

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

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| **Code assigned:** | **2022.004P** |  |
| **Short title:** Create eight new species (*Picornavirales*: *Secoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Secoviridae* Study Group |

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

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| Most members of the *Secoviridae* Study Group are supportive of the proposal |

**ICTV Study Group votes on proposal**

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| --- | --- | --- | --- |
| **Study Group** | **Number of members** | | |
| **Votes support** | **Votes against** | **No vote** |
| *Secoviridae* Study Group | 6 | 1 | 0 |
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**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)** | N |

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| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

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| Date first submitted to SC Chair | May 20, 2022 |
| Date of this revision (if different to above) | May 27, 2022 |

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

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

**Name of accompanying Excel module**

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| 2022.004P.N.v1\_Secoviridae\_8ns.xlsx |

**Abstract**

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| This taxonomic proposal considers the recognition of the following eight new virus species (Table 1) based on species demarcation criteria in the family *Secoviridae* of less than 75% amino acid sequence identity in the coat protein(s) and/or less than 80% amino acid sequence identity in the conserved Pro-Pol region (from the protease CG motif to the polymerase GDD motif), and/ordistinct plant hosts and biological properties: *Comovirus rapae* in the genus *Comovirus, Nepovirus stenotaphri* in the genus *Nepovirus,* *Nepovirus anemones* in the genus *Nepovirus, Sadwavirus betananas* in the genus *Sadwavirus,* subgenus *Cholivirus,* *Sadwavirus aciphyllae* in the genus *Sadwavirus*, subgenus *Stramovirus,* *Torradovirus codonopsis* in the genus *Torradovirus, Torradovirus manihotis* in the genus *Torradovirus*,and *Waikavirus camelliae* in the genus *Waikavirus*. |

