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**International Committee on Systematics of Prokaryotes
Subcommittee for the Taxonomy of *Rhizobium-Agrobacterium*
Minutes of the meeting, Budapest, August 25th, 2016**

Philippe de Lajudie, *Secretary.*

J. Peter W. Young, *Chairperson.*

Minute 1. Call to order. The subcommittee closed meeting was called to order by Philippe de Lajudie (secretary) at 14:45 on July 25th, 2016 just before the 12th Nitrogen Fixation Conference in Budapest, Hungary.

Minute 2. Record of attendance.

Present: Philippe de Lajudie (Secretary, Institut de Recherche pour le Développement, Montpellier, France), J. Peter W. Young (University of York, UK), Kristina Lindström (University of Helsinki, Finland), Chang-Fu Tian (China Agricultural University), Julie Ardley (Murdoch University, Australia).

Apologies for absence: Bertrand Eardly (Penn State Berks College Reading, USA), Esperanza Martinez-Romero (Chairperson, Universidad Nacional Autónoma de Mexico, Cuernavaca, Mor., Mexico), Xavier Nesme (Institut National de la Recherche Agronomique, University of Lyon I, France), Pablo Vinuesa (Universidad Nacional Autónoma de Mexico, Cuernavaca, Mor., Mexico), Gehong Wei (China), Anne Willems (University of Gent, Belgium).

JPW Young was designated as the chairperson of the meeting and, in the name of the whole subcommittee, warmly welcomed Julie Ardley and Chang-Fu Tian for their first attendance as subcommittee members.

Minute 3. 2014 Minutes approval. Minutes of our last meeting (7 September 2014 and online) were unanimously approved. They have since been published (de Lajudie and Martinez Romero, 2017).

Minute 4. Resignations and thanks. Prof. Esperanza Martinez-Romero resigned as the chairperson of the subcommittee; the subcommittee is grateful to her for the many years since the 1990s that she held this position and for the accomplished work. The subcommittee assumes that Dr. Rosemary Sylvester-Bradley must have resigned long ago since she has not attended a subcommittee meeting since the late 1990s nor responded to messages. The subcommittee expressed their warm thanks to Prof. Wenxin Chen (China Agricultural University) and to Dr. Peter Van Berkum (USDA, Beltsville, USA) for their long and active years of membership with appreciated contributions. The subcommittee also remembers, with gratitude, Dr. Gisèle Laguerre (1957-2013, INRA France) and Prof. Brion Jarvis (1928-2015, New Zealand), who both actively participated in the subcommittee for many years with significant inputs.

Minute 5. Election of a new chairperson of the subcommittee. JPW Young was unanimously elected by vote of the members present as the new chairperson of the subcommittee.

Minute 6. New members. The subcommittee decided to encourage new members to join in order to face new challenges, to cover more items, diversity of rhizobial taxonomic groups, scopes, technical and geographical concerns, i.e. representatives from all continents, experts of genome-based taxonomic approaches, *Agrobacterium*, beta-rhizobia and new rhizobial groups, etc. Several names were suggested and the Secretary agreed to contact them.

