Minutes

Daniel R. Brown, *Secretary*
Janet M. Bradbury, *Chairman*

International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of *Mollicutes*

Minutes of the meetings, 8, 9 and 14 July 2006, Cambridge, UK

Session 1 – Closed meeting

Minute 1. Call to order. The first session of the closed meeting was called to order at 16:30 on 8 July 2006 in the Castlereagh Room at St. John's College, Cambridge, UK, by the Chairman, J. Bradbury.

Minute 2. Record of attendance. Subcommittee members present were A. Bertaccini (Italy), A. Blanchard (France), J. Bradbury (Chairman; UK), D. Brown (Secretary; USA), G. Firrao (Italy), J. Frey (Switzerland), G. Gasparich (USA), R. Harasawa (Japan), B. Kirkpatrick (USA), T. Knight, Jr. (USA), K. Parham (UK), R. Rosenbusch (USA), K. Sachse (Germany) and R. Whitcomb (USA). Ex-officio members present were S. Levisohn (Israel) and D. Pitcher (UK). The invited guest present was N. Harrison (USA).

Minute 3. Apologies for absence. Apologies for absence were received from J. Bové (France), G. Christiansen (Denmark), M. Davidson (USA), J. Davis (USA), H. Neimark (USA), D. Pollack (USA), S. Razin (Israel), D. Taylor-Robinson (UK) and J. Tully (USA).

Minute 4. Adoption of agenda. The written agenda was presented and approved.

Minute 5. Minutes of the 2004 meeting. The minutes of the 2002 and 2004 meetings have been compiled by J. Bradbury following the resignation of the previous Secretary K.-E. Johansson.

Minute 6. Report of Chairman. The Chairman welcomed A. Bertaccini, D. Brown, G. Gasparich, T. Knight, Jr., and K. Parham as new members. D. Brown will replace K.-E. Johansson as Secretary. A majority of the eligible members present voted in favour of J. Bradbury and D. Brown continuing to serve as Chairman and Secretary, respectively, for two more years. J. Bradbury recorded the subcommittee's thanks to the organisers of the 16th Congress of the International Organization for Mycoplasma (IOM) for providing accommodation for the subcommittee meetings.

Minute 7. Report on International Committee on Systematics of Prokaryotes (ICSP) 2005 meeting plenary sessions. D. Brown attended the sessions to express concerns about Rule 27(3) and Rule 30(3b) of the

Bacteriological Code; these rules cover the deposition of type strains in culture collections. During the 1999 ICSP meeting, a recommendation by the Judicial Commission was adopted requiring that type strains be deposited in at least two different recognised culture collections in different countries. Contrary to the expectations of the ICSP leadership, the processing of type strains of mollicutes by culture collections has since been a problem. Therefore as an unintended consequence of Rule 27(3) and Rule 30(3b), it has since been extremely difficult to publish valid descriptions of novel mollicute taxa. It was suggested that *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) editors could consider ad hoc exemptions to the Rules, provided that strains are deposited in at least two different collections, even if the collections are in the same country. The consensus was in favour of the possibility of exemption and H. Trüper, Chairman of the Judicial Commission, agreed that the Judicial Commission would formulate specific language to allow such exemptions. Subsequently, when the decisions of the Judicial Committee were announced, the ICSP approved a three-member committee to be composed of the ICSP Chair, the IJSEM Editor-in-Chief, and the Judicial Commission Chair, which will consider requests for exemption from Rule 27(3) and Rule 30(3b). Unanimous agreement among the committee will be required for exemption. The committee will still prefer that authors show good-faith effort to deposit their strains in at least two different countries. This change will appear under Rule 30 of the *Bacteriological Code* and it will take effect from the date of publication of the minutes of the 2005 ICSP meeting. Authors of previously published descriptions that contravene Rule 27(3) and Rule 30(3b) will then be allowed a maximum of 3 years from that date to make efforts to conform to the Code.

Minute 8. Culture collection deposits. Worldwide support for microbial culture collections is steadily declining. Although it remains formally possible to deposit certain new strains with the American Type Culture Collection (USA) and the National Collection of Type Cultures (UK), only the Mollicutes Reference Collection at Purdue University (USA) readily accepts new deposits of mollicutes and specific antisera. J. Davis, curator, and M. Davidson, of the collection wrote to the subcommittee to announce that a formal external advisory group, including J. Bradbury, K. Dybvig, S. Levisohn and R. Rosenbusch,

has been formed to guide and assess the operations of the collection, which are anticipated to become financially unsustainable in the immediate future. E. Moore of the Culture Collection, University of Göteborg (Sweden) wrote to the subcommittee to express their willingness to consider deposits of mollicutes, but also indicated concerns about quality confirmation by collections that are inexperienced with comparatively fastidious strains intended for future distribution rather than mere archiving.

