






Review

# Nudiviruses in free-living and parasitic arthropods: evolutionary taxonomy

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The nudiviruses (family: *Nudiviridae*) are large double-stranded DNA (dsDNA) viruses that infect insects and crustaceans, and have most recently been identified from ectoparasitic members (fleas and lice). This virus family was created in 2014 and has since been expanded via the discovery of multiple novel viral candidates or accepted members, sparking the need for a new taxonomic and evolutionary overview. Using current information (including data from public databases), we construct a new comprehensive phylogeny, encompassing 49 different nudiviruses. We use this novel phylogeny to propose a new taxonomic structure of the *Nudiviridae* by suggesting two new viral genera (*Zetanudivirus* and *Etanudivirus*), from ectoparasitic lice. We detail novel emerging relationships between nudiviruses and their hosts, considering their evolutionary history and ecological role.

## Taxonomic history of the family *Nudiviridae*

dsDNA viruses (see Glossary) are enormously diverse and are divided over separate taxonomic realms [1]. One group of invertebrate-infecting dsDNA viruses sits in the class *Naldaviricetes* [2]. This includes the family *Nimaviridae* [3] and the order *Lefavirales*, housing the *Baculoviridae*, *Hytrosaviridae*, recently proposed *Filamentoviridae* [4], and the *Nudiviridae* [5,6]. The *Nudiviridae* comprise bacilliform viruses that infect hosts across the Pancrustacea [7], including insects (flies, moths, beetles, crickets, crane flies) and crustaceans (lobsters, shrimp, crabs, amphipods), with varying degrees of virulence and ecological impact [6].

Pathological and morphological data originally pointed towards the presence of baculoviruses in insect and crustacean hosts [8–10]. However, a baculovirus termed ‘Baculo-A’, derived from the swimming crab *Callinectes sapidus*, was described in the 1980s, but later revealed to be a nudivirus [11]. Similarly, the insect nudiviruses *Oryctes rhinoceros nudivirus* (OrNV), *Heliethis zea nudivirus* (HzNV-1), and *Gryllus bimaculatus nudivirus* were all originally described as baculoviruses [12–14]. Subtle morphological and pathological differences, including more obvious traits (i.e., lacking occlusion bodies leading to the term ‘non-occluded baculoviruses’; Box 1), supported the split from the other baculoviruses, leading to the suggestion of a baculoviral subfamily (‘Nudivirinae’) [15], which eventually became the family *Nudiviridae* [5]. Further pathological support was derived from the host specificity and tissue tropism of nudiviruses, such as those that infect the hepatopancreas of crustacean hosts [16,17], and those that manifest across the gut, fat-bodies and reproductive tissues of insects [6]. The localized nature of nudivirus infections within an infected individual stands as another primary distinction from baculoviruses, which typically induce systemic infections [18].

The *Nudiviridae* currently comprise four officially recognized genera: *Alphanudivirus* (including endogenous nudiviral elements), *Betanudivirus*, *Gammanudivirus*, and *Deltanudivirus* [5]. A fifth genus has been suggested for a group of divergent crustacean-infecting nudiviruses (‘Epsilon nudivirus’) [19] and two unofficial subfamilies have been considered, which would

## Highlights

An updated phylogenomic tree of the *Nudiviridae* is presented, including 49 species, proposing two genera associated with ectoparasitic insects.

Evolution of the family *Nudiviridae* is estimated to have occurred in the mid-Devonian, matching the timeline of the insect’s evolution.

All the crustacean-infecting nudiviruses have likely emerged from a single common ancestor (~143 million years ago), perhaps transmitted to Jurassic crustaceans by an insect host.

The *Nudiviridae* have a proclivity for ectoparasitic arthropods (i.e., lice and fleas), linked to multiple diversification events in the Triassic (early *Betanudivirinae* split), Jurassic (involving ectoparasitic hosts of *alphanudiviruses*), and Cretaceous periods (*Zeta/Etanudivirus* diversification).

Nudiviruses inhabit various geographical and climatic zones, including members from subpolar habitats.

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house the *Alphanudivirus* ('Alphanudivirinae') and the *Betanudivirus*, *Gammanudivirus*, and *Deltanudivirus* genera ('Betanudivirinae') [20]. An additional genus has been proposed for inclusion in the family *Nudiviridae* – the *Bracoviriform* (formerly 'Bracovirus') genus, which consists of endogenous nudiviruses (Box 2) from parasitic wasps, and which are currently classified as *Polydnaviriformidae* (formerly 'Polydnaviridae') (Figure 1) [21,22].

Transcriptomic and metagenomic studies in invertebrate hosts have hinted at the existence of yet uncharacterized nudiviruses in other arthropods – for example, nudivirus-like transcripts were found in two additional species in the order Decapoda: *Eriocheir sinensis* and a *Charybdis* sp. [23,24]. The bounty of next-generation sequencing data from arthropods (593 792 public datasets – 9 November 2023) in publicly available Sequence Read Archives (SRAs) [25] represents a major resource for discovering more nudiviruses in order to address questions regarding taxonomy and evolutionary timeline.

Nudivirus genomes consist of a single circular dsDNA molecule, ranging in size from approximately 96 to 232 kilobase pairs (kbp), encoding between 87 and 154 protein-coding genes (PCGs) [6]. All lefavirals encode homologues of genes involved in viral (late) gene transcription, including the four subunits of the DNA-directed RNA polymerase (*lef-4*, *lef-5*, *lef-8*, and *lef-9*). In nudiviruses, these four genes belong within a set of 28 'core' genes that can be found in all **exogenous** nudivirus genomes, whereas endogenous nudiviruses can have an incomplete repertoire of core genes [26,27]. Nudiviral core genes fulfil crucial functions in virus replication, infectivity, virion assembly and morphology, but several have unresolved functions [6].

### Underexplored nudiviral diversity

Numerous unclassified viral agents with nudivirus-like pathology and morphology exist. Two studies conducted in the 1970s identified nuclear viruses with nonoccluded, rod-shaped virions in *Solenopsis* sp. (Hymenoptera: Formicidae) and *Gyrinus natator* (Coleoptera: Gyrinidae). These viruses exhibited nudivirus-like symptoms [28,29], but molecular data are missing. Another example involves the *Tipula paludosa* nucleopolyhedrovirus (TpNPV) [30], that is, based on single gene homology, more closely related to *Tipula oleracea* nudivirus (ToNV) than to baculoviruses [31], emphasizing the possibility that unclassified viruses (some with occlusion bodies; Box 1) could be nudiviruses.

For the crustaceans, pathological studies have revealed nudivirus-like discoveries from (i) crabs: *Cancer pagurus* [16]; *Carcinus mediterraneus* (Tau-virus) [32]; *Paralithodes platypus* [33]; *Scylla serrata* [34]; and *Pinnotheres pisum* [35]; (ii) crayfish: *Astacus astacus* [36]; *Cherax quadricarinatus* [37]; *Pacifastacus leniusculus* [38]; *Cherax destructor* [39]; and *Astropotamobius*

### Glossary

**Ectoparasite:** a unicellular or multicellular organism that parasitizes its host externally.

**Endogenous:** in virology, refers to foreign genetic material that has integrated into the genome of a given organism (i.e., endogenous viral elements).

**Exogenous:** refers to viruses whose genetic material has not been integrated into the genome of its host but is packaged in infectious, replication-competent particles.

**Gene synteny:** the linear arrangement of genes across the genomic architecture of a given organism.

**Haematophagous:** the dietary trait of consuming blood.

**Molecular dating:** the process of analytically predicting the evolutionary timeline of a series of organisms using molecular data.

**Paleovirological:** descriptive term relating to paleovirology, which is the study of ancient viruses and how they have coevolved with their hosts, including the evolutionary inference of modern-day exogenous viruses.

**Pathological:** descriptive term relating to pathology, which is the study of diseases, their causes, processes, development, and consequences.

**Phylogeny:** involves the construction of phylogenetic trees to illustrate the evolutionary relationship and common ancestry of different species.

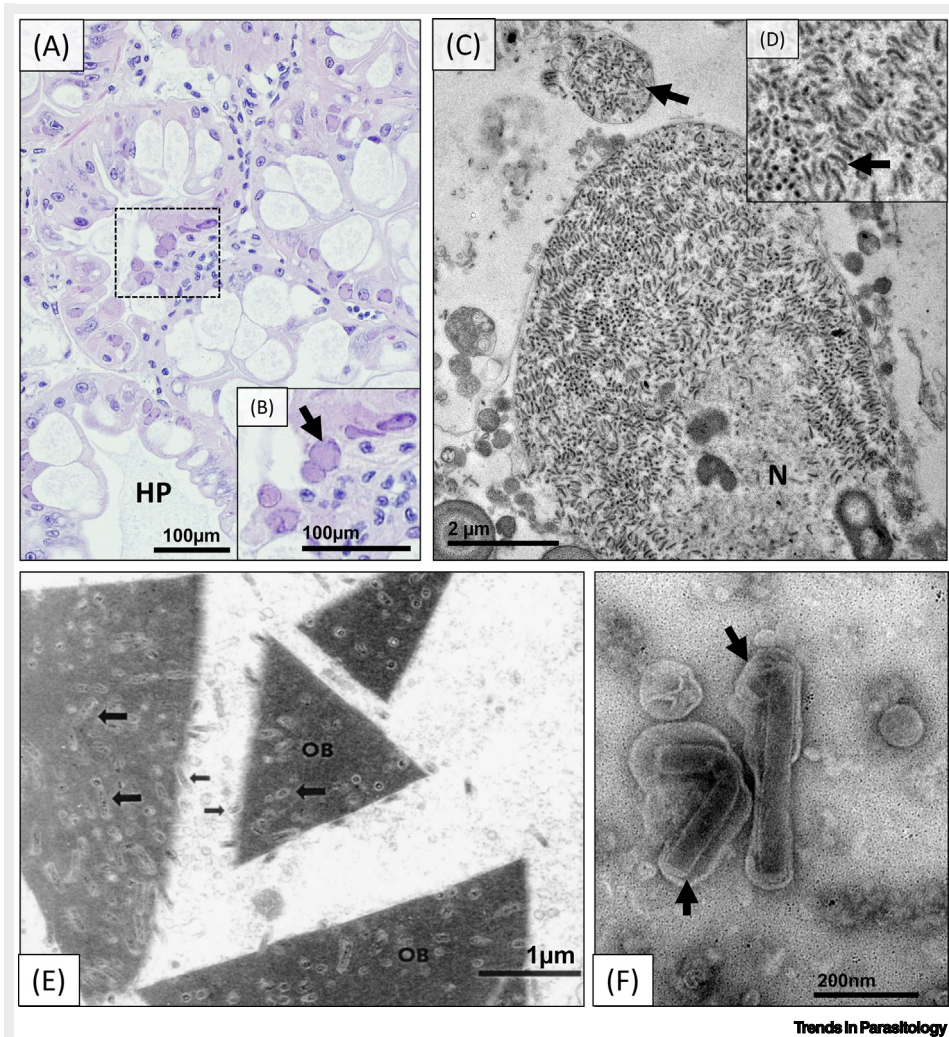
**Virulence:** describes the degree of disease a pathogen causes in its host.

**Viruses:** infectious mobile genetic elements that package their nucleic acid core in virions, assembled from at least one virus-encoded component.

#### Box 1. Not all nudiviruses are 'nudists': nudiviral occlusion bodies

Occlusion bodies (OBs) are crystalline structures assembled from viral proteins that are known for enwrapping the virions of certain insect viruses [105]. In baculoviruses, these dense protein structures protect the viral progeny from environmental stresses until they are ingested by their insect host. Despite their endurance towards many abiotic factors, baculovirus OBs easily dissolve under alkaline conditions to which they are exposed in the midgut of their hosts, leading to the release of virions responsible for primary infection [18].

