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:	***2013.016aV***	(to be completed by ICTV officers)

Short title: A new species in the genus *Tospovirus* isolated from *Polygonum convolvulus*
(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:

Massimo Turina (m.turina@ivv.cnr.it), Marina Ciuffo(m.ciuffo@ivv.cnr.it), Davide Pacifico (davide.pacifico@igv.cnr.it), Luciana Tavella (luciana.tavella@unito.it), Lara Bosco (lara.bosco@unito.it)

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 Chair Elliott, Richard M., rme1@st-andrews.ac.uk

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

Supported by Study Group

Date first submitted to ICTV: 12 june 2013
Date of this revision (if different to above):

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	***2013.016aV***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Tospovirus***
Subfamily:	
Family:	***Bunyaviridae***
Order:	

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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Polygonum ringspot virus	EU271753; EF445397

Reasons to justify the creation and assignment of the new species:

In two recent publications (1,2) we have provided a rather exhaustive characterization of a Tospovirus isolate provisionally named Polygonum ringspot virus, and of its relationship with its thrips vector, *Dictyothrips betae*. For the first time, a tospovirus species was isolated from a natural host (*Polygonum convolvulus* Fig. 1) that is not relevant in agriculture, which is instead a common weed. The vector belongs to a thrips genus for the first time shown to transmit a tospovirus (Fig 1). The virus isolate was found in many locations in Italy. Here we summarize the main biological and molecular features that warrant its “new species” status in the genus Tospovirus.

Classification of tospoviruses is based on serological and molecular characterization of the nucleocapsid combined with biological characters, like host range and thrips vector species. Criteria for establishing new species in the genus Tospovirus suggest that isolates sharing between 80 and 90% identity in the N protein should be identified as “strains of existing species” or as “distinct species” depending on additional criteria. Alignment of the N protein sequence with those of 28 other tospoviruses shows highest score (86% and 83% respectively, See Table 1 and Table 2) with Hippeastrum chlorotic ringspot (HCRV) (3) and Tomato yellow ring virus (TYRV) (4) and phylogenetic analysis (Fig. 2) confirms the cluster of PolRSV with TYRV HCRV and IYSV. None of the isolates in this cluster (HCRV, TYRV and PolRSV) are currently accepted species in the genus *Tospovirus*. As for serological relationships, a weak cross reactivity occurs between PolRSV and TYRV both in DAS-ELISA and Western blot. Nevertheless in support of the “new species” status for PolRSV, a number of distinctive biological characters should be outlined: i)TYRV has a wide natural host range, whereas PolRSV is naturally restricted to two species of the genus *Polygonum* (1); ii) the host range obtained through mechanical inoculation is also distinct (1); iii) TYRV is transmitted by *Thrips tabaci*, a polyphagous vector possibly responsible for its wide natural host range; PolRSV is not transmitted by *T. tabaci*, but instead is transmitted by *Dicthyothrips betae*, a monophagous thrips species (2); iv) PolRSV has also another unique molecular feature among other existing tospovirus species: the intergenic region of the S segment (but not of the M segment) is unusually short (123 nt) and does not present the typical self-complementary A-U-rich region, constantly shown in all the Tospovirus species so far characterized and present in the M segment of PolRSV (Fig. 3).

For the above mentioned distinctive molecular and biological characters, we propose that PolRSV is a new *Tospovirus* species. These comparisons also suggest that TYRV will probably be recognized as a distinct species in the future.

As for the status of the recently identified HCRV tospovirus isolate (3) we suggest that further biological data on HCRV should be provided before deciding if it is indeed a new species or a strain of PolRSV or of TYRV.

- 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

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Ciuffo, M., Tavella, L., Pacifico, D., Masenga, V., and Turina, M. (2008). A member of a new Tospovirus species isolated in Italy from wild buckwheat (Polygonum convolvulus). Arch. Virol. 153:2059–2068.

2. Ciuffo, M., Mautino, G. C., Bosco, L., Turina, M., and Tavella, L. (2010). Identification of Dictyothrips betae as the vector of Polygonum ring spot virus. Ann. Appl. Biol. 157:299–307.

3. Dong, J.H., Yin, Y.Y, Mcbeath, J.H., Zhang, Z.K. (2013). A new tospovirus causing chlorotic ring spot on Hippeastrum sp. In China. Virus Genes 46:567-570.

