Baraminic analysis of nucleocytoplasmic large DNA viruses

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The origin of nucleocytoplasmic large DNA viruses (NCLDVs) is very intriguing. While some researchers think they are large-sized viruses, there is proof that they might be degenerate bacteria. The Bible does not mention bacteria, viruses, or NCLDVs. Very little is known about their baraminology, so this analysis serves as a seminal attempt to discover what kinds of species relationships exist between them. Therefore, gene sequences were downloaded for 49 NCLDVs from the COG database, and BLASTed against one another to see whether NCLDVs form groups, such as the kinds of complex multicellular organisms, such as plants or animals. Here we employed a measure of gene content similarity called the Jaccard Coefficient Value (JCV) to measure the similarity between two species, and to see which species can be grouped together based on high gene content similarity between the individual members. Eight NCLDV clusters were found which have at least three members each. Although few species were analyzed, this study may serve as the basis for future baraminology studies on bacteria, viruses, or other microorganisms.

The origin of nucleocytoplasmic large DNA viruses, and where they come from, is still unclear. Some evolutionists have proposed that these microorganisms are large-sized viruses with very large genomes that broke out of the nuclei of eukaryotes, taking a host of genes along with them. On the other hand, they also carry a large number of genes from cellular organisms, raising the possibility that they are really degenerate bacterial species. Also, what is peculiar to these organisms is that they have a very high percentage of genes which do not have any homologs with any other organism,[1] supporting the view that NCLDVs have a separate origin from all other organisms.[2]

An important question to be addressed is whether baraminic analysis can be performed on NCLDVs. Since they have such a high proportion of unique genes, we can assume that they could form a possible apobaramin, which is defined as a group of one or more holobaramins independent from one another. The Bible does not mention microorganisms specifically, but if God created all living things, why would He create microorganisms in a different manner than plants or animals, after their kinds? If neither plants nor animals evolve, why would microorganisms evolve? Therefore, we can assume that transferring statistical baraminological methodology from complex organisms to microorganisms such as NCLDVs is warranted.

Principle of analysis

In this analysis, the number of common proteins were studied between 49 NCLDV species, whose protein sequences were available in the COG database.[3] Two different proteins were considered homologs if they had a minimal similarity of 40%, which is considered to be the lower limit of protein sequence homology.[4] A Jaccard Coefficient Value (JCV) was calculated to measure the degree of similarity between two NCLDV species. The higher the JCV, the higher the similar gene content between two species. As to what constitutes a high JCV, it depends on the kind of study being done. For example, here, the median JCV was 0.065. A matrix was made which contains the JCVs of all possible NCLDV species pairs, and can be seen in figure 1. Out of 1,176 possible species pairs, only 41 had a JCV ≥ 0.25, and only 8 pairs had a JCV ≥ 0.5. In figure 1 we can see 10 clusters of species, with 2–8 members each. A list of the species forming these clusters can be seen in table 1. According to our model, these clusters correspond to individual NCLDV baramins. If we lower the cutoff JCV to 0.1 (warranted due to the high rate of HGT in NCLDVs), we pick up three extra pairs of NCLDV species: Frog virus 3 and Singapore grouper Iridovirus (Iridoviridae), *Acanthocystis turfacea Chlorella* virus 1 and *Paramecium bursaria Chlorella* virus 1 NY2A (Phycodnaviridae), and *Ectocarpus siliculosus* virus 1 and *Feldmannia* species virus (Phycodnaviridae).

Description of different NCLDV baramins found in the analysis

Phaecocystis

The three *Phaecocystis* species all have JCVs greater than 0.98 with each other. However, notably, they also have JCVs of around 0.11 with Organic Lake phycodnaviruses, which belong to the same family. Therefore, these NCLDVs may be classified together into one baramin, thereby aggregating

clusters 1 and 6. Both Koonin *et al.*[5] and Santini *et al.*[6] have reported similar relationships between the genus *Phaecocytis* and the Organic Lake phycodnaviruses.

Prasinophyceae

Group 2 is made up of six Prasinophyceae species from the family Phycodnaviridae, a genetically diverse but morphologically similar group with an icosahedric capsid, which infect algal hosts from both fresh and marine waters.[7,8] They have few genes in common, and thus appear to be an apobaramin.

