

**Part 1:** **TITLE, AUTHORS, APPROVALS, etc**

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| **Code assigned:** | **2022.006S** |  |
| **Short title:** Create a new species in the genus *Cripavirus* (*Dicistroviridae*) |
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**Author(s) and email address(es)**

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**Corresponding author**

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| Steven M. Valles |

**List the ICTV Study Group(s) that have seen this proposal**

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| *Dicistroviridae*/*Iflaviridae* |

**ICTV Study Group comments and response of proposer**

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| ***Judy Chen (Chair)*****Comment 1:** Change abstract sentence to: “monopartite bicistronic RNA genome yielding two, non-overlapping ORFs separated by an intergenic region (IGR).”**Response 1:** The sentence has been changed as suggested.**Comment 2:** Do you want to add titles for columns of Host and species? **Response 2:** No, the main point of the phylogram is to show the relationship SINV-6 and the established Dicistroviridae genera (Triatovirus, Cripavirus, and Aparavirus), which is illustrated. ***Huoqing Zheng (SG Member)*****Comment 1:** Assign a different color or marker to highlight SINIV-6 in the phylogram.**Response 1:** The text for SINV-6 is now in red font as suggested.**Comment 2:** Were these results published?**Response 2:** No, the data are unpublished. I note this for the Table 1 caption.**Comment 3:** Use SINV-6 in the table.**Response 3:** This has been corrected in Table 1.***Andrew Firth (SG Member)*****Comment 1:** As you know, the dicistrovirus IGR-IRES is unusual in that it directs ORF2 initiation from a non-AUG codon, so the actual initiation site is not the AUG codon. I had a look at the IGR-IRES structure prediction by homology to those illustrated in Fig 3 of Nakashima & Uchiumi PMID 18621089. My prediction is in the attached pdf and would indicate that the actual ORF2 initiation site is probably the GCU codon at MH714708 nt 7085-7087. This would alter the reported coordinates and length of ORF2.**Response 1:** I have revised the genome section to read: “However, the dicistrovirus IGR-IRES is unusual in that it directs ORF2 initiation from a non-AUG codon, so the actual initiation site is likely not an AUG codon. Based on IGR-IRES structure prediction by homology to those illustrated in Nakashima & Uchiumi (2009) the actual ORF2 initiation site is probably the GCU codon at nucleotides 7085-7087 (MH714708). While empirical evidence for this conclusion has not been obtained, it would alter the reported coordinates (7085-9490) and length (2406 nts) of ORF2.”**Comment 2:** In the abstract, "identity with non-structural (capsid) proteins" -> "identity with structural (capsid) proteins".**Response 2:** Sentence is corrected as suggested.**Comment 3:** In Fig 1, I would suggest deleting "Aves" as there is no evidence that geese are the host of this virus - most likely it was just in some insects that the goose ate and defecated.**Response 3:** We agree that this virus was likely consumed and later defecated by the goose. The phylogram has been revised without Class information.***Members Declan Schroeder, Gabriela Echeverria, Eugene Ryabov, and Rhys Parry* had no comments.** |

**ICTV Study Group votes on proposal**

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| **Study Group** | **Number of members** |
| **Votes support** | **Votes against** | **No vote** |
| *Dicistroviridae*/*Iflaviridae* | 6 | 0 | 2 |
|  |  |  |  |

**Authority to use the name of a living person**

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| **Is any taxon name used here derived from that of a living person (Y/N)** | Yes |

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| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
| *Cripavirus porteri* | Sanford D. Porter | Yes |

**Submission dates**

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| --- | --- |
| Date first submitted to SC Chair  | 11/05/2022 |
| Date of this revision (if different to above) |  |

**ICTV-EC comments and response of the proposer**

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**Part 2:** **NON-TAXONOMIC PROPOSAL**

**Text of proposal**

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**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2022.006S.N.v1.Cripavirus\_1nsp.xlsx |

**Abstract**

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| I propose to assign Solenopsis invicta virus 6 (SINV-6) to a new species in the *Cripavirus* genus. SINV-6 exhibits characteristics consistent with those of other members of the *Cripavirus genus* (*Dicistroviridae*) including a single-stranded, monopartite, bicistronic RNA genome yielding two, non-overlapping ORFs separated by an intergenic region (IGR). The 5’-proximal ORF (5862 nts) sequence shares strong identity with non-structural components of dicistroviruses, including RdRp, protease, and helicase (RdRp-Pro-Hel). The 3’-proximal ORF predicted proteins exhibit identity with structural (capsid) proteins of dicistroviruses. SINV-6 was originally identified from a metatranscriptomics sequence project of fire ants, *Solenopsis invicta*. Two isolates were identified, namely *Formosa* (from Argentinean *Solenopsis invicta*), and *Hogtown* (from North American [Florida] *Solenopsis invicta).* Virus particles were purified from *Solenopsis invicta* fire ants and scanning electron microscopy revealed icosahedral particles with a mean diameter of 34.2 ±2.1 nm. Replicative genome strand was detected in worker ants of *Solenopsis invicta*. Virus was detected in other ant species suggesting a wider host range. |

