This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

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

Code assigned:	*2014.004aV*	(to be completed by ICTV officers)

Short title: create species *Iris yellow spot virus* in the genus *Tospovirus,* family *Bunyaviridae*
(e.g. 6 new species in the genus *Zetavirus*)

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

Author(s) with e-mail address(es) of the proposer:

Hanu R Pappu (hrp@wsu.edu) and Sudeep Bag (sudeepbag@wsu.edu)

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)	**Bunyaviridae Study Group**

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

EC comments: This proposal was conditionally approved. The tree should be modified to provide a phylogram built with neighbor joining or maximum likelihood methods with branches representing phylogenetic distance. The current cladogram is not acceptable.

Author response: Modified as suggested.

Date first submitted to ICTV: June 26, 2013
Date of this revision (if different to above): July 6, 2013

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	*2014.004aV*	(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:	*Tospovirus*
Subfamily:	
Family:	*Bunyaviridae*
Order:	*Unassigned*

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Iris yellow spot virus	FJ623474 FJ361359 AF001387

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

Taxonomic position of iris yellow spot virus

Genus *Tospovirus* characteristics:
- (Virus particle morphology):
  - Quasi-spherical morphology: 80-120 nm [11-12]
  - Glycoprotein envelope [3-4]

- Genome organization: [1]
  - Large (L) RNA is in negative sense [2]
  - Medium (M) RNA and Small (S) RNA are in ambisense gene arrangement [3-5]

- Thrips transmission: circulative and propagative manner. Transmitted by
  - *Thrips tabaci* [12,14]
  - *Frankliniella fusca* [18]

- Tospovirus species demarcation criteria (according to the Ninth Report of the ICTV):
  - Nucleoprotein (N-protein) amino acid sequence homologies with known tospoviruses should be less than 90%
  - Species are also defined based on host range and vector specificity

- Characteristics of IYSV compared to other tospoviruses
  - The N protein of iris yellow spot virus has less than 80% aa sequence identity with the most related tospovirus (see Annex and Table 1)
  - Exclusively transmitted by *Thrips tabaci* in circulative and propagative manner under natural conditions. Under greenhouse conditions, also transmitted by *Frankliniella fusca.*
  - IYSV is mainly confined to monocots whereas other tospoviruses cause diseases on both dicots and monocots.

The proposal for creation of a new species is supported by a phylogenetic tree based on the N-protein (Fig. 2)

MODULE 9: **<u>APPENDIX</u>**: supporting material

additional material in support of this proposal is included as Annex I

References:

Included in Annex I

Annex I:
Includes information necessary to support the proposal.

Iris yellow spot virus, a new species in the genus *Tospovirus*, family *Bunyaviridae*:
A proposal to ICTV

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Background

Iris yellow spot virus (IYSV) causes economic losses in a wide variety of

monocotyledonous plants, particularly onion seed and bulb crops in many parts of the world

[8,13, 15]. The virus is most damaging to onion crops where it can reduce the bulb size [9] and

may cause crop loss of up to 100% [16]. First reported in onion stalk from Brazil in 1981 [6]

referred to as "sapeca", the disease is characterized by symptoms of a chlorotic and necrotic eye

with green island at the center, known as the diamond eye [8]. Similar symptoms were observed

in onion growing regions of the Treasure Valley of Idaho and Oregon in the USA in 1989 [11],

and were referred to as "straw bleaching" [8]. Cortez et al., [5] described IYSV in the

Netherlands as a new tospovirus infecting iris (*Iris hollandica*) and named it Iris yellow spot

virus.

Genome Organization

The complete genome of the virus was sequenced. Like other tospoviruses of the genus,

IYSV consists of three segmented RNAs, namely large (L), medium (M) and small (S) single

stranded RNA molecules. The RNAs were either negative (L) or ambisense (M & S) coding orientation [1].

