Template for Taxonomic Proposal to the ICTV Executive Committee
Creating Species in an existing genus

Code[†] 2007.137V.04 To designate the following as species in the genus:

Orbivirus

belonging to the family° : ***Reoviridae***

Yunnan orbivirus
Yunnan orbivirus (YUOV)

[†] Assigned by ICTV officers
° leave blank if inappropriate or in the case of an unassigned genus

Author(s) with email address(es) of the Taxonomic Proposal

Houssam Attoui, SG co-chair Reoviridae: houssam.attoui@bbsrc.ac.uk

Peter Mertens, SG co-chair Reoviridae : peter.mertens@bbsrc.ac.uk

Old Taxonomic Order

Order
Family *Reoviridae*
Genus *Orbivirus*
Type Species *Bluetongue virus*
Species in the genus
African horsesickness virus
Bluetongue virus
Changuinola virus
Chenuda virus
Chogar Gorge
Corriparta virus
Epizootic hemorrhagic disease virus
Equine encephalosis virus
Eubenangee virus
Ieri virus
Great Island virus
Lebombo virus
Orungo virus
Peruvian horse sickness virus
Palyam virus

St Croix River virus
Umatilla virus
Wad Medani virus
Wallal virus
Warrego virus
Wongorr virus

Tentative Species in the Genus

Andasimbe virus
Codajas virus
Ife virus
Itupiranga virus
Japanaut virus
Kammavanpettai virus
Lake Clarendon virus
Matucare virus
Tembe virus
Tracambe virus
Yunnan orbivirus

Unassigned Species in the Family

New Taxonomic Order

Order
Family *Reoviridae*
Genus *Orbivirus*
Type Species *Bluetongue virus*
Species in the genus

African horsesickness virus
Bluetongue virus
Changuinola virus
Chenuda virus
Chogar Gorge
Corriparta virus
Epizootic hemorrhagic disease virus
Equine encephalosis virus
Eubenangee virus
Ieri virus
Great Island virus
Lebombo virus
Orungo virus
Peruvian horse sickness virus
Palyam virus
St Croix River virus
Umatilla virus
Wad Medani virus
Wallal virus
Warrego virus
Wongorr virus
Yunnan orbivirus

Tentative Species in the Genus	Andasimbe virus Codajas virus Ife virus Itupiranga virus Japanaut virus Kammavanpettai virus Lake Clarendon virus Matucare virus Tembe virus Tracambe virus

Unassigned Species in the Family

ICTV-EC comments and response of the SG

Species demarcation criteria in the genus

In common with the other genera within the family *Reoviridae*, the prime determinant for inclusion of virus isolates within a single *Orbivirus* species is compatibility for reassortment of genome segments during co-infection, thereby exchanging genetic information and generating viable progeny virus strains. However, data providing direct evidence of segment reassortment between isolates is limited and serological comparisons (primarily involving the immunodominant serogroup/species specific antigen VP7(T13)), form the usual basis of diagnostic assays for each of the virus species (serogroups).

Members of a single *Orbivirus* species may be identified by:

