Resolving deep evolutionary relationships within the RNA virus phylum Lenarviricota

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Abstract

The RNA virus phylum Lenarviricota is composed of the fungi-associated families Narnaviridae and Mitoviridae, the RNA bacteriophage Leviridae, and the plant and fungi-associated Botourmaviridae. Members of the Lenarviricota are abundant in most environments and boast remarkable phylogenetic and genomic diversity. As this phylum includes both RNA bacteriophage and fungi- and plant-associated species, the Lenarviricota likely mark a major evolutionary transition between those RNA viruses associated with prokaryotes and eukaryotes. Despite the remarkable expansion of this phylum following metagenomic studies, the phylogenetic relationships among the families within the Lenarviricota remain uncertain. Utilising a large data set of relevant viral sequences, we performed phylogenetic and genomic analyses to resolve the complex evolutionary history within this phylum and identify patterns in the evolution of virus genome organisation. Despite limitations reflecting very high levels of sequence diversity, our phylogenetic analyses suggest that the Leviridae comprise the basal lineage within the Lenarviricota. Our phylogenetic results also support the construction of a new virus family—the Narniviridae—comprising a set of diverse and phylogenetically distinct species, including a number of uniquely encapsidated viruses. We propose a taxonomic restructuring within the Lenarviricota to better reflect the phylogenetic relationships documented here, with the Botourmaviridae and Narnaviridae combined into the order Narnaviridae, the Mitoviridae remaining in the order Wolframvirales, and these orders combined into the single class, the Amabiliviricetes. In sum, this study provides insights into the complex evolutionary relationships among the diverse families that make up the Lenarviricota.

Key words: lenarviricota; mitoviridae; metatranscriptomics; phylogenetics; virus taxonomy; genome structure.

1. Introduction

The families Narnaviridae and Mitoviridae within the phylum Lenarviricota arguably comprise the simplest of all RNA viruses. They possess very small positive-sense single-stranded genomes (<4,000 nucleotides in length), usually encode a single protein— the RNA-dependent RNA polymerase (RdRp)—and uniquely lack the capsid protein often considered a defining feature of RNA viruses (Hillman and Cai 2013). Although originally discovered in fungal hosts, these families have recently been detected in other microbes, including protists (Akopyants et al. 2016; Grybchuk et al. 2018; Charon et al. 2019; Charon, Murray, and Holmes 2021) and diatoms (Urayama, Takaki, and Nunoura 2016). In addition, some narnaviruses and mitoviruses contain genes additional to the RdRp (Shi et al. 2016; Grybchuk et al. 2018; Charon et al. 2019; Wolf et al. 2020; Charon, Murray, and Holmes 2021).

The Narnaviridae and Mitoviridae were previously classified as two distinct genera (Narnavirus and Mitovirus, respectively) within the family Narnaviridae that could be differentiated by their site of replication. Narnavirus are restricted to the cell cytosol, while mitoviruses replicate within the cell mitochondria (Hillman and Cai 2013). Accordingly, not only do these families utilise different cell machinery in their replication cycle, but mitoviruses utilise the mitochondrial genetic code, in which the amino acid tryptophan is not only encoded by UGG, but also by UGA that results in a stop codon in the standard genetic code (Cole et al. 2000; Shackelton and Holmes 2008). The function of the UGA codon in the mitoviruses that infect fungi appears to match the bias in the mitochondrial genomes of their hosts (Nibert 2017). These factors, as well as more recent phylogenetic studies (Wolf et al. 2018), particularly utilising data from expansive metagenomic sequencing studies (for example, Shi et al. 2016; Wolf et al. 2020), have led to a taxonomic revision and their current status as two separate families classified into separate orders and classes within the Lenarviricota. Indeed, according to the most recent International Committee on Taxonomy of Viruses (ICTV) release, the Narnaviridae fall within the order Wolframvirales and class Amabiliviricetes, while the Mitoviridae are members of the order Cryppavirales, class Hauloviricetes (Walker et al. 2020).
Phylogenetic studies have also shown that the Narnaviridae are related to the plant and filamentous-fungi-infecting viruses of the family Botourmiaviridae (Shi et al. 2016; Wolf et al. 2018) that are classified within the order Ourmivirales, class Maviricetes of the Levanriviridae (Aylión et al. 2020; Walker et al. 2020). The Botourmiaviridae were initially classified as a single floating genus—‘Ourmavirus’—following the discovery of the type species, Ourmia melon virus (Aylión et al. 2020). Ourmaviuses, of which there are currently only three, are plant-infecting, capsidated, RNA viruses, whose seemingly chimeric genomes are arranged as three segments that encode a narnavirus-like RdRp, a picornavirus-like capsid protein, and a tombusvirus-like movement protein (Rastgou et al. 2009). The other genera currently placed within the Botourmiaviridae—Botoulivirus, Penoulivirus, Magoulivirus, Rhizoulivirus, and Scleroulivirus—each named after the fungal species in which they were discovered—have much smaller and simpler genomes than the ourmaviuses, ranging between 2 kb and 3.4 kb in length, and only encode an RdRp (Donaire, Rozas, and Ayllón 2016; Marzano et al. 2016; Illana et al. 2017; Nerva et al. 2019). These genera also differ in host range, infecting filamentous fungi as opposed to plants (Aylión et al. 2020).