L RNA is 8,880 nucleotides (nt) long in length and contains a single open reading frame of 8,621 nt in the viral complementary (vc) strand. The ORF codes for a protein of 2,873 aminoacids with a predicted molecular mass of 331.17 kDa and shares many of the features of the viral RNA-dependent RNA polymerase (RdRp) coded by L RNAs of known tospoviruses. The 5' and 3' termini of IYSV L RNA (vc) contain two untranslated regions of 33 and 226 nucleotides, respectively, and both termini have conserved terminal nucleotides, as common feature of tospovirus genomic RNAs [2].

The M RNA is 4,821 nucleotides long, with two ORFs in an ambisense arrangement. The smaller ORF of 935 nucleotides was located at the 5' end of the v-sense strand, potentially encoding a 311 amino-acid protein with a predicted molecular mass of 34.7 kDa, potentially a non-structural movement protein. The second ORF, the glycoprotein precursor (Gn/Gc), is in the vc-sense, was 3,410 nt in length potentially coding for 1,136 amino acid protein of 128.84 kDa. The two open reading frames are separated by a 380 nucleotide intergenic region [3-4].

The S RNA segment of IYSV is 3,105 nucleotides, codes two non-overlapping ORF in ambisense arrangement. The ORF in viral sense is 1,329 nt long coding for a potential 50.1 kDa non-structural protein (NSs), and the second ORF in vc-sense 816 nt long and potentially codes for a nucleoprotein of 30.5 kDa [5].

All three RNAs share some similar characteristics of other tospoviruses: the first eight nucleotides of the terminal sequences are identical at the 5' and 3' terminal of all the segments of RNA; the RNA segments have complementary ends in all the cases, forming stable panhandle

5

termini; and both coding ORFs of S and M RNAs are preceded by non-coding leader sequence and separated by intergenic regions.

Transmission

Under natural conditions, the virus is transmitted from plant-to-plant exclusively by thrips (Thysanoptera:Thripidae). Onion thrips (*Thrips tabaci* L.) is the confirmed vector for IYSV [5; 14] in natural field conditions and tobacco thrips (*Frankliniella fusca* (Hinds)) can also transmit IYSV to a limited extent under greenhouse experimental conditions [18].

Conclusions

IYSV has been reported from various weeds under natural conditions but mainly confined to the allium species, and it is continuously being reported from different allium growing regions, against other tospoviruses reported from various vegetable and oilseeds crops of economic importance. IYSV is mainly confined to monocots whereas other tospoviruses cause diseases on both dicots and monocots. Comparisons of N protein amino acid sequence of IYSV with other tospoviruses revealed that the IYSV is most closely related to TYRV with 72.9% identity (Table 1, Figure 1). It has been proposed that N protein sequence showing less than 90% identity with any other virus within the genus should be considered as a distinct species [10]. Based on the biological and molecular characterization we propose IYSV as a new *Tospovirus* species.

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Table 1: List of currently accepted and tentative tospovirus species (http://ictvonline.org/virusTaxonomy.asp?bhcp=1) and the GenBank accessions numbers used for comparisons.

No.	Species (Recognized-2013)	Abbreviation	S RNA	M RNA	L RNA
1	*Groundnut bud necrosis virus*	GBNV	U27809	U42555	AF025538
2	*Groundnut ringspot virus*	GRSV	AF251271(N) JN571117(NSs)	AF513220 (NSm) AY574055 (Gn/Gc)	-
3	*Groundnut yellow spot virus*	GYSV	AF013994	-	-
4	*Impatiens necrotic spot virus*	INSV	X66972	M74904	X93218
5	*Polygonum ringspot virus*	PolRSV	EF445397	EU271753	KJ541746
6	*Tomato chlorotic spot virus*	TCSV	JX244198	AF213674(NSm) AY574054(Gn/Gc)	HQ700667
7	*Tomato spotted wilt virus*	TSWV	AF020659	AF208497	AB190813
8	*Watermelon silver mottle virus*	WSMoV	U78734	U75379	AF133128
9	*Zucchini lethal chlorosis virus*	ZLCV	AF067069	AF213676 (NSm) AB274027 (Gn/Gc)	JN572104

