

## Letter to the Editor

### Reorganization and Monophyly of the Genus *Rickettsiella*: All in Good Time

The genus *Rickettsiella* of intracellular bacterial pathogens of arthropods currently comprises the three recognized species *Rickettsiella popilliae*, *R. grylli*, and *R. chironomi*, together with numerous further pathotypes or “subjective synonyms.” On the basis of ultrastructural data, these bacteria were originally assigned to the alphaproteobacterial order *Rickettsiales*. However, in 1997, the determination of a 16S rRNA-encoding sequence from *R. grylli* revealed the highest homology with the corresponding genes from *Coxiella burnetii* and related *Gammaproteobacteria* (12). Ten years later, a paper by Cordaux et al. (1) links up with this finding when asking “if only *R. grylli* has been misclassified” or “if the genus *Rickettsiella* is monophyletic,” in which case its taxonomic position “needs to be reassessed.” The study is based on three new 16S rRNA gene sequences from the crustacean-infecting pathotype “*R. armadillidii*.” From a phylogenetic analysis including these together with the aforementioned *R. grylli* sequence, the authors firstly conclude that “*Rickettsiella* bacteria overall appear to form a monophyletic group” and secondly suggest that “therefore, the genus *Rickettsiella* as a whole (not just *Rickettsiella grylli*) should be classified among the *Gammaproteobacteria* instead of the *Alphaproteobacteria*.” These claims surely deserve a closer look.

On the one hand, the above taxonomic suggestion simply comes late. During the last decade, 16S rRNA genes from several *Rickettsiella* pathotypes have been determined (e.g., 3, 7, 13). Despite the lack of sequences from both the species *R. popilliae* and *R. chironomi*, these data have motivated the previous taxonomic reorganization of the entire genus *Rickettsiella* in the class *Gammaproteobacteria*, order *Legionellales* (5), i.e., as suggested by Cordaux and colleagues. The authors use these additional sequences to calculate the evolutionary origin of *Rickettsiella* in the second part of their study but fail to mention the *R. grylli* genome sequence available since 2006 (provisional GenBank accession number NZ\_AAQJ00000000) that convincingly supports the reorganization of *R. grylli* by phylogenetic inference from beyond the 16S rRNA gene level (7a).

On the other hand, the claim of monophyly of the genus *Rickettsiella* appears premature. The authors’ contribution of 16S rRNA gene sequences from a further pathotype could, at best, trigger a negative result concerning “the monophyletic status of the genus *Rickettsiella*.” Positively, it can only add to the cumulative evidence provided by the aforementioned gammaproteobacterial rRNA gene sequences from *Rickettsiella* pathotypes. However, there is contradicting evidence as well, as several ultrastructurally well-characterized presumed *Rickettsiella* pathotypes have been found to carry chlamydial instead of alpha- or gammaproteobacterial 16S rRNA genes (e.g., see references 2, 4, 6, and 11). These findings appear relevant within the framework of a monophyly argument, and that they pass unmentioned is the more astonishing as Cordaux and colleagues have included other chlamydial sequences in their phylogenetic analysis. In contrast to the all-or-nothing scenario conjured by the authors, the emerging picture is therefore that the genus *Rickettsiella* has, with good reason, been removed from the *Alphaproteobacteria* and provisionally

been assigned to the *Gammaproteobacteria* but that it might, in its present state, comprise numerous misclassified pathotypes.

If so, then is there any sense in talking about monophyly of uncultured organisms as *Rickettsiella* bacteria with many of the ultrastructurally characterized specimens, among them the nomenclatural type strain, being no more available for molecular phylogenetic analysis? It might be seen as a reasonable minimal condition of the monophyly of this genus that—all loosely associated pathotypes aside—at least the recognized *Rickettsiella* species form a monophyletic group. As there has been no previous molecular evidence from either *R. chironomi* or *R. popilliae* and as the pathotype “*R. armadillidii*” is a subjective synonym of the latter species (5), the data assessed by Cordaux et al. in this sense make a considerable contribution to an elucidation of the problem under study. Curiously enough, the authors themselves seem to miss this point when stating incorrectly that “*R. armadillidii*” and *R. grylli* are considered synonymous species in the currently accepted nomenclature.’ Meanwhile, determined 16S rRNA-encoding sequences from further *R. popilliae*-synonymous pathotypes lend additional support to the taxonomic assignment of the genus *Rickettsiella* to the class *Gammaproteobacteria* (8–10). However, as long as there are no molecular data available from the third recognized species, *R. chironomi*, monophyly of the genus *Rickettsiella* remains a premature claim.

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### Authors' Reply

A. Leclerque appears to have no technical objection to our study (1), and he even ends up concluding that our “*Rickettsiella armadillidii*” 16S rRNA sequence data “make a considerable contribution to an elucidation of the problem under study.” Yet, Leclerque also argues that our results are merely confirmatory because previously determined 16S rRNA sequences from other *Rickettsiella* pathotypes (3, 6, 12) already led to the conclusion we drew. In fact, the sequence data cited by Leclerque are irrelevant to the question of the taxonomic assignment of *Rickettsiella* bacteria. This is because the 16S rRNA sequences reported in these studies (3, 6, 12) constituted the actual basis for retrospectively inferring the presence of *Rickettsiella* bacteria in the arthropod hosts analyzed, based on high sequence similarity with an *R. grylli* sequence (11). A circular reasoning subsequently consisting of using these sequences to investigate *Rickettsiella* taxonomic position would necessarily lead to support for the assignment of *Rickettsiella* bacteria to *Gammaproteobacteria* but would be undermined by ascertainment bias. By contrast, we performed an unbiased molecular characterization of *Rickettsiella* bacteria by only including samples for which *Rickettsiella* infection has been ascertained independently of molecular information (see Fig. 1 in reference 1).

Leclerque regrets that we “fail(ed) to mention the *R. grylli* genome sequence available since 2006 (provisional GenBank accession number NZ\_AAQJ00000000) that convincingly supports the reorganization of *R. grylli* by phylogenetic inference from beyond the 16S rRNA gene level.” We humbly note that Leclerque himself fails to mention any tangible result or publication that would actually support his claim. In any event, the reassignment of *R. grylli* to *Gammaproteobacteria* is already well accepted (11) and not questioned in our study (1).

Leclerque also argues that our suggestion of *Rickettsiella* monophyly is premature, as evidenced by two formerly presumed *Rickettsiella* pathotypes (4, 10) that have subsequently been shown to be related to *Chlamydiales* instead of the *Rickettsiella* genus sensu stricto and renamed accordingly (2, 5). Again, this argument is irrelevant because if these pathotypes are unrelated to *Rickettsiella* bacteria sensu stricto and had initially been misclassified as *Rickettsiella* members, then they

are not relevant anymore to the question of the monophyly of *Rickettsiella* bacteria sensu stricto. For example, it would seem absurd to include *Rickettsiella* bacteria in analyses addressing questions related to the monophyly of *Rickettsiales*, just because *Rickettsiella* bacteria used to be classified as *Rickettsiales* until they were shown to be *Gammaproteobacteria*.

Since the publication of our study (1), Leclerque's own work based on additional well-characterized *Rickettsiella* strains and markers has provided further molecular support for the monophyly of *Rickettsiella* bacteria sensu stricto (7–9). We agree with Leclerque that additional molecular data will help further clarify the evolutionary history of *Rickettsiella* bacteria. Meanwhile, assuming *Rickettsiella* monophyly constitutes the framework that is most consistent with the currently available molecular evidence.

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