While it was originally assumed that nudiviruses were non-occluded baculoviruses [106], cases of OB formations in members of the *Nudiviridae* have emerged, challenging the derivation of their name from the Latin term '*nudus*' (= naked or uncovered) [5]. Occluded nudiviruses are present in both insect (ToNV, *Tipula oleracea* nudivirus) and crustacean (PvNPV, *Penaeus vannamei* nucleopolyhedrovirus; PmNV, *Penaeus monodon* nudivirus) hosts. The OBs of ToNV and PmNV are irregularly shaped [31,93], while PvNPV occludes its virions into pyramid-shaped OBs [9] (Figure 1). Furthermore, OBs of insect- and crustacean-infecting nudiviruses seem to dissolve under different conditions. Congruent to baculoviruses in insects, the OBs of ToNV dissolved under alkaline treatment [107], while PmNV OBs showed resistance to high pH values, but dissociated when exposed to acidic conditions [93]. The dependency of OBs on *per os* transmission signifies the importance of coevolutionary adaptation, whereby nudiviral OBs might have been tailored to match their host's digestive environments.



**Figure 1. Histological and electron microscopic images of nudiviral ultrastructure and development.** (A) Multiple hepatopancreatic tubules (HPs) display infected hepatopancreatocytes with hypertrophic nuclei (dotted box), including a growing viroplasm (image obtained from *Faxonius rusticus*; [60]). (B) Inset image showing a higher magnified image of an infected nucleus (black arrow) (image obtained from *F. rusticus*; [60]). (C) A transmission electron micrograph of *Menippe mercenaria nudivirus* (MmNV), in the nucleus ('N') of an infected hepatopancreatic epithelial cell, including a virions-containing vesicle (black arrow) (image reproduced from [49]). (D) An enlarged view of the MmNV virions in the host nucleus (black arrow) (image reproduced from [49]). (E) An example of a nudivirus from *Penaeus* sp., which produces occlusion bodies (OBs) in pyramidal structures, containing virions (black arrows) (image reproduced from [9]). (F) Negatively stained rod-shaped virions of *Heliothis zea nudivirus 1* (HzNV-1) visualized via electron microscopy (purified from infected HZ-AM1 cells via sucrose cushion).

*pallipes* [40]; (iii) shrimp: *Farfantapenaeus* (= *Pandalus*) *montagui* [41]; *Penaeus japonicus* [42]; *Penaeus duorarum* [43]; *Penaeus plebejus* [44]; and (iv) various amphipods [45–48]. To date, all sequenced 'baculovirus-like' isolates in crustaceans belong to the family *Nudiviridae* [49].

### Evidence of nudiviruses hiding in cyberspace

In total, 2110 SRA datasets from arthropods were searched for nudivirus-like sequences (Files S1 and S2 in the supplemental information online). Ten SRA submissions included large, circular

### Box 2. Endogenous nudiviruses among insect hosts

Whole-genome sequencing techniques have revealed the presence of nudivirus-derived endogenous viral elements (EVEs) in various arthropods [108]. Although the circumstantial conditions for nudiviral genome integration is scarcely studied, it can be assumed that such integration is driven by two possible scenarios: an active integration mechanism employed by nudiviruses to establish persistent infections, as it was observed for HzNV-1 [109]; or indirect integration as a consequence of induced DNA damage and repair during replication in the host cell nucleus [110]. Evolutionarily, the integration of nudiviral genetic material into host genomes has resulted in the emergence of several endogenous and ‘functional’ nudiviruses – that is, the bracoviruses. These endogenized viruses serve as invaluable ‘fossil records’, offering insights into the evolutionary history of ancient viruses and their hosts [22]. Despite their ostensible frequency, nudiviral integration events produce only a few examples of functional endogenizations, and among those functional cases there are still varying degrees of benefit to the host. For instance, *Fopius arisanus* endogenous nudivirus (FaENV) and *Venturia canescens* endogenous nudivirus (VcENV) form virus-like particles (VLPs) in their parasitoid hosts (*Fopius arisanus* and *Venturia canescens*) that are injected together with the wasp eggs during parasitism-mediated oviposition. The particles of VcENV are considered to benefit the success of parasitism in its hymenopteran host (for instance via immune-protective coating of wasp eggs, carrying wasp-derived virulence proteins, and manipulation of parasitized host physiology), while FaENV particles carry DNA-empty nucleocapsids with unknown benefit to its parasitoid wasp. VLPs of VcENV and FaENV both possess viral envelopes with incorporated nudiviral proteins, but without a nucleic acid core. This stands in contrast to virions formed by bracoviruses, which are enveloped, and carry circular dsDNA molecules inside their nucleocapsids [6].







Next to these functional models of endogenous nudivirus-derived agents, there are scenarios where nudivirus integration events have led to nonfunctionality. The endogenous nudiviral agents of three hemipteran insects (*Nilaparvata lugens*: NIENV; *Melanaphis sacchari*: MsENV; *Aphis glycines*: AgENV), for instance, are unable to produce particles or at least have no evident function for the insect host [6,26,27]. Furthermore, a study by Cheng and coauthors [108] found a total of 359 nudivirus-like sequences in the genomes of various arthropods, the majority being present in Hemiptera and Hymenoptera, but with no confirmed functionalities.

dsDNA molecules derived from the nudivirus genomes (from insects: *Franciscoloa pallida* (Y. Dong, Y. PhD thesis, University of the Sunshine Coast, Queensland, 2022),

*Franciscoloa roseicapillae* (Y. Dong, Y. PhD thesis, University of the Sunshine Coast, Queensland, 2022), *Myrsidea ptilorhynchi* (Y. Dong, Y. PhD thesis, University of the Sunshine Coast, Queensland, 2022), *Heterodoxus spiniger*, *Cuculoecus africanus*, *Echinophilopterus claytoni*, *Lagopoecus perplexus* [50], three datasets from *Ctenocephalides orientis* [51]); and eight included partial/complete core gene sets (from insects: *Culex pipiens* [52], *Goniodes lagopi* [50]; from crustaceans: *Gammarus pulex*, *Gammarus fossarum*, *Echinogammarus berilloni*, *Astacus leptodactylus* [53], *Eriocheir sinensis*, *Charybdis* sp. [23]). Additionally, putative nudiviral sequences for which fragmented/incomplete core gene sequences were obtained from five species (insects: *Aleuroclava psidii* [54], *Dasineura brassicae* [55], *Phlebotomus chinensis* [56], *Trichocera saltator* [57,58]; crustacean: *Niphargus hrabei* [59]) (Files S1, S2, S3, and S4 in the supplemental information online).

Datasets from the oriental cat flea (*Ctenocephalides orientis*: Siphonaptera) led to the assembly of three distinct full nudivirus genomes that we refer to as *Ctenocephalides orientis* nudivirus isolates 1–3, CoNV1, CoNV2, and CoNV3, respectively (Genome range: 90 491–90 736 bp; PCGs: 86–87). Eight novel nudiviruses are from either the Amblycera or Ischnocera superfamily of ectoparasitic lice (Psocodea) (File S2 in the supplemental information online). Genomic data disclosed seven complete louse nudiviral genomes, reflecting *Franciscoloa pallida* nudivirus (FrpNV); *Franciscoloa roseicapillae* nudivirus (FrrNV); *Myrsidea ptilorhynchi* nudivirus (MpNV); *Heterodoxus spiniger* nudivirus (HxsNV); *Cuculoecus africanus* nudivirus (CafNV); *Echinophilopterus claytoni* nudivirus (EcNV); and *Lagopoecus perplexus* nudivirus (LpNV). *Goniodes lagopi* nudivirus (GlnV) presented as two contiguous sequences, but encoded homologues of all expected virus genes. Genome size ranges: 83 884–97 269 bp; PCGs: 88–104 proteins.

The common house mosquito (*Culex pipiens*: Culicidae) in the study by Feng *et al.* [52] provided 102 predicted nudivirus genes through transcriptomic data, which is considered to represent the

	Members	Genome	Hosts (Orders)	Biology
<b>Alphanudivirus</b>	<b>DiNV</b> <b>ENV</b> <b>GbNV</b> <u>Endogenous:</u> <b>KV</b> <b>MNV</b> NIENV <b>OrNV</b> FaENV <b>TNV</b> VcENV <b>PsNV</b> AgENV <b>AmNV</b> <b>OcNV</b> <b>DuhNV</b>	<u>Size (kbp):</u> ~97 to 183  <u>PCGs:</u> 87 to 139		<u>Transmission:</u> Ingestion of excrements or cadavers contaminated with non-occluded virions.  <u>Pathology:</u> Virus accumulates mostly in midgut and fat body tissue. Infections can manifest in lethargy and malformations as well as decreased fertility, growth and longevity.
<b>Betanudivirus</b>	<b>HzNV-1</b> <b>HzNV-2</b>	<u>Size (kbp):</u> ~228 to 232  <u>PCGs:</u> 113 to 154	 Lepidoptera	<u>Transmission:</u> Sexually during mating.  <u>Pathology:</u> Malformations in reproductive organs. "Waxy plug" filled with high quantities of non-occluded virions forms at genital opening.
<b>Gammanudivirus</b>	<b>PmNV</b> <b>HgNV</b> <b>CmNV</b> ApNV CsNV MrNV MmNV *PvNPV	<u>Size (kbp):</u> ~99 to 119  <u>PCGs:</u> 83 to 115	 Decapoda	<u>Transmission:</u> Presumably via ingestion of non-occluded virions, or occlusion bodies in few cases (i.a. PmNV and PvNPV).  <u>Pathology:</u> Virus accumulates in hepatopancreas.
<b>Epsilonudivirus</b>	DhNV FpNV FrNV FvNV CcNV	<u>Size (kbp):</u> ~119 to 147  <u>PCGs:</u> 97 to 129	 Amphipoda Decapoda	<u>Transmission:</u> Non-occluded virions are presumably ingested via contaminated urine.  <u>Pathology:</u> Virus accumulates in hepatopancreas.
<b>Deltanudivirus</b>	<b>ToNV</b>	<u>Size (kbp):</u> ~145  <u>PCGs:</u> 131	 Diptera	<u>Transmission:</u> Oral ingestion of occluded virions.  <u>Pathology:</u> Larvae display epidermal discoloration and increased mortality.
<b>Bracoviriform</b>	<b>CcBV</b> <b>CiBV</b> <b>MdBV</b> (in total 31 species recognized by ICTV)	Partial conservation of nudiviral core genes (varies among species).	 Hymenoptera	Endogenous viral agents that form replication-incompetent particles in the ovaries of parasitoid wasps. Particles are injected with wasp eggs during oviposition to ensure parasitism success.

Trends in Parasitology

Figure 1. Overview of the family *Nudiviridae* covering official (*Alphanudivirus*, *Betanudivirus*, *Gammanudivirus*, *Deltanudivirus*) and unofficial (*Epsilonudivirus*) genera as well as the related genus *Bracoviriform* (*Polydnaviriformidae*). Virus names in bold indicate official recognition by the International Committee on Taxonomy of Viruses (ICTV) with respective virus species names: DiNV, *Alphanudivirus droinnubilae*; ENV, *Alphanudivirus tertidromelanogasteris*; GbNV, *Alphanudivirus grybimaculati*; KV (*Alphanudivirus dromelanogasteris*); MNV (*Alphanudivirus quartidromelanogasteris*); OrNV (*Alphanudivirus oryrhinocerotis*); TNV (*Alphanudivirus alterdromelanogasteris*); HzNV-1/HzNV-2 (*Betanudivirus hezeae*); CmNV (*Gammanudivirus cameanadis*); CcNV (*Gammanudivirus cracrangonis*) is officially a gammanudivirus, but phylogenetic analyses support its assignment to Epsilonudivirus (blue arrow); HgNV (*Gammanudivirus hogamari*); PmNV (*Gammanudivirus pemonodonis*); ToNV (*Deltanudivirus tipoleraceae*). (\*) *Penaeus vannamei* nucleopolyhedrovirus (PvNPV) is currently assigned to the *Baculoviridae*, but shows clear phylogenetic relationship to the *Nudiviridae*. References for the respective virus taxa can be found in File S2 in the supplemental information online. Abbreviation: PCG, protein-coding gene.

entire collection of expressed genes for *Culex pipiens* nudivirus (CpNV). Transcriptomic data from three amphipods and three decapods also revealed new nudivirus-like sequences (File S2). We name these respectively, as: *Gammarus pulex* nudivirus (GpNV); *Gammarus fossarum* nudivirus (GfNV); *Echinogammarus berilloni* nudivirus (EbNV); *Astacus leptodactylus* nudivirus (AINV); *Eriocheir sinensis* nudivirus (EsNV) [24] and *Charybdis* nudivirus (CharyNV). These encode 62–96 proteins.

