

RESEARCH ARTICLE

Discovery of a novel coltivirus in a newly identified Bat Bug Species (Heteroptera: Cimicidae) in Cambodia

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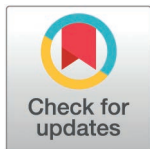
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Data availability statement: Accession numbers for the COI, 16S, and 18S gene sequences of *Stricticimex phnomsampovens* are provided in S1 Table. Sequencing data generated for *Stricticimex coltivirus* (SCCV) are publicly available in the NCBI Sequence Read Archive

Abstract

Bats and their ectoparasites are significant reservoirs and potential vectors of emerging zoonotic pathogens, yet the viral diversity within bat-associated arthropods remains poorly characterized. This study reports the identification of a novel coltivirus (order *Reovirales*), provisionally designated *Stricticimex coltivirus* (SCCV), in a newly described bat bug species, *Stricticimex phnomsampovens*, collected from cave-dwelling wrinkle-lipped free-tailed bats (*Mops plicatus*) in Cambodia. Metagenomic sequencing and phylogenetic analysis revealed that SCCV clusters within the *Coltivirus* genus, showing closest similarity to Tai Forest Reovirus (TFRV) previously isolated from African bats. SCCV was detected in 18.4% of examined bat bugs and successfully isolated in VeroE6 cells, with replication confirmed in multiple mammalian cell lines. The discovery of SCCV extends the known diversity and geographic range of coltiviruses and highlights bat ectoparasites as overlooked hosts of potentially zoonotic viruses. These findings underscore the importance of integrated One Health surveillance targeting both bats and their ectoparasites to better assess the risk of pathogen spillover in biodiverse regions with high human-animal contact.

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Author summary

In this study, we identify a novel coltivirus, named *Stricticimex coltivirus* (SCCV), in a newly described bat bug species collected from cave-dwelling bats in Cambodia. Using metagenomic sequencing and phylogenetic analysis, we find that SCCV is closely related to Tai Forest Reovirus, previously identified in African bats. We successfully isolated the virus in mammalian cell lines, suggesting potential to infect vertebrate hosts. This discovery not only expands the known diversity of coltiviruses but also underscores the role of bat ectoparasites as underexplored reservoirs of potentially zoonotic viruses. Our findings emphasize the importance of integrated One Health surveillance efforts targeting both bats and their ectoparasites to better assess the risk of virus spillovers in regions where human and wildlife habitats overlap.

Introduction

Bats, as one of the most diverse mammalian groups, are recognized as reservoirs for a wide range of pathogens, including viruses with zoonotic potential, and have therefore become a major focus of disease surveillance [1]. Their ecological characteristics—such as high species diversity, colony-based roosting, long lifespans, and frequent contact with humans and domestic animals—create opportunities for pathogen maintenance and spillover. At this wildlife–human interface, ectoparasitic arthropods associated with bats may play an important but underexplored role in pathogen transmission. While ectoparasitic arthropods are well-known vectors of human pathogens in other host systems, their role in pathogen transmission among bats, humans, and other species remains poorly understood [2]. Common bat-associated ectoparasites, including bat bugs (*Cimicidae* and *Polyctenidae*), bat flies (*Nycteribiidae* and *Streblidae*), fleas (*Ischnopsyllidae*), mites (*Acar*), and ticks (*Argasidae* and *Ixodidae*), are hematophagous and have been reported to occasionally feed on non-bat hosts, including domestic animals, wildlife, and humans [2]. Such cross-host feeding behavior may facilitate the transmission of pathogens between bats and other species, positioning these ectoparasites as potential bridge vectors. Consistent with this, multiple bat-associated ectoparasites have been shown to harbor zoonotic and potentially zoonotic pathogens [3,4], underscoring their potential relevance for public and animal health [2,5–9].

Cimicid insects (family *Cimicidae*) are hematophagous ectoparasites that primarily feed on mammals, including humans and bats. In Cambodia, the known cimicid fauna comprises six species (Table 1) [10,11]. Between the two species of bed bugs, only *Cimex hemipterus* (Fabricius, 1803) primarily feeds on humans [12–14]. The other cimicids reported from Cambodia, including *Aphrania thnotae* (Klein, 1970), *Aphrania vishnou* (Mathur, 1953), *Cimex insuetus* (Ueshima, 1968), *Crasscimex apsarae* (Klein, 1969), and *Stricticimex parvus* (Ueshima, 1968), are bat-associated ectoparasites that predominantly parasitize bats and are typically restricted to bat roosting environments, such as caves and roosts, like most cimicid species [14].

Table 1. Cimicidae species reported in Cambodia.

Subfamily	Species	Year of report	Location	Host
Cimicinae	<i>Cimex insuetus</i> Ueshima 1968 (Syn. <i>Cimex angkorae</i> Klein 1969)	1969 [11]	Angkor Wat, Siem Reap	<i>Taphozous melanopogon bicolor</i> Temminck [15]
	<i>Cimex hemipterus</i> (Fabricius, 1803) (Bed bug, cosmopolitan)	1969 [10]	Pochentong, Chroi Chang War (sic.; Chroy Chang War); Phnom Penh, Kompong Sela (sic.; Kampong Seila); Kampot	Humans
Cacodminae	<i>Aphrania thnotae</i> Klein, 1970	1970 [15]	Ang Sdock, Takeo province, Prek Takong Kandal province	<i>Scotophilus kuhli gairdneri</i> Kloss
	<i>Aphrania vishnou</i> (Mathur, 1953) (Syn. <i>Aphrania orientalis</i> Ferris & Usinger, 1953)	1969 [11]	Prek-Léap, Phnom Penh (Klein, 1969a); Barong region, Phnom Penh; Prek Phnau, Phnom Penh; Phnom Chiso (sic Phnom Chisor)	<i>Cynopterus sphinx</i> (Mathur, 1953), <i>Scotophilus kuhli gairdneri</i> Kloss (mentioned as <i>Scotophilus temmincki</i>), <i>Cynopterus brachyotis angulatus</i> ; humans [10]
	<i>Crassicimex apsarae</i> Klein, 1969	1969 [10]	Angkor Wat, Siem Reap [10]; Vat Nokor (sic. Wat Nokor) Kampong Cham province [15]	<i>Taphozous melanopogon bicolor</i> Temminck [10]
	<i>Stricimex parvus</i> Ueshima, 1968 (Syn. <i>Strictimex khmerensis</i> Klein, 1969)	1969 [10]	Vat Nokor in Kampong Cham [15]	<i>Taphozous melanopogon bicolor</i> Temminck [10], <i>Chaerephon plicatus</i> (Buchanan, 1800); Humans [15]

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Notably, two bat-associated species, *A. vishnou* (Mathur, 1953) and *S. parvus* (Ueshima, 1968), have been reported to readily feed on humans upon cave entry [10,15]. This capacity to feed on both bats and humans highlights their potential role as bridge vectors for pathogen transmission. In Cambodia, activities such as bat guano farming and bat ecotourism increase human exposure to bats and their ectoparasites, underscoring the need to better understand their potential public health implications.

Recent advancements in sequencing technologies have significantly increased virus discoveries, particularly in bats and arthropods due to their relevance in potential spillover events to humans and livestock [3,16–28]. One particularly intriguing interface is the bat-arthropod interaction, as these ectoparasites and their microbiome may influence the ecology of infested bats, affecting their health, survival, and behavior [3,29]. Although there is increasing evidence of pathogens in bat ectoparasites, surveillance efforts remain skewed toward viruses in bats themselves, while bacterial pathogens are more frequently studied in their ectoparasites [2,29,30]. Only a few zoonotic viruses have been identified in bat-associated ectoparasites, including Issyk-Kul and Kasokero viruses (Bunyaviridae) in bats and bat ticks [31–34]. Additionally, Kaeng Khoi virus, another bunyavirus, was found in *S.x parvus*, *C. insuetus* and free-tailed bats in Thailand, with neutralizing antibodies detected in cave guano miners, suggesting a potential vector role [35,36]. However, beyond these findings, our understanding of the virome of bat-associated ectoparasites, their feeding ecology, vectorial capacity, and public health risks remains limited.

One virus group that exemplifies these knowledge gaps in bat ectoparasite-associated zoonotic viruses is the genus of Coltivirus within the family of Spinareoviridae, order Reovirales. Currently, there are six member species of coltiviruses; Colorado tick fever virus (CTFV), Eyach virus (EYAV), Kundal virus (KUNDV), Tai Forest reovirus (TFRV), and Tarumizu

virus (TarTV) [37]. Coltiviruses are named after its first described member CTFV [38], and are segmented dsRNA viruses, usually transmitted by ticks. CTFV infections in humans are mainly reported in North America and are linked to flu-like symptoms, meningitis and encephalitis [39]. The other prominent Coltivirus, EYAV appears to be broadly distributed in Europe with isolations from ticks from Germany [40] and France [41] and associated with human neurological disease through serological evidence [40,42]. Except CTFV and EYAV, all Coltiviruses were identified through molecular surveillance in the last ten years. Most known Coltiviruses or Colti-like viruses were identified and isolated from ticks: *Dermacentor* sp. for CTFV, *Ixodes* sp. for EYAV as well as Shelly headland virus (SHLV) from Australia [17], and Gierle tick virus in Belgium [43], *Hyalomma* sp. for KUNDV from India [44] and Jeddah tick coltivirus from Saudi Arabia [45], and *Haemaphysalis* sp. for TarTV in Japan [46] and O'hara headland virus in Australia [47]. Exceptions are Lishui pangolin virus (LSPV) identified in China in 2018 from dead pangolins (*Manis javanica*) [48] and TFRV found 2016 in African free-tailed bats (*Chaerephon aloysiisabaudiae*) in Côte d'Ivoire [49]. Out of all Coltiviruses, only CTFV is well characterized including its environmental stability, clinical presentation [50] and transmission through ticks, blood transfusion and vertical transmission from mother to child [51]. Available serological diagnostics are limited to in-house ELISAs, IFA and neutralization tests for CTFV [52–56] and EAYV [42,57,58].

The discovery of novel viruses at the bat-arthropod interface remains a critical frontier in understanding zoonotic risk, particularly in biodiverse regions where human activities increasingly overlap with wildlife habitats. This study describes the identification of a previously unrecognized *Stricticimex* species in Cambodia and a novel coltivirus in Southeast Asia, expanding both the known diversity of bat ectoparasites and their associated virome. These findings highlight the ecological complexity of bat-ectoparasite-pathogen interactions and underscore the need for targeted surveillance to evaluate their potential role in pathogen transmission and emerging infectious disease threats.

Materials and methods

Ethics statement

The field mission was conducted in accordance with Cambodian guidelines and received formal approval from the Ministry of Agriculture, Forestry and Fisheries (MAFF), Ministry of Health National Ethics Committee for Health Research (NECHR #008), local governments, and land/site owners. All necessary permissions were obtained prior to data collection, ensuring compliance with local regulations and ethical standards. The study prioritized biosecurity measures, minimized disruption to the environment and wildlife, and upheld principles of transparency, respect, and responsible data handling.

