Comments on a recent paper introducing a non-phylogenetic approach to the establishment of relationships between higher taxa in Chlamydiae

This letter has been written as a reply to an article published in the International Journal of Systematic and Evolutionary Microbiology by Karin D. E. Everett and co-workers [Everett, K. D. E., Thao, M., Horn, M., Dyzynski, G. E. & Baumann, P. (2005). Int J Syst Evol Microbiol 55, 1581–1587: Novel chlamydiae in whiteflies and scale insects: endosymbionts ‘Candidatus Fritschea bemisiae’ strain Falk and ‘Candidatus Fritschea eriococci’ strain Elm]. In this article the authors made some statements that we feel need to be responded to.

The crucial issue in the paper published by Everett et al. (2005), which triggered our reply, is the proposed ‘combined’ tree (Fig. 2), which is supposed to delineate the relationships between chlamydiae at taxonomic levels higher than the species or genus. It is based on percentage identity of full- or nearly full-length 16S rRNA gene sequences, which were used to create a backbone for the tree, and this backbone was then supplemented with data from the 297-base long ‘so-called’ chlamydia-like 16S rRNA gene signature sequences. Although we agree that alternative approaches to handling available sequence data may improve our understanding of relationships between micro-organisms and could probably answer some of the as yet unresolved questions in bacterial systematics, we believe that the tree proposed by Everett et al. (2005) deviates from currently accepted knowledge on chlamydial systematics, based on phylogenetic approaches, and may therefore provoke nomenclature instability in the future.

In order to reproduce the results of Everett et al. (2005) depicted in the ‘combined’ tree (Fig. 2) and in the extended neighbour-joining tree (Supplementary Fig. S1 in IJSEM Online), showing the phylogenetic relationships between 331 chlamydial sequences based on the 297-base long signature sequence of the 16S rRNA gene, we applied several sequence analysis applications to the same datasets as the authors. However, we found it virtually impossible to be able to reproduce the results, due to the narrative description of the methods used by the authors, resulting in insufficient information on the datasets and sequence data analysis used.

Furthermore, the authors claim that the combined tree in Fig. 2 summarizes the diversity of 331 chlamydial sequences and that the backbone of the tree depicted as vertical bars represents the percentage identity of full-length or nearly full-length sequences. Despite the lack of information on the number of full- or nearly full-length sequences used in the analysis, we can assume that the backbone was most probably created from a much smaller dataset, since 51 of the 78 sequences depicted in Fig. 2 were shorter than 1300 nucleotides, whereas 31 of the sequences used were even shorter than 300 nucleotides. At this point it remains a mystery to us as to how the similarity between the sequences of different length was calculated and how the partial sequences, some even shorter than 300 nucleotides, were affiliated to the groups forming the backbone of the combined tree. We therefore find the proposed approach for the grouping of the sequences, which is based entirely on sequence similarity, inappropriate for the establishment of relationships between the higher chlamydial taxa or lineages represented by partial sequences only.