Based on previous phylogenetic analyses of the RdRp, the Narnaviridae, Botourmiaviridae, and Mitoviridae have been proposed as related to the bacteriophage-associated Leviviridae (Shi et al. 2016; Wolf et al. 2018). Members of the Leviviridae infect gram-negative bacteria including Enterobacter, Acinetobacter, Caulobacter, and Pseudomonas (King et al. 2012). Leviviruses are widespread and abundant in a range of environments, particularly animal faeces and sediment (Chen et al. 2021). Like the Narnaviridae and Mitoviridae, the Leviviridae are unenveloped and possess very small genomes (<4.3 kb in length). However, while most narnaviruses, botourmiaviruses, and mitoviruses are only composed of an RdRp, the Leviviridae genome is more complex and encodes a capsid protein, a maturation protein, and in some cases, a lysis protein. A read-through protein that extends the capsid protein through the suppression of the terminal UGA codon is also found in some cases (King et al. 2012).

As reflected by the narnaviruses and mitoviruses, the earliest discovered viruses within the phylum Levanriviricota were defined phenotypically and distinguished by host specificity. However, following the rise of metagenomic sequence data, they now comprise a large clade of diverse but distinct species previously classified as ‘narna-like’ viruses, some of which are encapsidated and which we propose might be considered a new family that we tentatively call the Narliviridae. Based on the phylogenetic patterns and genomic structures observed in this study, we also propose a taxonomic reorganization of these three families into the singular class Amabiliviricetes.

2. Methods

2.1 Data collection and processing

We analysed a database comprising 442 meta-transcriptomic libraries from soil, sediment, and animal faecal samples collected, sequenced, and assembled as described previously (Chen et al. 2021). Briefly, 442 RNA-sequencing libraries were generated from samples taken across a wide range of environments and geographical regions in China. These environments included forests, farmland, desert, water environments and sediments, and animal faeces. Total RNA was extracted using the RNasy® PowerSoil® Total RNA Kit (Qiagen), and each library was sequenced on the Illumina HiSeq X10 platform. The resulting reads were adaptor and quality-trimmed using Trimmomatic (Bolger, Lohse, and Usadel 2014) and assembled de novo using MEGAHIT (Li et al. 2015).

To identify viral hits the assembled contigs were compared to a database, curated in 2019, of Riboviria RdRp sequences available on GenBank using DIAMOND BLASTX (Buchfink, Xie, and Huson 2015). RdRp sequences were obtained by searching the National Center for Biotechnology Information (NCBI) non-redundant (nr) protein database for ‘RdRp’ and ‘RNA dependent RNA polymerase’ entries using Entrez Programming Utilities (https://www.ncbi.nlm.nih.gov/books/NBK25501). All contigs returning a match to a viral RdRp sequence were then run against the nr protein database using DIAMOND BLASTX (Buchfink, Xie, and Huson 2015) with a more sensitive e-value threshold of 1 × 10⁻5 to exclude false-positives. Those contigs returning a positive hit to a viral RdRp sequence and over 1,000 nucleotides in length were considered likely to be bona fide viral sequences and selected for further analysis, particularly expansive comparisons with other members of the Levanriviricota.

2.2 Sequence alignment and phylogenetic analysis

Contigs that had DIAMOND BLASTX (Buchfink, Xie, and Huson 2015) hits to members of the Mitoviridae and Narnaviridae and were over 1,000 nucleotides in length were imported into Geneious Prime (v2019.1.1). Sequences with multiple stop codons were translated using the mitochondrial genetic code and checked to ensure they resembled a viral RdRp, namely, the presence of viruses is hypothesised to have occurred during an endosymbiotic event potentially over 1.45 billion years ago in which the α-proteobacteria became intracellular symbionts (Martin and Mentel 2010), after which these mitochondrial viruses escaped to the cell cytosol and became what are now known as the narnaviruses (Wolf et al. 2018). Wolf et al. (2018) further suggest that the Mitoviridae gave rise to the plant-infecting ourmaviuses alongside the Narliviridae, making these sister clades with a mitovirus-like common ancestor.

