* Fritschea bemisiae"	"*Candidatus* Piscichlamydia salmonis"	*Simkania negevensis*	*Waddlia chondrophila*	*Waddlia malayensis*	"*Candidatus* Rhabdochlamydia crassificans"	"*Candidatus* Rhabdochlamydia porcellionis"	*Rhabdochlamydia* sp.	Unidentified *Chlamydiae*
Invertebrates																											
Insects	**Sweetpotato whitefly** (*Bemisia tabaci*)																		■								
	Imported elm bark louse (*Eriococcus spurius*)																	■									
	Oriental cockroach (*Blatta orientalis*)																							■			
Crustaceans	**Rough woodlouse** (*Porcellio scaber*)																								■		
Molluscs	**Pacific oyster** (*Crassostrea gigas*)				■																						
Protozoa	*Acanthamoeba* sp.													■		■											
	Hartmannella vermiformis											■															
	Naegleria lovaniensis																■										

[a]Evidence for the presence of chlamydiae by 16S rRNA analysis in combination with microscopic analyses (immunofluorescence, electron microscopy, histology) or for the recovery of the respective organism is indicated by dark blue boxes. Evidence for the presence of chlamydiae by only 16S rRNA analysis or serology without microscopic data is indicated by light blue boxes. Due to the revision of chlamydial taxonomy in 1999 (25), evidence for *Chlamydophila psittaci* (formerly *Chlamydia psittaci*) could also refer to *Chlamydophila abortus*, *Chlamydophila felis*, or *Chlamydophila caviae*. Follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org for a fully annotated table, including references.

rRNA sequences most similar to recognized chlamydiae have been detected in an impressive variety of habitats, including soils, sediments, aquatic environments, hydrothermal vent fluid, and engineered environments such as activated sludge and anaerobic bioreactors (**Supplemental Table 1** and **Supplemental Table 2**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). Owing to the varying length of the sequences obtained in the different studies (from <300 to ~1500 bp), it is impossible to estimate the true diversity of *Chlamydiae* from these data. However, if only sequences greater than 1000 nucleotides are considered, at least 11 operational taxonomic units (OTUs) at the family level and at least 27 OTUs at the genus level exist (corresponding to more than 90% and 95% 16S rRNA sequence similarity, respectively).

Taken together, there is molecular evidence of a previously unseen level of diversity of chlamydiae in the environment. *Chlamydiae* have a great and previously underestimated host range and show an almost ubiquitous distribution in the environment. It will be a major challenge to find, isolate and characterize those elusive microorganisms for which to date merely rRNA sequences exist.

CHLAMYDIA-LIKE BACTERIA: IMPLICATIONS FOR PUBLIC HEALTH?

Chlamydiae have been detected in diverse habitats; they are present in various animals and their major hosts, free-living amoebae, are ubiquitous. Therefore, humans are frequently exposed to chlamydia-like bacteria. Is this a concern for public health? Can chlamydia-like bacteria infect humans and cause disease, and thus should they be considered new emerging pathogens?

A number of reports suggest an association between chlamydia-like bacteria and human disease (14, 17, 18, 46, 86). Most recently, *Protochlamydia naegleriophila* was detected in a bronchoalveolar lavage sample of an immunocompromised pneumonia patient (9), and *Waddlia chondrophila* was implicated in a controlled, serology-based study with human fetal death (4). Accumulating data are available

rRNA: ribosomal RNA

for two chlamydia-like bacteria, *Parachlamydia acanthamoebae* and *Simkania negevensis*.

The Case of *Parachlamydia acanthamoebae*

In the laboratory, *P. acanthamoebae* infects and to a limited degree multiplies in simian and human cell lines (8, 12, 40). Proliferation of *P. acanthamoebae* in monocyte-derived macrophages, pneumocytes, and lung fibroblasts was, however, about three orders of magnitude slower than observed for the *Chlamydiaceae* or other pathogens such as *Legionella pneumophila* (8, 40). In addition, *P. acanthamoebae* showed major differences with respect to intracellular trafficking and host cell response compared with the *Chlamydiaceae* (36, 39).

P. acanthamoebae has been implicated primarily in respiratory disease (14), including community-acquired pneumonia, bronchitis, and aspiration pneumonia, but also in atherosclerosis (5, 18, 33, 34, 37, 79). The number of available studies is limited ($n = 5$), and the reported associations between *P. acanthamoebae* and human disease are based exclusively on serological (immunofluorescence) and molecular (PCR, nested PCR, real-time PCR) evidence. To date, *P. acanthamoebae* has not been isolated from a patient nor was its presence demonstrated at the site of infection.

The Case of *Simkania negevensis*

Simkania negevensis can thrive in a variety of human and simian cells, including Vero, BGM (buffalo green monkey), HeLa, human fibroblasts, McCoy, and HL cells (62), as well as in U973 macrophages, in epithelial cells originating from the respiratory or the genital tract, and in cells of vascular endothelial origin (61). However, *S. negevensis* takes longer to complete the developmental cycle than the *Chlamydiaceae* (7–12 versus 2–3 days) (60, 65). On the basis of serological and molecular evidence, *S. negevensis* has been associated with bronchiolitis and community-acquired pneumonia in children and with exacerbation of chronic obstructive pulmonary disease in adults (27, 32, 49, 63, 75, 76). A few studies also reported the isolation of *S. negevensis* from specimens of bronchiolitis and pneumonia patients (29, 64, 72). An association between *S. negevensis* and acute rejection in lung transplant recipients was supported not only by PCR but also by culturing the organism from bronchoalveolar lavage (56).

At first sight, these data appear to be convincing evidence of an association of *S. negevensis* with respiratory disease. However, the high seroprevalence of *S. negevensis* in healthy individuals, ranging from 39% to 68% and obviously increasing with age (29), shows that controlled studies are required for a reliable estimation of the contribution of *S. negevensis* to human disease. In one such study, no statistically significant association was found between respiratory disease and seropositivity or positive PCR for *S. negevensis* (72). Similarly, an association of *S. negevensis* with the onset of asthma in children was proposed but could not be confirmed in a controlled serology-based study (70). *S. negevensis* was cultured from 21 of 35 (60%) nasopharyngeal washing samples from healthy individuals and from 19 of 34 (56%) water samples analyzed in a recent study (64). Thus it is unclear whether an *S. negevensis*–positive culture from specimens such as bronchoalveolar lavage or nasopharyngeal washings indeed supports an association with disease or rather reflects the ubiquity of these bacteria in our surroundings.

Causal Relationships?