Even though there have been some detailed and profound debates on species concepts and bacterial species determination (e.g. Rosselló-Mora & Amann, 2001; Stackebrandt et al., 2002), there are no clear-cut recommendations for the delineation of prokaryote genera or other higher taxonomic ranks. They are usually created primarily based on the branching order of phylogenetically analysed sequences, after which sequence similarities can be used as circumstantial limits for taxa (Oren, 2004). The range of similarity values for higher taxa depends on the personal judgment of the bacteriologist alone and frequently refers to the phylogenetic depth of the clade. Despite the generally accepted approach in bacterial systematics described above, Everett et al. (2005) have given the advantage to sequence similarity over phylogenetic analysis, by creating the backbone of a tree based on sequence similarity, supplemented with phylogenetic analysis of partial 16S rRNA genes. As we are fully aware of the complexity of chlamydial diversity, which originates mainly from uncultured environmental samples, often represented only by partial 16S rRNA gene sequences, we agree that new and alternative approaches to handling the data might improve our understanding of the relationships and the natural history of chlamydiae. However, we find the approach depicted in Fig. 2 of Everett et al. (2005) misleading for the future classification of newly described taxa and inappropriate for establishing the relationships between the higher taxa of Chlamydiae. Our views are supported by several observed incongruities between the topology of the tree proposed in Fig. 2 and phylogenetic trees of Chlamydiae based on full-length as well as on appropriate partial signature sequences of the 16S rRNA gene, as was actually shown by the authors themselves in the tree depicted in Supplementary Fig. S1. For example, in Fig. 2 the ECL VII environmental group was placed in the family Parachlamydiaceae, whereas in fact it is more closely related to the family Chlamydiaceae, as has been shown previously by several authors (e.g. Horn & Wagner, 2001; Kostanješ et al., 2004; Corsaro et al., 2003) and also by Dr Everett and her colleagues themselves in
their Supplementary Fig. S1 (Everett et al., 2005). Similarly, the group containing ‘Candidatus Rhabdochlamydia porcellionis’ and the environmental group of sequences ECL VI have been placed in a higher taxonomic group containing organisms sharing 85–89 % identity, i.e. the family Parachlamydiaceae, the group of environmental clones ECL I and the taxonomic group containing the genus Waddlia and Waddlia-related environmental sequences. According to standard phylogenetic analysis, however, ‘Candidatus Rhabdochlamydia’ is most closely related to the group containing the genus Simkania and ‘Candidatus Fritschea’. Furthermore, the group Simkania and ‘Candidatus Fritschea’ were placed next to the Chlamydia/Chlamydiophila clade, which again contradicts the established phylogeny and, according to Supplementary Fig. S1, also the findings of the authors themselves.

Last but not least we would also like to mention some incorrect quotations that were made by Dr Everett and colleagues in their article and that need to be addressed. The authors claim that ‘Candidatus Rhabdochlamydia porcellionis’ was incorrectly placed in the family Simkaniacae, due to limited phylogenetic analysis using solely neighbour-joining analysis. In the original paper (Kostanjšek et al., 2004), the phylogenetic affiliation of ‘Candidatus Rhabdochlamydia porcellionis’ was described as ...an independent lineage within the order Chlamydiales, which is related most closely to cluster ECL VI and, somewhat more distantly, to Simkania negevensis Z1 (= ATCC VR-1471T) from the family Simkaniacae (Everett et al., 1999), which shared 86-7 % sequence similarity with the sequence of the intracellular hepatopancreatic bacteria’. The phylogenetic placement of ‘Candidatus Rhabdochlamydia porcellionis’ was based on neighbour-joining as well as on parsimony and maximum-likelihood treeing methods, which was explained in the ‘Methods’ and ‘Results and Discussion’ sections of the original paper. Furthermore, the phylogenetic relationships within chlamydiae were subsequently analysed using the ARB phylogenetic software package, and the phylogenetic positioning of ‘Candidatus Rhabdochlamydia porcellionis’ in the original paper was again confirmed. We believe that the scope of the paper by Everett et al. (2005) was to formally describe novel chlamydial symbionts of insects and to reanalyse their phylogenetic relationship, rather than proposing a novel classification system for chlamydiae. However, the way that Everett and co-workers approached the problem in Fig. 2 deviates from the current knowledge on chlamydial systematics, as has been shown by the authors of this discussion. Since we expect that many novel chlamydiae and/or their sequences will be discovered in the near future due to the expanded use of molecular techniques, we are convinced that the relationships depicted in the combined tree (Fig. 2) of Everett et al. (2005) might cause confusion.

We therefore propose that more caution should be used when dealing with limited datasets, e.g. the partial sequences of a single gene. Instead of using the sequence similarity of a single gene as a measure for deciding whether or not a novel genus should be considered a member of a known or a novel family and/or any other higher ranks, more established phylogenetic approaches should be implemented based on the analysis of (i) high quality, complete or almost-complete sequences and, where possible, secondary structure analysis, of appropriate molecular markers, followed by (ii) proper alignment of the sequence to reference sequences of the same quality, (iii) determination of the phylogenetic position and (iv) checking for the presence of signature nucleotides for members of a particular taxon (Stackebrandt et al., 1997).

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