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:** | ***2009.001a-II*** | (to be completed by ICTV officers) |

**Short title:** Hytrosaviridae: a proposal for classification and nomenclature of a new insect virus family
(e.g. 6 new species in the genus *Zetavirus*)

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

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

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a.m.m.abd-alla@iaea.org

**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) | Invertebrate Virus subcommittee - Hytrosaviridae Study Group |

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

The authors constitute the Hytrosaviridae Study Group

Date first submitted to ICTV:
Date of this revision (if different to above):

MODULE 2: **NEW SPECIES**

1-

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.

| Code | ***2009.001aI*** | (assigned by ICTV officers) |
|---|---|---|

**To create 1 new species within:**

| | |
|---|---|
| Genus: | ***Glossinavirus*** |
| Subfamily: | **unassigned** |
| Family: | **Hytrosaviridae (new)** |
| Order: | **unassigned** |

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.

**And name the new species:**

***Glossina hytrosavirus*** **(New Species)**

**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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Significant differences between GHV and MHV exist in genome size and gene content, tissue tropism beyond the salivary gland, virus transmission mode and virulence characteristics suggesting that the two viruses belong to distinct virus species.

**1- *Glossina hytrosavirus* (GHV)**

The distinct properties associated with GHV include: (i) the ability to infect a spectrum of insect species within the genus *Glossina*; (ii) the presence of a large circular dsDNA genome of about 190 kb and (iii) rod shaped virus particles, that measure 100 x 700-1000 nm; (iv) the ability to induce partial sterility in infected populations; (v) the widespread presence of asymptomatic infection in laboratory colonies; (vi) the inability to induce immediate development of SGH symptoms by feeding or injection of GHV preparations; (vii) in nature the virus is vertically transmitted, although horizontal transmission is assumed to occur at least within facilities used for mass rearing of tsetse flies[2]. The GenBank accession number for the genome sequence of *Glossina pallidipes* hytrosavirus (GpHV) (a member of this species) is **NC_010356.**

2-

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.

| Code | ***2009.001bI*** | (assigned by ICTV officers) |
|---|---|---|

**To create 1 new species within:**

| | |
|---|---|
| Genus: | ***Muscavirus*** |
| Subfamily: | **unassigned** |
| Family: | ***Hytrosaviridae*** **(new)** |
| Order: | **unassigned** |

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.

**And name the new species:**

***Musca hytrosavirus*** **(New Species)**

**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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Significant differences between GHV and MHV exist in genome size and gene content, tissue tropism beyond the salivary gland, virus transmission mode and virulence characteristics suggesting that the two viruses belong to distinct virus species.

***Musca hytrosavirus*** **(MHV)**

The distinct features of MHV members are: (i) the house fly as the only known host; (ii) the presence of a large circular dsDNA genome of about 124 kb and (iii) rod-shaped virus particles, that measure 80 x 680 nm; (iv) an infection that causes complete sterility of infected female flies; (v) overt symptoms as evidenced by hypertrophied salivary gland in all infected individual and the lack of asymptomatic infection as has been seen with GHV infections; (vi) the rapid development of SGH syndrome after artificial infection by feeding or injection; (vii) the exclusively horizontal transmission in the population by food contamination [9]. The GenBank accession number for the genome sequence of *Musca domestica* hytrosavirus (MdHV) (a member of this species) is **NC_010671.**

A further possible member of this family may be the *Merodon equestris hytrosavirus* (MeHV) described from the narcissus bulb fly (Fig. 2). The distinct features of this virus are (i) glandular proliferation; (ii) virus particles are rod shaped measuring 65 by x 650-700 nm [4]. The only known host is narcissus bulb fly. As no further information is yet available about this virus, it is too early to assign it as a member of the family Hytrosaviridae.

MODULE 3: **NEW GENUS**

1-

creating a new genus

Ideally, a genus should be placed within a higher taxon.

| Code | ***2009.001cI*** | (assigned by ICTV officers) |
|---|---|---|

**To create a new genus within:**

| | |
|---|---|
| Subfamily: | **unassigned** |
| Family: | ***Hytrosaviridae* (new)** |
| Order: | **unassigned** |

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 family is specified, enter "**unassigned**" in the family box

naming a new genus

| Code | ***2009.001dI*** | (assigned by ICTV officers) |
|---|---|---|

**To name the new genus: *Glossinavirus***

Assigning the type species and other species to a new genus

| Code | ***2009.001eI*** | (assigned by ICTV officers) |
|---|---|---|

**To designate the following as the type species of the new genus**

| | |
|---|---|
| ***Glossina hytrosavirus*(GHV) (New Species)** | Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered |

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

***1***

**Reasons to justify the creation of a new genus:**

Additional material in support of this proposal may be presented in the Appendix, Module 9

Significant differences between GHV and MHV exist in genome size and gene content, tissue tropism beyond the salivary gland, virus transmission mode and virulence characteristics suggesting that the two viruses belong to distinct virus species and even distinct virus genus.

**Origin of the new genus name:**

*Glossina spp.* which represent the virus host

**Reasons to justify the choice of type species:**

The first virus found and the most studied in term of prevalence, pathology, transmission and genome sequence.

**Species demarcation criteria in the new genus:**

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

The distinct properties associated with *Glossina hytrosavirus* (GHV) include: (i) the ability to infect a spectrum of insect species within the genus *Glossina*; (ii) the presence of a large circular dsDNA genome of about 190 kb and (iii) rod shaped virus particles, that measure 100 x 700-1000 nm; (iv) the ability to induce partial sterility in infected populations; (v) the widespread presence

| |
|---|
| of asymptomatic infection in laboratory colonies; (vi) the inability to induce immediate development of SGH symptoms by feeding or injection of GHV preparations; (vii) in nature the virus is vertically transmitted, although horizontal transmission is assumed to occur at least within facilities used for mass rearing of tsetse flies[2]; (viii) Ultra-thin section of the salivary gland hypertrophy show multilayer cells.. |

2-

creating a new genus

Ideally, a genus should be placed within a higher taxon.

| Code | ***2009.001fI*** | (assigned by ICTV officers) |
|---|---|---|

**To create a new genus within:**

| | |
|---|---|
| Subfamily: | **unassigned** |
| Family: | ***Hytrosaviridae* (new)** |
| Order: | **unassigned** |

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 family is specified, enter "**unassigned**" in the family box

naming a new genus

| Code | ***2009.001gI*** | (assigned by ICTV officers) |
|---|---|---|

**To name the new genus: *Muscavirus***

Assigning the type species and other species to a new genus

| Code | ***2009.001hI*** | (assigned by ICTV officers) |
|---|---|---|

**To designate the following as the type species of the new genus**

| | |
|---|---|
| ***Musca hytrosavirus* (MHV) (New Species)** | Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered |

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

***1***

**Reasons to justify the creation of a new genus:**

Additional material in support of this proposal may be presented in the Appendix, Module 9

| |
|---|
| Significant differences between GHV and MHV exist in genome size and gene content, tissue tropism beyond the salivary gland, virus transmission mode and virulence characteristics suggesting that the two viruses belong to distinct virus species and even distinct virus genus. |

**Origin of the new genus name:**

| |
|---|
| *Musca sp.* which represent the virus host |

**Reasons to justify the choice of type species:**

| |
|---|
| The first virus found and the most studied in term of prevalence, pathology, transmission and genome sequence. |

**Species demarcation criteria in the new genus:**

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

The distinct features of *Musca hytrosavirus* (MHV) members are: (i) the house fly as the only known host; (ii) the presence of a large circular dsDNA genome of about 124 kb and (iii) rod-shaped virus particles, that measure 80 x 680 nm; (iv) an infection that causes complete sterility of infected female flies; (v) overt symptoms as evidenced by hypertrophied salivary gland in all infected individual and the lack of asymptomatic infection as has been seen with GHV infections; (vi) the rapid development of SGH syndrome after artificial infection by feeding or injection; (vii) the exclusively horizontal transmission in the population by food contamination [9]: (viii) Ultra-thin section of the salivary gland hypertrophy show a hypertrophied monolayer cells.

MODULE 5: **NEW FAMILY**

creating and naming a new family

| Code | ***2009.001iI*** | (assigned by ICTV officers) |
|---|---|---|

**To create a new family containing the subfamilies and/or genera listed below within the Order: unassigned**

If there is no Order, write "**unassigned**" here.
If the Order has yet to be created (in Module 6) please write **"(new)"** after the proposed name.

| Code | ***2009.001jI*** | (assigned by ICTV officers) |
|---|---|---|

**To name the new family: *Hytrosaviridae***

assigning subfamilies, genera and unassigned species to a new family

| Code | | (assigned by ICTV officers) |
|---|---|---|

**To assign the following subfamilies (if any) to the new family:**
You may list several subfamilies here. For each subfamily, please state whether it is new or existing.

