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Original research article

Diversity and Dynamics: Virome Analysis of Two Honey Bee Species across Pakistan and Bangladesh

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Abstract Honey bee viruses remain largely unexplored in Pakistan and Bangladesh, where commercial apiculture plays an essential role in agricultural and economic sustainability. This study presents a comprehensive virome analysis of two key honey bee species, *Apis mellifera* and *A. cerana*, from these regions, utilizing high-throughput sequencing (HTS) technology. Our findings reveal the presence of nine honey bee viruses and seven plant viruses, including four novel viruses. Notably, Apis mellifera-associated partiti-like virus 2, first identified in South Korea, was consistently detected in all samples from Pakistan. Additionally, we identified a novel virus exhibiting minimal similarity to known members of the *Tobamovirus* and *Alphaendornavirus* genera. Moreover, we confirmed the complete genome sequence of Apis mellifera filamentous virus in *A. mellifera* samples from Bangladesh. This pioneering virome analysis highlights previously undetected viruses in honey bee populations of Pakistan and Bangladesh, providing critical insights for future research on honey bee health and viral ecology in these regions.

Keywords *Apis mellifera*, *Apis cerana*, High-throughput sequencing, Virome analysis, Honey bee virus, Pakistan, Bangladesh

INTRODUCTION

The *Apis mellifera* species is globally cultivated as a pollinator for numerous crops and is also economically significant to produce industrial goods like honey, royal jelly, wax, and propolis (Popovska Stojanov *et al*., 2021; Zacepins *et al*., 2021). *A. cerana* remains primarily managed in Asian countries (Theisen-Jones and Bienefeld, 2016). Both species are under constant threat from diseases, environmental changes, and pesticides, significantly impacting bee health and stability of bee colonies (Atkins, 1992; Allen and Ball, 1996; Le Conte and Navajas, 2008). A prominent issue for *A. mellifera* is Colony Collapse Disorder (CCD), which has led to considerable population declines worldwide. *A. cerana*, in contrast, has faced notable mortality from sacbrood virus (SBV) outbreaks in countries such as Korea, Vietnam, Thailand, India, and China (Bailey *et al*., 1983, Liu *et al*., 2010, Choe *et al*., 2012; Nguyen and Le, 2013).

Virological research on honey bees initially lagged behind other fields, with sacbrood virus identified as the first known honey bee virus (White, 1913). Interest in honey bee virology surged following the CCD phenomenon in 2007, with the number of identified viruses rising from just 17 prior to CCD to over 128 by 2023 (Chen and Siede, 2007; Oldroyd, 2007; Beaurepaire *et al*., 2020; Kwon *et al*., 2023a). Despite these advances, significant gaps remain, particularly in regions like Pakistan and Bangladesh, where honey bees are extensively managed but relatively under-researched.

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Beekeeping in Pakistan mainly involves the commercial beekeeping of two species, *A. mellifera* and *A. cerana*, with recent estimates indicating approximately 600,000 colonies managed by 10,000 beekeepers, producing about 12,000 tons of honey annually (Khan and Ghramh, 2023). Alongside virus threats, honey bees in Pakistan face various pests and diseases, including *Varroa destructor*, *Tropilaelaps clareae*, American foulbrood (AFB), and chalkbrood (CB) (Waghchoure-Camphor and Martin, 2009; Rehman *et al*., 2018; Khan and Ghramh, 2023). In Bangladesh, beekeeping encompasses five species, of which four (*A. cerana*, *A. dorsata*, *A. florea*, *Trigona* sp.) are native, including *A. cerana* (TBS, 2020). In 2023 survey, commercial apiculture is projected to involve around 70,000 colonies, maintained by 12,000−15,000 beekeepers with an annual honey yield of 5,000 tons (Haque, 2023). However, studies on honey bee pests and pathogens in Bangladesh are still limited.