### Extensive nudivirus phylogeny leads to new evolutionary perspectives

Complete virus genomes are necessary for formal taxonomic inclusion by the International Committee on Taxonomy of Viruses (ICTV); however, most studies apply just the conserved genes in their phylogenetic analyses [6,49]. By incorporating the core genes of incomplete viral genomes (or transcripts), we can achieve identical high-level taxonomic accuracy (Figure 2). We included 16 novel nudivirus isolates in our phylogenomic analysis, providing the first **phylogeny** of nearly 50 nudiviruses.

Our phylogeny supports the current genus demarcation (*Alphanudivirus* to *Deltanudivirus*) and emphasizes the proposed Epsilon nudivirus genus as a monophyletic group through the addition of GpNV, GfNV, and EbNV, as well as the most recently characterized crayfish nudiviruses *Faxonius rusticus* nudivirus (FrNV), *Faxonius propinquus* nudivirus (FpNV), and *Faxonius virilis* nudivirus (FvNV) [60] (Figure 2). The new decapod nudiviruses (AINV, EsNV, and CharyNV) cluster with the other decapod nudiviruses in the genus *Gammanudivirus*. The fundamental monophyletic groups of the nudivirus phylogeny remain stable and well supported, suggesting that the existing branches of the *Nudiviridae* family are resiliently defined using core proteins [5].

The CoNV isolates and CpNV group in the genus *Alphanudivirus*, which adds fleas and mosquitos to the already broad insect host range of this genus (Figures 1 and 2). The louse-infecting nudiviruses share a most recent common ancestor (MRCA) with the beta-, gamma-, delta- and epsilon nudiviruses, but branch off as a distinct clade that could be considered to form a novel genus in the proposed subfamily Betanudivirinae. The nudiviruses from amblyceran lice (FrpNV, FrrNV, MpNV) form their own monophyletic group, except for HxsNV, which clusters as a related group with four nudiviruses isolated from ischnoceran lice (CafNV, EcNV, GINV, LpNV).

The core **gene synteny** of alphanudiviruses is generally poorly conserved, with the exception of certain monophyletic groups, such as the *Drosophila*-infecting nudiviruses Esparto virus (ENV), *Kallithea* virus (KV), *Drosophila innubila* nudivirus (DiNV), and Mauternbach virus (MNV) (Figure 2). Nudiviral taxonomy must become united to determine whether gene synteny should be considered an appropriate taxonomic criterion, where in this case, the large genus *Alphanudivirus* may be broken into multiple additional genera. Accordingly, the core gene organisation within other genera was more congruent, with less drastic rearrangements, except for the expanded group of Epsilon nudivirus members, which shows ~five gene rearrangements (Figure 2). A similar observation could be made for the two monophyletic groups of louse-infecting nudiviruses (Figures 2 and 3). The group comprised of HxsNV, CafNV, EcNV, GINV, and LpNV shows nearly identical core gene synteny, though a slight gene shift can be observed in HxsNV due to multiple *pif-5* duplications. FrpNV and FrrNV share highly similar core gene synteny, while MpNV is most distinct in its gene organisation, relative to the other louse-infecting nudiviruses, perhaps indicating that MpNV may belong to a third clade of louse nudiviruses. Due to their monophyletic lineages and major differences in gene synteny (Figure 3B, C), the groups housing the louse nudiviruses might be considered as two novel genera for ICTV consideration, following the Greek alphabet – Zetanudivirus (FrpNV, FrrNV, MpNV) and Etanudivirus (HxsNV, CafNV, EcNV, GINV, LpNV).

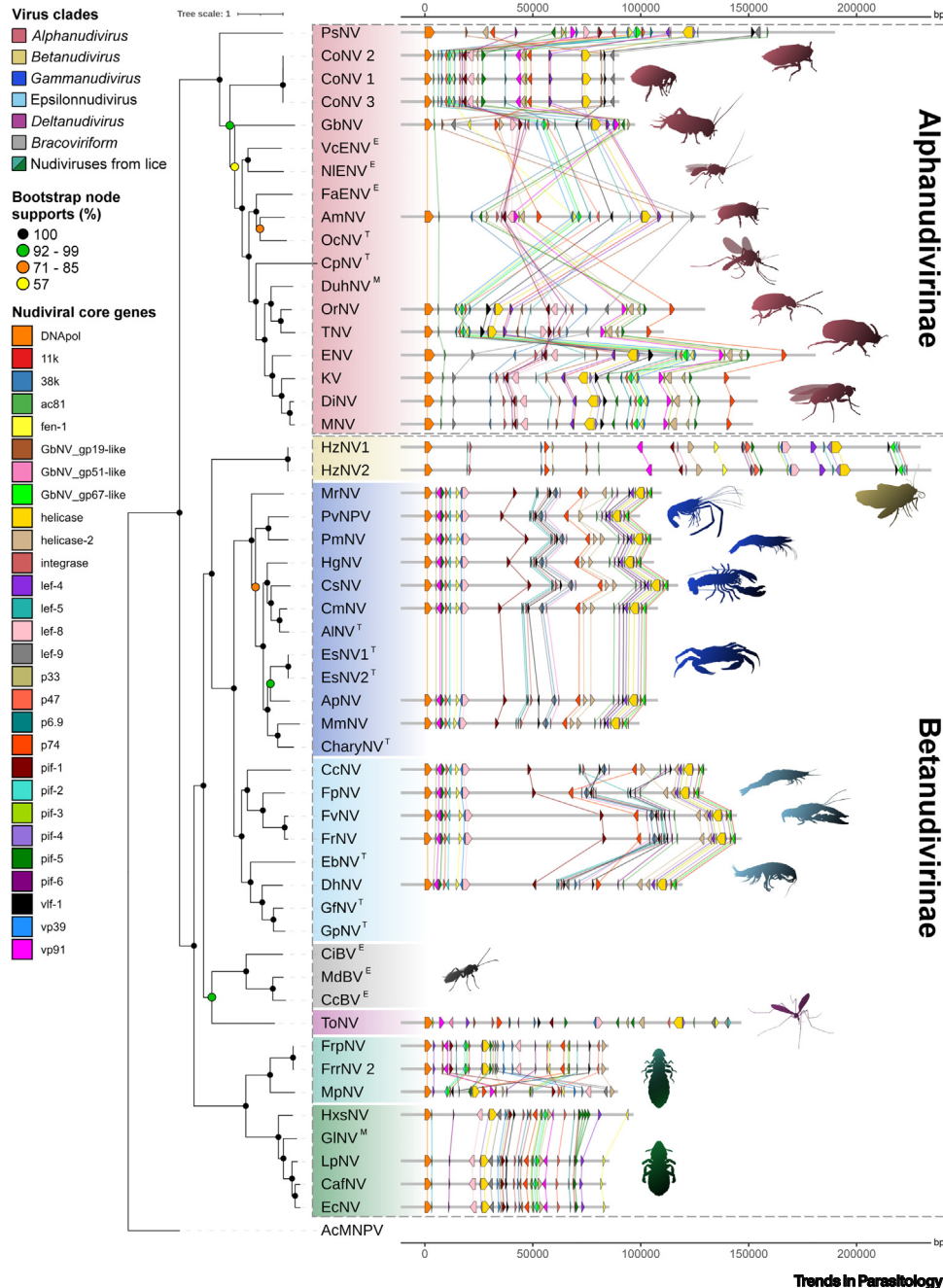


Figure 2. Phylogenetic tree with 49 nudiviruses, three bracoviruses, and the baculovirus *Autographa californica* nucleopolyhedrovirus (AcMNPV) as outgroup, coupled with intragenus core gene synteny compared among nudiviruses with complete genomes. The amino acid sequences of 17 nudiviral core genes were aligned with the MAFFT alignment tool (v7.490) of Geneious (v2023.2.1). Best-fit substitution models were determined separately for each core gene based on the Bayesian information criterion (BIC) generated in IQ-Tree [111] (File S2 in the supplemental information online). The multi-protein alignment file, containing 17 906 total sites, and the model information were subjected to maximum-likelihood (ML) phylogenetic analysis with 1000 bootstrap replicates. The inferred treefile was visualized with the online tool iTOL [112]. Percentage values of bootstrap supports are indicated as coloured circles. On the right, linear synteny plots generated with the R packages ggplot2 (v3.4.4) and gggenes (v0.5.1)

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### Molecular dating tree – nudiviral paleovirology

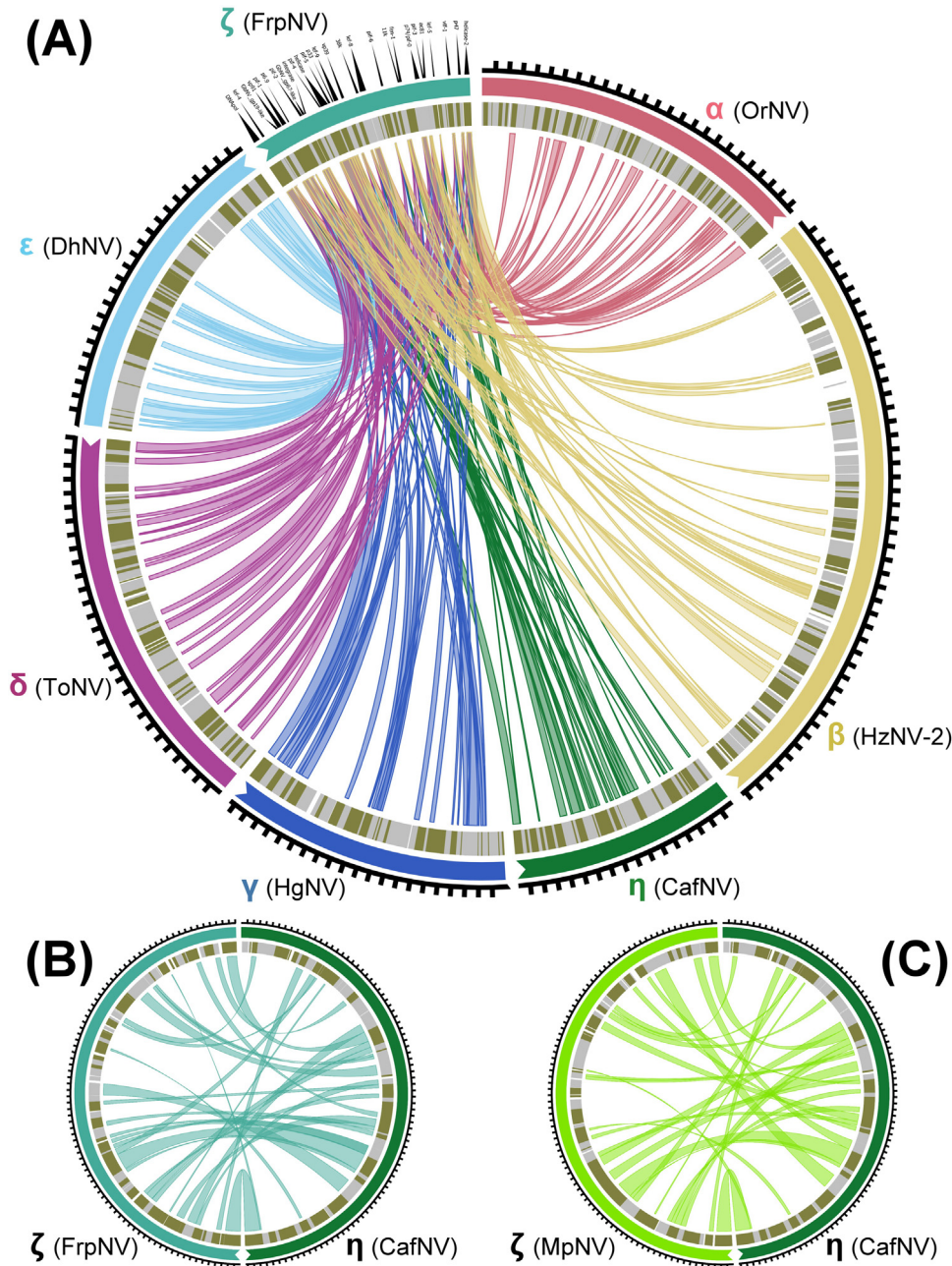
Using the available molecular information, we performed a **molecular dating** analysis with conserved nudiviral core genes from 49 nudiviruses, three bracoviruses, seven baculoviruses, and the hytrosavirus *Musca domestica* Salivary Gland Hypertrophy Virus (MdSGHV), to estimate the ages of viral evolutionary events from a **paleovirological** perspective. A time-dated tree was generated via the RelTime Branch-Lengths method implemented in MEGA11 [61]. The RelTime method is a non-Bayesian approach that operates on a relative rate framework (RRF), computing branch-specific relative rates directly from the branch lengths of inferred non-clock trees, while relaxing the molecular clock throughout the tree [62]. In contrast to Bayesian methods, RelTime does not require a tree prior and a clock model as parameters, and calculates confidence intervals (CIs) of divergence times using the Tao *et al.* method [63]. Next to the non-clock tree, a single calibration constraint of  $103.38 \pm 4.41$  million years ago (Mya) (red-circled Node 15; Figure 4A and Table 1) was used as input under a log-normal distribution, representing the MRCA of *Chelonus inanitus* bracovirus (CiBV) and *Cotesia congregata* bracovirus (CcBV) [64]. We accounted for the possibility of strongly divergent evolutionary rates of the endogenized bracoviruses compared with the exogenous nudiviruses by performing an evolutionary rate analysis with HyPhy (v2.5.61) [65] under the Fixed Effects Likelihood (FEL) model [66]. The analysis of eight nudiviral core genes conserved among four bracoviruses and 32 exogenous nudiviruses resulted in ratios of non-synonymous to synonymous substitutions (dN/dS) < 1, which indicates a strong negative selection across all nudiviral core genes (File S2 in supplemental information online). The bracoviral genes encoding virion components displayed higher evolutionary rates (dN/dS: 0.133–0.315) than those of exogenous nudiviruses (dN/dS: 0.048–0.118), which is congruent with findings from a previous study [67], while the transcription-associated genes evolve at more similar rates in both braco- and nudiviruses. Despite their endogenization, the nudiviral core genes of bracoviruses appear to have a more relaxed mutation rate than free-living nudiviruses and a possible underestimation of the age estimates in the molecular dating analysis needs to be considered.