- If the subfamily is new, it must be created in Module 4
- If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family

| Code | ***2009.001kI*** | (assigned by ICTV officers) |
|---|---|---|

**To assign the following genera to the new family:**
You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

**1- *Glossinavirus* (New)**

**2- *Muscavirus* (New)**

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

**Reasons to justify the creation of the new family:**
Additional material in support of this proposal may be presented in the Appendix, Module 9

Although sharing several important features with other large DNA viruses (baculoviruses, nudiviruses, ascoviruses, and nimaviruses) such as rod-shaped, enveloped virions, a nuclear site of replication and a large, circular, double-stranded DNA genome, GpHV and MdHV (and MeHV) differ significantly in several respects from these already-classified viruses. From a biological viewpoint, their host range is, so far, limited to order Diptera, and they are the only insect viruses to induce symptoms of salivary gland hypertrophy in adult insects **(Fig. 1)**. Salivary gland hypertrophy, although reducing fertility, does not impact fly survival. The general morphology of the long, enveloped, rod-shaped nucleocapsids is distinctly different from that of rod-shaped baculoviruses, nudiviruses, and ovoid-shaped ascoviruses or short rod-shaped nimaviruses **(Fig. 2)**. They also are excluded from the other large dsDNA viruses such

as Poxviridae and Iridoviridae that are not rod-shaped and which possess linear genomes. Most importantly, genome analysis of GpHV and MdHV revealed that not only do they differ from baculoviruses, nudiviruses, ascoviruses, and nimaviruses, but they cannot be assigned to any of the large dsDNA virus families known to infect invertebrates or vertebrates **[refs. 2 and 9].** The similarity between their genomes and those of other large DNA viruses is limited to a very small number of genes with well-known functions. Out of 108 putative genes, the MdHV genome has only 30 genes showing slight homology to database proteins that belong to nudiviruses (11), to baculoviruses (12), and to nimaviruses (6). Similarly, out of 160 putative genes of GpHV, only 37 display moderate homologies to genes from other viruses, including 11 to baculoviruses and nudiviruses, 16 to entomopoxviruses, 4 to nimaviruses, and 3 to ascoviruses. In the same comparison, MdHV and GpHV shared 37 and 42 genes, respectively [100]. The presence in both GpHV and MdHV genomes of genes homologous to baculoviruses and nudiviruses per os infectivity factors and several other structural genes suggests a common mechanism of virus entry via the insect alimentary tract and a possible common origin of these viruses. However, the phylogenetic trees of these genes clearly indicated that GpHV and MdHV are more closely related to each other than to any other baculovirus or nudivirus and form a strongly supported clade **(Fig. 3)**. Finally, phylogenetic analyses based on the DNA polymerase gene, which is frequently used to infer the relationship of dsDNA viruses, clearly separates hytrosaviruses from ten dsDNA virus families **(Fig. 4).** The unique pathology, tissue tropism, disease symptoms, virion structure, gene content, and DNA divergence exhibited by the hytrosaviruses provide criteria that support the classification of these viruses in a separate new virus family [3].

**Selected References**

2. Abd-Alla AMM, Cousserans F, Parker AG, Jehle JA, Parker NJ, Vlak JM, Robinson AS, Bergoin M (2008) Genome analysis of a *Glossina pallidipes* salivary gland hypertrophy virus (GpHV) reveals a novel large double-stranded circular DNA virus. J Virol 82: 4595-4611

3. Abd-Alla AMM, Vlak JM, Bergoin M, Maruniak JE, Parker AG, Burand JP, Jehle JA, Boucias DG (2008) Hytrosaviridae: a proposal for classification and nomenclature of a new insect virus family. Arch Virol Accepted.

9. Garcia-Maruniak A, Abd-Alla AMM, Salem T.Z., Parker AG, van Oers MM, Maruniak JE, Kim W, Burand JP, Cousserans F, Robinson AS, Vlak JM, Bergoin M, Boucias DG (2009) Two viruses that cause salivary gland hypertrophy in *Glossina pallidipes* and *Musca domestica* are related and form a distinct phylogenetic clade. J Gen Virol 90: 334-346

10. Garcia-Maruniak A, Maruniak JE, Farmerie W, Boucias DG (2008) Sequence analysis of a non-classified, non-occluded DNA virus that causes salivary gland hypertrophy of *Musca domestica*, MdHV. Virology 377: 184-196

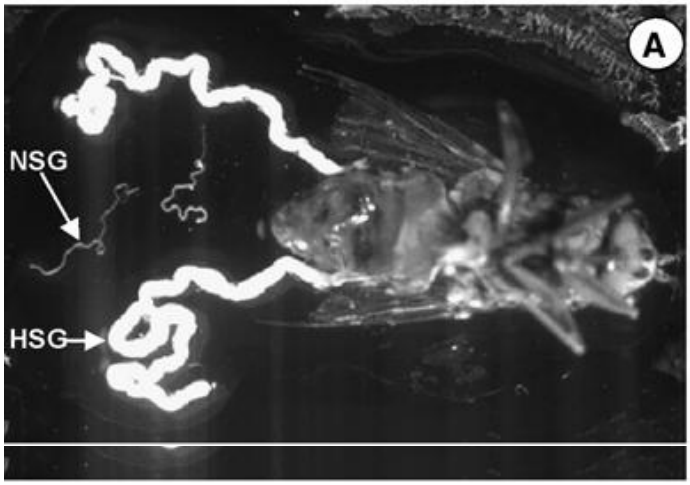


**Figure 1:** Normal (NSG) and hypertrophied salivary glands (HSG) of *Glossina pallidipes* (source [1]).

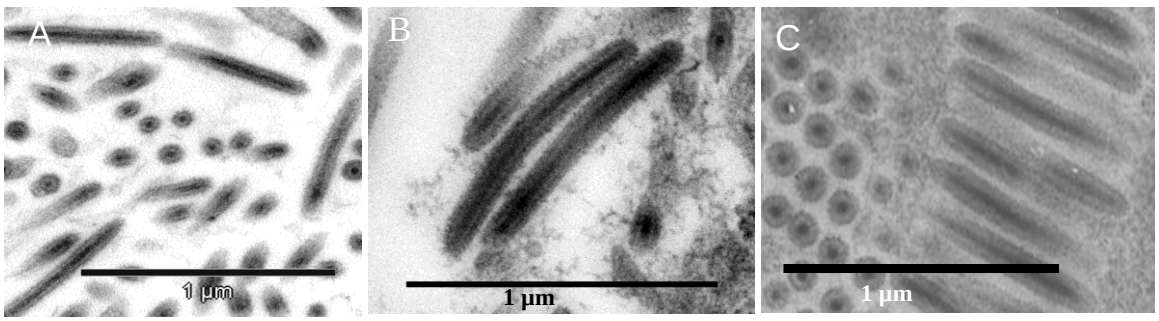


**Figure 2:** Ultrastructure of various members of the Hytrosaviridae A: thin section showing MdHV enveloped virus particles in the lumen of an infected salivary gland of *Musca domestica*. B: thin section of an infected salivary gland of *Glossina pallidipes* (GpHV), and C: thin section of an infected salivary gland of *Merodon equestris* (MeHV) (source Jean-Louis Duthoit, unpublished data).

