MODULE 1: **TITLE, AUTHORS, etc**

| Code assigned: | *2016.004a-cP* | (to be completed by ICTV officers) |
|---|---|---|

**Short title:**
One new species in the genus *Torradovirus*
One new species in the genus *Waikavirus*
One new unassigned species in the family *Secoviridae*

| **Modules attached** (modules 1 and 10 are required) | 1 ☒  2 ☒  3 ☐  4 ☐  5 ☐ 6 ☐  7 ☐  8 ☐  9 ☐  10 ☒ |
|---|---|

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| | |
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**List the ICTV study group(s) that have seen this proposal:**

| A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses) | Secoviridae |
|---|---|

**ICTV Study Group comments (if any) and response of the proposer:**



| Date first submitted to ICTV: | July 2016 |
|---|---|
| Date of this revision (if different to above): | |

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

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

| Code | *2016.004aP* | (assigned by ICTV officers) |
|------|--------------|------------------------------|

**To create 1 new species within:**

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

| | |
|---|---|
| Genus: | *Torradovirus* |
| Subfamily: | **-** |
| Family: | *Secoviridae* |
| Order: | *Picornavirales* |

| Name of new species: | Representative isolate: (only 1 per species please) | GenBank sequence accession number(s) |
|----------------------|------------------------------------------------------|---------------------------------------|
| *Squash chlorotic leaf spot virus* | squash chlorotic leaf spot virus (SCLSV) isolate Su12-10 | (RNA1) KU052530 (RNA2) KU052531 |

| Code | **2016.004bP** | | (assigned by ICTV officers) |
|------|----------------|--|-----------------------------|

**To create 1 new species within:**

| | | |
|--|--|--|
| Genus: | *Waikavirus* | |
| Subfamily: | **-** | |
| Family: | *Secoviridae* | |
| Order: | *Picornavirales* | |

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

| Name of new species: | Representative isolate: (only 1 per species please) | GenBank sequence accession number(s) |
|----------------------|-----------------------------------------------------|--------------------------------------|
| *Bellflower vein chlorosis virus* | bellflower vein chlorosis virus (BVCV) isolate CT1 | KT238881 |

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| Code | **2016.004cP** | | (assigned by ICTV officers) |
|------|----------------|--|-----------------------------|

**To create 1 new species within:**

| | | |
|--|--|--|
| Genus: | *unassigned* | |
| Subfamily: | **-** | |
| Family: | *Secoviridae* | |
| Order: | *Picornavirales* | |

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

| Name of new species: | Representative isolate: (only 1 per species please) | GenBank sequence accession number(s) |
|----------------------|-----------------------------------------------------|--------------------------------------|
| *Chocolate lily virus A* | Chocolate lily virus A (CLVA) isolate KP2 | (RNA1) JN052073 (RNA2) JN052074 |

**Reasons to justify the creation and assignment of the new species:**
- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Defined species demarcation:

1) CP aa sequence with less than 75% identity
2) Conserved Pro-Pol region aa sequences with less than 80% identity
3) Differences in antigenic reactions
4) Distinct vector specificity
5) Absence of cross-protection
6) For viruses with a bipartite genome, absence of re-assortment between RNA1 and RNA2

The criteria that have been met for each proposed virus are shown in table 1:

| virus | Acronym | isolate | Acc. no | Proposed allocation | 1. CP (<70%)[a] | 2. Pro-Pol (<80%)[a] | 3. Antigenic reaction | 4. Host range | 5. Transmission | 6. Cross-protection | 7. Reassortment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bellflower vein chlorosis virus | BVCV | CT1 | KT238881 | New species (Waika) | 44 | 62 | - | - | - | - | nt |
| Chocolate lily virus A | CLVA | KP2 | JN052073 JN052074 | New species | 22 | 46 | - | - | - | - | - |
| Squash chlorotic leaf spot virus | SCLSV | Su12-10 | KU052530 KU052531 | New species (Torrado) | 46 | 56 | - | Cucurbits | Mechanical-whitefly | - | - |

Table1. Listed species demarcation criteria for the Secoviridae with regard to the three new proposed species.

