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Chlamydiae have always been an enthralling group of organisms. Back in 1907, when the first *Chlamydia* was discovered (now named *Chlamydia trachomatis*), they were described as borderline organisms and only much later recognized as a bacterial phylum (Moulder, 1966). All the presently known *Chlamydiae* are associated with eukaryotic hosts and are obligate intracellular bacteria that undergo a distinctive biphasic life cycle inside cytoplasmic membrane-bound vacuole inclusions (Abdelrahman and Belland, 2005; Kuo et al., 2015). The developmental cycle is characterized by the alternation between two physiologically and morphologically distinct forms, elementary bodies (EBs) and reticulate bodies (RBs). The bacteria are transmitted as inert but highly infectious EBs that, once inside the inclusions, differentiate to metabolically active RBs. The latter undertake several division cycles and transform back to EBs, capable of evading the host cell and infecting others. The cycle is not synchronized i.e., although a young inclusion will consist only of elementary bodies, mature inclusions will most of the time display the different forms (Kuo et al., 2015). Physical changes in the nucleoid structure and in the outer membrane allow the distinction between EBs and RBs but the trigger causing the switch remains a conundrum (Abdelrahman and Belland, 2005; Kuo et al., 2015). Until recently, EBs were believed to be metabolically inactive and their characteristically highly condensed chromatin was thought to cause a complete shutdown of transcription (Barry et al., 1993). However, this dogma has been challenged by recent findings that have shown that transcription and protein biosynthesis takes place in host-free EBs and that major metabolic routes are active in these particles (Haider et al., 2010; Sixt et al., 2013; Omsland et al., 2014).

For a long time, *Chlamydiae* were considered a small bacterial phylum composed of one single family, the *Chlamydiaceae*, which included diverse well-known human and animal pathogens such as the previously mentioned *C. trachomatis*, the causative agent of trachoma and of the worldwide most common sexually-transmitted disease (Everett et al., 1999). But in recent years researchers have shown that *Chlamydiae* are a much more diverse group of bacteria than previously thought as eight new families (*Parachlamydiaceae, Waddliaceae, Simkaniaceae*, *Rhabdochlamydiaceae, Criblamydiaceae, Piscichlamydiaceae, Clavichlamydiaceae, Parilichlamydiaceae*) have been added to the phylum *Chlamydiae*, radically changing our perception of these organisms (Horn, 2008; Taylor-Brown et al., 2015). Furthermore, these findings show

that *Chlamydiae* are not only human or animal pathogens but that they encompass also diverse symbionts of a myriad of hosts such as protists, marine worms and arthropods. These newly discovered chlamydial families are often named "environmental chlamydia" given the range of habitats from where the isolates have been obtained from (Horn, 2008; Taylor-Brown et al., 2015). However, our current knowledge might be just the tip of the iceberg: a recent analysis of metagenomic and amplicon data suggested the existence of up to 181 putative *Chlamydiae* families comprising a large number of yet unidentified members and also of potential hosts and ecological niches (Lagkouvardos et al., 2014). The majority of the newly identified lineages derived from marine habitats, a commonly overlooked environment in the quest for new members of this phylum. Environmental *Chlamydiae* are more closely related to their pathogenic counterparts than to any other bacteria but, in contrast to what is observed for the *Chlamydiaceae*, the genomes of these environmental bacteria are diverse and display a large repertoire of genes likely associated with host-adaptation and ecology (Collingro et al., 2011; Domman and Horn, 2015). Interestingly, strains of environmental *Chlamydiae* have been shown to infect and thrive in human cells, suggesting a possible pathogenic role for some environmental chlamydia (Greub et al., 2003; Casson et al., 2006).

Chlamydiae have been the focus of several studies but the knowledge about this phylum remains limited, particularly for the environmental representatives. Their strict host-dependency has often hindered isolation and detection approaches, and the resulting impossibility to culture them in the laboratory hampered further studies on these bacteria. In this issue of EMI, Pizzetti and coworkers analyzed the chlamydial seasonal dynamics of the coastal lake Lago di Paola and used a new isolation approach that allowed the first co-isolation of a marine chlamydia (tentatively named *Neptunochlamydia vexilliferae*) with its natural host, an amoeba of the family *Vexillifera*. Remarkably, using a combination of a new high-throughput limited dilution approach that strongly limits the competition between protists (Schulz *et al.*, 2015) and super resolution microscopy, the authors directly observed and identified chlamydia-host associations (Figure 1). Besides the successful isolation of *N. vexilliferae* and its host, this procedure lead also to a much more accurate quantification of the chlamydial diversity and dynamics in this environment compared to a previous report (Pizzetti *et al.*, 2012). Curiously, this new isolate had not been detected in a polymerase chain reaction based study performed on Lago di Paola samples, perhaps because it does not belong to any of the major clusters of *Chlamydiae* previously identified in this lake (Pizzetti *et al.*,

2012). This method brings therefore not only significant advances for the isolation of new *Chlamydiae* and other amoeba-associated bacteria, but also for future quantification studies as it allows researchers to identify and study previously overlooked host-associated bacteria. Bearing in mind the strict obligate intracellular lifestyle of *Chlamydiae* and their low abundance in environmental samples compared to other free-living bacteria, analyzing host-associated bacteria will be of major importance for future studies of these organisms, particularly in previously unexploited environments and putative hosts. As highlighted by this study, the seasonality of chlamydia matched the dynamics of host abundance, showing once more the tight connection between the two partners. This work brings also attention to the fact that the observed peak of amoeba abundance matched the time of highest anthropogenic activity in the lake, which might have sanitary implications considering the putative pathogenic potential of some environmental chlamydia.

Noteworthy also, is the fact that Pizzetti and coworkers succeeded to routinely maintain the here identified environmental chlamydia species *N. vexilliferae* and its amoeba host in a laboratory culture. Most of the known *Chlamydiae* families are represented by only a few isolates and the lack of laboratory cultures poses a serious drawback for further studies. In contrast, the fact that Pizzetti and coworkers managed to culture both partners in the lab allows further studies and comparisons to be undertaken between chlamydia having different lifestyles and hosts. Such research is of key importance for in depth understanding of how this group of bacteria evolved, adapted to different hosts and surrounding environments, and emerged as pathogens for animals and humans.

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Figure 1.

Phylogenetic relationship of "Candidatus Neptunochlamydia vexilliferae" in the Chlamydiae and super-resolution microscopy and fluorescence in situ hybridization images showing the newly discovered bacterium (adapted from "Chlamydial seasonal dynamics and isolation of 'Candidatus Neptunochlamydia vexilliferae' from a Tyrrhenian coastal lake by Ilaria Pizzetti, Frederik Schulz, Tomáš Tyml, Bernhard M. Fuchs, Rudolf Amann, Matthias Horn, Stefano Fazi; DOI:10.1111/1462-2920.13111).