No.	Species (Tentative-2013)	Abbreviation	S RNA	M RNA	L RNA
10	Alstroemeria necrotic streak virus	ANSV	GQ478668	-	-
11	Bean necrotic mosaic virus	BeNMV	JN587268	JN587269	JF417980
12	Callalily chlorotic spot virus	CCSV	AY867502	FJ822961	FJ822962
13	Capsicum chlorosis virus	CaCV	DQ355974	DQ256125	DQ256124
14	Chrysanthemum stem necrosis virus	CSNV	AB600873	AF213675(NSm) AB274026 (Gn/Gc)	-
15	Groundnut ringspot virus-USA	GRSV-USA	HQ644140	HQ644141	HQ644142
16	Gloxinia tospovirus	GlaxRSV	AF059578	AF023172	-
17	Hippeastrum chlorotic ringspot virus	HCRV	JX833564	JX833565	-
18	Iris yellow spot virus	IYSV	AF001387	AF214014	FJ623474
19	Lisianthus necrotic ringspot virus	LNRV	AB852525	-	-
20	Melon yellow spot virus	MYSV	FJ386391	AB061773	AB061774
21	Melon severe mosaic virus	MSMV	EU275149	FJ157984	FJ157985
22	Pepper necrotic spot virus	PNSV	HE584762	-	-
23	Peanut chlorotic fan-spot virus	PCFV	AF080526	-	-
24	Physalis severe mottle virus	PhySMV	AF067151	-	-
25	Soybean vain necrosis virus	SVNV	GU722319	HQ728386	HQ728385
26	Tomato necrosis virus	TNeV	AY647437	-	-
27	Tomato necrotic ringspot virus	TNRV	FJ489600	FJ947152	-
28	Tomato yellow fruit ring virus	TYFRV/TYRV	DQ462163	JN560177	JN560178
29	Tomato zonate spot virus	TZSV	EF552433	EF552434	EF552435
30	Watermelon bud necrosis virus	WBNV	GU584184	GU584185	GU735408

Table 2: Similarity identity matrix among known tospoviruses, using the nucleocapsid (N) gene. The nucleotide and deduced amino acid sequences were obtained from the GenBank. Virus abbreviations were listed in Table 1. Above the diagonal is the identity in amino acid sequences and below the diagonal line is the identity based on nucleotide sequences. Percent identity of Iris yellow spot virus nucleocapsid protein amino acid sequence with those of known tospoviruses ranged from 16.4 % (PCFV) to 72.9% (TYRV), whereas at nucleotide level it is 32.6 % (GYSV) to 74.3% (TNRV) identical.

