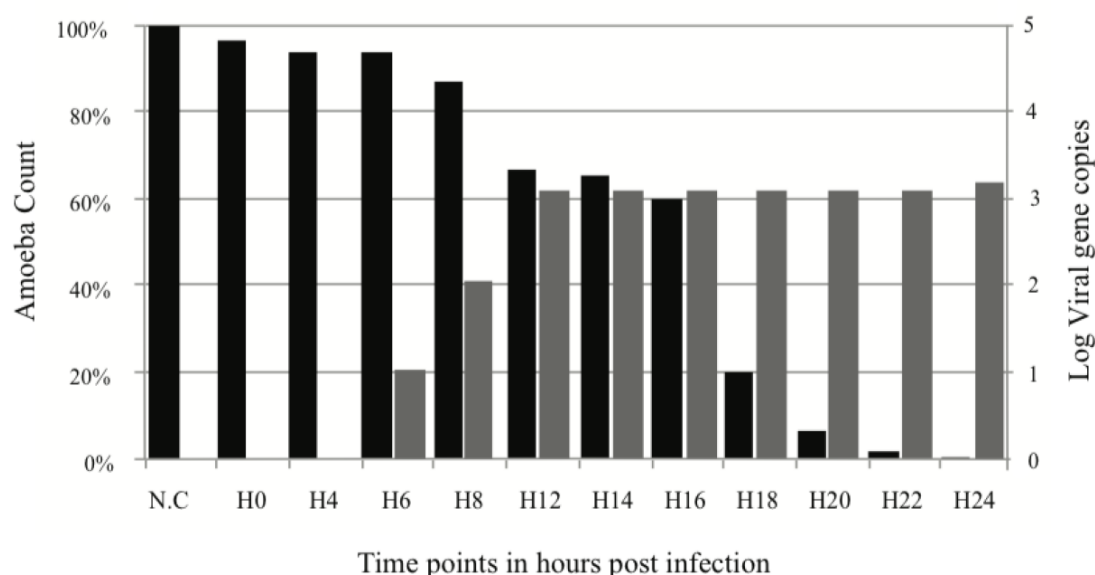


SUPPLEMENTAL INFORMATION

For article: DG Reteno, S Benamar, J Bou Khalil, J Andreani, N Armstrong, T Klose, M Rossmann, P Colson, D Raoult, B La Scola. **Faustovirus, an asfarvirus-related new lineage of giant viruses infecting amoebae.** Accepted in J Virol, 2015.

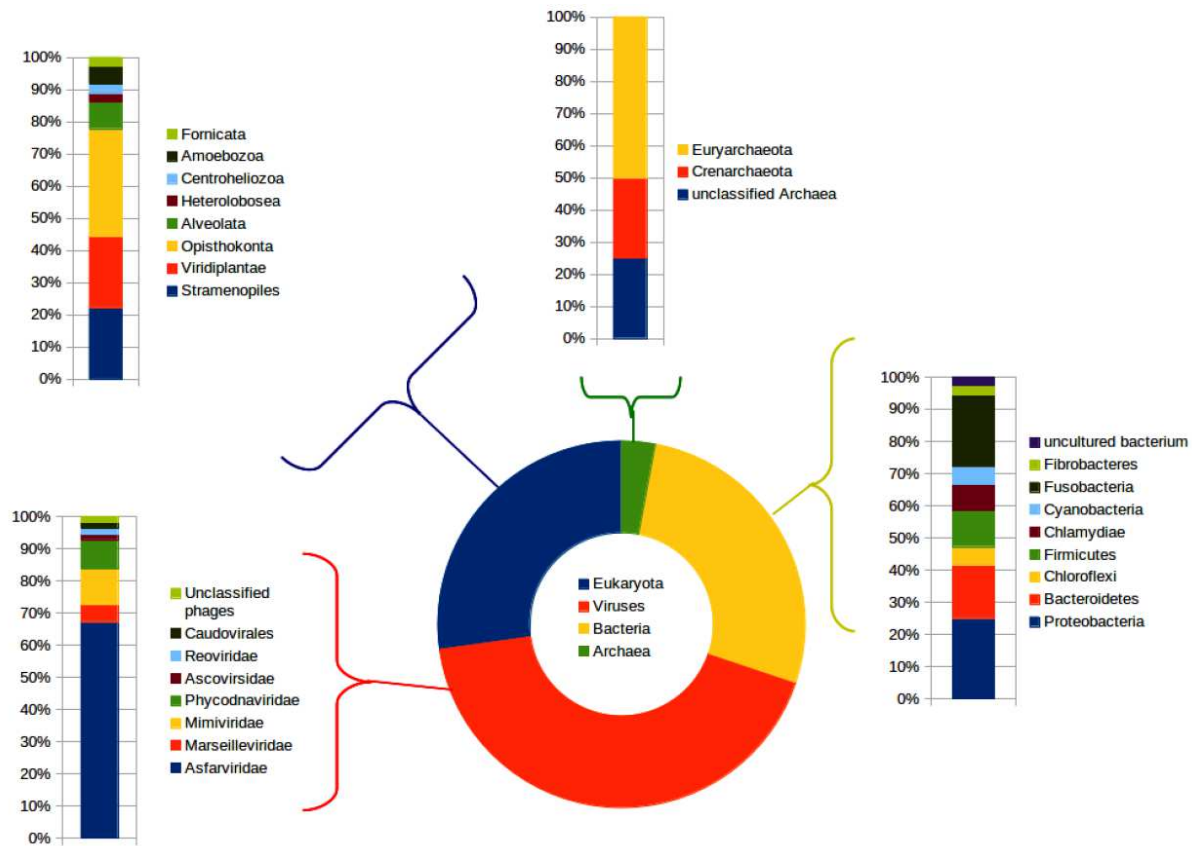
SUPPLEMENTAL FIGURES

Supplemental Figure S1. Histogram of Faustovirus cycle growth in *Vermamoeba vermiformis* measured by real-time PCR.

