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## Proposal to create an Order

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► **To cite this version:**

Peter Christian, Claude Fauquet, Alexander Gorbalenya, Andrew King, Nick J Knowles, et al.. Proposal to create an Order. [Technical Report] -. 2008. hal-02824169

**HAL Id: hal-02824169**

**<https://hal.inrae.fr/hal-02824169v1>**

Submitted on 6 Jun 2020

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# Template for Taxonomic Proposal to the ICTV Executive Committee

## To create a new Order

Code †  To create a new Order

Code †  To name the new order

Code †  To designate the following families as part of the new order:

- *Picornaviridae*
- *Dicistroviridae*
- *Marnaviridae*
- the unassigned genus *Iflavirus*
- *Sequiviridae*
- the unassigned genus *Cheravirus*
- the unassigned genus *Sadwavirus*
- *Comoviridae*

† Assigned by ICTV officers

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- Glyn Stanway (stanwg@essex.ac.uk)

## Old Taxonomic Order

- | Order | None   |
|-------|--|
|       | <ul style="list-style-type: none"><li>• Family <i>Picornaviridae</i></li><li>• Family <i>Dicistroviridae</i></li><li>• Family <i>Marnaviridae</i></li><li>• unassigned genus <i>Iflavirus</i></li><li>• Family <i>Sequiviridae</i></li><li>• unassigned genus <i>Cheravirus</i></li><li>• unassigned genus <i>Sadwavirus</i></li><li>• Family <i>Comoviridae</i></li></ul> |

## New Taxonomic Order

- | Order | Picornavirales   |
|-------|--|
|       | <ul style="list-style-type: none"><li>• Family <i>Picornaviridae</i></li><li>• Family <i>Dicistroviridae</i></li><li>• Family <i>Marnaviridae</i></li><li>• unassigned genus <i>Iflavirus</i></li><li>• Family <i>Sequiviridae</i></li><li>• unassigned genus <i>Cheravirus</i></li><li>• unassigned genus <i>Sadwavirus</i></li><li>• Family <i>Comoviridae</i></li></ul> |

## ICTV-EC comments and response of the SG

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## Argumentation to create a new order:

### Introduction:

When the first full sequences of the genomes of RNA viruses became available, it was noticed that some plant viruses had features very similar to those of animal, including human, viruses now classified within the family *Picornaviridae* (Argos et al., 1984; Franssen et al., 1984; Goldbach, 1986, 1987; Goldbach and Wellink, 1988): (i) icosahedral particles about 30nm in diameter packaging a single stranded RNA genome; (ii) the genome 3' polyadenylated and linked to a 5'-terminal protein, VPg; (iii) proteins expressed as polyprotein precursor(s), processed to mature proteins by one or more a virus-encoded proteinase(s); (iv) similarities in the sequences of the domains encoding NTPase and putative helicase (Hel), proteinase (Pro) and polymerase (Pol) domains, and (v) with these domains arranged in the same order, Hel-(VPg)-Pro-Pol, along the polyprotein.

Two main differences existed between these groups of viruses: (i) Some plant viruses were known to be bipartite, i.e. the genome is divided into two parts, each carried by a separate virus particle (Le Gall et al., 2005, 2005, 2005), whereas animal viruses are invariably monopartite. (ii) Their capsids were composed of either one capsid protein (CP; about 55kDa in typical nepoviruses) or two smaller CPs (35 and 20 kDa in comoviruses and fabaviruses) subunits, whereas the picornaviruses had 3 major CPs (and sometimes an additional smaller one). In 3-D structure, however, the capsids of comoviruses and picornaviruses proved to be strikingly similar, since both were assembled from three distantly related jelly-roll domains arranged with pseudo T=3 (P3) icosahedral symmetry (Hogle et al., 1985; Rossmann et al., 1985; Chen et al., 1989; Chandrasekar and Johnson, 1998; Tate et al., 1999) (Liljas et al. 59-84; Rossmann and Johnson, 1987). This similarity was all the more remarkable because such a structure had never previously been observed.

Further complete genome sequences have revealed several more families that share many, or all, of these features. This has led to the now commonly used concept of “picorna-like viruses” and picorna-like “superfamily/supergroup” (Franssen et al., 1984; Goldbach, 1986, 1987; Goldbach and Wellink, 1988; Gorbalenya and Koonin, 1993; Koonin and Dolja, 1993). Figure 1 shows the genomic organisations of all the picorna-like viruses for which genome sequences are available. The list includes, not only the proposed members of the new order, but also more distantly related picorna-like families that, for reasons discussed below, are considered too divergent to merit inclusion. Until now no formal proposal has been made to the ICTV to create a higher order taxon. This proposal is to create an Order, named the *Picornavirales*, to comprise the following taxonomic groupings: *Picornaviridae*, *Dicistroviridae*, *Marnaviridae*, *Sequiviridae*, *Comoviridae* and the unassigned genera *Iflavirus*, *Cheravirus* and *Sadwavirus*.

