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ANNOTATED SEQUENCE RECORD

A new member of the family *Totiviridae* associated with arboreal ants (*Camponotus nipponicus*)

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Abstract A putative new member of the family *Totiviridae* was identified in arboreal ants (*Camponotus nipponicus*). The viral dsRNA consisted of 5,713 nt with two overlapping open reading frames (ORFs). ORF1 encodes a putative capsid protein. ORF2 encodes a viral RNA-dependent RNA polymerase (RdRp). ORF2 could be translated as a fusion protein with the ORF1 product through a -1 frameshift in the overlapping ORF1. Phylogenetic analysis based on the RdRp revealed that the virus from *C. nipponicus* is closely related to Camponotus yamaokai virus, a member of the family *Totiviridae*, from another ant species. The name Camponotus nipponicus virus (CNV) is proposed for the new virus.

The family *Totiviridae* includes viruses that form small, non-enveloped, isometric particles with a double-stranded RNA (dsRNA) genome of 4.6–7.0 kbp in length [6]. The previously identified members of this family were detected in either parasitic protozoa or fungi. However, an unclassified member of the family *Totiviridae*, named penaeid shrimp infectious myonecrosis virus (IMNV) was found in the Pacific white shrimp [3]. Following the identification of IMNV, totiviruses have been reported in flies and mosquitoes, and all of those viruses were closely related to each other [2]. A totivirus that is closely related to Giardia lamblia virus was also found in salmon [1].

A virus belonging to the family *Totiviridae*, Camponotus yamaokai virus (CYV), was isolated from the arboreal ant *Camponotus yamaokai* [7]. CYV is the first totivirus found in ants. It shares the general characteristics of a totivirus in both genome composition and virus particle structure, but the genome sequence suggests that CYV is phylogenetically distinct from other arthropod totiviruses. In this study, we determined the complete genome sequence of a totivirus from another arboreal ant species, *C. nipponicus*.

C. nipponicus ants were collected from Shimoda, Shizuoka, Japan, in 2014. Total nucleic acids were extracted from worker ants using the phenol-chloroform method. Then, the dsRNA of the RNA virus was purified from the total nucleic acids and subjected to the fragmented and loop-primer ligated dsRNA sequencing (FLDS) method according to Urayama *et al.* [5].

A sequencing library was generated and analyzed by using IonPGM (Life Technologies), and the sequence reads were assembled using CLC workbench. As a result, the complete sequence of the dsRNA, 5,713 nt in length (DDBJ accession no. LC101918) was obtained. The sequence was predicted to contain two large ORFs estimated by Genetyx software. These two ORFs overlapped by 442 nt. The amino acid sequence of ORF2 had significant similarity to the RdRp of totiviruses and exhibited eight RdRP motifs similar to those of CYV (Supplementary Figure 1).

The presence of an overlapping region suggested that ORF2 is translated as a fusion protein with a product of

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Table 1 Comparison of the genome structures of CNV and CYV

		CNV	CYV	Identity (%)
Full length (nt)		5713	5704	56
5' UTR (nt)		263	203	50
ORF1	RNA (nt)	3018	3084	54
	aa (kDa)	111	116	48
Overlapping region (nt)		442	442	54
ORF2[a]	RNA (nt)	2715 (1896)	2715 (1977)	58 (61)
	aa (kDa)	102 (72)	103 (76)	52 (63)
Fusion protein (kDa)		198	203	51
3' UTR (nt)		159	144	64

[a] ORF2 was defined as the regions devoid of stop codons. Numbers in parentheses indicate regions starting from the first start codons after overlapping regions

ORF1 through a -1 ribosomal frameshift. Similar genomic arrangements have been described for several totiviruses [6]. Sequence analysis revealed the presence of a potential slippery heptamer AAAAAAC at nt position 2912–2918, that could facilitate a ribosomal -1 frameshift (Supplementary Figure 2). The heptamer is followed by a short "spacer" region and then a class 3 pseudoknot of 36 nt (at nt position 3054 – 3089).

The pairwise nucleotide and amino acid sequence identities of the novel RNA viral sequence and CYV are summarized in Table 1. A phylogenetic tree was constructed using the maximum-likelihood method with 1000 bootstrap replicates, based on the amino acid sequences of the RdRp domains (287–658 aa of ORF2) as described by Koyama *et al*. [7]. The phylogenetic analysis suggested that the viral sequence was closely related to, but distinct from, CYV (Fig. 1).

