Taxonomy of *Picornaviridae*: Current Situation and Future Proposals

Nick J. Knowles[1], Tapani Hovi[2], Timo Hyypiä[3], Andrew M.Q. King[4], A. Michael Lindberg[5], Philip D. Minor[6], Mark A. Pallansch[7], Ann C. Palmenberg[8], Tim Skern[9], Glyn Stanway[10], Teruo Yamashita[11] and Roland Zell[12]

(ICTV *Picornaviridae* Study Group)

1) Institute for Animal Health, Pirbright, UK; 2) Enterovirus Laboratory, KTL, Finland; 3) Dept. of Virology, University of Turku, Finland; 4) 'Sunfield', Dawney Hill, Pirbright, Woking, Surrey, UK; 5) Dept. of Chemistry and Biomedical Sciences, Univ. of Kalmar, Sweden; 6) National Institute for Biological Standards and Control, South Mimms, UK; 7) Enterovirus Team, CDC, Atlanta, USA; 8) Dept. of Animal Health and Biomedical Science, University of Wisconsin, USA; 9) Max F. Perutz Laboratories, Medical University of Vienna, Vienna, Austria; 10) Dept. of Biological Sciences, University of Essex, UK; 11) Dept. of Microbiology, Aichi Prefectural Institute of Public Health, Aichi, Japan; 12) Klinikum der Friedrich-Schiller-Universitaet, Institut fuer Virologie und Antivirale Therapie, Jena, Germany.

ABSTRACT

The family *Picornaviridae* currently consists of 23 species in 9 genera (*Enterovirus*, *Rhinovirus*, *Cardiovirus*, *Aphthovirus*, *Hepatovirus*, *Parechovirus*, *Erbovirus*, *Kobuvirus* and *Teschovirus*). Three new taxonomic proposals have been approved by the ICTV Executive Committee and await ratification by the ICTV membership. They are: i) to combine the enterovirus and rhinovirus genera, keeping the existing name *Enterovirus*; ii) to combine the species' *Poliovirus* and *Human enterovirus C*, retaining the latter name; and iii) to assign *Human enterovirus C* as the type species of the enterovirus genus. Two further proposals are in preparation: i) to create a new genus, *Sapelovirus*, consisting of three species, *Porcine enterovirus A*, Simian virus 2 and duck picornavirus TW90A (each to be renamed); and ii) to create a new unassigned species, *Seneca Valley virus*. Overall this will leave the number of genera unchanged, but result in the addition of two species. A number of issues are also being considered by the Study Group: i) the genus placement of *Seneca Valley virus*; ii) the position of avian encephalomyelitis virus as a tentative member of the genus *Hepatovirus*; iii) the proposal to place duck hepatitis virus type 1 (for which a number of genome sequences have recently been reported) in a new genus; iv) the proposal that bovine rhinovirus type 2 is classified as a new species in the genus *Aphthovirus*; and v) the proposal to divide bovine enteroviruses into two species. The *Picornaviridae* Study Group has a new website: www.picornastudygroup.com where the latest classification and proposals may be viewed.

Polioviruses are to be incorporated into the species *Human Enterovirus C* (which now becomes the *Enterovirus* type species)

It has been realised for many years that the sequences of members of the *Human enterovirus C* and *Poliovirus* species of the genus *Enterovirus* are very similar, particularly in the region encoding the non-structural proteins (Hughes *et al*., 1987; Hughes *et al*., 1989; Supanaranond *et al*., 1992). Recent complete nucleotide sequence analysis of representatives of all known serotypes contained within *Human enterovirus C* has definitively demonstrated this relationship (Brown *et al*., 2003; Newcombe *et al*., 2003). The species are not monophyletic in either of the regions used for *Picornaviridae* taxonomy, P1 and 2C+3CD, and in the 2C+3CD region amino acid identity is generally in excess of 90%. It has also been suggested in a number of studies that homologous recombination may take place between polioviruses and members of the *Human enterovirus C* species (e.g. Liu *et al*., 2003; Arita *et al*., 2005), which is one measure taken into account in the definition of picornavirus species. Although polioviruses have a distinct and specific receptor usage from other *Human enterovirus C* members, differences in receptor usage are also evident within the species *Human enterovirus B* and so this characteristic is currently not being considered equitably in *Enterovirus* taxonomy. These factors make the current division into two species untenable and the purpose of this proposal is to rationalize this situation by combining the *Human enterovirus C* and *Poliovirus* species, creating a single species.