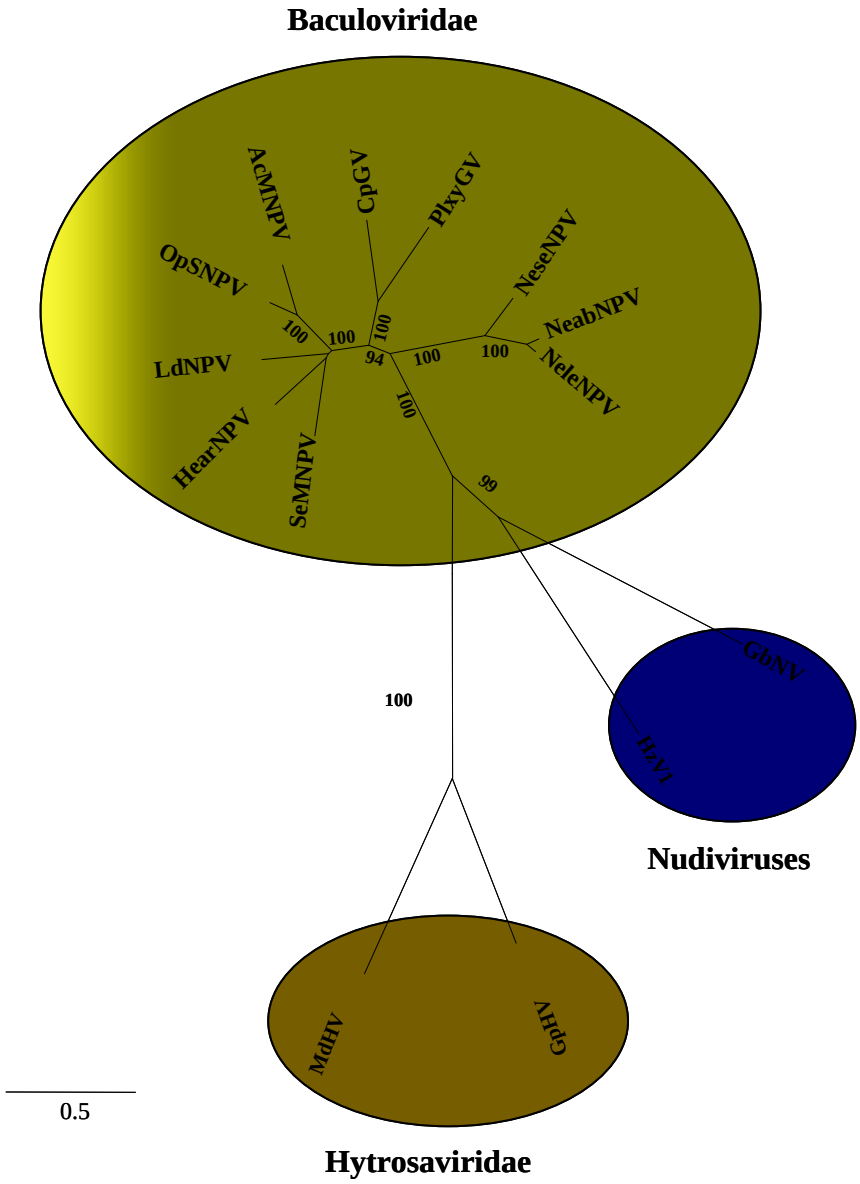


**Figure 3:** Neighbor-Joining (NJ) tree of the concatenated predicted amino acid sequences of

*p74*, *pif-1*, *pif-2* and *pif-3*, of hytrosaviruses (GpHV [NC_010356] and MdHV [NC_010671]) and their homologs in baculoviruses and nudiviruses. The following viruses (GenBank accession number in brackets) included: Alphabaculoviruses: *A. californica* (Ac) MNPV [NC_001623], *O. pseudotsugata* (Op) MNPV [NC_001875], *L. dispar* (Ld) MNPV [NC_001973], *S. exigua* (Se) MNPV [NC_002169], *H. armigera* (Hear) NPV [NC_002654]; Betabaculoviruses: *C. pomonella* (Cp) GV [NC_002816], *P. xylostella* (Plxy) GV [NC_002593]; Gammabaculoviruses: *N. sertifer* (Nese) NPV [NC_005905], *N. lecontei* (Nele) NPV [NC_005906], *N. abietes* (Neab) NPV [NC_008252], Nudiviruses: *H. zea* (Hz) NV-1 [NC_004156], *G. bimaculatus* (Gb) NV [NC_009240]. Distances were calculated using Poisson correction. Homogeneous substitution pattern among lineages with gamma distributed rate among sites (Gamma parameter 2.25) was employed for reconstruction of the trees. The robustness of the tree was tested using bootstrap analysis (500 replicates). (source [9]).

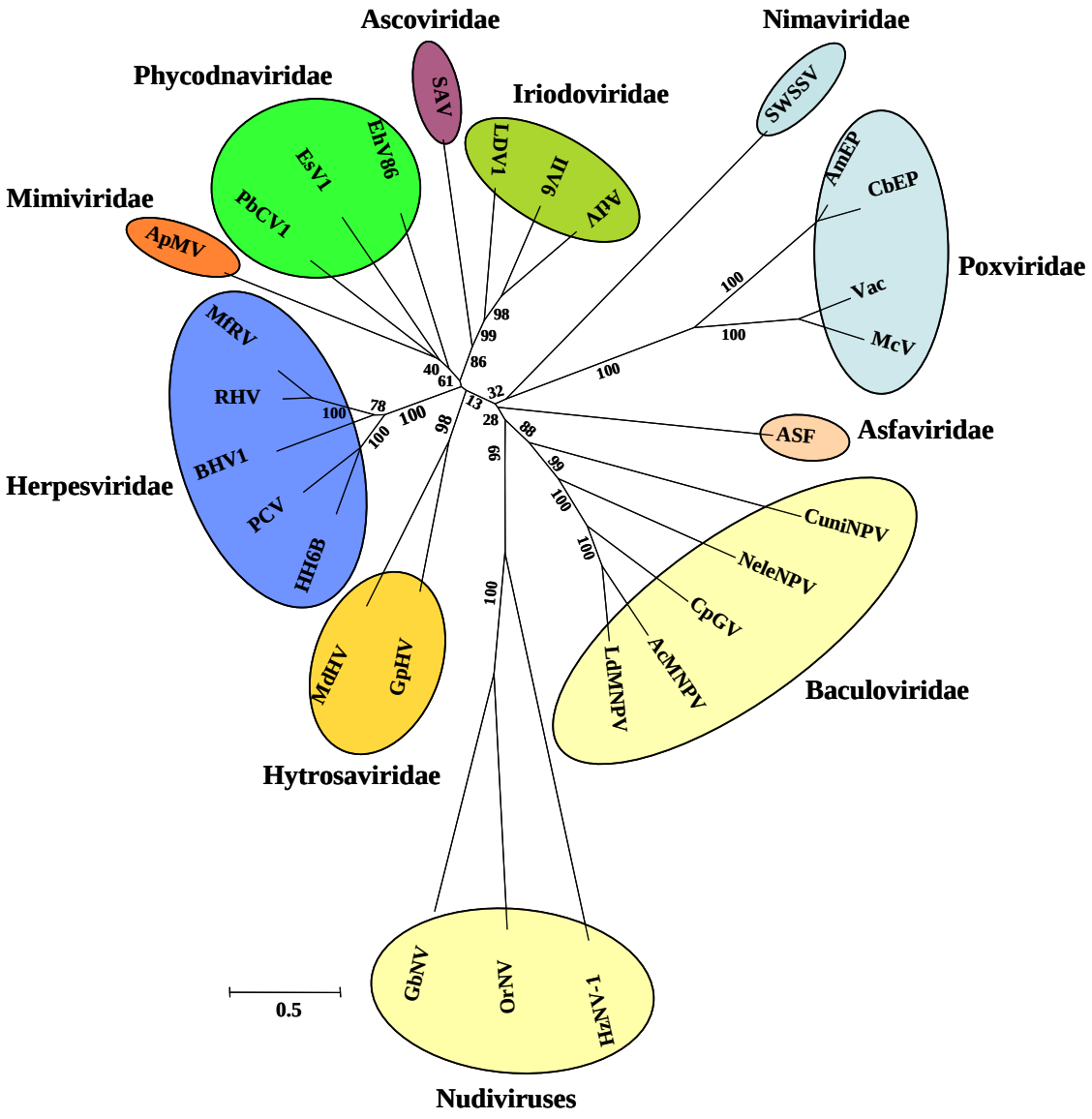

**Figure 4:** Neighbor-Joining (NJ) trees of predicted amino acid sequence of the DNA polymerase genes. The phylogenetic tree of DNA polymerase and its homologs is based on 2374 sites of DNA polymerase of 29 viruses from various families. Distances were calculated using Poisson correction. The robustness of the tree was tested using bootstrap analysis (500 replicates). Numbers on the nodes indicate bootstrap values. The name of the selected virus families are indicated on the tree. Asterisk indicates the proposed family name for the HVs. DNA polymerases used and their accession numbers in brackets: ASF: African Swine Fever virus [NP_042783], GpHV: *Glossina pallidipes* salivary gland hypertrophy virus [ABQ08852], MdHV: *Musca domestica* salivary gland hypertrophy virus [YP_001883329], HHB6: Human herpesvirus 6B [NP_050219], PCV: Porcine cytomegalovirus [AAF80107], BHV1: Bovine herpesvirus 1 [NP_045328], RHV: Retroperitoneal fibromatosis-associated herpesvirus [AAC57976], MfRV: *Macaca fuscata* rhadinovirus [YP_238317], ApMV: *Acanthamoeba polyphaga* mimivirus [YP_142676], PbCV1: *Paramecium bursaria* Chlorella virus 1 [AAC00532], EsV1: *Ectocarpus siliculosus* virus 1 [NP_077578], EhV86: *Emiliania huxleyi* virus 86 [AAL58859], SAV: *Spodoptera* ascovirus [AAC54632], LDV1: Lymphocystis disease virus 1 [NP_078724], IIV6: Invertebrate iridescent virus 6 [CAC19195], AtIV: *Aedes taeniorhynchus* iridescent virus [ABF82150], AmEPV: *Amsacta moorei* entomopoxvirus [NP_064832], CbEP: *Choristoneura biennis* entomopoxvirus [CAA40566], Vac: Vaccinia virus [NP_063712], McV: *Molluscum contagiosum* virus [AAL40129], WSSV: shrimp white spot syndrome virus [AAK77696], CuniNPV: *Culex nigripalpus* NPV [AAK13281], NeleNPV: *Neodiprion lecontei* NPV [YP_025217], CpGV: *Cydia pomonella* granulovirus [NP_148895], AcMNPV: *Autographa californica* nucleopolyhedrovirus [NP_054095], LdMNPV: *Lymantria dispar* nucleopolyhedrovirus [NP_047720], HzNV-1: *Heliothis zea* virus 1 [NP_690550], OrNV: *Oryctes rhinoceros* virus [ABF93350], GbNV: *Gryllus bimaculatus* nudivirus [YP_001111279] (source [9]).

