Minutes

G. Firrao, Secretary
D. R. Brown, Chairman

International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of Mollicutes

Minutes of the meetings, July 15th and 19th 2012, Toulouse, France

Session 1 - Closed meeting.

Minute 1. Call to order. The closed meeting was called to order at 09:00 on July 15th, 2012, in the Hotel Mercure, Toulouse, France, by the Chairman, D.R. Brown.

Minute 2. Record of attendance. Subcommittee members present were A. Bertaccini (Italy), A. Blanchard (France), J. Bradbury (UK), D. Brown (Chairman, USA), G. Firrao (Secretary, Italy), J. Frey (Switzerland), T. Knight Jr (USA), C. Knox (Australia), M. May (USA), L. Manso-Silván (France), L. Regassa (USA), K. Sachse (Germany), D. Volokhov (USA), The ex-officio member present was G. Browning (Australia).

Minute 3. Apologies for absence. Apologies for absence were received from G. Gasparich (USA), R. Harasawa (Japan), N. Harrison (USA), H. Neimark (USA) and D. Pollack (USA). The other members absent were J. Boveé (France), L. McAuliffe (UK), R. Rosenbusch (USA), the advisory members S. Razin (Israel), D. Taylor-Robinson (UK), J. Tully (USA), and the ex-officio members M. Davidson (USA) and J. Davis (USA).

Minute 4. Adoption of agenda. The written agenda was adopted by unanimous vote.

Minute 5. Minutes of the 2010 meeting. The minutes of the most recent meeting have been published [Int J Syst Evol Microbiol (2011) 61, 695–697] and are available online at http://ijs.sgmjournals.org/.

Minute 6. Report of Chairman. D. Brown recorded the Subcommittee’s thanks to C. Citti and the organizers of the 19th Congress of the International Organization for Mycoplasmology (IOM) for providing accommodation for the Subcommittee meetings. D. Brown recalled that although the Subcommittee has no formal relation with the IOM, meeting during that biennial event is very convenient for most Subcommittee members. The scheduled meetings of the Executive Board and Judicial Commission of the International Committee on Systematics of Prokaryotes (ICSP) to be held in association with the International Union of Microbiological Societies’ meeting in Sapporo, Japan, in September 2011, did not take place. Nevertheless the ICSP Executive Board has met online during the last biennium and an online plenary session is planned. Aharon Oren was selected to become the new Editor-in-Chief of the IJSEM following resignation of Peter Kämpfer. Revisions of the ICSP statutes are nearly complete, which was a necessary step before revisions to the Bacteriological Code can be undertaken.


Minute 8. New species of recognized genera and Candidatiae species and lineages. Since the last meeting of the Subcommittee the following species names have been effectively published: Mycoplasma neophronis (Suárez-Pérez et al., 2012, Int J Syst Evol Microbiol 62, 1321–1325); ‘Candidatus Mycoplasma haemocervae’ (Watanabe et al., 2010, J Vet Med Sci 72, 1527–1530); ‘Candidatus Mycoplasma erythrocervae’ (Watanabe et al., 2010, J Vet Med Sci 72, 1527–1530); ‘Candidatus Mycoplasma haemozalophi’ (Volokhov et al., 2011, Vet Microbiol 149, 262–268); ‘Candidatus Mycoplasma haemohominis’ (Steer et al., 2011, Clin Infect Dis 53, e147–e151); ‘Candidatus Mycoplasma aotii’ (Barker et al., 2011, Vet Microbiol 149, 478–481); ‘Candidatus Phytoplasma rubi’ (Malembic-Maher et al., 2011, Int J Syst Evol Microbiol 61, 2129–2134); ‘Candidatus Phytoplasma costaricanum’ (Lee et al., 2011, Int J Syst Evol Microbiol 61, 2822–2826); ‘Candidatus Phytoplasma sudamericanum’ (Davis et al., 2012, Int J Syst Evol Microbiol 62, 984–989). At the time of the meeting the papers describing the following species, that have subsequently appeared in print, had been published online only: ‘Candidatus Phytoplasma convolvuli’ (Martini et al., 2012, Int J Syst Evol Microbiol 62, 2910–2915); ‘Candidatus Phytoplasma malaysianum’ (Nejat et al., 2013, Int J Syst Evol Microbiol 63, 540–548); ‘Candidatus Phytoplasma balanitae’ (Win et al., 2013, Int J Syst Evol Microbiol 63, 636–640); ‘Candidatus Phytoplasma pruni’ (Davis et al., 2013, Int J Syst Evol Microbiol 63, 766–776). Novel DNA sequences of putative unnamed mycoplasmas and haemoplasmas were also reported in several papers published during the last biennium. Organisms doubtfully associated with the Tenericutes in GenBank taxonomy database during this period included Haloplasma contractile, Thermoplasma volcanium, ‘Candidatus Bacilloplasma’, ‘Candidatus Hepatoplasma crinochetorum’ and ‘Candidatus Lumbiricincola’. The Subcommittee remains concerned about the use of the category of Candidatus for organisms whose existence was only assessed by the recovery of DNA sequences in metagenomic studies.
Minute 9. Mollicute collection. Since M. Davidson, J. Davis and C. Wu relinquished their authority, the Mollicutes Collection of Cultures and Antisera (World Federation of Culture Collections TMC; World Data Centre for Microorganisms 858) has remained with no director nor curator. This valuable collection is presently unattended and no longer a functioning resource for the supply of material necessary for the identification and classification of Mollicutes or for deposit of antisera against proposed new species. The Subcommittee asked the Chair to submit the issue to the International Organization of Mycoplasmolology that may provide support for the maintenance of the Collection.