**Text of proposal**

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| |  | | --- | | **Creation of a novel species in the genus *Comovirus* of the family *Secoviridae*.** The genome sequence of two isolates of turnip ringspot virus (TuRSV) was previously reported (Koloniuk and Petrzik et al. 2009). This virus was described for the first time in turnip (*Brassica rapa*) in the U.S.A. (Rajakaruma et al. 2007). TuRSV isolate M12 from Chinese cabbage has a 6,078 nt long RNA1 and a 3,984 nt long RNA2, excluding the poly(A) tails. The genome organization of TuRSV is similar to that of other members of the genus *Comovirus* in the family *Secoviridae* (Figure 1). The molecular mass of the polyprotein encoded by RNA1 is 211 kDa and the polyprotein is predicted to be processed into five mature proteins, including a 32 kDa proteinase cofactor, a 58 kDa putative helicase, a 3.2 kDa viral genome linked protein (VPg), a 24 kDa proteinase, and a 87 kDa putative RNA dependent RNA polymerase. The polyprotein encoded by RNA2 has a 122 kDa molecular mass. It codes for a movement protein (52 kDa) and a large (41 kDa) and small (28 kDa) capsid protein. The CPs and conserved Pro-Pol region of TuRSV have 72% and 79.8% amino acid sequence identity with radish mosaic virus (RaMV), the closest related virus in the genus *Comovirus*, respectively. ML phylogenetic trees generated using the CPs (Figure 2) and conserved Pro-Pol sequences (Figure 3) of TuRSV and representative members of the family *Secoviridae* revealed the clustering of TuRSV in the genus *Comovirus*. Considering the species demarcation criteria for the family *Secoviridae* (Karasev et al. 2019, Sanfaçon et al. 2020, Thompson et al. 2017), we propose to classify turnip ringspot virus (TuRSV) as a member of a novel species named *Comovirus rapae* in the genus *Comovirus* of the family *Secoviridae* (Table 1).  **Creation of a first novel species in the genus *Nepovirus* of the family *Secoviridae*.** The genome sequence of a new nepovirus from *Stenotaphrum secundatum* (St Augustine grass or buffalo grass) was described in Australia by high-throughput sequencing (Tran et al. 2021). The virus was tentatively named Stenotaphrum nepovirus (SteNV). Icosahedral virions 25 nm in diameter were also observed in sap of infected *S. secundatum* plants by transmission electron microscopy (Tran et al. 2021). The genome sequence of SteNV was determined by RT-PCR and the 5’ and 3’ genomic extremities were determined by RACE. Given their diversity, nepoviruses have been informally divided into three subgroups (A, B and C) based on the size of RNA2 and immunological properties. This has not been recognized at the taxonomy level. A reevaluation of nepovirus relationships and the possibility of creating three or more subgenera to acknowledge the expanding diversity of nepoviruses is underway. For the purpose of this proposal, we refer to the three subgroups. The genome of SteNV was identical to the genome of members of genus *Nepovirus*, particularly of subgroup C members, in the family *Secoviridae* (Figure 1). The full-length SteNV RNA1 and RNA2 sequences are 7,824 and 7,104 nt in length, respectively (Tran et al. 2021). The 5’ untranslated regions are 41 nt for RNA1 and 140 nt for RNA2. The 3’ untranslated regions are 1,522 nt for RNA1 and 2,155 nt for RNA2. The two 3’ UTR share 99% nucleotide sequence identity. The genome organization of SteNV is similar to that of other nepoviruses from subgroup C in the family *Secoviridae* (Figure 1). The RNA1 polyprotein is processed *in cis* by the proteinase at putative H/G or H/S cleavage sites to produce a X1 protein of unknown function, the X2 protein, a transmembrane protein, an NTP-binding domain, a VPg, a cysteine protease, and an RNA-dependent RNA polymerase (Tran et al. 2021). The RNA2 polyprotein is processed *in trans* by the proteinase at putative H/S or L/S cleavage sites to produce a X3 protein of unknown function, a X4 protein of unknown function, the movement protein, and the coat protein (Tran et al. 2021). A diagnostic RT-PCR with primers designed in the RdRP coding region identified SteNV in 18% (22 of 122) grass samples from various regions in Australia. It is not known whether SteNV is pathogenic on grass (Tran et al. 2021). Sequence comparisons and phylogenetic analyses of amino acid sequences of the CP (Figure 2) and conserved Pro-Pol region (Figure 3) grouped