**Origin of the new family name:**

***Hytrosaviridae*** name is derived from *Hytrosa,* a sigla from the Greek ‘Hypertrophia’ for 'hypertrophy', ‘sialoadenitis’ for ‘salivary gland inflammation’ [3].

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

**References:**


1. Abd-Alla A, Bossin H, Cousserans F, Parker A, Bergoin M, Robinson A (2007) Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. J Virol Methods 139: 143-149
2. Abd-Alla AMM, Cousserans F, Parker AG, Jehle JA, Parker NJ, Vlak JM, Robinson AS, Bergoin M (2008) Genome analysis of a Glossina pallidipes salivary gland hypertrophy virus (GpSGHV) reveals a novel large double-stranded circular DNA virus. J Virol 82: 4595-4611
3. Abd-Alla AMM, Vlak JM, Bergoin M, Maruniak JE, Parker AG, Burand JP, Jehle JA, Boucias DG (2008) Hytrosaviridae: a proposal for classification and nomenclature of a new insect virus family. Arch. Virol. 154, 909-918.
4. Amargier A, Lyon JP, Vago C, Meynadier G, Veyrunes JC (1979) Discovery and purification of a virus in gland hyperplasia of insects. Study of Merodon equestris F. (Diptera, Syrphidae). C R Acad Sci D 289: 481-484
5. Burtt E (1945) Hypertrophied salivary glands in Glossina: evidence that G. pallidipes with this abnormality is particularly suited to trypanosome infection. Ann Trop Med Parasitol 39: 11-13
6. Coler RR, Boucias DG, Frank JH, Maruniak JE, Garcia-Canedo A, Pendland JC (1993) Characterization and description of a virus causing salivary gland hyperplasia in the housefly, Musca domestica. Med Vet Entomol 7: 275-282
7. Ellis DS, Maudlin I (1987) Salivary gland hyperplasia in wild caught tsetse from Zimbabwe. Entomol Exp Appl 45: 167-173
8. Feldmann U (1994) Guidelines for the rearing of tsetse flies using the membrane feeding technique. In: Ochieng'-Odero JPR (ed) Techniques of insect rearing for the development of integrated pest and vector management strategies. ICIPE Science Press, Nairobi, Kenya, pp 449-471
9. Garcia-Maruniak A, Abd-Alla AMM, Salem T.Z., Parker AG, van Oers MM, Maruniak JE, Kim W, Burand JP, Cousserans F, Robinson AS, Vlak JM, Bergoin M, Boucias DG (2009) Two viruses that cause salivary gland hypertrophy in Glossina pallidipes and Musca domestica are related and form a distinct phylogenetic clade. J Gen Virol 90: 334-346
10. Garcia-Maruniak A, Maruniak JE, Farmerie W, Boucias DG (2008) Sequence analysis of a non-classified, non-occluded DNA virus that causes salivary gland hypertrophy of Musca domestica, MdSGHV. Virology 377: 184-196
11. Geden CJ, Lietze VU, Boucias DG (2008) Seasonal prevalence and transmission of salivary gland hypertrophy virus of house flies (Diptera: Muscidae). J Med Entomol 45: 42-51
12. Gouteux JP (1987) Prevalence of enlarged salivary glands in Glossina palpalis, G. pallicera, and G. nigrofusca (Diptera: Glossinidae) from the Vavoua area, Ivory Coast. J Med Entomol 24: 268
13. Hu ZH, Arif BM, Jin F, Martens JW, Chen XW, Sun JS, Zuidema D, Goldbach RW, Vlak JM (1998) Distinct gene arrangement in the Buzura suppressaria single-nucleocapsid nucleopolyhedrovirus genome. J Gen Virol 79 ( Pt 11): 2841-2851
14. Jaenson TGT (1978) Virus-like rods associated with salivary gland hyperplasia in tsetse, Glossina pallidipes. Trans R Soc Trop Med Hyg 72: 234-238
15. Jaenson TGT (1986) Sex ratio distortion and reduced lifespan of Glossina pallidipes infected with the virus causing salivary gland hyperplasia. Entomol Exp Appl 41: 256-271
16. Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann

**References:**

DA, Thiem SM, Vlak JM (2006) On the classification and nomenclature of baculoviruses: a proposal for revision. Arch Virol 151: 1257-1266
17. Jura WGZO, Odhiambo TR, Otieno LH, Tabu NO (1988) Gonadal lesions in virus-infected male and female tsetse, Glossina pallidipes (Diptera: Glossinidae). J Invertebr Pathol 52: 1-8
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**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.

Hytroviruses (HVs) have been identified from different dipteran species, such as the tsetse fly *Glossina pallidipes* (GpHV), the house fly *Musca domestica* (MdHV) and the narcissus bulbfly *Merodon equestris* (MeHV). These viruses share the following characteristics: (i) they produce non-occluded, enveloped, rod-shaped virions that measure 550-1000 nm in length and 80-100 nm in diameter; (ii) they possess a large circular double-stranded DNA (dsDNA) genome ranging in size from 120-190 kbp and having G+C ratios ranging from 28-44%; (iii) they cause overt salivary gland hypertrophy symptoms in dipteran adults and partial to complete sterility. The available information on the complete genome sequence of GpHV and MdHV indicates significant co-linearity between the two viral genomes, whereas no co-linearity was observed with baculoviruses, ascoviruses, entomopoxviruses, iridoviruses and nudiviruses, other large invertebrate DNA viruses. The DNA polymerases encoded by the HVs are of the type B and closely related, but are phylogenetically distant from DNA polymerases encoded by other large dsDNA viruses. The great majority of HV ORFs could not be assigned by sequence comparison. Phylogenetic analysis of conserved genes clustered both HVs, but distantly from the nudiviruses and baculoviruses. On the basis of the available morphological, (patho)biological, genomic and phylogenetic data, we propose that the two viruses are members of a new virus family named *Hytrosaviridae.* This family currently comprises two unassigned species GpHV and MdHV and a tentative unassigned species MeHV. Here, we present the characteristics and the justification for establishing this new virus family.

Although numerous viruses have been reported to replicate in insect salivary glands [22], only three of them are known to induce overt salivary gland hypertrophy (SGH) symptoms [2, 10]. These viruses include the tsetse fly *G. pallidipes* salivary gland hypertrophy virus (GpHV), the house fly *M. domestica* salivary gland hypertrophy virus (MdHV) and possibly the narcissus bulb fly *M. equestris* salivary gland hypertrophy virus (MeHV)

Hypertrophy of the salivary glands **(Fig. 1)** was initially reported in wild tsetse flies in the 1930's [32]. In the late 1970's a virus infection was identified as the cause of this disease [14], and was then also associated with testicular degeneration and ovarian abnormalities [17, 19, 27, 28]. GpHV has since been detected in many tsetse fly species from different African countries [7, 12, 17, 18, 23, 24, 26, 30]. Similar viruses have been reported from the narcissus bulb fly *M. equestris* [4], and more recently in the house fly *M. domestica* [6]. All of these viruses share characteristics with viruses of other insect virus families, such as being specific to insect hosts, possessing enveloped rod-shaped virus particles (**Fig. 2**), containing large circular dsDNA genomes like *Baculoviridae* and being nonoccluded like the nudiviruses and *Nimaviridae*. The unique pathological property of this virus group is that virus infection induces major SGH in adult flies associated with a reduction in host fertility and fecundity [20]. Based on these properties, the three viruses GpHV, MdHV and MeHV seem to be related viruses. The recent comparative analysis of GpHV and MdHV genomes [9] supports this view, and we propose to accommodate these viruses in a new family, for which the name Hytrosaviridae is proposed [3]

**Virion properties**

Virions of the Hytrosaviridae are rod-like to filamentous in shape, have a regular symmetry, measure about 50 to 80 nm in diameter and range from 500 to 1000 nm in length

depending on the species. GpHV and MdHV band at a density of 1.153 g/cm$^3$ when subjected to 10-60 % Nycodenz gradient centrifugation at 30,000 g for 1 hour (unpublished data). The virion consists of an inner, 50 nm diameter rod-shaped nucleocapsid containing an electron dense DNA-protein core, an intermediate and a tight fitting outer trilaminar envelope. The nucleocapsids are about the same length as the virions **(Fig. 2).**

The nucleocapsid contains a single molecule of circular dsDNA with an approximate size range of 120 to 190 kbp. The G+C ratio among HVs examined to date varies between 28 and 44%. The genomes of GpHV and MdHV have been completely sequenced [2, 9]. The genome of GpHV (accession no. EF568108) consists of 190,032 bp with a G+C ratio of 28% that codes for 160 methionine-initiated putative open reading frames (ORFs) [2]. The genome of MdHV (accession no. EU522111) consists of 124,279 bp with a G+C ratio of 44% that codes for 108 putative ORFs [10]. The HV DNA contains repeat regions dispersed along the genome. A non-destructive PCR test for detection of GpHV has been developed [1].