As illustrated above, the ubiquity of chlamydia-like bacteria and their amoeba hosts poses a serious problem for investigating the role of chlamydia-like bacteria as emerging pathogens. In addition, the lack of standardized diagnostic tests further aggravates the assessment of the actual prevalence of chlamydia-like bacteria in clinical specimens. Therefore, despite much work that has been done in this field, the key question remains, Is there a causal relationship between chlamydia-like bacteria and human disease? To answer this, the guidelines

summarized by Fredericks & Relman (28) in their reconsideration of Koch's Postulates might be helpful. In brief, the putative pathogen should be present in most cases of an infectious disease and should be found preferentially in those anatomic sites known to be diseased. The pathogen should be visualized at the cellular level in these specimens, and it should be absent or less abundant in hosts or tissues without disease. With resolution of disease, the evidence of the presence of the pathogen should decrease. A causal relationship is more likely if the abundance of the pathogen correlates with severity of disease. The evidence of a causal relationship should be reproducible (28), i.e., concordant evidence should come from different approaches applied by different groups, at different time points, and in different places (3). To date, virtually none of these conditions has been fulfilled for chlamydia-like bacteria and human disease. Demonstrating a causal relationship thus remains a great challenge. Perhaps, chlamydia-like bacteria are opportunistic pathogens rather than a serious threat for healthy individuals.

Infections of Animals

Chlamydia-like bacteria infect a wide range of animals, in addition to humans (**Table 1**). In this context, the association of "*Candidatus* Piscichlamydia salmonis," "*Candidatus* Clavochlamydia salmonicola," and some unnamed chlamydia-like bacteria with epitheliocystis in fish and sea dragons seems to be supported particularly well (22, 68, 80). Although these organisms have not yet been obtained in cell culture, they were identified by sequencing their 16S rRNA genes, and they could be localized in epitheliocystis cysts in the gills using in situ hybridization and electron microscopy (22, 68). In addition, an association of *Waddlia chondrophila*, repeatedly isolated from aborted bovine fetuses (50, 94), with abortion in cattle was suggested (21), but more data are required to substantiate this. Future studies investigating the prevalence of chlamydia-like bacteria in animals and their role as potential veterinary pathogens are urgently needed.

BIOLOGY OF *CHLAMYDIA*-LIKE BACTERIA

Irrespective of their pathogenic potential, chlamydia-like bacteria deserve attention because they are the closest relatives of the *Chlamydiaceae*. Our knowledge about this diverse group is still scarce, primarily because of the obligate intracellular lifestyle, which hampers the ability to study these elusive microorganisms. Most available data come from the model organisms *Protochlamydia amoebophila*, *Parachlamydia acanthamoebae*, and *Simkania negevensis*.

Developmental Cycle

All chlamydia-like bacteria characterized so far show a developmental cycle similar to the *Chlamydiaceae*, with morphologically and physiologically distinct stages. Considerable diversity, however, exists with respect to the ultrastructure of the developmental forms. The coccoid EBs and RBs of *Parachlamydia acanthamoebae*, *Protochlamydia amoebophila*, *Neochlamydia hartmannellae*, *Simkania negevensis*, and *Waddlia chondrophila* resemble those of the *Chlamydiaceae*, whereas others like "*Candidatus* Clavochlamydia salmonicola," *Rhabdochlamydia* spp., and *Criblamydia sequanensis* show morphologically different developmental stages. In "*Candidatus* Clavochlamydia salmonicola" the proposed EBs were elongated and consisted of a pronounced head and tail region (68). *Rhabdochlamydia* spp. EBs were rod-shaped, possessed a five-layered cell wall, and showed elongate electron-translucent structures in the cytoplasm (15, 71). *C. sequanensis* EBs also possessed a five-layer cell wall but showed a star-like morphology (99).

The developmental cycle of *Parachlamydia acanthamoebae* and *Protochlamydia amoebophila* in their natural hosts, *Acanthamoeba* spp., starts with the uptake of EBs by phagocytosis (**Figure 3**, step A). Subsequently, EBs differentiate to RBs, which divide by binary fission to form multiple larger inclusions in the case of *P. acanthamoebae* (41, 81) or multiple single-cell inclusions in the case of *P. amoebophila* (13, 31)

(**Figure 3**, step B). Division of RBs can proceed coordinated with host cell division, leading to a long-term coexistence of the bacteria with their *Acanthamoeba* host (**Figure 3**, step D). At some point RBs redifferentiate back to EBs (**Figure 3**, step C) and are then released from the host cell within vesicles or by lysis of the amoebae to begin a new infection cycle (**Figure 3**, steps F and G) (41, 81). Lysis of the amoeba host is temperature dependent. *P. acanthamoebae* was lytic at temperatures above 32°C but showed a less cytopathic effect at temperatures between 25°C and 30°C, which are closer to the natural conditions in the environment (38). A third developmental form, the crescent body, has been observed for *P. acanthamoebae* and was mainly associated with prolonged incubation time (41). Like EBs, crescent bodies were also considered an infectious stage of *P. acanthamoebae*.

Although the inclusion is the preferred location of chlamydia-like bacteria in amoebae, *P. acanthamoebae* and *P. amoebophila* have been observed outside the inclusion too, residing directly in the cytoplasm (31, 41, 81). This was particularly pronounced for *N. hartmannellae*, in which no inclusion membrane was visible within the *Hartmannella* host (54). *P. acanthamoebae* largely inhibits cyst formation of its amoeba host (41, 81), whereas *P. amoebophila* and *S. negevensis* seem to survive the encystment and were also found within *Acanthamoeba* cysts (31, 59) (**Figure 3**, step E). *Acanthamoeba* cysts are stable and resistant against heat, desiccation, and disinfectants (78), thereby protecting intracellular bacteria from adverse environmental conditions and facilitating long-term survival in the environment (59). Developmental stages corresponding to persistent forms of the *Chlamydiaceae*, which have been implicated in chronic disease, have not been observed in acanthamoebae so far, but they were reported for *S. negevensis* in mammalian cells (61).

In summary, the existence of a developmental cycle is a feature conserved in all known chlamydiae, although differences with respect to developmental stages, their subcellular locations, and outcome are apparent. Time course analysis of gene expression using DNA microarrays has recently contributed to our understanding of the *Chlamydiaceae* developmental cycle (1). Future research should reveal the extent to which molecular mechanisms underlying the unique chlamydial developmental cycle are conserved in chlamydia-like bacteria.