SteNV into subgroup C nepoviruses with cherry leafroll virus (CLRV) as a closely related virus (Tran et al. 2021). The aa sequence identity between SteNV and CLRV is 62.6 % for the conserved Pro-Pol region and 34.6% for the CP region. Considering the species demarcation criteria for the family *Secoviridae* of less than 75% amino acid sequence in the CP and/or less than 80% amino acid sequence in the conserved Pro-Pol region (Karasev et al. 2019, Sanfaçon et al. 2019, Thompson et al. 2017), we propose to classify Stenotaphrum nepovirus (SteNV) as a member of a novel species named *Nepovirus stenotaphri* in the genus *Nepovirus* of the family *Secoviridae* (Table 1).  **Creation of a second novel species in the genus *Nepovirus* of the family *Secoviridae*.** The full-length genome sequence of a new nepovirus from *Anemone flaccida* from China was determined by high-throughput sequencing by Jo Y and Cho WK, Research Institute of Agriculture and Life Sciences, Seoul, Korea. The virus is tentatively named anemone nepovirus A (AnNVA). Sequences of AnNVA isolate Won were deposited in NCBI in 2018 as accession numbers MH898479 for RNA1 (7,680 nt) and MH898478 for RNA2 (6,854 nt). No record of AnNVA is available in the literature. The genome organization of AnNVA is similar to that of members of the genus *Nepovirus*, particularly of member belonging to the subgroup C, in the family *Secoviridae* (Figure 1). Sequence analyses suggested that the AnNVA RNA1 polyprotein is processed *in cis* by the proteinase at putative Q/G and Q/S cleavage sites to produce a X1 protein of unknown function, the X2 protein, an NTP-binding domain, a VPg, a cysteine protease, and an RNA-dependent RNA polymerase. The RNA2 polyprotein is hypothetically processed *in trans* by the proteinase at putative Q/S cleavage sites to produce a X3 protein of unknown function, a X4 protein of unknown function, the movement protein, and the coat protein. Sequence comparisons and phylogenetic analyses of aa sequences of the CP (Figure 2) and conserved Pro-Pol region (Figure 3) grouped AnNVA into subgroup C nepoviruses with tomato ringspot virus (ToRSV) as a closely related virus. The aa sequence identity between AnNVA and ToRSV is 64.4% for conserved the Pro-Pol region and 42.8% for the CP region. Considering the species demarcation criteria for the family *Secoviridae* of less than 75% amino acid sequence in the CP and/or less than 80% amino acid sequence in the conserved Pro-Pol region (Karasev et al. 2019, Sanfaçon et al. 2019, Thompson et al. 2017), we propose to classify anemone nepovirus A (AnNVA) as a member of a novel species named *Nepovirus anemones* in the genus *Nepovirus* of the family *Secoviridae* (Table 1).  **Creation of a first novel species in the genus *Sadwavirus* of the family *Secoviridae*.** The genome sequence of a new sadwavirus from pineapple (*Ananas comosus*) was described in Hawaii, USA by high-throughput sequencing (Larrea-Sarmiento et al. 2022). The virus was tentatively named pineapple secovirus B (PSVB). The genome organization of PSVB is identical to that of members of the genus *Sadwavirus*, subgenus *Cholivirus* in the family *Secoviridae* (Figure 1). The genome comprised two RNA molecules of 5,956 nt and 3,808 nt in length, excluding the poly(A) tails (Larrea-Sarmiento et al. 2022). The 5’ and 3’ UTR sequences were determined by RACE. The RNA1 polyprotein contains five conserved domains, including a protease cofactor, a helicase, a VPg, a protease, and an RNA-dependent RNA polymerase, and the RNA2 polyprotein contains the movement and coat proteins. The predicted cleavage sites were Q/S and E/G dipeptides. Sequence comparisons and phylogenetic analyses of aa sequences of the CP (Figure 2) and conserved Pro-Pol region (Figure 3) grouped PSVB with other members of the subgenus *Cholivirus* in the genus *Sadwavirus,* including the closely related pineapple sadwavirus A (PSVA) (Larrea-Sarmiento et al. 2022).Sequence identities of 45.1% and 23.6% were obtained in the conserved Pro-Pol regionand CPof PSVB and PSVA, respectively. A diagnostic RT-PCR with primers designed in the RdRP and CP coding regions identified PSVB in 30% (4 of 12) of the pineapple trees tested. No information is available on the role of PSVB in the etiology of mealybug wilt of pineapple (Larrea-Sarmiento et al. 2022). Considering the species demarcation criteria for the family *Secoviridae* of less than 75% amino acid sequence in the CP and/or less than 80% amino acid sequence in the conserved Pro-Pol region) (Karasev et al. 2019, Sanfaçon et al. 2019, Thompson et al. 2017), we propose to classify pineapple secovirus B (PSVB) as a member of a novel species named *Sadwavirus betananas* in the genus *Sadwavirus*, subgenus *Cholivirus* of the family *Secoviridae* (Table 1).  **Creation of a second novel species in the genus *Sadwavirus* of the family *Secoviridae*.** The genome sequence of a novel virus tentatively named surrounding non-legume associated secovirus (SnLaSV) was characterized from an unidentified weed species growing in proximity to pea plants in Germany by high-throughput sequencing (Gaafar et al. 2020). The genome of SnLaSV isolate Muenster\_17 comprised two RNA molecules of 6,775 nt (RNA1, GenBank accession number MN412739) and 5,291 nt (RNA2, GenBank accession number MN412740) in length, excluding the poly(A) tails. The genome organization of SnLaSV is identical to that of members of the genus *Sadwavirus*, subgenus *Stramovirus* in the family *Secoviridae* (Figure 1). Subsequently, SnLaSV was described in mountain celery (*Heracleum moellendorfii*), a perennial herb, exhibiting chlorotic spot symptoms in China by high throughput sequencing (Luan et al. 2021). Its genome was determined by RT-PCR and RACE and shown to consist of a 6.616 nt long RNA1 and a 5,356 nt long RNA2, excluding the poly(A) tails (Luan et al. 2021). Sequence analyses showed at 82% and 92% nt and aa sequence identity with the RNA1 and RNA2 of SnLaSV sequences previously determined, indicating that the virus from mountain celery is a strain of SnLaSV. Phylogenetic analyses of the aa sequences of the CP (Figure 2) and conserved Pro-Pol region (figure 3) grouped SnLaSV from China and Germany with lettuce secovirus 1 (LSV1) in a separate branch in the subgenus *Stramovirus* in the genus *Sadwavirus* (Luan et al. 2021). A diagnostic RT-PCR showed the presence of SnLaSV in 48% (11 of 23) of the mountain celeries with chlorotic spots but no in asymptomatic plants from the same field (Luan et al. 2021). The aa sequence identity between SnLaSV and LSV1 is 67.4% for the conserved Pro-Pol region and 42.8% for the CP region. Considering the species demarcation criteria for the family *Secoviridae* of less than 75% amino acid sequence in the CP and/or less than 80% amino acid sequence in the conserved Pro-Pol region met (Karasev et al. 2019, Sanfaçon et al. 2019, Thompson et al. 2017), we propose to classify non-legume associated secovirus (SnLaSV) as a member of a novel species named *Sadwavirus aciphyllae* in the genus *Sadwavirus*, subgenus *Stramovirus* of the family *Secoviridae* (Table 1).  **Creation of a first novel species in the genus *Torradovirus* of the family *Secoviridae*.** The genome sequence of a novel virus tentatively named cassava torrado-like virus (CsTLV) was characterized from cassava (*Manihot esculenta*) plants exhibiting root symptoms of cassava frogskin disease in Colombia by high-throughput sequencing on an Oxford Nanopore technology platform (Leiva et al. 2022). The 5’ and 3’ ends were determined by RACE. The genome organization of CsTLV is typical of members of the genus *Torradovirus* in the family *Secoviridae* (Figure 1). The RNA1 of CsTLV is 7,252 nt long and encodes a polyprotein with typical conserved replication torradovirus motifs, including a Maf/Ham1 domain (Leiva et al. 2022). The RNA2 of CsTLV is 4,469 nt long and contains two overlapping open reading frames coding a protein of unknown function, the movement protein, and three coat protein domains (Leiva et al. 2022). Sequence comparisons revealed the highest aa sequence identity to squash chlorotic leaf spot virus (SCLSV) with 37.8% identity for RNA1 and 45.5% identity for RNA2. For the CP and conserved Pro-Pol coding regions, the aa sequence identity was 46.6% and 53.6% between CsTLV and SCLSV. Phylogenetic analyses of the CP (Figure 2) and Pol-Pol (Figure 3) aa sequences confirmed the grouping of CsTLV with other members of the genus *Torradovirus* (Leiva et al. 2022). Considering the species demarcation criteria for the family *Secoviridae* of less than 75% amino acid sequence identity in the CP and/or less than 80% amino acid sequence identity in the conserved Pro-Pol region (Karasev et al. 2019, Sanfaçon et al. 2019, Thompson et al. 2017), we propose to classify cassava torrado-like virus (CsTLV) as a member of a novel species named *Torradovirus manihotis* in the genus *Torradovirus* of the family *Secoviridae* (Table 1).  **Creation of a second novel species in the genus *Torradovirus* of the family *Secoviridae*.**  The genome sequence of a novel virus tentatively named codonopsis torradovirus A (CoTVA) was characterized from a *Codonopsis lanceolata* plant exhibiting a stunted growth and malformed leaves in South Korea by high-throughput sequencing (Belete et al. 2021). Assembled contigs were annotated and BLASTx analysis revealed two large contigs that were most similar to sequences of RNA1 and RNA2 of motherwort yellow mosaic virus (MYMoV) of the genus *Torradovirus*. The genome sequence of CoTVA was determined by RT-PCR and the 5’ and 3’ end sequences were obtained by RACE. The genome organization of CoTVA is typical of members of the genus *Torradovirus* in the family *Secoviridae* (Figure 1). The RNA1 is 6,922 nt long and the RNA2 is 4,613 nt long, excluding the poly(A) tails (Belete et al. 2021). The 5’ and 3’ UTRs of RNA1 are 141 and 184 nt long. The polyprotein encoded by RNA1 contains conserved motifs for a superfamily 3 helicase, 3C-like protease and RdRP domains (Belete et al. 2021). The 5’ and 3’ UTRs of RNA2 are 153 and 202 nt long. The polyprotein encoded by RNA2 contains two ORFs with ORF1 encoding a protein of unknown function, and ORF2 encoding contains a conserved domain for the movement protein, and three coat proteins. Sequence comparisons between the CP and Pro-Pol aa sequences of CoTVA and MYMoV revealed 54% and 75% identity, respectively (Belete et al. 2021). Phylogenetic analyses of the aa sequences of the CP (figure 2) and conserved Pro-Pol region (Figure 3) confirmed the grouping of CoTSV with members of the genus *Torradovirus*. Considering the species demarcation criteria for the family *Secoviridae* of less than 75% amino acid sequence identity in the CP and/or less than 80% amino acid sequence identity in the conserved Pro-Pol region (Karasev et al. 2019, Sanfaçon et al. 2019, Thompson et al. 2017), we propose to classify codonopsis torradovirus A (CoTVA) as a member of a novel species named *Torradovirus codonopsis* in the genus *Torradovirus* of the family *Secoviridae* (Table 1).  **Creation of a novel species in the genus *Waikavirus* of the family *Secoviridae*.** The genome sequence of a novel virus tentatively named camellia virus A (CamVA) was characterized from *Camellia japonica* plants by high-throughput sequencing (Liao et al. 2021). The genome sequence of CamVA was determined by RT-PCR and the 5’ and 3’ RACE kits. The genome organization of CamVA is typical of members of the genus *Waikavirus* in the family *Secoviridae* (Figure 1). The complete monopartite genome of CaMVA is 12,570 nt long (GenBank accession number MW545173), excluding the poly(A) tail. The 5’ UTR is 641 nt long and the 3’ UTR is 564 nt long (Liao et al. 2021). The genome organization of CamVA is identical to that of members of the genus *Waikavirus*. The genome encodes three ORFs. ORF1 encodes a large polyprotein with conserved domains for a DNA double-strand break repair ATPase, capsid proteins, a RNA helicase, a proteinase, and an RNA-dependent RNA polymerase that is hypothetically process into seven mature protein products at the following predicted dipeptide cleavage sites: Q/T, Q/A, N/T, Q/P, N/K and Q/L (Liao et al. 2021). ORF2 overlaps ORF1 and shares homology with the corresponding protein of bellflower vein chlorotic virus (BVCV). ORF3 is located downstream of ORF1 and does not share significant similarity with any proteins in databases. Sequence comparisons shows CamVA closely related with persimmon waikavirus (PWaiV) with 47.7% and 51.5% aa sequence identity in the CP and conserved Pro-Pol region, respectively. Phylogenetic analyses of aa sequences of the CP (Figure 2) and conserved Pro-Pol region (Figure 3) confirmed the grouping of CamVA with members of the genus *Waikavirus* (Liao et al. 2021). A diagnostic RT-PCR identified CamVA in 68% (19 of 29) camellia trees tested but no consistent association with virus-like symptoms were found (Liao et al. 2021).Considering the species demarcation criteria for the family *Secoviridae*, we propose to classify camellia virus A (CamVA) as a member of a novel species named *Waikavirus camelliae* in the genus *Waikavirus* of the family *Secoviridae* (Table 1). | |