Minute 10. Eperythrozoon teganodes and haemotropic mycoplasmas. While summarizing the current efforts in the discovery of haemoplasmas and the clarification of their taxonomy D. Volokhov stressed the relevance of obtaining the entire 16S rRNA gene sequence. The subcommittee agreed to send a letter to the editors of major journals with a recommendation to require full-length 16S rRNA gene sequences in papers describing new haemoplasmas.

H. Neimark, who was unable to attend the meeting, wrote to document his efforts to clarify whether or not the organism reported as Eperythrozoon teganodes by Hoyte (1962; Parasitology, 52, 527–532) is the same as Bartonella sergenti reported by Adler & Ellenbogen (1934; J Comp Pathol 47, 219–220). To paraphrase Neimark, in 1962 Hoyte in Australia reported finding an eperythrozoon that he named Eperythrozoon teganodes occurring naturally in the blood of several different breeds of cattle in Queensland. When Adler and Ellenbogen had described Eperythrozoon wenyonii, they also identified a second erythrocyte-associated organism that they named Bartonella sergenti. Could Eperythrozoon teganodes be the second haemotropic mollicute they described? Bodies consistent with a haemoplasma are not evident in the blood smear photographs in Hoyte’s paper. Instead there are numerous polymorphic forms scattered throughout the plasma. Hoyte described the plasma parasites as slender rods, threads, rings and particularly a ‘not plentiful’ but characteristic form whose shape suggested ‘the outline of a frying pan’. The forms were seen in phase-contrast and dark-field preparations, not just in Giemsa-stained smears, and thus were not due to contaminated stain solutions.

Hoyte deposited Eperythrozoon teganodes reference blood smear slides in the Queensland Museum, Brisbane (slide no. G. 2533), the Wellcome Museum of Medical Science, London, and the Smithsonian Institute, Washington, DC (US National Museum) Helm. Coll. slide no. 59645. In response to a 2009 letter from Neimark to the University of Queensland, Mal Bryant, Collections Manager and Researcher, Parasitology Biodiversity Program, and Robert Adlard, Head of Marine Zoology and Senior Curator and Researcher, Parasitology Biodiversity Program, Queensland Museum, replied that Hoyte retired years ago and had died. They located his slide in the Queensland museum but understandably were reluctant to send their only specimen overseas; they kindly offered and generously made the effort to photograph several fields on the slide. The photos showed nothing resembling ‘frying pan’ forms, the plasma was mostly clear and the red cells appeared normal. The Smithsonian slide is now located in the National Parasite Collection, Animal Parasitic Diseases Laboratory, Agriculture Research Service, US Department of Agriculture. Through the efforts of Eric Hoberg, Chief Curator, and P. Pilitt the slide was loaned to Neimark for examination. The slide had the identification number scratched on the surface and the blood smear was sealed with a coverslip. The plasma space of the blood smear was essentially clear and the red cells, aside from some crenated cells, appeared normal. The third slide had been transferred from the Wellcome Museum to the Natural History Museum (NHM), London. Emily Barker and Sérénne Tasker from the University of Bristol sought to examine this slide, but the slide they received from the NHM ‘looked nothing like a blood smear, let alone a blood smear with haemoplasmas present’. The Wellcome collection had been sent over in a large batch, apparently in disarray, ‘there is every chance that the slide is mislabelled’ and there is no way to reasonably trace through the other slides. Barker also was told the NHM would not let anyone destroy even a part of any smear to purify DNA from it. Given that known leads have been exhausted, if someone were inclined to pursue this, it might be possible to persuade one of the other archive curators to permit removal of part of a smear for DNA analysis. A remote possibility is that a sample containing these forms exists and might be examined if, after 30 years, it is still possible to contact authors of reports finding Eperythrozoon teganodes in Argentina (1982, Vet Parasitol 9, 267–272).