Collection and processing of bat bugs

The cave of Ta Rumm is located in Sampeu hill, Banan district, Battambang province, north-western Cambodia (13.022 N, 103.095 E). This cave has two vertical entrances, accessible by descending 7–8 meters using a custom-made ladder installed by local guano miners (Fig 1A and 1B). The cave hosts a roost of nearly two million wrinkle-lipped free-tailed bats (*Mops plicatus*) (Fig 1C), the sole species documented to inhabit this location [59]. Bat guano is harvested in this cave on average twice a month using shovels and a custom pulley system.

In June 2022, the Institut Pasteur du Cambodge (IPC) field team entered the cave through the lower entrance wearing full biosafety level 3-adequate personal protective equipment to collect fresh environmental samples (guano and urine) from bats (Fig 1A–1B). During this mission, 13 bat bugs (Fig 1D) were opportunistically collected, seven were preserved in viral transport medium (VTM) consisting of 2.95% tryptose phosphate broth, 145 mM of NaCl, 5% gelatin, 54 mM Amphotericin B, 106/L U penicillin-streptomycin, 80 mg/L gentamicin (Sigma-Aldrich, Steinheim, Germany), while six bat bugs were stored in 70% ethanol. The bat bugs in VTM were transferred into liquid nitrogen within six hours for transport back to IPC and subsequently stored at -80°C until further analysis. The ethanol preserved bat bugs were kept on ice and handed over to the Medical and Veterinary Entomology Unit at IPC for morphological identification.

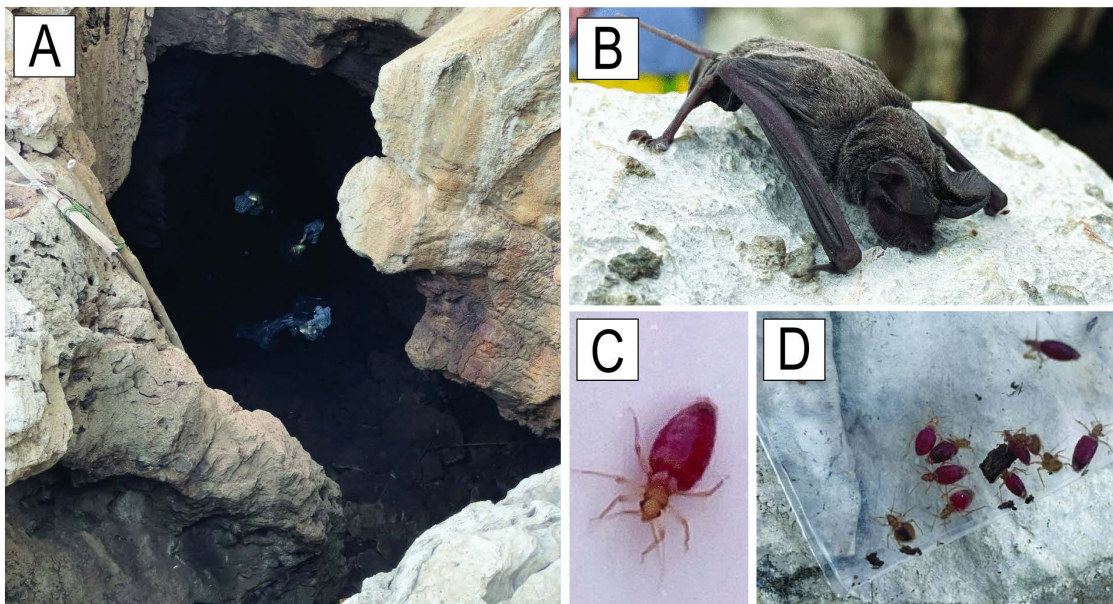


Fig 1. Ta Rumm cave in Phnom Sampov and bat bug collection. (A) IPC field team staff in personal protective equipment (B) Lower entrance of the Ta Rumm cave. IPC field team collecting bat guano and urine inside the cave. (C) Wrinkle-lipped free-tailed bat (*Chaerephon plicatus*). (D-E) blood engorged bat bugs (*Stricticimex phnomsampovenssis* Suor & Maquart n.sp.). (Photos: Jurre Y. Siegers/Institut Pasteur du Cambodge).

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The initial identification prompted the need for more specimens. Subsequently, we provided additional tubes to local bat guano collectors for further collection of bat bugs during a harvesting session in April 2023. In total, 80 specimens were stored in VTM, six in 70% ethanol and one was left in no medium for later mounting on a cover slide. These were transported on ice back to IPC within 24 hours and subsequently stored as previously described.

Morphological bat bug identification

The bat bugs were determined using the determination key provided by Usinger (1966) [60] and the descriptions provided in Ueshima (1968) [61], Klein (1969a, 1969b, 1970) [10,11,15].

Speciation (COI, 16s, and 18S) PCR and Sanger sequencing

Parasite species barcoding was performed using three PCR systems targeting invertebrate Cytochrome c oxidase subunit I (COI) [62], 16S and 18S rRNA genes [63–66]. Total nucleic acid of individual sample was extracted using Zymo Research Direct-zol RNA MiniPrep kit (Zymo Research, Cat # R2050, CA, USA) according to the manufacturer's instructions after a homogenization step (MagNA Lyser, Roche). Following reverse transcription using SuperScript III First-Strand Synthesis Super-Mix (Invitrogen, San Diego, CA) according to the manufacturer's instructions, cDNA was used for the different barcoding PCR protocols (Table 2).

The COI gene was targeted using primers LC01490-F and HC02198-R. During amplification, the following steps were used: 1 cycle of 94°C for 2 minutes, 40 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute, and a final extension of 1 cycle of 72°C for 10 minutes. The 16S rRNA gene was targeted with the primers 16S LR-J and 16S LR-N. Amplification was performed with the following conditions: 1 cycle of 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 48°C for 40 seconds, and 72°C for 60 seconds, and a final extension of 1 cycle of 72°C for 7 minutes. Finally, the 18S rRNA gene was targeted using pairs of primers 18S-1, 18S-3 and 18S-2, 18S-4. Following conditions were used

Table 2. Primers used for species identification.

Name	Sequence	Target	Size (bp)	Reference
LC01490-F	5'-GGTCAACAAATCATAAAGATATTGG-3'	COI	710	Folmer et al., 1994 [62]
HC02198-R	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'			
16S LR-J	5'-GCGAACATCGACAAGTCGAACGCACAT-3'	16S	415	Kambhampati et al., 1995; Simon et al., 1994 [64,66]
16S LR-N	5'-TTGTTTTCTCCGCTTATTAATATGCTTAA-3'			
18S-1	5'-CTGGTTGATCCTGCCAGTAGT-3'	18S	1,200	Tian et al., 2008
18S-3	5'-GGTTAGAACTAGGGCGGTATCT-3'			
18S-2	5'-AGATACCGCCCTAGTTCTAACC-3'		800	
	18S-4			
		5'-GATCCTTCTGCAGGTTACC-3'		

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for both PCR and nested PCR steps: 1 cycle of 94°C for 2 minutes, 40 cycle of 94°C for 30 seconds, 48°C for 60 seconds, and 72°C for 60 seconds, and final extension of 1 cycle of 72°C for 10 minutes.

All amplifications were performed using Invitrogen Platinum Taq polymerase and T100 Thermal Cycler, Bio-Rad. All PCR products were subsequently sequenced by Sanger sequencing (Macrogen, Inc., Seoul, Republic of Korea) in both forward and reverse directions using relevant primers from PCR systems (from the nested PCR for 18S amplification).

Phylogenetic analysis Cambodian bat bug

The obtained COI, 16S, and 18S sequences were concatenated and aligned with Geneious Prime 2023.2 (Geneious, <https://www.geneious.com>). Additional sequences of *Cimicidae* species and outgroups were obtained from the National Center for Biotechnology Information GenBank database (S1 Table). A Bayesian consensus tree was created using the MrBayes [67] Geneious plugin, with the General Time Reversible model with gamma distribution and invariant sites [68]. Chain length was set to 5,000,000, sampling frequency to 500, and burn-in length to 100,000. The random seed was set to 21,775. Phylogenetic trees were edited using TreeViewer.

Virus sequencing

Twist comprehensive viral research panel

The sequencing libraries were generated from homogenized, pooled (n=4) bat bugs using the combination of Twist Library Preparation Enzymatic Fragmentation (EF) kit 2.0 (#104211) and hybridization with Twist Comprehensive Viral Research Panel (#1035550), according to the manufacturer's protocol (Twist Total Nucleic Acids Library Preparation EF Kit 2.0 for Viral Pathogen Detection and Characterization Protocol and Twist Target Enrichment Standard Hybridization v1 protocol). Briefly, RNA was extracted using Direct-zol RNA Miniprep Kits (R2053) and the extracted nucleic acid was converted to cDNA using ProtoScript II First Strand cDNA Synthesis kit (E6560S) and Random Primer 6 (S1230S) from New England Biolab (NEB). The NEB Next Ultra II Non-Directional RNA second Strand Synthesis kit (E6111S) was subsequently used to convert the single-stranded cDNA to double stranded DNA (dsDNA). The Illumina TruSeq-compatible libraries were generated using the Twist Library Preparation EF kit and the generated libraries were pooled with 6 libraries per pool. Each library was measured for concentration and normalized to the same mass before pooling, with the total combined mass not exceeding 4 µg. The pooled libraries then underwent hybridization capture using the Twist Comprehensive Viral Research Panel. Following the enrichment, the enriched libraries were pooled again in equimolar ratios and sequencing with loading concentration of 6pM and spiked with 1% Phix control V3 using Illumina Miseq. The pooled library was diluted and denatured according to the standard Miseq System Denature and Dilute Libraries Guide (Document # 15039740v10), and sequenced to generate paired-end 75bp reads using a 150 cycle Miseq V3 reagent kit (illumina, MS-102–3001). After

sequencing, demultiplexed FASTQ files were generated and analyzed using Genome Detective [69] which performs quality control, de novo assembly of reads into contigs, and taxonomic assignment through similarity searches against custom made and curated viral databases. The pipeline employs a novel alignment approach that reconstructs viral genomes by reference-guided linking of de novo contigs, integrating both amino acid-level and nucleotide-level alignment scores. This combined scoring strategy improves detection and assembly of divergent viruses.

NovaSeq

Sequencing libraries were generated from Coltivirus infected Vero cell supernatants using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (New England Biolabs, MA, USA) following the manufacturer's instructions. First-strand cDNA synthesis was performed using the ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs, MA, USA), followed by second-strand synthesis with the NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module (New England Biolabs, MA, USA). The resulting double-stranded DNA was fragmented to an average size of approximately 400 bp before adapter ligation. Adapter-ligated DNA fragments were then amplified by PCR using the NEBNext Multiplex Oligos for Illumina. At each step, purification was performed using AMPure XP beads (Beckman Coulter), and DNA quantification was carried out with a Qubit 4.0 fluorometer. Final libraries were sequenced on an Illumina NovaSeq X platform (Macrogen, Inc.), generating paired-end 150 bp (PE150) reads.