**Supporting evidence**

**Table 1:** List of newly proposed virus species in the family *Secoviridae* with their names, genus and NCBI accession numbers.

Virus name Virus species Genus GenBank acc. No.

Turnip ringspot virus *Comovirus rapae* *Comovirus* RNA1 GQ222381

RNA2 GQ222382

Stenotaphrum nepovirus *Nepovirus stenotaphri Nepovirus* RNA1 MZ325761

RNA2 MZ325762

anemone nepovirus A *Nepovirus anemones Nepovirus* RNA1 MH898479

RNA2 MH898478

pineapple secovirus B *Sadwavirus betananas Sadwavirus* RNA1 OM777135

RNA2 OM777136

Surrounding non-legume associated secovirus *Sadwavirus aciphyllae Sadwavirus* RNA1 MN412739

RNA2 MN412740

Cassava torrado-like virus *Torradovirus manihotis Torradovirus* RNA1 OK040225

RNA2 OK040226

Codonopsis torradovirus A *Torradovirus codonopsis Torradovirus* RNA1 MZ325520 RNA2 MZ325521

Camellia virus A *Waikavirus camelliae Waikavirus* MW545173



**Figure 1.** Genome organization of representative members of the nine genera (*Comovirus*, *Fabavirus*, *Nepovirus*, *Stralarivirus*, *Cheravirus*, *Sadwavirus*, *Torradovirus*, *Sequivirus*, *Waikavirus*) in the family *Secoviridae*. Each RNA is shown with open reading frames (ORFs) represented with boxes. Circles at the 5' end of viral genomic RNA depict viral genome-linked proteins (VPg). Black circles represent VPg experimentally confirmed and open circles represent putative VPgs. The poly(A) tails at the 3' end of viral genomic RNAs are depicted with (An), when appropriate. Protein domains with conserved motifs for the putative NTP-binding domain protein (Hel, green), VPg (peach), 3C-like proteinase (Pro, dark blue), RNA-dependent RNA polymerase (Pol, light blue), movement protein (MP, orange) and coat protein(s) (CPs, red) are shown. Proteinase cleavage sites identified experimentally or predicted by sequence comparisons are indicated by solid vertical lines. The three sub-genera of sadwaviruses are indicated. Virus acronyms are: CPMV: cowpea mosaic virus; BBWV2: broad bean wilt virus 2; ArMV: Arabis mosaic virus (subgroup A of the genus *Nepovirus*); TBRV: tomato black ring virus (subgroup B of the genus *Nepovirus*); ToRSV: tomato ringspot virus (subgroup C of the genus *Nepovirus*); SLRSV: strawberry latent ringspot virus; CLRV: cherry rasp leaf virus: SMoV: strawberry mottle virus; CLVA: chocolate lily virus A; SDV: satsuma dwarf virus: ToTV: tomato torradovirus; PYVF: parsnip yellow fleck virus; and RTSV: rice tungro spherical virus.