The question remains of how Hoyte could have sent to reference collections at least two blood smears containing nothing like the bodies he described. It seems possible that the recently identified ‘Candidatus M. haemobovis’ [synonym haemobos] could also be synonymous with B. sergenti and/or E. teganodes because it too can occur as a co-infection with M. wenyonii, but according to D. Volokhov the issue could not be addressed in the absence of molecular data.

Minute 11. Establishment of a DNA bank for haemoplasmas. In consideration of the issues concerning the identification and classification, and the availability of future powerful means for DNA analysis, D. Volokhov suggested that a DNA bank will be established for the conservation of the nucleic acids of haemoplasmas. The following discussion focused on the availability of resources for the maintenance of such a bank. According to D. Volokhov there are sufficient resources among the researchers involved in the research on haemoplasmas. The Subcommittee endorses the proposal.

Minute 12. Serology in the description of new species in the class Mollicutes. G. Gasparich wrote in support of transitioning away from the requirement for serological testing in the description of new species. M. May led a lengthy discussion dealing with the present seriously restricted access...
to specific antisera against previously named species; the unequal potency of antisera; the present lack of a recognized collection to receive deposits of new antisera; the several cases of serological cross-reactivity between distantly related species; and the subjective nature of qualitative serological analyses such as the spiroplasma deformation test. Even when serological comparisons were restricted to one-way screening against strains with greater than 94% 16S rRNA gene sequence similarity, this necessitated testing against as many as 30 strains for several recent novel isolates of spiroplasmas. A number of recent publications have demonstrated that genome-based approaches such as multilocus sequence comparisons could be more reliable, cheaper and easier to perform. A number of loci including ATPase subunits, EF-Tu, gyrB, rpoB, the entire rRNA operon, other ribosomal proteins, and the mbc for ureaplasmas were mentioned but there was no consensus among the subcommittee members regarding which loci should be sequenced as a minimal genomic alternative to serology as a means to demonstrate species novelty. If it were known which proteins are effectively characterized by serology then it might be feasible to compare their gene sequences directly. After considerable discussion, the Subcommittee formally agreed to suspend immediately the requirement for serological testing for the description of new species. It is anticipated that future recommendations of minimal standards for descriptions of new species in the class Mollicutes will shift to genomics rather than serological analyses.

Minute 13. Ureaplasma taxonomy/classification. C. Knox informed the Subcommittee about the status of the taxonomy of the genus Ureaplasma. At present, the most critical issues are related to the definition of relationships among serovars. Horizontal gene transfer evidently confounds the serovar classification system, but associations with disease do correlate with serovar groupings.

Minute 14. Spiroplasma taxonomy. L. Regassa reported that new species are on the horizon as several laboratories are characterizing novel spiroplasma isolates from biting midges, the gypsy moth, leafhoppers, crustaceans and Tabanids. Strain deposition and antisera access continue to be major obstacles for the description of new species. A study on correlation between serology and 16S rRNA-based phylogeny in the Regassa lab revealed that while many serological groupings were consistent with phylogenetic relationships, the phylogenetic and serological trees were not congruent and DNA characters generated more parsimonious trees overall. With reference to possible substitutes for serology, L. Regassa stressed that many described species are likely to collapse if species delineation were based on ribosomal genes. Other regions with higher variability (e.g. ITS, rpoB) or a combination of variable and conserved genes may offer better resolution.

Minute 15. Phytoplasma taxonomy. According to A. Bertaccini an update of guidelines for species definition within the genus ‘Candidatus Phytoplasma’ is needed, as it is currently based primarily on 16S rRNA gene sequence supported by considerations of the type of transmission vector, the plant host range or the phytoplasma’s ‘behaviour’ within a plant, and possibly other molecular evidence of meaningful genetic or phenotypic diversity. The guidelines have known limitations and may generate name conflicts. G. Firrao recalled that there are no formal reference rules: the 97% rRNA similarity threshold for naming a new Candidatus species has been agreed among phytoplasmologists to prevent giving a different name to each of the several hundred of known phytoplasmas. Accordingly, strains similar but not identical are currently referred to as ‘related to’ named Candidatus species. Such taxonomic complexity seems contrary to the heterogeneity allowed in the current species concept for mollicutes (Brown et al., 2007, Int J Syst Evol Microbiol 57, 2703–2719). The Subcommittee acknowledged that in the last 15 years the adoption of this agreement has led to the introduction of about 35 Candidatus species names that may serve as provisional species. Other genes may be available to support Candidatus species definition. The Subcommittee encouraged the use of multiple genetic markers, minimally 16S rRNA and EF-Tu, for the circumscription of such provisional taxa to which strains should be assigned, therefore eliminating the requirement for the formula ‘related to’ when referring to strains highly similar but not identical to named Candidatus species.