Details for each virus and criterion are listed below.

**BVCV**
- 1) CP closest hit = 44% (40% coverage) to *Rice tungro spherical virus* (RTSV) (Acc. Q91PP5)

- 2) Pro-Pol closest hit = 62% (100% coverage) to RTSV (Acc. CAA67042)

Note – All information NGS derived + RACE. Complete sequence. Clear symptoms associated with infection in Bellflowers (*Campanula* spp.)(Seo et al., 2015)

**CLVA**
- 1) CP closest hit = 22% (77% coverage) to *Black raspberry necrosis virus* (BRNV) (Acc. CCE76876)

- 2) Pro-Pol closest hit = 46% (100% coverage) to *Strawberry mottle virus* (SMoV) (Acc. AMS36886)

Note – All information NGS derived (no RACE), sequence is near-complete and coding-complete (Wylie et al., 2012)

**SCLSV**
- 1) CP closest hit = 56% (100% coverage) to *Carrot torradovirus 1* (CTV1) (YP_009104369)

- 2) Pro-Pol closest hit = 46% (70% coverage) to cassava Torrado-like virus (acc. AHA91818) and 38% (98% coverage) to *Lettuce necrotic leaf curl virus* (acc. AGR55592)

- 4) Cucurbits species *Cucurbita pepo* [zucchini squash], *Cucumis melo* [melon], *Cucumis sativus* [cucumber] and *Citrullus lanatus* [watermelon] and tobacco species *Nicotiana benthamiana* and *N. clevelandii* were all infected via mechanical inoculation. The latter two species were asymptomatic. All cucurbits were symptomatic. Eighteen other species were inoculated and found uninfected - *Brassica rapa*, *Capsella bursa-pastoris*, *Capsicum annuum*, *Chenopodium amaranticolor*, *C. quinoa*, *Lactuca sativa*, *Lavatera trimestris*, *N. glutinosa*, *N. tabacum* cv. Xanthi, *Petunia hybrida*, *Phaseolus vulgaris*, *Physalis floridana*, *Pisum sativum*, *Salvia splendens*, *Solanum lycopersicum*, *Valerianella officinalis*, *Vigna sinensis*

and *Zinnia elegans*.

- 5) Mechanically transmissible to squash (*Cucurbita moschata*). Confirmed vectors – greenhouse whitefly (*Trialeurodes vaporariorum*), silverleaf whitefly (*Bemisia tabaci*).

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    Note – All information NGS derived + RACE. Complete sequence of both RNAs (Lecoq et al., 2016)