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**Figure 2.** Phylogenetic tree of the coat protein(s) amino acid sequence of the eight newly proposed species (depicted by a star) in the family *Secoviridae* and 90 representatives of the different genera in the family *Secoviridae.* In the cases that more than one CP domain is present, the two or three CP domains were combined. Alignments were performed by MUSCLE with default parameters implemented in MEGA X (Kumar et al. 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Le\_Gascuel\_2008 model (Le S.Q. and Gascuel O. (2008). The tree with the highest log likelihood (-52831.13) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (2 categories (+*G*, parameter = 1.3616)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 2.43% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 95 amino acid sequences. All positions with less than 90% site coverage were eliminated, i.e., fewer than 10% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). The final dataset consisted of a total of 412 positions. Sequence accession numbers are as follows: ToRSV (tomato ringspot virus, D12477), AnNVA (anemone nepovirus A, MH898478), SteNV (Stenotaphrum nepovirus, MZ325762), BRV (blackcurrant reversion virus, AF020051), GBLV (grapevine Bulgarian latent virus, FN691935), BLSV (blueberry latent spherical virus, AB649297), SLSV (soybean latent spherical virus, KX424572), PRMV (peach rosette mosaic virus, (KJ572573), CawYV (caraway yellow virus, MK492274), CLRV (cherry leaf roll virus, FR851462), GNVA (grapevine nepovirus A, MT507291), MMMoV (melon mild mottle virus, AB518486), RpRSV (raspberry ringspot virus, AY303788), PoLNVA (poaceae Liege nepovirus A, MW289236), MMLRaV (mulberry mosaic leaf roll-associated virus, KC904084), AeRSV (Aeonium ringspot virus, JQ670669), PBRSV (potato black ringspot virus, KC832892), TRSV (tobacco ringspot virus, AY363727), GDefV (grapevine deformation virus, AY291208), ArMV (Arabis mosaic virus, AY017339), GFLV (grapevine fanleaf virus, X16907), OLRSV (olive latent ringspot virus, AJ277435), PCMoV (petunia chlorotic mottle virus, KX812816), BRSV (beet ringspot virus, X04062), RCNA (red clover nepovirus A, MG253829), TBRV (tomato black ring virus, AY157994), AILV (artichoke Italian latent virus, LT608396), GARSV (grapevine Anatolian ringspot virus, AY291207), GCMV (grapevine chrome mosaic virus, X15163), CNSV (cycas necrotic stunt virus, AB073148), PVB (potato virus B, KX656671), GSPNeV (green Sichuan pepper nepovirus, MH323434), APMV (Andean potato mottle virus, L16239), CPSMV (cowpea severe mosaic virus, M83309), PvSMV (Phaseolus vulgaris severe mosaic virus, MN837499), BRMV (bean rugose mosaic virus, KP404603), BPMV (bean pod mosaic virus, U70866), TuRSV (turnip ringspot virus, GQ222382), CPMV (cowpea mosaic virus, X00729), ArLV1 (Arabidopsis latent virus 1, MH899121), RCMV (red clover mottle virus, M14913), BBTMV (broad bean true mosaic virus, GU810904), SqMV (squash mosaic virus, AB054689), PepMMV (pepper mild mosaic virus, MK990556), GFabV (grapevine fabavirus, KX241485), PrVF (prunus virus F, KX269871), CuMMV (cucurbit mild mosaic virus, EU881937), LMMV (lamium mild mosaic virus, KC595305), GeMV (gentian mosaic virus, AB084453), BBWV2 (broad bean wilt virus 2, AF225954), PLPaV (peach latent pitting-associated virus, KY867751), BBWV1 (broad bean wilt virus 1, AB084451), BRNV (black raspberry necrosis virus, DQ344640), SMoV (strawberry mottle virus, AJ311876), LSV1 (lettuce secovirus 1, KX925438), SDV (satsuma dwarf virus, AB009959), DMaV (dioscorea mosaic-associated virus, KU215539), SnLaSV (surrounding non-legume associated secovirus*,* MN412740), PSVA (pineapple secovirus A, MN809924), PSVB (pineapple secovirus B*,* OM777136), CLVA (chocolate lily virus A, JN052074), ALSV (apple latent spherical virus, AB030941), CuLV (currant latent virus, KT692953), CRLV (cherry leafroll virus, AJ621358), AVB (arracacha virus B, JQ581051), ToTV (tomato torrado virus, DQ388880), ToMarV (tomato marchitez virus, EF681765), MYMoV (motherwort yellow mottle virus, KM229701), CoTVA (Codonopsis torradovirus A*,* NC035220), CsTLV (cassava torrado-like virus, OK040226), LNLCV (lettuce necrotic leaf curl virus, KC855267), CaTV1 (carrot torradovirus 1, KF533720), SCLSV (squash chlorotic leaf spot virus, KU052531), LSMV (lettuce star mosaic virus, MT348706), PYFV (parsnip yellow fleck virus, D14066), AcYV1 (Actinidia yellowing virus 1, MN180070), PWaiV (persimmon waikavirus, LC488189), CamVA (camlelia virus A*,* MW545173), PolV1 (poaceae Liege virus 1, MW289237), BCWVA (blackcurrant waikavirus A, MN701059), BnRV1 (brassica napus RNA virus 1, MH844554), RCaV1 (red clover-associated virus 1, MH325329), CNDV (carrot necrotic dieback virus, EU980442), MCDV (maize chlorotic dwarf virus, U67839), RTSV (rice tungro spherical virus, M95497), BVCV (bellflower vein chlorosis virus, KT238881), LycMoV (lychnis mottle virus, KR011033), and SLRSV (strawberry latent ringspot virus, AY860979). The combined sequence of the three CPs from poliovirus (EVC, species *Enterovirus C*, NP\_041277, genus *Enterovirus*, family *Picornaviridae*) was used as an outgroup to root the tree.