Metabolism

Our current knowledge about the metabolism of chlamydia-like bacteria is based almost exclusively on comparative genome analysis of *Protochlamydia amoebophila*, to date the only chlamydia-like organism for which a genome sequence is available (51). Major differences may exist in other chlamydia-like bacteria.

The *Chlamydiaceae* have small (1 to 1.2 Mb), streamlined genomes, which are highly conserved in both gene content (sharing roughly 800 genes) and gene order (20, 51, 90, 96). The *Chlamydiaceae* are highly adapted to their intracellular lifestyle and they rely on their host cells with respect to a number of metabolic key intermediates. Despite the greater size of the *P. amoebophila* genome (2.4 Mb), a similar host dependence has been predicted (51, 52). Amino acid and cofactor biosynthesis pathways are largely absent in *P. amoebophila*, and most genes required for de novo synthesis of nucleotides are missing. Nevertheless, glycolysis and pentose phosphate pathways are encoded on the *P. amoebophila* genome, and in contrast to the *Chlamydiaceae* a complete gene set for the tricarboxylic acid cycle is present, suggesting that *P. amoebophila* is somewhat less dependent on its host cell compared with the *Chlamydiaceae* (51, 52). The respiratory chain predicted for *P. amoebophila* should be more versatile and efficient owing to the presence of a number of additional components (51, 52).

Although *P. amoebophila* is fully equipped to regenerate its own ATP, like the *Chlamydiaceae*, it encodes an ATP/ADP translocase. This transport protein catalyzes the import of host ATP into the bacterial cell in exchange for ADP and thus enables a life as energy parasite

(95). The ATP/ADP translocase of *P. amoebophila* has recently been characterized in detail and revealed the functional basis of bacterial energy parasitism by nucleotide transport proteins (100). The *P. amoebophila* ATP/ADP translocase is independent from the membrane potential and is stimulated by a high internal ADP/ATP ratio. However, it is functional only if the N terminus is directed toward the bacterial cytoplasm, thereby ensuring that it does not work in a mode detrimental to *P. amoebophila* (100). Four paralogs of this nucleotide transport protein are encoded in the *P. amoebophila* genome. Their concerted action facilitates the import of all RNA nucleotides and NAD^+ into *P. amoebophila* in a highly sophisticated manner (44, 45).

Host Cell Interaction

Attachment and entry are the earliest interactions between chlamydiae and their host cells during the developmental cycle, processes mediated primarily by the chlamydial cell envelope. In particular, the protein composition of the outer membrane of *P. amoebophila* seems fundamentally different from that of the *Chlamydiaceae* (48, 51, 52). Comparative genomics predicted only two conserved proteins, the cysteine-rich proteins OmcA and OmcB. Other important proteins present in the outer membrane of the *Chlamydiaceae*, including the major outer membrane protein (OmpA) and polymorphic membrane proteins, have no recognized homologs in *P. amoebophila*. These differences might reflect the different host range of the amoeba symbiont and its pathogenic counterparts, but further research is required to better characterize the cell envelope of *P. amoebophila* and other chlamydia-like bacteria.

The type three secretion system (T3SS) is another key mechanism of the *Chlamydiaceae* for host cell interaction (87). Analysis of the *P. amoebophila* genome and preliminary data from the *S. negevensis* genome sequence showed that it is well conserved among all chlamydiae (51, 52, 87). This complex, consisting of more than 20 different proteins, forms a molecular syringe that can be used to inject effector proteins into the host cytosol. Consistently, some effector proteins known from the *Chlamydiaceae* were also predicted in the *P. amoebophila* genome, for example, the serine/threonine protein kinase Pkn5, the inclusion protein IncA, and other proteins targeted to the host-derived inclusion membrane (51, 52). A few additional proteins that have been associated with pathogenicity of chlamydiae are also present in *P. amoebophila*, including the chlamydia protease-like activity factor (CPAF), whereas others such as the actin-recruiting protein Tarp and CADD (chlamydia protein associating with death domains) are notably absent.

Among proteins that were found in the genome of *P. amoebophila* but not in the *Chlamydiaceae* were a large number of leucine-rich repeat proteins, generally considered to be involved in protein-protein interactions and/or in recognition of bacterial motifs (24, 51, 52). *P. amoebophila* encodes a type four secretion system (T4SS) in a region with a G+C content significantly different from the genomic G+C content, suggesting that this genomic island has been acquired by lateral gene transfer (35, 51, 52). This is further supported by the finding of a similar transport system in the genomes of several *Rickettsia* species (6). The T4SS of *P. amoebophila* is most similar to that of the F plasmid of *Escherichia coli* (35) and could thus be used either for protein secretion or for conjugation. Experimental data for either hypothesis are not yet available.

Apart from the *Protochlamydia amoebophila* genome sequence, some in vitro data on host cell interaction is available for *Parachlamydia acanthamoebae* with human cells. Trafficking of *P. acanthamoebae* in macrophages (through the endocytic pathway) was fundamentally different from that of the *Chlamydiaceae* (39). Further differences between *P. acanthamoebae* and the *Chlamydiaceae* regarding cytokine production were observed. *P. acanthamoebae* did not induce an oxidative burst or the proinflammatory cytokines IL-6 and TNF-α in macrophages (36),

T3SS: type three secretion system

T4SS: type four secretion system

perhaps because of the differences in the cell envelope discussed above. However, in essence, despite large evolutionary differences between chlamydia-like bacteria and the *Chlamydiaceae* several basic mechanisms for host-cell interaction are conserved among all chlamydiae and are used by amoeba symbionts and human pathogens alike.

EVOLUTIONARY HISTORY OF THE *CHLAMYDIAE*

The evolution of the chlamydiae is intriguing. Although little is known about the early time points in their evolutionary history, the few hypotheses proposed have implications far beyond the chlamydiae.

A Last Common Intracellular Ancestor

Chlamydial genomes show hardly any evidence of recent lateral gene transfer (with the T4SS of *P. amoebophila* as a rare exception) (51, 90, 96). The genome sequence of *P. amoebophila*, twice as large as the genomes of the *Chlamydiaceae*, could thus be used to partially reconstruct the genome of the last common ancestor of this amoeba symbiont and its pathogenic counterparts (51, 52). On the basis of this analysis, the chlamydial ancestor already employed transport proteins, still present in all extant chlamydiae, such as the nucleotide transport proteins or a glucose-phosphate transporter. Development and conservation of such transport proteins can be explained most likely by an intracellular environment. It is thus most parsimonious that the last common ancestor of *P. amoebophila* and the *Chlamydiaceae* has already lived inside a eukaryotic host cell (51, 52). Consistent with this hypothesis, phylogenetic analysis of key mechanisms for host cell interaction, such as the T3SS or CPAF, shows that these mechanisms were already present in the last common chlamydial ancestor (51, 52). In the case of the T3SS, this is also supported by the highly conserved order of genes encoding this protein complex. In contrast to all other bacteria, chlamydial T3SS genes are located on multiple regions on the chromosome and are largely syntenic between the *Chlamydiaceae*, *P. amoebophila*, and *S. negevensis* (51, 87).

