This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document “Help with completing a taxonomic proposal”

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

**MODULE 1: TITLE, AUTHORS, etc**

<table>
<thead>
<tr>
<th>Code assigned:</th>
<th>2009.010a,bV (to be completed by ICTV officers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short title:</td>
<td>A new species in the genus Mastadenovirus: Murine adenovirus C (e.g. 6 new species in the genus Zetavirus)</td>
</tr>
<tr>
<td>Modules attached</td>
<td></td>
</tr>
<tr>
<td>(modules 1 and 9 are required)</td>
<td>1 ✗ 2 ✗ 3 4 5 ✗ 6 7 8 9 ✗</td>
</tr>
</tbody>
</table>

**Author(s) with e-mail address(es) of the proposer:**

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**Has this proposal has been seen and agreed by the relevant study group(s)?**

Please select answer in the box on the right

- Yes

**ICTV-EC or Study Group comments and response of the proposer:**

Date first submitted to ICTV: 08.05.2009
Date of this revision (if different to above):
MODULE 2: NEW SPECIES

Part (a) to create and name one or more new species.
If more than one, they should be a group of related species belonging to the same genus (see Part b)

**Code** 2009.010aV (assigned by ICTV officers)

To create one new species with the name:

*Murine adenovirus C*

Part (b) assigning new species to higher taxa

All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

**Code** 2009.010bV (assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

<table>
<thead>
<tr>
<th>Genus:</th>
<th>Mastadenovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subfamily:</td>
<td></td>
</tr>
<tr>
<td>Family:</td>
<td>Adenoviridae</td>
</tr>
<tr>
<td>Order:</td>
<td></td>
</tr>
</tbody>
</table>

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "(new)" after its proposed name.
- If no genus is specified, enter "unassigned" in the genus box.

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9
A novel adenovirus (AdV) was isolated from a striped field mouse (*Apodemus agrarius*) and named murine adenovirus 3 (MAdV-3)\(^1\). This virus shows an adequate evolutionary distance from all the other AdVs, and thus it is to be considered as the first member of a new adenovirus species.

The MAdV-3 genome had been fully sequenced (GenBank accession number EU835513)\(^1\). The highest similarities in genomic sequence were found to MAdV-1 (species: *Murine adenovirus A*). However, the differences between these two viruses are enough to classify these viruses into different species according to the following findings.

1. One of the species demarcation criteria in the *Mastadenovirus* genus is that the "properly calculated phylogenetic distance" must be higher than 0.1 (10%)\(^2\). Phylogenetic distance was performed for the four taxonomically relevant genes as proposed by ICTV – hexon, polymerase, protease, and pVIII – using different evolutionary models and software. The distance between MAdV-3 and MAdV-1 was in all four relevant genes higher than 0.1 and varied between 0.135 and 0.468, suggesting that MAdV-3 and MAdV-1 should be defined as different species (Table 1)\(^1\).

2. The novel virus was isolated from a striped field mouse (*Apodemus agrarius*)\(^1\), which is most likely its natural host, whereas MAdV-1 was isolated from *Mus musculus*.

3. In neutralization assays with MAdV-3, the anti-MAdV-3 serum pool showed a 16-fold higher neutralizing antibody titer than the anti-MAdV-1 serum pool (Table 2). In the neutralizing test with MAdV-1, the anti-MAdV-3 serum did not show any cross-neutralization at all, whereas the anti-MAdV-1 serum pool reached a neutralizing antibody titer of 1280 (Table 2)\(^1\). This is rather an argument that MAdV-3 and MAdV-1 can be regarded as different serotypes; however, it is a prerequisite to prove that two viruses isolated from very similar hosts and being candidates for members of different species are distinct also serologically.

4. MAdV-1 and MAdV-3 clearly differ in their organ tropism. While MAdV-1 is rather homogenously distributed in all organs including the brain, MAdV-3 shows a strong preference for myocardial tissue and is undetectable in the brain\(^1\).

5. There are also genome organization differences between MAdV-1 and 3, such as MAdV-3 lacking E4 ORFB, which is present in MAdV-1.
MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:


Annex:
Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders.
The use of Figures and Tables is strongly recommended.

**Table 1.** Genetic distance between MAdV-3 and MAdV-1 calculated for the four taxonomically relevant genes by different evolutionary models (first line) and software programs (in brackets).

<table>
<thead>
<tr>
<th>protein</th>
<th>Dayhoff(^a) (Protdist(^b))</th>
<th>Dayhoff(^a) (Tree-Puzzle(^b))</th>
<th>VT(^a) (Tree-Puzzle(^b))</th>
<th>no model(^a) (Bioedit(^b))</th>
</tr>
</thead>
<tbody>
<tr>
<td>pVIII</td>
<td>0.468447</td>
<td>0.36270</td>
<td>0.34771</td>
<td>0.303</td>
</tr>
<tr>
<td>protease</td>
<td>0.430186</td>
<td>0.35666</td>
<td>0.32742</td>
<td>0.283</td>
</tr>
<tr>
<td>polymerase</td>
<td>0.434361</td>
<td>0.36061</td>
<td>0.32794</td>
<td>0.282</td>
</tr>
<tr>
<td>hexon</td>
<td>0.175666</td>
<td>0.14820</td>
<td>0.14249</td>
<td>0.135</td>
</tr>
</tbody>
</table>

\(^a\) Evolutionary model used to calculate the genetic distance. In case of "no model", observed sequence difference (p-distance) is indicated as a proportion of positions in which the two sequences differ.

\(^b\) Software programs used to calculate the distances.
Table 2. Neutralizing antibody titers against MAdV-3 and MAdV-1 in cross-neutralization analysis of mice immune sera.

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>MAdV-3</th>
<th>MAdV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-MAdV-3</td>
<td>640</td>
<td>&lt;20</td>
</tr>
<tr>
<td>anti-MAdV-1</td>
<td>40</td>
<td>1280</td>
</tr>
</tbody>
</table>

\(^a\) Reciprocal end-point titers are given as determined by PRNT.
\(^b\) Reciprocal end-point titers are given as determined by immunofluorescent-focus reduction neutralization test.

Note on the proposed name.

Murine adenovirus 2 was shown in Report 8 as belonging to the “tentative species” Murine adenovirus B. While this designation is not a valid “taxon” anymore, the available data show that MAdV-2 indeed belongs to a separate species. Thus it is fully logical to keep the Murine adenovirus B designation for the future species to contain MAdV-2 and to give the next available letter (Murine adenovirus C) for the third recognized (and published) murine adenovirus. (The official proposal for species Murine adenovirus B is foreseen in the near future.) In this way, we can preserve also the logic of the continuous numbering and corresponding lettering (MAdV-1 to 3 belonging to Murine adenovirus A to Murine adenovirus C).

Fig. 1. Phylogenetic analysis of MAdV-3 (top of each tree) using amino acid sequences of genes coding for the following proteins: pVIII (A), protease (B), polymerase (C), and hexon (D). ML phylogenetic trees calculated with Tree-Puzzle software package (Dayhoff PAM model) are shown. Similar trees were obtained also when NJ and FM methods implemented in PHYLIP 3.67 software package were used. Atadenoviruses BAdV-4, OAdV-7, and DAdV-1 were used as the outgroup. The values at the tree branches represent the PUZZLE support values. The scale bar indicates an evolutionary distance of 0.1 substitution per position in the sequence. In the abbreviated virus names, the letter in front of AdV indicates the origin of the virus: B, bovine; C, canine; D, duck; H, human; M, murine; O, ovine; P, porcine; S, simian; and TS, tree shrew. Taxonomic species abbreviations are given in boldface.