**Figure 3.** Phylogenetic tree of the amino acid sequence of the conserved protease-polymerase (Pro-Pol) region (from the protease CG motif to the polymerase GDD motif) of the eight newly proposed species (depicted by a star) in the family *Secoviridae* and 87 representatives of the different genera in the family *Secoviridae.* Alignments were performed by MUSCLE with default parameters implemented in MEGA X (Kumar et al. 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Le\_Gascuel\_2008 model (Le and Gascuel, 2008). The tree with the highest log likelihood (-83836.29) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (3 categories (+*G*, parameter = 3.3665)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 99 amino acid sequences. All positions with less than 90% site coverage were eliminated, i.e., fewer than 10% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). The final dataset consisted of a total of 452 positions. Sequence accession numbers are as follows: ToRSV (tomato ringspot virus, L19655), AnNVA (anemone nepovirus A*,* MH898479), SteNV (Stenotaphrum nepovirus*,* MZ325761), BRV (blackcurrant reversion virus, AF368272), GBLV (grapevine Bulgarian latent virus, FN691934), CawYV (caraway yellow virus, MK494273), BLSV (blueberry latent spherical virus, AB649296), SLSV (soybean latent spherical virus, KX424571), PRMV (peach rosette mosaic virus, AF016626), CLRV (cherry leaf roll virus, FR851461), AYRSV (artichoke yellow ringspot virus, AM087671), GNVA (grapevine nepovirus A, MT507290), MMMoV (melon mild mottle virus, AB518485), RpRSV (raspberry ringspot virus, AY303787), PoLNVA (poaceae Liege nepovirus A, MW289235), MMLRaV (mulberry mosaic leaf roll-associated virus, KC904083), AeRSV (Aeonium ringspot virus, JX304792), PBRSV (potato black ringspot virus, KC832890), TRSV (tobacco ringspot virus, U50869), GDefF (grapevine deformation virus, [HE613269](https://www.ncbi.nlm.nih.gov/nuccore/HE613269)), ArMV (Arabis mosaic virus, AY303786), GFLV (grapevine fanleaf virus, [D00915](https://www.ncbi.nlm.nih.gov/nuccore/D00915)), PCMoV (petunia chlorotic mottle virus, KX812815), BRSV (beet ringspot virus, [D00322](https://www.ncbi.nlm.nih.gov/nuccore/D00322)), RCNA (red clover nepovirus A, MG253828), TBRV (tomato black ring virus, AY157993), AILV (artichoke Italian latent virus, LT608395), GARSV (grapevine Anatolian ringspot virus, [HE774604](https://www.ncbi.nlm.nih.gov/nuccore/HE774604)), GCMV (grapevine chrome mosaic virus, [X15346](https://www.ncbi.nlm.nih.gov/nuccore/X15346)), CNSV (cycas necrotic stunt virus, AB073147), PVB (potato virus B, [KX656670](https://www.ncbi.nlm.nih.gov/nuccore/KX656670)), GSPNeV (green Sichuan pepper nepovirus, MH323435), APMV (Andean potato mottle virus, MN148891), TuRSV (turnip ringspot virus, GQ222381), CPSMV (cowpea severe mosaic virus, M83830), PvSMV (phaseolus vulgaris severe mosaic virus, MN837498), BRMV (bean rugose mosaic virus, KP404602), BPMV (bean pod mosaic virus, U70866), CPMV (cowpea mosaic virus, [X00206](https://www.ncbi.nlm.nih.gov/nuccore/X00206)), ArLV1 (Arabidopsis latent virus 1, MH899120), RCMV (red clover mottle virus, X64886), BBTMV (broad bean true mosaic virus, GU810903), SqMV (squash mosaic virus, AB054688), PepMMV (pepper mild mosaic virus, MK990555), GFabV (grapevine fabavirus, KX241484), PcSMV (phaseolus vulgaris severe mosaic virus, MN837498), PrVF (prunus virus F, KX269870), CuMMV (cucurbit mild mosaic virus, EU881936), LMMV (lamium mild mosaic virus, KC595304), GeMV (gentian mosaic virus, AB084452), BBWV2 (broad bean wilt virus 2, AF225953), PLPaV (peach latent pitting-associated virus, KY867750), BBWV1 (broad bean wilt virus 1, AB084450), StPV (stocky prune virus, DQ143874), BRNV (black raspberry necrosis virus, DQ344639), SMoV (strawberry mottle virus, AJ311875), LSV1 (lettuce secovirus 1, KX925437), SDV (satsuma dwarf virus, AB009958), DMaV (dioscorea mosaic-associated virus, KU215538), PSVA (pineapple secovirus A, MN809923), PSVB (pineapple secovirus B*,* OM777135), SnLaSV (surrounding non-legume associated secovirus*,* MN412739), CLVA (chocolate lily virus A, JN052073), ALSV (apple latent spherical virus, AB030940), CuLV (currant latent virus, KT692952), CRLV (cherry rasp leaf virus, AJ621357), AVB (arracacha virus B, JQ437415), ToTV (tomato torrado virus, DQ388879), ToMarV (tomato marchitez virus, EF681764), MYMoV (motherwort yellow mottle virus, KM229700), CoTVA (Codonopsis torradovirus, NC035128), CsTLV (cassava torrado-like virus*,* OK040225), LNLCV (lettuce necrotic leaf curl virus, KC855266), CaTV1 (carrot torradovirus 1, KF533719), SCLSV (squash chlorotic leaf spot virus, KU052530), LSMV (lettuce star mosaic virus, MT348706), PYFV (parsnip yellow fleck virus, D14066), AcYV1 (Actinidia yellowing virus 1, MN180070), PWaiV (persimmon waikavirus, LC488189), CamVA (camellia virus A*,* MW545173), PolV1 (poaceae Liege virus 1, MW289237), BCWVA (blackcurrant waikavirus A, MN701059), BnRV1 (brassica napus RNA virus 1, MH844554), RCaV1 (red clover-associated virus 1, MH325329), CNDV (carrot necrotic dieback virus, EU980442), MCDV (maize chlorotic dwarf virus, U67839), RTSV (rice tungro spherical virus, M95497), BVCV (bellflower vein chlorosis virus, KT238881), LycMoV (lychnis mottle virus, KR011032), and SLRSV (strawberry latent ringspot virus, AY860978). The Pro-Pol sequence of poliovirus (EVC, species *Enterovirus C*, NP\_041277, genus *Enterovirus*, family *Picornaviridae*) was used as an outgroup to root the tree.

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