The Prasinoviruses however, appear to be a monophyletic clade, encompassing the genera *Bathycoccus*, *Ostreococcus*, and *Micromonas* based on alignments of the DNA *pol* protein.[9] Bellec *et al.*[9] found that the phylogenic position of *Emiliana huxleyi* 'virus' is close to that of Prasinophyceae,

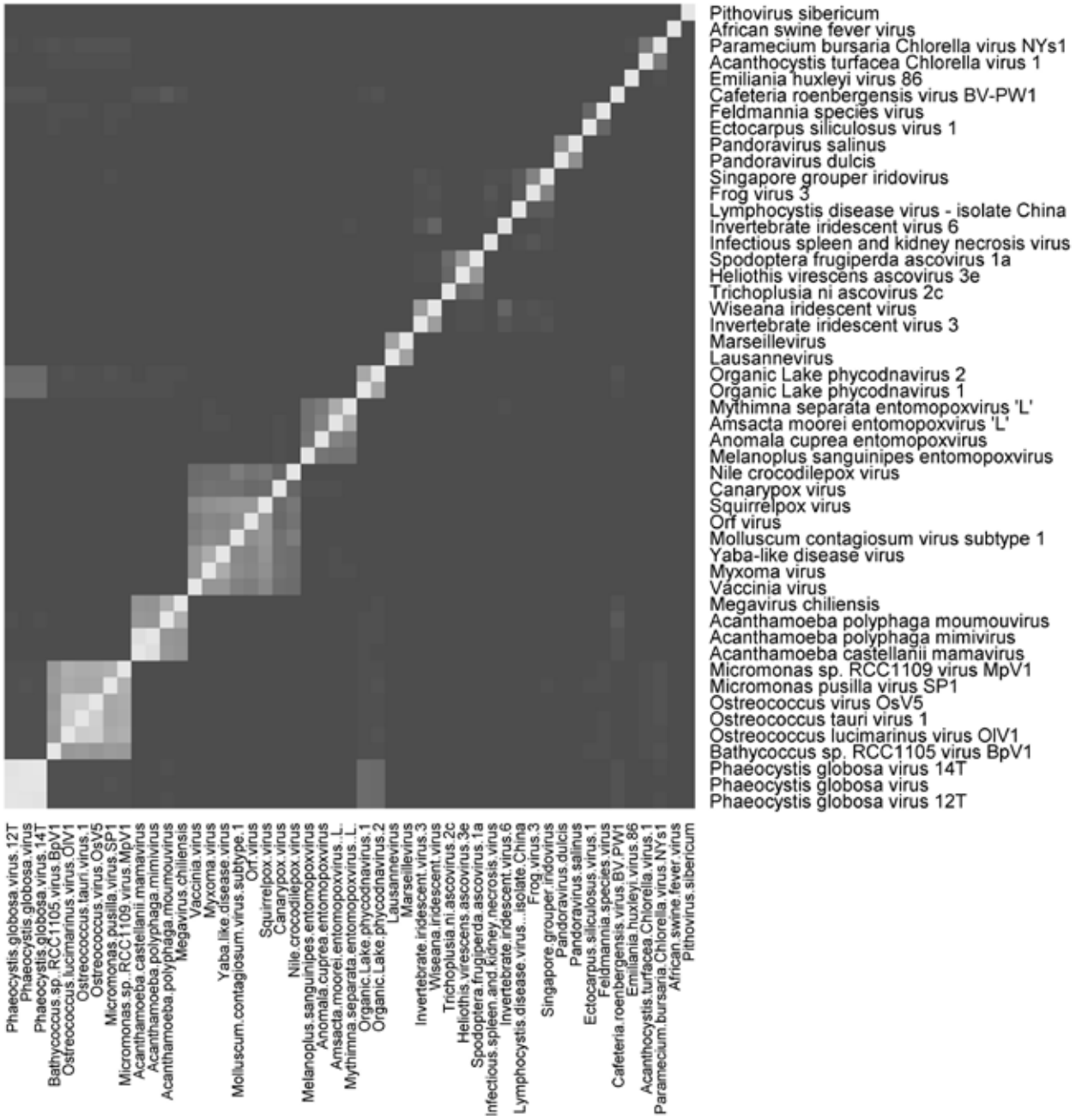

Figure 1. Heat map depicting Jaccard coefficients for pairs of NCLDV virus species which had protein sequences in the COG database. The Jaccard-Coefficient measures the common genes content between a given pair of NCLDV species. Lighter values correspond to higher values. Ten baramins can be seen on the heatmap with 2–8 members each, listed in table 4.

