The status of the genus *Seliberia* Aristovskaya and Parinkina 1963 (Approved Lists 1980) and the species *Seliberia stellata* Aristovskaya and Parinkina 1963 (Approved Lists 1980). Request for an Opinion

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The species *Seliberia stellata* was described in 1963 and the name validly published in 1980. Its type strain, INMI N-9⁷, was deposited in the VKM collection by one of the authors reporting its 5S rRNA gene sequence. Based on the analysis of this sequence, the currently distributed strains VKM B-1340 and CECT 7960 are not the original type strain of *Seliberia stellata*. A 16S rRNA gene sequence analysis of strain CECT 7960 had previously shown that this strain belongs to the species *Bradyrhizobium betae*, and this result was confirmed in the present paper by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS analysis for both CECT 7960 and VKM B-1340. Therefore, we propose that the Judicial Commission consider the following. (1) That the organism currently deposited as VKM B-1340 and CECT 7960 be recognized as a member of the species *Bradyrhizobium betae*. (2) That the organism deposited as VKM B-1340 and CECT 7960 does not represent the type strain of the species *Seliberia stellata*. (3) To place the species name *Seliberia stellata* Aristovskaya and Parinkina 1963 (Approved Lists 1980) on the list of rejected names if a suitable replacement strain, or a neotype, cannot be found within two years of publication of this Request (Rule 18c). (4) To place the genus name *Seliberia* Aristovskaya and Parinkina 1963 (Approved Lists 1980) on the list of rejected names (Recommendation 20d) if a suitable replacement type strain or a neotype for the type species of the genus *Seliberia* Aristovskaya and Parinkina 1963 (Approved Lists 1980) is not identified as indicated in point (3).

The species *Seliberia stellata* was described in 1963 and the original description of this species in either Russian or English did not indicate the type strain of this species (Aristovskaya & Parinkina, 1963). When this species was recorded in the 1974 edition of *Bergey’s Manual of Determinative Bacteriology* (Aristovskaya, 1974) it was stated that the type strain was INMI N-9⁷. This strain was deposited in the VKM culture collection by D. I. Nikitin under the accession code VKM B-1340 and *S. stellata* was included in the approved lists of bacterial names in 1980 (Skerman et al., 1980).

The VKM collection distributed this strain to the IAM and the JCM (two Japanese microbial culture collections that subsequently merged), where it is not longer available, and to the CECT (Spanish Type Culture Collection) where it was deposited as CECT 7960. Since the type strain of *S. stellata* is not available in the JCM collection, VKM and CECT are the only two culture collections currently having the strain recorded as the type strain of *S. stellata*.
Strain CECT 7960 was included in a project for sequencing the 16S rRNA genes of orphan species (Yarza et al., 2013; resulting GenBank accession no. HE795128), and from this date, the species *S. stellata* was added to the Ez-Taxon database (Kim et al., 2012). The close phylogenetic relationship between this 16S rRNA gene sequence and the genus *Bradyrhizobium* constitutes a serious problem because it appears in the output results from the Ez-Taxon server together with the other species of the genus *Bradyrhizobium*. Professor J. Euzeby and Dr A. Parte in the List of Prokaryotic Names with Standing in the Nomenclature (http://www.bacterio.net/seliberia.html) have therefore included a statement that *S. stellata* shows an unexpected affiliation with the family *Bradyrhizobiaceae* with a 100 % similarity in its 16S rRNA gene sequence with respect to *Bradyrhizobium betae*.

Therefore, the aim of this study was to analyse the two currently available cultures of the type strain of *S. stellata* deposited in the VKM and CECT collections in order to clarify their taxonomic status, to avoid confusion in 16S rRNA gene phylogenies when studying species of the genus *Bradyrhizobium*.

As a first step, we analysed the sequence of the 16S rRNA gene of strain VKM B-1340 according to Rivas et al. (2007). It was 100 % identical to that available in the

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**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain CECT 7960 (=VKM B-1340) within the genus *Bradyrhizobium*. Bootstrap values calculated for 1000 replications are indicated. Bar, 1 nt substitution per 100 nt.
GenBank database for strain CECT 7960 (GenBank accession no. HE795128). These sequences were compared with those available in the EzTaxon-e server (Kim et al., 2012), showing 100% sequence similarity with Bradyrhizobium betae PL7HG1T (Rivas et al., 2004). A phylogenetic analysis of these sequences and those of species of the genus Bradyrhizobium was performed using the CLUSTAL w program (Thompson et al., 1997). The distances were calculated according to Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were inferred using neighbour-joining analysis (Saitou & Nei, 1987). The MEGA5.0 software package (Tamura et al., 2011) was used for all the analyses. Bootstrap analysis was based on 1000 resamplings.

