This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections).

Code(s) assigned:	***2008.026P***	(to be completed by ICTV officers)

Short title: One new species in the genus Ophiovirus
(e.g. 6 new species in the genus *Zetavirus*; re-classification of the family *Zetaviridae* etc.)

Modules attached (please check all that apply):	**1** ☐ **2** ☐ **3** ☐ **4** ☐ **5** ☒ **6** ☐ **7** ☐

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ICTV-EC or Study Group comments and response of the proposer:

MODULE 5: **NEW SPECIES**

Code	***2008.026P***	(assigned by ICTV officers)

To create 1 new species assigned as follows:

Genus:	***Ophiovirus***
Subfamily:	-
Family:	***Ophioviridae***
Order:	-

Fill in all that apply. Ideally, species should be placed within a genus, but it is acceptable to propose a species that is within a Subfamily or Family but not assigned to an existing genus (in which case put "unassigned" in the genus box)

Name(s) of proposed new species:

Freesia sneak virus

Argument to justify the creation of the new species:
The genus *Ophiovirus* is currently comprised of five recognized species: *Citrus psorosis virus* (CPsV), *Ranunculus white mottle virus* (RWMV), *Tulip mild mottle mosaic virus* (TMMMV), *Mirafiori lettuce big-vein virus* (MLBVV) and *Lettuce ring necrosis virus* (LRNV), and one tentative species, provisionally named Freesia Ophiovirus (FOV). Species demarcation criteria for the Genus *Ophiovirus* are:

- Differing sizes of CP
- No or distant serological relationship between CPs of different species
- Natural host range
- Different number, organization and/or size of genome segments
- Despite being based on limited number of ophiovirus sequences available, % identity between intra-species CP amino acid sequences appears to be close to 100%, while

Argument to justify the creation of the new species:

between inter-species CP amino acid sequences ranges between 53-70%; there is about 80% identity between the C-terminal ends of TMMMV and MiLV CP, (where only partial sequence of TMMMV CP is available) that are already considered to be different species.

Morphology of **FreSV virus particles is indistinguishable from that characteristic of other Ophiovirus particles** [5]**,** being naked filamentous nucleocapsids about 3 nm in diameter forming circularized structures of different lengths (fig. 1)

OP1 and OP2 primers are genus-specific primers. Amplification of a 136-bp fragment of RNA 1 by RT-PCR and OP-1/OP-2 primers is obtained with all five ophiovirus species but not with species in other genera. **Total RNA extracted from FreSV-infected freesia was used as template for an RT-PCR and the expected 136 bp fragment was easily amplified**. A small DIG-labelled DNA probe was obtained by PCR using the 136 bp fragment amplified from infected freesia. This successfully hybridized in southern blots with the 136 bp fragments amplified from other ophioviruses, proving sequence similarity [5] (fig. 2). Despite the small size of the amplified fragment, alignment and phylogenetic study results based on its derived amino acid sequences entirely correlate with actual taxonomy in the Genus. FreSV therefore deserves recognition as a new species (fig. 3).

FreSV has been reported to infect two winter ornamental bulb plants, Freesia sp. (*Iridaceae*) and Lachenalia sp. (*Hyacinthaceae*) in Europe and South Africa, respectively [4, 5, 6, 8]. Neither genus, both of South African origin, was reported to be infected by other Ophiovirus species.

Natural transmission has been established for TMMMV, MLBVV and LRNV which are soil-borne, involving zoospores of the fungus *Olpidium brassicae*. **FreSV was demonstrated to be soil-transmitted** [5], although involvement of *O. brassicae* has not been studied.

FreSV CP protein (Freesia isolate) sequence alignments using EMBOSS-Align (EMBL-EBI website with default parameters) show **48.8% identity** (70.5% similarity) with MLBVV CP, **49% identity** (68.5% similarity) with LRNV CP and **30.6% identity** (53.9% similarity) with CPsV CP [3]. FreSV CP protein **identity between the Lachenalia isolate and the Freesia isolate is 97%** (98% similarity). Phylogenetic analysis of coat protein amino acid sequences comparisons **undoubtedly show FreSV isolates from Lachenalia and Freesia in a monophyletic branch,** supporting our proposal that FreSV deserves recognition as a new species [7] (Fig. 4)

The complete genome sequencing of a FreSV isolate from Lachenalia is in progress: 3202nt of RNA1, the complete sequence of RNA2 and 1382 nt of RNA3 (DQ885455) are known [3]. **FreSV genome organization is the same as that of the recognized Ophiovirus species; to date presence of an RNA4 has not been shown** using degenerate primer pairs.

The name proposed for the species was chosen owing to the virus ability to infect both bulbous host plants without easy detection and because an initial correlation between necrotic or other kinds of symptoms in both freesia and lachenalia and FreSV infection has not been validated in further study [1, 2, 3].

For the reasons listed above, we would like to propose renaming of the previously tentative FOV ophiovirus species, as a new official species under the name *Freesia sneak virus*.

