
First submission
Guidance from your Editor
Please submit by **27 May 2019** for the benefit of the authors (and your $200 publishing discount).

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data. Download from the materials page.

Image check
Check that figures and images have not been inappropriately manipulated.
Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.
Files

Download and review all files from the materials page.
5 Figure file(s)
1 Table file(s)
For assistance email peer.review@peerj.com

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. BASIC REPORTING
2. EXPERIMENTAL DESIGN
3. VALIDITY OF THE FINDINGS
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

Clear, unambiguous, professional English language used throughout.

Intro & background to show context. Literature well referenced & relevant.

✓

Structure conforms to PeerJ standards, discipline norm, or improved for clarity.

Figures are relevant, high quality, well labelled & described.

✓

Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

Original primary research within Scope of the journal.

✓

Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.

Rigorous investigation performed to a high technical & ethical standard.

Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.

All underlying data have been provided; they are robust, statistically sound, & controlled.

✓

Speculation is welcome, but should be identified as such.

Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

3

The best reviewers use these techniques

Tip

Example

Support criticisms with evidence from the text or from other sources

Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.

Give specific suggestions on how to improve the manuscript

Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).

Comment on language and grammar issues

The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.

Organize by importance of the issues, and number your points

1. Your most important issue
2. The next most important item
3. …
4. The least important points

Please provide constructive criticism, and avoid personal opinions

I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC

Comment on strengths (as well as weaknesses) of the manuscript

I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.

Automated, phylogeny-based genotype delimitation of the Hepatitis Viruses HBV and HCV

Dora Serdari [1] , **Evangelia Georgia Kostaki** [2] , **Dimitrios Paraskevis** [2] , **Alexandros Stamatakis** [1] , **Paschalia Kapli** Corresp. [3]

[1] The Exelixis Lab, Scientific Computing Group, Heidelberg Institute for Theoretical Studies, Heidelberg, Germany

[2] Department of Hygiene Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

[3] Centre for Life’s Origins and Evolution, Department of Genetics Evolution and Environment, University College London, University of London, London, United Kingdom

Corresponding Author: Paschalia Kapli
Email address: p.kapli@ucl.ac.uk

Background: The classification of hepatitis viruses still predominantly relies on ad hoc criteria, i.e., phenotypic traits and arbitrary genetic distance thresholds. Given the subjectivity of such practices coupled with the constant sequencing of samples and discovery of new strains, this manual approach to virus classification becomes cumbersome and impossible to generalize.

Methods: Using two well-studied hepatitis virus datasets, HBV and HCV, we assess if computational methods for molecular species delimitation that are typically applied to barcoding biodiversity studies can also be successfully deployed for hepatitis virus classification. For comparison, we also used ABGD, a tool that in contrast to other distance methods attempts to automatically identify the barcoding gap using pairwise genetic distances for a set of aligned input sequences.

Results - Discussion: We find that~~,~~ the mPTP species delimitation tool identified -even without adapting its default parameters- taxonomic clusters that~~,~~ either correspond to the currently acknowledged genotypes or to known subdivision of genotypes (subtypes or subgenotype). In the cases where the delimited cluster corresponded to subtype or subgenotype, there were previous concerns that their status ~~maybe~~ underestimated. The clusters obtained from the ABGD analysis differed depending on the parameters used. However, under certain values the results were very similar to the taxonomy and mPTP which indicates the usefulness of distance based methods in virus taxonomy under well informed parameters. The overlap of predicted clusters among methods and taxonomically ~~acknowledge~~ genotypes implies that virus classification can be successfully automated.

Title: Automatic, phylogeny based genotype delimitation of the Hepatitis Viruses HBV and HCV

Authors: Dora Serdari[1], Evangelia-Georgia Kostaki[2], Dimitrios Paraskevis[2], Alexandros Stamatakis[1,3], Paschalia Kapli[4*]

Affiliations

[1]Heidelberg Institute for Theoretical Studies, Heidelberg, Germany

[2]Department of Hygiene Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens

[3]Karlsruhe Institute of Technology, Institute for Theoretical Informatics, Karlsruhe, Germany

[4]Centre for Life's Origins and Evolution, Department of Genetics Evolution and Environment, University College London, London, United Kingdom

*Corresponding Author:
Paschalia Kapli
Darwin Building, Gower Street, London, WC1E 6BT, UK
Email address: p.kapli@ucl.ac.uk

Abstract

Background The classification of hepatitis viruses still predominantly relies on ad hoc criteria, i.e., phenotypic traits and arbitrary genetic distance thresholds. Given the subjectivity of such practices coupled with the constant sequencing of samples and discovery of new strains, this manual approach to virus classification becomes cumbersome and impossible to generalize.

Methods

Using two well-studied hepatitis virus datasets, HBV and HCV, we assess if computational methods for molecular species delimitation that are typically applied to barcoding biodiversity studies can also be successfully deployed for hepatitis virus classification. For comparison, we also used ABGD, a tool that in contrast to other distance methods attempts to automatically identify the barcoding gap using pairwise genetic distances for a set of aligned input sequences.

Results - Discussion

We find that~~,~~ the mPTP species delimitation tool identified ~~-~~even without adapting its default parameters~~-~~ taxonomic clusters that~~,~~ either correspond to the currently acknowledged genotypes or to known subdivision of genotypes (subtypes or subgenotype). In the cases where the delimited cluster corresponded to subtype or subgenotype, there were previous concerns that their status ~~maybe~~ underestimated. The clusters obtained from the ABGD analysis differed depending on the parameters used. However, under certain values the results were very similar to the taxonomy and mPTP which indicates the usefulness of distance based methods in virus taxonomy under well informed parameters. The overlap of predicted clusters among methods and taxonomically ~~acknowledge~~ genotypes implies that virus classification can be successfully automated.

Introduction

The continuous advances in next generation sequencing technologies lead to an increasingly easier and inexpensive production of genome and metabarcoding data. The wealth of available data has triggered the development of new models of molecular evolution, algorithms, and software, that aim to improve molecular sequence analyses in terms of biological realism, computational efficiency, or a trade-off between the two. In response to such technological and technical advancements, several fields of biology have undergone a substantial transformation. Sequence-based species delimitation and identification, in the framework of DNA-(meta)barcoding constitutes a representative example that revived taxonomy and systematics (*Tautz et al. 2003; Moritz & Cicero, 2004; Savoleinen et al., 2005; Waugh, 2007; Bucklin et al., 2010; Valentini et al., 2009; Li et al., 2014*), while ~~provide~~ a new mean of analysis in several fields (*Galimberti et al., 2013; Mishra et al., 2015; Lewray & Knowlton, 2015; Bell et al., 2016; Batovska et al., 2017*). Among others, the development of novel species delimitation tools has substantially advanced the study of biodiversity of microorganism that are often hard to isolate and study (*Taberlet et al., 2012; Gibson et al., 2014; Thomsen & Willerslev, 2015*). The sequencing of environmental samples in conjunction with algorithms for genetic clustering has led to the identification of a plethora of previously unknown organisms and a re-assessment of the microbial biodiversity in several settings.