The structure of the reconstructed genome sequence suggests that the virus is a member of the family *Totiviridae*. Phylogenetic analysis revealed that the sequence was closely related to CYV, a totivirus from another ant species. Accordingly, we conclude that the dsRNA in *C.*

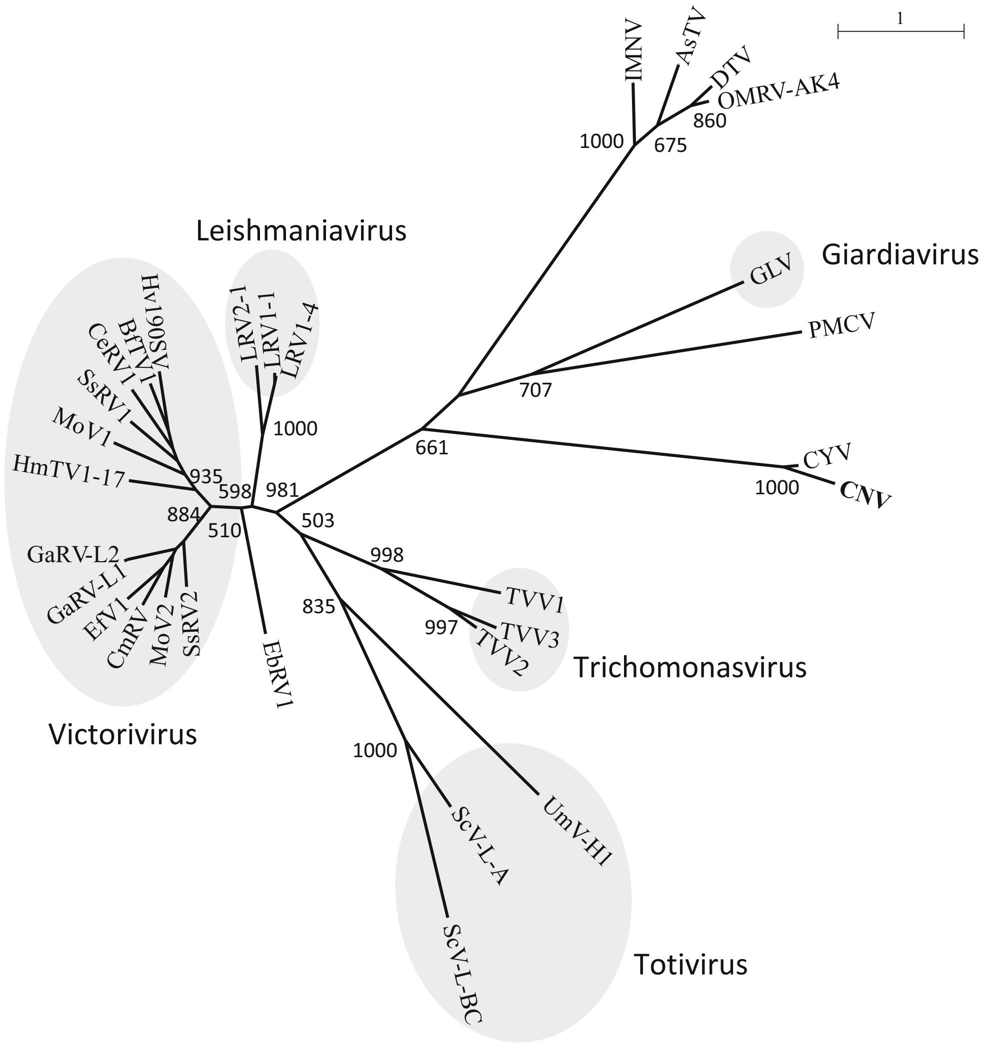

Fig. 1 Phylogenic tree of members of the family *Totiviridae*. Sequences used in this analysis are shown in reference [7]

nipponicus is derived from a new member of the family *Totiviridae* that is closely related to CYV. Therefore, the virus was named "Camponotus nipponicus virus" (CNV).

The significant similarity between CNV and CYV suggests two possibilities about the evolutionary relationship between the totiviruses infecting *C. nipponicus* and *C. yamaokai*, which belong to the subgenera *Colobopsis* and *Myrmamblys,* respectively [4]. First, because *C. nipponicus* and *C. yamaokai* share parts of their habitats [4], the totivirus might be transferred horizontally between host ants. Although totiviruses rarely undergo horizontal transmission [6], transmission between two ant species might have occurred over an evolutionary timescale. Second, an ancestral virus might have existed prior to the divergence of the two host ant species, with CNV and CYV later diverging and co-evolving with their hosts. In this case, the totiviruses in ants would have existed before the divergence of these subgenera. Some ant species must have lost their totiviruses, because no totiviruses have been found in ants of the subgenus *Myrmamblys*, such as *C. nawai* [7]. Future studies of the phylogeography of the host ants and that of the totiviruses will provide insight into the co-evolutionary relationship between the host insects and the viruses.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All applicable guidelines for the care and use of animals were followed.

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