Minute 9. New *Mycoplasma* and *Ureaplasma* species.

New mycoplasmas that had been proposed since the last subcommittee meeting were *Mycoplasma amphoriforme*, *Mycoplasma iguanae*, 'Mycoplasma sphenisci', *Mycoplasma testudineum*, 'Mycoplasma vulturi' and 'Mycoplasma zalophi'. Several of these remain to be validly described. It was discussed that reviewers and editors for some journals may be unaware of the minimum standards for the description of new species of mycoplasmas established by this subcommittee. The Chairman and Secretary intend to contact some of the journals that regularly publish reports of newly proposed species to draw their attention to the standards. No new ureaplasmas had been described since the last subcommittee meeting.

Minute 10. Phylogeny of the *Mycoplasma mycoides* cluster.

K. Sachse drew attention to a new analysis by L. Manso-Silvan and colleagues (16th IOM Congress abstract) based on a comparison of five conserved protein-coding sequences (*fusA*, *glpQ*, *gyrB*, *lepA* and *rpoB*). The investigators proposed combining *Mycoplasma mycoides* subsp. *mycoides* Large Colony with *Mycoplasma mycoides* subsp. *capri* as the single entity *Mycoplasma mycoides* subsp. *capri*, and the creation of a proposed new subspecies *Mycoplasma capricolum* subsp. *leachii* by combining *Mycoplasma capricolum* subsp. *capripneumoniae* with *Mycoplasma* sp. Bovine Group 7 of Leach, to clarify the phylogeny of the *Mycoplasma mycoides* cluster. The consensus of the subcommittee was that if a proposal for realignment is initiated by the investigators, it may be endorsed by the subcommittee following peer-reviewed publication.

Minute 11. New haemotrophic *Mycoplasma* species candidates.

D. Brown reported that there was no discussion during the 2005 ICSP plenary sessions regarding haemotrophic mycoplasma species candidates or the priority of any relevant nomenclature (e.g. *Eperythrozoon* and *Haemobartonella* species), nor any related announcement of decisions of the Judicial Commission. New candidate species described since the last subcommittee meeting were 'Candidatus *Mycoplasma haematoparvum*' and 'Candidatus *Mycoplasma turicensis*'.

Minute 12. Novel phylotype discovery through global 16S rRNA gene PCR.

D. Brown drew attention to new data of D. Relman and colleagues [*Science* **308** (2005), 1635–1638], and presented a phylogenetic analysis of their 16S rRNA gene sequences from at least ten unique

phylotypes among the human intestinal microbiota that cluster distinctly enough to suggest the existence of a previously undiscovered order within the class *Mollicutes*.

Minute 13. Novel *Spiroplasma* species.

Novel spiroplasmas described since the last subcommittee meeting were *Spiroplasma atrichopogonis*, *Spiroplasma leucomae* and *Spiroplasma penaei*. It was also reported that spiroplasmas have been isolated from the mitten crab *Eriocheir sinensis* and the crayfish *Procambarus clarkii* in China by W. Wang and colleagues [*FEMS Microbiol Lett* **249** (2005), 131–137]. These were the first reported isolates from crustaceans, but it is not yet known if they represent novel species.

Minute 14. Update on characterization of novel *Spiroplasma*.

L. Regassa from Georgia Southern University (USA) wrote to the subcommittee announcing that, in collaboration with F. French, their group is analysing over 200 diverse spiroplasma isolates from North America, Central America, South America and Australia. The isolates fall into 26 distinct serogroups, including 21 novel groups. Efforts are currently underway to characterize the putative novel species. Antisera have been produced for all of the 21 new candidate groups or species. The characterization of a large number of geographically diverse *Spiroplasma* species is certain to add to our overall understanding of spiroplasma biodiversity and biogeography, but it also presents some practical challenges. With the addition of many novel species and serogroups, the requirement of the minimum standards for descriptions of new species of the class *Mollicutes* that a putative novel spiroplasma be tested with antisera against all other *Spiroplasma* species or groups, becomes substantially more burdensome [International Committee on Systematic Bacteriology, Subcommittee on the taxonomy of *Mollicutes*. Revised minimum standards for descriptions of new species of the class *Mollicutes* (Division *Tenericutes*). *Int J Syst Bacteriol* **45** (1995), 605–612]. The serogroup system has played a valuable role in spiroplasma taxonomy and can continue to serve as a tool for preliminary screening and/or placement of isolates not classified at the species level.