Roughly estimated, *Protochlamydia* and the *Chlamydiaceae* split at least 700 mya—at some time point in the Precambrian, when unicellular eukaryotes were abundant. The last common chlamydial ancestor has therefore most likely lived in some kind of primordial protist (51). If this is true, then the major mechanisms still used today by symbiotic and pathogenic chlamydiae were developed during this early interaction. Ancient protozoa could thus have served as evolutionary training grounds for the development of the intracellular lifestyle of chlamydiae.

Chlamydiae and the Origin of Plants

All chlamydial genomes share an intriguing feature: They possess more plant- and cyanobacteria-like proteins than most other bacterial genomes. Even more puzzling, most plant-like proteins of chlamydiae are targeted to and function in plastids (7, 51, 89, 93, 96, 104). Among the chlamydiae, this is most obvious in *P. amoebophila*, which showed more than 150 proteins with highest sequence similarity to proteins of plants or cyanobacteria (51). Phylogenetic analysis of these proteins did not reveal a consistent picture of their evolutionary history but suggested a primordial association of chlamydiae with cyanobacteria, plastids, and/or plants, including complex ancestral gene transfers between these groups (51). Such an association is further supported by the presence of a group I intron in the 23S rRNA of *Simkania negevensis* and "*Candidatus* Fritschea bemisiae" and by evidence of a past presence of a group I intron in the *Chlamydiaceae* (26). Group I introns are widespread in algal chloroplasts, in lower eukaryotes, and archaea, but they are notably absent in most bacteria.

Different scenarios have been put forward to explain these observations. It has been suggested that the presence of plant-like genes in chlamydiae reflects an evolutionary

relationship between chlamydiae and the cyanobacterial ancestor of the chloroplast (7). Such a relationship is, however, not well supported by phylogenetic trees based on rRNA genes or ribosomal proteins (101). Alternatively, horizontal gene transfer events either from plants to chlamydiae (104) or from chlamydiae to plants (26, 93, 95) have been proposed, and it was speculated that chlamydiae participated in the ancient chimeric events that led to the formation of the plant and animal lineages (26). Indeed, recent phylogenomic analysis of the red alga *Cyanidioschyzon merolae* shed some light on this discussion and supported the latter hypothesis (55).

In this study, Huang & Gogarten detected 21 genes that were transferred between chlamydiae and *C. merolae* and green plants, with the donor most similar to *P. amoebophila*. They argued that the gene transfer events between chlamydiae and primary photosynthetic eukaryotes can be best explained by a stable association between these groups in the past, and they proposed a scenario in which a chlamydial symbiont similar to *P. amoebophila* facilitated the establishment of primary plastids (55). According to their hypothesis, the chlamydial symbiont entered a mitochondrion-containing eukaryote at about the same time the cyanobacterial ancestor of chloroplasts was captured. The eukaryotic host subsequently acquired transport proteins via lateral gene transfer from the chlamydial symbiont, which facilitated interactions between the cyanobacterial symbiont and its heterotrophic host cell. While the cyanobacterial symbiont was transformed to a photosynthetic organelle, the chlamydial symbiont might have gradually degenerated or transformed into an organelle not yet recognized in photosynthetic eukaryotes (55). Although it might not fully explain the observed high number of cyanobacteria-like proteins in chlamydial genomes, this is the most parsimonious scenario put forth. This hypothesis is intriguing because it implies that an ancient chlamydial symbiosis might have given rise to the first photosynthetic eukaryote, the ancestor of all extant plants on earth.

The Closest Free-Living Relatives

Even though chlamydiae have been detected in a wide variety of habitats by molecular methods, all known chlamydiae are obligate intracellular bacteria, and currently it seems questionable whether extant chlamydiae can live independent of a eukaryotic host cell. In view of the lack of such evidence, it might be worthwhile to search for the closest free-living relatives of chlamydiae. The phylum *Chlamydiae* is deeply branching in the domain Bacteria, and evolutionary relationships among most bacterial phyla are presently not resolved. Recent evidence, however, suggested a monophyletic grouping of the *Chlamydiae* with the phyla *Verrucomicrobia* and *Planctomycetes* (97, 101), as these groups share a number of unique features (101). A common ancestral origin of *Chlamydiae* and *Verrucomicrobia* is supported particularly well (42), indicating that the *Verrucomicrobia* are at present the closest free-living relatives of the *Chlamydiae*. The analysis of *Verrucomicrobia*, particularly of those members that live in association with eukaryotes, might reveal clues about evolutionary times at which chlamydiae were still free-living or facultative symbionts of eukaryotes.

PERSPECTIVES

From an evolutionary perspective, two chlamydia-like bacteria deserve special attention, the uncultured "*Candidatus* Piscichlamydia salmonis" and "*Candidatus* Clavochlamydia salmonicola." "*Ca.* Piscichlamydia salmonis" is particularly interesting because it currently represents the deepest branch in the *Chlamydiae* (**Figure 1**), i.e., it might still share features of the last common ancestor of all chlamydiae, which are absent in all other chlamydial lineages. "*Ca.* Clavochlamydia salmonicola" is the closest relative of the *Chlamydiaceae* (**Figure 1**) and might thus represent a transitional stage between the highly adapted human and animal pathogens of the *Chlamydiaceae* and all other chlamydia-like bacteria. Novel approaches and technologies such as whole-genome

amplification and pyrosequencing might facilitate genome analysis of these organisms in the near future.

Global transcriptional and proteomic analysis of chlamydia-like bacteria will help us to better understand their biology, as well as differences and similarities to the *Chlamydiaceae*. The use of the well-characterized amoeba *Dictyostelium discoideum* as a surrogate host for chlamydial symbionts might help to shed new light on host-bacteria interaction. Further investigation of the role of some chlamydia-like bacteria, particularly *P. acanthamoebae*, *S. negevensis*, and *W. chondrophila*, with respect to their pathogenic potential toward humans is warranted; an animal model might be helpful in this regard as well. Without doubt, recent studies have greatly increased our knowledge of diversity, evolution, and biology of the chlamydiae and their distribution in nature, but we are only beginning to understand one of the most enigmatic groups in the domain *Bacteria*.

