Isolation and genomic characterization of a novel orthoreovirus from a brown-eared bulbul (Hypsipetes amaurotis) in Japan

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Five species, Mammalian orthoreovirus, Avian orthoreovirus (ARV), Nelson Bay orthoreovirus (NBV), Baboon orthoreovirus and Reptilian orthoreovirus, have been identified in the genus Orthoreovirus. Their genomes each consist of 10 dsRNA segments. A novel orthoreovirus was isolated from the haemorrhagic intestine of a dead brown-eared bulbul (Hypsipetes amaurotis) in Japan. The virus formed syncytia in Caco-2 and Vero cells. Electron microscopy revealed non-enveloped capsids of ~70 nm diameter, which were characteristic of reoviruses. Complete genomic sequences were determined. The S1 segment was tricistronic and encoded three proteins, p10, p17 and sC, as in the two species ARV and NBV. Sequence and phylogenetic analyses showed that the virus was similar to ARV and NBV, but was located on a phylogenetic branch different from that of ARV and NBV. The virus had the closest phylogenetic relationship to two reovirus strains: SSRV from a Steller sea lion in Canada and PsRV Ge01 from a psittaciform bird in Europe. The 10 RNA segments had a 3′-pentanucleotide sequence (UCAUC-3′) conserved amongst all members of the genus Orthoreovirus, and a unique 5′ terminal heptasequence (5′-GCUUUUC) that was the same as those of SSRV and PsRV Ge01. These results suggested that the novel virus might form a new species with the two strains in the genus Orthoreovirus.

INTRODUCTION

Viruses of the family Reoviridae have dsRNA genomes and form two subfamilies: Sedoreovirinae, which contains six genera, and Spinareovirinae, which contains nine genera. The genus Orthoreovirus belongs to the subfamily Spinareovirinae and is composed of five species: Avian orthoreovirus (ARV), Baboon orthoreovirus (BRV), Mammalian orthoreovirus (MRV), Nelson Bay orthoreovirus (NRV) and Reptilian orthoreovirus (RRV) (Attoui et al., 2012; Benavente & Martinez-Costas, 2007; Duncan, 1999). Reoviruses have been found in many organisms, including vertebrates, invertebrates, fungi and plants. Orthoreoviruses can be divided into fusogenic ARV, BRV, NBV and RRV, which cause fusion of host cells, and non-fusogenic MRV, which infects mainly mammals (Attoui et al., 2012; Dermody et al., 2013). Recently, a sixth fusogenic Orthoreovirus species, Broome virus isolated from an Australian fruit bat, has been proposed (Thalmann et al., 2010). Orthoreoviruses are non-enveloped viruses of ~75 nm diameter. Their genome consists of 10 dsRNA segments in three size classes: large (L1–L3), medium (M1–M3) and small (S1–S4) (Dermody et al., 2013; Gouvea & Schnitzer, 1982).

At present, all orthoreoviruses of avian origin are classified as ARVs (Day, 2009). ARVs have been associated with different diseases in a variety of birds, including the chicken (viral arthritis/tenosynovitis, running-stunting syndrome, gastroenteritis, hepatitis, myocarditis and respiratory disease), turkey (infectious enteritis), goose, duck, pigeon, quail, parrot and several other wild birds (Jones, 2008). They have also been isolated from asymptomatic birds. ARVs fail to agglutinate erythrocytes, unlike MRVs (Deshmukh et al., 1969; Kawamura et al., 1965).

Phylogenetic analysis indicated that MRVs represent a major divergent clade, and that ARVs and NBVs form another clade (Duncan, 1999; Wellehan et al., 2009). This clade includes newly isolated strains: PsRV Ge01 from a psittacine bird in Germany (de Kloet, 2008), SSRV from a Steller sea lion in Canada (Palacios et al., 2011) and Tvärminne avian virus (TVAV) from a crow in Finland.
(Dandár et al., 2014). RRV and BRV and Broome virus form a third clade (Bányai et al., 2014; Thalmann et al., 2010; Yan et al., 2011).

