

## Genetic characterisation of *ompA*, *ompB* and *gltA* genes from *Candidatus Rickettsia rioja*

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Rickettsiosis are emerging and re-emerging infections distributed all over the world. In the last years 'new' *Rickettsia* species have been involved as human pathogens [1–3]. *Candidatus Rickettsia rioja* is a 'new' spotted fever group *Rickettsia* involved in *Dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL) or tick-borne lymphadenopathy (TIBOLA) cases from Spain [4,5]. In 2006, a fragment of the *ompA* gene from EDTA-treated blood of two patients who developed DEBONEL after being bitten by *Dermacentor marginatus* in La Rioja, was submitted to GenBank (GenBank accession no. EF028201). This *Rickettsia* species has also been amplified from ticks removed from patients with DEBONEL.

The aim of this study was to characterise the nucleotide sequence of *ompA*, *ompB*, *citrate sintase* (*gltA*) and 17 KDa genes from *Candidatus R. rioja*. As part of a retrospective study, 15 DNA extracts used for *ompA* PCR that showed 98% similarity with *Rickettsia raoultii* and with uncultured *Rickettsia* sp. RpA4, DnS14 and DnS28, were further analysed for the presence of *ompB*, *gltA* and 17 KDa. Specimens were obtained from two blood samples and nine *D. marginatus* removed from DEBONEL patients and from four *D. marginatus* removed from asymptomatic people.

For our 15 specimens, when *ompA* nucleotide sequences were compared with GenBank data, >99% similarity was found with *Candidatus Rickettsia rioja* (Fig. 1). Unfortunately, all attempts to amplify *ompB* and *gltA* genes from the two blood samples failed. However, PCR amplicons for *ompB* and *gltA* were obtained from 12/13 *D. marginatus* (92.3%). Subsequent sequence analysis of *ompB* PCR products showed highest

similarity (<98.9% for all cases) with the *ompB* gene from *R. raoultii*. For *gltA* amplicons, highest identity was also found with *R. raoultii* (<99.7% for all samples). Nucleotide sequences for partial *ompB* and *gltA* genes obtained in this study will be submitted to GenBank in order to characterise rickettsial genes of *Candidatus R. rioja*. By using the primers that amplify a fragment of the 17 KDa gene, we could not get any positive result.

Nucleotide sequences for the *ompA* gene from two blood samples and 13 *D. marginatus* showed >99% identity with *Candidatus R. rioja*. For the *ompB* and *gltA* amplicons obtained from 12 *D. marginatus*, highest identities for *ompB* and *gltA* were found with partial genes from *R. raoultii* (<98.9% and <99.7%, respectively). These comparisons suggest that *Candidatus R. rioja* can be considered a new *Rickettsia* species as soon as an established isolate is available.

### ACKNOWLEDGEMENTS

Financial support was provided in part by a grant from 'Instituto de Salud Carlos III' (EMER 07/033), Ministerio de Sanidad y Consumo (Spain).

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The authors have declared no potential conflicts.

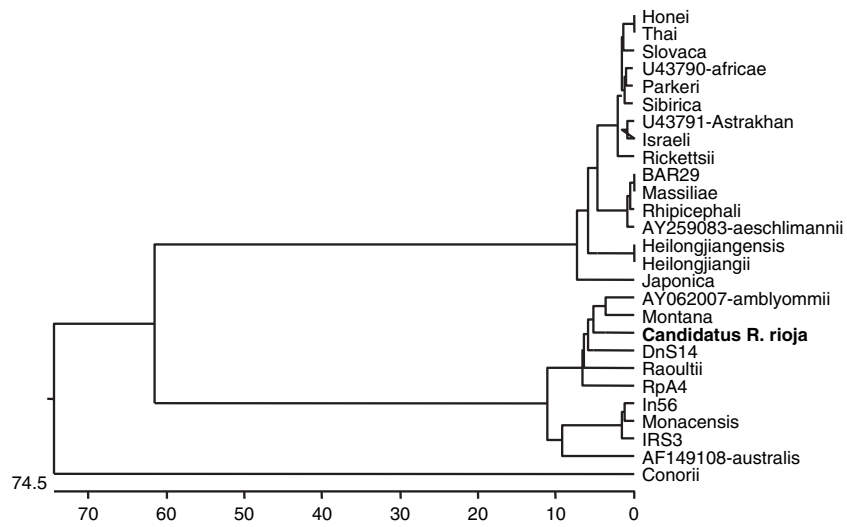


Fig. 1. Phylogenetic tree with different *Rickettsia* species.