In a similar context, genetic information has been a rich source of information for viral species. Several studies show how phylogenetic information can be deployed for identifying the spatial and temporal origin of a virus, potential factors that trigger its dispersal, and other key epidemiological parameters (*Stadler et al., 2012a; Stadler et al., 2014b; Gire et al., 2014*). In an era of high human mobility, such methods are important, as the increase of emerging and re-emerging epidemics is even more prominent than in the past (*Balcan et al., 2009; Meloni et al., 2011; Pybus et al., 2015*). Nevertheless, phylogenetic information is still not used in the context of virus species classification or identification. As we have witnessed for other microorganisms, using or adapting already available methods for fast and automated delimitation or identification of virus species can greatly contribute to better understand their evolution.

To date, the official taxonomy of viruses (ICTV, i.e., International Committee on Taxonomy of Viruses) has mainly been based on established biological classification criteria as used for other life forms, such as plants or animals. An analogous hierarchical classification system containing orders, families, subfamilies, genera, and species is being applied (*Simmonds, 2015*). The ICTV is typically based on phenotypic criteria, such as morphology, nucleic acid type (i.e., DNA or RNA), hosts, symptoms, mode of replication, geographical data, or presence of antigenic epitopes, to name a few. Generally, such criteria, despite being informative, can be subjective, require highly specialized knowledge, and are time consuming to apply. In contrast, sequence evolution takes into account the evolutionary history of life forms and, thus, may offer a more objective source of information for taxonomic classification. An important difference in viruses compared to other organisms is that they lack a common set of universal genes such as the 18S rRNA in eukaryotes or the 16S rRNA in prokaryotes. Therefore, we cannot infer a comprehensive virus tree of life (*Simmonds et al., 2017*), and, more importantly for species delimitation, we cannot rely upon barcoding markers that are universally suitable for all viruses. We can nonetheless gain valuable insights for their systematics by utilizing phylogenetic information at lower taxonomic ranks (e.g., families, genera, species), using appropriate genes for each dataset. In this context, methods using genetic-distance thresholds (*Bao et al., 2014, Lauber & Gorbalenya, 2012, Yu et al., 2013*) have been suggested as a complementary method to the traditional virus classification for accelerating new species identification.

In this study, we explore whether a recently developed algorithm for molecular species delimitation on barcoding or marker gene phylogenies can be deployed for ICTV. In contrast to genetic distance based methods the multi-rate Poisson Tree Processes (mPTP, *Kapli et al., 2017*) infers the number of genetic clusters given a phylogenetic input tree. Such trees can easily be inferred using both, Maximum Likelihood (*Stamatakis, 2014*), or Bayesian approaches (*Ronquist et al., 2012*) on single-gene or multi-gene multiple sequence alignments. The fundamental assumption of the model is that~~,~~ variance in the data, as represented by the phylogeny, is greater among species than within a species (*Zhang et al., 2013*). The additional assumption of mPTP, that the genetic variation may differ substantially among species allows to accurately delimit species in large (meta-) barcoding datasets comprising multiple species of diverse life histories (*Kapli et al., 2017*). Experiments using empirical data for several animal phyla (*Kapli et al., 2017*) and recently also viruses (*Thézé et al., 2018; Modha et al, 2018*) show that the method consistently provides extremely fast and sensible species estimates on ‘classic’ phylogenetic marker and barcoding genes.

To assess whether mPTP can be deployed as a quantitative ICTV method we analyze two medically important viruses, Hepatitis B (HBV) and Hepatitis C (HCV) that are a leading global causes of human mortality (*Stanaway et al., 2016*). Both viruses cause liver inflammation but are substantially different from each other. HBV has a partially double-stranded circular DNA genome with a length of about 3.2 kb while HCV is a single-stranded, positive-sense RNA virus, with a genome length of approximately 10kb (*Radziwill et al., 1990; Tang et al., 2001; Martell et al., 1992*). Both virus types comprise two taxonomic levels (HBV: genotypes, sub-genotypes; HCV: genotypes, subtypes). Besides the significance of the two viruses for human health, we selected them as test cases since due to the substantial amount of taxonomic research that has been conducted and that we can hence use to assess the efficiency of genetic clustering (e.g., *Simmonds et al., 2005; Schaefer, 2007; Smith et al., 2014; Messina et al., 2015*).

Materials and methods

Datasets

We obtained two previously published multiple sequence alignments (MSAs) corresponding to two virus types: HBV and HCV (*Kramvis, 2014; Smith et al., 2014*, respectively).

The HBV dataset comprises 110 sequences corresponding to eight genotypes (i.e., A-H) and 31 subgenotypes. The genotypes (A through D, F, and H) have been further divided into subgenotypes indexed by numbers for the corresponding genotype (e.g., A_1, A_2, B_1, B_2, B_3, etc.; *Kramvis et al., 2014*). The inter-genotypic and inter-subgenotypic divergence exceeds 8% and 4%-8%, respectively across the genome. No sub-genotypes have been reported for genotypes E, G and H which shows that they are of lower levels of genetic divergence than the rest. The distribution of HBV genotypes differ greatly with respect to the geographical origin. Moreover, they differ in their natural history, response to treatment and disease progression (*Huang, 2013; Biswas, 2013; Moura, 2013; Shi, 2013*). For our study we included the sequences of the eight genotypes (A-H) that form part of the oldest identified HBV groups.

The HCV dataset I) comprises 213 sequences corresponding to seven major taxonomic units named after genotypes (1, 2, 3, 4, 5, 6, and 7) and numerous subtypes (*Smith et al., 2014*). The HCV classification into genotypes and subtypes was based on genetic-distance thresholds that were verified by the fact that they formed monophyletic clades in an inferred phylogeny (*Smith et al., 2014)*. Therefore, the HCV classification serves as an appropriate test case for assessing whether a similar clustering can be identified with a more objective and automated method, such as mPTP, that does not require any user input apart from a phylogeny.

Genetic Cluster delimitation

To delimit the putative species, additionally to mPTP, we used the distance-based "*Automatic Barcode Gap Discovery*" tool (ABGD, *Puillandre et al., 2012*). ABGD is a popular distance-based barcoding method that, compared to other distance-based methods attempts to automatically identify the threshold value for the transition from intra-specific variation to inter-specific divergence (*Puillandre et al., 2012*).

For the mPTP delimitation, a fully binary (bifurcating) rooted phylogeny is required. Therefore, using the aligned sequences we inferred the phylogenetic relationships under the GTR+Γ model of nucleotide substitution using RAxML-NG (*Kozlov et al., 2018*). We rooted the phylogenetic trees according to the originally published phylogenies (i.e., using the branch leading to genotypes F/H for HBV and genotype 7 for HCV). Using heuristic search algorithms for finding the 'best' delimitation given the rooted phylogeny and without any further prior assumptions. We performed the mPTP delimitation under Maximum Likelihood (ML) and calculated the support of the delimited clusters using Markov-chain Monte Carlo (MCMC) sampling (*Kapli et al., 2017*). We conducted the MCMC sampling twice for 10^6 generations, to identify potential lack of convergence with a sampling frequency of 0.1.