Minute 7. The scope of the subcommittee. Originally restricted to the genera *Rhizobium* and *Agrobacterium*, the subcommittee concerns gradually extended to the taxonomy of « rhizobia », a general name covering all bacteria having the capacity to induce nodules on legumes where they fix dinitrogen. Therefore the subcommittee now deals with *Agrobacterium* and with all bacterial species nodulating legumes. These were all initially described as *Rhizobium* and later split in *Rhizobium sensu stricto*, *Azorhizobium*, *Bradyrhizobium*, *Sinorhizobium* (syn. *Ensifer*), *Mesorhizobium*, *Allorhizobium*, *Pararhizobium*, *Neorhizobium*, etc. Bacteria with this symbiotic ability have gradually also been discovered in other genera, i.e. *Methylobacterium*, *Devosia*, *Ciceribacter*, *Aminobacter*, *Bosea*, *Phyllobacterium*, *Ochrobactrum*, *Microvirga*, *Shinella*, *Labrys*, *Burkholderia*, *Paraburkholderia*, *Ralstonia*, *Cupriavidus*, *Herbaspirillum*. With « *Rhizobium* » having become “rhizobia”, including at least 21 genera in Alphaproteobacteria and Betaproteobacteria, the subcommittee discussed the opportunity to change its name accordingly. A longer name would not be convenient nor exhaustive either, and probably more rhizobia in more genera will be described in future, possibly in more proteobacterial classes. On the other hand, the general term “agrobacteria” covers all bacteria that form galls or root hypertrophies on plants by transfer of T-DNA, and are so far included in *Agrobacterium*, *Rhizobium*, *Pararhizobium* and *Allorhizobium*. Consequently, the subcommittee agreed to request a change in its name to the “**subcommittee for the taxonomy of rhizobia and agrobacteria**”, to reflect our growing knowledge of nitrogen-fixing microsymbionts of legumes and bacteria that transfer T-DNA to plants, consisting of diverse bacteria with similar accessory functions (nodule/gall forming ability), but the taxonomy of which follows the general bacterial rules, largely based on core genes/functions. There is precedent for a remit based on biological function rather than genera or families, such as the subcommittee for phototrophic bacteria. The subcommittee is aware that some rhizobia may fall within the taxonomic remit of another subcommittee, and agreed that species descriptions should have regard to the recommendations of that subcommittee as well as to any that are specific to rhizobia.

Thus the subcommittee understands its own main missions as:

1. To provide guidelines for describing new agrobacterial and rhizobial species,
2. To maintain information records about *Rhizobium*, *Agrobacterium*, agrobacterial and rhizobial species published,
3. To provide the list of taxa proposed since the last subcommittee meeting, and
4. To evaluate and comment on each genus/species validation, including rhizobia and agrobacteria described outside *Rhizobium* – *Agrobacterium*

Minute 8. New species. The number of rhizobial species increased exponentially in recent years. Table 1 lists the new taxa described since the last subcommittee meeting in September 2014, consisting of 2 new genera (*Neorhizobium*, *Pararhizobium*), 83 spp.

nov. (29 in *Rhizobium*, 24 in *Bradyrhizobium*, 12 in *Mesorhizobium*, 4 in *Ensifer*, 1 in *Pararhizobium*, 1 in *Agrobacterium*, 1 in *Bosea*, 1 in *Microvirga*, 1 in *Phyllobacterium*, 7 in *Burkholderia*, 2 in *Paraburkholderia*), 30 comb. nov. (3 in *Agrobacterium*, 6 in *Allorhizobium*, 3 in *Neorhizobium*, 4 in *Pararhizobium*, 12 in *Paraburkholderia*, 2 in *Ensifer*). *Rhizobium vignae* was found as a later synonym of (*Neo*)*rhizobium galegae* (Mousavi et al., 2014). *Rhizobium gei* (Shi et al., 2016) should be transferred as *Pararhizobium gei*. The rhizobial authentication of the 4 *Bosea* species (de Meyer and Willems, 2012 ; Safronova et al., 2015) may be regarded as pending as none of them was formally demonstrated to induce nodules on legumes so far, nor to harbor known nodulation genes (*nodA* and *nodC* could not be amplified) despite being isolated from nodules. As far as we know *Ciceribacter* is not confirmed to nodulate, despite having been isolated from the rhizosphere of chickpea. Authors publishing new species proposals outside IJSEM are encouraged to submit details of their papers to IJSEM for these species to appear in a validation list in that journal.