### Invariant properties of the proposed order:

It should be noted that not every member of the *Picornavirales* has been demonstrated to conform to all the taxonomic criteria listed below; for example, to confirm a jelly-roll protein structure “directly” would require the crystal structure to be determined. Nevertheless, direct evidence is available for a wide range of virus types, enabling such properties to be inferred in other cases from comparative sequence analysis, and other, evidence.

- Each virus particle comprises a non-enveloped capsid containing a single species of genomic RNA.
- The capsid, ~25-30 nm in diameter, is made up of 60 identical protomers, each protomer consisting of three similarly sized and distantly related domains each folded into an 8-stranded beta-barrel (jelly-roll fold). The capsid possesses T=1 icosahedral symmetry relative to protomers although the arrangement of the 3x60=180 constituent domains approximates to that of a T=3 icosahedron. The resulting pseudo-T3 (P3) symmetry is unique to members of this taxon.
- The 5' terminus of the genome, where this has been identified, is covalently attached to a 3-4 kDa virus-encoded protein (VPg).
- The genomic RNA also serves as the virus messenger RNA (mRNA); i.e. there are no subgenomic mRNAs.
- Each virus-encoded open reading frame (ORF) encoding multiple domains is expressed as a single polyprotein which is processed to the mature virus proteins exclusively by virus-encoded proteinase(s).
- Conserved order of the non-structural protein domains (defined below) in polyprotein, Hel-VPg-Pro-Pol.
- Conserved order of the three structural domains in polyprotein, their locations in the capsid being as follows: the N-terminal and middle domains (“VP2” and “VP3”, respectively, using picornavirus nomenclature) are located either side of the 3-fold icosahedral axis, the C-terminal domain (“VP1”) at the 5-fold.

Other properties which, while not invariant, are characteristic of the order:

- Each virus particle typically contains a single molecule of genomic RNA, although in some plant viruses with a bipartite genome two molecules of the small genome component can be encapsidated in a single virion.
- The genomic RNA is typically 3' polyadenylated, the sole exception being the genus *Sequivirus* (family *Sequiviridae*), where this feature appears to be absent (Turnbull-Ross et al., 1993).
- Viruses are typically monopartite (i.e. a single particle is infectious), the sole exception being several taxa of plant viruses (*Comoviridae*, *Cheravirus*, *Sadwavirus*), which have a bipartite genome.
- Each virus RNA molecule normally encodes a single multidomain ORF, the sole exception being the *Dicistroviridae* where, as the name suggests, each molecule encodes two ORFs.
- Individual domains of the capsid are normally cleaved proteolytically from each other to generate the three mature proteins typical of the *Picornavirales*. In the case of the family *Comoviridae* and (by analogy) probably the unassigned genus *Sadwavirus*, however, the processing of the CP precursor is not as extensive. This results in the jelly-roll domains remained fused in a single CP subunit: two in the large CP subunit (genera *Comovirus* and *Fabavirus* within the family *Comoviridae*; probably also unassigned genus *Sadwavirus* (Iwanami et al., 1999) or three in the single CP subunit (genus *Nepovirus*).
- the Hel, VPg, Pro, and Pol domains of the eighth groups that make up the *Picornavirales* can be characterized as follows:
  - Hel: a superfamily III helicase domain (Gorbalenya et al., 1990), with an “A” motif (GxxGxGK(S/T)), followed about 40 amino-acids downstream by a “B” motif (DD), followed about 30 amino-acids downstream by a “C” motif (KGxxxxSxxxx(S/T)(S/T)N). This domain occupies the central part of 2C protein in the *Picornaviridae* and 2C-like protein in other viruses of the *Picornavirales* and it is involved in NTP-binding and hydrolysis (Mirzayan and Wimmer 176-87; Pfister and Wimmer 1611-19)
  - VPg: a 3-4 kDa VPg with little or no characteristic sequence features except for the presence near its N-terminus of an amino-acid with a free hydroxyl group to which the RNA 5' end is covalently linked by a phosphodiester bond
  - Between Hel and VPg: an hydrophobic domain which may, or may not, be cleaved proteolytically from the Hel moiety
  - Pro: a proteinase resembling trypsin-like serine proteases in structure and having substrate specificity resembling that of the prototype picornavirus 3C proteinase (3C-like proteinase) . It contains a catalytic triad of histidine, aspartate/glutamate and typically cysteine, in place of the canonical serine of “serine” proteases, which serves as the catalytic nucleophile (Gorbalenya et al., 1986; Bazan and Fletterick, 1988; Gorbalenya et al., 1989; Allaire et al., 1994; Matthews et al., 1994). Interestingly, the only member of the family *Marnaviridae* to have been sequenced so far (Lang et al., 2004) appears to possess a serine at this position (AEG, unpublished observation). The catalytic cysteine/serine is located near the C-terminus of the Pro domain and is generally followed 10-20 amino-acids downstream by a GΦH motif (where Φ is a polar residue) that appears important for substrate specificity (Bazan and Fletterick, 1988; Cheah et al., 1990; Matthews et al., 1994; Hemmer et al., 1995; Miyashita et al., 1995).
  - Pol: a Type I Pol domain (Kamer and Argos, 1984; Koonin, 1991) with eight conserved motifs, motifs I (KxE), V (PSGΦΦΦTΦΦΦN(S/T)) and VI (YGDD) being the most conserved ones. The motifs V and VI are part of the catalytic center of this enzyme (Hansen, Long, and Schultz 1109-22)

