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***Ortervirales*: A new viral order unifying five families of reverse-transcribing viruses**

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Text

Reverse-transcribing viruses, which synthesize a copy of genomic DNA from an RNA template, are widespread in animals, plants, algae and fungi (1, 2). This broad distribution suggests ancient origin(s) of these viruses, possibly concomitant with the emergence of eukaryotes (3). Reverse-transcribing viruses include prominent human pathogens, such as human immunodeficiency viruses 1 and 2 (HIV-1/2) and hepatitis B virus, as well as plant pathogens that cause considerable economic losses (4).

The International Committee on Taxonomy of Viruses (ICTV) traditionally classified reverse-transcribing viruses into five families: *Caulimoviridae*, *Hepadnaviridae*, *Metaviridae*, *Pseudoviridae*, and *Retroviridae* (5). In 2018, the ICTV recognized an additional family, *Belpaoviridae*, which contains the genus *Semotivirus* (previously included in *Metaviridae (6)*). The infection cycles, nucleic acid types, genome organizations, and virion morphologies of these viruses are very diverse. Indeed, reverse-transcribing viruses are distributed between two Baltimore Classes of viruses. Belpaoviruses, metaviruses, pseudoviruses — better known as Bel/Pao, Ty3/Gypsy, and Ty1/Copia retrotransposons, respectively (1, 7) — and retroviruses typically have single-stranded RNA genomes (Table 1) and frequently integrate into the host genomes as part of their replication cycles (Baltimore Class VI). In contrast, members of the families *Caulimoviridae* and *Hepadnaviridae*, often referred to as "pararetroviruses" (8), encapsidate circular double-stranded DNA genomes and do not actively integrate into host chromosomes (Baltimore Class VII). However, capture of pararetroviral DNA in host genomes, presumably by illegitimate recombination, is commonplace, particularly in plants, giving rise to the corresponding endogenous elements (9, 10).

Mechanistic studies on the replication cycles of reverse-transcribing viruses of different families have revealed many similarities that have been reinforced by comparative genomics of the viral reverse transcriptases (RTs), the hallmark enzymes encoded by all reverse-transcribing viruses. Indeed, phylogenetic analyses support the monophyly of all viral RTs, to the exclusion of those encoded by non-viral retroelements from both eukaryotes and prokaryotes (11, 12). In addition to the evidence from the RT phylogeny, belpaoviruses, caulimoviruses, metaviruses, pseudoviruses, and retroviruses share several conserved features that hepadnaviruses lack (Table 1). In particular, the polymerase (Pol) polyproteins of belpaoviruses, metaviruses, pseudoviruses, and retroviruses possess similar domain architectures. These Pol polyproteins contain an aspartate protease, which is responsible for the processing of viral polyproteins, and an integrase of the DDE recombinase superfamily. The genomes of these viruses also share long terminal repeats (LTRs) (13). Within certain clades, Pol polyproteins of retroviruses and metaviruses share additional features, such as a dUTPase domain (14-16) and the GPY/F subdomain of the integrase (17, 18). Caulimoviruses also possess a homologous aspartate protease domain in their Pol polyprotein (19), but lack an integrase and LTR. However, RT-based phylogenies consistently place these plant-infecting viruses as a sister clade to the metaviruses (Figure 1), suggesting that among "pararetroviruses", encapsidation of a DNA genome is a homoplasious character and therefore not a reliable criterion for classification. The basal branches of the RT tree are not resolved and are presented as a multifurcation in Figure 1. This topology is at least compatible with placing the *Hepadnaviridae* clade outside the viral group that includes belpaoviruses, caulimoviruses, metaviruses, pseudoviruses, and retroviruses.

Belpaoviruses, caulimoviruses, metaviruses, pseudoviruses, and retroviruses share not only homologous proteins involved in genome replication and polyprotein processing, but also the two principal protein components of the virions, namely, the capsid and nucleocapsid proteins/domains (20-22), although the nucleocapsid domain appears to be absent in spumaretroviruses (family *Retroviridae*; Table 1). By contrast, hepadnaviruses encode an unrelated capsid protein (23). These findings suggest that belpaoviruses, caulimoviruses, metaviruses, pseudoviruses, and retroviruses have evolved from a common viral ancestor, rather than from distinct capsid-less retrotransposons (20).

Finally, similarities between belpaoviruses, caulimoviruses, metaviruses, pseudoviruses, and retroviruses extend to the mechanism of replication priming. All these viruses utilize host tRNA molecules as primers for genome replication by reverse transcription (24), whereas hepadnaviruses use a specific protein priming mechanism mediated by the polymerase terminal protein domain (25).

Taken together, the common complement of proteins required for genome replication, polyprotein processing, and virion formation, the topology of the RT phylogenetic tree, and mechanistic similarities in genome replication present strong evidence that belpaoviruses, caulimoviruses, metaviruses, pseudoviruses, and retroviruses share a common evolutionary origin. The hepadnaviruses, which typically branch out at the base of the viral RT clade (Figure 1), possess a unique capsid protein and employ a distinct replication mechanism, appear to be more distantly related to all these virus families. In recognition of these relationships, the ICTV has recently regrouped the families *Belpaoviridae*, *Caulimoviridae*, *Metaviridae*, *Pseudoviridae* and *Retroviridae* into an order *Ortervirales* (*orter*: an inversion of *retro*, which was derived from reverse transcription; *virales*: suffix for an order). This change in taxonomy acknowledges and formalizes the long-proposed evolutionary relationship among most groups of reverse-transcribing viruses (26). We note that although hepadnaviruses are not included in the order, they might be unified with other reverse-transcribing viruses at a higher taxonomic level in the future.

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Figure legend

Figure 1. Maximum likelihood phylogeny of viral reverse transcriptases. The tree includes sequences of 290 viruses belonging to all ICTV-recognized genera of reverse-transcribing viruses. The phylogeny was inferred using PhyML (30) with the LG+G+F substitution model and is rooted with sequences from non-viral retroelements (bacterial group II introns and eukaryotic LINE retroelements). Genomic organizations of selected representatives of reverse-transcribing viruses are shown next to the corresponding branches. Long terminal repeats (LTR) are shown as black triangles. Note that members of the virus families display considerable variation in gene/domain content (5), which is not captured in this figure. Abbreviations: 6, 6-kDa protein; ATF, aphid transmission factor; CA/CP, capsid protein; CHR, chromodomain (only present in the INT of particular clades of metaviruses of plants, fungi and several vertebrates); *gag*, group-specific antigen; *env*, envelope genes; SU, surface glycoprotein; TM, transmembrane glycoprotein; INT, integrase; MA, matrix protein; NC, MP, movement protein; nucleocapsid; *nef*, *tat*, *rev*, *vif*, *vpr*, and *vpu*, genes that express regulatory proteins via spliced mRNAs; TP, terminal protein domain; TT/SR, translation trans-activator/suppressor of RNA interference; P, polymerase; *pol*, polymerase gene; PR, protease; PreS, pre-surface protein (envelope); PX/TA, protein X/transcription activator; RH, RNase H; RT, reverse transcriptase; VAP, virion-associated protein.

Table 1. Features shared by reverse-transcribing viruses.

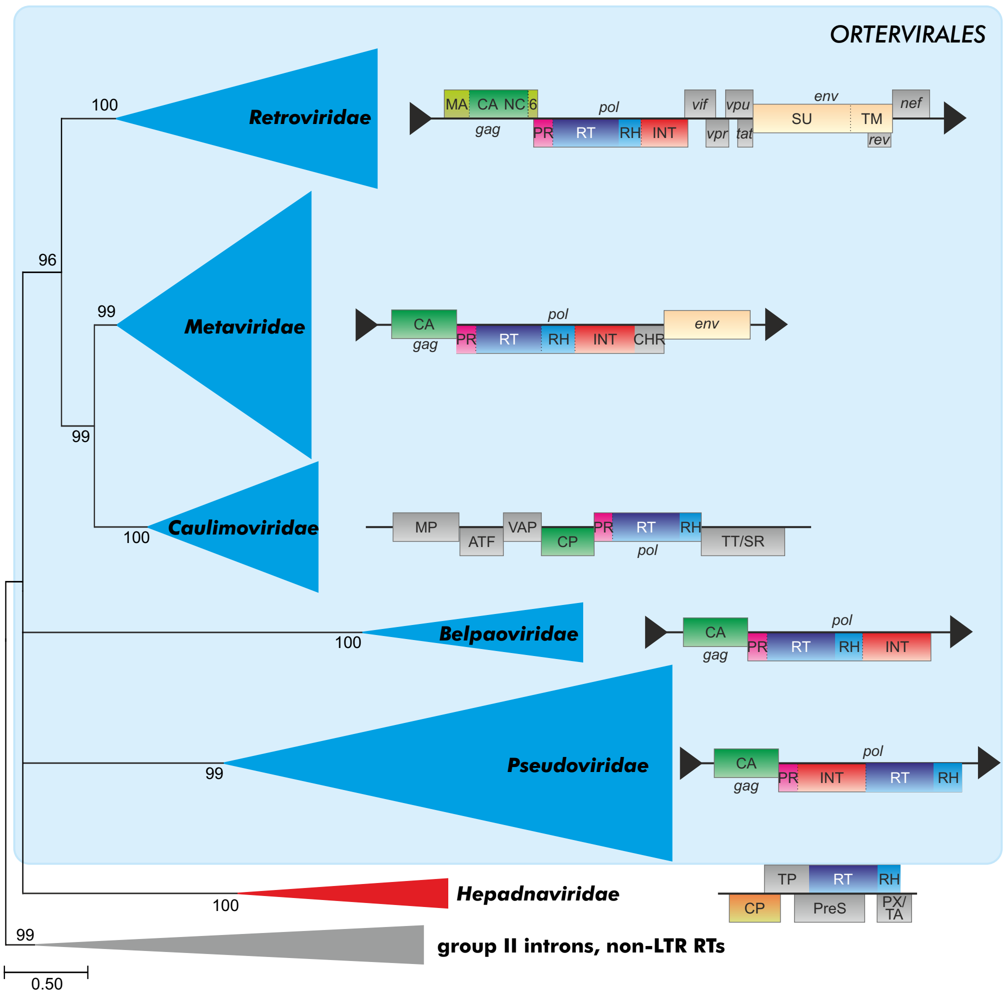
Family		*Retroviridae*		*Metaviridae*	*Pseudoviridae*	*Belpaoviridae*	*Caulimoviridae*	*Hepadnaviridae*
Subfamily		*Orthoretrovirinae*	*Spumaretrovirinae*					
Pol	RT-RH	+	+	+	+	+	+	+
	Protease	+	+	+	+	+	+	−
	Integrase	+	+	+	+	+	−	−
Gag	CA/CP	+	+	+	+	+	+	−
	NC	+	−	+	+	+	+	−
LTR		+	+	+	+	+	−$	−#
Priming		tRNA	tRNA	tRNA	tRNA	tRNA	tRNA	TP
Genome type		ssRNA	ssRNA/dsDNA*	ssRNA	ssRNA	ssRNA	dsDNA	dsDNA

* – Members of the subfamily *Spumaretrovirinae* contain both ssRNA and dsDNA in extracellular particles and reverse transcription occurs during virus assembly and disassembly.

$ – In the genus *Petuvirus* (*Caulimoviridae*) an inactivated integrase-like domain and quasi (long) terminal repeats have been identified (27, 28), suggesting that certain ancestral elements have been lost during the evolution of caulimoviruses.

– Upstream of the capsid protein gene, hepadnavirus genomes contain a sequence showing similarity to the U5 region of the retroviral LTR (29).

Abbreviations: CA/CP, capsid protein; Gag, group-specific antigen; LTR, long terminal repeats; NC, nucleocapsid protein; RH, RNase H; RT, reverse transcriptase; Pol, polymerase polyprotein; TP, terminal protein.


ORTERVIRALES
100
Retroviridae
MA
CA
NC
6
gag
PR
RT
RH
INT
pol
vif
vpr
vpu
tat
env
SU
TM
rev
nef
96
99
Metaviridae
CA
gag
PR
RT
RH
INT
CHR
pol
env
99
100
Caulimoviridae
MP
ATF
VAP
CP
PR
RT
RH
pol
TT/SR
100
Belpaoviridae
CA
gag
PR
RT
RH
INT
pol
99
Pseudoviridae
CA
gag
PR
INT
RT
RH
pol
100
Hepadnaviridae
TP
RT
RH
CP
PreS
PX/
TA
99
group II introns, non-LTR RTs
0.50