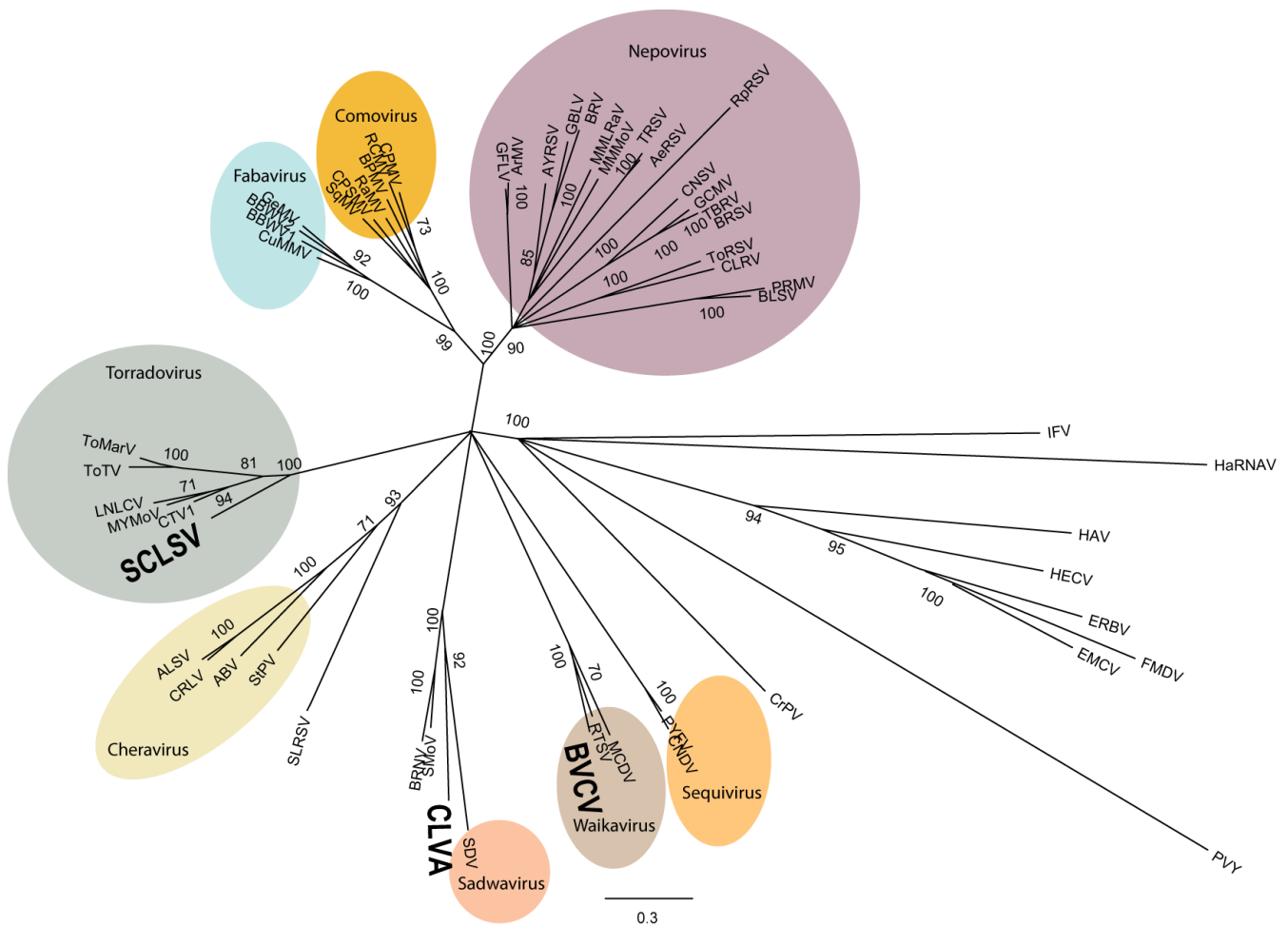Phylogenetically the three viruses are grouped as shown in Fig.1.



Fig. 1. Maximum likelihood (ML) inferred tree of the secovirids and related species using the Pro-Pol region. ML Method PhyML (Guindon et al., 2010), substitution model Blosum+G. Representative sequences were included for the family *Secoviridae* while only representative members of other families within the order *Picornavirales* were included.  Numbers on nodes show bootstrap values (1000 replicates) above 70%. The bar represents a genetic distance of 0.3.  All viruses grouped in the colored circles along with the unassigned SLRSV, BRNV and SMoV belong to the family *Secoviridae*. The GenBank accession numbers used for each virus are as follows: carrot torradovirus 1 (CaTV1, KF533719 = AHA85556), motherwort yellow mottle virus (MYMoV, KM229700 = AIT59085), apple latent spherical virus (ALSV, NC_003787 = AB030940), arabis mosaic virus (ArMV, NC_006057 = AY303786), arracacha virus B (AVB, JQ437415), artichoke yellow ringspot virus (AYRSV, AM087671), bean pod mottle virus (BPMV, NC_003496 = U70866), beet ringspot virus (BRSV, NC_003693 = D00322), black raspberry necrosis virus (BRNV, NC_008182 = DQ344639), blackcurrant reversion virus (BRV, NC_003509 = AF368272), broad bean wilt virus 1 (BBWV1, NC_005289 = AB084450), broad bean wilt virus 2 (BBWV2) (NC_003003 = AF225953), carrot necrotic dieback virus (CNDV,

