Taxonomy of endosymbiotic bacteria from a novel *Lupinus* sp. (*Lupinus mariae-josephi*) endemic of a limed-alkaline soil habitat in Southeastern Spain.

Luis Rey Navarro\(^a\), Carmen Sánchez Cañizares\(^a\), David Durán\(^a\), Francisco Temprano\(^b\), Paloma Sánchez Jiménez\(^a\), Juan Imperial \(^{a,c}\) and Tomás Ruiz-Argüeso\(^a\)

\(^a\)Departamento de Biotecnología, Universidad Politécnica de Madrid. Avenida Complutense s/n, 28040 Madrid. and Centro de Biotecnología y Genómica de Plantas, UPM, Campus de Montegancedo - Carretera M40 km 38, 28223 Pozuelo de Alarcón (Madrid)

\(^b\)IFAPA Las Torres-Tomejil. Carretera de Sevilla Cazalla, Km 12.2 41200-Alcalá del Río (Sevilla).

\(^c\)CSIC

E-mail: luis.rey@upm.es

*Lupinus mariae-josephi* is a recently described *Lupinus* species (Pascual 2004) endemic of a Southeastern area of Spain with soils singularly of high pH and active lime content where it is endangered due to the reduced size of its habitat. Ten isolates of *L. mariae-josephi* endosymbiotic bacteria were obtained using trap-plants and soils from five sampling points within a native plant population area in Llombai (Valencia, Spain). The microsymbionts are extra-slow (ultrabradytrophic) growing bacteria with phenotypic and symbiotic characteristics singularly different from *Bradyrhizobium* strains nodulating other *Lupinus* spp. thriving in the Iberian Peninsula and adapted to growth in acidic soils. Cross-inoculation experiments revealed that these *L. mariae-josephi* endosymbiotic bacteria isolates are unable to nodulate or efficiently fix nitrogen with other *Lupinus* spp. Their phylogenetic status was examined by a multilocus sequence analysis of four housekeeping genes (*16S rDNA, glnII, recA, atpD*) and the symbiotic *nodC* gene. The 16S rDNA phylogenetic analysis showed that *L. mariae-josephi* isolates are related to strains nodulating *Retama* spp. in northeastern Algeria (Boulila et al., 2009), *Phaseolus lunatus* from Peru (Ormeño-Orrillo et al., 2006), as well as to *B. elkanii, B. jicamae* and *B. pachyrhizi* species, forming a new clade (Clade I) within the *Bradyrhizobium* genus. All the single and concatenated *glnII+recA* and *glnII+recA+atpD* analyses consistently support the existence of Clade I, and also revealed that, within this clade, the *L. mariae-josephi* endosymbiotic bacteria belong to a single evolutionary lineage that also includes strains nodulating *Retama* spp. from northeastern Algeria. Within this new *Bradyrhizobium* lineage, the phylogenetic analyses performed showed essentially convergent results indicating that the tested *L. mariae-josephi* isolates nested in three sub-groups that might correspond to novel sister *Bradyrhizobium* species. *Bradyrhizobium* Clade I is highly differentiated from the *Bradyrhizobium* clade (Clade II) that includes currently named *Bradyrhizobium* species and well-delineated unnamed genospecies. Singularly, all the endosymbiotic bacteria from *Lupinus* species adapted to acid soils in the Iberian Peninsula and
tested in this study are included in Clade II. They are related either to strains of the *B. canariense* or *B. japonicum* lineages. The phylogenetic analysis based on the symbiotic *nodC* gene showed that *L. mariae-josephi* endosymbiotic bacteria define a novel branch in the *nodC* *Bradyrhizobium* tree. This branch groups together with a branch that gathers isolates from recently studied legume symbioses such as isolates from *Retama* spp., which suggests the existence of a common unique ancestor for the symbiotic genes of these two groups of bradyrhizobia. In contrast, the symbiotic genes of isolates from other *Lupinus* spp. from the Iberian Peninsula are clearly related to the *B. canariense* lineage. The allopatric (geographic) speciation of the *L. mariae-josephi* bradyrhizobia may result from the colonization of a singular habitat, such as the basic and high calcium carbonate soils of the Valencia area, by its unique legume host.

**References**

F. Boulila, G. Depret, A. Boulila, D. Belhadi, S. Benallaoua, & G. Laguerre (2009) *Retama* species growing in different ecological-climate areas of northeastern Algeria have a narrow range of rhizobia that form a novel phylogenetic clade within the *Bradyrhizobium* genus. *Systematic and Applied Microbiology* 32: 245-255


*This research was financed by Fundación del Banco de Bilbao Vizcaya Argentaria (FBBVA) 2009-2012*