



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**

<b>Code assigned:</b>	<b>2014.001aV</b>	(to be completed by ICTV officers)			
<b>Short title:</b> Bat hepatitis virus: a new species within the genus <i>Orthohepadnavirus</i> (e.g. 6 new species in the genus <i>Zetavirus</i> )					
<b>Modules attached</b> (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 6 <input type="checkbox"/>	2 <input checked="" type="checkbox"/> 7 <input type="checkbox"/>	3 <input type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	5 <input type="checkbox"/>

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

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**List the ICTV study group(s) that have seen this proposal:**

A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)

Hepadnaviridae & Hepatitis delta virus Study Group

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

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Date first submitted to ICTV:

02 October 2013

Date of this revision (if different to above):

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MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be “unassigned” within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	<b>2014.001aV</b>	(assigned by ICTV officers)
<b>To create 1 new species within:</b>		
Genus:	<b><i>Orthohepadnavirus</i></b>	Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ <b>(new)</b> ” after its proposed name. • If no genus is specified, enter “ <b>unassigned</b> ” in the genus box.
Subfamily:		
Family:	<b><i>Hepadnaviridae</i></b>	
Order:	<b><i>Unassigned</i></b>	
<b>And name the new species:</b>		<b>GenBank sequence accession number(s) of reference isolate:</b>
<i>Bat hepatitis virus</i>		JX941466-68

**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.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Within the genus *Orthohepadnavirus*, the species were demarcated based on the following ICTV criteria:

- 1) Full genome nucleotide sequence diversity of different species should be more than 15%, e.g. woodchuck hepatitis virus (WHV) / hepatitis B virus (HBV) 40%; ground squirrel hepatitis virus (GSHV) / WHV 15%; woolly monkey hepatitis B virus (WMHBV) / HBV 20%; WMHBV/WHV 30% [1].
- 2) Difference in host range: HBV is limited to infect primates [2, 3]. WMHBV was originally found in woolly monkey, but could be transmitted to spider monkey [4]. GSHV was originally found in ground squirrel [5], but could experimentally infect chipmunks and woodchucks [6, 7]. WHV was originally found in woodchuck and does not infect other rodents [7, 8].

In 2012 we first identified a new orthohepadnavirus in bats through metagenomic analysis followed by confirmation based on full genome sequencing and morphology observation [9].

The newly discovered bat hepatitis virus (BtHV) has 3,230 nt in full genome, which is close to the size of primate hepatitis viruses ( $\approx 3,200$  nt) but smaller than rodent hepatitis viruses ( $\approx 3,300$  nt). Under electron microscopy, the viral particles were observed, which were morphologically similar to cores/nucleocapsids or subviral particles (Fig.1). The genomic structure of BtHV contains the same circular and compact genome as other orthohepadnaviruses, comprising 4 open reading frames respectively encoding Pol, preS1/preS2/S, preC/C, and X protein in the same direction (Fig. 2). Here we propose classification of BtHV as a new species within orthohepadnavirus genus based on above criteria and following research data:

- 1) Genome sequence diversity: Nucleotide sequence diversity of BtHV full genome is 46.8-50.4% with HBV, 48.6-49.0% with WMHBV, 50.1-50.6% with WHV and 51.6-51.9% with GSHV (see annex Fig. 3 and table), more divergent than those between current species within the genus.
- 2) Host range: To date, this virus has only been found in long-fingered bats.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

**References:**

1. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, eds. Virus Taxonomy: Ninth Report of the International Committee of Taxonomy of Virus. Hepadnaviridae. San Diego, Calif: Elsevier Academic Press; 2012:445-455.
2. Bancroft WH, Snitbhan R, Scott RM, Tingpalapong M, Watson WT, et al. (1977) Transmission of hepatitis B virus to gibbons by exposure to human saliva containing hepatitis B surface antigen. *J Infect Dis* 135: 79-85.
3. Barker LF, Maynard JE, Purcell RH, Hoofnagle JH, Berquist KR, et al. (1975) Hepatitis B virus infection in chimpanzees: titration of subtypes. *J Infect Dis* 132: 451-458.
4. Robertson BH, Margolis HS (2002) Primate hepatitis B viruses - genetic diversity, geography and evolution. *Rev Med Virol* 12: 133-141.
5. Marion PL, Oshiro LS, Regnery DC, Scullard GH, Robinson WS (1980) A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. *Proc Natl Acad Sci U S A* 77: 2941-2945.
6. Trueba D, Phelan M, Nelson J, Beck F, Pecha BS, et al. (1985) Transmission of ground squirrel hepatitis virus to homologous and heterologous hosts. *Hepatology* 5: 435-439.
7. Seeger C, Marion PL, Ganem D, Varmus HE (1987) In vitro recombinants of ground squirrel and woodchuck hepatitis viral DNAs produce infectious virus in squirrels. *J Virol* 61: 3241-3247.
8. Seeger C, Baldwin B, Hornbuckle WE, Yeager AE, Tennant BC, et al. (1991) Woodchuck hepatitis virus is a more efficient oncogenic agent than ground squirrel hepatitis virus in a common host. *J Virol* 65: 1673-1679.
9. He B, Fan Q, Yang F, Hu T, Qiu W, et al. (2013) Hepatitis virus in long-fingered bats, Myanmar. *Emerg Infect Dis* 19(4): 638-640.