Our molecular dating analysis provided estimates of shared evolutionary events among members of the *Nudiviridae* and *Baculoviridae*. The MRCA of all nudiviruses, including the bracoviruses (~280 Mya; the Paleozoic Era, Permian) traces further back than the MRCA of baculoviruses (~222 Mya; the Mesozoic Era, Triassic), which is coherent with an earlier

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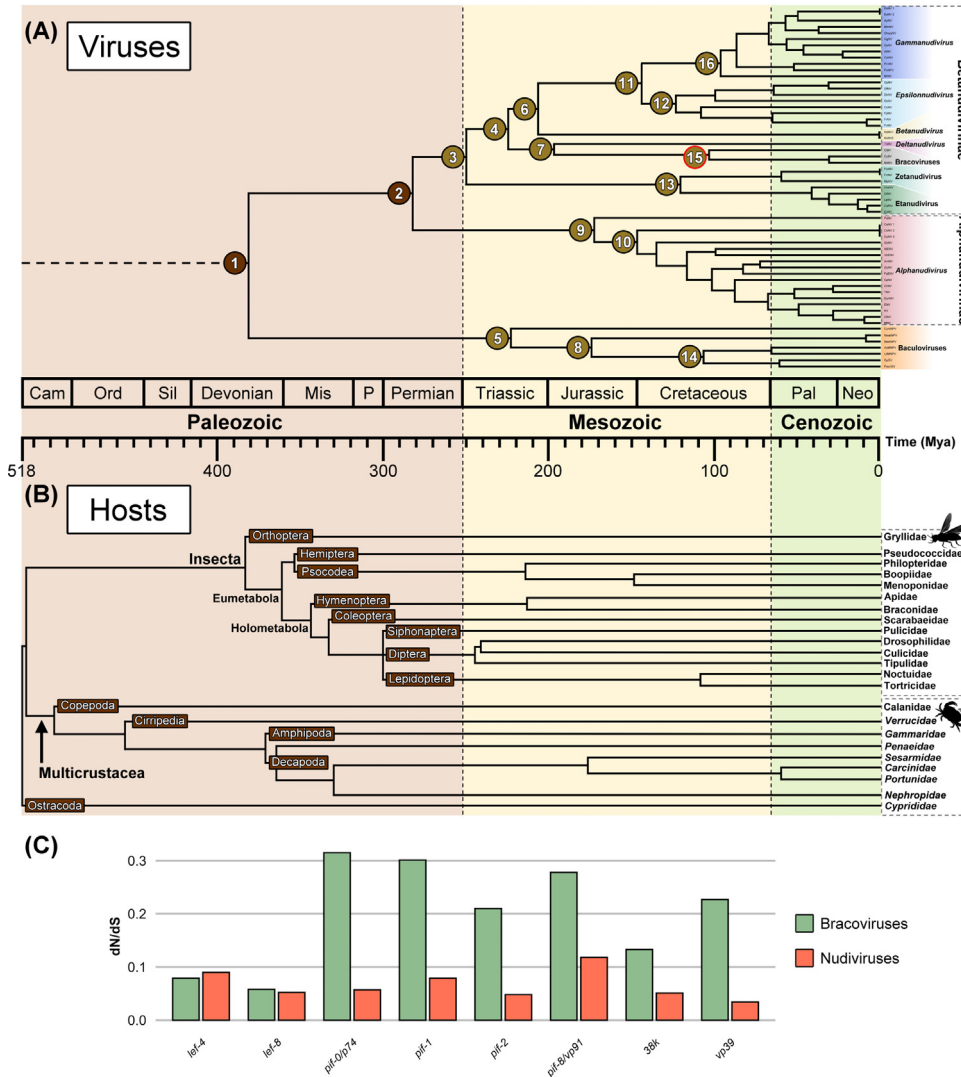
show core gene (coloured arrows) rearrangements compared among nudiviruses of the same genus in phylogenetic order. A scale bar (base-pairs) is presented at the top and bottom of the tree. Coloured lines between arrows connect homologous core genes of phylogenetic neighbors. Each dual synteny plot starts with the DNA polymerase of the respective virus. Virus species with superscript letters were not included in the gene synteny plot, either because they originate from transcriptomic data (T), are missing accurate genomes (M), or are endogenous (E). The original phylogenetic tree with node supports and additional references can be found in File S2, and accession numbers of genes used from each virus are included in File S1 in the supplemental information online. Abbreviations of virus names: AINV, *Astacus leptodactylus* nudivirus; AmNV, *Apis mellifera* nudivirus; ApNV, *Aratus pisonii* nudivirus; CafNV, *Cuculoecus africanus* nudivirus; CcBV, *Cotesia congregata* bracovirus; CcNV, *Crangon crangon* nudivirus; CharyNV, *Charybdis* nudivirus; CiBV, *Chelonus inanitus* bracovirus; CmNV, *Carcinus maenas* nudivirus; CoNV, *Ctenocephalides orientis* nudivirus; CsNV, *Callinectes sapidus* nudivirus; DhNV, *Dikerogammarus haemobaphes* nudivirus; DINV, *Drosophila innubila* nudivirus; EbNV, *Echinogammarus berilloni* nudivirus; EcNV, *Echinophilopterus claytoni* nudivirus; ENV, *Esparto* virus; EsNV, *Eriocheir sinensis* nudivirus; FaENV, *Fopius arisanus* endogenous nudivirus; FpNV, *Faxonius propinquus* nudivirus; FrNV, *Faxonius rusticus* nudivirus; FrpNV, *Franciscoloa pallida* nudivirus; FrrNV, *Franciscoloa roseicapillae* nudivirus; FvNV, *Faxonius virilis* nudivirus; GbNV, *Gryllus bimaculatus* nudivirus; GfNV, *Gammarus fossarum* nudivirus; GINV, *Goniodes lagopi* nudivirus; GpNV, *Gammarus pulex* nudivirus; HgNV, *Homarus gammarus* nudivirus; HxsNV, *Heterodoxus spiniger* nudivirus; HzNV1, *Heliothis zea* nudivirus 1; HzNV2, *Helicoverpa zea* nudivirus 2; KV, *Kallithea* virus; LpNV, *Lagopoeus perplexus* nudivirus; MdBV, *Microplitis demolitor* bracovirus; MmNV, *Menippe mercenaria* nudivirus; MNV, *Mauternbach* virus; MpNV, *Myrsidea ptilorhynchi* nudivirus; MrNV, *Macrobrachium rosenbergii* nudivirus; NIENV, *Nilaparvata lugens* endogenous nudivirus; OrNV, *Oryctes rhinoceros* nudivirus; PmNV, *Penaeus monodon* nudivirus; PsNV, *Phenacoccus solenopsis* nudivirus; PvnPV, *Penaeus vannamei* nucleopolyhedrovirus; TNV, *Tomelloso* virus; ToNV, *Tipula oleracea* nudivirus; VcENV, *Venturia canescens* endogenous nudivirus.





Trends in Parasitology

Figure 3. Inter-genus synteny of nudiviral core genes among representatives of nudivirus genera. (A) Circular plot showing the core gene synteny of nudivirus representatives from the genera *Alphanudivirus* ( $\alpha$ ), *Betanudivirus* ( $\beta$ ), *Gammanudivirus* ( $\gamma$ ), *Deltanudivirus* ( $\delta$ ), *Epsilonudivirus* ( $\epsilon$ ), and *Etanudivirus* ( $\eta$ ) compared with *Zetanudivirus* ( $\zeta$ ). (B) Comparative core gene synteny of *Zetanudivirus* [*Franciscolea pallida nudivirus* (FrpNV)] and *Etanudivirus* [*Cuculoecus africanus nudivirus* (CafNV)]. (C) Comparative core gene synteny of *Zetanudivirus* [*Myrsidea ptilorhynchi nudivirus* (MpNV)] and *Etanudivirus* (CafNV). The curved bars each represent the genome of the indicated nudivirus species, and are colored according to the genus they belong to. Sites with indented triangles represent the start of the genome (start codon of DNA polymerase). Gene directions are visualized as golden (positive strand) and silver (negative strand) rectangles. Ribbons represent genome connections between core gene homologs of the respectively colored nudiviruses. Distances between ticks represent 5 kb. The circular plots were generated with Circa ([www.omgenomics.com/circa/](http://www.omgenomics.com/circa/)).



