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**

Code assigned:	***2016.010a,bS***	(to be completed by ICTV officers)

Short title: Create 13 new species in the genus *Hepacivirus* and rename 1 species (family *Flaviviridae*)
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**2** ☒ **3** ☐ **4** ☐ **5** ☐ **6** ☐ **7** ☐ **8** ☐ **9** ☒ **10** ☐

Author(s):

Donald B. Smith, Paul Becher, Jens Bukh, Ernest A. Gould, Gregor Meyers, Thomas Monath, A. Scott Muerhoff, Alexander Pletnev, Rebecca Rico-Hesse, Jack T. Stapleton, Peter Simmonds

Corresponding author with e-mail address:

Donald Smith, Donald.smith.mail@gmail.com

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)	*Flaviviridae*

ICTV Study Group comments (if any) and response of the proposer:

The proposal is from the *Flaviviridae* Study Group

Date first submitted to ICTV: 23rd June 2016
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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	***2016.010aS***	(assigned by ICTV officers)

To create 13 new species within:

Genus:	***Hepacivirus***
Subfamily:	
Family:	***Flaviviridae***
Order:	

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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Hepacivirus A	NZP1 (equine)	KP325401
Hepacivirus B	GBV-B (primate)	U22304
Hepacivirus D	BWC08 (primate)	KC551800
Hepacivirus E	RHV-339 (rodent)	KC815310
Hepacivirus F	NLR-AP70 (rodent)	KC411784
Hepacivirus G	NrHV-1/NYC-C12 (rodent)	KJ950938
Hepacivirus H	NrHV-1/NYC-E43 (rodent)	KJ950939
Hepacivirus I	SAR-3 (rodent)	KC411806
Hepacivirus J	RMU10-3382 (rodent)	KC411777
Hepacivirus K	PDB-829 (bat)	KC796074
Hepacivirus L	PDB-112 (bat)	KC796077
Hepacivirus M	PDB-491.1 (bat)	KC796078
Hepacivirus N	463 (bovine)	KP641127

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 11

Phylogenetic relationships observed for two conserved genome regions and the host range of variants are consistent with the division of the *Hepacivirus* genus into 14 species which we propose should be named *Hepacivirus A to N* (Table 1). Hepacivirus sequences were aligned using MUSCLE and reduced to a set of those differing over their complete coding sequence by amino acid p-distances greater than 0.1. Since different genotypes of hepatitis C virus all differ by 0.23-0.31, this cut-off would be expected to include all variants likely to represent different species. A scan of mean amino acid p-distance over the coding region revealed two regions where p-distances were consistently less than 0.6; positions 1123-1566 and 2536-2959 (numbered relative to the *Hepacivirus* type species, M62321 (Choo *et al.*, 1989); Figure 1) and therefore most informative for phylogenetic comparisons. Phylogenies of *Hepacivirus* sequences in these regions were congruent apart from minor and non-bootstrap supported rearrangements of deep branches (Figure 2A, B). For the region 1123-1566 the distribution of amino acid p-distances was greater than 0.3 apart from distances between different genotypes of HCV which were 0.12-0.19 (Figure 2C). A more continuous distribution of amino acid p-distances was observed for the region 2536-2959, with discontinuities centred on distances of 0.35 and 0.45.

Demarcation between species is based upon amino acid p-distances of greater than 0.25 in the region 1123-1566 and greater than 0.3 in the region 2536-2959. The rationale for choosing these demarcation points is that they result in HCV and equine hepacivirus being separated into two species, as seems reasonable given their different hosts, while genotypes of HCV remain as members of the same species, reflecting their shared epidemiology and pathology. The only conflict that arises from these choices is that the rodent-derived sequences KC815310 (Kapoor *et al.*, 2013) and KC411784 (Drexler *et al.*, 2013) would be considered as two species by comparison of the region 1123-1566 (amino acid p-distance 0.30) but one species by comparison of the region 2536-2959 (amino acid p-distance 0.27). Since these sequences were obtained from different rodent species in the New and Old worlds, respectively, we prefer a demarcation point that separates these viruses into two species (*Hepacivirus E* and *Hepacivirus F*). The equivocal sequence distances of 0.30 and 0.32 in the region 2536-2959 derive from comparisons between the rodent species *Hepacivirus G* and *Hepacivirus E* and *F*; distances between these species in the region 1123-1566 (0.39, 0.40) are greater than those observed between *Hepacivirus A* and *Hepacivirus C*, (0.35-0.38) suggesting that their demarcation into species is appropriate.