SUMMARY POINTS

1. Members of the phylum *Chlamydiae* are a phylogenetically diverse group of obligate intracellular bacteria. *Chlamydiae* consist of at least eight recognized families. Molecular evidence suggests an additional yet unexplored diversity of chlamydiae in the environment.
2. All known chlamydiae show the characteristic chlamydial developmental cycle, even though morphology, number, and presumably also the physiology of their developmental stages vary between different chlamydiae.
3. Free-living amoebae, particularly of the genus *Acanthamoeba*, are frequent hosts of chlamydiae in the environment and might play an important role for the survival and dispersal of chlamydiae in nature. The host range of chlamydiae includes diverse representatives across the animal kingdom.
4. *Parachlamydia acanthamoebae* and *Simkania negevensis* have been associated with respiratory disease in humans, yet a causal link has not been established.
5. The amoeba symbiont *Protochlamydia amoebophila* is auxotrophic for a number of essential cell building blocks. It has retained several proteins associated with virulence of the *Chlamydiaceae*, including a T3SS.
6. Nucleotide transport proteins, particularly ATP/ADP translocases, are a hallmark of chlamydiae and catalyze a sophisticated interaction with their host cells.
7. *Chlamydiae* have acquired basic mechanisms for host cell interaction in ancient unicellular eukaryotes and have lived in association with eukaryotic hosts for several hundreds of millions of years. *Chlamydiae* might have been involved in the ancient symbiotic events that led to the emergence of the first photosynthetic eukaryote.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The author would like to sincerely apologize to authors of publications that were omitted for space considerations or accidentally. Susanne Haider, Eva Heinz, Jacqueline Montanaro, Stephan Schmitz-Esser, and Elena Toenshoff are greatly acknowledged for help with preparing figures and tables and for comments on the manuscript. The author is grateful to Tom Fritsche, who has initiated our work on chlamydia-like bacteria, and to Michael Wagner, an inspiring mentor and colleague. This work was supported by Austrian Science Fund (FWF) grant Y277-B03.

LITERATURE CITED

1. Abdelrahman YM, Belland RJ. 2005. The chlamydial developmental cycle. *FEMS Microbiol. Rev.* 29:949–59
2. Amann R, Springer N, Schonhuber W, Ludwig W, Schmid EN, et al. 1997. Obligate intracellular bacterial parasites of acanthamoebae related to *Chlamydia* spp. *Appl. Environ. Microbiol.* 63:115–21
3. Apfalter P. 2006. *Chlamydia pneumoniae*, stroke and serological associations. *Stroke* 37:756–58
4. Baud D, Thomas V, Arafa, Regan L, Greub G. 2007. *Waddlia chondrophila*, a potential agent of human fetal death. *Emerg. Infect. Dis.* 13:1239–43
5. Birtles RJ, Rowbotham TJ, Storey C, Marrie TJ, Raoult D. 1997. *Chlamydia*-like obligate parasite of free-living amoebae. *Lancet* 349:925–26
6. Blanc G, Ogata H, Robert C, Audic S, Claverie JM, Raoult D. 2007. Lateral gene transfer between obligate intracellular bacteria: evidence from the *Rickettsia massiliae* genome. *Genome Res.* 17:1657–64
7. Brinkman FS, Blanchard JL, Cherkasov A, Av-Gay Y, Brunham RC, et al. 2002. Evidence that plant-like genes in *Chlamydia* species reflect an ancestral relationship between *Chlamydiaceae*, cyanobacteria, and the chloroplast. *Genome Res.* 12:1159–67
8. Casson N, Medico N, Bille J, Greub G. 2006. *Parachlamydia acanthamoebae* enters and multiplies within pneumocytes and lung fibroblasts. *Microbes Infect.* 8:1294–300
9. Casson N, Michel R, Müller K-D, Aubert JD, Greub G. 2008. *Protochlamydia naegleriophila* as etiologic agent of pneumonia. *Emerg. Infect. Dis.* 14:168–72
10. Chappell CL, Wright JA, Coletta M, Newsome AL. 2001. Standardized method of measuring *Acanthamoeba* antibodies in sera from healthy human subjects. *Clin. Diagn. Lab. Immun.* 8:724–30
11. Chua PK, Corkill JE, Hooi PS, Cheng SC, Winstanley C, Hart CA. 2005. Isolation of *Waddlia malaysiensis*, a novel intracellular bacterium, from fruit bat (*Eonycteris spelaea*). *Emerg. Infect. Dis.* 11:271–77
12. Collingro A, Poppert S, Heinz E, Schmitz-Esser S, Essig A, et al. 2005. Recovery of an environmental chlamydia strain from activated sludge by cocultivation with *Acanthamoeba* sp. *Microbiology* 151:301–9
13. Collingro A, Toenshoff ER, Taylor MW, Fritsche TR, Wagner M, Horn M. 2005. '*Candidatus* Protochlamydia amoebophila', an endosymbiont of *Acanthamoeba* spp. *Int. J. Syst. Evol. Microbiol.* 55:1863–66
14. Corsaro D, Greub G. 2006. Pathogenic potential of novel *Chlamydiae* and diagnostic approaches to infections due to these obligate intracellular bacteria. *Clin. Microbiol. Rev.* 19:283–97
15. Corsaro D, Thomas V, Goy G, Venditti D, Radek R, Greub G. 2007. '*Candidatus* Rhabdochlamydia crassificans', an intracellular bacterial pathogen of the cockroach *Blatta orientalis* (Insecta: Blattodea). *Syst. Appl. Microbiol.* 30:221–28
16. Corsaro D, Valassina M, Venditti D. 2003. Increasing diversity within *Chlamydiae*. *Crit. Rev. Microbiol.* 29:37–78
17. Corsaro D, Venditti D, Le Faou A, Guglielmetti P, Valassina M. 2001. A new chlamydia-like 16S rDNA sequence from a clinical sample. *Microbiology* 147:515–16
18. Corsaro D, Venditti D, Valassina M. 2002. New parachlamydial 16S rDNA phylotypes detected in human clinical samples. *Res. Microbiol.* 153:563–67
19. De Bary A. 1879. *Die Erscheinung der Symbiose*. Strassburg: Verlag von Karl J. Trubner
20. Dean D, Myers GS, Read TD. 2006. Lessons and challenges arising from the 'first wave' of *Chlamydia* genome sequencing. In *Chlamydia: Genomics and Pathogenesis*, ed. PM Bavoil, PB Wyrick, pp. 1–24. Norfolk: Horizon Bioscience