Using a large data set of relevant viruses, we attempted to resolve the evolutionary relationships, and hence transitions, among the diverse virus families that comprise the phylum Levanriviricota. In addition, we provide insights into the complex phylogeny of the Narnaviridae and Botourmiaviridae, identifying a large clade of diverse but distinct species previously classified as ‘narna-like’ viruses, some of which are encapsidated and which we propose might be considered a new family that we tentatively call the Narliviridae. Based on the phylogenetic patterns and genomic structures observed in this study, we also propose a taxonomic restructuring of these three families into the singular class Amabiliviricetes.
conserved A (-DX-D-), B, and C (-GDD-) amino acid motifs in the palm domain of the RdRp (Jácome et al. 2015). These novel viruses were then aligned using Multiple Alignment using Fast Fourier Transform (MAFFT) (v7.450) (Katoh and Standley 2013) with reference RdRp sequences from the Lenarviricota (1,292 reference sequences). Five additional sequence alignments were constructed comprising the novel virus sequences, the Lenarviricota reference sequences, and established members of each of the following families that served as outgroups to root the phylogenies and hence infer the direction of evolutionary change: the Astroviridae (42 sequences), the Partitiviridae-Picobirnaviridae clade (321 sequences), Picornaviridae (176 sequences), Potyviridae (230 sequences), and Tombusviridae (233 sequences). These outgroups were chosen based on their phylogenetic proximity from a large-scale RdRp phylogeny (Wolf et al. 2018). A midpoint-rooted phylogenetic tree was also inferred. Reference sequences, a large proportion of which were described recently (Chen et al. 2021), were obtained by searching the NCBI nr protein database for relevant family and genera names within the Lenarviricota, as well as for the prefixes of all families (i.e. ‘narna’, ‘mito’, ‘levi’, and ‘ourmia’) to include unclassified sequences that contained ‘-like’ in their names. Reference sequence lists were checked manually to ensure that the top hit of each potentially novel virus was included.

The resultant amino acid sequence alignments were trimmed using trimAl (v1.4.1) with conservation thresholds between 3 and 8 per cent (Capella-Gutierrez, Silla-Martinez, and Gabaldon 2009) to remove any ambiguously aligned regions and retain only the most conserved 500–680 amino acid positions. The best-fit amino acid substitution model was determined using ModelFinder (Kalyaanamoorthy et al. 2017) and found to be the Dayhoff model in all cases (although topologically equivalent phylogenies were produced using the Le-Gascuel model; not shown). Maximum likelihood phylogenetic trees were then estimated on these data employing 1,000 Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) replicates in IQ-TREE (v1.6.12) (Nguyen et al. 2015). Three smaller ‘sub-trees’ were generated using the same method, the first only utilising the Amabiliviricetes (562 sequences), with the second and third based on an alignment of only the Mitoviridae (562 sequences) and Leviviridae (464 sequences), respectively. The unrooted Lenarviricota tree was visualised in FigTree (v1.4.4). All other trees were visualised in R (v4.1.0) using the packages ape (v5.5) (Paradis and Schliep 2019) and ggtree (v3.0.2) (Yu et al. 2017).

2.3 Sequence annotation
To identify possible links between genome structure and the evolutionary patterns within and between the families that comprise the Lenarviricota, we used Prokka (v1.14.5) (Seemann 2014) to annotate the genomes of all available narnavirus and narlivirus sequences, as well as twelve botourmiaviruses, forty mitoviruses, and eighty-nine leviviruses. The representative sequences from the latter groups were chosen based on the phylogenies estimated here to obtain an even distribution across all genera and/or major clades.

3. Results
Our analysis of 442 meta-transcriptomic sequencing libraries from soil, sediment, and animal faeces identified 236 novel mitoviruses utilising the mitochondrial genetic code: that is, when translated under the standard genetic code these viruses contained large numbers of internal UGA stop codons. These novel viruses were aligned with other members of the Lenarviricota (sequences ranging between 326 and 1,913 amino residues in length, final alignment length of 680 amino acids) to generate an RdRp phylogenetic tree from 1,542 RNA virus sequences. Branch lengths are scaled according to the number of amino acid substitutions per site, indicated by the scale bar.