Table 1. Clusters of NCLDV species with a relatively high proportion of common genes

Species in cluster	Virus family	Average ± std var. JCV	Genome size range
Phaecocystis globosa virus, *Phaecocystis globosa* virus 12T, *Phaecocystis globosa* virus 14T	Phycodnaviridae	0.97 ± 0.01	460 Kbp
Bathycoccus sp. RCC1105 virus BpV1, *Ostreococcus tauri* virus 1, *Ostreococcus virus* OsV5, *Ostreococcus lucimarinus* virus OlV1, *Micromonas sp.* RCC1109 virus MpV1, *Micromonas pusilla* virus SP1	Phycodnaviridae	0.49 ± 0.15	184–199 Kbp
Acanthamoeba castellanii mamavirus, *Acanthamoeba polyphaga* mimivirus, *Acanthamoeba polyphaga* moumouvirus, *Megavirus chiliensis*	Mimiviridae	0.46 ± 0.24; 0.6 ± 0.28	1.02–1.26 Mbp
Vaccinia virus, Myxoma virus, Yaba-like disease virus, *Molluscum contagiosum* virus subtype 1, Orf virus, Nile crocodilepox virus, Canarypox virus, Squirrelpox virus	Poxviridae	0.22 ± 0.1	140–360 Kbp
Melanoplus sanguinipes entomopoxvirus, *Anomala cuprea* entomopoxvirus, *Amsacta moorei* entomopoxvirus 'L', *Mythimna separata* entomopoxvirus 'L'	Poxviridae	0.22 ± 0.13	232–281 Kbp
Organic Lake phycodnavirus 1, Organic Lake phycodnavirus 2	Phycodnaviridae	0.46	n.a.
Lausannevirus, Marseillevirus	Marseillevirus	0.41; 0.55 ± 0.15	347–368 Kbp
Invertebrate iridescent virus 3, Wiseana iridescent virus	Iridoviridae	0.38	191–206 Kbp
Trichoplusia ni ascovirus 2c, *Heliothis virescens* ascovirus 3e, *Spodoptera frugiperda* ascovirus 1a	Ascoviridae	0.19 ± 0.14	157–186 Kbp
Pandoravirus dulcis, Pandoravirus salinus	Pandoraviridae	0.3; 0.32 ± 0.02	1.9–2.5 Mbp
Frog virus 3, Singapore grouper virus, Tiger frog virus, *Ambystoma tigrinum* virus	Iridoviridae	0.22; 0.52 ± 0.25; 0.71 ± 0.07	150–170 Kbp

but still not fully resolved. The average JCV between this species and that of Prasinophyceae is 0.0085, compared to the average JCV of 0.62 within this baramin. Similarly, we have an average JCV of 0.02 between the members of the baramin Prasinophyceae and *Paramecium bursaria Chlorella* 'virus' NY and *Acanthocystis turfacea Chlorella* 'virus' 1.

Figure 2 depicts a baraminological tree for the six species in this baramin based on the DNA polymerase protein. The species Yellowstone Lake phycodnavirus 2 (YLPV2) was chosen as an outlier (belonging to another baramin) based on BLAST results as the next most similar species. As we can see, YLPV2 is separate from the other six species on a long branch of its own. The gene similarity between this species and the others is 49%, whereas between the others the average similarity is 76.2%.

Mimiviridae

Mimiviridae is represented by four species: Mimivirus, Mamavirus, Moumouvirus, and *Megavirus chiliensis*. Considering some of their genes, these species could be considered to have originated from soil bacteria.[2] Of these four species, Mimivirus and Mamavirus had the highest JCV of 0.91. Both of these species had a lower JCV compared to Megavirus; about 0.32. Arslan *et al.*[10] suggest that Mimiviridae species all descended from a single ancestor through genome reduction, and have lost genes in a lineage-specific fashion, similar to what we see in other bacteria. Eighty-five percent of the 258 Megavirus genes[11] not present in the Mimivirus cluster towards the end of the Megavirus chromosome. Rost[4] calculated that orthologs and orthologous intergenic regions between these species are 65% similar, which is greater than the 40%