The results of the 16S rRNA gene phylogenetic analysis confirmed that the currently available type strains of S. stellata, CECT 7960 and VKM B-1340, belong to the genus Bradyrhizobium, with B. betae being the most closely related species (Fig. 1).

The identification of CECT 7960 and VKM B-1340 as strains of B. betae was confirmed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS analysis carried out as previously described (Sánchez-Juanes et al., 2013). The results of this analysis showed that both strains have identical spectra (Fig. S1, available in the online Supplementary Material) and that CECT 7960 and VKM B-1340 matched with B. betae PL7HG1T with more than 2.3 score values, indicating that these strains belong to this species according to Sánchez-Juanes et al. (2013).

After confirming that the two available type strains of S. stellata belong to the species B. betae, it is necessary to elucidate whether these strains correspond to the original type strain INMI N-9T. From the scarce data available for the original type strain of S. stellata, the most reliable one is the 5S rRNA gene sequence reported in the work of Chumakov (1987). This sequence was also included in the work of Bulygina et al. (1990), co-authored by D. I. Nikitin, who was the depositor of strain INMI N-9T in the VKM collection.

The 5S rRNA gene of S. stellata is currently available in the 5S rRNA database (http://rose.man.poznan.pl/5SData/). This allowed us to design specific primers for the 5S rRNA gene of S. stellata and another couple of specific primers for the genus Bradyrhizobium that were used to amplify this gene in the strains CECT 7960 and VKM B-1340 and in B. betae PL7HG1T. The amplification was carried out using the REDExtract-N-Amp, PCR kit (Sigma) under the following PCR conditions: pre-heating at 95 °C for 9 min; 35 cycles of denaturing at 95 °C for 1 min, annealing at 54 °C for 1 min and extension at 72 °C for 30 s; and a final extension at 72 °C for 7 min. The purification and sequencing of 5S rRNA genes, performed with the same primers used for amplification, were carried out as was previously described by Robledo et al. (2011). The sequence analysis was done as in the case of the 16S rRNA gene described above.

The results of the amplification of the 5S rRNA gene showed that the primers based on the 5S rRNA gene sequence of S. stellata did not amplify any DNA band of the expected size in any of the strains analysed (data not shown). However, the primers specific for the genus Bradyrhizobium allowed the amplification of a band of the expected size in strains CECT 7960 and VKM B-1340 as well as B. betae PL7HG1T. The phylogenetic analysis of 5S rRNA gene sequences corresponding to these bands (Fig. 2) showed complete identity among them, and that they were phylogenetically distant to S. stellata, although this species was also placed within the class Alphaproteobacteria as has been previously reported (Bulygina et al., 1990; Stackebrandt, 1992; Schmidt & Kelly, 1984).

Therefore, the results from analyses of 16S and 5S rRNA genes as well as those from MALDI-TOF MS showed that the currently available strains CECT 7960 and VKM B-1340 belong to the species B. betae and specifically the

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**Fig. 2.** Neighbour-joining phylogenetic tree based on 5S rRNA gene sequences showing the position of strain CECT 7960T (=VKM B-1340T) and Seliberia stellata INMI N-9T within the genus Bradyrhizobium (this last sequence has no GenBank accession no.). Bootstrap values calculated for 1000 replications are indicated. Bar, 2 nt substitution per 100 nt.
results of the 5S rRNA gene showed that these strains are not the original type strain of the species *S. stellata*.

Based on these results, the original type strain of species *Seliberia stellata* (Aristovskaya & Parinkina, 1963) is not currently available, and this species is therefore not represented by any type strain. Therefore, we propose that a search be made for a suitable replacement type strain, or a neotype should be designated according to Rule 18c of the International Code of Nomenclature of Bacteria. We suggest that if a suitable replacement strain, or a neotype, cannot be found within two years of publication of this Request, the Judicial Commission of the International Committee on Systematics of Prokaryotes place the name *Seliberia stellata* Aristovskaya and Parinkina 1963 (Approved Lists 1980) on the list of rejected names. Since the type and single species of the genus *Seliberia* Aristovskaya and Parinkina 1963 (Approved Lists 1980) is *Seliberia stellata* (Aristovskaya & Parinkina, 1963), which is currently not represented by any type strain, we suggest that the Judicial Commission should also place the genus name *Seliberia* Aristovskaya and Parinkina, 1963 (Approved Lists 1980) on the list of rejected names.

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**References**