4. Hassani-Mehraban, A., Saaijer, J., Peters, D., Goldbach, R., and Kormelink, R. (2005). A new tomato-infecting tospovirus from Iran. Phytopathology 95:852–858.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Fig.1

A) Symptoms of Polygonum ringspot virus on field *Polygonum convolvulus* from a natural infection transmitted by *Dictyothrips betae*

B) *Dictyothrips betae,* the vector species of Polygonum ringspot virus

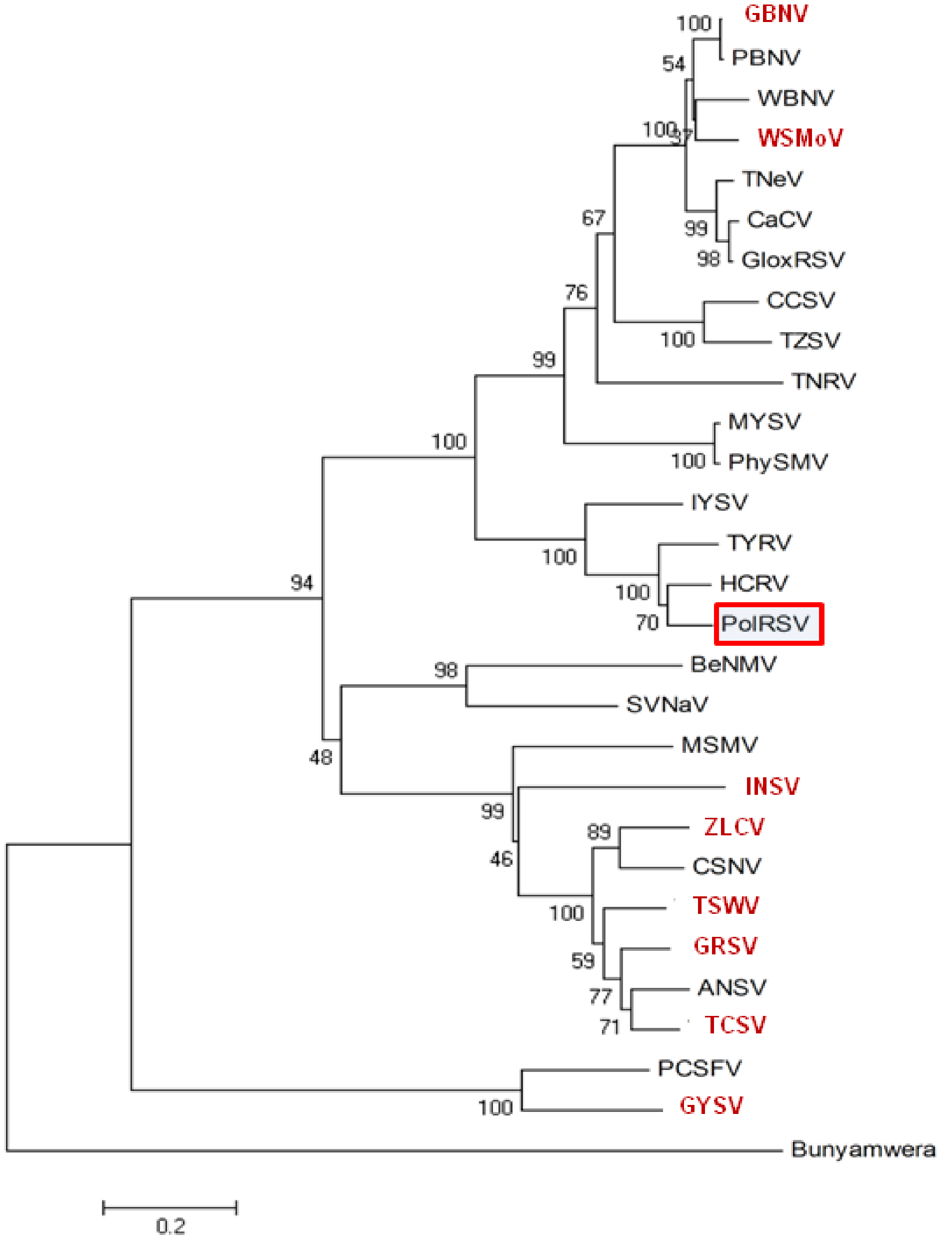
Table 1: Amino acid percentage identity of 29 aligned Tospovirus nucleocapsids