1) The ability to exchange genetic material by genome segment reassortment during dual infections, thereby producing viable progeny virus strains.
2) High levels of serological cross reaction by ELISA, or assays such as complement fixation (CF), or agar gel immunodiffusion (AGID), using either polyclonal sera, or monoclonal antibodies against conserved antigens such as VP7 (T13). For example in competition ELISA, at a test serum dilution of 1/5, a positive serum will show >50% inhibition of colour formation, while a negative control serum, or serum that is specific for a different species will normally produce <25% inhibition of colour compared to a no antibody control. Distinct but related species may show low level serological cross-reaction, which may be only 'one way'.
3) High levels of RNA sequence similarities in conserved genome segments. Viruses within the same species will normally show <24% sequence variation in genome segment encoding the major subcore structural protein, the T2 protein). Viruses in different species will normally contain >26% sequence variation in genome segment encoding the T2 protein; these differences are also reflected in the amino acid sequences of the viral proteins.
4) Relatively efficient cross hybridization of conserved genome segments (those not encoding outer capsid components, or other variable proteins) under high stringency conditions (>85% homology) (northern or dot blots, with probes made from viral RNA or cDNA).
5) PCR using primers to conserved genome regions or segments such as 3 or 7 can be coupled with cross hybridisation analysis (northern or dot blots).
6) Identification by virus serotype with a virus type already classified within a specific *Orbivirus* species. None of the serotypes from different species will cross neutralise.
7) Analysis of electropherotype by agarose gel electrophoresis but not by PAGE. (Viruses within a single species will show a relatively uniform electropherotype. However, a major deletion / insertion event may result in two distinct electropherotypes within a single species (for example EHDV) and some similarities can exist between more closely related species.
8) Identical conserved terminal regions of the genome segments (some closely related species can have identical terminal sequences on at least some segments).
9) Identification of vector or host species and the clinical signs produced. For example BTV is transmitted only by certain *Culicoides* species and will infect cattle and sheep producing clinical signs of varying severity but is not thought to infect horses. The reverse is true of AHSV.

Argumentation to justify the designation of new species in the genus

Yunnan orbivirus (YUOV) has been shown by initial electron microscopy studies to have structure similar to that of bluetongue virus and the other orbiviruses (double shelled capsid with a "non-turreted" core particle. It also has a ten-segmented dsRNA genome and appears to be transmitted between mammalian hosts by mosquitoes. These are characteristics, which clearly indicate that it is an orbivirus. It has been shown to replicate in a variety of mosquito cells and in animals including mice (Attoui *et al.*, submitted).

The full-length genome was cloned and sequenced completely (Attoui *et al.*, submitted).

The conserved terminal regions of the YUOV genome segments ar**e:** 5'-GUUAAA----$N^{A}/_{G/C}{}^{A}/_{G}$UAC-3' (N is A,U,G or C). These show considerable similarity (identical at the 5' end) to *Bluetongue virus* (the prototype *Orbivirus* species) but are not identical at the 3' end. They are also similar but different to those of the other orbiviruses that have been characterized (Mertens *et al.*, 2005).

Bluetongue virus (BTV, *Orbivirus* type species)	5'-GUUAAA----------ACUUAC-3'
African horse sickness virus (AHSV)	5'-GUU$^{A}/_{U}$A$^{A}/_{U}$----------AC$^{A}/_{U}$UAC-3'
Epizootic hemorrhagic disease virus (EHDV)	5'-GUUAAA----------$^{A}/_{G}$CUUAC-3'
Great Island virus (BRDV)	5'-GUAAAA----------A$^{A}/_{G}$GAUAC-3'
Palyam virus (CHUV)	5'-GU$^{A}/_{U}$AAA----------$^{A}/_{G}$CUUAC-3'
Equine encephalosis virus (EEV)	5'-GUUAAG----------UGUUAC-3'
St Croix River virus (SCRV)	5'-$^{A}/_{G}$UAAU$^{G}/_{A}/_{U}$---$^{G}/_{A}/_{U}{}^{C}/_{U}{}^{C}/_{A}$UAC-3'
Peruvian horse sickness virus (PHSV)	5'-GUUAAAA---------$^{A}/_{G}{}^{C}/_{G}{}^{A}/_{G}$UAC-3'
Yunnan orbivirus (YUOV)	5'-GUUAAA----------N$^{A}/_{G/C}{}^{A}/_{G}$UAC-3'

These data also indicate that although YUOV is an orbivirus, it does not belong to any of these other *Orbivirus* species.