References:

1. B Brandwagt, C van Eijk & S Heimovaara. Freesia leaf necrosis, the mystery remains. 12th ISVDOP, Haarlem, The Netherlands, April 20-24 2008.
2. E Meekes & M Verbeek. New insights in Freesia leaf necrosis disease. 12th ISVDOP, Haarlem, The Netherlands, April 20-24 2008.
3. AM Vaira & J Hammond. An update on Freesia sneak virus, a new species in the

References:

Ophiovirus genus. 12th ISVDOP, Haarlem, The Netherlands, April 20-24 2008.
4. AM Vaira, V Torok , GP Accotto, S Rapetti, M Vecchiati, V Masenga, HJ Vetten & RGMilne. Leaf necrosis in freesia: a new ophiovirus involved. Sixth IWGPVFV, Bologna, Italy, September 5-7 2005.
5. AM Vaira, V Lisa, A Costantini, V Masenga, S Rapetti & RG Milne. 2006. Ophioviruses infecting ornamentals and a probable new species associated with a severe disease in freesia. Acta Hort. 722:191-199.
6. AM Vaira, R Kleynhans, J Hammond. 2007. First report of Freesia sneak virus infecting Lachenalia cultivars in South Africa. Plant Dis 91, 6, p.770
7. AM Vaira & RG Milne. Ophiovirus. Encyclopedia of Virology. Third Edition. In press
8. M Verbeek, J Lindner, I Bowen, A Dullemans & R van der Vlugt. An Ophiovirus isolated from freesia with freesia leaf necrosis disease. 11th ISVDOP, Taichung, Taiwan. March 09-14, 2004.

Annexes:

Fig.1. EM image of Ophiovirus-like particles in crude freesia sap, 1% uranyl acetate (courtesy of R.G. Milne).

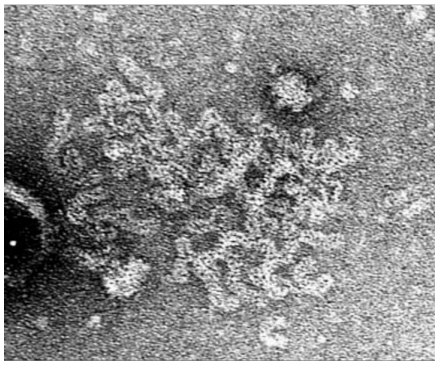

Fig. 2 upper part: amplification of the 136 bp fragment from RNA1 using genus-specific primers by RT-PCR. Most of the species are represented. m = mw marker.
Middle part: southern blot, probe FreSV, low stringency.
Lower part: the same but high stringency.

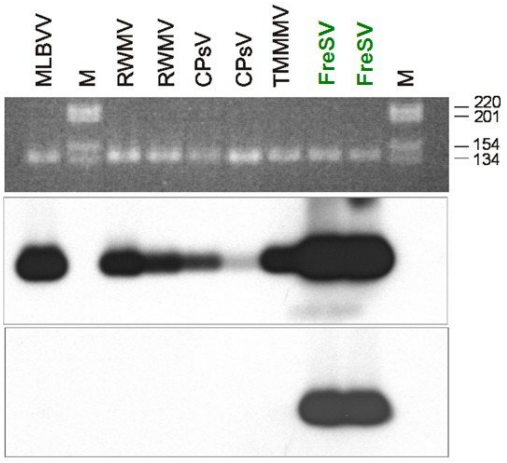

Fig. 3 Unrooted Phylogenetic tree based on predicted aa sequences encoded by the conserved 136 bp fragment from RdRP gene. (Present in 8th ICTV Report)

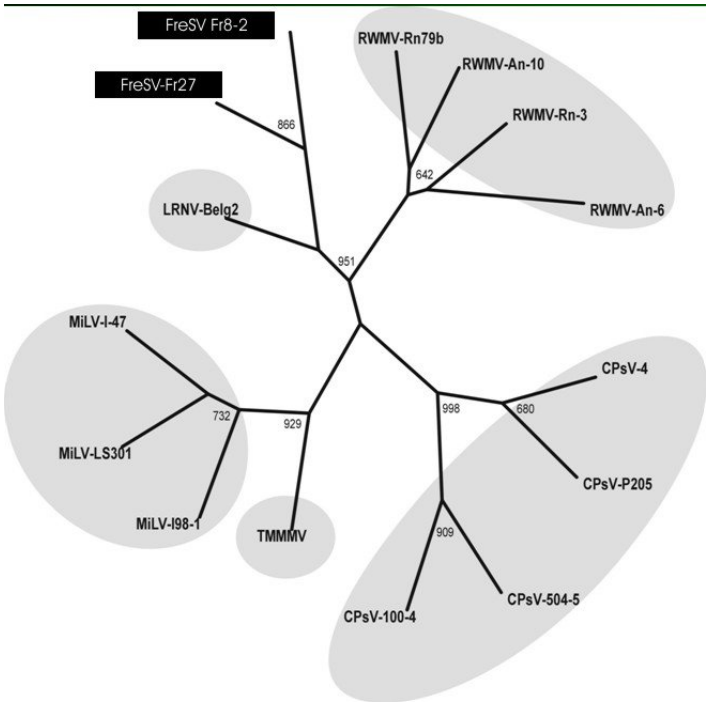

Fig.4 Phylogenetic tree (ClustalW – Megalign, DNASTAR) based on Ophiovirus CP aa sequences alignment.