#### Phylogenetic evidence:

The taxa listed above as candidate members of the Order *Picornavirales* appear to form a monophyletic lineage when the sequence similarity of the non-structural proteins is used to reconstruct in a hierarchical clustering analysis (Figures 3 and 5).

#### Divergent picorna-like viruses, not assigned to the order *Picornavirales*:

The level of structural, organisational and sequence divergence in the families *Caliciviridae*, *Potyviridae* and *Hypoviridae* were judged too high for them to be included in the order *Picornavirales*, for the following reasons:

- The family *Caliciviridae* have several characteristics atypical of the *Picornavirales*, as follows: (i) Genome encodes only a single jelly-roll domain. As a result, the icosahedral capsid of 180 jelly rolls is only superficially picorna-like, it exhibits true T3 symmetry (i.e. the 180 domains are identical to each other), rather than P3 symmetry. (ii) Each jelly-roll domain is linked to second, outwardly protruding, domain that is not found among the members of the *Picornavirales*. (iii) A major divergence can be seen in their strategy of gene expression, which involves the use of sub-genomic mRNAs. For these reasons, it was judged appropriate, on balance, to adopt a somewhat restrictive set of taxonomic criteria that exclude the *Caliciviridae*.

- The family *Potyviridae* has the following major deviations (i) the members employ a single capsid protein unrelated to the jelly-roll domain that forms capsid exhibiting helical, rather than icosahedral, symmetry ; (Shukla et al., 1994) and (ii) they have a Hel domain of superfamily II rather than III found in the *Picornavirales* viruses (Gorbalenya and Koonin 419-29).
- The members of the family *Hypoviridae* have the following specifics: they lack virions and 3C-like proteinase, have an atypical (double-stranded) RNA genome and a Hel domain of superfamily II, like the *Potyviridae* members with whom they phylogenically cluster.

#### Discussion:

The picornavirus supergroup comprises the eleven virus types listed in Fig 1, including three distantly related families, the *Caliciviridae*, *Potyviridae* and *Hypoviridae*, which differ markedly from the other eight. It should also be noted that there are other virus families, e.g. *Sobemoviridae*, *Astroviridae* and the order *Nidovirales*, which share certain properties with the *Picornavirales* viruses. The differences between these viruses and the *Picornavirales* are especially recurrent in relation to capsid proteins and virions (if any) they made. The caliciviruses appear the most picornavirus-like but, even in this case, there are major differences in the capsid organization, as well as the genome expression strategy, that separate this family from the other eight forming the *Picornavirales* (see above). As for the *Potyviridae* and *Hypoviridae*, these have deviated even more profoundly, including the lack of the icosahedral capsid. Despite these clear differences between the viruses included and not in the Order, we acknowledge that the choice of taxa to be assigned to the order *Picornavirales* fully depends on the criteria used. In our proposal, we have designed criteria using most conserved properties that characterize the prototype *Picornaviridae* and found that at least seven other virus groups pass this check. To include only one extra group, the closest *Caliciviridae*, in the Order, as was proposed before (Koonin and Dolja, 1993) two major criteria related to the virion architecture and genome expression would have needed to relax. We feel that this revision of the criteria would significantly “dilute” the common basis of the Order and, thus, it may not be justified for a relatively minor order expansion. We hope that the creation of the new Order, as defined in this proposal, will facilitate research on picorna-like viruses and knowledge dissemination. We also hope that this proposal will trigger further insights that in due course may lead to an appropriate higher order classification for other viruses of the supergroup that left outside the *Picornavirales*.