Figure 1. Unrooted phylogeny of the phylum Lenarviricota based on the RdRp domain from 1,542 RNA virus sequences. Branch lengths are scaled according to the number of amino acid substitutions per site, indicated by the scale bar.
occupied the basal position when the tree was midpoint rooted, (2) the Botourmiaviridae, and (3) the newly identified Narliviridae (Fig. 3, Supplementary Fig. S1). Notably, the three plant-infecting members of the genus Ourmiavirus did not fall within the Botourmiaviridae despite being classified in this family. Rather, they grouped with the Narliviridae in every phylogeny (Figs 1–3, Supplementary Fig. S1).

We next annotated the nucleotide sequences of several representative species or clades to identify how well differences in viral genome structure accorded with the overall evolutionary relationships (Figs 3–6). In particular, we carefully annotated the genomes of the Narnaviridae and Narliviridae within the Amabiliviricetes group (Fig. 3), for which the majority of sequences appeared to be complete. As expected, the majority of the traditional Narnaviridae had a single ORF encoding only an RdRp protein. There were four exceptions: Leptomonas seymouri narna-like virus, Sanxia water strider virus 1, Beihai narna-like virus 23, and Halia narna-like virus. These viruses did not group together, nor did they have similar genome structures, such that they comprised distinct and divergent narnaviruses with unique genome structures. The twelve representative botourmiaviruses and a large majority of the Narliviridae displayed similarly simple genomes to the Narnaviridae, only encoding an RdRp gene (Fig. 3). However, four distinct clades within the Narliviridae contained an additional protein that exhibited 25–82 per cent sequence similarity to viral capsids (Fig. 3). The first clade (Fig. 3, Point A) fell in a basal location within the family, although the associated bootstrap support was low (47.7 per cent) such that the branching position is uncertain. The second and third capsid gains appeared to have evolved more recently (Fig. 3, Points B and C). Most notably, the most recently diverged clade did not contain this additional capsid protein (Fig. 3, Point D), instead reverting to the single RdRp gene, although again with little bootstrap support such that the branching order is uncertain. The fourth occurrence of a capsid protein was within the three ourmiaviruses, each of which had a tri-segmented genome comprising the RdRp, movement protein, and capsid protein, respectively (Fig. 3).

Interestingly, the capsid genes in each of the four occurrences displayed sequence similarity to different sets of other viruses, although usually still with very high levels of divergence (<30 per cent amino acid sequence similarity) (Fig. 4). In the case of the most basal capsid clade (Point A), there was a sequence similarity to the capsid genes of Shahe tombus-like virus 2 and Changjiang narna-like virus 3, as well as to those from some tombusvirus-like and potyvirus-like viruses (Fig. 4). In contrast, the capsid genes at Point B all exhibited sequence similarity to Wenzhou narna-like virus 5. The final group of capsidated viruses (Fig. 4, Point C) had closest matches to Changjiang narna-like virus 2, Hubei narna-like viruses 9 and 10, Hubei tombus-like virus 33, and the nodavirus-like and weivirus-like capsid genes (Fig. 4, Point C). Although the phylogenetic history of these viruses is difficult to infer in places, it is possible that the capsid protein has evolved multiple times independently in the Narliviridae and may have also been lost in one clade.

We similarly annotated genomes within the Mitoviridae and Leviridae. Overall, thirty-six of the forty representative species within the Mitoviridae contained a single ORF encoding an RdRp (Fig. 5). The four mitoviruses containing additional genes—Daimones mito-like virus, Aiolos mito-like virus, Asopus mito-like virus, and Proteus mito-like virus—were all associated with microalgae, and the latter three displayed ambigrammatic genomes (Charon, Murray, and Holmes 2021); that is, genomes

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Figure 2. Phylogenies of the phylum Lenarviricota estimated using different groups of RNA viruses as potential outgroups: (A) Astroviridae, (B) Partitiviridae–Proobirnaviridae, (C) Picornaviridae, (D) Potyviridae, and (E) Tombusviridae. Finally, tree (F) is a midpoint-rooted phylogeny with no outgroup. The branch length scale bar represents 0.5 amino acid substitutions per site. Nodes with SH-aLRT support over 80 per cent are marked with circles. Each tree is rooted on its respective outgroup.
that contain a long, uninterrupted ORF spanning a large proportion of the reverse complement genome (DeRisi et al. 2019) (Fig. 5, Supplementary Fig. S2). In contrast, the majority of Leviviidae genomes contained three genes encoding a maturation protein, a capsid protein, and the viral RdRp (Fig. 6). In several species, a levivirus or levi-like virus lysis protein was also identified. Notably, the leviviruses encoding a lysis protein did not form a single monophyletic group, although they were only present in one of the two lineages within the phylogeny of this family (Fig. 6). Finally, one small group of four leviviruses contained a read-through protein alongside its maturation protein, capsid protein, and RdRp (Fig. 6).