For ABGD, the user has to define two important parameters, i) the prior maximum divergence of intraspecific diversity (P), which implies that the barcode gap is expected to exceed this value and should not be confused with the genetic thresholds assumed to define the inter-specific relationships, ii) a proxy for the minimum gap width (X), which indicates that the barcoding gap is expected to be X times larger than any intraspecific gap *(Puillandre et al., 2012*). For both, HBV, and HCV, we used 10 prior maximum thresholds in the range of $p = 0.001$ and $P = 0.05$. The proxy for the minimum gap width (X) was set to the default value ($X = 1.5$) for HCV, while for HBV the default value did not yield any delimitation and we therefore set it to a lower value ($X = 0.5$).

Results & Discussion

The biodiversity of viruses is tremendous and it is broadly accepted that our understanding of their ecology and evolution is constrained to a small fraction of species. In just a kilo of marine sediment there can be a million of different viral genotypes (*Breitbart & Rohwer, 2005*), while on a global scale the number of viruses is 10 million-fold higher than the number of stars in the universe (*Suttle, 2013*). The classification of such a diverse set of organisms constitutes a challenging task and is impossible to accomplish within reasonable time using phenotypic characters. Quantitative computational methods could provide a viable alternative, particularly for large scale clustering and fast identification of viral strains (*Simmonds et al., 2017; Modha et al., 2018*). Using empirical data of the HBV and HCV viruses we show that by applying phylogeny-aware and distance-based tools to classify the strains of the two virus types, the corresponding genetic clustering closely recovers their currently accepted taxonomy.

HCV Clustering

The current taxonomy of HCV comprises seven genotypes, while mPTP yielded 16 genetic clusters (Fig. 1, Suppl. Fig. 1, Suppl. Appendix). From the 16 clusters, five were congruent with the current taxonomy, i.e, genotypes 1, 2, 4, 5 and 7. On the contrary, genotype 3 and genotype 6 were further split into three and eight sub-clusters correspondingly (Fig. 1), which corroborates former views that divergent variants of these genotypes may qualify as separate major genotypes (*Simmonds et al., 2005*, *Smith et al, 2014*). In particular, the additional clusters identified by mPTP correspond to previously identified groups of subtypes (Suppl. Fig. 1). For genotype 6, these clusters consisted of the following subtype groups: 6a; 6b and 6xd; 6c, 6d, 6e, 6f, 6g, 6o, 6p, 6q, 6r, 6s, 6t, 6u, 6w, 6xc and 6xf; 6h, 6i, 6j, 6k, 6l, 6m, 6n, 6xb, 6xe; 6xa; 6v (~~uppl.~~ Fig. 1, Suppl. Appendix). Similarly, for genotype 3, the delimited clusters were i) 3g, 3b, 3i, 3a, 3e, 3d, ii) 3k, and iii) 3h and 3. All clusters were substantially supported by the MCMC sampling, except the split of 3k subtype from ~~it's~~ sister group (Fig. 1), which may be due to lack of adequate sequences for the subtype.

The number of clusters inferred with ABGD ranged from 1 to 208 depending on the value of the maximum intraspecific divergence threshold (Fig. 3). The most reasonable result (i.e., the one closest to the current standard taxonomy) comprised 19 clusters and was obtained for a minimum of intraspecific genetic diversity of 5.99% (i.e., p=0.0599). Under this threshold, the delimitation is largely identical to the delimitation obtained with mPTP (Fig. 1), with three differences: i) that genotype 3 was split into four clusters, instead of three, genotype six was divided into nine clusters instead of eight, and, iii) genotype 7 is divided into two clusters. When the prior intraspecific divergence was increased to a higher minimum of 10%, all sequences were grouped in a single cluster. When the threshold was set to a lower value (3.6%) the number of clusters increased to 135 (Fig. 3). Nevertheless, the delimitation with the 5.99% threshold is largely congruent to current taxonomy and the clusters obtained with mPTP, thus indicating the usefulness of distance based methods in virus taxonomy under well informed parameters.

The so far classification of HCV into genotypes and subtypes has been defined mostly by visual identification of clades in phylogenetic inference of HCV sequences (*Simmonds et al., 2005; Smith et al, 2014*). Specifically, the genotypes correspond to the seven major highly-supported phylogenetic HCV clusters while subtypes were defined as the secondary hierarchical clusters found within each genotype (*Smith et al, 2014*). This classification scheme has been widely adopted (*Combet et al., 2007*; *Yusim et al., 2015*) and has been shown to be robust (in terms of stability of the HCV phylogeny) and relevant for clinical practice, since response rates to immunomodulatory treatment for ~~the~~ chronic hepatitis C differs across genotypes. Nevertheless, new, unassigned lineages are often discovered from understudied areas (*Sulbaran et al., 2010; Nakano et al., 2011; Lu et al., 2013; Tong et al., 2015*) and it challenging to assign them a taxonomic status, given that the genetic distance cut-off among intra and inter-specific relationships is arbitrary and variable for different parts of the HCV phylogeny (*Simmonds et al., 2005*). The greatly overlapping mPTP and ABGD clusters with the HCV genotypes shows that the classification, and, consequently, the identification, of the genotypes can be easily automated utilizing objective, transparent, and unifying approaches. Embracing such alternatives can be crucial for viruses like HCV, taking into account that the correct identifying of the HCV genotypes is of clinical importance for providing the appropriate medical treatment (*Strader et al., 2004; Ge et al., 2009*).

HBV clustering

In the case of HBV, the mPTP clustering is almost identical to the current classification (*Norder et al., 2004; Kramvis et al., 2007*) of the virus that comprises eight genotypes, except for subgenotype C4 which formed a new cluster (Fig. 2, Suppl. Fig. 2, Suppl. Appendix). This is in line with the greater genetic divergence of C4 compared to the other subgenotypes due to its ancient origin in native populations in Oceania (*Paraskevis et al, 2013*). However, the split of C4 from its sister cluster (genotype C) is not supported by the MCMC sampling, potentially reflecting the lack of adequate sampling. On the other hand, the number of clusters identified by ABGD varied from 1 to 85 under different thresholds of minimum intraspecific divergence, while the delimitation for a threshold of 1.29% exactly matched the eight genotypes of the HBV classification (Fig. 2 and 3). Both ABGD and mPTP identified seven of the genotypes (A-F) as distinct genetic clusters. The only difference was that mPTP split genotype C into two distinct clusters (Fig. 2), i.e., subtype C4 was recovered as a distinct cluster from the remaining seven subtypes of genotype.

Conclusions

The application of mPTP to the HCV and HBV data sets shows that automated viral strain clustering using phylogeny-aware methods yields clusters that largely agree with the current standard taxonomy. The major advantage of mPTP over distance-based approaches is that it can be seamlessly applied to taxa of substantially different life histories (e.g., variable population sizes, evolution rates), as it does not require any input parameters except a phylogeny. On the contrary, the example of HCV and HBV~~,~~ shows that meaningful parameter values for distance based methods may differ substantially among datasets, and, therefore, establishing global thresholds is impossible. The ease-of-use of mPTP in conjunction with the computational efficiency on phylogenies with thousands of strains that are increasingly becoming available (*Modha et al., 2018; Paez- Espino et al. 2016*) render mPTP a useful tool for viral biodiversity estimates, initial classification of understudied taxa, and accelerating ~~the~~ viral species identification.

Acknowledgements

The authors gratefully acknowledge the support of the Klaus Tschira Foundation.