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### Origin of the proposed order name

- Derived from the name of the family *Picornaviridae*, to reflect the common expression “picorna-like virus”.
- The etymology of “Picorna” derives from “pico” (small) and “RNA”, to recall that these are small RNA viruses. This is a common property of all viruses in the proposed order.

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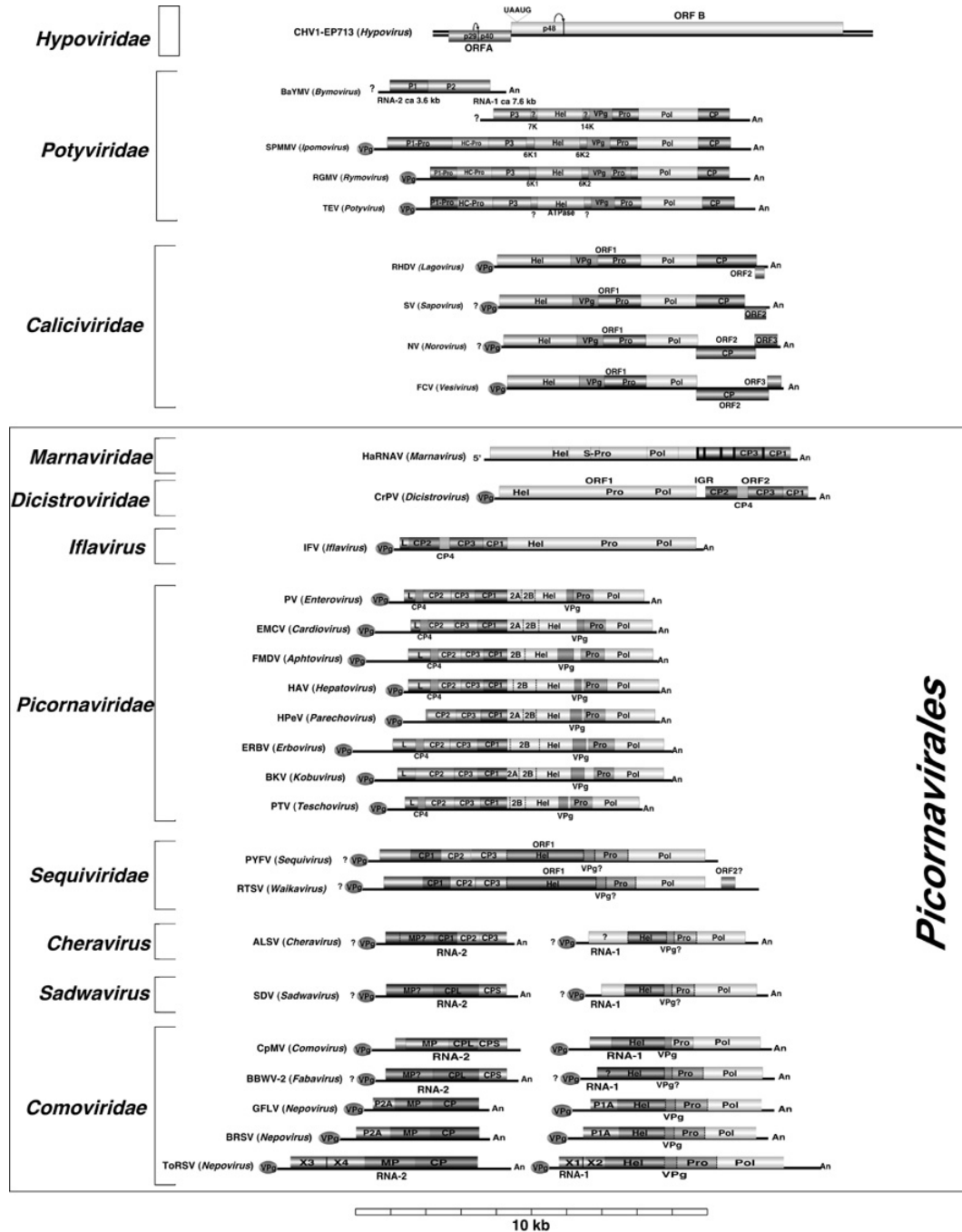
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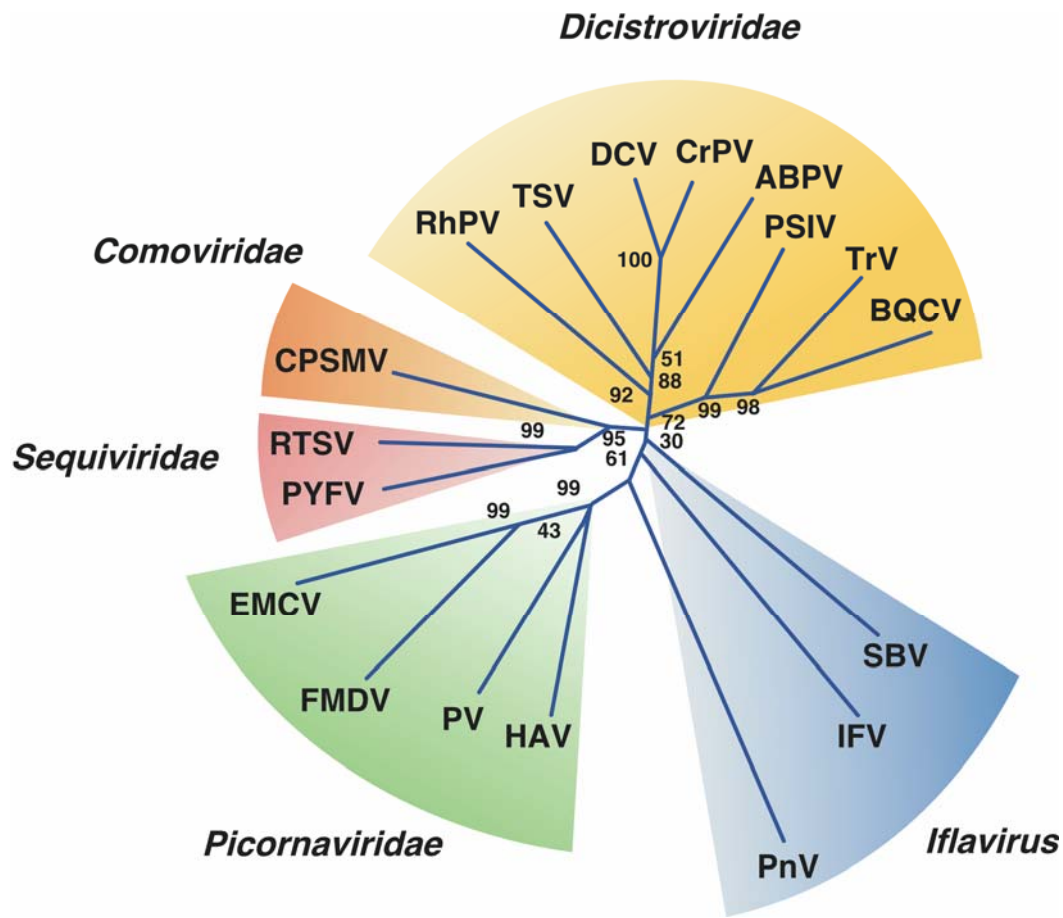
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Annexes:



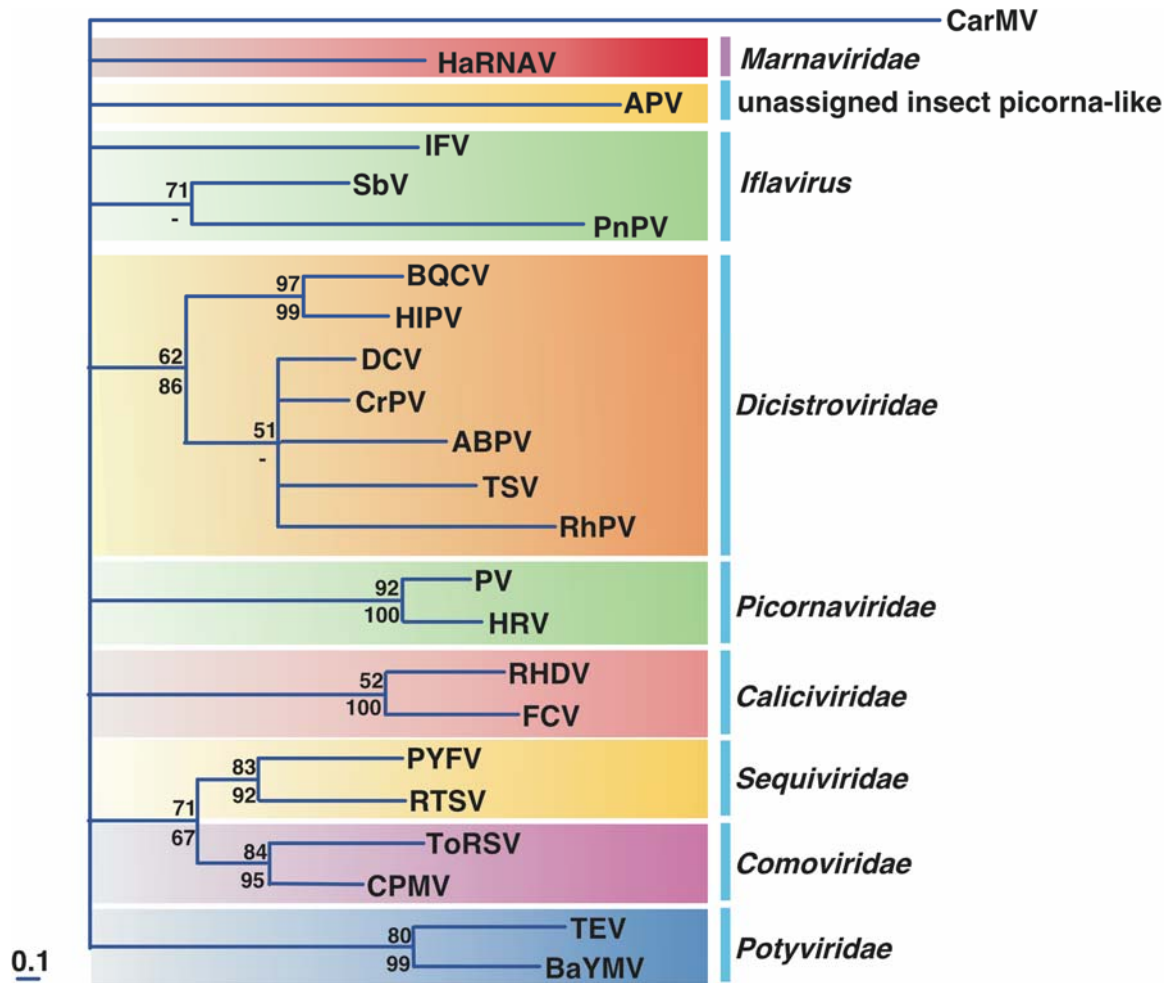
**Figure 1:** Genomic organization of the picorna-like viruses. The abbreviations are Hel: Helicase; Pro: Proteinase; Pol: Polymerase; CP: Capsid Protein; CP: Virion Protein; MP: Movement Protein. The figure includes, not only the members of the proposed order *Picornavirales* (boxed), but also the, more divergent, families not currently proposed for inclusion.