21. Dilbeck-Robertson P, McAllister MM, Bradway D, Evermann JF. 2003. Results of a new serologic test suggest an association of *Waddlia chondrophila* with bovine abortion. *J. Vet. Diagn. Invest.* 15:568–69

22. Draghi A 2nd, Popov VL, Kahl MM, Stanton JB, Brown CC, et al. 2004. Characterization of "*Candidatus* piscichlamydia salmonis" (order *Chlamydiales*), a chlamydia-like bacterium associated with epitheliocystis in farmed Atlantic salmon (*Salmo salar*). *J. Clin. Microbiol.* 42:5286–97

23. Essig A, Heinemann M, Simnacher U, Marre R. 1997. Infection of *Acanthamoeba castellanii* by *Chlamydia pneumoniae*. *Appl. Environ. Microbiol.* 63:1396–99

24. Eugster M, Roten CA, Greub G. 2007. Analyses of six homologous proteins of *Protochlamydia amoebophila* UWE25 encoded by large GC-rich genes (lgr): a model of evolution and concatenation of leucine-rich repeats. *BMC Evol. Biol.* 7:231

25. Everett KD, Bush RM, Andersen AA. 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.* 49:415–40

26. Everett KD, Kahane S, Bush RM, Friedman MG. 1999. An unspliced group I intron in 23S rRNA links *Chlamydiales*, chloroplasts, and mitochondria. *J. Bacteriol.* 181:4734–40

27. Fasoli L, Paldanius M, Don M, Valent F, Vetrugno L, et al. 2007. *Simkania negevensis* in community-acquired pneumonia in Italian children. *Scand. J. Infect. Dis.* 99999:1–4

28. Fredericks DN, Relman DA. 1996. Sequence-based identification of microbial pathogens: a reconsideration of Koch's Postulates. *Clin. Microbiol. Rev.* 9:18–33

29. Friedman MG, Kahane S, Dvoskin B, Hartley JW. 2006. Detection of *Simkania negevensis* by culture, PCR, and serology in respiratory tract infection in Cornwall, UK. *J. Clin. Pathol.* 59:331–33

30. Fritsche TR, Gautom RK, Seyedirashti S, Bergeron DL, Lindquist TD. 1993. Occurrence of bacteria endosymbionts in *Acanthamoeba* spp. isolated from corneal and environmental specimens and contact lenses. *J. Clin. Microbiol.* 31:1122–26

31. Fritsche TR, Horn M, Wagner M, Herwig RP, Schleifer KH, Gautom RK. 2000. Phylogenetic diversity among geographically dispersed *Chlamydiales* endosymbionts recovered from clinical and environmental isolates of *Acanthamoeba* spp. *Appl. Environ. Microbiol.* 66:2613–19

32. Greenberg D, Banerji A, Friedman MG, Chiu CH, Kahane S. 2003. High rate of *Simkania negevensis* among Canadian Inuit infants hospitalized with lower respiratory tract infections. *Scand. J. Infect. Dis.* 35:506–8

33. Greub G, Berger P, Papazian L, Raoult D. 2003. *Parachlamydiaceae* as rare agents of pneumonia. *Emerg. Infect. Dis.* 9:755–56

34. Greub G, Boyadjiev I, La Scola B, Raoult D, Martin C. 2003. Serological hint suggesting that *Parachlamydiaceae* are agents of pneumonia in polytraumatized intensive care patients. *Ann. N. Y. Acad. Sci.* 990:311–19

35. Greub G, Collyn F, Guy L, Roten CA. 2004. A genomic island present along the bacterial chromosome of the *Parachlamydiaceae* UWE25, an obligate amoebal endosymbiont, encodes a potentially functional F-like conjugative DNA transfer system. *BMC Microbiol.* 4:48

36. Greub G, Desnues B, Raoult D, Mege JL. 2005. Lack of microbicidal response in human macrophages infected with *Parachlamydia acanthamoebae*. *Microbes Infect.* 7:714–19

37. Greub G, Hartung O, Adekambi T, Alimi YS, Raoult D. 2006. *Chlamydia*-like organisms and atherosclerosis. *Emerg. Infect. Dis.* 12:705–6

38. Greub G, La Scola B, Raoult D. 2003. *Parachlamydia acanthamoebae* is endosymbiotic or lytic for *Acanthamoeba polyphaga* depending on the incubation temperature. *Ann. N. Y. Acad. Sci.* 990:628–34

39. Greub G, Mege JL, Gorvel JP, Raoult D, Meresse S. 2005. Intracellular trafficking of *Parachlamydia acanthamoebae*. *Cell Microbiol.* 7:581–89

40. Greub G, Mege JL, Raoult D. 2003. *Parachlamydia acanthamoebae* enters and multiplies within human macrophages and induces their apoptosis. *Infect. Immun.* 71:5979–85

41. Greub G, Raoult D. 2002. Crescent bodies of *Parachlamydia acanthamoebae* and its life cycle within *Acanthamoeba polyphaga*: an electron micrograph study. *Appl. Environ. Microbiol.* 68:3076–84

42. Griffiths E, Gupta RS. 2007. Phylogeny and shared conserved inserts in proteins provide evidence that *Verrucomicrobia* are the closest known free-living relatives of *Chlamydiae*. *Microbiology* 153:2648–54

22. Description of '*Candidatus* Piscichlamydia salmonis,' currently representing the deepest branch in the phylum *Chlamydiae*.