Minute 9. Type strains. The rule is that when a new species is validly described, the type strain should be deposited in two international collections (LMG, DSMZ, HAMBI, USDA, etc.) and available from them on request including a signed standard MTA (Material Transfer Agreement) form. Although the 'two collections rule' was not enforced rigorously in the past, recent examples show that it is being enforced: '*Allorhizobium taibaishanense*' comb. nov., '*Allorhizobium paknamense*' comb. nov. and '*Pararhizobium sphaerophysae*' comb. nov. (Mousavi et al., 2015) did not appear in IJSEM validation lists so far due to strain unavailability. In practice, some type strains may be difficult to obtain due to specific dissemination restrictions from authors in agreement with their specific national laws concerning biodiversity. In some countries the authorities do not allow any strain to be delivered abroad, but in general these restrictions vary from a simple additional declaration to the depositor, to the validation of an additional MTA by the depositor. Most of the restrictions imposed by MTAs, for example prohibitions on further distribution and commercial use, do not impede taxonomic study. However, a prohibition on the publication of the genome sequence of the type strain is problematic as it is now widely expected to have type strain genome sequences available, and the type strain genome sequence is likely to become mandatory for new species description quite soon. This issue may become a problem for taxonomists in countries with restrictions on genome sequencing to propose new species. Our subcommittee will initiate a discussion with the International Committee on Systematics of Prokaryotes on this sensitive case of national laws being problematic for taxonomists.

Minute 10. *Agrobacterium*. Recently, the number of agrobacterial species increased because of transferring the species *Rhizobium nepotum*, *Rhizobium pusense*, *Rhizobium skierniewicense* to the genus *Agrobacterium* (Mousavi et al, 2015; Table1) and also the description of a new species *Agrobacterium arsenijevicei* (Kuzmanović et al., 2016). *Agrobacterium tumefaciens* was found a later heterotypic synonym of *Agrobacterium radiobacter*, suggesting that *Ag. tumefaciens* should be amalgamated with *Ag. radiobacter* which has taxonomic priority over *Ag. tumefaciens* (Tindall, 2014). Even if treated as a later heterotypic synonym of *Ag. radiobacter*, the type species of the genus is *Ag. tumefaciens*, typified by the strain defined on the Approved Lists of Bacterial Names. This is the reason why it is recommended to retain ATCC 19358 (=LMG 140) as the type-

strain of *Ag. radiobacter* and B6 (=LMG 187=ATCC 23308) as the the type-strain of the genus *Agrobacterium*.

The genus *Agrobacterium* could be characterized by the presence of a large linear chromid [Ramirez-Bahena, 2014]. Based on the results of MLSA and genomic studies of the family *Rhizobiaceae* (Mousavi *et al.*, 2015 ; Ormeño-Orrillo *et al.*, 2015, unpublished data), the “*Rhizobium aggregatum* complex” consisting of the species *Agrobacterium albertimagni*, *R. rosettiformans*, *R. aggregatum*, *R. naphthalenivorans*, *R. selenitireducens*, and *R. daejeonense* should be considered as members of a separate genus, closely related to *Agrobacterium*. In addition, based on the results of MLSA (Mousavi *et al.*, 2014&2015), the species *Agrobacterium vitis* along with six *Rhizobium* species were transferred to the genus *Allorhizobium*. For other species that were named *Rhizobium* while genera were amalgamated the subcommittee recommends to use their original names (e.g. *Allorhizobium undicola*, *Agrobacterium larrymoorei* and *Ag. rubi*); *Rhizobium qilianshanense* (Xu *et al.*, 2013) should be transferred to *Allorhizobium*.

Minute 11. *Burkholderia*, *Paraburkholderia*, *Caballeronia*. The subcommittee discussed the particular case of *Burkholderia*. As currently circumscribed, *Burkholderia sensu lato* includes over 100 species, many of economic or clinical importance, that display remarkable diversity in their genome size, metabolism and ecological niches. Previous studies have established that *Burkholderia sensu lato* comprises three major clades: Clade I includes *Burkholderia cepacia* complex (Bcc) species, several plant pathogens and a group related to the animal pathogens *Burkholderia mallei* and *Burkholderia pseudomallei*; Clade II corresponds to the *Burkholderia glathei* clade; Clade III encompasses primarily environmental and plant-associated species, including rhizobial species and other members exhibiting beneficial plant growth promoting traits. In addition to the three major clades, *Burkholderia sensu lato* also includes several other deep-branching lineages, such as *Burkholderia rhizoxinica* and *Burkholderia endofungorum*; a group consisting of *Burkholderia caryophylli*, *Burkholderia symbiotica* and *Burkholderia soli*; and *Burkholderia andropogonis* (Depoorter *et al.*, 2016; Estrada-de los Santos *et al.*, 2016) and references therein). Informal proposals discussed splitting *Burkholderia sensu lato* into distinct genera, in particular to distinguish a plant-associated clade from a pathogen clade (Estrada-de los Santos *et al.*, 2013; Gyaneshwar *et al.*, 2011).