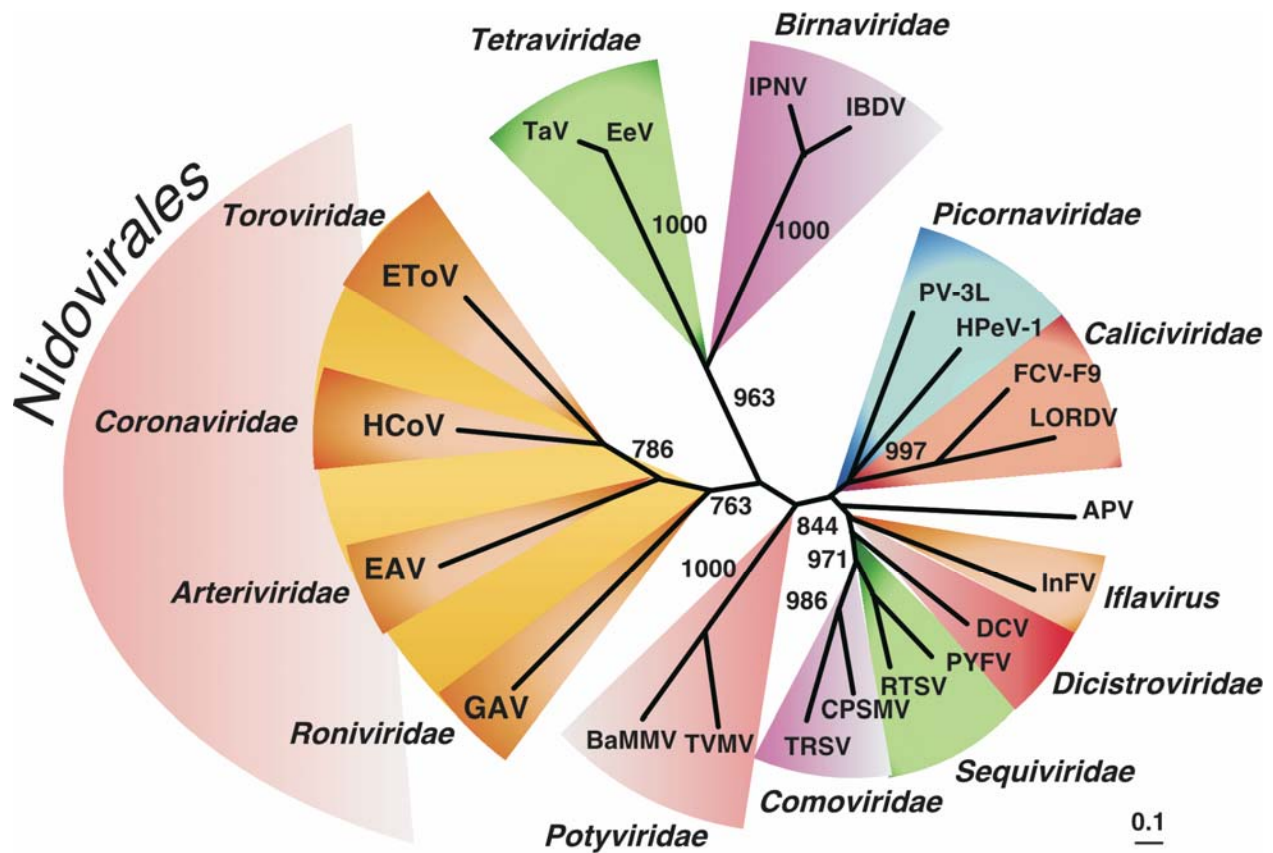
**Figure 2.** Unrooted phenogram showing the relationships of the CrPV-like viruses to representatives of the *Picornaviridae*, *Comoviridae*, *Sequiviridae* and the floating genus *Iflavirus*. The phenogram was constructed from an amino acid similarity matrix of the replicase (RdRP) region of the non-structural proteins using the neighbour-joining method. A bootstrap analysis was performed and the percentage values are indicated at the branching points. Viruses other than dicistroviruses included in the analysis, abbreviation ( ) and accession numbers [ ] are; Cowpea severe mosaic virus (CPSMV) [M83830], Encephalomyocarditis virus (EMCV) [M81861], Foot-and-mouth disease virus (FMDV) [X00871], Hepatitis A virus (HAV) [M14707], Infectious flacherie virus (IFV) [AB000906], Parsnip yellow fleck virus (PYFV) [D14066], Perina nuda virus (PnV) [AF323747], Poliovirus (PV) [J02281], Rice tungro spherical virus (RTSV) [M95497], Sacbrood virus (SBV) [AF469603]. Branch lengths are drawn to scale.