Trends in Parasitology

**Figure 4.** Molecular dating analysis of nudiviruses, baculoviruses, and bracoviruses in relation to evolutionary timeline of crustacean and insect host clades. (A) Molecular dating tree from double-stranded (ds)DNA viruses. Phylogenetic virus tree with optimized branch lengths served as input to compute the molecular dating tree with the MEGA11 (v11.0.10) software by using the RelTime-Branch Lengths option [61]. A single calibration constraint of 103.38 ± 4.41 million years ago (Mya) (red-circled Node 15) was specified with log-normal distribution, representing the most recent common ancestor (MRCA) of *Chelonus inanitus* bracovirus (CiBV) and *Cotesia congregata* bracovirus (CcBV) [64]. The age estimates and 95% confidence intervals (CIs) of the numbered nodes can be found in Table 1 in the main text. The original molecular dating tree from MEGA11, including error bars and additional details on the methodology, can be found in File S2 in the supplemental information online. (B) Timeline of insect and crustacean hosts. The web-based TimeTree of Life (TTOL5) resource [66] was used to build the host tree by providing a text file with relevant species names. The TTOL5 resource is a publicly accessible database that infers divergence times of taxa based on the median value of existing evolutionary studies. (C) Bar chart showing the non-synonymous to synonymous substitutions (dN/dS) values across eight nudiviral core genes from four bracoviruses and 32 exogenous nudiviruses. The dN/dS values were inferred from the codon-aligned nucleotide sequences with HyPhy (v2.5.61) under the Fixed Effects Likelihood (FEL) model (File S2).

evolutionary study [22]. Node 1 indicates the time at which the MRCA of both nudiviruses and baculoviruses is estimated at 378 Mya (Devonian period of the Paleozoic Era), while the divergence of holometabolous insects from polyneopteran insects is estimated at ~383 Mya

Table 1. Sixteen notable nodes from the molecular dating tree (see Figure 4 in the main text) together with their estimated divergence times and 95% CIs

Node	Time, Mya	95% CI, Mya	MRCA of <sup>a</sup>
1	378	344–415	Baculo-, braco-, and nudiviruses
2	280	257–304	Braco- and nudiviruses
3	248	223–275	$\beta$ -, $\gamma$ -, $\delta$ -, $\epsilon$ -, $\zeta$ -, $\eta$ -NVs, and bracoviruses
4	222	196–253	$\beta$ -, $\gamma$ -, $\delta$ -, $\epsilon$ -NVs, and bracoviruses
5	222	197–249	Baculoviruses
6	205	176–238	$\beta$ -, $\gamma$ -, and $\epsilon$ -NVs
7	195	167–228	$\delta$ -NVs and bracoviruses
8	173	148–203	$\alpha$ -, $\beta$ -, and $\gamma$ -BcVs
9	171	150–195	$\alpha$ -NVs
10	145	123–173	CoNV(1–3) and $\alpha$ -NVs excluding PsNV
11	143	118–172	$\gamma$ - and $\epsilon$ -NVs
12	122	100–149	$\epsilon$ -NVs
13	120	104–138	$\zeta$ - and $\eta$ -NVs
14	106	87–129	$\alpha$ - and $\beta$ -BcVs
15	102	99–105	Bracoviruses
16	96	78–118	$\gamma$ -NVs

<sup>a</sup>Abbreviations:  $\alpha$ -NV, *Alphanudivirus*;  $\beta$ -NV, *Betanudivirus*;  $\gamma$ -NV, *Gammanudivirus*;  $\delta$ -NV, *Deltanudivirus*;  $\epsilon$ -NV, *Epsilonnudivirus*;  $\zeta$ -NV, *Zetanudivirus*;  $\eta$ -NV, *Etanudivirus*;  $\alpha$ -BcV, *Alphabaculovirus*;  $\beta$ -BcV, *Betabaculovirus*;  $\gamma$ -BcV, *Gammabaculovirus*;  $\delta$ -BcV, *Deltabaculovirus*.

according to TimeTree [68]. Hence, the divergence of these viral families likely happened early in insect evolution.

Another Paleozoic hallmark involves the MRCA of all nudiviruses (~280 Mya), during the Permian, where the unofficial subfamilies Alphanudivirinae (including all alphanudiviruses) and Betanudivirinae (comprising all other nudiviruses, including the lice nudiviruses) split [20] (Figure 4A). In the evolutionary history of eumetabolous insects, hemipteran and psocodean insects diverged approximately 9 million years before the diversification of holometabolous insects (as inferred from Figure 4B). The emergence of the two nudiviral subfamilies could be linked to the diversification of these respective insect groups, with the theory that ancestral hemipteran and psocodean insects were hosts to the ancestors of the Alphanudivirinae and Betanudivirinae, respectively. This assumption is supported by the observation that the hemipteran *Alphanudivirus*, *Phenacoccus solenopsis* nudivirus (PsNV), and the psocodean nudiviruses (lice-infecting) branch off at the deepest nodes of their respective subfamilial clades (Figure 4A). This is further supported by the lack of hemipteran nudiviruses outside of the Alphanudivirinae, and no psocodean nudiviruses outside of the betanudivirinae, to date. Furthermore, the mosquito-infecting CpNV shares an MRCA with the other dipteran nudiviruses (e.g., ENV) and coleopteran nudiviruses (OrNV, *Oryctes rhinoceros* nudivirus; DuhNV, *Diabrotica undecimpunctata howardi* nudivirus), suggesting that the earlier divergence of CpNV does not follow the evolutionary lineage of the dipteran and coleopteran hosts. We do not yet have enough data on the diversity of beta- and deltanudiviruses in holometabolous insects to explore the emergence of these lineages further – one plausible option may be that these hosts (*Tipula* spp. and *Heliothis* spp.) attained these viruses from other, yet unidentified, host groups.

The MRCA between the CoNV isolates (*Alphanudivirus*) and the rest of the alphanudiviruses (excluding PsNV) was estimated at 145 Mya (Node 10; 95% CI: CI lower = 123 Mya, CI upper =

173 Mya), hinting that one of the oldest ancestors of alphanudiviruses might have been associated with an ancestral flea. Amniotes emerged ~325 Mya, during their colonization of terrestrial environments [69]. This coincides with the emergence of the Siphonaptera, which emerged ~300 Mya (Figure 4B) and have been termed ‘dinosaur fleas’, which are thought to have switched to mammals [70]. The late Jurassic and early Cretaceous sit within the 123–173 Mya period, a time when early mammals (e.g., marsupials) evolved alongside the diversification of boreid fleas [71]. The diversification of these flea nudiviruses, within the *Alphanudivirus* genus, could plausibly have occurred alongside the evolution of mammalian flea hosts.

The MRCA of all viruses in the Betanudivirinae clade can be traced back as the third deepest node among all observed events (Node 3; 248 Mya; Figure 4A), primarily including the early branching of the lice-infecting zeta- and etanudiviruses from the rest of the Betanudivirinae (Node 3). The diversification of the amblyceran zetanudiviruses and the ischnoceran etanudiviruses (HxsNV being the amblyceran exception) from one another is estimated between 104 and 138 Mya (Node 13; 120 Mya; Figure 4A). The radiation of those two nudivirus lineages is coherent with the estimated origin of the clade Phthiraptera (115 Mya) [72] and the MRCA of its subclades Amblycera and Ischnocera between 105 and 110 Mya [73]. Suggestively, the evolutionary split of zeta- and etanudiviruses may have been driven by the diversification of the Phthiraptera clade into amblyceran and ischnoceran lice during the early-mid Cretaceous Epoch. Given the time period (early-mid Cretaceous), and the age of bird-like dinosaur evolution and diversification events (90–170 Mya) [74], this virological event could have taken place on the back of a feathered dinosaur, mimicking this same ecological system we now see in modern day birds, infested with lice, and hyperparasitized by nudiviruses.

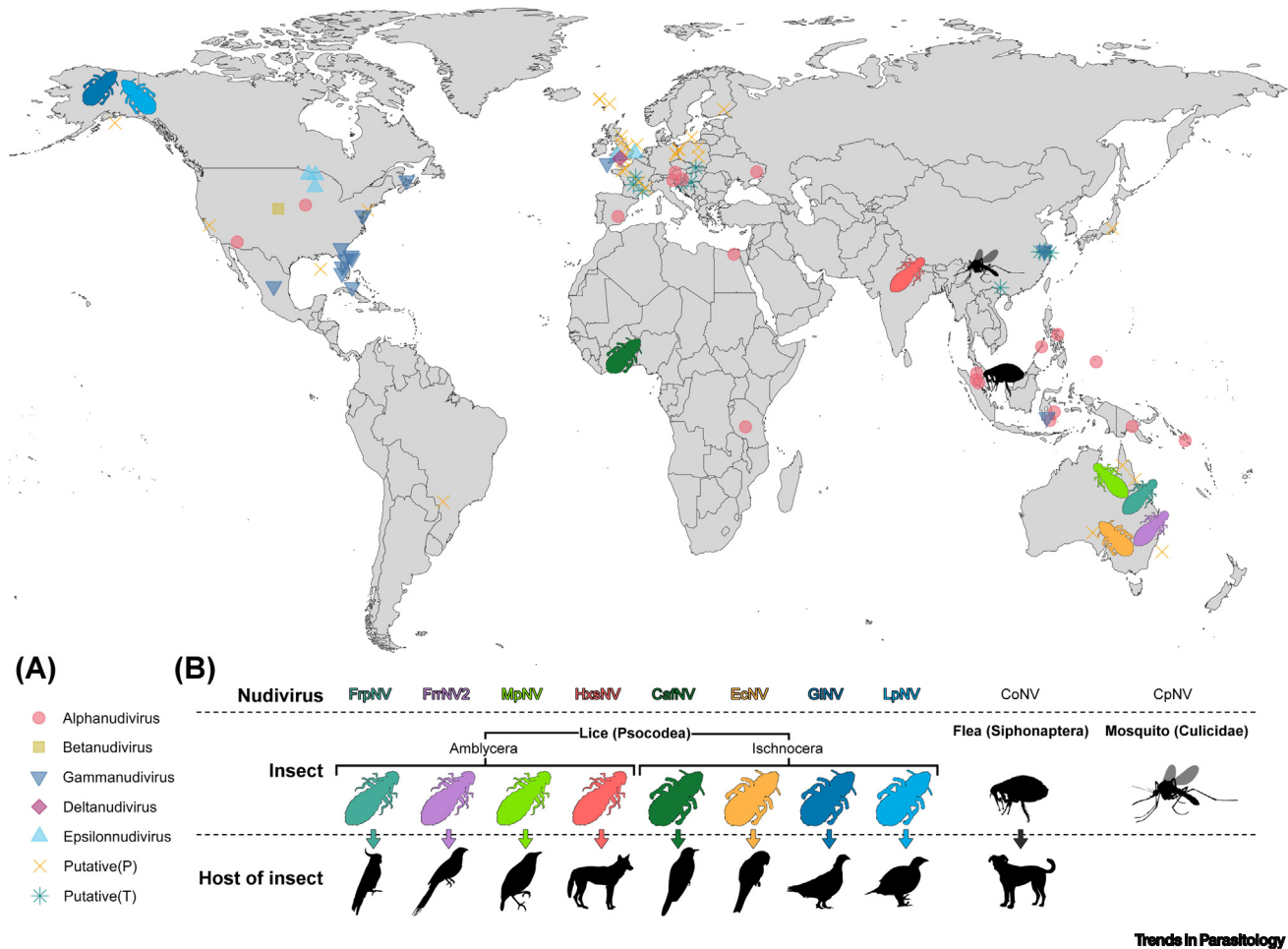
Within the Betanudivirinae, the divergence of gammanudiviruses represents a relatively recent event in nudivirus evolutionary history. Since the crustacean diversification occurred in the Ordovician (~500 Mya) [68], it seems that this group may have acquired nudiviruses via a zoonotic event, possibly from an insect. Given that all crustacean nudiviruses identified to date (gamma- and epsilon nudiviruses) have a single ancestor, estimated at ~140 Mya (Node 11; late Jurassic – early Cretaceous), it seems possible that a single host-switching event resulted in the crustacean infection capability that we see today. Perhaps the tissue tropism of crustacean-infecting compared with insect-infecting nudiviruses can explain this phenomenon. Crustacean nudiviruses target the gut, as do baculoviruses, but baculoviruses and other insect-infecting nudiviruses are also able to infect other tissue types – a trait missing in the *Gammanudivirus* and *Epsilon nudivirus* members. *Gammanudivirus* and *Epsilon nudivirus* appear to be a younger lineage of the *Nudiviridae*, beginning to parasitize the ancient arthropod group, Crustacea, during the late Jurassic.

### Global distribution of nudiviruses and their hosts

When all nudiviruses are considered, their distribution extends across all continents (Figure 5, Key figure). Marine, freshwater, and terrestrial hosts harbor nudiviruses – it seems that if insects or crustaceans occupy an area, nudiviruses likely occupy it as well. For insects, their environmental and ecological relationships are primarily associated with terrestrial systems, to a lesser extent freshwater systems, since we did not identify any nudiviruses from marine mammal-infesting insects. However, a handful of nudiviruses have been isolated from insects with a freshwater life stage (i.e., mosquitos), as well as a putative virus with nudivirus-like pathology from a water beetle (*G. natator*) [29]. Regarding terrestrial climatic zones, our findings complement the presence of nudiviruses across known biotopes (e.g., tropical: EcNV, FrpNV, FrrNV, MpNV; subequatorial: CpNV, HxsNV; equatorial: CafNV, CoNV), but also expand their habitat to the yet unrecognized sub-polar zones, with LpNV and GINV from Alaska.