The virions contain at least 35 polypeptides ranging in size from 10 to 200 kDa **(Fig. 3)**. In MdHV, 29 ORFs have been assigned to structural proteins identified by GeLC-MS/MS analysis of SDS-PAGE gel of purified virus particles. These proteins include baculovirus ODV-E66, PIFs and p74 homologs, and aminoacylase and dUTPase proteins [10]. The trilaminar viral envelope very likely contains lipids.

**Genome organization and replication**

The double-stranded, circular Hytrosavirus genomes, depending on the species, harbour 100 to 160 non-overlapping ORFs, that are almost equally distributed over both DNA strands [2, 9]. These ORFs encode among others for virion proteins [9], proteins involved in DNA replication (e.g. DNA polymerase, helicase) and protein-modifying enzymes (e.g. protein kinase). The vast majority of the ORFs are unassigned. Hytrosavirus genomes are further characterized by the presence of multiple regions of homologous repeats, which also exists in other large invertebrate DNA viruses such as baculoviruses and nimaviruses. Presently, very little is known about HV transcription except that the 5′ untranslated regions of the ORFs are highly enriched with a TAAG motif, which is identical to the canonical baculovirus late transcription initiation sequence [2, 21]. Replication of Hytrosavirus occurs in the nucleus of secretory epithelial cells of the salivary glands, whereas the virions are assembled in the nucleus and cytoplasm [11] **(Fig. 2)**. Genotypic variants of both the GpHV and the MdHV exist and can be distinguished by restriction fragment length polymorphism (RFLP) and on the basis of genomic sequencing.

**Biological properties**

Hytrosaviruses are restricted so far to the insect order *Diptera*, where they can be found in hematophagous flies (tsetse flies), filth flies (house flies), and sacchariphagous flies (narcissus bulb fly). Many species of tsetse flies have been reported to be infected with GpHV (**Table 1).** Feeding or injection of house flies with GpHV did not induce the typical SGH symptoms but the GpHV could be detected in the flies by PCR twenty days post infection (unpublished data). Host range studies have to date demonstrated that MdHV is specific to the host *M. domestica* (V-U. Lietze, unpublished) Various insect species, including adult Diptera (the sarcophagid carrion feeder, *Sarcophaga crassipalpis*, and the muscid horn fly, *Haematobia irritans*), the larvae of the citrus root weevil, *Diaprepes abbreviatus* (*Coleoptera: Curculionidae*), and the corn earworm, *Helicoverpa zea* (*Lepidoptera: Noctuidae*), were injected with a filter-sterilized virus preparations that induced 100% SGH in house flies. None of these injected insects produced detectable viral symptoms by 8-9 days post-injection. In addition to *in vivo* infection experiments, various insect cell lines including *Anopheles gambiae* 4e3A, *Aedes aegypti* Aag2e, *Aedes albopictus* C6/36, *Spodoptera frugiperda* SF9, and *Drosophila melanogaster* Kc167, S2, and S3 have been tested, but none appear to be competent for MdHV replication (V-U. Leitze, unpublished). These results

indicate that the tsetse and house fly HVs are distinct Hytrosaviruses that display a high degree of host specificity.

**Transmission**

Hytrosaviruses have been reported to be transmitted horizontally through feeding (*per os*, MdHV) as well as vertically from mother to offspring (GpHV) [14, 15, 18, 20].

In nature, the major mode of GpHV transmission in tsetse flies is believed to be vertical from mother to offspring either by transovum transfer of the virus to the embryo [18] or via virus-infected milk glands to the developing larvae [29]. Horizontal transmission is thought not to occur in the wild, but during colony rearing it is presumed that horizontal transmission may occur when the flies are fed *in vitro* [8]. Whilst all the flies in a tsetse colony may contain PCR detectable virus, only 5 – 15% display overt hypertrophy so that many GpHV infections are asymptomatic [1]. Asymptomatic flies are fertile and produce progeny where the GpHV can be detected by PCR in the pupa and unfed teneral flies. In *in vitro* fed tsetse fly colonies the virus is detected in blood only after fly feeding [1]. In symptomatic males around 5% still produce progeny when mated with asymptomatic females. Symptomatic tsetse fly females have reduced fecundity and produce only symptomatic progeny (unpublished data). The natural incidence of symptomatic GpHV infection is low; only 0.4 to 15.6% of the field-collected flies display SGH symptoms [7, 14, 17, 24-26].

In contrast, no asymptomatic MdHV infections have been detected in colonized house fly populations. Symptomatic females infected as young (previtellogenic) flies do not mate or develop eggs. Infected adults imbibe and digest food material that normally stimulates vitellogenesis in healthy females, however viral infection in these insects blocks production of female-specific proteins involved in egg maturation. Females that are infected later in life (after the initial oogenic cycle) deposit their initial batch of eggs, although they do not undergo additional oogenic cycles. The major mode of MdHV transmission within house fly populations is believed to be horizontal among adult flies via feeding on food sources contaminated by viremic conspecifics. The incidence of MdHV in feral house fly populations may reach up to 34% [11, 20].

**Geographical distribution**

GpHV has been detected in multiple tsetse fly populations of various tsetse species in Africa (Table 1). MdHV has been isolated only from house flies, however its distribution appears to be worldwide with strains of the MdHV being detected in Europe, North America, South America, Asia, and Australasia (D. Boucias, unpublished). The only isolation of the hitherto unassigned MeHV has been from adult narcissus bulb flies in France [4].

**Cytopathic effects**

The major target of the HVs is the salivary gland, although other tissues can also be affected (ovarioles, gonadal tissues). Following their replication in the nucleus and assembly in the nucleus and cytoplasm, virions are released into the lumen of the salivary gland. Infected flies show gross signs of gland enlargement (salivary gland hypertrophy), hence the name salivary gland hypertrophy viruses. HV infection does not cause heavy mortality, but results in significant reduction in fertility and fecundity. Induction of symptomatic infection in tsetse fly colonies results in total collapse of the colony within a few generations.

**Structural and phylogenetic relationships within the family**

GpHV and MdHV share 38 ORFs, including *DNA polymerase, DNA helicase, ODV-e66, per os* infectivity factors *pif-1*, *pif-2, pif-3,* and *p74* **(Table 2)**. The latter group of genes appears to be conserved over almost all large circular DNA viruses of invertebrates, suggesting a common mechanism of virus entry via the alimentary tract. GpHV and MdHV have at least 13 virion

proteins in common [9], and more are expected when the proteome of GpHV is known. Phylogenetic analyses of the *pif*/*p74* genes indicated that GpHV and MdHV are more closely related to each other than to any other baculovirus or nudivirus and form a strongly supported clade **(Fig. 4)**. Sequence information of MeHV is not available. The close relationship between GpHV and MdHV is further supported by the colinearity of their 38 common genes along the genome using Gene Parity Plot analysis **(Fig. 5)** [13].

**Relationship of Hytrosaviruses with other virus taxa**

Morphologically, the Hytrosaviridae virions and rod-shaped nucleocapsids resemble those of baculoviruses [16] and nudiviruses [31] **(Table 3)**. The presence of repeat regions dispersed over the DNA genome is a property shared with members of the families *Baculoviridae*, *Nimaviridae*, *Ascoviridae* and the yet unassigned group of nudiviruses. However, phylogenetic analyses based on the DNA polymerase gene, which is frequently used to infer the relationship of dsDNA viruses, did not assign the HVs to any of these viruses (Fig. 6). Though the resolution of this tree weakly resolves the interrelationship of different dsDNA virus families, it clearly indicates the lack of a close relationship of the proposed Hytrosviridae to any other known virus family.

**Conclusion**

Information on the morphology, (patho)biology, genome organization and single and concatenated gene phylogeny of GpHV and MdHV indicate that these two viruses have many characteristics in common. They are sufficiently distinct from other large vertebrate and invertebrate viruses based on the morphology of the mature virus particles, the symptoms they induce of hypertrophied salivary glands in their host, the genome organization and the single and concatenated gene phylogeny of GpHV and MdHV, and have sufficient characteristics in common to warrant the establishment of a new virus family, the proposed Hytrosaviridae **(Table 3)**. Within the family we propose the recognition of two HV species, GpHV and MdHV. As no molecular data of MeHV are available, this virus is presently considered as a tentative member of the family. This proposal for the establishment of the family Hytrosaviridae has been assembled by the Hytrosavirus Study Group and has been submitted to the ICTV for consideration.

