International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of mollicutes

Minutes of the interim meetings, 13 and 19 July 2000, Fukuoka, Japan

Session 1. Closed meeting

Minute 1. Call to order. The closed meeting was called to order at 09:00 on 13 July 2000 by the Chair, J. M. Bradbury.

Minute 2. Record of attendance. The members present were J. M. Bohé, J. M. Bradbury (Chair and Acting Secretary), G. Christiansen, G. Firrao, R. Harasawa, F. Laigret, R. J. Miles, J. D. Pollack, S. Razin, J. A. Robertson and R. F. Rosenbusch. Ex-officio members present were D. G. Pitcher of the Public Health Laboratory Service, London, and F. C. Minion, Chair of the Board of the International Organization of Mycoplasmology (IOM) International Research Programme on Comparative Mycoplasmology (IRPCM). Apologies for absence were received from J. Frey, K.-E. Johansson (Secretary), B. C. Kirkpatrick, H. C. Neimark, K. Sachse, J. G. Tully, R. F. Whitcomb, D. Taylor-Robinson and from ex-officio members M. K. Davidson and J. K. Davis.

Minute 3. Report of the Chair. J. M. Bradbury welcomed a new subcommittee member, Dr R. Harasawa from Japan, to the meeting. She also thanked Dr Sumio Arai and the organizing committee of the 13th International Congress of the IOM for their assistance in making the arrangements for the subcommittee meetings.

During the last biennium two members of the subcommittee, E. A. Freundt and R. H. Leach, tendered their resignations. Professor Eyvind Freundt had been a member of the subcommittee since its formation in 1967 and had served as either Chair or Secretary for eight meetings. Dr Leach joined the subcommittee in 1982 and attended every meeting thereafter until his retirement. The considerable input of both members was gratefully acknowledged.

During the 1998–2000 biennium, one new species was reported in each of the genera Acholeplasma, Entomoplasma and Spiroplasma. These were Acholeplasma vituli, Entomoplasma freundtii and Spiroplasma poulsonii respectively. In addition three new Candidatus Phytoplasma species were described: Candidatus Phytoplasma australasiae, Candidatus Phytoplasma japonicum and Candidatus Phytoplasma fraxini.

Minute 4. Spiroplasma taxonomy. J. M. Bohé reminded members that the order Entomoplasmatales contains mollicutes from insects, other arthropods and plants and is divided into two families, the Entomoplasmataceae and the Spiroplasmataceae. The former contains two genera, Entomoplasma and Mesoplasma and the latter contains the genus Spiroplasma. Following the complete sequencing of the 16S rDNA of 38 spiroplasma species, groups and strains, G. E. Gasparich and colleagues have undertaken a major study of the phylogeny of the genus Spiroplasma using these sequences together with 10 published earlier by Weisburg et al. [J Bacteriol 171 (1989), 6455–6467]. Thirteen phylogenetic analyses were performed by different methods and the same 12 groups were revealed by most analyses. Using maximum likelihood or parsimony these groups fit very well in general with the earlier serological groupings of the spiroplasmas. The genera Entomoplasma and Mesoplasma fall into a cluster with the Mycoplasma mycoides group but the root of this clade in the spiroplasma tree is not yet completely resolved.

Minute 5. Phytoplasma taxonomy. G. Firrao reported that many new phytoplasma 16S rRNA gene sequences have become available in the last biennium and approximately 80 sequences are now known. These will probably be organized into 15–20 different groups and B. C. Kirkpatrick was earlier asked to coordinate the production of a publication giving a general interpretation of the phylogenetic data, taking into account the diverse views of phytoplasma workers. The subcommittee urged that this publication be produced as soon as possible. The question of distance for separating the groups was discussed and a similarity value of around 97% was mentioned as a possible limit, but this is still open to discussion.

Hitherto, the strategy of the IRPCM Phytoplasma working team has been to describe one Candidatus species selected from each major phylogenetic group. The recent description of Candidatus Phytoplasma fraxini was discussed because the name has been assigned to a group of strains which, according to their 16S rRNA gene sequences, are not identical and a type strain has been identified to represent the group. The subcommittee considered that the assignment of Candidatus Phytoplasma fraxini to a group is not strictly in the spirit of the proposal of Murray & Schleifer [Int J Syst Bacteriol 44 (1994), 174–176]. A letter outlining this view will be sent to the Editorial Board of the International Journal of Systematic and Evolutionary Microbiology (IJSEM) on behalf of the subcommittee.