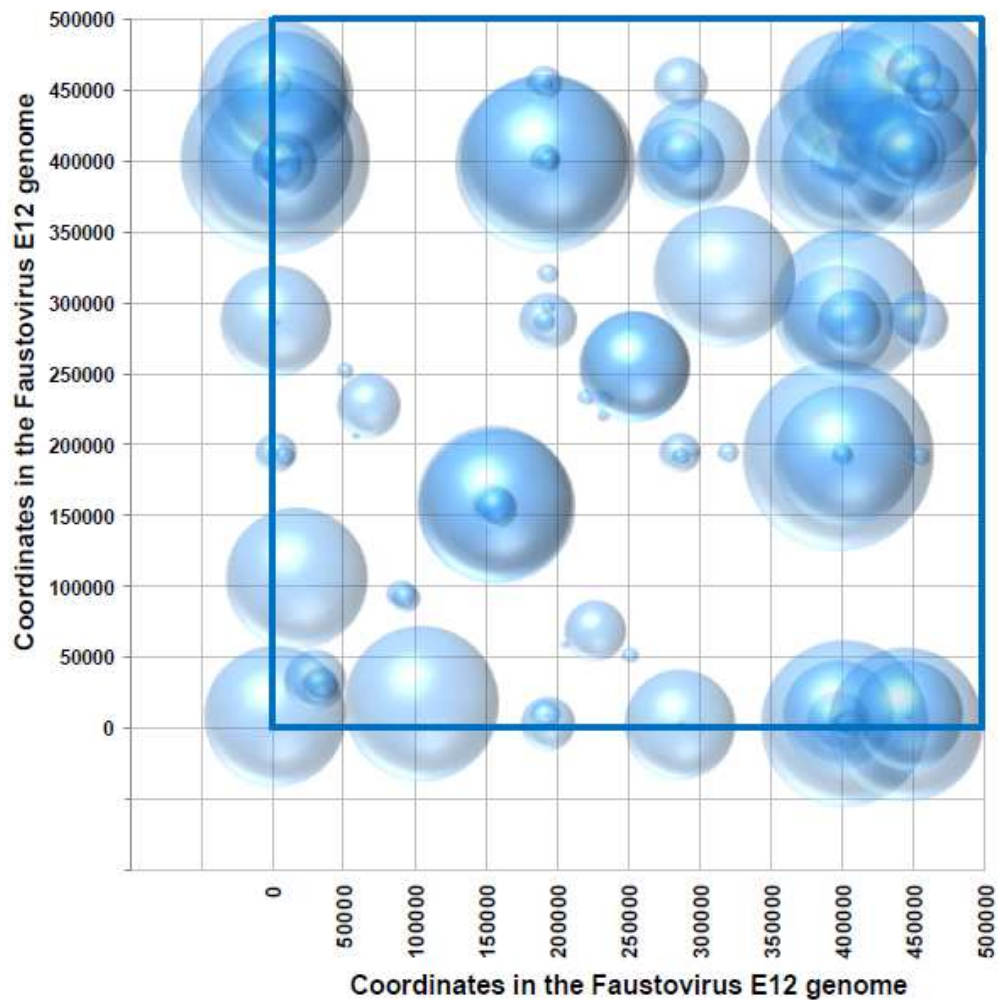


The log values are obtained after conversion of the Cycle threshold (ct) values based on standard curves realized with serial 1:10 dilution starting with 10^7 viral particles). The concentration of amoeba was quantified on kovalslides and by flow cytometry the 100% corresponds to the initial concentration of amoeba before infection; N.C corresponds to the negative control (non infected amoeba). This relative quantification by real-time PCR showed the increase in viral multiplication coming along with the decrease of the amoebal concentration. No viral DNA was detected from H0 till H4. Viral titers begin to be detected at H6 p.i; higher titers are at H12 with a plateau until H24 p.i.

Supplemental Figure S2. Taxonomic classification of best BLASTp hits for the Faustovirus E12 gene repertoire.