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**Table 1**: List of tsetse species in which, the hytrosaviruses (HVs) has been detected.

| Species | Location | Ref. |
|---|---|---|
| *G. pallidipes Austen* | Zululand, South Africa | [32][a] |
| *G. morsitans Westw.* | Kenya | [5][a] |
| *G. pallidipes Austen* | Kibwezi forest, Kenya | [14] |
| *G. pallidipes Austen* | Lab colony ICIPE, Kenya | [14] |
| *G. morsitans morsitans* | Lab colony ICIPE, Kenya | [7, 26] |
| *G. palpalis palpalis* | Vavoua, Ivory Coast | [12] |
| *G. pallicera pallicera* | Vavoua, Ivory Coast | [12] |
| *G. nigrofusca nigrofusca* | Vavoua, Ivory Coast | [12] |
| *G. pallidipes Austen* | Kiboko, Central Kenya | [23] |
| *G. pallidipes Austen* | Sindo, Kenya | [23] |
| *G. morsitans centralis* | Lab colony, ILRAD, Kenya | [30] |
| *G. brevipalpis* | Lab colony, ILRAD, Kenya | [30] |
| *G. morsitans submorsitans* | Lab colony, CIRDES, Burkina Faso | Personal observation[b] |
| *G. fuscipes fuscipes* | Lab colony, Seibersdorf | Personal observation[b] |
| *G. brevipalpis* | Lab colony, Seibersdorf | Personal observation[b] |
| *G. pallidipes* | Lab colony originating from Uganda held at Seibersdorf, Austria | Personal observation[b] |
| *G. pallidipes* | Lab colony originating from Ethiopia held at Seibersdorf, Austria | Personal observation[b] |
| *G. pallidipes* | Ethiopia | |
| *G. pallidipes* | Zambia | |
| *G. austeni* | South Africa | |
| *G. morsitans morsitans* | Zimbabwe | |
| *G. fuscipes* | Uganda | |

**[a] Correlation with virus infection not established in these articles.**

**[b] Personal communication Dr H. Bossin. Entomology Unit, FAO/IAEA Agriculture & Biotechnology Laboratory, IAEA Laboratories Seibersdorf, A-2444**

**Table 2**: Putative core genes of *Hytrosaviridae*

| Md HV ORFs | Gp HV ORFs | Genes |
|---|---|---|
| Putative Structural genes | | |
| 13 | 83 | |
| 22 | 93 | |
| 25 | 97, 96 | |
| 29 | 102 | *Pif-1* |
| 33 | 107, 108 | |
| 39 | 1 | *P74* |
| 47 | 5 | *ODV-E66* |
| 71 | 154 | |
| 72 | 43 | |
| 84 | 46 | |
| 86 | 50 | |
| 89 | 53 | *Pif-2* |
| 90 | 71 | |
| 96 | 65 | |
| 97 | 64 | |
| 102 | 72 | |
| 106 | 76 | *Pif-3* |
| DNA replication | | |
| 1 | 79 | *DNAPol* |
| 12 | 36 | *Thymidylate synthase* |
| 104 | 74 | *Helicase-2* |
| Auxilliary genes | | |
| 36 | 110, 111 | *Matrix metallo proteinase* |
| Unknown function | | |
| 4 | 82 | |
| 16 | 86 | |
| 17 | 88 | |
| 27 | 99 | |
| 30 | 104 | |
| 46 | 6 | |
| 55 | 41 | |
| 70 | 40 | |
| 73 | 44 | |
| 74 | 32, 33 | |
| 82 | 31, 30 | |
| 83 | 45 | |
| 87 | 51 | |
| 100 | 61 | |
| 107 | 77 | |
| 108 | 78 | *Ac81* |

Table 3: Comparison between Hytrosaviridae and large dsDNA invertebrate viruses

| **Virus** | **Morphology** | **Size (nm) (LxD)** | **Genome (kb)** | **Repli-cation** | **Occlusion Body** | **Symptoms/ Histopathology** | **Host Family** |
|---|---|---|---|---|---|---|---|
| *Ascoviridae* | Enveloped Ovoid | 220-240 x130 | Circular 160-190 | nucleus | No | Impairment of development, vesicle formation | Lepidoptera Hymenopera |
| *Baculoviridae* | Enveloped Rod | 200-450 x 30-100 | Circular 90-180 | nucleus | Yes | nuclear and cellular hypertrophy, | Lepidoptera, Hymenopera Diptera |
| *Iridoviridae* | Non-enveloped isometric | 160-350 | Linear 150-280 | nucleus | No | Iridescence | Lepidopterra Diptera Coleoptera |
| *Entomopoxvirinae* | Enveloped Ovid pleomorphic | 300-400 x170-250 | Linear 130-300 | cytoplas m | Yes | Spheroid (and spindle) formation, impairment of motility and development | Lepidoptera, Orthoptera Coleoptera Dipetera |
| *Nimaviridae* | Enveloped Rod | 270-290 x120-150 | Circular 300 | nucleus | No | White spots | Crustaceae |
| Nudiviruses | Enveloped Rod | 20-250 X 50-70 | Circular 125-220 | nucleus | Yes/No | Nuclear and cellular hypertrophy | Lepidoptera, Coleoptera Orthoptera |
| Hytrosaviridae | Enveloped Rod | 700-1000 x 50 | Circular 120-190 | nucleus | No | Hypertrophied Salivary gland | Diptera |

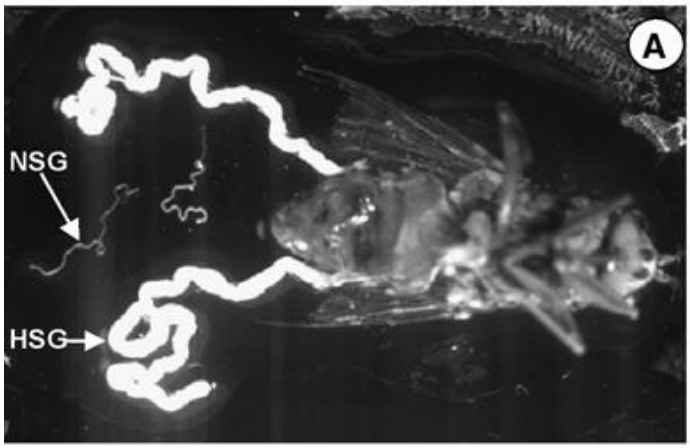


**Figure 1:** Normal (NSG) and hypertrophied salivary glands (HSG) of *Glossina pallidipes* (source [1]).

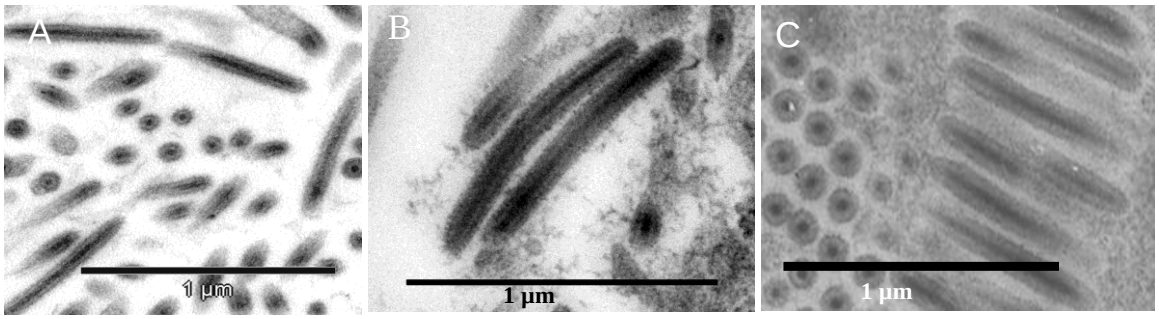


**Figure 2:** Ultrastructure of various members of the Hytrosaviridae A: thin section showing MdHV enveloped virus particles in the lumen of an infected salivary gland of *Musca domestica*. B: thin section of an infected salivary gland of *Glossina pallidipes* (GpHV), and C: thin section of an infected salivary gland of *Merodon equestris* (MeHV) (source Jean-Louis Duthoit, unpublished data).