4. Discussion

The phylum Lenarviricota is composed of RNA viruses that likely mark a major evolutionary transition event between RNA bacteriophage and early eukaryote-infecting RNA viruses. Members of this unique phylum are highly diverse, abundant in virtually every environment (Chen et al. 2021), and associated with a broad range of hosts including bacteria, protists, fungi, and plants. Here, we investigate the evolutionary relationships among the diverse families comprising the Lenarviricota, utilising a large data set of relevant viral sequences—including 236 novel mitoviruses identified in this study—the majority of which have been obtained from large-scale metagenomic studies.
Figure 4. Phylogeny of the Narliviridae (left) within the class Amabiliviricetes based on the RdRp domain. Collapsed clades - Botourmiaviridae (upper) and Narnaviridae (lower) - are shown as squares. Phylogenies estimated using the capsid protein sequences are shown in boxes on the right. Tip colours in the RdRp phylogeny and branch colours in the capsid phylogenies represent the closest capsid protein Blastx hits. The branch length scale bar represents 0.5 amino acid substitutions per site. Nodes with SH-aLRT support over 80 per cent are marked with circles in capsid protein phylogenies. The trees are midpoint rooted.
A key goal of our study was to resolve the phylogenetic history of the phylum Lenarviricota. Due to a trichotomy (and similar levels of divergence) between the Leuviridae, the Mitoviridae, and the Amabiliviricetes, the exact pattern of ancestor–descendent relationships among these viruses and hence between those viruses infecting prokaryotes and eukaryotes is difficult to determine. In addition, the long branches at the base of each group imply missing phylogenetic diversity that has yet to be identified. To help overcome these major issues in phylogenetic analysis, we rooted the Lenarviricota phylogeny using five different outgroups—the families Astroviridae, a Partitiviridae–Picobirnaviridae clade, Picornaviridae, Potyviridae, and Tombusviridae—as well as estimating a simple midpoint-rooted tree. Notably, however, this analysis did not result in a consistent tree topology. This is most clearly seen in the tree rooted on the Potyviridae, in which neither the Narviridae nor the Amabiliviricetes formed monophyletic groups and a group of divergent mitoviruses fell basal to the entire Lenarviricota phylum. Hence, the use of highly divergent outgroups cannot reliably resolve the evolution of the Lenarviricota. Indeed, it is clear that RNA viruses as a whole and likely the Lenarviricota, in particular, are too diverse to align with sufficient reliability to produce a robust phylogeny tree (Edgar 2021), with individual amino acid sites subject to extensive multiple substitution (Holmes and Duchêne 2019).

Despite these limitations, given that the codon bias in Mitoviridae genomes reflects that of their respective fungal hosts (Nibert 2017), the alternative codon usage by members of the Mitoviridae is likely a derived, adaptive function acquired after the origin of organisms containing the mitochondria. This means the mitoviruses are unlikely to be an ancestral group to RNA viruses as a whole as implied in the phylogeny using the Tombusviridae as an outgroup. In addition, the most common and perhaps likely phylogenetic pattern observed in this study (in three of the six phylogenetic trees) suggests that the Leuviridae is the basal lineage within the Lenarviricota, with the Mitoviridae and Amabiliviricetes falling as derived groups. This supports the most popular hypothesis for the evolutionary pathway of this phylum, in which a leuvivirus-like ancestral virus gave rise to the Mitoviridae that acquired the capacity to replicate in the newly emerged mitochondrion of early eukaryotic organisms (Koonin and Dolja 2014; Wolf et al. 2018).