Rhinovirus genus incorporated into the genus *Enterovirus*

Historically, the human pathogens enteroviruses and rhinoviruses have been classified into separate genera (*Enterovirus* and *Rhinovirus* respectively), largely on the basis of classical criteria such as pathogenicity and the acid stability of enteroviruses/ lability of rhinoviruses. Sequence analysis reveals that there are no significant differences in genome organisation or particle structure (Kitamura *et al*., 1981; Stanway *et al*., 1984; Laine *et al*., 2005). In both the standard regions used for picornavirus taxonomy (P1 and 2C+3CD) species representing these genera are much more closely related that those representing other distinct Picornaviridae genera. Also, in the P1 region, the genera are not monophyletic. Furthermore, it has recently been found that in sequence terms human rhinovirus 87 is in fact an acid labile member of the species *Human enterovirus D*, suggesting that acid stability/lability is not always a reliable classification criterion (Blomqvist *et al*., 2002; Savolainen *et al*., 2002). Thus, it is difficult to justify the current enterovirus/rhinovirus division and it is proposed to merge these genera, giving a single genus, *Enterovirus*.

Proposal to split bovine enteroviruses into two species, *Bovine enterovirus A* and *Bovine enterovirus B* (Zell *et al*., 2006).

It has recently been shown that the serotypes BEV-1 and BEV-2 are two distinct genetic clusters between which recombination is rare (possibly only occurring within the untranslated regions) and that each group contains a number of (sero)types. It has therefore been decided to submit a proposal to the ICTV Executive Committee to split the species into two.

New genus to be proposed: *Sapelovirus*

Porcine enterovirus A* to be renamed *Porcine Sapelovirus

Simian virus 2 and related viruses to be named *Simian sapelovirus A*

Duck picornavirus TW90A to be named *Avian sapelovirus*

Complete genome sequences of PEV-8 (Krumbholz *et al*., 2002), SV2 (Oberste *et al*., 2003) and DPV (Tseng & Tsai, unpublished) show these viruses to be related to, but distinguishable from, enteroviruses in the conserved genome regions (P1, 2C, 3C & 3D), however, they have a number of distinct genome features (hepatitis C-like IRES, a leader polypeptide, highly divergent 2A, 2B and 3A regions) which we consider warrants the creation of a new genus.

Your opinions are important to us: If you have any views on the classification/taxonomy of the *Picornaviridae* please contact one of the Study Group members.