Although evidence has been provided for recombination within (González-Candelas *et al.*, 2011) and between *Hepacivirus* species (Thézé *et al.*, 2015), the only known recombinant included in our dataset was the sequence KC796077 (Quan *et al.*, 2013) which is the single known representative of its clade; exclusion of this sequence did not affect the distribution of sequence distances or phylogenetic relationships between the other species (data not shown).

The genus *Hepacivirus* currently comprises the single species *Hepatitis C virus.* As detailed in module 9, we propose that this species be renamed *Hepacivirus* C. We have made this proposal since, although naming it *Hepacivirus* A would be consistent with it being the first member species to be described and assigned, to do so might cause confusion. All other species are named according to the date of publication of a complete coding sequence with the exception of *Hepacivirus B* which includes GBV-B (providing a memorable link) and *Hepacivirus A* (canine hepacivirus/non-primate hepacivirus).

According to this schema, the genus *Hepacivirus* contains the species *Hepacivirus A* including viruses first detected in dogs (canine hepacivirus) (Kapoor *et al.*, 2011), but which

subsequently have been detected more frequently in horses (non-primate hepacivirus, equine hepacivirus) (Burbelo *et al.*, 2012). There is much greater virus diversity between equine isolates than is currently described for canine isolates (Pybus & Thézé, 2015) with several studies demonstrating transmission and pathology of infection in the horse (Pfaender *et al.*, 2015; Ramsay *et al.*, 2015; Scheel *et al.*, 2015); these observations are consistent with the horse being the primary host and for this reason we have used an equine virus (NSP1, KP325401) as the type isolate. *Hepacivirus B* includes GBV-B, a virus initially detected in and capable of infecting new world primates, but that has not been isolated subsequently (Simons *et al.*, 1995). *Hepacivirus C* includes all currently known genotypes and subtypes of hepatitis C virus, all of which are confined to humans. *Hepacivirus D* includes sequences derived from colobus monkeys but about which there is no information for tropism, chronicity or pathogenicity (Lauck *et al.*, 2013). A similar lack of virological or biological information pertains to those species (*Hepacivirus E-J*) derived from rodents (Drexler *et al.*, 2013; Firth *et al.*, 2014; Kapoor *et al.*, 2013) and bats (*Hepacivirus K-M*) (Quan *et al.*, 2013) . We have retained KC796077 (Quan *et al.*, 2013) as the type species of *Hepacivirus L* although there is evidence that it is recombinant (Thézé *et al.*, 2015), since it groups separately from other species whether or not the recombinant region is included (Figure 2) and since it is the only representative of this clade with a complete coding region sequence. *Hepacivirus N* is represented by viruses isolated from cows and associated with a chronic but asymptomatic liver infection (Baechlein *et al.*, 2015; Corman *et al.*, 2015). We propose that when the next species of *Hepacivirus* is assigned it should be "P" rather than "O" in order to avoid confusion with the letter 0 (zero), and that species beyond X should be named XA, XB … , followed by YA, YB …. and ZA, ZB ….. ZZ.

MODULE 9: **RENAME**

Use this module to change the name of one or more existing taxa (but note that stability of nomenclature is encouraged wherever possible). Insert extra lines in the table if needed.

.

Renaming one or more taxa

Code	***2016.010bS***	(assigned by ICTV officers)

To rename the following taxon (or taxa):

Current name		**Proposed name**
Hepatitis C virus		***Hepacivirus C***

Reasons to justify the renaming: Explain why the taxon (or taxa) should be renamed
In developing a naming schema for the 13 additional species we propose to add to the genus *Hepacivirus,* we chose to use an alphabetical scheme using the format *Hepacivirus X*. The only current member of this genus, *Hepatitis C virus* could then retain its species name, but this would obscure its relationship to other species within the genus. Renaming it *Hepacivirus A* would reflect its positions as the first species to be discovered and named within the genus, but we considered that this might cause unnecessary confusion in the field. We agree as a study group that the best solution is to rename the species *Hepacivirus C* which therefore acknowledges its relation to other species within the genus while also avoiding a change in letter signifier. Hence, hepatitis C virus isolates would belong to the species *Hepacivirus C.*

additional material in support of this proposal

References:

Baechlein, C., Fischer, N., Grundhoff, A., Alawi, M., Indenbirken, D., Postel, A., Baron, A. L., Offinger, J., Becker, K. & other authors. **(2015).** Identification of a Novel Hepacivirus in Domestic Cattle from Germany. *J Virol* **89**, 7007–15.