References

Balcan, D., Colizza, V., Gonçalves, B., Hu, H., Ramasco, J. J., & Vespignani, A. (2009). Multiscale mobility networks and the spatial spreading of infectious diseases. *Proceedings of the National Academy of Sciences*, *106*(51), 21484-21489.

Bao, Y., Chetvernin, V., & Tatusova, T. (2014). Improvements to pairwise sequence comparison (PASC): a genome-based web tool for virus classification. *Archives of virology*, *159*(12), 3293-3304.

Batovska, J., Lynch, S. E., Cogan, N. O. I., Brown, K., Darbro, J. M., Kho, E. A., & Blacket, M. J. (2018). Effective mosquito and arbovirus surveillance using metabarcoding. *Molecular ecology resources*, *18*(1), 32-40.

Bell, K. L., Burgess, K. S., Okamoto, K. C., Aranda, R., & Brosi, B. J. (2016). Review and future prospects for DNA barcoding methods in forensic palynology. *Forensic Science International: Genetics*, *21*, 110-116.

Biswas, A., Panigrahi, R., Pal, M., Chakraborty, S., Bhattacharya, P., Chakrabarti, S., & Chakravarty, R. (2013). Shift in the hepatitis B virus genotype distribution in the last decade among the HBV carriers from eastern India: possible effects on the disease status and HBV epidemiology. *Journal of medical virology*, *85*(8), 1340-1347.

Bolotov, I. N., Vikhrev, I. V., Kondakov, A. V., Konopleva, E. S., Gofarov, M. Y., Aksenova, O. V., & Tumpeesuwan, S. (2017). New taxa of freshwater mussels (Unionidae) from a species-rich but overlooked evolutionary hotspot in Southeast Asia. *Scientific Reports*, *7*(1), 11573.

Breitbart, M., & Rohwer, F. (2005). Here a virus, there a virus, everywhere the same virus? *Trends in microbiology*, *13*(6), 278-284.

Bucklin, A., Hopcroft, R. R., Kosobokova, K. N., Nigro, L. M., Ortman, B. D., Jennings, R. M., & Sweetman, C. J. (2010). DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition. *Deep Sea Research Part II: Topical Studies in Oceanography*, *57*(1-2), 40-48.

Combet, C., Garnier, N., Charavay, C., Grando, D., Crisan, D., Lopez, J., A., Dehne-Garcia, C., Geourjon, E., Bettler, C., H., P., Le Mercier, R., Bartenschlager, H., Diepolder, D., Moradpour, J.-M., Pawlotsky, C., M., Rice, C., Trépo, F., Penin, G., Deléage. euHCVdb: the European hepatitis C virus database. *Nucleic Acids Res* 2007; 35: D363- D366.

Galimberti, A., De Mattia, F., Losa, A., Bruni, I., Federici, S., Casiraghi, M., Martelos, S., & Labra, M. (2013). DNA barcoding as a new tool for food traceability. Food Research International, 50(1), 55-63.

Ge, D., Fellay, J., Thompson, A. J., Simon, J. S., Shianna, K. V., Urban, T. J., Heinzen, E.L., Qiu, P., Bertelsen, A.H., Muir, A.J., & Sulkowski, M. (2009). Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*, *461*(7262), 399.

Gibson, J., Shokralla, S., Porter, T. M., King, I., van Konynenburg, S., Janzen, D. H., Hallwachs, W., & Hajibabaei, M. (2014). Simultaneous assessment of the macrobiome and microbiome in a bulk sample of tropical arthropods through DNA metasystematics. *Proceedings of the National Academy of Sciences*, *111*(22), 8007-8012.

Gire, S. K., Goba, A., Andersen, K. G., Sealfon, R. S., Park, D. J., Kanneh, L., Jalloh, S., Momoh, M., Fullah, M., Dudas, G., Wohl, S., Moses, L. M., Yozwiak, N. L., Winnicki, S., Matranga, C.B., Malboeuf, C. M., Qu, J., Gladden, A. D., Schaffner, S. F., Yang, X., Jiang, P., Nekoui, M., Colubri, A., Coomber, M. R., Fonnie, M., Moigboi, A., Gbakie, M., Kamara, F. K., Tucker, V., Konuwa, E., Saffa, S., Sellu, J., Jalloh, A. A., Kovoma, A., Koninga, J., Mustapha, I., Kargbo, K., Foday, M., Yillah, M., Kanneh, F., Robert, W., Massally, J. L. B., Chapman, S.B., Bochichio, J., Murphy, C., Nusbaum, C., Young, S., Birren, B. W., Grant, D. S., Scheiffelin, J. S., Lander, E. S., Happi, C., Gevao, S. M., Gnirke, A., Rambaut, A., Garry, R. F., Khan, S. H., & Sabeti, P. C. (2014). Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. *Science*, *345*(6202), 1369-1372.

Huang, C. C., Kuo, T. M., Yeh, C. T., Hu, C. P., Chen, Y. L., Tsai, Y. L., Chen, M. L., Chang, C., & Chang, C. (2013). One single nucleotide difference alters the differential expression of spliced RNAs between HBV genotypes A and D. *Virus research*, *174*(1-2), 18-26.

Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., & Flouri, T. (2017). Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics*, *33*(11), 1630-1638.

Kozlov, A., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2018). RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *bioRxiv*, 447110.

Kramvis, A., & Kew, M. C. (2007). Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. *Hepatology Research*, *37*, S9-S19.

Kramvis, A. (2014). Genotypes and genetic variability of hepatitis B virus. *Intervirology*, *57*(3-4), 141-150.

Lauber, C., & Gorbalenya, A. E. (2012). Genetics-based classification of filoviruses calls for expanded sampling of genomic sequences. *Viruses*, *4*(9), 1425-1437.

Leray, M., & Knowlton, N. (2015). DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences*, *112*(7), 2076-2081.

Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2015). Plant DNA barcoding: from gene to genome. *Biological Reviews*, *90*(1), 157-166.

Lu, L., Li, C., Yuan, J., Lu, T., Okamoto, H., & Murphy, D. G. (2013). Full-length genome sequences of five hepatitis C virus isolates representing subtypes 3g, 3h, 3i and 3k, and a unique genotype 3 variant. *The Journal of general virology*, *94*(Pt 3), 543.

Martell, M., Esteban, J. I., Quer, J., Genesca, J., Weiner, A., Esteban, R., Guardia, J., & Gomez, J. (1992). Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. *Journal of virology*, *66*(5), 3225-3229.

Meloni, S., Perra, N., Arenas, A., Gómez, S., Moreno, Y., & Vespignani, A. (2011). Modeling human mobility responses to the large-scale spreading of infectious diseases. *Scientific reports*, *1*, 62.

Messina, J. P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G. S., Pybus, O. G., & Barnes, E. (2015). Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology*, *61*(1), 77-87.

Mishra, P., Kumar, A., Nagireddy, A., Mani, D. N., Shukla, A. K., Tiwari, R., & Sundaresan, V. (2016). DNA barcoding: an efficient tool to overcome authentication challenges in the herbal market. *Plant biotechnology journal*, *14*(1), 8-21.