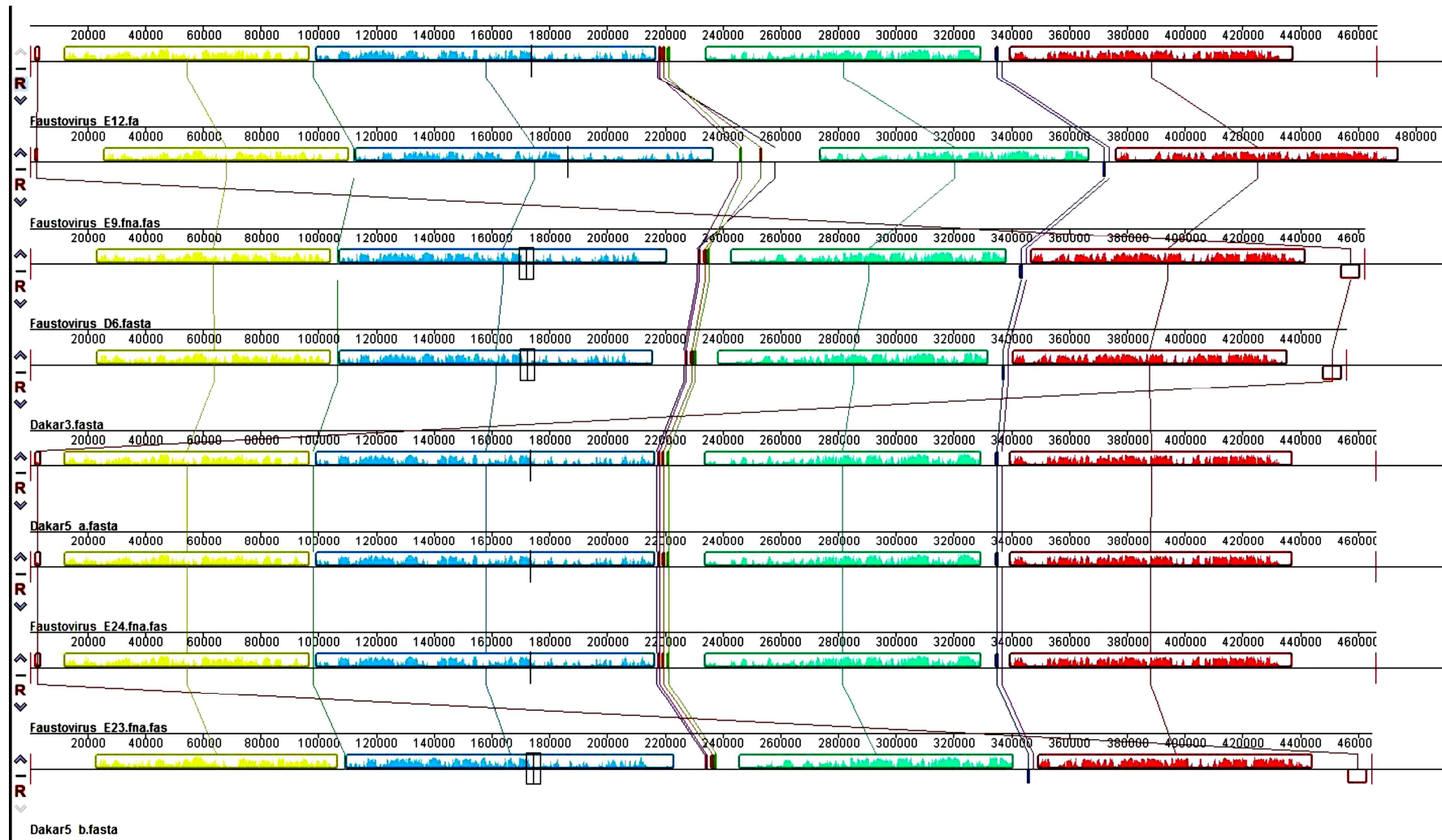


Supplemental Figure S3. Genomic dot-plot of the Faustovirus E12 genome against itself. The dot plot is based on a BLASTp analysis using $1e-5$ as the e-value threshold. Each circle represents a pair of paralogs; diameters are proportional to the BLASTp e-values.



29 **Supplemental Figure S4.** Comparison of the genomes from the eight faustovirus isolates.

