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REVIEW

Picornaviridae—the ever-growing virus family

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Abstract Picornaviruses are small, nonenveloped, icosahedral RNA viruses with positive-strand polarity. Although the vast majority of picornavirus infections remain asymptomatic, many picornaviruses are important human and animal pathogens and cause diseases that affect the central nervous system, the respiratory and gastrointestinal tracts, heart, liver, pancreas, skin and eye. A stunning increase in the number of newly identified picornaviruses in the past decade has shown that picornaviruses are globally distributed and infect vertebrates of all classes. Moreover, picornaviruses exhibit a surprising diversity of both genome sequences and genome layouts, sometimes challenging the definition of taxonomic relevant criteria. At present, 35 genera comprising 80 species and more than 500 types are acknowledged. Fifteen species within five new and three existing genera have been proposed in 2017, but more than 50 picornaviruses still remain unassigned.

Introduction

Picornaviruses are ubiquitous and globally distributed, and they pose a threat to the health of humans and livestock. Picornavirus infections may induce diseases of the central nervous system, of the respiratory and gastrointestinal tracts, and of the heart, liver, pancreas, skin and eye [26, 92, 95, 98]. For most picornaviruses, the majority of infections are asymptomatic, and only a fraction of infections produce severe clinical symptoms, but due to their ubiquitous prevalence and the great number of types, hundreds of millions of infections occur annually, causing morbidity and mortality as well as substantial costs to national health care systems and economic losses [19, 25].

The family *Picornaviridae* is one of only two virus families that were listed in the First Report of the International Committee on Nomenclature of Viruses (ICNV) in 1971 [101]. Before that, in 1963, the Subcommittee on Nomenclature of Viruses (SCNV) had adopted the term 'picornavirus group' following a proposal of the International Enterovirus Study Group, then chaired by Joseph L. Melnick [35]. André Lwoff, Nobel Prize winner of 1965 and elected member of the ICNV, groused about the name 'picornavirus': "*Pico is a prefix in the metric system meant to indicate a submultiple of a unit, namely* 10^{-12}*, and rna stands for RNA. Thus, picorna means* 10^{-12} *ribonucleic acid. Moreover, it is stated in the minutes of the subcommittee that the initial letters of picorna may be taken to refer to poliomyelitis, insensitivity to ether, Coxsackie, orphan, and rhinovirus. A disease, a chemical property, a virus, a state, and again a virus. This is ridiculous.*" [62]. However, despite such criticism, the term 'picornavirus' gained general acceptance in the scientific community, and today the family *Picornaviridae* presents itself as a highly diverse virus family comprising 80 species divided in 35 genera [110]; https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/picornavirales/w/picornaviridae). In 2017, an additional 15 species within five new and three existing genera have been proposed, and more than 50 unassigned picornaviruses

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are awaiting classification. The fast-growing field of picornavirology may be illustrated by the following fact: as of September 2017, the nucleotide sequence database of NCBI (GenBank) registers c. 100,000 entries with the search term '[organism] Picornaviridae', and this number increases by roughly 1000 entries each month. This review aims to give a very brief overview of picornaviruses and their life cycle and focuses on picornavirus diversity and the state of picornavirus taxonomy in 2017.

Picornavirus genome and capsid structure

Picornaviruses are small, non-enveloped RNA viruses [88]. Their genomes have a positive-strand polarity [32] with lengths ranging from 6.7 kilobases (seal picornavirus) to 10.1 kilobases (goose megrivirus). The 5′ end of the viral RNA is covalently linked to the tyrosine-3 residue of a small virus-encoded peptide, termed 3A or VPg (viral peptide, genome-associated) [1, 23, 54, 86]. The 3′ end has a poly(A) tail [17, 108]. The typical picornavirus genome codes for a single polyprotein [36, 41] that is co- and posttranslationally processed by virus-encoded proteinases to yield the capsid proteins [63] and a number of nonstructural proteins as well as various intermediate proteins that are also necessary for replication of viral RNA [64, 93]; reviewed in [73]. Only the recently discovered dicipiviruses have a dicistronic genome organisation with one capsid-protein-encoding open reading frame (ORF) and one ORF coding for the nonstructural proteins [102].

A first, simple model of a picornavirus, poliovirus, based on x-ray diffraction patterns was presented by [21], but the near-atomic three-dimensional structure of poliovirus at a 2.9 Å resolution did not follow until 1985 [21, 31]. Meanwhile, more than 200 crystal structures and cryoelectron microscopy structures of picornaviruses representing seven genera and 17 species have been deposited in the RCSB Protein Databank. The icosahedral T=1/pseudoT=3 capsid is composed of 60 protomers, each comprising either one copy of the four capsid proteins 1A (VP4), 1B (VP2), 1C (VP3) and 1D (VP1), or—if the 1AB precursor remains uncleaved—of 1AB (VP0), 1C and 1D [85].

A systematic nomenclature of picornavirus proteins was proposed on basis of an idealized genome layout, called the L434 diagram (Fig. 1). The L434 convention was agreed on at the third meeting of the European Study Group on the Molecular Biology of Picornaviruses [87] and refers to an idealized polyprotein and the picornaviral polyprotein processing paradigm, which summarized the knowledge of that time: the idealized polyprotein is cleaved into a leader protein (L) and three polyproteins—P1, P2, P3—in the primary processing steps. The next processing steps generate three capsid proteins from the precursor P1 (i.e., 1AB, 1C, 1D), three nonstructural

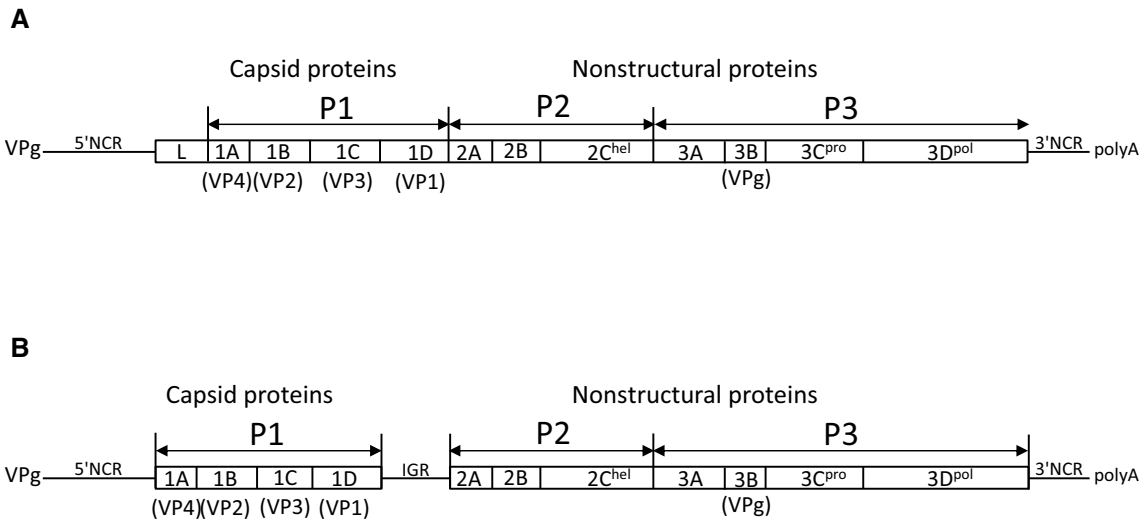

Fig. 1 Schematic diagram of an idealized picornavirus genome (A) and of the dicistronic *Dicipivirus* genome (B). Genome lengths vary from 6.7 kb to 10.1 kb. Genomic RNA is polyadenylated and linked to a virus-encoded VPg peptide at its 5′ end. It consists of a 5′ noncoding region (NCR) up to 1450 nt (sometimes with a poly(C) tract), one (A) or two (B) open reading frames, and a 3′-NCR of variable length (25-795 nt). The 5′-NCR contains one of five known IRES types; the intergenic region (IGR) of dicipiviruses (B) is thought to contain another, yet undefined IRES type. All picornaviruses except dicipiviruses encode a single polyprotein ranging from 2050 to 2886 amino acids; dicipiviruses code for two polyproteins. Primary polyprotein processing yields the leader protein L, if present, and three polypeptides, P1, P2, P3. Further processing steps are carried out by the viral 3C proteinase ($3C^{pro}$) and generate the mature proteins 1AB, 1C, 1D, 2A, 2B, 2C, 3A, 3B, 3C, and 3D. A final processing step, the maturation cleavage of 1AB, occurs in some picornaviruses, either after assembly of empty capsids or after RNA packaging, presumably by autocatalytic mechanisms

proteins from the midpiece polyprotein P2 (2A, 2B, 2C), and four nonstructural proteins from P3 (3A, 3B, 3C, 3D). A final cut of 1AB yields the mature capsid proteins 1A and 1B. This occurs in some picornaviruses after RNA incorporation. The maturation cleavage is thought to be an RNA-catalyzed step [5, 29]; reviewed in [96] and induces the formation of stable, infectious virions [28]. It was further agreed that the designations VP4, VP2, VP3, VP1, VP0 and VPg for 1A, 1B, 1C, 1D, 1AB and 3B may be retained [87]. Noteworthily, the systematic nomenclature denotes the picornavirus proteins according to their order in the polyprotein, whereas the former designations of the capsid proteins, VP1 to VP4, refer to their apparent molecular weights in SDS polyacrylamide gel electrophoresis, with VP1 being the largest protein and VP4 the smallest (Fig. 1). For many of the newer picornaviruses, experimental and biological data are lacking, and only sequence data are available. In these cases, putative proteins have been proposed solely on the basis of their similarity to known picornavirus proteins. This has lead to a considerable variation in the sizes of the proposed proteins compared to their reference proteins with available biochemical data. Accordingly, designations like VP1, VP2, etc. may no longer correspond to the sizes as originally intended. Therefore, it is recommended by the *Picornaviridae* Study Group to prefer the systematic nomenclature. An idealized picornavirus genome layout may be described as VPg+5′UTR[(L/)1A-1B-1C-1D/2A-2B-2C/3A-3B-3C-3D]3′UTR-poly(A), with "[" and "]" indicating the open reading frame. The "/" symbols indicate primary cleavage sites leading to the three polypeptides P1, P2, P3, and "-" symbols indicate the final processing sites. Functions of the nonstructural proteins or characteristic sequence motifs may be presented (e.g., L^{pro}, $2A^{pro}$, $2A^{npgp}$, $2A^{H\text{-}box/NC}$, $2A^{NPTase}$). If multiple copies of a protein are present, these copies are numbered (e.g., 2A1, 2A2, 2A3, etc.). If certain proteins are not present in all members of a genus, the respective genome region is presented in round brackets. There are many exceptions to the idealized genome layout, e.g., the absence of a leader protein in many genera, an uncleaved 1AB precusor (VP0), the presence of several distinct 2A proteins, or the presence of additional copies of 3B (VPg).

The capsid proteins 1B, 1C, 1D as well as 2C (a helicase), 3C (a chymotrypsin-like cysteine proteinase) and 3D (a RNA-dependent RNA polymerase) are orthologous and thus conserved in all picornaviruses. Due to their marked divergence, the proteins 1A, 2B, 3A, 3B often lack detectable similarity, although there may be a functional analogy and possibly a common evolutionary origin with their cognates of other picornaviruses. At least the proteins L and 2A may have different evolutionary origins in the various picornavirus genera. The functions of the processed picornavirus proteins and some intermediates are summarized in Table 1.

