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1 **Environmental treasures: co-isolation of the first marine *Chlamydiae* and**  
2 **its protozoan host**

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44 *Chlamydiae* have always been an enthralling group of organisms. Back in 1907,  
45 when the first *Chlamydia* was discovered (now named *Chlamydia trachomatis*), they  
46 were described as borderline organisms and only much later recognized as a bacterial  
47 phylum (Moulder, 1966). All the presently known *Chlamydiae* are associated with  
48 eukaryotic hosts and are obligate intracellular bacteria that undergo a distinctive  
49 biphasic life cycle inside cytoplasmic membrane-bound vacuole inclusions  
50 (Abdelrahman and Belland, 2005; Kuo et al., 2015). The developmental cycle is  
51 characterized by the alternation between two physiologically and morphologically  
52 distinct forms, elementary bodies (EBs) and reticulate bodies (RBs). The bacteria are  
53 transmitted as inert but highly infectious EBs that, once inside the inclusions,  
54 differentiate to metabolically active RBs. The latter undertake several division cycles  
55 and transform back to EBs, capable of evading the host cell and infecting others. The  
56 cycle is not synchronized i.e., although a young inclusion will consist only of elementary  
57 bodies, mature inclusions will most of the time display the different forms (Kuo et al.,  
58 2015). Physical changes in the nucleoid structure and in the outer membrane allow the  
59 distinction between EBs and RBs but the trigger causing the switch remains a  
60 conundrum (Abdelrahman and Belland, 2005; Kuo et al., 2015). Until recently, EBs were  
61 believed to be metabolically inactive and their characteristically highly condensed  
62 chromatin was thought to cause a complete shutdown of transcription (Barry et al.,  
63 1993). However, this dogma has been challenged by recent findings that have shown  
64 that transcription and protein biosynthesis takes place in host-free EBs and that major  
65 metabolic routes are active in these particles (Haider et al., 2010; Sixt et al., 2013;  
66 Omsland et al., 2014).

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

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

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

110 2012). This method brings therefore not only significant advances for the isolation of new  
111 *Chlamydiae* and other amoeba-associated bacteria, but also for future quantification studies as  
112 it allows researchers to identify and study previously overlooked host-associated bacteria.  
113 Bearing in mind the strict obligate intracellular lifestyle of *Chlamydiae* and their low  
114 abundance in environmental samples compared to other free-living bacteria, analyzing  
115 host-associated bacteria will be of major importance for future studies of these  
116 organisms, particularly in previously unexploited environments and putative hosts. As  
117 highlighted by this study, the seasonality of chlamydia matched the dynamics of host  
118 abundance, showing once more the tight connection between the two partners. This work  
119 brings also attention to the fact that the observed peak of amoeba abundance matched the time  
120 of highest anthropogenic activity in the lake, which might have sanitary implications  
121 considering the putative pathogenic potential of some environmental chlamydia.

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

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186 **Figure 1.**

187 Phylogenetic relationship of “*Candidatus Neptunochlamydia vexilliferae*” in the Chlamydiae  
188 and super-resolution microscopy and fluorescence in situ hybridization images showing the  
189 newly discovered bacterium (adapted from “Chlamydial seasonal dynamics and isolation of  
190 ‘*Candidatus Neptunochlamydia vexilliferae*’ from a Tyrrhenian coastal lake by Ilaria Pizzetti,  
191 Frederik Schulz, Tomáš Tým, Bernhard M. Fuchs, Rudolf Amann, Matthias Horn, Stefano  
192 Fazi; DOI:10.1111/1462-2920.13111).