Sawana *et al.* (2014) were first to formally propose splitting *Burkholderia* into two genera, a revised *Burkholderia* retaining all pathogenic members and *Paraburkholderia* gen. nov. consisting of all plant-associated ones, with 62 new combinations. The genus *Paraburkholderia* was rapidly validated in IJSEM (Oren & Garrity, 2015b; Oren & Garrity, 2015c) and is now in use in databases.

In particular, symbiotic legume-nodulating *Burkholderia* species were transferred to *Paraburkholderia* with corresponding new combinations: the *Mimosa* symbionts as *Paraburkholderia tuberum*, *P. mimosarum*, *P. phymatum*, *P. nodosa*, *P. sabiae*, *P. symbiotica* and *P. diazotrophica*; *Lebeckia* symbionts as *P. sprentiae* comb. nov. and *P. dilworthii* comb. nov.; *Rhynchosia* symbionts as *P. rhynchosiae* comb. nov.; *Aspalathus* symbionts as *P. aspalathi* comb. nov.; *Phaseolus* symbionts as *P. caballeronis* comb. nov.. *Burkholderia sensu lato* was further split, with the concomitant publication of *Caballeronia* gen. nov. to accommodate twelve species of *Burkholderia* and *Paraburkholderia* (Dobritsa & Samadpour, 2016).

The taxonomic scheme of Sawana *et al.* (2014) was based on 16S rRNA phylogeny, as well as the percentage guanine plus cytosine content and conserved indels in whole

genome sequences of some 25 formally named *Burkholderia* species and several unclassified strains. However, Eberl and Vandamme (2016) criticized the lack of several type strains in the study by Sawana *et al.* and noted that the authors ignored phylogenetic heterogeneity, as revealed by 16S rRNA-based divergence and by differences in the distribution of 22 additional conserved sequence indels, in their simplistic inclusion of all species outside Clade I (*Burkholderia sensu stricto*) within the novel genus *Paraburkholderia*. According to this scheme, the deep-branching lineages of *B. rhizoxinica*, *B. endofungorum*, *B. caryophylli*, *B. symbiotica*, *B. soli* and *B. andropogonis* are all to be included within *Paraburkholderia*.

Similarly, Dobritsa & Samadpour (2016) proposed *Caballeronia* gen. nov. for species within the *B. glathei*-like Clade II, on the basis of 16S rRNA phylogeny and conserved indels, but have omitted a number of type strains in their study, including those of *B. caryophylli*, *B. soli* and *B. symbiotica*. We would further note a recent paper, which ascribed a strain to a novel species of *Burkholderia* on the basis of a phylogenetic analysis of 16S rRNA genes that did not include sequences from all type strains (Weber & King, 2017).

It is the opinion of this subcommittee that the studies describing *Caballeronia* gen. nov. and *Paraburkholderia* gen. nov. do not represent best taxonomic practice, and lead to confusion. Already, the phylogenetic classification of "*P. andropogonis*" within *Burkholderia* has been reassessed, with the result that it is now described as a new genus, *Robbsia andropogonis* gen. nov., comb. nov. (Lopes-Santos *et al.*, 2017).

While remaining within the Principles of the Bacterial Code (Parker *et al.*, 2015), in particular Principle 1(4) regarding freedom of taxonomic thought, we would note that implications exist outside prokaryotic taxonomy circles in the renaming of bacterial taxa, especially in the case of a genus of economic and clinical importance such as *Burkholderia*. In this regard, the following principles of the Code of Nomenclature of Prokaryotes should be considered:

Principle 1 - The essential points in nomenclature are as follows.

1. Aim at stability of names.
2. Avoid or reject the use of names which may cause error or confusion.
3. Avoid the useless creation of names.