**Text of proposal**

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| Solenopsis invicta virus 6 (SINV-6) was originally described from genome sequence alone (Valles and Rivers 2019). This proposal seeks to assign the virus as a new species, *Cripavirus porteri* in the *Dicistroviridae* family, *Cripavirus* genus. Two isolates have been identified, ***Formosa*** (from Argentinean *Solenopsis invicta* fire ants: accession MH714707) and ***Hogtown*** (from North American *Solenopsis invicta* fire ants: accession MH714708). We propose to assign the SINV-6 Formosa as the exemplar for this species. Phylogenetic analysis of the non-structural and structural proteins places the sequences within the *Cripavirus* clade of the *Dicistroviridae*. According to the ICTV species demarcation rules for dicistroviruses, the virus is undoubtedly a unique addition to the *Dicistroviridae*/*Cripavirus* group. Structural and non-structural protein sequences show less than 50% and 42% identity with currently assigned members of the *Cripavirus* genus, respectively. Therefore, sequences are below the 90% identity threshold and meet the criterion for recognition as a separate species. The isolates, *Hogtown* and *Formosa*, share 99.5% and 99.7% amino acid sequence identity for ORF1 and ORF2, respectively and can therefore both be assigned to the new proposed species.The proposed species name is *Cripavirus porteri* after the entomologist who collected the fire ants used as source material for the sequencing effort, Dr. Sanford D. Porter. In addition, the new name adheres to the new binomial nomenclature adopted by the ICTV. Dr. Porter has provided permission to use his name in this regard (see accompanying letter). No overt symptoms were observed among SINV-6-infected fire ants |

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**Supporting evidence**

***Phylogenetic Analysis***

BLAST analysis of the structural and non-structural translated ORFs of SINV-6 revealed sequence homology to other members of the *Dicistroviridae* family. When phylogenetic analysis of the non-structural ORF predicted protein sequence was conducted with formally accepted dicistrovirus species and related unclassified virus sequences, SINV-6 groups within the *Cripavirus* clade (Fig. 1).

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**Figure 1.** Mid-point rooted maximum likelihood phylogenetic tree of sequences of currently assigned dicistroviruses, related unclassified virus sequences identified from BLAST analysis, and the SINV-6 sequences (Formosa and Hogtown isolates). Genetic distance is indicated in the lower left and the genera comprising the *Dicistroviridae* are indicated on the left and highlighted with different colors (*Triatovirus* = purple; *Cripavirus* = blue; *Aparavirus* = salmon). GenBank accession numbers follow each virus name, except for SINV-6. These accession numbers are MH714707 (Formosa isolate) and MH714708 (Hogtown isolate). Values on the internal nodes represent the number of nonparametric bootstrap samples out of 100 with the same topology. Adapted from Valles and Rivers 2019.

***Genome structure***

The RNA genome of both isolates (*Formosa* and *Hogtown*) was 9793 nucleotides in length, excluding the polyadenylated 3’ terminus. Two large non-overlapping ORFs were predicted in the sense orientation flanked and separated by untranslated regions (Fig. 2). ORF1 (5’-proximal) was 5862 nucleotides and ORF2 (3’-proximal) was 2085 nucleotides based on a canonical start codon at genome position 7406. However, the dicistrovirus IGR-IRES is unusual in that it directs ORF2 initiation from a non-AUG codon, so the actual initiation site is likely not the AUG codon. Based on IGR-IRES structure prediction by homology to those illustrated in Nakashima & Uchiumi (2009) would indicate that the actual ORF2 initiation site is probably the GCU codon at nucleotides 7085-7087 (MH714708). While empirical evidence for this conclusion has not been obtained, it would alter the reported coordinates (7085-9490) and length (2406 nts) of ORF2. BLAST analysis of each ORF revealed significant identity to non-structural proteins (ORF1) and structural (capsid) proteins (ORF2) of viruses in the *Dicistroviridae*. The RdRp region of ORF1 exhibited significant identity with Cricket paralysis virus—the type species for the Cripavirus genus. The IRES bulge sequence (UGAUCU) found in cripaviruses was detected intact within the 5’UTR and partially (UGAUC) within the intergenic region of both isolates (Jang and Jan 2010, Pfingsten et al. 2007).

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**Hel Pro RdRp**

**Capsid**

5’

AAA 3’

5’UTR 1-731

3’IGR 6597-7084/7405

3’UTR 9494…

ORF1

ORF2

**Figure 2.** Genome architecture of SINV-6.

***Purification of Virus***

SINV-6 was purified by isopycnic ultracentrifugation from the worker caste of *Solenopsis invicta* previously shown to be positive for the virus by RT-PCR (and negative for other known viruses of the ant host). Virus particles migrated to a mean density of 1.308 ±0.0085 g/ml. This fraction was stained with 2% phosphotungstic acid, pH 7, and examined by electron microscopy at an accelerating voltage of 75kV. Uninfected worker ants were prepared under the same conditions and used as a negative control. Electron microscopy revealed isometric particles (Fig. 3) with a mean diameter of 34.2 ±2.1 nm in *SINV-6*-infected ants. No virus particles were detected in negative controls. This structure, diameter, and sedimentation rate are all consistent with viruses in the *Dicistroviridae*.