Modha, S., Thanki, A. S., Cotmore, S. F., Davison, A. J., & Hughes, J. (2018). ViCTree: an automated framework for taxonomic classification from protein sequences. *Bioinformatics*, *34*(13), 2195-2200.

Moritz, C., & Cicero, C. (2004). DNA barcoding: promise and pitfalls. *PLoS biology*, *2*(10), e354.

Moura, I. F., Lopes, E. P., Alvarado-Mora, M. V., Pinho, J. R., & Carrilho, F. J. (2013). Phylogenetic analysis and subgenotypic distribution of the hepatitis B virus in Recife, Brazil. *Infection, Genetics and Evolution*, *14*, 195-199.

Nakano, T., Lau, G. M., Lau, G. M., Sugiyama, M., & Mizokami, M. (2012). An updated analysis of hepatitis C virus genotypes and subtypes based on the complete coding region. *Liver international*, *32*(2), 339-345.

Norder, H., Courouсé, A. M., Coursaget, P., Echevarria, J. M., Shou-Dong, L., Mushahwar, I. K., & Magnius, L. O. (2004). Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology*, *47*(6), 289.

Paez-Espino, D., Eloe-Fadrosh, E. A., Pavlopoulos, G. A., Thomas, A. D., Huntemann, M., Mikhailova, N., Rubin, E., Ivanova, N. N. & Kyrpides, N. C. (2016). Uncovering Earth's virome. *Nature*, *536*(7617), 425.

Paraskevis, D., Magiorkinis, G., Magiorkinis, E., Ho, S. Y., Belshaw, R., Allain, J. P., & Hatzakis, A. (2013). Dating the origin and dispersal of hepatitis B virus infection in humans and primates. *Hepatology*, *57*(3), 908-916.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular ecology*, *21*(8), 1864-1877.

Pybus, O. G., Tatem, A. J., & Lemey, P. (2015). Virus evolution and transmission in an ever more connected world. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1821), 20142878.

Radziwill, G., Tucker, W., & Schaller, H. (1990). Mutational analysis of the hepatitis B virus P gene product: domain structure and RNase H activity. *Journal of virology*, *64*(2), 613-620.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology*, *61*(3), 539-542.

Rulik, B., Eberle, J., von der Mark, L., Thormann, J., Jung, M., Köhler, F., Apfel, W., Weigel, A., Kopetz, A., Köhler, J., Fritylar, F., Hartmann, M., Hadulla, K., Schmidt, J., Hörren, T., Krebs, D., Theves, F., Eulity, U., Skale, A., Rohwedder, D., Kleeberg, A., Astrin, I. I., Geiger, M. F., Wägele, W., Grobe, P., & Ahrens, D. (2017). Using taxonomic consistency with semi-automated data pre-processing for high quality DNA barcodes. *Methods in Ecology and Evolution*, *8*(12), 1878-1887.

Savolainen, V., Cowan, R. S., Vogler, A. P., Roderick, G. K., & Lane, R. (2005). Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1805-1811.

Schaefer, S. (2007). Hepatitis B virus genotypes in Europe. *Hepatology Research*, *37*, S20-S26.

Shi, W., Zhang, Z., Ling, C., Zheng, W., Zhu, C., Carr, M. J., & Higgins, D. G. (2013). Hepatitis B virus subgenotyping: history, effects of recombination, misclassifications, and corrections. *Infection, genetics and Evolution*, *16*, 355-361.

Simmonds, P. (2015). Methods for virus classification and the challenge of incorporating metagenomic sequence data. *Journal of General Virology*, *96*(6), 1193-1206.

Simmonds, P., Adams, M. J., Benkő, M., Breitbart, M., Brister, J. R., Carstens, E. B., Davidson, A.J., Delwart, E., Gorbalenya A. E., Harrach, B., Hull, R., King, A. M. Q., Koonin, E. V., Krupovic, M., Kuhn, J. H., Lefkowity E. J., Nibert, M. L., Orton, R., Roossinck, M. J., Sabanadyovic, S., Sullivan, M. B., Suttle, C. A., Tesh, R. B., van der Vlugt, R. A., Varsani, A., & Zerbini, F. M. (2017). Consensus statement: virus taxonomy in the age of metagenomics. *Nature Reviews Microbiology*, *15*(3), 161.

Simmonds, P., Bukh, J., Combet, C., Deléage, G., Enomoto, N., Feinstone, S., Halfon, P., Inchauspe, G., Kuiken, C., Maertens, G., Miyokami, M., Murphy, D. G., Okamoto, H., Pawlotsky, J. M., Penin, F., Sablon, E., Shin-I, T., Stuyver, L. J., Thiel, H. J., Viayov, S., Weiner, A. J., & Widell, A. (2005). Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology*, *42*(4), 962-973.

Smith, D. B., Bukh, J., Kuiken, C., Muerhoff, A. S., Rice, C. M., Stapleton, J. T., & Simmonds, P. (2014). Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology*, *59*(1), 318-327.

Strader, D. B., Wright, T., Thomas, D. L., & Seeff, L. B. (2004). Diagnosis, management, and treatment of hepatitis C. *Hepatology*, *39*(4), 1147-1171.

Stadler, T., Kouyos, R., von Wyl, V., Yerly, S., Böni, J., Bürgisser, P., Klimkait, T., Joos, B., Rieder, P., Xie, D., Günthard, H. F., Drummond, A. J., & Bonhoeffer, S., (2011). Estimating the basic reproductive number from viral sequence data. *Molecular biology and evolution*, *29*(1), 347-357.

Stadler, T., Kühnert, D., Rasmussen, D. A., & du Plessis, L. (2014). Insights into the early epidemic spread of Ebola in Sierra Leone provided by viral sequence data. *PLoS currents*, *6*.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312-1313.

Stanaway, J. D., Flaxman, A. D., Naghavi, M., Fitzmaurice, C., Vos, T., Abubakar, I., Abu-Raddad, L., Assadi, R., Bhala, N., Cowie, B., Forouzanfour, M. H., Groeger, J., Hanafiah, M., Jacobsen, K. H., James, S. L., MacLachlan, J., Malekyadeh, R., Martin, N. K., Mokhad, A. A., Mokdad, A. H., Murray, C. J. L., Plass, D., Rana, S., Rein, D. B., Richardus, J. H., Sanabria, J., Sazyan, M., Shahraz, S., So, S., Vlassov, V. V., Weiderpass, E., Wiersma, S. T., Younis, M., Yu, C., El Sayed Zaki, M., & Cooke, G. S.(2016). The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *The Lancet*, *388*(10049), 1081-1088.

Sulbaran, M. Z., Di Lello, F. A., Sulbaran, Y., Cosson, C., Loureiro, C. L., Rangel, H. R.,Cantaloube, J.F., Campos, R.H., Moratorio, G., Cristina, H.,& Pujol, F. H. (2010). Genetic history of hepatitis C virus in Venezuela: high diversity and long time of evolution of HCV genotype 2. *PloS one*, *5*(12), e14315.

Suttle, C. A. (2013). Viruses: unlocking the greatest biodiversity on Earth. *Genome*, *56*(10), 542-544.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular ecology*, *21*(8), 2045-2050.

Tang, H., & McLachlan, A. (2001). Transcriptional regulation of hepatitis B virus by nuclear hormone receptors is a critical determinant of viral tropism. *Proceedings of the National Academy of Sciences*, *98*(4), 1841-1846.