Principle 9 - The name of a taxon should not be changed without sufficient reason based either on further taxonomic studies or on the necessity of giving up a nomenclature that is contrary to the Rules of this Code.

Notwithstanding that there are valid taxonomic reasons for splitting *Burkholderia sensu lato* into several genera, the members of this group cannot be described as pathogens or non-pathogens solely on the basis of their affiliation to a particular clade (Eberl & Vandamme, 2016). However, many strains, particularly rhizobial and plant beneficial strains from Clade III, show great potential as biofertilisers and bioremediants in sustainable agricultural systems. Research efforts directed towards robust characterisation and taxonomy of *Burkholderia sensu lato* species can help in realising this agricultural potential (Estrada-de los Santos *et al.*, 2016). Clearly, large-scale phylogenomic study is required for resolving these taxa.

Minute 12. The subcommittee home page. The sub-committee home page

(<http://edzna.ccg.unam.mx/rhizobial-taxonomy/node/2>) refers to LPSN

(<http://www.bacterio.net/>) for validly published bacterial species names and focuses on the taxonomy of rhizobia, their distribution and phylogenetic relationships with their close bacterial relatives, host plants, nodulation capacity and some features. The

subcommittee noticed the problem of obsolete genus and species affiliation in molecular sequence databases where the affiliation (genus, species) of the strain is forever retained as it was given at the time of its sequence deposit, with no further update of their later identification or genus/species transfers. The subcommittee will produce a list of the taxonomic transfer history and related sequence accession numbers for type strains to make this information available on its website.

Minute 13. Guidelines and Recommendations. The subcommittee recognizes the urgent need to update the last formal guidelines published for rhizobial species description 25 years ago (Graham et al., 1991) and will reactivate the process of publishing recommendations in IJSEM. There is a need of clarification regarding the minimal standards required to describe novel bacterial taxa and to better inform researchers and so seek to avoid errors in the classification of bacteria. It would appear, for example, that effective publication of a bacterial taxon does not have to be in print, and there is no longer a requirement for a new genus to be distinguishable by phenotypic characteristics.

Minute 14. Next meeting. Next meetings were planned to be held during the 20th International Congress on Nitrogen Fixation, Granada, Spain, 3-7 September 2017 and during the 13th European Nitrogen Fixation Conference (ENFC) in Stockholm, Sweden (2018).

Minute 15. Adjournment. The meeting was adjourned at 17:00 on August 25th 2016. The meeting continued online. Online discussion was adjourned on 18 April 2017.

Note Added in Preparation

Minute 16 (online) New members.

Several scientists were later contacted by the subcommittee secretary and Seyed Abdollah Mousavi (University of Helsinki, Finland), Alvaro Peix (Consejo Superior de Investigaciones Científicas, Instituto de Recursos Naturales y Agrobiología de Salamanca, Spain), Emma Steenkamp (University of Pretoria, South Africa), Jerri Zilli (Empresa Brasileira de Pesquisa Agropecuária, Seropédica, Brazil), Lionel Moulin (Institut de Recherche pour le Développement, Montpellier, France), Mitchell Andrews (Lincoln University, New Zealand), Ridha Mhamdi (Centre of Biotechnology of Borj-Cédria, Hammam-Lif, Tunisia) and Tomasz Stepkowski (Warsaw Life Sciences University, Warsaw, Poland) were further elected as members, and contributed in the online discussion to prepare the present minutes.

Minute 17. Current membership. The current members of the subcommittee are J.P.W. Young (UK, Chairperson), P. de Lajudie (France, Secretary), M. Andrews (New Zealand), J. Ardley (Australia), B. Eardly (USA), K. Lindström (Finland), R. Mhamdi (Tunisia), E. Martinez-Romero (Mexico), L. Moulin, (France), A. Mousavi, (Finland / Iran), X. Nesme (France), A. Peix, (Spain), E.T. Steenkamp (South Africa), T. Stepkowski (Poland), C.F. Tian (China), P. Vinuesa (Mexico), G. Wei (China), A. Willems (Belgium), J. Zilli, (Brazil).

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