The T2 protein of orbiviruses correlates with species (serogroup) within genus *Orbivirus* (Mertens *et al.*, 2005). Analysis of VP2 of YUOV showed it to be the "T2" protein, which forms the subcore shell of the orbivirus core [VP2(T2) of BRDV (Moss and Nuttall, 1994), VP2(T2) of SCRV (Attoui *et al.*, 2001) and VP3(T2) of BTV (Grimes *et al.*, 1998), Mertens *et al.*, 2005]. As a consequence of its important functional role in virus protein / RNA structure and assembly, the T2 protein is highly conserved (Grimes *et al.*, 1998; Gouet *et al.*,1999) exhibiting very high levels of sequence identity. T2 aa identity within a single *Orbivirus* species (serogroup) is over 91% and this value could be used for delineation of species (Attoui *et al.*, 2001). The level of aa identity that was detected in the "T2" protein between YUOV and the other orbiviruses ranges between 24 and 52 %, showing that YUOV is a member of a new species within genus *Orbivirus*. A phylogentic tree for the T2 proteins of orbiviruses is presented in figure 1. This tree identifies YUOV as a distinct species.

Within genus *Orbivirus*, amino acid identity detected between the VP1(Pol) sequences from the insect-borne species AHSV, BTV and PALV are 55 to 64% and those between these viruses and the tick-borne SCRV are ~35%. The VP1(Pol) of YUOV is ~47% identical to insect borne orbiviruses and 36% to SCRV. Accordingly, YUOV is only distantly related to both the insect and tick transmitted *Orbivirus* species described to date and represents a new *Orbivirus* species (figure 2).
Host and vector range, together with terminal sequence and coding region comparisons are consistent with classification of YUOV within a new *Orbivirus* species.

List of created Species in the genus

References

Houssam ATTOUI, Fauziah MOHD JAAFAR, Mourad BELHOUCHET, Nicolas ALDROVANDI, Sanju TAO, Boquan CHEN, Guodong LIANG, Robert B. TESH, Philippe de MICCO and Xavier de LAMBALLERIE. The *Yunnan orbivirus*, a new orbivirus species isolated from *Culex tritaeniorhynchus* mosquitoes in China. *J Gen Virol* **86**, 3409-3417.

Attoui, H., Stirling J. M., Munderloh, U. G., Billoir, F., Brookes, S. M., Burroughs, J. N., de Micco, P., Mertens, P. P. C. & de Lamballerie, X. (2001). Complete sequence characterisation of the genome of the St. Croix River Virus, a new orbivirus isolated from Ixodes scapularis cells. *J Gen Virol* **82**, 795-804.

Gouet, P., Diprose J. M., Grimes J. M., Malby. R., Burroughs, J. N., Zientara, S., Stuart, D. I., & Mertens, P. P. C. (1999). The Highly ordered double-stranded RNA genome of bluetongue virus revealed by crystallography. *Cell* **97**, 481-490.

Grimes, J. M., Burroughs, J. N., Gouet, P., Diprose, J. M., Malby, R., Zientara, S., Mertens, P. P. C. & Stuart, D. I. (1998). The atomic structure of the bluetongue virus core. *Nature* **395**, 470-478.

Mertens, P.P.C., Attoui, H., Duncan, R. & Dermody, T.S. (2005). *Reoviridae*. In *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses,* pp. 447-454. Edited by C. M Fauquet,. M. A. Mayo, J. Maniloff, U. Desselberger & L. A. Ball. London: Elsevier/Academic Press.

Moss, S. R. & Nuttall, P. A. (1994). Subcore and core-like particles of Broadhaven virus (BRDV), a tick-borne orbivirus, synthesized from baculovirus expressed VP2 and VP7, the major core proteins of BRDV. *Virus Res* **32,** 401-407.

Annexes:

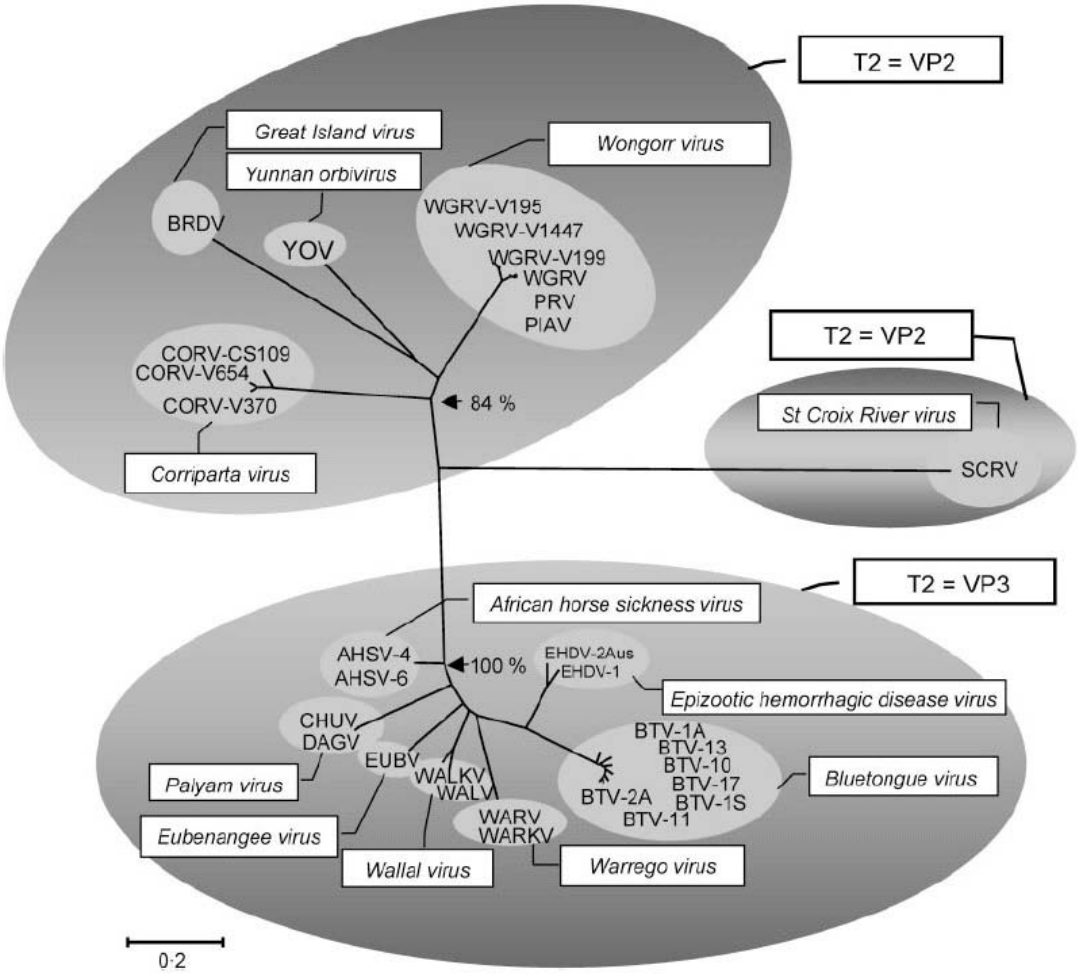

Figure 1: Neighbour-joining phylogenetic tree (using the Poisson correction algorithm or the gamma distribution) of the T2 proteins (the major component of the subcore shell) of YUOV and other orbivirus species. This protein is equivalent to the VP3 (T2) protein of BTV, the prototype species of the genus Orbivirus, and to the VP2 (T2) of two tick-borne orbiviruses, St Croix River virus (SCRV) and Broadhaven virus (BRDV). Many of the available sequences are incomplete; therefore, the analysis (presented as a radial tree) is based on partial sequences (aa 393–548 relative to the BTV-10 sequence; GenBank accession no. P12435). Two clusters that are supported by bootstrap values >80% are identified: the cluster of viruses with the T2 protein encoded by segment 2 (VP2) and the cluster of viruses with the T2 protein encoded by segment 3 (VP3). SCRV dissects the tree and forms a distinct phylogenetic group.