Figure taken from the VIIIth ICTV report.



**Figure 3:** Phylogenetic analysis of picorna-like RdRp domain protein sequences. CLUSTAL\_X alignments were done with residues 1362-1619 of the HaRNAV polyprotein that represent the conserved regions I-VIII (defined in Koonin and Dolja (1993)) and the corresponding regions from the other viruses included. The tree is based on maximum likelihood distances generated with TREE-PUZZLE. The sequence from the Carnation mottle virus (CarMV) was used as an outgroup. Support values based on 10,000 puzzling steps are shown above the branches. Bootstrap values (based on 1,000 replicates) for branches that are supported by >50% by neighbor-joining analysis are labeled below the branches (a dash indicates there was no corresponding branch in the neighbor-joining tree). The maximum likelihood scale bar is shown.

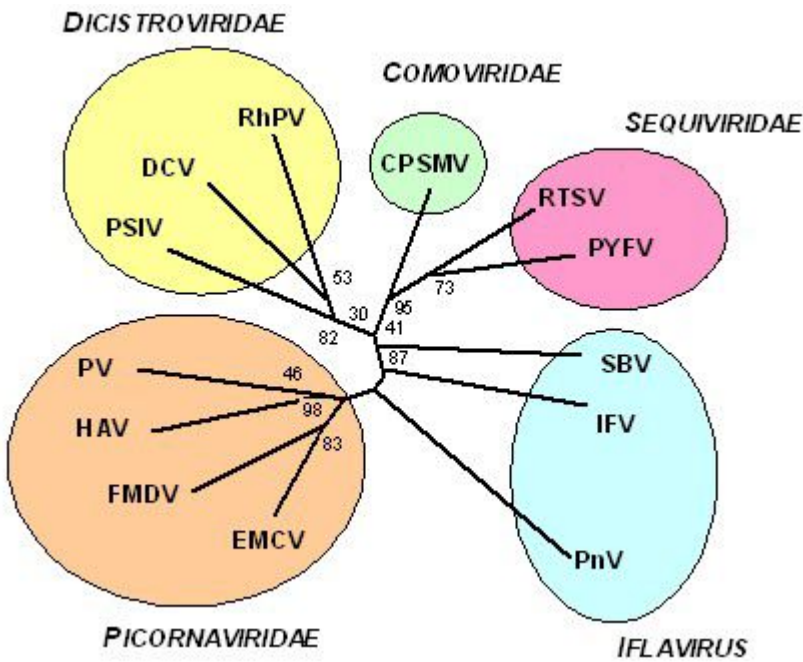
Figure taken from the VIIIth ICTV report.