Phylogenetic analysis coltivirus

A total number of 96 Reovirales including species in the genus, *Mycoreovirus*, *Cypovirus*, *Dinovernavirus*, *Oryzavirus*, *Fijivirus*, *Aquareovirus*, *Orthoreovirus*, unclassified Reovirales, and all species of *Coltiviruses* were obtained from NCBI GenBank (assessed on 2025-01-26). A full list of the GenBank protein accession numbers is provided as [S3 Table](#). Sequences were aligned with MAFFT v.7.490 [70], option L-INS-i and trimmed using trimAL [71], option -gappyout. Phylogenetic trees were constructed using IQ-TREE v.2.0.3 [72] using ModelFinder with the best-fit amino acid substitution model Q.pfam+F+I+R6 for RdRp and LG+F+R3 for VP2 chosen according to Bayesian Information Criterion (BIC), with 1,000 bootstrap replicates used to assess branch support. Trees were visualized and annotated using FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) and Adobe Illustrator 2024.

Genome annotation

Coltivirus genomes were annotated with Geneious Prime v2025.1.2 (Biomatters Ltd., New Zealand). Briefly, all available Coltivirus sequences were downloaded from the NCBI database (accessed 29 October 2025). Each newly generated segment was aligned to annotated Coltivirus reference sequences using the Annotate from Database function implemented in Geneious. Start and stop codons as well as reading frames were manually inspected and adjusted where necessary. Final annotations were verified by visual inspection of translated coding sequences and exported in GenBank format for downstream analyses and submission to GenBank. The viral sequences were deposited in GenBank under accession numbers PZ358652–PZ358681.

Real-Time quantitative PCR (RT-qPCR)

A duplex real-time quantitative PCR (RT-qPCR) system was developed to target the viral RNA-dependent RNA polymerase (RdRp) gene. The sequence of this gene was aligned using CLC Genomics Workbench 5.5 with the closest available Coltivirus sequences, - Tai Forest reovirus (accession: KX989543.1) and Kundal virus (accession: NC_055248.1). Based on this alignment, two probes were designed: one targeting the newly identified *Stricticimex coltivirus* sequence and another targeting the Tai Forest reovirus sequence ([Table 3](#)). Prior to RNA extraction, samples underwent homogenization using a MagNA Lyser instrument (Roche) with 1.2–1.4 mm ceramic beads (Saint-Gobain, China). Viral RNA

Table 3. Primers and probe designed to detect *Stricticimex coltivirus* (SCCV).

Name	Sequence	Target	Size (bp)
Colti_F3 (Forward)	5' TGAAAAGTTTGGATGGGGTCA 3'	RdRp	105
Colti_R3 (Reverse)	5' ACAGCCTCGTAATTAACCA 3'		
Colti_P3_Kh (Probe 1)	5'-FAM GATGGTTGGTATGCTTGGCTT 3'-BHQ1		
Colti_P3_Tai (Probe 2)	5'-HEX CGTAGCTAACTCATTAGAG 3'-BHQ2		

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was extracted from individual samples using the Direct-zol RNA MiniPrep Kit (Zymo Research, Cat #R2050, CA, USA), following the manufacturer's instructions. Bat bug RNA samples were screened using the developed duplex real-time PCR. A 25 µL reaction mix contained 5 µL of cDNA, 12.5 µL of 2x reaction buffer provided with the Superscript III one-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany) containing 0.5 µL of a 50mM MgSO₄ solution (Invitrogen) and 0.3 µL of 20-40U/µL of RNasin ribonuclease inhibitor (Promega). Primers and probes were used at final concentrations of 200nM. PCR cycling conditions were as follows: 50°C for 15 minutes followed by 95°C for 5 minutes before 45 cycles of 95°C for 15 seconds, 56°C for 30 seconds and 72°C for 1 minute, using CFX9 Real-Time PCR detection system (Bio-Rad).

Cell lines

All cell lines were obtained from either the American Type Culture Collection (ATCC) or the European Collection of Cell Cultures (ECACC), except for the Rhileki cell line, which was provided by G.J. Smith (Duke-NUS, Singapore), and the Aag-2 cell line, provided by J. Pompon (Duke-NUS, Singapore). The Rhileki cell line is a spontaneously immortalized, clonal cell line derived from kidney tissue of a *Rhinolophus lepidus* bat (NUS-IACUC B01/12), as previously described [73]. The invertebrate cell lines Aag-2 (*Aedes aegypti*) and C6/36 (*Aedes albopictus*, CRL-1660) were maintained in Leibovitz L-15 medium (Sigma-Aldrich) supplemented with 5% fetal calf serum (FCS; Gibco), 10% tryptose phosphate broth (Sigma-Aldrich), 2 mM L-glutamine (Gibco), and 100 U/mL penicillin-streptomycin (Pen/Strep; Gibco) at 28°C. Vertebrate cell lines were cultured at 37°C with 5% CO₂. Most of these, including BHK-21 (baby hamster kidney, *Mesocricetus auratus*, CCL-10C) and VeroE6 (simian kidney, *Cercopithecus aethiops*, C1008), were grown in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich) supplemented with 10% FCS and 100 U/mL Pen/Strep. Caco-2 cells (human adenocarcinoma, HTB-37) were additionally supplemented with 1 mM HEPES (Gibco), while Rhileki cells were supplemented with 1% non-essential amino acids (Gibco) and 1 mM sodium pyruvate (Gibco). HeLa (human adenocarcinoma, CRM-CCL-2) and MDCK (Madin-Darby canine kidney, *Canis familiaris*, CCL-34) cells were maintained in Minimum Essential Medium (MEM; Sigma-Aldrich) supplemented with 10% FCS and 100 U/mL Pen/Strep. For viral infection experiments, the FCS content in the culture medium was reduced to 3% for invertebrate cell lines and 5% for vertebrate cell lines (Table 3).

Virus isolation and propagation

For virus isolation, homogenized bat bug samples were sterile-filtered using a 0.45 µm filter (Millipore, Burlington, MA, USA) and inoculated to cell monolayers in six-well plates at approximately 80% confluence. Inoculated cells were maintained in DMEM supplemented with 2% FCS, Pen/Strep, and 0.25 µg/mL amphotericin B (Gibco) for seven days. Cell morphology and cytopathic effects were automatically monitored every six hours using an IncuCyte S3 live-cell imaging system (Sartorius, Göttingen, Germany). At the end of the incubation period, cell supernatants were collected by centrifugation and cells were harvested by trypsinization with 0.05% trypsin-EDTA (Gibco). All samples were subsequently

analyzed by RT-qPCR. Following initial isolation in VeroE6 cells, the virus was further passaged in this cell line, and virus-containing VeroE6 culture supernatants were used for inoculating growth kinetic experiments.

Results

Description of *Stricticimex phnomsampovens*

Upon examination, the *Stricticimex* specimens appeared to belong to a new species, closely related to *Stricticimex parvus* and are described below. The updated list of bat bugs in Cambodia, their references, location and known hosts are provided in [Table 1](#). The type series is kept in the collection of the Institut Pasteur du Cambodge, Phnom Penh.

Holotype. [Fig 2](#). Male. Head: length: 0.44, slightly longer than wide (0.41), interocular space 5.3 times as wide as eye; Head with 7 setae forming a “y” shape, joining together in the vertex; labrum with 10 pairs of bristles; 5 bristles (3 longer and 2 medium) along inner margin of each eye in addition to about 3 pairs of vertex. Antennae measuring 1.7mm long, always less than 2mm long; Segments I-IV: 0.15; 0.42; 0.7; 0.43. Second antennal segment as long as width of head, 0.42: 0.41. Second and fourth segments are subequal. Rostrum 0.48 size of segments I-III: 0.2:0.18:0.1. Pronotum 0.6 wide, twice as wide as long (0.6: 0.28) and straight on the inner sides, narrowed and rounded laterally and posteriorly. Longest bristles at sides as long as those on pronotum. Hemelytral pads are almost twice as large as long (0.37:0.19) and

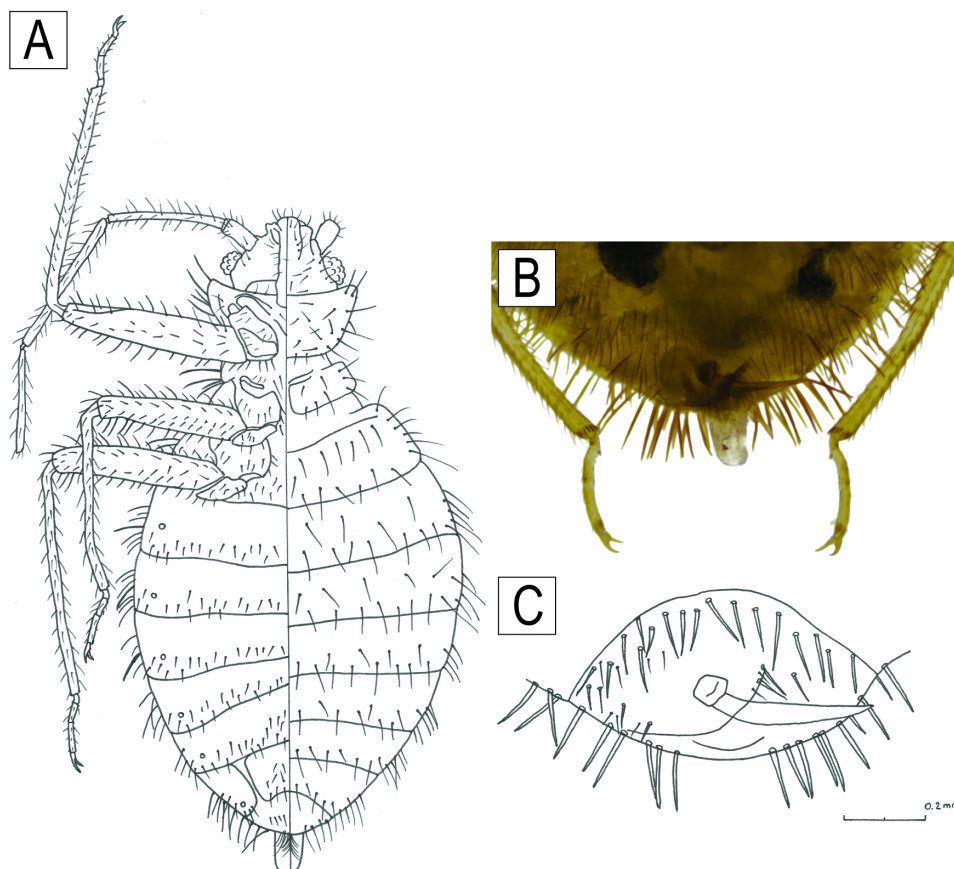


Fig 2. *Stricticimex phnomsampovens* Suor & Maquart n.sp description. (A) Drawing of *Stricticimex phnomsampovens* Suor & Maquart n.sp lateral view (left) and dorsal view (right) (B) Photo Male genital (C) Male genital drawing.

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straight on inner sides, narrowed and rounded laterally and posteriorly. Longest bristles at sides about as long as those on the pronotum. Abdomen suboval; second and third segments with 3–4 ill-defined rows of bristles, remaining segments with 2 rows of bristles and with cluster of bristles on terminal segment. Ventral surface with much finer and numerous bristles on the posterior part of each segment. Base of paramere forming an acute angle, then continuing by curving sideways, paramere half as long as the genital segment at base (Fig 2). Legs long and slender; hind femora about 4,5 times as long as the greatest width, 0.79:0.18; tibiae 2 times longer than femora, 1.6:0.79.