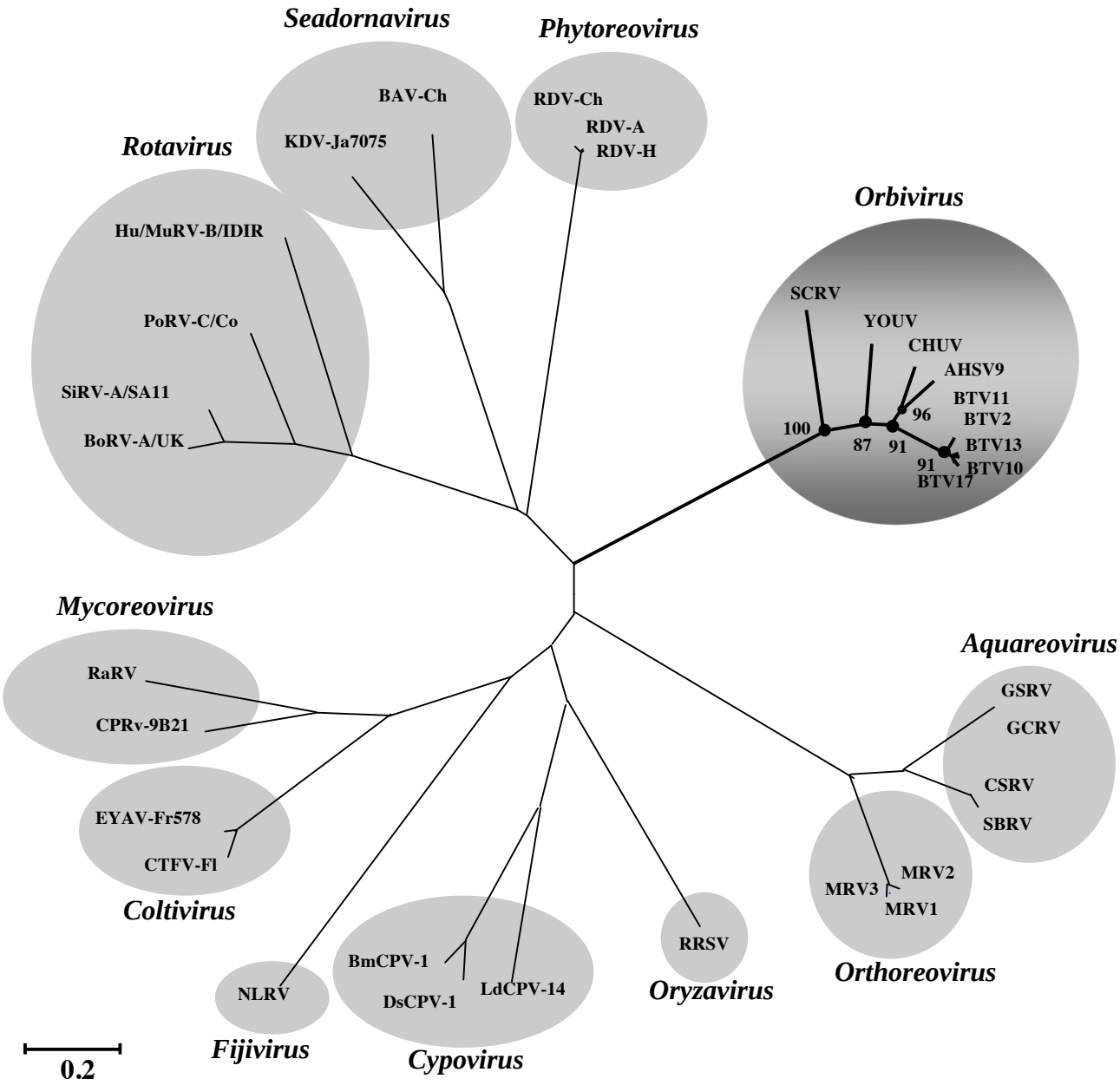

Figure 2 : Phylogenetic comparison of the viral polymerase VP1(Pol) proteins of YUOV, other orbivirus species and members of other genera within the family *Reoviridae*. The analysis (presented as radial tree) was constructed with the help of the MEGA3 program using the p-distance algorithm. The cluster of orbiviruses is presented at the upper right of the tree and bootstrap values higher than 85% support the branching within this cluster. Accession numbers and further detail of the sequence and viruses used are from Attoui *et al*., 2001.