Advancements in sequencing technologies, from Sanger sequencing to second-generation high-throughput sequencing (HTS), have greatly expanded virological research by reducing analysis time and costs (Collins *et al*., 2003; Mardis, 2011; Pareek *et al*., 2011). Additionally, several third- and fourth-generation analysis platforms are now in use (Feng *et al*., 2015; Rhoads and Au, 2015; Deamer *et al*., 2016). These advancements have significantly reduced the extensive time required for analysis by Sanger sequencing and the cost of analysis by more than 100-fold (Schloss, 2008). HTS has catalyzed progress in bioinformatics, especially virome analysis, which comprehensively examines viral communities in a sample, including known, novel, and asymptomatic viruses (Ercolini, 2013; Kapoor and Lipkin, 2014; Conesa *et al*., 2016; Sekse *et al*., 2017; Kwon *et al*., 2023b). In honey bees, virome analysis has illuminated the viral diversity affecting bees, such as the discovery of Lake Sinai virus (LSV) species and the tripartite classification of deformed wing virus (DWV) associated with CCD (Runckel *et al*., 2011; Roberts *et al*., 2018a; Bonilla-Rosso *et al*., 2020; Daughenbaugh *et al*., 2021; Kadlečková *et al*., 2022; Li *et al*., 2022). Since then, several researchers have conducted virome analyses, confirming that LSV group is a virus complex with multiple species (Šimenc *et al*., 2020; Daughenbaugh *et al*., 2021; Čukanová *et al*., 2022; Kadlečková *et al*., 2022). Research has also demonstrated that DWV, which is associated with CCD, can be classified into three types (Mordecai *et al*., 2016; Natsopoulou *et al*., 2017). In some instances, plant viruses have been detected in honey bees (Granberg *et al*., 2013; Li *et al*., 2014). Interestingly, virome studies have also identified plant viruses in bees, suggesting honey bees may play a role in plant virus transmission during pollination (Darzi *et al*., 2018; Roberts *et al*., 2018b).

This study aims to explore the viral landscape within *A. mellifera* and *A. cerana* populations in Pakistan and Bangladesh. By employing virome analysis through HTS, we seek to identify the diversity of viruses present, including novel species, and provide a foundation for future virological research and conservation efforts in these regions.

MATERIALS AND METHODS

1. Honey bee sample collection

Honey bee samples were collected from multiple apiaries across Pakistan and Bangladesh (Fig. 1). In Pakistan, honey bees were obtained from the regions of Fateh Jang (33°33′59.6″N 72°36′51.1″E; 33°35′28.9″N 72°40′48.2″E), Jand (33°25′57.1″N 72°00′57.1″E; 33°25′44.0″N 72°01′40.2″E; 33°26′12.8″N 72°00′32.4″E), Khunda (33°27′43.8″N 72°23′55.4″E; 33°27′31.8″N 72°23′49.3″E), and PindiGheb (33°14′58.1″N 72°16′19.4″E; 33°14′26.8″N 72°16′20.2″E; 33°13′56.5″N 72°16′36.0″E). In Bangladesh, samples were collected from Tangail (24°14′59.4″N 89°54′59.6″E). From each location, a minimum of 50 honey bees were collected, preserved in 70% ethanol within 50 mL conical tubes, and stored at 4°C until RNA extraction.

2. Total RNA extraction

Ten honey bees from each sample were air-dried with tissue paper to remove residual ethanol and ground into a fine powder using a sterile pestle and mortar. Total RNA extraction was carried out using the TRIzol® reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. RNA concentration and purity were measured using a NanoPhotometer® NP80 (IMPLEN, Munich, Germany), and samples were subsequently stored at −80°C until further processing for high-throughput sequencing.

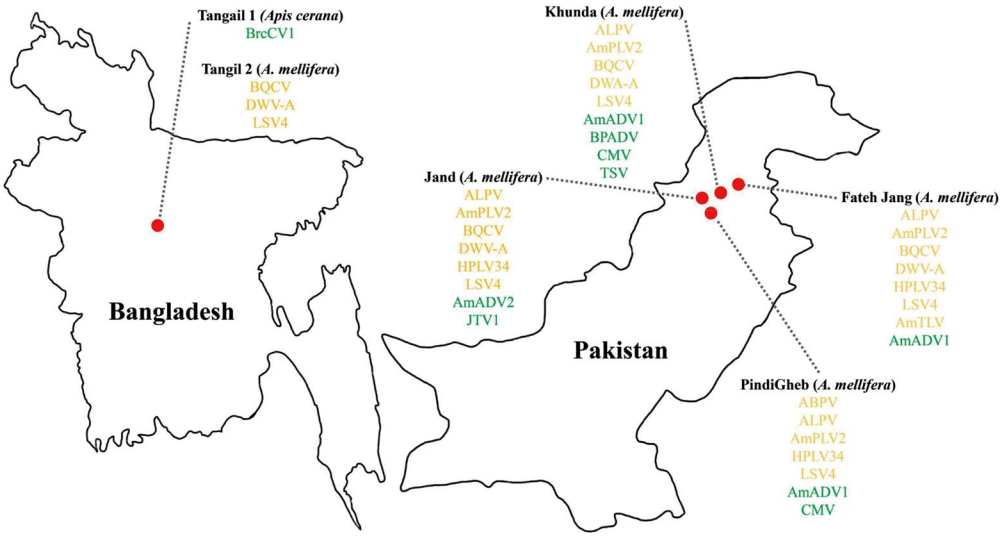


Fig. 1. Geographic distribution of viruses identified in honey bee samples from Bangladesh and Pakistan. Red circles indicate sampling locations on the map. The list of detected viral taxa from virome analysis is shown beside each country. Viruses are color-coded based on host specificity: green for plant viruses and yellow for honey bee viruses.

3. High-throughput sequencing

For Pakistan samples, total RNA was pooled across collection sites before library preparation. Quality control of RNA was confirmed using the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Libraries were then constructed using the TruSeq Stranded Total RNA with Ribo-Zero H/M/R Gold kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Library quality was verified again on Bioanalyzer 2100. High-throughput sequencing was performed using the Novaseq 6000 platform (Illumina) to generate raw FASTQ files.

4. Virome analysis

Raw FASTQ files were processed using the CLC Genomics Workbench (version 23.0.2, QIAGEN, Hilden, Germany). Low-quality sequences, chimeric reads, and adaptor sequence were trimmed to ensure data quality. Host-related reads were removed by mapping against *Apis mellifera* and *A. cerana* reference genomes downloaded from NCBI (NCBI, 2023a, 2023b) with sequences displaying $\geq$80% similarity excluded. Viral reads were identified by mapping against the NCBI viral genome database (NCBI, 2022), retaining sequences with $\geq$80% similarity.

To identify potential novel viruses and mutations, *de novo* assembly was performed on the host-filtered reads, generating contigs for further analysis. Each contig was compared against viral databases using tBLASTx, selecting sequences with E-values $\leq$1e-10. Confirmed viral contigs were further validated using BLASTn to refine virus identification. For suspected novel viruses with low similarity to known sequences, the assembled contigs were used directly as references to confirm complete viral genomes.

5. Phylogenetic analysis

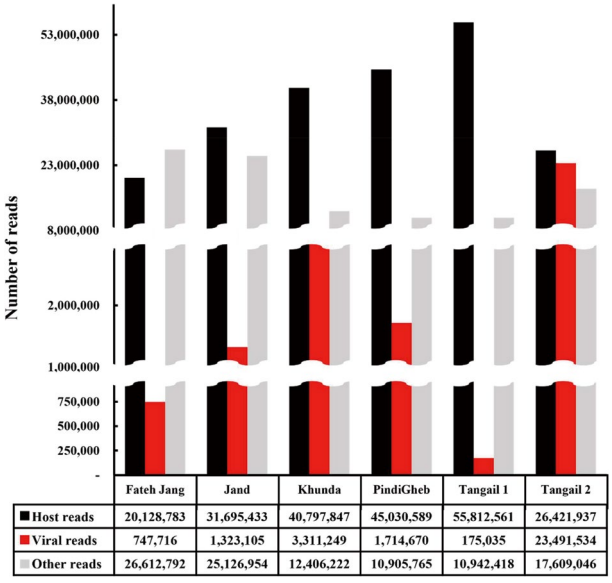
Phylogenetic relationships of detected viruses were analyzed using IQ-TREE (Minh *et al*., 2020). Viral sequences, including complete viral genomes from global isolates in the NCBI database, were aligned, and a phylogenetic tree was constructed using the maximum likelihood method with 1,000 bootstrap replicates under the automatically selected best model.

RESULTS

1. High-throughput sequencing overview

The average number of reads generated per sample was 64,236,804. Notably, the Fateh Jang sample had approximately 10 million fewer reads than other samples,

though all samples maintained significant Q30 values, ensuring high data quality. Tangail 1 had the highest host reads (55,812,561), while Tangail 2 held the highest viral reads count, with 23,491,534 reads (Fig. 2, Table S1). Thirteen viruses spanning seven families, along with an unclassified DNA virus, were identified (Fig. 3). Among the sixteen identified viruses, nine were honey bee viruses, while seven were plant viruses, including four novel viruses (Table 1). Apis mellifera filamentous virus (AmFV) showed the highest viral read count in Tangail 2, with approximately 20 million reads. Khunda recorded the highest virus diversity, with eight viruses identified. Plant viruses were detected in most samples, except for Fateh Jang and Tangail 2.



	Fateh Jang	Jand	Khunda	PindiGheb	Tangail 1	Tangail 2
Host reads	20,128,783	31,695,433	40,797,847	45,030,589	55,812,561	26,421,937
Viral reads	747,716	1,323,105	3,311,249	1,714,670	175,035	23,491,534
Other reads	26,612,792	25,126,954	12,406,222	10,905,765	10,942,418	17,609,046

Fig. 2. Distribution of reads following quality trimming for high-throughput sequencing. Black bars represent host-related reads, red bars represent viral reads, and gray bars represent other reads. Samples from Fateh Jang, Jand, Khunda, PindiGheb, and Tangail 1 correspond to *Apis mellifera*, while the Tangail 1 sample represents *A. cerana*.

2. Honey bee viruses

1) *Dicistroviridae*

Aphid latent paralysis virus (ALPV) sequences from PindiGheb (PP971556) had 96.98% similarity with MF458892 from Kenya, while sequences from Fateh Jang (PP971553) and Jand (PP971554) showed 98.51% and 97.26% similarity with MW063131 from South Africa (Fig. S1A). Acute bee paralysis virus (ABPV) identified in PindiGheb (PP971549) showed 94.52% similarity with MZ821785 reported in China (Fig. S1B). Black queen cell virus (BQCV) was detected in four samples, with highest homology values of 91.88% (Fateh Jang, PP971562), 93.54% (Jand, PP971563), 93.88% (Tangail 2, PP971589), and 95% (Khunda, PP971564) to MW397638 identified in Israel (Fig. S1D). A partial sequence from the *Triatovirus* genus was identified in Fateh Jang (PP971572), showing 87% coverage and 80.64% identity to Vespula vulgaris picorna-like virus 2 (VvPLV2, QZZ63316) and was classified as a novel virus named "Apis mellifera associated triato-like virus (AmTLV)" (Table 2).

2) *Partitiviridae*

The Hubei partiti-like virus 34 (HPLV34) virus detected in PindiGheb (PP971577) showed 95.34% similarity

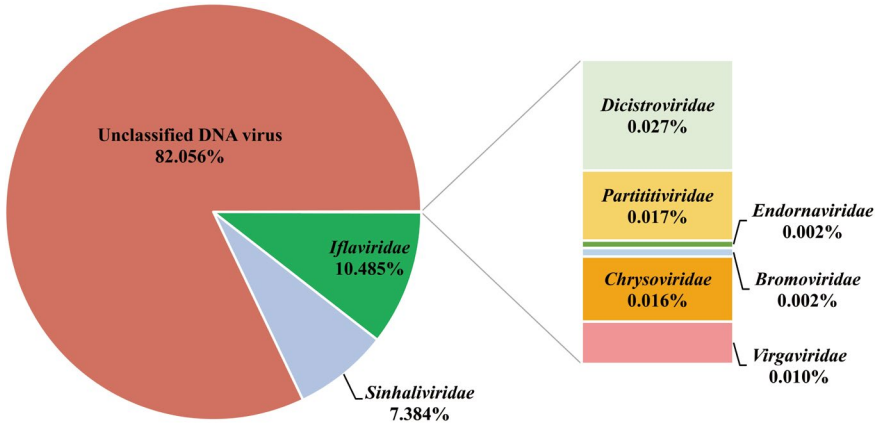


Fig. 3. Virus read counts by family across detected viral sequences. This analysis identified eight well-known viral families and one unclassified DNA virus family from the honey bee virome samples.

Table 1. Virus read counts from virome analysis in honey bee samples

Viruses		Pakistan				Bangladesh	
		Fateh Jang	Jand	Khunda	PindiGheb	Tangail 1	Tangail 2
Honey bee viruses	ABPV	–	–	–	355	–	–
	ALPV	125	108	129	457	–	–
	AmPLV2	2,046	373	77	1,026	–	–
	BQCV	1,071	1,516	247	–	–	2,487
	DWV	4,431	126	727	–	–	2,651,144
	HPLV34	140	51	–	659	–	–
	LSV4	28	4,983	1,853,444	608	–	11,738
	AmTLV	405	–	–	–	–	–
	AmFV	–	–	–	–	–	20,789,671
Plant viruses	AmADV1	1,586	–	5,492	8,181	–	–
	AmADV2	–	2,904	–	–	–	–
	BPADV	–	–	468	–	–	–
	BrcCV1 RNA1	–	–	–	–	1,949	–
	BrcCV1 RNA2	–	–	–	–	1,164	–
	BrcCV1 RNA3	–	–	–	–	935	–
	CMV RNA1	–	–	66	34	–	–
	CMV RNA2	–	–	71	25	–	–
	CMV RNA3	–	–	169	107	–	–
	TSV RNA1	–	–	98	–	–	–
	TSV RNA2	–	–	133	–	–	–
	TSV RNA3	–	–	166	–	–	–
	JTV1	–	2,904	–	–	–	–

to ON648754 from Slovenia, while sequences from Fateh Jang (PP971576) and Jand (PP971578) showed similarities of 95.48% and 97.63% to MT747982 from Italy (Fig. S1C). The Apis mellifera associated partiti-like virus 2 (AmPLV2), previously reported in South Korea, was detected in Pakistani samples, with sequences from Fateh Jang (PP971557) and PindiGheb (PP971559) showing 96.53% and 96.39% similarity to OR496402, and sequences from Jand (PP971558) and Khunda (PP971560) showing 96.61% and 96.24% similarity to OR496403.

3) *Iflaviridae*

Deformed wing virus (DWV) was identified in Fateh Jang (PP971573), and Tangail 2 (PP971590), showing 92.76%, 92.85%, and 93.39% similarity with MT940255 from the USA. The sequence in Jand (PP971574) exhibited 96.09% similarity with MN607197 from Viet Nam (Fig. S1E).

4) *Sinhaliviridae*

Lake Sinai virus 4 (LSV4) was detected in all samples except Tangail 1. The sequences showed similarities of 90.05% (Khunda, PP971580), 90.12% (Jand, PP971579), 89.30% (PindiGheb, PP971581), and 89.22% (Tangail 2, PP971591) with MZ821913 from China. The Fateh Jang (PP971582) sample yielded a partial sequence overlapping the ORF1 and RdRp regions, showing 98% similarity with LSV4 (Fig. S1F).

5) Unclassified DNA virus

The complete genome sequence of Apis mellifera filamentous virus (AmFV) was confirmed in Tangail 2 from Bangladesh (PP971592). This is the fourth country where AmFV has been fully sequenced, following China (OK 392616), Hungary (OR644609-OR644611, OR270109), and Switzerland (NC_027925).

3. Plant viruses

1) *Endornaviridae*

Bell pepper alphaendornavirus (BPADV) was identified in Khunda (PP971561), showing 97.66% similarity

to MH182675 from *Capsicum annuum* in China (Fig. S1M). Four novel virus sequences were detected, with two classified under the *Alphaendornavirus* genus. Alphaendornavirus 1 (provisional) showed 65–70% identity and 3–5% coverage with known sequences, while alphaendornavirus 2 (provisional) had 70% identity and 15–18% coverage (Table 2). These novel viruses, with 40% amino acid sequence similarity to previously reported viruses, were named "Apis mellifera associated alphaendornavirus 1 (AmADV1)" and "Apis mellifera associated alphaendornavirus 2 (AmADV2)", and their sequences were uploaded to NCBI GenBank (AmADV1; PP 971550, PP971551, AmADV2; PP971552) (Fig. S1O).

2) *Bromoviridae*

Three genome segments of cucumber mosaic virus (CMV) were identified in both the Khunda and PindiGheb samples. For segment RNA1, the sequence identified in Khunda (PP971565) showed a 99.41% similarity to OQ993352, previously reported in *Cucumis sativus* from Canada, while the sequence from PindiGheb (PP971566) showed a 99.50% similarity to OP722596, identified in *Pelargonium peltatum* in Germany (Fig. S1G). Segment RNA2 showed similarities of 98.72% and 99.31% with MG882751, reported in *Cucurbita pepo* from Poland, in Khunda (PP971567) and PindiGheb (PP971568), respectively (Fig. S1H). For segment RNA3, the sequence identified in Khunda (PP971569) had 98.01% similarity to OQ993354 from *C. sativus* in Canada, and the sequence from PindiGheb (PP971570) had 98.60% similarity to ON13912 identified in *Solanum lycopersicum* in Greece (Fig. S1I).

Tobacco streak virus (TSV) was found in Khunda, with three RNA segments. RNA1 (PP971583) (Fig. S1J), RNA2 (PP971584) (Fig. S1K), and RNA3 (PP971585) (Fig. S1L) showed similarities of 98.72%, 99.52%, and 99.55% with Indian isolates from *Gossypium barbadense*, *Parthenium hysterophorus*, and *Gomphrena globosa*, respectively.

3) *Chrysoviridae*

Brassica campestris chrysovirus 1 (BrcCV1), previously reported only in China, was identified in Tangail 1. Complete genome sequences of RNA1 (PP971586), RNA2 (PP971587), and RNA3 (PP971588) showed similarities of 97.3%, 98.08%, and 98.43% with NC_043661, NC_043660, and NC_043659, respectively.

Table 2. Summary of BLAST analysis results for novel viruses identified in this study

Virus	Location	BLAST type	Scientific name	Query cover	E value	Per. Ident	Acc. Len	Accession
Apis mellifera associated alphaendornavirus 1 (AmADV1)*	Khunda	BLASTn	Plant associated alphaendornavirus 1	5%	3.00E-15	69.87%	14445	OL472078.1
			Plant associated alphaendornavirus 1	3%	3.00E-08	65.74%	14445	OL472077.1
	PindiGheb		Plant associated alphaendornavirus 1	5%	6.00E-17	70.31%	14445	OL472078.1
			Plant associated alphaendornavirus 1	3%	3.00E-08	65.74%	14445	OL472077.1
Apis mellifera associated alphaendornavirus 2 (AmADV2)†	Jand		Basella alba alphaendornavirus 1	18%	0	70.44%	14027	AB844264.1
			Basella alba alphaendornavirus	15%	0	70.61%	14022	OM108480.1
Jand tobamovirus 1 (JTV1)‡	Jand		Youcai mosaic virus	54%	6.00E-65	63.54%	6304	OK655842.1
			Youcai mosaic virus	50%	3.00E-63	63.48%	6114	MH427309.1
Apis mellifera associated triato-like virus (AmTLV)§	PindiGheb	BLASTp	*Picornavirales* sp.	90%	0	88.75%	2776	XCO48961.1
			Triatovirus sp.	90%	0	88.57%	1605	WMV69912.1

*,†*Alphaendornavirus* genus demarcation criteria: Members of different species have an overall nucleotide sequence identity below 75%.
‡*Tobamovirus* genus demarcation criteria: Most of the sequenced tobamoviruses of different species have considerably less than 90% sequence identity
§*Triaovirus* genus demarcation criteria: Sequence identity at the amino acid level between the capsid proteins of isolates and strains of a species is above 90%.

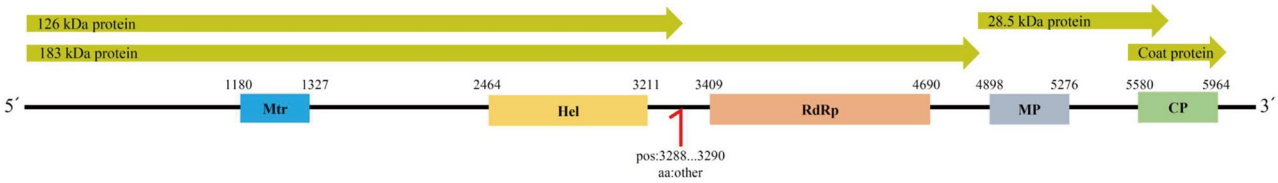


Fig. 4. Genomic structure of Jand tobamovirus 1 (JTV1), showing the open reading frames (ORFs) and corresponding functional domains. Domains were annotated based on nucleotide positions, including Mtr (Methyltransferase), Hel (Helicase), RdRp (RNA-dependent RNA polymerase), MP (Movement protein), and CP (Coat protein).

4) *Virgaviridae*

One of the four novel virus sequences was classified under the *Tobamovirus* genus. Comparative analysis of the complete genome nucleotide sequence using BLASTn, according to ICTV genus demarcation criteria, showed 80–99% identity with previously reported tobamovirus sequences, though the coverage was limited to 1–2%. Despite the low coverage, this virus sequence contained all five conserved domains characteristic of the *Tobamovirus* genus (Fig. 4). Each domain showed approximately 50% similarity to known tobamovirus sequences in BLASTp analysis (Table 2). Based on these findings, this novel virus was classified as a new member of the *Tobamovirus* genus and named "Jand tobamovirus 1 (JTV1)." Its sequence has been deposited in NCBI GenBank (PP 971571) (Fig. S1N).

DISCUSSION

Since the emergence of CCD in 2007, interest in honey bee viruses has surged, driving research efforts worldwide (Beaurepaire *et al*., 2020). HTS-based virome analyses have been conducted in many regions, revealing diverse viral profiles across Europe, North America, South America, Africa, the Middle East, and Asia (Granberg *et al*., 2013; Tozkar *et al*., 2015; Remnant *et al*., 2017; Galbraith *et al*., 2018; Haddad *et al*., 2018; Regan *et al*., 2018; Roberts *et al*., 2018a; Thaduri *et al*., 2018; Kraberger *et al*., 2019; Bonilla-Rosso *et al*., 2020; Bovo *et al*., 2020; Deboutte *et al*., 2020; Gebremedhn *et al*., 2020; Roberts *et al*., 2020; Šimenc *et al*., 2020; Daughenbaugh *et al*., 2021; da Silva *et al*., 2023; Kwon *et al*., 2023b; Lee *et al*., 2023; Li *et al*., 2023; Shojaei *et al*., 2023). Our study represents the first comprehensive virome analysis of two honey bee species, *Apis mellifera* and *A. cerana*, from Pakistan and Bangladesh, contributing valuable data on honey bee viruses in these countries on the Indian subcontinent.

Our results revealed the presence of multiple honey bee and plant viruses, including several previously unreported in these countries. Although the sample size is limited and may not capture the full viral diversity across all seasons and regions, this study lays the groundwork for future research. Broader, seasonal, and geographically extensive sampling is expected to reveal a more comprehensive viral landscape, potentially aiding in predicting and managing future honey bee health challenges in these regions.

Notably, we identified Apis mellifera-associated partitivirus 2 (AmPLV2), previously reported only in South Korea (Kwon *et al*., unpublished data, NCBI GenBank accession OR496401-OR496403), in honey bees from Pakistan. Future research should investigate the routes of transmission of this virus and its potential impact on bee health. Two novel viruses within the *Alphaendornavirus* genus were also identified: AmADV1 in Jand, Khunda, and PindiGheb, and AmADV2 in Jand. Given that alphaendornaviruses are pollen-transmissible (Moriyama *et al*., 1996), it is possible that these viruses were introduced through pollen carried by the bees. This finding underscores the potential role of honey bees as vectors for plant viruses, though it remains challenging to determine the exact plant host without further analysis.

We also detected a novel tobamovirus in honey bees from Jand. The limited sequence similarity between this virus and previously reported tobamoviruses suggests an unexplored diversity of viruses affecting plants in Pakistan (Dombrovsky *et al*., 2017; Zhang *et al*., 2022; Spiegelman and Dinesh-Kumar, 2023). Expanding virome studies to include plant hosts in Pakistan could yield new insights into viral diversity and potential reservoirs.

The viral profiles of the two bee species from Tangail, Bangladesh, showed notable differences. In *A. cerana*,

only one plant virus was detected, while *A. mellifera* harbored four honey bee viruses but no plant viruses. This study also marks the first identification of Apis mellifera filamentous virus (AmFV) in *A. mellifera* samples from Bangladesh. Although AmFV has been linked to DWV, SBV, and BQCV in other studies (Hartmann *et al*., 2015), our findings provide limited data to establish relationships among these viruses. Further investigation is warranted to explore possible interactions among these pathogens.

In conclusion, this study presents the first virome analysis of honey bees in Pakistan and Bangladesh, identifying both known and novel viruses that may influence honey bee health. These findings provide foundational data for future research on honey bee viruses in these countries.

AUTHOR CONTRIBUTIONS

E-JK and CJ conceived and designed the study. MFK, MN-u-A prepared the samples. MK performed the experiments. E-JK and MK conducted bioinformatics and data analyses. MK and E-JK wrote the manuscript. All authors contributed to manuscript revision and have read and approved the submitted version.

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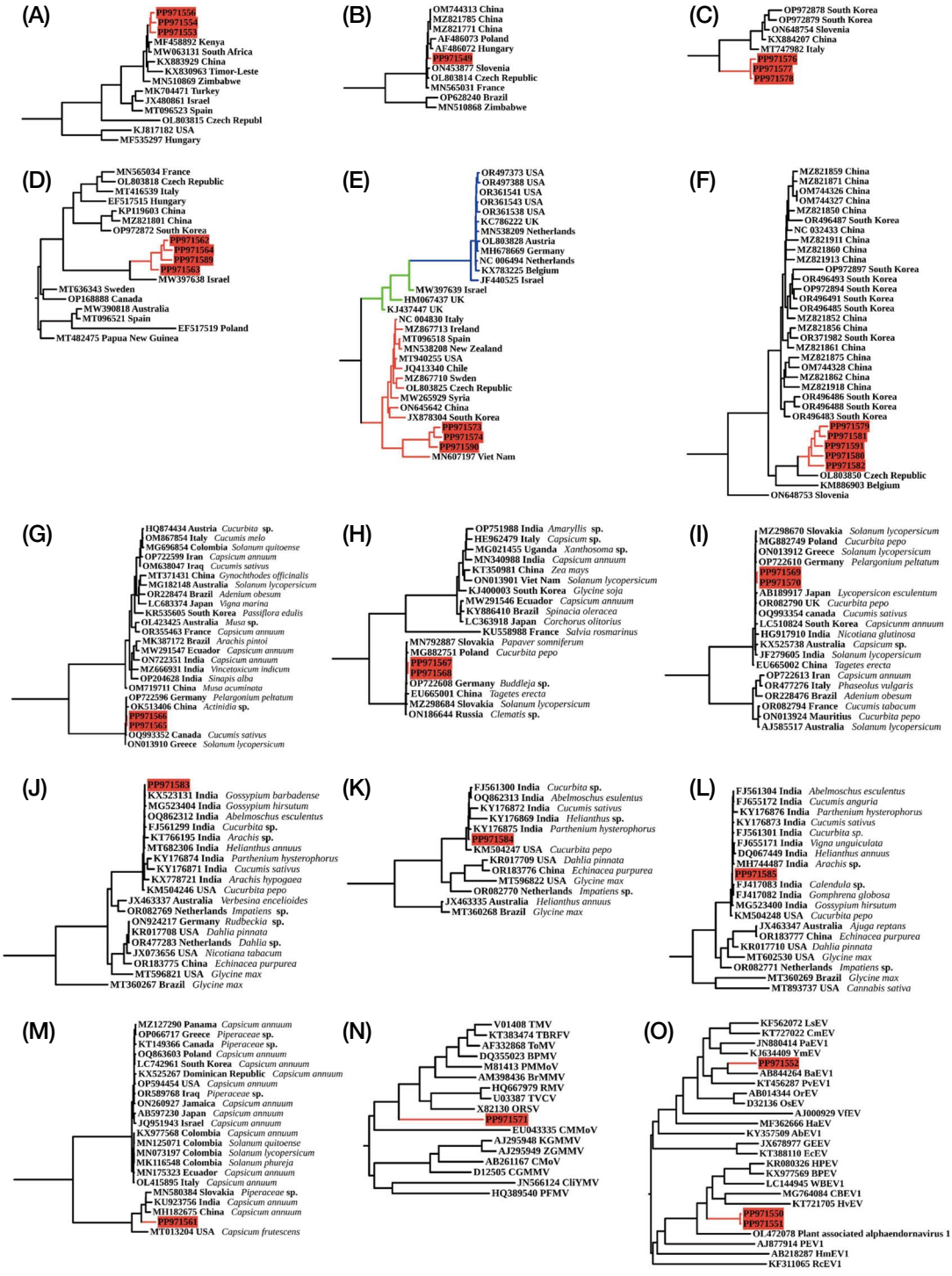

Fig. S1. Maximum likelihood phylogenetic trees constructed using IQ-TREE2 with 1,000 bootstrap replicates. The models were selected based on the Bayesian Information Criterion (BIC): (A) Aphid latent paralysis virus (ALPV) using the TIM2 + F + I + G4. (B) Acute bee paralysis virus (ABPV) using the TIM2 + F + G4 model. (C) Hubei partiti-like virus 34 (HPLV34) using the HKY + F + I model. (D) Black queen cell virus (BQCV) using the GTR + F + I + G4 model. (E) Deformed wing virus (DWV) using the TIM2 + F + I + G4 model. DWV-A (red line), DWV recombinant (green line), DWV-B (blue line). (F) Lake Sinai virus 4 (LSV4) using the TN + F + I + G4 model. (G) Cucumber mosaic virus (CMV) RNA1 using the GTR + F + I + G4 model. (H) CMV RNA2 using the TIM2 + F + G4 model. (I) CMV RNA3 using the TIM3 + F + G4 model. (J) Tobacco streak virus (TSV) RNA1 using the TVM + F + G4 model. (K) TSV RNA2 using the TIM2 + F + G4 model. (L) TSV RNA3 using the TVMe + G4 model. (M) BPADV using the TIM2 + F + G4 model. (N) Jand tobamovirus 1 (JTV1) using the GTR + F + I + G4 model. (O) Apis mellifera associated alphaendornavirus (AmADV) using the GTR + F + I + G4 model. Bootstrap support values are provided at key nodes, demonstrating high confidence in the tree topology. Viral sequences identified in this study are highlighted in red.

Table S1. Summary of high-throughput sequencing (HTS) results, detailing the number of reads, quality control metrics, and host and viral read distributions for each sample

Species	Country	Location	Size (Gb)	Total bases (bp)	Total reads	GC (%)	Q20 (%)	Q30 (%)
Apis cerana	Bangladesh	Tangail 1	3.17	6,759,939,494	66,930,094	41.2	98.4	95.3
		Tangail 2	3.37	6,819,800,376	67,522,776	44.1	97.6	93.8
Apis mellifera	Pakistan	Fateh Jang	2.43	5,417,648,080	53,640,080	55.9	98.3	95.4
		Jand	3.2	6,750,175,218	66,833,418	55	98.3	95
		Khunda	3.08	6,477,234,838	64,131,038	54.6	98.4	95.3
		PindiGheb	3.19	6,702,705,218	66,363,418	54.3	98.1	94.8