Eighteen of 35 picornavirus genera exhibit highly divergent L proteins, but only for members of the genera *Aphthovirus*, *Cardiovirus* and *Erbovirus* has their function been elucidated. The aphthovirus and erbovirus L protein is a papain-like cysteine proteinase [27] that cleaves at its C-terminus to release itself from the nascent polyprotein. In addition, the L^{pro} of FMDV is involved in cleavage of eukaryotic elongation factor 4G, leading to a host cell shut-off of translation [14, 43]. The cardiovirus L proteins inhibits the assembly of stress granules and nucleocytoplasmic transport [4, 7, 79]. The function of all other L protein is obscure. Various versions of 2A exist, two of which release the P1 polypeptide either by proteolytic cleavage ($2A^{pro}$ of *Enterovirus* and possibly *Sapelovirus* and *Rabovirus*) or by a translation elongation arrest followed by a re-initiation of translation at the next in-frame codon ($2A^{npgp}$ of *Aphthovirus*, *Aquamavirus*, *Avihepatovirus*, *Avisivirus*, *Cardiovirus*, *Cosavirus*, *Erbovirus*, *Hunnivirus*, *Kunsagivirus*, *Limnipivirus*, *Mischivirus*, *Mosavirus*, *Parechovirus B-D*, *Pasivirus*, *Potamipivirus*, *Rosavirus*, *Senecavirus*, *Teschovirus* and *Torchivirus* plus several unclassified picornaviruses) [16]. A third type of 2A, $2A^{H\text{-}box/NC}$, has similarity to the H-rev107 family of proteins and the N-terminus of the viral polyprotein (pfam08405) of caliciviruses [34], but its function in the virus life cycle is unclear. The $2A^{H\text{-}box/NC}$ protein is found in members of the genera *Avihepatovirus*, *Avisivirus*, *Gallivirus*, *Kobuvirus*, *Megrivirus*, *Parechovirus*, *Passerivirus*, *Potamipivirus*, *Sakobuvirus*, *Salivirus*, *Sicinivirus* and *Tremovirus*. Members of the genera *Avihepatovirus* and *Avisivirus* have a fourth type of 2A. This protein, $2A^{NTPase}$, shares similarity with the AIG1 (avrRpt2-induced gene 1)-type guanine binding domain found in P-loop NTPases; one characteristic feature is its NTP-binding motif GxxGxGKS [15]. In addition, putative 2A proteins without similarity to any known protein in the GenBank database and of unknown function have been described in members of the genera *Ampivirus, Aquamavirus, Dicipivirus, Harkavirus, Hepatovirus, Kunsagivirus, Megrivirus, Oscivirus* and *Pasivirus*.

The coding region of picornaviruses is flanked by 5′ and 3′ nontranslated regions, which give rise to RNA structures. Some of these RNA structures promote cap-independent initiation of translation [60] and—at least for enteroviruses—initiation of negative- and positive-strand RNA synthesis [2]. Five distinct types of internal ribosome entry sites (IRESs), which recruit ribosomes and other host factors to direct initiation of translation, have been shown in picornaviruses [37]. The type I IRES is

Table 1 Picornavirus proteins

Protein	Function
L, L^{pro}	Leader protein, i.e., the region of the polyprotein preceding the capsid proteins, often with unknown function; in *Aphthovirus, Erbovirus* and possibly in *Mosavirus*, it is a papain-like proteinase (L^{pro}) that releases itself from the poly-protein and targets host proteins
1A (VP4)	Capsid protein, located at the inner surface of the capsid, may be very short like in *Hepatovirus*
1B (VP2)	Capsid protein with jellyroll fold (8-stranded β-barrel) and conserved drug-binding site
1AB (VP0)	Capsid protein, precursor of 1A and 1B, remains uncleaved in members of the genera *Ampivirus, Aquamavirus, Avihepatovirus, Avisivirus, Gallivirus, Kobuvirus, Kunsagivirus, Limnipivirus, Megrivirus, Mischivirus, Oscivirus, Parechovirus, Pasivirus, Passerivirus, Potamipivirus, Rosavirus, Sakobuvirus, Salivirus,* and *Sicinivirus* and several unclassified picornaviruses
1C (VP3	Capsid protein with jellyroll fold (8-stranded β-barrel) and conserved drug-binding site
1D (VP1)	Capsid protein with jellyroll fold (8-stranded β-barrel) and conserved drug-binding site
$2A^{pro}$	Chymotrypsin-like cysteine proteinase in *Enterovirus* and possibly in *Sapelovirus* and *Rabovirus*, cleaves the polyprotein at its N-terminus and targets various host proteins
$2A^{npgp}$	Mediates cotranslational processing by a translation elongation arrest and re-initiation at the next in-frame codon in members of the genera *Aphthovirus, Aquamavirus, Avihepatovirus, Avisivirus, Cardiovirus, Cosavirus, Erbovirus, Hunnivirus, Kunsagivirus, Limnipivirus, Mischivirus, Mosavirus, Parechovirus B, C, D, Pasivirus, Potamipivirus, Rosavirus, Senecavirus, Teschovirus,* and *Torchivirus* and several unclassified picornaviruses; the lengths vary from 17 to >150 amino acids; up to six proteins with NPGP motifs may be present in a single genome; for some picornaviruses, it is unclear whether it is cleaved off the P1 polyprotein
$2A^{H\text{-}box/NC}$	2A protein with the sequence motif of H-box/NC proteins found in the H-rev107 family of proteins and a nonstructural protein of caliciviruses, found in members of the genera *Avihepatovirus*, *Avisivirus*, *Gallivirus*, *Kobuvirus*, *Megrivirus*, *Parechovirus*, *Passerivirus*, *Potamipivirus*, *Sakobuvirus*, *Salivirus*, *Sicinivirus* and *Tremovirus*, function unclear
$2A^{NTPase}$	2A protein with similarity to the AIG1-type guanine-binding domain (P-loop NTPase with GxxGxGKS motif) found in members of the genera *Avihepatovirus* and *Avisivirus*, function unclear
2A	2A protein without similarity to known proteins and unknown function found in members of the genera *Ampivirus, Aquamavirus, Dicipivirus, Harkavirus, Hepatovirus, Kunsagivirus, Megrivirus, Oscivirus* and *Pasivirus*
2B	Membrane-associated, functions as viroporin (increase of plasma membrane permeability), inhibition of host secretory pathways
$2C^{hel}$	Helicase, P-loop NTPase with GxxGxGKS motif, membrane-associated
2BC	Membrane-anchored protein, involved in formation of the replication organelle, precursor of 2B and 2C proteins
3A	Key protein involved in membrane remodelling; although lacking detectable similarity in the various genera, all 3A proteins exhibit a C-terminal hydrophobic domain, membrane associated
3B (VPg)	Viral peptide, genome-associated, with conserved tyrosine-3 residue
$3C^{pro}$	Chymotrypsin-like cysteine proteinase with GxCG motif, involved in polyprotein processing, targets numerous host proteins, interacts with a cloverleaf-like structure at the 5′ end of the positive-strand RNA of coxsackievirus and rhinovirus
$3D^{pol}$	RNA-dependent RNA polymerase with conserved KDE, PSG, YGDD and FLKR motifs
$3CD^{pro}$	Precursor of 3C and 3D, poliovirus $3CD^{pro}$ executes many processing steps and interacts with the cloverleaf-like structure at the 5′ end of the positive-strand RNA

found in members of the genera *Enterovirus* and *Harkavirus*, type II in members of the genera *Aphthovirus*, *Avisivirus*, *Cardiovirus*, *Cosavirus*, *Erbovirus*, *Gallivirus*, *Hunnivirus*, *Mischivirus*, *Mosavirus*, *Parechovirus*, *Rabovirus*, *Rosavirus*, and *Sicinivirus*, and type III in members of the genus *Hepatovirus*. The type IV IRES is similar to the hepatitis C virus IRES and is present in members of the genera *Aquamavirus*, *Avihepatovirus*, *Kunsagivirus*, *Limnipivirus*, *Megrivirus*, *Pasivirus*, *Sakobuvirus*, *Sapelovirus*, *Senecavirus*, *Teschovirus* and *Tremovirus*. Type V has been described in members of the genera *Kobuvirus*, *Oscivirus* and *Salivirus*. Due to its dicistronic genome, the only known member of the genus *Dicipivirus* presumably has two IRESs. The first one is a type II IRES, whereas the IRES type of the intergenic region is unknown. Also unknown is the IRES type of the ampiviruses, passeriviruses, potamipiviruses and torchiviruses.

Picornavirus replication cycle

Picornaviruses enter their host cells by receptor-mediated endocytosis. Various mechanisms have been described that lead to endosome formation followed by alteration of capsid structures and penetration of the membrane by the viral RNA (reviewed in [96]). In the cytoplasm, viral RNA is translated to yield one major polyprotein (all genera except *Dicipivirus*), which is co- and posttranslationally processed into the L protein (if present), capsid proteins (1AB, 1C, 1D), mature nonstructural proteins, and some stable intermediates, namely 2BC, 3AB and 3CD (Fig. 1). Not only the

RNA-dependent RNA polymerase [3] but most if not all nonstructural proteins are necessary for replication of viral RNA. They modify the cellular environment and promote synthesis of negative-strand RNA molecules, which in turn serve as template for positive-strand RNA production. VPg molecules are uridylated at cis-replicative elements (cre) and VPg-pU-pU serves as a primer for both positive- and negative-strand RNA synthesis (reviewed in [77]). It is assumed that viral RNA synthesis occurs at a multimeric replication complex comprising several of the viral nonstructural proteins and their precursors as well as host factors. The components of the replication complex are concentrated and assembled at the surface of the viral replication organelle (RO). Presumably, the RO protects viral RNA from host nucleases and cytosolic defense mechanisms of the innate immunity. Several membrane-associated viral proteins (2B, 2C and 3A) are involved in generation of an RO of the protrusion type, which is achieved by the massive virus-induced rearrangement of intracellular membranes. This membrane rearrangement presumably involves Golgi components and autophagic mechanisms. ROs are highly dynamic structures and consist initially of a network of single-membrane vesicles, which develop into double-membrane and, at late stages, multilamellar agglomerations. Picornaviruses have developed various mechanisms to accumulate sterols, glycerophospholipids and sphingolipids at the ROs. For sterols, some mechanisms involve the viral 3A protein and phosphatidylinositol 4-kinases (PI4Ks), phosphatidylinositol 4-phosphate (PI4P), and oxysterol-binding protein (OSBP), as shown for enteroviruses, Aichi virus, encephalomyocarditis virus, and Saffold virus. Other viruses are independent of OSBP (foot-and-mouth disease virus, hepatitis A virus, human parechovirus). Virus-induced membrane contact sites (vMCSs) are formed to direct lipids from the endoplasmic reticulum to viral membranes and modulate the lipid composition of the RO membranes, which is necessary for their function (reviewed in [69, 93, 97]).

Progeny picornavirus particles are generally released by lysis; however, for hepatitis A virus, poliovirus and coxsackievirus B3, alternative, non-lytic mechanisms of viral spread have been described [6, 13, 20, 84].

Picornavirus taxonomy

A virus species is defined as *"a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria"* (International Code of Virus Classification and Nomenclature, ICVCN; https://talk.ictvonline.org/information/w/ictv-information/383/ictv-code). Accordingly, a picornavirus species contains viruses that are phylogenetically related and share a significant degree of homology of all proteins. They have essentially identical genome maps and thus are thought to have a significant degree of compatibility in proteolytic processing, replication, encapsidation and genetic recombination. Picornavirus genera—the next taxonomic level—may subsume species that cluster on the same branch in phylogenetic trees and share a significant degree of homology of the orthologous proteins P1 (divergence <0.66), $2C^{hel}$, $3C^{pro}$ and $3D^{pol}$ (divergence each < 0.64). Thus they conform to the ICVCN rule that a virus genus comprises *"a group of species sharing certain common characters"*. The many picornavirus genera differ by distinctive features of their genome maps and exhibit significant divergence (number of differences per site between sequences) of the orthologous proteins. The remaining proteins (L, 2A, 2B, 3A, 3B) usually lack detectable similarity. For sequence comparisons, the *Picornaviridae* Study Group recommends to align only the orthologous genome regions of picornavirus sequences that share significant similarity to each other. Species demarcation rules vary depending on the genus under investigation and consider the observed intra- and interspecies variability; moreover, they are based on current sequence data and may be amended when additional data are available in the future. Usually, the amino acid sequence divergence within a genus may vary from 0.4 to c. 0.65 depending on the protein. Intergenus comparisons, however, yield divergences greater 0.65. Within a species, sequence variation of the VP1 gene region may be used to define genetic types. For *Enterovirus*, it has been shown that genetic types correspond to the antigenic serotypes [66, 71]. However, typing is species-specific; for *Enterovirus A, B, C*, the border was estimated with a nucleotide sequence divergence of 0.25 (corresponding to an amino acid sequence divergence of 0.12), whereas the nucleotide sequence divergence thresholds of *Rhinovirus A, B, C* were 0.12-0.13 (0.095-0.1 amino acid sequence divergence). Since the available picornavirus sequence data meanwhile include isolates collected in a period of eight decades, a considerable accumulation of substitutions over time can be observed. Whether this genetic drift also affects serological properties is unknown.

In recent years, the binomial nomenclature of virus names has been adopted for all but one of the picornavirus genera. A typical picornavirus species name is composed of the genus name and the adjunct capital letters A, B or C etc. (e.g., genus *Ampivirus*, species *Ampivirus A*). Exceptions are the genera *Dicipivirus*, with the species *Cadicivirus A*, genus *Enterovirus* with species *Enterovirus A* to *J* and *Rhinovirus A* to *C*, genus *Kobuvirus* with the six species *Aichivirus A* to *F*, and genus *Megrivirus* with the species *Melegrivirus A*. Genus *Aphthovirus* is the only picornavirus genus that does not conform the International Committee on Taxonomy of Viruses (ICTV) nomenclature rules, as the names of the four species were derived from the host species and/or the disease

they induce (*Foot-and-mouth disease virus*, *Equine rhinitis A virus*, *Bovine rhinitis A virus*, *Bovine rhinitis B virus*).

Since the invention of next-generation sequencing techniques, the majority of novel picornaviruses have been identified in metagenomics studies rather than by classical virus isolation methods and subsequent experimental characterization. Based on typical hallmarks of picornaviruses, i.e., the presence of an IRES, three capsid protein domains with drug-binding sequence motifs (so-called rhv domains), a helicase with an NTP-binding site (GxxGxGKS/T), a VPg with a tyrosine-3 residue, a chymotrypsin-like proteinase with a $GxCGx_{10\text{-}18}GxH$ motif, and an RNA-dependent RNA polymerase with KDE, PSG, YGDD, FLKR motifs, picornaviruses can reliably be identified solely on basis of their sequences, even if sometimes the one or other feature may be absent. Moreover, bioinformational techniques may help to identify the possible hosts of new picornaviruses. Metagenomics studies investigating the intestinal viromes of humans and various animals have revealed numerous viruses that are likely of dietary origin (e.g., [55, 57, 112]). Nucleotide composition analysis (NCA), or demonstration of characteristic sequence patterns may provide evidence of the identity of the picornavirus host. Whereas NCA can clearly distinguish between mammal, insect, and plant hosts, discrimination of mammal, avian and fish viruses is less conclusive [49, 107]. An example of a host-specific sequence pattern is the apical "8" structure of the type IV IRES of many avian picornaviruses, which is either a part of domain III (megriviruses, unclassified sapelo-like viruses) or part of a separate domain I (avihepatoviruses, aaliviruses) (reviewed in [9]). Because picornavirus sequences can be reliably identified, the *Picornaviridae* Study Group supports proposals that are premised on sequence data, phylogeny, divergence metrics and similarity detection. This practice conforms to the requirements of the ICTV. Correspondingly, in 2016, the Executive Committee of the ICTV endorsed a consensus statement of an expert panel that proposed a classification pipeline based on metagenomic sequence data [90].

Progress in sequencing techniques not only led to a remarkable increase in the number of known picornaviruses but has also revealed an unexpected degree of diversity. As of 2017, 35 genera and 80 species have been approved by the ICTV. More than 500 types can be distinguished genetically or by serological means. Moreover, the *Picornaviridae* Study Group of the ICTV has recently proposed five novel genera with six species plus nine new species within existing genera; Table 2 summarizes these picornaviruses. An additional 56 picornaviruses have been described but are yet unassigned (Table 3). In order to improve the clarity of this fast-growing family, there is a need to define subfamily criteria.

Table 2 New picornavirus genera and species proposed in 2017

Genus[1]	Species	Virus	Exemplar	GenBank acc. no.
Aalivirus	Aalivirus A	duck aalivirus	duck/GL/12/China/2012	KJ000696
Bopivirus	Bopivirus A	bovine picornavirus	cattle/TCH6/2013/USA	KM589358
Crohivirus	Crohivirus A	shrew crohivirus	shrew/ZM54/Zambia/2012	AB937989
	Crohivirus B	bat crohivirus	bat/Cameroon/2014	KX644937
Enterovirus	Enterovirus K	rodent picornavirus	rodent/Ee/PicoV/NX2015	KX156158
	Enterovirus L	simian enterovirus gx	Macaca mulatta/SEV-gx/2014/China	KU587555
Orivirus	Orivirus A	chicken orivirus 1	chicken/Pf-CHK1/2013/HUN	KM203656
			chicken orivirus 2	KT880667
Kunsagivirus	Kunsagivirus B	bat kunsagivirus	bat/CAM/KuV-P2/2013	KX644936
	Kunsagivirus C	bakunsavirus	baboon/M27-KuV/1986/TAN	KY670597
Megrivirus	Megrivirus A	duck megrivirus	LY	KC663628
		goose megrivirus	W18	KY369299
		goose megrivirus	HN56	KY369300
	Megrivirus B	mesivirus 1	HK21	KC876003
		mesivirus 2	pigeon/GALII5-PiMeV/2011/HUN	KC811837
	Megrivirus C	chicken megrivirus	chicken/B21-CHV/2012/HUN	KF961186
		chicken megrivirus	chicken/CHK-IV-CHV/2013/HUN	KF961187
		chicken proventriculitis virus	CPV/Korea/03	KJ690629
		chicken picornavirus	27C	KF979336
	Megrivirus D	harrier picornavirus 1	MR-01/HUN/201	KY488458
	Megrivirus E	penguin megrivirus	penguin/KGI-BEL-P5/2015	MF405436
Shanbavirus	Shanbavirus A	bat picornavirus	bat/BtMf-PicoV-1/Sax2011	KJ641698

[1]approved taxa are printed in *italics*

Table 3 List of unassigned picornaviruses

Virus	Genus	GenBank acc. no.	Reference	Comment[1]
lesavirus 1 [Mis101308/2012]	new	KM396707	[59]	complete genome
lesavirus 2 [Nai108015/2012]	new	KM396708	[59]	complete genome
livupivirus [newt/II-5-Pilis/2014/HUN]	new	KX463670	[75, 76]	complete genome
poecivirus [BCCH-449]	new	KU977108	[113]	complete genome
porcine picornavirus Japan [Tottori-WOL]	new	LC113907	[70]	complete genome
Rhinolophus affinis picornavirus [bat/China/2010]	new	JQ814853	[104]	par (P1)
Rhinolophus ferrumequinum picornavirus [BtRf-PicoV-2/YN2012]	new	KJ641684	[104]	par
rodent picornavirus [rodent/Ds/PicoV/IM2014]	new	KX156153	[18]	complete genome
rodent picornavirus [rodent/Rn/PicoV/SX2015_1]	new	KX156154	[18]	complete genome
rodent picornavirus [rodent/CK/PicoV/Tibet2014]	new	KX156155	[18]	complete genome
tortoise rafivirus [tortoise/UF4/USA/2009]	new	KJ415177	Ng et al., unpubl.	complete genome
Cosavirus C1 [5152]	*Cosavirus*	FJ438895, FJ442995	[38]	par (5′-NCR, nonstructural proteins)
hedgehog dicipivirus [hedgehog/H14/2015/HUN]	*Dicipivirus*	MF188967	[82]	complete genome
chicken gallivirus 1 [518C]	*Gallivirus*	KF979337	[52]	complete genome
hunnivirus 05VZ-75-RAT099	*Hunnivirus*	KT944214	Nguyen et al. unpubl.	complete genome
hunnivirus 83GR-70-RAT106	*Hunnivirus*	KT944212	Nguyen et al. unpubl.	complete genome
hunnivirus 83GR-70-RAT051	*Hunnivirus*	KT944201	Nguyen et al. unpubl.	par (VP1)
bat/TM001k/USA/2008	*Kobuvirus*	HM228881	[55, 57]	par (P1)
caprine kobuvirus [12Q108]	*Kobuvirus*	KF793927	[72]	complete genome
Norway rat kobuvirus 2 [rat/NYC-D21a/USA/2013]	*Kobuvirus*	KJ950970	[22]	par (VP1)
canine picornavirus [A128thr/USA/1968]	*Mischivirus*	KY512802	Norby et al. unpubl.	complete genome
Manhattan parechovirus [rat/NYC-A11/USA/2013]	*Parechovirus*	KJ950935, KJ950936, KJ950913	[22]	par (5′-NCR, VP1, $3D^{pol}$)
falcon parecho-like virus [falcon/HA18_080/2014/HUN]	*Parechovirus*	KY645497	[75, 76]	complete genome
Rosavirus B [rat/RNCW0602091R/HKN/2009]	*Rosavirus*	KX783423	Lau et al. unpubl.	complete genome
Rosavirus C [rat/RATLC11A/HKN/2009]	*Rosavirus*	KX783425	Lau et al. unpubl.	complete genome
Rosavirus C [rat/RASK8F/HKN/2008]	*Rosavirus*	KX783424	Lau et al. unpubl.	complete genome
Rosavirus C [rat/NCGX12IN/CHA/2010]	*Rosavirus*	KX783432	Lau et al. unpubl.	complete genome
Rosavirus C [rodent/RL/PicoV/FJ2015]	*Rosavirus*	KX156156	[18]	complete genome
fur seal sakobuvirus [ATROP12/BR/2012]	*Sakobuvirus*	KR072975	[45]	par
sapelo-like bat picornavirus [NC16A]	sapelo-like	HQ595340	[50]	complete genome
sapelo-like bat picornavirus [LMH22A]	sapelo-like	HQ595341	[50]	complete genome

Table 3 (continued)

Virus	Genus	GenBank acc. no.	Reference	Comment[1]
sapelo-like bat picornavirus [MH9F]	sapelo-like	HQ595342	[50]	complete genome
sapelo-like bat picornavirus [SK17F]	sapelo-like	HQ595343	[50]	complete genome
sapelo-like bat picornavirus [TLC5F]	sapelo-like	HQ595344	[50]	complete genome
sapelo-like bat picornavirus [TLC21F]	sapelo-like	HQ595345	[50]	complete genome
sapelo-like bat picornavirus [BtRh-PicoV/SC2013]	sapelo-like	KJ641693	[105]	complete genome
sapelo-like bat picornavirus [BtMf-PicoV-2/SAX2011]	sapelo-like	KJ641699	[105]	complete genome
sapelo-like bat picornavirus [BtMf-PicoV/FJ2012]	sapelo-like	KJ641687	[105]	complete genome
sapelo-like bat picornavirus [BtMf-PicoV-1/GD2012]	sapelo-like	KJ641690	[105]	par
sapelo-like bat picornavirus [BtRf-PicoV-1/YN2012]	sapelo-like	KJ641685	[105]	complete ORF
sapelo-like bat picornavirus [BtRlep-PicoV/FJ2012]	sapelo-like	KJ641688	[105]	par
sapelo-like bat picornavirus [BtRa-PicoV/JS2013]	sapelo-like	KJ641692	[105]	par
sapelo-like bat picornavirus [BtNv-PicoV/SC2013]	sapelo-like	KJ641697	[105]	complete genome
sapelo-like bat picornavirus [BtVs-PicoV/SC2013]	sapelo-like	KJ641696	[105]	complete genome
sapelo-like bat picornavirus [BtMa-PicoV/FJ2012]	sapelo-like	KJ641689	[105]	par
sapelo-like bat picornavirus [BtRs-PicoV/YN2010]	sapelo-like	KJ641694	[105]	complete genome
sapelo-like Ia io picornavirus	sapelo-like	JQ814852	[104]	complete genome
sapelo-like bovine picornavirus [Bo-11-39/2009/JPN]	sapelo-like	LC006971	[68]	complete genome
sapelo-like bovine picornaviruses	sapelo-like	LC036579-LC036582	[68]	complete genomes
bat sapelovirus [CAM/Sap-P24/2013]	sapelo-like	KX644938	[107]	complete genome
California sea lion sapelovirus [1162]	sapelo-like	JN420368	[56]	par
canine sapelovirus [dog/Hong Kong/325F/2008]	sapelo-like	JN831356	[103]	complete genome
feline sapeloviruses	sapelo-like	JN572115-JN572119	[51]	complete genomes
phacovirus [chicken/Pf-CHK1/PhV]	sapelo-like	KT880670	[8]	complete genome
sapelo-like pigeon picornavirus A [pigeon/Norway/03/603-7/2003]	sapelo-like	FR727145	[47]	par
sapelo-like pigeon picornavirus B [pigeon/Norway/03/641/ 2003]	sapelo-like	FR727144	[47]	complete genome
sapelo-like pigeon picornavirus B [pigeon/GAL-7/2010/Hungary]	sapelo-like	KC560801	Reuter et al. unpublished	complete genome
sapelo-like quail picornavirus [quail/Hungary/2010]	sapelo-like	JN674502	[74]	complete genome

[1] abbreviations: NCR, noncoding region; ORF, open reading frame; par, partial genome; P1, P1 region; VP1, capsid protein VP1

Genus *Ampivirus*

Number of species: 1 (*Ampivirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: KP770140).

Genome layout: VPg+5′UTR^{IRES}[1AB-1C-1D/2A-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: 9246 nt (5′-UTR: 970 nt; ORF: 8142 nt; 3′-UTR: 134 nt), length of polyprotein: 2713 aa.

Genus *Aphthovirus*

Number of species: 4 (*Bovine rhinitis A virus*, 2 types; *Bovine rhinitis B virus*, 5 types; *Equine rhinitis A virus*, 1 type; *Foot-and-mouth disease virus*, 7 types; GenBank acc. nos. of reference strains or exemplars: KP236128, EU236594, X96870, AY593829).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[L^{pro}/1A-1B-1C-1D-2A^{npgp}/2B-2C/3A-3B(-3B2-3B3)-3C-3D]3′UTR-poly(A).

Genome length: 7250-8203 nt (5′-UTR: up to 1112 nt with poly(C) tract; ORF: 6657-7020 nt; 3′-UTR: 32-110 nt); length of polyprotein: 2218-2339 aa.

Genus *Aquamavirus*

Number of species: 1 (*Aquamavirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: EU142040).

Genome layout: VPg+5′UTR$^{IRES\text{-}IV}$[1AB-1C-1D-2A1^{npgp}/2A2-2B-2C/3A-3B1-3B2-3C-3D]3′UTR-poly(A).

Genome length: c. 6700 nt (5′-UTR: 506 nt, ORF: 6154 nt, 3′-UTR: 34 nt); length of polyprotein: 2050 aa. Aquamaviruses have the shortest known picornavirus genomes and polyproteins.

Genus *Avihepatovirus*

Number of species: 1 (*Avihepatovirus A*, 3 types; GenBank acc. no. of reference strain or exemplar: DQ226541).

Genome layout: VPg+5′UTR$^{IRES\text{-}IV}$[1AB-1C-1D-2A1^{npgp}/2A2^{NTPase}-2A3$^{H\text{-}box/NC}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: up to 7792 nt (5′-UTR: 625-655 nt, ORF: 6750-6756 nt, 3′-UTR: c. 318 nt); length of polyprotein: 2249-2251 aa.

Genus *Avisivirus*

Number of species: 3 (*Avisivirus A*, 1 type; *Avisivirus B*, 1 type; *Avisivirus C*, 1 type; GenBank acc. nos. of reference strains or exemplars: KC465954, KF979333, KF979334).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[1AB-1C-1D-(2A1^{npgp})/2A2^{npgp}/2A3^{NTPase}-2A4$^{H\text{-}box/NC}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: > 7167-7532 nt (5′-UTR: > 457-609; ORF: 6441-6720; 3′-UTR: 194-269 nt); length of polyprotein: 2146-2239 aa.

Genus *Cardiovirus*

Number of species: 3 (*Cardiovirus A*, 2 types; *Cardiovirus B*, 15 types; *Cardiovirus C*, 2 types; GenBank acc. nos. of reference strains or exemplars: M81861, X56019, JQ864242).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[L/1A-1B-1C-1D-2A^{npgp}/2B-2C/3A-3B-3C-3D]3′UTR-poly(A) Despite a significant similarity, the 2A protein of *Cardiovirus C* lacks the NPGP motif.

Genome length: c. 7730-8530 nt (5′-UTR: up to 1451 nt with poly(C) tract, ORF: 6879-6924 nt, 3′-UTR: 121-144 nt); length of polyprotein: 2293-2308 aa. Cardioviruses C have the longest known poly(C) tracts of picornaviruses.

Genus *Cosavirus*

Number of species: 5 (*Cosavirus A*, 24 types; *Cosavirus B*, 1 type; *Cosavirus D*, 5 types; *Cosavirus E*, 2 types; *Cosavirus F*, 1 type; GenBank acc. nos. of reference strains or exemplars: FJ438902, FJ438907, FJ438908, FJ555055, JN867758). There is one tentative cosavirus species with one type (compare Table 3).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[1A-1B-1C-1D-2A^{npgp}/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7632-7802 nt (5′-UTR: up to 1361 nt; ORF: 6366-6402 nt; 3′-UTR: 75-93 nt); length of polyprotein: 2113-2133 aa.

Genus *Dicipivirus*

Dicipiviruses are the only known picornaviruses with a dicistronic genome layout. Two open reading frames encoding a capsid protein precursor and a nonstructural polyprotein are separated by an intergenic region thought to have IRES function.

Number of species: 1 (*Cadicivirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: JN819202). There is one tentative dicipivirus (compare Table 3).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[1A-1B-1C-1D]-IGR^{IRES}[2A-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 8785 nt (5′-UTR: at least 982 nt; ORF1: 2535 nt; IGR: 588 nt; ORF2: 4221 nt; 3′-UTR: 549 nt); lengths of polyproteins: 845 aa (P1), 1407 aa (P2-P3).

Genus *Enterovirus*

Number of species: 13 (*Enterovirus A*, 25 types; *Enterovirus B*, 63 types; *Enterovirus C*, 23 types; *Enterovirus D*, 5 types; *Enterovirus E*, 5 types; *Enterovirus F*, 7 types; *Enterovirus G*, 20 types; *Enterovirus H*, 1 type; *Enterovirus I*, 1 type; *Enterovirus J*, 6 types; *Rhinovirus A*, 80 types; *Rhinovirus B*, 32 types; *Rhinovirus C*, 55 types; GenBank acc. nos. of reference strains or exemplars: AY421760, M16560, V01149, AY426531, D00214, DQ092770, AF363453, AF326759, KP345887, FJ007373, FJ445111, DQ473485, EF077279). There are two tentative species with 3 types (compare Table 2).

Genome layout: VPg+5′UTR$^{\text{IRES-I}}$[1A-1B-1C-1D/2A$^{\text{pro}}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7100-7450 (5′-UTR: 610-822 nt; ORF: 6417-6645 nt; 3′-UTR: 37-99 nt); length of polyprotein: 2138-2214 aa. Recently, porcine enteroviruses (*Enterovirus-G*) have been identified in the USA and Germany that express a torovirus-like proteinase inserted between 2C and 3A [12, 46, 89], in press). The function and implication of this insertion for the viral life cycle are still unclear, ditto its prevalence.

Genus *Erbovirus*

Number of species: 1 (*Erbovirus A*, 3 types; GenBank acc. no. of reference strain or exemplar: X96871).

Genome layout: VPg+5′UTR$^{\text{IRES-II}}$[L$^{\text{pro}}$/1A-1B-1C-1D-2A$^{\text{npgp}}$/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 8828 nt (5′-UTR: up to 905 nt; ORF: 7752-7770 nt; 3′-UTR: 164 nt); length of polyprotein: 2583-2589 aa.

Genus *Gallivirus*

Number of species: 1 (*Gallivirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: JQ691613). Additionally, there is one tentative type (compare Table 3).

Genome layout: VPg+5′UTR$^{\text{IRES-II}}$[L/1AB-1C-1D/2A$^{\text{H-box/NC}}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 8500 nt (5′-UTR: 761 nt; ORF: 7425 nt; 3′-UTR: 310 nt); length of polyprotein: 2474 aa.

Genus *Harkavirus*

Number of species: 1 (*Harkavirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: KP230449).

Genome layout: VPg+5′UTR$^{\text{IRES-I}}$[1A-1B-1C-1D/2A-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: 8003 nt (5′-UTR: 673 nt; ORF: 7263 nt; 3′-UTR: 67 nt); length of polyprotein: 2420 aa.

Genus *Hepatovirus*

Number of species: 9 (*Hepatovirus A*, 1 type; *Hepatovirus B*, 1 type; *Hepatovirus C*, 2 types; *Hepatovirus D*, 2 types; *Hepatovirus E*, 1 type; *Hepatovirus F*, 2 types; *Hepatovirus G*, 2 types; *Hepatovirus H*, 3 types; *Hepatovirus I*, 1 type; GenBank acc. nos. of reference strains or exemplars: M14707, KR703607, KT452742, KT452637, KT452735, KT452685, KT452730, KT452691, KT452658).

Genome layout: VPg+5′UTR$^{\text{IRES-III}}$[1A-1B-1C-1D-2A/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: up to 7810 nt (5′-UTR: c. >225-773 nt; ORF: 6597-6909, nt; 3′-UTR: 35-257 nt); length of polyprotein: 2199-2303.

Genus *Hunnivirus*

Number of species: 1 (*Hunnivirus A*, 6 types; GenBank acc. no. of reference strain or exemplar: JQ941880). There is one tentative species (Table 3).

Genome layout: VPg+5′UTR$^{\text{IRES-II}}$[L/1A-1B-1C-1D-2A$^{\text{npgp}}$/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: 7469-7588 nt (5′-UTR: > 600-732 nt; ORF: 7432-6822 nt; 3′-UTR: > 14-122 nt); length of polyprotein: 2244-2274 aa.

Genus *Kobuvirus*

Number of species: 6 (*Aichivirus A*, 6 types; *Aichivirus B*, 3 types; *Aichivirus C*, 2 types; *Aichivirus D*, 2 types; *Aichivirus E*, 1 type; *Aichivirus F*, 2 types; GenBank acc. nos. of reference strains or exemplars: AB010145, AB084788, EU787450, LC055961, KT325853, KJ641686). There are three tentative kobuviruses (Table 3).

Genome layout: VPg+5′UTR$^{\text{IRES-V}}$[L/1AB-1C-1D/2A$^{\text{H-box/NC}}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: up to 8280 nt (5′-UTR: up to 744 nt; ORF: 7299-7467 nt; 3′-UTR: 136-240 nt); length of polyprotein: 2432-2488 aa. Porcine kobuviruses (*Aichivirus C*) have no type V IRES but have a type IV IRES.

Genus *Kunsagivirus*

Number of species: 1 (*Kunsagivirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: KC935379). There are two tentative species with 2 types (Table 2).

Genome layout: VPg+5′UTR$^{\text{IRES-IV}}$[1AB-1C-1D-2A1$^{\text{npgp}}$/2A2-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7092-7429 nt (5′-UTR: 393-532 nt; ORF: 6639-6729 nt; 3′-UTR: 25-177 nt); length of polyprotein: 2213-2248 aa. Kunsagiviruses A have the shortest known 3′-UTR of picornaviruses.

Genus *Limnipivirus*

Number of species: 3 (*Limnipivirus A*, 1 type; *Limnipivirus B*, 1 type; *Limnipivirus C*, 1 type; GenBank acc. nos. of reference strains or exemplars: JX134222, KF306267, KF183915).

Genome layout: VPg+5′UTR$^{\text{IRES-IV}}$[1AB-1C-1D-2A1$^{\text{npgp}}$/2A2$^{\text{npgp}}$/-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7700-8050 nt (5′-UTR: > 501-712 nt; ORF: 6810-7011 nt; 3′-UTR: 312-363 nt); length of polyprotein: 2270-2337 aa.

Genus *Megrivirus*

Number of species: 1 (*Melegrivirus A*, 3 types; GenBank acc. no. of reference strain or exemplar: KF961188). A revision of megrivirus taxonomy was proposed in 2017 as sequence data indicate that all known members of *Melegrivirus A*1 are interspecies recombinants. There are five tentative species with 11 types (compare Table 2).

Genome layout: VPg+5′UTR$^{\text{IRES-IV}}$[(L/)1AB-1C-1D/(2A0-2A1-2A2-)2A3$^{\text{H-box/NC}}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A). The tentative megriviruses exhibit a considerable diversity of the 2A1 and 2A2 proteins. The tentative 'Megrivirus D' has a type II IRES.

Genome length: 9040-9840 nt (5′-UTR: > 461-663 nt; ORF: 8235-8520 nt; 3′-UTR: > 175-669 nt); length of polyprotein: 2745-2886 aa. The goose megriviruses have the longest known genomes and polyproteins of picornaviruses.

Genus *Mischivirus*

Number of species: 3 (*Mischivirus A*, 1 type; *Mischivirus B*, 1 type; *Mischivirus C*, 1 type; GenBank acc. no. of reference strain or exemplar: JQ814851, KP054273, KY512802). There is one tentative new species (Table 3).

Genome layout: VPg+5′UTR$^{\text{IRES-II}}$[L/1AB-1C-1D-2A$^{\text{npgp}}$/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 8457 nt (5′-UTR: 1407 nt; ORF: 6846 nt; 3′-UTR: 204 nt); length of polyprotein: 2282 aa.

Genus *Mosavirus*

Number of species: 1 (*Mosavirus A*, 2 types; GenBank acc. no. of reference strain or exemplar: JF973687).

Genome layout: VPg+5′UTR$^{\text{IRES-II}}$[L/1A-1B-1C-1D-2A$^{\text{npgp}}$/2B-2C/3A-3B1-3B2-3C-3D]3′UTR-poly(A).

Genome length: c. 8385 nt (5′-UTR: > 646 nt; ORF: 7653 nt; 3′ UTR: 86 nt); length of polyprotein: 2551 aa.

Genus *Oscivirus*

Number of species: 1 (*Oscivirus A*, 2 types; GenBank acc. no. of reference strain or exemplar: GU182408).

Genome layout: VPg+5′UTR$^{\text{IRES-V}}$[L/1AB-1C-1D/2A-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7640-7678 nt (5′-UTR: 636-646 nt; ORF: 6765-6774 nt; 3′ UTR: 238-256 nt); length of polyprotein: 2255-2258 aa.

Genus *Parechovirus*

Number of species: 4 (*Parechovirus A*, 19 types; *Parechovirus B*, 5 types; *Parechovirus C*, 1 type; *Parechovirus D*, 1 type; GenBank acc. nos. of reference strain or exemplar: L02971,AF327920, HF677705, KF006989). There are two tentative parechoviruses (Table 3).

Genome layout: VPg+5′UTR$^{\text{IRES-II}}$[1AB-1C-1D-(2A$^{\text{npgp}}$/)2A$^{\text{H-box/NC}}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: 7339-7608 (5′-UTR: 710-730 nt; ORF: 6543-6753; 3′-UTR: 87-111 nt); length of polyprotein: 2180-2250 aa.

Genus *Pasivirus*

Number of species: 1 (*Pasivirus A*, 3 types; GenBank acc. no. of reference strain or exemplar: JQ316470).

Genome layout: VPg+5′UTR$^{\text{IRES-IV}}$[1AB-1C-1D-2A1$^{\text{npgp}}$/-2A2-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 6938 nt (5′-UTR: c. 420 nt; ORF: c. 6402 nt; 3′ UTR: 136 nt); length of polyprotein: 2133 aa.

Genus *Passerivirus*

Number of species: 1 (*Passerivirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: GU182406).

Genome layout: VPg+5′UTR$^{\text{IRES}}$[L/1AB-1C-1D/2A$^{\text{H-box/NC}}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 8035 nt (partial) (5′-UTR: > 415 nt; ORF: 7287 nt; 3′ UTR: 334 nt); length of polyprotein: 2428 aa.

Genus *Potamipivirus*

Number of species: 1 (*Potamipivirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: KC843627).

Genome layout: VPg+5′UTR^{IRES}[1AB-1C-1D-2A1^{npgp}/2A2$^{H\text{-}box/NC}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7496 nt (partial) (5′-UTR: > 488 nt; ORF: 6777 nt; 3′ UTR: 231 nt); length of polyprotein: 2259 aa.

Genus *Rabovirus*

Number of species: 1 (*Rabovirus A*, 2 types; GenBank acc. no. of reference strain or exemplar: KJ950883).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[L/1A-1B-1C-1D/2A^{pro}-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: 7834 nt (5′-UTR: 751 nt; ORF: 7005 nt; 3′-UTR: 78 nt); length of polyprotein: 2335 aa.

Genus *Rosavirus*

Number of species: 1 (*Rosavirus A*, 3 types; GenBank acc. no. of reference strain or exemplar: JF973686). There are two tentative new species with 4 types (Table 3).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[1AB-1C-1D-2A^{npgp}/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 8931 nt (5′-UTR: up to 828 nt; ORF: 7413 nt; 3′ UTR: up to 795 nt); length of polyprotein: 2468 aa. Rosaviruses have the longest known 3′-UTR of picornaviruses.

Genus *Sakobuvirus*

Number of species. 1 (*Sakobuvirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: KF387721). There is one tentative sakobuvirus (Table 3).

Genome layout: VPg+5′UTR$^{IRES\text{-}IV}$[L/1AB-1C-1D/2A$^{H\text{-}Box/NC}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7800 nt (5′ UTR: 591 nt; ORF: 7059 nt; 3′-UTR: 157 nt); length of polyprotein: 2352 aa.

Genus *Salivirus*

Number of species: 1 (*Salivirus A*, 2 types; GenBank acc. no. of reference strain or exemplar: GQ179640).

Genome layout: VPg+5′UTR$^{IRES\text{-}V}$[L/1AB-1C-1D/2A$^{H\text{-}Box/NC}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: 7982-8021 nt (5′-UTR: 709-763 nt; ORF: 7110-7125 nt; 3′ UTR: 148 nt); length of polyprotein: 2374 aa.

Genus *Sapelovirus*

Number of species: 3 (*Avian sapelovirus*, 1 type; *Sapelovirus A*, 1 type; *Sapelovirus B*, 3 types; GenBank acc. nos. of reference strains or exemplars: AF406813, AY064708, AY563023).

Genome layout: VPg+5′UTR$^{IRES\text{-}IV}$[L/1A-1B-1C-1D/2A^{pro}-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7491-8226 nt (5′-UTR: up to 741 nt; ORF: 6969-7566 nt; 3′ UTR: 82-235 nt); length of polyprotein: 2322-2521 aa. A great diversity of the three species plus the availability of c. 30 sapelo-like viruses urge a major revision of this genus. Creation of several new genera is indicated.

Genus *Senecavirus*

Number of species: 1 (*Senecavirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: DQ641257).

Genome layout: VPg+5′UTR$^{IRES\text{-}IV}$[L/1A-1B-1C-1D-2A^{npgp}/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7310 nt (5′-UTR: 666 nt; ORF: 6546 nt; 3′-UTR: 71 nt); length of polyprotein: 2181 aa.

Genus *Sicinivirus*

Number of species: 1 (*Sicinivirus A*, 5 types; GenBank acc. no. of reference strain or exemplar: KF741227).

Genome layout: VPg+5′UTR$^{IRES\text{-}II}$[L/1AB-1C-1D/2A$^{H\text{-}box/NC}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: up to 9809 nt (5′-UTR: up to 939 nt; ORF: 8595; 3′-UTR: 308 nt); length of polyprotein: 2864 aa.

Genus *Teschovirus*

Number of species: 1 (*Teschovirus A*, 13 types; GenBank acc. no. of reference strain or exemplar: AF231769).

Genome layout: VPg+5′UTR$^{IRES\text{-}IV}$[L/1A-1B-1C-1D-2A^{npgp}/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: > 7110 nt (5′-end missing) (5′-UTR: >335 nt with poly(C) tract; ORF: 6600-6711; 3′-UTR: 67 nt); length of polyprotein: 2199-2236 aa.

Genus *Torchivirus*

Number of species: 1 (*Torchivirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: KM873611).

Genome layout: VPg+5′UTR^{IRES}[L/1A-1B-1C-1D-2A^{npgp}/2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: 7077 nt (5′-UTR: 192 nt; ORF: 6654 nt; 3′-UTR: 231 nt); length of polyprotein: 2218 aa.

Genus *Tremovirus*

Number of species: 1 (*Tremovirus A*, 1 type; GenBank acc. no. of reference strain or exemplar: AJ225173).

Genome layout: VPg+5′UTR$^{IRES\text{-}IV}$[1A-1B-1C-1D/2A$^{H\text{-}box/NC}$-2B-2C/3A-3B-3C-3D]3′UTR-poly(A).

Genome length: c. 7032 nt (5′-UTR: 494 nt; ORF: 6405; 3′-UTR: 135 nt); length of polyprotein: 2134 aa.

Recombination and the consequences for picornavirus taxonomy

Evidence of RNA recombination in poliovirus and foot-and-mouth disease virus was first presented in the 1960s when cells were infected with mixtures of virus variants and progeny virus showed the wild-type phenotypes [30, 53, 80]. RNA recombination occurs during negative-strand RNA synthesis. The most likely mechanism is described by the "copy choice" hypothesis, which assumes that the RNA polymerase initiates RNA synthesis on one viral RNA molecule, then switches the template to continue replication on another RNA molecule [44]. Intertypic recombination was first demonstrated by King et al. [42], but the vast extent of natural recombination was not figured out until two decades later (reviewed by Lukashev [61]).

RNA recombination occurs in all investigated picornavirus genera and seems to be a very efficient mechanism to create chimeric RNA molecules with new combinations of features, of which the advantageous ones will be selected. Analysis of the speed of adaptation in poliovirus variants with and without the ability to support recombination revealed that the accumulation of beneficial alleles was reduced while detrimental mutations were increased in recombination-defective viruses [106]. It is tempting to assume that new virulent enterovirus variants emerge more efficiently through the interplay of recombination and substitution than by the mere accumulation of exchanges over time, and it is compelling that the high frequency of picornavirus coinfections is one driving force of this mechanism of picornavirus evolution. Several reports describing salient recombinant virus strains support this view (e.g., [24, 40, 48].

Whereas intraspecies/intertypic recombination between picornaviruses is quite common, recombination between members of different species or genera seems to be less frequent. There are a few sequence features that may be interpreted as remnants of previous interspecies recombination events: (i) Whereas the coding region of EV-C109 supports grouping this virus with members of the species *Enterovirus C*, its 5′-noncoding region has similarity to those of members of the species *Enterovirus A* [109]. Clustering of 5′ noncoding sequences of pairs of enteroviruses of different species has been observed for *Enterovirus A* and *B, Enterovirus C* and *D, Enterovirus E* and *F* and *Rhinovirus A* and *C* [33, 65, 67, 111]. Accordingly, recombination in the 5′ noncoding region has been shown for *Rhinovirus A* and *C*, *Enterovirus A* and *C* and *Enterovirus A* and *D* [33], McIntyre et al. [67, 91]. It has been suggested that extensive recombination occurred during earlier stages of type diversification (McIntyre et al. [67]. (ii) EV-G strains isolated from sheep and goats exhibit the typical 5′ noncoding region of bovine enteroviruses (*Enterovirus E, F*) [10, 99]. (iii) Interspecies recombinants with recombination breakpoints in the P2 region have been described by Huang et al. [33] and McIntyre et al. [65] for *Rhinovirus A/C* recombinants and by Kapusinszky et al. [39] for a *Cosavirus D/E* recombinant. (iv) Genome sequencing of several megriviruses has revealed evidence of interspecies recombination and prompted the *Picornaviridae* Study Group to propose a revision of the megrivirus taxonomy in 2017. Available sequence data suggest that *Melegrivirus A* (*MelV-A*) comprises three types. Additional megriviruses have been detected in pigeons, chickens, geese, ducks, harriers and penguins [9, 11, 58, 78, 100]; Zell et al. unpublished). Their sequences can be grouped into a total of five species and 11 types. All strains of *MelV-A1* were revealed to be recombinant, as well as one of two known goose megriviruses. In order to avoid a recombinant exemplar strain of the genus *Megrivirus*, the proposed revision intends to abolish the previous species *Melegrivirus A*. Instead, five proposed species, '*Megrivirus A*' (MeV-A) to '*E*', will be created, each with a nonrecombinant strain as an exemplar. The recombinant turkey hepatitis viruses are moved to the proposed species '*Megrivirus C*', consistent with the phylogenetic relationship of their P3 region. To qualify either origin of the presumed picornavirus recombinants, a novel terminology was also proposed ($A1_{CP}$-C_{pol}), where the relationship to both parental viruses is indicated: the capsid proteins (CP) cluster with MeV-A and the polymerase (pol) with MeV-C. Likewise, goose megrivirus 2 can be described by $B3_{CP}$-A_{pol}. (Suppl. Figs. 1 and 2).

There are also hints suggesting intergenus recombination events: (i) the presence of a type IV IRES in porcine kobuviruses (*Aichivirus C*) rather than a type V IRES [81] and (ii) the similarity of the hunnivirus IRES to the human parechovirus IRES while the hunnivirus polyprotein is more similar to the teschovirus polyprotein [83]. The presence of the HCV-like IRES, the 2A$^{H\text{-}box/NC}$, and torovirus-like proteinase in picornaviruses [12, 46, 89], in press) suggests additional recombination events involving non-picornavirus donors.

Phylogenetic analysis as the main tool of picornavirus taxonomy

Phylogenetic analysis requires alignments of homologous nucleotide or amino acid sequences. For intergenus comparisons of picornaviruses, a reasonable degree of similarity is only detected in the orthologous proteins P1, $2C^{hel}$, $3C^{pro}$ and $3D^{pol}$ or their corresponding gene regions. Concatenated 3C-3D or 2C-3C-3D sequences yield more-robust results due to the lengths of the investigated regions and the compensatory effects of variable and conserved sequence stretches. Problems arise if picornavirus sequences are investigated without the knowledge of precise processing sites. The 3C proteinases of cardioviruses and entero-/rhinoviruses preferably use glutamine-glycine (Q/G) cleavage sites [73]. This lead to the assumption that Q/G sequences are also the cleavage sites of the 3C proteinases of other picornaviruses. However, aphthoviruses and hepatitis A virus use a number of other dipeptide sequences [73]. Due to the lack of biochemical data for many newer picornaviruses, putative processing sites have been proposed on the basis of sequence comparisons of highly divergent viruses. Such predictions have to be treated with caution until reliable experimental results are available.

Phylogenetic trees of the P1 region (Fig. 2) and the 3CD region (Fig. 3) reveal consistent arrangements of many picornavirus genera, suggesting the existence of subfamilies. For the time being, such clusters are designated as 'supergroups' in order to avoid an allusion to a taxonomic relevance of this observation. Five supergroups were proposed originally (Nick J. Knowles; http://www.picornaviridae.com/unassigned/unassigned.htm) and modified with the acknowledgement of new genera. Supergroup (SG) 1 comprises members of the genera *Aphthovirus, Cardiovirus, Cosavirus, Erbovirus, Hunnivirus*, *Mischivirus, Mosavirus, Senecavirus,* and

Fig. 2 Phylogenetic analysis of the picornavirus P1 genome region. One hundred ninety-nine picornavirus P1 nucleotide sequences representing all approved and proposed picornavirus species plus unassigned picornaviruses were aligned with MEGA5 and adjusted manually. Bayesian MCMC tree inference was conducted with MrBayes3.2 using an optimal substitution method (GTR+G+I). Convergence was reached after 4,000,000 generations. Presented are 35 acknowledged genera (printed in italics) plus five proposed genera (prop.) plus unassigned picornaviruses (unass.). Five picornavirus supergroups (SGs) are indicated in different colours. *Ampivirus*, *Dicipivirus, Harkavirus, Megrivirus* and *Rosavirus* (printed in black) do not match the 5-supergroup scheme, suggesting the existence of further supergroups. Supplementary Figure 1 presents details of the same phylogenetic tree

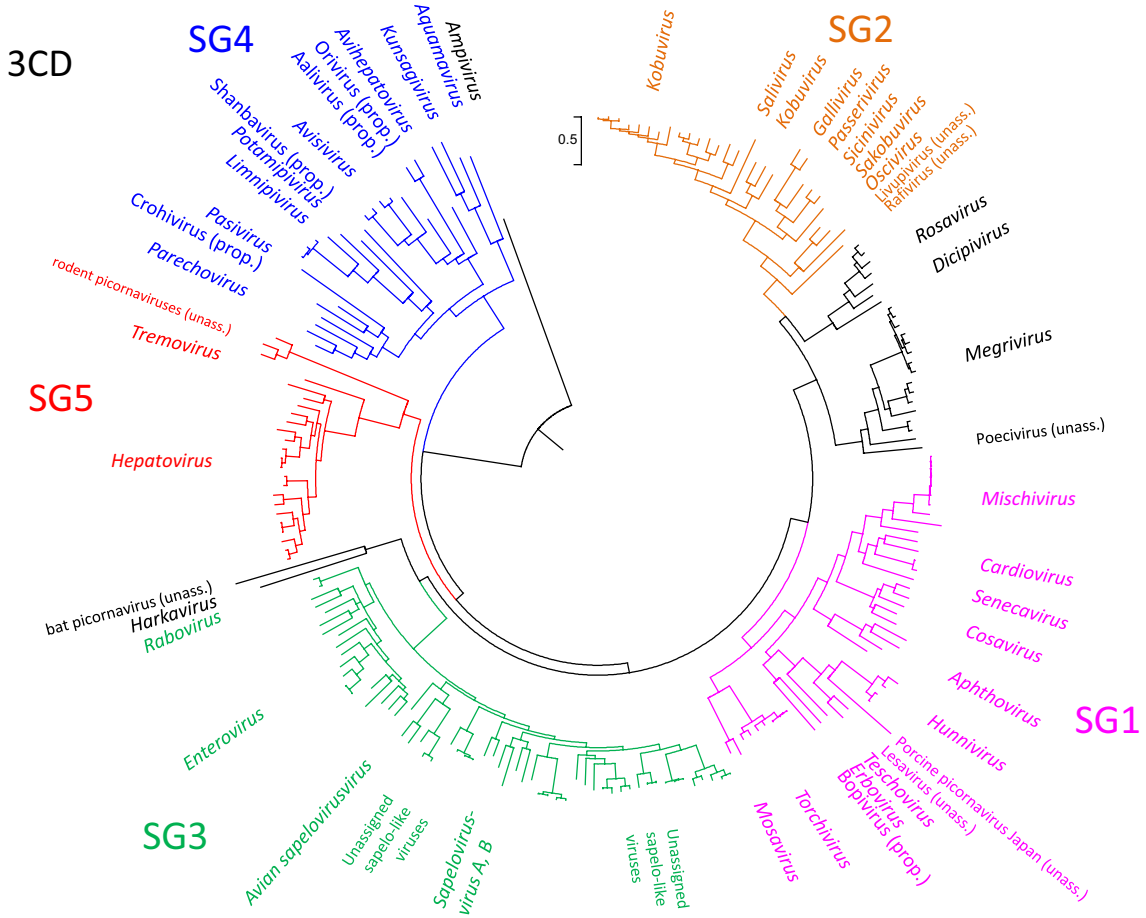

Fig. 3 Phylogenetic analysis of the picornavirus 3CD genome region. Two hundred six picornavirus 3CD nucleotide sequences representing all approved and proposed picornavirus species plus unassigned picornaviruses were aligned with MEGA5 and adjusted manually. Bayesian MCMC tree inference was conducted with MrBayes3.2 using an optimal substitution method (GTR+G+I). Convergence was reached after 6,500,000 generations. Presented are 35 acknowledged genera (printed in italics) plus five proposed genera (prop.) plus unassigned picornaviruses (unass.). Five picornavirus supergroups (SGs) are indicated in different colours. *Ampivirus*, *Dicipivirus, Harkavirus, Megrivirus, Rosavirus,* an unassigned bat picornavirus and poecivirus (printed in black) do not match the 5-supergroup scheme, suggesting the existence of further supergroups. Supplementary Figure 2 presents details of the same phylogenetic tree

Teschovirus. SG2 includes the genera *Gallivirus, Kobuvirus, Oscivirus, Passerivirus, Sakobuvirus, Salivirus,* and *Sicinivirus*. SG3 contains the genera *Enterovirus, Rabovirus,* and *Sapelovirus*. SG4 includes the genera *Aquamavirus, Avihepatovirus, Avisivirus, Kunsagivirus, Limnipivirus, Pasivirus, Parechovirus,* and *Potamipivirus*. SG5 includes the genera *Hepatovirus* and *Tremovirus*. Members of the genera *Ampivirus*, *Dicipivirus*, *Harkavirus, Megrivirus, Rosavirus* and a few unclassified viruses do not fit this scheme, suggesting the existence of further supergroups. As sequences of the five supergroups cluster in robust P1 and 3CD trees (Figs. 2 and 3), the supergroups are possible candidates for the creation of picornavirus subfamilies. Noteworthily, supergroups are supported by phylogenetic analysis only; other features, such as common genome layouts or the consistent presence/absence of certain IRES types, L proteins, or 2A variants, have not been identified. Whether the supergroup hypothesis is a sustainable concept has to be seen. The available sequences of several novel picornaviruses, however, fit well into this scheme, e.g., bopivirus, the unassigned lesaviruses, and porcine picornavirus Japan (SG1), the unassigned rafivirus and livupivirus (SG2), numerous unassigned sapelo-like viruses (SG3), aalivirus, crohiviruses, oriviruses, shanbaviruses (SG4) and the unassigned rodent picornaviruses (SG5) (Figs. 2 and 3).

Outlook

By now, picornaviruses have been detected in five of seven vertebrate classes, but not in invertebrates, fungi, plants, protozoans or procaryotes. If, in future, this observation proves to be true, important conclusions regarding the virus life

cycle, dependence on host factors and co-evolution may be drawn. Methodological and computational advances allow virologists to determine a virus structure by cryo-electron microscopy in very short time. It is likely that the number of picornavirus structures will substantially increase in the near future and improve our knowledge of virus capsids, interaction of viruses with certain antivirals or their receptors, or RNA penetration. Tools have to be established to isolate and propagate in cell culture those viruses which are known only from their sequences. Presently, there is a frustrating disparity in our capabilities of virus isolation and genome sequencing. Without viruses at hand, biochemical and other molecular analyses are hampered. Most annoying is the lack of knowledge of the precise polyprotein processing sites. Likewise, it is crucial to characterize the nonstructural proteins (e.g., by proteomics approaches), many of which have been identified by their sequence motifs only, while their function in the viral life cycle is obscure. Another important task will be the improvement of picornavirus diagnostics. Many picornaviruses have been identified in stools of pets (dogs, cats) and bats, birds and various lower vertebrates in individual cases, raising questions regarding their prevalence in host populations and environmental samples as well as their zoonotic potential, spill-over infections and transmission routes. An improved knowledge of the diversity and prevalence of pathogens will be important for risk assessment and sanitation of animal flocks. Finally, the expansion and further development of picornavirus taxonomy is essential for keeping pace with this ever-growing virus family.

Compliance with ethical standards

Conflict of interest The author chairs the *Picornaviridae* Study Group of the International Committee on Taxonomy of Viruses (ICTV).

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by the author.

Informed consent Not applicable.