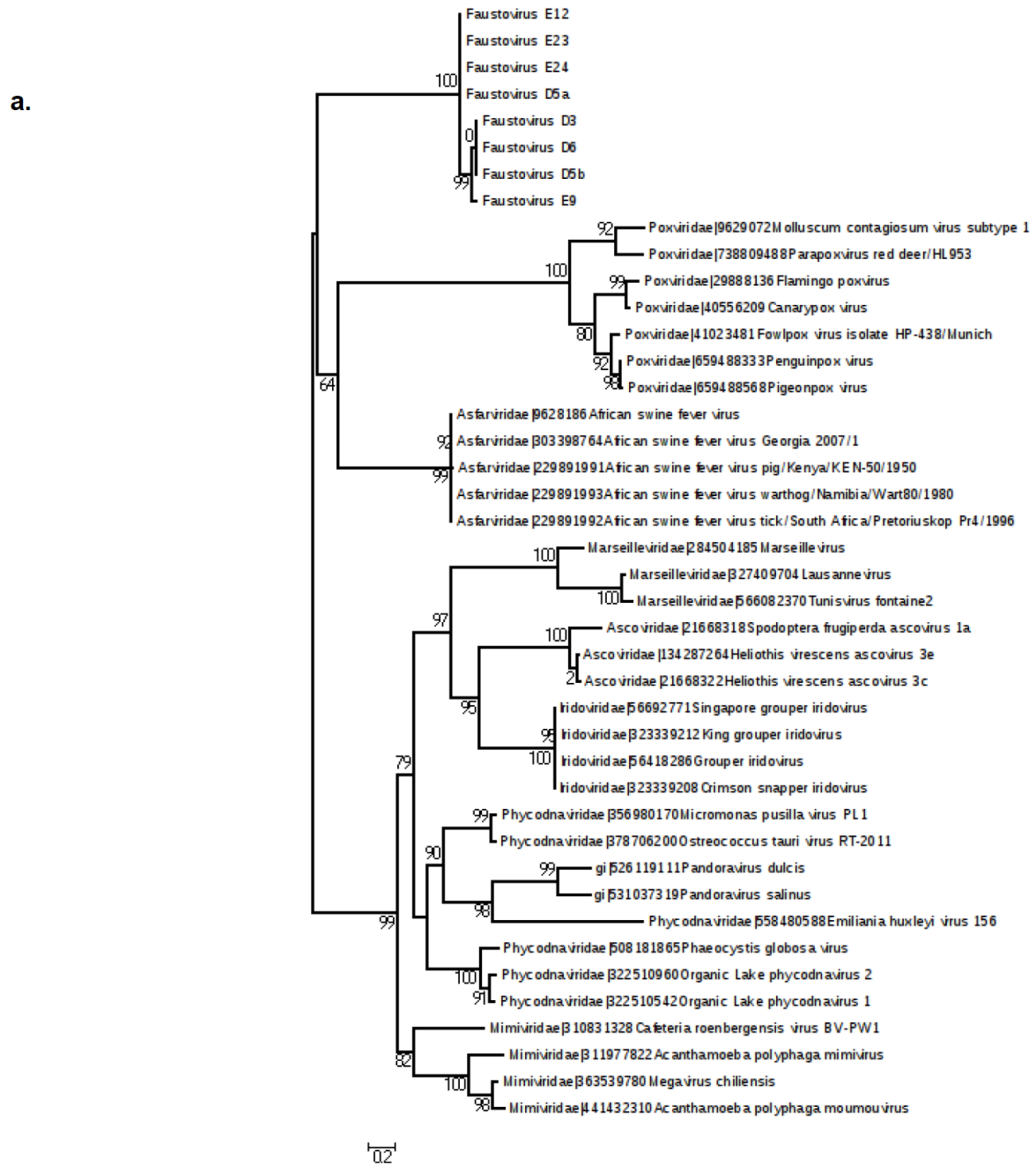
30 Genome comparison was performed using the Mauve software (1). The Faustovirus E12 genome is at the top.



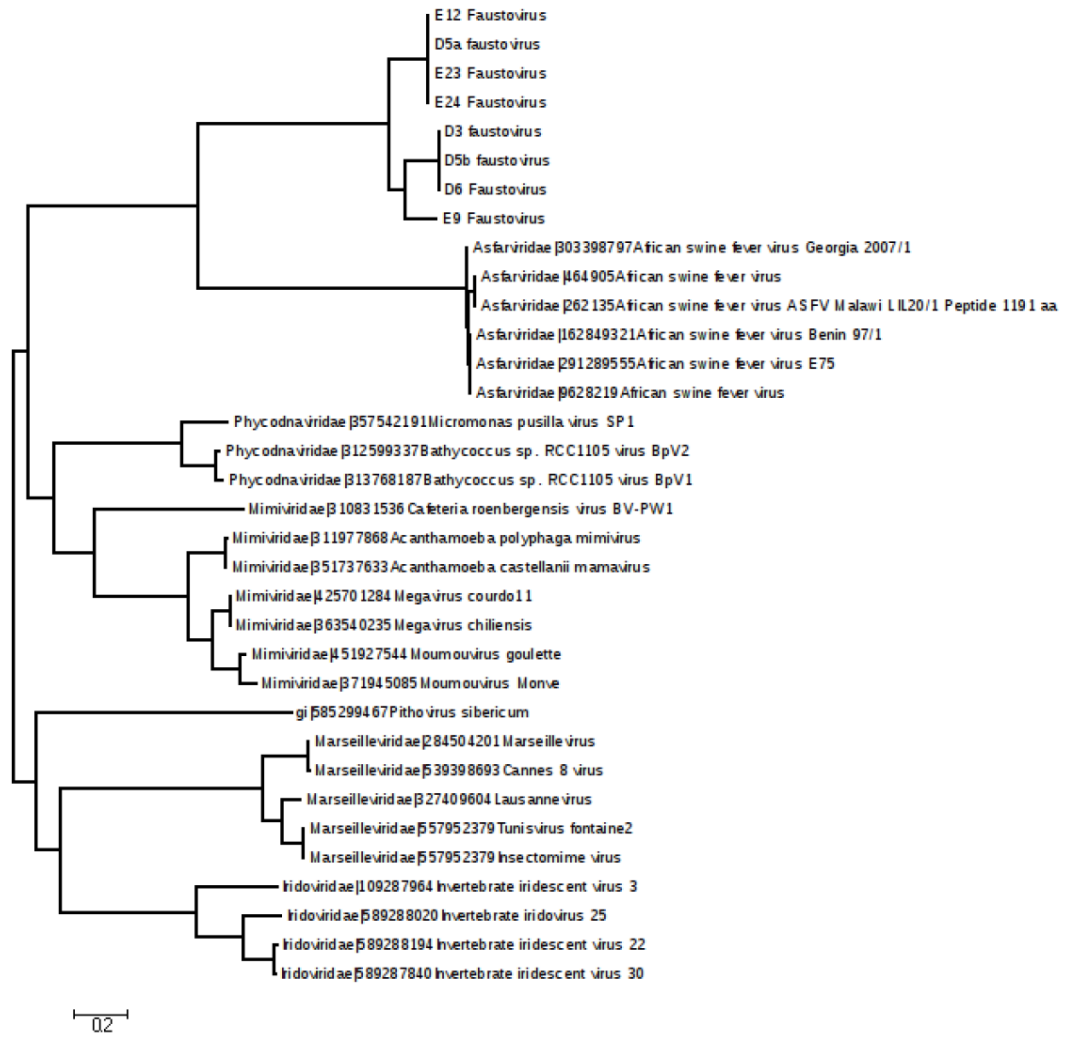
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Supplemental Figures S5. Phylogeny reconstruction performed for the eight faustovirus isolates (including the isolate Faustovirus E12) and other *Megavirales* members. Phylogeny reconstruction were performed using the Maximum Likelihood method and were based on A32-like packaging ATPase (a); DNA topoisomerase (b); and D5-like helicase-primase (c).