Burbelo, P. D., Dubovi, E. J., Simmonds, P., Medina, J. L., Henriquez, J. A., Mishra, N., Wagner, J., Tokarz, R., Cullen, J. M. & other authors. **(2012).** Serology-enabled discovery of genetically diverse hepaciviruses in a new host. *J Virol* **86**, 6171–8.

Choo, Q., Kuo, G., Weiner, A., Overby, L., Bradley, D. & Houghton, M. (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science (80-)* **244**, 359–362.

Corman, V. M., Grundhoff, A., Baechlein, C., Fischer, N., Gmyl, A., Wollny, R., Dei, D., Ritz, D., Binger, T. & other authors. **(2015).** Highly divergent hepaciviruses from African cattle. *J Virol* **89**, 5876–82.

Drexler, J. F., Corman, V. M., Müller, M. A., Lukashev, A. N., Gmyl, A., Coutard, B., Adam, A., Ritz, D., Leijten, L. M. & other authors. **(2013).** Evidence for novel hepaciviruses in rodents. *PLoS Pathog* **9**, e1003438 (D. Wang, Ed.). Public Library of Science.

Firth, C., Bhat, M., Firth, M. A., Williams, S. H., Frye, M. J., Simmonds, P., Conte, J. M., Ng, J., Garcia, J. & other authors. **(2014).** Detection of zoonotic pathogens and characterization of novel viruses carried by commensal Rattus norvegicus in New York City. *MBio* **5**, e01933–14.

González-Candelas, F., López-Labrador, F. X. & Bracho, M. A. (2011). Recombination in hepatitis C virus. *Viruses* **3**, 2006–24. Molecular Diversity Preservation International.

Kapoor, A., Simmonds, P., Scheel, T., Hjelle, B., Cullen, J., Burbelo, P., Chauhan, L., Duraisamy, R., Sanchez, L. M. & other authors. **(2013).** Identification of Rodent Homologs of Hepatitis C Virus and Pegiviruses. *MBio* **4**, e00216–13.

Kapoor, A., Simmonds, P., Gerold, G., Qaisar, N., Jain, K., Henriquez, J. A., Firth, C., Hirschberg, D. L., Rice, C. M. & other authors. **(2011).** Characterization of a canine homolog of hepatitis C virus. *Proc Natl Acad Sci U S A* **108**, 11608–11613.

Lauck, M., Sibley, S. D., Lara, J., Purdy, M. A., Khudyakov, Y., Hyeroba, D., Tumukunde, A., Weny, G., Switzer, W. M. & other authors. **(2013).** A novel hepacivirus with an unusually long and intrinsically disordered NS5A protein in a wild Old World primate. *J Virol* **87**, 8971–81.

Pfaender, S., Cavalleri, J. M. V, Walter, S., Doerrbecker, J., Campana, B., Brown, R. J. P., Burbelo, P. D., Postel, A., Hahn, K. & other authors. **(2015).** Clinical course of infection and viral tissue tropism of hepatitis C virus-like nonprimate hepaciviruses in

References:

horses. *Hepatology* **61**, 447–59.

Pybus, O. G. & Thézé, J. (2015). Hepacivirus cross-species transmission and the origins of the hepatitis C virus. *Curr Opin Virol* **16**, 1–7.

Quan, P.-L., Firth, C., Conte, J. M., Williams, S. H., Zambrana-Torrelio, C. M., Anthony, S. J., Ellison, J. A., Gilbert, A. T., Kuzmin, I. V & other authors. (2013). Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proc Natl Acad Sci U S A* **110**, 8194–8199.

Ramsay, J. D., Evanoff, R., Wilkinson, T. E., Divers, T. J., Knowles, D. P. & Mealey, R. H. (2015). Experimental transmission of equine hepacivirus in horses as a model for hepatitis C virus. *Hepatology* **61**, 1533–46.

Scheel, T. K. H., Kapoor, A., Nishiuchi, E., Brock, K. V, Yu, Y., Andrus, L., Gu, M., Renshaw, R. W., Dubovi, E. J. & other authors. (2015). Characterization of nonprimate hepacivirus and construction of a functional molecular clone. *Proc Natl Acad Sci U S A* **112**, 2192–7.

Simons, J. N., Pilot-Matias, T. J., Leary, T. P., Dawson, G. J., Desai, S. M., Schlauder, G. G., Muerhoff, A. S., Erker, J. C., Buijk, S. L. & Chalmers, M. L. (1995). Identification of two flavivirus-like genomes in the GB hepatitis agent. *Proc Natl Acad Sci* **92**, 3401–3405.