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1: Bunyamwera	100.00	13.30	14.75	16.52	12.50	15.62	14.16	12.61	14.10	14.10	13.22	12.33	10.57	11.45	11.45	13.22	13.22	11.01	11.89	13.39	14.80	16.14	15.70	17.94	19.28	15.25	16.59	13.51	15.25
2: PCSFV	13.30	100.00	62.96	16.48	18.70	18.32	19.85	19.85	18.66	19.40	20.08	21.21	20.45	20.83	21.21	20.75	20.75	19.55	19.48	19.92	24.10	22.04	22.45	23.27	23.27	22.27	22.27	19.69	20.00
3: PYSV	14.75	62.96	100.00	21.67	21.07	20.25	23.14	20.41	21.22	20.82	20.90	23.36	22.13	22.95	21.31	20.49	20.90	20.00	19.59	21.67	23.85	20.68	22.59	23.43	22.18	22.59	22.18	18.91	20.50
4: IYSV	16.52	16.48	21.67	100.00	67.65	66.91	65.19	45.72	51.48	51.11	44.98	48.33	47.58	47.58	44.24	46.30	46.30	48.51	47.21	30.16	33.99	35.18	33.47	33.47	33.47	31.62	32.81	32.42	34.50
5: TYRV	12.50	18.70	21.07	67.65	100.00	82.12	80.88	46.86	48.16	47.79	42.80	44.65	43.91	43.91	43.17	43.75	43.75	46.67	43.54	31.50	31.76	33.20	34.39	34.78	33.20	32.55	31.37	31.01	34.23
6: HCRV	15.62	18.32	20.25	66.91	82.12	100.00	84.19	46.86	48.53	48.53	42.80	44.65	43.91	43.17	42.80	43.75	43.75	44.44	42.80	28.74	33.33	32.41	33.20	32.81	30.83	32.94	31.76	34.11	36.15
7: PolRSV	14.16	19.85	23.14	65.19	80.88	84.19	100.00	45.96	47.79	47.43	41.91	42.65	41.54	41.54	41.54	42.28	42.28	43.75	42.28	30.31	32.16	32.41	34.39	33.99	33.60	33.33	32.16	33.46	34.35
8: TNRV	12.61	19.85	20.41	45.72	46.86	46.86	45.96	100.00	51.80	52.16	55.64	57.82	57.82	58.55	56.73	58.55	58.55	56.32	55.40	26.85	28.46	33.20	28.91	30.47	30.08	28.29	29.07	32.20	33.58
9: MYSV	14.10	18.66	21.22	51.48	48.16	48.53	47.79	51.80	100.00	98.92	58.18	58.55	57.82	57.45	57.09	59.06	59.06	57.04	57.55	27.45	32.17	29.92	29.13	30.31	28.74	29.69	30.86	30.68	33.21
10: PhySMV	14.10	19.40	20.82	51.11	47.79	48.53	47.43	52.16	98.92	100.00	58.18	58.18	57.82	57.09	57.09	59.06	59.06	57.40	57.91	27.84	32.17	29.92	29.13	30.31	29.13	30.08	31.25	30.68	33.58
11: WBNV	13.22	20.08	20.90	44.98	42.80	42.80	41.91	55.64	58.18	58.18	100.00	80.00	80.00	80.73	83.64	86.18	86.18	64.96	63.64	29.02	33.98	32.68	31.89	32.68	29.92	30.47	30.47	30.65	35.36
12: TNeV	12.33	21.21	23.36	48.33	44.65	44.65	42.65	57.82	58.55	58.18	80.00	100.00	92.36	93.45	85.82	85.45	85.82	62.77	62.91	30.20	33.20	35.83	31.89	33.46	32.28	32.03	32.42	29.50	33.84
13: CaCV	10.57	20.45	22.13	47.58	43.91	43.91	41.54	57.82	57.82	57.82	80.00	92.36	100.00	98.18	86.91	84.73	84.36	64.23	62.91	30.98	32.81	35.43	31.50	33.86	31.89	31.64	31.25	29.12	34.22
14: GloxRSV	11.45	20.83	22.95	47.58	43.91	43.17	41.54	58.55	57.45	57.09	80.73	93.45	98.18	100.00	86.91	85.82	85.45	63.50	62.91	30.59	33.20	35.43	31.50	33.86	31.89	31.64	31.25	29.12	34.22