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., & Vogler, A. P. (2003). A plea for DNA taxonomy. *Trends in ecology & evolution*, *18*(2), 70-74.

Thézé, J., Lopez-Vaamonde, C., Cory, J. S., & Herniou, E. A. (2018). *Viruses*, *10*(7), 366

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity. *Biological conservation*, *183*, 4-18.

Tong, Y. Q., Liu, B., Liu, H., Zheng, H. Y., Gu, J., Song, E. J., ... & Li, Y. (2015). Accurate genotyping of hepatitis C virus through nucleotide sequencing and identification of new HCV subtypes in China population. *Clinical microbiology and infection*, *21*(9), 874-e9.

Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in ecology & evolution*, *24*(2), 110-117.

Waugh, J. (2007). DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays*, *29*(2), 188-197.

Yu, C., Hernandez, T., Zheng, H., Yau, S. C., Huang, H. H., He, R. L., Yang, J., & Yau, S. S. T. (2013). Real time classification of viruses in 12 dimensions. *PloS one*, *8*(5), e64328.

Yusim, K., Korber, B. T., Brander, C., Barouch, D., de Boer, R., Haynes, B. F., & Watkins, D. (2016). *HIV Molecular Immunology 2015* (No. LA-UR-16-22283). Los Alamos National Lab.(LANL), Los Alamos, NM (United States).

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, *29*(22), 2869-2876.

Figure 1(on next page)

Clustering of the HCV samples into genotypes

Clustering of the HCV samples into genotypes; the first bar of colors corresponds to the genotypes currently acknowledged by ICTV, the second to the mPTP clustering and the third to the ABGD clustering (p=0.0599, X=1.5). The numbers on the nodes are the support values obtained by the MCMC sampling under the mPTP model. The phylogenetic relationships were inferred using RAxML under the GTR+Γ model.