28. Revision of Koch's Postulates to meet current methods for the identification of pathogens.

30. First morphological description of chlamydia-like bacteria as symbionts in free-living amoebae.

40. First report showing survival and multiplication of *P. acanthamoebae* in human cells.

43. Guindon S, Lethiec F, Duroux P, Gascuel O. 2005. PHYML online—a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res.* 33:W557–59
44. Haferkamp I, Schmitz-Esser S, Linka N, Urbany C, Collingro A, et al. 2004. A candidate NAD^+ transporter in an intracellular bacterial symbiont related to *Chlamydiae*. *Nature* 432:622–25
45. Haferkamp I, Schmitz-Esser S, Wagner M, Neigel N, Horn M, Neuhaus HE. 2006. Tapping the nucleotide pool of the host: novel nucleotide carrier proteins of *Protochlamydia amoebophila*. *Mol. Microbiol.* 60:1534–45
46. Haider S, Collingro A, Walochnik J, Wagner M, Horn M. 2008. Chlamydia-like bacteria in respiratory samples of community-acquired pneumonia patients. *FEMS Microbiol. Lett.* 281:198–202
47. Halberstädter L, Prowazek SV. 1907. Über Zelleinschlüsse parasitärer Natur beim Trachom. *Arbeiten aus dem Kaiserlichen Gesundheitsamte, Berlin* 26:44–47
48. Hatch TP. 1999. Developmental biology. In *Chlamydia*, ed. RS Stephens, pp. 29–67. Washington, DC: ASM
49. Heiskanen-Kosma T, Paldanius M, Korppi M. 2008. *Simkania negevensis* may be a true cause of community acquired pneumonia in children. *Scand. J. Infect. Dis.* 40:127–30
50. Henning K, Schares G, Granzow H, Polster U, Hartmann M, et al. 2002. *Neospora caninum* and *Waddlia chondrophila* strain 2032/99 in a septic stillborn calf. *Vet. Microbiol.* 85:285–92
51. Horn M, Collingro A, Schmitz-Esser S, Beier CL, Purkhold U, et al. 2004. Illuminating the evolutionary history of chlamydiae. *Science* 304:728–30
52. Horn M, Collingro A, Schmitz-Esser S, Wagner M. 2006. Environmental chlamydia genomics. In *Chlamydia: Genomics and Pathogenesis*, ed. PM Bavoil, PB Wyrick, pp. 25–44. Norfolk, UK: Horizon Bioscience
53. Horn M, Wagner M. 2001. Evidence for additional genus-level diversity of *Chlamydiales* in the environment. *FEMS Microbiol. Lett.* 204:71–74
54. Horn M, Wagner M, Muller KD, Schmid EN, Fritsche TR, et al. 2000. *Neochlamydia hartmannellae* gen. nov., sp. nov. (*Parachlamydiaceae*), an endoparasite of the amoeba *Hartmannella vermiformis*. *Microbiology* 146:1231–39
55. Huang J, Gogarten J. 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biol.* 8:R99
56. Husain S, Kahane S, Friedman MG, Paterson DL, Studer S, et al. 2007. *Simkania negevensis* in bronchoalveolar lavage of lung transplant recipients: a possible association with acute rejection. *Transplantation* 83:138–43
57. Israelsson O. 2007. Chlamydial symbionts in the enigmatic *Xenoturbella* (Deuterostomia). *J. Invertebr. Pathol.* 96:213–20
58. Jaenicke L. 2001. Stanislaus von Prowazek (1875–1915)—prodigy between working bench and coffee house. *Protist* 152:157–66
59. Kahane S, Dvoskin B, Mathias M, Friedman MG. 2001. Infection of *Acanthamoeba polyphaga* with *Simkania negevensis* and *S. negevensis* survival within amoebal cysts. *Appl. Environ. Microbiol.* 67:4789–95
60. Kahane S, Everett KD, Kimmel N, Friedman MG. 1999. *Simkania negevensis* strain ZT: growth, antigenic and genome characteristics. *Int. J. Syst. Bacteriol.* 49(Pt. 2):815–20
61. Kahane S, Fruchter D, Dvoskin B, Friedman MG. 2007. Versatility of *Simkania negevensis* infection in vitro and induction of host cell inflammatory cytokine response. *J. Infect.* 55:e13–21
62. Kahane S, Gonen R, Sayada C, Elion J, Friedman MG. 1993. Description and partial characterization of a new chlamydia-like microorganism. *FEMS Microbiol. Lett.* 109:329–34
63. Kahane S, Greenberg D, Friedman MG, Haikin H, Dagan R. 1998. High prevalence of "Simkania Z," a novel *Chlamydia*-like bacterium, in infants with acute bronchiolitis. *J. Infect. Dis.* 177:1425–29
64. Kahane S, Greenberg D, Newman N, Dvoskin B, Friedman MG. 2007. Domestic water supplies as a possible source of infection with *Simkania*. *J. Infect.* 54:75–81
65. Kahane S, Kimmel N, Friedman MG. 2002. The growth cycle of *Simkania negevensis*. *Microbiology* 148:735–42
66. Kahane SEM, Friedman MG. 1995. Evidence that the novel microorganism 'Z' may belong to a new genus in the family *Chlamydiaceae*. *FEMS Microbiol. Lett.* 126:203–8

45. Comprehensive biochemical analysis of nucleotide transport proteins of *P. amoebophila*.

47. The original report on the discovery of *Chlamydia trachomatis* as an agent of trachoma.

51. Analysis of the first genome sequence of a chlamydia-like organism, *Protochlamydia amoebophila* UWE25.

55. Elaborate analysis of chlamydia-like proteins in red algae and green plants, with implications for the origin of the first photosynthetic eukaryote.