Key figure

World map of confirmed and putative locations where nudivirus have been identified



**Figure 5.** Coordinates (latitude and longitude) of nudivirus-related find spots were used as input for the R packages maps (v3.4.2) and ggplot2 (v3.4.4) with support by the packages dplyr (v1.1.4) and ggpubr (v0.6.0). (A) Differently coloured shapes represent already identified nudiviruses with fully sequenced genomes, or putative yet uncharacterized nudiviruses based on pathological (P) or transcriptomic implications (T) from literature and personal data. (B) Newly characterized nudiviruses found in mosquito and ectoparasitic insects (flea and lice). Nudivirus names are shown at the top with their respectively coloured insect hosts below (separated by broken lines). For the lice and flea, the warm-blooded animals that those ectoparasitic insects infest are displayed at the bottom (left to right): *Eolophus roseicapilla*, *Centropus phasianinus*, *Ptilonorhynchus violaceus*, *Canis lupus*, *Chrysococcyx cupreus*, *Northiella haematogaster*, *Lagopus lagopus/muta*, *Tympanuchus phasianellus*, *Canis familiaris*. The list of nudiviruses and their coordinates can be found in File S1 in the supplemental information online. Abbreviations: CafNV, *Cuculoecus africanus* nudivirus; CoNV, *Ctenocephalides orientis* nudivirus; CpNV, *Culex pipiens* nudivirus; EcnNV, *Echinophlopterus claytoni* nudivirus; GINNV, *Goniodes lagopi* nudivirus; FfpNV, *Franciscoloa pallida* nudivirus; FrrNV2, *Franciscoloa roseicapillae* nudivirus 2; LpNV, *Lagopoecus perplexus* nudivirus; MpNV, *Myrsidea ptilorhynchi* nudivirus; HxsNV, *Heterodoxus spiniger* nudivirus.

Insect hosts of nudiviruses from across Asia include fleas (*C. orientis*) from infested dogs in Perak (Malaysia) [51]; the hemipteran, *A. psidii*, which infested guava trees (*Psidium guajava*) in Xinyang, China [54]; *H. spiniger* lice from wolves in India; and *C. pipiens* and *P. chinensis* from China [52,56]. Siphonaptera, Hemiptera, and Diptera samples had been collected across both Asia and Europe, while the lice hosts (Psocodea) cover a broader continental range. Most lice samples

were gathered from bird hosts in New South Wales and Queensland (Australia; *F. pallida*, *F. roseicapillae*, *M. ptilorhynchi*, *E. claytoni*), while *L. perplexus* and *G. lagopi* were sampled in Alaska (North America), and another (*C. africanus*) in Ghana (Africa; Figure 5B). Given the ability of **ectoparasites** to traverse diverse environments by hitchhiking on the animals on which they feed (including birds), the widespread distribution of nudiviruses could be explained by migratory birds through evolutionary history.

In addition to the geographical dispersal, the observed nudiviral host diversity highlights further layers of ecological complexity (Figures 5 and 6). Next to their importance as pathogens of commercially important arthropods, and as biocontrol agents against certain insect pests (i.e., OrNV [75]; bracoviruses [76]), their presence in **haematophagous** (blood-feeding) insects emphasizes a yet undescribed ecological niche this virus family fills. The eight psocodean species, the mosquitoes *C. orientis* and *C. pipiens*, as well as the sandfly *P. chinensis* all feed on the host tissue or blood of endothermic animals, including humans. However, *C. pipiens* and *P. chinensis* can

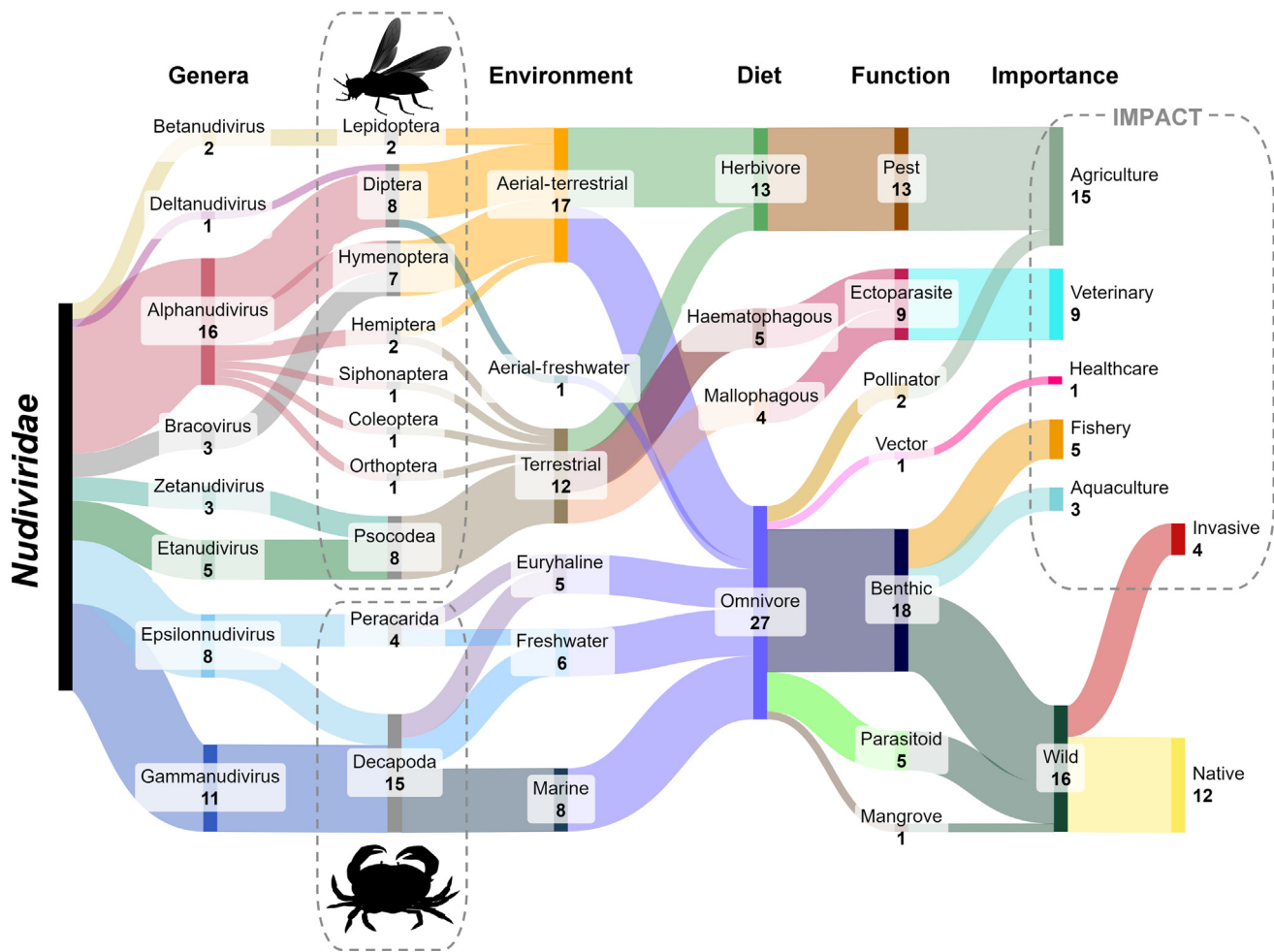


Figure 6. A Sankey diagram, elaborating on the host diversity, environmental diversity, host diet, ecological role, and potential impact associated with nudiviruses and their hosts (Crustacea and Hexapoda). The numbers indicate how many nudiviruses are associated with the respective trait. The noted haematophagous lice (Amblycera) also consume host skin and feathers (mallophagy), in contrast to the exclusively mallophagous ischnoceran lice. The Sankey diagram was generated with the web-based online tool (sankeymatic.com), supported by File S1 in the supplemental information online.

complete their life cycles without the need of hematophagy [77,78], while obligate ectoparasites such as lice and fleas spend most of their lives on the hosts from which they feed [79,80]. The proximate interaction between ectoparasites and their hosts are usually accompanied by a long history of coevolutionary adaptations [81,82]. The discovery of these large dsDNA viruses in lice and fleas introduces a yet undescribed trophic level to these ectoparasite–host systems. Since viruses are intracellular parasites [83], one may consider this tripartite relationship a form of hyperparasitism [84–86] or, at least, showcase another parasitic strategy that members of the *Nudiviridae* have converged upon [87]. Another tripartite model is known from parasitoid wasps and their endogenized bracoviruses, whose nudiviral ancestor integrated into an ancestral wasp genome ~100 Mya and coevolved with the wasp to become a domesticated mutualist [88]. Our findings emphasize an expanding societal relevance of nudiviruses that might eventually reach beyond applications in biocontrol and arthropod farming, to the sectors of healthcare and veterinary medicine (Figure 6). Where phage therapy is used to control bacterial infection, perhaps ‘nuditherapy’ might help animals to get rid of lice and flea pests in the future, providing that empirical data are obtained one day to show these newly discovered nudiviruses are pathogenic to their insect hosts.

Crustacean-infecting nudiviruses were found across marine and freshwater systems of China, Australia, Europe, inland USA, the Atlantic and Pacific coasts of Canada and the USA, as well as the Gulf of Mexico (Figure 5). The host diversity of these groups includes Decapoda and Amphipoda; however, all these hosts are omnivores (primarily detritivorous) in their freshwater, euryhaline, or marine habitat (Figure 6). Five crustacean hosts of nudiviruses are commonly present in fisheries, and three are involved in aquaculture efforts, highlighting the importance of understanding the interactions between crustacean and virus, including their economic impact (Figure 6). Examples include *C. sapidus* (‘blue crab fishery’) [89,90]; *Homarus gammarus*, where HgNV has only been identified from hatcheries to date [91]; and the more intensive *Penaeus monodon* aquaculture systems across Asia [92], where PmNV causes ‘wasting disease’, lowering yield via a reduction in juvenile shrimp growth [93]. The remaining ten nudiviruses have been sampled from wild environments, where some are native components of co-evolved ecosystems [45,94], while other nudiviruses infect invasive species [17,19].

Ecologically, the ‘wild’ crustaceans that host nudiviruses all form part of the aquatic benthos. For example, amphipods are considered important nutrient recyclers [95] and the mangrove crab, *Aratus pisonii*, is an essential part of mangrove ecosystems as a detritivorous and semi-terrestrial species [96]. The four invasive non-native species that harbor nudiviruses include: the globally invasive *Carcinus maenas* (aka. invasive green crab), *Dikerogammarus haemobaphes* (aka. the demon shrimp), and the invasive crayfish *Faxonius propinquus* and *Faxonius rusticus* [60,97–99]. Some of the fishery and aquaculture species are also considered invasive species in certain locations, such as *C. sapidus* in Europe [100]; however, nudiviruses have not been screened-for in these invasive populations. The nudiviruses in invasive crustacean populations have the potential to increase in prevalence and they may to some extent modify host behavior [98,101]; however, there remains a need for broader ecological studies to untangle how these viruses might influence biological invasions – that is, by potential transmission of nudiviruses to native crustacean populations, comparable with what happened when gray squirrels introduced squirrelpox to red squirrel populations [102]. Finally, there is one putative crustacean host with a parasitic lifestyle. The pea crab, *P. pisum*, parasitizes mussels, and specimens with a histologically demonstrated nudivirus infection were collected from the south coast of the UK [35]. However, no genomic data have been collected to confirm the relationship with the *Nudiviridae*.

Overall, a reasonable diversity of known nudiviruses are associated with facultative or obligate ectoparasitic/parasitoid hosts – fleas, lice, wasps, mosquito, pea crabs. Most terrestrial

nudiviruses have been detected in insect hosts involved with agricultural systems, but their occurrence is likely much broader, whereas several crustacean hosts are associated with fisheries and aquaculture. For the majority, these viruses sit within insect/crustacean taxa with an herbivorous or omnivorous lifestyle. The ecological role of the latter group is often prey species, or have a role in nutrient recycling.