15: WSMoV	11.45	21.21	21.31	44.24	43.17	42.80	41.54	56.73	57.09	57.09	83.64	85.82	86.91	86.91	100.00	88.00	87.64	63.14	62.18	30.59	34.77	33.86	32.28	33.86	30.71	32.03	31.64	30.27	33.46
16: GBNV	13.22	20.75	20.49	46.30	43.75	43.75	42.28	58.55	59.06	59.06	86.18	85.45	84.73	85.82	88.00	100.00	99.28	65.33	63.27	29.80	34.38	33.46	32.68	33.07	31.10	32.81	32.03	29.50	34.98
17: PBNV	13.22	20.75	20.90	46.30	43.75	43.75	42.28	58.55	59.06	59.06	86.18	85.82	84.36	85.45	87.64	99.28	100.00	65.69	63.27	29.41	34.38	33.46	32.68	33.07	31.10	32.81	32.03	29.89	35.36
18: CCSV	11.01	19.55	20.00	48.51	46.67	44.44	43.75	56.32	57.04	57.40	64.96	62.77	64.23	63.50	63.14	65.33	65.69	100.00	79.42	25.49	29.84	29.53	29.92	29.13	27.56	28.52	28.52	29.92	33.58
19: TZSV	11.89	19.48	19.59	47.21	43.54	42.80	42.28	55.40	57.55	57.91	63.64	62.91	62.91	62.91	62.18	63.27	63.27	79.42	100.00	23.92	28.29	30.31	27.56	29.13	27.17	26.95	27.73	29.17	33.58
20: INSV	13.39	19.92	21.67	30.16	31.50	28.74	30.31	26.85	27.45	27.84	29.02	30.20	30.98	30.59	30.59	29.80	29.41	25.49	23.92	100.00	52.33	55.47	54.69	53.91	53.52	52.71	54.65	36.90	34.78
21: MSMV	14.80	24.10	23.85	33.99	31.76	33.33	32.16	28.46	32.17	32.17	33.98	33.20	32.81	33.20	34.77	34.38	34.38	29.84	28.29	52.33	100.00	57.36	59.69	59.69	59.30	59.62	60.77	37.65	37.50
22: TSWV	16.14	22.04	20.68	35.18	33.20	32.41	32.41	33.20	29.92	29.92	32.68	35.83	35.43	35.43	33.86	33.46	33.46	29.53	30.31	55.47	57.36	100.00	79.30	78.91	80.47	72.87	76.74	37.45	36.90
23: ANSV	15.70	22.45	22.59	33.47	34.39	33.20	34.39	28.91	29.13	29.13	31.89	31.89	31.50	31.50	32.28	32.68	32.68	29.92	27.56	54.69	59.69	79.30	100.00	80.62	82.17	76.36	74.81	37.85	38.10
24: GRSV	17.94	23.27	23.43	33.47	34.78	32.81	33.99	30.47	30.31	30.31	32.68	33.46	33.86	33.86	33.86	33.07	33.07	29.13	29.13	53.91	59.69	78.91	80.62	100.00	84.88	76.36	73.26	36.65	36.90
25: TCSV	19.28	23.27	22.18	33.47	33.20	30.83	33.60	30.08	28.74	29.13	29.92	32.28	31.89	31.89	30.71	31.10	31.10	27.56	27.17	53.52	59.30	80.47	82.17	84.88	100.00	75.58	74.42	37.85	37.30
26: ZLCV	15.25	22.27	22.59	31.62	32.55	32.94	33.33	28.29	29.69	30.08	30.47	32.03	31.64	31.64	32.03	32.81	32.81	28.52	26.95	52.71	59.62	72.87	76.36	76.36	75.58	100.00	79.62	37.94	37.80
27: CSNV	16.59	22.27	22.18	32.81	31.37	31.76	32.16	29.07	30.86	31.25	30.47	32.42	31.25	31.25	31.64	32.03	32.03	28.52	27.73	54.65	60.77	76.74	74.81	73.26	74.42	79.62	100.00	38.74	37.01
28: BeNMV	13.51	19.69	18.91	32.42	31.01	34.11	33.46	32.20	30.68	30.68	30.65	29.50	29.12	29.12	30.27	29.50	29.89	29.92	29.17	36.90	37.65	37.45	37.85	36.65	37.85	37.94	38.74	100.00	54.10
29: SVNaV	15.25	20.00	20.50	34.50	34.23	36.15	34.35	33.58	33.21	33.58	35.36	33.84	34.22	34.22	33.46	34.98	35.36	33.58	33.58	34.78	37.50	36.90	38.10	36.90	37.30	37.80	37.01	54.10	100.00