The brown-eared bulbul (Hypsipetes amaurotis) (order Passeriformes, family Pycnonotidae) is distributed widely in Japan, far-eastern Russia, north-eastern China, South Korea, Taiwan and northern Philippines (Clements, 2007). In Japan, it is regarded as harmful as it causes damage to agricultural products (Matsuoka, 1994; Yamaguchi, 2004). The birds are either resident or migratory (Karasawa, 1997; Takano, 1981). In spring and autumn, flocks ranging in size from ~10 to several hundred individuals migrate inside Japan (Hamada et al., 2009; Hirata et al., 2009; Nakamura, 2008).

In this study, a novel orthoreovirus strain, named Pycno-1, was isolated from the haemorrhagic intestine of a dead brown-eared bulbul in Japan. Its whole genomic sequence was determined and compared with those of other species of the genus Orthoreovirus.

**RESULTS**

**Virus isolation and virological characterization**

In a bacterial examination, significant bacteria could not be isolated from any of various organ samples, including intestine samples, of three dead brown-eared bulbuls. However, amongst the intestine samples of the three dead brown-eared bulbuls, one homogenate of the haemorrhagic intestine showed a cytopathic effect (CPE), characterized by syncytium formation, in Caco-2 cells 4 days after inoculation. The supernatant of Caco-2 cells showing syncytia also caused the formation of syncytia in Vero cells. As syncytium formation was observed more clearly in Vero cells than in Caco-2 cells, Vero cells were used for further analyses of the isolated agent.

Electron microscopic examination of the syncytium-forming Vero cells revealed non-enveloped particles with a mean diameter of ~70 nm in the cytoplasm (data not shown).

Genome segments of the CPE agent revealed at least eight segments of <6 kbp, estimated by a DNA size marker, which showed similar electrophoretotypes of dsRNAs of ARV and NBV within the genus Orthoreovirus (Benavente & Martínez-Costas, 2007) (Fig. 1). dsRNA normally exhibits a lower electrophoretic mobility than that of dsDNA having the same number of base pairs (Gast & Sänger, 1994). These observations suggested that the CPE agent might belong to ARV or NBV. The isolate was named Pycno-1. Similar to ARV, Pycno-1 virus failed to haemagglutinate chicken erythrocytes (data not shown).

**Genetic analysis of the isolate Pycno-1**

In the process to determine nucleotide sequences of the isolate Pycno-1, it appeared that both the largest segment and the smallest segment contained two segments. Therefore, Pycno-1 had 10 dsRNA segments, as in the genus Orthoreovirus. Sequences of the full-lengths of the 10 RNA segments (L1–L3, M1–M3 and S1–S4) of Pycno-1 were determined. The genomic sequences of Pycno-1 were deposited in the DDBJ database under accession numbers AB914760–AB914769, in the order of segments L1–S4. The nucleotide sizes of segments L1–S4 and the deduced lengths of the encoded proteins from each segment are shown in Table 1. The total nucleotide number of the 10 segments was 23 593. Individual genome segment sizes ranged from 2.0–4.3 kbp, estimated by a DNA size marker, which showed similar electropherotypes of dsRNAs of ARV and NBV. The 3' termini of Pycno-1 RNAs were identical to those of the SSRV strain isolated from a Steller sea lion in Canada (Palacios et al., 2011) and the PsRV Ge01 strain detected in a psittacine bird in Germany (de Kloet, 2008) (Table 2). However, the 5' termini of Pycno-1 RNA segments differed from those of the 10 segments of all five species, including ARV and NBV. The 3' termini of Pycno-1 RNAs were identical to those of other orthoreoviruses. The lengths of the 5' UTRs of the 10 segments were conserved (5' end, 5'-GCUUUUC; 3' end, UCAUC-3'). The 5' and 3' termini of Pycno-1 segments were identical to those of the SSRV strain isolated from a psittacine bird in Germany (de Kloet, 2008) (Table 2). However, the 5' termini of Pycno-1 RNA segments differed from those of the 10 segments of all five species, including ARV and NBV. The 3' termini of Pycno-1 RNAs were identical to those of other orthoreoviruses. The lengths of the 5' UTRs of the 10 segments and 3' UTRs of eight segments (excluding L2 and M2) of Pycno-1 were identical to those of the corresponding segments of SSRV (data not shown). The differences in the lengths of the 3' UTRs of the two segments L2 and M2 between Pycno-1 and SSRV were only 1 or 2 nt (data not shown). The lengths of the 5' UTRs of seven segments and 3' UTRs of two segments were the same as those of the corresponding segments of ARV. The differences in nucleotide numbers of unmatched segments were 1–4 nt in 5' UTRs and 1–24 nt in 3' UTRs (data not shown). BLAST
Table 1. Characteristics of genome segments in Pycno-1

| Genomic segment | Genome size (nt) | UTR (nt) | ORF (nt) | Sequence at the termini | Protein encoded protein | Protein locations 

*Protein locations were adopted from Benavente & Martínez-Costas (2007).

Table 2. Conserved terminal sequences of orthoreovirus genome segments

<table>
<thead>
<tr>
<th>Virus species or strain</th>
<th>Sequence at the termini</th>
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<tbody>
<tr>
<td>MRV</td>
<td>5'-GCUA UCAUC-3'</td>
</tr>
<tr>
<td>ARV</td>
<td>5'-GCUUUUUC UCAUC-3'</td>
</tr>
<tr>
<td>NBV</td>
<td>5'-GCUU UA UCAUC-3'</td>
</tr>
<tr>
<td>BRV</td>
<td>5'-GUA AUC UCAUC-3'</td>
</tr>
<tr>
<td>RRV</td>
<td>5'-GUUA UU UCAUC-3'</td>
</tr>
<tr>
<td>Pycno-1</td>
<td>5'-GUCAA UCAUC-3'</td>
</tr>
<tr>
<td>SSRRV</td>
<td>5'-GCCUUUUC UCAUC-3'</td>
</tr>
<tr>
<td>PsRV Ge01</td>
<td>5'-GCCUUUUC UCAUC-3'</td>
</tr>
<tr>
<td>TVAV</td>
<td>5'-GCCUUUUC UCAUC-3'</td>
</tr>
</tbody>
</table>

analysis revealed that nine protein-coding regions of Pycno-1 except for three proteins, p10 and σC in the S1 segment and σA in the S2 segment, had the highest amino acid sequence identities of 71–95% to those of SSRV (Table 1). The other three protein-coding regions of Pycno-1 had the highest amino acid sequence identities of 75–97% to cognate regions of the PsRV Ge01 genome.

Phylogenetic trees based on nucleotide sequences of each protein-coding region are shown in Fig. 2. cDNA sequence data for all orthoreovirus genes from the GenBank database used in this study are shown in Table S1 (available in the online Supplementary Material). All 12 protein-coding sequences of Pycno-1 were located in the same clade as that of ARV and NBV, but in different clusters. Pycno-1 was closely related to SSRV and PsRV Ge01 in all phylogenetic trees, and formed a new cluster that was independent of ARV and NBV. TVAV isolated from a crow (Dandár et al., 2014) was located at the same cluster as that of Pycno-1 in phylogenetic trees of eight out of 12 protein-coding nucleotide sequences: λA, λB, λC, μA, μB, p10, σC and σB.

All 12 protein-coding nucleotide sequences and amino acid sequences of Pycno-1 were compared with those of SSRV, PsRV Ge01, TVAV, ARV (strain 138) and NBV (strain Pulau), which were located in the same clade (Table 3). In terms of nucleotide sequence identities of the more conservative inner core proteins, λA, λB, μA and σA, Pycno-1 showed high values against SSRV and PsRV Ge01 (75–88%), and lower values against TVAV, ARV and NBV, in that order. In terms of nucleotide sequence identities of the more divergent outer capsid proteins, λC, μB, σC and σB, Pycno-1 also showed high values against SSRV and PsRV Ge01 (67–77%), and lower values against TVAV, ARV and NBV. In terms of amino acid sequence identities of the more conservative inner core proteins, Pycno-1 showed high values against SSRV and PsRV Ge01 (91–97%), and lower values against TVAV, ARV and NBV, in that order. In terms of amino acid sequence identities of the more divergent outer capsid proteins, Pycno-1 also showed high values against SSRV and PsRV Ge01 (73–92%), and lower values against TVAV, ARV and NBV.
All orthoreoviruses except for MRV have a fusogenic small transmembrane (FAST) protein that was named p10 in ARV and NBV (Barry et al., 2010; Clancy & Duncan, 2009; Shmulevitz & Duncan, 2000). The predicted structure of Pycno-1 p10 is similar to those of the FAST proteins of other orthoreoviruses, especially those of SSRV and PsRV (Fig. 3).

**DISCUSSION**

A new fusogenic orthoreovirus was isolated from a dead wild brown-eared bulbul in Japan and was named Pycno-1. Nucleotide sequence analysis revealed that the virus contained a FAST protein in the S1 segment, as in ARV and NBV, and that the structure of the Pycno-1 FAST protein was similar to those of fusogenic orthoreoviruses. Phylogenetic analysis of all 12 genes revealed that Pycno-1 is located in a cluster with ARV and NBV, suggesting their common evolutionary history. ARV and NBV have already been shown to form a clade (Duncan, 1999; Wellehan et al., 2009). SSRV from a Steller sea lion appears to be an additional member of this clade. In phylogenetic trees of eight out of 12 genes, Pycno-1 also clustered with TVAV isolated from a crow. However, the other four phylogenetic trees of proteins showed that TVAV was only distantly related to the cluster of Pycno-1.

Modern species demarcation for orthoreoviruses is based on several criteria, including reassortment of genome segments between isolates of the same species (but not those of different species), nucleotide sequence identity of homologous genome segments (in most cases >75% nucleotide sequence identity within species versus <60% between species) and amino acid sequence identity of proteins encoded by homologous genome segments (for more conservative core proteins, >85% within species and <65% identity between species; for outer and more divergent capsid proteins, >55 versus <35%) and identification of conserved terminal genomic RNA sequences within a species (absolute conservation of the 5'- and 3'-terminal 4-8 nt) (Attoui et al., 2012). In the absence of any evidence for genome segment reassortment, the designation of two isolates as distinct species must be inferred from other polythetic criteria (Duncan, 1999; Mayo, 1996). According to these criteria, except for reassortment, nucleotide sequence identities of seven out of 12 genes between Pycno-1 and SSRV and those of four out of six genes between Pycno-1 and PsRV Ge01 were between 75 and 88%. However, nucleotide sequence identities of all genes of Pycno-1 with ARV, NBV and TVAV did not exceed 72% (35-72%). For more conservative core proteins, amino acid sequence identities of Pycno-1 against SSRV and PsRV Ge01 were >85% (91-97%), but those of Pycno-1 against ARV, NBV and TVAV did not exceed 82% (50-82%). For divergent outer capsid proteins, amino acid sequence identities of Pycno-1 against SSRV and PsRV Ge01 were >55% (73-92%), but those of three out of four

<table>
<thead>
<tr>
<th>Protein</th>
<th>Nucleotide (amino acid) sequence identity (%)</th>
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<tbody>
<tr>
<td>lA</td>
<td>77 (95)</td>
</tr>
<tr>
<td>lB</td>
<td>77 (94)</td>
</tr>
<tr>
<td>lC</td>
<td>67 (76)</td>
</tr>
<tr>
<td>lM</td>
<td>75 (91)</td>
</tr>
<tr>
<td>lB</td>
<td>76 (92)</td>
</tr>
<tr>
<td>lNS</td>
<td>71 (81)</td>
</tr>
<tr>
<td>p10</td>
<td>74 (86)</td>
</tr>
<tr>
<td>p17</td>
<td>70 (71)</td>
</tr>
<tr>
<td>p10</td>
<td>70 (73)</td>
</tr>
<tr>
<td>p10</td>
<td>79 (95)</td>
</tr>
<tr>
<td>p17</td>
<td>77 (86)</td>
</tr>
<tr>
<td>p10</td>
<td>77 (91)</td>
</tr>
</tbody>
</table>

NA, Not available.
proteins of Pycno-1 against ARV, NBV and TVAV did not exceed 56% (20–56%). Furthermore, the nucleotide sequence at the 5′ UTR region of Pycno-1 coincided completely with those of SSRV and PsRV Ge01, but not with those of ARV, NBV and TVAV. The mechanism of reassortment of orthoreoviruses is not fully understood. Involvement of the differences of conserved 5′ ends in reassortment should be examined by the use of, for example, a reverse genetics system (Boehme et al., 2011; Kobayashi et al., 2010). On these grounds, Pycno-1, SSRV and PsRV Ge01 should be considered to be in a different species from ARV and NBV.

Although Pycno-1, SSRV and PsRV Ge01 are closely related phylogenetically, the places where they were isolated and their hosts were different. Pycno-1, SSRV and PsRV Ge01 were isolated in Japan, Canada and Europe, respectively. Pycno-1 was isolated from a brown-eared bulbul that was a resident or a migratory bird in Japan. Therefore, it is difficult to consider that those three similar viruses evolved uniquely in those areas. One possibility is that wild birds transmit a similar virus to those hosts. In wader or shorebird flyways, Japan is included in the East Asian–Australasian Flyway (Boere & Stroud, 2006). Europe and Canada are included in the East Atlantic Flyway and Pacific Americas Flyway, respectively. The breeding grounds of these three flyways are in the same place – the Arctic. Infection of a brown-eared bulbul in Japan and psittacine birds in Europe might be associated with transmission by a wild bird along the migratory bird flyway. As the Steller sea lion does not eat birds, one possibility is that it was infected through viral-excreted faeces of wild birds when it was on the ground.

![Fig. 3.](http://vir.sgmjournals.org) Multiple sequence alignment of various strains of orthoreovirus p10. The overlines indicate the locations of conserved structural motifs, and the first and last amino acid numbers of each motif in the Pycno-1 sequence (Barry et al., 2010; Shmulevitz & Duncan, 2000). Residues identical to the Pycno-1 amino acid sequence are indicated by asterisks. Arrows indicate the locations of the four conserved cysteine residues. CR, conserved region.

Although Pycno-1 was isolated from a dead brown-eared bulbul with a haemorrhagic region in the intestine, the pathology of Pycno-1 is obscure. As the first step to clarify the pathology of Pycno-1 in birds, we plan to perform experimental infection in chicks. PsRV Ge01 was detected in the liver of a psittacine bird that died, and showed severe splenomegaly and hepatomegaly with multifocal acute necrosis (de Kloet, 2008). PsRV infections were shown to be associated with a high rate of mortality in the order Psittaciformes in The Netherlands (van den Brand et al., 2007). SSRV was isolated from an aborted mid-gestational male foetus and associated placenta of a Steller sea lion in British Columbia, Canada. Although a causal relationship between SSRV infection and abortion was not proven, it was suggested that SSRV has the potential for interspecies transmission. Viruses related to NBV have recently been isolated from humans. These viruses, including Melaka virus, Kampar virus, HK23629/07 and Miyazaki-Bali/2007 (Cheng et al., 2009; Chua et al., 2007, 2008; Yamanaka et al., 2014), were identified as causative agents for respiratory tract infection in humans. However, MRVs were isolated from European bats, but the pathology of those viruses in bats is unclear (Kohl et al., 2012). From these observations, Pycno-1 could potentially infect various mammals, such as the Steller sea lion. These ecological features might provide the opportunity for the transmission of a Pycno-1-like virus via the migration of wild birds.

In summary, a novel orthoreovirus was isolated from a dead brown-eared bulbul in Japan, and the isolated virus formed a new cluster with SSRV and PsRV Ge01 that was different from clusters of ARV and NBV. A new species...
with these three viruses named *Wild bird orthoreovirus* is proposed.

**METHODS**

**Tissue sampling.** Many wild brown-eared bulbuls died in Wakayama Prefecture in Japan in the winter of 2011. Three dead birds were transported to Rakuno Gakuen University for bacterial and virological examinations. Representative samples of organs were cultured for bacteria. The homogenate of a small intestine was used for virus isolation. One intestine sample of the three dead birds showed haemorrhage.

**Cells and virus isolation.** Human epithelial colorectal adenocarcinoma (Caco-2) cells and African green monkey kidney (Vero) cells were used. Caco-2 cells were cultured in minimum essential medium (MEM) supplemented with 10% FCS, 1% non-essential amino acids (NEAs), antibiotics and amphotericin B at 37 °C, 5% CO₂. Vero cells were cultured in the same medium without NEAs at 37 °C, 5% CO₂. In the maintenance medium, the concentration of FCS was reduced to 4%. Homogenates of small intestines were inoculated into Caco-2 cells grown in 25 cm² flasks (BD Falcon). After 1 h adsorption at 37 °C, 5% CO₂, the maintenance medium was added and cultured at 37 °C, 5% CO₂, and observed daily for signs of CPE.

**Haemagglutination (HA) test.** The supernatants of virus-infected Vero cells were each mixed with an equal volume of 0.5% chicken red blood cells. The mixtures were left to stand for 60 min at room temperature. Newcastle disease virus was used as HA-positive control.