### Concluding remarks

Histology and electron microscopy identified the first nudiviruses, and now genomic data provide us with a vast resource of nudivirus diversity across invertebrate hosts. The complete genomes of eight nudiviruses (CafNV, CoNV, EcNV, FrpNV, FrrNV, HxsNV, LpNV, MpNV) were hidden in cyberspace from pre-published studies, while an additional eight nudiviruses were identified as partial gene sets or from transcriptomic data (AINV, CharyNV, CpNV, EbNV, EsNV, GINV, GpNV, GfNV). Five other resources hint at the presence of putative nudiviruses, but with insufficient assembly recovery (File S2). These new nudiviruses, coupled with the isolates sequenced between 2007 and 2023 [11, 17, 19, 20, 31, 49, 60, 91, 93, 94, 103], have allowed for a new taxonomic perspective, suggesting two new genera ('Etanudivirus' and 'Zetanudivirus') to house nudiviruses from ectoparasitic lice. Nudiviruses first emerged around 280 Mya – crustacean nudivirus infections may have once been considered the older lineage; however, they appear to have the youngest evolutionary history. Insect-infecting nudiviruses appear to be the first to emerge, including their potential evolution and diversification in lice on early birds in the Cretaceous period (see [Outstanding questions](#)). Future studies are required to address questions regarding the morphology and pathology of these new nudiviruses, which may in turn reveal their ecological importance and new prospects for biocontrol of deleterious mammalian and avian ectoparasites, where chemical resistance is common [104] (see [Outstanding questions](#)). Our findings merely scratch the surface, but we are hopeful that our study will act as a steppingstone for forthcoming exploration into nudiviral taxonomic reasoning to develop delineation criteria and explore further nudivirus diversity across the Arthropoda.

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### Declaration of interests

All authors declare no conflict of interests.

### Supplemental information

Supplemental information associated with this article can be found online at <https://doi.org/10.1016/j.pt.2024.06.009>.

### References

- Koonin, E.V. *et al.* (2020) Global organization and proposed megataxonomy of the virus world. *Microbiol. Mol. Biol. Rev.* 84, e00061–19
- Van Oers, M.M. *et al.* (2023) Correction to: developments in the classification and nomenclature of arthropod-infecting large DNA viruses that contain pif genes. *Arch. Virol.* 168, 255
- Wang, H.-C. *et al.* (2019) ICTV virus taxonomy profile: Nimaviridae. *J. Gen. Virol.* 100, 1053–1054
- Guinet, B. *et al.* (2024) A novel and diverse family of filamentous DNA viruses associated with parasitic wasps. *Virus Evol.* 10, veae022
- Harrison, R.L. *et al.* (2020) ICTV virus taxonomy profile: Nudiviridae. *J. Gen. Virol.* 101, 3–4
- Petersen, J.M. *et al.* (2022) The naked truth: an updated review on nudiviruses and their relationship to bracoviruses and baculoviruses. *J. Invertebr. Pathol.* 189, 107718
- Hegna, T.A. *et al.* (2020) The fossil record of the Pancrustacea. *Nat. History Crustacea* 8, 21–52
- Anderson, I. and Prior, H. (1992) Baculovirus infections in the mud crab, *Scylla serrata*, and a freshwater crayfish, *Cherax quadricarinatus*, from Australia. *J. Invertebr. Pathol.* 60, 265–273

### Outstanding questions

Several examples of 'baculoviruses' have been pathologically defined in arthropods, but will these cases become identifiable as nudiviruses once genomic sequencing is conducted?

The ectoparasitic hosts of nudiviruses have chewing mouthparts (lice) or piercing mouthparts (flea) to feed on their warm-blooded hosts. Is it possible that nudiviruses of ectoparasites are orally transmitted like the majority of known nudiviruses, or might they be sexually transmitted like the unconventional betanudiviruses, or follow a yet undescribed transmission route, for example, by circulating in bloodstreams from which they can be taken up again by other ectoparasites?

If the nudiviruses of fleas and lice are as detrimental to their ectoparasitic hosts as other members of *Nudiviridae*, what potential value could they hold for ecological associations and for biological control of ectoparasitic infestations?

Our phylogenomic review highlights that novel taxonomic rankings may be needed to better explore diversity in the *Nudiviridae*. Does the presence of these new taxa indicate novel features and patterns that could promote feasible delineation criteria for (re)defining nudivirus species?

Are the evolutionary pressures guiding nudivirus evolution greater governed by biotic (e.g., trophic, host biology) or abiotic (e.g., environmental) factors?

We identify that the Crustacea appear likely to have acquired nudiviruses from insect hosts. Is it possible that other insectivorous arthropods (i.e., the Arachnida) might also house nudiviruses, which have jumped across host phyla?



9. Bonami, J.-R. *et al.* (1997) The polyhedra of the occluded baculoviruses of marine decapod crustacea: a unique structure, crystal organization, and proposed model. *J. Struct. Biol.* 120, 134–145
10. Groff, J. *et al.* (1993) Detection of a nonoccluded baculovirus in the freshwater crayfish *Cherax quadricarinatus* in North America. *J. Aquat. Anim. Health* 5, 275–279
11. Bojko, J. *et al.* (2022) Rediscovering ‘Baculovirus-A’ (Johnson, 1976): the complete genome of ‘*Callinectes sapidus nudivirus*’. *J. Invertebr. Pathol.* 194, 107822
12. Boucias, D. *et al.* (1989) Characterization of a non-occluded baculovirus (subgroup C) from the field cricket, *Gryllus rubens*. *Arch. Virol.* 106, 93–102
13. Crawford, A.M. and Sheehan, C. (1985) Replication of *Oryctes* baculovirus in cell culture: viral morphogenesis, infectivity and protein synthesis. *J. Gen. Virol.* 66, 529–539
14. Kelly, D. *et al.* (1981) Induction of a nonoccluded baculovirus persistently infecting *Heliothis zea* cells by *Heliothis armigera* and *Trichoplusia ni* nuclear polyhedrosis viruses. *Virology* 112, 174–189
15. Wang, Y. and Jehle, J.A. (2009) Nudiviruses and other large, double-stranded circular DNA viruses of invertebrates: new insights on an old topic. *J. Invertebr. Pathol.* 101, 187–193
16. Bateman, K. and Stentiford, G. (2008) Cancer pagurus bacilliform virus (CpBV) infecting juvenile European edible crabs *C. pagurus* from UK waters. *Dis. Aquat. Org.* 79, 147–151
17. Bateman, K.S. *et al.* (2021) Identification and full characterisation of two novel crustacean infecting members of the family Nudiviridae provides support for two subfamilies. *Viruses* 13, 1694
18. Ros, V.I.D. (2020) Baculoviruses: general features (Baculoviridae). In *Reference Module in Life Sciences* (Zuckerman, M. and Bamford, D.H., eds), pp. 739–746, Elsevier
19. Allain, T.W. *et al.* (2020) A novel nudivirus infecting the invasive demon shrimp *Dikerogammarus haemobaphes* (Amphipoda). *Sci. Rep.* 10, 14816
20. Liu, S. *et al.* (2021) Nudivirus sequences identified from the Southern and Western corn rootworms (Coleoptera: Chrysomelidae). *Viruses* 13, 269
21. Bézier, A. *et al.* (2009) Polydnavirus hidden face: the genes producing virus particles of parasitic wasps. *J. Invertebr. Pathol.* 101, 194–203
22. Thézé, J. *et al.* (2011) Paleozoic origin of insect large dsDNA viruses. *Proc. Natl. Acad. Sci. U. S. A.* 108, 15931–15935
23. Porter, A.F. *et al.* (2019) Diversity and evolution of novel invertebrate DNA viruses revealed by meta-transcriptomics. *Viruses* 11, 1092
24. Shen, Z. *et al.* (2021) Metatranscriptomic analysis reveals an imbalance of hepatopancreatic flora of Chinese mitten crab *Eriocheir sinensis* with hepatopancreatic necrosis disease. *Biology* 10, 462
25. Leinonen, R. *et al.* (2010) The sequence read archive. *Nucleic Acids Res.* 39, D19–D21
26. Cheng, R.-L. *et al.* (2014) Brown planthopper nudivirus DNA integrated in its host genome. *J. Virol.* 88, 5310–5318
27. Liu, S. *et al.* (2020) Endogenous viral elements integrated into the genome of the soybean aphid, *Aphis glycines*. *Insect Biochem. Mol. Biol.* 123, 103405
28. Avery, S. *et al.* (1977) Virus-like particles in a fire ant, *Solenopsis* sp., (Hymenoptera: Formicidae) from Brazil. *Florida Ent.* 60, 17–20
29. Gouranton, J. (1972) Development of an intranuclear nonoccluded rod-shaped virus in some midgut cells of an adult insect, *Gyrinus natorator* L. (Coleoptera). *J. Ultrastruct. Res.* 39, 281–294
30. Smith, K.M. and Xeros, N. (1954) An unusual virus disease of a dipterous larva. *Nature* 173, 866–867
31. Bézier, A. *et al.* (2015) The genome of the nucleopolyhedrosis-causing virus from *Tipula oleracea* sheds new light on the Nudiviridae family. *J. Virol.* 89, 3008–3025
32. Pappalardo, R. *et al.* (1986)  $\tau$  (tau) virus infection of *Carcinus mediterraneus*: histology, cytopathology, and experimental transmission of the disease. *J. Invertebr. Pathol.* 47, 361–368
33. Johnson, P.T. (1988) The rod-shaped nuclear viruses of crustaceans: gut-infecting species. *Dis. Aquat. Org.* 4, 123–141
34. Owens, L. *et al.* (2010) Intranuclear bacilliform virus and hepatopancreatic parvovirus (PmergDNV) in the mud crab *Scylla serrata* (Forsk.) of Australia. *Aquaculture* 310, 47–51
35. Longshaw, M. *et al.* (2012) Parasites and pathogens of the endosymbiotic pea crab (*Pinnotheres pisum*) from blue mussels (*Mytilus edulis*) in England. *J. Invertebr. Pathol.* 109, 235–242
36. Edgerton, B.F. *et al.* (2004) Understanding the causes of disease in European freshwater crayfish. *Conserv. Biol.* 18, 1466–1474
37. Edgerton, B. and Owens, L. (1997) Age at first infection of *Cherax quadricarinatus* by *Cherax quadricarinatus* bacilliform virus and *Cherax giardiavirus*-like virus, and production of putative virus-free crayfish. *Aquaculture* 152, 1–12
38. Anderson, L.G. *et al.* (2021) Patterns of infection in a native and an invasive crayfish across the UK. *J. Invertebr. Pathol.* 184, 107595
39. Edgerton, B. *et al.* (1996) Description of a bacilliform virus from the freshwater crayfish, *Astacus astacus*. *J. Invertebr. Pathol.* 68, 187–190
40. Edgerton, B. *et al.* (2002) An intranuclear bacilliform virus associated with near extirpation of *Austropotamobius pallipes* Lereboullet from the Nant watershed in Ardèche, France. *J. Fish Dis.* 25, 523–531
41. Bateman, K. and Stentiford, G. (2017) A taxonomic review of viruses infecting crustaceans with an emphasis on wild hosts. *J. Invertebr. Pathol.* 147, 86–110
42. Takahashi, Y. *et al.* (1994) Electron microscopic evidence of bacilliform virus infection in kuruma shrimp (*Penaeus japonicus*). *Fish Pathol.* 29, 121–125
43. Couch, J.A. (1974) An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure, prevalence, and enhancement. *J. Invertebr. Pathol.* 24, 311–331
44. Lester, R. *et al.* (1987) Light and electron microscope evidence of baculovirus infection in the prawn *Penaeus plebejus*. *Dis. Aquat. Org.* 3, 217–219
45. Bojko, J. and Ovcharenko, M. (2019) Pathogens and other symbionts of the Amphipoda: taxonomic diversity and pathological significance. *Dis. Aquat. Org.* 136, 3–36
46. Bojko, J. *et al.* (2013) Baseline histopathological survey of a recently invading island population of ‘killer shrimp’, *Dikerogammarus villosus*. *Dis. Aquat. Org.* 106, 241–253
47. Warren, D.A. *et al.* (2022) Histopathological survey for parasite groups in *Gammarus varsoviensis* (Amphipoda). *Dis. Aquat. Org.* 149, 47–51
48. Warren, D.A. *et al.* (2023) Histopathological screening of *Pontogammarus robustoides* (Amphipoda), an invader on route to the United Kingdom. *J. Invertebr. Pathol.* 200, 107970
49. Bojko, J. *et al.* (2023) A new member of the Nudiviridae from the Florida stone crab (*Menippe mercenaria*). *Virology* 588, 109910
50. Sweet, A.D. *et al.* (2020) Lousy grouse: comparing evolutionary patterns in Alaska gallform lice to understand host evolution and host–parasite interactions. *Ecol. Evol.* 10, 8379–8393
51. Beliauskaja, A. *et al.* (2023) Metagenomics of culture isolates and insect tissue illuminate the evolution of *Wolbachia*, *Rickettsia* and *Bartonella* symbionts in *Ctenocephalides* spp. fleas. *Microb. Genom.* 9, mgen001045
52. Feng, Y. *et al.* (2022) A time-series meta-transcriptomic analysis reveals the seasonal, host, and gender structure of mosquito viromes. *Virus Evol.* 8, veac006
53. Tom, M. *et al.* (2013) Crustacean oxi-reductases protein sequences derived from a functional genomic project potentially involved in ecdysteroid hormones metabolism – a starting point for function examination. *Gen. Comp. Endocrinol.* 194, 71–80
54. Song, N. *et al.* (2022) The mitogenome of *Aleuroclava psidii* (Singh, 1931) (Hemiptera: Aleyrodidae) and increased number of mitochondrial gene rearrangements in whiteflies. *Front. Biosci. Landmark* 27, 154
55. Sana, S. *et al.* (2023) *De novo* transcriptome assemblies of five major European oilseed rape insect pests. *BMC Genom.* Data 24, 15
56. Wang, J. *et al.* (2022) Total RNA sequencing of *Phlebotomus chinensis* sandflies in China revealed viral, bacterial, and