Thézé, J., Lowes, S., Parker, J. & Pybus, O. G. (2015). Evolutionary and Phylogenetic Analysis of the Hepaciviruses and Pegiviruses. *Genome Biol Evol* **7**, 2996–3008.

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.

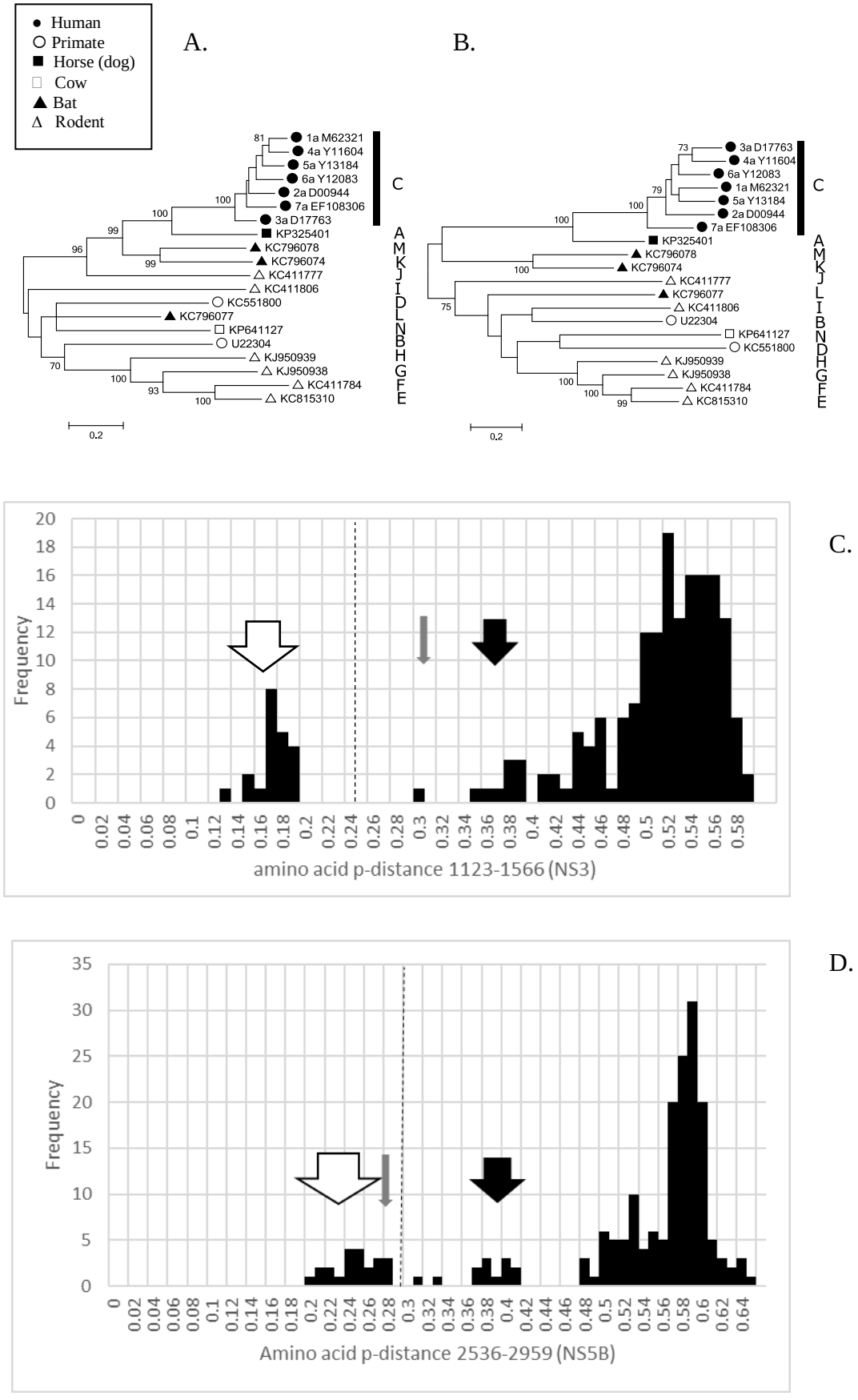

Figure 1 Amino acid divergence across the *Hepacivirus* polyprotein. Mean amino acid p-distances were calculated for 26 aligned *Hepacivirus* polyprotein sequences that differed by > 0.1 of amino

acid positions using a sliding window of 50 amino acids incremented by 10 residues and plotted against the amino acid position of the start of the fragment. The x-axis scale is uneven because of gaps in the reference sequence (M62321). Two regions with distances consistently < 0.6 are indicated by bars. A schematic representation of the *Hepacivirus* polyprotein is shown to scale below.