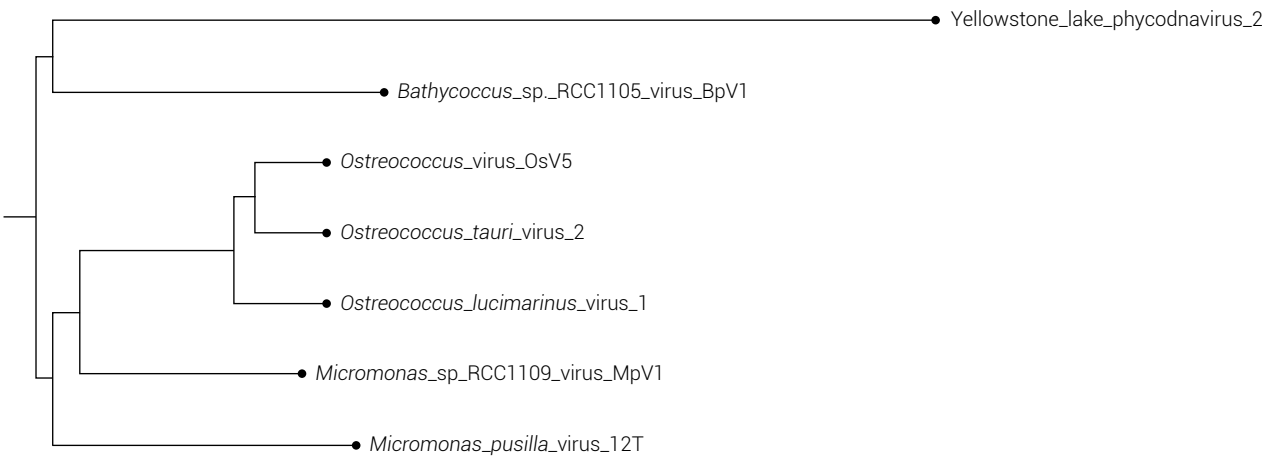

Figure 2. Baraminological tree of *Ostreococcus*, *Micromonas*, and *Bathycoccus* species

limit which serves as a lower limit for protein homology. Boughalmi *et al.*[12] described a new member of the family Mimiviridae from the medicinal leech, *Hirudo medicinalis*, called Hirudovirus, with a genome represented by two scaffolds, 1.16 Mbp and 25.7 Kbp long, each.[13] This species has 998 ORFs, 47% of which had orthologs in other species. The number of common genes, JCVs with the previous four species can be seen in table 2. Hirudovirus is most similar to Mimivirus and Mamavirus, possibly only a new strain. With the inclusion of this new species, the average JCV increases to 0.6.

Though *Cafeteria roenbergensis* virus is considered to be a distant member of the Mimiviridae family, Yoosuf *et al.*[14] found ≤ 5% of its genes to be in common with two strains of Mimiviridae, the Terra1 and Terra2 viruses. Indeed, when compared to the four members of this group, we see that the average JCV is 0.03, which is very low, thus we should exclude *Cafeteria roenbergensis* virus from this group.

The Poxviridae apobaramin

The Poxviridae are another group of highly variable NCLDVs, similar to Phycodnaviridae, made up of two subfamilies, and also of several genera. The Entomopoxvirinae (EPV) infect insect hosts, whereas the Chordopoxvirinae (ChPV) infect vertebrates. While these NCLDVs infect a wide range of hosts, each species infects a narrow range of hosts. Genetically, these organisms have a genome size range of 133–360 kbp, and from 133–328 genes, and also vary in AT%. Cluster 4 is comprised of Chordopoxvirinae species, whereas cluster 5 is made up of Entomopoxvirus species. Lefkowitz *et al.*[15] also did pairwise sequence comparison (PASC) on DNA polymerase for all Poxviridae species, and found that comparisons between the two subfamilies yielded a sequence identity of 23–31%, which is below the protein homology threshold of 40%.[4] This is strong evidence that the ChPVs and the EPVs belong to different baramins. Species in separate EPV genera also "show almost as much divergence between themselves as they do with ChPVs".[15]

Table 2. Jaccard Coefficient Values between Hirudovirus and members of the Mimivirus baramin

Species	Number of common genes	JCV
Acanthamoeba castellanii mamavirus	975	0.99
Acanthamoeba polyphaga mimivirus	982	0.69
Acanthamoeba polyphaga moumouvirus	772	0.96
Megavirus chiliensis	835	0.65

Marseilleviridae

Marseilleviridae consists of NCLDVs discovered in water sources in France, Tunisia[16] and Senegal.[17] These species form a compact baramin, with the majority of their genomes being collinear with each other, and also share a large number of orthologous proteins. Even their GC% content ranges only between 43–45%, which reflects a very similar genomic makeup. Approximately two thirds of their genes are ORFans.[16,18] An analysis of six Marseillevirus species showed that the pan-genome contained a total of 608 genes of which 233 genes were common to all six species. The genomes of these organisms, which are particularly stable despite varying between distinctly different ecological niches, further support the stability of baranomes (the created genome within a specific kind). Recently, the Port-Miou virus was discovered, which is 99% similar to Lausannevirus, exhibiting only one indel per 1,000 bp.[19]