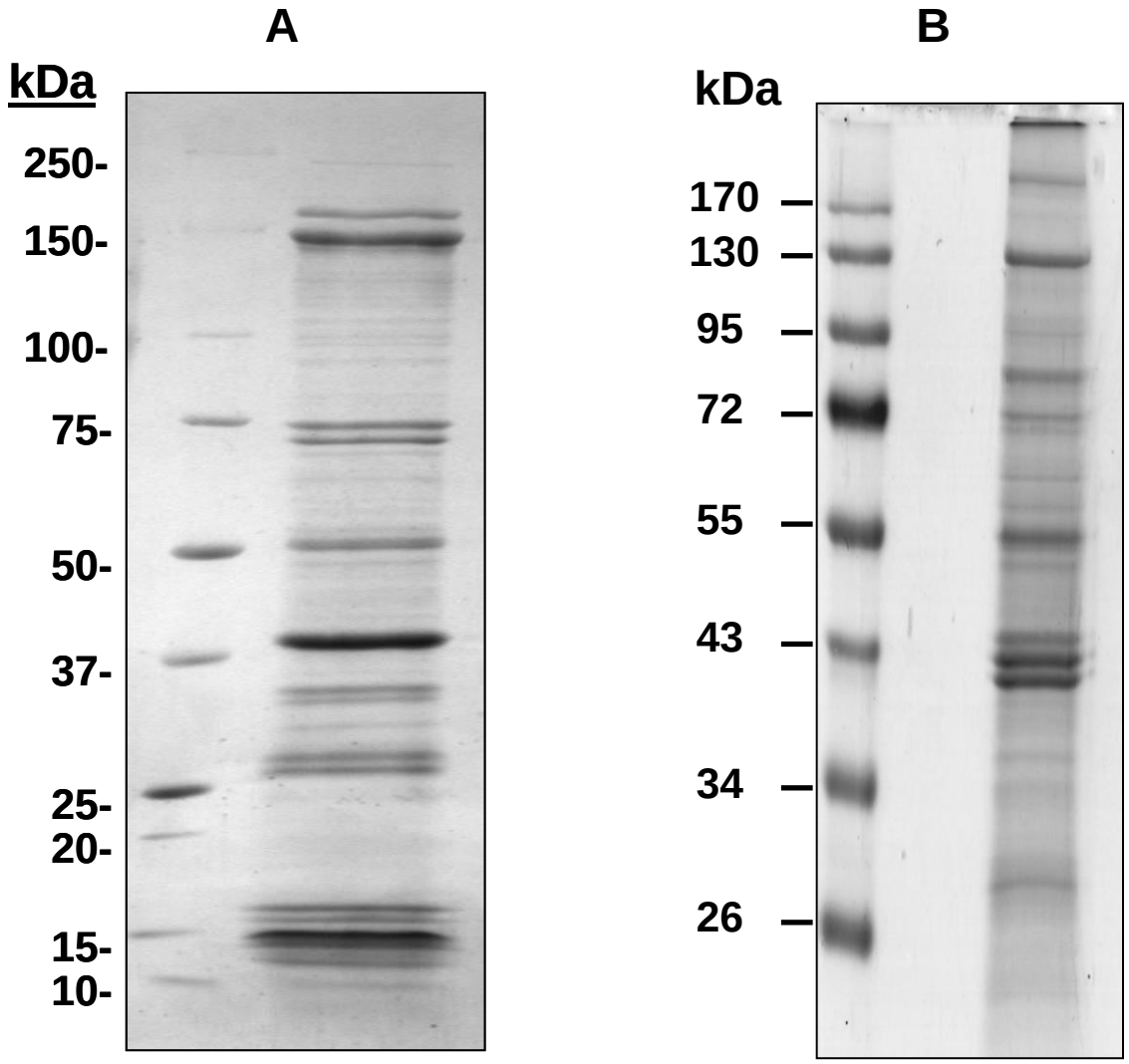


**Figure 3**: SDS-PAGE of (A) MdHV and (B) GpHV stained with Coomassie Brilliant Blue. Molecular masses (kDa) of the Bio-Rad markers are denoted on the left side of the gel (source of A [10]).

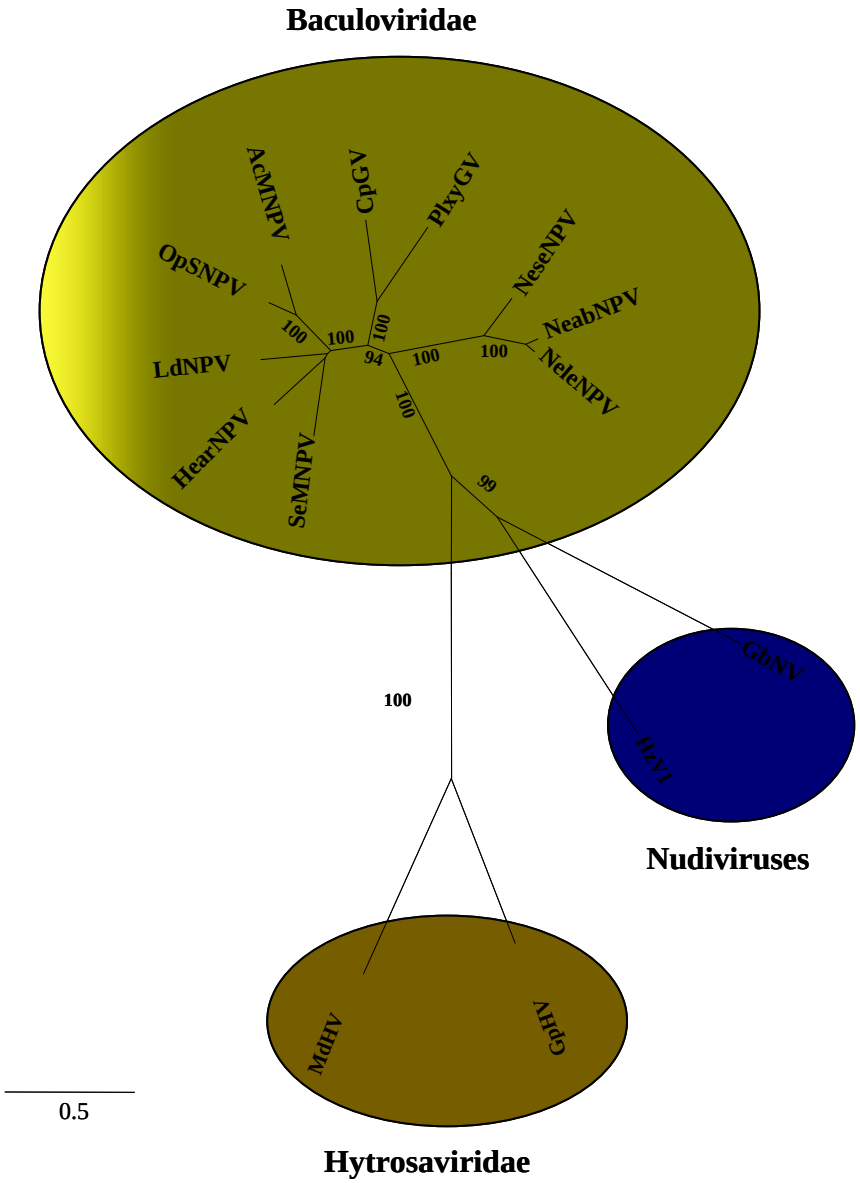


**Figure 4:** Neighbor-Joining (NJ) tree of the concatenated predicted amino acid sequences of *p74*, *pif-1*, *pif-2* and *pif-3*, of hytrosaviruses (GpHV [NC_010356] and MdHV [NC_010671]and their homologs in baculoviruses and nudiviruses. The following viruses (GenBank accession number in brackets) included: Alphabaculoviruses: *A. californica* (Ac) MNPV [NC_001623], *O. pseudotsugata* (Op) MNPV [NC_001875], *L. dispar* (Ld) MNPV [NC_001973], *S. exigua* (Se) MNPV [NC_002169], *H. armigera* (Hear) NPV [NC_002654]; Betabaculoviruses: *C. pomonella* (Cp) GV [NC_002816], *P. xylostella* (Plxy) GV [NC_002593]; Gammabaculoviruses: *N. sertifer* (Nese) NPV [NC_005905], *N. lecontei* (Nele) NPV [NC_005906], *N. abietes* (Neab) NPV [NC_008252], Nudiviruses: *H. zea* (Hz) NV-1 [NC_004156], *G. bimaculatus* (Gb) NV [NC_009240]. Distances were calculated using Poisson correction. Homogeneous substitution pattern among lineages with gamma distributed rate among sites (Gamma parameter 2.25) was employed for reconstruction of the trees. The robustness of the tree was tested using bootstrap analysis (500 replicates). (source [9]).