Tab 2. GenBank accession numbers of the nucleocapsid amino acid sequences used for the alignment and phylogenetic analysis: in italics accepted species in the genus *Tospovirus*; *Bunyamwera virus* accession number is also provided as outgroup in the tree

Species	Acc. Number
Groundnut bud necrosis virus (GBNV)	AAR24021
Groundnut ringspot virus (GRSV)	AAB25255
Groundnut yellow spot virus (GYSV)	AAB94022
Impatiens necrotic spot virus (INSV)	AAA47944
Tomato chlorotic spot virus (TCSV)	AAG23654
Tomato spotted wilt virus (TSWV)	BAA03025
Watermelon silver mottle virus (WSMoV)	AAW64930
Zucchini lethal chlorosis virus (ZLCV)	AAF04198
Alstromeria necrotic streak virus (ANSV)	ACZ18222
Bean necrotic mosaic virus (BeNMV)	AER23986
Calla lily chlorotic spot virus (CCSV)	AAW58115
Capsicum chlorosis virus (CaCV)	ABC86907
Chrysanthemum stem necrosis virus (CSNV)	AAF04197
(Gloxinia tospovirus) GloxRSV	AAQ83791
Hippeastrum chlorotic ringspot virus (HCSV)	AGC54619
Iris yellow spot virus (IYSV)	AAF75556
Melon severe mosaic virus (MSMV)	ABX72231
Melon yellow spot virus (MYSV)	BAB79455
Peanut bud necrosis virus (PBNV)	AAM76043
Peanut chlorotic fan-spot virus (PCSFV)	AAC99405
Physalis severe mottle virus (PhySMV)	AAD34201
Polygonum ringspot virus (PolRSV)	ABO31117
Soybean vein necrosis virus (SVNaV)	ADX96066
Tomato necrosis virus (TNeV)	AAT68025
Tomato necrotic ringspot virus (TNRV)	ACK99533
Tomato yellow (fruit) ring virus (TYRV)	ABF59486
Tomato zonate spot virus (TZSV)	YP_001740044
Watermelon bud necrosis virus (WBNV)	ABD39046
Bunyamwera virus	NP_047213

Fig.2



Evolutionary relationships of 29 tospovirus taxa

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 7.26042723 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 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 and are in the units of the number of amino acid substitutions per site. There were a total of 210 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [2]. In red accepted species in the genus

1. Saitou N & Nei M (**1987**) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406-425.
2. Tamura K, Dudley J, Nei M & Kumar S (**2007**) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**:1596-1599.

Fig 3

A

PolRSV S IR 5’-
TTGTGTCTACAACTTGCTGCTGCAAGTTT
GTGTGAGTGTTAGTGCTGTGCTGCAGTTA
TGTTAAGTTAAGTTATTTTAGTAATTACT
TAAGGTATTGTT**AAATAAAATAAATAAAA**
ACAAATAAAAACTAATAAAATAAAAACAC
AAAAAAAGATAAAGTTAAAGCAAGCATTA
AGAAATCATTCA
-3’

B

IR medium segemnet 5’-
TTTCCTTTTCTTGTCATTTAAGCTTTATCGCAA
AAT**AAAACAAAAAAAGAAAACAACAAAAAAAAGAG**
GAAAAGAAAATAAAACAAAAGAAAAAAAGAGAAA
AGAAAAGAAAAGAAAAGAAAAAAAGAAGGAATGT
ATATAAATAAGGCCAAGGCCAACCTTGGCCTTT
TGGGGCTC**TTTTTGGGTTTTGTTTGTTTTATTT**
ATTTTTATTTCTTTTTGTTTGTTTATTTGTTTG
TCTCCTTTAACTTGT-3’

C

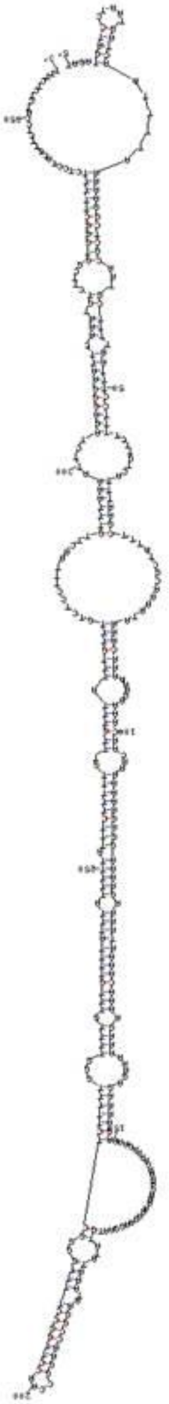


Fig. 3. A) Sequence of PolRSV Intergenic Region of small segment. In bold and underlined the series of A. B) sequence of PolRSV Intergenic Region (IR) of medium segment. In bold and underlined the series of A and T, able to form the hairpin structure. C) secondary structure of IR of medium segment calculated with the software M Fold.