Allotype female. Like male. Paragenital sinus sinuate broadly on hind margin of third tergite sublaterally. Ectospermalegae broad at opening, directed inwardly and then bent downward. Pronotum width/length: 0,68/0.3. Hemelytras width/length: 0.20/0.38. Hind tibia/femur: 1,86/0,94.

Etymology. The name refers to the location where this new species was found (Phnom Sampov).

Ecology. This species was found in a cave dominated by *Mopsalicatus* (Buchanan, 1800), probably feeding off the bats, but they were aggressive, readily feeding on humans entering in the cave (Fig 1E).

Differential diagnosis. The new species, close to *S. parvus* differs by the following characters: (i) Clypeus short and stout while it is more elongated and pointing forward in *S. parvus*; (ii) Hemelytrals pads twice as long as wide, where it is square-shaped in *S. parvus*; (iii) Hind tibiae 2 times longer than femora, while it is 1,5 times longer in *S. parvus*; (iv) Shape of paramere, usually straight or directed downward for *S. parvus* but curving sideways on the left for *S. phnomsampovens* with a more acute angle (obtuse angle for *S. parvus*) at the phallobase.

Authorship. The authors of the new taxon, *Stricticimex phnomsampovens*, are Suor and Maquart, and are different from the authors of this article, in accordance with Article 50.1 and Recommendation 50A of the International Code of Zoological Nomenclature (ICZN, 2012, <https://www.iczn.org/the-code/the-code-online/>).

Updated Key to the Species of *Stricticimex* (revision of Usinger's 1966 key in Ueshima, 1968)

- 1.Fore femora slightly longer than tibiae. Size small, pronotum 0.6 mm wide. India.....pattoni (Horvath)
Fore femora is slightly shorter than tibiae. Size medium to large, pronotum 0.7 mm or more in width...2
- 2.Second antennal segment shorter or as long as than width of head.....3
Second antennal segment much longer than width of head.....6
- 3.Hind femora less than 4 times as long as wide. South Africa..... *transversus* Ferris & Usinger
Hind femora 4 times or more as long as wide.....4
- 4.Second antennal segment as long as the length of the pronotum at median line or longer.....5
Second antennal segment shorter than length of pronotum. Interocular space wider than the length of second antennal segment. Size medium. Pronotum 1.0 mm wide. Egypt. *namru* Usinger
- 5.Clypeus pointing forward, hemelytrals pads as long as wide, hind tibiae 1,5 times longer than femora. Obtuse angle at the base of the paramere, continuing almost in a straight line or slightly downward..... *parvus* Ueshima
Clypeus short and stout, hemelytrals pads twice as long as wide, hind tibiae 2 times longer than femora. Base of paramere forming an acute angle, curving sideways. *phnomsampovens* **n.sp.**
- 6.Third antennal segment more than twice as long as fourth. Size large, pronotum 1.0 mm or more in width. South Africa.....antennatus Ferris & Usinger

- Third antennal segment less than twice as long as fourth. Size smaller, pronotum less than 1 mm wide.....7
7. Last antennal segment longer than width of head. Longest bristles at sides of pronotum, wing pads and abdomen about 0.37 mm. Kenya.....intermedius Ferris & Usinger
- Last antennal segment subequal to width of head. Longest bristles at sides of pronotum, wing pads and abdomen about 0.31 mm. Democratic Republic of Congo. brevispinosus Usinger

Phylogenetic analysis of *Stricticimex phnomsampovens*

A BLAST search of the COI sequences obtained from bat bugs collected in Ta Rumm Cave revealed the closest match to *Cyanolicimex patagonicus* (*Haemosiphoninae*), with a percentage identity of 82.77% and query coverage of 98% (GenBank: MG596833). The 16S sequences showed similarity to *Leptocimex inordinatus* (*Cacodminae*), with a 100% percentage identity but low query coverage (65–66%; GenBank: KT592539), indicating potential genetic divergence.

Analysis of the 18S gene fragment revealed high similarity to multiple species across different subfamilies, including *Cimex latipennis* (Cimicinae, 98.9% identity, 100% query coverage; GenBank: KF018720), *Latrocimex spectans* (Latrocimicinae, 98.7% identity, 99–100% query coverage; GenBank: MZ378786), and *Cimex* sp. (Cimicinae, 98.5–98.8% identity, 99% query coverage; GenBank: EU683122). Notably, trimmed sequence comparisons showed 100% similarity among our specimens.

The Bayesian consensus tree, constructed using concatenated sequences of COI, 16S, and 18S rRNA gene fragments, provides strong support for the classification of this newly discovered species within the *Cacodminae* subfamily (Fig 3). This phylogenetic placement, combined with the genetic divergence observed, suggests *Stricticimex phnomsampovens* represents a novel species within bat-associated *Cimicidae* (Fig 3).

Identification and Phylogenetic analysis of a Novel *Coltivirus* in Cambodian Bat Bugs

A novel coltivirus, *Stricticimex coltivirus* (SCCV), was identified in *Stricticimex phnomsampovens* collected from Ta Rumm Cave. The high level of amino acid identity between proteins of SCCV and Tai Forest reovirus (TFRV) and other coltivirus species suggest that SCCV belongs to the same genus (Table 4). Phylogenetic analysis of the available viral genomes revealed that all identified proteins were most closely related to members of the genus *coltivirus*, which includes the tick-borne pathogenic CTFV, other tick-associated viruses, and TFRV, previously identified in African free tailed bats (Figs 4 and 5). Further in-depth analysis of the RNA-dependent RNA polymerase (RdRp) gene, based on amino acid sequences, showed the highest similarity to TFRV, with nucleotide identity ranging from 74.6% to 75.1% and amino acid similarity between 88.8% and 89.0% (Fig 4 and Table 4). Comparisons of other identified viral proteins revealed amino acid similarities exceeding 70% for VP5, >80% for VP1, VP3, and VP10, and >90% for VP2, VP8, VP9, and VP11, all of which were most similar to TFRV. Notably, VP4 displayed a partially shared similarity with Kundal (Table 4). Due to the lack of available nucleotide or protein sequences for VP6, VP7, and VP12 from TFRV, no contigs were generated for these gene segments, leaving their genomic composition unresolved.

Phylogenetic analyses of the conserved RdRp and VP2 protein sequences revealed that the SCCV clusters within a distinct clade alongside known *coltivirus* and *coltivirus*-like viruses detected in bats (Figs 4 and 5). Notably, SCCV is most closely related to *Tai Forest reovirus* (TFRV), originally isolated in 2006 from free-tailed bats (*Mops aloysiisabaudiae*) in Côte d'Ivoire. More distantly, and with greater uncertainty due to partial RdRp and VP2 sequences, SCCV also shares similarities with *coltivirus*-like viruses detected in *Taphozous melanopogon* bats detected in Guangxi, China in 2017.

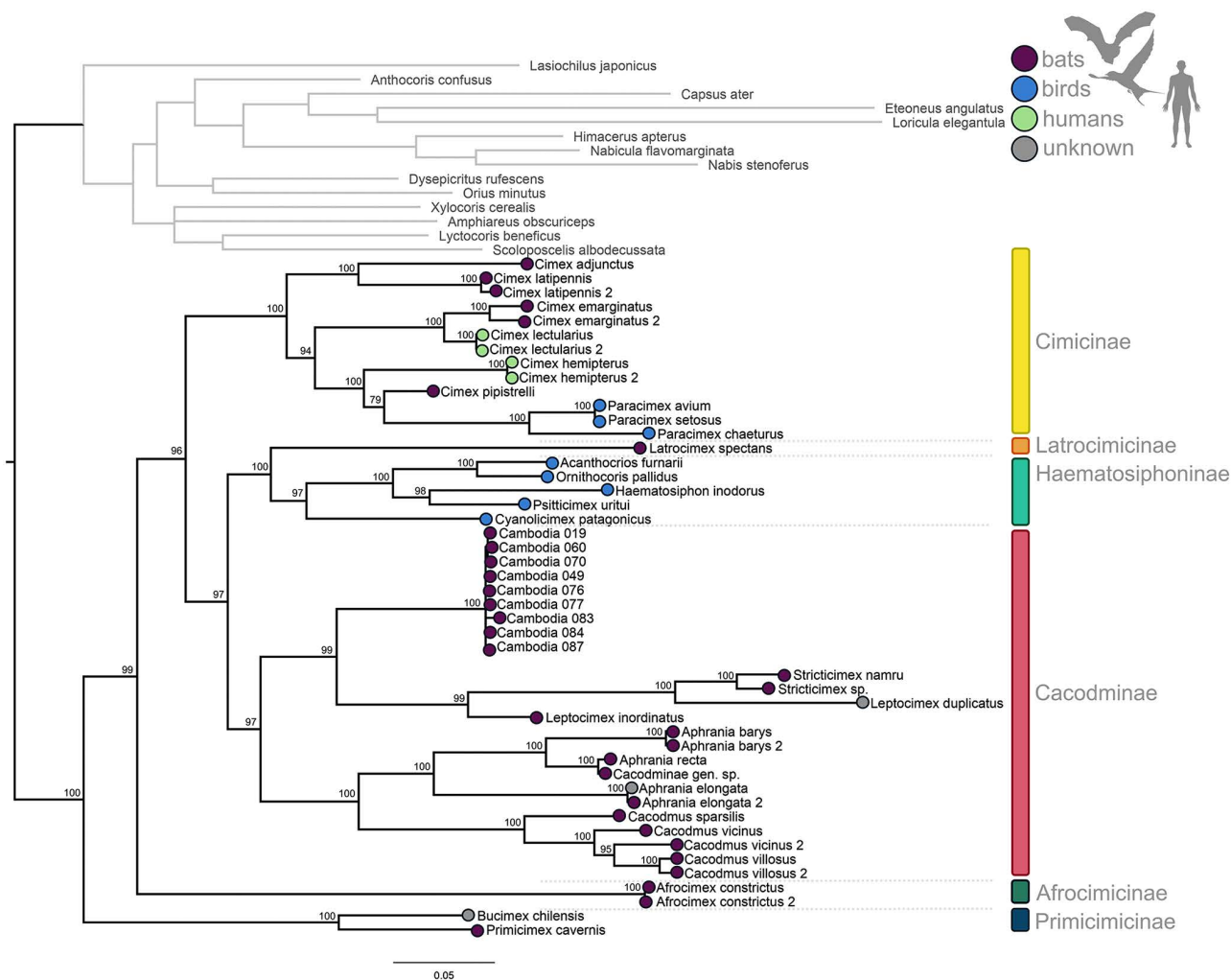


Fig 3. Bayesian consensus tree of family Cimicidae (including all six described subfamilies) based on concatenated sequences of COI, 16S, and 18S rRNA gene fragments. GenBank accession numbers are indicated in [S1 Table](#). The scale bar indicates the number of substitutions per site. Main host groups of each species are indicated with circles (purple: bats; blue: birds; green: humans; gray: unknown host). Animal silhouettes used in this figure were sourced from PhyloPic (<https://www.phylopic.org/>).