With regard to Candidatus species, the subcommittee wished to record its view that, in the event of such an organism being cultivated and fully described, due recognition be given to the original workers and that the proposed Latin binomial should be retained. At present there is no absolute requirement for this but this view is in accord with that of Murray & Schleifer [Int J Syst Bacteriol 44 (1994), 174–176], who made the initial proposal for the Candidatus rank in order to record the properties of putative new taxa.

Minute 6. Ureaplasma taxonomy. J. A. Robertson reminded the subcommittee that there were 14 Ureaplasma urealyticum serovars but that the homogeneity of the strains within a single species had not been demonstrated. The strains clearly fall into two groups as indicated by many criteria. These included DNA homologies, the similarity of their proteins and polypeptides, genome sizes and 16S rDNA sequences. For the 16S rRNA genes the sequence difference between the two groups is around 1%. This division into two groups is now supported by other properties including sequence data for the 16S–23S rDNA intergenic spacer region genes, the urease genes and the multibanded antigen (MBA) genes. These have all been confirmed independently...
by Kong et al. [Int J Syst Evol Microbiol 49 (1999), 1879–1889]. MBAs are putative virulence factors but there is no conclusive evidence of pathogenicity differences between the two groups.

The subcommittee has discussed the division of the strains into two groups at an earlier meeting [Int J Syst Bacteriol 47 (1997), 911–914] and a manuscript proposing a new species, ‘Ureaplasma parvum’, for the group with the smaller genome will be submitted in the near future. However, the organism selected for genome sequencing by J. I. Glass and co-workers was a strain of the proposed ‘Ureaplasma parvum’. In order to avoid future confusion in the literature, the subcommittee has requested that the authors make reference to this fact when this genome sequence is published in Nature. Mention of this in the abstract was felt to be particularly important. [Note added after the meeting: the authors agreed to insert the term ‘parvum biovar’ in parentheses after the first mention of Ureaplasma urealyticum in the abstract since the Editors of Nature will not permit the use of the name ‘Ureaplasma parvum’ before it is validly published.]

Minute 7. Taxonomy of the haemotrophic mollicutes. A paper by Neimark et al. has been published [Int J Syst Evol Microbiol 51 (2001), 895–899] proposing the transfer of two members of the genus Haemobartonella (Haemobartonella felis and Haemobartonella muris) and two of the genus Eperythrozoon (Eperythrozoon wenyonii and Eperythrozoon suis) to the genus Mycoplasma on the basis of their 16S rRNA sequence similarities. The respective new designations are ‘Candidatus Mycoplasma haemofelis’, ‘Candidatus Mycoplasma haemomuris’, ‘Candidatus Mycoplasma wenyonii’ and ‘Candidatus Mycoplasma haemosuis’. These noncultivable organisms form a new phylogenetic cluster within the pneumoniae group and share some properties with members of this group and with one another. They represent a new group of parasitic mollicutes whose mode of parasitism appears to differ from that of other known mollicutes in that they are closely associated with erythrocytes and under certain circumstances may give rise to anaemia. The affiliation of the other named species of the Haemobartonella and Eperythrozoon is uncertain.

Minute 8. Update on 16S rDNA sequencing of the genus Mycoplasma. A report by K.-E. Johansson indicated that 28 new 16S rDNA sequences from members of the genus Mycoplasma were deposited in GenBank in the last biennium, leaving only 15 such sequences remaining to be deposited and published in order to complete all the recognized species in this genus. The type strain of M. gallisepticum will also be sequenced. The subcommittee commended the team working on this large-scale project, whose goal should be achieved by the end of the year 2001.

Minute 9. Use of 16S rRNA sequence data as a replacement for large-scale serology. D. G. Pitcher raised the possibility of revising the minimal standards for the description of new species of Mollicutes in order to reduce the large numbers of serological tests currently required for characterizing putative new species of the genus Mycoplasma. The subcommittee agreed that when the 16S rDNA sequences of all known Mycoplasma species were published, it should then be possible to identify the phylogenetic clade to which a potential new species belongs, and to restrict serological cross-testing to members of that clade. Serological tests were still necessary because 16S rDNA sequences alone have not proved to be completely reliable for distinguishing between certain species (e.g. Mycoplasma gallisepticum and Mycoplasma imitans which show 99-9% identity of the 16S rRNA genes [J. F. Boyle, BSc Hons thesis. University of Melbourne (1993)] but only 40–46% total DNA–DNA homology [Bradbury et al. Int J Syst Bacteriol 43 (1993), 721–728]. Similar discrepancies have been demonstrated previously within other genera [Fox et al. Int J Syst Bacteriol 42 (1992), 166–170].