Minute 15. The *Spiroplasma* group system.

In 1976, a group of spiroplasma taxonomists agreed to develop tests for differentiation of spiroplasma strains. A serogroup classification system for members of the genus *Spiroplasma* was in place by 1980, and formal criteria for the serogroup system were proposed in 1987. Since 1987, many of the serogroups have been converted into species. The last of several revisions of the *Spiroplasma* groups was published in 1998. Because the minimum standards for description of new species of mollicutes are currently being revised, following the last subcommittee meeting R. Whitcomb has convened an *ad hoc* committee to review the spiroplasma serogroup system. L. Regassa is the Chairman. The committee currently includes F. French, S.

Hogenhout, U. Melcher, L. Nunan, G. Gasparich, W. Wang, R. Whitcomb and D. Williamson, who are all currently active in spiroplasma work. Additional committee members are anticipated.

Minute 16. Taxonomy of *Acholeplasmatales*: *Acholeplasma pleciae* (Tully et al. 1994) Knight, Jr. 2004 (basonym *Mesoplasma pleciae* Tully et al. 1994) and *Mycoplasma feliminutum*. T. Knight, Jr. explained his proposal following the last subcommittee meeting that *M. pleciae* be reclassified as *Acholeplasma pleciae* comb. nov. based on an analysis of 16S rRNA and *gyrB* gene sequences [Knight, Jr, T. *Int J Syst Evol Microbiol* **54** (2004), 1951–1952]. The consensus of the subcommittee was in support of the reclassification. The subcommittee also urged that similar analyses should be conducted to achieve proper reclassification of *Mycoplasma feliminutum*.

Minute 17. New ‘*Candidatus* Phytoplasma’ species candidates. New strains described since the last subcommittee meeting were ‘*Candidatus* Phytoplasma allocasuariae’, ‘*Candidatus* Phytoplasma asteris’, ‘*Candidatus* Phytoplasma caricae’, ‘*Candidatus* Phytoplasma cynodontis’, ‘*Candidatus* Phytoplasma fragariae’, ‘*Candidatus* Phytoplasma graminis’, ‘*Candidatus* Phytoplasma mali’, ‘*Candidatus* Phytoplasma pini’, ‘*Candidatus* Phytoplasma prunorum’, ‘*Candidatus* Phytoplasma pyri’, ‘*Candidatus* Phytoplasma rhamni’, ‘*Candidatus* Phytoplasma spartii’ and ‘*Candidatus* Phytoplasma trifolii’. At least seven additional new candidate species have been incidentally cited but not yet formally described.

Minute 18. Adjournment. The first session of the closed meeting was adjourned at 19:30 on 8 July 2006.

Session 2 – Closed meeting

Minute 19. Call to order. The second session of the closed meeting was called to order at 08:00 on 9 July 2006 in the Castlereagh Room at St. John’s College, Cambridge, UK, by the Chairman, J. Bradbury.

Minute 20. Record of attendance. Subcommittee members present were A. Bertaccini (Italy), A. Blanchard (France), J. Bradbury (Chairman; UK), D. Brown (Secretary; USA), G. Firrao (Italy), J. Frey (Switzerland), G. Gasparich (USA), R. Harasawa (Japan), B. Kirkpatrick (USA), T. Knight, Jr. (USA), K. Parham (UK), R. Rosenbusch (USA), K. Sachse (Germany) and R. Whitcomb (USA). Ex-officio members present were S. Levisohn (Israel) and D. Pitcher (UK). The invited guest present was N. Harrison (USA).