Minute 16. Update on Mollicutes genomics. A. Blanchard reports that 45 genomes belonging to 32 species are currently available for analysis on the MolliGen database and this figure is increasing rapidly. However, this becomes a challenge as genome sequencing becomes cheaper, so in the future finished genomes will be prioritized over draft genome sequences, keeping the quality of annotation as high as possible. The Subcommittee agreed that MolliGen represents a highly valuable resource for taxonomic purposes, as the availability of an annotated genome collection greatly facilitates recognition of discriminating phylogenetic markers.

Minute 17. Prospects for genotaxonomy of Mollicutes. D. Brown reported that also non-phylogenetic genomics based methods, such as average nucleotide identities (ANI; Goris et al., 2007, Int J Syst Evol Microbiol 57, 81–91) have been proposed as substitutes for DNA–DNA hybridization for the circumscription of species. This suggests the possibility that a well-chosen type species of a genus would have consistently high ANI values with other species within that genus. The accepted boundary between species of 70% DNA–DNA hybridization empirically equates to about 95–96% ANI. An analysis of representatives of the class Mollicutes using ANI was carried out by M. May and D. Brown and the results were circulated among members. The purpose was to evaluate the possible use of ANI as additional new taxonomic information especially regarding the anomalous mycoides cluster. The software package JSpecies (Richter & Rosselló-Móra 2009, Proc Natl Acad Sci USA 106, 19126–19131) was used to calculate ANI values
by pairwise comparisons of the complete genome sequences of 20 species of mollicutes representing six families within the class. Only Order Anaeroplasmatales was not represented. ANIm was calculated by the NUCmer algorithm of MUMmer and ANIb was calculated by reciprocal BLASTN. The alignment-free tetranucleotide usage frequency correlation coefficient was also calculated. The ANIm, ANIb and Tetra matrices were converted into Newick format and visualized as unrooted trees using the DRAWTREE algorithm of PHYLIP. For comparison, the similarity matrix of 16S rRNA gene sequences of these species was visualized as an unrooted tree using the Ribosomal Database Project’s Tree Builder algorithm. The Subcommittee unanimously agreed that trees built on ANI scores are not congruent with the shared view of the taxonomic structure of the class Mollicutes. G. Firrao added that ANI and similar analyses, as it is also the case with DNA–DNA hybridization, are not suitable for the determination of supraspecific relationships, although they may be valuable for species circumscription.

Minute 18. Available data for phytoplasma genotaxonomy. G. Firrao reported that the availability of phytoplasma genome data had greatly increased in the last biennium due to the production of genome drafts using second generation sequencing approaches. A phylogenetic analysis based on putative protein data consisting of about 30 000 aa residues and an ANI analysis of about 500 000 nt from each of 10 phytoplasmas were shown to provide results highly congruent with 16S rRNA based taxonomy. The Subcommittee encourages the use of genomic methods for the classification of uncultivable mollicutes.

Minute 19. Relationship with IRPCM. The chair of the IOM’s International Research Programme on Comparative Mycoplasmology, G. Browning, reported on the activity of the program and on the interest of IRPCM teams in the taxonomy of the Mollicutes.

Minute 20. Current membership. The current membership of the subcommittee is: A. Bertaccini (Italy), A. Blanchard (France), D. Brown (Chairman; USA), G. Firrao (Secretary; Italy), J. Frey (Switzerland), G. Gasparich (USA), R. Harasawa (Japan), N. Harrison (USA), T. Knight, Jr (USA), C. Knox (Australia), L. Manso-Silván (France), M. May (USA), L. McAuliffe (UK), L. Regassa (USA), R. Rosenbusch (USA), K. Sachse (Germany), and D. Volokhov (USA).

Ex-officio member: Glenn Browning (Australia).
Emeritus members: J. Bové (France), H. Neimark (USA), D. Pollack (USA), S. Razin (Israel), D. Taylor-Robinson (UK) and J. Tully (USA).

Minute 21. Next meeting. The next subcommittee meeting will be in June, 2014 at Blumenau, Brazil, in conjunction with the 20th Congress of the IOM.

Minute 22. Adjournment. The meeting was adjourned at 12:00 on July 15th, 2012.

Session 2 - Open meeting.

Minute 23. Summary of activities. During the final session of the 19th Congress of the IOM, at 17:00 on July 19th, 2012, D. Brown described the organization and responsibilities of the ICSP and its subcommittees and summarized the closed session. Approximately 200 delegates attending the Congress were present.

Minute 24. Adjournment. The open meeting was adjourned at 17:30 on July 19th, 2012.