b.

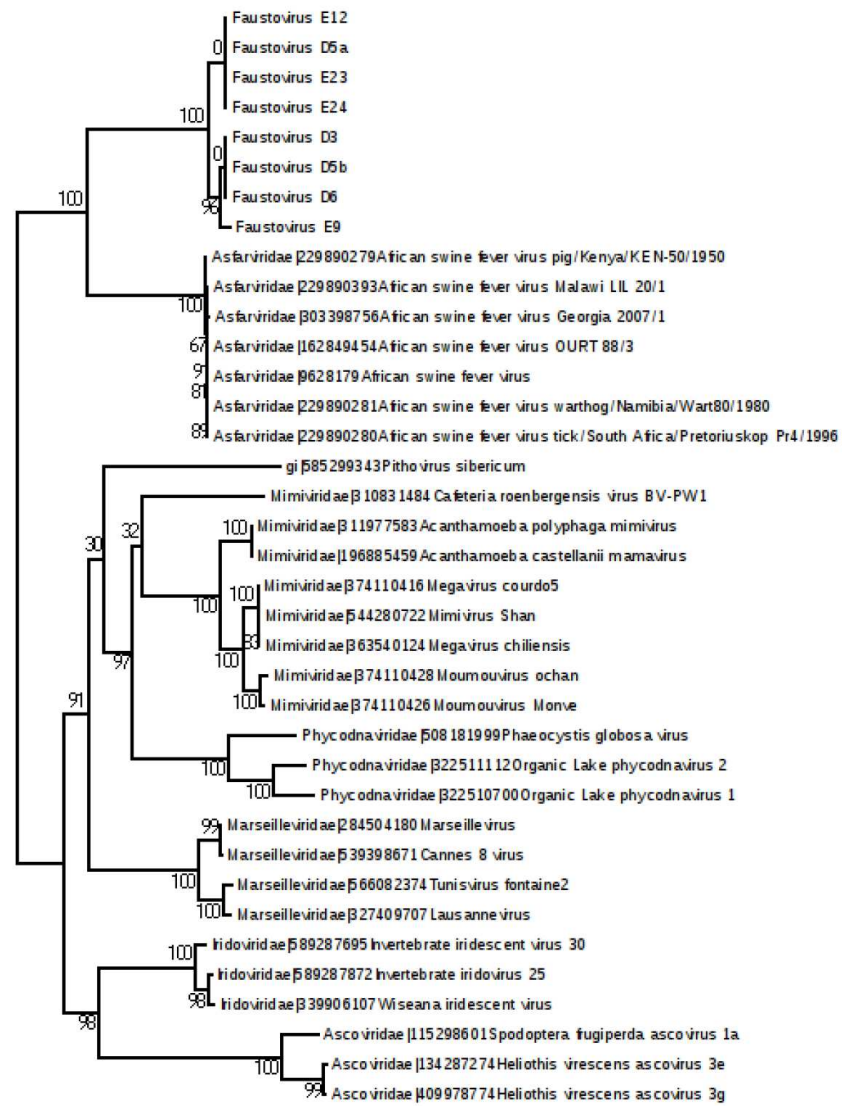


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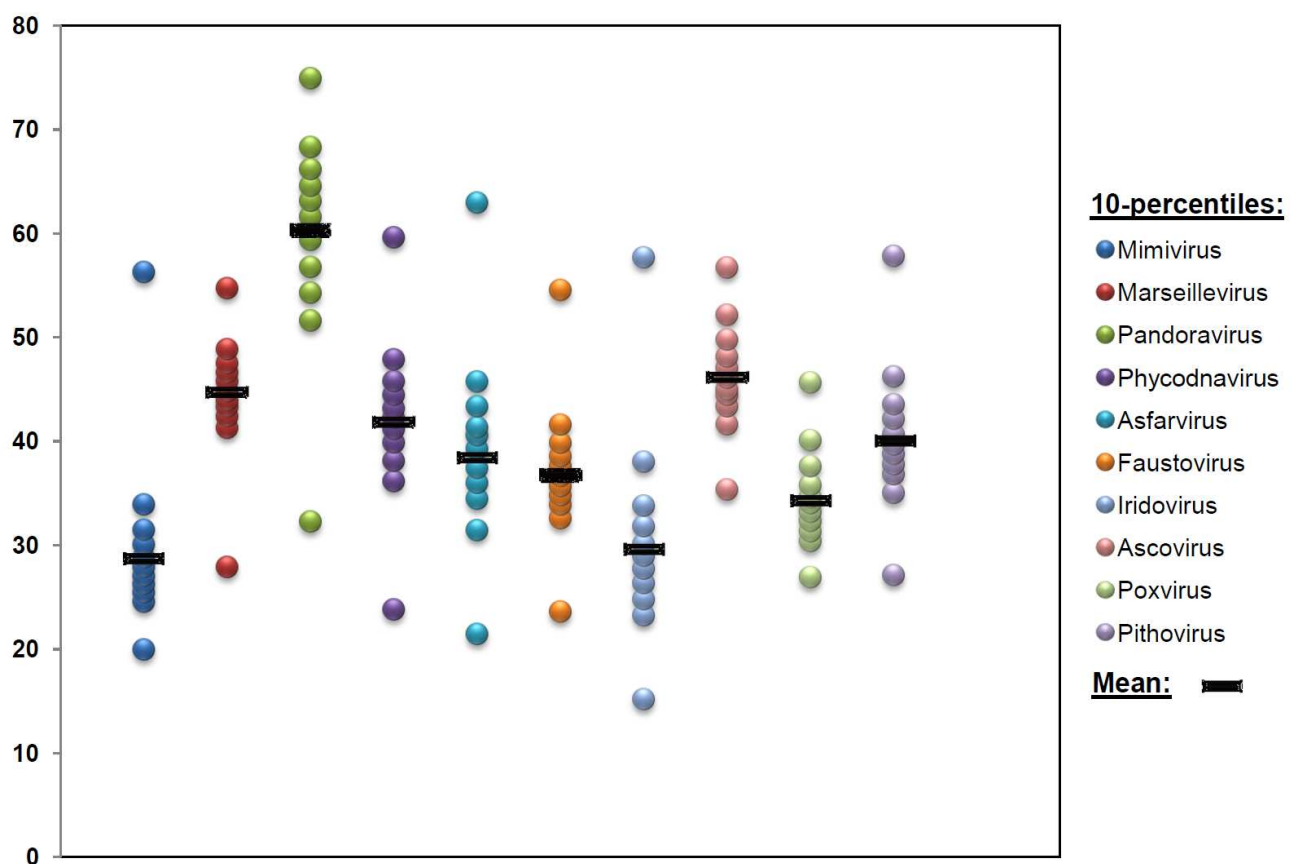
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C.