Minute 21. Sense of the subcommittee regarding recognition of the order *Phytoplasmatales*, family *Phytoplasmataceae*, and genus *Phytoplasma*. G. Firrao reviewed the evidence that phytoplasmas have been described in sufficient detail to warrant elevation from

their provisional ‘*Candidatus*’ status and justify establishment of a new taxon. Many species have been proposed and their taxonomic positions in a distinct cluster within the *Mollicutes* are firmly established based on 16S rRNA gene sequence analyses supported by phenotypic data. At present, two peer-reviewed phytoplasma genome sequences have been published, two others are finished and are awaiting publication and at least six others are being carried out. These sequences provide information equivalent or superior to that obtainable by the DNA–DNA reassociation measurements which have been traditionally regarded as the gold standard for species definition. A genome sequence constitutes a description of a type, but a ‘description, preserved (non-viable) specimen, or illustration’ presently may not serve as the nomenclatural type of a taxon [De Vos, P. & Trüper, H.G. *Int J Syst Evol Microbiol* **50** (2000), 2239–2244]. Phytoplasmas are not likely to be cultivated in the near future, but after considerable discussion among the subcommittee members, the unanimous opinion was that concepts of order, family, and genus are necessary for phytoplasmas, and that the time for the proposal of the normal genus *Phytoplasma* has come. It was recommended that the forthcoming revision of the minimum standards for descriptions of new species of the class *Mollicutes* include a forward-looking statement to that effect. However, there was very little discussion regarding the assignment of the phytoplasmas to either an existing or a new family and order within the class and no consensus about such assignments was reached.

Minute 22. *Bergey’s Manual of Systematic Bacteriology* status report. J. Tully wrote to the subcommittee that the new edition of Volume 3, which was to include the *Mollicutes*, will not become available in the foreseeable future and he has withdrawn from the project. Many co-authors contributed to the revised section on mollicutes and more than five updates were submitted to the Editorial Office. The subcommittee expressed its gratitude to Dr Tully for his enduring dedication to that work and also its opinion that some other means should be found for timely dissemination of the information including future updates.

Minute 23. Non-compliance with minimum standards for description of novel species of the class *Mollicutes*. R. Whitcomb drew attention to three recently-published peer-reviewed descriptions of novel species which did not conform to the current minimum standards and also cited the description of *Mycoplasma amphoriforme* as an ideal. Non-compliance threatens to make the minimum standards meaningless. Strain designation or strain identifiers should be used rather than specific epithets when publishing descriptions that do not fulfil the standards. Referring to Minute 9, the subcommittee further discussed mechanisms to communicate the necessity of upholding minimum standards to editors and reviewers of the *IJSEM* and other journals, such as by

providing a checklist for minimum standards compliance. K. Parham made a useful suggestion that authors should be encouraged to approach a member of the subcommittee for advice on intended descriptions of novel species.

Minute 24. Revised minimum standards status report.

R. Whitcomb, J. Bradbury and D. Brown drafted a revision of the minimum standards for description of new species of mollicutes, which was circulated to all subcommittee members before the meeting. The manuscript was prepared principally by R. Whitcomb, with input on key points from J. Bradbury and some other members of the subcommittee. The revision is intended to reflect recent advances in molecular systematics and the species concept for prokaryotes. It consists of a summary and introduction, a description of the class *Mollicutes*, a review of the species concept in *Mollicutes*, an overview of the usual steps in characterization of new species and a short list of mandatory requirements for the descriptions of new species. Despite concerns that a mandatory requirement to include serological testing has become a substantial technical and financial burden to investigators, the clear consensus of the subcommittee was to retain that requirement, as described in the newly revised minimum standards document, until such time as robust and readily-available equivalent alternative methods for the characterization of new species of *Mollicutes* justify its elimination. The laboratory of K. Parham seems currently to be the only one among the international community of mollicute researchers with the facilities and expertise necessary to conduct DNA–DNA reassociation measurements. Corrections and final suggestions for refinements of the minimum standards were considered by the subcommittee and the manuscript will be submitted for publication in the *IJSEM*.

Minute 25. Matters arising. As mentioned under Minute 8, a review of the current status of the Mollicutes Collection at Purdue University was submitted by J. Davis and M. Davidson. It has proved impossible thus far to obtain funding from within the USA to support this growing collection of cultures and antisera, in part because its small scale and scope contrast with other recognized culture collections. The start-up funds provided by Purdue University are now depleted and there is no possibility of replacing equipment or replenishing antisera under the present financial circumstances. Advisors representing the IOM Board of Directors, the Board of the IOM's International Research Programme on Comparative Mycoplasmaology committee and this ICSP subcommittee were invited to report on this situation at their respective meetings. In the context of the difficulty in persuading many recognized culture collections to accept deposits of new mollicutes experienced recently by several investigators, this is perceived by the subcommittee as a very serious problem. The subcommittee expressed sympathy and

support for this valuable collection and, having no funding resources itself, would strongly endorse any financial help that could be obtained from other sources.