References

1. Ambros V, Baltimore D (1978) Protein is linked to the 5′-end of poliovirus RNA by phosphodiester linkage to tyrosine. J Biol Chem 253:5263–5266
2. Andino R, Rieckhof GE, Baltimore D (1990) A functional ribonucleoprotein complex forms around the 5′ end of poliovirus RNA. Cell 63:369–380
3. Baltimore D, Franklin RM, Eggers HJ, Tamm I (1963) Polio-induced RNA polymerase and the effects of virus-specific inhibitors on its production. Proc Natl Acad Sci USA 49:843–849
4. Bardina MV, Lidsky PV, Sheval EV, Fominykh KV, van Kuppeveld FJM, Polyakov VY, Agol VI (2009) Mengovirus-induced rearrangement of the nuclear pore complex: Hijacking cellular phosphorylation machinery. J Virol 83(7):3150–3161
5. Basavappa R, Syed R, Flore O, Icenogle JP, Filman DJ, Hogle JM (1994) Role and mechanism of the maturation cleavage of VP0 in poliovirus assembly: structure for the empty capsid assembly intermediate at 2.9 Å resolution. Protein Sci 3:1651–1669
6. Bird SW, Maynard ND, Covert MW, Kirkegaard K (2014) Nonlytic viral spread enhanced by autophagy components. Proc Natl Acad Sci USA 111:13081–13086
7. Borghese F, Michiels T (2011) The leader protein of cardioviruses inhibits stress granule assembly. J Virol 85(18):9614–9622
8. Boros A, Pankovics P, Adonyi A, Fenyvesi H, Day JM, Phan TG, Delwart E, Reuter G (2015) A diarrheic chicken simultaneously co-infected with multiple picornaviruses: Complete genome analysis of avian picornaviruses representing up to six genera. Virology 489:63–74
9. Boros A, Pankovics P, Knowles NJ, Nemes C, Delwart E, Reuter G (2014) Comparative complete genome analysis of chicken and turkey megriviruses (family *Picornaviridae*): long 3′ untranslated regions with a potential second open reading frame and evidence of possible recombination. J Virology 88(11):6434–6443
10. Boros A, Pankovics P, Knowles NJ, Reuter G (2012) Natural interspecies recombinant bovine/porcine enterovirus in sheep. J Gen Virol 93:1941–1951
11. Boros A, Pankovics P, Matics R, Adonyi A, Bolba N, Phan TG, Delwart E, Reuter G (2017) Genome characterization of a novel megrivirus-related avian picornavirus from a carnivorous wild bird, western marsh harrier (*Circus aeruginosus*). Arch Virol. doi:10.1007/s00705-017-3403-4
12. Bunke J, Receveur K, Oeser AC, Fickenscher H, Zell R, Krumbholz A (2017) High genetic diversity of porcine enterovirus G in Schleswig-Holstein, Germany. Arch Virol. doi:10.1007/s00705-017-3612-x
13. Chen YH, Du W, Hagemeijer MC, Takvorian PM, Pau C, Cali A, Brantner CA, Stempinski ES, Connelly PS, Ma HC, Jiang P, Wimmer E, Altan-Bonnet G, Altan-Bonnet N (2015) Phosphatidylserine vesicles enable efficient en bloc transmission of enteroviruses. Cell 160:619–630
14. Devaney MA, Vakharia VN, Lloyd RE, Ehrenfeld E, Grubman MJ (1988) Leader protein of foot-and-mouth disease virus is required for cleavage of the p220 component of the cap-binding protein complex. J Virol 62:4407–4409
15. Ding C, Zhang D (2007) Molecular analysis of duck hepatitis virus type 1. Virology 361:9–17
16. Donnelly MLL, Luke G, Mehrota A, Li X, Hughes LE, Gani D, Ryan MD (2001) Analysis of the aphthovirus 2A/2B polyprotein 'cleavage' mechanism indicates not a proteolytic reaction, but a novel translational effect: a putative ribosomal 'skip'. J Gen Virol 82:1013–1025
17. Dorsch-Haesler K, Yogo Y, Wimmer E (1975) Replication of picornaviruses. I. Evidence from in vitro RNA synthesis that poly(A) of the poliovirus genome is genetically coded. J Virol 16:1512–1517
18. Du J, Lu L, Liu F, Su H, Dong J, Sun L, Zhu Y, Ren X, Yang F, Guo F, Liu Q, Wu Z, Jin Q (2016) Distribution and characteristics of rodent picornaviruses in Chin. Sci Rep 6:34381
19. Fendrick AM, Monto AS, Nightengale B, Sarnes M (2003) The economic burden of non-influenza-related viral respiratory tract infections in the United States. Arch Intern Med 163:487–494
20. Feng Z, Hensley L, McKnight KL, Hu F, Madden V, Ping L, Jeong SH, Walker C, Lanford RE, Lemon SM (2013) A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature 496:367–371
21. Finch JT, Klug A (1959) Structure of poliomyelitis virus. Nature 183:1709–1714

22. Firth C, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, Conte JM, Ng J, Garcia J, Bhuva NP, Lee B, Che X, Quan PL, Lipkin WI (2014) Detection of zoonotic pathogens and characterizsation of novel viruses carried by commensal *Rattus norvegicus* in New York City. mBio 5:e01933-14
23. Flanegan JB, Pettersson RF, Ambros V, Hewlett MJ, Baltimore D (1977) Covalent linkage of a protein to a defined nucleotide sequence at the 5′-terminus of virion and replicative intermediate RNAs of poliovirus. Proc Natl Acad Sci USA 74:961–965
24. Gaunt E, Harvala H, Österback R, Sreenu AB, Thomson E, Waris M, Simmonds P (2015) Genetic characterization of human coxsackievirus A6 variants associated with atypical hand, foot and mouth disease: a potential role of recombination in emergence and pathogenicity. J Gen Virol 96:1067–1079
25. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators (2016) Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388(10053):1545–1602
26. Grubman MJ, Baxt B (2004) Foot-and-mouth disease. Clin Microbiol Rev 17:465–493
27. Guarné A, Tormo J, Kirchweger R, Pfistermueller D, Fita I, Skern T (1998) Structure of the foot-and-mouth disease virus leader protease: a papain-like fold adapted for self-processing and eIF4G recognition. EMBO J 17:7469–7479
28. Guttman N, Baltimore D (1977) Morphogenesis of poliovirus. IV. Existence of particles sedimenting at 150S having the properties of provirions. J Virol 23:363–367
29. Hindiyeh M, Li QH, Basavappa R, Hogle JM, Chow M (1999) Poliovirus mutants at histidine 195 of VP2 do not cleave VP0 into VP2 and VP4. J Virol 73:9072–9079
30. Hirst GK (1962) Genetic recombination with Newcastle disease virus, polioviruses and influenza. Cold Spring Harbor Symp Quant Biol 27:303–308
31. Hogle JM, Chow M, Filman DJ (1985) Three-dimensional structure of poliovirus at 2.9 A resolution. Science 229:1358–1365
32. Holland JJ, McLaren LC, Hoyer BH, Syverton JT (1960) Enteroviral ribonucleic acid. II. Biological, physical, and chemical studies. J Exp Med 112:841–864
33. Huang T, Wang W, Bessaud M, Ren P, Sheng J, Yan H, Zhang J, Lin X, Wang Y, Delpeyroux F, Deubel V (2009) Evidence of recombination and genetic diversity in human rhinoviruses in children with acute respiratory infection. PLoS One 4(7):e6355
34. Hughes PJ, Stanway G (2000) The 2A proteins of three diverse picornaviruses are related to each other and to the H-rev107 family of proteins involved in the control of cell proliferation. J Gen Virol 81:201–207
35. International Enterovirus Study Group, Melnick JL, Cockburn WC, Dalldorf G, Gard S, Gear JHS, Hammon WM, Kaplan MM, Nagler FP, Oker-Blom N, Rhodes AJ, Sabin AB, Verlinde JD, Von Magnus H (1963) Picornavirus group. Virology 19:114–116
36. Jacobson MF, Baltimore D (1968) Polypeptide cleavages in the formation of poliovirus proteins. Proc Natl Acad Sci USA 61:77–84
37. Jan E, Mohr I, Walsh D (2016) A cap-to-tail guide to mRNA translation strategies in virus-infected cells. Annu Rev Virol 3(1):283–307
38. Kapoor A, Victoria J, Simmonds P, Slikas E, Chieochansin T, Naeem A, Shaukat S, Sharif S, Alam MM, Angez M, Wang C, Shafer RW, Zaidi S, Delwart E (2008) A highly prevalent and genetically diversified Picornaviridae genus in South Asian children. Proc Natl Acad Sci USA 105:20482–20487
39. Kapusinszky B, Phan TG, Kapoor A, Delwart E (2012) Genetic diversity of the genus *Cosavirus* in the family *Picornaviridae*: a new species, recombination, and 26 new genotypes. PLoS One 7(5):e36685
40. Karrasch M, Fischer E, Scholten M, Sauerbrei A, Henke A, Renz DM, Mentzel HJ, Böer K, Böttcher S, Diedrich S, Krumbholz A, Zell R (2016) A severe pediatric infection with a novel enterovirus A71 strain, Thuringia, Germany. J Clin Virol 84:90–95
41. Kiehn ED, Holland JJ (1970) Synthesis and cleavage of enterovirus polypeptides in mammalian cells. J Virol 5:358–367
42. King AMQ, McCahon D, Slade WR, Newman JWI (1982) Recombination in RNA. Cell 29:921–928
43. Kirchweger R, Ziegler E, Lamphear BJ, Waters D, Liebig HD, Sommergruber W, Sobrino F, Hohenadl C, Blaas D, Rhoads RE, Skern T (1994) Foot-and-mouth disease virus leader proteinase: purification of the Lb form and determination of its cleavage site on eIF-4 gamma. J Virol 68:5677–5684
44. Kirkegaard K, Baltimore D (1986) The mechanism of RNA recombination in poliovirus. Cell 47:433–443
45. Kluge M, Campos FS, Tavares M, de Amorim DB, Valdez FP, Giongo A, Roehe PM, Franco AC (2016) Metagenomic survey of viral diversity obtained from feces of subantarctic and South American fur seals. PLoS One 11:e0151921
46. Knutson TP, Velayudhan BT, Marthaler DG (2017) A porcine enterovirus G associated with enteric disease contains a novel papain-like cysteine protease. J Gen Virol 98:1305–1310
47. Kofstad T, Jonassen CM (2011) Screening of feral and wood pigeons for viruses harbouring a conserved mobile viral element: characterization of novel astroviruses and picornaviruses. PLoS One 6:e25964
48. Krumbholz A, Egerer R, Braun H, Schmidtke M, Rimek D, Kroh C, Hennig B, Groth M, Sauerbrei A, Zell R (2016) Analysis of an echovirus 18 outbreak in Thuringia, Germany: insights into the molecular epidemiology and evolution of several enterovirus species B members. Med Microbiol Immunol 205:471–483
49. Lange J, Groth M, Fichtner D, Granzow H, Keller B, Walther M, Platzer M, Sauerbrei A, Zell R (2014) Virus isolate from carp: genetic characterization reveals a novel picornavirus with two ahphthovirus 2A-like sequences. J Gen Virol 95:80–90
50. Lau SK, Woo PC, Lai KK, Huang Y, Yip CC, Shek CT, Lee P, Lam CS, Chan KH, Yuen KY (2011) Complete genome analysis of three novel picornaviruses from diverse bat species. J Virol 85:8819–8828
51. Lau SK, Woo PCY, Yip CCY, Choi GKY, Wu Y, Bai R, Fan RYY, Lai KKY, Chan KH, Yuen KY (2012) Identification of a novel feline picornavirus from the domestic cat. J Virol 86:395–405
52. Lau SK, Woo PC, Yip CC, Li KS, Fan RY, Bai R, Huang Y, Chan KH, Yuen KY (2014) Chickens host diverse picornaviruses originated from potential interspecies transmission with recombination. J Gen Virol 95:1929–1944
53. Ledinko N (1963) Genetic recombination with poliovirus type 1. Studies of crosses between a normal horse serum-resistant mutant and several guanidine-resistant mutants of the same strain. Virology 20:107–119
54. Lee YF, Nomoto A, Detjen BM, Wimmer E (1977) A protein covalently linked to poliovirus genome RNA. Proc Natl Acad Sci USA 74:59–63
55. Li L, Kapoor A, Slikas B, Oderinde BS, Wang C, Shaukat S, Alam MM, Wilson ML, Ndjango JB, Peeters M, Gross-Camp ND, Muller MN, Hahn BH, Wolfe ND, Triki H, Bartkus J, Zaidi SZ, Delwart E (2010) Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. J Virol 84:1674–1682
56. Li L, Shan T, Wang C, Cote C, Kolman J, Onions D, Gulland FM, Delwart E (2011) The fecal flora of California sea lions. J Virol 85:9909–9917
57. Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart E (2010) Bat guano virome: predominance of dietary