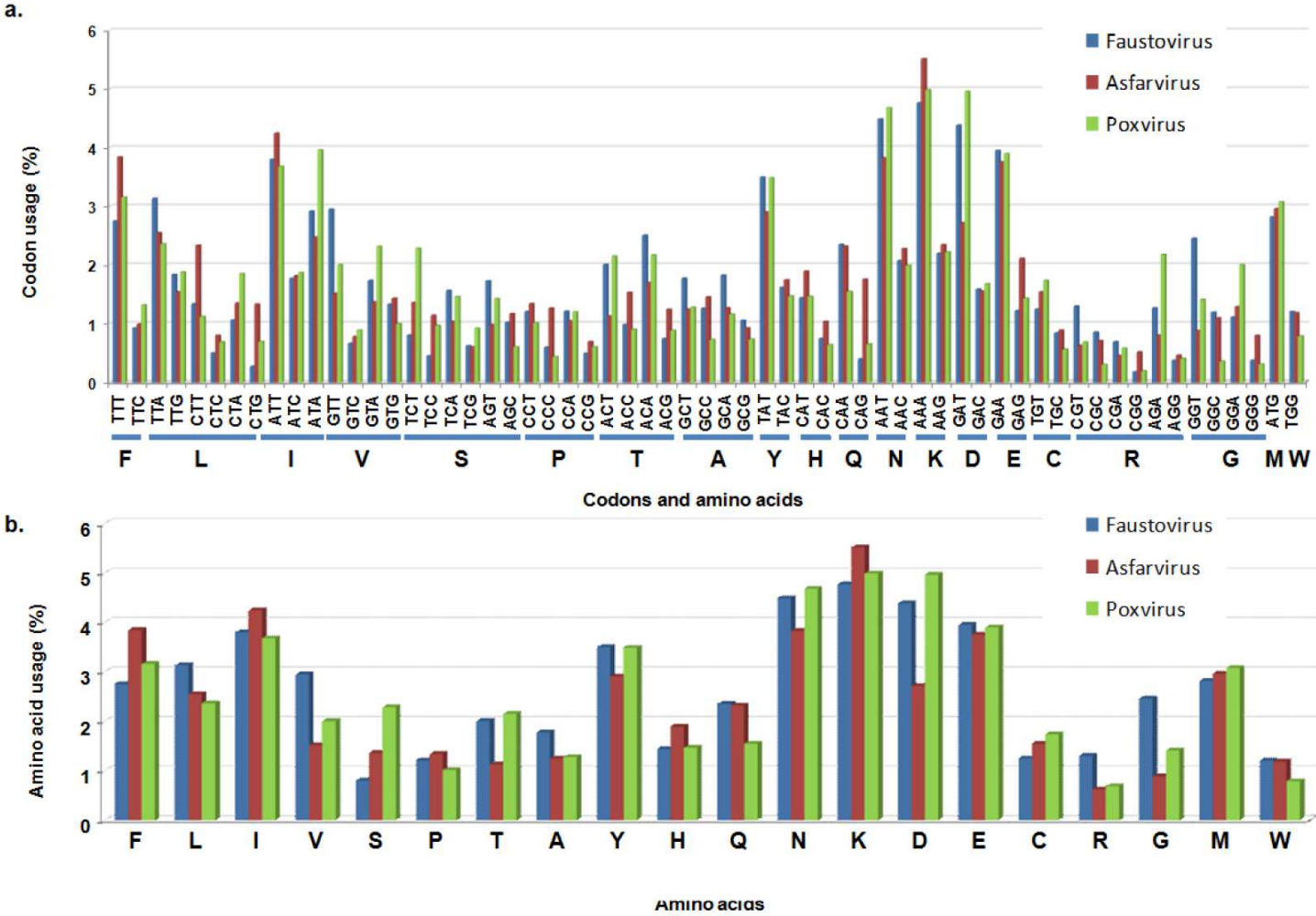


Supplemental Figure S6. G+C content of Faustovirus E12 and representative members from the seven previously described families or putative families.

Analysed sets of genes were from Mimivirus (NC_014649.1), Marseillevirus (NC_013756.1), *Pandoravirus salinus* (NC_022098.1), *Paramecium bursaria* Chlorella virus NY2A (NC_009898.1), African swine fever virus (NC_001659.1), Faustovirus E12, Invertebrate