Based on data from several studies, JCVs were calculated for for Marseillevirus, Lausannevirus, Tunis virus, Cannes 8 virus,[20] and Insectomime virus.[12] The JCVs can be seen in table 3. Based on this, the revised average JCV for the Marseilleviridae is 0.55, indicating they make up a single baramin.

Wiseana iridescent virus and Invertebrate iridescent virus 3

Group 8 is made up of two species, which belong to the family of Iridoviridae, which is a genetically diverse group of viruses. Not much is known about the relationship between these two viruses, but their JCV is significant enough to group them together.

Ascovirus

Group 9 is made up of three Ascovirus species: *Trichoplusia ni* ascovirus 2c (TnAV), *Heliothis virescens* ascovirus 3e (HvAV), and *Spodoptera frugiperda* ascovirus 1a (SfAV). As of date only five Ascovirus species in the genus Ascovirus have been recognized by the International Committee on Taxonomy of Viruses,[21] so not much is known about them. They mainly infect lepidopteran insects, and induce reorganization of the host cell nucleus, resulting in lysis.

This group has the lowest average JCV value for all species pairs, 0.19, therefore these species could probably form an apobaramin. There were 119 protein sequences available for a fourth Ascovirus species, *Diadromus pulchellus* ascovirus 6a (DpAV), so JCV values were calculated with the other three species, but were much lower than 0.19 (0.021, 0.01, and 0.022). This supports the idea that *D. pulchellus* may be a member of another Ascovirus baramin. In fact, Stasiak *et al.*[22] analyzed the PolIII-PolI regions of the δ DNA polymerase gene between these four species, and found that they were very divergent (with a similarity of less than 50%), thereby separating these four species into two groups, with TnAV, HvAV, and SfAV in one group, and DpAV in the other. Interestingly, these two groups are divided based on how their host organisms (wasps) infect their prey (caterpillars).

Table 3. Jaccard Coefficient Values between members of the family Marseilleviridae

	Lausannevirus	Tunis virus	Cannes 8 virus	Insectomime virus
Marseillevirus	0.41	0.43	0.76	0.43
Lausannevirus		0.59	0.48	0.59
Tunis virus			0.42	0.86
Cannes 8 virus				0.5

Pandoraviridae

The small group Pandoraviridae is made up of two species, *Pandoravirus salinus*, discovered in marine water near Chile, and *Pandoravirus dulcis*, discovered in fresh water near Melbourne. A third species has also been discovered, *P. inopinatum*, with a genome size of 2.24 Mbp. The ORFan content for *P. salinus* is 84% (its most abundant virion protein is an ORF), yet only three species have been discovered, so this intrabaraminic ORFan gene content may decrease with more newly discovered *Pandoravirus* species. However, one characteristic which separates these species from other NCLDVs is that it lacks a major capsid protein which is present in all other groups.[23] JCVs were calculated between *P. inopinatum* and the other two *Pandoravirus* species, resulting in a revised average JCV of 0.32 ± 0.02. It was also found that the average JCV between *Pandoravirus* and *Pithovirus sibericum* is only 0.008, therefore *P. sibericum* should be separated into another baramin. Similarly, an average JCV of 0.04 ± 0.01 was calculated between the three *Pandoravirus* species and *Mollivirus sibericum*, another possible NCLDV species which is also held to be similar to Pandoraviridae. This low-protein content is similar to results described in Abergel *et al.*[23] (89% of 18% of its genes similar to Pandoraviridae). Due to the low JCVs it is also suggested that this species be separated from Pandoraviridae.

Other species pairs

Besides the aforementioned 10 virus clusters there are three pairs of viruses who show a faint similarity with each other. *Paramecium bursaria* Chlorella virus NYs1 and *Acanthocystis turfacea* Chlorella virus 1 (both Phycodnaviruses) have a JCV of 0.19, similar to the three *Ascovirus* species of group 9. *Feldmannia* virus and *Ectocarpus siliculosus* virus 1 (Phycodnaviruses) have a JCV of 0.098. According to Park *et al.*[24], their DNA adenine methyltransferase proteins have an identity of 36%.

