

# Template for Taxonomic Proposal to the ICTV Executive Committee

## Creating Species in an existing genus

Code †  To designate the following viruses as species in the genus:

belonging to the family<sup>°</sup> :

† Assigned by ICTV officers

° leave blank if inappropriate or in the case of an unassigned genus

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### New Taxonomic Order

**Order**

**Family** *Reoviridae*

**Genus** *Orbivirus*

**Type Species** *Bluetongue virus*

**List of 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*
- Palyam virus*
- Peruvian horse sickness virus***
- St Croix River virus*
- Umatilla virus*
- Wad Medani virus*
- Wallal virus*
- Warrego virus*
- Wongorr virus*

**List of 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

**List of Unassigned Species in the Family**  
(Fungal viruses)

Rosellina necatrix mycoreovirus (proposed)

## Argumentation to justify the designation of new species in the genus 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 A conserved genome segments. Viruses within the same species will normally show <24% sequence variation in genome segment 3 (encoding the major subcore structural protein, VP3 (T2)). Viruses in different species will normally contain >26% sequence variation in genome segment 3; these differences are also reflected in the amino acid sequences of the viral proteins.
- 4) Relatively efficient cross hybridization of A 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 A 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

Peruvian horse sickness virus (PHSV) 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 'spiked' 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 isolated from horses and other mammalian species in Peru but is only thought to cause significant (severe) disease in horses.

Full-length cDNA clones have been generated for all ten genome segments. Approximately 80% of the genome has now been sequenced (manuscript in preparation).

The conserved terminal regions of the PHSV genome segments are: 5'GUUAAAA .....<sup>A/G</sup>/<sub>G</sub><sup>C/G</sup>/<sub>G</sub><sup>A/G</sup>UAC3', which 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 characterised

Bluetongue virus (the prototype <i>Orbivirus</i> species)	5'-GUUAAAA.....ACUUAC-3',
<i>African horse sickness virus</i>	5'-GUU <sup>A/U</sup> A <sup>A/U</sup> .....AC <sup>A/U</sup> UAC-3',
<i>Epizootic hemorrhagic disease virus</i>	5'-GUUAAAA..... <sup>A/G</sup> CUUAC-3',
<i>Great Island virus</i> (BRDV)	5'-GUAAAA.....A <sup>A/G</sup> GAUAC-3',
<i>Palyam virus</i> (CHUV)	5'-GU <sup>A/U</sup> AAA..... <sup>A/G</sup> CUUAC-3'
<i>Equine encephalosis virus</i> (segment 10)	5'-GUUAAG.....UGUUAC-3'
<i>St Croix River virus</i>	5' <sup>A/G</sup> UAAU <sup>G/A/U</sup> ..... <sup>G/A/U</sup> <sup>C/U</sup> <sup>C/A</sup> TAC-3'

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

Comparisons the nucleotide sequences for the genome segment coding for the internal sub-core shell protein (equivalent to the VP3(T2) protein of BTV) showed highest levels of identity to Chuzan virus (55% identity : *Palyam* species of *Orbivirus* ) and an EHDV (54% identity). The other *Orbivirus* species for which data is available, including the other known equine viruses (*African horsesickness virus* and *Equine encephalosis virus*) all gave lower levels of identity in this gene. These levels of similarity are consistent with PHSV belonging to a distinct orbivirus species.

Comparison of the viral polymerase amino acid sequences (VP1(Pol) of BTV) showed approximately 30% amino acid identity with viruses from several different *Orbivirus* species, with 29.8% identity to that of the AHSV polymerase. Comparisons of the whole protein sequences derived from other genome segments have identified partial matches with proteins from several orbiviruses, sufficient in most cases to indicate protein function. However, the level of homology detected is sufficiently low, in each case to indicate that PHSV does not belong to any of the other genera for which sequence data are currently available. In particular comparisons to EEV and AHSV (the two other orbiviruses known to cause significant disease in horses: but both transmitted by *Culicoides* rather than mosquito species) showed only relatively low levels of homology. Host and vector range, together with terminal sequence and coding region comparisons are consistent with classification of PHSV within a new *Orbivirus* species.

## List of created Species in the genus

*Peruvian horse sickness virus* (PHSV)

## References

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## Annexes: