Molecular characterization of a novel picobirnavirus in a chicken

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Abstract

Picobirnaviruses (PBVs) are bisegmented viruses with a wide geographical and host species distribution. The number of novel PBV sequences has been increasing with the help of the viral metagenomics. A novel picobirnavirus strain, pbv/ CHK/M3841/HUN/2011, was identified by viral metagenomics; the complete segment 1 (MH327933) and 2 (MH327934) sequences were obtained by RT-PCR from a cloacal sample of a diseased broiler breeder pullet in Hungary. Although the conserved nucleotide (e.g., ribosome binding site) and amino acid motifs (e.g., ExxRxNxxxE, S-domain of the viral capsid and motifs in the RNA-dependent RNA polymerase) were identifiable in the chicken picobirnavirus genome, the putative segment 1 showed low (< 30%) amino acid sequence identity to the corresponding proteins of marmot and dromedary PBVs, while segment 2 showed higher (< 70%) amino acid sequence identity to a wolf PBV protein sequence. This is the first full-genome picobirnavirus sequence from a broiler breeder chicken, but the pathogenicity of this virus is still questionable.

The GenBank[/EMBL/DDBJ] accession number for the study sequence: MH327933, MH327934.

Since 1988, when the first picobirnaviruses (PBVs) were dis- covered [1, 2], many different novel PBVs have been identified [1, 3–22]. PBVs are bisegmented, double-stranded (ds) RNA viruses belonging to the family *Picobirnaviridae*. Segment 1 encodes two hypothetical proteins (ORF1 and ORF2) with unknown function and a viral capsid protein (ORF3) [23]. Segment 2 encodes the RNA-dependent RNA polymerase (RdRp, ORF1) [8]. Due to the segmented nature of the PBV genome, the process of reassortment is also observed [15]. PBVs are thought to be able to coinfect vertebrates and have been associated with watery diarrhoea, gastroenteritis outbreaks, and sporadic cases of disease. They are potential opportunistic pathogens with a wide geographic distribution [23]. However, the presence of Shine-Dalgarno-like sequences in recently described PBV genomes may suggest a prokaryotic host origin [24, 25].

In this study, the first complete genome sequence of a novel PBV from a cloacal sample of a broiler breeder pullet in Hungary is presented.

The diseased 18-week old broiler breeder chicken was part of a broiler breeder chicken colony in which the number of dead animals suddenly increased. Hepato-renopathy and liver rupture were found by routine pathohistological investigation, suggesting a toxic origin rather than an infectious pathogen. Known bacterial pathogens could not be isolated. Total RNA was extracted from a cloacal sample by the TRIzol method. The complete genome of pbv/CHK/ M3841/HUN/2011 (MH327933, MH327934) was identified by viral metagenomics [26, 27] and amplified by adapter- ligated RT-PCR [4].

Segments 1 and 2 are 2532 nt and 1700 nt long, respectively (Fig. 1). The conserved GUAAA and ACUGC nt sequences are identifiable in the 5' and 3' ends of segments 1 and 2, respectively. Segment 1 contains three open reading frames, with ORF1 and ORF2 encoding two hypothetical proteins with unknown function, whereas ORF3 encodes the viral capsid protein (VCP). The conserved motif of the ribosomal binding site (RBS) nucleotide sequence (AGG AGG) is present upstream of the ORF2 and ORF3 AUG start codon [28]. Three-copies of the conserved **E**xx**R**x**N**xxx**E** aa motif and the conserved aa motifs of the VCP S-domain are identifiable in ORF2 and ORF3, respectively [29, 30] (Fig. 1). The ORF2 and ORF3 aa sequences showed 27% and 30% identity to the corresponding proteins of marmot (KY928777) and dromedary (KM573793) PBVs, respectively, but no significant hits were found in the case of ORF1 using Blast(p). Segment 2 contains an ORF1 encoding the viral RdRp gene [8]. Interestingly, the ORF starts with the alternative start codon UUG instead of AUG, and an optimal RBS was found upstream with a spacing of 7 nucleotides, (Fig. 1). The conserved aa motifs are also identifiable in the RdRp coding region of segment 2 (Fig. 1) [31, 32]. The RdRp shares 69% aa sequence identity with the corresponding protein of wolf PBV (KT934307).

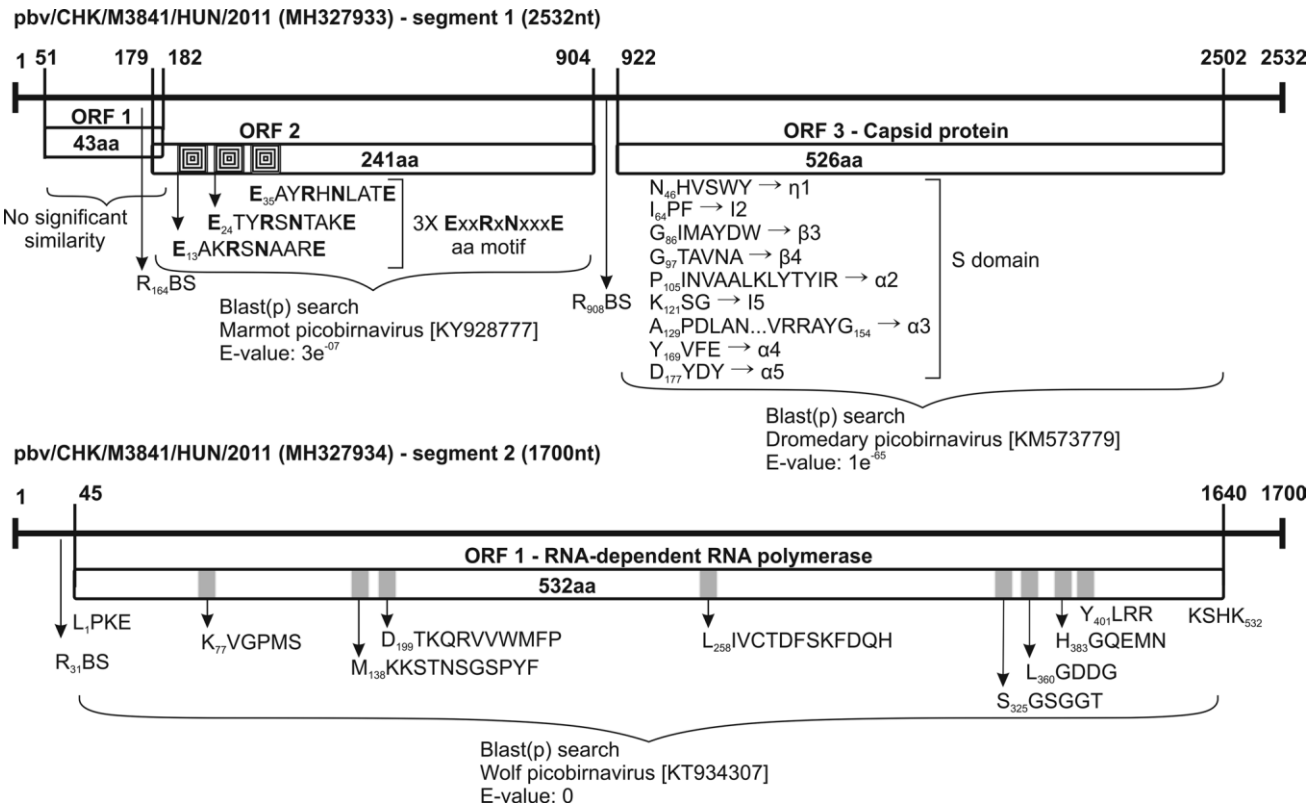

Fig. 1 Genome organization of the novel chicken picobirnavirus strain pbv/CHK/M3841/HUN/2011 from a chicken cloacal sample. Segment 1 encodes two hypothetical proteins (ORF1 and ORF2) with unknown function and the viral capsid protein (ORF3). Segment 2 encodes the viral RNA-dependent RNA polymerase (RdRp). The ribosome binding sites (RBS), the **E**xx**R**x**N**xxx**E** amino acid (aa) motifs, the conserved aa motifs of the S-domain of the capsid protein, and the conserved aa motifs of the RdRp are marked. The most significant relatives are identified based on the lowest E-scores in a Blast(p) search. The ORFs were identified using ORFfinder (NCBI, https://www.ncbi.nlm.nih.gov/orffinder/), allowing the use of "ATG" and alternative initiation codons. The conserved nucleotide sequences and amino acid motifs were identified manually using GeneDoc [33]

Phylogenetic analysis based on the deduced aa sequences VCP (segment 1) and RdRp (segment 2) of the study strain and other representative PBV showed that the novel chicken picobirnavirus strain pbv/CHK/M3841/HUN/2011 occupies a divergent phylogenetic position among PBVs (Fig. 2).

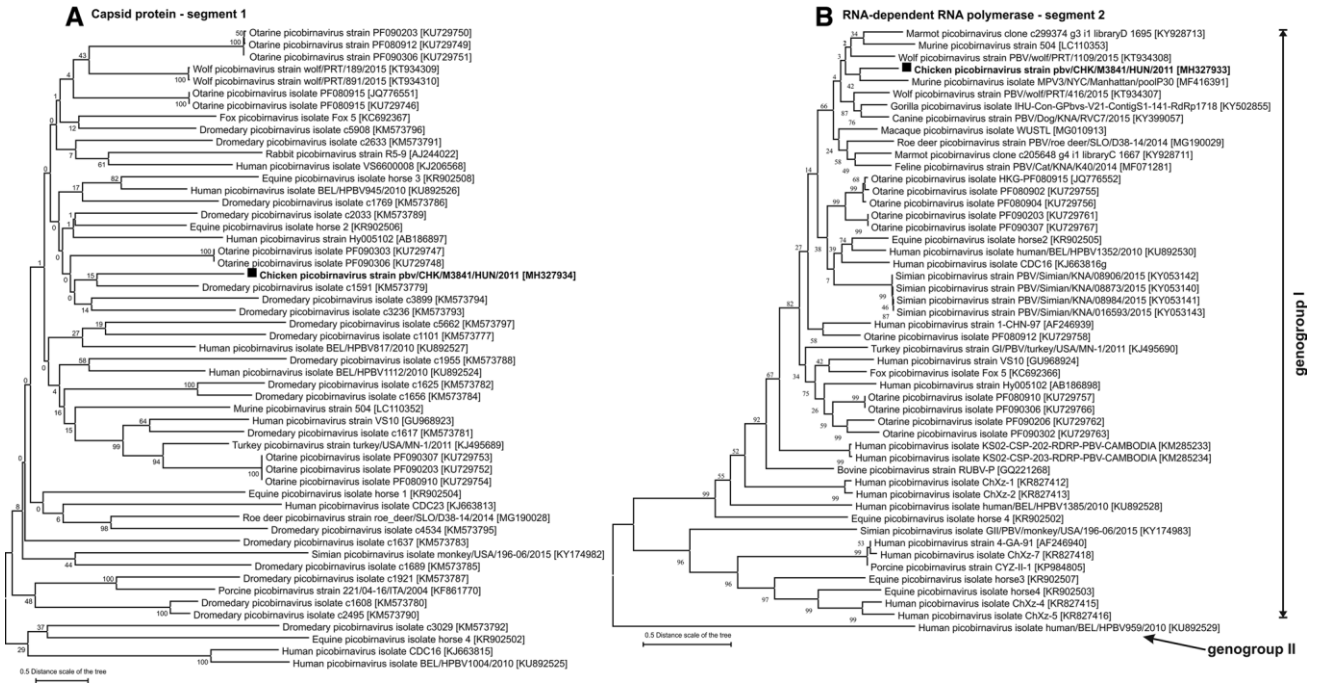

Fig. 2 Phylogenetic reconstruction of the **A)** viral capsid (segment 1) and **B)** RNA-dependent RNA polymerase (segment 2) proteins of the novel chicken picobirnavirus strain PBV/CHK/M3841/HUN/2011 (**bold**) and representative picobirnaviruses. The complete amino acid (aa) sequences were aligned using the MUSCLE algorithm, and the aa sequence alignment was pre-tested by the maximum-likelihood (ML) method. Phylogenetic trees were constructed in MEGA 6 [34] using the ML method based on the LeGascuel [35] with Freqs. (+F) model. A discrete gamma distribution (+G5) was used to model evolutionary rate differences among sites and the rate variation model allowed for some sites to be evolutionarily invariable [+I]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Despite the wide distribution of PBVs, there is still no convincing evidence of their pathogenicity in vertebrates. In this study, a novel picobirnavirus was detected in a cloacal sample from a broiler breeder chicken by viral metagenomics and molecular techniques, but the role of the virus in this infection was not confirmed, and its pathogenicity is still questionable. Further experimental studies are needed to clarify the virulence or synergistic effect of PBVs in vertebrates.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals or animal samples were followed.

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