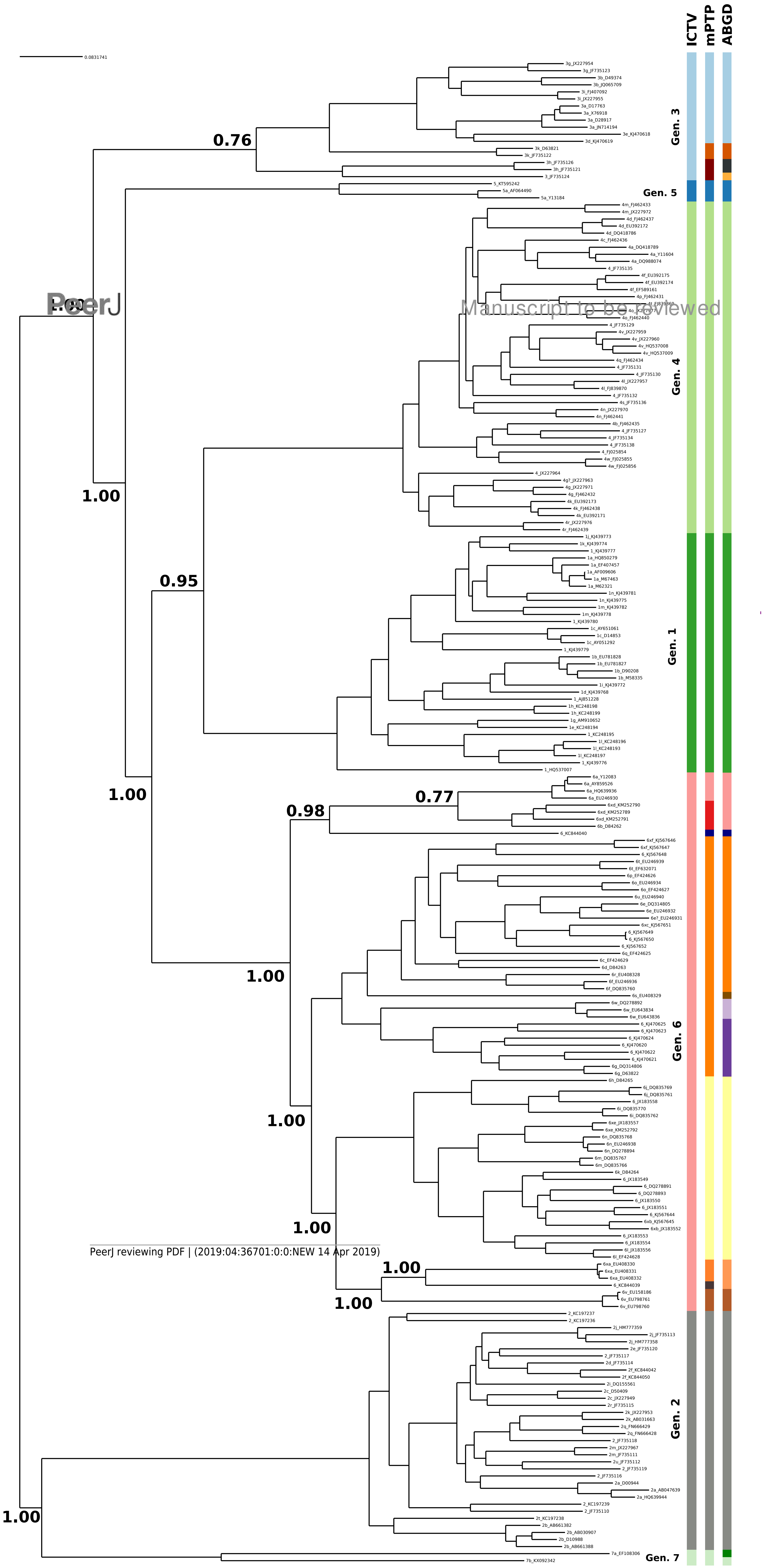

0.0831741
ICTV
mPTP
ABGD
0.76
1.00
1.00
0.95
1.00
0.98
0.77
1.00
1.00
1.00
1.00
1.00
1.00
3g_JX227954
3g_JF735123
3b_D49374
3b_JQ065709
3i_FJ407092
3i_JX227955
3a_D17763
3a_X76918
3a_D28917
3a_JN714194
3e_KJ470618
3d_KJ470619
3k_D63821
3k_JF735122
3h_JF735126
3h_JF735121
3_JF735124
Gen. 3
5_KT595242
5a_AF064490
5a_Y13184
Gen. 5
4m_FJ462433
4m_JX227972
4d_FJ462437
4d_EU392172
4d_DQ418786
4c_FJ462436
4a_DQ418789
4a_Y11604
4a_DQ988074
4_JF735135
4f_EU392175
4f_EU392174
4f_EF589161
4p_FJ462431
4t_FJ839869
4o_JX227977
4o_FJ462440
4_JF735129
4v_JX227959
4v_JX227960
4v_HQ537008
4v_HQ537009
4q_FJ462434
4_JF735131
4_JF735130
4l_JX227957
4l_FJ839870
4_JF735132
4s_JF735136
4n_JX227970
4n_FJ462441
4b_FJ462435
4_JF735127
4_JF735134
4_JF735138
4_FJ025854
4w_FJ025855
4w_FJ025856
4_JX227964
4g?_JX227963
4g_JX227971
4g_FJ462432
4k_EU392173
4k_FJ462438
4k_EU392171
4r_JX227976
4r_FJ462439
Gen. 4
1j_KJ439773
1k_KJ439774
1_KJ439777
1a_HQ850279
1a_EF407457
1a_AF009606
1a_M67463
1a_M62321
1n_KJ439781
1n_KJ439775
1m_KJ439782
1m_KJ439778
1_KJ439780
1c_AY651061
1c_D14853
1c_AY051292
1_KJ439779
1b_EU781828
1b_EU781827
1b_D90208
1b_M58335
1i_KJ439772
1d_KJ439768
1_AJ851228
1h_KC248198
1h_KC248199
1g_AM910652
1e_KC248194
1_KC248195
1l_KC248196
1l_KC248193
1l_KC248197
1_KJ439776
1_HQ537007
Gen. 1
6a_Y12083
6a_AY859526
6a_HQ639936
6a_EU246930
6xd_KM252790
6xd_KM252789
6xd_KM252791
6b_D84262
6_KC844040
6xf_KJ567646
6xf_KJ567647
6_KJ567648
6t_EU246939
6t_EF632071
6p_EF424626
6o_EU246934
6o_EF424627
6u_EU246940
6e_DQ314805
6e_EU246932
6e?_EU246931
6xc_KJ567651
6_KJ567649
6_KJ567650
6_KJ567652
6q_EF424625
6c_EF424629
6d_D84263
6r_EU408328
6f_EU246936
6f_DQ835760
6s_EU408329
6w_DQ278892
6w_EU643834
6w_EU643836
6_KJ470625
6_KJ470623
6_KJ470624
6_KJ470620
6_KJ470622
6_KJ470621
6g_DQ314806
6g_D63822
6h_D84265
6j_DQ835769
6j_DQ835761
6_JX183558
6i_DQ835770
6i_DQ835762
6xe_JX183557
6xe_KM252792
6n_DQ835768
6n_EU246938
6n_DQ278894
6m_DQ835767
6m_DQ835766
6k_D84264
6_JX183549
6_DQ278891
6_DQ278893
6_JX183550
6_JX183551
6_KJ567644
6xb_KJ567645
6xb_JX183552
6_JX183553
6_JX183554
6l_JX183556
6l_EF424628
6xa_EU408330
6xa_EU408331
6xa_EU408332
6_KC844039
6v_EU158186
6v_EU798761
6v_EU798760
Gen. 6
2_KC197237
2_KC197236
2j_HM777359
2j_JF735113
2j_HM777358
2e_JF735120
2_JF735117
2d_JF735114
2f_KC844042
2f_KC844050
2i_DQ155561
2c_D50409
2c_JX227949
2r_JF735115
2k_JX227953
2k_AB031663
2q_FN666429
2q_FN666428
2_JF735118
2m_JX227967
2m_JF735111
2u_JF735112
2_JF735119
2_JF735116
2a_D00944
2a_AB047639
2a_HQ639944
2_KC197239
2_JF735110
2t_KC197238
2b_AB661382
2b_AB030907
2b_D10988
2b_AB661388
Gen. 2
7a_EF108306
7b_KX092342
Gen. 7

Figure 2(on next page)

Clustering of the HBV samples into genotypes

Clustering of the HBV samples into genotypes; the first colored bar corresponds to the genotypes currently acknowledged by ICTV, the second to the mPTP clustering and the third to the ABGD clustering (p=0.0129, X=0.5). The numbers on the nodes are the support values obtained by the MCMC sampling under the mPTP model. The phylogenetic relationships were inferred using RAxML under the GTR+Γ model