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The strong bootstrap support for the clustering of SCCV sequences with TFRV indicates a relatively close evolutionary relationship (Figs 4 and 5). Phylogenetic analysis of the RdRp and VP2 proteins further reveals that bat-associated colt-iviruses form a monophyletic clade within the coltivirus genus. However, the presence of partial protein sequences introduces some uncertainty at certain branching points and may not fully capture the true evolutionary relationships.

Taken together, the sequence data, gene segment identities, and phylogenetic placement strongly support SCCV as a novel *coltivirus* species, expanding our understanding of bat-associated reoviruses. Further studies are needed to fill phylogenetic gaps and refine its evolutionary history and clarify its relationship to other *Coltivirus* members and whether bats and bat-associated ectoparasites occupy a phylogenetic niche within the *Coltivirus* genus.

Table 4. Nucleotide and amino acid similarities between Stricticimex coltivirus, Tai Forest Reovirus and Kundal virus.

N/A: not available

SCCV: Stricticimex coltivirus

TFRV: Tai Forest Reovirus

KUNV: Kundal Coltivirus

*: based on ICTV

#: Manual BLASTp data for VP3 AA identity

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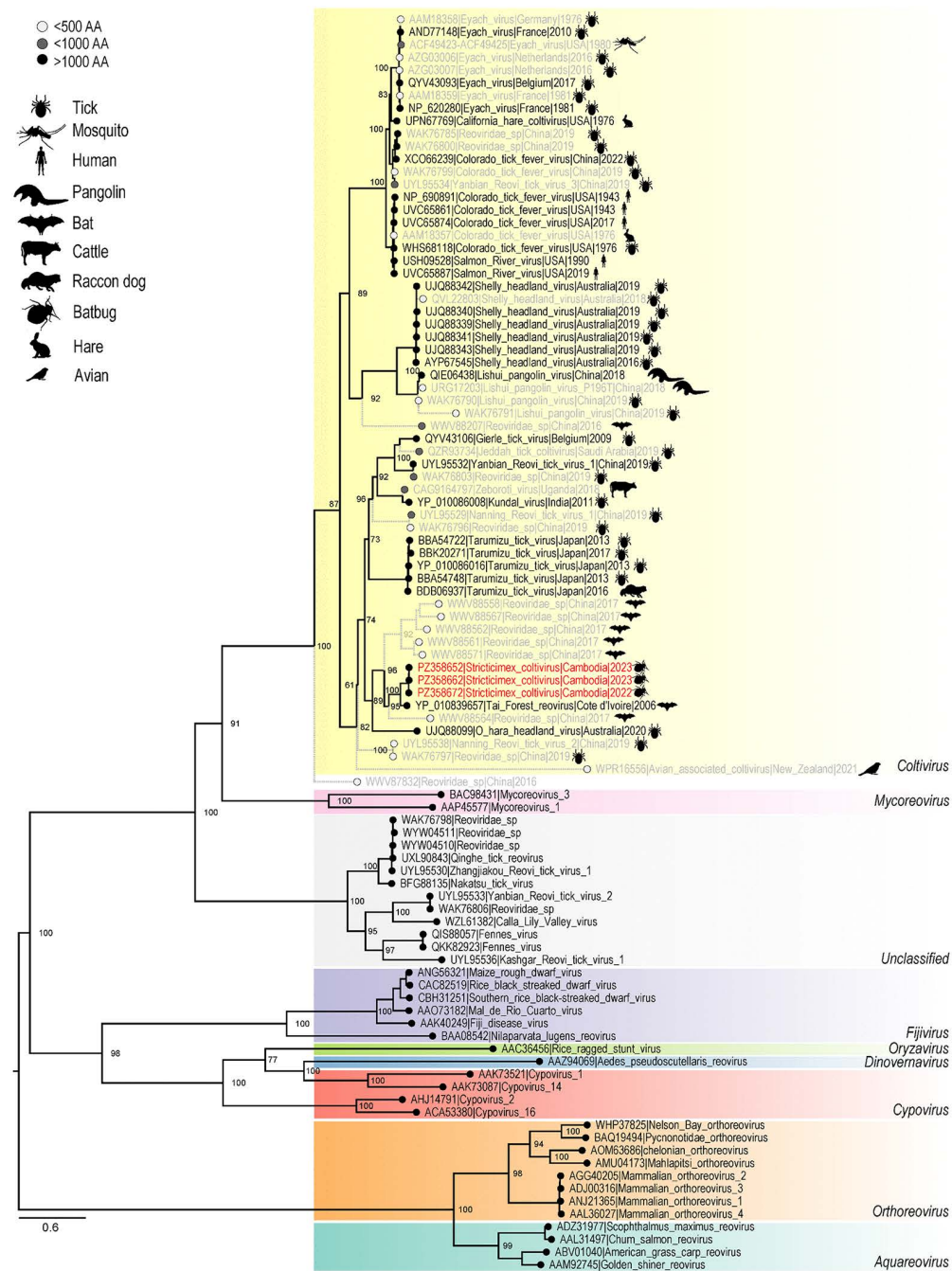


Fig 4. Maximum likelihood tree of the RdRp protein from Coltiviruses and other Reovirales. Bootstrap values are indicated at key nodes. Stricticimex coltivirus (SCCV) in bold red. Shading corresponding to the different Reovirales genera. Tip shading and branch style reflect amino acid lengths of the sequence used to generate phylogenetic tree. Animal symbols within the Coltivirus genus represent the isolation host. Animal silhouettes used in this figure were sourced from PhyloPic (<https://www.phylopic.org/>).

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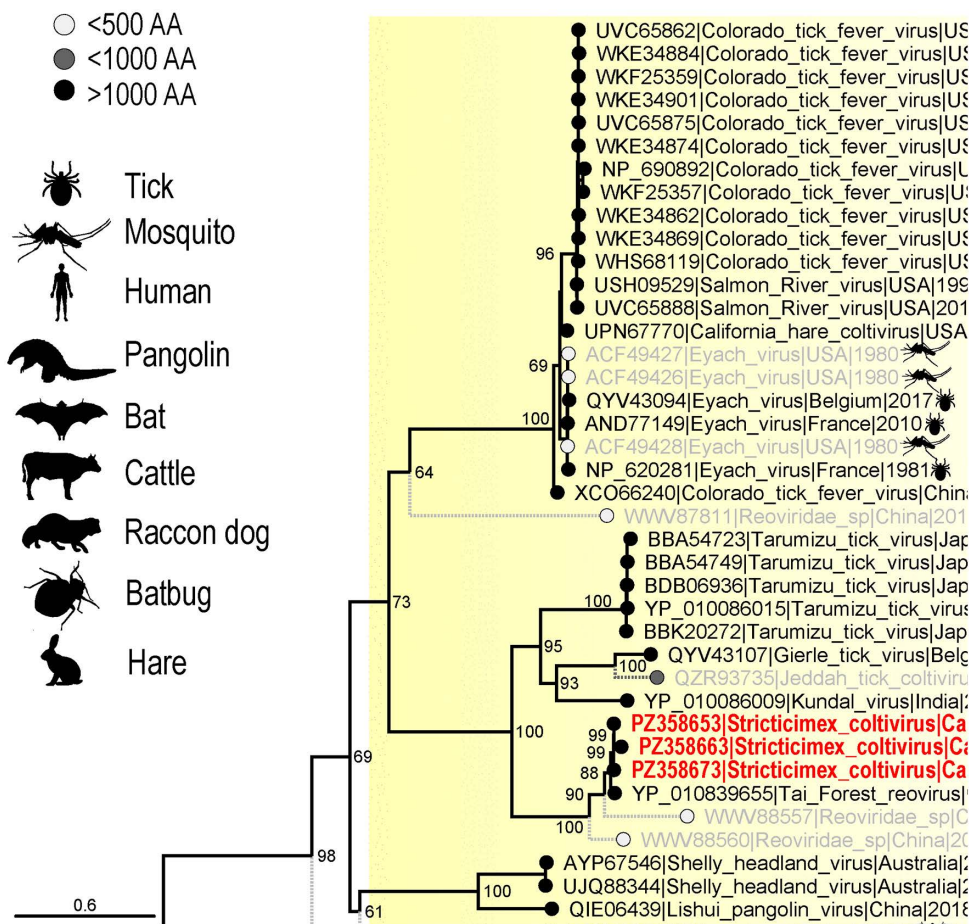


Fig 5. Maximum likelihood tree of the VP2 protein from Coltiviruses and other Reovirales. Bootstrap values are indicated at key nodes. Stricticimex coltivirus (SCCV) in bold red. Shading corresponding to the different Reovirales genera. Tip shading and branch style reflect amino acid lengths of the sequence used to generate phylogenetic tree. Animal symbols within the Coltivirus genus represent the isolation host. Animal silhouettes used in this figure were sourced from PhyloPic (<https://www.phylopic.org/>).

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Coltivirus prevalence in Cambodian bat bugs

A total of 87 individual bat bugs collected from Ta Rumm Cave in April 2023 were screened for coltivirus using a newly developed real-time PCR assay targeting the RdRp gene. Sixteen (18.4%) tested positive, with Ct values ranging from 23.02 to 40.58 (mean Ct: 30.18). All positive samples were identified as the SCCV based on a specifically designed probe, while no samples tested positive for Tai Forest reovirus (TFRV).

Virus Isolation

Virus isolation was attempted using homogenized bat bugs to inoculate C6/36 (*Aedes albopictus*), BHK-21 (*Mesocricetus auratus*), Rhileki (*Rhinolophus lepidus*), and VeroE6 (*Chlorocebus sabaeus*) cell lines. Initial inoculations included samples with Ct values ranging from 23.02 to 40.58. Successful virus isolation was achieved in VeroE6 cells from samples with Ct values of 23.02 and 25.29. The presence of the virus was confirmed in the culture supernatant across three subsequent passages using the SCCV specific RT-PCR assay.

Virus growth kinetics

The replication dynamics of one SCCV isolate was assessed in invertebrate (Aag-2, C6/36) and vertebrate (BHK-21, Caco-2, MDCK, Rhileki, VeroE6) cell lines over 12 days. Culture supernatants and cells were analyzed by RT-PCR to detect the presence of SCCV (Fig 6). Viral entry was observed in all tested cell lines at some point during the experiment (Fig 6B). However, productive viral replication, indicated by the release of virus into the culture supernatant, was only detected in Caco-2, HeLa, BHK-21, and VeroE6 cells. Among these, BHK-21 and VeroE6 cells exhibited the highest viral titers, with replication increasing over time (Fig 6A).