- eukaryotic microbes potentially pathogenic to humans. *Emerg. Microbes Infect.* 11, 2080–2092
57. Käfer, S. *et al.* (2019) Re-assessing the diversity of negative strand RNA viruses in insects. *PLoS Pathog.* 15, e1008224
  58. Misof, B. *et al.* (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346, 763–767
  59. Pérez-Moreno, J.L. *et al.* (2018) Transcriptomic insights into the loss of vision in Molnár János Cave's crustaceans. *Integr. Comp. Biol.* 58, 452–464
  60. Stratton, C.E. *et al.* (2024) North American crayfish harbour diverse members of the Nudiviridae. *SSRN*, Published online April 12, 2024. <https://doi.org/10.2139/ssrn.4790364>
  61. Tamura, K. *et al.* (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38, 3022–3027
  62. Tamura, K. *et al.* (2018) Theoretical foundation of the RelTime method for estimating divergence times from variable evolutionary rates. *Mol. Biol. Evol.* 35, 1770–1782
  63. Tao, Q. *et al.* (2020) Reliable confidence intervals for RelTime estimates of evolutionary divergence times. *Mol. Biol. Evol.* 37, 280–290
  64. Murphy, N. *et al.* (2008) Phylogeny of the parasitic microgastroid subfamilies (Hymenoptera: Braconidae) based on sequence data from seven genes, with an improved time estimate of the origin of the lineage. *Mol. Phylogenet. Evol.* 47, 378–395
  65. Kosakovsky Pond, S.L. *et al.* (2020) HyPhy 2.5 – a customizable platform for evolutionary hypothesis testing using phylogenies. *Mol. Biol. Evol.* 37, 295–299
  66. Kosakovsky Pond, S.L. and Frost, S.D. (2005) Not so different after all: a comparison of methods for detecting amino acid sites under selection. *Mol. Biol. Evol.* 22, 1208–1222
  67. Herniou, E.A. *et al.* (2013) When parasitic wasps hijacked viruses: genomic and functional evolution of polydnviruses. *Philos. Trans. R. Soc. B, Biol. Sci.* 368, 20130051
  68. Kumar, S. *et al.* (2022) TimeTree 5: an expanded resource for species divergence times. *Mol. Biol. Evol.* 39, msac174
  69. Brocklehurst, N. and Benson, R.J. (2021) Multiple paths to morphological diversification during the origin of amniotes. *Nat. Ecol. Evol.* 5, 1243–1249
  70. Watson, V. and Rothschild, B. (2021) Deep origin of parasitic disease in vertebrates. In *The Evolution and Fossil Record of Parasitism: Coevolution and Paleoparasitological Techniques* (De Baets, K. and Huntley, J.W., eds), pp. 317–358, Springer
  71. Zhu, Q. *et al.* (2015) Fleas (Siphonaptera) are Cretaceous, and evolved with Theria. *Mol. Phylogenet. Evol.* 90, 129–139
  72. De Moya, R.S. *et al.* (2021) Phylogenomics of parasitic and nonparasitic lice (Insecta: Psocodea): combining sequence data and exploring compositional bias solutions in next generation data sets. *Syst. Biol.* 70, 719–738
  73. Zhang, Y. *et al.* (2024) Stem chewing lice on Cretaceous feathers preserved in amber. *Curr. Biol.* 34, P916–922 E1
  74. Yu, Y. *et al.* (2021) Deep time diversity and the early radiations of birds. *Proc. Natl. Acad. Sci.* 118, e2019865118
  75. Etebari, K. *et al.* (2020) Transcription profile and genomic variations of *Oryctes rhinoceros nudivirus* in coconut rhinoceros beetles. *J. Virol.* 94, e01097–20
  76. Cusumano, A. and Volkoff, A.-N. (2021) Influence of parasitoid-associated viral symbionts on plant–insect interactions and biological control. *Curr. Opin. Insect Sci.* 44, 64–71
  77. Haba, Y. and McBride, L. (2022) Origin and status of *Culex pipiens* mosquito ecotypes. *Curr. Biol.* 32, R237–R246
  78. Safyanova, V. (1964) Laboratory cultivation of sandflies (Diptera: Phlebotominae). *Bull. World Health Organ.* 31, 573
  79. Ahmad, A. *et al.* (2021) Insecta: Phthiraptera. In *Faunal Diversity of Biogeographic Zones of India: North-East* (Chandra, K. and Raghunathan, C. and Kosygin, L. and Gupta, D., eds), pp. 271–280, Zoological Survey of India
  80. Zhang, Y. *et al.* (2022) Mitochondrial phylogenomics provides insights into the taxonomy and phylogeny of fleas. *Parasit. Vectors* 15, 223
  81. Appelgren, A.S. *et al.* (2018) Gene flow and adaptive potential in a generalist ectoparasite. *BMC Evol. Biol.* 18, 1–13
  82. Harnos, A. *et al.* (2017) Size matters for lice on birds: coevolutionary allometry of host and parasite body size. *Evolution* 71, 421–431
  83. Gelderblom, H.R. (1996) Structure and classification of viruses. In *Medical Microbiology* (Baron, S., ed.), University of Texas Medical Branch at Galveston, Galveston (TX). Chapter 41, Available from: <https://www.ncbi.nlm.nih.gov/books/NBK8174/>.
  84. Sandhu, S.K. *et al.* (2021) Revisiting the role of hyperparasitism in the evolution of virulence. *Am. Nat.* 197, 216–235
  85. Togebaye, B.S. *et al.* (2014) Ultrastructure and development of *Nosema podocotyloides* n. sp. (Microsporidia), a hyperparasite of *Podocotyloides magnatestis* (Trematoda), a parasite of *Parapristipoma octolineatum* (Teleostei). *Parasite* 21, 44
  86. Wood, J. and Ashby, B. (2023) Hyperparasitism and the evolution of parasite virulence. *Evolution* 77, 2631–2641
  87. Poulin, R. and Randhawa, H.S. (2015) Evolution of parasitism along convergent lines: from ecology to genomics. *Parasitology* 142, S6–S15
  88. Burke, G.R. *et al.* (2014) Widespread genome reorganization of an obligate virus mutualist. *PLoS Genet.* 10, e1004660
  89. Perry, H.M. *et al.* (2022) Status and management of the blue crab fishery in the Gulf of Mexico. *N. Am. J. Fish Manag.* 42, 164–179
  90. Walters, E.A. *et al.* (2023) Salinity and temperature affect the symbiont profile and host condition of Florida USA blue crabs *Callinectes sapidus*. *J. Invertebr. Pathol.* 198, 107930
  91. Holt, C.C. *et al.* (2019) The first clawed lobster virus *Homarus gammarus nudivirus* (HgNV n. sp.) expands the diversity of the Nudiviridae. *Sci. Rep.* 9, 10086
  92. Arulmoorthy, M. *et al.* (2020) Major viral diseases in culturable penaeid shrimps: a review. *Aquac. Int.* 28, 1939–1967
  93. Yang, Y.-T. *et al.* (2014) The genome and occlusion bodies of marine *Penaeus monodon* nudivirus (PmNV, also known as MBV and PemoNPV) suggest that it should be assigned to a new nudivirus genus that is distinct from the terrestrial nudiviruses. *BMC Genomics* 15, 628
  94. Bojko, J. *et al.* (2022) Pathology and genetic connectedness of the mangrove crab (*Aratus pisonii*) – a foundation for understanding mangrove disease ecology. *Anim. Dis.* 2, 8
  95. Ritter, C.J. and Bourne, D.G. (2024) Marine amphipods as integral members of global ocean ecosystems. *J. Exp. Mar. Biol. Ecol.* 572, 151985
  96. Sandoval, L.A. *et al.* (2022) Leaf consumption and experimental discrimination of stable isotopes between mangrove leaves and the tree-climbing crab (Brachyura: Sesamidae: *Aratus pisonii*). *Estuar. Coast. Shelf Sci.* 274, 107906
  97. Bojko, J. *et al.* (2021) Invasive non-native crustacean symbionts: diversity and impact. *J. Invertebr. Pathol.* 186, 107482
  98. Bojko, J. *et al.* (2018) Green crab *Carcinus maenas* symbiont profiles along a North Atlantic invasion route. *Dis. Aquat. Org.* 128, 147–168
  99. Wood, L. *et al.* (2021) Options for the control of *Dikerogammarus villosus* (killer shrimp) and other invasive amphipods: invasive amphipod control. *Manag. Biol. Invasion.* 12, 662–684
  100. Mancinelli, G. *et al.* (2021) A global occurrence database of the Atlantic blue crab *Callinectes sapidus*. *Sci. Data* 8, 111
  101. Bojko, J. *et al.* (2019) Pathogens of *Dikerogammarus haemobaphes* regulate host activity and survival, but also threaten native amphipod populations in the UK. *Dis. Aquat. Org.* 136, 63–78
  102. Shuttleworth, C.M. *et al.* (2022) An opportunistic assessment of the impact of squirrelpox disease outbreaks upon a red squirrel population sympatric with grey squirrels in Wales. *Animals* 12, 99
  103. Wang, Y. *et al.* (2007) The genome of *Gryllus bimaculatus nudivirus* indicates an ancient diversification of baculovirus-related nonoccluded nudiviruses of insects. *J. Virol.* 81, 5395–5406
  104. Rust, M.K. (2020) Recent advancements in the control of cat fleas. *Insects* 11, 668
  105. Chiu, E. *et al.* (2012) Insect virus polyhedra, infectious protein crystals that contain virus particles. *Curr. Opin. Struct. Biol.* 22, 234–240
  106. Francki, R. (1991) Fifth report of the International Committee on Taxonomy of Viruses. *Arch. Virol. Suppl.* 2, 1–450

107. Keown, J.R. *et al.* (2023) Atomic structure of a nudivirus occlusion body protein determined from a 70-year-old crystal sample. *Nat. Commun.* 14, 4160
108. Cheng, R.-L. *et al.* (2020) Nudivirus remnants in the genomes of arthropods. *Genome Biol. Evol.* 12, 578–588
109. Lin, C.-L. *et al.* (1999) Persistent Hz-1 virus infection in insect cells: evidence for insertion of viral DNA into host chromosomes and viral infection in a latent status. *J. Virol.* 73, 128–139
110. Weitzman, M.D. and Fradet-Turcotte, A. (2018) Virus DNA replication and the host DNA damage response. *Annu. Rev. Virol.* 5, 141–164
111. Minh, B.Q. *et al.* (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37, 1530–1534
112. Letunic, I. and Bork, P. (2021) Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 49, W293–W296