iridescent virus 6 (NC_003038.1), *Heliothis virescens* ascovirus 3e (NC_009233.1), vaccinia virus (NC_006998.1) and *Pithovirus sibericum* (NC_023423.1).



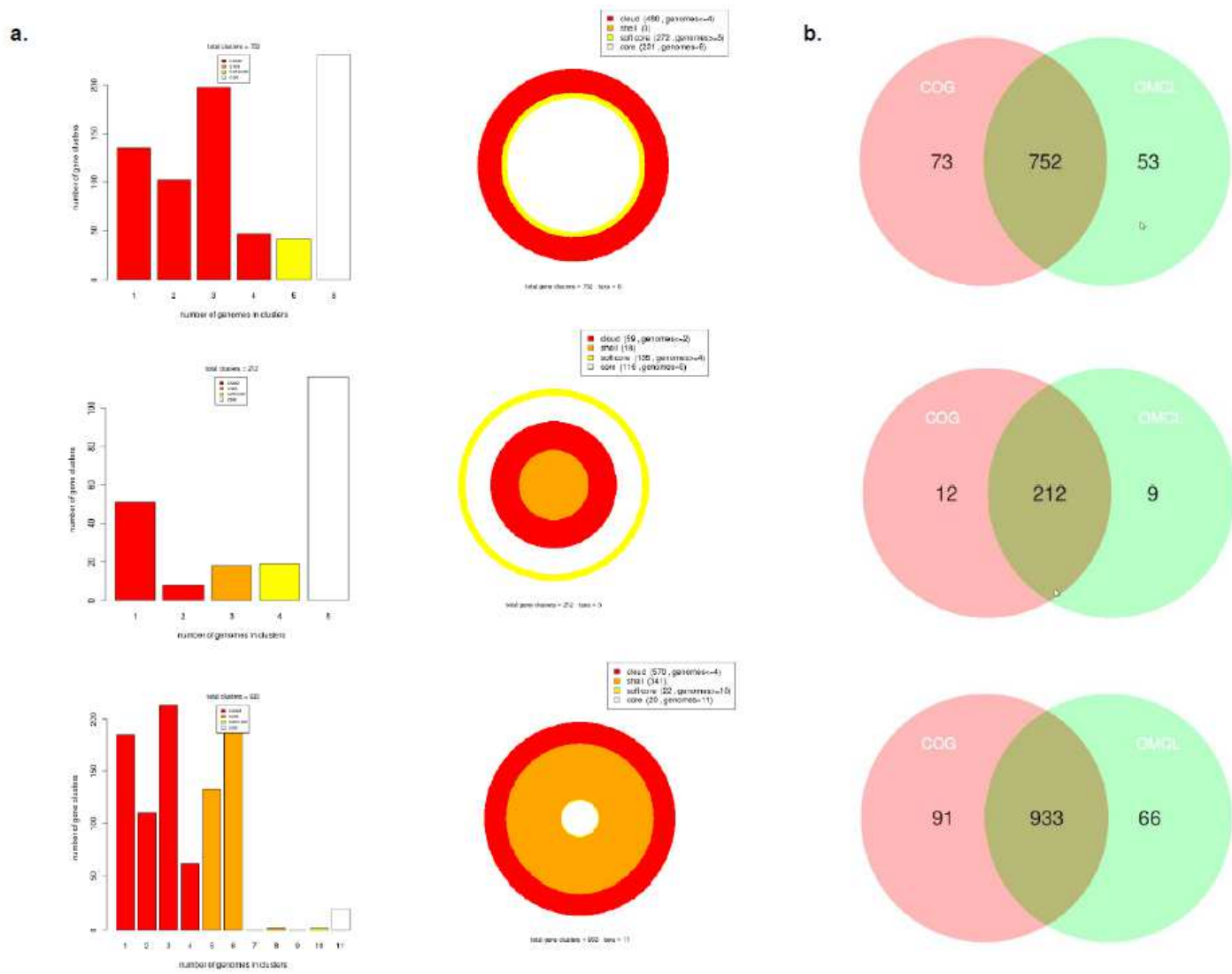
53 **Supplemental Figure S7.** Codon and amino acid usage of Faustovirus E12, and a representative member of the families *Asfarviridae* (African
54 swine fever virus (NC_001659.1)) and *Poxviridae* (vaccinia virus (NC_006998.1)).