The Botourmiaviridae were previously considered to be a monophyletic, phylogenetically distinct sister clade to the Narnaviridae (Ayllón et al. 2020). However, the phylogeny of the Narnaviridae, Botourmiaviridae, and sequences classified as 'narna-like' at their time of discovery—that we now term as the Narliviridae—has changed considerably with the periodic addition of a huge number of diverse viruses found in invertebrates, soil, and marine samples.
In all phylogenies estimated here using an alignment containing sequences from the Amalibiviricetes, the non-encapsidated, filamentous-fungi-infecting genera within Botourmiaviridae (Botoulivirus, Magaolivirus, Penoulivirus, Rhizoulivirus and Scleroulivirus) remained monophyletic. However, according to our phylogenetic analysis the family no longer includes the plant-infecting ourmiaviruses, which instead appear to have evolved from an entirely different lineage within the Nariliviridae. Importantly, this contradicts previous phylogenetic analyses and thus challenges the family’s current taxonomic organisation (Ayllón et al. 2020). Currently, the Narnaviridae and Botourmiaviridae are separated at the class level: the Narnaviridae in the Amalibiviricetes and the Botourmiaviridae in the Miairicetes (Ayllón et al. 2020; Walker et al. 2020). In contrast, the phylogeny produced in this study suggests the Narnaviridae, Botourmiaviridae, and Narliiviridae likely fall within a single taxonomic class. Hence, we propose that the Botourmiaviridae and newly classified Narliiviridae should be combined into one order—the Ourlivirales, while the Narnaviridae remain in the order Wolfnavirales and that both orders be combined into one class—the Amalibiviricetes. Importantly, this taxonomic distinction is robust to all the phylogenetic trees presented in this paper.

The family Narnaviridae has traditionally been defined as having a remarkably simple genome of a single ORF encoding only the viral RdRp (Hillman and Cai 2013), although some recently identified members of this family appear to contain additional genes and multiple ORFs or ambigrammatic genomes (Shi et al. 2016; Grybchuk et al. 2018; Charon et al. 2019; Chiapello et al. 2020; Wolf et al. 2020; Charon, Murray, and Holmes 2021). Notably, large-scale metagenomic studies have suggested the presence of a capsid protein in assembled sequences resembling narnaviruses (Shi et al. 2016, Wolf et al. 2020), all of which appear to fall within the newly proposed Narliiviridae. The genome structures of viruses within the Amalibiviricetes also support the taxonomic distinction between the families Narnaviridae and Narliiviridae. While the vast majority of species within the Narnaviridae do indeed have the typical narnavirus genome comprising a single RdRp gene, the Narliiviridae appear to have gained a capsid gene at multiple distinct points and lost it at one, suggesting that they may possess more flexible genomes than those of the Narnaviridae. These diverse capsid genes show some sequence similarity to the capsids of tombusviruses, nodaviruses, and sobemoviruses with the picona-like single jelly-roll fold (Koonin et al. 2008), suggesting frequent and independent instances of horizontal gene transfer between these plant and animal-associated virus families and the Narliiviridae. This has been proposed as a mechanism for how ourmiaviruses gained their capsid and movement proteins (Koonin and Dolja 2014). The presence of capsid genes within this family shows that despite these viruses having similarity to the narnavirus RdRp (Shi et al. 2016; Chen et al. 2021), they instead likely comprise a new family with variable genome structures.

Further metagenomic studies will inevitably increase the number of viruses within this phylum, although the identification of potential ‘intermediate’ species alone may not resolve their evolutionary history. Large-scale virus discovery projects are identifying viruses so diverse that even the most conserved regions of their genomes (i.e. the RdRPs) are difficult to align with currently available computational tools. Hence, if RNA virus taxonomy continues to increasingly depend on RdRp phylogenies, it is likely to be continually disrupted by the inevitable discovery of diverse viral species. In contrast, protein structures are considerably more conserved than primary sequences (Illergård, Ardell, and Elofsson 2019, Černý et al. 2014), with polymerases exhibiting relatively high levels of conservation reflecting their central function in the viral life cycle. This makes structural analysis an attractive tool for the discovery of highly divergent viruses (Ortiz-Baez et al. 2020). With both the growing availability of structural data and advances in protein modelling (Kelley et al. 2015), it is likely that uncovering the evolutionary history of RNA viruses will rely increasingly on structure-based phylogenies.

Supplementary data
Supplementary data are available at Virus Evolution online.

Funding
Australian Research Council Australian Laureate Fellowship (FL170100022) to E.C.H.; National Natural Science Foundation of China (31930001 and 32130002) to Y.Z.Z.

Conflict of interest: None declared.

Data availability
Sequence reads are available at the NCBI Sequence Read Archive database under BioProject accession PRJNA716119. Novel viral sequences identified in this study are available in GenBank under the accession numbers ON001450–ON001685.

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