Discussion

We report on the identification of a novel coltivirus species in a previously unknown bat ectoparasite, *-stricticimex-*, species in Cambodia highlighting the importance of understanding potentially zoonotic viruses associated with bat ectoparasites. In recent years, several coltiviruses have been identified in Asia [74], however, to the best of our knowledge, the coltivirus described in this study marks the first of its genus to be identified in Southeast Asia. Given the fact that some members of the coltivirus genus can cause human disease highlights the importance of understanding potentially zoonotic viruses associated with bat ectoparasites. Phylogenetic analyses suggest that the *Stricticimex coltivirus* and *Tai*

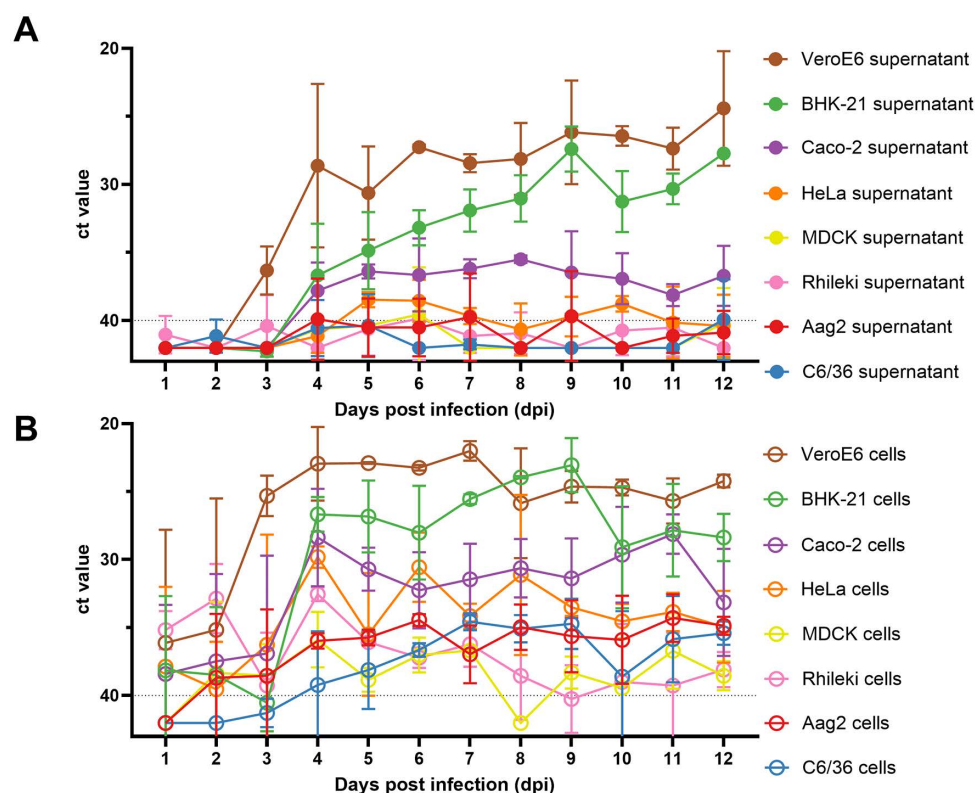


Fig 6. In vivo growth kinetics of *Stricticimex coltivirus* (SCCV). Various invertebrate (Aag2 in red; C6/36 in blue and shades of green) and vertebrate cell lines (Vero E6 in brown; BHK-21 in green; Caco-2 in purple; HeLa in orange; MDCK in yellow and shades of blue), including a bat-derived cell line (in pink/violet), were inoculated with the SCCV isolate (passage 3 in Vero E6) for 1 hour at 37 °C. Supernatants (A) and cells (B) were collected every 24 hours and analyzed by RT-PCR. Experiments were conducted in biological duplicates. The Ct values are plotted inversely to intuitively demonstrate viral growth.

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Forest Reovirus (TFRV) may share a recent common ancestor, showing limited evolutionary divergence at the protein level despite significant geographic separation. TFRV was isolated from the blood of African free-tailed bats (*Chaerephon aloysiisabaudiae*) in Côte d'Ivoire in 2006 and capable of causing cytopathic effects on C6/36 insect cells and on various mammalian cell lines (VeroE6, human cell lines MRC-5 and Hep2, and a fruit bat cell line originating from *Rousettus aegyptiacus* (R05T)) [49]. Although both TFRV and SCCV were able to infect and replicate in several mammalian, -including human-, cells, it remains to be determined whether both viruses are able to infect, replicate and cause disease in humans.

The *Stricticimex* coltivirus was identified in bat bugs present at a large colony of cave dwelling wrinkle lipped free-tailed bats. Bat parasites are often considered to be host specific due to the ecological isolation of bats or the associated life history strategies of these parasites [75] highlighting the possibility of a similar virus-host dynamic as for TFRV. TFRV is the only coltivirus reported from bats, with no detection in ticks so far, suggesting a potentially unique virus-host relationship. The prevalence of the SCCV in the collected bat bugs from Ta Rumm cave was notably high at nearly 20%. Given that this study was limited to two sampling events within one year, more extensive surveillance is needed to determine if similar prevalence rates are observed in other caves and during different seasons, and to understand if these rates are influenced by factors such as the bat birthing period or seasonal variations in ectoparasite infestations, similar to patterns observed with coronaviruses [76] and bat flies in other studies [77]. As SCCV was identified only in bat bugs, it remains to be investigated if this is primarily a bat or a bat bug virus and whether it has the potential to infect other cave-dwelling species such as rodents. In addition, Ta Rumm cave as well as other caves in the area are used for guano harvesting increasing the chance of zoonotic pathogen transmission to humans either directly from bats or from bat ectoparasites. Future serological studies should elucidate whether guano farming practices increase the risk of coltivirus exposure. In addition, bat ecotourism, such as observation of bat mega colonies exodus at dusk, visits to pagodas inside bat caves, can increase the risk of zoonotic pathogen exposure and transmission by bringing humans into close proximity with bat colonies, thereby facilitating opportunities for direct or indirect contact with bats and bat excreta that may harbor zoonotic pathogens. The induction of CPE in VeroE6 cells and SCCV's close phylogenetic relationship to human pathogenic viruses suggest its potential to infect humans. Febrile illnesses, like those caused by CTFV, are common in tropical regions and often go undiagnosed. In remote areas in Cambodia, where guano collectors enter bat-inhabited caves, limited healthcare access and a lack of diagnostic testing could mean that an SCCV-like disease remains undetected. Together, this necessitates further research to explore the virus's reservoir, host range, cross-species transmission capabilities and serological studies into human exposure.

Merely identifying these viral sequences is not sufficient for comprehensive risk assessments. Detailed *in vitro* and *in vivo* studies are required to evaluate their cross-species transmissibility, prior exposure in humans and other animals, and pathogenicity. Such efforts should adopt a One Health approach, integrating human, animal, and environmental health to achieve a holistic understanding of virus ecology. A proactive surveillance, rather than waiting for outbreaks to occur, can potentially detect and prevent future zoonotic spillovers, thereby safeguarding global health. Additionally, this work highlights the importance of traditional and molecular taxonomy of hematophagous parasites, which could further enhance our understanding of potential vectors for animal and human pathogens. Particularly in tropical regions, there are still a number of bat-associated parasites likely undescribed, which could serve as vectors for both known and unknown animal and human-associated diseases. Future studies should pay particular attention to those closely related to species that occasionally feed on humans or other non-chiropteran hosts, such as *Leptocimex* species. These behavioral traits may indicate a broader host range in related species, including *Stricticimex* spp., suggesting their potential role in interspecies pathogen transmission.

The bat bugs analyzed in this study belong to the Cacodminae subfamily, with *S. phnomsampovens* exhibiting a close phylogenetic relationship to other *Stricticimex* and *Leptocimex* species, as supported by both molecular and morphological data. These phylogenetic findings align with previous studies that clarify relationships among Cimicoidea subfamilies

[14,65,78]. Species within Cacodminae, including *Leptocimex* and *Stricticimex*, are obligate blood-feeding ectoparasites primarily associated with bats from the Molossidae and Vespertilionidae families across Asia, Africa, and Europe [79]. The species of Cacodminae subfamily, including species such as *Leptocimex* and *Stricticimex*, are blood-feeding ectoparasites predominantly targeting bats of the Molossidae and Vespertilionidae families across various regions including Asia, Africa and Europe [79]. Given the broader context, where tens of thousands of new arthropod species are described annually [80], approximately 14,000 species of arthropods across over 400 genera are known to have developed the capacity to feed on vertebrate blood, including specific groups like certain mosquitoes, ticks, fleas, and some flies, such as sand-flies and horseflies. This represents a small fraction of the total arthropod species, indicating that blood-feeding (hematophagy), is a relatively rare trait among arthropods [81]. However, the arthropods known for hematophagy have been intensely studied due to their importance in transmitting pathogens.

Several limitations should be considered when interpreting our findings. First, although morphological characterization strongly supports the placement of *Stricticimex phnomsampovens* within the genus *Stricticimex*, molecular phylogenetic analyses based on concatenated COI, 16S, and 18S rRNA gene fragments do not unambiguously resolve genus-level boundaries within *Cacodminae*. The limited availability and occasional misidentification of reference sequences for bat-associated cimicids in public databases constrain phylogenetic resolution at this taxonomic depth, and expanded, better-curated molecular datasets will be required to refine genus-level relationships. Second, while SCCV was detected in bat bugs and demonstrated productive replication in mammalian cell lines *in vitro*, the natural transmission cycle remains unknown. The present study does not establish whether bats serve as vertebrate hosts or whether the virus is transmitted between bat bugs, bats and humans. Addressing this question would require host range studies and serological investigations in bats and exposed human populations. Finally, three genome segments of SCCV were not recovered. Sequencing of segmented RNA viruses can exhibit substantial segment imbalance due to library preparation bias, amplification efficiency, and sequence divergence, as reflected in the nucleotide and amino acid similarities reported in Table 4. Consequently, certain segments may assemble poorly or fall below similarity detection thresholds despite adequate overall read depth ultimately limiting full genome reconstruction.

The discovery of a novel bat bug species and its associated virus underscore the critical need for One Health surveillance, emphasizing the interconnectedness of ecosystems and the potential for zoonotic disease emergence. This study demonstrates that with field access, modern analytical methods, and cross-disciplinary collaboration, pathogen detection in high-risk areas can proactively identify emergence risks. By fostering a comprehensive understanding of pathogen presence, emergence, and spread, One Health surveillance supports proactive public health strategies, mitigating outbreak risks and enhancing global health security [82].