| Nt \AA	GBNV	INSV	GYSV	TCSV	TSWV	WSMoV	ZLCV	ANSV	BeNMV	CCSV	CaCV	CSNV	GRSV	GlaxRSV	HCRV	IYSV	LNRV	MYSV	MSMV	PNSV	PCFV	PhySMV	PolRSV	SVNV	TneV	TNRV	TYRV	TZSV	WBNV
GBNV	100	26.7	17	26.3	26.6	85.8	26.6	26.6	27.9	64.5	83.3	26.6	27.4	82.7	42.1	43.2	17.8	59.1	29.5	26.6	19.5	59.1	42.1	31.1	84.7	56.3	43.2	62.3	84.7
INSV	39.3	100	17	53	53.4	27.5	51.8	53.8	32.9	22.5	27.9	54.1	54.5	27	24.4	24.8	17	23.7	50.7	53.4	15.5	23.7	25.5	30.4	27.2	23	26.5	21.7	25.7
GYSV	35.5	35.1	100	16.4	15.7	17.5	15.9	16.4	14.9	15.3	17.5	18.2	17.2	18.7	16.1	17.2	13.2	17.3	19.5	17.6	60	17.3	17.5	16	19.6	15.1	15.7	15.2	16.4
TCSV	40.9	57.7	35.6	100	78.2	26.4	75	81.3	32.9	23.1	27.5	73	87.9	26.9	27.2	28.2	20.5	25.4	58.7	84.1	16	25.4	29.6	32.8	27.8	25.4	29.6	23.4	26
TSWV	42.1	58.2	36.7	83.1	100	27.5	73.4	78.6	32.9	23.4	28.9	74.6	78.6	28	27.9	30	20.1	24	55.3	80.6	15	24	27.5	31.8	28.9	27.5	28.6	24.4	26.7
WSMoV	79.6	40.7	34.7	40.1	41.5	100	26.4	26.7	27.5	62.9	86.5	26.7	28.2	84.1	42.1	41.4	18.2	57.3	28.8	27.1	18.4	57.7	41.7	29	85.4	54.8	42.1	61.5	83.6
ZLCV	40.7	57.3	35.9	73.8	75	40.6	100	75.7	35.5	23.8	26	79.2	76.5	26.5	28.6	27.2	17.9	25	59.1	77.6	15.7	25	28.9	33.5	26.4	24.3	28.2	22.3	25.7
ANSV	39.4	56.7	36	77.6	77.6	40	74.9	100	34	24.9	26	73.4	82.1	26.9	28.6	27.9	19	24.3	58.7	87.5	16	24.3	30.3	33.2	26.4	24.3	29.3	23.4	26
BeNMV	40.5	41.4	31.8	43.4	43.2	42.2	42.7	43.2	100	28	26.8	35.8	32.6	26.3	30.3	28.2	20.8	27.8	33.6	34.7	16.1	28.1	29.6	52.1	27.2	29.5	27.9	27.2	27.9
CCSV	63.2	37.2	30.6	38.7	39.6	65.1	38.8	39.4	39.7	100	64	23.8	24.1	62.9	42.7	45.9	17.4	56.9	25.1	24.5	16.9	56.9	43.4	28.8	62.5	55.8	44.8	80.5	64.7
CaCV	77.6	39.6	35.8	41	41.6	80.6	41.5	38.4	40.2	64.4	100	26.4	28.2	91.3	42.5	44.6	17.5	58.4	27.7	27.5	18.4	58.7	41.7	29.7	92.3	56.5	43.5	62.2	81