The subcommittee will initiate revision of the minimal standards document for discussion at the next meeting in 2002. It was agreed that preliminary cloning of strains should be retained as an essential step in order to avoid the common pitfall of working with mixed cultures. The need to use polyclonal antisera rather than monoclonal antisera for the serological tests will also be heavily stressed. Antiserum prepared against new species will still be needed so that other workers can identify isolates in situations where sequencing is not practical.

The possibility of including a special paragraph on methods for describing phytoplasmas is to be considered in view of the discussions in Minute 5 above.

Minute 10. Taxonomic status of some members of the Mycoplasma mycoides cluster. R. J. Miles raised some taxonomic issues on behalf of the former European Union COST 826 action on Ruminants’ Mycoplasmoses. This group was seeking opinions on the possibility of combining Mycoplasma mycoides subsp. capri and Mycoplasma mycoides subsp. mycoides LC strains into one taxon on the basis of sequence analysis of their 16S rRNA genes [Pettersson et al. Int J Syst Bacteriol 178 (1996), 4131–4142]. The proposal is also supported by sequence analysis of a putative membrane-protein gene [Thiaucourt et al. Vet Microbiol 72 (2000), 251–268]. These reassignments would avoid future confusion between Mycoplasma mycoides subsp. mycoides SC and LC strains, since the latter are apparently more closely related to the ‘capri’ subspecies than to the SC strains. There followed some discussion about the retention of subspecies status for these organisms, but D. Pitcher intimated that the subspecies rank is not favoured by the Editorial Board of the IJSEM. One possible solution was to propose the combination of Mycoplasma mycoides subsp. capri and Mycoplasma mycoides subsp. mycoides LC into the single taxon Mycoplasma capri, as suggested previously by Pettersson et al. [Int J Syst Bacteriol 178 (1996), 4131–4142]. As a consequence Mycoplasma mycoides subsp. mycoides SC might become simply M. mycoides although the possible regulatory complications of these reassignments should be very carefully considered.

The taxonomic position of bovine serogroup 7 was discussed. Several outbreaks of arthritis and pneumonia due to this organism had occurred in Australia (J. Frey & S. Djordjevic, unpublished) and for practical reasons it would be useful to place members of this group into a species or subspecies. 16S rDNA analysis reveals that this serogroup is more closely related to the two subspecies of Mycoplasma capricolum than to the subspecies of M. mycoides [Pettersson et al. J Bacteriol 178 (1996), 4131–4142] and this group might logically become a subspecies of M. capricolum. However, in view of the editorial policy of IJSEM referred to above, the view was that bovine serogroup 7 might be established as a new species, provided that the minimal standards were met. The merits of assigning species status to the M. capricolum subspecies (M. capricolum subsp. capricolum and M. capricolum subsp. capripneumoniae) was discussed since it was clear that these two organisms are biologically very different. The implications of all these possible nomenclatural changes should be considered...
further and ideally should also include the views of absent subcommittee members as well as the IRPCM Ruminant working team.

Minute 11. Phylogenetic relationship of the ovine/caprine group 11 mycoplasmas to M. bovigenitalium. A further taxonomic issue raised by R. J. Miles on behalf of the former European Union COST 826 action on Ruminants’ Mycoplasmoses was the status of the ovine/caprine group 11 mycoplasmas. R. A. J. Nicholas and colleagues (unpublished) have demonstrated that the 16S rDNA sequences of eight field strains of group 11 mycoplasmas isolated from diseased sheep show very high homology with Mycoplasma bovigenitalium strains. Cross-testing by immunofluorescence and growth inhibition confirms a very close relationship and the subcommittee concurred with the views of these workers who are of the opinion that the ovine/caprine group 11 strains should be incorporated into the species M. bovigenitalium. R. F. Rosenbusch will discuss the practical implications of this with the IRPCM Ruminant working team.

Minute 12. Intraspecific variation of different genes in M. hominis. G. Christiansen and co-workers have studied variability within four housekeeping genes of M. hominis from different parts of the genome. They selected the gyrB, tuf, ftsY and gap genes, looked at the least conserved parts of the gene, and observed low intraspecies variability within these genes. However, there was theoretical (statistical) evidence of recombination within the gap gene and between the other genes although this has still to be demonstrated by biological methods. This had already been suspected for the membrane-protein genes.

Minute 13. Are additional genes besides 16S rRNA genes needed for taxonomic purposes? Correspondence from J. Davis and M. K. Davidson, who are ex-officio members of the subcommittee, raised the possibility of using additional gene sequences to enable differentiation of the major groups of Mollicutes since their Gainesville laboratory was well placed to assist with sequencing nominated genes. J. D. Pollack reported that kinases were being investigated with this in mind, but the general feeling was that it was too soon to make decisions on which genes were most suitable. The gap gene or other housekeeping genes were cited as possibilities, but J. M. Bové suggested that species-specific genes, which are not in databases, might prove more useful; however, no recommendations could be made in the light of present knowledge. F. C. Minion reported that the IRPCM Molecular Genetics working team had been consulted but felt that the issue was a taxonomic one and should be decided by the subcommittee once more gene sequences were known.