**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 but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

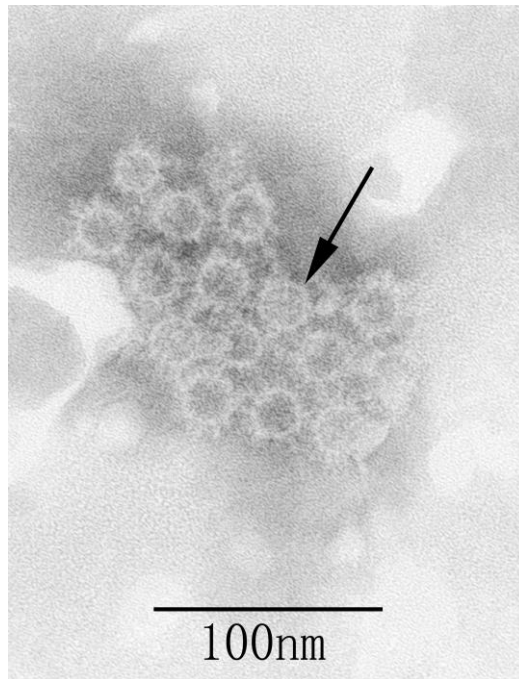


Figure 1. Electron microscopy of negative-stained BtHV particles (arrow), which were morphologically similar to either cores/nucleocapsids or subviral particles. Bar scale represents 100 nm. [from He et al. (2013) *Emerg Infect Dis.*19(4): 638-640].

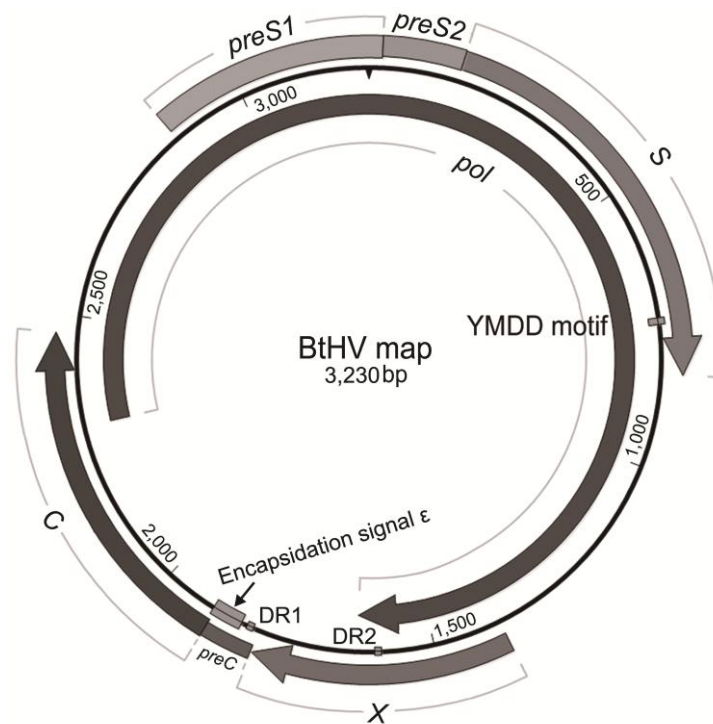


Figure 2. Schematic of genome organization of the BtHV. Boxes and arrows represent the ORFs encoding the main proteins: Pol gene (2,305 to 1,636), preS1/S2 and S gene from (2,864 to 833), preC/C gene (1,815 to 2,468) and X gene (1,378 to 1,812). Two 12 nt direct repeat sequences (DR1 from 1,825 to 1,836 and DR2 from 1,594 to 1,605), the encapsidation signal  $\epsilon$  (1,848 to 1,903) and YMDD domain (734 to 745) are also depicted in the map. [from He et al. (2013) *Emerg Infect Dis.* 19(4): 638-640].