Supporting information

S1 Table. COI, 16S and 18S Sequences of Cimicidae species and outgroups obtained from NCBI GenBank.
(DOCX)

S2 Table. Body measurements of *Stricticimex phnomsampovens* Suor & Maquart sp. Nov.
(DOCX)

S3 Table. GenBank accession numbers.
(DOCX)

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References

1. Letko M, Seifert SN, Olival KJ, Plowright RK, Munster VJ. Bat-borne virus diversity, spillover and emergence. *Nat Rev Microbiol*. 2020;18(8):461–71. <https://doi.org/10.1038/s41579-020-0394-z> PMID: 32528128
2. Szentiványi T, Szabadi KL, Görföl T, Estók P, Kemenesi G. Bats and ectoparasites: exploring a hidden link in zoonotic disease transmission. *Trends Parasitol*. 2024;40(12):1115–23. <https://doi.org/10.1016/j.pt.2024.10.010> PMID: 39516134
3. Ortiz-Baez AS, Jaenson TGT, Holmes EC, Pettersson JH-O, Wilhelmsson P. Substantial viral and bacterial diversity at the bat-tick interface. *Microb Genom*. 2023;9(3):mgen000942. <https://doi.org/10.1099/mgen.0.000942> PMID: 36862584
4. Szentiványi T, Heintz A-C, Markotter W, Wassef J, Christe P, Glaizot O. Vector-borne protozoan and bacterial pathogen occurrence and diversity in ectoparasites of the Egyptian Roussette bat. *Med Vet Entomol*. 2023;37(2):189–94. <https://doi.org/10.1111/mve.12639> PMID: 36625469
5. Sandor AD, Mihalca AD, Domsa C, Peter A, Hornok S. Argasid ticks of Palearctic bats: distribution, host selection, and zoonotic importance. *Frontiers in Veterinary Science*. 2021;8:684737.
6. Gill JS, Rowley WA, Bush PJ, Viner JP, Gilchrist MJR. Detection of human blood in the bat tick *Carios (Ornithodoros) kelleyi* (Acari: Argasidae) in Iowa. *J Med Entomol*. 2004;41(6):1179–81. <https://doi.org/10.1603/0022-2585-41.6.1179> PMID: 15605658
7. Péter Á, Barti L, Corduneanu A, Hornok S, Mihalca AD, Sándor AD. First record of *Ixodes simplex* found on a human host, with a review of cases of human infestation by bat tick species occurring in Europe. *Ticks Tick Borne Dis*. 2021;12(4):101722. <https://doi.org/10.1016/j.ttbdis.2021.101722> PMID: 33865178
8. Vlaschenko A, Răileanu C, Tauchmann O, Muzyka D, Bohodist V, Filatov S, et al. First data on bacteria associated with bat ectoparasites collected in Kharkiv oblast, Northeastern Ukraine. *Parasit Vectors*. 2022;15(1):443. <https://doi.org/10.1186/s13071-022-05582-3> PMID: 36434644
9. Reeves WK, Beck J, Orlova MV, Daly JL, Pippin K, Revan F. Ecology of bats, their ectoparasites, and associated pathogens on Saint Kitts Island. *J Med Entomol*. 2016;53(5):1218–25.
10. Klein J-M. Nouvelles punaises du Cambodge: *Crassimex apsarae* n. sp. et *Stricticimex khmerensis* n. sp. *Bulletin de la Société entomologique de France*. 1969;:87–96.
11. Klein JM. *Cimex angkorae* n. sp., une nouvelle Punaise du Cambodge [Hem. Cimicidae]. *Bulletin de la Société entomologique de France*. 1969:139–45.
12. Reinhardt K, Siva-Jothy MT. Biology of the bed bugs (Cimicidae). *Annu Rev Entomol*. 2007;52:351–74. <https://doi.org/10.1146/annurev.ento.52.040306.133913> PMID: 16968204
13. Hamlili FZ, Bérenger JM, Parola P. Cimicids of Medical and Veterinary Importance. *Insects*. 2023;14(4):392. <https://doi.org/10.3390/insects14040392> PMID: 37103207
14. Roth S, Balvín O, Siva-Jothy MT, Di Iorio O, Benda P, Calva O, et al. Bedbugs Evolved before Their Bat Hosts and Did Not Co-speciate with Ancient Humans. *Curr Biol*. 2019;29(11):1847–1853.e4. <https://doi.org/10.1016/j.cub.2019.04.048> PMID: 31104934
15. Klein JM. Cimicides du Cambodge. (III). Description des mâles de *Crassimex apsarae stricticimex parvus* et *Aphrania thnotae* n. sp. [Hemiptera, Cimicidae]. *Annales de la Société entomologique de France (NS)*. 1970;6(3):713–9.
16. Liu Z, Li L, Xu W, Yuan Y, Liang X, Zhang L, et al. Extensive diversity of RNA viruses in ticks revealed by metagenomics in northeastern China. *PLoS Negl Trop Dis*. 2022;16(12):e0011017. <https://doi.org/10.1371/journal.pntd.0011017> PMID: 36542659
17. Harvey E, Rose K, Eden J-S, Lo N, Abeyasuriya T, Shi M, et al. Extensive Diversity of RNA Viruses in Australian Ticks. *J Virol*. 2019;93(3):e01358–18. <https://doi.org/10.1128/JVI.01358-18> PMID: 30404810
18. Damian D, Maghembe R, Damas M, Wensman JJ, Berg M. Application of Viral Metagenomics for Study of Emerging and Reemerging Tick-Borne Viruses. *Vector Borne Zoonotic Dis*. 2020;20(8):557–65. <https://doi.org/10.1089/vbz.2019.2579> PMID: 32267808
19. Yan X, Liu Y, Hu T, Huang Z, Li C, Guo L, et al. A compendium of 8,176 bat RNA viral metagenomes reveals ecological drivers and circulation dynamics. *Nat Microbiol*. 2025;10(2):554–68. <https://doi.org/10.1038/s41564-024-01884-7> PMID: 39833544
20. Ge X, Li Y, Yang X, Zhang H, Zhou P, Zhang Y, et al. Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *J Virol*. 2012;86(8):4620–30. <https://doi.org/10.1128/JVI.06671-11> PMID: 22345464
21. Zheng X-Y, Qiu M, Guan W-J, Li J-M, Chen S-W, Cheng M-J, et al. Viral metagenomics of six bat species in close contact with humans in southern China. *Arch Virol*. 2018;163(1):73–88. <https://doi.org/10.1007/s00705-017-3570-3> PMID: 28983731
22. Wang J, Pan Y-F, Yang L-F, Yang W-H, Lv K, Luo C-M, et al. Individual bat virome analysis reveals co-infection and spillover among bats and virus zoonotic potential. *Nat Commun*. 2023;14(1):4079. <https://doi.org/10.1038/s41467-023-39835-1> PMID: 37429936
23. Feng KH, Brown JD, Turner GG, Holmes EC, Allison AB. Unrecognized diversity of mammalian orthoreoviruses in North American bats. *Virology*. 2022;571:1–11. <https://doi.org/10.1016/j.virol.2022.03.012> PMID: 35421704

24. Van Brussel K, Holmes EC. Zoonotic disease and virome diversity in bats. *Curr Opin Virol*. 2022;52:192–202. <https://doi.org/10.1016/j.coviro.2021.12.008> PMID: 34954661
25. Wang D, Yang X, Ren Z, Hu B, Zhao H, Yang K, et al. Substantial viral diversity in bats and rodents from East Africa: insights into evolution, recombination, and cocirculation. *Microbiome*. 2024;12(1):72. <https://doi.org/10.1186/s40168-024-01782-4> PMID: 38600530
26. Bolatti EM, Viarengo G, Zorec TM, Cerri A, Montani ME, Hosnjak L. Viral metagenomic data analyses of five new world bat species from Argentina: identification of 35 novel DNA viruses. *Microorganisms*. 2022;10(2).
27. Albuquerque NK, Silva SP, Aragão CF, Cunha TCAS, Paiva FAS, Coelho TFSB, et al. Virome analysis of *Desmodus rotundus* tissue samples from the Amazon region. *BMC Genomics*. 2024;25(1):34. <https://doi.org/10.1186/s12864-023-09950-w> PMID: 38177994
28. Shi M, Lin X-D, Tian J-H, Chen L-J, Chen X, Li C-X, et al. Redefining the invertebrate RNA virosphere. *Nature*. 2016;540(7634):539–43. <https://doi.org/10.1038/nature20167> PMID: 27880757
29. Szentivanyi T, McKee C, Jones G, Foster JT. Trends in Bacterial Pathogens of Bats: Global Distribution and Knowledge Gaps. *Transbound Emerg Dis*. 2023;2023:9285855. <https://doi.org/10.1155/2023/9285855> PMID: 40303798
30. Lei BR, Olival KJ. Contrasting patterns in mammal-bacteria coevolution: bartonella and leptospira in bats and rodents. *PLoS Negl Trop Dis*. 2014;8(3):e2738. <https://doi.org/10.1371/journal.pntd.0002738> PMID: 24651646
31. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, et al. Kasokero virus: a new human pathogen from bats (*Rousettus aegyptiacus*) in Uganda. *Am J Trop Med Hyg*. 1986;35(2):387–92. <https://doi.org/10.4269/ajtmh.1986.35.387> PMID: 3082234
32. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. *Emerg Infect Dis*. 2020;26(12):2944–50. <https://doi.org/10.3201/eid2612.202411> PMID: 33219649
33. Cholleti H, de Jong J, Blomström A-L, Berg M. Investigation of the Virome and Characterization of Issyk-Kul Virus from Swedish *Myotis brandtii* Bats. *Pathogens*. 2022;12(1):12. <https://doi.org/10.3390/pathogens12010012> PMID: 36678360
34. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, et al. First detection of bat-borne Issyk-Kul virus in Europe. *Sci Rep*. 2020;10(1):22384. <https://doi.org/10.1038/s41598-020-79468-8> PMID: 33361773
35. Neill WA, Kading RC. Viral Ecology and Natural Infection Dynamics of Kaeng Khoi Virus in Cave-Dwelling Wrinkle-Lipped Free-Tailed Bats (*Chaerophon plicatus*) in Thailand. *Diseases*. 2021;9(4):73. <https://doi.org/10.3390/diseases9040073>
36. Williams JE, Imlarp S, Top FH Jr, Cavanaugh DC, Russell PK. Kaeng Khoi virus from naturally infected bedbugs (cimicidae) and immature free-tailed bats. *Bull World Health Organ*. 1976;53(4):365–9. PMID: 1086729
37. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Alfenas-Zerbini P, et al. Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses (2022). *Arch Virol*. 2022;167(11):2429–40. <https://doi.org/10.1007/s00705-022-05516-5> PMID: 35999326
38. Florio L, Stewart MO, Mugrage ER. The Etiology Of Colorado Tick Fever. *J Exp Med*. 1946;83(1):1–10. <https://doi.org/10.1084/jem.83.1.1> PMID: 19871512
39. Fagre A, Lehman J, Hills SL. Colorado Tick Fever in the United States, 2013–2022. *Am J Trop Med Hyg*. 2024;111(3):598–602.
40. Rehse-Küpper B, Casals J, Rehse E, Ackermann R. Eyach—an arthropod-borne virus related to Colorado tick fever virus in the Federal Republic of Germany. *Acta Virol*. 1976;20(4):339–42. PMID: 9824
41. Chastel C, Main AJ, Couatarmanac'h A, Le Lay G, Knudson DL, Quillien MC, et al. Isolation of Eyach virus (Reoviridae, Colorado tick fever group) from *Ixodes ricinus* and *I. ventralis* ticks in France. *Arch Virol*. 1984;82(3–4):161–71. <https://doi.org/10.1007/BF01311160> PMID: 6508529
42. Málková D, Holubová J, Kolman JM, Marhoul Z, Hanzal F, Kulková H, et al. Antibodies against some arboviruses in persons with various neuropathies. *Acta Virol*. 1980;24(4):298. PMID: 6106383
43. Vanmechelen B, Merino M, Vergote V, Laenen L, Thijssen M, Martí-Carreras J, et al. Exploration of the *Ixodes ricinus* virosphere unveils an extensive virus diversity including novel coltiviruses and other reoviruses. *Virus Evol*. 2021;7(2):veab066. <https://doi.org/10.1093/ve/veab066> PMID: 34532065
44. Yadav PD, Whitmer SLM, Sarkale P, Fei Fan Ng T, Goldsmith CS, Nyayanit DA, et al. Characterization of Novel Reoviruses Wad Medani Virus (Orbivirus) and Kundal Virus (Coltivirus) Collected from *Hyalomma anatolicum* Ticks in India during Surveillance for Crimean Congo Hemorrhagic Fever. *J Virol*. 2019;93(13):e00106–19. <https://doi.org/10.1128/JVI.00106-19> PMID: 30971476
45. Zakham F, Albalawi AE, Alanazi AD, Truong Nguyen P, Alouffi AS, Alaoui A, et al. Viral RNA Metagenomics of *Hyalomma* Ticks Collected from Dromedary Camels in Makkah Province, Saudi Arabia. *Viruses*. 2021;13(7):1396. <https://doi.org/10.3390/v13071396> PMID: 34372602
46. Fujita R, Ejiri H, Lim C-K, Noda S, Yamauchi T, Watanabe M, et al. Isolation and characterization of Tatumizu tick virus: A new coltivirus from *Haemaphysalis flava* ticks in Japan. *Virus Res*. 2017;242:131–40. <https://doi.org/10.1016/j.virusres.2017.09.017> PMID: 28964878
47. Gofton AW, Blasdel KR, Taylor C, Banks PB, Michie M, Roy-Dufresne E, et al. Metatranscriptomic profiling reveals diverse tick-borne bacteria, protozoans and viruses in ticks and wildlife from Australia. *Transbound Emerg Dis*. 2022;69(5):e2389–407. <https://doi.org/10.1111/tbed.14581> PMID: 35502617
48. Gao W-H, Lin X-D, Chen Y-M, Xie C-G, Tan Z-Z, Zhou J-J, et al. Newly identified viral genomes in pangolins with fatal disease. *Virus Evol*. 2020;6(1):veaa020. <https://doi.org/10.1093/ve/veaa020> PMID: 32296543