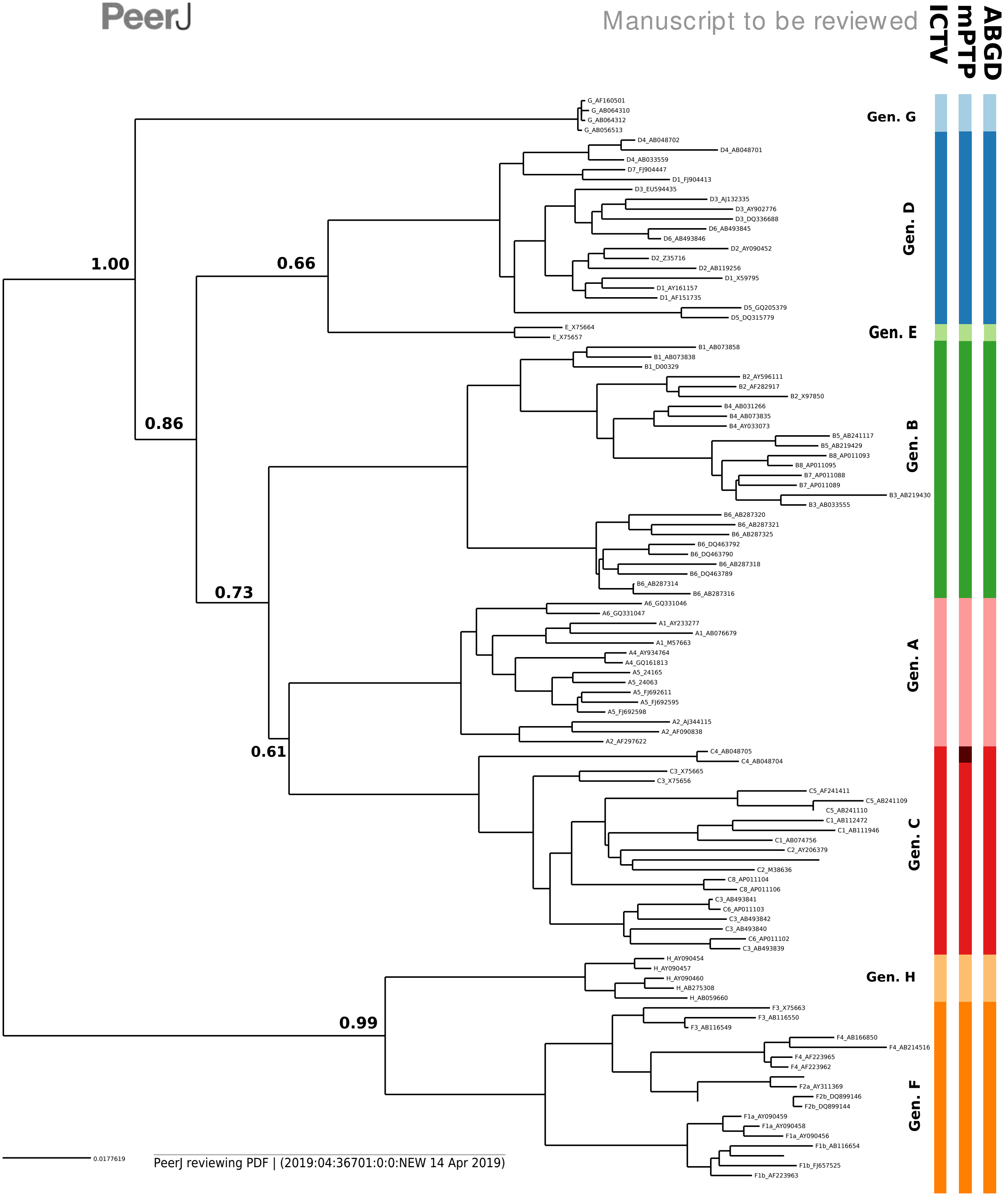
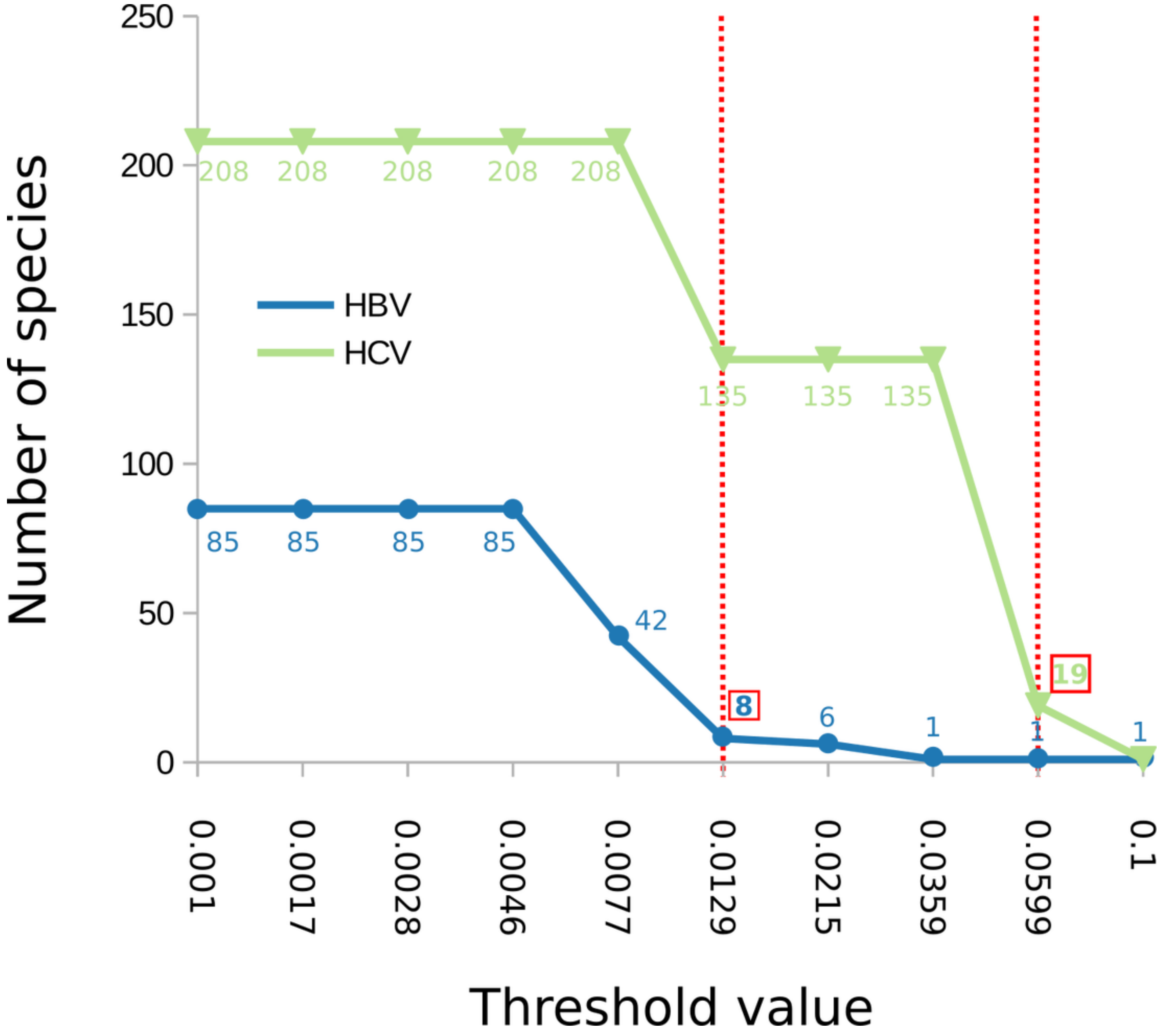

ICTV
mPTP
ABGD
G_AF160501
G_AB064310
G_AB064312
G_AB056513
Gen. G
D4_AB048702
D4_AB048701
D4_AB033559
D7_FJ904447
D1_FJ904413
D3_EU594435
D3_AJ132335
D3_AY902776
D3_DQ336688
D6_AB493845
D6_AB493846
D2_AY090452
D2_Z35716
D2_AB119256
D1_X59795
D1_AY161157
D1_AF151735
D5_GQ205379
D5_DQ315779
Gen. D
E_X75664
E_X75657
Gen. E
B1_AB073858
B1_AB073838
B1_D00329
B2_AY596111
B2_AF282917
B2_X97850
B4_AB031266
B4_AB073835
B4_AY033073
B5_AB241117
B5_AB219429
B8_AP011093
B8_AP011095
B7_AP011088
B7_AP011089
B3_AB219430
B3_AB033555
B6_AB287320
B6_AB287321
B6_AB287325
B6_DQ463792
B6_DQ463790
B6_AB287318
B6_DQ463789
B6_AB287314
B6_AB287316
Gen. B
A6_GQ331046
A6_GQ331047
A1_AY233277
A1_AB076679
A1_M57663
A4_AY934764
A4_GQ161813
A5_24165
A5_24063
A5_FJ692611
A5_FJ692595
A5_FJ692598
A2_AJ344115
A2_AF090838
A2_AF297622
Gen. A
C4_AB048705
C4_AB048704
C3_X75665
C3_X75656
C5_AF241411
C5_AB241109
C5_AB241110
C1_AB112472
C1_AB111946
C1_AB074756
C2_AY206379
C2_M38636
C8_AP011104
C8_AP011106
C3_AB493841
C6_AP011103
C3_AB493842
C3_AB493840
C6_AP011102
C3_AB493839
Gen. C
H_AY090454
H_AY090457
H_AY090460
H_AB275308
H_AB059660
Gen. H
F3_X75663
F3_AB116550
F3_AB116549
F4_AB166850
F4_AB214516
F4_AF223965
F4_AF223962
F2a_AY311369
F2b_DQ899146
F2b_DQ899144
F1a_AY090459
F1a_AY090458
F1a_AY090456
F1b_AB116654
F1b_FJ657525
F1b_AF223963
Gen. F
1.00
0.86
0.66
0.73
0.61
0.99
0.0177619

Figure 3

Number of delimited clusters for ABGD with respect to input parameters

The graph shows the change of the number of delimited clusters (y axis) with respect to the minimum intraspecific threshold ("p") assumed by ABGD (x axis). The threshold that yielded the most sensible clustering for HBV was p= 0.0129 while for HCV was p=0.0599, both are shown with a dotted red line in the figure; the corresponding number of clusters is indicated in a red box.


250
200
150
100
50
0
Number of species
HBV
HCV
208
208
208
208
208
135
135
135
19
85
85
85
85
42
8
6
1
1
1
0.001
0.0017
0.0028
0.0046
0.0077
0.0129
0.0215
0.0359
0.0599
0.1
Threshold value