CSNV	40.5	57	36.8	72.9	73	41.5	77.1	75.4	44.3	39.2	40.6	100	73.4	26.5	28.2	28.2	22.6	27.9	59.9	74.6	17.5	27.9	28.6	32.1	27.1	25.4	28.2	24.1	26.4
GRSV-USA	42.1	58.3	36.5	83.3	98.1	41.9	74.8	77.2	42.9	40.3	42	72.7	100	27.6	28.2	28.6	18.7	25	58.3	86	16.4	25	29.6	32.5	27.8	25.4	30.3	24.4	26.7
GlaxRSV	29.2	26.6	23.9	28.2	28.4	28.3	28.5	27.2	28.4	28.4	27.4	26.5	28.5	100	42.1	43.2	17.4	57.2	27.5	26.9	20.4	57.6	41.8	28.8	90.6	55.8	43.2	61.5	78.3
HCRV	53.6	40.9	31.9	42.9	42.5	53.7	42.1	41.1	41.7	50.4	54.3	42	42.5	29.6	100	69.7	23.3	46.6	28.9	28.6	17.2	46.6	86.1	31.4	43.2	44.4	84.6	40.9	41.4
IYSV	51.8	38.4	32.6	41	41.6	53.6	39.7	41	41.7	52.3	54.3	41.5	41.6	27.9	71.3	100	19.7	48	28.2	27.9	16.4	48	69.7	30.4	45.3	43	72.9	44.1	42.8
LNRV	31.3	29.9	27.7	31.5	31.4	31.3	31.9	31.4	27.5	29.5	31	32.8	31.9	25	28.1	28.6	100	18.5	19.7	19.4	12.8	19.2	19	20.3	17.8	17.9	19.7	18	18.2
MYSV	63.4	36.9	30.4	37.6	38.5	63.2	36.9	37.4	41	60.4	62.6	37.1	38.4	28.9	53.1	56.1	30.7	100	26.1	25.4	17.7	99.2	46.2	30	58.7	50	46.9	56.9	59.1
MSMV	43.3	58.7	36.4	61.8	61.9	42.7	62.6	61.7	43.9	40	42.4	64.1	62.1	26.8	42.5	39.5	32.1	39	100	61	17.5	26.1	27.5	32.5	27.7	25	27.9	24.3	28.4
PNSV	40.6	56.5	36.1	80.1	80.1	40.4	76.3	84.4	44.4	38	41.7	76.1	80	27.3	40.7	39.7	32.9	36.7	62.6	100	15.3	25.4	29.6	33.5	27.5	23.6	29.6	24.4	26.7
PCFV	36.4	36.2	60.8	35.1	35.3	36.7	33.4	35.9	34.3	34.8	36.7	35.2	35.7	28	33.5	34.5	29.1	35	35.9	35.7	100	17.3	17.5	16.5	18.7	17	16.4	16.9	18
PhySMV	62.9	36.9	30.2	37.5	38.9	63.4	37.1	37.3	41.1	60.1	62.6	36.9	38.5	28.6	53.4	56.1	28.7	97.5	39.4	36.8	34.8	100	46.2	30	59.1	50	46.9	56.9	59.4
PolRSV	53.1	41.1	32.4	42.5	42.2	52.5	41.4	43.1	41.3	48.8	52.2	41.9	42	28.4	79.1	70.5	28.7	54	40.7	42.1	33.4	54.1	100	30.4	42.8	44.4	85	40.9	41.7