49. Weiss S, Dabrowski PW, Kurth A, Leendertz SAJ, Leendertz FH. A novel Coltivirus-related virus isolated from free-tailed bats from Côte d'Ivoire is able to infect human cells in vitro. *Virol J*. 2017;14(1):181. <https://doi.org/10.1186/s12985-017-0843-0> PMID: [28923111](#)
50. Goodpasture HC, Poland JD, Franci DB, Bowen GS, Horn KA. Colorado tick fever: clinical, epidemiologic, and laboratory aspects of 228 cases in Colorado in 1973–1974. *Ann Intern Med*. 1978;88(3):303–10. <https://doi.org/10.7326/0003-4819-88-3-303> PMID: [204240](#)
51. Attoui H, Mohd Jaafar F, de Micco P, de Lamballerie X. Coltiviruses and seadornaviruses in North America, Europe, and Asia. *Emerg Infect Dis*. 2005;11(11):1673–9.
52. Calisher CH, Poland JD, Calisher SB, Warmoth LA. Diagnosis of Colorado tick fever virus infection by enzyme immunoassays for immunoglobulin M and G antibodies. *J Clin Microbiol*. 1985;22(1):84–8. <https://doi.org/10.1128/jcm.22.1.84-88.1985> PMID: [2991332](#)
53. Attoui H, Billoir F, Bruey JM, de Micco P, de Lamballerie X. Serologic and molecular diagnosis of Colorado tick fever viral infections. *Am J Trop Med Hyg*. 1998;59(5):763–8. <https://doi.org/10.4269/ajtmh.1998.59.763> PMID: [9840594](#)
54. Mohd Jaafar F, Attoui H, Gallian P, Biagini P, Cantaloube J-F, de Micco P, et al. Recombinant VP7-based enzyme-linked immunosorbent assay for detection of immunoglobulin G antibodies to Colorado tick fever virus. *J Clin Microbiol*. 2003;41(5):2102–5. <https://doi.org/10.1128/JCM.41.5.2102-2105.2003> PMID: [12734255](#)
55. Dobler G, Wölfel R, Schmüser H, Essbauer S, Pfeffer M. Seroprevalence of tick-borne and mosquito-borne arboviruses in European brown hares in Northern and Western Germany. *Int J Med Microbiol*. 2006;296 Suppl 40:80–3. <https://doi.org/10.1016/j.ijmm.2005.12.007> PMID: [16530475](#)
56. Emmons RW, Dondero DV, Devlin V, Lennette EH. Serologic diagnosis of Colorado tick fever. A comparison of complement-fixation, immunofluorescence, and plaque-reduction methods. *Am J Trop Med Hyg*. 1969;18(5):796–802. <https://doi.org/10.4269/ajtmh.1969.18.796> PMID: [4980381](#)
57. Karabatsos N, Poland JD, Emmons RW, Mathews JH, Calisher CH, Wolff KL. Antigenic variants of Colorado tick fever virus. *J Gen Virol*. 1987;68 (Pt 5):1463–9. <https://doi.org/10.1099/0022-1317-68-5-1463> PMID: [3572369](#)
58. Dobler G. Arboviruses causing neurological disorders in the central nervous system. *Imported Virus Infections*. Springer Vienna. 1996. 33–40. https://doi.org/10.1007/978-3-7091-7482-1_4
59. Furey NM, Racey PA, Ith S, Touch V, Cappelle J. Reproductive Ecology of Wrinkle-Lipped Free-Tailed Bats *Chaerephon plicatus* (Buchanan, 1800) in Relation to Guano Production in Cambodia. *Diversity*. 2018;10(3):91. <https://doi.org/10.3390/d10030091>
60. Usinger RL. Monograph of Cimicidae (Hemiptera - Heteroptera). College Park, Maryland: Entomological Society of America; 1966. i-ix, 1–585
61. Ueshima N. New species and records of Cimicidae with keys (Hemiptera). *The Pan-Pacific Entomologist*. 1968;44:264–79.
62. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*. 1994;3(5):294–9. PMID: [7881515](#)
63. Tian Y, Zhu W, Li M, Xie Q, Bu W. Influence of data conflict and molecular phylogeny of major clades in Cimicomorphan true bugs (Insecta: Hemiptera: Heteroptera). *Mol Phylogenet Evol*. 2008;47(2):581–97. <https://doi.org/10.1016/j.ympev.2008.01.034> PMID: [18396064](#)
64. Kambhampati S, Smith PT. PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Mol Biol*. 1995;4(4):233–6. <https://doi.org/10.1111/j.1365-2583.1995.tb00028.x> PMID: [8825760](#)
65. Szentiványi T, Hornok S, Kovács ÁB, Takács N, Gyuranecz M, Markotter W, et al. Polyctenidae (Hemiptera: Cimicoidea) species in the Afrotropical region: Distribution, host specificity, and first insights to their molecular phylogeny. *Ecol Evol*. 2022;12(10):e9357. <https://doi.org/10.1002/ece3.9357> PMID: [36203641](#)
66. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America*. 1994;87(6):651–701. <https://doi.org/10.1093/aesa/87.6.651>
67. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. 2012;61(3):539–42. <https://doi.org/10.1093/sysbio/sys029> PMID: [22357727](#)
68. Nei M, Kumar S. Molecular evolution and phylogenetics. New York: Oxford University Press. 2000.
69. Vilsker M, Moosa Y, Nooij S, Fonseca V, Ghysens Y, Dumon K, et al. Genome Detective: an automated system for virus identification from high-throughput sequencing data. *Bioinformatics*. 2019;35(5):871–3. <https://doi.org/10.1093/bioinformatics/bty695> PMID: [30124794](#)
70. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 2013;30(4):772–80. <https://doi.org/10.1093/molbev/mst010> PMID: [23329690](#)
71. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*. 2009;25(15):1972–3. <https://doi.org/10.1093/bioinformatics/btp348> PMID: [19505945](#)
72. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol Biol Evol*. 2020;37(5):1530–4. <https://doi.org/10.1093/molbev/msaa015> PMID: [32011700](#)
73. Mah MG, Linster M, Low DHW, Zhuang Y, Jayakumar J, Samsudin F, et al. Spike-Independent Infection of Human Coronavirus 229E in Bat Cells. *Microbiol Spectr*. 2023;11(3):e0348322. <https://doi.org/10.1128/spectrum.03483-22> PMID: [37199653](#)
74. Hu Z, Zhang J, Liu Y, Liu L, Tang F, Si G, et al. First Genomic Evidence of California Hare Coltivirus from Natural Populations of Ixodes persulcatus Ticks in Northeast China. *Pathogens*. 2024;13(8):614. <https://doi.org/10.3390/pathogens13080614>
75. Dick CW, Patterson BD. Against all odds: explaining high host specificity in dispersal-prone parasites. *Int J Parasitol*. 2007;37(8–9):871–6. <https://doi.org/10.1016/j.ijpara.2007.02.004> PMID: [17382332](#)

76. Cappelle J, Furey N, Hoem T, Ou TP, Lim T, Hul V, et al. Longitudinal monitoring in Cambodia suggests higher circulation of alpha and betacoronaviruses in juvenile and immature bats of three species. *Sci Rep*. 2021;11(1):24145. <https://doi.org/10.1038/s41598-021-03169-z> PMID: [34921180](#)
77. Sándor AD, Corduneanu A, Hornok S, Mihalca AD, Péter Á. Season and host-community composition inside roosts may affect host-specificity of bat flies. *Sci Rep*. 2024;14(1):4127. <https://doi.org/10.1038/s41598-024-54143-4> PMID: [38374243](#)
78. Hornok S, Szentiványi T, Takács N, Kovács ÁB, Glaizot O, Christe P, et al. Latrocimicinae completes the phylogeny of Cimicidae: meeting old morphologic data rather than modern host phylogeny. *Parasit Vectors*. 2021;14(1):441. <https://doi.org/10.1186/s13071-021-04932-x> PMID: [34479609](#)
79. Hornok S, Szőke K, Boldogh SA, Sándor AD, Kontschán J, Tu VT, et al. Phylogenetic analyses of bat-associated bugs (Hemiptera: Cimicidae: Cimicinae and Cacodminae) indicate two new species close to Cimex lectularius. *Parasit Vectors*. 2017;10(1):439. <https://doi.org/10.1186/s13071-017-2376-1> PMID: [28934957](#)
80. Cobb NS, Gall LF, Zaspel JM, Dowdy NJ, McCabe LM, Kawahara AY. Assessment of North American arthropod collections: prospects and challenges for addressing biodiversity research. *PeerJ*. 2019;7:e8086. <https://doi.org/10.7717/peerj.8086> PMID: [31788358](#)
81. Graça-Souza AV, Maya-Monteiro C, Paiva-Silva GO, Braz GRC, Paes MC, Sorgine MHF, et al. Adaptations against heme toxicity in blood-feeding arthropods. *Insect Biochem Mol Biol*. 2006;36(4):322–35. <https://doi.org/10.1016/j.ibmb.2006.01.009> PMID: [16551546](#)
82. Carroll D, Morzaria S, Briand S, Johnson CK, Morens D, Sumption K, et al. Preventing the next pandemic: the power of a global viral surveillance network. *BMJ*. 2021;372:n485. <https://doi.org/10.1136/bmj.n485> PMID: [33712471](#)