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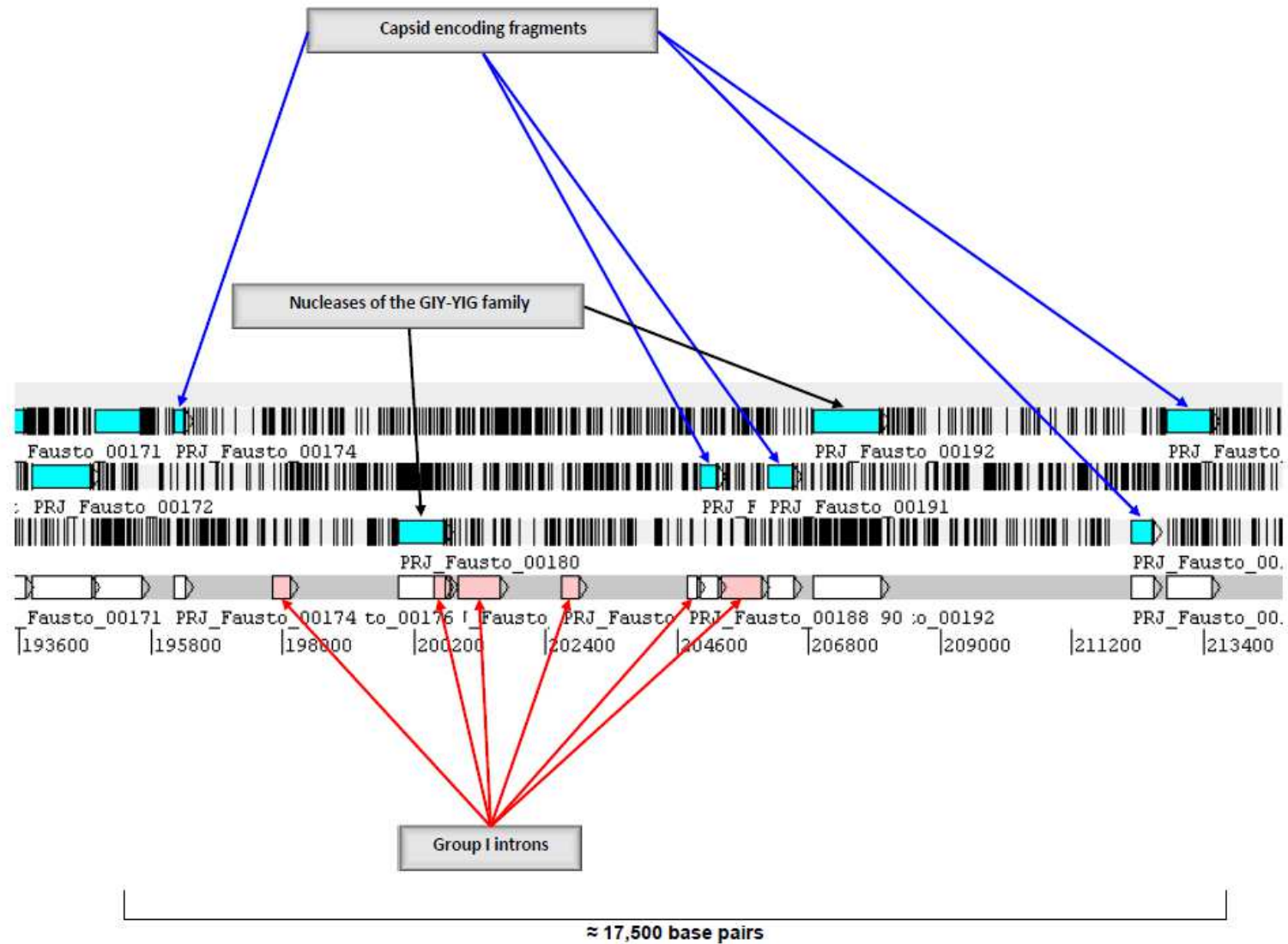
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57 **Supplemental Figure S8.** Core (a) and pan-(b) genome of the faustoviruses (upper), asfarviruses (middle) and combined faustoviruses and
 58 asfarviruses (bottom).



60 **Supplemental Figure S9.** Schematic of the Faustovirus E12 genome region encoding capsid fragments.

61 The $\approx 17,000$ -base pair-long fragment was represented using the Artemis tool (2)



62

Reference List

1. **Darling AC, Mau B, Blattner FR, Perna NT.** 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* **14**:1394-1403.
2. **Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J.** 2005. ACT: the Artemis Comparison Tool. *Bioinformatics.* **21**:3422-3423. doi:bt553 [pii];10.1093/bioinformatics/bti553 [doi].