Ranaviruses

A third pair of species, Singapore grouper iridovirus (SGIV), and Frog virus 3 (FV3) come from the genus *Ranavirus*, within the family Iridoviridae. Their JCV is 0.22. These viruses infect mainly amphibians and/or fish.[25] The proteomes for three other Ranaviruses were downloaded (Epizootic hematopoietic necrosis virus (EHNV), Tiger frog virus (TFV) and *Ambystoma tigrinum* virus (ATV)), and compared to the other two virus proteomes. It was found that with these

Table 4. Jaccard Coefficient Values between members of the genus Ranavirus. ATV = *Ambystoma tigrinum* virus, EHNV = Epizootic hemtopoietic necrosis virus, FV3 = Frog virus 3, SGIV = Singapore grouper iridovirus, TFV = Tiger frog virus.

	SGIV	TFV	ATV	EHNV
FV3	0.22	0.67	0.63	0.67
SGIV		0.22	0.26	0.27
TFV			0.81	0.74
EHNV				0.74

four species, the average JCV was 0.52 ± 0.25, meaning that it is possible that these species form a baramin. The JCVs for these five species can be seen in table 4. It might be possible that these four species group together into a baramin due to their common host range.

The Singapore grouper is a fish, which leads us to the question that since the host belongs to a different order (fish as compared to amphibians), then does it also belong to a different baramin? Holopainen *et al.*[25] studied the sequence similarity of the major capsid protein (MCP), DNA polymerase, and neurofilament triplet H1-like protein (NF-H1) between a number of Iridoviruses, and found that SGIV is always an outlier compared to the other four species mentioned here. If we exclude SGIV from the other four species of Ranaviruses, the average JCV rises to 0.71 ± 0.07.

Table 5. Jaccard Coefficient Values calculated for two groups of Ranaviruses, according to data from Eaton *et al.* [26]. ATV = *Ambystoma tigrinum* virus, FV3 = Frog virus 3, GIV = grouper iridovirus, SGIV = Singapore grouper iridovirus, TFV = Tiger frog virus.

First species	No. genes	Second species	No. genes	No. common genes	JCV
Amphibian Ranaviruses					
ATV	91	FV3	97	86	0.88
ATV	91	TFV	103	91	0.84
FV3	97	TFV	103	92	0.85
Fish vs Amphibian Ranaviruses					
ATV	91	GIV	139	72	0.46
ATV	91	SGIV	139	72	0.46
FV3	97	GIV	139	72	0.44
FV3	97	SGIV	139	72	0.44
GIV	139	TFV	103	72	0.42
SGIV	139	TFV	103	72	0.42
Fish Ranaviruses					
GIV	139	SGIV	139	138	0.99

Eaton *et al.*[26] arrived at similar results. They compared FV3, TFV, and ATV with SGIV and grouper iridovirus (GIV). The two grouper iridoviruses had a JCV of 0.99, whereas an average JCV of 0.86 for FV3, TFV and ATV. However, the average JCV dropped to 0.44 when comparing between these two groups (see table 5).

Also, for example, if we exclude SGIV from the other four species of Ranaviruses, the average JCV rises to 0.71±0.07 for that group. This is interesting, since this way, the standard error of the JCVs is reduced 3.6-fold (0.25 to 0.07). This is a useful method of subtractive evidence in excluding species from a baramin, if these groups can be classified this way.

African Swine Fever Virus

African Swine Fever Virus (ASFV) is the single species in the family Asfarviridae. It has a genome size of 170–193 Kbp depending on the isolate, which encodes 150–167 ORFs. It is icosahedral in shape, and replicates in the cytoplasm of infected cells.[27] It is not very much related to any other NCLDV family. It has an average JCV of 0.0023 ± 0.001 with other species in our analysis, showing high discontinuity; therefore, it belongs to its own baramin.

Summary and conclusion

This study was a preliminary analysis of 49 NCLDV species, based on the assumption that microorganisms follow the same kind of speciation patterns as seen in different kinds of created animals and plants. Here eight clusters were found, or groups with at least three species each. Since there are likely several hundreds or even thousands of NCLDV species in nature, this study is only a preliminary analysis, especially since the baraminology of NCLDVs and microorganisms in general is very much unknown.