SVNV	39.6	43	32.2	44	43.5	39.9	44.9	45.7	56.3	39.8	39.9	44.4	44	29	42	41.8	29.4	39.1	42.1	44.2	33.2	39.3	41.7	100	29.4	29.9	30.7	28.7	30.7
TneV	79.9	38.9	34.9	40.9	41.9	78.9	42.5	39.9	41.1	63.4	85.7	41.6	41.7	29.4	53.7	54.4	30	62.2	41.6	41.1	35.7	62.5	54.7	39.2	100	56.5	43.9	62.2	80.3
TNRV	54.7	41.1	31.7	42.2	43.3	54.8	42.4	41.5	39.6	51.9	53.6	42.5	43.2	27.4	78.9	74.3	27.6	54.8	40.9	41.1	33.2	55	81.6	40.9	54.1	100	46.8	54.8	54.4
TYRV	59.2	36.9	31.6	39	38.8	58.2	38.9	38	37.5	56.1	59.9	39.4	38.8	27.9	52.1	51	28.1	56.6	39	38.5	35	56.8	49.1	38.1	58.6	51.8	100	42.7	42.8
TZSV	64.2	35.6	32.2	36.2	39.3	63.2	38.5	37.7	39.1	78.8	64.3	38.7	39.1	28.6	48.4	51.5	29.3	62.7	38.9	38	34.2	62.2	49.8	39.1	63.3	50.6	56.7	100	62.9
WBNV	79.5	39	35.3	40.2	41	78.1	40.6	40.7	41.9	63.1	78	40.8	40.9	29.7	52.8	51.9	30.3	62.6	42.8	42.1	38.2	63.2	51.9	40.8	77.7	52.3	59.2	64.7	100
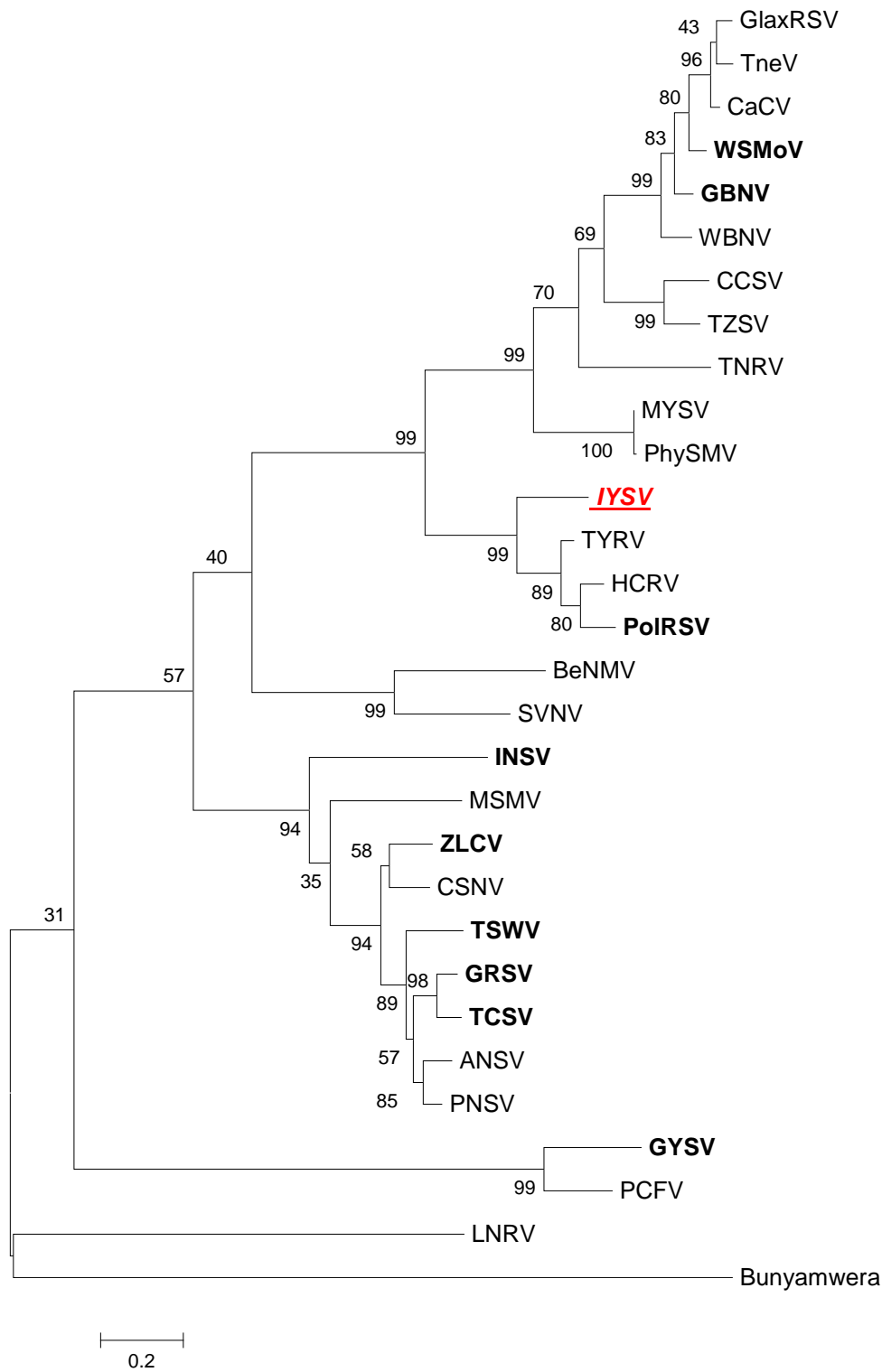
Fig 1: The evolutionary history was inferred using the Neighbor-Joining method [17]. The optimal tree with the sum of branch length = 7.13048396 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [7]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method [20] and are in the units of the number of amino acid substitutions per site. The analysis included amino acid sequences of the nucleopcapsid protein of 30 tospoviruses and one Bunyamwera as out-group of coat protein gene.

All positions containing gaps and missing data were eliminated. There were a total of 206 positions in the final dataset. Evolutionary analyses were conducted using MEGA5 [19]. Abbreviations and accession numbers were defined in Table 1. Currently accepted *Tospovirus* species are highlighted in bold face. Accession number for *Bunyamwera* coat protein gene is BAA00261.