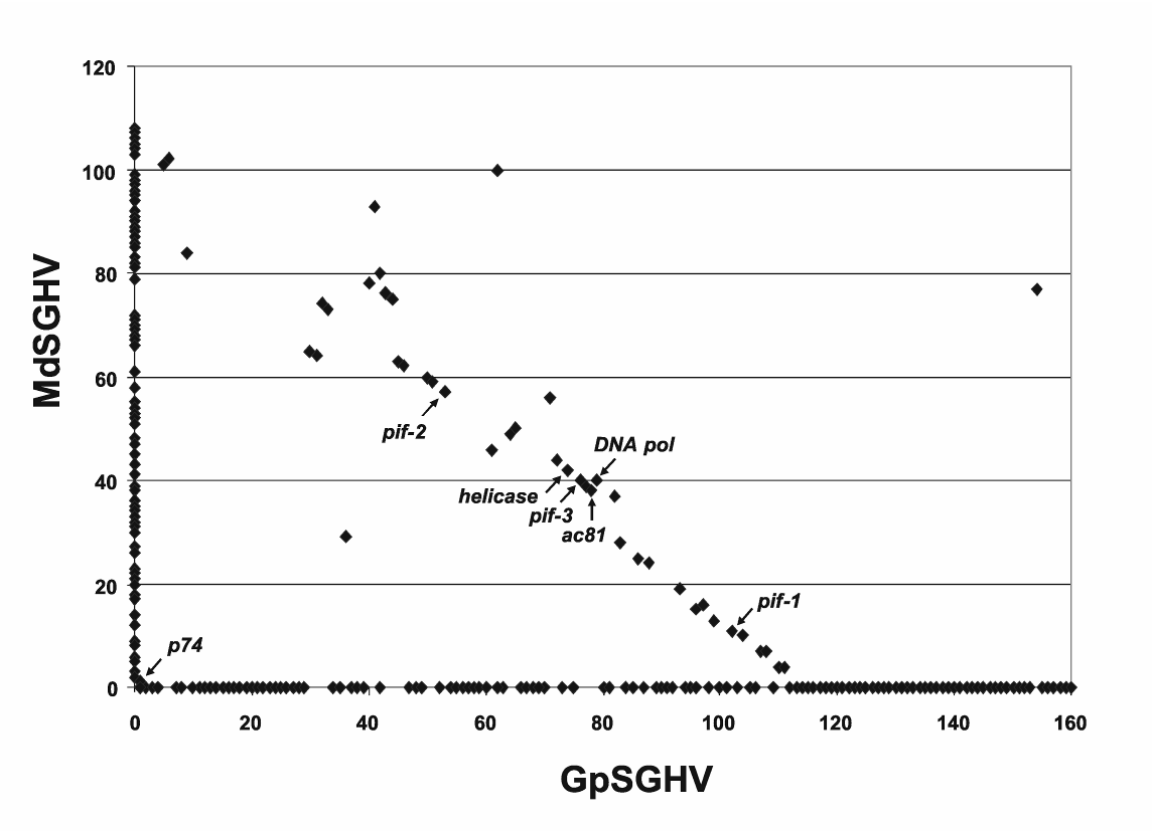


**Figure 5:** Gene parity plot comparing gene order of MdHV and GpHV (modified from [9]. Gene order was 'aligned' by choosing the *p74* gene homologs as the starting point for the plot. Unique ORFs of GpHV and MdHV are on the x- and y-axes, respectively.

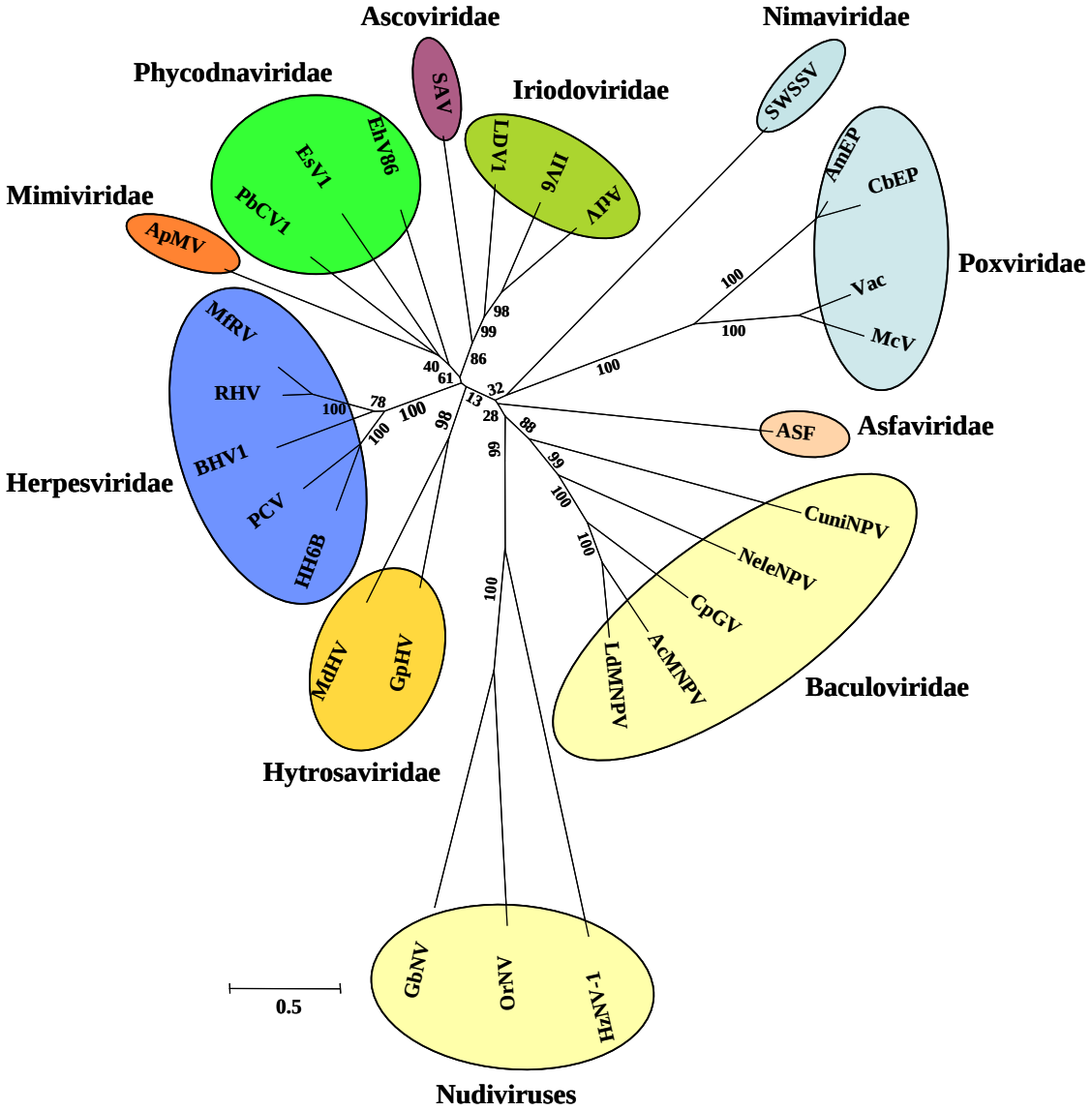


**Figure 6:** Neighbor-Joining (NJ) trees of predicted amino acid sequence of the DNA polymerase genes. The phylogenetic tree of DNA polymerase and its homologs is based on 2374 sites of DNA polymerase of 29 viruses from various families. Distances were calculated using Poisson correction. The robustness of the tree was tested using bootstrap analysis (500 replicates). Numbers on the nodes indicate bootstrap values. The name of the selected virus families are indicated on the tree. Asterisk indicates the proposed family name for the HVs. DNA polymerases used and their accession numbers in brackets: ASF: African Swine Fevervirus [NP_042783], GpHV: *Glossina pallidipes* salivary gland hypertrophy virus [ABQ08852], MdHV: *Musca domestica* salivary gland hypertrophy virus [YP_001883329], HHB6: Human herpesvirus 6B [NP_050219], PCV: Porcine cytomegalovirus [AAF80107], BHV1: Bovine herpesvirus 1 [NP_045328], RHV: Retroperitoneal fibromatosis-associated herpesvirus [AAC57976], MfRV: *Macaca fuscata* rhadinovirus [YP_238317], ApMV: *Acanthamoeba polyphaga* mimivirus [YP_142676], PbCV1: *Paramecium bursaria* Chlorella virus 1 [AAC00532], EsV1: *Ectocarpus siliculosus* virus 1 [NP_077578], EhV86: *Emiliania huxleyi* virus 86 [AAL58859], SAV: *Spodoptera* ascovirus [AAC54632], LDV1: Lymphocystis disease virus 1 [NP_078724], IIV6: Invertebrate iridescent virus 6 [CAC19195], AtIV: *Aedes taeniorhynchus* iridescent virus

[ABF82150], AmEPV: *Amsacta moorei* entomopoxvirus [NP_064832], CbEP: *Choristoneura biennis* entomopoxvirus [CAA40566], Vac: Vaccinia virus [NP_063712], McV: *Molluscum contagiosum* virus [AAL40129], WSSV: shrimp white spot syndrome virus [AAK77696], CuniNPV: *Culex nigripalpus* NPV [AAK13281], NeleNPV: *Neodiprion lecontei* NPV [YP_025217], CpGV: *Cydia pomonella* granulovirus [NP_148895], AcMNPV: *Autographa californica* nucleopolyhedrovirus [NP_054095], LdMNPV: *Lymantria dispar* nucleopolyhedrovirus [NP_047720], HzNV-1: *Heliothis zea* virus 1 [NP_690550], OrNV: *Oryctes rhinoceros* virus [ABF93350], GbNV: *Gryllus bimaculatus* nudivirus [YP_001111279] (source [9]).