T. Knight, Jr. drew attention to current research at the Venter Institute and elsewhere which suggests that soon this subcommittee may have to accommodate a system of nomenclature and classification for entirely synthetic species of prokaryotes.

Minute 26. Current membership. The current membership of the subcommittee is as follows: A. Bertaccini (Italy), A. Blanchard (France), J. Bové (France), J. Bradbury (Chairman; UK), D. Brown (Secretary; USA), G. Christiansen (Denmark), G. Firrao (Italy), J. Frey (Switzerland), G. Gasparich (USA), R. Harasawa (Japan), B. Kirkpatrick (USA), T. Knight, Jr. (USA), H. Neimark (USA), K. Parham (UK), R. Rosenbusch (USA), K. Sachse (Germany) and R. Whitcomb (USA). Current advisory members are: K.-E. Johansson (Sweden), D. Pollack (USA), S. Razin (Israel), D. Taylor-Robinson (UK), and J. Tully (USA). Current ex-officio members are: M. Davidson (USA), J. Davis (USA), S. Levisohn (Israel) and D. Pitcher (UK). A majority of the eligible members present voted in favour of inviting N. Harrison (USA) and the *IJSEM* editor for mollicutes to join as new members of the subcommittee.

Minute 27. Next meeting. The next meeting will be held in 2008 in Tianjin, China, at the 17th Congress of the IOM.

Minute 28. Adjournment. The meeting was adjourned at 13:00 on 9 July 2006.

Session 3 – Open meeting

Minute 29. Call to order. The open meeting was called to order by the Chairman, J. Bradbury, at 09:00 on 14 July 2006 in the Palmerston Room, St John's College, Cambridge, UK, during the 16th Congress of the IOM.

Minute 30. Record of attendance. Subcommittee members in attendance were J. Bradbury, G. Firrao, J. Frey, G. Gasparich, K. Parham, D. Pitcher, R. Rosenbusch, K. Sachse, D. Taylor-Robinson and R. Whitcomb. Approximately 50 of the delegates attending the IOM Congress were present.

Minute 31. Summary of activities. J. Bradbury explained for the benefit of the congress delegates that the ICSP, a committee within the International Union of Microbiological Societies, is the parent body to which the subcommittee reports. The ICSP is responsible for publication of the International Code of Nomenclature of Bacteria (the *Bacteriological Code*) and the *IJSEM*. She indicated the general role various ICSP subcommittees have in giving advice in response to queries regarding the nomenclature and taxonomy of specific groups of

prokaryotes and in recommending minimum standards for the description of new taxa. She also indicated that the subcommittees have an obligation to meet at regular intervals and that the subcommittee on the taxonomy of Mollicutes has permission from the ICSP to meet in conjunction with the IOM Congresses.

Minute 32. Summary of major issues discussed during the closed meeting. J. Bradbury pointed out that D. Brown had attended the ICSP 2005 meeting plenary sessions and raised the concerns felt by mycoplasmaologists regarding recent amendment to the *Bacteriological Code* which now requires cultures of a novel species to be deposited in two recognized collections in two different countries. He reported that the ICSP's Judicial Commission, after some discussion, had agreed to provide a mechanism for appeals for exemption from this rule. Another major issue was the agreement of the subcommittee on new minimum standards for the description of new species of the class *Mollicutes* that would reflect advances in this area. The revised standards were reviewed by the subcommittee and will be submitted for publication

in the *IJSEM*. G. Firrao proposed that sufficient data had now been assembled regarding the characterization of phytoplasmas to enable a formal classification to be introduced. The case for this would be constructed by him and his colleagues. The subcommittee endorses this concept. Problems within the Bergey's Trust have brought the production of a revised Volume 3 of *Bergey's Manual of Systematic Bacteriology*, which would include the *Mollicutes*, to a standstill. The subcommittee recorded its extreme disappointment in this news, especially since J. Tully and his co-authors had expended major efforts in preparing and updating the relevant material. There was considerable support for exploring an alternative route for publishing this work. The valuable Mollicutes Collection at Purdue University and financial problems that threaten its continuity were discussed by the subcommittee. Collaborative discussions with the IOM and IRPCM Boards were instrumental in raising awareness of the problem and possible solutions.

Minute 33. Adjournment. The open meeting was adjourned at 10:00 on 14 July 2006.