viruses from insects and plants plus novel mammalian viruses. J Virol 84:6955–6965
58. Liao Q, Zheng L, Yuan Y, Shi J, Zhang D (2014) Genomic characterization of a novel picornavirus in Pekin ducks. Vet Microbiol 172:78–91
59. Lim ES, Deem SL, Porton IJ, Cao S, Wang D (2015) Species-specific transmission of novel picornaviruses in lemurs. J Virol 89:4002–4010
60. Lozano G, Fernandez N, Martinez-Salas E (2016) Modeling three-dimensional structural motifs of viral IRES. J Mol Biol 428(5):767–776
61. Lukashev AN (2010) Recombination among picornaviruses. Rev Med Virol 20(5):327–337
62. Lwoff A, Torunier P (1966) The classification of viruses. Annu Rev Microbiol 20:45–74
63. Maizel JV (1963) Evidence for multiple components in the structural protein of type 1 poliovirus. Biochem Biophys Res Commun 13:483–489
64. Maizel JV, Summers DF (1968) Evidence for differences in size and composition of the poliovirus-specific polypeptides in infected HeLa cells. Virology 36:48–54
65. McIntyre CL, McWilliam Leitch EC, Savolainen-Kopra C, Hovi T, Simmonds P (2010) Analysis of genetic diversity and sites of recombination in human rhinovirus species C. J Virol 84(19):10297–10310
66. McIntyre CL, Knowles NJ, Simmonds P (2013) Proposals for the classification of human rhinovirus species A, B, and C into genotypically assigned types. J Gen Virol 94:1791–1806
67. McIntyre CL, Savolainen-Kopra C, Hovi T, Simmonds P (2013) Recombination in the evolution of human rhinovirus genomes. Arch Virol 158:1497–1515
68. Nagai M, Omatsu T, Aoki H, Kaku Y, Belsham GJ, Haga K, Naoi Y, Sano K, Umetsu M, Shiokawa M, Tsuchiaka S, Furuya T, Okazaki S, Katayama Y, Oba M, Shirai J, Katayama K, Mizutani T (2015) Identification and complete genome analysis of a novel bovine picornavirus in Japan. Virus Res 210:205–212
69. Nagy PD, Strating JRPM, van Kuppeveld FJM (2016) Building viral replication organelles: close encounters of the membrane types. PLOS Pathog 12:e1005912
70. Naoi Y, Kishimoto M, Masuda T, Ito M, Tsuchiaka S, Sano K, Yamasato H, Omatsu T, Aoki H, Furuya T, Katayama Y, Oba M, Okada T, Shirai J, Mizutani T, Nagai M (2016) Characterization and phylogenetic analysis of a novel picornavirus from swine feces in Japan. Arch Virol 161:1685–1690
71. Oberste MS, Maher K, Kilpatrick DR, Pallansch MA (1999) Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. J Virol 73:1941–1948
72. Oem JK, Lee MH, Lee KK, An DJ (2014) Novel kobuvirus species identified from black goat with diarrhea. Vet Microbiol 172:563–567
73. Palmenberg AC (1990) Proteolytic processing of picornaviral polyprotein. Annu Rev Microbiol 44:603–633
74. Pankovics P, Boros A, Reuter G (2012) Novel picornavirus in domesticated common quail (*Coturnix coturnix*) in Hungary. Arch Virol 157:525–530
75. Pankovics P, Boros A, Matics R, Kapusinszky B, Delwart E, Reuter G (2017) Ljungan/Sebokele-like picornavirus in birds of prey, common kestrel (*Falco tinnunculus*) and red-footed falcon (*F. verspertinus*). Inf Genet Evol 55:14–19
76. Pankovics P, Boros A, Toth Z, Phan TG, Delwart E, Reuter G (2017) Genetic characterization of a second novel picornavirus from an amphibian host, smooth newt (*Lissotriton vulgaris*). Arch Virol 162:1043–1050
77. Paul AV, Wimmer E (2015) Initiation of protein-primed picornavirus RNA synthesis. Virus Res 206:12–26
78. Phan TG, Vo NP, Boros A, Pankovics P, Reuter G, Li OTW, Wang C, Deng X, Poon LLM, Delwart E (2013) The viruses of wild pigeon droppings. PLOS One 8(9):e72787
79. Porter FW, Brown B, Palmenberg AC (2010) Nucleoporin phosphorylation triggered by the encephalomyocarditis virus leader protein is mediated by mitogen-activated protein kinases. J Virol 84(24):12538–12548
80. Pringle (1965) Evidence of genetic recombination in foot-and-mouth disease virus. Virology 25:48–54
81. Reuter G, Boldizsar A, Pankovics P (2009) Complete nucleotide and amino acid sequences and genetic organization of porcine kobuvirus, a member of a new species in the genus *Kobuvirus*, family *Picornaviridae*. Arch Virol 154:101–108
82. Reuter G, Boros G, Földvari G, Szekeres S, Matics R, Kapusinszky B, Delwart E, Pankovics P (2017) Dicipivirus (family Picornaviridae) in wild Northern white-breasted hedgehog (*Erinaceus roumanicus*). Arch Virol. doi:10.1007/s00705-017-3565-0
83. Reuter G, Pankovics P, Knowles NJ, Boros A (2012) Two closely related novel picornaviruses in cattle and sheep in Hungary from 2008 to 2009, proposed as members of a new genus in the family *Picornaviridae*. J Virol 86:13295–13302
84. Robinson SC, Tsueng G, Sin J, Mangale V, Rahawi S, McIntyre LL, Williams W, Kha N, Cruz C, Hancock BM, Nguyen DP, Sayen MR, Hilton BJ, Doran KS, Segall AM, Wolkowicz R, Cornell CT, Whitton JL, Gottlieb RA, Feuer R (2014) Coxsackievirus B exits the host cell in shed microvesicles displaying autophagosomal markers. PLoS Pathog 10:e1004045
85. Rossmann MG, Johnson JE (1989) Icosahedral RNA virus structure. Annu Rev Biochem 58:533–573
86. Rothberg P, Harris T, Nomoto A, Wimmer E (1978) The genome-linked protein of picornaviruses. V. O^4-(5′uridylul)-tyrosine is the bond between the genome-linked protein and the RNA of poliovirus. Proc Natl Acad Sci USA 75:4868–4872
87. Rueckert R, Wimmer E (1984) Systematic nomenclature of picornavirus proteins. J Virol 59:957–959
88. Schwerdt CE, Schaffer FL (1955) Some physical and chemical properties of purified poliomyelitis virus preparations. Ann N Y Acad Sci 61:740–750
89. Shang P, Misra S, Hause B, Fang Y (2017) A naturally occurring recombinant enterovirus expresses a torovirus deubiquitinase. J Virol 91:e00450-17
90. Simmonds P, Adams MJ, Benko M, Breitbart M, Brister JR, Carstens EB, Davison AJ, Delwart E, Gorbalenya AE, Harrach B, Hull R, King AMQ, Koonin EV, Krupovic M, Kuhn JH, Lefkowitz EJ, Nibert ML, Orton R, Roossinck MJ, Sabanadzovic S, Sullivan MB, Suttle CA, Tesh RB, van der Vlugt RA, Varsani A, Zerbini FM (2017) Virus taxonomy in the age of metagenomics. Nat Rev Microbiol 15:161–168
91. Smura T, Blomqvist S, Paananen A, Vuorinen T, Sobotova Z, Bubovica V, Ivanova O, Hovi T, Roivainen M (2007) Enterovirus surveillance reveals proposed new serotypes and provides new insight into enterovirus 5′-untranslated region evolution. J Gen Virol 88:2520–2526
92. Stanway G, JokiKorpela P, Hyypiä T (2000) Human parechoviruses—biology and clinical significance. Rev Med Virol 10:57–69
93. Strating JRPM, van Kuppeveld FJM (2017) Viral rewiring of cellular lipid metabolism to create membranous replication compartments. Curr Opin Cell Biol 47:24–33
94. Summers DF, Maizel JV, Darnell JE (1965) Evidence for virus-specific noncapsid protein in poliovirus-infected HeLa cells. Proc Natl Acad Sci USA 54:505–513
95. Tapparel C, Siegrist F, Kaiser L (2013) Picornavirus and enterovirus diversity with associated human diseases. Infect Genet Evol 14:282–293

96. Tuthill TJ, Groppelli E, Hogle JM, Rowlands DJ (2010) Picornaviruses. Curr Top Microbiol Immunol 343:43–89
97. van der Schaar HM, Dorobantu CM, Albulescu L, Strating JRPM, van Kuppeveld FJM (2016) Fat(al) attraction: Picornaviruses usurp lipid transfer at membrane contact sites to create replication organelles. Trends Microbiol 24:535–546
98. Vaughan G, Goncalves Rossi LM, Forbi JC, de Paula VS, Purdy MA, Xia G, Khudyakov YE (2014) Hepatitis A virus: host interactions, molecular epidemiology and evolution. Infect Genet Evol 21:227–243
99. Wang M, He J, Lu H, Liu Y, Deng Y, Zhu L, Guo C, Tu C, Wang X (2017) A novel enterovirus species identified from severe diarrheal goats. PLoS One 12:e0174600
100. Wang F, Liang T, Liu N, Ning K, Yu K, Zhang D (2017) Genetic characterization of two novel megriviruses in geese. J Gen Virol 98:607–611
101. Wildy P (1971) Classification and nomenclature of viruses. First report of the International Committee on Nomenclature of Viruses. Monogr Virol 5:1–81
102. Woo PCY, Lau SKP, Choi GKY, Huang Y, Teng JLL, Tsoi HW, Tse H, Yeung ML, Chan KH, Jin DY, Yuen KY (2012) Natural occurrence and characterization of two internal ribosome entry site elements in a novel virus, canine picodicistrovirus, in the picornavirus-like superfamily. J Virol 86:2797–2808
103. Woo PCY, Lau SK, Choi GK, Yip CC, Huang Y, Tsoi HW, Yuen KY (2012) Complete genome sequence of a novel picornavirus, canine picornavirus, discovered in dogs. J Virol 86:3402–3403
104. Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, Zhang J, Dong J, Sun L, Du J, Liu L, Xue Y, Wang J, Yang F, Zhang S, Jin Q (2012) Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces. J Virol 86:10999–11012
105. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, Qian Z, Dong J, Sun L, Zhu Y, Du J, Yang F, Zhang S, Jin Q (2016) Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. ISME J 10:609–620
106. Xiao Y, Rouzine IM, Bianco S, Acevedo A, Faul Goldstein E, Farkov M, Brodsky L, Andino R (2016) RNA recombination enhances adaptability and is required for virus spread and virulence. Cell Host Microbe 19:493–503
107. Yinda CK, Zell R, Deboutte W, Zeller M, Conceicao-Neto N, Heylen E, Maes P, Knowles NJ, Ghogomu SM, Van Ranst M, Matthijnssens J (2017) Highly divers population of *Picornaviridae* and other members of the *Picornavirales*, in Cameroonian fruit bats. BMC Genomics 18:249
108. Yogo Y, Wimmer E (1972) Polyadenylic acid at the 3′-terminus of poliovirus RNA. Proc Natl Acad Sci USA 69:1877–1882
109. Yozwiak NL, Skewes-Cox P, Gordon A, Saborio S, Kuan G, Malmaseda A, Ganem D, Harris E, DiRisi JL (2010) Human enterovirus 109: a novel interspecies recombinant enterovirus isolated from a case of acute pediatric respiratory illness in Nicaragua. J Virol 84:9047–9058
110. Zell R, Delwart E, Gorbalenya AE, Hovi T, King AMQ, Knowles NJ, Lindberg AM, Pallansch MA, Palmenberg AC, Reuter G, Simmonds P, Skern T, Stanway G, Yamashita T, ICTV Report Consortium (2017) ICTV virus taxonomy profile: *Picornaviridae*. J Gen Virol 98:2421–2422
111. Zell R, Krumbholz A, Dauber M, Hoey E, Wutzler P (2006) Molecular-based reclassification of the bovine enteroviruses. J Gen Virol 87:375–385
112. Zhang T, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SW, Hibberd ML, Liu ET, Rohwer F, Ruan Y (2006) RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS Biol 4:e3
113. Zylberberg M, Van Hemert C, Dumbacher JP, Handel CM, Tihan T, DeRisi JL (2016) Novel picornavirus associated with avian keratin disorder in Alaskan birds. mBio 7:e00874-16