The present study shows that NCLDV species from different groups do not have too many genes in common. Thus, if two NCLDV species happen to have a high number of common genes, it suggests they belong to the same group (kind) of organisms. Different NCLDV groups had an average JCV of 0.45, or 0.23 or even 0.18. These relatively low numbers of common genes even within groups could be due to the propensity of NCLDVs to have a high ratio of unique genes, possibly due to HGT. Comparing total gene/protein content is a simpler, robust and holistic way of determining species relationships than creating dozens of contradicting evolutionary trees based on single genes. Thus the theory of a 'fourth domain' of life is not well founded.

In the analysis of 49 NCLDV species, only a number of cases indicated groups equal to the level of genus (*Phaecocystis*, *Prasinophyceae*, *Pandoraviruses*, *Ranaviruses*), suggesting that this might be a good boundary for the limits of NCLDV groups (or kinds). Similarly, for

other microorganisms, such as viruses, van Regenmortel[28] also described a lack of higher taxonomic classification than the family.

In conclusion, it seems that baraminic analysis can be performed on microorganisms such as NCLDVs, however, as of yet, only a few species have been analyzed. Though this analysis serves as a starting point, much more careful thought is needed to proceed in taxonomic analysis of microorganisms from a biblical perspective.

Materials and methods

NCLDV protein sequences were retrieved from the COG website: ftp://ftp.ncbi.nih.gov/pub/wolf/COGs/NCVOG. All 20,086 protein sequences from 49 species were BLASTed (BLASTP) against each other, with an e-score < 1e-4. A Jaccard Coefficient Value J was calculated between each possible pair of 1,176 species, where $0 \leq J \leq 1$, and where $J = |A \cap B|/(|A|+|B|+|A \cap B|)$ for species A and B. The Jaccard Coefficient Values (JCVs) were put into a matrix and visualized by the heatmap function in R, version 3.1.3. Lighter colours mean higher JCVs. The JCV is a measure of how similar the gene content of two genomes. A JCV of 1 means that the two organisms have the exact same genes. The lower the JCV, the less related two viruses are to each other. Protein sequences for Epizootic hematopoietic necrosis virus, *Pandoravirus inopinatum*, *Mollivirus sibericum*, *Diadromus pulchellus* ascovirus 6a, *Ambystoma tigrinum* virus, Tiger frog virus, Tunis virus, Cannes 8 virus, and Insectomime virus were downloaded from NCBI. Figure 2 was generated by using CLC Genomics, version 8. The JCV values for all NCLDV species pairs can be found in Supplementary data file 1.

Acknowledgements

I would hereby like to thank Robin M. Wyle for critically reading the manuscript.