**Electron microscopic analysis.** Virus-infected Vero cells were prepared for thin sectioning. Briefly, each specimen was fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h, rinsed with three changes of phosphate buffer and post-fixed with 1% osmium tetroxide in phosphate buffer for 1 h. After being washed, the specimen was dehydrated in a graded series of ethanol and embedded in epoxy resin, following the standard protocol. Ultrathin sections were collected on 200 mesh copper grids, and stained with 1% uranyl acetate and 1% lead citrate. Electron microscopy grids were screened at 80 kV in a JEM-1220 transmission electron microscope (JEOL).

**Preparation of dsRNA.** dsRNA was extracted from virus-infected Vero cells using TRIzol reagent (Invitrogen) according to the protocol described by Attoui et al. (2000). Briefly, dsRNA was separated from contaminating ssRNA by precipitating the latter in 2 M lithium chloride at 4 °C overnight. The dsRNA in the supernatant was precipitated by addition of 1 vol. 2-propanol and 0.25 vol. 7.5 M ammonium acetate at −20 °C for 2 h and pelleted by centrifugation at 14,000 r.p.m. for 10 min followed by washing with 75% ethanol. The dsRNA was resuspended in RNase-free water. The genomic segments were separated by 10% SDS-PAGE at 100 V for 5.5 h according to Laemmli′s method (Laemmli, 1970). HindIII digests of λ DNA were used as Mₚ markers. The gel was stained with 0.5 μg ethidium bromide ml⁻¹ and dsRNAs were visualized under UV light.

**Full-length amplification of cDNAs.** A self-priming anchor-primer was used to ensure full-length first-strand cDNA synthesis in the absence of free-floating primers, preventing any mispriming and non-specific amplification (Maan et al., 2007). The anchor-primer consisted of 35 base oligonucleotides that had a spacer9 instead of a terminal nucleotide equivalent to 5’ end of the positive (forward primer) or negative (reverse primer) avian orthoreovirus RNA strands (forward primer, Phased-F 5’-AGAATTCCTAGAGGTGCTTTT-3’; reverse primer, Phased-R 5’-AGAATTCCTAGAGGTGCTTTT-3’). Sequencing was conducted by Hokkaido System Science using the phased primers and walking primers (Table S2). All primer locations were completely sequenced. The normal read lengths of these primers were ~700-800 nt. There were no mixed nucleotide positions. About 53% on both strands were sequenced. The sequence data were checked on their electrophorograms one by one to obtain accurate sequences.

**Viral genome sequencing.** Both termini of the PCR products were identical. The 5′ and 3′ termini of each ARV genome segment ended in six and five conserved base pairs, respectively. These were conventionally represented as (5’-GCUUUU-.........UCAUCC-3’) for the positive strands. These sequences were exploited in the design of universal-sequencing primers (phased primers) (Maan et al., 2007). The phased primers consisted of the sequence of primer 5-15-1 without the BamHI restriction site and with six or five 3′ terminal nucleotides equivalent to the conserved 5′ end of the positive (forward primer) or negative (reverse primer) avian orthoreovirus RNA strands (forward primer, Phased-F 5’-AGAATTCCTAGAGGTGCTTTT-3’; reverse primer, Phased-R 5’-AGAATTCCTAGAGGTGCTTTT-3’). Sequencing was conducted by Hokkaido System Science using the phased primers and walking primers (Table S2). All primer locations were completely sequenced. The normal read lengths of these primers were ~700–800 nt. There were no mixed nucleotide positions. About 53% on both strands were sequenced. The sequence data were checked on their electrophorograms one by one to obtain accurate sequences.

**Sequence and phylogenetic analyses.** Sequence analyses were conducted by DNASS Pro (Hitachi Software Engineering) and a BLAST search was used to identify homologous genes amongst sequences deposited in GenBank (Altschul et al., 1990). Phylogenetic analysis of the nucleotide sequences of cognate genes was conducted by using MEGA5 with 1000 bootstrap replicates of the neighbour-joining method (Saitou & Nei, 1987; Tamura et al., 2011). Evolutionary distances were estimated according to the Kimura two-parameter method (Kimura, 1980). The GenBank database accession numbers of all orthoreoviruses used in this study are provided in Table S1.

**REFERENCES**

Novel orthoreovirus from a brown-eared bulbul