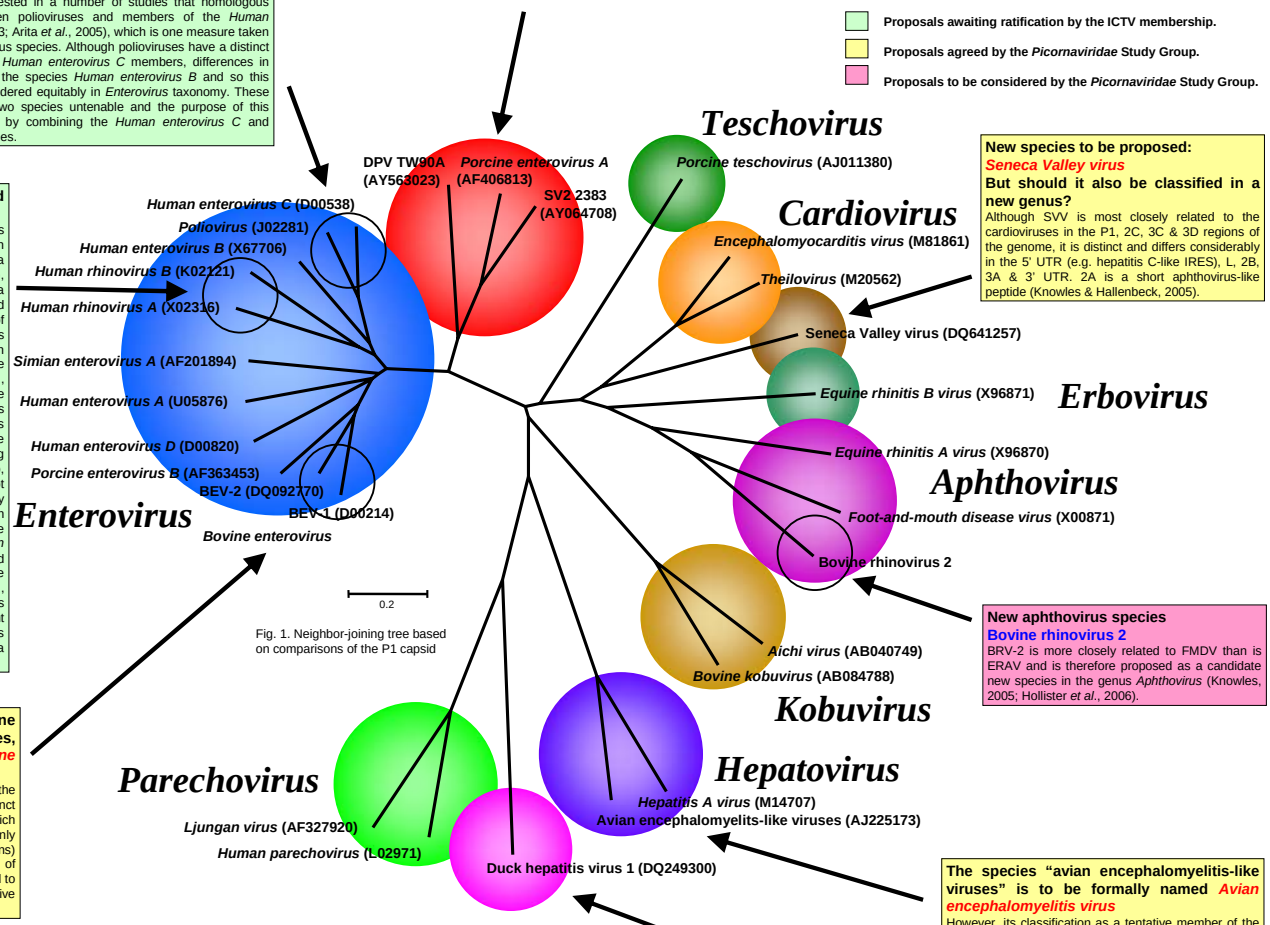

Fig. 1. Neighbor-joining tree based on comparisons of the P1 capsid

New species to be proposed: *Seneca Valley virus*

But should it also be classified in a new genus?

Although SVV is most closely related to the cardioviruses in the P1, 2C, 3C & 3D regions of the genome, it is distinct and differs considerably in the 5' UTR (e.g. hepatitis C-like IRES), L, 2B, 3A & 3' UTR. 2A is a short aphthovirus-like peptide (Knowles & Hallenbeck, 2005).

New aphthovirus species

Bovine rhinovirus 2

BRV-2 is more closely related to FMDV than is ERAV and is therefore proposed as a candidate new species in the genus *Aphthovirus* (Knowles, 2005; Hollister *et al*., 2006).

The species "avian encephalomyelitis-like viruses" is to be formally named *Avian encephalomyelitis virus*

However, its classification as a tentative member of the genus *Hepatovirus* will be discussed by the Study Group. It has a number of genome features which distinguish it from hepatitis A virus, e.g. hepatitis C-like IRES, parechovirus-like 2A.

New species & genus?

Duck hepatitis virus 1

The complete genome sequences of five isolates of DHV-1 have recently been described (Kim *et al*., 2006; Tseng *et al*., 2006; Ding and Zhang, unpublished; Zhang et al., unpublished). They are most closely related to the parechoviruses, but genetic distances are large.

REFERENCES

Arita, M., Zhu, S.L., Yoshida, H., Yoneyama, T., Miyamura, T. & Shimizu, H. (2005). A Sabin 3-derived poliovirus recombinant contained a sequence homologous with indigenous human enterovirus species C in the viral polymerase coding region. J. Virol. 79: 12650-12657.

Blomqvist S., Savolainen C., Raman L., Roivainen M. & Hovi T. (2002). Human rhinovirus 87 and enterovirus 68 represent a unique serotype with rhinovirus and enterovirus features. J. Clin. Microbiol. 40:4218-4223.

Brown B., Oberste M.S., Maher K. & Pallansch M.A. (2003). Complete genomic sequencing shows that polioviruses and members of human enterovirus species C are closely related in the noncapsid coding region. J. Virol. 77:8973-8984.

Ding, C.Y. & Zhang, D.B. Molecular analysis of duck hepatitis virus type 1. Unpublished.

Hollister, J.R., Vagnozzi, A., Knowles, N.J. & Rieder, E. (2006). Molecular analysis of bovine rhinovirus type 2 shows a close relationship to the aphthoviruses. *EUROPIC 2006: XIVth Meeting of the European Study Group on the Molecular Biology of Picornaviruses*, Saariselkä, Inari, Finland, 26th November-1st December 2006.