**Figure 4.** Unrooted phenogram showing the relationships of the RdRps of the *Nidovirales* lineages with viruses of the families of the “Picornavirus-like” supergroup, *Tetraviridae* and *Birnaviridae*. The most conserved part of RdRps from representative viruses in the *Picornaviridae*, *Dicistroviridae*, *Sequiviridae*, *Comoviridae*, *Caliciviridae*, *Potyviridae*, *Coronaviridae*, *Roniviridae*, *Arteriviridae*, *Birnaviridae*, *Tetraviridae* and unclassified insect viruses was aligned. The RdRps of *Thosea asigna* virus (TaV), *Euprosterina elaeasa* virus (EeV) and the birnaviruses were converted into the canonical ABC form before the analysis. Using an extended, gap-free version of the alignment containing 332 informative characters, an unrooted neighbor-joining tree was inferred by the ClustalX1.81 program. All bifurcations with support in > 700 out of 1000 bootstraps are indicated. Different groups of viruses are highlighted. Note that the relative positions of members of the families *Arteriviridae* and *Roniviridae* within the *Nidovirales* are not resolved in this tree.

Virus families and groups, viruses included in the analysis, abbreviations ( ) and the NCBI protein (unless other specified) IDs [ ] are as follows: *Picornaviridae*: Human poliovirus type 3 Leon strain (PV-3L) [130503] and parechovirus 1 (HpeV-1) [6174922]; *Iflavirus*: Infectious flacherie virus (InFV) [3025415] and Unclassified insect viruses: *Acyrtosiphon pisum* virus (APV) [7520835]; *Dicistroviridae*: *Drosophila C* virus (DCV) [2388673]; *Sequiviridae*: Rice tungro spherical virus (RTSV) [9627951] and Parsnip yellow fleck virus (PYFV) [464431]; *Comoviridae*: Cowpea severe mosaic virus (CPSMV) [549316] and Tobacco ringspot virus (TRSV) [1255221]; *Caliciviridae*: Feline calicivirus F9 (FCV-F9) [130538] and Lordsdale virus (LORDV) [1709710]; *Potyviridae*: Tobacco vein mottling virus (TVMV) [8247947] and Barley mild mosaic virus (BaMMV) [1905770]; *Coronaviridae*: Human coronavirus 229E (HCoV) [12175747] and Berne torovirus (BEV) [94017]; *Arteriviridae*: Equine arteritis virus (EAV) [14583262]; *Roniviridae*: Gill-associated virus (GAV) [9082018]; *Tetraviridae*: *Thosea asigna* virus (TaV) [AF82930; nt sequence] and *Euprosterina elaeasa* virus (EeV) [AF461742; nt sequence]; *Birnaviridae*: Infectious pancreatic necrosis virus (IPNV) [133634] and Infectious bursal disease virus (IBDV) [1296811]. (Tree was modified from Gorbalenya *et al.*, (2002), with permission).

Figure taken from the VIIIth ICTV report.



**Figure 5:** Unrooted phenogram derived from the RdRp domain of the viral non-structural proteins showing the relationships of representative picornaviruses, sequiviruses, dicistroviruses and the three members of the genus *Iflavirus* (IFV, PnV and SBV). Taxa used (with sequence accession numbers shown in brackets [ ]) were Cowpea severe mosaic virus (CPSMV) [M83830], Drosophila C virus (DCV) [AF014388], Encephalomyocarditis virus (EMCV) [M81861], Foot-and-mouth disease virus (FMDV) [X00871], Hepatitis A virus (HAV) [M14707], Poliovirus (PV) [J02281], Parsnip yellow fleck virus (PYFV) [D14066], Plautia stali intestine virus (PSIV), Rhopalosiphum padi virus (RhPV) [AF022937], and Rice tungro spherical virus (RTSV) [M95497]. A bootstrap analysis was performed and values obtained are shown next to the branching points. Branch lengths are proportional to distance.

Figure taken from the VIIIth ICTV report.



**Figure 6:** Hierarchical clustering based on the region between the Pro (CG) and the Pol (GDD) sequence motifs. The sequences were aligned using Clustal using the Neighbor-Joining approach, and p-distances are represented. For each sequence, the GenBank accession number, the virus acronym and a shortened description of the taxonomical status are given directly in the dendrogram. The outgroup used is a chimeric sequence reconstituted from the bovine trypsin C-terminus and the *Tobacco mosaic tobamovirus* RdRp N-terminus.