66. First molecular identification of a chlamydia-like organism, *Simkania negevensis*.

67. Kalayoglu MV, Byrne GI. 2006. The genus *Chlamydia*—medical. In *The Prokaryotes*, ed. M Dworkin, S Falkow, E Rosenberg, K-H Schleifer, E Stackebrandt, pp. 741–54. New York: Springer
68. Karlsen M, Nylund A, Watanabe K, Helvik JV, Nylund S, Plarre H. 2007. Characterization of '*Candidatus* Clavochlamydia salmonicola': an intracellular bacterium infecting salmonid fish. *Environ. Microbiol.* 10:208–18
69. Khan NA. 2006. *Acanthamoeba*: biology and increasing importance in human health. *FEMS Microbiol. Rev.* 30:564–95
70. Korppi M, Paldanius M, Hyvarinen A, Nevalainen A. 2006. *Simkania negevensis* and newly diagnosed asthma: a case-control study in 1- to 6-year-old children. *Respirology* 11:80–83
71. Kostanjsek R, Strus J, Drobne D, Avgustin G. 2004. '*Candidatus* Rhabdochlamydia porcellionis', an intracellular bacterium from the hepatopancreas of the terrestrial isopod *Porcellio scaber* (Crustacea: Isopoda). *Int. J. Syst. Evol. Microbiol.* 54:543–49
72. Kumar S, Kohlhoff SA, Gelling M, Roblin PM, Kutlin A, et al. 2005. Infection with *Simkania negevensis* in Brooklyn, New York. *Pediatr. Infect. Dis. J.* 24:989–92
73. Kuo C-C, Horn M, Stephens RS. 2008. The order *Chlamydiales*. In *Bergey's Manual of Systematic Bacteriology: The Planctomycetes, Spriochaetes, Fibrobacteres, Bacteriodetes and Fusobacteria*, ed. B Hedlund, NR Krieg, W Ludwig, BJ Paster, JT Staley, et al. New York: Springer. In press
74. Letunic I, Bork P. 2007. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* 23:127–28
75. Lieberman D, Dvoskin B, Lieberman DV, Kahane S, Friedman MG. 2002. Serological evidence of acute infection with the *Chlamydia*-like microorganism *Simkania negevensis* (Z) in acute exacerbation of chronic obstructive pulmonary disease. *Eur. J. Clin. Microbiol. Infect. Dis.* 21:307–9
76. Lieberman D, Kahane S, Lieberman D, Friedman MG. 1997. Pneumonia with serological evidence of acute infection with the *Chlamydia*-like microorganism 'Z'. *Am. J. Respir. Crit. Care Med.* 156:578–82
77. Ludwig W, Strunk O, Westram R, Richter L, Meier H, et al. 2004. ARB: a software environment for sequence data. *Nucleic Acids Res.* 32:1363–71
78. Marciano-Cabral F, Cabral G. 2003. *Acanthamoeba* spp. as agents of disease in humans. *Clin. Microbiol. Rev.* 16:273–307
79. Marrie TJ, Raoult D, La Scola B, Birtles RJ, de Carolis E. 2001. *Legionella*-like and other amoebal pathogens as agents of community-acquired pneumonia. *Emerg. Infect. Dis.* 7:1026–9
80. Meijer A, Roholl PJM, Ossewaarde JM, Jones B, Nowak BF. 2006. Molecular evidence for association of *Chlamydiales* bacteria with epitheliocystis in leafy seadragon (*Phycodurus eques*), silver perch (*Bidyanus bidyanus*), and barramundi (*Lates calcarifer*). *Appl. Environ. Microbiol.* 72:284–90
81. Michel R, Hauröder-Philippczyk B, Müller K-D, Weishaar I. 1994. *Acanthamoeba* from human nasal mucosa infected with an obligate intracellular parasite. *Eur. J. Protistol.* 30:104–10
82. Michel R, Müller KD, Zöller L, Walochnik J, Hartmann M, Schmid EN. 2005. Free-living amoebae serve as a host for the *Chlamydia*-like bacterium *Simkania negevensis*. *Acta Protozool.* 44:113–21
83. Michel R, Steinert M, Zöller L, Hauröder B, Henning K. 2004. Free-living amoebae may serve as hosts for the chlamydia-like bacterium *Waddlia chondrophila* isolated from a aborted bovine foetus. *Acta Protozool.* 43:37–42
84. Moulder JW. 1964. *The Psittacosis Group as Bacteria*. New York: Wiley. 95 pp.
85. Ochman H, Elwyn S, Moran NA. 1999. Calibrating bacterial evolution. *Proc. Natl. Acad. Sci. USA* 96:12638–43
86. Ossewaarde JM, Meijer A. 1999. Molecular evidence for the existence of additional members of the order *Chlamydiales*. *Microbiology* 145:411–17
87. Peters J, Wilson DP, Myers G, Timms P, Bavoil PM. 2007. Type III secretion a la *Chlamydia*. *Trends Microbiol.* 15:241–51
88. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 35:7188–96
89. Read TD, Brunham RC, Shen C, Gill SR, Heidelberg JF, et al. 2000. Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39. *Nucleic Acids Res.* 28:1397–406

90. Read TD, Myers GS, Brunham RC, Nelson WC, Paulsen IT, et al. 2003. Genome sequence of *Chlamydophila caviae* (*Chlamydia psittaci* GPIC): examining the role of niche-specific genes in the evolution of the *Chlamydiaceae*. *Nucleic Acids Res.* 31:2134–47
91. Rodriguez-Zaragoza S. 1994. Ecology of free living amoebae. *Crit. Rev. Microbiol.* 20:225–41
92. Rourke AW, Davis RW, Bradley TM. 1984. A light and electron microscope study of epitheliocystis in juvenile steelhead trout, *Salmo gairdneri* Richardson. *J. Fish Dis.* 7:301–9
93. Royo J, Gimez E, Hueros G. 2000. CMP-KDO synthetase: a plant gene borrowed from gram-negative eubacteria. *Trends Genet.* 16:432–33
94. Rurangirwa FR, Dilbeck PM, Crawford TB, McGuire TC, McElwain TF. 1999. Analysis of the 16S rRNA gene of microorganism 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.* 49:577–81
95. Schmitz-Esser S, Linka N, Collingro A, Beier CL, Neuhaus HE, et al. 2004. ATP/ADP translocases: a common feature of obligate intracellular amoebal symbionts related to *Chlamydiae* and *Rickettsiae*. *J. Bacteriol.* 186:683–91
96. Stephens RS. 1999. Genomic autobiographies of chlamydiae. In *Chlamydia*, ed. RS Stephens, pp. 9–27. Washington, DC: ASM
97. Strous M, Pelletier E, Mangenot S, Rattei T, Lehner A, et al. 2006. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature* 440:790–94
98. Thao ML, Baumann L, Hess JM, Falk BW, Ng JC, et al. 2003. Phylogenetic evidence for two new insect-associated *Chlamydia* of the family *Simkaniaceae*. *Curr. Microbiol.* 47:46–50
99. Thomas V, Casson N, Greub G. 2006. *Criblamydia sequanensis*, a new intracellular *Chlamydiales* isolated from Seine River water using amoebal coculture. *Environ. Microbiol.* 8:2125–35
100. Trentmann O, Horn M, Van Scheltinga ACT, Neuhaus HE, Haferkamp I. 2007. Enlightening energy parasitism by analysis of an ATP/ADP transporter from *Chlamydiae*. *PLoS Biol.* 5:1938–51
101. Wagner M, Horn M. 2006. The *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae* and sister phyla comprise a superphylum with biotechnological and medical relevance. *Curr. Opin. Biotechnol.* 17:241–49
102. WHO. 2001. *Global Prevalence and Incidence of Curable STIs*. Geneva: WHO
103. WHO. 2008. *Priority eye diseases*. **http://www.who.int/blindness/causes/priority/en/index2.html**
104. Wolf YI, Aravind L, Koonin EV. 1999. Rickettsiae and chlamydiae: evidence of horizontal gene transfer and gene exchange. *Trends Genet.* 15:173–75

100. Detailed biochemical analysis of the ATP/ADP translocase of *Protochlamydia amoebophila* revealing the molecular mechanism of energy exploitation.

Annual Review of Microbiology
Volume 62, 2008

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Microbiology* articles may be found at http://micro.annualreviews.org/