Hughes, P.J., John, A., Minor, P.D. and Stanway, G. (1987). The sequence of the polymerase gene of coxsackievirus A21 indicates a remarkably close relationship to the polioviruses, Arch. Virol. 94:141-147.

Hughes P.J., North C., Minor P.D. & Stanway G. (1989). The complete nucleotide sequence of coxsackievirus A21. J. Gen. Virol. 70:2943-2952.

Kim, M.-C., Kwon, Y.-K., Joh, S.-J., Lindberg, A.M., Kwon, J.-H., Kim, J.-H. & Kim, S.-J. (2006). Molecular analysis of duck hepatitis virus type 1 reveals a novel lineage close to the genus Parechovirus in the family *Picornaviridae*. *J. Gen. Virol.* **87:** 3307-3316.

Kitamura N., Semler B.L., Rothberg P.G., Larsen G.R., Adler C.J., Dorner A.J., Emini E.A., Hanecak R., Lee J.J., Der Werf S., Anderson C.W. & Wimmer E. (1981). Primary structure, gene organization and polypeptide expression of poliovirus RNA. Nature 291:547-553.

Knowles, N.J. (2005). Molecular identification of all three bovine rhinovirus serotypes as members of the genus *Aphthovirus*. *EUROPIC 2005: XIIIth Meeting of the European Study Group on the Molecular Biology of Picornaviruses*, Lunteren, The Netherlands, 23-29th May 2005. Abstract A18.

Knowles, N.J. & Hallenbeck, P.L. (2005). A new picornavirus is most closely related to cardioviruses. *EUROPIC 2005: XIIIth Meeting of the European Study Group on the Molecular Biology of Picornaviruses*, Lunteren, The Netherlands, 23-29th May 2005. Abstract A14.

Krumbholz, A., Dauber, M., Henke, A., Birch-Hirschfeld, E., Knowles, N.J., Stelzner, A. & Zell, R. (2002). Sequencing of porcine enterovirus groups II and III reveals unique features of both virus groups. *J. Virol.* **76:** 5813-5821.

Laine P., Savolainen C., Blomqvist S. & Hovi T. (2005). Phylogenetic analysis of human rhinovirus capsid protein VP1 and 2A protease coding sequences confirms shared genus-like relationships with human enteroviruses. J. Gen. Virol. 86:697-706.

Liu, H.M., Zheng, D.P., Zhang, L.B., Oberste, M.S., Kew, O.M. & Pallansch, M.A. (2003). Serial recombination during circulation of type 1 wild-vaccine recombinant polioviruses in China. J. Virol. 77: 10994-11005.

Newcombe N.G., Andersson P., Johansson E.S., Au G.G., Lindberg A.M., Barry R.D. & Shafren D.R. (2003). Cellular receptor interactions of C-cluster human group A coxsackieviruses. J. Gen. Virol. 84:3041-3050.

Oberste, M.S., Maher, K. & Pallansch, M.A. (2003). Genomic evidence that simian virus 2 and six other simian picornaviruses represent a new genus in *Picornaviridae*. *Virology* **314:** 283-293.

Savolainen C., Blomqvist S., Mulders M.N. & Hovi T. (2002). Genetic clustering of all 102 human rhinovirus prototype strains: serotype 87 is close to human enterovirus 70. J. Gen. Virol. 83:333-340.

Stanway G., Hughes P.J., Mountford R.C., Minor P.D. & Almond J.W. (1984). The complete nucleotide sequence of a common cold virus: human rhinovirus 14. Nucleic Acids Res. 12:7859-7875.

Supanaranond K., Takeda N. & Yamazaki S. (1992). The complete nucleotide sequence of a variant of Coxsackievirus A24, an agent causing acute hemorrhagic conjunctivitis. Virus Genes 6:149-158.

Tseng, C.-H. & Tsai, H.-J. Sequence analysis of a duck picornavirus isolate indicates that it together with porcine enterovirus type 8 and simian picornavirus type 2 should be assigned a new picornavirus genus. Unpublished.

Tseng, C.-H., Knowles, N.J. & Tsai, H.-J. (2006). Molecular analysis of type 1 duck hepatitis virus indicated that it should be assigned to a new genus. *Virus Research*, Oct 24; [Epub ahead of print].

Zell, R., Krumbholz, A., Dauber, M., Hoey, E. & Wutzler, P. (2006). Molecular-based reclassification of the bovine enteroviruses. *J. Gen. Virol.* **87:** 375-385.

Zhang, X.F., Zhao, R.H. & Wen, L.B. Unpublished.