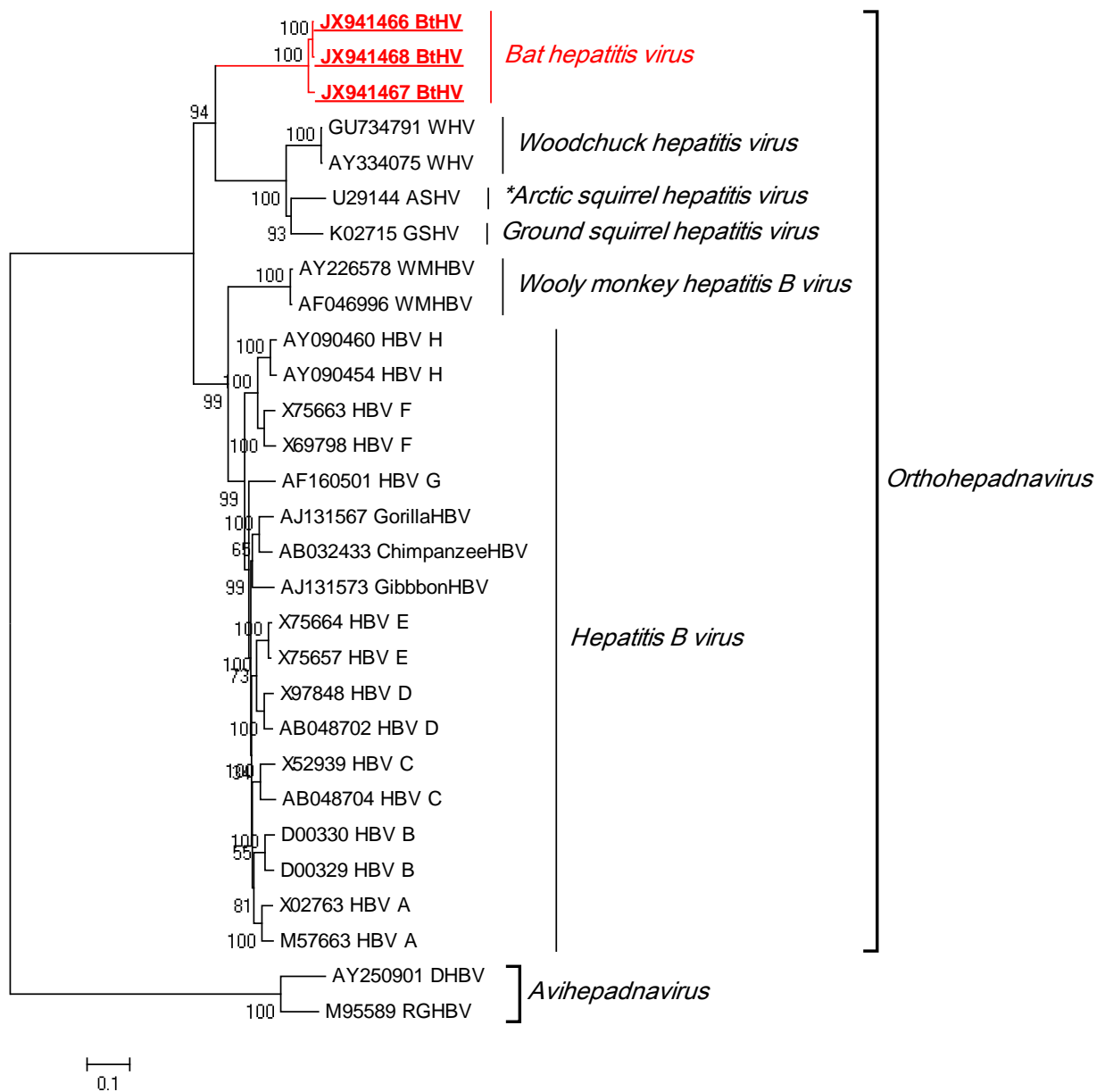


Figure 3: Phylogenetic analysis based on full genomic sequences of BtHVs and other representatives of current species within family *Hepadnaviridae*. These sequences were aligned using Clustal W, and the alignment was tested with the neighbour-joining method. Calibration bar: substitution per site. The classifications of 3 BtHV isolates are highlighted in red color. \*: Arctic squirrel hepatitis virus may be a member of the genus *Orthohepadnavirus* but has not been approved as species.

Table. The length statistic of genes and the percent diversity between BtHV and other hepadnaviruses <sup>a</sup>

Virus	Pol. gene				preS1/preS2/S gene				preC/C gene				X gene			
	nt	D.	aa	D.	nt	D.	aa	D.	nt	D.	aa	D.	nt	D.	aa	D.
BtHV776	2562	/	853	/	1200	/	399	/	654	/	217	/	435	/	144	/
HBV	2532	37	843	43	1203	37	400	41	639	35	212	34	465	39	154	51
WMHBV	2508	37	835	45	1176	36	391	40	636	35	211	37	459	34	152	50
WHV	2640	34	879	44	1281	34	426	49	678	31	225	29	426	33	141	56
ASHV	2634	33	877	47	1284	33	427	48	654	32	217	29	417	31	138	48
DHBV	2526	59	841	70	1104	47	367	70	888	58	295	78	a	/	a	/

a: nt, the nucleotide length of gene; D., the percent diversity of nucleotide sequence and amino acid sequence between BtHV and the others; aa, the amino acids length of gene; a, X gene not available for DHBV; the accession numbers of HBV, WMHBV, WHV, ASHV and DHBV are D00329, AF046996, AY344076, U29144 and EU429324.