Minute 14. Liaison with IRPCM. F. C. Minion summarized the relevant activities of the IRPCM over the last biennium. Current interactions with the subcommittee are limited to issues relevant to taxonomy and are mostly in the hands of the IRPCM Molecular Genetics working team (see Minute 13). Since the Mollicutes are so well studied, he felt that there was an ideal opportunity for the subcommittee and the members of the IRPCM between them to set taxonomic standards for the microbiological community.

Minute 15. Status of the mollicute reference collection at the University of Florida. A statement from J. K. Davis and M. K. Davidson indicated that the extensive reagent collection donated by J. G. Tully had now been fully transferred to Florida. Inventories were being prepared of the collections donated by R. F. Whitcomb and M. F. Barile.

It is hoped that a searchable database will be available on the World Wide Web in future and will be linked to the IOM and IRPCM web pages. The reagents are available to researchers for a modest fee but are limited in amount.

Minute 16. Future meetings. The next meeting of the subcommittee will be held in connection with the IOM meeting in Vienna, Austria, 7–12 July 2002. The meeting in the year 2004 will take place in Athens, Georgia, USA.

Minute 17. Election of officers and membership changes. A ballot will be held during the next biennium.

Minute 18. Any other business. A statement received from J. G. Tully, an editor of Volume 3 of the next edition of Bergey’s Manual of Systematic Bacteriology, indicated that most sections of the updated Bergey’s Manual were well on schedule and that publication is planned for 2001. [Note added after the meeting: due to delays in the publication of Volumes 1 and 2, the publication of Volume 3 is also likely to be delayed until 2002]. D. G. Pitcher updated the subcommittee on the editorial policies and funding situation of the IJSEM. Despite a large increase in submissions, subscriptions for the journal had decreased since its transfer from the American Society for Microbiology to the Society for General Microbiology. This has resulted in a need for strict page limits, for extreme brevity in manuscripts and a very strict editorial policy. The prime purpose will be to publish the official descriptions of new species and high-quality evolution and phylogeny papers. Finally, the hope was expressed that the importance of the subcommittee meetings would be better recognized by future IOM congress organizing committees. The time allocations at the last two meetings had proved too short for proper discussion of some items and at least an extra hour has been requested for the 2002 meeting.

Minute 19. Adjournment. The closed meeting was adjourned at 12:30 on 13 July 2000.

Session 2. Open meeting

Minute 20. Call to order. An open meeting of the subcommittee was called to order at 16:00 on 19 July 2000 by the Chair, J. M. Bradbury.

Minute 21. Record of attendance. The members present were J. M. Bové, J. M. Bradbury (Chair and Acting Secretary), G. Christiansen, G. Firraro, F. Laigret, R. J. Miles, J. D. Pollack, S. Razin, J. A. Robertson and R. F. Rosenbusch. Ex-officio members present were D. G. Pitcher of the Public Health Laboratory Service, London, and F. C. Minion, Chair of the Board of the International Organization of Mycoplasmology (IOM) International Research Programme on Comparative Mycoplasmology (IRPCM).

Minute 22. J. M. Bradbury welcomed approximately 50 attendees to the meeting and briefly described the functions of the subcommittee. She acknowledged the valuable contributions of two retiring members, E. A. Freundt and R. H. Leach, and stressed that the subcommittee would welcome the input of newer workers in the field who had an interest in taxonomy. It was noted that there had been one new member of each of the genera Acholeplasma, Entomoplasm and Spiroplasma validly described in the last biennium. Three ‘Candidatus’ Phytoplasma’ species had been published and a paper had been submitted proposing the transfer of four haemotrophic bacteria from the order Rickettsiales to the genus Mycoplasma [this proposal has since been published:...

Minute 23. Spiroplasma taxonomy. J. M. Bové presented a synopsis of the current classification, indicating that a new phylogenetic study fitted well with the previously established serological groups.

Minute 24. Phytoplasma taxonomy. G. Firrao summarized the discussions of the subcommittee over the recent assignment of a Candidatus species name to a group of non-identical phytoplasmas. The subcommittee recommends that each future description of a ‘Candidatus Phytoplasma’ species should refer to a single, unique 16S rRNA gene sequence.

Minute 25. Ureaplasma taxonomy. J. A. Robertson outlined the current situation with regard to the proposed division of the two biovars of U. urealyticum into two species. The validity of the proposal has been reinforced by studies published during the last biennium. It was noted that the genome being sequenced is actually that of the ‘parvum’ biovar and a discussion then ensued on the ethics of patenting gene sequences. J. M. Bové pointed out that gene sequences themselves are not patentable, but only innovations resulting from them.

Minute 26. 16S rDNA sequencing of members of the genus Mycoplasma and use of the sequences in the characterization of new species. The project to establish the 16S rDNA sequences of all members of the genus Mycoplasma will be completed by K.-E. Johansson’s and B. Pettersson’s groups, hopefully by the end of 2001. Once these sequences are available to the scientific community, the minimal standards document could be revised to incorporate the use of this sequence data in the description of new species. It should be possible to define the taxonomic cluster to which a putative new species belongs and to restrict serological cross-testing to members of this cluster, thus considerably reducing the large-scale serology that is currently needed. The subcommittee considered that serology will still be essential for the near future since experience has shown that all species cannot be safely determined using 16S rDNA sequences alone. In view of the antigenic variation shown by these organisms, such serology must be carried out with polyclonal, not monoclonal, antisera. The vital importance of cloning to ensure a pure culture at the outset of such studies was stressed.

Minute 27. Use of additional genes to the 16S rRNA gene for taxonomic purposes. It is generally acknowledged that it would be useful to confirm taxonomic groupings resulting from 16S rDNA gene sequencing by using other gene sequences. Housekeeping genes or species-specific genes might be considered but it is too early to nominate any particular gene as being of value in this respect.

Minute 28. Taxonomic status of some bovine/ovine/caprine mycoplasmas. Several taxonomic issues have been raised for discussion by the former European Union COST 826 action on Ruminants’ Mycoplasmoses in view of the phylogenetic data resulting from 16S rDNA sequences. R. J. Miles reported that the subcommittee favoured the combination of M. mycoides subsp. capri and M. mycoides subsp. mycoides LC strains into one taxon since the LC strains are more closely related to M. mycoides subsp. capri than to the SC strains of M. mycoides. This would leave M. mycoides comprising only the SC strains. Bovine group 7 mycoplasmas may be assigned to a new species, provided that the minimal standards are fulfilled and ovine/caprine group 11 may be incorporated into the species M. bovigenitalium. The implications of all these possible changes should be fully discussed by relevant parties.

Minute 29. Intraspecific variation of different genes in M. hominis. Preliminary statistical evidence of recombination events in housekeeping genes of M. hominis was reported by G. Christiansen. This may have implications for future gene-based taxonomic schemes.

Minute 30. Reference collections. J. M. Bradbury summarized the status of the mollicute reference collection at the University of Florida. The large collection of reagents donated by J. G. Tully was now available and collections donated by R. F. Whitcomb and M. F. Barile were being inventoried. A web site with details of these reagents would eventually be available to IOM members.

Minute 31. Election of Officers. A ballot will be held during the forthcoming biennium to replace the members who retired during the last biennium.

Minute 32. Current membership. The current membership of the subcommittee is as follows: J. M. Bradbury, Liverpool, UK (Chair); K.-E. Johansson, Uppsala, Sweden (Secretary); J. M. Bové, Villenave d’Ornon, France; G. Christiansen, Aarhus, Denmark; J. Frey, Berne, Switzerland; G. Firrao, Udine, Italy; R. Harasawa, Tokyo, Japan; B. C. Kirkpatrick, Davis, CA, USA; F. Laigret, Villenave d’Ornon, France; R. J. Miles, London, UK; H. C. Neaimark, NY, USA; J. D. Pollack, Columbus, OH, USA; S. Razin, Jerusalem, Israel; J. A. Robertson, Edmonton, Canada; R. F. Rosenbusch, Ames, IA, USA; K. Sachse, Jena, Germany, J. G. Tully, Frederick, MD, USA; R. F. Whitcomb, Tucson, AZ, USA. Advisory member is D. Taylor-Robinson, Buckinghamshire, UK. Ex officio members are K. Waites (IOM IRPCM Chair Elect); M. K. Davidson and J. K. Davis, Gainesville, FL, USA; and D. G. Pitcher, London, UK.

Minute 33. Adjournment. J. M. Bradbury thanked all who had attended the open meeting and those who had contributed to the discussions. The particular help of the Secretary K.-E. Johansson and of J. G. Tully in preparations for the meeting was acknowledged. The open meeting was adjourned at 17:00 on 19 July 2000.

J. M. Bradbury, Acting Secretary