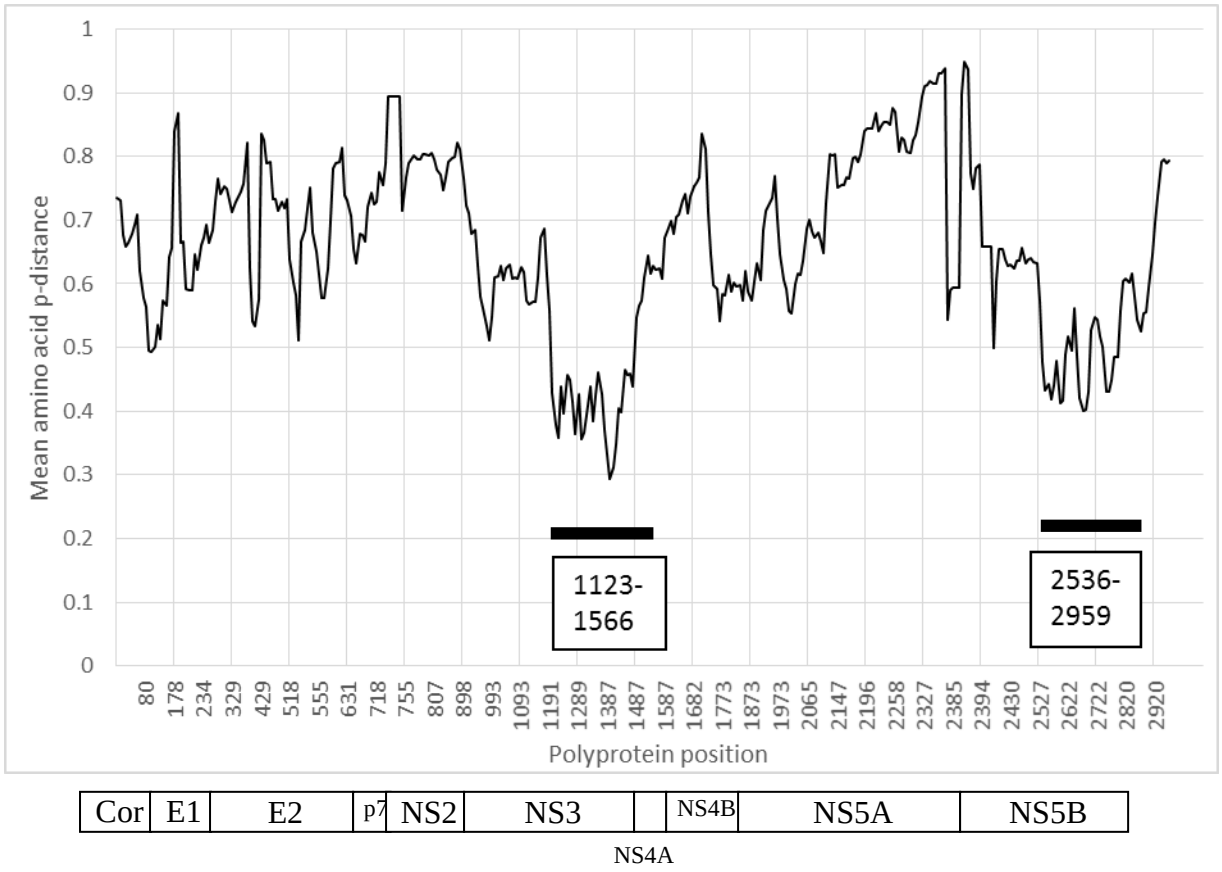

Figure 2. Analysis of *Hepacivirus* conserved regions. Maximum likelihood trees were produced using MEGA 6 for (A) positions 1123-1566 and (B) 2536-2959 of the virus polyprotein using the Le and Gascuel (LG) model and a gamma distribution of variation with invariant sites. Branches observed in >70% of bootstrap replicates are indicated. Proposed *Hepacivirus* species assignments are indicated by single letters to the right of each branch. (C) Frequency histograms of amino acid p-distance between *Hepacivirus* sequences in the region 1123-1566 and (D) the region 2536-2959. The range of distances between different genotypes of *Hepacivirus C* (hepatitis C virus) is indicated by an open arrow, between *Hepacivirus G* and *Hepacivirus H* a shaded arrow and between *Hepacivirus A* and *Hepacivirus C* a black arrow. The distance that demarcates different species is indicated by a dotted line.