References

1. Claverie, J.M. and Ogata, H., Ten good reasons not to exclude giruses from the evolutionary picture, *Nat. Rev. Microbiol.* **7**(8):615, 2009.
2. O'Micks, J., Creation perspective of nucleocytoplasmic large DNA viruses, *J. Creation* **30**(3):110–117, 2017.
3. Yutin, N., Wolf, Y.I., Raoult, D. and Koonin, E.V., Eukaryotic large nucleo-cytoplasmic DNA viruses: clusters of orthologous genes and reconstruction of viral genome evolution, *Virol. J.* **6**:223, 2009.
4. Rost, B., Twilight zone of protein sequence alignments, *Protein Eng* **12**(2): 85–94, 1999.
5. Koonin, E.V., Krupovic, M. and Yutin, N., Evolution of double-stranded DNA viruses of eukaryotes: from bacteriophages to transposons to giant viruses, *Ann. N.Y. Acad. Sci.* **1341**:10–24, 2015.
6. Santini, S., Jeudy, S., Bartoli, J. *et al*., Genome of Phaeocystis globosa virus PgV-16T highlights the common ancestry of the largest known DNA viruses infecting eukaryotes, *Proc. Natl. Acad. Sci. USA* **110**(26):10800–10805, 2013.
7. Dunigan, D.D., Fitzgerald, L.A. and Van Etten, J.L., Phycodnaviruses: a peek at genetic diversity, *Virus Res.* **117**(1):11932, 2006.
8. Derelle, E., Ferraz, C., Escande, M.L., Eychenié, S. and Cooke, R., Life-cycle and genome of OtV5, a large DNA virus of the pelagic marine unicellular green alga *Ostreococcus tauri*, *PLoS One* **3**(5):e2250, 2008.
9. Bellec, L., Grimsley, N., Moreau, H. and Desdevises, Y., Phylogenetic analysis of new Prasinoviruses (Phycodnaviridae) that infect the green unicellular algae Ostreococcus, Bathycoccus and Micromonas, *Environ. Microbiol. Rep.* **1**(2): 114–123, 2009.
10. Arslan, D., Legendre, M., Seltzer, V., Abergel, C. and Claverie, J.M., Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae, *Proc. Natl. Acad. Sci. USA* **108**(42):17486–17491, 2011.
11. Legendre, M., Arslan, D., Abergel, C. and Claverie, J.M., Genomics of Megavirus and the elusive fourth domain of life, *Commun. Integr. Biol.* **5**(1): 102–106, 2012.
12. Boughalmi, M., Pagnier, I., Aherfi, S. *et al*., First isolation of a Marseillevirus in the Diptera Syrphidae Eristalis tenax, *Intervirology* **56**(6):386–394, 2013a.
13. Boughalmi, M., Pagnier, I., Aherfi, S. *et al*., First isolation of a giant virus from wild Hirudo medicinalis leech: Mimiviridae isolation in Hirudo medicinalis, *Viruses* **5**(12):2920–2930, 2013b.
14. Yoosuf, N., Pagnier, I., Fournous, G., Robert, C., Raoult, D. *et al*., Draft genome sequences of Terra1 and Terra2 viruses, new members of the family Mimiviridae isolated from soil, *Virology* **452–453**:125–32, 2014.
15. Lefkowitz, E.J., Wang, C. and Upton, C., Poxviruses: past, present and future, *Virus Res.* **117**(1):105–118, 2006.
16. Aherfi, S., Boughalmi, M., Pagnier, I., *et al*. Complete genome sequence of Tunisvirus, a new member of the proposed family Marseilleviridae. *Arch Virol* **159**(9):2349–2358, 2014a.
17. Colson, P., Fancello, L., Gimenez, G., Armougom, F. and Desnues, C., Evidence of the megavirome in humans, *J. Clin. Virol.* **57**(3):191–200, 2013.
18. Aherfi, S., La Scola, B., Pagnier, I., Raoult, D. and Colson, P., The expanding family Marseilleviridae, *Virology* **466–467**:27–37, 2014b.
19. Doutre, G., Arfib, B., Rochette, P. *et al*., Complete Genome Sequence of a New Member of the Marseilleviridae Recovered from the Brackish Submarine Spring in the Cassis Port-Miou Calanque, France, *Genome Announc.* **3**(6): e01148-15, 2015, doi:10.1128/genomeA.01148-15.
20. Aherfi, S., Pagnier, I., Fournous, G. *et al*., Complete genome sequence of Cannes 8 virus, a new member of the proposed family 'Marseilleviridae', *Virus Genes* **47**(3):550–555, 2013.
21. Wei, Y.L., Hu, J., Li, S.J. *et al*., Genome sequence and organization analysis of Heliothis virescens ascovirus 3f isolated from a Helicoverpa zea larva, *J. Invertebr. Pathol.* **122**:40–3, 2014.
22. Stasiak, K., Demattei, M.V., Federici, B.A. and Bigot, Y., Phylogenetic position of the Diadromus pulchellus ascovirus DNA polymerase among viruses with large double-stranded DNA genomes, *J. Gen. Virol.* **81**(12):3059–3072, 2000.
23. Abergel, C., Legendre, M. and Claverie, J.M., The rapidly expanding universe of giant viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus, *FEMS Microbiol. Rev.* **39**(6):779–796, 2015.
24. Park, Y., Kim, G.D. and Choi, T.J., Molecular cloning and characterization of the DNA adenine methyltransferase gene in Feldmannia sp. Virus, *Virus Genes* **34**(2):177–183, 2007.
25. Holopainen, R., Ohlemeyer, S., Schütze, H., Bergmann, S.M. and Tapiovaara, H., Ranavirus phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofilament triplet H1-like protein genes, *Dis. Aquat. Organ.* **85**(2):81–91, 2009.
26. Eaton, H.E., Metcalf, J., Penny, E. *et al*., Comparative genomic analysis of the family Iridoviridae: re-annotating and defining the core set of iridovirus genes, *Virol. J.* **4**:11, 2007.
27. Dixon, L.K., Chapman, D.A., Netherton, C.L. and Upton, C., African swine fever virus replication and genomics, *Virus Res.* **173**(1):3–14, 2013.
28. Van Regenmortel, M.H.V., Introduction to the species concept in virus taxonomy; in: Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R. and Wickner, R.B. (Eds.), Virus Taxonomy, *Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, San Diego, CA, pp. 3–16, 2000.

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