EU980442), cherry leaf roll virus (CLRV, NC_015414 = FR851461), cherry rasp leaf virus (CRLV, NC_006271 = AJ621357), cowpea mosaic virus (CPMV, NC_003549 = X00206), cowpea severe mosaic virus (CPSMV, NC_003545 = M83830), cricket paralysis virus (CrPV, NC_003924 = AF218039), cucurbit mild mosaic virus (CuMMV, FJ194941), cycas necrosis stunt virus (CNSV, NC_003791 = AB073147), encephalomyocarditis virus (EMCV, NC_001479 = M81861), equine rhinitis B virus 1 (ERBV, NC_003983 = X96871), foot-and-mouth disease virus- type C (FMDV, NC_002554 = AF274010), gentian mosaic virus (GeMV, BAD99001), grapevine Bulgarian latent virus (GBLV, NC_015492 = FN691934), grapevine chrome mosaic virus (GCMV, NC 003622 = X15346), grapevine fanleaf virus (GFLV, NC_003615 = D00915), hepatitis A virus (HAV, NC_001489 = M14707), Heterosigma akashiwo RNA virus (HaRNAV, NC_005281 = AY337486), human enterovirus C (HECV, NC_002058.3 = V01149), infectious flacherie virus (IFV, NC_003781 = AB000906), lettuce necrotic leaf curl virus (LNLCV, KC8552566), maize chlorotic dwarf virus (MCDV, NC_003626 = U67839), melon mild mottle virus (MMMoV, AB518485), motherwort yellow mottle virus (MYMoV, KM229700), parsnip yellow fleck virus (PYFV, NC_003628 = D14066), peach rosette mosaic virus (PRMV, AF016626), radish mosaic virus (RaMV, NC_010709 = AB295643), raspberry ringspot virus (RpRSV, NC_005266 = AY303787), red clover mottle virus (RCMV, NC_003741 = X64886), rice tungro spherical virus (RTSV, NC_001632 = M95497), satsuma dwarf virus (SDV, NC_003785 = AB009958), squash mosaic virus (SqMV, NC_003799 = AB054688), stocky prune virus (StPV, DQ143874), strawberry latent ringspot virus (SLRSV, NC_006964 = AY860978), strawberry mottle virus (SMoV, NC_003445 = AJ311875), tobacco ringspot virus (TRSV, NC_005097 = U50869), tomato black ring virus (TBRV, NC_004439 = AY157993), tomato marchitez virus (ToMarV, NC_010987 = EF681764), tomato ringspot virus (ToRSV, NC_003840 = L19655), tomato torrado virus (ToTV, NC_009013 = DQ388879).

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59(3), 307-321.

Lecoq, H., Verdin, E., Tepfer, M., Wipf-Scheibel, C., Millot, P., Dafalla, G., Desbiez, C., 2016. Characterization and occurrence of squash chlorotic leaf spot virus, a tentative new torradovirus infecting cucurbits in Sudan. Arch Virol 161(6), 1651-1655.

Seo, J.K., Kwak, H.R., Lee, Y.J., Kim, J., Kim, M.K., Kim, C.S., Choi, H.S., 2015. Complete genome sequence of bellflower vein chlorosis virus, a novel putative member of the genus Waikavirus. Arch Virol 160(12), 3139-3142.

Wylie, S.J., Luo, H., Li, H., Jones, M.G., 2012. Multiple polyadenylated RNA viruses detected in pooled cultivated and wild plant samples. Arch Virol 157(2), 271-284.