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**Figure 3.** Electron micrograph of SINV-6 particles.

**Characteristics of SINV-6 infection**

*SINV-6* was first identified from the red imported fire ant, *Solenopsis invicta*, and detected in South and North American populations. The negative genome strand was detected in the worker caste of *Solenopsis invicta* indicating that the virus replicates in this stage. Therefore, *Solenopsis invicta* serves as host for SINV-6. The virus was detected in all developmental stages, except eggs. Early and late instar larvae, pupae, adults (workers, male and female winged reproductives) and queens were host to the virus. Field surveys showed that SINV-6 was quite prevalent in *Solenopsis invicta* with 75 ±25% testing positive by RT-PCR. Perfunctory host specificity tests were conducted by examining twenty additional ant species in four subfamilies collected directly from the field for the presence of the virus. SINV-6 was detected in two additional ant species, *Monomorium pharaonis* and *Dorymyrmex medeis*. Table 1 summarizes the ant species evaluated. It appears that the host range for SINV-6 is not limited to *Solenopsis invicta*. Cripaviruses typically exhibit expanded host ranges (Christian and Scotti 2008).

**Table 1.** Ant species examined by RT-PCR for the presence of SINV-6 (unpublished data, SMV).

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|  **Ant species** |  **Subfamily** |  ***n*** |  **Presence of SINV-6(%)** |
| *Solenopsis invicta* | Myrmicinae | 30 | 75.2 ±24.5 |
| *Solenopsis papuana* | Myrmicinae | 13 | 0 |
| *Solenopsis richteri* | Myrmicinae | 5 | 0 |
| *Solenopsis carolinensis* | Myrmicinae | 1 | 0 |
| *Solenopsis molesta* | Myrmicinae | 3 | 0 |
| *Solenopsis carolinensis* | Myrmicinae | 1 | 0 |
| *Solenopsis xyloni* | Myrmicinae | 6 | 0 |
| *Solenopsis aurea* | Myrmicinae | 2 | 0 |
| *Pheidole obscurithorax* | Myrmicinae | 3 | 0 |
| *Monomorium floricola* | Myrmicinae | 3 | 0 |
| *Tetramorium sp.* | Myrmicinae | 1 | 0 |
| *Monomorium pharaonis* | Myrmicinae | 1 | 100 |
| *Wasmannia auropunctata* | Myrmicinae | 2 | 0 |
| *Aphaenogaster sp.* | Myrmicinae | 1 | 0 |
| *Pseudomyrmex graciles* | Pseudomyrmicinae | 1 | 0 |
| *Dorymyrmex bureni* | Dolichoderinae | 3 | 0 |
| *Dorymyrmex medeis* | Dolichoderinae | 2 | 100 |
| *Tapinoma melanocephalum* | Dolichoderinae | 1 | 0 |
| *Nylanderia fulva* | Formicinae | 2 | 0 |
| *Brachymyrmex obscurior* | Formicinae | 3 | 0 |
| *Camponotus floridanus* | Formicinae | 7 | 0 |

**Impact of SINV-6**

Solenopsis invicta colonies infected with SINV-6 did not exhibit a significant difference in brood production or queen egg production compared with uninfected control colonies. Furthermore, no obvious overt pathological symptoms or mortality were detected in Solenopsis invicta colonies infected with the virus.

**Conclusions**

SINV-6 exhibits characteristics consistent with viruses within the Cripavirus genus of the Dicistroviridae. The virus possesses a single-stranded RNA virus that predicts two open reading frames flanked and separated by untranslated regions. The 5’-proximal ORF encodes for non-structural proteins and the 3’-proximal ORF for structural (capsid) proteins. Phylogenetic analyses place SINV-6 within the *Cripavirus* clade with high probability. The sedimentation rate for the virus is consistent with those in the Picornavirales and the form and size of the virus are also consistent with dicistroviruses. The host range of the virus appears to extend to several ant species. No overt symptoms or obvious colony impacts were observed in *Solenopsis invicta* ant colonies infected with the virus.

**References**

Christian, PD and Scotti, PD. 2008. Encyclopedia of Virology (third edition). Mahy, WJ and Van Regenmortel MHV (editors).

Nakashima, N.; Uchiumi, T. Functional analysis of structural motifs in dicistroviruses. *Virus Res* **2009**, *139*, 137-147, doi:10.1016/j.virusres.2008.06.006.

Jang, CJ and Jan, JE. 2010. Modular domains of the Dicistoviridae intergenic internal ribosome entry site. RNA. 16: 1182-1195.

Pfingsten, JS, Contantino, DA, Kieft, JS. 2007. Conservation and diversity among the three-dimensional folds of the Dicistroviridae intergenic region IRESes. Journal of Molecular Biology. 370: 856-869.

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